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Mutation and selection in bacterial populations: Alternatives to the hypothesis of directed mutation

(population genetics/evolution/acquired characteristics/jackpot distribution)

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ABSTRACT **Bacterial populations have served as model systems for studying evolutionary processes ever since the classic experiments of Luria and Delbrück, which demonstrated the occurrence of mutations prior to selection for the traits they conferred. However, several authors have recently presented experiments suggesting that bacteria may have mechanisms for directing which mutations occur, such that the rate of adaptive mutations is enhanced. Before the hypothesis of directed mutation is accepted, it is imperative to consider alternative hypotheses that might account for the same observations. To this end, we expand upon existing mathematical theory of the dynamics of mutation and selection in clonal populations for two cases of particular interest. The first case concerns selection against mutants before plating; this selection occurs as the result of differences in growth rate between mutants and nonmutants. We demonstrate that this selection model gives rise to distributions of mutants, obtained by plating from sister cultures, that are very similar to those expected when some mutations are induced by the selective environment. The second case concerns the sequential incorporation of two mutations as the result of selection for an intermediate genotype after plating. We demonstrate that this two-step mutation model also yields distributions that are similar to those expected when some mutations are induced by the selective environment. These two cases therefore provide alternatives to the hypothesis of directed mutation. We suggest experiments that might be used to examine our alternative hypotheses. We also contrast the hypothesis of directed mutation with the notion of inheritance of acquired characteristics.**

Luria and Delbrück (1) showed that the distribution of mutants obtained by selective plating from sister cultures will have a jackpot distribution, which is characterized by a high ratio of variance to mean, when the following conditions are met. Each sister culture starts from a small inoculum of some fixed number of cells (N_0), such that no mutants are present when the sister cultures are founded. Each sister culture then grows exponentially at some rate (r) for a fixed period of time (t). During each time interval, there is some probability (u) that a cell mutates. Once a mutation has occurred in a cell lineage, it has no effect on the subsequent growth of that lineage. Some or all of each sister culture is then plated on a medium that permits each mutant cell to grow into a visible colony, but prevents the growth of nonmutant cells. Lea and Coulson (2) have shown that the jackpot distribution is described by a single parameter (m) that represents the expected number of mutational events per culture: $m = u(N_t - N_0)/r$, where $N_t = N_0e^{rt}$. By contrast, if mutations occur only after cells have been plated on the selective medium, with some low probability, then the expected distribution of mutants obtained from sister cultures will be the Poisson distribution, in which the variance is equal to the mean (1).

However, several authors (3–5) have published experiments that suggest that certain mutations occur more frequently in the presence of the environmental factors that favor their subsequent growth than in the absence of those factors. This hypothesis of directed mutation rests on two types of observations. (*i*) The distribution of mutants obtained by selective plating from sister cultures is sometimes less variable than expected on the basis of mutations that occur at random during the growth of independent cultures. The observed distribution appears to fit a hybrid model, in which some mutations occur at random prior to plating, whereas other mutations are induced by the selective environment (3). (*ii*) Some mutants appear after a long delay following selective plating. The delay is inconsistent with the rapid growth of these mutants on the plates, and the number of late mutants is too high, given the rate of mutation estimated from cells cultured under nonselective conditions (3–5).

If the hypothesis of directed mutation is, indeed, correct, it has onerous implications for bacterial genetics and, in particular, for the use of bacterial populations as model systems for the study of evolutionary processes. Before such a hypothesis is accepted, it is imperative to exclude alternative hypotheses that might account for these types of observations. In this paper, we expand upon existing mathematical theory of the dynamics of mutation and selection in clonal populations for two cases of particular interest. The first concerns selection against mutants prior to plating, which occurs as the result of differences in growth rate between mutants and nonmutants. The second concerns the sequential incorporation of two mutations as the result of selection for an intermediate genotype after plating. We suggest experiments that might be used to distinguish these alternative hypotheses from the hypothesis of directed mutation. We also contrast the hypothesis of directed mutation with the notion of inheritance of acquired characteristics.

Selection Against Mutants Before Plating

The jackpot distribution assumes that mutants and nonmutants have equal growth rates during growth in the sister cultures (i.e., prior to selective plating). However, many mutants that are selected under one set of environmental conditions grow more slowly under other conditions (6–9), owing to pleiotropic effects of the mutations. If mutants grow more slowly than nonmutants, then mutations that occur by chance earlier in some sister cultures than in others will contribute less to the number of mutants at some later time than if mutants grow at the same rate as nonmutants. This has the effect of reducing the variation among sister cultures (8, 10–12).

Koch (8) has already modified the equations of Lea and Coulson (2) to allow for selection against mutants before

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plating. He did so by assuming that mutants had a different growth rate from nonmutants and letting g be the ratio of the two growth rates. We repeated Koch's extension of Lea and Coulson's analysis; we did not obtain the solutions in closed form, but the resulting equations were easy to solve numerically to obtain the distribution of mutants from sister cultures as a function of g and m. We were then able to compare this selection model with the hybrid mutation model presented by Cairns *et al.* (3), in which some fraction of the mutations are induced by the selective environment. As shown in Fig. 1, the distributions under the selection model are almost identical to the hybrid mutation model when the parameters are chosen to provide good agreement to the experimental data of Cairns *et al.* (In our numerical analysis of the selection model, we also considered the possibility that there were two or more classes of mutants with different growth rates. We found that, to a close approximation, the distribution of mutants obtained from sister cultures could be predicted from the weighted average of the growth rates for the different mutant classes.)

One can falsify the hypothesis of selection before plating by demonstrating that mutants and nonmutants do not differ in their growth rate by an amount sufficient to account for the observed distribution. By means of appropriate experimental designs and analyses (6, 7, 9, 13–15), it is possible to detect or reject even small differences in growth rate between two clones.

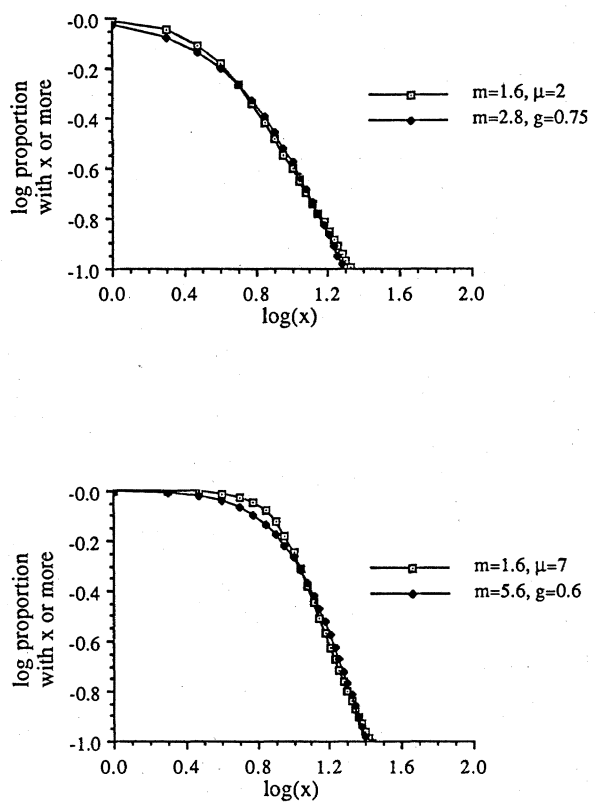

FIG. 1. Model of selection against mutants before plating is contrasted with the hybrid mutation model of Cairns *et al.* (3). The plots indicate the expected distributions of mutants obtained by plating from sister cultures. Because the distributions are cumulative, the proportion of cultures with x or more mutants must decline with increasing x. For both models, m is the mean number of mutations that occur randomly during the growth of a culture. For the hybrid mutation model (⊡), μ represents the mean number of directed mutations, which occur only when the last generation of cells is tested on the selective medium. (μ is different from u; the latter is the rate of random mutation, and m is a function of u, as described in the text). For the selection model (◆), g indicates the growth rate of the mutant genotype relative to the nonmutant genotype before plating. The parameters used for the hybrid mutation model in the two panels correspond to those used by Cairns *et al.* to fit the observed distributions of Lac$^+$ mutants obtained for two different strains of *E. coli*. The selection model generates very similar distributions.

Sequential Incorporation of Two Mutations, with Selection for an Intermediate Genotype After Plating

The jackpot distribution also assumes that colonies observed on selective medium differ from the genotype used to found the sister cultures by only a single mutation. But certain adaptations occur by two (or more) mutations [refs. 4, 16, 17 (pp. 561–562), and 18 (pp. 137–139)], which may be incorporated into a lineage either simultaneously or, more commonly, sequentially. Selection that favors an intermediate genotype clearly accelerates the sequential incorporation of two mutations. However, we are unaware of any previous work that has addressed the problem of how selection for an intermediate would affect the distribution of double mutants obtained by selective plating from sister cultures. We have therefore developed the following model for haploid genotypes. A is used to found the sister cultures; A cannot grow at all after selective plating. A mutates to A' at the rate u; A' grows slowly after selective plating. A' mutates to B at the rate v; B grows quickly on the selective plates.

In our analysis, we make two simplifying assumptions. (*i*) We assume that the rate of direct mutation from A to B is negligible; this allows us to focus upon the two-step transition through the intermediate A'. (*ii*) We assume that A and A' grow at the same rate in the sister cultures; therefore, the distribution of A' cells immediately after plating is characterized by the single parameter m of the jackpot distribution (cf. ref. 19).

Each A' cell that is present on the selective plate forms the center of a "microcolony," which grows at the rate c. Hence, t' time units after plating, each microcolony contains $K = e^{ct'}$ cells. The probability that at least one mutation from A' to B has occurred in a microcolony is expressed by $p = 1 - e^{-vK}$. (If there is some upper limit on the size of microcolonies, as might arise by deterioration of the selective medium with time, then p will remain <1 even as t' increases indefinitely. Between the first mutation to B and the formation of a readily visible colony, there will be, of course, some additional time elapsed that depends upon the growth rate of B on the selective medium.)

Under this two-step model, the distribution of mutants obtained by selective plating from sister cultures depends critically on the time between selective plating and counting of visible colonies. In general, many colonies [i.e., "late" mutants (3)] will appear after delays that seem inconsistent with the rate of growth of genotype B on the selective medium, as shown in Fig. 2. Moreover, when $p < 1$, the distribution of mutants can be similar to the distribution expected under the hybrid mutation model of Cairns *et al.* (3), as shown in Fig. 3.

It is quite easy to imagine situations in which the sequential incorporation of two mutations might occur and yet go undetected. For example, if one plates 10^9 cells from each sister culture, then the presence of 10^3 microcolonies of A', each containing 10^5 cells, would have little effect on the total population on the plate. Yet, given these numbers, secondary mutations could be quite common. Moreover, because the secondary mutations must invariably occur within these microcolonies, the B cells quickly overgrow their A' progenitors, making detection of these intermediates even more difficult.

The hypotheses of directed mutation and of sequential incorporation of two mutations might be distinguished on the basis of direct genetic evidence. Alternatively, under the latter hypothesis, there must occur on the selective medium

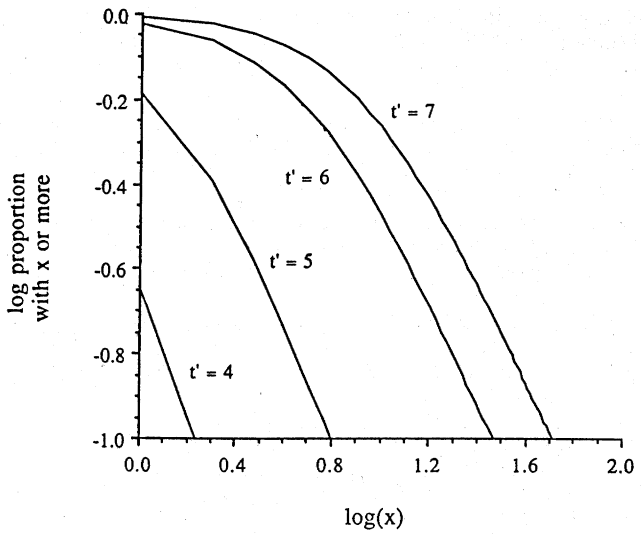

FIG. 2. Accumulation of secondary mutants (genotype B) with time (t') on selective plates according to the two-step model. $m = 4$ is the mean number of mutations from A to A' during growth of sister cultures before plating; $c = 2$ per unit time is the rate of increase in the number of cells in microcolonies of A' on the selective plates; and $v = 10^{-5}$ is the rate of mutation from A' to B. Visible colonies of genotype B are seen after a further interval that depends upon the rate of growth of B on the selective plates. For definition of x, see the legend for Fig. 1.

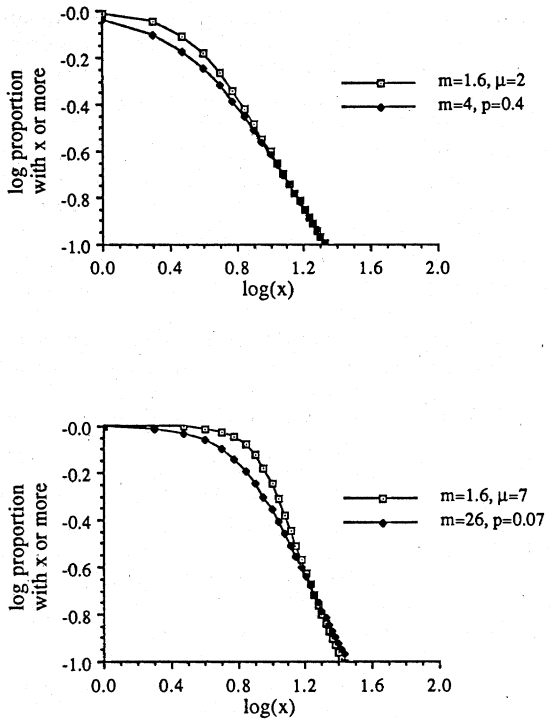

FIG. 3. Model of sequential incorporation of two mutations is contrasted with the hybrid mutation model. For the two-step model (♦), m represents the mean number of mutations from A to A' that occur randomly during the growth of a culture, and p indicates the proportion of microcolonies of A' that have experienced at least one mutation to B, which quickly forms visible colonies. The parameter p is a function of the rate of mutation from A' to B and of the number of cells in each microcolony, which, in turn, depends upon the growth rate of A' on the plates and the time elapsed since plating. The hybrid mutation model (⊡) of Cairns *et al.* (3) and the definition of x are described in the legend to Fig. 1. The model of sequential incorporation of two mutations generates distributions similar to the hybrid mutation model.

a change in clonal composition that leads to an increased likelihood of mutation to the readily observed genotype B. One can determine whether or not such a change has occurred as follows. First, one must sample a large number of clones from the selective plates after several days or weeks have elapsed. Any clones of genotype B can be easily excluded by testing their ability to grow rapidly on the selective medium. Then, the remaining clones must be cultured independently (and from small inocula) under nonselective conditions, and these quasi-sister cultures must be plated on selective medium once again. (In essence, one repeats the Luria and Delbrück fluctuation test, but using clones harvested from a population that has been exposed to the selective environment for an extended time period.) If the population on the original plates contained a mixture of genotypes A and A', and if A' has increased in frequency relative to A, and if A' mutates to B at a higher rate than does A, then the numbers of colonies of genotype B obtained from the quasi-sister cultures should be greater, on average, than the numbers obtained for true sister cultures founded exclusively by genotype A. If mutations to B occur as the consequence of exposure to the selective environment, however, any increase in the rate of mutation should be eliminated by the many generations of growth under nonselective conditions. (Forty to sixty generations will occur during colony formation plus growth in liquid medium before the second plating.) Moreover, if the resulting distributions support the hypothesis of sequential incorporation of two mutations, then one can isolate the A' intermediate from among those quasi-sister cultures that produced the most B mutants, and one can demonstrate that the higher rate of mutation is indeed a reproducible property of a particular clone (16).

Inheritance of Acquired Characteristics

The alternatives to the hypothesis of directed mutation that we have presented involve selection acting at the level of individuals within populations. By contrast, the processes hypothesized by Cairns *et al.* (3) and by Stahl (20) require selection acting on molecular variants within cells. There is an extensive literature in evolutionary biology concerning the potential for selection to act at different levels of organization (21–25). We do not reject outright the potential for selection acting on molecular variation within cells to produce changes in gene frequency. Rather, we argue that any plausible hypothesis to account for the types of observation reported by Cairns *et al.* (3), Hall (4), and Shapiro (5) must distinguish between the generation of variation and selection, at whatever levels of biological organization.

Cairns *et al.* (ref. 3, p. 145) assert that directed mutation in bacteria (if it is demonstrated to exist) "could, in effect, provide a mechanism for the inheritance of acquired characteristics." We disagree with this claim; we also view it as potentially harmful in that it may seem to give credence to prescientific claims that have been thoroughly disproved. The inheritance of acquired characteristics is a notion associated with Jean Baptiste Lamarck (1744–1829), whose second law for explaining adaptation by animals says, "*Everything which nature has caused individuals to acquire or lose as a result of the influence of environmental conditions* to which their race has been exposed over a long period of time . . . is conveyed by generation to new individuals descended therefrom" (ref. 26, p. 113, our emphasis).

The case against the inheritance of acquired characteristics was made by August Weismann (1834–1914), who observed that, in animals, the hereditary material is contained in the nucleus: "heredity is brought about by the transmission from one generation to another of a substance with a definite

chemical . . . constitution" (27). Moreover, Weismann had noticed that the germ-line cells of animals are segregated early in the life of the individual, and he argued that there is no mechanism by which acquired modifications of the somatic cells could be transmitted to the nuclei of the germ cells. The matter was further clarified in 1909 when Wilhelm Johannsen (1857–1927) coined the terms genotype and phenotype to differentiate the genetic constitution of an individual from its appearance. Clearly, Cairns *et al.* (3) are not proposing that all phenotypic modifications are inherited, nor are they denying that heredity in bacteria is mediated by DNA.

It might seem that the environmentally directed mutation hypothesis of Cairns *et al.* (3), if correct, could still resemble Lamarck's view of how organisms acquire adaptive characteristics. But this also is not so. According to Lamarck, adaptive somatic changes are acquired by organisms in response to their environment, but this change occurs through the effects of use and disuse of organs and other parts. Thus he expresses in his first law: "In every animal. . . frequent and sustained use of any organ gradually strengthens, develops, and enlarges that organ . . .; while the constant disuse of such an organ . . . weakens it . . . until it finally disappears" (26, p. 113). Therefore, even if directed mutation were demonstrated in bacteria, this would not support in any manner whatsoever the traditional notions of Lamarck and his followers concerning the acquisition of adaptive characteristics.

Nothing is gained but confusion by using the same phrase, inheritance of acquired characteristics, with two disparate meanings: (*i*) mutations adaptively induced by the environment but ingrained and transmitted in the DNA of bacteria, as hypothesized by Cairns *et al.* (3), and (*ii*) the traditional meaning that ignores the definite chemical basis of heredity as well as the difference between genotype and phenotype and that attributes the origin of adaptations to use and disuse. The semantic distinction is of scientific and historical importance. Moreover, any confusion between the two may perpetuate mistaken beliefs concerning heredity that are still widely held outside scientific circles.

Conclusions

We have formulated two explicit and testable alternatives to the hypothesis of directed mutation. As these alternative hypotheses rest upon well-known processes, we believe that it is premature to conclude that novel mechanisms are needed to explain the types of data that have been reported thus far. Further work on this subject is needed. Appropriate mathematical models and experimental designs will clearly be required to decide among the various hypotheses. Regardless of the outcomes of further research, the hypothesis of directed mutation should not be equated with the notion of inheritance of acquired characteristics.

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