Shifts in mutation bias promote mutators by altering the distribution of fitness effects

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Abstract

Recent experimental evidence demonstrates that shifts in mutational biases, for example increases in transversion frequency, can change the distribution of fitness effects of mutations (DFE). In particular, reducing or reversing an existing bias can increase the probability that a *de novo* mutation is beneficial. It has also been shown that mutator bacteria are more likely to emerge if the beneficial mutations they generate have a larger effect size than observed in the wildtype. Here, we connect these two results, demonstrating that mutator strains that reduce or reverse an existing bias have a positively-shifted DFE, which in turn dramatically increases their emergence probability. Since changes in mutation rate and bias are often coupled through the gain and loss of DNA repair enzymes, our results demonstrate that shifts in mutation bias can facilitate the invasion of mutator strains due to their improved access to previously undersampled beneficial mutations.

Introduction

Foundational to all genetic diversity and every adaptive innovation across evolutionary history, mutation is "the most important of all genetic processes" (Hodgkinson and Eyre-Walker, 2011). Increasing evidence supports an evolutionary fine-tuning of mutation rates (Wei et al., 2022) both among taxa (Lynch et al., 2016) and across the genome (Hodgkinson and Eyre-Walker, 2011; Martincorena and Luscombe, 2013).

Mutations, however, occur in many forms, and single nucleotide substitutions are frequently classified as transitions, transversions, specific base-to-base substitutions or substitutions in *n*-mer contexts, while insertions, deletions, or larger genome rearrangements are also important (Hodgkinson and Eyre-Walker, 2011). Mutation accumulation studies have allowed for detailed analyses of the rates and contexts of specific types of mutations, the "mutation spectrum" (Katju and Bergthorsson, 2019).

These data have also brought to light the fact that in many species, the mutation spectrum is biased, in other words, certain classes of mutations occur more frequently than others (Foster et al., 2015; Katju and Bergthorsson, 2019). A wealth of recent work has demonstrated that this underlying mutation bias influences and is ultimately echoed in the spectrum of adaptive substitutions (Soares et al., 2021; Stoltzfus and McCandlish, 2017; Stoltzfus and Yampolsky, 2009), even at high mutation rates (Gomez et al., 2020), as demonstrated for fitness landscapes in transcription factor binding sites (Cano and Payne, 2020), for antibiotic resistance (Payne et al., 2019), for convergent mutations in protein sequences (Storz et al., 2019), and for thousands of amino acid changes observed in natural and experimental microbial populations (Cano et al., 2022; Stoltzfus and Norris, 2015). Taken together, this body of work demonstrates that in multiple evolutionary contexts, the spectrum of likely mutations is strongly reflected in the spectrum of adaptive substitutions.

What are the implications of this result? Taking transitions and transversions as an example, the result above implies that beneficial transitions will be more likely to fix, during adaptation,

in a population with transition-biased mutation. If there is no *a priori* reason to expect that beneficial mutations are more likely to be transitions or transversions, this implies that beneficial transversions will be comparatively undersampled as adaptation proceeds. Thus, after a period of adaptation with a transition bias, reducing the bias to sample more transversions, or even reversing it to oversample transversions can offer access to previously unsampled beneficial mutations. This effect has been recently demonstrated in bacteria, in which shifts in the mutation bias increased the fraction of new mutations that were beneficial (Sane et al., 2021). Simulations of adaptive walks likewise demonstrated a robust effect in which, after a period of adaptation, any reduction or reversal in the existing mutation bias altered the distribution of fitness effects of mutations (DFE), increasing the fraction of beneficial mutations (Sane et al., 2021). This idea, that changing the mutation spectrum could in some way increase the beneficial fraction of the DFE, has been previously suggested (MacLean et al., 2010; Maharjan and Ferenci, 2017), and changes in the DFE have been demonstrated for bacterial mutators evolving antibiotic resistance (Couce et al., 2013).

Mutators, strains that increase the mutation rate by one or several orders of magnitude, are frequently observed both in experimental (Miyake, 1960; Shaver et al., 2002; Sniegowski et al., 1997; Treffers et al., 1954; Wielgoss et al., 2012) and natural (Giraud et al., 2001; LeClerc et al., 1996; Matic et al., 1997; Oliver et al., 2000; Richardson et al., 2002) microbial populations; the mutator phenotype is also prevalent in human tumors, which reproduce asexually as well (Fox et al., 2013). This phenomenon is understood to occur in asexual evolution because a mutation that increases the mutation rate (typically the loss of function of a mismatch repair enzyme (Denamur and Matic, 2006)) hitchhikes with the *de novo* beneficial mutation produced in the mutator strain (Maynard Smith and Haigh, 1974). The dynamics of mutation rate modifiers have been well-studied both analytically and in simulation; mutators are disfavoured in the presence of genetic exchange (Johnson, 1999; Tenaillon et al., 2000), but favoured in fluctuating environments (Leigh, 1970; Travis and Travis, 2002), or when multiple mutations are required for adaptation either simultaneously (Taddei et al., 1997; Tenaillon et al., 1999) or in sequence (Tanaka et al.,

2003). On smooth fitness landscapes, the complex interplay among genetic drift, deleterious load and competition between wildtype and mutator strains is well-understood analytically (Kessler and Levine, 1998; Wylie et al., 2009).

Couce et al. (2013) studied two mutator strains of *E. coli*, each of which increased specific classes of transversions. These changes in mutation spectrum resulted in changes to the DFE for both strains relative to the wildtype. To investigate whether these changes in spectrum could influence mutator emergence, populations were simulated in which the fraction of beneficial mutations was held constant, and every beneficial mutation had fixed effect size s in the wildtype and effect size σs in mutators. Results predicted that if the effect size of the beneficial mutation in a mutator strain was increased relative to the wildtype, even by a modest amount, the emergence of mutator strains was substantially increased (Couce et al., 2013).

Here, we use theory and simulations to explicitly investigate how a shift in bias affects the DFE, changing both the fraction of mutations that are beneficial and the mean selective effect of beneficial mutations. Using full population simulations, we demonstrate robust benefits of reducing or reversing the mutation bias after a period of adaptation. We investigate the fate of mutations that change the bias, change the mutation rate, or change both the bias and mutation rate. We demonstrate that mutations that reduce or reverse the bias and increase the mutation rate are most likely to emerge, and that this effect is non-linear; a bias shift can dramatically improve the chances of mutator fixation, through the resulting changes in the DFE. Since many loss-of-function mutations affect both mutation rate and bias, our results suggest that shifts in mutation bias powerfully facilitate the invasion of mutator strains.

Methods

The Distribution of Fitness Effects

Suppose an individual with fitness W has an offspring that carries a mutation. If the offspring has fitness W', the fitness effect of the mutation is defined as $s = W'/W - 1$, where s can be negative,

zero, or positive. The distribution of fitness effects (DFE) can then be defined as the distribution of such values of s from all possible mutations, for a single ancestor genome. This definition, however, assumes that the organism has equal access to all mutations, whereas in reality certain classes of mutations are typically over- or under-represented. It's therefore important to draw the distinction between the DFE of all possible mutations ($\mathrm{DFE}_{\mathrm{all}}$), which is often of theoretical interest, and the observed or realized DFE ($\mathrm{DFE}_{\mathrm{obs}}$) which is "seen" by the mutation process and observed experimentally, for example in mutation accumulation experiments. In theoretical models, $\mathrm{DFE}_{\mathrm{obs}}$ is computed when $\mathrm{DFE}_{\mathrm{all}}$ is weighted by the probability of occurrence of each mutation.

A second definition that requires clarification is the concept of unbiased mutation. In each individual, a particular locus in the genome exists in a particular state (for example, as a particular nucleotide, but the argument extends to amino acids or more general "alleles"). A number of other alternate states are possible at that locus. The mutation process is unbiased if each of these alternate states is accessed by mutation with equal probability. Thus unbiased mutation does not depend on the underlying biochemistry of mutation or neutrality of effect; it depends only on the possible alternative states. As an example, a transition fraction of 1/3 is unbiased, because for any nucleotide, 1/3 of the alternate states are reached via transitions. We define mutation classes as over- or under-sampled relative to this unbiased expectation. (We also note that for some classes of mutations, such as GC→AT versus AT→GC, the unbiased genome-wide mutation rate will depend on genome content; we will not treat these cases here.)

In the Introduction, we provided a verbal argument that a reduction or reversal in an existing mutation bias increases the beneficial fraction of the $\mathrm{DFE}_{\mathrm{obs}}$. Although the logic is straightforward, in the remainder of this section we demonstrate this effect mathematically, which allows us to carefully define the conditions and assumptions under which this expectation holds.

Consider an evolving population in which each individual has M possible mutations, in two distinct classes (e.g. transitions and transversions). (The approach below can be generalized to any number of mutation classes but we use two for clarity.) Let α be the fraction of possible

mutations of type 1, such that there are αM mutations in the first class and $(1-\alpha)M$ in the second. In the case of transitions as class one and transversions as class two, the value of α is 1/3.

Consider a particular "ancestor" genotype. Let B denote the total number of beneficial mutations, out of the M possible mutations for the ancestor. Thus the fraction of beneficial mutations in the ancestor is $fb_a = B/M$. Now suppose that we have no *a priori* reason to assume that mutations of one type or another are more likely to be beneficial, nor do they have different beneficial effect sizes. Under these assumptions, we expect αB beneficial mutations in the first class, and $(1-\alpha)B$ in the second.

Now assume that after a period of adaptive evolution, b beneficial mutations have fixed. For clarity, we will assume that mutations fix sequentially without competition, i.e. the strong-selection weak-mutation regime, but this will be relaxed in the simulations to follow. If no bias exists, then the two types of mutations have been sampled according to the unbiased ratio $(\alpha : 1-\alpha)$, so that we expect αb fixations in the first mutation class and $(1-\alpha)b$ in the second. On the other hand, if mutation bias has lead to a different sampling ratio of mutations, say $(\beta : 1-\beta)$, then we would observe βb fixations in the first class, and $(1-\beta)b$ in the second.

Without loss of generality, assume $\alpha < \beta$, such that class one is the over-sampled class. As a realistic example, we consider a transition-biased organism, such that $\beta > 1/3$ of fixed mutations are transitions. This implies that mutation bias has lead to the fixation of more beneficial mutations of type 1, and fewer of type 2, than expected under the theoretically-defined unbiased mutation process (see Cano et al. (2022) for recent empirical examples).

At this point we must impose further assumptions regarding the fitness landscape. In reality, due to epistasis, each beneficial substitution may change the remaining number of beneficial mutations, both in its own class and in others. Even in the absence of epistasis, a given nucleotide could have two possible beneficial mutations, for example both possible transversions could be beneficial, and after one transversion has fixed, the remaining transversion (now a transition) may or may not provide a further benefit. These complexities are captured in the simulation studies

that follow. For analytical tractability, however, we will assume a smooth fitness landscape, and that beneficial mutations are rare. In particular, each locus has at most a single beneficial mutation available.

In this simplified fitness landscape, it is straightforward to compute the fraction of remaining mutations in each class that are beneficial, fb_1 and fb_2, by simply subtracting the number of beneficial substitutions that have occurred. We find:

$$fb_1 = \frac{\alpha B - \beta B}{\alpha M} = fb_a - \frac{\beta}{\alpha}\left(\frac{b}{M}\right)$$

And similarly

$$fb_2 = fb_a - \frac{(1-\beta)}{(1-\alpha)}\left(\frac{b}{M}\right)$$

Since $\alpha < \beta$, it is clear that $fb_1 < fb_2$; the beneficial fraction for mutations in the over-sampled class is less than the beneficial fraction in the under-sampled class.

We can also compare the two DFEs defined above: (1) DFE_{all}, the DFE of all possible mutations that assumes equal access to all mutations, and (2) DFE_{obs}, the observed DFE that takes the bias into account. Let fb_{all} and fb_{obs} denote the fractions of beneficial mutations in DFE_{all} and DFE_{obs}, respectively, after this period of evolution. Then

$$fb_{\text{all}} = \frac{\alpha fb_1 + (1-\alpha) fb_2}{fb_1 + fb_2}, \quad fb_{\text{obs}} = \frac{\beta fb_1 + (1-\beta) fb_2}{fb_1 + fb_2}.$$

Again, since $\alpha < \beta$ and $fb_1 < fb_2$, we find that $fb_{\text{all}} > fb_{\text{obs}}$. Hence, if the bias stays the same, the organism will access a smaller fraction of beneficial mutations than is potentially available.

This might change, however, if the mutation bias shifts. If the mutation bias shifts to β', then the observed DFE changes and the fraction of beneficial mutations becomes $fb'_{\text{obs}} = (\beta' fb_1 + (1-\beta') fb_2)/(fb_1 + fb_2)$. There are two possibilities: (1) the new bias reduces or reverses the previous bias. When $\alpha < \beta$, a bias reduction occurs when $\alpha < \beta' < \beta$, while a reversal happens when β' is even lower that α. In either case (reduction or reversal), we find that $fb'_{\text{obs}} > fb_{\text{obs}}$, in other words, the new bias increases access to beneficial mutations. In contrast, (2) the new bias could reinforce the original bias, which occurs when $\beta' > \beta$. This yields $fb'_{\text{obs}} < fb_{\text{obs}}$,

implying reduced access to beneficial mutations. Moreover, it is clear that these effects will be stronger when the difference $|\beta - \beta'|$ is bigger, i.e., when the reversal or the reinforcement has a greater magnitude.

In the simulations to follow, we illustrate examples of these effects where mutations are classified as transitions and transversions, and in which we relax the assumptions of a smooth fitness landscape (no epistasis), of rare beneficial mutations, and of strong-selection weak-mutation evolutionary dynamics.

Hitchhiking probabilities

Some simple "back of the envelope" calculations offer critical insights into the evolution of the mutation bias. We follow a heuristic argument described by Lenski (2004) and developed more formally by Wylie et al. (2009). Suppose a fraction p_μ of a population has an F-fold higher mutation rate, but is otherwise identical to the wildtype. The chance that the next beneficial mutation that sweeps through the population occurs in the mutator strain is then simply approximated by $p_\mu F$. Lenski gives a quantitative estimate of this factor for *E. coli* mutators that segregate at an estimated frequency of 1×10^{-4} and have a 100-fold increase in mutation rate, predicting that about 1% of beneficial substitutions will occur in the mutator.

This argument naturally assumes that the DFE is identical in the wildtype and mutator. In contrast, suppose a fraction p_{bs} of a population has a shift in mutation bias, but has the same mutation rate as the wildtype. If the bias shift reduces or reverses the wildtype bias, the bias-shifted strain will experience a G-fold higher beneficial fraction:

$$G = \frac{fb_{\text{bs}}}{fb_{\text{wt}}}$$

where fb_{wt} and fb_{bs} denote the beneficial fraction of the DFE_{obs} in the wildtype and bias-shifted strain respectively. Assuming the effect size of beneficial mutations and the deleterious load are unchanged with the bias shift, the chance that the next beneficial mutation carries the bias shifted strain to fixation is approximated by $p_{\text{bs}}G$.

If, in addition, the beneficial effect size is increased in the bias-shifted strain, then the expected fixation probability of a beneficial mutation in this strain, π_{bs}, will exceed the analogous expectation in the wildtype, π_{wt}, such that beneficial mutations have an H-fold higher fixation probability if they occur in the bias-shifted background, where:

$$H = \frac{\pi_{\text{bs}}}{\pi_{\text{wt}}}.$$

Overall, the fraction of beneficial fixations that occur in the bias-shifted strain is then $p_{\text{bs}}GH$.

A quantitative estimate of this factor is challenging. In bacteria, both environmental stress and the loss of function of specific DNA repair enzymes can cause bias shifts without a substantial change in mutation rate (Foster et al., 2015; Maharjan and Ferenci, 2017; Sane et al., 2021; Shewaramani et al., 2017), however it is difficult to estimate the frequency at which such mutations might segregate in natural populations. Nonetheless, in both experimental (Sane et al., 2021) and simulated fitness landscapes as seen in the Results to follow, both factors G and H (the relative advantage of bias-shifted strains) are modest, typically in the range of one to two. In contrast, F, the mutation rate multiplier, can be a factor of 100 or more. We conclude that unless bias-shifted strains are maintained by the mutation-selection balance at frequencies that are several orders of magnitude higher than mutator strains, they will be much less likely than mutators to fix during adaptation.

However, suppose a fraction $p_{\mu\text{bs}}$ of a population has *both* an increased mutation rate and a bias-shift. This is in fact the most likely situation for loss-of-function mutations in DNA repair (Foster et al., 2015; Sane et al., 2021). The chance that a beneficial mutation carries this strain to fixation would then be $p_{\mu\text{bs}}FGH$, in other words, the effects combine multiplicatively. We thus predict, in agreement with Couce et al. (2013), that bias shifts could powerfully affect the invasion rate of mutator strains.

The reasoning above neglects the effects of changes to the deleterious load when the bias or mutation rate is altered. Recall that for a mutation rate μ per genome and observed deleterious fraction fd, the load is approximated by the product μfd, and is in fact independent of the

deleterious effect size (Ewens, 2012). Thus a strain that increases the mutation rate only, by factor F, will experience an increased load of $F\mu fd$. In contrast, for strains in which only a bias shift occurs, the deleterious fraction, fd, will be reduced whenever fb is increased (although this reduction is exactly concomitant only in very large populations in which the neutral fraction of the DFE is negligible). Thus any bias shift that increases the beneficial fraction will reduce the load.

For a mutator strain in which both the mutation rate and bias are altered, both of these effects come into play. Depending on the relative magnitudes of the increase in mutation rate and reduction in fd, the mutator strain may carry a greater or lesser load than the wildtype.

The magnitude of deleterious load will ultimately affect the fixation probability for beneficial mutations on the mutator background, reducing (and possibly reversing) the fixation advantage. For a mutator with an increased mutation rate $F\mu$, load overwhelms the expected selective advantage of new beneficial mutations when $F\mu fd > s_b$, or when the mutation rate multiple exceeds the critical value

$$F^*_\mu = \frac{s_b}{\mu fd} \ . \tag{1}$$

In constrast, consider a bias shift that increases the expected beneficial effect by a factor $(1+\delta)$, and also reduces the deleterious fraction by a factor $(1-\epsilon)$. For a mutator in which both the bias and mutation rate change, load overwhelms the selective advantage when $F\mu fd(1-\epsilon) > s_b(1+\delta)$, or when F exceeds

$$F^*_{\mu bs} = \frac{s_b(1+\delta)}{\mu fd(1-\epsilon)} \ . \tag{2}$$

Since $F^*_{\mu bs} > F^*_\mu$, these results predict that bias reductions and reversals will not only increase the invasion probability of mutator strains, but will extend the range of mutation rates over which invasion is favoured. Both of these predictions are verified in the simulation results to follow.

Simulation model

We simulated population evolution on the well-studied NK fitness landscape (Macken and Perelson, 1989; Stoltzfus, 2006). Each genotype is represented by a genomic sequence of length N, composed of four bases (A, C, G, T) such that mutations can be classified as transitions or transversions.

In an NK fitness landscape, the fitness of a sequence is simply calculated by adding the fitness contributions of all its loci. To incorporate epistasis, however, the fitness contribution of each locus depends not only on the state of the locus, but on the states of K other randomly assigned loci. Thus K determines the degree of epistasis, i.e., the number of loci epistatically coupled to each locus. For instance, for $K = 2$ and a given locus m, there are $4^{K+1} = 64$ different possible combinations for that locus and its two neighbours: AAA, AAC, AAG, ... TTT. Every such combination is randomly assigned a uniformly distributed value from the interval $(0, 1)$ which defines the fitness contribution of locus m.

Each individual in the population is assigned the following: (1) a genome sequence, which determines fitness; (2) a transition:transversion bias $Ts : Tv$, where $Ts = 1 - Tv$ gives the probability that a *de novo* mutation is a transition; and (3) a mutation rate μ per genome per generation. In some simulations the bias and/or mutation rate are fixed, while in other scenarios they are manipulated.

The population is initialized in generation 0 with a population size n_0, typically seeded with a number of random genotypes. Generations are discrete; the number of offspring for the ith genotype in generation j is Poisson distributed with mean

$$\lambda_{ij} = r_j \, n_{ij} \, \frac{w_{ij}}{\bar{w}_j}$$

where n_{ij} and w_{ij} represent the number of individuals and the fitness of the ith genotype in generation j, respectively, while $\bar{w}_j$ is the mean fitness of the population in generation j. The variable $r_j = exp(1 - \frac{n_j}{\kappa})$ is the Ricker factor in generation j, which limits overall population growth by comparing the total number of individuals in generation j, n_j, to the carrying capacity

κ.

In every generation, mutations occur according to each genotype's mutation rate, μ_i. When a mutation occurs, it affects a randomly chosen nucleotide in the sequence, and the mutation is a transition or a transversion according to that genotype's bias, i.e., it is a transition with probability Ts_i. If the mutation is a transversion, one of the two possibilities (e.g. $T \to G$ or $T \to A$) is chosen at random. Mutations occur during reproduction and when they occur, a single individual with the new genotype is added to the next generation, where the new individual inherits the parental mutation rate and bias.

In the results to follow, we perform invasion tests for "mutator" strains that change only the mutation bias but not the mutation rate, mutators that increase the mutation rate, and mutators that change both the rate and bias, as illustrated in Figure 1. Invasion tests are initiated by replacing a fixed fraction, typically 5%, of the population with the potentially invading subpopulation.

Populations in these simulations are genetically diverse; in some parameter regimes the average number of segregating strains is of order 100. We perform invasion tests starting with an invading subpopulation at 5%, rather than with a single individual, for computational efficiency, to avoid simulating the (very many) replicates in which a single lineage goes extinct even when beneficial. However this choice necessitates the choice of a genotype for the invading strain. Since the most common genotype in the population is typically also the fittest, using this genotype for the invading strain biases the results in favour of invasion. Instead, we initiate the mutator subpopulation by changing the mutation rate and/or bias in a random 5% of individuals in the population. Thus, the invading sub-population has on average the same genetic diversity (and, critically, the same genetic load) as the ancestor population.

Similar to a competition experiment, we then track whether this sub-population invades. We perform invasion tests in populations at various degrees of adaptive potential, which we estimate based on the remaining fraction of beneficial mutations in the $\mathrm{DFE}_{\mathrm{obs}}$ for the most common genotype. The invasion probability is estimated as the fraction of n_{reps} replicates in which a

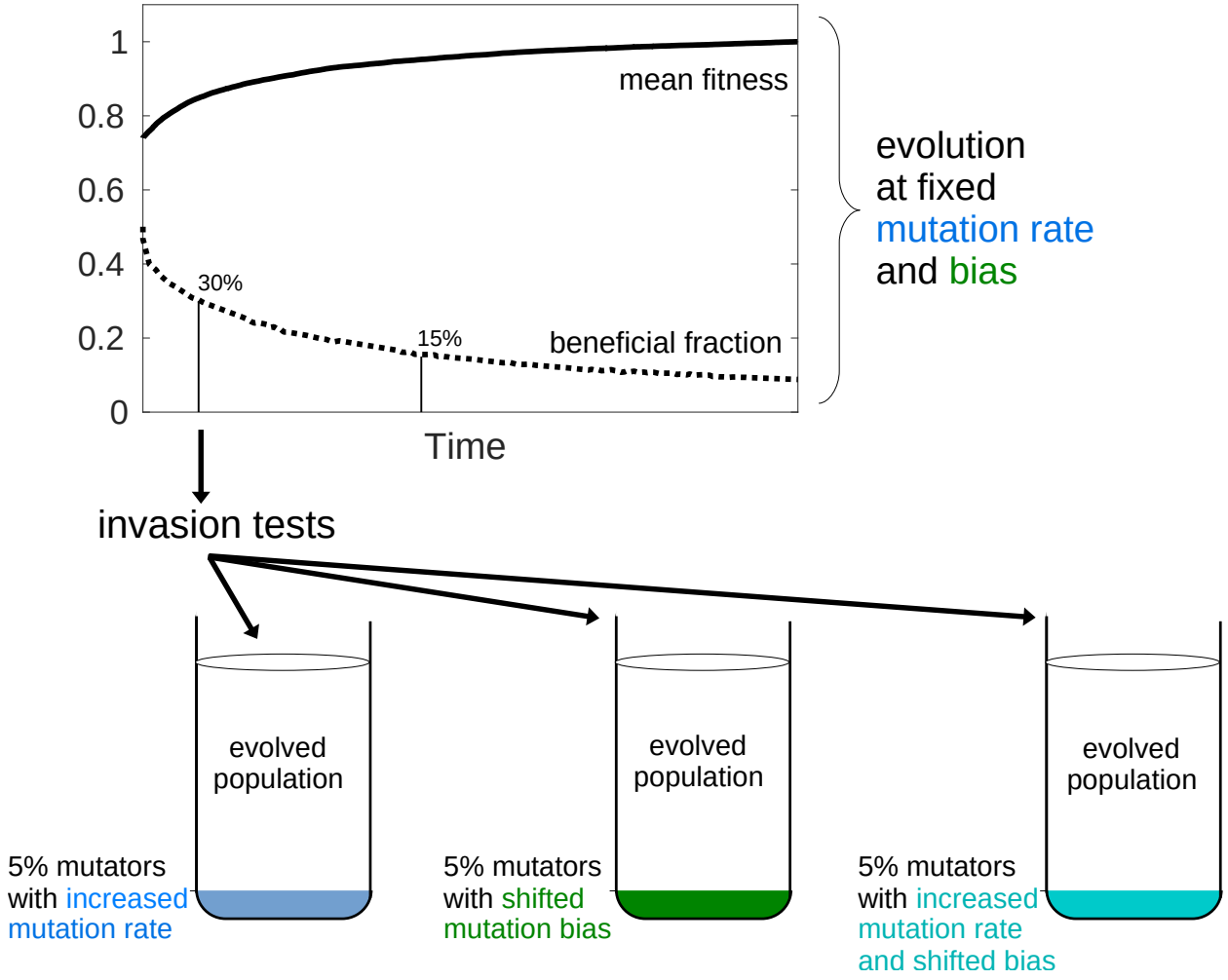

Figure 1: Schematic diagram of simulated invasion tests. Populations of initially random genotypes evolve with a fixed mutation rate and fixed transition:transversion bias. The mean population fitness (solid line) increases over time, while the fraction of mutations in the observed DFE that are beneficial (dotted line) declines. When the beneficial fraction reaches 30%, we perform the three invasion tests illustrated. The same tests are repeated in new simulations but when the beneficial fraction in the evolving population reaches 15%. For each 'replicate', we create a new fitness landscape, initiate a new population, and evolve starting at time zero.

lineage from the original subpopulation becomes the most common genotype in the population. For an estimated invasion probability p, the standard deviation of the estimate is computed as $\sqrt{p(1-p)/n_{\text{reps}}}$.

Parameter values

Unless otherwise indicated, our simulations used a sequence of length $N = 100$ and epistasis parameter $K = 0$ (no epistasis) or $K = 1$ or 2. We used a carrying capacity of $\kappa = 5000$ and initialized simulations with 50 random genotypes, each with 100 individuals. We note that for a randomly generated genotype in the NK-model, 50% of mutations are expected to be beneficial.

The default sequence mutation rate in the simulations was $\mu = 0.0001$ per genome per generation, the order of magnitude of *E. coli* K12 (Sane et al., 2021).

Invasions were initiated at a population frequency of 5%, and invasion success is determined after 5000 generations. Note that our results were not sensitive to this final time, as long as it was sufficiently large.

Results

The fraction of beneficial mutations in the over-sampled mutation class rapidly declines

Our analytical work predicts that beneficial mutations in over-sampled mutation classes fix more frequently than those in under-sampled classes, reducing the beneficial fraction of the DFE for over-sampled classes. We confirm this effect in full populations by simulating the evolution of a transition-biased population, recording the beneficial fraction of all possible transitions, fb_{Trs}, and all possible transversions, fb_{Trv}, for the most common genotype in the population at any time.

Although the beneficial fraction is initially the same in both mutational classes, as evolution

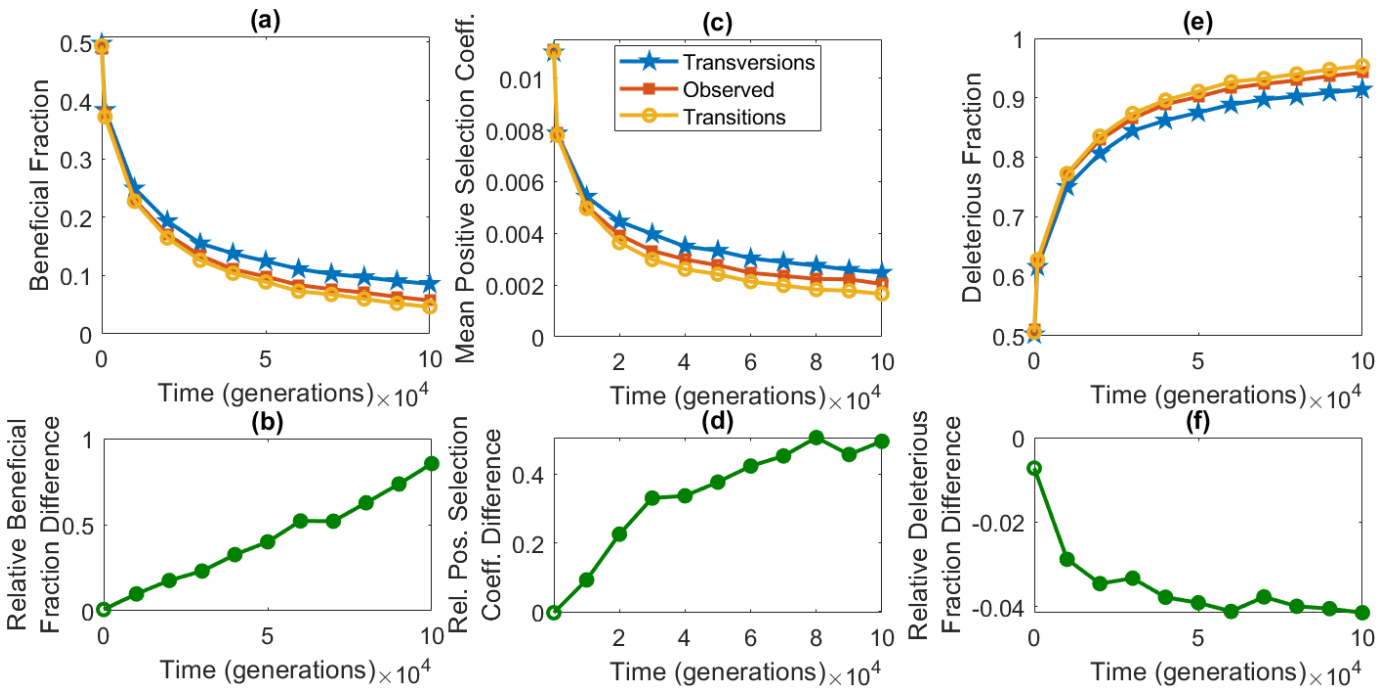

Figure 2: Beneficial fractions and mean beneficial effect sizes decline during the evolution of a transition-biased population, while deleterious fractions increase. (a) The fraction of beneficial transitions (the over-sampled class, yellow circles) decreases over time, falling more rapidly than the fraction of beneficial transversions (blue stars). The overall observed beneficial fraction of the DFE (red squares) falls between these two extremes (closer to fb_{Trs} as expected since the transition bias $\beta = 0.7$). (b) As a result of their different decay rates, the relative difference in the beneficial fraction, $(fb_{\text{Trv}} - fb_{\text{Trs}})/fb_{\text{Trs}}$ increases over time; filled circles indicate that the difference $fb_{\text{Trv}} - fb_{\text{Trs}}$ is significantly different from zero at that time (t test, $p < .05$). Panels (c) and (d) show analogous results for the mean positive selection coefficients of transitions and transversions. (e) As the beneficial fraction decreases, the deleterious fraction increases. The fraction of deleterious transitions (yellow circles) increases more rapidly than the fraction of deleterious transversions (blue stars). (f) The relative difference in the deleterious fraction increases in magnitude over time, but the effect is modest (less than 5% difference). In all cases, means of 100 simulation replicates are shown for $\beta = 0.7$ and epistatis degree $K = 2$; DFEs are computed for the most common genotype in the population at each time point. Error bars in top panels are smaller than symbol heights and are omitted.

proceeds, fb_{Trs} decays more quickly than fb_{Trv} (Fig. 2(a)). The observed beneficial fraction de-
294 clines but remains between fb_{Trs} and fb_{Trv}. The difference between fb_{Trs} and fb_{Trv} increases with time as beneficial transitions fix more often than beneficial transversions, and is significantly different from zero except at very early timepoints (panel (b)). After 100,000 generations, there
297 are roughly twice as many beneficial transversions as transitions (panel (b)); this factor of two also reflects the maximum possible increase in the beneficial fraction for a bias-shifted strain, equivalent to the factor G in the heuristic argument above, for an extreme bias shift from tran-
300 sitions to transversions. Although this factor could increase further at later times, the simulated populations have little remaining adaptive potential at the end of the simulation time.

In addition, transversions not only exceed transitions in their beneficial fraction, but also in
303 the mean magnitude of the positive selection coefficient; in other words, as evolution progresses a single beneficial transversion is expected to be more advantageous than a single beneficial transition (Fig. 2(c) and (d)). At the end of the simulation time, the effect size of a beneficial
306 transversion was about 1.5-fold higher than a beneficial transition, corresponding to factor H in the heuristic argument.

As evolution proceeds and the beneficial fraction of the DFE is reduced, the deleterious frac-
309 tion concomitantly increases. Again, the deleterious fraction for both mutational classes is initially 50%, but the deleterious fraction for the oversampled class, fd_{Trs}, increases more quickly than fb_{Trv} (Fig. 2(e)). The difference between fd_{Trs} and fd_{Trv} increases in magnitude over
312 time (panel f), but the effect is more modest than the changes observed in the beneficial fraction. After 10,000 generations, the difference in the deleterious fraction is less than 5%. Overall, the observed increase in the deleterious fraction predicts a modest increase in the deleterious load
315 for an over-sampled mutational class.

We note for completeness that the mean negative selection coefficient changes only negligibly during evolution (not shown); differences between the deleterious selective effect for transitions
318 and transversions were at most 2% and were not significantly different from zero at all time points (t tests; p-values in the range .07 to .98) except the final time of 10^5 generations ($p < .03$).

Bias reversals increase the invasion probability of mutators, and can reverse the expected outcome of invasion

Given the potential increase in beneficial fraction afforded by a bias shift, we next asked whether strains with a bias shift would invade a wildtype population. Figure 3 shows results for both smooth (blue) and epistatic (red) fitness landscapes, for populations at two degrees of adaptive potential (30% and 15% remaining beneficial mutations, left and right panels respectively), and for the limiting case of a full bias shift ($\beta = 1 \rightarrow \beta' = 0$, top panels) and a second case of a strong bias shift ($\beta = 0.9 \rightarrow \beta' = 0.1$, lower panels).

As a comparison, we tested the invasion probability of mutator strains with an F-fold increase in mutation rate (x-axis), but no change in the mutation spectrum (solid lines). On the left edge of each panel (y-intercepts), we confirm that strains that change neither the mutation rate nor the bias have a 5% invasion probability when seeded at an initial frequency of 5%, the neutral expectation. As the mutation rate multiple, F, is increased, mutator strains are initially favoured (have an invasion probability higher than neutral), particularly in populations with more adaptive potential (left panels). Further increases in mutation rate become disfavoured, however, as the deleterious load overwhelms the benefit of accessing beneficial mutations more easily. In general we see that mutators are strongly favoured when many beneficial mutations are available (left panels), but high mutation rates are disadvantageous when the population is closer to the fitness peak (right panels). We also note that mutators are favoured on epistatic landscapes (solid red versus solid blue), presumably because of an enhanced ability to cross fitness valleys on rugged landscapes.

We then tested shifts in mutation bias (dashed lines). The left edge of each panel thus shows the invasion probability for a strain that has a bias shift (dashed lines) but no change in mutation rate; in all cases the bias shift increases the invasion probability above the neutral expectation, but even in the most extreme case ($fb_{\text{obs}} = 15\%$ and $\beta = 1 \rightarrow \beta' = 0$), the advantage of the bias-shifted strain is rather modest.

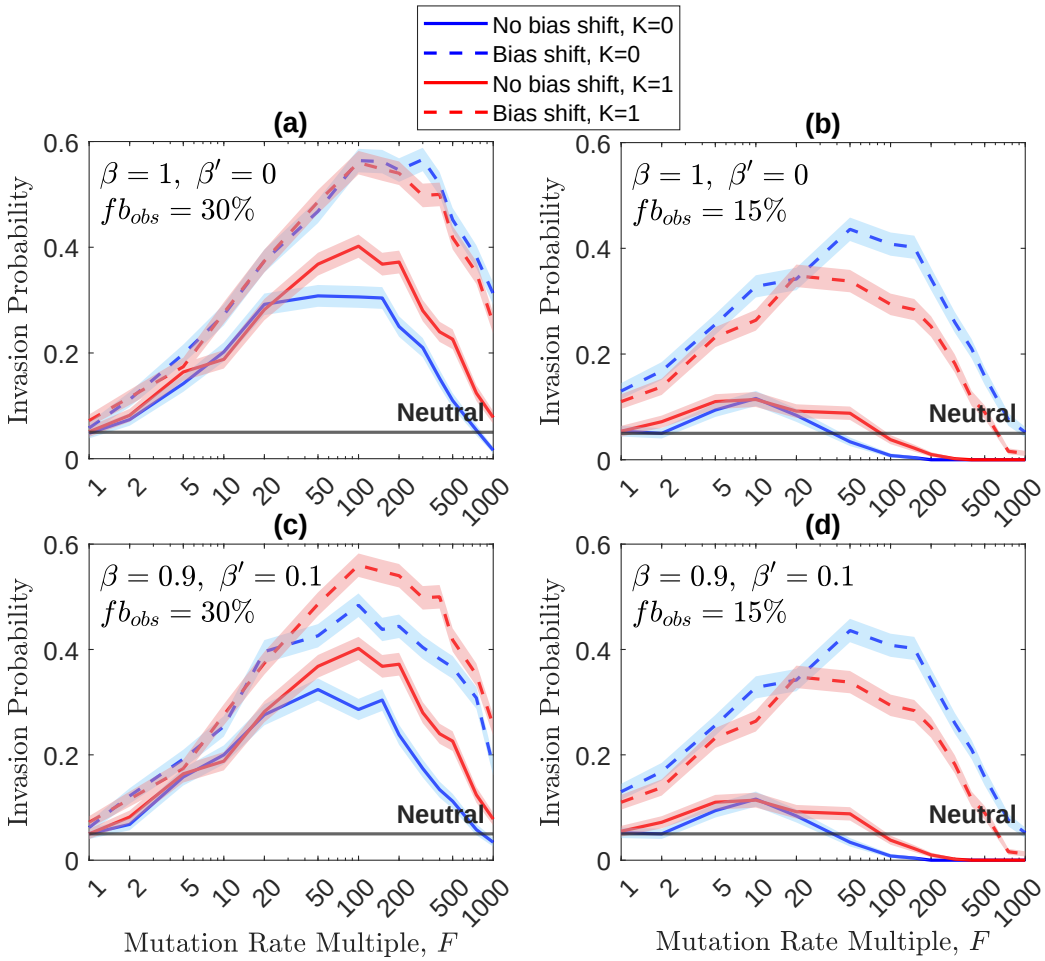

Figure 3: Invasion probabilities for strains with both bias shifts and increased mutation rates (dashed lines) exceed invasion probabilities for strains that increase the mutation rate only (solid lines). Simulation results are shown in which each invading strain is initiated at 5% of the population and has an F-fold increase in the mutation rate (x-axis); we compare no change in bias (solid lines), with a full change in bias ($\beta = 1 \rightarrow \beta' = 0$, top row), or a strong change in bias ($\beta = 0.9 \rightarrow \beta' = 0.1$, bottom row). Invasion tests occur for populations at two levels of adaptive potential, $fb_{\text{obs}} = 30\%$ (left panels) and $fb_{\text{obs}} = 15\%$ (right panels). The case of epistasis ($K = 1$) is shown in red, while the blue lines show the case of no epistasis ($K = 0$). The horizontal lines represent the neutral expectation 0.05, which represents the invasion probability of a 5% subpopulation in the absence of phenotypic change. Results of 500 replicates are shown; shaded regions indicate $\pm$ one standard deviation.

The effect of a bias shift can be dramatically increased, however, for mutator strains (dashed lines versus solid lines at $F > 1$). In all cases, mutators have substantially higher invasion probabilities if they also reverse the mutation bias. As predicted theoretically, in all cases the bias shift extends the range of mutation rates over which mutators are favoured. For mutator strains with very high mutation rates, the invasion probability can change from below the neutral expectation or near zero, without the bias shift, to values as high as 20-40%. We also point out that the effect of combining an increase in mutation rate with a bias shift, in these full population simulations, is highly nonlinear. If we take as an example the smooth fitness landscape (blue lines) in Figure 3(b), a 100-fold increase in mutation rate, alone, reduces the invasion probability below the neutral expectation, while the bias shift, alone, increases the invasion probability by a factor of just over two (y-intercept). However a strain that both shifts the bias and increases the mutation rate 100-fold has an invasion probability that is 8-fold higher than neutral.

The direction of the bias shift relative to an unbiased spectrum determines the effect of the shift

Having established that bias shifts, at least in these extreme cases ($1 \to 0$ and $0.9 \to 0.1$), are more likely to emerge when coupled with changes in mutation rate, we now investigate bias shifts at a range of directions and magnitudes on the fate of mutator strains.

As described in the *Methods* section, the effect of a bias shift depends on the unbiased mutation frequency, α, the bias with which the population has been evolving prior to the shift, β, and the shifted bias β'. Figure 4 shows how this applies to the transition-transversion ratio, where $\alpha = 1/3$. We first investigate changes in the existing bias, β. In particular, after populations have evolved with various values of β, we quantify the invasion probability of a mutator strain with a 50-fold increase in mutation rate, comparing the fate of this mutator strain without a bias shift (solid lines) to its fate with a bias shift to $\beta' = 0.1$ (dashed lines). When the population is initially transition biased (to the right of the vertical line), a shift to $\beta' = 0.1$ reduces or reverses

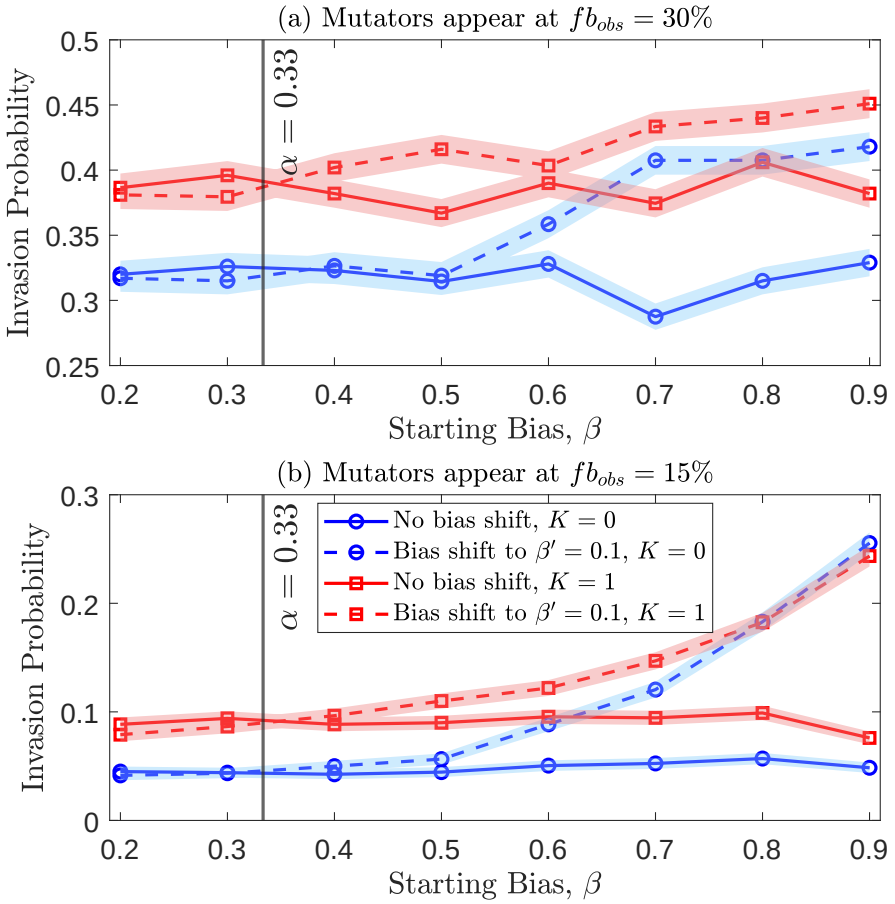

Figure 4: Invasion probabilities of mutators increase when the bias is reversed and decrease when it is reinforced. Populations evolved with a fixed starting bias (x-axis) until the beneficial fraction of the most common genotype reached (a) 30% or (b) 15%. An invasion test was then simulated for a strain with a 50-fold increase in mutation rate. Two cases are compared: (1) the mutators either have the same bias as the initial population (solid lines), or (2) have a bias shift to $\beta' = 0.1$ (dashed lines). The vertical line at $\alpha = 1/3$ represents the unbiased transition frequency. For a starting bias less than α, a shift to $\beta' = 0.1$ tends to reduce the invasion probability; the same bias shift increases the invasion probability if the starting bias exceeds α. Results of 2000 replicates are shown; shaded regions indicate $\pm$ one standard deviation.

the previous bias, and the invasion probability is increased by the bias shift; in contrast, if the population is initially transversion biased (to the left of the vertical line), the bias shift reinforces the existing bias, and tends to reduce the invasion probability. Results are shown for populations at two degrees of adaptive potential (30% and 15% remaining beneficial mutations, panels (a) and (b) respectively) and for both smooth and epistatic fitness landscapes.

Stronger bias reversals increase the invasion probability

While the direction of the bias shift determines whether it is beneficial, the magnitude of the shift modifies the magnitude of the benefit. Figure 5 shows that stronger reversals have a higher invasion probability, again in the presence or absence of epistasis and for populations relatively early and later in adaptation. In contrast with Figure 4, here we fix the starting bias, $\beta = 0.9$, and shift the bias to various values of β', all for mutator strains with a 50-fold increase in the mutation rate.

Discussion

Previous work has provided compelling evidence that changes in mutation spectrum can drive both the emergence and decay of mutators (Couce et al., 2017, 2013; Couce and Tenaillon, 2019). Here, we provide a mechanistic and general explanation of why, and under what conditions, these effects might occur. In particular, we demonstrate that when a spectrum change either reduces or reverses an existing bias, that is, a bias that has persisted over a period of adaptation, that spectrum change will both increase the beneficial fraction of the observed DFE and increase the mean selective effect of beneficial mutations.

Our analytical approach, while necessarily simplified, demonstrates mathematically that after a period of adaptation, bias reductions or reversals are expected to increase the beneficial fraction of the DFE, and that this effect will be stronger with larger magnitude reversals. This prediction is confirmed in simulations that relax several simplifying assumptions, treating full populations

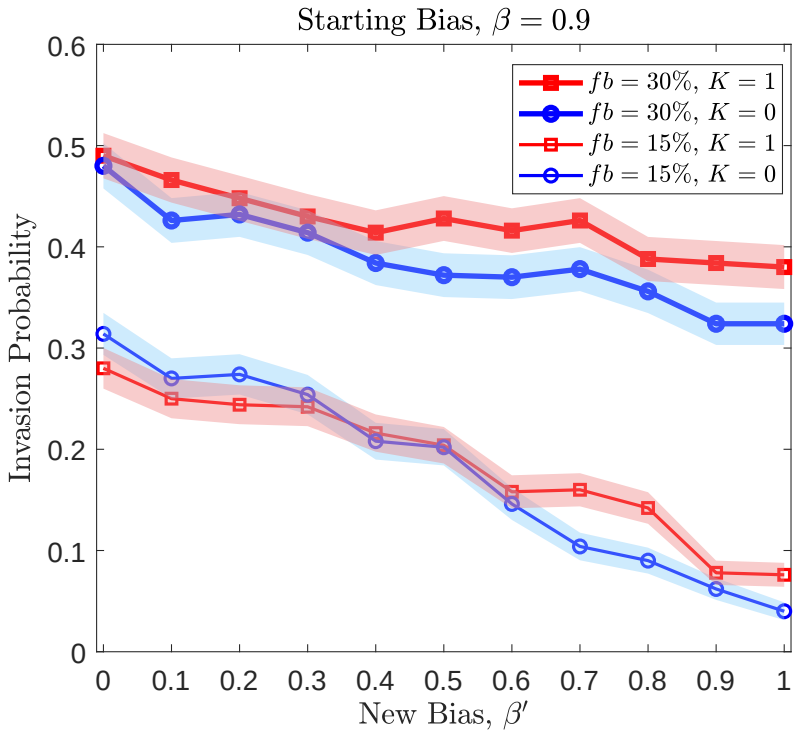

Figure 5: In populations that have evolved with a transition bias of $\beta = 0.9$, the invasion probability of mutators is increased for larger magnitude bias reversals, and reduced when the bias is reinforced ($\beta' = 1$). Invasion tests were initialized for a mutator strain with a 50-fold increase in mutation rate and a bias shift to β' as indicated (x-axis). Larger reversals increased the invasion probability whether the invasion test occurred at an observed beneficial fraction of 30% (bold lines) or 15% (thin lines), on either smooth ($K = 0$, blue circles) or epistasic landscapes ($K = 1$, red squares). Results of 500 replicates are shown; shaded regions indicate $\pm$ one standard deviation.

under both clonal interference and epistasis.

Despite these robust changes to the DFE when the mutation bias is reduced or reversed, a surprising result of our study is that in the absence of changes to mutation rate, the invasion probability of bias-shifted strains is only modestly increased, even under very strong bias reversals (Fig. 3, y-intercepts). This is because increases in both the beneficial fraction of the DFE and increases in the positive selective effect are of the order of several fold, while mutation rate increases may be 50- or 100-fold or more (Denamur and Matic, 2006). For example, a bias shift that doubles the beneficial fraction and increases the positive selective effect by 50% (Fig. 2) alters the DFE substantially, but would confer only a three-fold advantage over the wildtype in generating beneficial fixations.

In contrast, as observed for mutations with increased beneficial effect (Couce et al., 2013), bias shifts can dramatically alter the fate of mutator strains (Fig. 3). When a shift in the bias is coupled with an increase in mutation rate, the invasion probability of the strain can be vastly increased, including cases in which a disfavoured increase in the mutation rate becomes strongly favoured if coupled with a bias reduction or reversal. This is due to three effects: (1) the expected value of the selection coefficient for beneficial mutations is increased by the bias shift; (2) the fraction of mutations that are beneficial is increased; and (3) as the beneficial fraction is increased, the fraction of mutations that are deleterious is concomitantly reduced. Of these three effects, in our simulated landscapes the increase in beneficial fraction had the greatest magnitude, while the reduction in deleterious load was modest. Further work on empirical fitness landscapes that quantify the DFE for bias-shifted strains (Couce et al., 2013; Sane et al., 2021) is necessary to determine which effects might dominate in natural settings.

We also note that after a change in mutation rate, load accrues gradually, thus mutator strains in our invasion tests may invade before the equilibrium load is realized. This extends the range of mutation rates over which the mutator strain invades beyond the theoretical prediction, which assumed equilibrium load. Since load would also accrue gradually in natural settings after a loss of DNA-repair function, our theoretical prediction is conservative, underestimating the success

of bias-shifted mutators. More accurate predictions could include the dynamics of mutational load after a sudden change in mutation rate.

The effects we describe are relevant to asexual evolution, in which changes to mutation rate and/or bias remain linked to the beneficial mutations they generate for sufficient time to reach fixation. Previous work suggests that for mutation rate modifiers, modest levels of horizontal gene transfer or recombination substantially reduce the mutator advantage (Johnson, 1999; Tenaillon et al., 2000). Since DNA repair enzymes in bacteria are frequently gained and lost (Denamur and Matic, 2006; Sane et al., 2021), invasion simulations that couple bias shifts and mutation rates estimated in specific bacterial knock-out strains are another clear avenue for future work.

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Statement of Authorship

MT, DC, RNG and LMW designed the study. LMW, MT and SV wrote the simulation code. MT and SV generated simulation data; MT, DC, RNG and LMW analysed and interpreted simulation data. MT and LMW derived the theory and drafted the manuscript. All authors edited and finalized the manuscript.

Data and Code Accessibility

Code and data can be accessed at `doi:10.5061/dryad.qz612jmk2`

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