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Stress-induced mutagenesis and complex adaptation

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Because mutations are mostly deleterious, mutation rates should be reduced by natural selection. However, mutations also provide the raw material for adaptation. Therefore, evolutionary theory suggests that the mutation rate must balance between *adaptability*—the ability to adapt—and *adaptedness*—the ability to remain adapted. We model an asexual population crossing a fitness valley and analyse the rate of complex adaptation with and without stress-induced mutagenesis (SIM)—the increase of mutation rates in response to stress or maladaptation. We show that SIM increases the rate of complex adaptation without reducing the population mean fitness, thus breaking the evolutionary trade-off between *adaptability* and *adaptedness*. Our theoretical results support the hypothesis that SIM promotes adaptation and provide quantitative predictions of the rate of complex adaptation with different mutational strategies.

1. Introduction

There is experimental, clinical and theoretical evidence that high mutation rates increase the rate of adaptation and that during adaptive evolution, constitutive mutators—alleles that constitutively increase the mutation rate—can rise in frequency because of the beneficial mutations they generate [1–3]. However, during evolution in a stable environment, constitutive mutators become associated with poor genetic backgrounds due to increased accumulation of deleterious mutations; this was evidenced both in the laboratory [4] and in the clinic [5]. Classical models suggest the 'reduction principle', which states that natural selection reduces the mutation rate in a stable environment [6,7]. But many adaptations require new beneficial mutations, especially in asexual populations. This tension between the effects of beneficial and deleterious mutations leads to 'the rise and fall of the mutator allele' [8], where mutator alleles increase in frequency in a maladapted population, only to be eliminated by natural selection when the population is well adapted. This dynamic was studied using experimental evolution [9,10], mathematical analysis and simulations [11–13].

Thus, the mutation rate must balance between two evolutionary traits, as Leigh [14] suggested: *adaptability*—the capacity to adapt to new environmental conditions—and *adaptedness*—the capacity to remain adapted to existing conditions.

Stress-induced mutagenesis (SIM)—the increase of mutation rates in stressed or maladapted individuals—has been demonstrated in several species, including both prokaryotes and eukaryotes [15]. SIM has been observed in laboratory strains [16,17] and natural populations of *Escherichia coli* [18] (but see [19]), and in other species of bacteria such as pseudomonads [20], *Helicobacter pylori* [21], *Vibrio cholera* [22] and *Streptococcus pneumonia* [23]. SIM has also been observed in yeast [24,25], algae [26], nematodes [27], flies [28] and human cancer cells [29]. Several stress responses regulate the mutation rate in bacteria by shifting replication to error-prone DNA polymerases [30] and by inhibiting the mismatch repair system [31]. These stress responses include the SOS DNA-damage response, the RpoS-controlled general or starvation stress response, and the RpoE membrane protein stress response [32].

It is still not clear how SIM affects evolution and adaptation. Some authors have proposed that SIM has a significant impact on *adaptability* or *evolvability* [17,33,34], but there is no theoretical treatment of this impact. On the other hand, the effect of SIM on *adaptedness* was studied with deterministic [35]
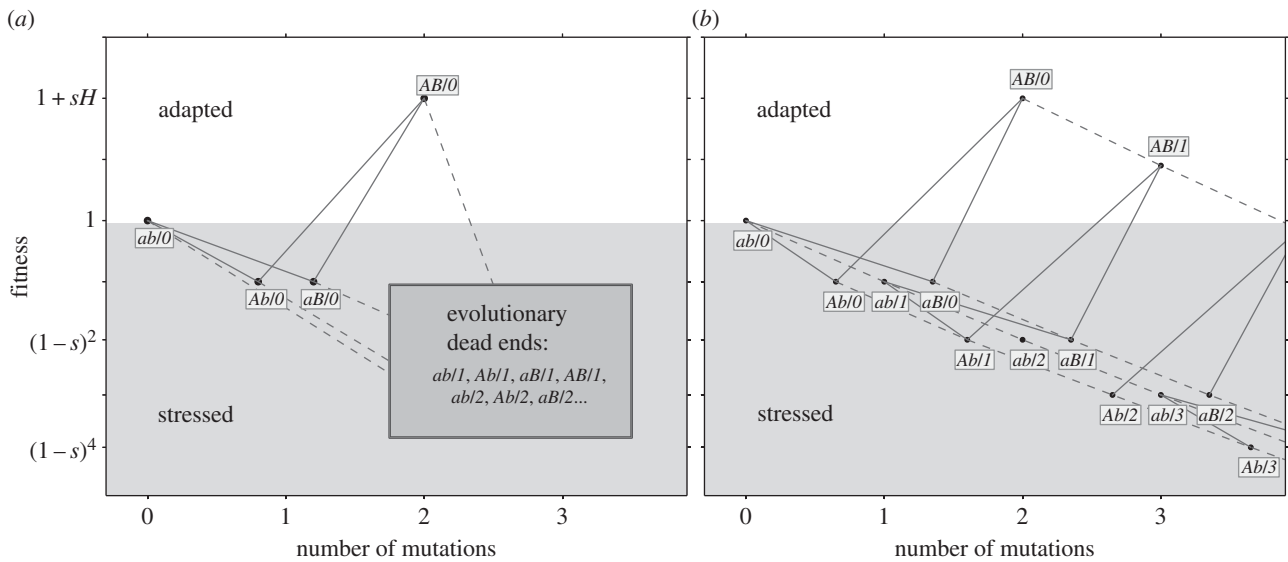
Figure 1. Adaptation on a rugged fitness landscape. The figure shows the fitness of the possible genotypes, which are represented by the allele combination at the specific loci (*ab*, *Ab*, *aB* and *AB*) and the number of deleterious alleles across the genome following the forward-slash ('/'). The *y*-axis represents fitness: the wild-type *ab/0* has fitness 1; the fittest genotype *AB/0* has fitness $1 + sH$; deleterious alleles, either at the *A/a* and *B/b* loci, or at the non-specific loci, reduce fitness by $1 − s$. The *x*-axis represents the number of accumulated mutations (genotypes are jittered to increase separation). Solid lines represent mutations at the *a/A* and *b/B* loci, occurring with probability μ. Dashed lines represent deleterious mutations in the rest of the genome, occurring with rate *U*. Mutagenesis is induced in stressed genotypes with fitness less than 1 (grey background). Fit genotypes, with fitness more than or equal to 1, do not hypermutate (white background). (*a*) In the analytic model, genotypes with deleterious alleles in non-specific loci are considered 'evolutionary dead ends' and do not contribute to adaptation. (*b*) In the simulations, individuals can accumulate up to 25 deleterious alleles (the figure only shows three). Multiple mutations can occur simultaneously but are not shown for simplicity of the illustration.

and stochastic [36] models. These models showed that without beneficial mutations SIM does not affect the mean fitness of asexual populations in stable environments, in contrast with constitutive mutagenesis (CM), which decreases the population mean fitness. More recently, we have shown that with rare beneficial mutations, if maladapted individuals increase their mutation rate then the population mean fitness of asexual populations increases [37].

Complex traits, coded by multiple genes, present an open evolutionary problem, first described by Sewall Wright in 1931 [38,39]: if different alleles are separately deleterious but jointly advantageous, how can a population evolve from one co-adapted gene complex to a fitter one, crossing a less fit 'valley'? Wright suggested the 'shifting balance theory of evolution'. His solution is valid [40–42], but possibly limited to specific parameter ranges [43–46]. As a result, other mechanisms have been proposed: increased phenotypic variance after population bottlenecks [47], environmental fluctuations [48], environmental heterogeneity [49], fitness-associated recombination [50], stochastic tunnelling in large asexual populations [51] and intermediate recombination rates [52].

Here, we analyse population genetics models of adaptive evolution to explore the rate of complex adaptation on rugged fitness landscapes, in which adaptations require two separately deleterious mutations. We develop analytic approximations and stochastic simulations, and compare normal, constitutive and stress-induced mutagenesis. We show that SIM can break the trade-off between *adaptability* and *adaptedness* by increasing the rate of complex adaptation without decreasing the population mean fitness.

2. Model

We model a population of *N* haploid asexual individuals with a large number of loci in full linkage. The model includes the effects of mutation, selection and genetic drift. Individuals are characterized by their genotype in two specific bi-allelic loci—*ab*, *Ab*, *aB* and *AB*—and by the number of deleterious mutations they carry in the rest of the non-specific loci. For example, *aB/3* is the *aB* genotype with three additional deleterious mutations in non-specific loci.

We focus on adaptation to a new rugged fitness landscape. The fitness of the wild-type *ab/0* is 1, the fitness of the single mutants *Ab/0* and *aB/0* is $1 − s$, and the double mutant *AB/0* has the highest fitness $1 + sH$, where *s* is the selection coefficient and *H* is the relative advantage of the double mutant. This is the simplest case of a rugged fitness landscape: the single mutants *Ab/0* and *aB/0* are fitness valleys between the local and global fitness peaks *ab/0* and *AB/0* (figure 1). We do not consider smooth fitness landscapes in which single mutants have intermediate fitness $(1 + sH > 1 − s > 1)$. We have already shown that SIM has higher mean fitness in changing environments on smooth landscapes [37]; however, analysis of the effect of SIM on the adaptation rate on smooth landscapes will be the subject of future efforts.

Deleterious mutations in the non-specific loci independently (multiplicatively) reduce the fitness of the individual by $1 − s$. Mutations occur in the specific loci with probability μ. The number of new mutations per replication in the rest of the genome (the non-specific loci) is Poisson distributed with an average *U*. The model neglects back-mutations and compensatory mutations due to their insignificant short-term effects.

We consider three mutational strategies: normal mutagenesis (NM), where there is no increase in the mutation rate; CM, where all individuals always increase their mutation rate by τ, the mutation rate fold increase; and SIM, where only stressed or maladapted individuals increase their mutation rate by τ. Individuals are considered stressed if their fitness is below a specific threshold, so stress can be caused by a deleterious mutation (either in the specific *A/a*

and B/b loci or in non-specific loci). Evidence shows that numerous stress responses induce mutagenesis in bacteria [16,32]. These responses can be activated due to deteriorating environmental conditions (see §3e) or due to mutations that impair important cell functions, thereby reducing fitness and inducing a stress response. For example, a frameshift mutation in the *lac* gene causes cells to starve on lactose, thus inducing mutagenesis via a stress response [17,53].

The main analysis assumes that SIM induces mutagenesis in individuals less fit than the wild-type; that is, the mutation rate U of individuals with fitness ω is

$$U(\omega) = \begin{cases} U, & \omega \geq 1 \\ \tau U, & \omega < 1. \end{cases} \qquad (2.1)$$

This equation models a scenario in which an environmental change (i.e. appearance of a new ecological niche or a new carbon source) provides an opportunity for adaptation without affecting the fitness of the wild-type ($ab/0$). We also study a different scenario in which the environmental change reduces the absolute fitness of the wild-type so that it is also stressed (see §3e). The assumption of a threshold relationship between fitness and the mutation rate in equation (2.1) is relaxed in the electronic supplementary material, appendix E, in which we explore continuous relationships between fitness and the mutation rate. The results are robust to this relaxation (see §3c).

We are interested in calculating the adaptation rate of a population homogeneous for each of the above mutational strategies (NM, CM or SIM). The adaptation process is separated into two distinct stages. In the first stage, a double mutant AB appears in the population, usually in a single copy. In the second stage, the double mutant either goes to extinction or avoids extinction, increases in frequency and goes to fixation.

We analysed this model with two methods. The first is analytic (figure 1a), in which we assume that: (i) genotypes with deleterious backgrounds (deleterious alleles in the non-specific loci) do not contribute to the adaptation process, and (ii) the number of deleterious alleles per individual before the appearance of a double mutant is at a mutation-selection balance (MSB) and is Poisson distributed with mean U/s [54]. The former assumption requires that mutation is weaker than selection ($U \ll s$); the later assumption only requires that mutation is not much stronger than selection. Specifically, the expected number of mutation-free individuals is at least one: $Ne^{-U/s} > 1 \Rightarrow U < s \cdot \log N$ [55].

The second method is a stochastic Wright–Fisher simulation with selection, mutation and genetic drift (figure 1b), in which (i) individuals with a deleterious background can contribute to adaptation, and (ii) a mutation-free population evolves towards a MSB without assuming a Poisson distribution of the number of deleterious alleles.

(a) Wright–Fisher simulations

We track the number of individuals in each genotype class: ab/x, Ab/x, aB/x and AB/x, where $x \geq 0$ is the number of deleterious alleles in non-specific loci. The simulations start with a single-peak smooth fitness landscape (the fitness of AB/x is $(1-s)^{2+x}$) and a mutation-free population (all individuals start in the optimal $ab/0$ genotype with fitness 1) that accumulates deleterious mutations over the first 5000 generations of the simulation. With $s = 0.05$ and 0.005, 180

Table 1. Model parameters and estimated values for *E. coli*.

symbol	name	estimate	references
s	selection coefficient	$0.001 - 0.03$	[57,58]
H	double mutant advantage	$1 - 10$	[58]
U	genomic deleterious mutation rate	$0.0004 - 0.003$	[59,60]
μ	site-specific mutation rate	$U/5000$	[58]
τ	fold increase in mutation rate	$1 - 100$	[18,61]
N	population size	$10^5 - 10^{10}$	[62,63]

and 1800 generations are enough for the average number of deleterious alleles per individual to reach 99.99% of its MSB value, U/s [56].

After 5000 generations, the fitness landscape changes to a rugged one, making AB the optimal genotype with fitness $1 + sH$ (figure 1b). The simulation then proceeds until an AB genotype appears and either fixates in the population or goes extinct (either all or no individuals are in the AB classes, respectively). Therefore, each simulation provides one sample of the waiting time for the appearance of a double mutant and one sample of the probability of fixation of a double mutant. At least 1000 simulations were performed for each parameter set.

Table 1 summarizes the model parameters with estimated values for *E. coli*.

3. Results

(a) Appearance of a double mutant

We are interested in the waiting time for the appearance of a double mutant AB either by a double mutation in a wild-type individual ab, or via a single mutation in a single mutant Ab or aB (figure 1a). Denoting the population size by N, we note that (i) if $Ne^{-U/s}(\mu/s)^2 > 1$, then double mutants are already expected at the MSB and adaptation will not require new mutations, and (ii) if $Ne^{-U/s}\mu/s < 1$, then no single mutants are expected at the MSB and double mutants must be generated by a double mutation in a wild-type individual. In this case, increasing the mutation rate of individuals with fitness below 1 will have no effect on the appearance of the double mutant and there is no point in analysing the effect of SIM.

Combining the two constraints, we get this constraint on the population size N: $e^{U/s}s/\mu < N < e^{U/s}(s/\mu)^2$. This constraint is reasonable for bacterial populations (see table 1).

The frequencies of wild-type (ab) and single mutants (aB and Ab combined) that are mutation-free at the MSB are roughly $e^{-U/s}$ and $2\mu/s \cdot e^{-U/s}$, respectively. The probability that an offspring of a wild-type or single mutant parent is a double mutant AB is μ^2 and μ, respectively. The probability that such an offspring is also mutation-free in the rest of its genome (the only mutations that occurred were at the specific loci) is e^{-U}. Therefore, the probability q that a random

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offspring is a double mutant, given there are no double mutants in the current generation, is approximated by

$$q = \mu^2 e^{-U/s-U} + 2\frac{\mu^2}{s}e^{-U/s-U} \approx 2\frac{\mu^2}{s}\left(1 - \frac{U}{s}\right). \quad (3.1)$$

The first expression assumes that individuals with a deleterious background do not contribute to adaptation and that the MSB distribution of deleterious alleles is Poisson. The second expression also assumes that mutation is much weaker than selection: $U \ll s$.

With SIM, the mutation rate of single mutants is increased τ-fold and the probability that a random offspring is a double mutant is

$$q_{SIM} = \mu^2 e^{-U/s-U} + 2\frac{\tau\mu^2}{s}e^{-U/s-\tau U} \approx q \cdot \tau(1 - \tau U). \quad (3.2)$$

These expressions use the same assumptions as in equation 3.1. The second expression also assumes that $\tau U < 1$.

Appendix A in the electronic supplementary material includes full derivations of the above equations and figure S1 compares them with simulation results.

(b) Fixation probability of the double mutant

Assuming an advantage to the double mutant ($H > 1$) and a large population size (see the above constraint on N), a double mutant has two possible fates after its appearance: fixation or extinction. Following Eshel [64], the fixation probability ρ of the double mutant (see the electronic supplementary material, appendix B) is

$$\rho \approx 2\frac{sH}{1 + sH} \approx 2sH. \quad (3.3)$$

That is, the fixation probability of the double mutant is roughly twice its adaptive advantage. This is a classic result of population genetics theory [65,66].

The fixation probability with SIM equals that of NM and CM because the mutation rate of the wild-type ab equals that of the double mutant AB (but see an exception in §3e).

(c) Adaptation rate

From the probability q that a random offspring is a double mutant, we can derive the probability that one or more double mutants appear in the next generation: $1 - (1 - q)^N \approx Nq$. This is a good approximation because Nq is very small due to the constraint on N. Once a double mutant appears, it goes to fixation with probability ρ.

When fixation is much faster than appearance of the double mutant AB, the time for adaptation T can be approximated by the waiting time for a double mutant that goes to fixation. This waiting time follows a geometric distribution with rate $Nq\rho$, and therefore the adaptation rate ν (the inverse of the waiting time for adaptation) is approximately

$$\nu = E[T]^{-1} \approx Nq\rho. \quad (3.4)$$

Plugging equations (3.1)–(3.3) in equation (3.4), we get these approximations:

$$\nu_{NM} = 2NH\mu^2 e^{-U/s-U}(2 + s) \approx 4NH\mu^2\left(1 - \frac{U}{s}\right), \quad (3.5)$$

$$\nu_{CM} = \nu_{NM} \cdot \tau^2 e^{-(\tau-1)U(1+s)/s} \approx \nu_{NM} \cdot \tau^2\left(1 - \frac{\tau U}{s}\right) \quad (3.6)$$

and

$$\nu_{SIM} = \nu_{NM} \cdot \frac{2\tau e^{-(\tau-1)U} + s}{2 + s} \approx \nu_{NM} \cdot \tau(1 - \tau U). \quad (3.7)$$

The middle expression in each equation is the full approximation, which assumes a Poison distribution and no contribution of deleterious genotypes to adaptation. The right-hand sides are first-order approximations that assume mutation is much weaker than selection ($U \ll s$ for NM and SIM, $\tau U \ll s$ for CM) and that $1 < \tau < 1/U$. See table 1 for description of model parameters and an article by Weinreich & Chao [67] for a result similar to equation (3.5).

The main conclusions from equations (3.5)–(3.7): first, adaptation with CM is faster than with NM. Second, adaptation with SIM is also faster than with NM, but not as fast as with CM because the mutation-free wild-type ($ab/0$) does not hypermutate.

If mutation is weaker than selection ($U \ll s$), then the adaptation rate with CM increases with τ^2 and the adaptation rate with SIM increases with τ. In addition, because the fixation probability is the same for NM, CM and SIM, the differences in the adaptation rate are due to differences in the appearance probability q (electronic supplementary material, figure S1); see §3e for a different scenario in which SIM also increases the fixation probability.

Figure 2 compares the analytic approximations with simulation results for the weak mutation regime ($U \ll s$; see the electronic supplementary material, appendix G, for results with strong mutation $U = s/10$). This regime is relevant for asexual microbes in which the deleterious mutation rate is generally 10^{-4} to 10^{-3} mutations per genome per generation and selection coefficients are estimated to be between 10^{-1} and 10^{-2} (see table 1). When the mutation rate fold increase τ is high (more than 10), the approximations slightly overestimate the adaptation rate because the double mutant AB is more likely to appear on a deleterious background ($AB/1$ instead of $AB/0$). Because the fitness of $AB/1$ is higher than that of the wild-type $ab/0$ (this happens because $1 + sH > (1 - s)^{-1} \approx 1 + s$), the double mutant can go to fixation even when it appears on a deleterious background, sweeping the deleterious alleles with it to fixation in a process called 'genetic hitch-hiking' [68]. However, these sweeps result in a lower fixation probability for the double mutant (electronic supplementary material, figure S2).

The adaptation rate of SIM with continuous relationships between fitness and mutation rate (electronic supplementary material, appendix E) is comparable to that of SIM with threshold relationships (electronic supplementary material, figure E2). This is because the main factors determining the adaptation rate are the mutation rates of the wild-type and the single mutants (ab, aB and Ab), as individuals with more than a single mutation do not have a significant contribution to adaptation. Therefore, our results are robust to the choice of the relationship between fitness and the mutation rate.

(d) The trade-off between *adaptability* and *adaptedness*

Next, we explore how different mutational strategies (NM, CM and SIM) balance between *adaptability*—the ability to adapt to new conditions—and *adaptedness*—the ability to remain adapted to current conditions. For this purpose, we define *adaptedness* as $\bar{\omega}$, the population mean fitness in a stable environment, and *adaptability* as ν, the rate of complex adaptation.
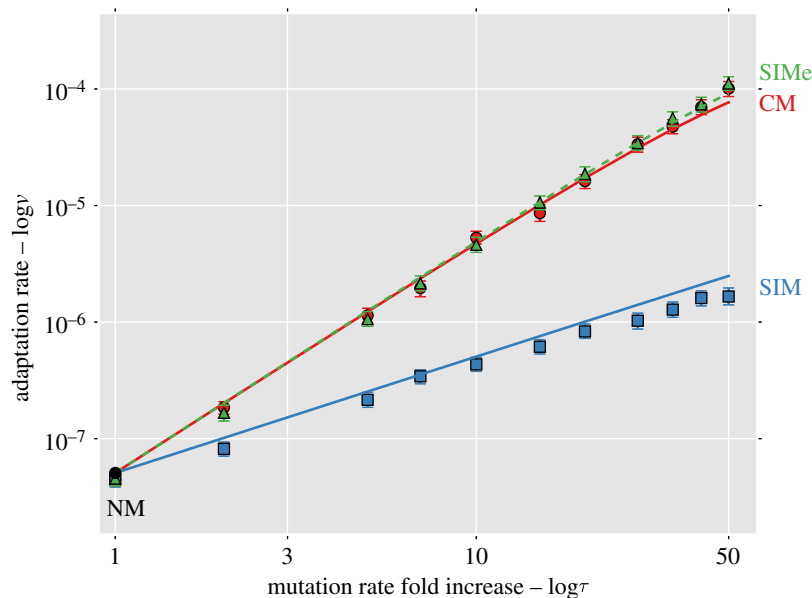
Figure 2. Complex adaptation with different mutational strategies. The figure shows the adaptation rate ν as a function of the mutation rate fold increase τ (both in log scale). The black circle is NM ($\tau = 1$); solid line with circles is CM; solid line with squares is SIM; dashed lines with triangles is SIM with environmental stress (SIMe; see §3e). Lines are analytic approximations. Markers are the means of stochastic simulation results. Error bars represent 95% CI of the mean (at least 1000 simulations per point; computed with bootstrap with 1000 samples per point). Parameters (see table 1): $U = 0.0004$, $s = 0.05$, $\beta = 0.0002$, $H = 2$, $N = 10^6$. (Online version in colour.)

We used the above approximations (equations (3.5)–(3.7)) to calculate the rate of complex adaptation of populations with NM, CM and SIM. We also extended an existing model [37] to calculate the population mean fitness at the MSB. This extended model includes rare back or compensatory mutations (which have a stronger effect on MSB dynamics than on adaptive dynamics) and allows more than one mutation to occur in the same individual and generation. The details of this model and the calculation of population mean fitness with various mutational strategies are given in the electronic supplementary material, appendix D.

The mutation rate with CM is constant and uniform across the population, and the population mean fitness mainly depends on the fitness and mutation rate of the fittest individuals. Therefore, the population mean fitness decreases when the mutation rate increases; this decrease is due to generation of deleterious mutations in the fittest individuals. The adaptation rate, however, increases with the mutation rate (equation (3.6)). This trade-off between *adaptability* and *adaptedness* constrains the population: after a long period of environmental stability it can lose the potential for adaptation, and after a long period of environmental change the population can be susceptible to reduced fitness and mutational meltdowns [69].

However, this trade-off between *adaptability* and *adaptedness* can be broken if mutation rates are not uniform across the population. Increased mutation rates in unfit individuals increase the population mean fitness, as long as beneficial (or compensatory) mutations can occur [37]. Figure D1 in the electronic supplementary material shows this advantage of SIM over NM in terms of the difference in population mean fitness ($\bar{\omega}_{SIM} - \bar{\omega}_{NM}$). Moreover, increased mutation rates in unfit individuals also increase the adaptation rate (equation (3.7); figure 2). Therefore, SIM breaks the trade-off between *adaptability* and *adaptedness*.

Figure 3 shows the adaptation rate and population mean fitness of CM and SIM compared to NM for different values of τ, the mutation rate fold increase.

Any realistic rate of adaptation ν can be realized using both CM and SIM. The highest mean fitness will always be attained with SIM, which has a small advantage over NM (it cannot be seen in figure 3, but see the electronic supplementary material, figure D1) due to the increased generation of beneficial mutations in individuals with low fitness. If, for some rate of adaptation, the mutation rate fold increase τ required by SIM is too high (i.e. $\tau U > s$), the same adaptation rate can be realized by a mixed strategy (dashed line in figure 3). For example, a 96-fold increase in adaptation rate can be achieved with CM with $\tau = 10$, with SIM with $\tau = 96$ or with a mixed strategy with $\tau_{CM} = 7$ and $\tau_{SIM} = 2$ in which all individuals increase their mutation rate sevenfold and stressed individuals further increase their mutation rate twofold. However, these increases in adaptation rates have a price: the mutational load will decrease the population mean fitness from 0.9996 with NM to 0.996 with CM and 0.9972 with the mixed strategy. This price in not paid by populations with SIM because the mean fitness mainly depends on the mutation rate of fit individuals.

(e) Environmental stress

So far, we have considered the case in which the environmental change creates an opportunity for adaptation without affecting the absolute fitness of the population—for example, a new ecological niche can be favourable without affecting the well-being of the current population. In that scenario, the wild-type *ab* was not stressed and did not hypermutate.

Next, we consider a different scenario in which an environmental change affects the well-being of the entire population: for example, exposure to an antibiotic drug or a host immune response. In this case, the environmental change does not just create an opportunity for adaptation but also causes stress in the entire population. We use a subscript 'e' to denote quantities related with this scenario.

As before, the double mutant *AB* is resistant to the stress (i.e. the drug or immune response) and therefore has a higher
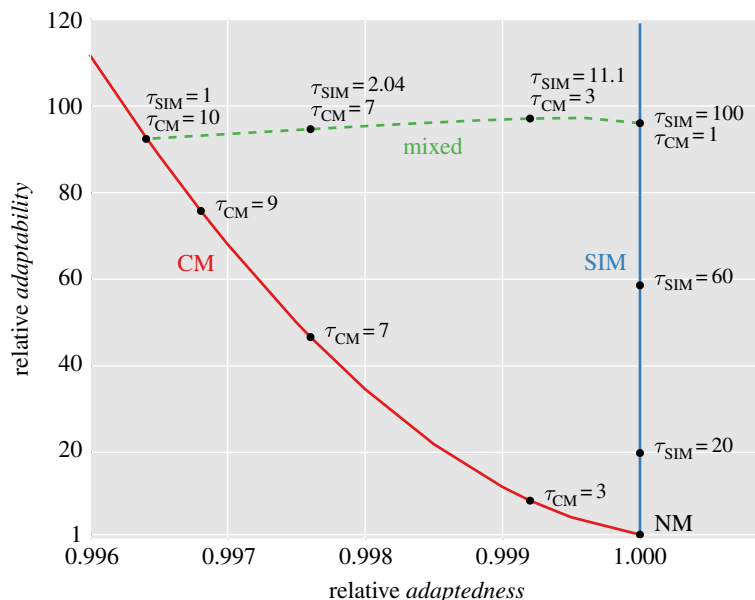
Figure 3. The trade-off between *adaptedness* and *adaptability*. The figure shows the relative *adaptedness* and the relative *adaptability* of different mutational strategies in comparison to NM. *Adaptedness* is defined by the population mean fitness at MSB, $\bar{\omega}$ (see the electronic supplementary material, appendix D). *Adaptability* is defined by the rate of complex adaptation, ν (equations (3.5)–(3.7)). CM increases the mutation rate of all individuals τ_{CM}-fold; SIM increases the mutation rate of stressed individuals τ_{SIM}-fold; mixed strategies (dashed line) increase the mutation rate of all individuals τ_{CM}-fold and of stressed individuals an additional τ_{SIM}-fold. SIM breaks off the *adaptability–adaptedness* trade-off of CM, increasing the *adaptability* without compromising the *adaptedness* of the population. Parameters (see table 1): $N = 10^6$, $U = 0.0004$, $\beta = 0.0002$, $s = 0.05$, $H = 2$, $\tau \ll s/U$. (Online version in colour.)

fitness than either the wild-type or the non-resistant single mutants. However, in this scenario, the wild-type *ab* is also stressed and therefore hypermutates with SIM (compare with equation (2.1))

$$U_e(\omega) = \begin{cases} U, & \omega > 1 \\ \tau U, & \omega \leq 1. \end{cases} \tag{3.8}$$

This scenario has an important biological relevance, as SIM has been implicated in the evolution of drug resistance in bacteria and yeast [34,70,71], and could be involved in the evolution of pathogen virulence and the evolution of drug resistance and progression in cancer cells [72].

We assume that after the environmental change, the SIM$_e$ population has reached a new MSB [56] with mutation rate τU, before the appearance of the double mutant (with $s = 0.05$ and $U = 0.0004$, for example, the average number of deleterious mutations is $0.99 \cdot U/s$ after 90 generations, whereas the adaptation time is well over 1000 generations). Under this assumption, the adaptation rate with SIM$_e$ is (see the electronic supplementary material, appendix C, for full derivation)

$$\nu_{SIM_e} \approx \nu_{CM}\left(1 + \frac{U(\tau - 1)}{sH}\right). \tag{3.9}$$

That is, adaptation with SIM$_e$ is faster than with CM (figure 2*a*). The fixation probability of double mutants is higher with SIM$_e$ than with CM, because the mutation rate of double mutants is lower than that of the rest of the population. This difference in mutation rates confers an additional selective advantage to the double mutants (see the electronic supplementary material, appendix C), which increases their fixation probability

$$\rho_{SIM_e} \approx \rho\left(1 + \frac{U(\tau - 1)}{sH}\right). \tag{3.10}$$

This additive advantage increases linearly with τ with a slope of U/sH and can be significant: for $s = 0.05$, $H = 2$ and $U = 0.0004$, increasing the mutation rate of stressed individuals 10-fold

increases the fixation probability by 3.6%. The increased fixation probability was verified by simulations (electronic supplementary material, figure S2).

4. Discussion

We studied the effect of SIM on both *adaptability* (the capacity of populations to adapt to new complex conditions) and *adaptedness* (the ability of populations to stay adapted to existing conditions) [14]. We showed that SIM breaks the trade-off between *adaptability* and *adaptedness*, allowing rapid adaptation to complex environmental challenges without compromising the population mean fitness in a stable environment.

In addition to the pure strategies of CM and SIM, our model also considers a mixed mutational strategy. There are two examples of such a mixed strategy. First, if individuals have incomplete information regarding their condition (this is the case in most realistic biological scenarios), then we expect errors in the induction of mutagenesis: induction of mutagenesis without stress and failure to induce mutagenesis under stress. In this case, the population would, on average, use a mixed strategy. Second, a mutator allele can increase the mutation rate constitutively and further increase it under stress—for example, a recent study with *Pseudomonas aeruginosa* found that although the *mutS*, *mutY* and *mutM* mutator alleles always increase the mutation rate in comparison with the wild-type, the level of this increase depends on the level of stress the cell experiences [73].

We do not assume direct fitness costs for any of the mutational strategies. A 'cost of DNA replication fidelity' [74]—the energy and time expended in order to maintain a low mutation rate—could make both CM and SIM more successful. The 'cost of fidelity' may require further study, but empirical evidence suggests that it does not play an

important role in the evolution of the mutation rate [75–78]. Another fitness cost might be associated with the regulation of the mutation rate: for individuals to determine whether their condition calls for the induction of mutagenesis, they must invest resources and energy in costly sensory mechanisms. However, such mechanisms already exist for various unrelated purposes, such as the maintenance of cell cycle and homeostasis. Therefore, we consider these mechanisms as 'free' in terms of fitness costs. For example, in *E. coli* mutagenesis is induced by several stress responses that serve other cellular functions [16,32], and this is probably the case in other organisms as well.

This article focuses on asexual populations, ignoring recombination, segregation and sexual reproduction. These mechanisms are important for adaptation on a rugged fitness landscape both because they help to cope with deleterious mutations and because they allow different single mutants to produce double mutants without an increased mutation rate. Recombination may reduce the advantage of SIM over NM in terms of population mean fitness [35], direct competitions [79] and adaptation rate (due to the Fisher–Muller effect).

Mean fitness and adaptation rate are both population-level traits. But simply because SIM has the most efficient balance between these traits does not mean it will necessarily evolve, because individual-level selection and population-level selection can act in opposing directions. In a previous article, we have demonstrated that second-order selection can lead to the evolution of SIM [37]: in an asexual population evolving on a smooth fitness landscape, selection favoured SIM over both NM and CM. In the current article, we show that selection also favours SIM on a rugged fitness landscape (electronic supplementary material, appendix F).

Several authors have suggested that the mutation rate must balance between *adaptability* and *adaptedness*: Kimura [6] found a mutation rate that balances between mutational and substitutional load; Johnson & Barton [80] found an optimal mutation rate that balances the generation of beneficial and deleterious mutations during adaptation; Leigh [81] found an optimal mutation rate that balances the generation of deleterious mutations and maintenance of standing variation in a fluctuating environment; Komarova & Wodarz [82] found an optimal rate of chromosome loss that balances the unmasking of recessive alleles and genetic load during carcinogenesis; Komarova *et al.* [83] and Agur *et al.* [84] found a time-dependent mutation rate strategy that optimizes carcinogenesis and adaptive immune response, respectively. By contrast, we find that SIM breaks, rather than balances, the trade-off between *adaptability* and *adaptedness*: it allows individuals to switch between rates optimized for stressful and benign conditions according to the circumstances.

Mutators have been suggested to play a role in cancer [85–87]. Furthermore, there is evidence that cancer cells increase their mutation rate in response to stresses such as hypoxia [88,89]. Our results suggest that such increases can have an important effect on the emergence of drug resistance, progression and metastasis of tumours [87,90].

Our model of complex adaptation on rugged fitness landscapes is similar to that of Weinreich & Chao [67], but our model includes various mutational strategies and the effects of stress and deleterious mutations. Our results (figure 2) suggest that SIM can help resolve the problem of fitness valley crossing by reducing the time required for a population to shift an adaptive peak.

Our results provide theoretical basis to the conjecture that SIM facilitates adaptation. This conjecture can be tested experimentally; for example, with *E. coli*, where it is possible to interfere with the regulation of mutagenesis [34]. The adaptation time with and without SIM can be measured in an experimental population adapting on a two-peak fitness landscape [91]. These measurements can then be compared to our analytic approximations to determine the relative advantage and disadvantage of the different mutational strategies.

5. Conclusion

SIM has been implicated as a driver of adaptive evolution for several decades. We provide theoretical treatment of this concept. Our results show that SIM increases the rate of complex adaptation and that in contrast to CM it does not jeopardize the fitness of populations under stable conditions. Because mutation is a fundamental force in every biological system, these results have important implications for many fields in the medical and life sciences, including epidemiology, oncology, ecology and evolutionary biology.

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