OPINION

Evaluating evolutionary models of stress-induced mutagenesis in bacteria

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Abstract | Increased mutation rates under stress allow bacterial populations to adapt rapidly to stressors, including antibiotics. Here we evaluate existing models for the evolution of stress-induced mutagenesis and present a new model arguing that it evolves as a result of a complex interplay between direct selection for increased stress tolerance, second-order selection for increased evolvability and genetic drift. Further progress in our understanding of the evolutionary biology of stress and mutagenesis will require a more detailed understanding both of the patterns of stress encountered by bacteria in nature and of the mutations that are produced under stress.

It has been argued that exposure to stress is associated with increased mutation rates in a wide variety of eukaryotes, bacteria and archaea[1–7]. In eukaryotes, the underlying mechanistic causes of this phenomenon remain obscure. In microorganisms, positive associations between stress and mutation have been attributed to two distinct classes of mechanism. First, stress can lead to a direct increase in the mutation rate: for example, this can occur when toxic molecules such as reactive oxygen and alkylating agents directly damage DNA or inhibit enzymes that are involved in maintaining a high fidelity of DNA replication and repair[8]. In this article, we refer to this first mechanism as stress-associated mutagenesis (SAM), because the relationship between stress and mutagenesis is purely coincidental: that is, there is no a priori reason why exposure to stress should lead to an elevated mutation rate. By contrast, stress could indirectly increase mutation rates when exposure to stress results in changes in the expression of genes that elevate the mutation rate by, for example, inducing the expression of error-prone DNA polymerases[9–13] or by repressing the expression of genes involved in the repair of DNA[9].

We refer to this phenomenon as stress-induced mutagenesis (SIM), because it reflects mutagenesis that is induced, but not directly caused, by stress. The term SIM has frequently been used in the literature but often without critical evaluation. Our use of terminology emphasizes the distinction between mechanisms in which there is and in which there is not clear evidence that stress, per se, results in altered mutation rates. The juxtaposition of the terms is novel in its emphasis and is key to our Perspective.

> “Mechanisms that couple stress to an elevated mutation rate have been investigated in detail, but less attention has been given to understanding how natural selection acts on genes involved in SIM”

We define a stressor as any environmental variable that leads to a decrease of bacterial growth rate or competitive ability, such as elevated temperature, exposure to toxins or unfavourable pH.

Experiments conducted using several different bacteria, stress-response mechanisms and stressors have reported results that are consistent with the existence of SIM (BOX 1). It is important to point out, however, that the existence of SIM is controversial, and it has been argued that mechanisms other than SIM could explain results such as those presented in BOX 1 (for example, see REF. 14). Although we emphasize the importance of using appropriate experimental designs and mutation rate estimation to test for SIM (BOX 1), the premise of this article is that SIM occurs.

Mechanisms that couple stress to an elevated mutation rate have been investigated in detail, but less attention has been given to understanding how natural selection acts on genes involved in SIM, even though SIM could have a crucial role in accelerating adaptation by natural selection to environmental stressors, including antibiotics[15–19]. For example, the SOS pathway, which is associated with the expression of error-prone polymerases[19–22], increases the ability of both *Escherichia coli* and *Mycobacterium tuberculosis* to evolve resistance to antibiotics in mice[23,24]. Although it is intuitively reasonable that the proximate consequence of these increased mutation rates is an increase in evolvability under stressful conditions, it does not follow that the ultimate evolutionary cause of SIM is selection for increased mutagenesis[25]. Because most new mutations are deleterious, even under stressful conditions[26,27], it is challenging to explain the evolution of any mechanism that leads to an elevation of the mutation rate[25,28]. Two main explanations have been put forward to explain SIM[10,11,14,18,29]. First, it has been argued that an increase in the mutation rate is the unavoidable price that bacteria pay for mechanisms that increase survival under stress. More controversially, it has been argued that SIM exists because selection favours the evolution of mechanisms that couple the evolutionary demand for novelty imposed by stress to the rate of production of novelty provided by mutation. We review and evaluate experimental tests of these hypotheses in a wide range of bacterial model systems and then introduce a novel hypothesis based on

genetic drift to explain SIM, and we show that comparative evidence supports the drift hypothesis. Although it is convenient to discuss each of the different proposed mechanisms of SIM, we emphasize that they are not mutually exclusive. Rather, we envisage an interplay for which the complexity is reflected in the variety and different time frames of the interactions between bacteria and environmental stressors.

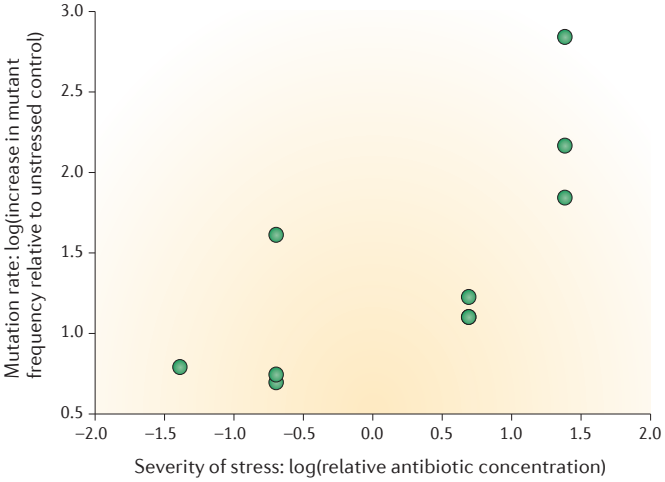
The trade-off hypothesis

The simplest explanation for the origin of SIM is that natural selection favours genes that increase fitness under stressful conditions by, for example, increasing survival

Box 1 | **Measuring stress-induced mutagenesis**

In a seminal paper published in 1943, Luria and Delbrück[66] described a simple experimental method, known as a fluctuation test, for estimating bacterial mutation rates that is based on scoring the presence or absence of mutants with an easily selectable phenotype, such as antibiotic or phage resistance, in bacterial populations. In a fluctuation test, a bacterial strain is used to set up numerous independent cultures that have a low enough population density that they do not initially contain any of the mutations of interest. After some period of growth, the number of resistant mutants in a sample of *N* cells is then determined for each culture. Because the number of mutants per culture follows a Poisson distribution, Luria and Delbrück[66] argued that mutation rate (μ) per cell division can be estimated as $\mu = (-\ln(p(0)) / N)$, where $p(0)$ is the proportion of mutant free cultures, and *N* is the number of cells sampled from each culture. The downside of this method is that large numbers of replicate cultures are required to estimate the mutation rate, especially if $p(0)$ is close to 0 or 1. Maximum-likelihood-based methods have been developed to use data on the frequency distribution of mutants per culture, instead of just the proportion of mutant-free cultures, to estimate the mutation rate[59,67,68]. Unfortunately, many papers estimate changes in the mutation rate by simply estimating changes in the mean or median mutant frequency; this method provides a poor estimate of the mutation rate[59,68].

The ideal experiment to test for stress-induced mutagenesis using a fluctuation test is as follows. First, it is necessary to measure the mutation rates in the presence and absence of a specific stressor to test the hypothesis that stress increases the mutation rate. Second, it is necessary to measure the mutation rate of both a wild-type strain and a mutant lacking a gene that is thought to be involved in stress-induced mutagenesis (SIM); without this crucial control, it is not possible to distinguish between SIM and stress-associated mutagenesis (SAM). It is also important to use a selectable marker phenotype that is selectively neutral in both the presence and the absence of the stressor being investigated. If the marker being used to estimate mutation rates is subject to selection, then changes in marker frequency that are used to infer differences in the mutation rate will be driven by both selection and mutation[58]. Examples of studies that have used this approach are given in the table.



These studies show that the increase in mutation rate associated with SIM can show profound variation. Although the causes of this variation are not fully understood, one factor that is likely to have a key role in determining the extent of SIM is the severity of stress, as demonstrated by the fact that the increase in mutation rate associated with SIM on exposure to antibiotics in *Escherichia coli* strongly correlates with antibiotic dose, as shown in the figure. A study[69] measured changes in the frequency of rifampicin-resistant mutants when populations of *E. coli* were exposed to ten different antibiotics (not including rifampicin) that were administered at different relative doses. Across antibiotics, the increase in mutant frequency strongly correlates with the strength of antibiotic-imposed stress (correlation coefficient (r^2) = 0.52; $F_{1,8}$ = 8.7; P = 0.02). A *recA* mutant did not show an increase in mutation frequency in response to any of these treatments, demonstrating that stress-induced mutagenesis is driven by the SOS pathway, which controls the expression of several error-prone polymerases in *E. coli*. Data in the figure are from REF. 72.

Bacterial species	Increase in mutation frequency associated with SIM	Stress factor	Reporter system	Tested SIM mechanism	Refs
Bacillus subtilis JJS149	5.4×	UV irradiation (40 J m^{-2} or 60 J m^{-2})	Rif resistance (10 μg mL^{-1})	YqjW (Pol Y2 homologue)	70
Caulobacter crescentus NA1000	3×	UV irradiation (90 J m^{-2})	Rif resistance (100 μg mL^{-1})	ImuA and ImuB; DnaE2	71
B. subtilis YB955	6.5×	UV irradiation (83 W m^{-2})	His+ revertants	Pol IV (DinB homologue; also known as YgjH)	72
Mycobacterium smegmatis	10–50× (mutation rate, not frequency)	UV irradiation (70 mJ cm^{-2})	Rif resistance (2 μg mL^{-1})	SOS	24
Staphylococcus aureus 8325	29.6×	UV irradiation (8.6 J m^{-2})	Rif resistance (100 μg mL^{-1})	SOS	20
S. aureus 8325	11.7×	UV irradiation (8.6 J m^{-2})	Strep resistance (500 μg mL^{-1})		20
E. coli K-12 ME12	2.0–17.1×	Ten different antibiotics	Rif resistance (100 μg mL^{-1})	SOS	69
E. coli K-12 ME12	2.0–7.9×	Eight different antibiotics	Fos resistance (10 mg L^{-1})	SOS	69

Fos, fosfomycin; His+ revertants, histidine-positive revertants (that is, bacteria carrying mutations that overcome histidine deficiency); Pol, DNA polymerase; Rif, rifampicin; Strep, streptomycin; UV, ultraviolet; SOS, SOS response to DNA damage.

rate or competitive ability. According to this hypothesis, an elevated mutation rate is simply the unavoidable, pleiotropic by-product of the mechanisms that directly improve fitness under stress. The key prediction of this trade-off hypothesis is that genes involved in SIM carry a fitness benefit under stress that can be directly linked to their mutagenic activity.

Perhaps the best support for the trade-off hypothesis comes from the repression of DNA repair in starving, stationary-phase populations of *E. coli*. When *E. coli* enters stationary phase, it downregulates the expression of *mutS*, which encodes a key component of the methyl-directed mismatch repair pathway (MMR pathway) that produces a strong mutator phenotype when deleted (namely, a ~100-fold increase in mutation rate)[30]. The survivorship of *E. coli* strains that downregulated *mutS* expression in stationary phase was compared with a mutant strain carrying *mutS* under the control of an inducible promoter so that it continued to be expressed at levels similar to those found in exponentially growing populations[9]. Overexpression of *mutS* in stationary phase led to a dramatic decrease in survivorship, directly demonstrating a pleiotropic trade-off between survival and mutagenesis under stress. Further evidence to support this conclusion comes from the fact that a second gene involved in the MMR pathway, called *mutL*, which is not normally downregulated during stationary phase, does not cause any decline in cell viability when overexpressed in stationary phase[9].

One general response to DNA damage is that bacteria upregulate the expression of alternative, low-fidelity DNA polymerases that carry out *trans*-lesion synthesis of damaged DNA[13,29]. These polymerases include 'Y-family' polymerases[13,31], such as the highly mutagenic DNA polymerase (Pol) IV (encoded by *dinB*)[22], and DnaE2, which functions as an alternative catalytic subunit of DNA Pol III[21,24,32]. Structural studies show that alternative DNA polymerases have fairly open active sites, and it has been argued that this allows *trans*-lesion synthesis polymerases to increase survival under stress by allowing bacteria to accommodate bulky DNA lesions at a cost of an increased mutation rate owing to a reduction in the strength of geometric constraints that favour correct Watson–Crick base pairs in essential polymerases, such as Pol III[33,34]. Consistent with this idea, it has been shown that mutants lacking functional Y-family polymerases and DnaE2 show decreased survival under stress in a wide range of bacteria[29,35].

In summary, available evidence shows that genes involved in SIM can generate a direct fitness benefit under stress. This finding is hardly surprising, given that many genes that are induced by stress (including DNA damage) have a clear role in providing protection against stress[12]. From a theoretical perspective, the existence of a trade-off between fitness and mutagenesis under stress is sufficient to explain the evolution of SIM, and the trade-off hypothesis provides a useful null model against which more complex models of SIM evolution can be compared.

The evolvability hypothesis

A more controversial explanation for the origin of SIM is that natural selection favours the evolution of mechanisms that couple the demand for innovation imposed by stress to the supply of novelty provided by mutation: the evolvability hypothesis. Although most new mutations are deleterious, bacterial populations clearly have an incredible potential to adapt to stress by fixing novel mutations that increase stress resistance. Theoretical models of mutation rate evolution show that when beneficial mutations are potentially available to natural selection, any gene that increases the mutation rate — for example, a gene that causes constitutive[25,28,36] or stress-induced[15,17] increases in the mutation rate — has an increased likelihood of generating a beneficial mutation. Providing that the recombination rate is low enough for the mutator allele to remain linked to the beneficial mutation, positive selection then results in the fixation of both the mutator allele and the beneficial mutation; this hitch-hiking effect is often called second-order selection. For example, several studies have shown that mutations that lead to a constitutive increase in the mutation rate, often as a result of defects in the MMR pathway, become fixed when bacterial populations adapt to constant environments as a result of second-order selection[37–40]. Although no studies that we are aware of have demonstrated the *de novo* evolution of stress-induced mutagenesis, two studies have reported results that provide direct evidence that this stress can generate efficient second-order selection for SIM.

In a pioneering study of the evolutionary biology of SIM, Yeiser and colleagues[41] challenged populations of *E. coli* lacking alternative, error-prone polymerases and a wild-type control population with long-term starvation. Under these conditions, it is known that *E. coli* populations evolve as a result of successive selective sweeps of beneficial mutations that allow for continued growth after the onset of stationary phase (known as GASP mutants)[42]. Pure cultures of polymerase mutants showed population dynamics that were similar to those of the wild type, suggesting that error-prone polymerases do not provide any direct fitness benefit under these experimental conditions. However, when polymerase mutants were placed in the same culture as a wild-type strain, the wild-type strain eventually replaced all of the mutants, suggesting that the wild-type strain eventually prevailed as a result of second-order selection. Using a model of *E. coli* infection in mouse thighs, it was found that the SOS response, which includes the expression of genes involved in DNA repair and of error-prone translesion polymerases, does not provide any increase in survival in the presence of ciprofloxacin or rifampicin (which are antibiotics that are potent inducers of the SOS response)[23]. However, this study found that the SOS response was required for the evolution of antibiotic resistance *in vivo*[23]. In this example, the SOS response is associated with an important indirect benefit in terms of increased evolvability in a scenario in which this pathway does not provide any apparent direct benefit.

Both of these experimental studies report that SIM is not associated with any direct fitness benefits, but it does not follow that second-order selection is necessary to explain the evolution of the mechanisms of SIM investigated in these studies. First, both of these studies tested for direct benefits associated with SIM by measuring the survivorship of pure cultures of isogenic SIM-proficient and SIM-deficient strains under stress. Although survivorship is an important component of fitness, it is not a direct measure of fitness, which would require competition experiments between mixed cultures of SIM-proficient and SIM-deficient strains under stress. Second, the stressors used in these studies (namely, long-term culture in a rich medium and exposure to a synthetic antibiotic) are undoubtedly different from the stressors that bacteria encounter in nature, and it is conceivable that the mechanisms of SIM investigated in these studies evolved as a result of direct selection to stressors encountered in nature.

Further evidence to support the evolvability hypothesis comes from studies

that have demonstrated that some stress-response pathways not classically associated with SIM appear to have co-opted SIM genes that are typically associated with stress-response pathways associated with elevated mutation rates. For example, it was found that the envelope stress response of *E. coli*, which is induced by exposure to β-lactam antibiotics, triggers the expression of the mutagenic SOS pathway by DpiA-mediated blockage of DNA replication[43]. This mechanism of SOS pathway induction does not increase long-term survival in the presence of β-lactams, suggesting that this link between the envelope and SOS stress-response pathways may have evolved to increase mutagenesis in the presence of cell envelope stress. A second example of this phenomenon is provided by the GroE-mediated increase in the activity of the mutagenic translesion polymerase DNA Pol IV (also known as DinB) in *E. coli*[44]. Exposure to heat shock induces a large increase in the expression of GroE, which is an important chaperone protein, and this increases the mutation rate by increasing the stability of the Pol IV protein. Pol IV is highly mutagenic, but it does not have any known role in providing protection against thermal stress, suggesting that GroE may have evolved the ability to stabilize Pol IV as a result of second-order selection.

In summary, numerous studies have reported evidence that is consistent with the hypothesis that second-order selection can favour SIM even in the absence of direct selection for SIM. One important caveat is that none of these studies has considered the influence of homologous recombination: second-order selection can drive the evolution of SIM only if tight linkage exists between the beneficial mutations and genes that are involved in SIM; homologous recombination erodes this linkage, and theoretical models show that modest levels of recombination are sufficient to prevent second-order selection for elevated mutation rates[45]. Rates of homologous recombination in bacteria remain poorly characterized, but it is clear that the rate of recombination relative to mutation widely varies across bacteria[46]. If second-order selection is the main evolutionary driver of SIM, then SIM should be especially prevalent in species with fairly low rates of recombination. It has also been reported that stress can actually lead to an increase in the recombination rate in *E. coli*[47], raising the intriguing possibility that stress-induced increases in recombination rate may limit the efficacy of second-order selection for stress-induced mutagenesis. Future theoretical and experimental work in this area should investigate the links between SIM and recombination.

The drift hypothesis

The stress-response literature shows that the most widespread mechanism of SIM is the stress-induced expression of alternative, low-fidelity DNA polymerases. All else being equal, should we expect polymerases that are expressed only at high levels under stress to copy DNA with a fidelity as high as that of polymerases that are expressed under normal conditions of growth?

> “because stress is the exception rather than the norm, any genes that are specialized for replicating and repairing DNA under stress will be subject to less effective selection to function as efficient enzymes”

Because most spontaneous mutations are deleterious, selection favours mechanisms that result in a high accuracy of DNA replication and repair. We argue that because stress is the exception rather than the norm, any genes that are specialized for replicating and repairing DNA under stress will be subject to less effective selection to function as efficient enzymes[48], and this will ultimately result in an elevation of the mutation rate under stress; we call this the drift hypothesis. For example, a mutation that doubles the mutation rate will incur a much greater cost when it occurs in a polymerase that is used to replicate DNA regularly than it will incur when it occurs in a polymerase that is used to replicate DNA only 1% of the time. One important limitation of this hypothesis is that it can only explain how rarely used mechanisms of SIM degenerate over time and cannot explain the evolutionary origin of mechanisms that specifically replicate and repair DNA under stressful conditions.

To test the drift hypothesis, we carried out two basic tests of the efficacy of selection that acts on two polymerases — DnaE and DnaE2 — in bacteria from the genus *Pseudomonas*. DnaE2 is a stress-induced polymerase, and so we compared the efficiency of selection on DnaE2 to DnaE, which carries out the same function in the absence of stress. Like many other rapidly growing bacteria, *Pseudomonas aeruginosa* exhibits strong codon bias, such that highly expressed genes tend to be enriched for a particular subset of codons that is presumably best recognized by the most abundant tRNA species[49]. By comparing the strength of codon bias between DnaE and DnaE2, it is therefore possible to gain some insights into the strength of selection for translational efficiency that operates on the two polymerases. DnaE exhibits an ~8% higher frequency of preferred codons than does DnaE2 (paired-sample $t_{12} = 2.25$; $P = 0.044$) (FIG. 1a). This suggests that selection has more efficiently optimized the expression of DnaE, as would be expected if stress were the exception rather than the norm in this species. As a second test of the efficacy of selection on DnaE and DnaE2, the relative rate of nonsynonymous to synonymous mutations (dN / dS) was measured across *Pseudomonas* spp. (see Supplementary information S1 (box) for further details). The underlying rationale of this test is that synonymous mutations are subject to weak selection relative to nonsynonymous mutations, implying that measuring the rate of fixation of nonsynonymous mutations (dN) relative to synonymous mutations (dS) can provide a measure of how selection acts on nonsynonymous mutations. If dN / dS is equal to 1, nonsynonymous mutations fix at the same rate as synonymous mutations, implying that selection on nonsynonymous mutations is weak. Conversely, dN / dS values that are <1 are indicative of a tendency towards negative selection against amino-acid-changing mutations. Values of dN / dS for DnaE2 are, on average, close to 1 (mean = 0.87; standard error (s.e.) = 0.07; $t_5 = 1.82$; $P = 0.13$), implying weak selection on amino acid substitutions for this protein. By contrast, values of dN / dS for DnaE are much smaller (mean = 0.05; s.e. = 0.01) than those for DnaE2 (mean difference = 0.81; $t_5 = 12.9$; $P < 0.0001$), implying strong purifying selection against nonsynonymous mutations in this protein (FIG. 1b).

In summary, simple tests of the efficacy of selection on the catalytic component of DNA Pol III (namely, DnaE) and its stress-induced counterpart (namely, DnaE2) show that the efficacy of selection on the stress-induced polymerase is fairly low, suggesting that genetic drift is likely to have a role in the evolution of SIM. However, it remains an open question the extent to which the changes in mutation rate caused by the expression of stress-induced polymerases (see the figure in BOX 1) can be

accounted for by differences in the strength of purifying selection that acts on canonical and stress-induced polymerases. For this reason, future work should focus on testing whether patterns of variation in the strength of SIM are consistent with variation in the relative strength of purifying selection that acts on stress-induced polymerases. For example, if drift is an important driver of SIM, then the increase in mutation rate caused by SIM should be greatest in species with a low effective population size; this prediction could be tested by comparing the strength of SIM in species that undergo regular population bottlenecks, such as endosymbionts and obligate pathogens, relative to species that are free-living. A second prediction of the hypothesis is that the expression rate of stress-induced polymerases should negatively correlate with their mutation rate; this could be tested by comparing the strength of SIM associated with polymerases that are regulated by multiple stress-response pathways relative to more highly specialized alternative polymerases[10,13].

Conclusions

Environments vary across space and time, implying that positive selection acting on novel beneficial mutations is required for populations to remain well-adapted to their environment. This ubiquity of positive selection, combined with the fitness costs associated with accurately replicating DNA[50], ensures that the selection favours a mutation rate that is greater than zero, even though most mutations are deleterious[25,28]. When should organisms increase the mutation rate? Optimization arguments suggest that one possible answer is to couple the rate of appearance of evolutionary novelty provided by mutation to the demand for evolutionary innovation imposed by environmental stress. Widespread SIM in bacteria (see the table in BOX 1) implies that this strategy is the norm rather than the exception in this domain of life, and the evolutionary consequence of SIM is that bacteria are able to adapt rapidly to stressful environments, including those that contain clinical doses of antibiotics. Eukaryotes ranging from yeast to humans have also been found to carry alternative error-prone polymerases[13], suggesting that eukaryotes may also engage in SIM. For example, several recent studies in *Drosophila melanogaster* have shown that mutation rates are higher in poor-quality individuals[51,52], which is consistent with the idea that stress induces increases in the mutation rate.

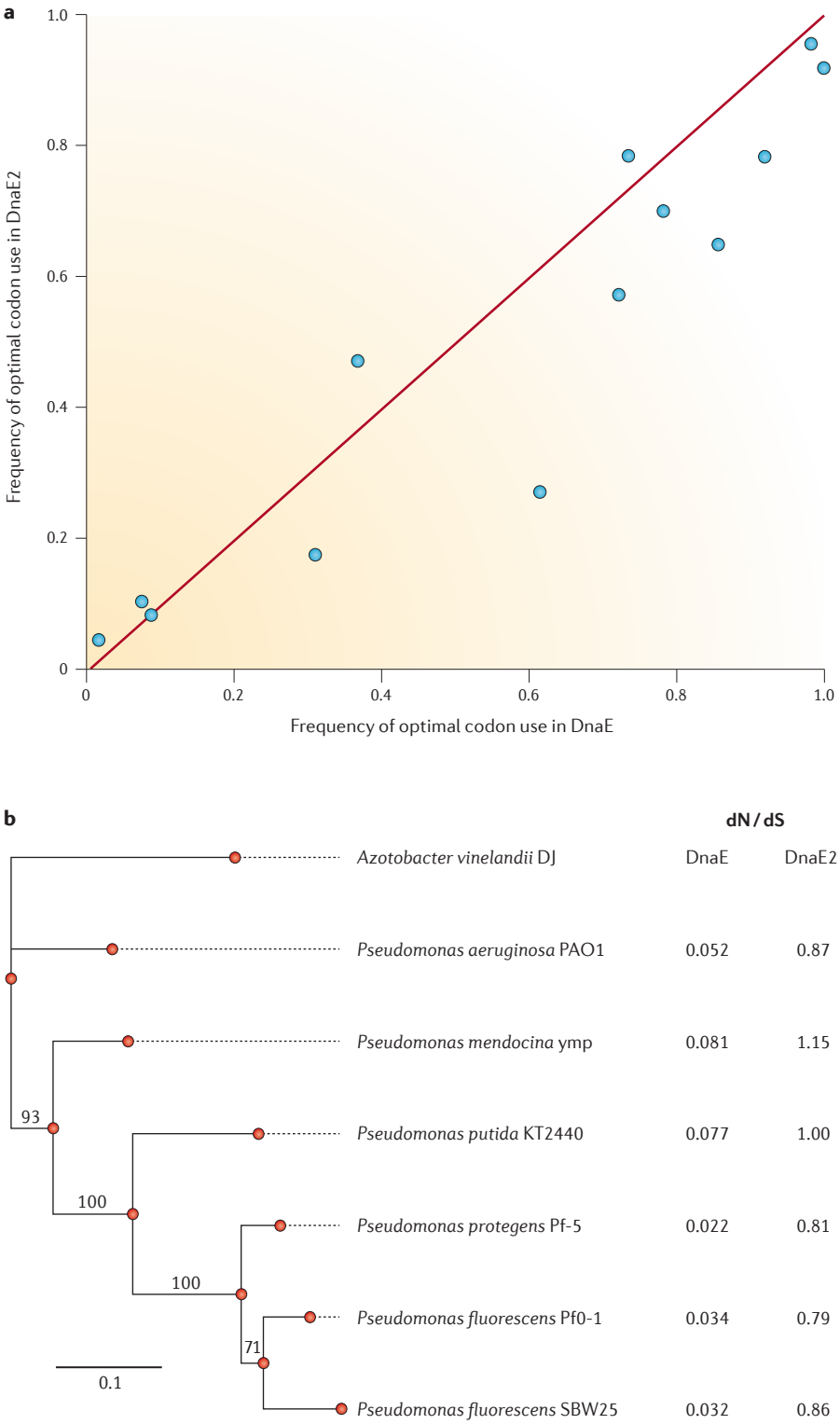


Figure 1 | **Tests of the drift hypothesis in *Pseudomonas* spp.** **a** | Each point shows the frequency of preferred codons for DnaE and DnaE2 in *Pseudomonas aeruginosa* PAO1 for an amino acid with a preferred codon. Preferred codons were taken from REF. 73. **b** | Shows the rate of molecular evolution of DnaE and DnaE2 measured as the rate of nonsynonymous substitution (dN) to synonymous substitution (dS) across *Pseudomonas* spp. We reconstructed the phylogeny of *Pseudomonas* spp. using the nucleotide sequence of *rpoB*: numbers indicate the percentage of bootstrap replicates ($n = 100$) that support each branch on the phylogeny, and the scale bar shows the number of substitutions per site. Further details of this test are available in Supplementary information S1 (table).

One of the central themes that we have discussed is that even if the proximate consequence of SIM is increased evolvability, it does not follow that the ultimate evolutionary cause of SIM is second-order selection for increased evolvability. Instead, we have argued that the evolution of SIM can also be explained by: direct selection for increased survival or competitive ability under stress, coupled with a trade-off between stress resistance and mutation rate; or by neutral genetic drift as a result of the reduced efficacy of selection on mechanisms that repair and replicate DNA under conditions of rare stress. Throughout this article, we have separately discussed these three hypotheses, but it is important to emphasize that there does not need to be a single and universally applicable explanation for the evolution of SIM. Instead, the existing literature suggests that it is possible that all three of these mechanisms apply but that different evolutionary forces drive SIM under different conditions. One factor that is likely to have a key role in determining the relative importance of selection and drift is time. Exposure to stress will immediately generate direct selection for SIM if it increases survival on a physiological timescale of <1 generation. Continued exposure to stress (that is, >1 generation) will result in selection for novel stress-resistance mechanisms, creating second-order selection for SIM. Finally, over long evolutionary timescales (that is, thousands to millions of generations), an absence of stress will result in the stochastic decay of stress-specific mechanisms of DNA replication and repair.

In our opinion, progress in our understanding of the evolutionary biology of SIM will require a much more detailed understanding of patterns of stress and the mutations that are induced by stress. Research on stress in bacteria has heavily focused on the genetics and biochemistry of stress responses, resulting in a vast and detailed literature but, paradoxically, the nature of stress under natural conditions remains largely unknown. For example, the microbial stress literature does not provide answers to simple questions, such as "is stress rare or common?" or "how severe are bouts of stress?" We argue that we cannot hope to explain the contributions of selection and drift to the evolution of SIM without answers to these questions. The literature on antimicrobial pharmacodynamics[53] provides some excellent examples of spatial and temporal variation in the intensity of stress in a clinical context, but the clinical use of antibiotics represents a recent and extreme example of stress, whereas stress is an evolutionary pressure that doubtlessly has existed since the dawn of life. Hopefully, researchers will use some of the concepts and techniques that are accessible to clinicians to measure stressors under natural conditions.

A second important line of research will be to determine how SIM affects the rate and range of mutations that are accessible to natural selection. Research on SIM has focused on using simple reporter phenotypes, such as antibiotic resistance or lactose metabolism, to estimate changes in the mutation rate under stress. There are several important limitations associated with this approach. First, this method uses mutations at a small number of sites in a tiny fraction of bacterial populations as a reporter for changes in the genomic mutation rate, but there is growing evidence that the mutation rate shows substantial variation both among individuals in a population[54,55] and across the genome[56,57]. Furthermore, it has been argued that some commonly used phenotypic markers for mutation are systematically biased because of, for example, the occurrence of gene duplications[14] or because the marker is subject to positive selection under conditions of stress[58]. Second, because most mutation rate estimates rely on measuring rare mutations in a small number of populations, this method produces estimates of the mutation rate that are associated with considerable error[59]. Next-generation sequencing technologies now provide researchers with the ability to measure mutation rates using a method that is both unbiased and comprehensive[60,61], and by using this approach it should be possible to investigate an entire range of mutations produced by SIM. The only study to date that has used this approach shows that expression of RulAB, a Y-family stress-induced polymerase, biases the range of mutations produced and that RulAB leads to the greatest increase in mutation rate near the origin of replication[62]. Crucially, this variation in the rate and spectrum of mutations available to selection may have a profound consequence on the rate and fitness effects of beneficial mutations produced as a consequence of SIM, and any such variation will then have an impact on the efficacy of second-order selection for SIM. For example, several researchers have argued that stress targets mutations to regions of the genome that are required for adaptation to stress[14,63–65], and genome-wide estimates of the consequences of SIM will allow this controversial hypothesis[29,61–63] to be tested in a powerful and decisive manner.

Glossary

Endosymbionts
Organisms that obligately live within other host species.

Error-prone DNA polymerases
DNA polymerases that lead to a high mutation rate owing to a low fidelity of DNA replication. Examples of error-prone polymerases include Y-family polymerases, such as DNA polymerase IV and DnaE2, which is an alternative catalytic subunit of DNA polymerase III.

Methyl-directed mismatch repair pathway
(MMR pathway). A highly conserved pathway for repairing errors that occur during DNA replication. The MMR pathway recognizes mismatched base pairs that are the product of mutation, excises DNA containing the mismatch in the newly synthesized daughter strand of DNA and then resynthesizes the excised DNA using the parental strand as a template.

Mutator phenotype
Bacteria that have a constitutively elevated mutation rate often as a result of mutations in genes involved in DNA replication and repair.

Reactive oxygen
Ions or small molecules, including oxygen ions, free radicals and inorganic and organic peroxides, that are highly reactive owing to the presence of unpaired valence shell electrons. Reactive oxygen species are highly toxic owing to their ability to damage proteins, lipids and nucleic acids.

SOS pathway
A bacterial global stress-response pathway that is expressed in response to DNA damage. Different sets of genes are SOS-regulated in different bacterial species, but it appears that expression of this pathway is universally regulated by RecA and LexA and that there is a common core of SOS-regulated genes, which primarily have roles in DNA replication and repair and which are common to all bacteria.

Survivorship
The proportion of individuals that survive exposure to stress.

***Trans*-lesion synthesis**
Occurs when specific DNA polymerases replicate across non-instructive or misinstructive lesions in DNA, such as single- and double-strand breaks. Translesion DNA polymerases can bypass several different types of DNA lesions in a mutagenic translesion process.

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Competing interests statement

The authors declare no competing financial interests.

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