# Are Some Mutations Directed?

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*A fundamental tenet of modern genetics is that mutations occur spontaneously, without regard to their effects on the organism's fitness. However, several authors have recently published evidence that suggests that the bacterium* E. coli *can respond to novel environmental conditions by inducing or directing specifically those mutations that enhance fitness in such environments. These claims of directed mutation have been met with alternative explanations for the reported observations.*

At times, it seems as though molecular geneticists and population geneticists have little in common in their scientific methods, assumptions and outlook. But of course this is not true. There are certain fundamental principles that all geneticists accept. For example, all mutations are supposed to occur spontaneously, without regard to their selective value for the organism. Right?

Not according to Cairns, Overbaugh and Miller[1], Hall[2] and Shapiro[3], who present evidence that certain mutations in bacteria occur more frequently in the presence of environmental agents that favor the resulting mutants than in the absence of these agents. It is known that there is variation among bacterial genotypes in mutation rates, and indeed selection may act to raise or lower mutation rates under certain circumstances[4–7]. However, it is an entirely different matter to suggest that mutations at particular loci are induced by specific environmental stimuli in an adaptive fashion.

### A classic experiment supporting the hypothesis of spontaneous mutation

The principles of bacterial heredity were largely ignored or misunderstood until the 1940s[8]. This ignorance stemmed from the fact that genetic variation among individual bacterial cells was essentially impossible to detect except by selecting on a population of cells. Thus, it was not obvious whether preexisting genetic variation had been selected or whether the selective agent somehow directed or induced the acquisition of an appropriate genetic change. The first study to resolve this issue clearly was published by Luria and Delbrück[9] in 1943. Like Mendel's work with pea plants, the experiments of Luria and Delbrück rested upon quantitative reasoning to distinguish among clearly formulated alternative hypotheses.

According to the hypothesis of spontaneous mutation, mutations occur at random at some low rate during the growth of a culture, even though they are only detected by virtue of their ability to form colonies when grown on a selective medium that prevents growth of the nonmutant genotype. According to the hypothesis of induced or directed mutation, however, the mutant genotype appears, with some low probability, as the direct result of its exposure to the selective environment. Luria and Delbrück[9] devised the following 'fluctuation test' to distinguish between these alternatives.

The investigator grows a number of 'sister' cultures, each founded from a few individuals of the same genotype, to a final density of which some fixed fraction is plated on the selective medium. Under the hypothesis of directed mutation, the number of *mutants* (as reflected by colonies on the selective plates) should be distributed among the sister cultures according to the Poisson distribution (such that the expected ratio of variance to mean is one), as illustrated in Fig. 1a. Under the hypothesis of spontaneous mutation, the number of *mutations* should be distributed according to the Poisson, but the number of mutants will have a much greater variance-to-mean ratio. This occurs because mutations will, by chance, occur earlier in some cultures than others; these early (or 'jackpot') mutations are represented in many descendants, as shown in Fig. 1b. Luria and Delbrück used this experimental design to examine the distribution among sister cultures of mutants that were resistant to a certain virus, and they observed the extreme 'fluctuations' (i.e. variance) expected if mutants had arisen prior to encountering the virus.

Later workers derived more precisely the 'jackpot' distribution of mutants obtained from sister cultures that is expected under the spontaneous mutation hypothesis[10]. In 1952, Lederberg and Lederberg[11] devised another type of experiment to distinguish between the spontaneous and directed hypotheses of mutational origins. Their replica plating experiment did not rely on the statistical machinations of the fluctuation test, but rather allowed them to demonstrate directly that mutants existed prior to selection.

### New evidence supporting the hypothesis of directed mutation

Let us now consider the major experimental evidence that has been used to revive the hypothesis of directed mutation. Before proceeding, however, it should be emphasized that none of the authors claims that all mutations are directed, but rather that previous studies demonstrating that certain bacterial mutations occur spontaneously have been inappropriately generalized.

Cairns *et al.*[1] examined a strain of *E. coli* that has a nonsense mutation in one gene of the *lac* operon, so that the cell cannot grow on minimal lactose medium. They performed a fluctuation test to observe the distribution among sister cultures of Lac$^+$ mutants. (The Lac$^+$ mutants were of two types: revertants and nonsense suppressors, wherein the cell reads through one class of stop codons.) Although the authors present no statistical analyses, their data appear to fit distributions expected under a hybrid model in which some of the mutations occur spontaneously during the growth of the culture, whereas others occur only after the cells have been plated.

Shapiro[3] studied a strain of *E. coli* in which a regulatory element from one operon (*ara*) controls expression of the products of another operon (*lac*), provided that a transposable bacteriophage Mu is excised. The excision generates cells that are phenotypically Lac(Ara)$^+$; i.e. they can grow on lactose only if arabinose is also present as an inducer. Shapiro observed that most Lac(Ara)$^+$ colonies were detected only after cells had been exposed to minimal lactose–arabinose medium for many days or even weeks. However, if the plates were seeded with small numbers of cells with the Lac(Ara)$^+$ phenotype, colonies appeared much sooner. Because the long delays are inconsistent with the demonstrably rapid growth of the derived genotype on that medium, it suggests once again that the mutations occurred in the selective environment, and were not simply observed as the result of selection for pre-existing mutants.

The results of Hall[2] are the most surprising, because they seem to imply that bacterial mutations can be induced not only when they immediately enhance fitness, but also when they create a future benefit in combination with some other forthcoming mutation! Hall worked with a strain of *E. coli* that required two mutations to make the *bgl* operon functional, such that it could use salicin as a substrate. These were excision of the

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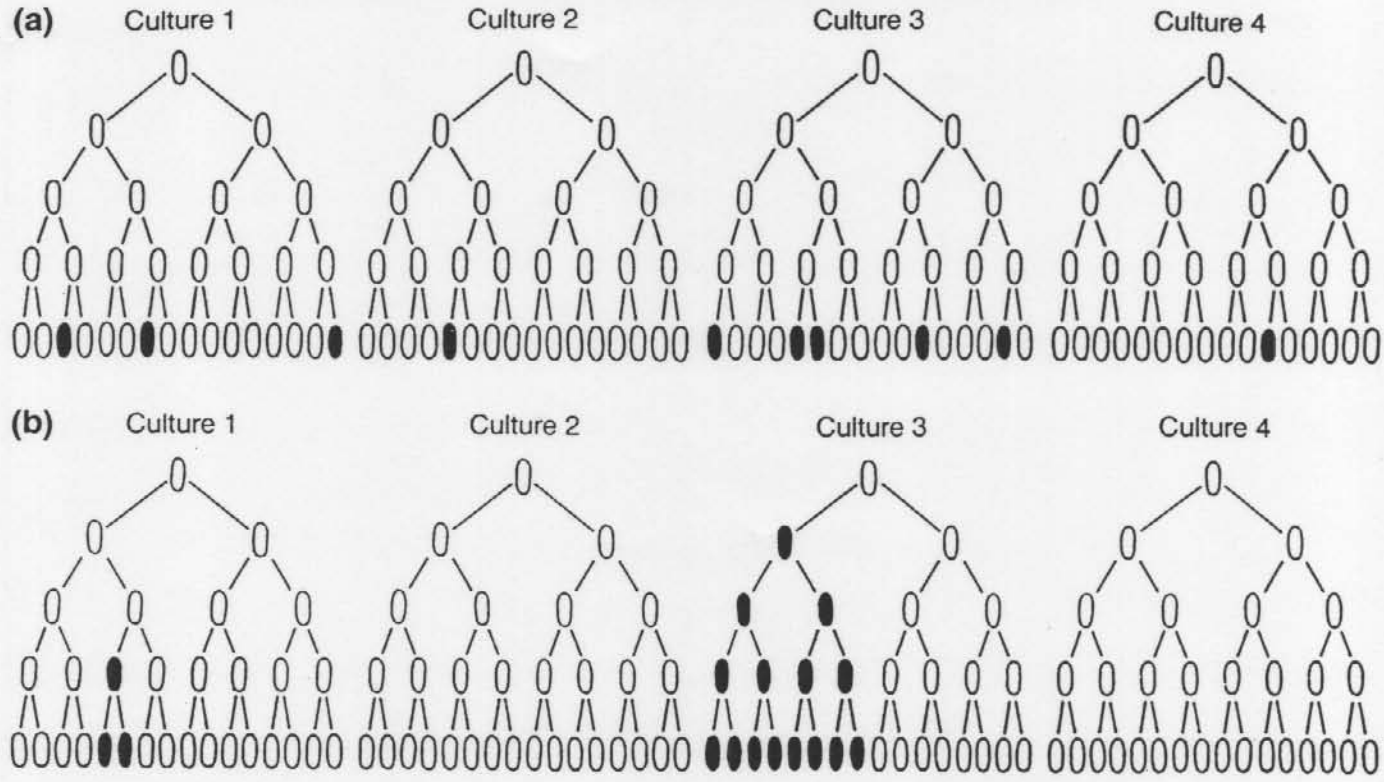


**Fig. 1.** Schematic representation of the appearance of mutants in four sister cultures; the final row indicates colonies formed on selective plates. (a) Typical distribution expected under the hypothesis of directed or induced mutation. (b) Typical distribution expected under the hypothesis of spontaneous mutation. *Redrawn from Ref. 8, with permission.*

insertion sequence IS*103* from the *bglF* structural gene (*bglF*::IS*103* to *bglF*$^+$) and mutation in the *bglR* regulatory gene (*bglR*$^0$ to *bglR*$^+$). Hall observed that the rate of excision of IS*103* in a *bglR*$^+$ strain was exceedingly low in the absence of salicin. However, when *bglR*$^0$ *bglF*::IS*103* cells were grown in the presence of salicin, *bglF*$^+$ excision mutants accumulated at a high rate, even though this mutation by itself did not confer the ability to grow on salicin. Hall even seeded salicin-containing plates with mixtures of *bglR*$^0$ cells with and without the excision mutation in order to determine whether there was some unanticipated advantage associated with the intermediate genotype, but he could find none.

### Possible mechanisms of directed mutation and alternative hypotheses

What could account for all of these results? One fairly trivial explanation is that there might be some general acceleration of mutation rates as cells sit starving on medium in which they cannot grow. Cairns *et al.*[1] and Hall[2] sought to rule out this explanation by demonstrating that another mutation (which if accelerated would not confer any growth advantage) did not accumulate under these conditions. However, this comparison may not provide a sufficiently tight control, because of possible differences in the effects of stress on different classes of mutations, such as reversion, nonsense suppression, and excision of transposable elements[12–14].

What mechanisms do the authors of these papers suggest could account for their claims of directed mutation? Hall[2] and Shapiro[3] do not propose any specific mechanism. However, they do argue that there is no reason that feedbacks could not exist that regulate the probability of specific mutations in response to appropriate stimuli, at least for organisms (like bacteria) in which there is no distinction between soma and germline.

Cairns *et al.*[1] are bolder, and propose a rather detailed scenario in which: (1) each gene produces variable RNA messages, which in turn produce variable proteins; (2) the cell somehow monitors the proteins and identifies the 'best' protein *and* the RNA message from which it was translated; and (3) that message is somehow sequestered and reverse transcribed into the DNA. Thus, 'individual cells [could] subject a subset of their informational macromolecules to the forces of natural selection. [This process] could, in effect, provide a mechanism for the inheritance of acquired characteristics.'[1] Interestingly, Lampson *et al.* have recently found a gene encoding a reverse transcriptase in a clinical isolate of *E. coli* [15].

Whether one believes that selection (albeit among molecules rather than individuals) acting on randomly generated variants (albeit among messages rather than genes) is more lamarckian or more darwinian[16–18], the mechanism proposed by Cairns *et al.*, if correct, would radically alter our views of the molecular genetics of bacteria. (A separate controversy[19] has arisen over whether Cairns *et al.* failed properly to cite an earlier hypothesis that reverse transcription could give rise to quasi-lamarckian inheritance.)

Aren't there any simpler explanations? Stahl[20] offers another explanation for how selection might act at the molecular level, indeed directly on the DNA. Mutation is a two-step process. 'Incipient' mutations arise through mistakes or damages to DNA, and cause a mismatch between the two DNA strands. Most incipient mutations are corrected by specific enzymes before the DNA is replicated. Once replication occurs, however, the tell-tale mismatch is obliterated and the mutation is stabilized. Thus, whether or not an incipient mutation can ever be observed depends on the relative rates of correction and replication. Stahl suggests that an incipient mutation might cause the transcribed DNA strand to encode a functional product, thereby allowing a cell to replicate where it otherwise could not, and thus stabilizing the mutation. This explanation, while still suggesting an unanticipated correlation between the likelihood of *observable*

mutations and their utility to the organism, would probably not profoundly alter either molecular or population genetic orthodoxies.

In addition to explanations that rely on phenomena at the molecular level, there have been alternative hypotheses that invoke phenomena acting at the level of individuals within populations. Several discussions[12,17,21,22] of the experiments of Cairns *et al.*[1] have pointed out that the distribution of mutants among sister cultures expected under the hypothesis of spontaneous mutation depends upon the assumption that mutants and non-mutants have equal fitnesses *prior* to selective plating. Indeed, Koch[23] has shown previously that if mutants are disadvantaged prior to plating, this has the consequence of reducing the variation among sister cultures. Many of the Lac$^+$ mutants obtained by Cairns *et al.* were nonsense suppressors, which often have deleterious effects on fitness[24].

Another population-level alternative is that the mutant genotypes detected in these experiments may differ from the original genotypes by two mutations[17,25], rather than the one that is assumed by Cairns *et al.*[1] and by Shapiro[3]. It is known that excision of certain transposable elements (including Mu, used in the Shapiro study) is usually imprecise, so that it often takes two steps to restore a fully functional genotype[26]. If some of the intermediates can grow even slightly under selective conditions, then this two-step process could explain the appearance of mutant colonies after delays that are inconsistent with the subsequent rapid growth of these genotypes in the selective medium[17].

None of these alternatives provides much of an explanation for Hall's results[2]. There are two mutations, but Hall could not detect any selection to account for accumulation of the intermediate (although Symonds[14], for one, is unconvinced by the robustness of this conclusion). Stahl's[20] model also fails because the first incipient mutation would not permit the cell to replicate and thereby stabilize that mutation. The model of Cairns *et al.*[1], and indeed the general idea of regulation of specific mutations put forth by Hall[2] and Shapiro[3], cannot easily create the sort of feedback that would increase the likelihood of a mutation that was beneficial only in combination with some future mutation.

One puzzling aspect of Hall's data was noted by Lenski *et al.*[25] The intermediate genotype (i.e. with IS*103* excised) was not randomly distributed across sister populations exposed to the selective medium, as one would expect for an induced mutation, but rather was clustered, as one would expect if excision mutants had arisen spontaneously. Other sources of variation among sister populations might account for this clustering. But of course Luria and Delbrück[9] did not invoke *ad hoc* variation of this sort.

Exactly what evidence would convincingly demonstrate directed mutation? At the very least, it is now clear that such studies must (1) rule out artifacts due to 'cryptic' selection, including the differential growth of mutants and non-mutants prior to plating and the enrichment of intermediates subsequent to plating; and (2) demonstrate that defined classes of mutations are stimulated by particular environmental factors *only if* they cause a selective advantage. When such evidence has been gathered, we will know whether (and how greatly) we must alter the current dogmas of molecular and population genetics.

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