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MUTATION AND ADAPTATION: The Directed Mutation Controversy in Evolutionary Perspective

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ABSTRACT

A central tenet of evolutionary theory is that mutation is random with respect to its adaptive consequences for individual organisms; that is, the production of variation precedes and does not cause adaptation. Several recent experimental reports have challenged this tenet by suggesting that bacteria (and yeast) "may have mechanisms for choosing which mutations will occur" (6, p. 142). The phenomenon of nonrandom mutation claimed in these experiments was initially called "directed mutation" but has undergone several name changes during its brief and controversial history. The directed mutation hypothesis has not fared well; many examples of apparently directed mutation have been rejected in favor of more conventional explanations, and several reviews questioning the validity of directed mutation have appeared (53, 54, 59–61, 79, 80). Nonetheless, directed mutation has recently been reincarnated under the confusing label "adaptive mutation" (5, 23, 24, 27, 35, 74). Here we discuss the many experimental and conceptual problems with directed/adaptive mutation, and we argue that the most plausible molecular models proposed to explain "adaptive mutation" are entirely consistent with the modern Darwinian concept of adaptation by natural selection on randomly occurring variation.

In the concluding section of the paper, we discuss the importance of an informed evolutionary approach in the study of the potential adaptive significance of mutational phenomena. Knowledge of the molecular bases of muta-

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tion is increasing rapidly, but rigorous evolutionary understanding lags behind. We note that ascribing adaptive significance to mutational phenomena (for example, "adaptive mutation") is beset with some of the same difficulties as ascribing adaptive significance to features of whole organisms (29). We consider some examples of mutational phenomena along with possible adaptive and nonadaptive explanations.

INTRODUCTION: THE HISTORICAL RELATIONSHIP BETWEEN VARIATION AND ADAPTATION

> Any heuristic can be treacherous, but a Darwinian explanation is the first I would seek in explaining a biological enigma. I do not insist that it will always last, but it has had enormous power in bringing us to our present understanding.
>
> J. Lederberg (49, p. 398)

We begin by providing a brief historical sketch of theories and evidence concerning the relationship between heritable variation and adaptation. Our purpose is not to present a formal or comprehensive historical analysis of this subject. Rather, we wish to place the directed mutation controversy in perspective by illustrating some of the ways in which variation and adaptation have repeatedly been confounded and then sorted out since Lamarck. We acknowledge that a brief historical synopsis such as this risks oversimplifying the rich history of scientific ideas and debate. Readers should consult comprehensive treatments of the history of biology (e.g. 63) for more detail.

Lamarck and Darwin

Lamarck (47) theorized that heritable adaptive variation arises in individual organisms as a consequence of needs and activities stimulated by environmental conditions. In the Lamarckian view, the origin of heritable variation and the origin of evolutionary adaptation are one and the same. Darwin, in contrast, conceived of a separation between variation and adaptation. According to Darwin, heritable variation arises continually as a result of (unknown) processes; evolutionary adaptation occurs as a consequence of natural selection acting on this heritable variation. Mayr has succinctly contrasted the evolutionary theories of Lamarck and Darwin: "The crucial difference between Darwin's and Lamarck's mechanisms of evolution is that for Lamarck the environment and its changes had priority. They produced needs and activities in the organism, and these, in turn, caused adaptational variation. For Darwin random variation was present first, and the ordering activity of the environment ('natural selection') followed afterwards" (63, p. 354). The primacy of natural selection in Darwin's theory of adaptation is illustrated by the following passage, which concludes Chapter Five of *The Origin of Species* (14, p. 170):

> Whatever the cause may be for each slight difference in the offspring from their parents—and a cause for each must exist—it is the steady accumulation, through natural selection, of such differences, when beneficial to the individual, that gives rise to all the more important modifications of structure, by which the innumerable beings on the face of this earth are enabled to struggle with each other, and the best adapted to survive.

From Darwin to the Modern Synthesis

The blending mechanism of heredity widely accepted in Darwin's time posed problems for natural selection (42). Blending swamps variation, eroding both the hereditary differences among individuals that are necessary for natural selection and any heritable differences that might accumulate by natural selection. Under the assumption of blending, an enormous input of new variation is required at each generation to maintain distinct variants in an interbreeding population. Darwin argued both for the existence of variation and for the evolutionary transformation of old forms into new by natural selection, but he did not demonstrate a source of variation or a hereditary mechanism that could withstand the effects of blending. To account for the problem of blending, Darwin included roles for such Lamarckian factors as "the effects of use and disuse of parts" in the generation of new variation and in adaptation, both in the *Origin of Species* and in his pangenesis theory of inheritance (15).

Support for Lamarckian evolution, however, began to erode in the late 1880s. Weismann (93) made a forceful case against Lamarckism, arguing, among other things, that the observed separation of germline and soma in many organisms was inconsistent with the inheritance of acquired characters. Further doubt was cast on both Lamarckian evolution and blending inheritance by the rediscovery of Mendelism around 1900 (e.g. 68). Mutations in Mendelian factors were found to be infrequent and usually deleterious, contrary to the requirement of blending inheritance for tremendous inputs of variation and also inconsistent with what would be expected if mutations were to direct evolution by arising in response to adaptive need.

During the 1920s and 1930s, the emerging science of genetics and the theory of natural selection were incorporated into a comprehensive view of evolution—the modern synthesis (39). The modern synthetic theory identifies natural selection as the sole evolutionary force responsible for the adaptation of organisms to their environment. (There remain, of course, debates as to the levels of selection necessary to explain apparently adaptive phenomena; see, e.g. 94). A central tenet of the modern synthetic theory, therefore, is that mutation is random with respect to the adaptive needs of individual organisms.

As we show in the next section, direct experimental evidence indicating that mutation is random with respect to its adaptive consequences was not available prior to the publication of several classic experiments with bacteria in the

1940s and 1950s. Nonetheless, by the 1930s the randomness of mutation (in the sense given above) was widely accepted among geneticists and evolutionary theorists (e.g. 22, 88). Circumstantial evidence clearly favored random mutation; it also seems that the need to invoke an adaptive role for mutation had been effectively eliminated by the perceived potential of natural selection to explain adaptation.

Lamarckism and Bacterial Adaptation

Long after natural selection on randomly arising variation had gained wide acceptance as the mechanism of adaptation in higher organisms, debate continued over the relationship between variation and adaptation in bacteria (41, 85). In contrast to the situation in higher organisms, it was impossible to observe the origin of an individual bacterial variant in circumstances in which it was disfavored; the only way to isolate a specific bacterial variant was by altering the environment so as to favor its phenotype. Bacteria also had no separation of germline and soma as found in most higher organisms. Early experiments had shown that pure cultures of bacteria would quickly adapt to a selective agent when challenged, but it was unclear whether such adaptation should be attributed to the mass conversion of cells from one state to another or to selection on randomly occurring genetic variation. In retrospect, some of these cases may have been the result of physiological adaptation, i.e. the regulation of gene expression. But in 1934, Lamarckian inheritance in bacteria remained a definite possibility (Lewis in Ref. 57, p. 636):

> The subject of bacterial variation and heredity has reached an almost hopeless state of confusion. Almost every possible view has been set forth, and there seems no reason to hope that any uniform concensus of opinion may be reached in the immediate future. There are many advocates of the Lamarckian mode of bacterial inheritance, while others hold to the view that it is essentially Darwinian.

Indeed, it was unclear whether bacteria even had genes analogous to those of higher organisms. Julian Huxley was careful to exclude bacteria from the modern synthesis in 1942 (39, pp. 131–132).

Bacteria Enter the Modern Synthesis

In the 1940s and 1950s, elegant experiments opening the way to modern bacterial genetics provided strong support for the Darwinian view of bacterial adaptation, and, in fact, provided the first direct demonstrations, in any organism, of the random nature of mutation. The logic and methodology of these experiments have proven important in the recent debate over directed mutation, and so we describe them briefly here.

THE FLUCTUATION AND RESPREADING TESTS In 1943, Luria & Delbrück formulated and tested two competing hypotheses to account for the appearance of cells resistant to viral infection in populations of *Escherichia coli* that were

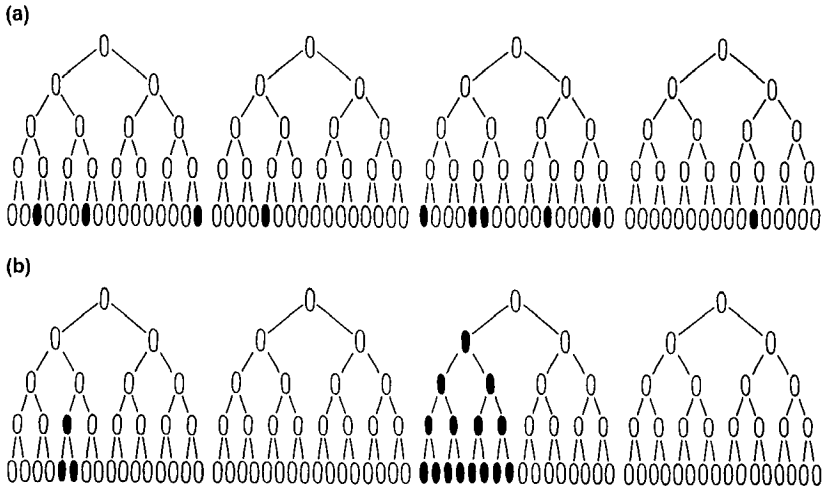

Figure 1 Schematic representation of the Luria-Delbrück fluctuation test. Distributions of mutants (filled symbols) across four populations, each founded from a single progenitor cell, expected under the hypotheses of (*a*) acquired hereditary immunity and (*b*) spontaneous mutation prior to exposure to the selective agent. The final row of cells represents the generation that is exposed to the selective agent. (Reprinted with permission from reference 85.)

previously sensitive to infection (58). The "acquired hereditary immunity" hypothesis supposed that each bacterium has a certain small probability of surviving exposure to the virus, and survival confers immunity that is inherited. In contrast, the "mutation" hypothesis supposed that each bacterium has a small probability of mutating spontaneously to viral resistance even in the absence of the virus, and that each descendant of a resistant mutant is itself resistant.

Luria & Delbrück deduced that the expected distribution of resistant mutants among independent cultures (each grown from a few sensitive cells) was markedly different under these two hypotheses. Under acquired hereditary immunity, resistance that arises with small probability per cell upon exposure to the virus should result in a Poisson distribution of resistant cells among cultures, with the expected variance equal to the mean (Figure 1a). Under the mutation hypothesis, however, occasional cultures in which resistant clones arose several generations before selection are expected to contain large numbers of resistant cells ("jackpots") compared with the average. The mutation hypothesis, therefore, predicts a clumped distribution of mutants among cultures, with variance greater than the Poisson expectation (Figure 1b). By spreading many cultures on agar plates containing the virus, Luria & Delbrück observed that resistant mutants were in fact distributed in jackpot fashion. This

result was consistent with the mutation hypothesis but not with the hypothesis of acquired hereditary immunity.

A related test was presented by Newcombe in 1949 (71). In the respreading test, thousands of sensitive bacteria were allowed to grow from single cells into nearly confluent lawns of microcolonies on agar plates. Control plates were sprayed with the selective virus without disrupting the colonies, while other plates were sprayed with the virus and then the colonies were respread around the plate. Because respreading does not change the number of cells present on a plate, the hypothesis of acquired hereditary immunity predicted that control and respread plates would show equal numbers of resistant colonies. However, Newcombe observed a large increase in the numbers of resistant colonies on respread plates relative to controls. This result indicated that clones of resistant mutants had arisen spontaneously during growth on the plate before exposure to the virus and had then been dispersed around the plate by respreading.

REPLICA PLATING AND SIB SELECTION The fluctuation test and the respreading test relied on quantitative reasoning to demonstrate the preexistence of bacterial variants resistant to selection. Neither test actually enabled an investigator to isolate variants without first exposing bacteria to a selective agent. Skeptics were not convinced (e.g. 37). In the 1950s, two additional tests—replica plating and sib selection—succeeded in demonstrating resistant variants in bacterial cultures never exposed to viruses or antibiotics.

In the replica plating test reported in 1952 by J. and E. Lederberg (50), cells were grown into a nearly confluent lawn of microcolonies on a nonselective agar plate. A piece of velveteen was then used to transfer a spatially ordered inoculum of cells from this "master plate" to several "replica plates" containing the selective agent, on which only resistant cells could form colonies (Figure 2). The Lederbergs observed a striking correspondence in the locations of resistant colonies on replica plates made from the same master plate, indicating that resistant cells had arisen by spontaneous mutation and increased in number by clonal growth on the master prior to selection. Furthermore, by pursuing a succession of master and replica plates with cells from suspected locations of resistant clones on each successive master plate, the Lederbergs were able to establish pure cultures of resistant bacteria without ever exposing them to the selective agent.

Cavalli-Sforza and J. Lederberg presented the related method of sib selection by limit sampling in 1956 (7). In this method, a primary culture containing a small number of presumptive mutants resistant to an antibiotic was divided into several equal subcultures, resulting in a chance increase in the proportion of resistant mutants in some subcultures. In essence, this procedure employs random genetic drift (i.e. founder effect) to increase nonselectively the proportion of mutants in certain subcultures. For example, if a primary culture

containing one mutant is divided into ten equal subcultures, the subculture that receives the single mutant has a proportion of mutant cells that is tenfold higher than the original proportion in the primary culture. Cavalli-Sforza & Lederberg recognized that this increased proportion of mutants should be roughly maintained upon regrowth of this subculture in fresh medium, provided the growth rate of the mutants in nonselective medium is comparable to that of the nonmutant cells. Selective plating of samples from each regrown subculture allowed Cavalli-Sforza & Lederberg to determine retrospectively which subculture contained the increased proportion of mutants. This regrown subculture was then subjected to a new round of subculturing and regrowth. By repeating this cycle several times, Cavalli-Sforza & Lederberg were able to isolate pure cultures of antibiotic-resistant bacteria from cells that had never been exposed to antibiotics.

CONCLUSIONS FROM THE CLASSIC EXPERIMENTS The experiments of Luria & Delbrück, Newcombe, Cavalli-Sforza, and J. and E. Lederberg showed that

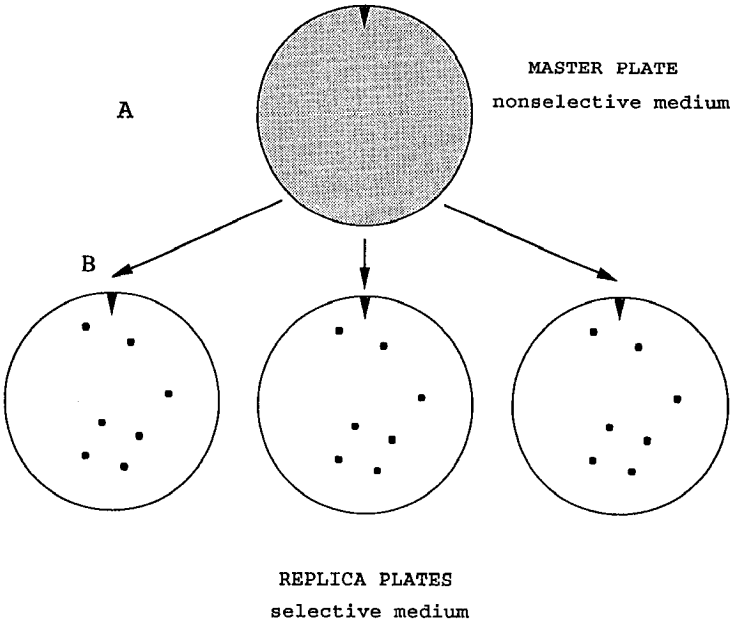

Figure 2 The logic of replica plating. (*A*) A large number of cells is grown into a nearly confluent lawn on a plate containing nonselective agar. A piece of velveteen (not shown) is then used to transfer spatially structured inocula from this master plate to replica plates containing selective agar. (*B*) The correspondence in location of colonies resistant to the selective agent on the replica plates indicates the spontaneous origin and clonal growth of resistance mutants on the master plate prior to selection. Sampling from the master plate using the spatial information in the replica plates allows the isolation of mutants that were never exposed to the selective agent.

heritable variants resistant to lethal agents could arise in bacterial populations before selection was applied; selection, therefore, could not have caused the occurrence of such variants. This finding suggested in turn that bacteria, as well as higher organisms, possess stable hereditary factors—genes—and that evolutionary adaptation in bacteria also proceeds via the action of natural selection on spontaneously arising genetic variation.

In retrospect, these experiments provided the first *direct* demonstrations of the random nature of mutation in any organism. These demonstrations were made possible by the ability of bacteriologists to manipulate and quantify vast clonal populations under controlled environmental circumstances, features not available to students of higher organisms. Bacteriology, the last major stronghold of Lamarckism, provided the textbook examples against Lamarckism.

THE DIRECTED MUTATION CONTROVERSY

Origin of the Controversy

In 1988, Cairns et al argued that the "classical experiments could not have detected (and certainly did not exclude) the existence of a non-random, possibly product-oriented form of mutation" (6, p. 142). They maintained that, because the classic experiments had employed lethal selective agents (viruses and antibiotics), the possibility that bacteria might adapt to nonlethal selective agents by some directed mutational process had been ignored. To that end, Cairns et al investigated cases "where the selective pressure rewards mutants by letting them multiply but allows all the other, non-mutant cells to survive so that they can at least have the opportunity to perform directed mutation" (6, p. 142). They concluded that the most plausible explanations for their experimental results resided in mechanisms that would confer on cells the capacity to adapt through the "inheritance of acquired characteristics" (6, p. 145). This and subsequent claims of "directed mutation" challenged the generality of the classic experiments demonstrating spontaneous mutation and raised a new controversy over the possibility of non-Darwinian adaptation.

Certain geneticists (e.g. 21, 83) seem to have found the evidence for directed mutation convincing. After all, claims of directed mutation emerged from the same powerful experimental system as the original demonstrations of random mutation. It is important to emphasize, however, that two significant aspects of the classic experiments have largely been overlooked in the directed mutation controversy. First, the authors of the classic experiments were careful about the assumptions of their tests. For example: in its simplest form, the sib selection experiment assumes that putative mutants and their progenitors grow at equal rates (are equally fit) in the absence of a selective agent. If, instead, mutants grow more slowly (are less fit), then the results of this experiment

will deviate from randomness in a manner suggestive of directed mutation. Rather than immediately invoke directed mutation on such evidence, Cavalli-Sforza & Lederberg (7) considered and quantitatively tested the alternative hypothesis of differential growth rate. By contrast, the failure to consider and test alternative hypotheses led to harsh criticisms of the recent experiments that claim to demonstrate directed mutation.

Second, the observation that some mutations occur after cells are exposed to a selective agent does not indicate that those mutations are caused by selection. To imply that postselection mutations per se challenge the Darwinian view of adaptation (46) is to confuse the method of the classic experiments (showing that variation arises before the imposition of selection) with the logical interpretation of their results (variation is not caused by selection).

We discuss the important evidence and ideas in the directed mutation controversy in the remainder of this section. As we show, several apparent cases of directed mutation have been undermined by subsequent demonstrations that experimental problems gave rise to artifactual results. Furthermore, the most plausible mechanisms proposed to explain remaining cases of apparently directed mutation are entirely consistent with the modern Darwinian view that genetic variation arises without regard to adaptive need; that is, variation precedes adaptation.

Initial Claims of Directed Mutation Advanced by Cairns et al

"POISSON-LIKE" DISTRIBUTIONS OF LAC+ REVERTANTS IN FLUCTUATION TESTS

The first modern case of apparently directed mutation involved the appearance of Lac^+ revertants in cultures of a Lac^- (*lacZ*$_{am}$ *uvrB*) strain of *E. coli* starving in a medium containing only lactose as a potential carbon source (lactose minimal medium). Cairns et al (6) reported that when numerous cultures of this strain were grown under permissive conditions and subsequently plated onto lactose minimal medium, the observed distribution of Lac^+ mutants per culture was markedly different from the jackpot distribution expected in a fluctuation test if mutants arose only before plating. In particular, substantial numbers of mutants appeared some days after plating on the lactose minimal medium, giving rise to a hybrid, "Poisson-like" distribution of mutants; such a distribution might be expected if these late-arising mutants occurred during starvation specifically in response to lactose. Cairns et al tested for the dependence of the late-arising mutants on lactose by plating cells onto medium containing no carbon source and adding lactose later; they found that Lac^+ revertants did not begin accumulating until after lactose was added. Furthermore, Cairns et al observed that mutants to a phenotype (valine resistance) unrelated to the lactose selection did not appear during starvation on lactose

minimal plates. To Cairns et al this result indicated that mutation rates were not generally elevated in the starving cultures.

Cairns et al argued that the occurrence of Poisson-like distributions, the appearance of late-arising Lac$^+$ mutants only after the addition of lactose, and the lack of increased mutation at an unselected locus all were consistent with the hypothesis of directed mutation to Lac$^+$ in the presence of lactose. However, numerous authors subsequently noted that these results were also consistent with spontaneous mutation. Many questioned the appropriateness of valine-resistance mutations as a control for elevation of the general mutation rate (13, 21, 38, 53, 54, 59, 61). Mutations to valine resistance can arise in several loci and by many types of sequence alteration, and they therefore may not be comparable to the reversion or suppression of an amber mutation in *lacZ*. MacPhee (60) showed that the assay conditions Cairns et al had used to detect mutations to valine resistance in starving cells actually suppressed the occurrence of those mutations. Many authors also pointed out that Poisson-like distributions can result from violations of various assumptions of the fluctuation test; hence, the appearance of Poisson-like distributions of mutants need not indicate directed mutation (10, 54, 55, 86, 87, 89). Indeed, a number of earlier authors had noted that discrepancies between fluctuation test results and the predicted jackpot distribution of mutants were not sufficient to reject the hypothesis of spontaneous mutation (44, 48, 75). For example, if mutants grow more slowly than nonmutants before exposure to the selective agent, then the distribution of mutants observed when the replicate cultures are plated on selective medium will be less variable than the jackpot expectation. Several authors noted that among the late-arising Lac$^+$ phenotypes observed by Cairns et al were many amber suppressor mutants, which are likely to grow slowly in permissive medium as a consequence of altered transcription (10, 38, 54, 55). Indeed, Cairns et al noted that these suppressor mutants produced characteristically small colonies on permissive agar plates (6).

No further experimental evidence has appeared for or against directed mutation to Lac$^+$ in the *lacZ*$_{am}$ *uvrB* strain investigated by Cairns et al, and this case must thus be regarded as unresolved. However, Cairns acknowledged the potential problems with the case, noting that "if these had been the only experiments, the [1988] paper would not have been written" (4, p. 527). Ironically, the case that Cairns regarded as stronger evidence for directed mutation has fared much worse.

A CASE OF DIRECTED MUTATION THAT SEEMED PARTICULARLY STRONG IS REJECTED: EXCISION OF PROPHAGE MU In *E. coli* strain MCS2 (76), part of the *ara* operon including a regulatory region has been joined to structural genes from the *lac* operon by bacteriophage Mu DNA containing transcription terminating signals. With this prophage intact, MCS2 cannot grow on either

lactose or arabinose. However, upon excision of the prophage in a suitable reading frame, MCS2 is phenotypically Lac(Ara)$^+$; it can grow on lactose if arabinose is present as an inducer. Shapiro (76) had noted that Lac(Ara)$^+$ excision mutants almost never arise in MCS2 cultures that are actively growing on glucose or glycerol, but that substantial numbers of Lac(Ara)$^+$ excision mutants appear in MCS2 cultures that have been starved for several days on medium containing only lactose and arabinose as potential carbon sources. In their 1988 paper (6), Cairns et al reported further experiments in which they were unable to recover Lac(Ara)$^+$ mutants from cultures starving on media not containing lactose and arabinose. These results led them to conclude that Lac(Ara)$^+$ mutants arose only when MCS2 cells were starving in the presence of lactose and arabinose, so that the occurrence of Mu excisions in MCS2 seemed a particularly clear case of directed mutation.

Mittler & Lenski (66) confirmed Shapiro's observations that Lac(Ara)$^+$ mutants almost never occur in growing cultures but do occur at high frequency when cells are starved on medium containing lactose and arabinose. However, in contrast to Cairns et al, Mittler & Lenski found that Lac(Ara)$^+$ mutants also occur in starving cultures on media that do not contain lactose and arabinose. The latter result suggested that Lac(Ara)$^+$ mutants are not directed by the presence of lactose and arabinose but instead are induced by starvation. Mittler & Lenski further showed that the frequency of Lac(Ara)$^+$ mutants detected in cultures of MCS2 starved without lactose and arabinose is stable when those cultures are regrown in glucose (66). This result clearly did not support the existence during starvation of unstable Mu excision intermediates that rapidly convert to the Lac(Ara)$^+$ phenotype only upon exposure to lactose and arabinose. Nonetheless, some proponents of directed mutation were skeptical of Mittler & Lenski's results (23, 78). Foster (23) implied that use of the classical methods of detecting preexisting mutations was necessary to confirm or reject directed mutation in MCS2.

Fluctuation analysis, sib selection, and replica plating have now all been used to test the directed mutation hypothesis in MCS2. All three approaches uphold Mittler & Lenski's finding that Lac(Ara)$^+$ mutations occur in starving cultures regardless of whether lactose and arabinose are present. Foster & Cairns (26) employed the fluctuation test to show that a jackpot distribution of mutants was obtained when replicate MCS2 cultures starved in liquid medium without lactose and arabinose were regrown and plated on medium containing lactose and arabinose. This result implies the existence of Lac(Ara)$^+$ excision mutants before the exposure of cultures to lactose and arbinose. Maenhaut-Michel & Shapiro (62) used sib selection to enrich the proportion of Lac(Ara)$^+$ excision mutants in starved MCS2 cultures, and they obtained pure cultures of Lac(Ara)$^+$ mutants without ever exposing the progenitor cells to lactose and arabinose. Finally, Sniegowski (81) used replica plating to show

that nearly all the Lac(Ara)$^+$ mutants detected when a starved MCS2 culture was exposed to lactose and arabinose were preexisting.

The Many Potential Flaws in Claims of Directed Mutation

In an earlier review, Lenski & Mittler (54) identified several effects that have the potential to mislead experimenters into concluding that directed mutation is occurring when, in fact, it is not. The *lacZ*$_{am}$ and Mu cases indeed illustrate two of these effects; others will be brought out below when we discuss subsequent cases of apparently directed mutation. The "Poisson-like" distributions of *lacZ*$_{am}$ revertants that Cairns et al observed in fluctuation tests were quite plausibly due to slow growth of some revertants, particularly amber suppressors, prior to selective plating. In the case of Mu excision, starvation and the presence of selective substrates were confounded; the observed discrepancy between rates of mutation to Lac(Ara)$^+$ during growth and during starvation on lactose-arabinose medium was a nonspecific consequence of starvation rather than a specific response to the presence of lactose and arabinose. Indeed, the case of Mu excision illustrates the general point, made earlier, that the occurrence of mutations after the imposition of a selective agent does not demonstrate that the selective agent is the cause of those mutations.

Subsequent Cases of Apparently Directed Mutation

We next consider several cases of apparently directed mutation that were reported after the 1988 paper by Cairns et al. We focus upon cases for which detailed experimental reevaluation has supported alternative explanations consistent with the modern Darwinian view of adaptation. We acknowledge that not all cases of apparently directed mutation have been so examined (see, e.g., 33, 84). Given the general nature of the potentially misleading effects in directed mutation experiments, some of these other cases may have explanations similar to those described below. We do not speculate here on such possible alternative explanations, except to note that no case of apparently directed mutation has received a full mechanistic explanation that supports a non-Darwinian process of adaptation. At the end of this section, we describe the most studied remaining case of apparently directed mutation, the so-called "adaptive" reversion of a *lac* frameshift in *E. coli*. After discussing recent results in this case, we consider the molecular models that have been invoked to explain it and other cases of apparently directed mutation. We stress that mechanisms in the most plausible models, though inherently fascinating and potentially important to the study of mutagenesis, are consistent with the modern Darwinian view that variation precedes adaptation.

EVENTS GIVING RISE TO DOUBLE MUTANTS IN THE *Bgl* OPERON: ANTICIPATORY MUTATION? Hall (31) studied an *E. coli* K12 strain in which two mutations in the *bgl* operon are apparently required for growth on salicin: excision of an insertion sequence, *IS*150, from a structural gene, *bglF*, and a mutation in a regulatory region, *bglR*. Hall observed that *IS*150 excision almost never occurred in growing cultures of this strain; consequently, salicin-utilizing (Sal^+) double mutants did not arise at detectable frequencies during growth on some other substrate. However, Hall detected large numbers of Sal^+ cells in cultures subjected to prolonged incubation on agar supplemented with salicin as the only available growth substrate. Hall reported that *IS*150 excision-mutant intermediates accumulated in these cultures before the appearance of Sal^+ double mutants, but that an excision mutant clone was incapable of growth on salicin without the second mutation in *bglR*. On this basis, Hall argued that the observed increase in the frequency of excision-mutant intermediates was the result of anticipatory directed mutation to produce a population of cells large enough to acquire the second, random mutation in *bglR* that would allow growth on salicin.

Hall's extraordinary claim of anticipatory directed mutation was challenged by Mittler & Lenski (67). Contrary to Hall's claim, these authors found that many excision mutants, including the one tested by Hall, are in fact capable of some growth on salicin. The growth of these excision-mutant intermediates increases the expected number of fully Sal^+ double mutants on selective salicin agar by many orders of magnitude, such that there is no need to invoke anticipatory directed mutation.

Hall has acknowledged that some excision mutants are capable of growth on salicin without the second mutation in *bglR*, and that such growth can explain his previous results in the *bgl* system without the need to invoke anticipatory directed mutation (34a). At the same time, however, Hall has made a further claim that *IS*150 excision is nonetheless directed in a genetic background in which no other mutations are required for full utilization of salicin (34a). To date, this new claim has not been challenged experimentally.

ENHANCED RATE OF APPEARANCE OF TRP^+ CELLS DURING STARVATION OF A *trpA trpB* DOUBLE MUTANT FOR TRYPTOPHAN Hall (32) also claimed that the appearance of Trp^+ cells in cultures of a *trpA trpB* strain of *E. coli* starved for tryptophan is "selection-induced," in that $trpA^+$ $trpB^+$ cells arise at far higher rates than expected from the product of the reversion rates of single *trpA* and *trpB* mutants in similar circumstances. Foster, however, suggested that single-mutant *trpA* $trpB^+$ intermediates might be able to grow on indole, a tryptophan precursor that can accumulate in medium without tryptophan as a result of excretion by $trpA^+$ *trpB* intermediates or breakdown of indoleglycerol phosphate excreted by the *trpA trpB* progenitor (25). As in the *bgl* case, the

accumulation, by growth, of an intermediate genotype could explain the increased occurrence of double mutants in starving *trpA trpB* populations without the need to invoke directed mutation. Further experiments by Hall have in fact revealed substantial growth of *trpA trpB*$^+$ cells in mixed culture with *trpA trpB* cells on selective medium, and Hall now acknowledges that selective enrichment of *trpA trpB*$^+$ intermediates may explain the increase in *trpA*$^+$ *trpB*$^+$ double revertants on medium without tryptophan (34).

BIASED RECOVERY OF DEX$^+$ MUTANTS Benson et al (2) examined mutation in an *E. coli* strain that lacks the LamB outer membrane protein and thus is unable to grow on large maltodextrins (Dex$^-$). Mutations in genes for two other membrane proteins, OmpC and OmpF, can give rise to Dex$^+$ phenotypes in this strain. Benson et al observed that when Dex$^-$ populations were starved on a medium containing only maltodextrins as a potential carbon source, OmpF$^+$ mutations apparently occurred at a much higher frequency than did OmpC$^+$ mutations (2), as though a process of directed mutation were taking place at the ompF locus. Upon further investigation, however, Benson et al discovered that OmpF$^+$ mutants overgrew their Dex$^-$ progenitors much more quickly than did OmpC$^+$ mutants, leading to a bias in the recovery, rather than the occurrence, of the OmpF$^+$ mutation (1).

REVERSION TO LEUCINE PROTOTROPHY IN *SALMONELLA TYPHIMURIUM* Dijkmans et al (18) observed Poisson-like distributions of Leu$^+$ revertants in fluctuation tests with a Leu$^-$ strain of *S. typhimurium*. The growth rates of Leu$^+$ mutants on permissive media prior to selective plating were similar to that of the Leu$^-$ progenitor, and this seemed to rule out one frequently suggested alternative to directed mutation. However, many Leu$^+$ clones consisted of cells that were 10- to 100-fold larger than nonmutant cells. Dijkmans et al postulated that the transition from nonmutant Leu$^-$ to much larger Leu$^+$ mutant cells is likely to involve a substantial initial delay in cell division as mutant daughter cells increase in size; this delay appears to be responsible for the observed Poisson-like distribution of seemingly directed, late-arising Leu$^+$ mutants on selective plates. Consistent with this hypothesis, Dijkmans et al observed that Leu$^+$ mutants that gave rise to jackpots on selective plates had normal cell sizes, in contrast to the late-arising mutants (18).

The Case of "Adaptive Mutation"

Despite the setbacks in the cases described above, directed mutation has recently garnered renewed publicity (27, 35, 74) under the guise of "adaptive mutation," a term that sits uneasily between Lamarckian and Darwinian connotations. DNA sequence data have suggested the involvement of known molecular mechanisms in this case. In a later section, we argue that the suggested mechanistic basis for the phenomenon of "adaptive mutation" is

entirely consistent with the modern Darwinian view that adaptation is a consequence of natural selection, not mutation. However, we first describe the important features in this case.

In 1991, Cairns & Foster reported that a strain of *E. coli* unable to grow on lactose because of a *lacI* frameshift polar on the *lacZ* region would revert to Lac$^+$ during prolonged incubation on lactose minimal medium (5). Unlike the case of Mu excision described above, this case was not a clearcut candidate for directed mutation; some Lac$^+$ mutations occurred in populations of this strain growing in permissive (nonselective) medium. However, Cairns & Foster showed that Lac$^+$ revertants did not accumulate in this strain during starvation when lactose was absent or when lactose was present but another growth requirement was unfulfilled. (The latter finding implies that lactose per se is not sufficient to promote recovery of the *lac* frameshift revertants. This observation is critical when it comes to considering the mutational mechanisms that may be involved and their implications, as we discuss further below.) Foster (24) has examined and apparently rejected many potential artifactual explanations similar to those we have described in conjunction with other cases of apparently directed mutation and concluded that the presence of lactose is necessary for Lac$^+$ mutations to occur during starvation.

DNA sequencing of Lac$^+$ revertants recovered during starvation of the *lac* frameshift strain on lactose minimal medium revealed that the majority of these are the result of single-base deletions in short mononucleotide repeats (27, 74). In contrast, sequencing of revertants recovered from growing cultures indicates a broader spectrum of mutational events, including duplications, deletions, and insertions that are many nucleotides in length. This change in the relative frequencies of recovered mutations suggests that certain mutational events occur more frequently in *lac* frameshift cells starving in the presence of lactose than in growing cells. (Artifactual explanations, however, have not been completely ruled out. For example, selection may favor certain mutants over others, as in the case of biased recovery of Dex$^+$ mutants.) Very recently, it has unexpectedly been shown that replication and possibly conjugal transfer of the plasmid carrying the defective *lac* gene may be involved in "adaptive mutation" (27a, 73a). These findings, while intriguing, further illustrate the lack of a clear understanding of the molecular mechanisms and population dynamics underlying apparently directed mutation in this system.

MOLECULAR MODELS PROPOSED TO EXPLAIN APPARENTLY DIRECTED MUTATION

> ... only a vitalist Pangloss would consider that the genes know how and when it is good for them to mutate.
>
> Th. Dobzhansky (19, p. 92)

Here we consider several molecular models that have been proposed to explain "adaptive mutation" and other cases of apparently directed mutation. We identify two major categories of model: 1. neo-Lamarckian models in which individual cells are postulated to possess the capacity to monitor their own fitness and somehow increase the probability of mutations conferring higher fitness; and 2. non-Lamarckian models. We argue that the mechanisms invoked in the second category of model are the more plausible, but we caution that no model has received experimental confirmation.

Neo-Lamarckian Models

SPECIFIC REVERSE TRANSCRIPTION OF mRNAS ENCODING SUCCESSFUL PROTEINS

In conjunction with the original claims of directed mutation, Cairns et al suggested that "the cell could produce a highly variable set of mRNA molecules and then reverse-transcribe the one that made the best protein" (6, p. 145). In other words, if a cell could somehow monitor protein variants and reverse transcribe the specific message that encoded the most successful one, then the result truly would be directed mutation. It is a fact that a single allele yields variable mRNA molecules as a consequence of transcription errors; upon translation, such variable mRNAs can produce variable proteins. Also, reverse transcriptase is present in some *E. coli* strains. However, the specific reverse transcription model postulates the existence of a heretofore unknown cellular component that can somehow monitor the effect of variant proteins on fitness and choose the appropriate mRNA for reverse transcription.

Foster (23) has argued that the specific reverse transcription model incorporates selection, in that cells first generate variable mRNAs and proteins and only successful ones are reverse transcribed. Clearly, however, the model invokes a non-Darwinian process in which an individual cell somehow assesses its own fitness and selects the appropriate protein, RNA, and ultimately mutation. It seems very unlikely that a cell can assess its own fitness dependably in this manner. Fitness is the cross-generational product of survival and reproductive success, and it need not correlate predictably (monotonically) with the activity of specific proteins or with the ability of cells to utilize specific growth substrates. How is an organism, in this case a cell, to assess its own fitness?

There is little current support for the specific reverse transcription model. Reverse transcriptase has not been discovered in the particular *E. coli* K12 strains used to study directed mutation. In addition, suppressor mutations that occur outside the transcribed gene also accumulate when Lac$^-$ cells are incubated on media containing lactose, a phenomenon not predicted by the model (25). Foster & Cairns have acknowledged the lack of evidence supporting the specific reverse transcription model, concluding that "selective condition does

not play an 'instructional' role in determining which DNA sequence changes arise" (25, p. 785).

NONRANDOM AMPLIFICATION OF BENEFICIAL MUTANT GENES Cairns & Foster (5) showed that RecA function is required for "adaptive" reversion of a *lac* frameshift mutant in *E. coli*, and they suggested that this finding implicates gene amplification as part of a mechanism for directed mutation. In essence, gene amplification could create a large target for mutation, consisting of an array of many copies of the relevant gene. Any gene in the array that garnered a mutation conferring growth would allow a cell to escape starvation, after which the amplified region could be resolved by a RecA-dependent process.

The problem with this model is that it can produce a high bias in favor of beneficial mutations only if such mutations can be identified by the cell and preferentially amplified further within an array. Otherwise, any favorable mutant sequence within an array has as high a probability as any other sequence of being lost when the array is resolved back to a single copy by random recombinational processes. Stahl (83) has noted that there is no known process that would allow a cell to treat one sequence in an array differently from another. In fact, the preferential amplification model suffers from the same general difficulty as that described above for the reverse transcriptase model: There is no known molecular mechanism that would allow a cell to assess its own fitness and preferentially generate or retain the mutation it needs.

Non-Lamarckian Models

A number of models have invoked mechanisms in which a cell need not assess the effects of different genetic variants on its own fitness. Instead, these models propose to explain apparently directed mutation either as a consequence of increased random mutation at specific loci induced by the selective agent (mutagenic transcription model) or of differential proliferation of genetic variants arising during limited DNA replication in nongrowing cells (incipient mutation models). In these non-Lamarckian models, as in the neo-Lamarkian models, the initial variation is proposed to occur at random. There is a crucial distinction between these two categories of models, however. The neo-Lamarckian models require that an individual cell somehow be able to scrutinize variants and select the most appropriate mutation. The non-Lamarckian models, on the other hand, simply assume that randomly arising genetic variants proliferate differentially as a consequence of their fitness effects. These models, therefore, actually invoke natural selection to explain apparently directed mutation (56, 80).

MUTAGENIC TRANSCRIPTION Davis (16) hypothesized that transcription might be mutagenic, such that the presence of a selective substrate (e.g. lactose) that

induces transcription increases the mutation rate at the selected locus. Such a mechanism could give the appearance of directed mutation, because beneficial mutations would arise at a higher rate in the presence of the substrate. However, the model also predicts that nonbeneficial (misdirected) mutations should arise at a higher rate in the presence of the substrate, and so it does not imply that mutation would be systematically beneficial at the selected locus (54).

Experimental evidence published to date has not supported the transcriptional mutagenesis model as an explanation for apparently directed mutation. Although addition of the gratuitous inducer IPTG to growing populations of an inducible Lac$^-$ strain does slightly increase the number of Lac$^+$ mutants observed, this effect is absent when IPTG is added to starving Lac$^-$ populations, contrary to the prediction of the model (16). In addition, the model does not explain why starving cells that constitutively transcribe the *lac* operon apparently accumulate mutations only in the presence of lactose (5, 6, 25).

INCIPIENT MUTATION MODELS Earlier, we alluded to an important finding in studies of "adaptive mutation": revertants of a Lac$^-$ frameshift mutant do not accumulate in the presence of lactose when there is a second, unfulfilled growth requirement (5). Evidently, lactose is not sufficient to promote apparently directed mutations in this system. The requirement for cell growth offers support for another category of molecular models for "adaptive mutation" first proposed by Stahl (82). These models invoke random DNA sequence alterations in starving cells, which we call "incipient mutations," to explain apparently directed mutation (Figure 3). If a coding strand in a starving cell should be altered so as to encode a variant sequence that can be transcribed and translated, and if the resulting protein allows the cell to grow and replicate its DNA, then one of the two daughter cells could possess a mutation at the site of the sequence alteration in the parent cell. If, however, the incipient mutation does not allow replication and cell growth (e.g. if there is a second, unfulfilled growth requirement), then nonmutagenic mismatch repair may eventually restore the original sequence, or the cell may die as a consequence of unrepaired damage. In either of the latter cases, a mutation will not be detected.

In response to the initial Cairns et al paper, Stahl (82) and Boe (3) proposed that the methyl-directed mismatch repair system might act more slowly during starvation than during growth, allowing unrepaired sequence alterations to be made permanent by chromosome replication if they enable cells to grow. Mismatch repair-deficient strains do show elevated mutation rates under selective conditions (3). However, the slow repair model predicts that uncorrected mismatch mutations should accumulate when a mismatch repair-deficient strain is starved, regardless of whether selection is applied. This is not the case: Lac$^+$ mutants apparently do not accumulate in Lac$^-$ strains deficient in repair when lactose is absent (25).

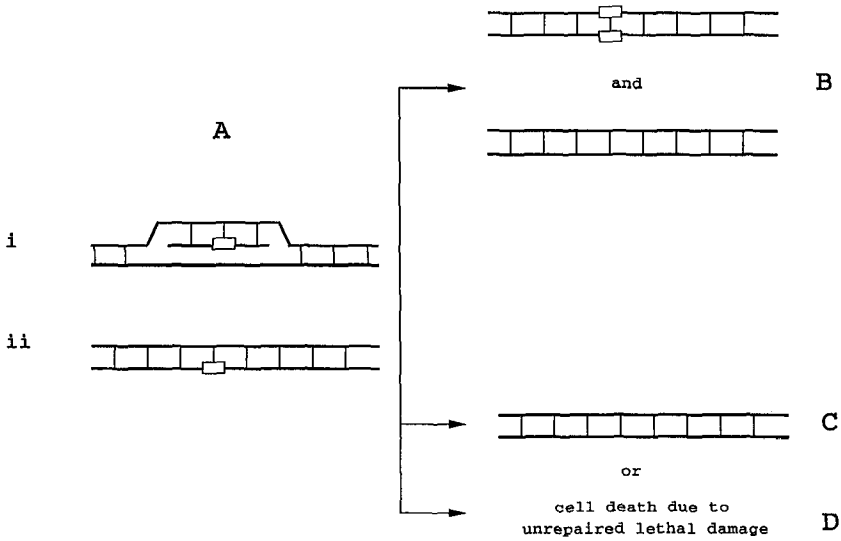

Figure 3 Hypothetical "incipient mutation" model to explain why certain mutations might be recovered only in environments in which they are advantageous. Polymerase error during limited DNA synthesis (A_i) or slow repair of DNA damage (A_{ii}) alters the genetic sequence originally present in a cell. By chance, the altered sequence encodes a functional gene (such as *lacZ*); before the altered sequence can be (correctly) repaired, it is transcribed and translated. (*B*) In an environment where the gene product allows the cell to grow and replicate its DNA (in the case of *lacZ*, where lactose is the sole carbon source), one of the daughter cells could inherit a mutation at the site where the original sequence alteration occurred. (*C*, *D*) In an environment where the gene product is superfluous (lactose is absent) or insufficient for cell growth (some other nutritional requirement is unfulfilled), then either nonmutagenic mismatch repair may restore the original sequence (*C*) or the cell may die as a consequence of unrepaired damage (*D*).

The discovery by Foster & Cairns that "adaptive mutation" is RecA dependent (25) led Stahl subsequently to suggest a second incipient mutation model. This model invokes a form of DNA synthesis, called "stable DNA replication" (17), which occurs in nondividing cells and is RecA dependent (95). Stahl suggested that such replication might ordinarily halt at the D-loop stage during starvation (Figure 3, part A_i), with subsequent degradation of the incipient strand. However, if a growth-enabling sequence change on the incipient strand could be transcribed and translated, then a full replication fork might form and the useful mutation could be transmitted to a daughter cell (Figure 3B).

We emphasize that there is presently no evidence to confirm any incipient mutation model. Nonetheless, mutations associated with limited DNA replication are implicated by some recent results on "adaptive" mutation (27, 74). As mentioned above, revertants recovered during starvation of the *lac*

frameshift strain on lactose minimal medium are mostly the result of single-base deletions in short mononucleotide repeats. Such sequence changes implicate polymerase errors, possibly associated with strand slippage during recombination, repair, or replication, as the cause of these mutations (27, 74).

It will be interesting to see whether an incipient mutation model such as those described above can be experimentally confirmed. It seems unlikely, however, that such a finding would alter evolutionary theory. As we have argued repeatedly in this paper, the key feature of the modern Darwinian theory of adaptation is that genetic variation arises at random with regard to its effects on fitness, such that adaptation occurs solely as a consequence of natural selection on this variation. According to the incipient mutation models, discrepancies between the two DNA strands arise at random with respect to their adaptive utility; the systematic difference in the proliferation of variant strands that these models invoke is due to natural selection. In contrast to the neo-Lamarckian models, the individual cell does not select, choose, or instruct anything in the incipient mutation models.

MUTATION AND ADAPTATION IN EVOLUTIONARY PERSPECTIVE: EVALUATING THE POTENTIAL ADAPTIVENESS OF MUTATIONAL PHENOMENA

> Beneath the imposing building called 'Heredity' there has been a dingy basement called 'Mutation.' Lately the searchlight of genetic analysis has thrown a flood of illumination into many of the dark recesses there, revealing some of them as ordinary rooms in no wise different from those upstairs, that merely need to have their blinds flung back, while others are seen to be subterranean passageways of a quite different type.
>
> H. J. Muller, 1921 (70, p. 106)

Throughout this chapter, we have argued that the evidence for directed mutation does not warrant a revision or qualification of the modern Darwinian theory that evolutionary adaptation occurs solely as a consequence of natural selection acting on randomly occurring variation. We have shown that many purported examples of directed mutation have alternative explanations of a more conventional nature. In addition, we have argued that the most plausible molecular models proposed to explain the current incarnation of directed mutation ("adaptive mutation") are fully consistent with the modern Darwinian theory of adaptation.

By arguing that mutation is random, we have not meant to imply that mutation occurs at equal rates at all loci or in all environments, or that mutations do not have definable, proximate causes. Rather, we argue that environmental factors (proximate causes) do not induce specifically those mutations that are beneficial. In this final section, we shift our focus and briefly consider

Table 1 Several mutational phenomena, their hypothesized adaptive significance, and possible alternative explanations.

Mutational phenomenon	Hypothesized adaptive significance	Alternative explanation
Starvation-induced mutagenesis	May occasionally allow an organism that is physiologically stressed, and which presumably has little to lose, to acquire the ability to use some available resource (e.g. 32).	See text.
Transposon activity	May promote complex variation not accessible by point mutation (64, 77).	Mutation may be an indifferent consequence of the activities of selfish DNA (9, 20, 73); causes mostly deleterious mutations
Transcription-induced mutagenesis	May allow an organism to improve particular genes under specific ecological conditions where the gene product is required for growth (16).	May be an unavoidable consequence of mechanistic constraints during transcription; may increase deleterious mutations in essential genes (54).
Hypermutable loci	May allow an organism to increase variation in certain "contingency" genes without increasing load of deleterious mutations in essential "housekeeping" genes (69).	Variation in rates among loci may have arisen for reasons unrelated to postulated adaptive value. Requires confirmation using comparative and experimental methods (69).
Mutation rate disparity between leading and lagging strands during DNA replication	May provide a balance between novelty and conservatism superior to what can be achieved by having both strands equally mutable (92).	May be an unavoidable consequence of DNA replication machinery (92).

the possibility that various mutational phenomena are nonetheless adaptive in the sense that they have been "designed" or maintained by natural selection (ultimate causes) because the random variation they produce increases evolutionary flexibility. As more is known about mechanisms causing mutation, speculation increases about the possible adaptive significance of these mechanisms as sources of variation (see Table 1 for some examples). Here we emphasize that notions about the adaptive significance of mutational phenomena must be regarded as evolutionary hypotheses, which require rigorous testing and independent confirmation.

The process of adaptation by natural selection is the cornerstone of modern

evolutionary theory, and so it is natural to look for adaptive explanations for organismal traits. But not all traits are the result of adaptation by natural selection. Students of morphology and behavior were given a sharp reminder of this in 1979 when Gould & Lewontin (29) labelled the uncritical invocation of adaptive explanations for various organismal traits as "adaptationism." Gould & Lewontin provided numerous alternative explanations for the existence of any particular trait, including the random fixation of alleles by genetic drift, developmental or mechanistic correlations among traits, phylogenetic inertia and constraints, and so on.

In the context of this paper, we suggest that, while a given mutation may sometimes have beneficial effects (e.g. the Lac$^+$ mutant that can grow in an environment where the cell would otherwise starve), it is not the case that the mechanism that causes mutation is necessarily adaptive. One finding from studies of the directed mutation phenomenon that is well supported is that certain mutagenic processes (for example, Mu excision) are increased in starving bacterial cells. One might assume that a starving cell has nothing to lose, and that an elevated rate of mutation during starvation is adaptive (beneficial) because a cell might thereby stumble on a good mutation that allowed it to grow on a substrate that happened to be available. But there is also an evolutionary downside, which is the possibility that a cell might acquire a deleterious mutation that would prevent it from growing in the event that the environment later became more favorable for growth (prior to death by starvation). In addition, the very mechanism of mutagenesis itself could involve the risk of cell death through unrepaired DNA damage (35).

Perhaps increased mutation in response to starvation is not an adaptation at all, but rather is symptomatic of a cell that is falling apart and losing control over its genetic integrity. (Indeed, in the case of Mu excision, a plausible evolutionary hypothesis is that the Mu bacteriophage has evolved the capacity to detect when its host is dying, and, as a consequence, to leave in search of a new host (65).) Consider the SOS response, in which an elevated mutation rate is induced by environmental stresses, such as UV irradiation, that cause damage to DNA (45). The increased mutations result from the action of enzymes that bypass DNA damage (e.g. pyrimidine dimers) that would otherwise block replication. This replicative bypass introduces mutations, and these mutations might occasionally have beneficial consequences. Even if the vast majority of mutations are detrimental, however, it is clearly more evolutionarily advantageous to repair the damage mutagenically than not to repair it at all, since the alternative is failure to replicate. Perhaps, then, the mutagenic effects of the SOS response are the best that can be made of a bad situation (e.g. see 27a, p. 510).

Given the above criticisms, some might throw up their hands at the apparent difficulty of determining whether a mutational phenomenon is or is not adap-

tive. Certainly the task is not likely to be easy. Several approaches, however, may allow this question to be addressed (69). One is theoretical analysis, which examines the costs and benefits of one evolutionary strategy relative to another. Such an approach can establish the conditions under which an adaptive explanation is feasible, and it may suggest variables that could be measured to shed further light on this feasibility. For example, in the case of starvation-induced mutation, the feasibility of the adaptive explanation may hinge on the relative rates of death due to starvation and environmental change that relieves starvation. There already exists a substantial theoretical literature on the evolution of mutation rates (e.g. 28, 40, 43, 51, 52), which may provide a framework for further analyses to address specific issues. A second approach is comparative. In essence, one tests the correlation between organismal traits and features of their environments. Although the comparative approach is very old, important methodological advances have recently been made that reflect the importance of phylogenetic considerations in developing appropriate statistical criteria for accepting or rejecting an association (36). A third approach, for which bacteria are particularly well suited, is experimental. The idea here is to devise selective regimes that would be expected to favor, for example, an increase in the trait of interest under one hypothesis but not under an alternative. Several experimental studies have examined the evolutionary adjustment of mutation rates (8, 11, 12, 72, 90, 91); the methodology of these experiments can provide a foundation for future research. Of course, the most compelling cases of adaptation are those that can be supported by careful theoretical, comparative, and experimental analyses.

Our point in criticizing adaptive explanations for various mutational mechanisms and phenomena is not to imply that these explanations are wrong or implausible. We believe, however, that such explanations should be regarded as evolutionary hypotheses until sufficient evidence is provided to corroborate or refute them. The rapidly advancing field of molecular genetics is sure to provide more intriguing possibilities of the kind listed in Table 1. We suggest that studies reflecting an informed evolutionary perspective will be essential to a comprehensive understanding of such phenomena. Such studies may further enrich the modern Darwinian perspective on mutation and adaptation.

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