Mutators and sex in bacteria: Conflict between adaptive strategies

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Edited by John Maynard Smith, University of Sussex, Brighton, United Kingdom, and approved July 3, 2000 (received for review February 11, 2000)

Bacterial mutation rates can increase and produce genetic novelty, as shown by *in vitro* and *in silico* experiments. Despite the cost due to a heavy deleterious mutation load, mutator alleles, which increase the mutation rate, can spread in asexual populations during adaptation because they remain associated with the rare favorable mutations they generate. This indirect selection for a genetic system generating diversity (second-order selection) is expected to be highly sensitive to changes in the dynamics of adaptation. Here we show by a simulation approach that even rare genetic exchanges, such as bacterial conjugation or transformation, can dramatically reduce the selection of mutators. Moreover, drift or competition between the processes of mutation and recombination in the course of adaptation reveal how second-order selection is unable to optimize the rate of generation of novelty.

Bacteria are numerous and are continuously adapting to various environments (1). Therefore, genetic systems allowing rapid evolution are potentially under selection among these organisms. The spread of alleles increasing the mutation rate (2, 3), referred to as mutators (4), is an illustration of such a selection. In asexual populations, mutator alleles remain associated with the alleles they generate; therefore ''they are held responsible for the consequences of their action'' (5). They are selected against because they generate deleterious and lethal mutations (6–8). Despite their being selected against, during adaptation, mutator alleles in a population can increase in frequency up to 100% because of their association with the selective advantage of the beneficial mutations (genetic hitchhiking) (9) that are generated more frequently than in nonmutator genotypes (3, 10). Consequently, selection of favorable alleles (first-order selection that increases bacterial ''skills'' in a particular environment) leads to adaptation, but also promotes indirectly (although hitchhiking) the mutator alleles as a genetic strategy for producing more diversity. Such an indirect selection of a genetic system creating diversity is referred to as second-order selection.

The existence of this selection in the wild is supported by the frequency of mutators in nature. In several studies (11, 12), strong mutators (strains with mutator alleles increasing the mutation rate at least 50-fold compared with other strains) were shown to represent a few percent of natural isolates, a frequency higher than the one expected when only the effect of deleterious mutations is considered (10, 13). In addition to this indirect selection, mutators could also be directly selected for because of the reduced time and energy costs of lower fidelity replication (14). Now that the mechanisms selecting for mutators are better understood, a new question arises: what prevents mutators from spreading at a much higher frequency? Indeed, experiments and simulations have shown that adaptation of asexual populations could easily lead to takeover of mutators in more than 25% of populations (2, 3, 10, 15). Hence, in a model of continued evolution (16), one would expect mutators to invade all populations, as they invade up to 25% of populations in which no mutators are initially present and win competitions with nonmutators when their frequency at the outset is above 10^{-4} (17).

Several explanations can be proposed for the discrepancies between what is observed in nature and what is obtained experimentally in the laboratory:

(*i*) Bacteria may be very well adapted to most environments, and therefore most of the time mutators pay the cost of their increased mutation load, and beneficial mutations are too infrequent or are of too little advantage to compensate for this cost.

(*ii*) Natural environments are much more complex than experimental ones and require any given strain to survive in a variety of niches (for example, to be able to pass from one host to another). Rapid genetic adaptation to a given niche could lead by selection or drift to the loss of some ability required to survive in other niches (18).

(*iii*) In all laboratory experiments and simulations, populations were asexual and therefore adaptation was dependent on the successive acquisition of favorable alleles by mutation. However, the existence of genetic exchanges could modify the dynamics of adaptation and limit the success of mutator alleles.

We tested this last hypothesis using simulation models that include the rate of genetic exchange as a parameter. Indeed, in nature, most bacteria are able to exchange genetic information by transformation, conjugation, or transduction (19). In *Escherichia coli*, the genetic exchanges occur through conjugation and transduction, at a very low frequency (less than 10^{-4} per gene per generation for chromosomal markers) compared with eukaryote gene exchanges, and depend on conjugative plasmids and phages, respectively. Exchanges have been observed among natural isolates (20), and their evolutionary consequences are illustrated by the spread of antibiotic resistance genes (21), as well as the mosaicism of surface antigens (22–24), as a consequence of the strong selection imposed, respectively, by the use of antibiotics and by the immune system.

Previous analytical models (5, 14) have already considered sexual populations but not the case of adaptation involving multiple beneficial mutations segregating at the same time. Such an assumption is clearly a limitation for the study of microorganisms, because their populations are usually large enough for several favorable mutations to be present at the same time (25). The simulation model we designed considers finite populations of bacteria under directional selection and takes into account several mutations having various fitness effects. We show that even under conditions in which mutators have been shown to be

This paper was submitted directly (Track II) to the PNAS office.

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Article published online before print: *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.180063397. Article and publication date are at www.pnas.org/cgi/doi/10.1073/pnas.180063397

easily selected for (large populations and colonization by a single individual) (10), rare genetic exchanges have a dramatic effect on the fixation of mutators.

Model

To test the effect of genetic exchanges on the fate of mutators, models simulating evolving populations of bacteria were used. Two models simulating different kinds of genetic exchanges were created, one simulating the process of conjugation, the other, the process of transformation. Those models are similar to the density-based model described previously (10).

Simulated Genomes and Sexual Exchange Procedures. The simulated genome was composed of one mutator locus and six loci that could be the site of a favorable mutation. In the transformation model, all loci were treated independently, assuming that only one locus will be involved in the process at one time. Consequently, the relative positions of loci are not relevant. Hence, in addition to the mutator locus and the six loci potentially carrying favorable alleles, we considered a pool of 1,000 loci that could carry a deleterious mutation. For all bacteria, the gene transfer process was simulated by choosing one or several independent loci from among all existing ones (the number of genes received was randomly drawn using a Poisson law), after each new round of replication and mutation. The determination of the allele received at a chosen locus depended solely on its frequency in the population and not on its effect on the death rate as proposed by Redfield (26). It is considered that the recipient bacteria integrate new alleles into their genome. The rate of chromosomal gene transfer was constant throughout the simulations, which supposed a constant density of cells.

We also simulated the process of genetic exchanges through mobilization of the chromosome by a conjugative plasmid. In this model, all loci reside on a specified genetic map. Conjugation and transformation models were tested under the same conditions, with an increasing rate of sex and an increasing frequency of favorable mutations. The times of adaptation and the frequencies of mutator fixation were similar in the two models. Nevertheless, the conjugation model was much more time-consuming than the other model. Therefore additional simulations were performed with the transformation model, and all of the presented results were obtained with this model.

Mutation Rates and Fitness Effects. As we wanted to analyze the decrease in the probability of mutator fixation, we chose initial conditions that were slightly favorable for mutator allele fixation: no diversity was present before adaptation, and therefore the process of mutation was necessary for populations to evolve. Hence, we modeled the colonization of a virgin environment by a single cell with neither favorable alleles nor deleterious ones. The probability of each favorable mutation is 10^{-8}, the genomic deleterious mutation rate (27) was 10^{-4}, and the lethal mutation rate was 10^{-5}. The mutator allele increased all mutation rates by 100-fold, an effect similar to that of the most common strong mutators found in nature (11, 12), the mutation rate toward the mutator genotype was 5×10^{-6} (28), and reversion was $100 \times 5 \times 10^{-10}$. The fitness was set to 1 at the beginning. The effects on fitness were additive, as in previous studies (10); however, multiplicative effects gave the same patterns and amplitudes in all tested conditions (data not shown). Favorable alleles yield an increase in fitness of 0.06, 0.04, 0.04, 0.03, 0.03, and 0.03, respectively. To decrease simulation time, we chose a cost of 0.05 for each deleterious mutation and a genomic deleterious mutation rate of 10^{-4}, rather than a cost of 0.01 and a genomic rate of 1.7×10^{-4} (27) in most simulations. However, we ran some simulations with the latter parameters to confirm that the observed effects were still valid under these conditions.

Table I. Criteria defining mutator allele fixation or contribution

Fixation: Mutator frequency $> 50\%$
Contribution: Mutator frequency $< 50\%$. At least one mutation appearing in the mutator background is shared by more than 50% of the population at the end of adaptation

Simulation Output. We calculated the time of adaptation as the number of generations required for 95% of the population to carry all favorable alleles. We estimated the mean frequency of mutator fixation at the end of the process of adaptation over at least 1,000 simulations. To test whether beneficial mutations and mutator background became decoupled, in some simulations we have labeled the mutations that occurred in a mutator background. The mutator was considered to have positively contributed to the adaptation without becoming very frequent (mutator frequency $< 50\%$) when at the end of a simulation, at least one beneficial mutation that had been generated by mutator activity was present in more than 50% of the population (Table 1). The mutator was considered fixed when its frequency had reached 50% in the course of adaptation (Table 1). A 95% threshold gave the same results (data not shown) but disturbed the calculation of the contribution when the mutator allele frequency was between 50% and 95%. Frequencies of mutator fixation were calculated over at least 1,000 independent simulations, and each simulation corresponded to the adaptation of one population.

Results

The effect of genetic exchanges on the fate of mutator alleles was studied by measuring the frequency of their fixation and the time needed for the population to possess all of the favorable alleles required for adaptation to different conditions.

Genetic Exchanges Reduce the Frequency of Mutator Alleles Fixation. When the rate of genetic exchange increased from null to 10^{-4} per gene per generation, the probability of fixation of the mutator allele decreased from 60% to 2% (Fig. 1*A*)., Such a decrease in mutator fixation could be due to the separation, by genetic exchange, of favorable alleles from the mutator loci that created them. In such a case, the beneficial alleles that would have led the mutator to fixation in an asexual population would be transferred into a nonmutator background and fixed in such a background, where they would be residing in genomes unburdened by the overproduction of deleterious mutations. Tagging the mutations generated in the mutator background and following them through genetic exchanges could reveal whether this process is likely to occur. Indeed, this analysis showed that in up to 5% of the simulations (Fig. 1*A*) mutator alleles contribute to adaptation (Table 1). Because such populations adapted somewhat faster (2%) than those in which mutators neither contributed nor became fixed, mutators can be seen in such a case as "adaptive altruists." It is worth noticing that such a "behavior" could be avoided in the simulations by forbidding genetic exchanges between mutator and nonmutator cells. Even in this case, mutator alleles did not become fixed as often as they would in the absence of recombination (7% instead of 60% with a 10^{-8} mutation rate toward beneficial mutations and 0.3% instead of 16% with a 10^{-9} rate). This finding reveals that sex mainly reduced mutator fixation by a process other than separation of the mutator allele from the associated beneficial mutations, as was observed in infinite asexual populations through the reversion of mutators by reverse mutation (3). To understand why sex reduced mutator fixation, we had to look for some other effects of genetic exchanges on the dynamics of adaptation.

Sex Accelerates Adaptation. Another effect of sex is the acceleration of the process of adaptation (Fig. 1*B*) by allowing favorable

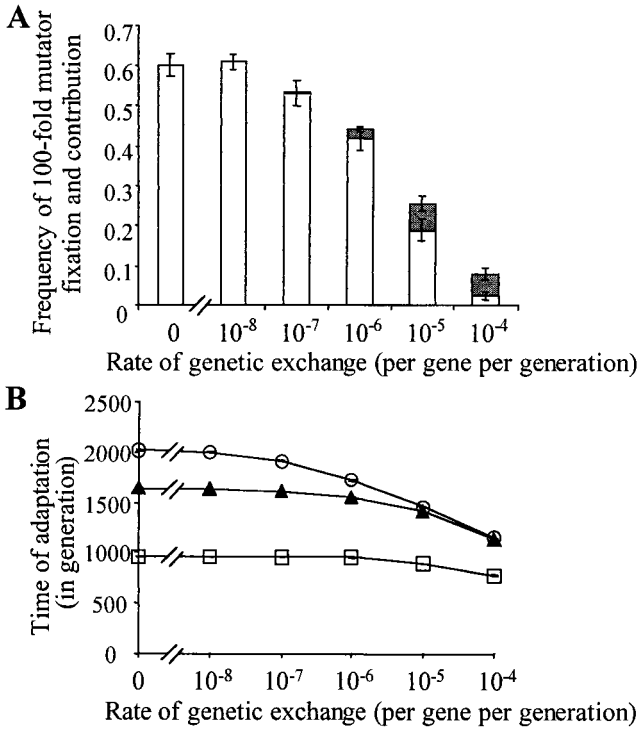

Fig. 1. Genetic exchanges reduce the frequency of mutator fixation, although faster adaptation is always occurring in a mutator background. (*A*) Frequency of 100-fold mutator allele fixation (white) and contribution (gray) in a large population (10^9 bacteria) (1,000 simulations per point). (*B*) Difference in adaptation times between mutator and antimutator backgrounds in large populations (over 200 simulations). ○, The mean adaptation time of populations with a fixed low mutation rate; □, populations with a fixed high mutation rate (100-fold higher than the previous). ▲, The mean adaptation time of populations with an initially low mutation rate that could possibly fix a 100-fold mutator during adaptation.

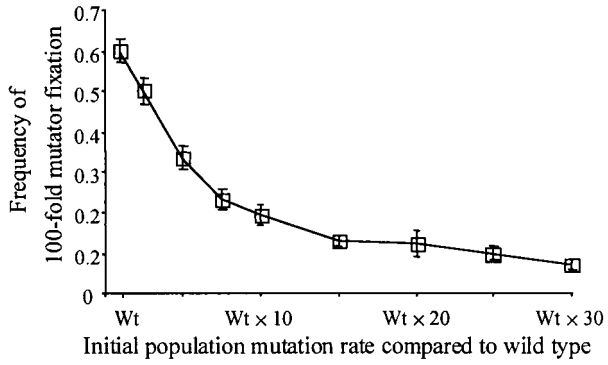

Fig. 2. The presence of moderate mutator alleles reduces the probability of fixation of a strong mutator allele. Fixation probability (1,000 simulations per point) of a 100-fold mutator in an asexual population of 10^9 cells is presented as a function of the strength of the moderate mutator allele fixed in the population before adaptation (mutator allele strengths are relative to wild type).

mutations produced in different individuals to be combined in the same genome (29, 30). Inasmuch as mutator alleles are fixed, because they are able to generate a succession of favorable mutations faster than the wild type, their advantage is much less obvious in populations evolving rapidly because of genetic exchanges. Hence, a new hypothesis is that the reduction of mutator fixation with an increasing rate of genetic exchange could be due to the accelerated population adaptation. We tested the effect of a faster adaptation by increasing the background mutation rate in the population, i.e., the mutation rate of the whole nonmutator population. Under these conditions (equivalent, for example, to the adaptation of a population having fixed a 10-fold mutator before this adaptation) the fixation probability of the 100-fold mutator was much lower (Fig. 2), which is similar to what was seen with sex.

Dynamics of Adaptation and the Effect of Sex on Mutator Fixation. The impact of sex on the fixation of mutators being the strongest with a rate of genetic exchange of 10^{-4}, we used this level to examine various parameters that are highly variable in natural populations and to see their effects on the action of sex on the fate of mutators. (Additional simulations were performed with a rate of genetic exchange of 10^{-6} to determine the amplitude of the effect of sex on mutator allele fixation with such a rate of genetic exchange). We varied (*i*) the size of the adapting population (Fig. 3), (*ii*) the frequency of favorable mutations (Fig. 4), (*iii*) and the selective advantage conferred by beneficial mutations (Fig. 5).

(*i*) The larger an asexual population is, the more frequent is the fixation of mutator alleles. In sexual populations, the frequency of mutator fixation first decreased and then increased when population size increased. Therefore the effect of sex on the fate of mutators is most pronounced for a population size of about 3×10^8 individuals (Fig. 3*A*). The adaptation time is reduced for larger population size (Fig. 3*B*). Moreover, populations with a fixed mutator mutation rate adapted faster than nonmutators, and sexual populations adapted faster than asexual. The increase in adaptation rate due to mutator genotype diminishes with increasing population size (Fig. 3*C*). In comparison, the increase in adaptation rate due to genetic exchanges first increased and then decreased with increasing population size (Fig. 3*C*). Both the effect of sex on the speed of adaptation (Fig. 3*C*) and the effect of sex on the fate of mutator (Fig. 3*A*) were at maximum for a population size of about 10^8 individuals.

(*ii*) The lower the frequency of favorable alleles, the less frequently mutators became fixed (Fig. 4). When the frequency of favorable alleles was increased, the effect of sex on fixation of mutator alleles was similar to the effect observed with increasing population size (Fig. 3*A*): it reduced the frequency of mutator allele fixation by up to 100-fold for a rate of genetic exchange of 10^{-4} and by up to 13-fold for a rate of 10^{-6}. Interestingly, the effect was at maximum for a mutation rate toward beneficial mutations of about 10^{-9}, a value near the estimated ones in experimental work on bacteria (31).

(*iii*) To determine the effect of the strength of selective advantage of the beneficial mutation on the fixation of mutator alleles, we modified the strength of the selection by multiplying the fitness advantages conferred by all favorable alleles by factors greater than (higher selection) and less than (weaker selection) 1 (Fig. 5). As the selective pressure diminishes, the fixation of mutator alleles diminishes, but the effect of sex in reducing the fixation of mutator alleles is more severe.

Deleterious Mutation Rates and Costs and the Effect of Sex on Mutator Fixation. As proposed by Johnson (13), the probability of fixation of mutator alleles is dependent on the mean cost of deleterious mutations. We therefore varied this parameter, using a rate of deleterious mutation of 1.7×10^{-4} (27). In the absence of genetic exchanges, the mutator fixation frequency decreased from 95% to 12% when the cost of deleterious mutation increased from 0.1% to 5% (15% for a cost of 1%). The effect of genetic exchanges (with a rate of 10^{-4}) on the fixation on mutator alleles varied from a 5-fold decrease to a 50-fold decrease over the same

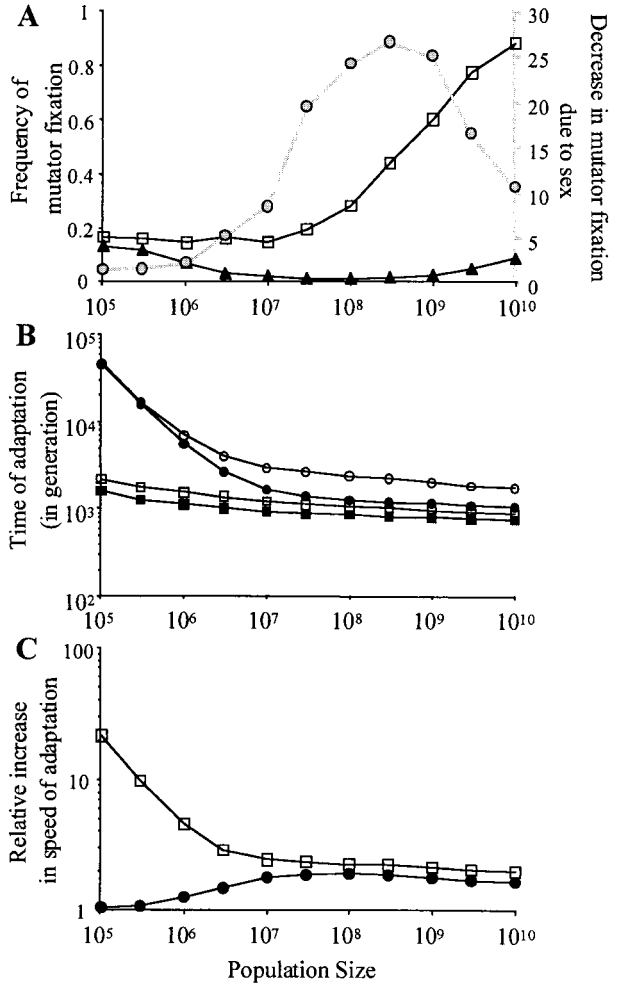

Fig. 3. Population size modifies the effect of genetic exchanges on the fixation of mutators. (*A*) The fixation frequency (1,000 simulations per point) of a 100-fold mutator allele, with a rate of sex of 10^{-4} per gene per generation (▲) or without genetic exchanges (□) as a function of the population size. The effect of sex on the fate of the mutator (◎) is the ratio of the frequency of mutator fixation without sex to the frequency of mutator fixation with sex. (*B*) Difference in adaptation times between mutator and antimutator backgrounds in large populations (over 200 simulations). ○, ●, The mean adaptation time of populations with a fixed low mutation rate, with a rate of sex of 10^{-4} per gene per generation (●) or without genetic exchanges (○). □, ■, Populations with a fixed high mutation rate (100-fold higher than the previous population) with a rate of sex of 10^{-4} per gene per generation (■) or without sex (□). (*C*) Increase in the speed of adaptation due to a mutator background or to the presence of genetic exchanges. □, The factor by which mutator background accelerates adaptation compared with nonmutator background in asexual population; ●, the factor by which genetic exchanges (at a rate of 10^{-4} per gene per generation) accelerate adaptation compared with asexual populations in the nonmutator background.

range. For a cost of deleterious mutation of 1% as estimated for *E. coli* (27), the effect of sex was a 17-fold decrease in the probability of fixation of the mutator allele.

Discussion

Considering a simple model of evolution in which the selection of mutators is favored (mutation as the only original source of diversity), we have shown that even rare genetic exchanges can lead to a large decrease in the probability of mutator fixation (Fig. 1*A*). This decrease is due to two phenomena that we will discuss in turn. First, recombination separates mutator alleles

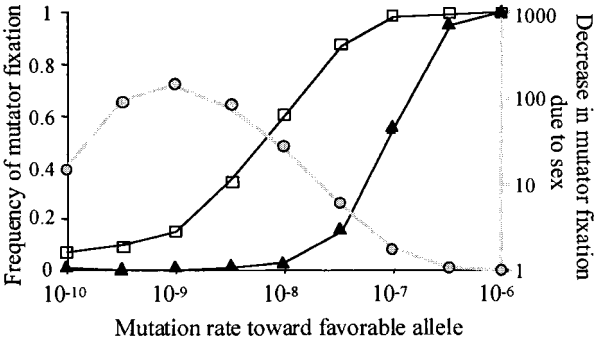

Fig. 4. At high rates of beneficial mutations, genetic exchanges cannot prevent the fixation of mutators. The fixation probability (1,000 simulations per point) of a 100-fold mutator allele, with a rate of sex of 10^{-4} per gene per generation (▲) or without genetic exchanges (□). (◎), Ratio of the frequency of mutator fixation without sex to the frequency of mutator fixation with sex. The population size is 10^9 bacteria.

from the favorable and deleterious alleles generated by mutator activity. In this study, the rate of genetic exchange and the rate of deleterious mutations are too low for the transfer of deleterious alleles to have an effect on the fate of mutator alleles. The rate of genetic exchanges considered here does not allow enough separation of mutator alleles from the deleterious alleles they generate to affect the frequency of mutator alleles at equilibrium without adaptation (data not shown). On the contrary, as beneficial mutations determine the future of the population, whether or not a mutator allele remains associated with them is crucial in determining its fate. The separation of mutator alleles from favorable alleles by recombination was already studied in several theoretical papers (5, 14, 32), but these have only considered the fixation of one favorable allele at a time. They were therefore limited only to the effect of separation of the beneficial allele from the mutator allele through sex, thus omitting another important effect of sex, which is to recombine into the same genome favorable alleles generated in different nonmutator individuals.

This second effect, known as the Fisher–Müller effect (29, 30), leads to an acceleration of the speed of adaptation and seems to be of major importance in the reduction of mutator

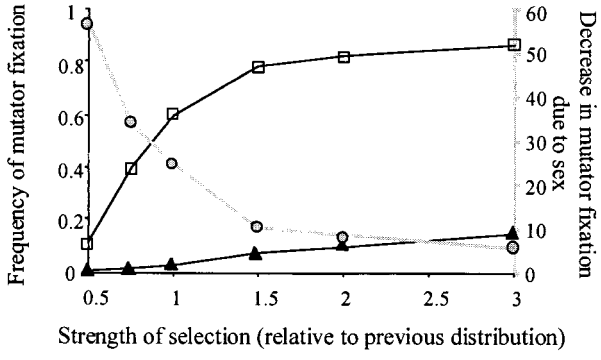

Fig. 5. Weak selection increases the effect of sex on mutator fixation. The fixation probability (1,000 simulations per point) of a 100-fold mutator allele, with a rate of sex of 10^{-4} per gene per generation (▲) or without genetic exchanges (□), is presented as a function of the strength of selection. All selective values of favorable alleles were multiplied by 0.5, 0.75, 1, 1.5, 2, and 3. The effect of sex on the fate of mutators (◎) is the ratio of the frequency of mutator fixation without sex to the frequency of mutator fixation with sex. The population size is 10^9 bacteria.

fixation among sexual bacterial populations (Fig. 1). Moreover, this latter effect can be regarded as a kind of competition between different strategies for the generation of diversity (second-order competition). As the selection of mutators is based on their ability to generate a succession of favorable mutations faster than nonmutators, any system that helps nonmutators adapt faster would reduce the relative advantage of mutators and therefore would reduce the probability of their fixation. Hence, mutator alleles in some cases cannot spread because of competition with recombination (Fig. 1) or other mutational strategies (Fig. 2) for finding favorable genetic combinations.

The outcome of the "sex versus mutator" competition can be understood by comparing the time needed to generate combinations of multiple beneficial mutations, either by recombination (33) or by a mutator activity. When mutation is limiting ($N \times \mu$ is small, where N is the population size and μ is the beneficial mutation rate), sex is unable to improve adaptation speed, because only one favorable mutation at a time is being fixed (Fig. 3*C*). Independently of the presence of sex, mutators spread sometimes when, by drift, they reach high frequencies that increase their probability of producing a favorable mutation (10, 17) (Fig. 3*A*). For intermediate values of $N \times \mu$, sex is efficient in accelerating evolution and prevents mutators from becoming fixed (Figs. 3*A*, 3*C*, and 4). Similarly, when the selective advantage conferred by beneficial alleles is weak (Fig. 5), favorable alleles will spread slowly through the population, increasing the chances for recombination to bring them together in a single individual. This phenomenon causes sex and recombination to have a stronger negative effect on the fixation of mutators. For high values of $N\mu$, mutators are numerous enough to generate a succession of favorable *de novo* mutations more rapidly than recombination in a nonmutator background (Fig. 3*C*). For instance, if $N \times \mu = 1{,}000$ ($n = 10^9$, $\mu = 10^{-6}$), mutators would always become fixed during adaptation in a sexual population (Fig. 4).

The competition between adaptive processes alters the selection for optimal strategies. As an illustration, even if the presence of sex and mutators would have been the best means of accelerating evolution (Fig. 1*B*), recombination prevents mutator alleles from spreading. In addition to competition, drift can deeply influence second-order selection. Although the fixation of a mutator allele would have a huge impact on the adaptation time of small populations, their fixation probability is much lower in such a context than in large populations (Fig. 3*A*), where their impact on the speed of adaptation is about 20-fold less (Fig. 3*C*) (34). This reduced impact is due to the way the dynamic of genetic hitchhiking constrains second-order selection: to increase in frequency, a mutator allele must be preferentially associated with favorable mutations. This combination of two alleles (mutator allele and beneficial mutation) is expected to occur less frequently than a simple beneficial allele, making second-order selection even more sensitive to population size than first-order selection. Being sensitive to drift and competition, second-order selection could be inefficient in the selection of optimal genetic systems at the population level. This possible inefficiency demonstrates that the strength of the selection for a genetic system and the strength of its effect on the "fitness" of the group are not correlated. Therefore the selected mutation rates in nature are not necessarily optimal.

Although we have focused on negative interactions between recombination and newly arising mutations, Fig. 4 reveals also some positive interactions between different mutational strategies. Genes under selective pressure exhibiting a high basal mutation rate have been described as an adaptive strategy (35–37). For instance, in a pathogenic panmictic (38) species such as *Helicobacter pylori*, surface antigen genes encoding proteins that are recognized by the immune system are known to be hot spots of mutation (35, 39) (contingency loci). Fig. 4 suggests that the existence of such hot spots of mutations can increase the selection for mutators, even in panmictic species. This selection engenders populations that are well equipped to face antibiotics or the immune system, combining the advantages of high rates of mutation and genetic exchanges.

For other species such as *E. coli*, in which no contingency loci have been found (40), genetic exchanges could play an important role in limiting the spread of mutators. Under conditions in which mutators are less likely to be selected, e.g., rare favorable alleles with low selective advantage, the effect of sex on the fate of mutators is of major importance (Figs. 4 and 5). It seems, therefore, that sex could severely reduce the number of environments in which mutators can be selected for and thus limit the ability of mutators to invade all environments.

The bacterial rates of genetic exchange are much lower than those of eukaryotic species, but they may have a substantial effect on the pathways of adaptation of bacterial populations. Recombination accelerates evolution and by this process might limit the fixation of mutator alleles. This limit on fixation could provide sex with a long-term advantage, because by limiting the spread of mutator alleles, it could reduce the deleterious mutation rate, which is harmful for most bacterial populations, because they often experience bottlenecks that could erode bacterial genomes in a mutator background (18). Moreover, when sex is inefficient in stopping the fixation of mutator alleles, it might still limit the cost of a high mutation rate once adaptation is achieved by accelerating the transition from a high to a low mutation rate through the reacquisition of an efficient repair system via sexual exchange with nonmutator migrants. Sex would therefore help bacteria approach an optimized mutation rate strategy composed of shifts toward high mutation rates in phases of adaptation under strong selective pressure and rapid recovery of a low mutation rate once adaptation is achieved.

When bacteria face various challenges, their genetic systems may evolve mechanisms of increasing evolvability. In addition to the moderate mutators that represent up to 10% of natural isolates of *E. coli* (12), other mutational strategies such as transposons (41) and inducible mutators (42) have been described in bacteria. Whether these different molecular strategies are present as the by-products of different functions (such as selfish behavior or DNA repair) or selected for because of their mutagenic potential remains to be determined in rigorous studies involving population genetics approaches. In any case, our results suggest that these alternative mutational systems could reduce the spread of strong constitutive mutators and help explain their low frequency among natural isolates. More generally, depending on ecological factors such as population size or strength of selection, the presence of one system for generating variability may greatly influence the fate of the others. Hence, a given composition of the molecular toolbox (36, 43, 44) that generates genetic variability may influence the selection of other such tools, illustrating the role of historical constraints on evolvability.

We thank M. Radman and P. H. Gouyon for their support and suggestions and E. Stewart, M. Vulic, M. Le Thierry, and anonymous referees for constructive comments and for improving this paper. This work has been funded by the Ministère de l'Environnement, the Bureau des resources génétiques, Association pour la Recherche sur le Cancer and Ligue contre le Cancer, Direction Générale de l'Armement, Program de Recherche Fondamentale en Microbiologie et Maladies Infectieuses et Parasitaires du Ministère de l'Education Nationale de la Recherche et de la Technologie, "Program Environnement et Santé 1999—Ministère de l'Aménagement du Territoire et de l'Environnement."

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