Evolution, Experimental

Richard E. Lenski
Michigan State University

Glossary

Adaptation Match between a particular feature of an organism and its environment, which results from natural selection

Evolution Change in the genetic properties of populations and species over generations, which requires the origin of variation (by mutation and/or mixis) as well as the subsequent spread or extinction of variants (by natural selection and/or genetic drift)

Fitness Average reproductive success of a genotype in a particular environment, usually expressed relative to another genotype

Genetic drift Changes in gene frequency caused by the random sampling of genes during transmission across generations, rather than by any detrimental or beneficial effects of those genes

Mixis Production of a new multilocus genotype by recombination of genes from two sources

Natural selection Changes in gene frequency caused by specific detrimental or beneficial effects of those genes

Population Group of individuals belonging to the same species and living in close proximity, so that individuals may potentially recombine, compete for limiting resources, or otherwise interact

EXPERIMENTAL EVOLUTION is the study, in the laboratory, of the fundamental processes of evolutionary change. These processes include spontaneous mutation and adaptation by natural selection, and they give rise to various patterns of genetic diversity within and between populations. Microorganisms have proven to be useful subjects for this research as a consequence of their large population sizes and short generation times, the ease with which their environments and genetic systems can be manipulated, and other desirable properties. Experimental studies of microbial evolution have generally confirmed the basic principles of modern evolutionary theory, while also providing new insights into the genetics, physiology, and ecology of microorganisms.

I. Review of Evolutionary Theory

Evolutionary theory seeks to explain the patterns of biological diversity in terms of a relatively few fundamental evolutionary processes. These processes are presumed not only to have operated in the past, but also to continue to operate today. Therefore, they can be studied by direct experimentation in the laboratory. Before discussing experiments that have used microorganisms to examine evolutionary processes, the major elements of evolutionary theory will be reviewed.

A. Evolutionary Patterns

Three of the most conspicuous products of organic evolution are (1) the wealth of genetic variation that exists within almost every species, (2) the apparent divergence of populations and species from one another and from their common ancestors, and (3) the manifest adaptation, or fit, of organisms to the environments in which they live.

1. Genetic Variation

The existence of extensive genetic variation within species has been demonstrated by a variety of means. Variation in certain traits, such as seed shape in pea plants and blood type in humans, can be

Encyclopedia of Microbiology, Volume 2 J. Lederberg, ed.

shown to have a genetic basis by careful examination of pedigrees. For many other traits, such as milk production in cows or body weight in humans, quantitative genetic analyses are required to partition the phenotypic variation that is due to genetic versus environmental influences. Biochemical and molecular techniques have also revealed extensive variation in DNA sequences and the proteins they encode.

2. Divergence and Speciation

All biological species differ from one another in some respects. It is generally possible to arrange species hierarchically, depending on the extent and nature of their similarities and differences. This hierarchy is reflected in the taxonomic classification scheme of Linnaeus (species, genus, family, and so on). This hierarchical arrangement also suggests a sort of "tree of life," in which the degree of taxonomic relatedness reflects descent with modification from some common ancestor in the more or less distant past.

Investigating the origins of particular traits and the relationships of taxa requires an historical approach, which is not amenable to direct experimentation. Even so, historically based hypotheses can often be tested using phylogenetic and comparative methods, which utilize data on the distribution of character states across various taxa and environments, sometimes supplemented with information from the fossil record or biogeography.

The extent of evolutionary divergence that is necessary for two groups of organisms to be regarded as distinct species is embodied in the biological species concept, according to which "species are groups of actually or potentially interbreeding populations, which are reproductively isolated from other such groups" (E. Mayr, 1942, "Systematics and the Origin of Species," Columbia University Press). Speciation thus refers to the historical events by which groups of organisms have become so different from one another that they no longer can interbreed. However, many organisms (including most microorganisms) reproduce primarily or exclusively asexually, and the preceding species definition is not applicable. For such organisms, the extent of evolutionary divergence that corresponds to distinct species is somewhat arbitrary and often more a matter of convenience than of scientific principle. [*See* TAXONOMIC METHODS.]

3. Adaptation

The various features of organisms often exhibit an exquisite match to their environments. For example, the bacteria that live in hot springs have special physiological and biochemical properties that allow them to survive and grow at very high temperatures, which would kill most other bacteria; often these thermophiles cannot grow at all under the much more benign conditions where most other bacteria thrive. Nevertheless, organisms are by no means *perfectly* adapted to the environments in which they live. Evidence for the imperfection of adaptation can be seen when species go extinct, usually as a consequence of some change in the environment.

B. Evolutionary Processes

Biological evolution occurs whenever the genetic composition of a population or species changes over a period of generations. Four basic processes contribute to such change: mutation, mixis, natural selection, and genetic drift. Selection and drift cannot act unless genetic variation exists among individuals.

1. Sources of Genetic Variation

Genetic variation among individuals is generated by two distinct processes: mutation and mixis. In terms of evolutionary theory, these processes are usually distinguished as follows: Mutation refers to a change at a single gene locus from one allelic state to another (e.g., $abcd \rightarrow Abcd$), whereas mixis refers to the production of some new multilocus genotype by the recombination of two different genotypes (e.g., $abcd + ABCD \rightarrow aBcD$).

a. Mutation

There are many different types of mutations, including point mutations, rearrangements, and transposition of mobile genetic elements from one site in the genome to another. Some mutations cause major changes in an organism's phenotype; for example, a bacterium may become resistant to attack by a virus (bacteriophage) as the result of a mutation that alters a receptor on the cell surface. Other mutations have little or even no effect on an organism's phenotype: Many point mutations have absolutely no effect on amino acid sequence (and, hence, protein structure and function) because of the redundancy that exists in the genetic code. [*See* TRANSPOSABLE ELE-

MENTS; *ESCHERICHIA COLI* AND *SALMONELLA TYPHIMURIUM*, MUTAGENESIS.]

Any number of factors may affect mutation rates, including both environmental agents (e.g., intensity of ultraviolet irradiation) and the organism's own genetic constitution (e.g., presence or absence of transposons). Evolutionary theory makes almost no assumptions about the rates of mutations or their biophysical bases, with one exception: *Mutations are assumed to occur spontaneously, i.e., irrespective of their beneficial or harmful effects on the organism.*

Although particular mutations are assumed to occur without regard to their selective value for the organism, it is quite possible that organims have evolved characteristic mutation rates, which may reflect a balance between beneficial and harmful effects of mutations. I shall return to this point later.

b. Mixis

Recombination among genomes can occur by a number of different mechanisms. The most familiar mechanism is eukaryotic sex, which arises from Mendelian segregation (meiosis) and reassortment of chromosomes (fertilization). Many eukaryotic microorganisms, including fungi and protozoa, engage in sexual mixis. Bacteria generally reproduce asexually but may undergo mixis via conjugation (plasmid-mediated), transduction (virus-mediated), or transformation. Even viruses may recombine when two or more co-infect a single host cell. [*See* CONJUGATION, GENETICS; PLASMIDS.]

Unlike mutation, these various mechanisms do not necessarily produce organisms with new genes; instead, they may produce organisms that possess new *combinations* of genes. This can have very important consequences in evolutionary theory. In the absence of mixis, two or more mutations can be incorporated into an evolving population only if they occur *sequentially* in a single lineage (Fig. 1a). With mixis, however, mutations that occur in separate lineages can be incorporated *simultaneously* into an evolving population (Fig. 1b). Thus, mixis may accelerate the rate of adaptive evolution, at least in some circumstances, by bringing together favorable combinations of alleles.

2. Natural Selection

One of the most conspicuous features of biological evolution is the evident "fit" (adaptation) of organisms to the environments in which they live. For

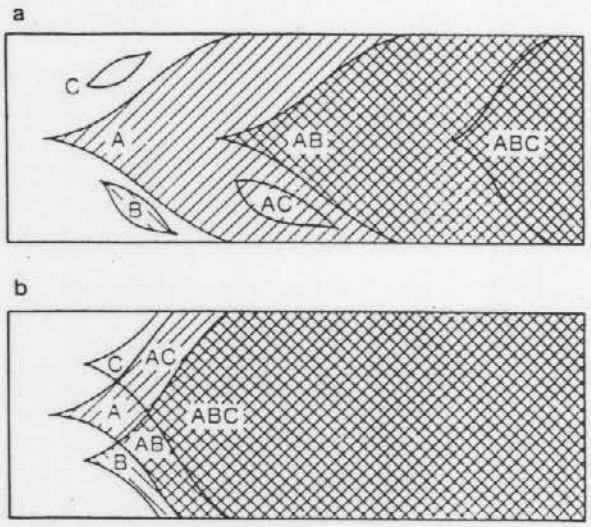

Figure 1 Substitution of advantageous mutations in large populations of asexual (a) and sexual (b) organisms. The variously hatched and shaded areas indicate the changing frequencies of mutant alleles at three loci with time. Recombination of genes from different individuals allows the favored alleles at these loci to be substituted simultaneously. In the absence of mixis, however, the favored alleles must be fixed sequentially in a single lineage. Sexuality and other forms of mixis may thereby accelerate the rate of adaptive evolution. [From Crow, J. F., and Kimura, M. (1965). *Am. Nat.* **99**, 439–450. The University of Chicago Press, Chicago.]

many centuries, this match between organism and environment was taken as evidence for the design of a Creator. But in 1859, Charles Darwin published "The Origin of Species," in which he set forth the principle of adaptation by natural selection. This principle follows logically from three simple premises. First, variation among individuals exists for many phenotypic traits. Second, these phenotypic traits influence individual survival and reproductive success. Third, phenotypic variation in those characters that affect survival and reproductive success is heritable, at least in part. (Many phenotypic traits are subject to both genetic and environmental influences.) Hence, individuals in later generations will tend to be better adapted to their environment than were individuals in earlier generations, provided that the environment itself has not changed too much in the intervening time. (Environments do sometimes change, of course, and when this happens a population or species may go extinct if it cannot adapt to these changes.)

Darwin himself did not know about the material basis of heredity (DNA and chromosomes), nor did

he even understand the precise causes of heritable variation among individuals (mutation and mixis). What he clearly understood, however, was that this heritable variation did exist and its causes (whatever they were) could be logically separated from its consequences for the reproductive success of individuals and the resulting adaptation of species to their environments.

Darwin's theories were influenced, in part, by his observations on the practices of breeders of domesticated animals and plants. These practices are now commonly referred to as artificial selection. It is useful to distinguish between artificial and natural selection and to relate this distinction to experimental evolution in the laboratory. Under artificial selection, individual organisms are chosen directly by a breeder, who allows some but not all individuals within a population to survive and reproduce. Individuals are thus selected on the basis of particular traits that are desirable to the breeder. By contrast, under natural selection, no one consciously chooses which individuals within a population will survive and reproduce and which will not. Instead, the match between organismal traits and environmental factors determines whether or not a particular individual will survive and reproduce.

At first glance, one might regard all laboratory studies of selection as studies of artificial selection, because they are necessarily performed under unnatural environmental conditions. Such usage, however, would not reflect the critical distinction between artificial and natural selection that I have outlined earlier, i.e., whether a breeder or the environment determines which individuals will survive and reproduce. In experimental evolution, an investigator typically manipulates environmental factors, such as temperature and resource concentration, but he or she does not directly choose which individuals within an experimental population will survive and reproduce. Instead, *natural selection in the laboratory, like natural selection in the wild, depends on the match between organismal traits and environmental factors.*

3. Genetic Drift

The process of adaptation by natural selection has sometimes been criticized because a "just-so story" can be offered to explain the value of almost any phenotypic trait. In fact, the frequency of genes within populations, and hence also the distribution of phenotypic traits, may change not only as the result of natural selection, but also as a consequence of the random sampling of genes during transmission across generations.

In practice, it can be difficult to distinguish between natural selection and genetic drift. This difficulty is especially evident when the only available data consist of static distributions of gene frequencies or phenotypic traits. What is usually needed to resolve this problem is some independent method for directly assessing the effects of particular genes or phenotypic traits on survival and reproductive success.

By using microorganisms to study evolution experimentally, it is possible to compare the survival and reproductive success of different genotypes that are placed in direct competition with one another. With proper replication of such experiments, it becomes possible to distinguish systematic differences in survival and reproductive success from chance deviations that are due to random genetic sampling.

II. Experimental Tests of Fundamental Principles

Two of the most important principles of modern evolutionary theory are the spontaneity of mutation and adaptation by natural selection. According to the former, mutations occur irrespective of any beneficial or harmful effects they may have on the individual. According to the latter, individuals in later generations will tend to be better adapted to their environment than were individuals in earlier generations, provided that the necessary genetic variation exists and the environment itself does not change.

A. Spontaneous Mutation

For many years, it was known that bacteria could adapt to various environmental challenges. For example, the introduction of bacteriophage into a population of susceptible bacteria often caused the bacterial population to become resistant to further viral infection. It was unclear, however, whether the mutations that were responsible for bacterial adaptation were caused directly by exposure to the selective agent, or this adaptation was the result of spontaneous mutation and subsequent natural selection. Two elegant experiments were performed during the 1940s and 1950s, which demonstrated that mutations existed *prior to* exposure to the selective agent, so that these mutations could not logically have been *caused by* that exposure.

1. Fluctuation Test

The first of these experiments was published by Salvador Luria and Max Delbrück in 1943 and relied on subtle mathematical reasoning. Imagine a set of bacterial populations, each of which is allowed to grow from a single cell to some larger number of cells (N); the founding cells are identical in all of the populations. If exposure to the selective agent causes a bacterial cell to mutate with some low probability (p), then the number of mutants in a population is expected to be, on average, pN. Although this probability is the same for each of the replicate populations, the exact number of mutants in each population may vary somewhat due to chance (just as the number of heads and tails in 20 flips of a fair coin will not always equal exactly 10). If the hypothesis that exposure to the selective agent causes these mutations is correct, then mathematical theory shows that the expected *variance* in the number of mutants among the set of replicate populations is equal to the average number of mutants. A typical outcome expected under this hypothesis is shown in Fig. 2a.

Now imagine this same set of bacterial populations, but assume that mutations occur spontaneously, i.e., independent of exposure to the selective agent. During each generation of binary fission, there is a certain probability that one of the two daughter cells is a mutant. A mutant cell's progeny are themselves also mutants, and so on. According to mathematical theory, under this hypothesis the expected variance in the number of mutants among the set of replicate populations is much greater than the average number of mutants. This large variance comes about because mutations will, by chance, occur earlier in some replicate populations than in others, and these early ("jackpot") mutations will leave numerous mutant progeny owing to the geometric growth of the population. Figure 2b shows a typical outcome expected under the hypothesis of spontaneous mutation.

Luria and Delbrück designed experiments that allowed them to compute both the average and the variance of the number of mutants in a set of bacterial populations. In these experiments, the observed variances were much greater than expected under the hypothesis that exposure to the selective agent caused the mutations. Hence, Luria and Delbrück's results provided strong evidence in support of the hypothesis of spontaneous mutation.

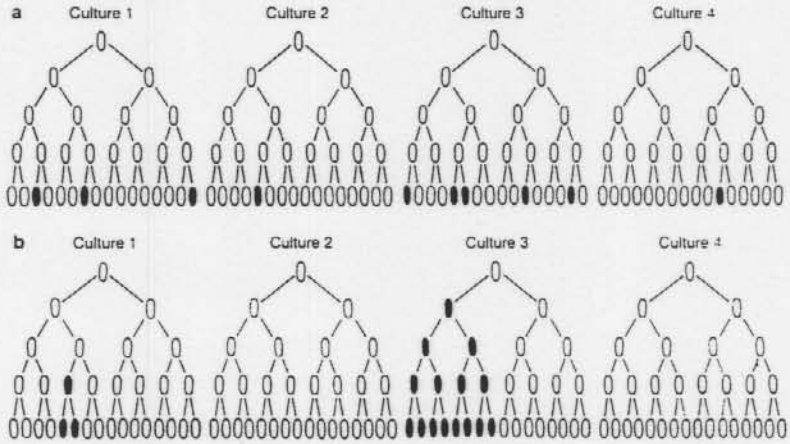

Figure 2 Schematic illustration of the origin of mutants in a set of four cultures of bacteria. Each culture is initiated from a single cell, and cells are spread on a selective medium after the four generations of binary fission that correspond to the bottom row. (a) Typical distribution expected under the hypothesis that exposure to the selective agent causes bacterial cells to mutate with some low probability. (b) Typical distribution expected under the hypothesis that mutations occur spontaneously, i.e., independent of exposure of the cells to the selective agent. The two distributions are not distinguished by the mean number of mutants in the replicated cultures but, rather, by the ratio of the variance to the mean. [From Molecular Genetics: An Introductory Narrative by Gunther S. Stent and Richard Calendar. Copyright (c) 1971, 1978, by W. H. Freeman and Company, New York. Reprinted by permission.]

2. Replica-Plating Experiment

Joshua and Esther Lederberg devised a more direct demonstration of the spontaneous origin of mutations, which they published in 1952. In their experiment, cells are spread on an agar plate that does not contain the selective agent, so that each cell grows until it produces a discrete colony (master plate). Cells from each of these colonies are then transferred onto several other agar plates that contain the selective agent, which prevents the growth of colonies *except* by those cells that have the appropriate mutation (replica plates). If mutations are caused by exposure to the selective agent, then there should be no tendency for mutant colonies detected on the replica plates to be derived from a restricted subset of the colonies on the master plate. But if mutations occur during the growth of the colony on the master plate (i.e., prior to the cells' exposure to the selective agent), then those master colonies that give rise to mutant colonies on one replica plate should also give rise to mutant colonies on the other replica plates. Indeed, Lederberg and Lederberg observed that master colonies giving rise to mutants on one replica plate gave rise to mutants on the other replica plates, thus demonstrating that the mutations had occurred spontaneously during the growth of the colony on the master plate.

B. Adaptation by Natural Selection

In addition to demonstrating the spontaneous occurrence of mutations, both the fluctuation test and the replica-plating experiment demonstrate adaptation by natural selection. Two other types of experiments also demonstrate adaptation by natural selection.

1. "Periodic" Selection

When a population is propagated in a constant environment, classes of mutant genotypes that are selectively neutral will tend to accumulate owing to recurring mutations. Early studies of bacterial populations in chemostats documented the expected accumulation with time of certain mutants that were readily scored by the investigator. However, these studies, as well as other more recent studies, also showed that the frequency of mutants did not increase continuously but, instead, exhibited a sawtooth trajectory of steady increases punctuated by sudden declines (Fig. 3). What causes these unusual dynamics? [*See* PERIODIC SELECTION.]

The mutation that is readily scored by the investigator can be designated as *a* to *A*. Now consider a mutation at another locus, which is not scored by the investigator but which is highly advantageous to the organism, designated as *b* to *B*. If the frequency of

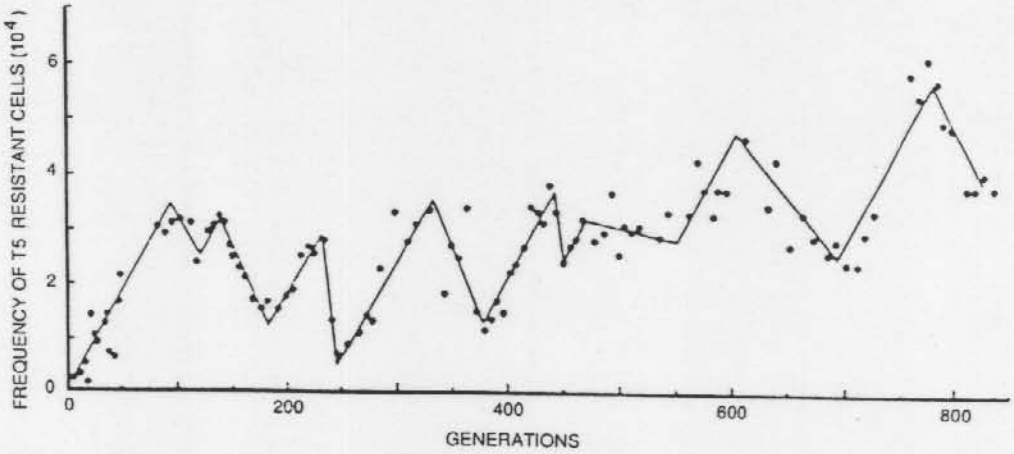

Figure 3 Changes in the frequency of T5-resistant mutants in a population of *E. coli* propagated for 800 generations in a glucose-limited chemostat. T5-resistant mutants are selectively neutral in this environment, but they increase in frequency owing to recurring mutations. Periods of steady increase are interrupted, however, by sudden declines in the frequency of these mutants. These declines result from selection for favorable mutants at other loci, which tend to occur in the numerically dominant T5-sensitive (or wild-type) portion of the population. As these favorable mutants increase in frequency, they competitively exclude their progenitors, including also the T5-resistant derivatives thereof. With time, T5-resistant mutants accumulate anew in the now dominant genetic background that contains the favorable mutant allele. This cycle may occur repeatedly and is commonly referred to as periodic selection. [From Helling, R. B., Vargas, C. N., and Adams, J. (1987). *Genetics* **116**, 349–358. Genetics Society of America.Chapel Hill, North Carolina.]

the *a* allele is much greater than the frequency of the *A* allele (as is the case before too much time has elapsed in such an experiment), then a mutation from *ab* to *aB* is much more common than a mutation from *Ab* to *AB*. Because the *aB* genotype is more fit than either *ab* or *Ab* (the first locus being selectively neutral), the *aB* genotype will outcompete the others. As this happens, cells containing the mutant *A* alleles, which had been accumulating in the *b* background, will be purged. With time, however, new mutations from *a* to *A* will begin accumulating in the *B* background. Subsequent favorable mutations at other loci will give rise to additional "periodic" reversals in the accumulation of *A*. (Note that this explanation depends on the asexuality of bacterial reproduction, which causes linkage disequilibrium between the alleles at the two loci.) In other words, each saw-tooth corresponds to the substitution of a favorable allele by natural selection.

One can test this explanation further by isolating clones from both before and after sharp down-turns in the frequency of *A* and then placing these clones in direct competition with one another under the same culture conditions from which they were isolated. Such tests have been performed repeatedly and confirm adaptation by natural selection.

2. Direct Estimation of Fitness Relative to an Ancestor

It is also possible to demonstrate adaptation by natural selection without tracking the dynamics at any particular locus. To do this, a population is founded using an *ancestral* clone, which is also stored in a nongrowing state (usually at a very low temperature). The population is then propagated under defined environmental conditions, and *derived* clones are isolated from it at arbitrary intervals. A derived clone is placed in direct competition with the ancestral clone under these same defined environmental conditions, after each clone has been allowed to acclimate physiologically to these conditions. If, in competition, the derived clone's population density increases relative to the ancestral clone's density in a systematic and statistically reproducible fashion, then the derived clone has become more fit than its ancestor, in the particular experimental conditions, as the result of mutation and natural selection (Fig. 4).

To distinguish the derived and ancestral clones from one another in a competition experiment, it is usually necessary to introduce a genetic marker that can be scored into one (or both) of the clones. This genetic manipulation necessitates an appropriate control experiment to estimate the effect of the genetic marker on fitness.

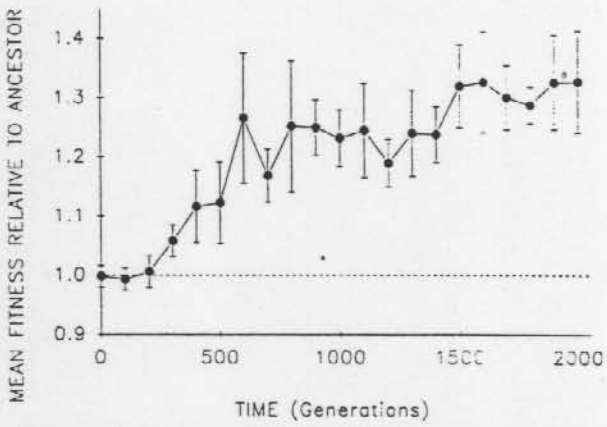

Figure 4 Changes in the mean fitness of 12 populations of *E. coli* during serial propagation for 2000 generations in a glucose-limited minimal medium. Fitness is expressed relative to the ancestral clone, which has been stored at −80°C; relative fitnesses were estimated by competing ancestral and derived strains in this same medium. Error bars indicate 95% confidence intervals about the sample means. The mean fitness of the derived strains relative to their ancestor increased by more than 30% during these 2000 generations of experimental evolution. [From Lenski, R. E., Rose, M. R., Simpson, S. C., and Tadler, S. C. (1991). *Am. Nat.* **138**, 1315–1341. The University of Chicago Press. Chicago.]

III. Genetic and Physiological Bases of Fitness

The fact that one clone may be more fit than another in a particular environment usually tells us little about the causes of that difference. It is interesting to know why one clone is more fit than another in terms of their genotypes and their physiological properties. There are two distinct approaches that have been employed in trying to elucidate the genetic and physiological bases of differences in fitness. The "bottom-up" approach uses clones that are well characterized genetically and seeks to determine the consequence of their genetic differences for physiological performance and for relative fitness. By contrast, the "top-down" approach uses clones that have been derived from some ancestor by propagation in a defined environment and seeks to elucidate the physiological and even genetic changes that have occurred as the result of this experimental evolution. Each approach has its strengths and limitations. The bottom-up approach permits more direct causal inferences to be drawn

with respect to the effects of particular genetic differences, provided that proper care is taken to ensure that clones are otherwise isogenic (genetically identical). The bottom-up approach is limited, however, in that it cannot easily address whether or not these defined genetic differences are representative of the genetic changes that are available to an evolving population. The top-down approach uses precisely those genetic changes that have been important during the evolution of a particular population. However, many of these genetic changes are very difficult to analyze using standard microbial genetic methods because they cause phenotypic changes that cannot be scored in a simple "either–or" manner.

A. Fitness Effects due to Possession of Unused Functions

A number of studies have used well-characterized bacterial genotypes to examine the effects on fitness caused by the carriage and expression of superfluous gene functions. These studies have measured the relative fitnesses of (1) bacteria with constitutive (high-level) and repressed (low-level) expression of enzymes for catabolism of carbon sources in media where those resources are not available; (2) prototrophic bacteria (which produce an amino acid or other required nutrient) and auxotrophic mutants (which cannot produce the required nutrient) in media where the required nutrients are supplied; (3) phage-sensitive bacteria and phage-resistant mutants in environments where phages are absent; and (4) bacteria with plasmid-encoded resistance to antibiotics and isogenic plasmid-free bacteria in media that contain no antibiotics.

These studies have often, but not always, demonstrated substantial fitness disadvantages associated with possession of unnecessary gene functions. In many of the cases where such disadvantages have been detected, they are much greater than can be explained on the basis of the energetic costs associated with the synthesis of unneeded proteins and other metabolites. For example, one study found that the fitness disadvantage associated with synthesis of the amino acid tryptophan, when it was supplied in the medium, was 1000-fold greater than could be explained on the basis of energetic costs. Evidently, the expression of superfluous functions can sometimes have strong indirect effects, which may arise through the disruption of other physiological processes.

B. Effects due to Variation in Essential Metabolic Activities

It is clear that the expression of unnecessary metabolic functions is often disadvantageous to a microorganism. An equally important issue concerns the relationship between fitness and the level of expression of metabolic functions that are *required* for growth in a particular environment. This latter issue is generally much more difficult to address experimentally, because it necessitates detailed analyses of subtle differences between strains in biochemical activities rather than the mere manipulation of the presence or absence of some function.

Daniel Dykhuizen, Anthony Dean, and Daniel Hartl performed a pioneering study to examine the relationships among genotype, biochemical activities in a required metabolic pathway, and fitness. Their study examined growth on lactose by genotypes of *Escherichia coli* that varied in their levels of expression of the permease that is required for active transport of lactose into the cell and the β-galactosidase that is required for hydrolysis of the lactose. Given that both enzymes are necessary for growth on lactose, how do the activities at each step affect the net flux through this metabolic pathway? And how does net flux affect fitness?

Using metabolic control theory, Dykhuizen and

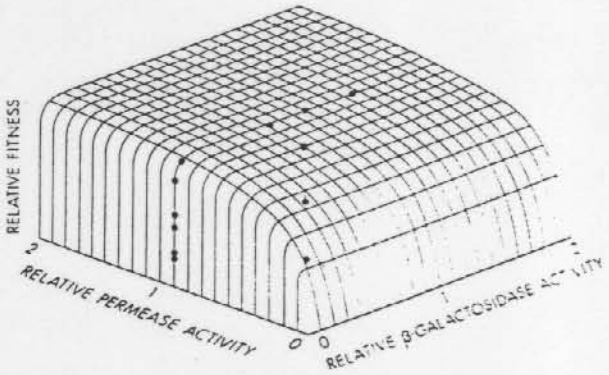

Figure 5 Relative fitnesses of *E. coli* expressing different levels of permease and β-galactosidase activities in the lactose operon. The fitness surface is predicted from metabolic control theory using estimates of the biochemical activities of the two enzymes. Estimates of relative fitnesses are shown as points above or below the fitness surface; these were obtained by competing strains with different enzyme activities in chemostats in which lactose was the sole source of energy. [From Dykhuizen, D. E., Dean, A. M., and Hartl, D. L. (1987). *Genetics* **115**, 25–31. Genetics Society of America, Chapel Hill, North Carolina.]

his co-workers could predict how the equilibrium flux through this pathway would depend jointly on the activities of the permease and β-galactosidase enzymes. They estimated these activities for their genotypes using appropriate biochemical methods. They then predicted that the relative fitness of any two strains would be directly proportional to their relative fluxes whenever lactose provided the sole energy source.

To test the model and its predictions, Dykhuizen and his colleagues estimated the relative fitnesses of the various genotypes in a medium in which lactose provided the sole source of energy for growth. The observed relative fitnesses were extremely close to those predicted from the model, as shown in Fig. 5. Interestingly, their results also show that a small change in permease activity from the wild-type level (=1, after standardization) has a much greater effect on fitness than does a comparable change in β-galactosidase activity. This difference may indicate that the permease activity is suboptimal for lactose transport, perhaps owing to some opposing selective pressure on the permease.

C. Effects of Genetic Background

It is obvious that the fitness effects caused by particular genetic differences depend strongly on the environment into which an organsim is placed. For example, the same antibiotic-resistance gene function that is essential for survival and replication of a bacterium in the presence of antibiotic may hinder growth in an antibiotic-free environment. Similarly, the fitness effects that are due to particular gene functions may often depend on the genetic background in which those genes are found.

For example, one study showed that several different alleles at the 6-phosphogluconate dehydrogenase locus in *E. coli* had very similar fitnesses in a gluconate-limited medium, provided that these alleles were introduced into a genetic background that also encoded an alternative metabolic pathway for 6-phosphogluconate utilization. In a genetic background where this alternative pathway was defective, however, these several alleles had quite variable fitnesses in the gluconate-limited medium.

In another study with *E. coli*, it was observed that the selective disadvantage associated with resistance mutations in a bacteriophage-free environment was reduced by about 50% over the course of several hundred generations of experimental evolution. This improvement resulted from secondary mutations in the genetic background, which compensated for the maladaptive side effects of the resistance mutations but had no effect on the expression of resistance itself.

IV. Genetic Variation within and between Populations

In nature, genetic variation is abundant in most species, including microorganisms. Some of this variation exists within local populations, while other variation may distinguish one population from another. In this section, we will consider studies that have addressed the various processes that influence the maintenance of genetic variation within a population as well as those that may contribute to the divergence of populations.

A. Transient Polymorphisms

A population can be said to be polymorphic whenever two or more genotypes are present in the population. A polymorphism exists, for example, while an advantageous mutation is increasing in frequency relative to the ancestral allele. This type of polymorphism is said to be transient, because eventually the favored genotype will exclude the ancestral genotype. Transient polymorphisms must necessarily exist during any substitution of one allele for another by natural selection.

B. Selective Neutrality

At the other extreme, some polymorphisms may exist almost indefinitely precisely because the alleles that are involved have little or no differential effect on fitness. Such selectively neutral alleles are subject only to genetic drift. Experimental studies have sought to determine whether some polymorphic loci in natural populations of *E. coli* might exist because of selective neutrality, or other explanations are needed. To that end, naturally occurring alleles at particular loci were transferred into a common genetic background, and the fitness effects associated with the various alleles were determined. Even when the bacteria were grown under conditions where growth was directly dependent on the particular enzymatic steps encoded by these loci, there were in many cases no discernible effects on fitness due to the different alleles. These studies

thus support the hypothesis that random mutation and genetic drift may be responsible for a substantial amount of the genetic variation that is present within natural populatons. (It should be noted, however, that this does not imply that most *substitutions* of one allele for another are due to genetic drift.)

C. Frequency-Dependent Selection

To this point, we have implicitly assumed that the fitness of one genotype relative to another is independent of their relative abundances. But this may not always be the case. In the course of growth and competition in a particular environment, microorganisms modify their environment through the depletion of resources, the secretion of metabolites, and so on. When this happens, the relative fitnesses of genotypes may depend on the frequency with which they are represented in a population, and the process of selection is said to be frequency-dependent. Frequency-dependent selection can give rise to several different patterns of genetic variation within and between populations, as will now be discussed.

1. Stable Equilibria

Two (or more) genotypes can coexist indefinitely when each genotype has some competitive advantage that disappears as that genotype becomes more common. In that case, each genotype can invade a population consisting largely of the other genotype but cannot exclude that other genotype, so that a stable equilibrium results.

A number of different ecological interactions between genotypes can promote these stable equilibria. For example, an environment may contain two different carbon sources. If one genotype is better at exploiting one resource and the other genotype is superior in competition for the other resource, then whichever genotype is rarer will tend to have more resource available to it, thereby promoting their stable coexistence. In some cases, a resource that is essential for one genotype may actually be produced as a metabolic by-product of growth by another genotype; such interactions are termed cross-feeding. Stable coexistence of genotypes in one population can also occur when the environment contains a population of predators (or parasites); predator-mediated coexistence requires that one of the prey genotypes be better at exploiting the limiting resource while the other prey genotype is more resistant to being exploited by the predator. The evolution of stably coexisting bacterial genotypes from a single ancestral genotype has been demonstrated in several experiments involving both cross-feeding and predator–prey interactions.

2. Unstable Equilibria

Those ecological interactions that promote the stable coexistence of two or more genotypes contribute to the maintenance of genetic variation in populations. However, certain frequency-dependent ecological interactions can actually give rise to *un*stable equilibria. An unstable equilibrium exists when each of two genotypes can prevent the other genotype from increasing in number.

One type of ecological interaction that can give rise to an unstable equilibrium is interference competition. Interference competition occurs when one genotype produces an allelopathic (toxic) substance, which inhibits the growth of competing genotypes; it is distinguished from exploitative competition, which occurs simply by the depletion of resources. Many microorganisms secrete allelopathic compounds, including fungi, which produce antibiotics. Certain strains of *E. coli* produce colicins, which kill competing strains of *E. coli* but to which the colicinogenic (colicin-producing) strain is immune. Colicinogenic genotypes, when common, produce so much toxin that they can exclude a colicin-sensitive genotype that is otherwise more efficient in exploitative competition for a limiting resource. When the colicinogenic genotype is rare, however, the cost of colicin synthesis is greater than the benefit of the resource that is made available to it by the killing of colicin-sensitive cells, and so the colicinogenic genotype loses out to the more efficient colicin-sensitive competitor. (The outcome of competition between colicinogenic and colicin-sensitive genotypes also depends on the physical structure of the environment. In particular, the advantage shifts to the colicinogenic bacteria on surfaces, even when they are rare, because the resources made available by the killing action of the colicins accrue locally, rather than being dispersed as in liquid.)

Ecological interactions that give rise to unstable equilibria do not promote genetic polymorphisms within a particular population. However, they may contribute to the maintenance of genetic differences between populations, because neither type can invade a resident population of the other type.

3. Nontransitive Interactions

In some cases, frequency-dependent selection may give rise to nontransitive competitive interactions. Nontransitivity exists, for example, when genotype *A* out-competes genotype *B* and genotype *B* out-competes genotype *C*, but genotype *C* out-competes genotype *A*. Nontransitive interactions among genotypes were demonstrated in one study with populations of the yeast *Saccharomyces cerevisiae* evolving in a chemostat, in which glucose was supplied as the sole carbon source. Mathematical models of competition for a single growth rate-limiting resource in a spatially and temporally homogeneous environment (such as a chemostat) predict strictly transitive interactions among genotypes, so that the demonstration of nontransitivity apparently indicates the involvement of other limiting factors, such as the accumulation of allelopathic metabolites in the culture medium.

Nontransitive interactions can give rise to situations in which the mean fitness of an evolving population relative to some distant ancestor may actually decline with time, even though each genotype has increased fitness relative to its immediate predecessor. Nontransitive interactions may also maintain genetic diversity within populations over time by recycling genotypes that would otherwise be lost.

D. Divergence of Populations

One very interesting issue is the extent to which experimental evolution in the laboratory is reproducible. If identical genotypes are introduced into identical environments, will the replicate populations adapt to their environment in similar or different ways? One might evaluate similarities and differences in adaptation by several criteria: the extent of improvement in ecological performance (e.g., fitness relative to a common ancestor), the physiological bases of enhanced performance (e.g., increased transport of some limiting nutrient into the cell), or the genetic changes underlying adaptation (e.g., the particular genes in which the beneficial mutations occur). Few studies have addressed this issue directly, and none so far has systematically examined divergence at all of these different levels. Nonetheless, several studies suggest that the process of adaptation by natural selection can be remarkably reproducible, given its dependence on the generation of variation by random mutation. In one study, it was observed that 12 replicate populations of *E. coli* increased in mean fitness *relative to their common ancestor* by >30% during 2000 generations of experimental evolution, and yet during this time the evolving populations diverged in mean fitness *relative to one another* by only 3% or so. Several other studies provide evidence for the similarity of the physiological and genetic changes underlying adaptations in replicate populations. For example, replicated populations of *E. coli* placed in chemostats in which lactose provides the sole energy source almost invariably evolve constitutive expression of the enzymes encoded by the lactose operon.

V. Coevolution of Interacting Genomes

Microorganisms in nature rarely, if ever, exist as single species, as they are usually studied in the laboratory. Rather, they exist in complex natural communities that contain many interacting populations. Some interactions are exploitative: One population makes its living by parasitizing or preying upon another population. Other interactions are mutualistic, so that each population obtains some benefit from its association with the other. In many cases, these interactions can be quite plastic both genetically and ecologically. For example, a single mutation in a bacterium may render it resistant to lethal infection by a bacteriophage. And a plasmid that confers antibiotic resistance may be beneficial to its bacterial host in an antibiotic-laden environment but detrimental in an antibiotic-free environment.

As a consequence of this variability, microorganisms have proven very useful for investigating experimentally the ecological and genetic factors that shape the coevolution of interacting populations. Are there evolutionary "arms races" between host defenses and parasite counterdefenses? Why are some parasites so virulent to their hosts, whereas others are relatively benign? How can mutualistic interactions evolve, if natural selection favors inherently "selfish" genes?

A. Exploitative Interactions

A number of studies have demonstrated the stable coexistence of virulent bacteriophage (lytic viruses) and bacteria in continuous culture. In these studies, the virus population may hold the bacterial population in check at a density that is several orders of

magnitude below the density that would be permitted by the available resource if viruses were not present. Typically, however, bacterial mutants eventually appear that are resistant to the virus, and these mutants have a pronounced selective advantage over their virus-sensitive progenitors. The proliferation of bacteria that are resistant to infection by the original virus provides a selective advantage to host-range viral mutants, which are capable of infecting these resistant bacteria. Thus, one can imagine, in principle, an endless "arms race" between resistant bacteria and extended host-range viruses.

In fact, however, there appear to be important constraints that prevent the realization of this outcome. Bacterial mutants may appear, sooner or later, against which it is difficult or impossible to isolate corresponding host-range viral mutants. This asymmetry may arise because bacterial resistance can occur via mutations that cause either the alteration or the complete loss of certain receptors on the bacterial surface, whereas viral host-range mutations can counter only the former. Despite this asymmetry, the virus population persists if the virus-resistant bacterial mutants are less efficient than their sensitive progenitors in competing for limiting resources. In such cases, a dynamic equilibrium is obtained in which the growth-rate advantage of the sensitive bacterium relative to the resistant bacterium is offset by death due to viral infection. Such trade-offs between competitiveness and resistance commonly occur, in fact, because the receptors that are used by viruses to adsorb to the cell surface often serve also to transport nutrients into the cell or to maintain the structural integrity of the cell envelope.

A commonly held belief is that a predator or parasite that is too efficient or virulent will drive its prey or host population extinct, thereby causing its own demise. We have just seen, however, that virulent bacteriophage can stably coexist with bacteria, despite the fact that successful reproduction of the viral genome is necessarily lethal to the infected bacterium. Moreover, the process of natural selection neither requires nor permits foresight, so that the mere *prospect* of extinction cannot deter the evolution of more efficient predators or more virulent parasites. Nevertheless, there do indeed exist many viruses (lysogenic and filamentous bacteriophages) that are replicated alongside the host genome and whose infections, although deleterious, are not necessarily lethal. These viruses, as well as conjugative plasmids, have life cycles that include both horizontal (infectious) and vertical (intergenerational) transmission.

At present, the evolutionary factors that favor these alternative modes of transmission are not fully understood. One factor that is likely to be important, however, is the density of hosts. If susceptible hosts are abundant, then the opportunity for horizontal transmission is correspondingly great. In such circumstances, selection may favor those parasites that replicate and infectiously transmit themselves most rapidly, regardless of the consequences of these activities for the host's fitness. But if susceptible hosts are scarce, then the potential for horizontal transmission is limited. Vertical transmission, by contrast, does not depend on the parasite or its progeny "finding" another host. Instead, the success of a vertically transmitted parasite is inexorably linked to the success of its infected host. The greater the burden that such a parasite imposes on its host, the slower that host will be able to reproduce its own genome and that of the parasite. Hence, when the density of susceptible hosts is low, selection may favor those parasites that minimize their replicative and infectious activities, thereby minimizing their deleterious effects on the host. It is hoped that more studies will address this interesting problem in the future.

B. Mutualistic Interactions

It has often been suggested that many mutualisms have evolved from formerly exploitative interactions. Indeed, the hypothesis advanced above implies that, at sufficiently low host densities, genetic elements such as plasmids and phage can persist *only* if they are actually beneficial to the host. Many plasmids do encode functions that are useful to their bacterial hosts, including resistance to various antibiotics, restriction immunity to certain phages, production of bacteriocins, and so on. And some of these plasmids are incapable of conjugation, instead relying exclusively on vertical transmission. Moreover, several studies have demonstrated unexpected competitive advantages for bacteria that are infected by plasmids, transposons, and even temperate phage, relative to cells that are not infected but are otherwise genetically identical.

Two studies have even demonstrated the evolution of mutualistic interactions from formerly antagonistic associations. In one study, the growth rate of a strain of *Amoeba proteus* was shown initially to be severely reduced by a virulent bacterial infection.

The harmful effects of the bacteria were diminished by propagation of the infected amebae for several years, and the amebae eventually became dependent on the bacterial infection for their viability. In the other study, a plasmid initially reduced the fitness of its bacterial host in antibiotic-free medium; after 500 generations had elapsed, however, the plasmid enhanced the fitness of its host in this same medium. Interestingly, the genetic change responsible for the newly evolved mutualistic interaction was in the host chromosome, not in the plasmid genome. Both of these studies demonstrate that hosts can become dependent on, or otherwise benefit from, formerly parasitic genomes, thus giving rise to mutualistic interactions.

VI. Evolution of New Metabolic Functions

Microorganisms exhibit a tremendous diversity of metabolic activities, some of which function in biodegradative pathways (catabolism) while others work in biosynthetic pathways (anabolism). How has this diversity evolved? One major avenue of research in the field of experimental evolution seeks to elucidate the various processes by which microorganisms can acquire new metabolic functions. This research is particularly timely as humans seek to harness microorganisms that can be used, for example, to degrade toxic pollutants in the environment. [*See* BIOREMEDIATION.]

A. Acquisition by Genetic Exchange

Perhaps the simplest way in which a microorganism can acquire some new metabolic function is by genetic exchange with another microorganism that already possesses that function. For example, antibiotic resistance functions are frequently encoded by plasmids, which are transmitted from donors to recipients by conjugation. Acquisition by genetic exchange is not always so simple a solution, however. Effective biodegradation of certain recalcitrant compounds may require complex coordination of several steps in a biochemical pathway, which are encoded by complementary genes from two (or more) different microorganisms. The acquisition of activities that depend on such pathways may typically require not only genetic exchange, but also subsequent refinement of the new function by mutation and natural selection.

B. Changes in Regulatory and Structural Genes

In several cases, microorganisms have been shown to acquire new metabolic activities without any genetic exchange. Instead, the acquisition of a new metabolic function may occur by selection for one or more mutations in existing regulatory or structural genes, which normally have some other function. For example, the bacterium *Klebsiella aerogenes* cannot normally grow on the sugar D-arabinose, although it does possess an enzyme, isomerase, that is capable of catalyzing the conversion of D-arabinose into an intermediate, D-ribulose, which can be further degraded to provide energy to the cell. This isomerase is normally expressed at a very low level, however, which does not permit growth on D-arabinose. Mutations that increase the level of expression of this isomerase are sufficient to enable growth by *K. aerogenes* on D-arabinose. The ability of this bacterium to grow on D-arabinose may be further improved by certain mutations in the structural gene, which change the amino acid sequence of the isomerase in such a way as to improve the efficiency of the catalytic conversion of D-arabinose to D-ribulose.

In essence, the evolution of new metabolic activities may depend on the microorganism "borrowing" gene products that were previously used for other metabolic activities. It is perhaps not surprising that this process may sometimes also encroach upon those gene products' previous metabolic activities. Such encroachment could, in turn, favor gene duplication, a type of mutation whereby a single copy of an ancestral gene gives rise to two homologous copies, each of which may subsequently evolve toward different metabolic capabilities.

C. Reactivation of Cryptic Genes

Selection for novel metabolic activities has occasionally revealed the existence of "cryptic" genes, which are apparently nonfunctional but can be made functional by one or a few mutations. Cryptic genes are presumably derived from once-active genes, which have been silenced by mutations that destroyed their functions. In the course of experimental evolutionary studies, the existence of such cryptic genes has been revealed by selection for new mutations that reverse or suppress these earlier mutations, thus restoring the lost metabolic activities.

VII. Evolution of Genetic Systems

The process of adaptation by natural selection requires genetic variation in those characters that influence the survival and reproduction of organisms. The two sources of genetic variation are mutation and mixis. In general, rates of mutation and mixis depend not only on environmental factors (e.g., the intensity of ultraviolet irradiation), but also on the properties of the "genetic system" intrinsic to the organism in question. Here, genetic system is taken to mean all those aspects of the physiology, biochemistry, and reproductive biology of an organism that influence rates of mutation and mixis. For example, organisms have mechanisms of varying efficacy to promote the accurate replication and repair of their DNA. And while sex is an integral part of reproduction for some organisms, many others (including numerous microorganisms) reproduce asexually, so that the resulting progeny are usually genetically identical to their parent and to one another.

Among the most interesting questions in evolutionary biology are those that concern the adaptive significance and evolutionary consequences of alternative genetic systems. Why do some organisms reproduce sexually, whereas others reproduce asexually? If mutation generates variation that is necessary for adaptation by the species, but most mutations have deleterious effects on the individual organism, then what mutation rate is optimal? Might organisms somehow be able to choose only those mutations that are beneficial to them, given their present ecological circumstances?

A. Sexuality and Mixis

The hypothesized advantages for sexuality depend, in one way or another, on the genetic variation that is produced by mixis. Efforts to address these hypotheses have been based primarily on mathematical models and on phylogenetic and ecological patterns of the distribution of sexual versus asexual life cycles. Only a few studies have examined experimentally the evolutionary consequences of mixis, and most of these have used microorganisms, for which it is often possible to manipulate the extent of intergenomic recombination. For example, mixis in bacterial viruses can be manipulated by varying the multiplicity of infection (MOI) of host cells, since recombination of viral genotypes can occur only if two or more viruses infect the same host cell. One study compared the rate of adaptive evolution of a bacterial virus at high and low MOI: the total size of the viral population was standardized for both treatments. The average fitness increased more rapidly under the high MOI (=high recombination) treatment than under the low MOI (=low recombination) treatment. This result is consistent with the hypothesis that sexual populations can adapt more rapidly than asexual populations because two or more advantageous mutations can be incorporated simultaneously in the former, but only sequentially in the latter (see Fig. 1).

Some experiments have suggested that another advantage of mixis may arise when the overall rate of deleterious mutation is high and the effective population size is very small. Such conditions may apply to microorganisms with high error rates during replication (e.g., RNA viruses) or those with relatively large genomes (e.g., protozoa), if their populations are also subject to periodic "bottlenecks." In these cases, deleterious mutations tend to accumulate indefinitely in asexual lineages, a process called "Muller's ratchet" (after the geneticist H. J. Muller, who first described this phenomenon). However, even occasional mixis can purge lineages of their accumulated load of deleterious mutations. This effect occurs because two recombining genomes may each complement the deleterious mutations that are present in the other, thereby producing some progeny with a reduced load of deleterious mutations (as well as other progeny with an increased load, which will tend to be removed by natural selection).

In still other cases, mixis appears to be less an adaptation to recombine genes per se than a coincidental consequence of the movement between cells of parasitic entities. In many bacteria, for example, recombination of chromosomal genes occurs only when cells are infected by viruses (transduction) or plasmids (conjugation). The new combinations of chromosomal genes that may result from such infections will occasionally be advantageous. One need not regard phages and plasmids as benevolent agents of bacterial carnal pleasure, however, because their effects are more often deleterious to the host.

B. Evolutionary Effects of Mutator Genes

"Mutator" genes increase the rate of mutation elsewhere in the genome by disrupting aspects of DNA replication and repair. Mobile genetic elements may

also behave like mutator genes, as their physical transposition in the genome can alter the expression of other genes. Several studies have investigated the effects of mutator genes, including transposons, on the evolution of bacteria. These studies have revealed a pattern that seems, at first glance, rather curious (Fig. 6). When a mutator gene is introduced into a population above a certain initial frequency (e.g., 0.01%), it tends to increase in frequency over the long-term. But if that mutator gene is introduced at a frequency below that threshold, then it tends to be out-competed and go extinct over the long-term.

What causes this threshold phenomenon? In a sense, there is an evolutionary race between two clones, one with and one without the mutator gene, to see which one gets the next advantageous mutation. The rate of appearance of advantageous mutations for each clone depends on the product of its population size, N, and its corresponding mutation rate, u. So when the ratio of the mutation rates of the mutator and nonmutator clones, u'/u, is greater than the inverse ratio of their population sizes, N/N', then the mutator clone is more likely to have the next favorable mutation. But when u'/u, is less than N/N', the nonmutator clone, by virtue of its greater numbers, is likely to produce the next beneficial mutation.

This explanation, while almost certainly the correct one for these laboratory experiments, presents two difficulties for understanding the possible evolution of mutator genes in nature. First, if mutator genes are advantageous only when they are common, then how do they *become* common? Second, for how long can this process continue before a mutator clone "uses up" its advantageous mutations? The answer to this second question almost certainly depends on the extent of environmental variability. In particular, it has been hypothesized that the advantage of a mutator clone will progressively deteriorate in a constant environment, as the mutations that produce further improvement in fitness in that environment are exhausted. As a consequence, the ratio of beneficial to harmful mutations caused by the mutator gene will decline, and its effect will become progressively more deleterious with time.

Thus, we see that aspects of genetic systems that increase variation—whether by mutation or mixis—may accelerate adaptive evolution. But mutation and mixis can also break down genotypes that are already well adapted to particular environments. The evolution of genetic systems may therefore represent a balance between these opposing pressures.

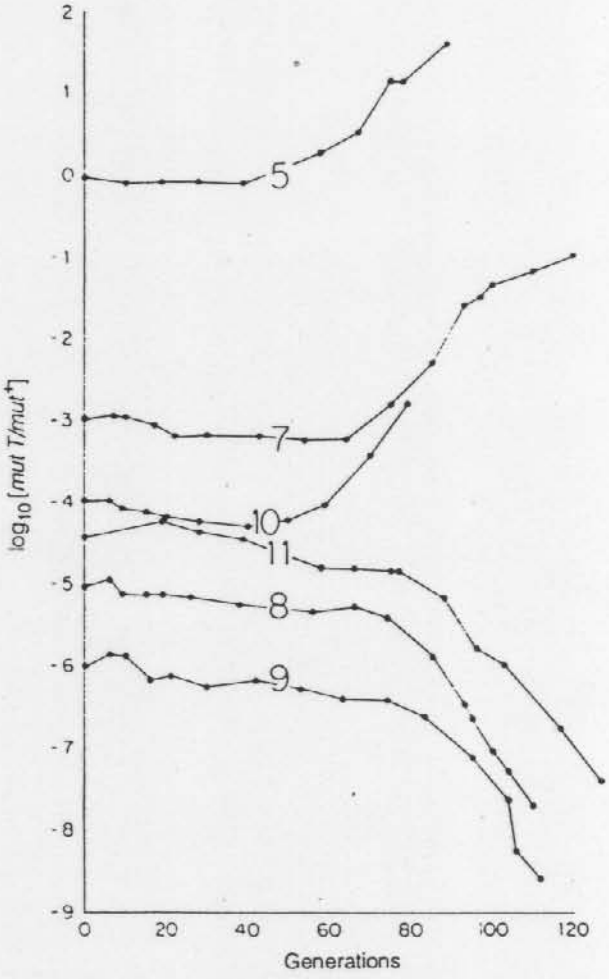

Figure 6 Changes in the frequency of an *E. coli* clone expressing a mutator gene during competition with a nonmutator (or wild-type) clone. The several numbered lines indicate separate populations in which the initial frequency of the clone with the mutator gene was varied over several orders of magnitude. When the clone expressing the mutator gene is initially present above a certain threshold frequency, it is likely to get a highly advantageous mutation before the nonmutator clone gets such a mutation. When the initial frequency of the mutator gene is below this threshold, however, the greater numbers of the nonmutator clone are more than sufficient to offset the higher mutation rate of the mutator clone. [From Chao, L., and Cox, E. C. (1983). *Evolution* **37**, 125–134. Society for the Study of Evolution, Santa Barbara, California.]

C. Directed Mutation: A Controversy

We have seen already how the fluctuation test of Luria and Delbrück and the replica-plating experiment of the Lederbergs were used to demonstrate that bacterial mutations arose prior to the cells' exposure to the selective agent and, hence, could not

have been caused by the organism's response to that agent. Recently, the generality of spontaneous mutation has been called into question, however, as several studies have purported to show that certain bacterial mutations occur only (or more often) when the mutants are favored. Two lines of evidence may suggest the existence of these so-called "directed" mutations. (1) The distribution of certain mutants among replicate cultures has a variance that is lower than that expected under the Luria–Delbrück hypothesis of spontaneous mutation. (2) Certain mutants appear on selective media after delays that are inconsistent with the mutations having occurred prior to this exposure, contrary to the Lederbergs' test.

At this time, however, it is unclear whether or not these claims of directed mutation will withstand further scrutiny. With respect to the first type of evidence, it has been shown that many processes other than directed mutation can produce similar deviations from the Luria–Delbrück hypothesis. For example, if mutants grow more slowly than nonmutants prior to selective plating, then this also reduces the expected variance of the distribution of mutants among replicate cultures. Similarly, the fact that certain mutations occur after plating on selective medium does not necessarily imply that the cell perceives the selective agent and mutates accordingly. For example, the rates of certain classes of mutation may increase sharply as cells starve, irrespective of the presence or absence of the selective agent that permits growth of the mutants. General acceptance of the directed mutation hypothesis will require more careful experiments to exclude these alternative hypotheses, as well as demonstration of a physiological mechanism that permits directed mutation.

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