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Evolutionary significance of stress-induced mutagenesis in bacteria

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Mutagenesis is often increased in bacterial populations as a consequence of stress-induced genetic pathways. Analysis of the molecular mechanisms involved suggests that mutagenesis might be increased as a by-product of the stress response of the organism. By contrast, computer simulations and analyses of stress-inducible phenotypes among natural isolates of *Escherichia coli* suggest that stress-induced mutagenesis (SIM) could be the result of selection because of the beneficial mutations that such a process can potentially generate. Regardless of the nature of the selective pressure acting on SIM, it is possible that the resulting increased genetic variability plays an important role in bacterial evolution.

Stress is a disturbance of the normal functioning of a biological system that is provoked by environmental factors, the amplitude and persistence of which cause reduced growth rate or increased mortality. Different stresses, such as starvation, oxidation or UV irradiation, can increase the mutation rates of bacteria. Mutations can result from the direct alteration of the DNA molecule under stress conditions or from a genetic program that is induced under stress (Box 1). Most mutations are known to be deleterious, therefore the existence of genetically controlled pathways that are involved in the production of mutations as a result of stress has been quite puzzling for both microbiologists and evolutionary biologists. The selective pressures that lead to such a phenotype remain a subject of intense debate [1–4]. Although some researchers view stress-induced mutagenesis (SIM) as the unavoidable by-product of mechanisms that are involved in growth or survival under stress (the pleiotropic hypothesis), others see the upsurge in mutation rate as an adaptive strategy that could potentially enhance the chances of surviving the stress (the second-order selection hypothesis). The latter hypothesis is popular because it reflects the adaptationist view that bacteria modulate their mutation rate to enhance the potential for adaptation when needed (e.g. under stressful conditions). In support of this controversial adaptationist hypothesis was the discovery that SIM might target genes that are directly involved in promoting stress survival. This discovery also challenged some of the most basic tenets of neo-Darwinian theory, such as the independence of mutation from selection [5]. However, recent progress in understanding the mechanisms involved in SIM and also the selection acting on systems that modulate mutation rates have reframed the debate in a fully neo-Darwinian paradigm [6], in which mutations appear without any knowledge of their potential effect on growth or survival.

In this review, we consider the previously mentioned hypotheses and discuss the recent developments in the study of SIM in bacteria to understand the evolutionary causes and consequences of genetic pathways that are capable of increasing mutation rates under stress.

Experimental observations of SIM

Studies of stress-induced increases in mutation rate can be classified into two classes on the basis of the assay used: those in which the scored mutational events provide a response to stress and those in which they do not. Studies belonging to the first class of experiments are not the main focus of this paper, as the molecular mechanisms that lead to a preferential generation of adaptive mutations in the target gene are very specific to the system used and it is therefore difficult to assess their evolutionary relevance (Box 2). The second class of experiments, which showed that stressful conditions could lead to a genetically controlled increase in mutagenesis [7–10], revealed that such an increase is genome-wide. In these studies, the genes used to estimate the mutation rate were not involved in stress resistance (e.g. genes involved in antibiotic resistance). Using similar assays, studies of adaptive mutagenesis have also shown that the mutation rate increase is not restricted to genes that are involved in the stress response [9,11].

Most of the knowledge that exists regarding the genetic control of SIM has been derived from studies in which cells were stressed using chemical and physical treatments [12]. UV irradiation was among the first types of stress to be described that led to a mutation rate increase as a result of a genetic program, known as the SOS response (Box 1) [12]. From the time of the discovery of adaptive mutagenesis in 1988 [5], studies on starvation-induced mutagenesis have provided valuable data for understanding the evolutionary role for a genetic control of SIM. The advantage of starvation as a stress is that it is one of

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Box 1. Molecular mechanisms associated with stress-induced mutagenesis (SIM)

In *Escherichia coli*, different stresses can result in the increased generation of mutations by different mechanisms (Figure I):

(1) Various chemical and physical agents can generate mutagenic miscoding DNA structures that cause replication errors. For example, ionizing radiation generates 8-hydroxy-guanine, whereas methylating agents generate O^6-methyl-guanine [12].

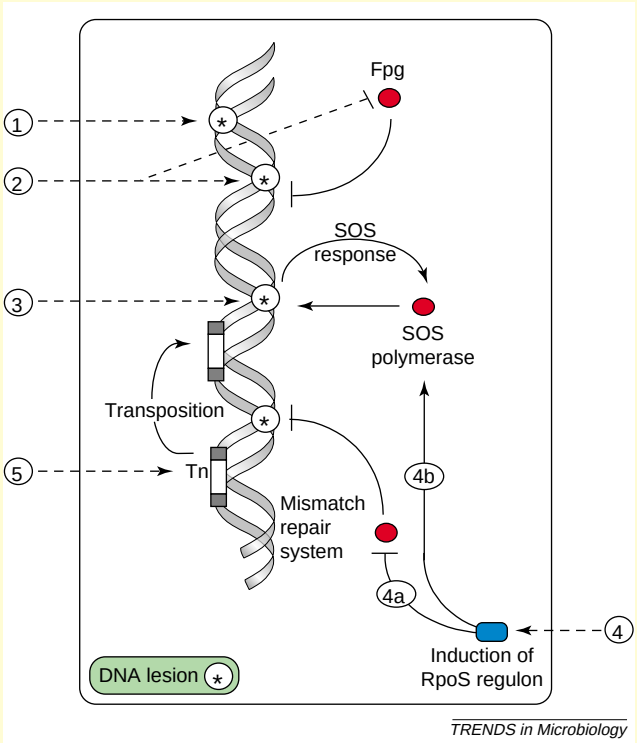

Figure I. The different molecular mechanisms that are associated with stress-induced mutagenesis (SIM).

(2) Some environmental agents affect DNA and also inhibit anti-mutator DNA repair enzymes, therefore increasing mutation rates. For example, the nitric oxide produced by macrophages damages DNA and inhibits Fpg DNA glycosylase, O^6-methyl-guanine-DNA methyltransferase and DNA ligase [40–42].

(3) DNA lesions (e.g. pyrimidine dimers produced by UV irradiation) that block replicative DNA polymerases induce the SOS system. Translesion synthesis polymerases that are controlled by the SOS system have the capacity to bypass pyrimidine dimers, but they have a low fidelity and often produce mutations [43].

(4) Different stresses, such as starvation, high osmolarity, low temperature and low pH, induce the RpoS regulon [44]. The induction of this regulon results in various morphological and physiological modifications that increase the capacity of cells to resist different stresses and survive. However, RpoS-dependent downregulation of the anti-mutator mismatch repair system (MRS) (Figure I, 4a) [15] and induction of the *dinB* gene that codes for the error-prone translesion synthesis polymerase (Figure I, 4b) [45] result in increased mutagenesis [4,7,46], revealing the conflict between priorities during the stress response.

(5) Stresses have also been shown to induce the mobility of transposons and insertion sequences, which can lead to gene inactivation or activation [47,48].

Three of the above mechanisms (3–5) imply genetic control of the mutation rate increase. Regarding the selective pressure acting on such mechanisms, the case of transposons is peculiar. It has been shown that transposons could be selected for as mutator genes [49] through second-order selection by the mutations they produce (see Box 2 in the main text). Nevertheless, it is hard to tell whether transposon mobility is the result of a selection acting to enhance the chance of survival of the bacterial strain carrying them, or if it is the result of the inherently selfish nature of successful transposons. Transposons can increase their opportunity for transmission to other bacteria by increasing their copy number in the chromosome, conjugative plasmid and prophage genomes of their bacterial host. Bacteria will probably die under stress, but their transposon-infested DNA can be transmitted before or after cellular death.

those that is most frequently experienced in the natural environment [13].

The study of starvation-induced mutagenesis among hundreds of *Escherichia coli* natural isolates showed that SIM occurs frequently and that its intensity varies drastically between isolates [7]. On average, a sevenfold increase in mutagenesis was observed; 20% of strains showed an increase of greater than 100-fold and 1.5% showed an increase of greater than 1000-fold (Figure 1). These variations did not show a correlation with strain phylogenetic relatedness, but they did correlate to the environment from which the strains were isolated, specifically the host and its diet. This pattern of correlation suggests a quick modulation of SIM on an evolutionary timescale. Another interesting observation suggesting that SIM might be under selection is the existence of a negative correlation between the rates of SIM and constitutive mutations [7].

Mutations as the price to pay for improved survival and growth: the pleiotropic hypothesis

The generation of mutations under stress conditions, in a genetically controlled manner, could be the by-product of genetic pathways that operate to optimize growth and survival. The association of SIM with SOS polymerases or the mismatch repair system (MRS) suggests that this could be the case (Box 1).

To date, the *E. coli* PolV SOS polymerase is the best illustration of how molecular constraints on survival functions can lead to mutagenesis (Box 1) [14]. PolV can bypass non-coding lesions that modify the structure of the DNA (e.g. UV-induced thymine-dimers) and block replicative polymerase PolIII. Translesion synthesis allows survival, however, as it is performed with low fidelity it introduces mutations. Therefore, it appears that mutations produced by PolV are the price to pay for survival. Why have such polymerases not evolved to be error-free and to add the proper nucleotide opposite the damaged DNA they recognize? There are two possible explanations. First, because the same lesion bypass polymerase is used to recognize several types of lesions, reduced fidelity could be the optimal solution for the trade-off between the recognition of different lesions and the fidelity of the bypass polymerization. If this is the case, the fact that PolV recognizes thymine-dimers and is biased towards adding an adenine residue onto the opposite

Box 2. Adaptive mutagenesis in the *Escherichia coli* Lac system

The phenomenon of adaptive mutagenesis is most frequently studied using the *Escherichia coli* strain lacking the chromosomal *lac* gene, but carrying a plasmid with a non-functional *lacZ* gene that could be reactivated by a mutation. When such a strain is starved on a minimum media plate with lactose, the number of $LacZ^+$ revertant colonies increases with time [6,50]. The increasing number of $LacZ^+$ revertants suggests that the increase in mutagenesis is due to carbon starvation. This mutagenesis often results in the exact mutational event that allows resumption of growth on lactose. It had therefore been assumed that mutagenesis under stress was adaptive, meaning that it was directed preferentially towards beneficial mutations. Other experiments using similar systems in which either a non-functional gene product can be reactivated by point mutation, or in which a mutation can modify an existing enzyme enabling the strain to use a new carbon source, resulted in the same kind of observation [6,50].

The analysis of genetic requirements for adaptive mutagenesis revealed the existence of mechanisms that could result in an increase in the appearance of lac^+ genes without increased mutagenesis. The challenge made by adaptive mutagenesis on classical random mutagenesis models relied heavily on the assumption that there was no bacterial growth under the experimental conditions used. Years of study have revealed that this might not be the case and that residual growth plays an important role in this phenomenon [6,50].

The experiment uses a strain with a gene (*lacZ* in many experiments) carrying a single mutation that inactivates the gene product activity. Nevertheless, some of the proteins produced from the mutated gene will be functional owing to errors in the process of transcription and translation that restore protein function. These functional proteins permit some cell divisions to occur, leading to an overestimation of the mutation rate. Additionally, this residual growth can be further enhanced if the number of copies of the mutated gene is increased, a mechanism known as gene amplification, which is further magnified if the mutated gene is on the plasmid [51]. In some experiments, up to 100 copies of *lacZ* have been found within a cell [3]. The probability of producing a mutation that restores a wild-type *lacZ* gene is increased by an increase in the number of copies of the amplified mutant gene. The understanding of adaptive mutagenesis targeted to the *lacZ* gene relies on the interplay between selection and mutagenesis.

Whether gene amplification (and its diverse consequences) is the only mechanism involved in the observed increase in mutation rate under starvation, or whether there are other mechanisms involving genome-wide increases in mutation rate is still a subject of debate [2–4]. The answer might be organism specific; experiments suggest an exclusive role for gene amplification in *Salmonella* [51], whereas experiments suggest the existence of some other pathways of mutagenesis in *E. coli* [6]. Given the variation in stress-induced mutagenesis (SIM) observed among *E. coli* isolates [7], such variations between species could be possible.

Could the mechanisms that produce adaptive mutations, such as gene amplification, have been selected for? The complexity of the requirements for such phenomena makes it difficult to assess their evolutionary relevance. To be selected for, such mechanisms need a recurrent presence in the media of a partly consumable substrate, such as a substrate on which a gene product can have some residual activity that can be largely enhanced by mutation. This is possible but it is hard to predict whether such conditions occur frequently enough in nature to allow the evolution and maintenance of such specific functions, or if they are just a by-product of the intrinsic instability of the genomic machinery.

strand suggests that such lesions are among the most frequent lesions that PolV encounters. Another possible explanation is that the cost of the deleterious mutations that are produced is not high when compared with the selective cost associated with the activity of error-free DNA repair systems. Therefore, there is no strong selective pressure to reduce the error-rate.

Another example of molecular constraints leading to mutagenesis is RpoS-dependent induction of the *dinB* gene, which is specific to the stationary phase. DinB codes for the error-prone PolIV SOS polymerase and is not dependent on canonical regulation of SOS genes (Box 1). Such regulation of DNA repair genes might help cells to process certain DNA damage during prolonged stationary phases that lack new protein synthesis. Notably, RpoS is also involved in MRS downregulation, which causes increased mutagenesis (Box 1) [7,15]. MRS downregulation might be selected for because MRS enzymes require energy to be produced and also for their activity. Furthermore, the MRS repair process might threaten cell survival (i.e. it can damage DNA by initiating repair processes without finishing them owing to energy depletion). Such costs could be much higher than the cost that is associated with the production of deleterious mutations.

Can the pleiotropic hypothesis explain the high levels of SIM polymorphisms that are observed among natural isolates (Figure 1)? One example is the variability of the stationary-phase-specific MRS downregulation, which has been observed in two different *E. coli* strains [16] and might be a consequence of the high variability of *rpoS* alleles observed in natural bacterial populations [17]. *rpoS* polymorphism results in different capacities of stress resistance and therefore potentially also in mutation rate variability. Because protection and repair processes are costly in terms of energy, if the ecological niche is only

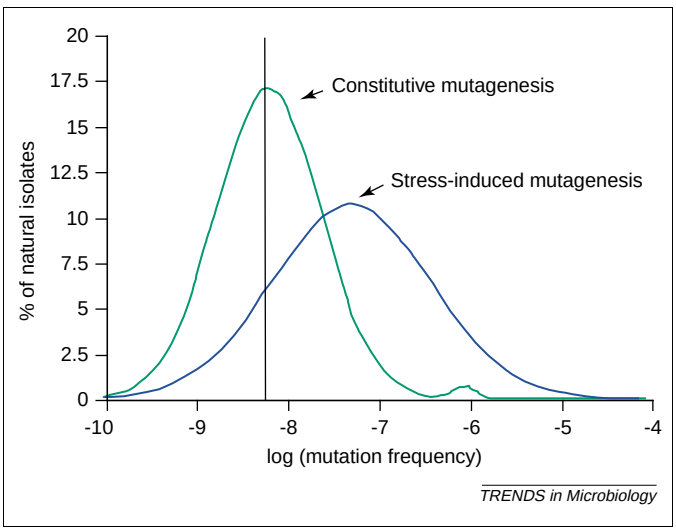

Figure 1. Polymorphism of mutation rates among *Escherichia coli* natural isolates. The green line represents the distribution of constitutive mutation rates, whereas the blue line represents the distribution of stress-induced mutation rates. Mutator phenotypes have been estimated by measuring the capacity of different strains to generate mutations conferring resistance to rifampicin in one-day-old colonies (for constitutive mutagenesis) and seven-day-old colonies (for stress-induced mutagenesis). The mutagenesis was measured using a collection of 800 natural isolates [7]. The median of constitutive mutagenesis (5.8×10^{-8}) is presented as a vertical black line.

mildly stressful then such energy investment could be of negligible benefit or even deleterious (Figure 2). Therefore, a high level of protection and repair could be counter-selected, resulting in higher cell vulnerability to new environmental stresses. Consequently, the observed polymorphism in mutation rate could reflect the modulation of the protection and repair functions to optimize survival strategies within different ecological niches (Figure 2).

SIM is selected for by the mutations it produces: the second-order selection hypothesis

Experimental, theoretical and ecological studies of bacterial constitutive mutation rates have shown that a higher mutation rate can be selected for during adaptation [18–20]. Despite the fact that a majority of mutations are deleterious, a small fraction of beneficial mutations can lead to the selection of the constitutive mutator allele that has generated them [21–30] through a process known as second-order selection [18] (Box 3). Can SIM be selected for through second-order selection? Computer simulations show that as long as the stresses are frequent or if periods of stress last long enough, the selection of stress-induced mutator systems will be almost as efficient as the selection of constitutive mutator alleles [7] (Figure 3). Under this selection, both the stress-induced mutator and the constitutive mutator evolve to have the same average phenotypic effect.

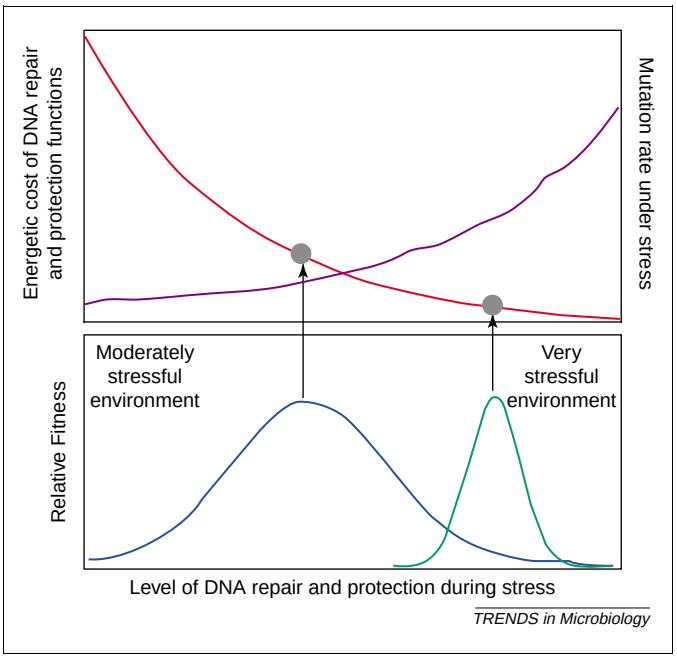

Figure 2. Selection of different mutation rates under the trade-off model of pleiotropic hypothesis. DNA is an important target of damaging agents during stress, and maintenance of the genome requires both protection and repair mechanisms. Higher levels of DNA repair and protection are associated with higher energetic costs (purple line) and a lower mutation rate under stress (red line). In a very stressful environment the physical integrity of the DNA molecule is endangered, hence increased protection and repair are necessary for survival. As a consequence of the selection based on survival, a population will evolve towards low mutation rates (green line). In a moderately stressful environment, only the integrity of the informational content of the DNA molecule is hindered. In the balance between the long-term effects of the production of deleterious mutations and the direct effect of the energetic cost associated with repair and protection, it is probable that direct effect will predominate in the selection regime. Therefore, a less costly repair and protection system will be selected even if it leads to an increased mutation rate (blue line). Different environments can therefore lead to different mutation rates and explain the diversity of stress-induced mutation rates observed among natural isolates.

Do we have any evidence that second-order selection plays a role in shaping the observed mutation rate polymorphism (Figure 1)? Some indirect evidence might reside in the negative correlation observed between constitutive mutation rates and SIM among natural isolates [7]. Simulations suggest that once one mutator allele is selected within a population, it limits the selection of others. Therefore, the existence of high SIM is expected to be associated with lower constitutive mutagenesis and vice versa, as has been observed among natural isolates.

How do the molecular mechanisms involved in SIM fit this second-order selection hypothesis? The downregulation of the repair system or cryptic polymorphism within the repair genes that are revealed under stress could be selected as mechanisms inducing mutation rate [31,32]. Similarly, the error-prone nature of SOS polymerases could be the result of selection rather than constraint. The fact that different SOS polymerases have different error rates could reflect the fact that some polymerases have been selected to produce mutations whereas others have not.

What are the benefits and drawbacks of these constitutive and stress-inducible mutator alleles? If stresses are recurrent, stress-induced mutators can be selected during the process of long-term adaptation. During periods of stress, stress-induced mutator alleles over-produce mutations that can be selected at a later date. Computer simulations have shown that under these conditions the selection acting on stress-induced mutator alleles is very similar to the selection acting on an equivalent constitutive mutator allele, such as a constitutive mutator allele that produces the same number of mutations as the stress-induced mutator allele, averaged over stress and non-stress periods [7] (Figure 3). However, because the production of stress-induced mutations is restricted to stress periods, SIM alleles produce more mutations within these periods than their equivalent constitutive mutator allele; therefore, beneficial mutations have a greater probability of being lost in genomes loaded by deleterious mutations [2,33]. In addition, the appearance of stress-induced mutations depends on the existence of the stress period; therefore, constitutive mutator alleles have an advantage over stress-induced mutator alleles because there is a higher probability that they will produce the beneficial mutations earlier. If the selection of stress-induced mutator alleles is less efficient than that acting on constitutive mutators, it would be more difficult to explain the much larger fraction of stress-induced mutators compared with constitutive mutators. Notably, only MRS-deficient strains have been found as constitutive mutators among natural isolates [34,35], whereas several genetic pathways have been found to be involved in starvation-induced mutagenesis [7,8,36]. Therefore, the higher frequency of stress-induced mutators could be explained by the greater probability to generate stress-induced mutator alleles by mutation, as several pathways can be involved in SIM.

The second-order selection hypotheses can also explain the variation in SIM according to the life-style of different natural isolates. Different *E. coli* ecotypes are subject to

Box 3. Second-order selection acting on constitutive mutation rates

Any allele that modifies the mutation rate can be subject to indirect selection due to the mutations it produces. The following two-allele two-locus model explains this concept (Figure I). At the locus under primary selection, allele A (with fitness $1 + s$) is favored over allele a (with fitness 1). The other locus is under secondary selection and influences the mutation rate at the previous locus: individuals having allele m mutate from a to A with rate μ, whereas individuals with allele M (a mutator allele) mutate from a to A at an increased rate μ_X. This locus has no direct effect on fitness. In such a system, if a population is composed of half (m, a) and half (M, a) at generation 0, after the process of mutation, $0.5 \times \mu$ individuals have genotype (m, A) and $0.5 \times \mu \times X$ genotype (M, A). There is therefore linkage disequilibrium. After the selection process, the global ratio of M over m alleles has increased from 1 to $(1 + \mu \times X \times s)/(1 + \mu \times s) > 1$ as a result of the linkage disequilibrium between M and A. Because of its higher mutation rate, M has increased in frequency. Consequently, this mutator allele has hitchhiked with the allele A to higher frequency.

In this case, the selection of mutator alleles depends on the mutations that they generate. As most mutations are neutral or deleterious, mutator alleles should be strongly counter-selected; however, computer simulations and some mathematical models have shown that a tiny fraction (fewer than 1 in 10^4) of beneficial mutations is enough to allow selection for, and in some cases the fixation of, mutator alleles [25–28,52–54].

Because mutator allele selection is possible only if the mutator subpopulation is large enough to generate some beneficial mutations, such selection depends on the population size and on the rate at which mutator alleles appear [25,28]. Strong effect beneficial mutations allow the selection of strong mutator alleles, the cost of which in terms of deleterious mutations would otherwise be too high. As mutator alleles are selected for because they produce more beneficial mutations than the wild-type population, mutator allele selection is also affected by the ability of the population to produce beneficial mutations. For example, recombination, which accelerates the generation of combinations of favorable alleles, limits the selection of mutator alleles [55]. Similarly, the existence of other systems producing mutations, such as stress-induced mutator alleles, reduces selection of constitutive mutator alleles [7].

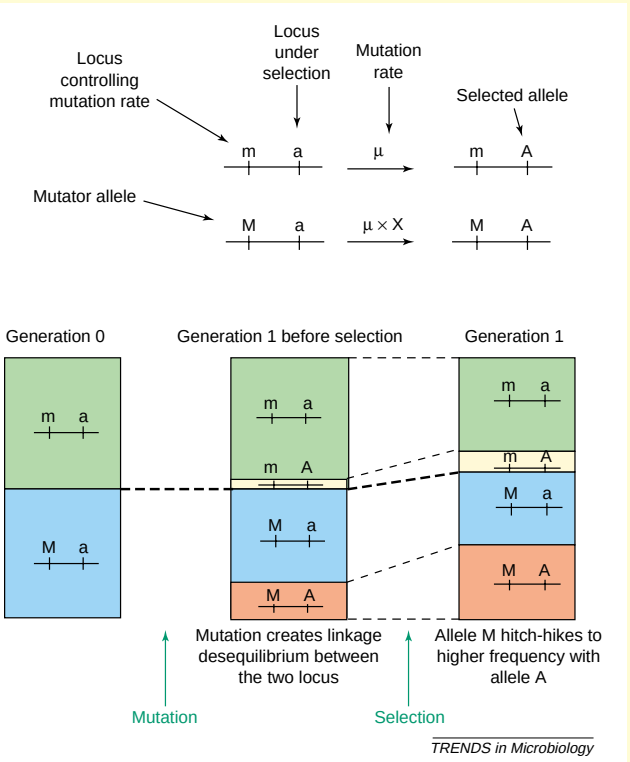

Figure I. An explanation for the concept that any allele that modifies the mutation rate can be subject to indirect selection as a result of the mutations it produces.

different stresses, each having a different nature, strength and frequency. Computer simulations show that both the intensity of selection [25] and the frequency of stresses influence which different stress-induced mutator allele (of different mutator strength) will be selected for (Figure 3). Strong selection and a low frequency of stresses favor strong stress-induced mutators. Hence, the observed variability of SIM could reflect local environmental variations that result in the selection of different strengths of stress-induced mutators.

If second-order selection can rapidly select for mutator alleles, it is important to emphasize that such selection does not necessary mean that the optimal mutation rate is selected. A strong increase in the mutation rate can be selected for with a few beneficial mutations, even if many deleterious mutations are also produced. The price of the production of these deleterious mutations is paid as soon as the supply of beneficial mutation is limiting [26]; high mutator strains are then rapidly counter-selected [37]. Taking this into account, the polymorphism observed in SIM and in constitutive mutators (Figure 1) should be viewed as a snapshot of a dynamic process in which strong constitutive and stress-induced mutators appear and disappear. As the selection or counter-selection for small-effect mutators alleles occurs over much longer periods [26], these mutator alleles might have a greater effect on bacterial evolution; this is supported by the correlation observed between small increases in mutation rate and levels of resistance to antibiotics [38,39].

Concluding remarks

Recent progress on understanding SIM from both a mechanistic and evolutionary perspective suggests that both hypotheses proposed to explain the appearance, maintenance and distribution of SIM phenotypes among natural isolates might be valid (Figures 2 and 3). The nature (activity and regulation) of molecular mechanisms that are involved in SIM is in agreement with the pleiotropic hypothesis: SIM could just be a by-product of selection for improved survival, either due to constraints at the molecular level or a trade-off between survival functions and the costs of repair and protection functions. Simulation models show that stress-induced mutator phenotypes can be selected for in the same way as constitutive mutator alleles are selected for (Box 3; Figure 3), which is in agreement with the second-order selection hypothesis. However, current models do not take into account the potential constraints and pleiotropic effects that are associated with an increased mutation rate, and therefore cannot stipulate that second-order selection is the only selective pressure acting on stress-induced mutator alleles. In conclusion, SIM phenomena can be explained by both hypotheses, and to date, there are no strong arguments to reject either. It is probable that

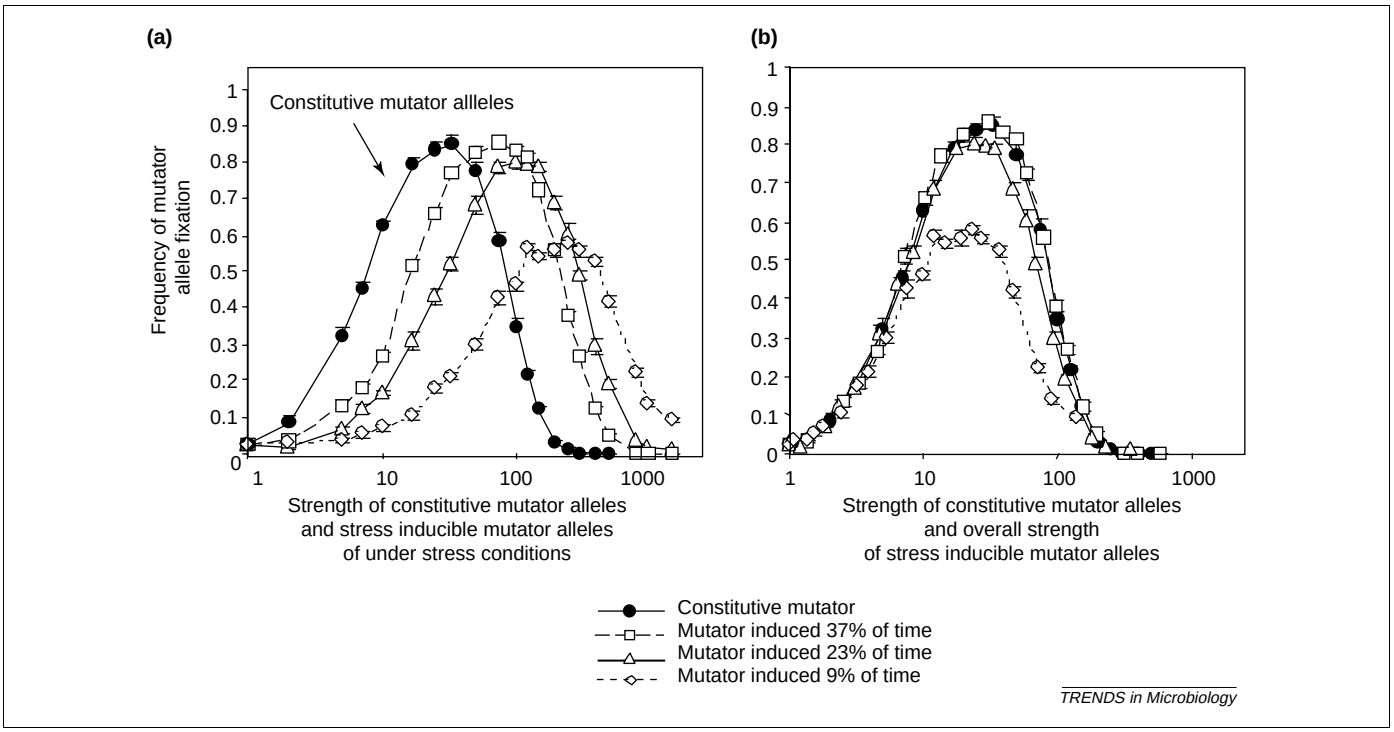

Figure 3. Selection of different mutation rates under the second-order selection hypothesis. Simulation models show that stress-induced mutagenesis (SIM) can be selected for in long-term adaptation. **(a)** The fraction of mutator alleles that are fixed in the population is presented as a function of mutator effects (filled circles correspond to the constitutive mutator; open symbols correspond to stress-induced mutators in environments in which stress occurs). For a given intensity of selection, for all type of mutators, intermediate phenotypes are selected most frequently. Stronger effects on mutation rates are selected for among stress-induced mutators. Moreover, the mutator strength of stress-induced mutator alleles selected depends on the frequency of stresses. When stresses are rare (open diamonds), occurring 9% of the time, stronger stress-induced mutator alleles are selected. Therefore, different frequencies of stresses in the different niches can result in a wide distribution of stress-induced mutator alleles among natural isolates. **(b)** The fraction of mutator alleles that become fixed is presented as a function of the effect of the alleles on mutation rate averaged over stress and non-stress periods. The global mutation rate selected is the same for both constitutive and inducible mutators, but when stresses are rare, stress-induced mutators are less efficiently selected than constitutive mutators.

both scenarios act simultaneously but to a different extent in shaping SIM in nature.

Independent of the nature of the selective pressure that acts to shape the distribution of SIM, mutations produced by stress-induced mutator alleles can represent a large fraction of the mutations produced by a clone during its evolution. Even a very modest control of constitutive mutation rates can significantly influence bacterial evolution, as was shown for the evolution of resistance to antibiotics in populations of *E. coli* strains that were isolated during urinary tract infections [38]. Therefore, because SIM varies among natural isolates to a much greater extent than constitutive mutation rates it is expected that it would have a greater impact on bacterial evolution.

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