

## MINI- REVIEW

# The fate of microbial mutators

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### Mutation rate evolution

The separation of sex and reproduction in bacteria and most other microbes makes their evolutionary adaptation primarily dependent on mutation as the 'raw material'. At first sight, producing as many mutations as possible may thus seem a profitable strategy for microbes, because it would allow them to respond rapidly to changing environmental conditions. However, mutations come in many forms and only very few are beneficial for adaptation of the organism to its environment, while many more have deleterious effects (Sturtevant, 1937). This begs the question to what level the mutation rate should evolve to balance the necessity of adaptive change with the cost of deleterious mutations (Sniegowski *et al.*, 2000). Whatever the best mutation rate might be theoretically, actual changes of the mutation rate come about by natural selection acting on mutants with different mutation rates. Since these forces can be different for *individual* mutation-rate mutants within a population and entire *populations* of such mutants (see below), the observed mutation rate does not always reflect a single theoretical optimum. Actual mutation rates are rather a product of the interplay between short- and long-term evolutionary forces acting on these mutants, where short-term forces affect the frequency of individual mutation-rate mutants within populations, and long-term forces affect the frequency of populations of these mutants relative to other populations. The clonal structure of most microbial populations causes competition between populations to be a relevant force.

The best known class of mutation-rate mutants is that of the so-called 'general mutators', which have increased mutation rates throughout their genome due to defects in DNA proofreading and repair functions. These mutants have constitutively higher mutation rates, in contrast to transient mutators, where the increase of the mutation rate depends on environmental conditions (Taddei *et al.*, 1995; Rosenberg *et al.*, 1998; Foster, 1999). For the sake of simplicity and because their fate depends on very much the same evolutionary costs and benefits as those of transient mutators, I will concentrate

on the evolutionary fate of genetically stable mutators only.

Mutators are found among both natural and laboratory populations of bacteria (Jyssum, 1960; Gross & Siegel, 1981; LeClerc *et al.*, 1996; Mao *et al.*, 1997; Sniegowski *et al.*, 1997; Matic *et al.*, 1997; Boe *et al.*, 2000; Oliver *et al.*, 2000; Giraud *et al.*, 2001a; Denamur *et al.*, 2002). Most spontaneous mutator mutants appear defective in mismatch repair pathways, often because the *mutS* gene is inactivated (Miller, 1996; LeClerc *et al.*, 1996; Matic *et al.*, 1997; Sniegowski *et al.*, 1997; Funchain *et al.*, 2001). Depending on the type of function that is defective, mutators can have mutation rates that are moderately (~10-fold) to strongly (100–10000-fold) increased (Miller, 1996). Anti-mutator mutants with lower than normal mutation rates have also been observed (reviewed by Sniegowski *et al.*, 2000), albeit at much lower frequencies than mutators (Drake *et al.*, 1998). However, within mutator populations, mutants with lower mutation rates (but higher than wild-type) can evolve (Tröbner & Piechocki, 1984; Giraud *et al.*, 2001a). These studies show that evolution of the mutation rate is possible in evolving populations of bacteria. Here, I review a number of recent studies that shed light on the short- and long-term fate of microbial mutators, and provide new insights into the mechanisms and dynamics of mutation-rate evolution.

### Short-term fate

Theoretically, a mutator mutant could directly benefit from its lack of DNA proofreading and repair by saving time and energy and consequently having a higher rate of replication. However, the evidence for such direct fitness benefits is at best indirect (reviewed by Sniegowski *et al.*, 2000). Moreover, direct competitions between mutators and their repair-proficient equivalents reveal a small growth rate disadvantage for mutators (Chao & Cox, 1983; Tröbner & Piechocki, 1984; Boe *et al.*, 2000), presumably due to the increased production of deleterious mutations in their offspring. The magnitude

of this direct cost depends on mutator strength (Drake, 1991; Tenaillon *et al.*, 1999). Therefore, the finding of bacterial mutators at frequencies much higher than those at which they are maintained by mutation and selection – i.e.  $\sim 10^{-5}$  (Mao *et al.*, 1997; Drake *et al.*, 1998; Boe *et al.*, 2000) to  $10^{-3}$  (Johnson, 1999) – cannot be explained by direct fitness benefits. These intrinsically deleterious mutator mutants apparently provide indirect benefits, related to their increased production of rare beneficial mutations.

### **Hitch-hiking with beneficial mutations**

How might such indirect benefits of an increased mutation rate be realized? Because mutator mutants introduce more mutations in their offspring than the rest of the population, they will also generate more beneficial mutations. In asexually reproducing populations, a mutator allele (the modifier) and the mutations it produces (the effect) remain physically linked; their fates are thus tied. Once natural selection causes a beneficial mutation in a mutator background to increase in frequency, the mutator allele will hitch-hike to high frequency along with it (Maynard-Smith & Haigh, 1974; Chao & Cox, 1983). Natural selection favouring mutators is thus indirect.

For a mutator to increase in frequency within a population of finite size, it needs only to produce a successful beneficial mutation in one of its descendants before it occurs in the remaining wild-type majority of the population. To compensate for its numerical disadvantage, a mutator subpopulation needs a mutation rate which is increased by more than the inverse of its numerical disadvantage to have a better chance to produce the *next* beneficial mutation (Chao & Cox, 1983). Given that the relative size of a mutator subpopulation is smaller than  $10^{-3}$ , a more than 1000-fold increase of the mutation rate would be needed to win the race to produce the next beneficial mutation. The fact is, however, that a mutator subpopulation has an increased per capita probability of producing beneficial mutations, which is equal to its strength. Therefore, if adaptation involves the sequential selection of *several* beneficial mutations, these chance events add up to a robust benefit for the mutator subpopulation to win the competition and hitch-hike to high frequency (Mao *et al.*, 1997; Miller *et al.*, 1999; Tenaillon *et al.*, 1999).

### **Conditions for hitch-hiking**

The indirect selective benefit of mutator mutants thus depends on opportunities for adaptation. The fraction of mutations that improve adaptation depends on the evolutionary history of the population in the present environment. If the population is already well adapted, then most if not all mutations will have negative or at best neutral effects on fitness, and the mutator subpopulation lacks opportunities for hitch-hiking. Instead, it

will pay the cost of the increased production of deleterious mutations. However, if the environment is in some respect novel, adaptation is not perfect and the mutator subpopulation may outcompete the wild-type majority by its association with a higher per-cell number of beneficial mutations. Novel environmental conditions are abundant if environments (i) change rapidly in time, as for pathogens coping with host defence mechanisms such as the immune system, or (ii) are heterogeneous and consist of multiple ecological niches. For example, *Pseudomonas aeruginosa* living in the lungs of cystic fibrosis patients is thought to cope with rapidly changing conditions due to host defence mechanisms and therapeutic treatment, as well as with a heterogeneous environment (Oliver *et al.*, 2000). Consistent with the abundance of opportunities for hitch-hiking is the high frequency of mutators found in cystic fibrosis lungs (Oliver *et al.*, 2000).

Hitch-hiking of mutator alleles with beneficial mutations depends on the physical linkage between modifier (mutator allele) and effect (beneficial mutation). If modifier and effect are separated regularly, as in sexual populations, mutators are not expected to profit from their indirect beneficial effect (Tenaillon *et al.*, 2000). As a consequence, mutators are not expected to be as frequent in sexual as in asexual populations, a premise that needs to be verified empirically. However, other forms of constitutive mutators *can* evolve in sexual populations. One example are transposable elements, such as transposons and insertion sequences, which can be considered mutator genes due to their mutagenic properties (Chao *et al.*, 1983). The absolute linkage of transposable elements to their effect allows them to hitch-hike to high frequency even in sexual populations. Another form of mutator that may evolve in sexual populations are so-called ‘contingency genes’ with increased mutation rate (Moxon *et al.*, 1994; Metzgar & Wills, 2000). Here, again, modifier and effect are tightly linked. The presence of short-sequence DNA repeats in the promoter or coding region of a gene, or local cassette-like mechanisms to activate alternative alleles of a gene are among the mechanisms that can increase the mutation rate at specific loci only. This way, only genes involved in adaptation to unpredictable aspects of the environment might profit from an increased production of beneficial mutations, while the costs of producing a high load of deleterious mutations is very much limited. Examples of such ‘local mutators’ include virulence genes of pathogenic bacteria (Moxon *et al.*, 1994; Metzgar & Wills, 2000).

Some mutator alleles (e.g. with deficient mismatch-repair genes *mutS* and *mutL*) not only increase the mutation rate, but also tend to have a hyper-recombination phenotype (Rayssiguier *et al.*, 1989). On the one hand this additional effect offers further opportunities for genetic hitch-hiking, i.e. along with beneficial recombinants. A recent study showed that selection for recombinants of a mating between *Escherichia coli* and *Salmonella enterica* indirectly selected for mismatch-repair-deficient mutators (Funchain *et al.*, 2001). On the

other hand, the hyper-recombination phenotype of mutators might frustrate the process of hitch-hiking by rapidly separating modifier and effect. However, the hyper-recombination phenotype of such mutators particularly involves recombination between unrelated DNA molecules (Vulic *et al.*, 1997), and thus will not interfere with the process of hitch-hiking within a clonal population.

### Long-term fate

The final consequence of indirect selection of spontaneous mutator mutants is the 'fixation' of a mutator allele in the population, causing the entire population of cells to become mutator. What then is the evolutionary fate of such a mutator population? This depends on: (i) within population forces to restore a low mutation rate, and (ii) the competitive ability of a mutator population relative to low-mutation-rate populations. The short-term restoration of a low mutation rate by back mutation is not very likely. The mutation rate to restore a DNA-repair function is much lower than the rate to new repair defects, because there are many more ways to cause a defect in any of a number of repair genes than to restore one specific defect in one specific function. If the mutator mutation consists of a (partial) deletion of a repair gene, the back-mutation rate is even zero. A more likely route to re-establish a low mutation rate is by horizontal transfer of a functional copy of the gene from a related population (e.g. Denamur *et al.*, 2000; Brown *et al.*, 2001). Provided they do arise in the population, the direct fitness advantage of DNA-repair-proficient mutants – and hence the chance that they take over the population – is still small (Chao & Cox, 1983; Tröbner & Piechocki, 1984; Boe *et al.*, 2000). The probability that a population will quickly lose its mutator phenotype once it has been fixed is thus not very high. Consistent with this is the observation that three *E. coli* populations which evolved a mutator phenotype during evolution in the laboratory (Sniegowski *et al.*, 1997) have retained high mutation rates for at least 11500 further generations (Cooper & Lenski, 2000). Since adaptation had slowed down considerably during this period, these mutators were maintained in the face of growing fitness costs due to their increased production of deleterious mutations. Note, however, that these populations had no ability to restore proficient repair genes by horizontal transfer from a wild-type population.

Because a mutator population has limited possibilities to re-establish a low mutation rate, its fate largely depends on the outcome of competition with non-mutator populations. Competition experiments between mutator and wild-type populations have revealed that mutator populations can be competitively superior under certain conditions (Cox & Gibson, 1974; Chao & Cox, 1983; Tröbner & Piechocki, 1984; Giraud *et al.*, 2001a). What then determines the competitive ability of a mutator population? Any benefit of a mutator population must rely on its greater adaptability fuelled by a higher production of beneficial mutations. However,

faster adaptation of a mutator population is only possible if further adaptation is (i) possible at all, and (ii) limited by the population's supply rate of mutations. As I will show, these premises largely depend on the population's size and adaptedness.

### Extinction by mutation accumulation

For the first prerequisite to be met, the population needs to be not well adapted (to allow beneficial mutations), as well as sufficiently large (to produce these rare mutations and to allow efficient selection against deleterious mutations). Once a population becomes too small, it risks the irreversible accumulation of deleterious mutations by genetic drift, a process called Muller's ratchet (Muller, 1964; Haigh, 1978). This risk must be higher for a mutator population. Muller's ratchet depends on the interplay between mutation rate and population size. For example, when mutator (*mutS*) *E. coli* populations were passaged through single-cell bottlenecks, Funchain *et al.* (2000) observed the extinction of 4% of the lineages after 1000 generations. Similarly, mutator yeast populations (*msh2*, i.e. defective in a mismatch-repair function, resulting in ~30-fold increased mutation rate) which were regularly passaged through bottlenecks of a single cell accumulated deleterious mutations at an increased rate relative to non-mutator populations (Zeyl & de Visser, 2001; Wloch *et al.*, 2001). In another study, similar *msh2* and wild-type yeast populations were grown in liquid medium by the daily transfer of a constant small volume, containing ~25 cells, to fresh medium (Zeyl *et al.*, 2001). By generation 2900 (after 175 bottlenecks), two of the 12 mutator populations had gone extinct due to the accumulation of deleterious mutations in a mutational meltdown process. A mutational meltdown results from the accelerated accumulation of deleterious mutations caused by their effect of reducing population size (Lynch & Gabriel, 1990). In contrast, all 12 wild-type populations had retained their initial fitness. These studies affirm that the costs of an elevated mutation rate strongly depend on population size.

### Clonal interference

Provided that adaptive evolution is possible (i.e. adaptation is not maximal and the population is sufficiently large), when is adaptation limited by the population's supply of beneficial mutations? The population's supply rate of *beneficial* mutations (Beneficial Mutation Supply Rate, BMSR) is the product of (i) its supply rate of *all* mutations (Mutation Supply Rate, MSR; de Visser *et al.*, 1999), and (ii) the fraction of mutations that are beneficial. The MSR is simply the product of mutation rate and population size, while the fraction of beneficial mutations depends on the population's level of adaptedness. Thus a mutator population will have a high BMSR if the population is large and not well adapted, and a low BMSR if the population is small and already well adapted. At high BMSR, several beneficial mutants are

present in the population simultaneously, all in different genetic backgrounds. Due to the clonal structure of most microbial populations, these contending mutants (Gerrish, 2001) will then compete with each other, a process that has been dubbed ‘clonal interference’ (Gerrish & Lenski, 1998). Clonal interference on the one hand increases the predictability of adaptive evolution by causing: (i) the fixation of beneficial mutations in decreasing order of their fitness effect (i.e. large ones first; Gerrish & Lenski, 1998; Miralles *et al.*, 1999), and (ii) a low temporal variation between, or strong ‘rhythm’ of, fixations (Gerrish, 2001). On the other hand, it causes the rate of adaptation to increase in a decelerating way with increasing BMSR, leading to an effective ‘speed limit’ on the rate of adaptive evolution in asexual populations (Gerrish & Lenski, 1998; de Visser *et al.*, 1999; Miralles *et al.*, 1999, 2000).

Thus large or not well-adapted mutator populations may not adapt faster than similar populations with low mutation rate due to the hampering effect of clonal interference. Only when BMSR is intermediate (e.g. due to relatively small population size) may mutator populations profit from their higher BMSR by adapting faster. Under these conditions, beneficial mutations are extremely rare and mutator populations shorten the waiting time between mutations. For example, pathogens might profit from a mutator phenotype by allowing faster adaptation, because they experience severe bottlenecks during the initial stages of infection (Moxon & Murphy, 1978). The above predictions were tested in a laboratory experiment with evolving *E. coli* populations that differed in mutation rate, population size and initial adaptedness (de Visser *et al.*, 1999). Consistent with predictions, the results showed diminishing returns from the MSR on the rate of adaptation if the population’s founders were not well adapted, and hence BMSR was high. If, however, the founding strains were already well adapted, adaptation appeared indeed limited by the MSR, so that mutator populations would adapt faster. The experiment also showed that well-adapted populations may benefit from an increased mutation rate, but that this benefit may be small. The reasons are that (i) adaptive events have become exceedingly rare and, moreover, (ii) clonal interference causes these later beneficial mutations to be small in effect, because the big-effect mutations will then have been used up (Gerrish & Lenski, 1998).

#### **Loss of functions needed in future environments**

There are yet more limitations to the competitive ability of mutator populations. Their long-term competitive ability may be hindered by the rapid loss of functions, not essential in the present, but important in future environments, if environmental conditions change frequently (Funchain *et al.*, 2000; Cooper & Lenski, 2000; Giraud *et al.*, 2001a). Many mutations which improve functions needed at present have negative effects on functions that are essential elsewhere, a principle referred to as ‘antagonistic pleiotropy’

(Cooper & Lenski, 2000). Other mutations are neutral in the selective environment but show negative effects in secondary environments, and may accumulate by genetic drift (Funchain *et al.*, 2000). Since mutator populations are thought to accumulate both classes of mutations at increased rate, they should rapidly specialize in exploiting the present environment at the expense of functions acquired previously, a process that has been dubbed ‘genetic amnesia’ (Giraud *et al.*, 2001b). Thus rapidly changing environments provide a selective advantage to mutator mutants within populations in the short term, while they may deprive mutator populations of adaptive flexibility in the long run. However, these studies have not been able to attribute the poor long-term performance of mutators to any particular mechanism, i.e. antagonistic pleiotropy, mutation accumulation, or just low BMSR.

#### **Adaptive landscape**

A final peculiarity of mutator populations that may affect their long-term fitness has recently been observed in digital organisms evolving in computers (Wilke *et al.*, 2001). In this study, pairs of digital organisms with the same ancestor but a fourfold difference in mutation rate were allowed to evolve for 1000 generations by mutation and selection processes similar to those of biological organisms. Several organisms that evolved with a high mutation rate appeared to evolve lower fitness (measured as rate of replication) than their low-mutation-rate equivalent if both populations had a low mutation rate during competition. However, if both populations had a high mutation rate during competition, the organisms that had also evolved with a high mutation rate would win. Apparently, these mutator digital organisms had adapted to lower but flatter fitness peaks in the adaptive landscape (Wright, 1931), making them more robust to the effects of deleterious mutations at the expense of a lower replication rate (Wilke *et al.*, 2001). Hence mutator populations not only rapidly adapt to the latest resources they encounter, but they even adapt to the effects of their increased mutation rate itself. Their lower replication rates, however, make them inferior competitors in direct competition with organisms that evolved with lower mutation rates. To what extent these results can be expected in real microbes is an empirical question that begs experimental testing.

#### **Conclusions**

We have a relatively good understanding of why mutator mutants are found at higher than expected frequencies in laboratory and natural populations of bacteria: because they profit from their increased production of beneficial mutations by genetic hitch-hiking when populations adapt to novel environments. However, once the mutator allele is fixed and the entire population has become mutator, the fate of a mutator mutant is less clear. The fate of a mutator population largely depends on its competitive ability relative to low-mutation-rate populations. Several recent studies have shown that the

long-term fitness of mutator populations may be low for a number of reasons. First, mutator populations living in a changing environment may suffer from a reduced niche breadth by the mutational loss of functions not needed at present, but essential in future environments. Second, even if the environment does not change, mutator populations only outcompete non-mutator populations under few conditions. If the population is too small, mutators run the risk of mutational extinction. If, instead, the supply rate of beneficial mutations is too high, due to large population size or low initial adaptedness, then clonal interference reduces the mutator's benefit of faster adaptation. Third, work on digital organisms suggests that mutator populations may be prone to evolve genomes that are robust against the effects of deleterious mutations at the expense of a lower replication rate. In sum, populations of mutators have – in evolutionary terms – a high birth rate but a short life. Their short evolutionary life may explain why mutators are found only in a fraction of the microbial populations, as well as why they do not seem to affect the long-term rate of molecular evolution (Whittam *et al.*, 1998). [However, alternative explanations for the latter observation are possible. For instance, even if mutators would exist longer they may have no impact on the rate of molecular evolution due to the clearing of genetic variation during adaptive sweeps, which occur with strong 'rhythm' and are independent of the mutation rate of the population (Gerrish, 2001).]

Studies addressing the exact short- and long-term costs and benefits of mutators are needed for a more conclusive view on the evolutionary causes and consequences of mutators. What in particular should be done? Little is known about the mutational spectra of general mutators. A comparison between the distribution of mutational fitness effects – especially the ratio of beneficial to deleterious mutation numbers – of mutators and DNA-repair-proficient strains is fundamental for understanding the basic costs and benefits involved. Recently, protocols have become available that use microbes for this purpose (Imhof & Schloetterer, 2001; D. E. Rozen, J. A. G. M. de Visser & P. J. Gerrish, unpublished results). Emphasis should also be given to the long-term evolutionary persistence of mutators. To study their long-term fate, competition experiments over many generations between mutators and wild-type strains should be performed under conditions applied to test the present hypotheses. This way, the long-term fate of mutators in fluctuating or heterogeneous environments versus constant and homogeneous environments can be empirically tested. Finally, by varying the population size in competition experiments, the balance between adaptive and maladaptive evolution can be explored, as well as its impact on the long-term evolutionary fate of mutators.

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