

Mutators, Population Size, Adaptive Landscape and the Adaptation of Asexual Populations of Bacteria

Olivier Tenaillon,^{*,1} Bruno Toupance,^{†,1} Hervé Le Nagard,^{*} François Taddei^{*} and Bernard Godelle^{†,‡}

Laboratoire de Mutagenèse, Institut J. Monod, CNRS Université Paris 7, F75251 Paris, France, [†]Université Paris-Sud CNRS, Laboratoire Evolution et Systématique, F91405 Orsay, France and [‡]Institut National Agronomique de Paris-Grignon, F75005 Paris, France

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ABSTRACT

Selection of mutator alleles, increasing the mutation rate up to 10,000-fold, has been observed during *in vitro* experimental evolution. This spread is ascribed to the hitchhiking of mutator alleles with favorable mutations, as demonstrated by a theoretical model using selective parameters corresponding to such experiments. Observations of unexpectedly high frequencies of mutators in natural isolates suggest that the same phenomenon could occur in the wild. But it remains questionable whether realistic *in natura* parameter values could also result in selection of mutators. In particular, the main parameters of adaptation, the size of the adapting population and the height and steepness of the adaptive peak characterizing adaptation, are very variable in nature. By simulation approach, we studied the effect of these parameters on the selection of mutators in asexual populations, assuming additive fitness. We show that the larger the population size, the more likely the fixation of mutator alleles. At a large population size, at least four adaptive mutations are needed for mutator fixation; moreover, under stronger selection stronger mutators are selected. We propose a model based on multiple mutations to illustrate how second-order selection can optimize population fitness when few favorable mutations are required for adaptation.

SOME alterations in mechanisms ensuring the maintenance of genetic information result in mutator genotypes that exhibit increased mutation rates. The effect on mutation rate, also called “mutator strength,” can reach a 10,000-fold increase (Miller 1996). Several studies on natural isolates (Jyssum 1960; Gross and Siegel 1981; Leclerc *et al.* 1996; Matic *et al.* 1997) have shown that mutators are present in up to 15% of the *Escherichia coli* populations. This frequency is too high to be explained only by a balance between *de novo* generation of mutator genotypes by mutation of DNA repair genes and selection against these variants suffering from higher mutation rates that accumulate lethal and deleterious mutations. Previous models of the evolution of mutation rate predicted that in stable environments, mutation should follow a “reduction principle” (Lieberman and Feldman 1986; Kondrashov 1995), as do other components of the genetic system (*e.g.*, recombination or migration) and that a minimal mutation rate should be selected. Conversely, in oscillating environments, infinite populations could have a non-minimal mutation rate at equilibrium (Leigh 1970, 1973; Ishii *et al.* 1989). The overrepresentation of muta-

tor alleles in natural populations suggests the existence of conditions that favor increased mutation rates. Moreover, the existence of a polymorphism in mutation rate suggests that natural populations are not at equilibrium. Laboratory evolution experiments (Chao and Cox 1983; Mao *et al.* 1997; Sniegowski *et al.* 1997) and theoretical approaches (Taddei *et al.* 1997b) indeed show that mutator genotypes could be transiently selected for in populations undergoing adaptation, *i.e.*, in populations in which the acquisition of favorable mutations conferring fitness advantage is needed for adaptation. Under such adaptive conditions, mutator alleles, despite the mutation load they generate, can become fixed in populations by hitchhiking with favorable alleles they produce [as could happen with neutral alleles (Maynard-Smith and Haigh 1974)]. This phenomenon was demonstrated under well-defined and relatively stable laboratory conditions. However, because of the variability of natural environments encountered by bacteria, the relevance of such results for natural isolates remains to be demonstrated and eventually quantified.

The two main parameters that can vary across events of bacterial adaptation are (i) the population size and (ii) the number and selective advantage of favorable mutations needed for bacteria to be adapted. In comparison with the limited set of conditions explored in laboratory experiments, natural microbiological environments are extremely variable with respect to these two

Corresponding author: Olivier Tenaillon, Laboratoire de Mutagenèse, Institut Jacques Monod, CNRS Université Paris 7, 2 Place Jussieu, F75251 Paris, France. E-mail: tenaillon@ijm.jussieu.fr

¹ These authors contributed equally to this work.

parameters. Regarding population sizes, we may note the following:

1. In a given environment, population sizes of different bacterial species may be extremely variable. For example, *Bacteroides* is typically found at 10^{11} – 10^{12} cells/g of intestinal content of adult humans, whereas *E. coli* is present only at 10^7 – 10^8 cells/g (Savage 1977).
2. As a single species can have several hosts of various body sizes, the size of the total bacterial population per host organism must vary (Savage 1977). For example, population sizes per host organism for *E. coli* (found in mice as well as in whales) range from 10^8 to 10^{13} cells.
3. Within the same host organism, *E. coli* concentration ranges from 10^3 to 10^8 cells/g, depending on localization in the gastrointestinal tract or in the infected organs (Savage 1977).
4. *E. coli* is found at very low concentrations in secondary environments such as water and soil (Hartl and Dykhuizen 1984).

These very different environments associated with varying maximal bacterial population sizes may also require different adaptations, *i.e.*, different adaptive alleles defining new adaptive peaks. As changes in human physiology are sufficient to change the composition of one's intestinal microflora and thus the interactions between gastrointestinal commensal species (Goldin 1986), we can assume that different adaptations are required for colonization of different hosts of the same species or different hosts belonging to different species. Moreover, within the same host, the adaptations to stomach, intestine, urinary tract, and blood are completely different, and migrations of bacteria among these different environments impose different selective pressures (Gulig 1996). In addition to variation in the abiotic environment, bacteria are constantly subjected to the pressure of competition with other species, leading to a constant need for adaptation [the "Red Queen" metaphor (Van Valen 1973)].

Large variations in both population size and adaptive landscape are expected to greatly affect the adaptive process, as it can be predicted from the well-known influence of drift on the fate of rare advantageous mutants (Gale 1990) and as previous studies of population genetics have shown in some particular cases [interaction between population size and deleterious mutations (Lande 1994); influence of epistatic interactions on the probability of fixation of favorable mutations (Michalakis and Slatkin 1997) or compensatory mutations (Stephan 1996); and clonal competition when only one favorable mutation can become fixed at a time (Gerish and Lenski 1998)]. However, little is known about the process of adaptation in a finite population in the course of fixation of several advantageous mutations at the same time.

The aim of this article is to quantify theoretically the influences of population size and height and steepness of the adaptive peak on the adaptation of bacterial populations to new environments and on the probability of fixation of mutator alleles under varying conditions. As we focus on simple adaptation events, we study the evolution in a single-peak adaptive landscape, *i.e.*, from a valley to the top of a peak.

MODELS AND METHODS

We simulated the colonization of an unknown virgin environment by considering an initial inoculum of a single individual, followed by exponential growth of the population (population size doubled each generation). Once the maximum capacity of the environment was reached, the size of the population remained constant. Each generation consisted of selection, mutation, and sampling. By mutation, the genome could accumulate deleterious alleles (up to 20) and favorable ones (up to 24, depending on the environment). The organism was haploid and asexual, so that different loci did not need to be assigned a position on a genetic map and could be pooled into classes with respect to their effect on fitness; only the number of alleles in a given class had to be counted (number of accumulated favorable and deleterious alleles). The phenotype of an individual directly resulted from these numbers (see *Selection*). In the course of the adaptation process, a mutator allele could appear by mutation at a modifier locus, so that mutation rates were affected in subsequent generations in the mutants. The initial colonizing cell was assumed to be nonmutator.

We used a density-based model for populations of $<10^{10}$ cells and a frequency-based model for larger and infinite populations. Models were conceived independently and the differences between the two models for the frequency of fixation of mutator alleles were not significant, as judged by a *t*-test (1000 simulations) comparing the results of both models for populations of 10^9 cells. In the frequency-based model the frequencies of all possible genotypes were stored in an array, whereas in the density-based model, the numbers of cells of the existing genotypes were stored in memory. The frequency-based model was faster than the density-based model for large and infinite populations, whereas it was the reverse for small population sizes.

Beginning with a reference population of 10^9 bacterial cells adapting to a new environment requiring 12 favorable alleles with a 3% additive fitness advantage each, we explored the effect of each parameter (population size, number of loci, fitness advantage) one at a time. We simulated adaptive processes by varying (i) population sizes [from 10^4 up to 10^{20} cells, which is close to the likely overall number of *E. coli* cells on earth (Whitman *et al.* 1998), the estimated effective population size ranging from 10^5 to 10^{10} (Berg 1996; Pupo

and Richardson 1995)]; (ii) the number of favorable alleles required for complete adaptation (from 2 to 24); or (iii) their additive selective effect on fitness (from 0.5 to 10%). Experimental directional adaptation indeed showed that selective effect reached 10% in the first stages of adaptation, while further increase in fitness was lower (Lenski and Travisano 1994). For each simulation, we followed the mutator frequency and the adaptation time, *i.e.*, the number of generations needed for the whole population to acquire all favorable alleles needed for complete adaptation. This allowed us to calculate for each set of simulations (defined by a given population size, a mutator strength, and an adaptive landscape) an average adaptation time (time until $1 - 10^{-9}$ cells have all favorable alleles) and a probability of fixation of the mutator allele, *i.e.*, the percentage of populations in which the mutator frequency had reached 95%. A minimum of 100 independent simulations was done, and up to 1000 when required.

We also simulated more complex adaptive peaks, composed of favorable mutations having different effects on fitness. More precisely, we considered an exponential distribution for favorable mutations. In this case, the computational time was dramatically increased as compared with conditions where all favorable mutations have the same fitness effect.

Mutation rates: Mutations occurred at constant rates per replicaton: 10^{-5} for lethal mutations, 10^{-8} for each favorable mutation as well as for the reversion of deleterious mutations, and 10^{-4} for deleterious mutations, which is in the range of estimated values [the rate of deleterious mutations is about 2×10^{-4} , with an average cost of 1.2%, as estimated by Kibota and Lynch (1996)]. In the mutator genotype, all these mutation rates were increased by a given factor, m (the strength of the mutator allele). The mutation producing the mutator phenotype occurred at a constant rate of 5×10^{-7} , as estimated in Ninio (1991). This probably underestimated value is a conservative hypothesis concerning the probability of fixation of mutator alleles. The reversion toward a nonmutator phenotype occurred at a rate of $m \times 5 \times 10^{-10}$. In each simulation, a single mutator strength was considered. Simulations with a nonmutator allele (onefold mutator) were performed as a control. A Poisson distribution was used to distribute simple and multiple mutations.

Selection: The effects of favorable and deleterious alleles on fitness were additive, the fitness of the population before adaptation being 1. Deleterious alleles always conferred a 0.05 decrease in fitness. When a constant fitness effect of favorable mutations was considered, each favorable mutation conferred an increase in fitness of 0.005, 0.01, 0.03, 0.05, or 0.1, depending on simulation conditions. The acquisition of the mutator allele had no direct influence on fitness. In the density-based model a 0.01 fitness advantage corresponded to a 0.02 chance to produce three cells instead of two.

When a distribution of favorable mutations was considered, each favorable mutation was assigned to one of five discrete classes of effect on fitness. The number of mutations and the mutation rate in each of the five classes were chosen so that the constructed distribution was exponential (*i.e.*, the probability density function of the selective effect, s , was $\alpha e^{-\alpha s}$): 10, 6, 4, 2, and 1 mutations with a respective selective effect of 1, 3, 5, 10, and 15% and a respective mutation rate of 8.2×10^{-9} , 9.1×10^{-9} , 9.2×10^{-9} , 6.9×10^{-9} , and 5×10^{-9} for a distribution with $\alpha = 20$, and 12, 8, 5, 3, and 1 mutations with a respective selective effect of 1, 2, 3, 5, and 10% and a respective mutation rate of 4.1×10^{-9} , 4.3×10^{-9} , 4.9×10^{-9} , 4.0×10^{-9} , and 2.0×10^{-9} for a distribution with $\alpha = 35$, which corresponds to the distribution calculated after experiments of laboratory evolution (Lenski and Travisano 1994; Gerrish and Lenski 1998).

Sampling procedure used to model drift: In each simulation, the growth of the population simulated the colonization of a new environment by a single cell. There was an exponential increase in population size until the maximum capacity of the environment was reached (*i.e.*, the fixed population size), after which a sampling procedure was used at each generation to keep the population size constant. For populations of $>10^5$ cells, genotypes found in <100 individuals were sampled with a Poisson sampling procedure, whereas the sample size of more common genotypes were the expected ones (because the probability of losing a genotype with >100 representatives by drift is $<10^{-40}$). For populations of 10^4 cells, we used a binomial sampling procedure (to avoid the limits of the Poissonian approximation).

Algorithm: The computation of the evolution of the population from generation t to generation $t + 1$ can be summarized in three phases as shown in Figure 1: (1) a replication-selection process, (2) a mutation process, and (3) a random sampling process. The population at generation t was subdivided into K different genotypes defined by their numbers of favorable and deleterious alleles and their mutator status (mutator or nonmutator). The density-based model considered the numbers of individuals of each genotype i (n_i) present in the population, whereas the frequency-based model considered the frequencies (f_i) of all possible genotypes. In Figure 1, the size of the box associated with each genotype represents its relative representation in the population.

Replication-selection process: In the density-based model, the number of individuals of genotype i (n_i) after selection was randomly drawn using a Poisson law, whereas in the frequency-based model, the frequency of individuals of genotype i (f_i) was set to its expected value. The fitness coefficient of genotype i (s_i) was calculated from its numbers of favorable and deleterious alleles. The size of the population could either increase or decrease, depending on the average fitness of its members.

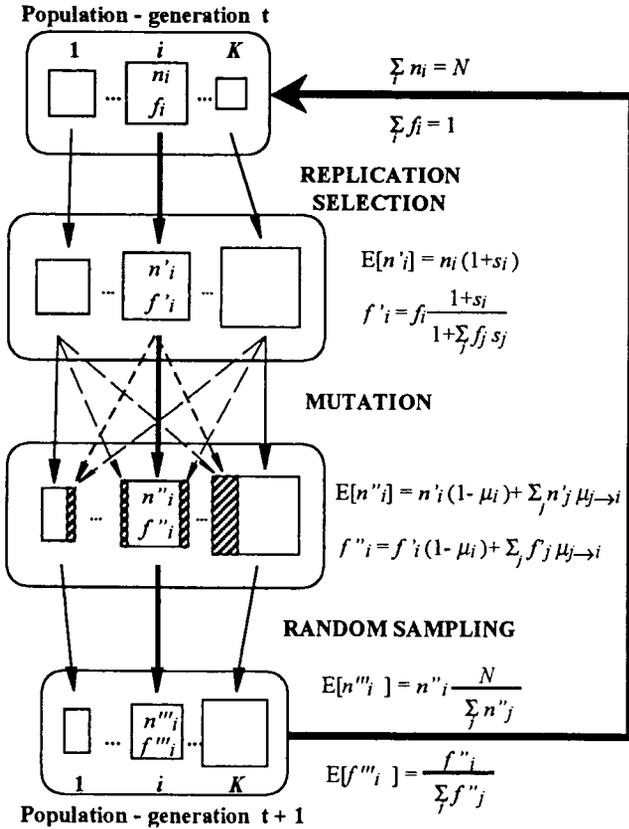


Figure 1.—Description of the algorithms used in the simulation models. See discussion of algorithm in text.

Mutation process: In the density-based model, the number of individuals of genotype i (n'_i) after mutation was randomly drawn using a Poisson law. In the frequency-based model, the frequency of genotype i (f'_i) was set to its expected value, except that the frequencies were not normalized. New genotypes could be generated by mutation (others could also disappear), so that the number of present genotypes (K) could actually vary in the density-based model. In Figure 1, white boxes represent nonmutants (they were present in the previous time step), whereas hatched boxes represent new mutants.

Random sampling process: In the density-based and the frequency-based models, genotypes were randomly drawn from a Poisson distribution to generate a population of size N (the carrying capacity of the environment). Note that at the beginning of each simulation, exponential growth brought the population size from one individual to the carrying capacity (N), using the same algorithm except for sampling procedure.

Note that in the density-based model, random processes were used for the three steps (replication-selection, mutation, and random sampling), whereas random processes were used only for the random sampling step in the frequency-based model. For large populations (*i.e.*, when the number of present genotypes was close to the number of all possible genotypes), the frequency-based model was used, whereas for small popula-

tions (*i.e.*, when the number of present genotypes was small), the density-based model was used.

RESULTS

Influence of population size: We followed the probability of the fixation of mutator alleles and the adaptation time of populations varying in size from 10^4 to 10^{20} cells, adapting to a single-peak adaptive landscape composed of 12 favorable mutations, each with a 3% advantage in fitness. The adaptation time was affected by population size: the larger the population, the shorter the adaptation time (Figure 2a). At a given population size, adaptation time was affected by the presence of the mutator allele (Figure 2b). Mutators of 100-fold increased the speed of adaptation up to 30%, and 10-fold mutators up to 13%, over the range of population sizes studied. Strong mutator alleles (*i.e.*, increasing mutation rates 1000-fold or more) had an effect on adaptation time only for a population size $>10^{18}$ cells.

Figure 2c shows the percentage of populations in which the mutator allele became fixed during the course of adaptation as a function of population size. Under this particular adaptive landscape, strong mutator alleles (*i.e.*, those increasing mutation rates 1000-fold or more) did not reach high frequencies in either finite or infinite populations. The probability of fixation of weaker mutator alleles (*i.e.*, those increasing mutation rates by 10-fold and 100-fold) was strongly influenced by population size, the overall pattern being a sigmoidal increase. As expected from infinite population simulations, these mutator alleles always became fixed in large populations ($>10^{18}$ and 10^{11} cells for 10-fold and 100-fold mutator alleles, respectively). The pattern of increase in the probability of fixation of 10-fold mutator alleles was shifted to larger population sizes as compared with that of 100-fold mutator alleles: this increase spanned from 10^7 to 10^{11} cells for 100-fold mutator alleles and from 10^{10} to 10^{18} cells for 10-fold ones. Interestingly, the probability of fixation of the mutator allele in small populations (*i.e.*, 10^4 and 10^5 cells) was not null but $\sim 4\%$ for both 10-fold and 100-fold mutator alleles. The influences of population size on the probability of fixation of mutator alleles and on the speed of adaptation were identical when considering a distribution for favorable mutational effects; *i.e.*, all the patterns described above were retained (data not shown).

Influence of the height and steepness of the adaptive peak: *The fitness advantage of favorable alleles:* At moderate population sizes, *i.e.*, from 10^7 to 10^{14} cells, the probability of fixation of 100-fold mutator alleles was much higher than that of 10-fold mutator alleles (Figure 2a). As adaptive landscapes can influence probabilities of fixation, we varied the fitness advantage of favorable alleles from 0.5 to 10% and the mutator strength from 5-fold to more than 1000-fold. The result confirmed the influence of the shape of adaptive peaks on the

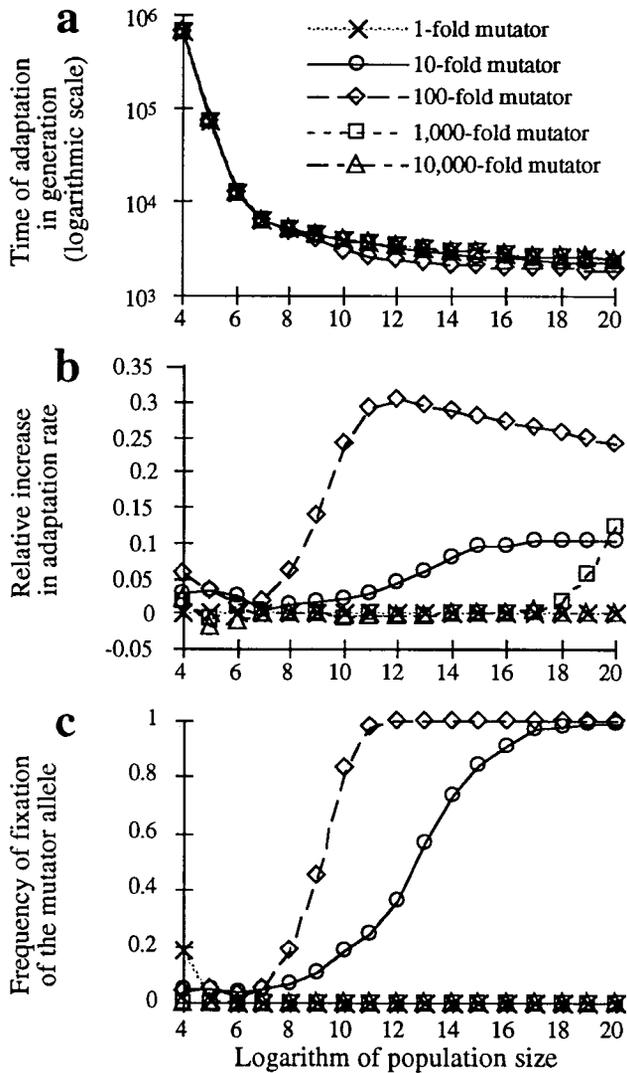


Figure 2.—Population size and mutators. (a) Adaptation time of populations in generations as a function of population size and mutator allele strength. (b) Relative increase in adaptation rate for populations in which a mutator allele (10-fold to 10,000-fold) can appear relative to populations without mutator allele (1-fold). The increase is calculated as $(t_{non-mut} - t_{mut}) / t_{non-mut}$ where $t_{non-mut}$ and t_{mut} are the mean times until adaptation is reached, respectively, for populations with a 1-fold mutator allele (nonmutator allele) and populations with a stronger mutator allele (10-fold to 10,000-fold, whether population has a fixed mutator allele or not). Differences are significant when exceeding 2% for population size of 10^4 and 10^5 cells and 1% for larger populations. (c) Probability of fixation of the mutator allele as a function of population size. The probability of fixation is the percentage of populations in which the frequency of the mutator allele at the end of adaptation reaches 95% (over 300 independent simulations for a population size superior to 10^9 and over 1000 for the others). The single peak adaptive landscape is defined by 12 novel favorable alleles, each conferring a 3% additive effect on fitness. In a nonmutator genotype, deleterious or lethal mutations and reversion of deleterious mutations occur at 10^{-4} , 10^{-5} and 10^{-8} , respectively; each favorable mutation occurs at 10^{-8} . Mutation conferring the mutator phenotype occurs at 5×10^{-7} and its reversion at $m \times 10^{-10}$ (m being the strength of the mutator).

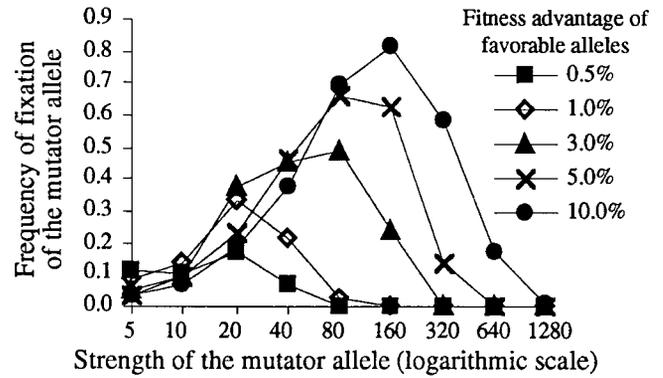


Figure 3.—Steepness of the adaptive peak and mutators. Probability of fixation of the mutator allele (>100 simulations) as a function of its strength for fitness advantages of adaptive alleles ranging from 0.5 to 10%. Parameter values as in Figure 2 except for the size of the adapting population, which was fixed to 10^9 cells.

probability of fixation of different mutators (Figure 3). The strength of the mutator with the highest fixation frequency increased with the advantage conferred by the adaptive mutations. For example, at a fitness advantage of 1%, 160-fold mutator alleles were never fixed, whereas at an advantage of 10%, they were fixed in 80% of populations.

The number of favorable alleles: As the composition of adaptive peaks could favor the fixation of different mutator alleles, we next studied the influence of the number of adaptive alleles required for complete adaptation on the probability of fixation of the mutator allele (Figure 4). Strong and very strong mutator alleles (1000-fold and 10,000-fold) never became fixed, whereas the percentage of populations in which weaker mutator alleles (10-fold and 100-fold) became fixed increased with the number of favorable alleles to acquire. Under conditions where favorable mutations were not limiting (population size was 10^9 cells), there was a threshold (four adaptive alleles) below which mutator alleles never became fixed in populations.

How do mutator alleles hitchhike? We simulated an adaptive peak with a single favorable mutation conferring 3% advantage in fitness, occurring as frequently as the 12 mutations in the previous adaptive peak, *i.e.*, at a rate of 12×10^{-8} . In adapting populations of 10^5 and 10^9 individuals, respectively, 0.4 and 0.0% of populations did fix the 100-fold mutator allele (1000 simulations were done).

Considering a distribution for favorable mutational effects ($\alpha = 35$) and a mutation rate toward mutators of 5×10^{-6} without reversion of the mutator allele, we followed the 100-fold mutator allele frequency in 27 populations in which mutators became fixed in <3000 generations (Figure 5; this arbitrary threshold was chosen for the sake of better graphical representations). The evolution of the mutator frequency in the 23 popu-

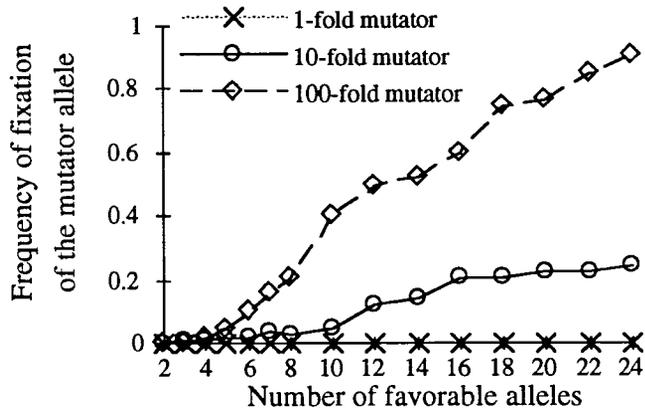


Figure 4.—Height of the adaptive peak and mutators. Probability of fixation of mutator alleles of different strengths (10-fold to 10,000-fold; >100 simulations) as a function of the number of favorable alleles that can be acquired, each favorable allele conferring a 3% increase of fitness. Other parameter values as in Figure 3.

lations of 10^9 individuals showed a multistep dynamic with several phases of increase and decrease, while the mutator frequency in the 4 populations of 10^5 individuals showed a very stochastic behavior around its equilibrium frequency and then reached 1 in a single phase. Moreover, in adapting populations of 10^5 and 10^9 individuals, respectively, 35.3 and 88.0% of populations did fix the 100-fold mutator allele (500 simulations were done). But if we considered that adaptation was reached when a single mutation was fixed (effect on fitness of favorable mutations being nonadditive), we found that

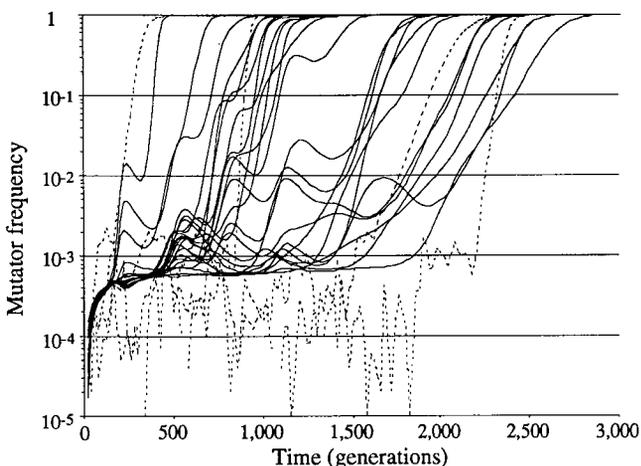


Figure 5.—Evolution of 100-fold mutator allele frequency in 27 independent simulations in which the mutator becomes fixed in <3000 generations. The adaptive peak comprises 12, 8, 5, 3, and 1 mutations with respective selective effects of 1, 2, 3, 5, and 10%, and respective mutation rates of 4.1×10^{-9} , 4.3×10^{-9} , 4.9×10^{-9} , 4.0×10^{-9} , and 2.0×10^{-9} . The mutation rate toward nonreversible mutator genotype is 5×10^{-6} . The solid and dashed lines show the evolution of the mutator frequency to fixation in adapting populations of 10^9 cells and 10^5 cells, respectively.

0.32 and 0.0% of populations fixed the 100-fold mutator allele in populations of 10^5 and 10^9 cells, respectively.

DISCUSSION

In this work, we examine how mutator alleles may influence the process of adaptation of finite asexual bacterial populations, corresponding, for example, to the colonization of a new environment. Such an event is modeled considering that the population accumulates several favorable mutations under directional selection pressure. The adaptation time is the number of generations until populations acquire the maximum number of favorable alleles. In infinite-sized populations all possible adaptive genotypes are present at the beginning of colonization. Therefore adaptation time represents the time until the fittest genotype becomes fixed, starting from very low frequency. In finite-sized populations, mutation is a limiting factor, and thus the time until the appearance of new favorable genotypes by mutation forms a large part of the adaptation time. In other words, the process of adaptation is greatly influenced by the stochasticity of mutations. Evolution can be considered to proceed in two phases: stochastic phases, during which new mutations appear and may be lost by drift, and deterministic phases, during which a “lucky” mutation, having reached sufficiently high frequency, can go to fixation nearly deterministically (Gale 1990).

In addition, some events that occur in models of infinite size populations may be nearly impossible in finite populations. For example, adaptation in infinite-sized populations is more rapid in the presence of strong mutator alleles (conferring 1000-fold increase in mutation rate) even if mutator frequency remains very low (Taddei *et al.* 1997a). This effect is due to recurrent generation of rare favorable nonmutator genotypes by reversion at the mutator locus following the generation of favorable alleles in the mutator background. In finite populations, such events are too infrequent [$5 \times 10^{-7} \times (10^{-8} \times 1000) \times (5 \times 10^{-10} \times 1000) = 2.5 \times 10^{-18}$; probability of becoming a mutator, generating a favorable allele, and reversing to nonmutator background for a 1000-fold mutator] to influence the evolution of populations whose size is inferior to 10^{19} (already larger than those expected under natural conditions; Figure 2b).

Considering the fate of moderate-strength (*i.e.*, 10-fold or 100-fold) mutator alleles in finite-size populations, Taddei *et al.* (1997a) showed that they could become fixed as a result of hitchhiking with favorable mutations. However, the generality of such a result and the process of mutator fixation remained to be established more precisely. The dynamics of mutator evolution can be understood as a balance between the induced advantages and disadvantages of such alleles on their bearers.

The mutator alleles, frequently generating lethal or

deleterious mutations, yield an average fitness cost. Thus, if the population is at equilibrium, mutator genotypes are expected to be very rare, *i.e.*, at their mutation/counterselection equilibrium frequency. Let μ_d and μ_l be the mutation rate for deleterious and lethal mutations, respectively, μ_m the mutation rate toward mutator genotypes, and m the strength of the mutator allele. Neglecting mutator genotypes associated with deleterious mutations (because they are evolutionary dead-ends), the equilibrium frequency of the mutator is approximately $\mu_m/[m \times (\mu_d + \mu_l)]$. For a 100-fold mutator it is then $5 \times 10^{-7}/[100 \times (10^{-4} + 10^{-5})] \approx 5 \times 10^{-5}$, which results in an average cost of 1% in fitness. Numerical estimates were consistent with this analytical formula except for very strong mutators; *e.g.*, the simulated equilibrium frequency for a 1000-fold mutator was about twice that expected (data not shown).

On the other hand, mutators have the advantage of producing favorable mutations. To analyze this capacity, let u be the mutation rate toward a favorable allele in a nonmutator genotype. An m -fold mutator genotype generates n additional favorable alleles with the probability $(m \times u)^n$ instead of u^n in nonmutator genotypes. Mutator alleles being at frequency p , the relative contribution of mutator *vs.* nonmutator background in generating n favorable mutations is

$$\frac{p(m \times u)^n}{(1 - p)u^n} = m^n \frac{p}{1 - p}. \quad (1)$$

Hence the probability of fixation of mutator alleles is linked with their ability to generate several favorable alleles either simultaneously (multiple mutation events) or successively within a few generations (several single mutation events) more frequently than nonmutator alleles. The disadvantage of being a mutator [initially, $p/(1 - p) \ll 1$] can be compensated for by the generation of numerous favorable alleles (because m^n increases rapidly with n). Thus, if the genotypes are classified according to fitness, there exists, at least in some conditions, a fitness value for which the frequency of mutator genotypes is higher than the frequency of their nonmutator counterparts. For a 100-fold mutator this value is reached by generating three favorable alleles [with $p = 5 \times 10^{-5}$, $(100 \times 10^{-8})^3 \times (5 \times 10^{-5}) = 5 \times 10^{-23} > 10^{-24} = (10^{-8})^3$]. Unless considered over time, those values are not relevant for natural populations. However, sequential single mutations within a few generations, and not only the multiple mutations in a single generation, should be taken into account. The acquisition of a first favorable allele allows mutator genotypes to increase in frequency and thus increases their probability of fixation by a positive feedback loop between the number of mutator genotypes and their advantage over nonmutator genotypes, conferred by their increased ability for generating favorable alleles (Figure 5). Therefore, increasing the number of favorable al-

les needed for adaptation increases the probability of fixation of the mutator allele. When mutation is not limiting (at population size of 10^9 cells) the fixation of mutators cannot be explained by a simple multiplication of single independent hitchhiking effects (Figure 3, and Figure 5) as shown by the fact that no fixation was observed in single mutation simulations either with the standard model, or with a distribution of favorable mutational effect ($\alpha = 35$) with a higher frequency of mutation toward nonreversible 100-fold mutator genotype (5×10^{-6}). Hence, when favorable mutations are not limiting, if adaption requires fewer than three adaptive alleles, mutator alleles never go to fixation (Figure 4). Moreover, as strong and very strong mutator alleles have too high a cost to be able to hitchhike with the first favorable mutation they generate (10 and 100% are the first approximations of the average cost of 1000-fold and 10,000-fold mutators, respectively), they cannot enter the positive feedback loop, and they never go to fixation.

However complex the dynamic of this positive feedback loop, Equation 1 gives us a qualitative understanding of the results. First, increase in population size increases the probability of multiple mutation events and thus favors the fixation of mutator alleles (Figure 2c). The larger the population size, the higher the likelihood of a mutator becoming fixed and increasing the rate of evolution. However, at small population size, mutators still become fixed with a nonnegligible frequency (Figure 2c). Drift is stronger at small population size and the cost of the mutator allele is due only to the possible generation of deleterious and lethal mutations. This average cost is instantaneously paid only if the mutator subpopulation is large enough to generate some of those mutations at each generation. Consequently, in small populations, mutator alleles are effectively neutral and may increase in frequency by drift (Figure 5). The maximum frequency of the 10-fold mutator indeed reached 10% in every simulation with a 10^4 population size (data not shown). Furthermore, at small population sizes, the generation of one favorable allele leads to fixation, fixation time being shorter than the time until the generation of a second adaptive allele (Figure 5). Mutations being limiting, the fixation of mutator is the product of (i) single independent hitchhiking effects and (ii) the number of selective sweeps occurring during adaptation. For example, at a population of 10^{-5} cells, the probability of fixation of mutator alleles observed in single mutation simulations (0.4%) is similar to the one observed in multiple mutation simulations (4%) divided by the number of selective sweeps (12): $0.4\% \approx 0.33\% = 4\%/12$. Mutator alleles can therefore become fixed in 4% of populations (Figure 2c), a value that is lower than the probability of fixation of a neutral allele (onefold mutator becomes fixed with a 19% probability, thus expressing the cost of mutator alleles).

Another aspect of mutator cost and advantage is re-

vealed by adaptive landscapes and mutator strength variation. Very strong and very weak mutator alleles do not become fixed with high frequency, the former because of their high cost and the latter because of their inability to generate enough favorable alleles. It follows that for a given environment and for a given population size, there is a corresponding mutator allele of optimal strength that becomes fixed with frequency higher than that of any other mutator allele (Figure 3). The strength of this selected mutator increases with the advantage conferred by the favorable mutations. The higher the advantage of favorable alleles, the better the mutator cost can be compensated through hitchhiking, thus selecting for this higher ability of generating a succession of favorable mutations.

Further discussion: The parameters of the model are derived from parameters measured for *E. coli*. Nevertheless, as we have used a very wide range of parameters, the results can be applied more generally to asexual populations. As somatic cells can be understood as evolving populations, cancerous cells showing a high mutation rate (Jackson and Loeb 1998, and references therein) could be the products of an adaptive process involving several adaptive mutations. When the mutation was not limiting (Figure 4), at least four adaptive mutations were required for the mutator to become fixed. Interestingly, this threshold fits with the estimated number of mutations required to generate a cancerous cell. Cell turnover could favor the clones bearing mutations conferring shorter generation time or better survival. This local selection could lead to a local fixation of mutator allele(s), which would subsequently greatly facilitate transition to a cancerous state.

In bacterial populations within the human gastrointestinal tract the incidence of mutator alleles should be higher within dominant species such as *Bacteroides* (10^{11} – 10^{12} cells/g of intestinal content) than within less-represented ones such as *E. coli* (10^7 – 10^8 cells/g of intestinal content), all else being equal. The same trend would also be expected when comparing the frequency of mutator alleles within a species, concerning populations of different sizes in different host organisms, such as *E. coli* populations in humans and mice.

The entire world population of *E. coli* does not represent a single well-mixed population of 10^{20} cells exposed to the same stress. If it did, mutator alleles would always become fixed. Clearly the world population is structured into many smaller populations, which fits with the observation of variation for mutator allele frequency. Nevertheless, small populations can also fix mutator alleles. This could explain the loss of some repair genes in some species such as intracellular parasites, *e.g.*, mycobacteria (Woese 1984; Himmelreich *et al.* 1996).

E. coli is known to be locally asexual but globally sexual (Maynard-Smith *et al.* 1993; Milkman 1996, 1997). Replacement of mutator alleles could then occur either by reversion or by genetic exchange. During adaptation,

sex would break the linkage between mutator alleles and favorable mutations, thus limiting the possibility for mutators to hitchhike and to become fixed in populations. This phenomenon would probably tend to reduce the incidence of mutators in natural environments.

Concerning the adaptive landscape, bacterial populations exposed to strong stress should fix mutator alleles (Mao *et al.* 1997). This phenomenon could also lead to the loss of antimutator repair alleles at the species level (Eisen 1998). Therefore, a strong stress such as repeated antibiotic treatment would not only lead to the spread of resistance but also to the selection of a high mutation rate, leading consequently to a decreased efficiency of new treatments.

Furthermore, the nature of the selected mutator allele seems to be influenced by the conditions of adaptation. This might explain why in laboratory evolution experiments, where the environment is highly controlled and constant, 3 out of 12 *E. coli* populations exhibiting increased mutation rates at the end of adaptation had fixed mutator alleles of the same strength (100-fold; Sniegowski *et al.* 1997), even though mutators of varying strength are found in natural populations (Matic *et al.* 1997).

Second-order selection (Arber 1993) on the genetic tools generating diversity therefore defines the rate of adaptation and the adaptability of a species. As a second-order selection system, the ability to increase the mutation rate via generation of a mutator seems a way to adapt more rapidly to new environments. The probability of fixation and the strength of the selected mutator depend on the adaptation conditions, leading in some cases to rapid adaptation.

However, once the mutator allele is fixed by hitchhiking with favorable alleles, the generation of numerous deleterious mutations has a cost for the population. Reversion of the mutator allele is then advantageous. The cost, characterized by the fixation of deleterious alleles [Muller's ratchet (Muller 1932)], can be negligible in large populations but not in small ones or those passing through strong bottlenecks. Hence, increased mutation rate could be a dangerous genetic system of adaptation in the long run. Still, there are other ways to modulate mutation rate that could avoid this kind of cost. Inducible mutator alleles that would increase mutation rate only under stress conditions would not be costly after adaptation (Taddei *et al.* 1995). Another strategy could be contingency loci, loci showing increased mutation rates that encode for surface antigen proteins (Moxon *et al.* 1994). Those loci are subject to recurrent selection and counterselection via the immune system of the host. A local increase in mutation rate avoids the cost of high mutation rate on housekeeping genes.

Genetic exchange is another second-order selection genetic system that helps adaptation (Otto and Bar-

ton 1997). The interactions between a mutator strategy and contingency loci, inducible mutator, and sex are not yet described and could be either competitive or synergistic. To better understand the adaptation of cell populations we now have to take into account those possible interactions and to accumulate data in different bacterial species, each species having probably evolved its own combination of adaptive strategies.

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