EVOLUTION

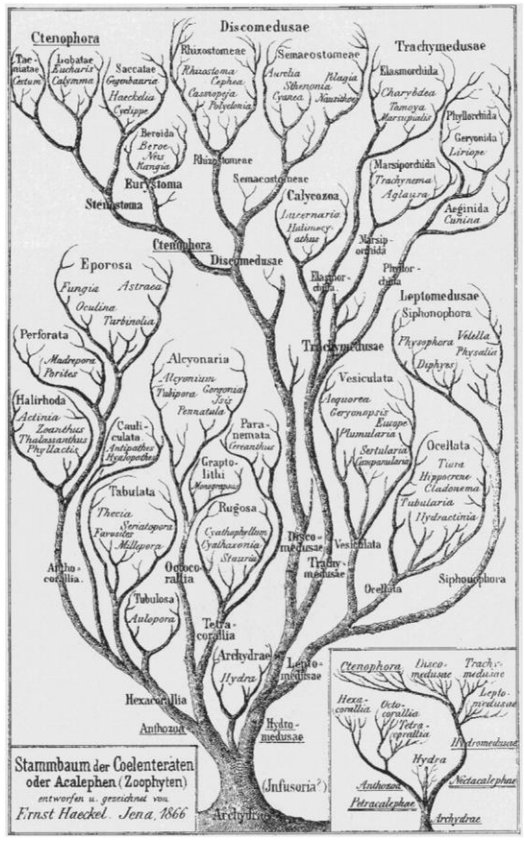
Biology Recapitulates Phylogeny

David M. Hillis

Shortly after the publication of Darwin's *The Origin of Species*, biologists were enamored with the concept of phylogeny. In 1866, Ernst Haeckel (who coined the term "phylogeny") published a collection of detailed phylogenetic trees that depicted much of what was known about the evolutionary history of life (*1*). But over much of the next century, biologists' interest in phylogeny was gradually replaced by new emphases on the processes and mechanisms of genetics, physiology, development, and evolution. By the 1940s and 1950s, study of phylogeny had greatly diminished, even in evolutionary biology. Three papers in this issue (on pages 227, 253, and 256) illustrate the complete reversal of this trend over the past few decades and emphasize the key role of phylogenetic analysis in comparative biological studies of every description.

The reemphasis on phylogenetic perspectives in biology began in the 1960s and 1970s, with the accumulation of new phylogenetic data (especially from molecular biology), the development of explicit and objective methods for phylogenetic inference, and the construction of computer hardware and software sufficient to the task of applying the new methods to the new data (*2*). As phylogenetic analyses became commonplace in the 1980s, several groups [for example (*3*)] emphasized what should have been obvious all along: Units of study in biology (from genes through organisms to higher taxa) do not represent statistically independent observations, but rather are interrelated through their historical connections. Therefore, almost any comparative statistical analysis in biology requires information on phylogeny if we are to interpret (rather than simply describe) the results. Suddenly, phylogenetic analyses are everywhere in biology, with new applications appearing all the time.

One area of recent advancement has been the rapid development of statistical approaches to evaluate and incorporate phylogenetic information. Huelsenbeck and Rannala (*4*) discuss new applications of likelihood-ratio tests in phylogenetic analysis. These tests represent a family of approaches for comparing alternative phylogenetic hypotheses, as well as for testing the veracity and robustness of evolutionary models (especially of DNA substitution) in a phylogenetic context. These approaches have been used to examine problems as varied as the probability of multiple infection of individuals with human immunodeficiency virus, to estimating the extent of coevolution of host-parasite systems, to comparing competing hypotheses about the age and origin of life on Earth.



Original phylogeny. In 1866, Ernst Haeckel coined the word "phylogeny" and presented phylogenetic trees for most known groups of living organisms. His tree of coelenterate relationships is shown here.

For most phylogenetic applications, statistical evaluation of the significance of likelihood ratios requires generation of expected distributions through parametric bootstrapping (*5*). This technique consists of the simulation of replicate data sets based on a model of evolution derived from the original data. These simulated replicates are then used to estimate the expected distributions of a test statistic of interest (such as a likelihood ratio). Huelsenbeck and Rannala demonstrate that such likelihood-ratio tests have considerable power for a wide range of applications in phylogenetics. However, the parametric bootstrap approach is quite general, and if expected distributions are generated in this manner, there is no reason that the tests need be limited to evaluation of likelihood ratios. Similar evaluations of virtually any other measures of goodness-of-fit (such as those derived from parsimony or minimum evolution analyses) are also possible. Such extensions are likely to relieve the computational burden that prevents the application of likelihood-ratio tests to highly complex phylogenetic problems. This is especially important because the trend in phylogenetic analysis is toward the analysis of very large data sets, such as hundreds or thousands of DNA sequences, each of which typically are thousands of nucleotides in length (*6*).

The demand for new statistical tests is driven by new applications of phylogenies. For instance, Pierce and Crawford (*7*) demonstrate how information on phylogenetic relationships can be used to tease apart alternative explanations for metabolic regulation by enzyme concentrations. One of several competing models holds that the activity of a single enzyme is used to control a given metabolic pathway, whereas other theories suggest that the activity of many enzymes works synergistically to control metabolism (*8*). If all species had evolved independently, it would be straightforward to ask if the concentration (a parameter that affects activity) of one or many enzymes covaried with temperature (or some other environmental variable closely related to metabolic rate) across species. However, environmental temperature and enzyme concentrations both also covary to some extent with phylogeny, so the evolutionary nonindependence of the sampled species must be taken into account. Pierce and Crawford use two different methods to accomplish this task and find that although much of the covariation among enzyme concentrations across species can be explained by phylogenetic constraints, the correlations between the concentrations of several enzymes and environmental temperature are far greater than can be explained by phylogenetic relationships alone. Thus, their data support the view that many enzymes work

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together to help modulate metabolic flux.

Phylogeny is also an integral part of the interpretation of any coevolutionary system, such as host-parasite or large-term symbiotic interactions. For instance, in the coevolution of a group of insects and their host plants, the plants evolve chemical defenses against insects, and the insects evolve resistance to the defenses. Just as the Red Queen in Lewis Carroll's *Alice Through the Looking Glass* had to keep running just to stay in the same place, so too do the plants and insects have to keep evolving new defenses or ways of coping with the new defenses just to stay even in the evolutionary race against each other (*9*). However, since the universe of possible defensive compounds is limited, many different plants may evolve similar chemical defenses, so much parallelism and convergence is expected in the plants' defensive systems. Are the insects that feed on the plants more likely to track a lineage of plants through time as it evolves new defenses, or will they "cheat" in the race by switching to a related host plant that contains chemical compounds to which they are already adapted? Becerra (*10*) asked this question of a group of beetles that specialize on the strongly aromatic plants of the genus *Bursera*. If the beetles coevolve with the plants as the plants evolve new chemical defenses, then the phylogeny of the beetles would be expected to match the phylogeny of the plants. On the other hand, if the beetles switch hosts to take advantage of their existing resistance to particular chemical defenses, then the beetle phylogeny would be expected to show a closer match to the plants' chemistry than to their phylogeny. Becerra found significant congruence between the beetle phylogeny and the plant chemistry, but not between the beetle phylogeny and the plant phylogeny. Thus, it appears that the beetles would rather switch than fight when it comes to coping with their host plants.

These few examples sample only a small range of the recent applications of phylogenetic analyses. Phylogenetic analyses have become increasingly important in studies of human diseases: for epidemiological investigations (*11*), for identifying and characterizing newly discovered pathogens (*12*), and for identifying and tracking natural reservoirs of zoonotic diseases (*13*). Recently, phylogenetic analyses have been found admissible as evidence in a criminal court case involving an alleged purposeful viral transmission (*14*). Phylogenetic analysis of molecular sequences is also one of the principal interpretive tools for understanding the organization and evolution of genes and genomes (*15*). Behavioral ecologists have used phylogeny to reconstruct and study the evolution of behaviors (*16*). At the same time, phylogeny has solidified its more traditional role as the criterion for organizing and classifying life (*17*). One can only wonder if Darwin and Haeckel would have ever believed that the fruits of their ideas would come to all of this.

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CELL BIOLOGY

Journey Across the Osteoclast

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Despite its persistence after death, the living vertebrate skeleton is a dynamic enterprise. Bone is continuously forming and being resorbed, starting in the embryo and continuing throughout adult life (*1*). This process is accomplished by precise coordination of two cell types: osteoblasts, which deposit the calcified bone matrix, and osteoclasts, which resorb it. Osteoclasts are large, multinucleated cells that are derived from the same hematopoietic precursor as macrophages (*2*). As in most animal cells, the osteoclast plasma membrane is divided into multiple domains (*3*). One of these, the ruffled border, faces the bone surface (see figure, left panel) and is surrounded by a sealing zone, which forms a tight seal against the bone surface. At the ruffled border, the osteoclast secretes acid and lysosomal enzymes that digest the mineral and protein components of the underlying bone (*4*). A leakproof seal is required to maintain the low pH in the compartment next to the bone, but this presents a disposal problem for the cell—how to remove the soluble degradation products of bone? Now in this issue, Nesbitt and Horton on page 266 (*5*) and Salo *et al.* on page 270 (*6*) show that degraded bone proteins and inorganic matrix components are transcytosed in vesicles to the free surface of the osteoclast opposite the ruffled border and released.

The best-known examples of polarized membrane domains are the apical and basolateral surfaces of epithelial cells (see figure, right panel) (*7*). Proteins reach these surfaces by two pathways. Newly made proteins can travel from the trans-Golgi network directly to the apical or basolateral surface. Alternatively, proteins can reach one surface, generally the basolateral, and then be endocytosed and transcytosed to the opposite surface. Transcytosis is found universally in all epithelial cells examined to date and in some epithelial cells is the only pathway for apical delivery of proteins.

It was once thought that the osteoclast's ruffled border corresponds to the apical plasma membrane domain of epithelial cells and that the free surface is the basolateral

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