The term preadaptation is inappropriate. A preadaptation is an adaptation for a role earlier in time (e.g., early tetrapod forelimbs for terrestrial locomotion) that would in future be coopted by selection for a different role in a descendant lineage (digging in moles). This is similar to an exaptation except that exaptations need not have been adaptations in their original role—they may have been nonaptations. Preaptation does not apply to the coopted character in its new role, nor does it encompass the possibility that nonaptations may also be coopted by future selection regimes.

Exaptations may be more important in the history of life than commonly appreciated. There may be redundant genetic material, the products of which can be exapted into novel roles. Numerous examples of regulatory elements and genes with new roles have been identified. At the level of phenotypes, Darwin foresaw that "almost every part of each living being has probably served, in a lightly modified condition, for diverse purposes, and has acted in the living machinery of many ancient and distinct specific forms" (Jacob, 1983, p. 131).

Current methods for testing hypotheses of adaptation involve analysis of the phylogenetic context and comparisons of the locations in the phylogeny of the changes in characters, in selective regimes, and in functional capabilities.

[*See also* Adaptation; Evolution; Natural Selection.]

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— ELISABETH S. VRBA

EXONS. *See* Genes; Introns

EXPERIMENTAL EVOLUTION

[This entry comprises two articles. The first article provides a summary overview contrasting experimental and comparative/historical approaches for studying evolution; the second article provides a case study of an evolutionary experiment in which populations of E. coli *have been propagated and monitored for 20,000 generations. For related discussions, see* Adaptation; Artificial Selection; Bacteria and Archaea; Comparative Method; Fitness; Genetic Drift; Natural Selection; *and* Senescence.]

An Overview

Experimental evolution is a scientific method in which populations of organisms are introduced into novel environments, and changes within those populations are then observed over many generations. In other words, it is a method for directly observing evolutionary change, including adaptation.

Experimental evolution is an alternative to the most common method for studying evolutionary adaptation, the comparative or historical approach. [*See* Comparative Method.] The latter examines characters in organisms from different natural environments and tries to understand the pattern of change that the descendants of a common ancestor might have followed in diversifying into their current forms. In essence, the comparative approach attempts to look backwards in time, given information about the current diversity of organisms. It necessarily has to make many assumptions about phylogenetic relationships among groups and the probable course of evolution.

In contrast, the experimental method creates an ancestral population and then watches, in real time, adaptation and diversification of descendant populations in one or more environments. It does not make assumptions about evolution, or constraints on it, but rather observes the adaptive solutions that appear within the evolving populations. The two approaches are complementary, each with its strengths and limitations. The comparative approach investigates evolution in populations of all kinds of organisms in the natural world, which is full of complexity and compromise. Experimental evolution is limited to strictly controlled conditions in the laboratory and is feasible for only certain types of organisms. However, this approach permits the application of the experimental scientific method to evolutionary studies, including control, replication, and repeatability. The comparative method attempts to understand events that are unique, at least in certain respects, which imposes limitations and assumptions on the application of statistical tests to comparative data. The two methods provide different insights into evolutionary adaptation to the environment.

An evolutionary experiment might proceed in the following way. A large population of organisms is created and placed in a controlled environment in the labora-

tory. It is important that a great number of individuals (certainly hundreds, preferably millions) be used, to limit the establishment of particular alleles (alternative forms of the same gene) by chance alone. [*See* Genetic Drift.] This population is then kept under these conditions for many generations, permitting adaptation to the general laboratory conditions. After this initial period this population is then used as the ancestral population for the experiment itself. To that end, the ancestral population is randomly divided into experimental (selected) and control groups of populations, each of which is replicated ideally several times. The control populations are kept under the same conditions in which the ancestral population evolved; they serve to indicate the direction and amount of evolutionary change that can be attributed to further laboratory adaptation. The selected populations are placed in one or more novel environments, in which a single variable of interest (e.g., temperature, water or nutrient availability, breeding time, amount or duration of light exposure) is altered and all other conditions are maintained identical to those of the ancestral population. Both the selected and the control populations are then permitted to evolve, preferably for hundreds or even thousands of generations.

Types of Evolutionary Experiments. There are several forms that selection in an evolutionary experiment might take. The first is laboratory natural selection, in which replicated populations are exposed to a novel environment and changes within the populations over many generations are measured and analyzed, as described above. The experimenter provides the environment but does not otherwise directly select any character or choose individuals for differential breeding. The populations are left to their own devices to evolve solutions to the environmental challenge. The second variety is artificial truncation selection, in which only organisms possessing certain characters (or extreme character values) are permitted to reproduce the next generation. [*See* Artificial Selection.] This artificial selection is the familiar form used in animal and plant breeding, and it can sometimes quickly result in the creation of new types of organisms (e.g., breeds of dogs). However, by imposing a single desirable factor or set of factors, it may constrain the pathways along which evolution can proceed to solve a more general problem. The third approach is laboratory culling. In this design, a more extreme environmental condition (e.g., high temperature) is imposed every generation, and only a small proportion of the population is permitted to survive to reproduce the next generation. This type of selection has elements of the other two designs. It permits only a small fraction of the population to breed, in contrast to most laboratory natural selection protocols. However, it does not specify that survivors must possess certain characteristics, as does artificial truncation selection; consequently, a diversity of pathways might be used in evolving solutions to the environmental challenge.

Though not absolutely necessary, it is very desirable that two basic measurements can be undertaken during an evolutionary experiment: ancestral comparison and relative fitness. For some types of organisms, the ancestral population can be preserved in a condition (typically frozen) from which it can be revived and compared directly to its descendants, both selected and control populations. Such comparisons permit a direct determination of the type and amount of evolutionary change that the descendants have undergone. In addition, preservation of samples of the selected populations during the course of the experiment allows later analysis of the time of appearance of novel adaptive traits. It is also highly useful to be able to measure the fitness of the descendant populations relative to their ancestor in both the novel and ancestral environments. [*See* Fitness.] Fitness can be measured by placing both the ancestral and a selected (or control) population in a common environment and measuring the number of offspring produced by each. A readily scored, but preferably neutral, genetic marker is often used to distinguish the ancestral and derived types. Differential reproduction serves as a quantitative measure of the adaptive improvement of an evolving population and is the single best measure of evolutionary adaptation.

Obviously, such evolutionary experiments cannot be undertaken on all kinds of organisms. Large organisms with long generation times are not feasible objects for such studies. Organisms that reproduce rapidly and can be grown easily in large numbers in the laboratory are the best subjects. Viruses or unicellular organisms such as bacteria, protists, and yeasts are ideal for experimental evolution, not only because of their short generation times and large population sizes in culture, but also because many are well characterized genetically. In such organisms, it may be possible to determine the exact genetic alterations that underlie adaptive change. Evolutionary experiments can also be undertaken on multicellular animals such as fruit flies and even mice, but these are more challenging because of the difficulty in maintaining large population sizes and many replicated populations.

Advantages of Experimental Evolution. Part of the utility of experimental evolution is the production of biological novelty, literally building a better mouse for some particular environmental condition. The creation of lineages of organisms with known evolutionary and environmental histories provides a valuable resource for future study. It may even be possible to analyze the genetic and functional bases underlying the observed adaptive changes. An issue of great interest in that regard is the degree of repeatability in evolution, that is, whether certain genetic changes would occur repeat-

edly in similar populations adapting to a novel environment or whether there are a large number of potential mechanisms of adaptive change. The replicated populations within an experimental group provide a means of examining the number of potential adaptive solutions to a common environmental condition and whether the same changes occur repeatedly.

Another advantage of this method is that it provides an experimental way to test general evolutionary assumptions and predictions. It is frequently assumed, for instance, that adaptive gains in a new environment are accompanied by a decline in function in other environments. In evolutionary experiments, it is possible to test for such "trade-offs" directly: a common assumption is thereby turned into a testable hypothesis. The importance of having replicated populations within each experimental group is that each population can be considered an independent observation in testing the hypothesis. Standard statistical methods can thus be applied in the analysis of the experiment, and the evolutionary hypothesis can be rejected or provisionally supported by the observations. Experimental evolution permits evolutionary studies to move beyond retrospective comparative analyses.

Review of Studies. There are many examples of experimental evolutionary studies, and only a few can be mentioned here; more are detailed in Bell (1997) and Bennett and Lenski (1999). In regard to testing evolutionary theory, a classic series of studies by Michael Rose and his coworkers (1990) examined predicted trade-offs between early reproduction and longevity. Using populations of fruit flies, and selecting some for early reproduction and preventing others from reproducing until later in life, they found that life span increased significantly in the latter group, as predicted by theory. Interestingly, and unanticipated, the longer-lived populations also evolved greater resistance to some types of environmental stress, including desiccation and starvation. When selection for delayed reproduction was removed, longevity decreased, as did stress resistance. These studies have greatly influenced thinking concerning aging and its evolutionary and physiological bases.

Another study of particular interest is that of J. Swallow and colleagues (1998), who selected populations of mice on the basis of their voluntary running behavior. Mice that ran the most in cage wheels were bred to similar mice, and these lineages were compared to controls that were bred at random. After ten generations, voluntary running behavior approximately doubled. Maximum oxygen consumption in the selected mice also increased at the same time. From a mechanistic viewpoint, it is now possible to examine which portions of the oxygen transporting and utilization systems responded to this selection and whether these changes were consistent across selected populations. These populations can also be used to test more general evolutionary theories concerning performance trade-offs, models for the evolution of endothermy, and constraints on organismal design.

In a study on the repeatability of evolution, Jim Bull and coworkers (1997) examined the adaptation of replicated lineages of a bacteriophage (virus that attacks bacteria) to a moderately high and stressful temperature, which greatly inhibited its growth rate. During adaptation, growth rates improved as much as four thousand fold. Because a bacteriophage has a small genome, it was possible to analyze the DNA of each of the different replicates; it was found that approximately half of the changes that occurred were identical in more than one of the lineages. These experiments indicate that the same evolutionary changes may appear repeatedly in a population exposed to a novel environmental stress.

In a final example, Mike Travisano and colleagues (1995) performed an experiment to examine the relative roles of adaptation, chance, and historical contingency in shaping phenotypic diversity among populations adapting to similar environments. Bacterial populations were permitted to evolve in two different environments, a novel sugar nutrient and low temperature. These populations initially differed among themselves in relative fitness and cell size. During evolution, relative fitness improved in both novel environments, and differences in fitness among populations were greatly decreased as a result of adaptation. The remaining differences attributable to the initial fitness condition (history) and chance divergence in fitness were very small. In contrast, chance and history were far more significant factors influencing the final diversity of cell size among the populations. These results indicate that historical condition and chance may have longer lasting influences on characters that do not strongly affect fitness, such as cell size, than on fitness itself.

[*See also* Adaptation; Natural Selection, *article on* Natural Selection in Contemporary Human Society; Senescence.]

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— ALBERT F. BENNETT

A Long-Term Study with *E. coli*

The paleontologist Stephen Jay Gould envisioned a thought experiment of "replaying life's tape" to explore the predictability, or repeatability, of evolution. Gould (1989) argued that evolution is not repeatable: "Any replay of the tape would lead evolution down a pathway radically different from the road actually taken . . . no finale can be specified at the start, and none would ever occur a second time in the same way, because any pathway proceeds through thousands of improbable stages." No one can replay evolution on the vast scale imagined by Gould. But on a much smaller scale, the following experiment examines the same issue by monitoring multiple populations, all founded from the same ancestor, as they evolve in identical environments for thousands of generations.

Using a homogeneous clone of the bacterium *Escherichia coli*, Richard Lenski started twelve populations in 1988. These populations have been propagated side by side for more than 20,000 cell generations in a simple, glucose-limited environment in the laboratory. This environment is a novel one for the bacteria, insofar as it differs in important respects from the conditions that have prevailed during their evolution in nature. Consequently, there is considerable scope for the bacteria to improve their functioning in the laboratory environment. Meanwhile, cells of the ancestral strain were stored in a nonevolving state (in a deep freezer), and samples from the evolving populations likewise have been stored at periodic intervals.

By simultaneously reviving ancestral and derived cells from the freezer, one can compare them directly to measure the evolutionary changes that occurred during the experiment. Changes in ecological, physiological, and genomic properties have been quantified in this manner. Using a genetic marker that allows two strains to be distinguished when they are mixed, it is possible even to compete the derived cells against their ancestor, and thereby measure their relative fitness. In effect, this approach is comparable to resurrecting fossil hominids such as Neanderthals—not merely their bones or even DNA, but actual beings—and comparing their performance capacities with those of modern humans.

Every day, the populations have been diluted 100-fold into fresh medium. The population size, after the cells have exhausted the daily supply of glucose, is about 3×10^8 individuals. The particular strain of *E. coli* used in this experiment lacks viruses and plasmids (which can promote intergenomic recombination in bacteria). Hence, these experimental populations are completely asexual, and spontaneous mutation provides the only source of genetic variation. The total mutation rate for *E. coli* is about 3×10^{-3} mutations per genome replicated (for DNA repair–proficient replication). This rate, coupled with the population size, implies that each population has had almost a million mutations every day. Mutation therefore generates abundant variation on which natural selection and genetic drift can act.

During 20,000 generations, the competitive fitness of the derived bacteria improved by about 75 percent on average, relative to their common ancestor (see Figure 1). The rate of improvement was much greater early in the experiment than later on; as the populations became better adapted to the experimental environment, further gains became progressively slower. Notice also that the populations evolved along roughly similar, though not identical, fitness trajectories. Thus, with respect to competitive performance, evolution appears to have been fairly repeatable.

The results in the preceding paragraph were based on competition assays under conditions identical to those that prevailed during the experimental evolution. Additional competitions were run in other environments, in which different resources (such as maltose) were substituted for glucose (the only sugar provided during the evolution experiment). The fitness of the derived lines relative to their ancestor tended to be lower in these "foreign" environments than in their "home" environment. The multiple derived lines were also more heterogeneous (diverse) in their response to foreign environments than in their performance in the glucose-limited environment in which they evolved. This diversity in foreign environments indicates that the twelve populations had diverged physiologically and genetically, despite their similarity in the selective environment.

The populations changed relative to their ancestor, and diverged phenotypically from one another, in other aspects as well. All twelve evolved lines produced larger individual cells than did the ancestor, but the extent of