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MicroReview

Evolution of mutation rates in bacteria

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Summary

Evolutionary success of bacteria relies on the constant fine-tuning of their mutation rates, which optimizes their adaptability to constantly changing environmental conditions. When adaptation is limited by the mutation supply rate, under some conditions, natural selection favours increased mutation rates by acting on allelic variation of the genetic systems that control fidelity of DNA replication and repair. Mutator alleles are carried to high frequency through hitchhiking with the adaptive mutations they generate. However, when fitness gain no longer counterbalances the fitness loss due to continuous generation of deleterious mutations, natural selection favours reduction of mutation rates. Selection and counter-selection of high mutation rates depends on many factors: the number of mutations required for adaptation, the strength of mutator alleles, bacterial population size, competition with other strains, migration, and spatial and temporal environmental heterogeneity. Such modulations of mutation rates may also play a role in the evolution of antibiotic resistance.

Newly arisen mutations can have very different impacts on the fitness of the organism, ranging from deleterious through neutral to beneficial. However, they appear at very different rates. For example, for *Escherichia coli* K-12, the rate of deleterious mutations per genome per replication is, at least, $2–8 \times 10^{-4}$ (Kibota and Lynch, 1996; Boe *et al*., 2000), while that of beneficial mutations is, at least, 2×10^{-9} (Imhof and Schlotterer, 2001). The rate of spontaneous mutations results from a balance between the effects of deleterious mutations and the metabolic costs of further reducing mutation rates (Drake *et al*., 1998). Any variant that has increased mutation rates is expected to have reduced fitness due to increased production of deleterious mutations. However, contrary to these intuitive predictions, strains having high mutation rates (mutators) are not rare in natural bacterial populations. They have been found in populations of *E. coli* (Matic *et al*., 1997), *Salmonella enterica* (LeClerc *et al*., 1996), *Neisseria meningitidis* (Richardson *et al*., 2002), *Haemophilus influenzae* (Watson *et al*., 2004), *Staphylococcus aureus* (Prunier *et al*. 2003), *Helicobacter pylori* (Bjorkholm *et al*., 2001), *Streptococcus pneumoniae* (del Campo *et al*., 2005) and *Pseudomonas aeruginosa* (Oliver *et al*., 2000), with frequencies ranging from 0.1% to above 60%. Experimental (Mao *et al*., 1997; LeClerc *et al*., 1998) and theoretical (Boe *et al*., 2000) studies indicate that the frequency of mutators observed among natural isolates is much higher than expected from mutation/selection equilibrium, which suggests that there are situations in nature where being a mutator confers a selective advantage. In this review, we will discuss the role of different factors involved in shaping mutation rates in natural bacterial populations, as well as their role in the evolution of pathogenicity and antibiotic resistance.

Mismatch repair-deficient mutators

Mutator phenotypes in general result from alterations in genes coding for DNA repair enzymes and for proteins that assure accuracy of DNA replications. These mutant genes are called mutator alleles. The majority of strong mutators found in the laboratory [*E. coli* (Sniegowski *et al*., 1997); *S. enterica* serovar Typhimurium (LeClerc *et al*., 1998)] and in nature [*E. coli* (Matic *et al*., 1997); *S. enterica* (LeClerc *et al*. (1996); *N. meningitidis* (Richardson *et al*., 2002); *P. aeruginosa* (Oliver *et al*., 2002)] have a defective mismatch repair system due to the inactivation of *mutS* or *mutL* genes. This repair system controls the fidelity of DNA replication by eliminating biosynthetic errors (Schofield and Hsieh, 2003), and by participating in processing DNA lesions during transcription-coupled repair (Mellon and Champe, 1996). In addition, the mismatch repair system is involved in the maintenance of chromosomal structural integrity and in the control of horizontal gene transfer by preventing recombination

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between non-identical DNA sequences. The mismatch repair system involves several proteins, of which two, MutS and MutL, have been highly conserved during evolution. MutS protein recognizes seven of eight possible base pair mismatches, C-C mismatches (the least frequent replication error) being refractory. In addition, MutS protein binds up to four unpaired bases, allowing for repair of frameshift errors. The efficiency with which different mismatches are repaired is determined by the affinities of MutS protein for various mismatches. MutL plays the role of 'molecular matchmaker' between MutS–mismatch complexes and other proteins involved in the repair process. The inactivation of *mutS* or *mutL* genes results in a strong mutator phenotype, with a 10^2-fold increased rate of transition (G:C→A:T and A:T→G:C) and 10^3-fold increased rate of frameshift mutations. In addition, *mutS* or *mutL* knockout mutants have a strong hyper-recombination effect, resulting in a 10^1–10^3-fold increase in the rate of chromosomal rearrangements. Molecular characterization of *E. coli* and *P. aeruginosa* natural *mutS* and *mutL* mutants has revealed that these genes are inactivated by a variety of mechanisms: frameshifts, insertions, premature stop codons and deletions (Oliver *et al.*, 2002; Li *et al.*, 2003).

Any bacterial population is expected to harbour a subpopulation of mismatch repair mutants due to spontaneous mutations in mismatch repair genes. The frequency of mismatch repair-deficient mutators in cultures of *E. coli* K-12 that are not subjected to any selective pressure was estimated to be less than 3×10^{-5} (Mao *et al.*, 1997; Boe *et al.*, 2000), which corresponds to a mutation rate from non-mutators to mutators of 5×10^{-6} per cell per generation (Boe *et al.*, 2000). For *S. enterica* serovar Typhimurium, the frequency of mutators in the unselected population is even lower, $1–4 \times 10^{-6}$ (LeClerc *et al.*, 1998). How can mismatch repair-deficient mutators outgrow non-mutator cells when they produce such vastly increased numbers of mutations that are predominantly deleterious? There are two possibilities: (i) mismatch repair mutators have higher rates of replication than non-mutators due to the absence of the metabolic load imposed by DNA repair enzymes, or (ii) cells with a high mutation rate have a selective advantage because they adapt faster than non-mutators. These two hypotheses have been experimentally and theoretically tested and the first one was rejected. If there is a selective advantage due to decreased metabolic load, then this advantage should be independent of the initial ratio of mutator to non-mutator cells. However, this is not the case. The mutator outgrows the non-mutator strain only when the ratio of mutator : non-mutator population size is above a certain threshold. This was observed for *mutS* (Fig. 1 and Giraud *et al.*, 2001; Labat *et al.*, 2005) and *mutT* (Chao and Cox, 1983) mutators. This threshold is determined by the ratio

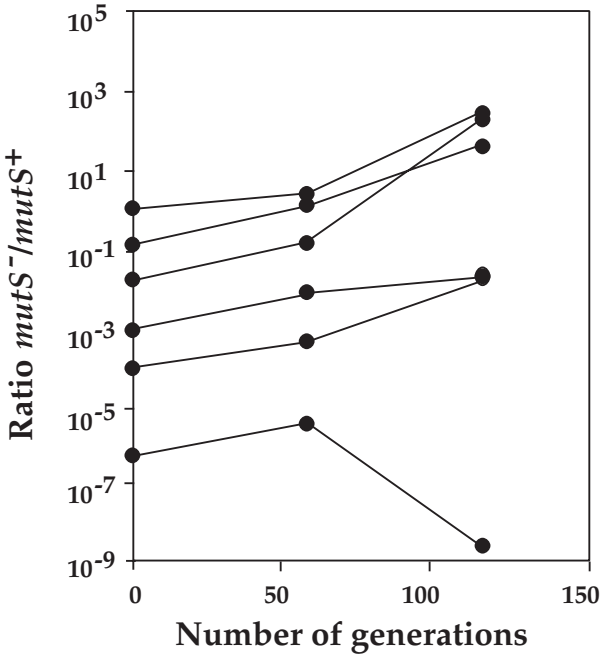

Fig. 1. Batch-culture competitions between *E. coli* CFT073 uropathogenic strain (*mutS*$^+$) and its mutator (*mutS*) derivative at varying starting ratios in human urine. Once a day, overnight cultures were diluted 10^3-fold into fresh urine. This regime permits ≈ 10 ($\log_2$ 1000) cell generations per day. The results show that mutator strain wins the competition only when the ratio of mutator : non-mutator population size is above certain threshold. This figure is adapted from Labat *et al.* (2005).

of the frequency of mutants carrying beneficial alleles in mutator versus non-mutator population. In each population, the frequency of these mutants depends on the mutation rate and the population size (Chat *et al.*, 2006). These studies strongly suggest that mutators are selected because they produce more adaptive mutations. This selective advantage occurs despite the fact that mismatch repair-defective mutators start off with a small selective disadvantage (about 1%) relative to non-mutators (Tröbner and Piechocki, 1984a; Boe *et al.*, 2000). The selective advantage of mutators over non-mutator strains is not restricted to *mutS* mutants, but is also observed for *mutL* mismatch repair-deficient mutants (Tröbner and Piechocki, 1981).

Selection of high mutation rates

Natural selection favours mutator cells by favouring beneficial mutations generated in these cells due to its mutator phenotype. However, it also indirectly favours the mutator allele due to its physical linkage with beneficial mutations. The linkage between beneficial mutations and mutator alleles is particularly strong in bacteria because the rate of gene exchange in these asexual organisms is, in general, very low. Mutators can increase in frequency

even when their initial population size is very small (see above) when several beneficial mutations are required for adaptation (Mao *et al*., 1997; Giraud *et al*., 2002). For example, if the probability of generating each beneficial mutation is 10^2-fold higher in mutators than in non-mutators, then the probability that two beneficial mutations will be generated in mutators is 10^4-fold higher than in non-mutators. The hitchhiking of mutator alleles with beneficial mutations is possible only when fitness gain counterbalances the fitness loss due to generation of deleterious mutations (Taddei *et al*., 1997a).

Selection of mutator alleles also depends on many other phenomena. For example, the increase in frequency of mutators depends on total population size (Tenaillon *et al*., 1999), on mutator strength [i.e. increase of mutator mutation rate relative to non-mutator mutation rate (Taddei *et al*., 1997a)], and on the rate of gene exchange (Tenaillon *et al*., 2000). Furthermore, it depends on the stability of the environment. For example, mutator alleles are particularly advantageous upon a shift in environmental conditions (Travis and Travis, 2002; Tanaka *et al*., 2003). The dynamics of selection of mutators depends also on environmental spatial heterogeneity, which will allow or prevent the competition between the cells carrying different adaptive mutations. Theoretical modelling predicts that mutators will be particularly favoured in temporally and spatially heterogeneous environmental conditions (Travis and Travis, 2004).

Counter-selection of mutators

Experimental and theoretical studies showed that the frequency of mutator strains in a population could rapidly increase to almost 100%. However, the majority of natural isolates are not mutators. A major factor that diminishes the fitness of mutators is the continuous production of deleterious mutations, particularly in a stable environment, once adaptation is achieved (Giraud *et al*., 2001). A second factor is that neutral, beneficial and deleterious mutations can have very different impacts on fitness in different environments. Consequently, no single genotype is optimally adapted to all environments. For example, an adaptive mutation in one environment can be deleterious in another [a phenomenon called 'antagonistic pleiotropy' (Cooper and Lenski, 2000)]. Therefore, migration from one environment to another might contribute to the reduction of fitness of mutators in natural populations, as observed in *in vivo* laboratory experiments for *E. coli mutS* (Giraud *et al*., 2001) and *S. enterica* serovar Typhimurium *mutS* (Nilsson *et al*., 2004) mutators. Finally, continuous passages through strong bottlenecks result in the accumulation of deleterious mutations due to genetic drift. This phenomenon, called Muller's ratchet, is particularly deleterious for strong mutator populations. For example, when wild-type and *mutS* defective cells were passaged through single-cell bottlenecks, only 3% of the wild-type lineages had phenotypically detectable mutations after 40 cycles. On the contrary, 4% of *mutS* lineages had died out, 55% had auxotrophic requirements, 70% had defects in at least one sugar or catabolic pathway, 33% had a defect in cell motility, and 26% were either temperature-sensitive or cold-sensitive lethals (Funchain *et al*., 2000). The loss of fitness of mutator strains should be positively correlated to the mutator strength (Taddei *et al*., 1997a).

Therefore, in the long run, fitness cost associated with high mutation rates is expected to cause the loss of adaptive mutations from bacterial populations, with consequential elimination of the mutator genome. However, some adaptive mutations generated in mutator backgrounds can be saved either by their horizontal transfer to a non-mutator background or by a reduction in the mutation rate of the adapted mutator strain before the load of deleterious mutations becomes too high. The reduction of mutation rate might be achieved by several mechanisms: reversion of the mutator mutation, acquisition of suppressor mutations [e.g. in populations of *mutT* mutators (Tröbner and Piechocki, 1984b)], or by reacquisition of a wild-type allele of anti-mutator gene from non-mutator bacteria via horizontal gene exchange (see below and Denamur *et al*., 2000).

Some bacterial species, like *H. influenzae* and *N. meningitides* (Bayliss *et al*., 2001; Meyers *et al*., 2003), possess mechanisms allowing them to permanently maintain high mutation rates at some loci, avoiding fitness costs associated with high genome-wide mutation rates. The hypermutability of these loci results from the mutational properties of repetitive DNA sequences located within the gene or within its controlling elements. These genes code for evasins, lipopolysaccharide biosynthesis enzymes, adhesins, iron acquisition proteins, and restriction–modification systems. Repetitive DNA sequences experience high rates of insertion and deletion mutations through replication slippage, which results in alternating loss-of-functions and reversions. Such mutagenesis can increase bacteria fitness by allowing evasion of the host's immune system. However, the type of variation produced by localized mutator activity might not always be sufficient for adaptation, as suggested by the presence of strong generalized mutators in natural populations of *N. meningitides* and *H. influenzae* (Richardson *et al*., 2002; Watson *et al*., 2004).

Why are mismatch repair-deficient mutants so frequent?

Low spontaneous mutation rates are maintained by the activity of many molecular mechanisms that protect and repair DNA, as well as by the mechanisms that assure

high-fidelity DNA replication. Inactivation of over 20 different *E. coli* genes can confer mutator phenotypes of different strengths (for review see Horst *et al*., 1999). So, why do nearly all strong mutators found in nature and in the laboratory have defective mismatch repair systems? One explanation for this phenomenon is that inactivation of the other genes involved in important aspects of DNA or RNA metabolism might have too high a cost to be compensated by advantageous mutations. For example, competition experiments in chemostats have shown that *E. coli mutT* mutators can also be selected for by beneficial mutations they generate (Chao and Cox, 1983). However, *mutT* mutators were never found in *E. coli* natural populations. This may be explained by the fact that inactivation of *mutT* gene, which codes for the protein that eliminates 8-oxo-G from the nucleotide pool (Horst *et al*., 1999), increases replication, but also transcriptional errors (Taddei *et al*., 1997b), which might considerably reduce fitness of the mutant cell.

The specific advantage of mismatch repair-deficient strains over other mutator alleles, which might also explain their abundance in nature, is their hyper-recombination phenotype. Recombination can also increase adaptability by increasing genetic variability. Consequently, genotypes with increased recombination rates might be selected for by virtue of the favourable genotypes they generate by allowing the association of beneficial mutations that have appeared in different individuals. Indeed, the frequency of mismatch repair-deficient mutants has been reported to increase rapidly in *E. coli* populations by hitchhiking with the recombination events they generate (Funchain *et al*., 2001). Irrespective of whether selection of mismatch repair mutators is due to their hyper-recombination or mutator phenotypes, the cost of high genetic instability remains the same. However, when selective pressure for increased genetic variability is no longer present, the hyper-recombination phenotype of mismatch repair-deficient strains might facilitate the reacquisition of the functional mismatch repair genes via horizontal gene transfer. This can 'save' the adapted mutator genome from being overburdened with deleterious mutations and allows survival. Such events seem to happen frequently during *E. coli* evolution (Fig. 2; Denamur *et al*., 2000).

Mutators, pathogenicity and antibiotic resistance

Although mutator strains have been found in commensal populations (Matic *et al*., 1997), most of them were isolated from populations of pathogenic bacteria. Furthermore, pathogens are exposed to changing stressful environments due to host defences and antibiotic treatments, i.e. conditions expected to favour mutators. Therefore, mutator and hyper-recombination phenotypes might accelerate the evolution of pathogenic strains by, e.g. increasing variation of surface antigens, as well as by facilitating acquisition of pathogenic determinants and antibiotic resistance (Taddei *et al*., 1997c). Indeed, the highest frequency of mutators within *E. coli* species was found among isolates from urinary tract infections (Denamur *et al*., 2002) and *N. meningitidis* strains displaying high phase variation rates (Richardson *et al*., 2002). Also, antibiotics resistance was significantly higher in mutator than in non-mutator *P. aeruginosa* pathogenic isolates (Oliver *et al*., 2000). In addition to these epidemiological studies, several experimental studies have confirmed the link between mutators, pathogenicity and antibiotic resistance.

For example, the measurements of the fitness of the uropathogenic *E. coli* CFT073 strain relative to its mismatch repair-defective derivative showed that the mutator was advantaged, *in vitro*, in urine (Fig. 1), and *in vivo*, in the late stages of urinary tract infection in a mouse model (Labat *et al*., 2005). Hence, increased mutagenesis could play an important role in the development of persistent reservoirs of uropathogenic *E. coli* observed in the mammalian bladder epithelial cells (Mulvey *et al*., 2000).

In vitro and *in vivo* studies have also provided clear evidence that antibiotic treatment can contribute to the selection of the mutators (Mao *et al*., 1997; Giraud *et al*., 2002). Mutators are favoured under such conditions because they generate antibiotic-resistance-conferring mutations at a higher rate than non-mutators (Fig. 3). This phenomenon is not specific to one antibiotic or to one family of antibiotics, suggesting that mutations can contribute to various modes of evolution of antibiotic resistance. Indeed, whenever mutations can confer or increase resistance to the antibiotics, or reduce the biological cost of resistance on bacterial fitness, it is more likely that they will appear in the populations of cells with higher mutation rates. Furthermore, when resistance is associated with the acquisition of several mutations, the advantage of being a mutator increases significantly (Tenaillon *et al*., 1999). For example, the sequential appearance of several mutations responsible for the evolution of extended spectrum β-lactamases is favoured by high mutation rates (Orencia *et al*., 2001). Therefore, the presence of mutator bacteria is a risk factor in the treatment of infectious diseases, as they might be responsible for therapeutic failures.

Fine tuning of mutation rates

A positive correlation between multiple-antibiotic resistance and high mutation rates is expected to be frequent in natural populations of bacterial pathogens (Blazquez, 2003). Such a correlation was found for *P. aeruginosa* isolated from lungs of patients with cystic fibrosis, bronchiectasis and chronic obstructive pulmonary disease

A

Phylogenetic group	Strain	*mutS* gene
A	ECOR20	TGCCCCTCACGCCCTAATCTCCGATCGCAGCCTACCGCATGCTAGCTGCAGTGACGTCT
	ECOR23	TGCCCCTCACGCCCTAATCTCCGATCGCAGCCTACCGCATGCTAGCTGCAGTGACGTCT
	ECOR4	TGCCCCTCACGACCTAATCTCCGATCGCAGCCTACCGCATGCTAGCTGCAGTGACGTCT
	ECOR1	TGCCCCTCACGCCCTAATCTCCGATCGCAGTCTACCACGTACTAGCCGCGGTGATATCT
	ECOR10	TGCCCCTCACGCCCTAATCTCCGATCGCAGTCTACCACGTACTAGCCGCGGTGATATCT
	ECOR13	TGCCCCTCACGCCCTAATCTCCGATCGCAGTCTACCACGTACTAGCCGCGGTGATATCT
	ECOR24	TGCCCCCCACGCCCTAATCTCCGATCTCAGTCTACCACGTACTAGCCGCGGTGATATCT
B1	ECOR17	TGCTCCTCGTGCTCCGCGTCTCGATCTCAGTCTACCGCGCGTCACCTGCGGTAGCGCCT
	ECOR70	TGCTTTCCACGCCCTAATCTCCGATCTCAGTCTACCACGTACTAGCCGCGGTGATATCT
	ECOR26	TGCTTTCCACGCCCTAATCTCCGATCTCAGTCTACCACGTACTAGCCGCAGTGACGCCT
	ECOR27	TGCTTTCCACGCCCTAATCTCCGATCTCAGTCTACCACGTACTAGCCGCAGTGACGCCT
	ECOR68	TGCTTTCCACGCCCTAATCTCCGATCTCAGTCTACCACGTACTAGCCGCAGTGACGCCT
	ECOR45	TGTCCCTCACGCCCTAATCTCCGATCTCAGTCTACCACGTACTAGCCGCAGTGACGCCT
	ECOR58	TGTCCCTCGTACTCCGCGTCTCGATCTTGGCCTATCATATGCTAGCTGCGGTGACGCCT
	ECOR34	TGTCCCTCGTACTCCGCGTCTCGATCTTGGCCTATCATATGCTAGCTGCGGTGACGCCT
D	ECOR35	CGTCCCTCACGCTCCGCGTCTCGTCTATAGCCCGCGGCGTGTTAGCTGCTGTGATGCTC
	ECOR41	CGCCCTTCGTGGTCCGCGCCTCGACTATAGCCTACCATGTGCTAGCTACGACAGCGTCT
	ECOR40	CGCCCTTCGTGGTCCGCGCCTCGACTATAGCCTACCATGTGCTAGCTACGACAGCGTCT
	ECOR46	CGTCCTTTACGCCCTAATCTCGAGTTATAACCTACCGCACGTCGGCTGCGGTAGTGCCT
	ECOR47	CGTCCTTTACGCCCTAATCTCGAGTTATAACCTACCGCGCGTTAGCTGCTGTGATGTTT
	ECOR49	CGTCCTTTACGCCCTAATCTCGAGTTATAACCTACCGCACGTCGGCTGCGGTAGTGCCT
	ECOR50	CGTCCTTTACGCCCTAATCTCGAGTTATAACCTACCGCACGTCGGCTGCGGTAGTGCCT
	ECOR60	CGTCCTTCGTGGTCCGCGTCTTCGTTTTAGCCTACCATGTGCTAGTTATGACAGCGTCT
	ECOR59	CGTCCTTCGTGGTCCGCGTCTTCGTTTTAGCCTACCATGTGCTAGTTATGACAGCGTCT
B2	ECOR62	CGCCCCTCGTGGTTCGGGCTCTCGTTTTAGCTCGCCACGTGTCAGCTGTGACAGCGTCT
	ECOR64	CGTCCTTCGTGGTCCGCGTCTTCGTTTTAGCCTACCATGTGTTAGCTGTGACAGCGTCT
	ECOR57	CATCCTTCGTGGTCCGCGTCTTTGTTTTAGCCTACCACGTGCTAGCTATGACAGCGTCT
	ECOR52	CATCCTTCGTGGTCCGCGTCTTTGTTTTAGCCTACCACGTGCTAGCTATGACAGCGTCT
UG	ECOR51	CATCCTTCGTGGTCCGCGTCTTTGTTTTAGCCTACCACGTGCTAGCTATGACAGCGTCT
	ECOR37	TGCGTTTCACGCTCCGCGTCTGAGTTTTAGCTCGCCGCGCGTCAGCTGCGGTGGCGCCT

B

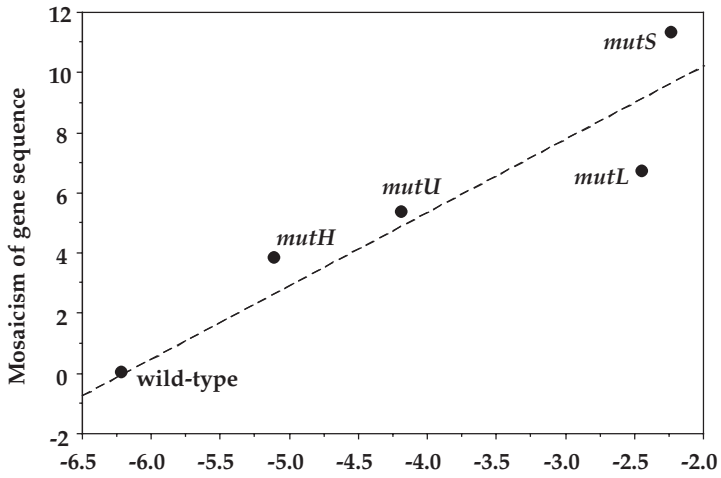

Fig. 2. A. Alignment of the informative sites of *E. coli mutS* genes from the ECOR reference collection based on the strain phylogeny showing sequence mosaicism. A given *mutS* gene sequence is considered mosaic when it is composed of segments of *mutS* genes from different phylogenetic groups as a consequence of horizontal gene transfers between strains belonging to different phylogenetic groups (A, B1, D, B2, UG). Transferred segments among different genes were defined by at least three bases delineating a stretch of DNA which is different from that of the sequences of the other strains belonging to the same phylogenetic group, but which is identical to those of another phylogenetic group. It was considered that one transfer event is more parsimonious than at least three independent mutational events occurring within the corresponding DNA stretch. Transferred segments are indicated by the same colour as that of the phylogenetic group of their origin. Black colour indicates sequence of unknown origin.

B. The relationship between mosaicism of *E. coli* mismatch repair genes and one housekeeping gene, *trpB* (wild type) resulting from horizontal gene transfer between different phylogenetic groups and hyper-recombination phenotypes of mismatch repair mutants. Mosaicism is expressed as the relative number of horizontal gene transfers between strains belonging to different phylogenetic groups, calculated as the number of inferred transfers (e.g. see panel A) divided by the maximum number of possible transfers (Denamur *et al.*, 2000). Recombination phenotypes were measured using *E. coli* Hfr × *E. coli* F^-, and *S. enterica* serovar Typhimurium Hfr × *E. coli* F^- conjugational crosses. These results suggest that mismatch repair functions have been repeatedly lost and reacquired during the evolutionary history of *E. coli* and that the restoration of mismatch repair anti-mutator functions often occurred via genetic exchange which gave rise to mosaic alleles. Panel B is adapted from Denamur *et al.* (2000).

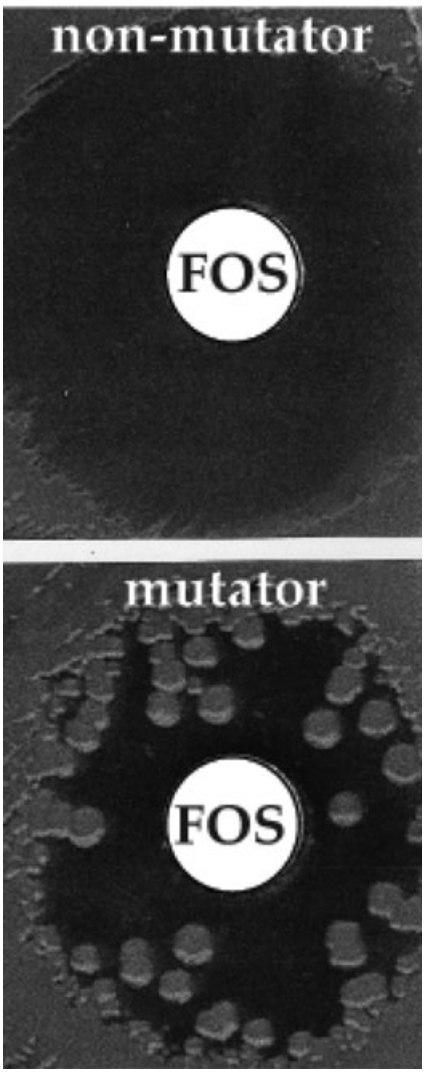

Fig. 3. Diffusion test for antibiotic susceptibility of *E. coli* natural isolates using commercial fosfomycin discs. The mutator strain (natural *mutS*$^{-}$ mutant), which generates mutations conferring resistance to the antibiotic at a high rate, is clearly differentiated from the non-mutator natural isolate by the presence of squatter colonies inside growth inhibition zone.

(Oliver *et al*., 2000; Macia *et al*., 2005). This is probably due to extensive antibiotic therapy and chronicity of the infection as well as to strong compartmentalization and low migration rates between hosts that limit competition between strains. These variables would therefore increase the probability of persistence of strong mutators. Indeed, the prevalence of *P. aeruginosa* mutator strains increases with time in the lungs of cystic fibrosis patients (Ciofu *et al*., 2005). However, this correlation was not observed in *P. aeruginosa* isolated from acutely infected patients (Gutierrez *et al*., 2004). The explanation for this discrepancy is provided by studies of the relationship between antibiotic resistance and mutation rates in *E. coli* natural populations (Gustafsson *et al*., 2003; Lindgren *et al*., 2003; Baquero *et al*., 2005; Denamur *et al*., 2005).

The correlation between multiple-antibiotic resistance and high mutation rates was also absent in *E. coli* natural populations, despite the capacity of strong *E. coli* mutators to generate mutations conferring resistance to antibiotics *in vivo* and *in vitro* (Fig. 3; Mao *et al*., 1997; Tanabe *et al*., 1999; Denamur *et al*., 2002; Giraud *et al*., 2002). Among *E. coli* natural isolates, the highest frequency of antibiotic resistance was found among weak mutators, while strains with high mutation rates had significantly lower antibiotic resistance (Denamur *et al*., 2005). The results of several other studies also suggested that even modest increase in mutation rate can significantly influence the evolution of antibiotic resistance (Gustafsson *et al*., 2003; Lindgren *et al*., 2003; Baquero *et al*., 2005).

The absence of the correlation between high mutation rates and antibiotic resistance can be explained by the dynamics of selection and counter-selection of mutator alleles. Selection acting on mutator alleles is modulated by many factors including changes in the environment and the opportunity for competition between strains. Mutator clones can rapidly specialize to one environment, which renders them less fit in other environments (Giraud *et al*., 2001). The initial gain and long-term loss of fitness is proportional to the strength of the mutator allele. Computer simulations predict that weak mutators will be selected less rapidly than strong mutators but, once selected, they will persist much longer than strong mutators (Taddei *et al*., 1997a). This may explain why there is around 1% of strong mutators and around 10–30% of weak mutators in *E. coli* natural populations (Matic *et al*., 1997; Baquero *et al*., 2004). Therefore, in bacterial species that cycle between different environments, e.g. *E. coli*, weak mutator strains might have more chance to accumulate multiple-antibiotic resistance than the strong mutators (Denamur *et al*., 2005). In conclusion, spatial and temporal environmental variations, together with the nature and the strength of selective pressures, constantly adjust mutation rates to levels that favour adaptation to each unique environment.

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