Mathematical Issues Arising From the Directed Mutation Controversy

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ABSTRACT

During the past 14 years or so a large body of new evidence that supposedly supports the directed mutation hypothesis has accumulated. Interpretation of some of the evidence depends on mathematical reasoning, which can be subtler than it appears at first sight. This article attempts to clarify some of the mathematical issues arising from the directed mutation controversy, thereby offering alternative interpretations of some of the evidence.

A long-held fundamental tenet in evolutionary biology posits that mutations occur at random, regardless of fitness consequences to the resulting mutants; in nature it is selection that decides which mutations will be preserved. This so-called random mutation hypothesis has been repeatedly challenged and defended since early the last century, but experiments conducted since 1988 presented decisively new findings, supposedly lending stronger support to an alternative hypothesis that mutations are more likely to occur when the environment favors the survival of the resulting mutants. These new findings impinged on evolutionary thinking with long-lasting repercussions. However, a few mathematical issues inseparably intertwined with the controversy have so far received surprisingly little attention. This neglect has caused considerable confusion, severely impeding progress in resolving a fundamental controversy in modern history of evolutionary biology. This article attempts to address some of these issues.

The foregoing alternative hypothesis has acquired numerous names in the course of the controversy, but it is most commonly called the directed mutation hypothesis. To avoid nomenclature difficulties, we first articulate an interpretation of the random mutation hypothesis: The rate of occurrence of mutation is independent of fitness that the mutation confers to the mutants. This interpretation serves as a contrast by which the directed mutation hypothesis can be more easily comprehended. Thus, a mutation mediated by a newfound mechanism does not automatically qualify as a directed mutation, for the existence of an undocumented mutational mechanism itself is not a deviation from the random mutation hypothesis. However, if this mutation can be proven to occur only under some specific environmental conditions that favor the survival of the resulting mutants, then that mutation can be a possible example of directed mutation. A less stringent criterion is whether a certain type of mutation occurs more often under conditions favorable to the survival of the resulting mutants.

That is the viewpoint from which this article reexamines some of the new evidence produced since 1988 to support the directed mutation hypothesis. Mutations occurring in nondividing cells characterize the new evidence. It is worth noting that Hendrickson et al. (2002) have recently conducted a carefully designed experiment to show that some seemingly replication-independent mutations may actually be mediated by cell replication. However, this article accepts the assumption that not all mutations are mediated by DNA replication and focuses on replication-independent mutations that were relatively poorly documented. We begin our discussion by reiterating an important principle that once played a prominent role in the controversy some 60 years ago.

THE MUTATION-MUTANT PRINCIPLE

In the 1940s the focus of the controversy was whether bacterial mutants resistant to a virus can arise spontaneously. Luria and Delbrück (1943) answered that question by experimentally proving that such mutants could arise before a cell population came into contact with the virus. By first growing cells in a liquid medium that reduces the possibility of cell clustering and by subsequently plating cells on a solid, selective medium that immobilizes cells, one can in theory conduct a fluctuation experiment in which all mutational events are registered on a plate in one of two distinct ways: A preplating mutation is registered by as many colonies as the mutant cells spawned before plating by that mutation, and a postplating mutation is registered just as a single colony. If spontaneous mutation does occur, one can choose experimental conditions such that the number of postplating mutations is negligible compared to the number of mutant cells resulting from preplating mutations, thereby observing mutant colonies obeying a jackpot distribution (Luria and Delbrück 1943; Lea and Coul-
son 1949). [If the preplating incubation period is not long enough, the distribution may resemble a Poisson distribution (ZHENG 1999).] On the other hand, if spontaneous mutation does not occur, one finds only colonies due to postplating mutations, thereby observing Poisson-distributed colonies. The Poisson distribution arising in this context is merely a consequence of the long-held axiom that cells mutate with small probability and they do so independently of one another. We call this distinction the mutation-mutant principle, which LEDERBERG (1989, p. 395) put thus: “[W]hile the distribution of mutational events should follow a Poisson distribution over a series of similar cultures, the distribution of mutant cells would show occasional jackpots.”

NEWCOMBE’s (1949) spreading experiment exemplifies the pith of the mutation-mutant principle.

In light of the mutation-mutant principle, the number of mutant colonies due to postplating mutations shall follow a Poisson distribution, regardless of possible mechanisms by which postplating mutations arise. Only when spontaneous mutations cease to occur shortly after plating (e.g., when selection is lethal) one assume that Poisson-distributed colonies may be an indicator of directed mutation. To ignore this point amounts to assuming that spontaneous mutation can be inhibited by any selective agent, lethal or nonlethal. This is logi-cally unwarranted, although the central question of the debate was often posed in that spirit, as is evident from the way the “old question” was paraphrased: “What proves that the mutants detected by some selective procedure were present in the population before it had been subjected to selection?” (FOSTER AND CAIRNS 1994, p. 5240). In retrospect, the key question can be more aptly phrased as follows: What proves that at least a portion of the mutations detected by some selective procedure would not have occurred without the selective condition?

RYAN’S DISCOVERY AND ITS RAMIFICATIONS

Almost half a century ago RYAN (1955) made a discovery that “mutation can take place in nondividing bacteria.” Ryan’s discovery stems from his meticulous experimental studies of an Escherichia coli strain (His−) that had lost its capacity to synthesize histidine. He showed experimentally that wild-type (His+) cells arose in a population of His− cells that were not dividing. Ryan made quantitative observations about the phenomenon that are still very illuminating today.

Let \( N_e \) denote the initial size of a nondividing cell population and \( \delta \) denote the cell death rate (per cell per unit time). If cells undergo mutation at a constant rate \( \mu \) (per cell per unit time), then at time \( t \) the size of the nonmutant population is expressible by \( N(t) = N_0 e^{-(\delta + \mu)t} \). Because \( N(t) \) was large in Ryan’s experiments, the occurrence of mutations can be viewed as a Poisson process having rate \( \mu N(t) \). Therefore, the average number of mutations accumulated in a time period between \( t_1 \) and \( t_2 \) is

\[
m(t_1, t_2) = \int_{t_1}^{t_2} \mu N_0 e^{-(\delta + \mu)s} ds = \frac{\mu N_0}{\delta + \mu} (e^{-(\delta + \mu)t_2} - e^{-(\delta + \mu)t_1}).
\]

(1)

Furthermore, if \( \delta \) is small, a useful approximation valid for moderate \( t_1 \) and \( t_2 \) is

\[
m(t_1, t_2) \approx \mu N_0 (t_2 - t_1).
\]

(2)

Equation 1 was essentially known to RYAN (1955), although he used a deterministic model in place of a Poisson process. Two important corollaries can be drawn when the cell death rate \( \delta \) is small. First, mutation will continue to occur in accord with a Poisson process for a long time. Second, during a long initial period, the expected number of mutations is approximately proportional to the duration of the process. These observations cannot be accounted for by the classic Luria-Delbrück type model, as emphasized by FOSTER (1994).

For example, Ryan found in his experiments that the rate of His− → His+ reversion was \( \mu \approx 1.2 \times 10^{-3} h^{-1} / \) His− cell and His− cells die at the rate \( \delta \approx 0.018 h^{-1} / \) cell. If a plate initially contains \( N_0 = 2.4 \times 10^9 \) nondividing His− cells, then even at the 10th day the daily average number of mutations is \( m(216, 240) = 0.086 \).

Another important finding of Ryan is that, in terms of physical time, mutation due to cell division is 40 times as fast as mutation occurring in nondividing cells. This finding explains why in laboratory replication-independent mutations are rare compared to replication-dependent mutations. Under laboratory conditions, cell division is immensely accelerated, and so is the rate of replication-dependent mutation when measured in physical time. But other types of mutation (e.g., mutation mediated by insertion sequence) may not be as easily accelerated by increased growth. A 4-day period is equivalent to \( \approx 192 \) generations, during which time a single cell can theoretically expand to \( \approx 6.28 \times 10^{27} \) cells. Because end-product toxicity will reach a lethal level long before the cell population can attain such a large size, the time period during which replication-independent mutations are allowed to occur is short. One obvious way of lengthening this period is nutrition control, prolonged starvation being the most efficient. The fact that dividing cells also undergo replication-independent mutation was ingeniously demonstrated by NOVICK AND SZILARD (1958), who showed that certain mutations occur at a constant rate (in physical time), irrespective of cell growth rate. We thus conjecture that in nature replication-independent mutation may not be as rare as in laboratory—bacterial generation time in nature can be as long as a day, \( \approx 40 \) times longer than that in laboratory.
THE SCOPE OF FLUCTUATION TEST

The first experimental example that rekindled interest in the directed mutation hypothesis is the observation that in a fluctuation experiment the number of mutant colonies appearing on a plate may follow a Poisson-like distribution, not a Luria-Delbrück jackpot distribution (Cairns et al. 1988). This observation was made when an E. coli strain (designated lacZam) that had lost its capacity to use lactose due to an amber mutation in the lacZ gene was subjected to a fluctuation test. The experiment was conducted with an important modification to the classic fluctuation test: The postplating incubation period was prolonged by using a nonlethal selective agent starvation in the presence of lactose. The lacZam cells were thought to be more prone to reverse to Lac+ cells in the presence of lactose, partly because the distribution of the number of Lac+ colonies looked like a composite of two distributions, a jackpot and a Poisson distribution, “as if there are two periods when mutations are occurring—first during the period of growth . . . then in stationary phase . . .” (Cairns et al. 1988, p. 143).

In light of the mutation-mutant principle, the Poisson-distributed Lac+ colonies appearing on a plate of lacZam cells is a phenomenon to be expected. As long as cells are immobilized on a solid medium, the number of Lac+ colonies occurring in any time interval is Poisson distributed. Furthermore, if birth and death of lacZam cells are negligible, then the expected number of Lac+ colonies should be approximately proportional to the length of postplating incubation, according to Equation 2. The status of starvation and the availability of a potentially usable carbon source might be purely coincidental factors. The key question is whether these two concomitant factors actually enhance mutation rate, but to answer this question is clearly out of the scope of the Luria-Delbrück fluctuation test.

Several explanations have been put forward for the appearance of Poisson-distributed colonies. For instance, Lenski et al. (1989) cited differential growth rates as a cause, which has been mathematically proven (Pakes 1993; Zheng 2002). But most explanations emphasize preplating factors, which might have inadvertently reinforced the notion that postplating mutations are directed mutations. The two distinct periods that Cairns et al. (1988) found exhibiting differential distributional patterns were caused by loss of cellular mobility after cells were plated on a solid medium. A manifestation of two distinct mutation periods thus may not be an indicator of directed mutation. Some investigators tend to think so-called late-arising colonies are a marker of directed mutation, partly because most cells still viable a few days after plating are in stationary phase and under starvation stress. To determine whether starvation stress is responsible for late-arising colonies, one has to encounter an unanticipated dilemma.

THE STARVATION DILEMMA

The occurrence of a mutation is directly responsible for only a single mutant cell (or at most two mutant cells when the mutation is replication mediated); many environmental factors (e.g., temperature, pH, and nutrition) are responsible for allowing a first-generation mutant to grow into a detectable colony. In laboratory, a mutant colony can be detected mainly for two reasons: Either appropriate conditions cause a first-generation mutant to grow into a colony or a large number of first-generation mutants cluster together to give the appearance of a colony. The second possibility is highly implausible. The first possibility poses an unexpected dilemma in the context of experiments like that involving lacZam cells. Lactose plays two roles in such an experiment—as a nutrient necessary for a Lac+ revertant to grow into a colony and as a supposed environmental factor responsible for “directing” mutations. Without a carbon source, all Lac− → Lac+ mutations would remain invisible; with a carbon source of lactose, the origin of mutations becomes moot; with any carbon source other than lactose, there might be no starvation stress.

The difficulties in overcoming the starvation dilemma were evident in many experiments involving auxotrophic bacteria. For example, Cairns et al. (1988) applied isopropyl-thiogalactoside (IPTG) to plates with lacZam cells to see whether IPTG can accelerate the appearance of Lac+ colonies. That addition of IPTG cannot increase the number of Lac+ colonies is not totally unexpected, for even if the Lac+ revertants can be fooled into producing β-galactosidase, no substrate would be available for the enzyme. The Lac+ revertants cannot use IPTG as a carbon source for clonal expansion. The observation that “the accumulation of late Lac+ mutants occurred only in the presence of lactose” (Cairns et al. 1988, p. 144) might well be restated as follows: The detection of Lac+ colonies occurs only in the presence of a carbon source usable by Lac+ revertants. This point was more aptly illustrated by control experiments concerning mutations that confer valine resistance (Valr). Cairns et al. (1988) overlaid lactose plates with agar containing valine and glucose, and they found that Valr colonies did not appear as rapidly as Lac+ colonies would under similar circumstances. As MacPhee (1993) later suggested experimentally, glucose may not be a carbon source usable by Valr mutants, for significantly more Valr colonies were detected when glucose was replaced with glycerol, a carbon source that can be more readily used by Valr mutants.

THE CAUSES OF THE MU EXCISION

In E. coli strain MCS2, part of the ara operon including a regulatory region has been joined with the lacZ gene by a DNA segment of phage Mu. Because the phage
Mu segment contains transcription terminating signals, MCS2 cells can use neither lactose nor arabinose for growth. (Intact ara and lac operons are missing in MCS2 cells.) However, the inability of MCS2 cells to use lactose can be circumvented by their excising properly the Mu phage DNA. This Mu excision creates a hybrid coding sequence, enabling MCS2 cells to use lactose with arabinose as an inducer. We denote the latter phenotype by Lac\(^{-}\)\textit{Ara} and the phenotype before Mu excision by Lac\(^{-}\)\textit{Ara}. A key question has been whether Mu excisions occur more often when Lac\(^{-}\)\textit{Ara} cells are starving and exposed to both lactose and arabinose.

A major obstacle to unraveling the mystery of this case is the starvation dilemma. The experiment conducted by \textsc{Mittler and Lenski} (1990) aimed at overcoming this dilemma. Mittler and Lenski incubated Lac\(^{-}\)\textit{Ara} cells in the absence of lactose and arabinose for prolonged periods and then sprayed these Lac\(^{-}\)\textit{Ara} cells and possible revertants with lactose and arabinose. They found that Lac\(^{+}\)\textit{Ara} colonies appearing within 2 days of spraying increased steadily as a function of the low-concentration carbon source available before the time that Lac\(^{-}\)\textit{Ara} cells were incubated in the absence of lactose and arabinose. This finding suggests that Lac\(^{+}\)\textit{Ara} revertants can occur without the presence of lactose and arabinose. However, an interesting question arose naturally: “[D]id Mittler & Lenski actually prove that the [Lac\(^{+}\)\textit{Ara}] fusion occurred in the absence of selection for it? The answer is clearly no. Mittler & Lenski did not confirm the preexistence of [Lac\(^{+}\)\textit{Ara}] mutants...” (\textsc{Foster} 1993, p. 481). This argument is theoretically correct; to overcome the starvation dilemma, one must transport any first-generation Lac\(^{-}\)\textit{Ara} cell to a source of lactose and arabinose immediately after its occurrence, an experimental feat unlikely to be accomplished in the foreseeable future. Additional approaches were needed to complement the Mittler-Lenski experiment. Using ideas from the classic replica-plating technique (\textsc{Lederberg} and \textsc{Lederberg} 1951), Sniegowski (1995) concentrated on this simple question: If starving Lac\(^{-}\)\textit{Ara} cells are incubated for 7 days without a carbon source, and if all the cells extant at the end of the 7-day incubation are exposed to lactose and arabinose for the ensuing 2 days, which period would be more responsible for the Lac\(^{+}\)\textit{Ara} colonies observed after the spraying of lactose and arabinose? Sniegowski’s experiment suggested that a great majority of the 103 Lac\(^{-}\)\textit{Ara} colonies were likely to be spawned by Lac\(^{+}\)\textit{Ara} ancestors that occurred during the 7-day period.

The originators of both experiments postulated that starvation nonselectively enhanced mutation rates. There seems to be an alternative conjecture. The primary role of starvation may be to allow Lac\(^{-}\)\textit{Ara} cells sufficient time to mutate at their intrinsic mutation rates. The first evidence supporting this conjecture is the observation that continued aeration allowed more Lac\(^{-}\)\textit{Ara} cells to be detected than static incubation allowed (\textsc{Maenhaut-Michel and Shapiro} 1994), possibly because aeration delays or reduces crowding effects. Second, considering a random lag between the occurrence of a mutation and the appearance of a colony representing the mutation, the linear trend exhibited in the experimental data (\textsc{Mittler and Lenski} 1990, Figure 2) is roughly consistent with the prediction of Equation 2. Third, because replication-independent mutations can also occur in dividing cells, but replication-dependent mutations might not occur in stationary phase cells, one would expect the mutation spectra of dividing cells to be richer than those of stationary phase cells; experimental evidence supporting this reasoning does exist (\textsc{Foster and Trimarchi} 1994). Fourth, when \textsc{Maenhaut-Michel and Shapiro} (1994) repeated the Mittler-Lenski experiment, they found that presence of glucose at low concentrations (e.g., 0.2 and 0.4%) before spraying helped them to detect more Lac\(^{+}\)\textit{Ara} colonies. This observation seems to be consistent with the conjecture, for Lac\(^{+}\)\textit{Ara} cells arising before spraying can use glucose for survival or for growth. In other words, a low-concentration carbon source available before the spraying of lactose and arabinose can enhance the chance of a Lac\(^{+}\)\textit{Ara} cell proliferating into a detectable colony. The conjecture is finally supported by the fact that some types of possibly directed mutation also occur in slowly dividing cells (\textsc{Yang et al.} 2001).

\section*{The Puzzle of Double Mutants}

Earlier investigators have apparently pondered the mechanisms by which multiple mutants arise (\textsc{Luria} 1946). \textsc{Opadia-Kadima} (1987) seems the first to view the appearance of double mutants as evidence for directed mutation. Opadia-Kadima made the observation that an \textit{E. coli} strain that has lost its ability to use lactose can regain this ability by undergoing two or more mutations. Opadia-Kadima then concluded that the two mutations cannot have occurred by chance, because, assuming the probability of one mutation occurring is \(10^{-7}\), the probability of two mutations occurring by chance is \(10^{-14}\). He was thus compelled to believe that the two mutations occurred simultaneously, orchestrated by the availability of lactose to the starving cells. A flaw in this argument is the neglect of two factors essential for estimating probabilities of mutation: the length of the organism’s evolutionary history and the changing population size of the organism. Bacterial mutation has been going on for as long as bacteria have been on earth, and bacteria exist in astronomical quantities. It may not be uncommon in nature for an organism to acquire double or multiple mutations sequentially and randomly. The same can be true in laboratory. To appreciate both claims, it suffices to examine two recent experiments that were more elaborately conducted than those cited by Opadia-Kadima.

In wild-type \textit{E. coli} the \textit{bgIR} gene is cryptic in the sense that the gene cannot be expressed unless it undergoes
a certain type of mutation. In *E. coli* strain χ342LD the *bglF* gene is also inactive due to the presence of an insertion sequence. Thus two mutations are necessary for a χ342LD cell to gain the ability to use salicin for growth: one to activate the *bglF* gene and one to rid the *bglF* gene of the insertion sequence (Hall 1988). Fluctuation experiments have shown (Parker et al. 1988) that rates of the two mutations are \(6 \times 10^{-8}\) and \(< 2 \times 10^{-10}\) (it was later changed to \(2 \times 10^{-12}\)) per cell division, respectively. On the other hand, χ342LD cells were starved for 20 days in the presence of salicin, and only 7 of the 27 colonies (each consisting of \(\sim 10^6\) cells) tested contained no *Sal* + cells. This was considered a case of directed mutation in the following computations: (1) If both mutations occur at random, then the probability that a double revertant appears is \(6 \times 10^{-8} \times 2 \times 10^{-10} = 1.2 \times 10^{-17}\) and (2) because the mean number of cells in the 7 colonies decreased to \(6.7 \times 10^7\) at the end of the 20th day, the mutation rate per cell is \(-\log(7/27)/(6.7 \times 10^7) = 2 \times 10^{-5}\). Therefore, “the frequency of the *Sal* + double mutants is about 12 orders of magnitude higher than expected on the basis of the mutation rates measured during growth” (Hall 1988, p. 890). Such acceleration of mutation was thought to be the organism’s response to starvation stress. Alternative explanations have been suggested (Symonds 1989). The example was also criticized on the grounds that the analysis did not account for the fact that single revertants (excision revertants) can grow on salicin (Müller and Lenski 1992). Ironically, a more basic issue has escaped attention: What was the basis for this conclusion? Here logical ambiguities were later avoided by a more elaborate type of comparison, of which we offer a mathematical analysis.

The FCY6 strain of *E. coli* harbors two mutations in the *trp* operon, one in the *trpB* gene and one in the *trpA* gene. Thus an FCY6 cell must acquire two reversion mutations to recover its ability to synthesize tryptophan. By experiments with cells that harbor one of the two types of mutation, it was found that the two mutation rates are \(\mu_1 = 5.2 \times 10^{-10}\) and \(\mu_2 = 6.4 \times 10^{-10}\) cell/day, respectively. On the other hand, 9254 colonies of FCY6 cells were grown to about the size of \(2.8 \times 10^8\) cells/culture and were then starved for 30 days. At the end of 37 of the 9254 colonies were believed to contain double revertants. It was further inferred that FCY6 cells in starvation produce Trp + double revertants at the rate of \(9.9 \times 10^{-10}\) cell/day, which is \(\sim 10^8\) times the quantity \(\mu_1 \times \mu_2 = 3.3 \times 10^{-19}\). Therefore, FCY6 cells under starvation stress seemed to produce double revertants “10^8 times more often than expected,” the most plausible explanation being that the two types of mutation occurred simultaneously in response to the selective stress (Hall 1991). A drawback of this analysis is omission of intervening growth of *trpA trpB* + cells. As suggested by R. Kolter to Hall (1993), *trpAB* double mutants can synthesize indole-glycerol-phosphate, which may break down spontaneously to excrete indole. Because *trpA trpB* + cells can grow on indole to enlarge the pool of *trpA trpB* + single revertants, the probability of having Trp + double revertants is enhanced. This possibility was quantitatively confirmed: *trpA trpB* + cells grow at the rate \(\beta = 0.64\) cell/day at least for the first 10 days, and *trpAB* cells die at the rate \(\delta = 0.14\) cell/day. Even with this additional information, it was found that “the observed number of double revertants is still considerably more than would be expected if the mutations were independent” (Hall 1993, p. 6413). In fact, the role of *trpA trpB* + cell growth is more important than it might appear at first sight. A detailed mathematical analysis of the experiment can be laborious, but a simple stochastic two-compartment model is a helpful tool for catching a glimpse of the effects of intervening growth. By a trivial modification of an existing mutation model (Zheng 1997), it can be shown (see Appendix for details) that the probability of at least one double revertant appearing by time \(t\) in a colony originally consisting of \(N_0\) double mutants is

\[
f_h(t) = \exp\left[\mu_1 \mu_2 N_0 e^{-\beta t} \int_0^t \frac{e^s - \exp\left[(\beta + \mu_2)s\right]}{\beta + \mu_2 e^s[(\beta + \mu_2)s]} ds\right].
\]

Here \(\mu_1\) and \(\mu_2\) are the rates of two sequentially occurring mutations. Let us consider a slightly simplified case in which *trpA trpB* + cells grow at the rate of \(\beta = 0.54\) cell/day for an entire 30-day period. This simplification still gives a biologically possible scenario. For example, if a *trpA trpB* + cell occurs at the outset of the experiment, it may expand to \(\sim 1.1 \times 10^5\) cells; but if a *trpA trpB* + cell occurs at the 10th day, it may grow to only \(\sim 4.9 \times 10^9\) cells. We now replace other parameters in Equation 3 with experimental values, e.g., \(\delta = 0.12\) and \(N_0 = 2.8 \times 10^6\). The probability of one or more double mutants in a particular colony by day 10 is \(5.7 \times 10^{-3}\), but the probability increases to 0.0028 by day 30. Figure 1 depicts the time course of the process, which bears a striking resemblance to actual experimental data (Hall 1991, Figure 1B).

**CONCLUDING REMARKS**

This article does not attempt to refute the directed mutation hypothesis. However, from a mathematical point of view, this article suggests that some of the evidence accumulated to date to support the directed mutation hypothesis is weaker than was originally thought and may be invalid. In summary, we have offered several conjectures to explain some of the evidence. Mutations
mediated by mechanisms other than DNA replication are not peculiar to cells under starvation stress, and in nature such mutations may occur spontaneously at rates possibly comparable to those of replication-dependent mutations. In laboratory starvation or another nutritional control procedure can allow an organism to acquire replication-independent mutations at their intrinsic rates, and physiological changes caused by starvation stress may play a secondary role. In nature it is common for an organism to acquire multiple mutations in a random and sequential manner; even in laboratory it is not uncommon that a cell in a plate acquires two mutations sequentially and randomly in a matter of a few weeks.

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Figure 1.—A simplified case adapted from an experiment with TrpAB double mutants. There are initially $2.8 \times 10^8$ TrpAB double mutants, which die at the rate of $0.12/\text{cell/day}$. Single revertants ($\text{trpA trpB}$) are assumed to grow at the rate of $0.54/\text{cell/day}$. The two mutation rates are $5.2 \times 10^{-10}$ and $6.4 \times 10^{-10}/\text{cell/day}$, respectively. The probability of there being at least one Trp$^+$ double revertant is computed using Equation 3. The time lag between a mutation and its formation of a colony is ignored.

LITERATURE CITED


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APPENDIX: DERIVATION OF EQUATION 3

Consider a two-compartment model. Let the first compartment represent trpA trpB+ cells, and the second compartment Trp+ cells. Following Ryan (1955), we assume that mutation rates are negligible compared to cell death rates. We further assume that trpA trpB+ cells enter the first compartment as a Poisson stream having rate \( \mu_1 N_0 e^{-\beta t} \), and cells in the first compartment replicate at rate \( \beta \). In addition, cells in the first compartment migrate to the second compartment at rate \( \mu_2 \). Since this model is a slight variation of a so-called \( A_0/0/0 \) mutation model, the reader is referred to Zheng (1997) for mathematical details.

From a standard procedure it follows readily that the probability generating function \( G(z_1, z_2; t) \) for this model satisfies the partial differential equation

\[
\frac{\partial G}{\partial t} + [ - \beta z_1^2 + (\beta + \mu_2) z_1 + \mu_2 z_2 ] \frac{\partial G}{\partial z_1} = \mu_1 N_0 e^{-\beta t} (z_1 - 1) G,
\]

subject to the initial condition \( G(z_1, z_2; 0) = 1 \). Because we are interested only in \( p_h(t) = G(1, 0; t) \), we set \( z_2 = 0 \) in Equation A1 to yield a simplified partial differential equation

\[
\frac{\partial G}{\partial t} + [ - \beta z_1^2 + (\beta + \mu_2) z_1 ] \frac{\partial G}{\partial z_1} = \mu_1 N_0 e^{-\beta t} (z_1 - 1) G.
\]

To solve for \( p_h(t) \), we resort to a so-called characteristic curve \( z(s) \) defined by

\[
z'(s) = -\beta z(s)^2 + (\beta + \mu_2) z(s), \\
z(t) = 1.
\]

This device reduces the above partial differential equation to an ordinary differential equation

\[
\frac{d}{ds} \log G(z(s), 0, s) = \mu_1 N_0 e^{-\beta s} (z(s) - 1).
\]

That is,

\[
\log p_h(t) = \int_0^t \mu_1 N_0 e^{-\beta s} (z(s) - 1) ds.
\]

Solving Equation A2 yields

\[
z(s) = \frac{\beta + \mu_2}{\mu_2 e^{\beta t} + \mu_3 e^{(\beta + \mu_2)(t-s)}}.
\]

Therefore,

\[
\log p_h(t) = \mu_1 N_0 \int_0^t e^{-\beta s} (1 - \exp[(\beta + \mu_2)(t-s)]) ds.
\]

Making a change of variable \( y = t-s \), we get

\[
\log p_h(t) = \mu_1 N_0 \int_0^t e^{\beta y} (1 - \exp[(\beta + \mu_2) y]) dy.
\]

whence we obtain Equation 3.