

THE EVOLUTION OF STRESS-INDUCED HYPERMUTATION IN ASEQUAL POPULATIONS

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Received August 17, 2011

Accepted January 3, 2012

Numerous empirical studies show that stress of various kinds induces a state of hypermutation in bacteria via multiple mechanisms, but theoretical treatment of this intriguing phenomenon is lacking. We used deterministic and stochastic models to study the evolution of stress-induced hypermutation in infinite and finite-size populations of bacteria undergoing selection, mutation, and random genetic drift in constant environments and in changing ones. Our results suggest that if beneficial mutations occur, even rarely, then stress-induced hypermutation is advantageous for bacteria at both the individual and the population levels and that it is likely to evolve in populations of bacteria in a wide range of conditions because it is favored by selection. These results imply that mutations are not, as the current view holds, uniformly distributed in populations, but rather that mutations are more common in stressed individuals and populations. Because mutation is the raw material of evolution, these results have a profound impact on broad aspects of evolution and biology.

KEY WORDS: Mathematical models/simulations, population genetics, phenotypic plasticity, mutagenesis, genetic variation, evolvability.

Hypermutation—an increase in the genomic mutation rate—is a surprising phenomenon, as most mutations are deleterious, so mutators (alleles that induce hypermutation) will usually be surrounded by poor genetic backgrounds and experience a decrease in fitness (Sturtevant 1937; Kimura and Maruyama 1966; Funchain et al. 2000; Montanari et al. 2007). Consequently, selection against mutator alleles should drive the mutation rate to its lower limit (Kimura 1967; Tröbner and Piechocki 1984; Liberman and Feldman 1986; Drake 1991; but also see Dawson 1998; Johnson 1999; Lynch 2010). However, mutations can also help individuals escape stress. Theory (Kimura 1967; Leigh 1970, 1973; Ishii et al. 1989; Sniegowski et al. 2000) and evolutionary experiments in vivo (Gibson et al. 1970; Sniegowski et al. 1997; Oliver 2000; Giraud et al. 2001; Loh et al. 2010; Gentile et al. 2011) and in silico (Taddei et al. 1997; Tenaillon et al. 1999; Heo and Shakhnovich 2010) show that in maladapted populations and in changing environments, mutators can increase in frequency when they “hitchhike” with the beneficial mutations they generate, and therefore the mutation rate evolves to a significantly higher level than the one predicted in well-adapted populations

at a mutation-selection balance. Nevertheless, after adaptation is complete and the balance is restored, mutators decrease in frequency due to accumulation of deleterious mutations (Taddei et al. 1997; Sniegowski et al. 1997; Denamur and Matic 2006), thereby restoring the population-wide mutation rate to a lower level.

Most models of the evolution of the mutation rate consider only mutators that constitutively increase mutation rates (reviewed in [Sniegowski et al. 2000], but see below for exceptions). Here we focus on stress-induced mutators (SIMs)—alleles that increase the mutation rate in response to stress and cause individuals that are maladapted to their environment to hypermutate. In a study by Matic and co-workers (Bjedov et al. 2003), 40% out of 787 natural isolates of *Escherichia coli* exhibited at least a 10-fold increase in mutation rates under stress encountered in aging colonies in comparison with only 3% that exhibited an equivalent increase constitutively, that is, independent of stress. Such stress-induced hypermutation in *E. coli* has been documented over the last two decades in numerous studies and some of the regulating genetic pathways involved have been demonstrated

(Hall 1990; Taddei et al. 1995; McKenzie et al. 2000; Rosenberg 2001; Bjedov et al. 2003; Lombardo et al. 2004; Ponder et al. 2005; Foster 2007; Galhardo et al. 2007; Gibson et al. 2010; Shee et al. 2011). This phenomenon has also been studied in other species of bacteria such as *Bacillus subtilis* (Sung and Yasbin 2002; Debora et al. 2010), *Pseudomonas putida* (Kivisaar 2010), *Listeria monocytogenes* (van der Veen et al. 2010), *Staphylococcus aureus* (Cirz et al. 2007), and *Mycobacterium tuberculosis* (Boshoff et al. 2003), and in several eukaryote species: *Saccharomyces cerevisiae* (Hall 1992; Steele and Jinks-Robertson 1992; Heidenreich 2007), *Chlamydomonas reinhardtii* (Goho and Bell 2000), and *Drosophila melanogaster* (Agrawal and Wang 2008). Furthermore, cancer cells experiencing hypoxia enter a hypermutation state in which mutations that lead to tumor progression and resistance to chemotherapeutic drugs are more probable (Huang et al. 2007; Bristow and Hill 2008; Ruan et al. 2009).

There are two explanations for stress-induced hypermutation (Sniegowski and Lenski 1995; Sniegowski et al. 2000; Tenaillon et al. 2004; Denamur and Matic 2006; Saint-Ruf and Matic 2006). (1) The adaptive hypothesis, or second-order selection hypothesis, suggests that hypermutation in times of stress is favored by selection because it increases variation and allows adaptation to stress. (2) The nonadaptive explanation, or pleiotropic hypothesis, claims that hypermutation is an inevitable by-product of stress that is caused by lack of energy and resources that are needed to maintain replication fidelity or by some other causes.

The adaptive explanation can be illustrated in the following way. When bacteria with nonmutator (NM) alleles experience stress, many generations may pass before a beneficial mutation will occur and allow adaptation to the environmental conditions. Constitutive mutators (CMs) are alleles that induce a constant state of hypermutation, causing bacteria to generate mutations at an increased rate. Therefore, bacteria with CMs will generate a beneficial mutation that will lead to adaptation in just a few generations, and because bacteria experience little, if any, recombination, the mutator will “hitchhike” with the beneficial mutation and spread in the population. However, most mutations are deleterious and reduce fitness, and because hypermutation continues after adaptation, more deleterious mutations are generated and the growth rate diminishes, eventually leading to the replacement of the CMs by NM alleles. Bacteria with SIMs will hypermutate when stressed, resulting in rapid adaptation similar to CMs. But unlike CMs, bacteria with SIMs will be able to stay at the adaptive peak once it is reached, because the mutation rate of the adapted individuals will return to a lower level and deleterious mutations will not be overproduced.

We present here a theoretical basis that can explain the evolution of stress-induced hypermutation. We use both deterministic and stochastic models. Our deterministic models study the evolution of infinite populations under selection and muta-

tion in constant environments. We demonstrate that, contrary to classic theory (Liberman and Feldman 1986), selection acts to reduce only the mutation rates of fit individuals, while the mutation rates of unfit individuals are expected to increase due to selection. Previous work on the mean fitness of populations with SIMs in constant environments focused on the effect of deleterious mutations (Agrawal 2002; Shaw and Baer 2011) and found that stress-induced hypermutation does not affect the population mean fitness. In contrast, our analysis includes both deleterious and beneficial mutations and shows that stress-induced hypermutation can be favored in constant environments. Our stochastic models include finite-size populations, genetic drift, and changing environments in addition to selection and mutation and allow us to show that SIMs can be favored by selection over CMs and NM alleles in adaptive evolution, both at the individual and at the population level.

Deterministic Models

Consider an infinite asexual population of bacteria subject to mutation and selection. Assume these bacteria have a single mutator locus and n loci affecting fitness. Selection acts at reproduction. The relative growth rate of bacteria with x harmful alleles is $\frac{\omega_x}{\bar{\omega}}$, f_x is the frequency of bacteria with x harmful alleles, and $\bar{\omega} = \sum_{x=0}^n f_x \omega_x$ is the population mean fitness.

A cell with x harmful alleles mutates at reproduction with probability m_x , and mutations are uniformly distributed over the genome and are deleterious, beneficial, or neutral with probabilities δ , β , and $1-\delta-\beta$, respectively. In addition, our model does not consider mutations at the mutator locus and double mutations.

The change in frequencies during a single time unit is described by the following system of equations:

$$\begin{aligned} \bar{\omega} f'_x &= (1 - m_x(\beta + \delta))\omega_x f_x + m_{x-1}\delta\omega_{x-1}f_{x-1} \\ &+ m_{x+1}\beta\omega_{x+1}f_{x+1}, \quad \forall x \geq 0, \end{aligned} \tag{1}$$

where we define for convenience $m_{-1} = m_{n+1} = 0$, or equivalently by the matrix equation:

$$\bar{\omega} f' = Mf, \tag{2}$$

where M is the $n+1$ by $n+1$ tridiagonal matrix of transitions resulting from mutation and selection, and $f = (f_0, \dots, f_n)$ is the frequencies vector. In our main model, we assume that the beneficial and deleterious mutation probabilities, β and δ , are independent of x , the number of harmful alleles (when β equals zero mutation is unidirectional and deleterious).

At mutation-selection balance $f' = f$ and $\bar{\omega}$ is equal to the leading eigenvalue of M (Perron–Frobenius theorem, see [Otto and Day 2007, p. 709]). Therefore, the change in $\bar{\omega}$ can be

formulated by (Hermisson et al. 2002):

$$\frac{\partial \bar{\omega}}{\partial m_x} = v \frac{\partial M}{\partial m_x} f, \tag{3}$$

where v is the unique positive left eigenvector that satisfies $\sum_{x=0}^n v_x f_x = 1$. Equation 3 allows us to obtain a general expression for the sign of the partial derivative of the population mean fitness with respect to the mutation rate of individuals with x harmful alleles (Appendix A):

$$\text{sign} \frac{\partial \bar{\omega}}{\partial m_x} = \text{sign}(\bar{\omega} - \omega_x). \tag{4}$$

This result means that an increase in the mutation rate of individuals with x harmful alleles increases the population mean fitness if and only if the fitness of these individuals is below the population mean fitness.

In addition, we used *Mathematica* (Wolfram 2010) to numerically calculate the population mean fitness—the leading eigenvalue of M . This allowed us to calculate the relative difference in mean fitness between a population carrying a mutator allele and a NM population.

In our numerical calculations we used a multiplicative fitness function, $\omega_x = (1 - s)^x$, with s as the selection coefficient. For bacteria with a NM allele, we set the mutation rate to a minimal level $m_x = \mu = 0.003$ mutations per genome per generation (Drake 1991; Drake et al. 1998), independently of x , the number of harmful alleles. For bacteria with CMs $m_x = \tau \cdot \mu$, where τ is the mutator’s mutation rate increase, which we set to conservative values between one- and 10-fold (Sniegowski et al. 1997; Bjedov et al. 2003; Loh et al. 2010). For bacteria with SIMs, we assume a sensitivity threshold π that determines the minimal number of harmful alleles required to induce hypermutation, so

$$\text{that } m_x = \begin{cases} \mu, & x < \pi \\ \tau \cdot \mu, & x \geq \pi \end{cases}$$

We have also analyzed two alternative models of stress-induced hypermutation. In the first, the relationship between mutation rate and fitness is a continuous function rather than a threshold function and is given by $m_x = \tau\mu - (\tau\mu - \mu)(1 - s)^{xk}$, where k is a shape parameter (Fig. S2). This model is similar to the one used by Agrawal (2002), but includes beneficial mutations. In the second model, which corresponds to our stochastic model, we set the beneficial and deleterious mutation probabilities to be dependent on x : $\beta = \frac{x}{n}\phi$ and $\delta = 1 - \frac{x}{n}$. Hence, a mutation at a locus with a favorable allele will always change it into a harmful allele, while a mutation at a locus with a harmful allele will change it into a favorable allele with probability ϕ . Our results were robust to the specific model of stress-induced hypermutation, as long as the NMs mutation rate, μ , was not too high (Figs. 2, 3, S1, S3, S4).

STRESS-INDUCED HYPERMUTATION IS ADVANTAGEOUS IN CONSTANT ENVIRONMENTS

Our results show a drastic difference between the performance of CMs and SIMs—our analysis shows that an increase in m_x , the mutation rate of individuals with x harmful alleles, increases the population mean fitness if and only if the fitness of these individuals, ω_x , is lower than the population mean fitness (Eq. 4).

For mutation-free individuals ($x = 0$), $\bar{\omega}$ is smaller than ω_0 for any positive mutation rate, and therefore CM populations are less fit than their NM counterparts, a disadvantage that increases with τ , the mutation rate increase caused by the mutator allele (Fig. 1B). This result is in accord with classic theory (see section Discussion).

In contrast, increasing the mutation rate of individuals carrying harmful alleles can be favored. When m_0 is low, the fitness of individuals with a single harmful allele, ω_1 , is lower than the population mean fitness and increasing the mutation rate of these individuals ($m_{x \geq 1}$) increases the population mean fitness. Hence, SIM populations are fitter than NM, an advantage that increases with τ (Fig. 1A) and with β , the fraction of beneficial mutations (Fig. 2). This advantage is present as long as β is positive, that is, as long as back mutations occur, even if rarely. When selection is too weak or mutation rates are too high, so that ω_1 is higher than $\bar{\omega}$, it is still advantageous to increase the mutation rate of individuals with more than a single harmful allele ($m_{x \geq 2}$), that is, SIM with sensitivity threshold $\pi = 2$ is fitter than NM (Fig. 3, black dashed curve). If beneficial mutations do not occur at all, the mean fitness of SIM and NM is the same (Fig. 1A, the curve labeled “ $\beta = 0$ ”), consistent with the results of Agrawal (2002) and Shaw and Baer (2011) who investigated models that focus on the effect of deleterious mutations. In addition, as π , the stress sensitivity threshold, increases, SIM is advantageous for a wider range of parameters, but the magnitude of this advantage diminishes.

We further extended our analysis (see Appendix B) to study if a mutator that increases the mutation rate of individuals with below average fitness and as well as individuals with above average fitness can still increase the population mean fitness. We found that such a mutator can be advantageous, as long as it increases mostly the mutation rates of unfit individuals and beneficial mutations are not too rare (Fig. 3).

Using SIM with a continuous relationship between mutation rate and fitness rather than a threshold (Fig. S2) resulted in a similar advantage of SIM over NM, as long as beneficial mutations were allowed and selection was stronger than mutation (Figs. 2, 3, S3). When the continuous relationship was highly concave ($k \gg 1$), continuous SIM was similar to threshold SIM with $\pi = 1$ (Fig. S2, S3).

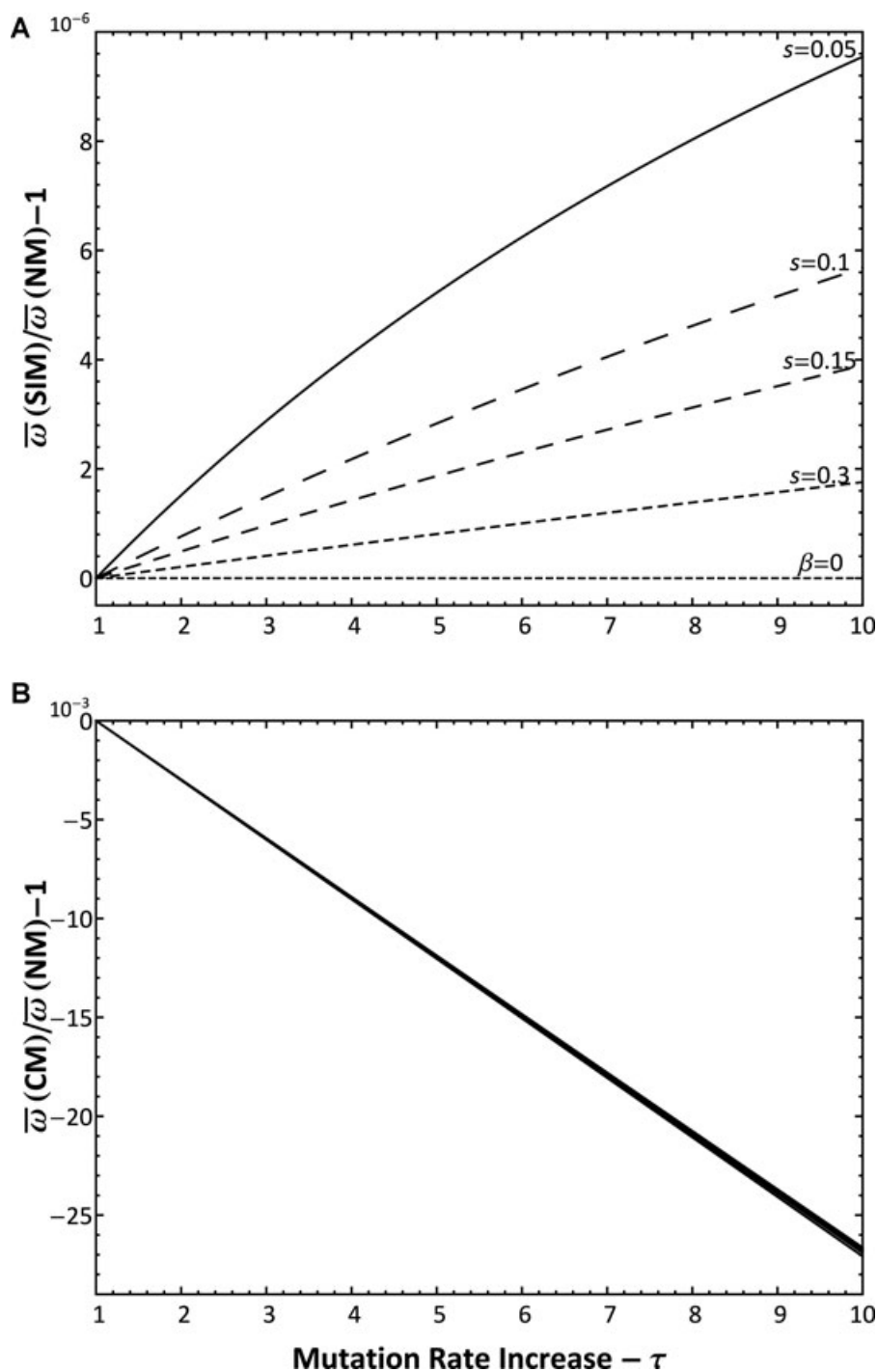


Figure 1. The advantage of SIM: deterministic model, constant environment. The relative difference in population mean fitness between mutator (stress-induced or constitutive) and nonmutator (NM) alleles as a function of the mutators' mutation rate increase, τ , at mutation-selection balance. (A) Stress-induced mutators (SIMs) have a higher mean fitness than NM alleles unless there are no beneficial mutations ($\beta = 0$). (B) The population mean fitness of constitutive mutators (CMs) is lower than that of NM and decreases as τ increases. Five different CM curves ($\beta = 0.01, 0.005, 0$ with $s = 0.1$ and $\beta = 0.01$ with $s = 0.5$ or 0.05) are plotted but the curves overlap. Parameters used unless noted otherwise: number of loci $n = 100$, selection coefficient $s = 0.1$, NM mutation rate $\mu = 0.003$ (Drake 1991; Drake et al. 1998), beneficial mutations fraction $\beta = 0.01$ (see Figs. 2 and S1 for relaxations of this assumption). CM always increases the mutation rate τ -fold and SIM increases the mutation rate τ -fold when with one or more harmful alleles. Note that the y-axis scale in panels A and B is different.

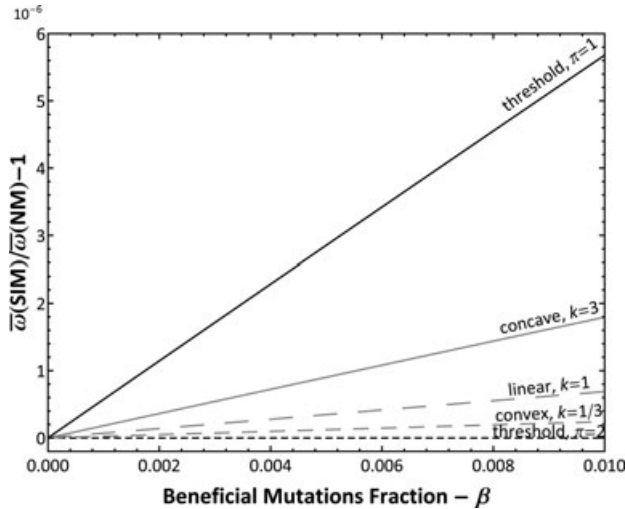


Figure 2. The role of beneficial mutations in the advantage of stress-induced mutators (SIM): deterministic model, constant environment. The relative difference in population mean fitness between SIMs and nonmutators (NMs) as a function of β , the beneficial mutations fraction. The figure shows that when $\beta > 0$, SIM is advantageous (the advantage of SIM with threshold $\pi = 2$ is too small for the scale used here: it is roughly 2×10^{-11} for $\beta = 0.01$). The gray curves represent mutators with continuous stress-induction functions (Agrawal 2002) rather than threshold functions, which are represented by black curves (see Fig. S2 for a detailed comparison). These curves demonstrate that the advantage of SIM is robust to the specific form of stress induction. Parameters used: number of loci $n = 100$, selection coefficient $s = 0.1$, NM mutation rate $\mu = 0.003$ (Drake 1991; Drake et al. 1998), mutation rate increase $\tau = 10$. SIM with sensitivity threshold π increases the mutation rate τ -fold when with π or more harmful alleles. Continuous SIM with parameter k mutates at a rate of $\tau\mu - (\tau\mu - \mu)(1 - s)^{kx}$ when with x harmful alleles.

Stochastic Models

We used individual-based simulations to model the evolution of bacterial populations in stationary-phase growth (source code available on request). Population size was kept constant at 100,000 individuals. Each simulation step is divided to four stages: genetic drift, selection, mutation, and environmental change. At the first stage, which models the effect of random genetic drift, a random individual dies. At the second stage, a random individual is chosen for reproduction with probability proportional to its fitness. This stage models the effect of natural selection—fitter individuals have a higher probability to reproduce. Note that reproduction is clonal and does not involve sex, that is, there is no recombination or segregation. At the third stage, the genome of the new individual may undergo mutation. At the final stage, environmental changes may occur. The model has overlapping generations, but we use the term “generation” to refer to 100,000 simulation steps (see [Otto and Day 2007, p. 581] for more details on Moran models).

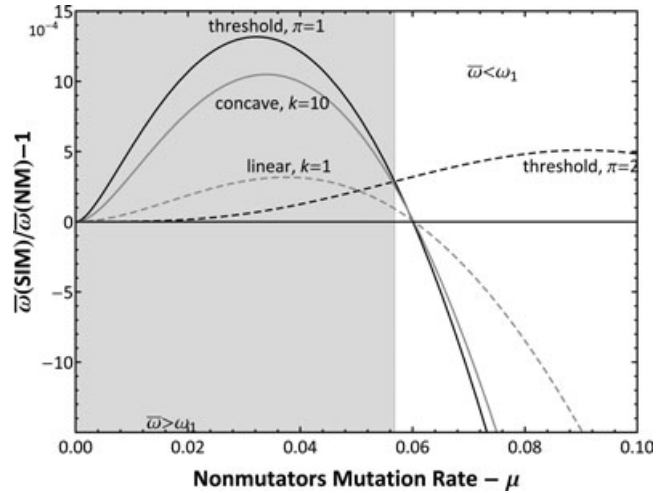


Figure 3. The effect of stress-induced mutator (SIM) under high baseline mutation rate: deterministic model, constant environment. The relative difference in population mean fitness between SIMs and nonmutators (NMs) as a function of the mutation rate of NMs. SIM with sensitivity threshold $\pi = 1$ (black curve) is fitter than NM when $\bar{\omega} > \omega_1$ (shaded area). When this threshold is crossed (nonshaded area), SIM with $\pi = 1$ is still advantageous as long as the condition in eq. B3 holds. When this condition is violated, SIM with $\pi = 1$ is disadvantageous, but SIM with $\pi = 2$ (dashed black curve) is still advantageous, because $\bar{\omega} > \omega_2$ (although the advantage of SIM with $\pi = 2$ is smaller—roughly 3×10^{-6} for $\mu = 0.06$). The gray curves represent mutators with continuous stress-induction functions (Agrawal 2002; see Fig. S2 for a detailed comparison). The range of NMs mutation rate in which continuous mutators are advantageous over NM is smaller than that of threshold SIM, but is still biologically relevant (Figs. 2, S3). Parameters used: number of loci $n = 100$, selection coefficient $s = 0.05$, mutation rate increase $\tau = 10$, beneficial mutations fraction $\beta = 0.1$ (see Fig. S5 for a similar figure with $\beta = 0.0001$). SIM with sensitivity threshold π increases the mutation rate τ -fold when with π or more harmful alleles. Continuous SIM with parameter k mutates at a rate of $\tau\mu - (\tau\mu - \mu)(1 - s)^{kx}$ when with x harmful alleles.

Individuals are defined by their haploid genome, which has 1000 loci that affect fitness. Half of these loci are “housekeeping genes”—their effect on fitness does not depend on the environment. We model these as biallelic loci. At each locus there may be a favorable allele denoted by A or a harmful allele denoted by a . The other loci are “environmental genes”—the identity of the favorable allele at these loci may change when the environment changes. We model “environmental genes” as triallelic loci, with alleles A_1, A_2 , and a . The a allele is always harmful, but the other two switch—in some environments A_1 is favorable and A_2 is harmful, and in some environments A_2 is favorable and A_1 is harmful. The fitness function, which we use to select individuals for reproduction, is $\omega = (1 - s)^x$, with x as the number of harmful alleles in the genome and s as the selection coefficient.

The number of mutations occurring at reproduction is drawn from a Poisson distribution with expectation μ , the mutation rate. The positions of the mutations are drawn from a uniform distribution across the genome. Mutations are bidirectional—the current allele changes to one of the alternatives. In “housekeeping genes”, A changes to a and vice versa; in “environmental genes”, a changes to either A_1 or A_2 with equal probability, A_1 changes to either a or A_2 , and so on. In all our simulations, the majority of the loci are occupied by favorable alleles, so most mutations are deleterious, substituting favorable alleles with harmful ones. Nevertheless, after an environmental change or a deleterious mutation, a beneficial mutation may occur and substitute a harmful allele with a favorable one (in these cases the fraction of beneficial mutations is between 0.00075 and 0.002).

The mutation rate is determined by the mutator locus, which is unaffected by mutations and does not directly affect fitness. Bacteria with NM alleles mutate at a constant low-level mutation rate of $\mu = 0.003$ mutations per genome per replication (Drake 1991; Drake et al. 1998). Bacteria with CM alleles constantly increase their mutation rate τ -fold. Bacteria with SIM alleles increase their mutation rate τ -fold only when with π or more harmful alleles. Therefore, two sister cells with the same π and τ combination may have different mutation rates if one of them has experienced a beneficial or a deleterious mutation. In addition, the exact same genotype may experience different mutation rates before and after an environmental change. Note that mutations only occur at birth, so the genome does not change between birth and reproduction. Thus, the model assumes a lag between the occurrence of an environmental change and its effect on individual mutation rates.

POPULATIONS OF SIMs ARE FITTER IN CHANGING ENVIRONMENTS

When environmental changes occur once every 100 generations (equivalent to 10^7 simulation steps), populations of bacteria with SIM have a significantly higher mean fitness than populations with NM or weak to intermediate CM (Fig. 4A). However, strong CMs, with a 10-fold mutation rate increase, have a population mean fitness as good as SIM does. In these rapidly changing environments the population is always in need of adaptation, and strong mutators (SIM or CM) are more fit on average, because they adapt faster through the beneficial mutations they generate.

When environmental changes are less frequent, occurring once every 500 generations, SIM is significantly fitter than any CM (Fig. 4B). In these environments the environmental changes are sparse enough to allow the population to reach a mutation-selection balance, even if for a short while, which highlights the advantage of SIM over strong CM—a higher mean fitness at mutation-selection balance (Fig. 1).

These results, together with the results of the deterministic model of constant environments (Fig. 1), suggest that SIM has a population-level advantage over both NM and CM, conditional on the frequency of environmental changes. In constant and slowly changing environments, SIM is considerably more successful than CM and slightly more successful than NM. In rapidly changing environments, SIM is considerably more successful than NM and at least as good as CM. Because the population mean fitness is a good approximation to selective advantage in asexual organisms, and because realistic environments are somewhere between constant and rapidly changing, we expect SIM to be favored by selection in natural populations.

SIMs ARE FAVORED BY SELECTION IN CHANGING ENVIRONMENTS

We simulated invasions of SIM into populations of NM and CM to determine if SIM is indeed favored by selection. The simulations started with the introduction of a SIM allele to 5% of a population at mutation-selection balance. Then the environment was allowed to change at random (the interval between consecutive environmental changes was geometrically distributed with an average of 100 generations, equivalent to 10^7 simulation steps) and the population was allowed to evolve. The frequency of the mutator alleles was monitored and the simulation was terminated when the mutator allele reached fixation or extinction, or after 500 generations, in which case we assumed the mutator allele reached extinction (at least 90% of the simulations finished with fixation or extinction of the mutator allele). The fixation probability (estimated by the fraction of simulations that end in fixation) of a neutral allele is expected to be its frequency in the population at the beginning of the simulation. A favorable allele, on the other hand, is expected to have a fixation probability that is higher than its frequency in the population. Hence, we checked if the fixation probability of SIM was significantly higher than 5%.

Figure 5 shows that SIM successfully invades populations of both NM and CM in changing environments. All four SIM alleles are significantly successful against all six competitor alleles, with fixation probabilities ranging between 12% and 42% (Figs. 5, 7). In addition, SIM is more successful than CM in invading NM populations (Fig. 6). These results suggest that SIMs are favored by selection over both NMs and CMs during adaptive evolution. Furthermore, the results indicate that SIMs are likely to invade and fixate in populations with constant mutation rates even when starting at a low frequency, and are therefore expected to spread in natural populations.

Discussion

We studied the evolution of stress-induced hypermutation in bacteria using deterministic models and stochastic individual-based

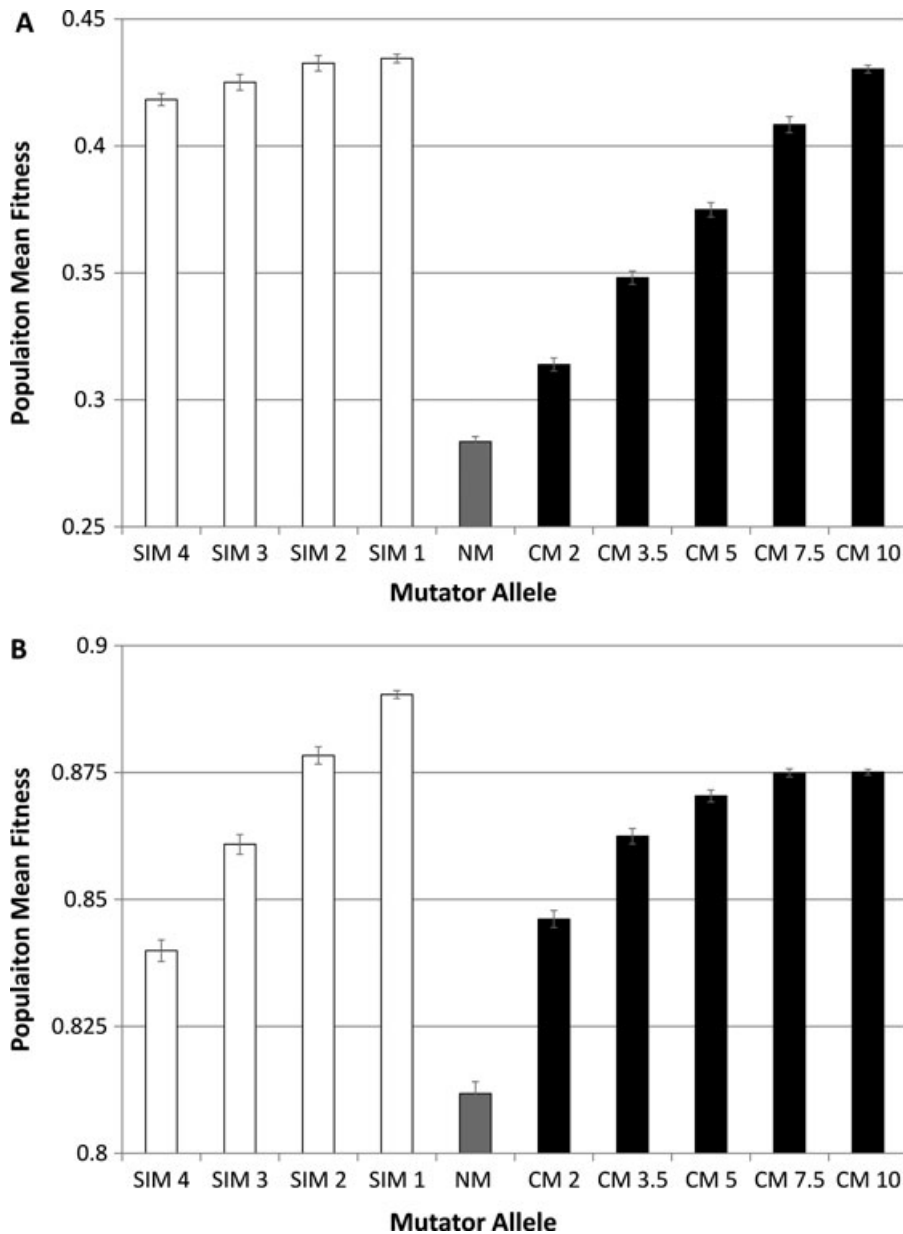


Figure 4. The population-level advantage of stress-induced mutators (SIM): stochastic model, changing environment. Populations of either SIMs (white), nonmutators (NMs; gray), or constitutive mutators (CMs; black) evolve in changing environments. (A) Environmental changes occur every 100 generations. SIM with sensitivity threshold $\pi = 1$ and SIM with $\pi = 2$ have a significantly higher population mean fitness than NM and CM (Kruskal–Wallis with Bonferroni correction: $P < 9 \cdot 10^{-4}$), except CM with $\tau = 10$, because bacteria with SIM adapt more rapidly than bacteria with NM or weak to moderate CM. CM with $\tau = 10$ was not significantly more successful than any SIM. Note that the effect of the mutation rate fold increase of CM on the population mean fitness in changing environments is opposite to its effect in constant environments (Fig. 1B). The average mutation rate increase of SIM was between 9.5- and 10-fold (averaged over time). (B) Environmental changes occur every 500 generations. SIM with $\pi = 1$ has a significantly higher population mean fitness than NM and CM ($P < 10^{-6}$) because when the environment changes slowly, the population spends roughly half of the time around the mutation-selection balance, in which SIM is fitter than CM (Fig. 1). The average mutation rate increase of SIM was between two- and fivefold (averaged over time). The population mean fitness is averaged over the entire time span of the simulation—1,000 generations (equivalent to 10^8 simulation steps). Parameters used: NM mutation rate $\mu = 0.003$ mutations per genome per replication (Drake, 1991; Drake et al., 1998), selection coefficient $s = 0.1$, population size $N = 100,000$, # replicates ≥ 100 , error bars: ± 1 SEM. SIM π is a mutator allele that increases the mutation rate 10-fold when with π or more harmful alleles. CM τ is a mutator allele that always increases the mutation rate τ -fold.

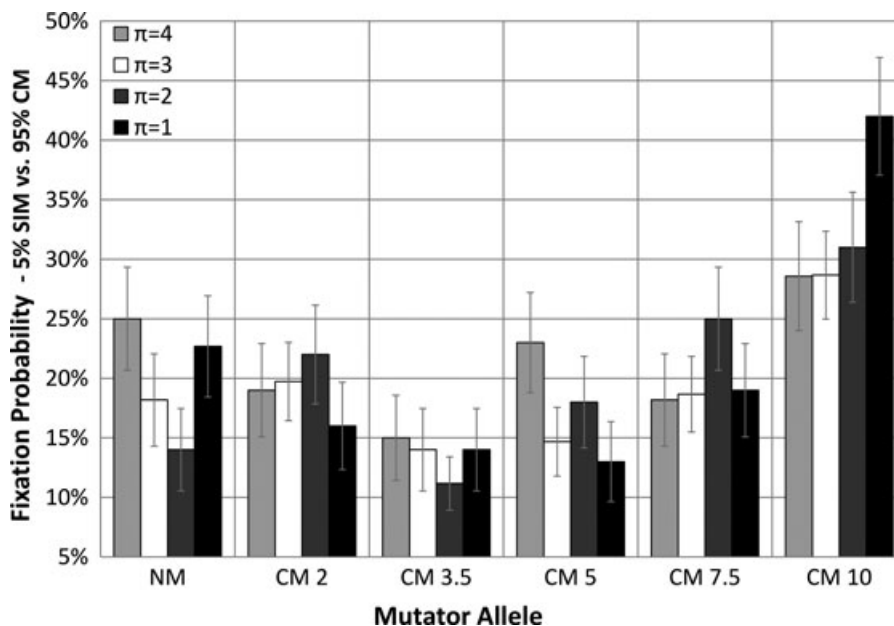


Figure 5. The individual-level advantage of stress-induced mutators (SIM): stochastic model, changing environment. Simulations started with 5% SIMs versus 95% of either nonmutators (NMs) or constitutive mutators (CMs) at mutation-selection balance, and continued until one of the alleles reached 100% of the population or until reaching 500 generations (equivalent to $5 \cdot 10^7$ simulation steps). Over 90% of the simulations reached fixation of one of the alleles. The environment changed at random approximately once every 100 generations, switching the identity of the favorable allele at four loci. The fixation probability of all SIM alleles is significantly higher than 5% (binomial test: $P < 5 \cdot 10^{-4}$), indicating that SIM is favored by selection over both NM and CM in changing environments, and that it is likely to invade to and reach fixation in bacterial populations. The results were qualitatively the same when SIM was initially introduced to 50% of the population (results not shown). Parameters used: NM mutation rate $\mu = 0.003$ mutations per genome per replication, selection coefficient $s = 0.1$, population size $N = 100,000$, # replicates ≥ 100 , error bars: ± 1 SEM. The average mutation rate increase of SIM was between 5.6- and 7.6-fold (averaged over time). SIM π is a mutator allele that increases the mutation rate 10-fold when with π or more harmful alleles. CM τ is a mutator allele that always increases the mutation rate τ -fold.

multilocus simulations of finite populations. We showed that selection favors stress-induced mutators over both constitutive mutators and nonmutator alleles (Fig. 5). Considering the long-term success of the population, we found that populations of SIMs are fitter than populations with a constant mutation rate both in constant (Fig. 1) and in changing environments (Fig. 4). We used biologically realistic values for all the model parameters and our results were robust to the magnitude of the increase in mutation rates of CMs competing with the SIMs.

These results have several implications. First, hypermutation in bacteria is more likely to arise and be maintained if it is induced by stress and is negatively correlated with the individual's condition. Second, stress-induced hypermutation is advantageous both for adaptation to new environmental conditions and for coping with deleterious mutations in constant environments, thus significantly relaxing the constraints on the evolution of high mutation rates in bacteria. Third, the high frequencies of SIMs attained in our model are due to selection, not just drift. Finally, even if there is no immediate growth advantage or physiological reason for hypermutation to be induced by stress, a modifier of mutation rates that induces such correlation will be

selected due to its linkage to the fitter genotypes in the population. This supports the adaptive hypothesis, which suggests that stress-induced hypermutation is not just a by-product of stress but a mechanism that is selected for its adaptive advantages (Sniegowski and Lenski 1995; Sniegowski et al. 2000; Tenailon et al. 2004; Denamur and Matic 2006; Saint-Ruf and Matic 2006).

Classical theory predicts that in asexual populations with constant mutation rates the mean fitness can be estimated by $e^{-\mu}$, which is a decreasing function of the mutation rate, μ (Kimura and Maruyama 1966) and that selection acts to reduce the mutation rate. This effect has been referred to as the "Reduction Principle" (Liberian and Feldman 1986, Altenberg 2011). Our analysis shows that when the rate of beneficial mutations is positive this is not necessarily true. If mutation rates are regulated by the level of stress an individual experiences, then selection acts to reduce only the mutation rates of individuals whose fitness is above the population mean. In contrast, selection acts to increase the mutation rates of individuals whose fitness is below the population mean, even in a constant environment (Eq. 4). Our results demonstrate for the first time that hypermutation can increase the

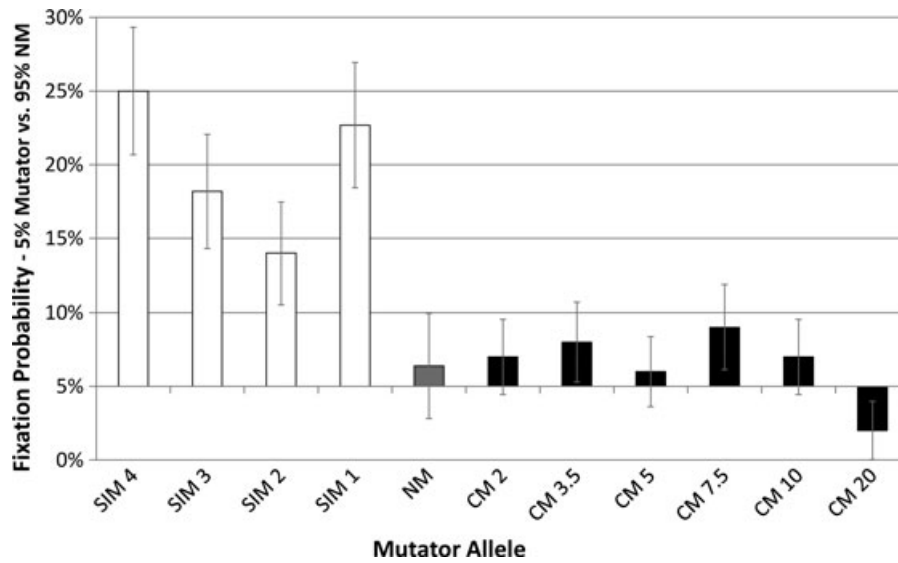


Figure 6. Stress-induced mutator (SIM) versus constitutive mutator (CM) versus nonmutator (NM): stochastic model, changing environment. Invasions of SIMs (white) or CMs (black) to populations of NMs (gray). All SIMs are successful against NM alleles (binomial test: $P < 0.0002$). However, CM with mutation rate increase between two- and 100-fold (50 and 100 not shown) are not significantly more successful than NM, which served as control ($P > 0.025$). SIM with $\pi = 1$ and $\pi = 4$ are significantly more successful than all CM (t-test: $P < 0.005$ and 0.0005 , respectively), suggesting that the advantage of SIM over CM is not limited to direct competitions between these mutator alleles. The results were qualitatively the same when SIM and CM were introduced to 50% of the population (results not shown). Unless otherwise noted, all parameters are identical to those in Figure 5.

mean fitness of a population in a constant environment, as long as back mutations occur, even rarely.

In a study by Matic and co-workers (Bjedov et al. 2003), simulations were used to examine the role of SIMs in adaptation to a new environment. Their results showed that SIMs are selected almost as efficiently as CMs. We extended this work by modeling both changing environments and constant ones using simulations and mathematical analysis. Furthermore, we expressed stress intrinsically as a result of a mismatch between genome and environment, rather than as an extrinsic event experienced by the entire population. Thus, we allowed each individual to regulate its own mutation rate according to the stress it experiences. Our results show that this individual-level plasticity allows SIMs to be selected more efficiently than CMs both in adaptive evolution (Fig. 6) and at mutation-selection balance (Fig. 1).

Bacteria sense their condition and the environment through a variety of extra-, intra-, and intercellular signals (such as growth rate, energy balances, nutrient concentrations, temperature, and pH) and use these signals to regulate distinct expression profiles consisting of hundreds of genes via signal transduction mechanisms and stress responses (Boor 2006; Keller and Surette 2006). An implicit assumption of our models is that bacteria can use this information to estimate how far their genotype is from the environmental optimum. But such estimations are not always accurate and stress is not always correlated with maladaptation. A more realistic assumption would be that these estimations are

error-prone, and therefore it would be interesting to investigate more detailed models in which the error in condition estimation is handled explicitly, and correlation between maladaptation and stress is limited to a subset of the genome.

As our models do not include a cost for DNA replication fidelity, the only selection on stress-induced hypermutation is the selection acting on the mutations it generates. While a cost of fidelity might increase the advantage of SIMs over NMs, it might also increase the success of CMs in competitions versus SIMs and NM alleles, as CMs will constitutively benefit from not paying the cost of fidelity (Dawson 1998; Sniegowski et al. 2000). However, recent empirical studies suggest that the cost of fidelity does not play an important role in the evolution of CMs (Giraud et al. 2001; Loh et al. 2010; Gentile et al. 2011; Shee et al. 2011).

Although asexual, bacteria do engage in recombination by horizontal gene transfer. Recombination tends to disrupt the evolutionary advantage of hypermutation. It reduces the “hitchhiking” effect by separating favorable alleles from the mutators that generated them and allows NMs to acquire favorable alleles without paying the price of hypermutation. In addition, recombination is an adaptation strategy and as such it competes with hypermutation by allowing multiple favorable alleles, generated by different individuals, to combine in the same individual, thus accelerating adaptation (Tenaillon et al. 2000). Therefore, even though we expect stress-induced hypermutation to remain advantageous at the

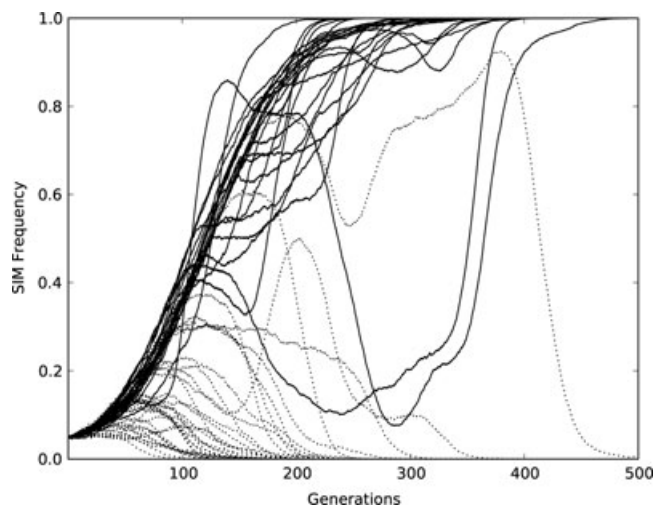


Figure 7. Dynamics of SIM invasions to CM populations: stochastic model, changing environment. This figure describes 60 of 100 simulations that were used for the right-most column in Figure 5—invasions of SIM with $\pi = 1$ and $\tau = 10$ to populations of CM with $\tau = 10$. The x-axis shows the time in generations (each generation is equivalent to 100,000 simulation steps); The y-axis shows the frequency of the SIM allele. Bold and dashed lines represent invasions that ended in fixation or extinction of SIM, respectively. SIM reached fixation in 25 of 60 invasions in this figure (roughly 41%; binomial test: $P < 10^{-15}$). Notice the highly stochastic nature of the simulations, which include a number of random processes: random genetic drift, changing environments, and mutation. Nevertheless, it is apparent that SIM is very successful in invading the CM population, despite its unfavorable initial frequency. This is due to natural selection favoring the SIM allele, which induces an adaptive distribution of the mutation rates in the population—mutations are more likely to occur in individuals that are likely to benefit from them and less likely to occur in individuals that have more to lose.

population level in the presence of recombination, its short-term advantage might be diminished.

CONCLUDING REMARKS

Previous studies demonstrated induction of hypermutation by stress in natural and laboratory strains of several species of bacteria, as well as in yeast and cancer cells. Because this induction is genetically regulated and its strength is highly variable, it was suggested that it is under selective pressure. We showed that stress-induced hypermutation can have an evolutionary advantage over constant mutation rates both in changing environments and in constant ones.

As mutation is the ultimate source for novel adaptations, these results are relevant to broad aspects of biology. Emergence of drug resistance in bacteria (Cirz et al. 2005; Cirz and Romesberg 2006, 2007); evolution of cancer cells and the emergence of chemotherapeutic resistance (Huang et al. 2007; Bristow and Hill 2008; Ruan et al. 2009); evolution of pesticide resistance

in commercial crops (Gressel 2010); industrial applications using bacteria in stressful environments (Machielsen et al. 2010); host–parasite coevolution (Pal et al. 2007; Morgan et al. 2010; Racey et al. 2010); and evolution of pathogen virulence (Oliver et al. 2000; Mérino et al. 2002; Boshoff et al. 2003; Gibson et al. 2010).

But most importantly, our work contributes to the ongoing shift in the understanding of mutation—one of the basic notions in evolution. Contrary to the classical view of mutation as an inevitable copying error, kept at bay by accurate replication and error-correction mechanisms, it becomes increasingly evident that mutation rates are controlled and regulated. We provide a theoretical foundation for the observation that more mutations are generated in individuals that are likely to benefit from them and that populations have a higher mean mutation rate when maladapted to their environment.

ACKNOWLEDGMENTS

We thank A. F. Agrawal, T. Beker, N. Goldenfeld, A. Gueijman, J. Hermisson, U. Obolski, P. Reuven, S. M. Rosenberg, and N. Roseth for their insightful comments on the manuscript. This research has been supported in part by the Israel Science Foundation 840/08 (LH) and by Marie Curie reintegration grant 2007–224866 (LH).

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Associate Editor: J. Hermisson

Appendix A

PROPOSITION

Let f_x , m_x , ω_x be the frequency, the mutation rate, and the fitness of individuals with x harmful alleles. Let δ and β be the probabilities that a mutation is deleterious or beneficial. Denote the population mean fitness by $\bar{\omega}$. Then

$$\frac{\partial \bar{\omega}}{\partial m_x} = \frac{f_x v_x}{m_x} (\bar{\omega} - \omega_x).$$

And therefore

$$\text{sign} \frac{\partial \bar{\omega}}{\partial m_x} = \text{sign} (\bar{\omega} - \omega_x).$$

PROOF

The system equilibrium is defined by (with $m_{-1} = 0$):

$$\bar{\omega} f_x = (1 - m_x (\beta + \delta)) \omega_x f_x + m_{x-1} \delta \omega_{x-1} f_{x-1} + m_{x+1} \beta \omega_{x+1} f_{x+1}, \quad \forall x \geq 0$$

or by the matrix equation:

$$\bar{\omega} f = Mf$$

by defining the vector $f = (f_0, f_1, \dots)$ and the infinite tridiagonal matrix (for an n by n matrix simply define $\omega_{x \geq n} = 0$):

$$M = \begin{pmatrix} (1 - m_0 \delta) \omega_0 & m_1 \beta \omega_1 & 0 & \dots \\ m_0 \delta \omega_0 & (1 - m_1 (\beta + \delta)) \omega_1 & m_2 \beta \omega_2 & \ddots \\ 0 & m_1 \delta \omega_1 & (1 - m_2 (\beta + \delta)) \omega_2 & \ddots \\ \vdots & \ddots & \ddots & \ddots \end{pmatrix}.$$

By the Perron–Frobenius theorem, $\bar{\omega}$ is the leading eigenvalue of M and f is the unique nonnegative right eigenvector with $\sum_{x \geq 0} f_x = 1$, that is, f is the stationary frequencies vector.

Let us denote by $v = (v_0, v_1, \dots)$ the unique nonnegative left eigenvector of M that satisfies

$$\bar{\omega} v_0 = (1 - m_0 \delta) \omega_0 v_0 + m_0 \delta \omega_0 v_1 \tag{A1}$$

$$\bar{\omega} v_x = (1 - m_x (\beta + \delta)) \omega_x v_x + m_x \beta \omega_x v_{x-1} + m_x \delta \omega_x v_{x+1}, \quad \forall x > 0 \tag{A2}$$

$$v \cdot f = \sum_{x \geq 0} v_x f_x = 1. \tag{A3}$$

Now, because f and v are right and left eigenvectors, a simple expression for the partial derivative of the eigenvalue is obtained by application of eq. A3 and the product rule:

$$v \cdot \bar{\omega} f = v M f \Rightarrow \frac{\partial \bar{\omega}}{\partial m_x} = v \frac{\partial M}{\partial m_x} f. \tag{A4}$$

Equation A4 allows us to find the partial derivative of the population mean fitness with respect to the mutation rate of individuals with x harmful alleles.

Before we continue we must obtain an expression for $\frac{v_{x+1}}{v_x}$:

$$(A1) \Rightarrow \frac{v_1}{v_0} = 1 + \frac{\bar{\omega} - \omega_0}{m_0 \delta \omega_0} \quad (A5)$$

$$(A2) \Rightarrow \frac{v_{x+1}}{v_x} = 1 + \frac{\bar{\omega} - \omega_x}{m_x \delta \omega_x} - \frac{\beta}{\delta} \left(\frac{v_{x-1}}{v_x} - 1 \right), \quad \forall x > 0. \quad (A6)$$

We can now find an expression for $\frac{\partial \bar{\omega}}{\partial m_x}$. We start with $x = 0$. Deriving M we obtain (note that the indices (0, 0) represent the entry at the first column of the first row):

$$\left(\frac{\partial M}{\partial m_0} \right)_{i,j} = \begin{cases} -\delta \omega_0, & (i, j) = (0, 0) \\ \delta \omega_0, & (i, j) = (1, 0) \\ 0, & \text{otherwise} \end{cases}$$

and therefore,

$$\begin{aligned} \frac{\partial \bar{\omega}}{\partial m_0} &= v \frac{\partial M}{\partial m_0} f \\ &= (v_0, v_1, v_2, \dots) \cdot \begin{pmatrix} -\delta \omega_0 & 0 & 0 \\ \delta \omega_0 & 0 & 0 \\ 0 & 0 & 0 \end{pmatrix} \cdot \begin{pmatrix} f_0 \\ f_1 \\ f_2 \\ \dots \end{pmatrix} \\ &= \delta \omega_0 f_0 v_0 \left(\frac{v_1}{v_0} - 1 \right). \end{aligned}$$

Plugging in eq. A5 we find that

$$\frac{\partial \bar{\omega}}{\partial m_0} = \delta \omega_0 f_0 v_0 \left(\frac{\bar{\omega} - \omega_0}{m_0 \delta \omega_0} \right) = \frac{f_0 v_0}{m_0} (\bar{\omega} - \omega_0).$$

For $x > 0$:

$$\left(\frac{\partial M}{\partial m_x} \right)_{i,j} = \begin{cases} \beta \omega_x, & (i, j) = (x-1, x) \\ -(\beta + \delta) \omega_x, & (i, j) = (x, x) \\ \delta \omega_x, & (i, j) = (x+1, x) \\ 0, & \text{otherwise} \end{cases} \Rightarrow$$

$$\begin{aligned} \frac{\partial \bar{\omega}}{\partial m_x} &= v \frac{\partial M}{\partial m_x} f \\ &= \omega_x f_x v_{x-1} \left(\beta \left(1 - \frac{v_x}{v_{x-1}} \right) - \delta \frac{v_x}{v_{x-1}} \left(1 - \frac{v_{x+1}}{v_x} \right) \right). \end{aligned}$$

We plug in eq. A6 to obtain a general expression for the partial derivative:

$$\frac{\partial \bar{\omega}}{\partial m_x} = \omega_x f_x v_{x-1} \frac{v_x}{v_{x-1}} \frac{\bar{\omega} - \omega_x}{m_x \omega_x} = \frac{f_x v_x}{m_x} (\bar{\omega} - \omega_x), \quad \forall x > 0.$$

Appendix B

We turn our attention to a modifier that regulates the mutation rates of individuals with π or more harmful alleles. The derivative of the transition matrix with respect to the mutation rate induced by such a modifier is

$$\left(\frac{\partial M}{\partial m_{x \geq \pi}} \right)_{i,j} = \sum_{x \geq \pi} \left(\frac{\partial M}{\partial m_x} \right)_{i,j}.$$

By the same method as above:

$$\frac{\partial \bar{\omega}}{\partial m_{x \geq \pi}} = v \sum_{x \geq \pi} \frac{\partial M}{\partial m_{x \geq \pi}} f = \frac{1}{m_{x \geq \pi}} \sum_{x \geq \pi} f_x v_x (\bar{\omega} - \omega_x). \quad (B1)$$

And as we are interested in the sign of the derivative:

$$\frac{\partial \bar{\omega}}{\partial m_{x \geq \pi}} > 0 \Leftrightarrow \sum_{x \geq \pi} f_x v_x (\bar{\omega} - \omega_x) > 0. \quad (B2)$$

A special case is that of a modifier that affects the mutation rates of individuals that have a below-average fitness as well as individuals that have an above-average fitness:

Suppose that $\omega_\pi > \bar{\omega}$ and $\forall x > \pi, \omega_x < \bar{\omega}$, we can rearrange eq. B2 to get:

$$\frac{\partial \bar{\omega}}{\partial m_{x \geq \pi}} > 0 \Leftrightarrow \sum_{x > \pi} \frac{f_x v_x}{f_\pi v_\pi} (\bar{\omega} - \omega_x) > \omega_\pi - \bar{\omega}. \quad (B3)$$

Figures 3 and S5 show the fate of such a modifier for $\pi = 1$. In most of these cases the derivative $\frac{\partial \bar{\omega}}{\partial m_{x \geq \pi}}$ is negative, but not always. We find a range where an increase in $m_{x \geq \pi}$ will lead to an increase in $\bar{\omega}$, which could sometimes increase enough so that $\omega_\pi < \bar{\omega}$. Then $\frac{\partial \bar{\omega}}{\partial m_{x \geq \pi}}$ will remain positive by the above proposition, which will lead to further increases in $m_{x \geq \pi}$.

Supporting information

The following supplementary material is available for this article:

Figure S1. The advantage of SIM: alternative deterministic model, constant environment.

Figure S2. Comparison of stress-induction functions.

Figure S3. The advantage of SIM with continuous stress-induction: deterministic model, constant environment.

Figure S4. The role of beneficial mutations in the advantage of SIM: alternative deterministic model, constant environment.

Figure S5. The effect of SIM under high baseline mutation rate: deterministic model, constant environment.

Supporting Information may be found in the online version of this article.

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