Hidden role of mutations in the evolutionary process

Alexandre de Aquino Soares* and Lucas Wardil
Departamento de Física, Instituto de Ciências Exatas (ICEx), Universidade Federal de Minas Gerais (UFMG), 31270-901 Belo Horizonte, Minas Gerais, Brazil

Louis Bernard Klaczko
Departmento de Genética, Evolução, Microbiologia e Imunologia, Instituto de Biologia, Universidade Estadual de Campinas (Unicamp), C. P. 6109, 13083-970 Campinas, São Paulo, Brazil

Ronald Dickman
Departamento de Física and National Institute of Science and Technology for Complex Systems, Instituto de Ciências Exatas (ICEx), Universidade Federal de Minas Gerais (UFMG), C. P. 702, 30123-970 Belo Horizonte, Minas Gerais, Brazil

(Received 1 February 2021; revised 5 October 2021; accepted 5 October 2021; published 25 October 2021)

Mutations not only alter allele frequencies in a genetic pool but may also determine the fate of an evolutionary process. Here we study which allele fixes in a one-step, one-way model including the wild type and two adaptive mutations. We study the effect of the four basic evolutionary mechanisms—genetic drift, natural selection, mutation, and gene flow—on mutant fixation and its kinetics. Determining which allele is more likely to fix is not simply a question of comparing fitnesses and mutation rates. For instance, if the allele of interest is less fit than the other, then not only must it have a greater mutation rate, but also its mutation rate must exceed a specific threshold for it to prevail. We find exact expressions for such conditions. Our conclusions are based on the mathematical description of two extreme but important regimes, as well as on simulations.

DOI: 10.1103/PhysRevE.104.044413

I. INTRODUCTION

The evolution of biological populations—the changing frequencies of genetic types over time—is driven by four principal forces: genetic drift, natural selection, mutation, and gene flow [1]. Genetic drift corresponds to fluctuations in frequencies with no preferred direction, as in unbiased diffusion. Natural selection drives the composition toward the fittest genotype. Mutation creates new types, guaranteeing genetic diversity. Last, gene flow consists of the exchange of genetic types between populations. In the standard view, mutations introduce novelty but do not determine the direction of evolution [2–4]. Once mutation has created new genetic types, the latter compete in a race determined by fitness, that is, the tendency to increase in frequency in subsequent generations, reflecting fertility, viability, and other factors given the present environment [5].

Nevertheless, mutation can have a more prominent role in evolution. Yampolsky and Stoltzfus [6] proposed a model in which one mutant is fitter while the other arises with a greater likelihood and showed that the prevailing type can shift when mutability (an overall factor multiplying all mutation rates) is varied.

Interplay between mutation rates and fitnesses has been studied via experiment and simulation, as well as analytically [7–11]. As an example of mutation having a determing role, we may cite the class of point mutations, called transitions (mutations from purine to purine or pyrimidine to pyrimidine) which occur at a higher frequency than transversions (mutations from a purine to a pyrimidine, or vice versa).

Stoltzfus and Norris [11] analyzed the hypothesis that transitions are overrepresented because they are less effective in altering proteins. They show, however, that this effectiveness is weak and thus the prevalence of transitions is not likely to be explained as selection on proteins, favoring the hypothesis that ratios between mutation rates (that is, mutation biases), may direct evolution.

In the context of adaptive evolution (the process by which fitness increases), Stolzfus *et al.* [7] found that transitions are overrepresented among adaptive mutations. More specifically, in Ref. [9] they studied the molecular basis of convergent increases in the affinity of hemoglobin for oxygen in high-altitude birds and identified causative substitutions for the increased affinity. The dinucleotide consisting of a cytosine followed by a guanine in the same strand (CpG), depending on their frequent modification by methylation, is more prone to mutation than other dinucleotides. They find that a disproportionate number of causative amino acid replacements are attributable to CpG, and because this dinucleotide is associated with a higher mutation rate, their results suggest that mutation bias influences the outcome of molecular adaptation.

Mutational dynamics is commonly analyzed using a stochastic birth-and-death process with a finite population. Because the population is finite, it will eventually become homogeneous; homogeneity can be violated if one or more mutations are possible. The probability of attaining a homogeneous state is called the *fixation probability*. Thus, if mutation rates have an effect on the direction of evolution, then they should affect fixation probabilities.

*asoares@fisica.ufmg.br

There are many stochastic models that can be applied to population genetics [12–27]. Here we analyze the Wright-Fisher model in a population starting with the wild type and evolving to one of two mutants. We study which mutation prevails as the mutation rates are varied and show that mutation rates, fitness, population size, and gene flux are all relevant to this process. We investigate the kinetics of the process as mutability is varied, focusing on two regimes, rare mutations and frequent mutations, and find analytical expressions for specific regimes, establishing thresholds for changes in prevalence.

Furthermore, if we suitably redefine the concept of prevalence in the presence of continuous migration, then the competition between mutants (up to a certain value of the rate of exchange of the population with an external wildtype population) is weaker the greater the gene flow. We study these phenomena using computer simulation and analytical approximations that become exact in specified limits. Additionally, we analyze the time evolution of the composition of the population before fixation and how it depends on the evolutionary parameters. Interestingly, coexistence of the two mutants (or clonal interference [28,29]) favors fixation of the fittest type, whereas if both mutants are quasineutral, then the higher mutation rate may dictate which type wins. The larger the population, the stronger the effect of clonal interference.

This work is organized as follows: In Sec. II we define the model. Then, in Sec. III, we demonstrate that all four mechanisms may lead to distinct evolutionary steps. Following this, we concentrate on the mechanisms of drift, selection, and mutation, for which we have robust analytical and numerical results. In the analytical study, we examine limits of the probabilities of absorption of the mutants, and the conditions for one mutant to prevail over the other in different regimes. Numerical results complement the analysis and are used to probe nonextreme regimes. Finally, in Sec. IV, we summarize our main results, compare our work with the literature, and offer perspectives.

II. THE MODEL

We study a generalization of the Wright-Fisher model [30]. Let N_W^t, N_A^t, and N_B^t be the numbers of individuals of type W (the wild type), A, and B (the two mutants), respectively, at generation t. We set $N_W^t + N_A^t + N_B^t = N$, the fixed population size. The corresponding numbers at generation $t+1$ are drawn from a multinomial distribution with probabilities proportional to:

$$\begin{aligned} p_W^{t+1} &\propto N_W^t(1 - r_A - r_B) + \left(w_A N_A^t + w_B N_B^t\right) f, \\ p_A^{t+1} &\propto (1-f) w_A N_A^t + N_W^t r_A, \\ p_B^{t+1} &\propto (1-f) w_B N_B^t + N_W^t r_B, \end{aligned} \tag{1}$$

which are normalized by their sum. The variables p_W^t, p_A^t, and p_B^t are the probabilities of the alleles W, A, and B at time t to be sampled at the next generation. $r_A = r$ and $r_B = r/\rho$ are the mutation rates for mutants A and B, r is the mutation rate or *mutability*, and ρ is the mutation bias. w_A and w_B are the fitnesses of types A and B, and f is the rate of exchange of the population with an external wildtype population. Let us fix $w_B \geqslant w_A \geqslant w_W$; the fitness of the wild type, w_W, is set to unity. Note that in this article we deal only with average to high values of the fitnesses of the mutants. It is out of our scope to study quasineutral mutations [31–33], which would demand significant changes to our model. It is usual to recast the fitness of the mutants as $w_i = 1 + s_i$, where s_i is called coefficient of selection, with $s_i > 0$, corresponding to adaptive mutations. The actual probability of mutation is equal to $r\Delta t$. But, because the process is discrete with time interval equal to unity, we sometimes refer to r_A and r_B as mutation *rates*.

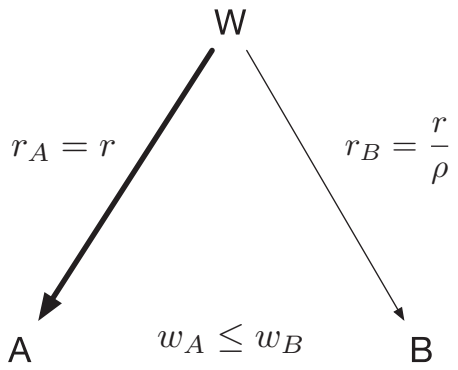

FIG. 1. Model for mutations of wildtype haploid individuals at a single locus. The wild type W can mutate to types A or B. The mutation rate to A (thicker line) is greater than to B, but the fitness of B is higher, $w_W \leqslant w_A \leqslant w_B$, in which w_W, the fitness of the wild type, is set to unity. The mutation bias, $\rho > 1$, is set so that $r_A/r_B = \rho$.

The basic model is sketched in Fig. 1. The initial, homogeneous population has only wild types. In the presence of one-way mutations and absence of gene flow ($f = 0$), which is the scenario we focus on, the population will certainly reach a state in which all individuals are of type A or all are of type B. Once an all-A or all-B state is attained, there are no further mutations, and the population remains in this state, i.e., the all-A and all-B states are *absorbing*. After the first mutation takes place and before reaching an absorbing state, the population can return, one or more times, to the initial all-W state.

The *fixation probability* of an allele in a heterogeneous population is defined as the probability that the frequency of this allele reaches unity. Because the all-W state may be revisited repeatedly before one of the absorbing states is reached, we make the following distinction between the fixation and the absorption probabilities. The former is the probability to reach a homogeneous state *for the first time* after the first mutation takes place. We denote by π_f^A, π_f^B, and π_f^W the fixation probabilities of each type. The time it takes from the first mutation until fixation is called the *fixation time*. Complementarily, we define the *absorption probability* as the probability to reach an absorbing state (all-A or all-B). We denote by π_a^A, π_a^B, and π_a^W the absorption probabilities of each type, with $\pi_a^W = 0$ and $\pi_a^A + \pi_a^B = 1$. Table I lists the symbols used in this work.

We study this model via simulation, and obtain results for all regimes of mutations, including rare and frequent mutations. Subsequently, we model analytically the latter two regimes, and investigate approximations based on these results.

TABLE I. Symbols for the evolutionary parameters and variables.

$\pi_f^W, \pi_f^A, \pi_f^B$	Fixation probability of types W, A, and B, respectively.
$\pi_a^W, \pi_a^A, \pi_a^B$	Absorption probability of types W, A, and B, respectively. $\pi_a^W = 0$.
t_f	Fixation time.
N	Population size.
N_W^t, N_A^t, N_B^t	Size of subpopulations W, A, and B, respectively, at time t.
p_W^t, p_A^t, p_B^t	Probability of an individual to become type W, A, and B, respectively, at time t.
r	Reference mutation rate. Also the mutability, a common factor that multiplies all mutation rates.
r_A, r_B	Reference mutation rates from the wild type to types A and B, respectively. $r_A = r$.
ρ	Ratio of r_A to r_B, i.e., $r_B = r_A/\rho$.
θ_A, θ_B	Mutation supply of types A and B, equal to $N\ m\ r$ and $N\ m\ r/\rho$, respectively, in a wildtype population
w_W, w_A, w_B	Fitnesses of types W, A, and B, respectively.
s_A, s_B	Selection coefficients of types A and B. $w_A = 1 + s_A$ and $w_B = 1 + s_B$.
s	Reference selection coefficient. $s_A = s$.
$\omega_A, \omega_B, \omega$	Equal to e^{-2s_A}, e^{-2s_B}, and $\omega_A/\omega_B = e^{2(s_B - s_A)}$, respectively
f	Fraction of mutants that emigrate, and are replaced by wildtype individuals.

In simulations, each realization of the process begins with N wildtype individuals. The generation at time $t + 1$ is formed by sampling independently with replacement with probabilities of sampling the types W, A, and B given by Eqs. (1) (after normalization). The simulation ends when one of the absorbing states (all-A or all-B) is attained. If a type has infinite fitness, then it fixes the generation after it appears. We calculate means and uncertainties over samples of 2^{21} ($\gtrsim 2 \times 10^6$) independent realizations. Population sizes varied from a few individuals up to 10^9. The sampling algorithm for the multinomial distribution corresponds to the command "gsl_ran_multinomial" from the GNU Scientific Library [34].

Last, we can show that if we define the selection coefficient of a single mutant allele of an n-ploid under additive fitness and we fix the number of chromosomes, then the fixation probability and fixation time are invariant with the ploidy [30]. Thus, our results remain valid for diploids and haploids if we make the transformation $2N \to N$, $s \to 2s$ to go from diploid to haploid organisms.

III. RESULTS AND DISCUSSION

This section is organized as follows. In Sec. III A we analyze the absorption probability and in Sec. III B the fixation probability and fixation times. Next, in Secs. III C and III D, respectively, we develop analytical approximations for two limiting regimes, rare and frequent mutations, and find the conditions under which the least-fit mutant prevails. Finally, in Sec. III E we extend the basic model to include more than two mutants or a slightly deleterious mutation.

A. Absorption probability

Simulation results illustrating the effects of the mutation rate and population size on the absorption probability, as well as the effect of fitness and genetic flux on the steady-state prevalence of the least-fit mutant are shown in Fig. 2. The absorption probability of a type with higher mutation rate and smaller fitness (in this case, type A) is typically close to unity if the mutation rate is low but tends to zero as the mutation rate attains higher values. The reason is that, at high mutation rates, the two types can coexist and the advantage of the fitter type may dominate evolution, depending on how close are the fitnesses of the mutants. Interestingly, even if the fittest type B has an infinite fitness value, the type A with greater mutation rate and lower fitness still may have an absorption probability close to unity for low mutation rates, as shown in Fig. 2(a). On the other hand, type A no longer has an advantage if the population size is large. In large populations, the number of individuals of the fitter type B that appear due to mutation is higher and, consequently, they take over the population with higher probability, as shown in Fig. 2(b). Figure 2(c) illustrates that increasing the fitness of A (increasing s_A) in the rare-mutation regime increases its absorption probability, π_a^A, as expected.

In the presence of migration, there is a continuous flux of W types and hence no absorbing states. The dominant mutant can be defined as the more populous one, more specifically, the one that first reaches more than half of the total population size. Figure 2(d) shows that the less-fit type is benefited by an increase in the migration rate. The frequent gene flow removal of mutants is compensated by the higher mutation rate of type A.

To better understand the simulation results, let us develop an analytical approximation for the fixation probabilities of the mutants. This approximation can then be inserted in the relation,

$$\pi_a^i = \frac{\pi_f^i}{\pi_f^A + \pi_f^B}, \tag{2}$$

where $i \in \{A, B\}$, to obtain the absorption probabilities. Suppose that all mutations that may happen take place at a single generation, an assumption that simplifies the analysis. The probability of a set of subpopulations, (N_W, N_A, N_B), in the presence of mutation and drift in this same generation is

$$p(N_W, N_A, N_B) = \frac{N!}{N_W!N_A!N_B!}(1 - r - r/\rho)^{N_W} r^{N_A} (r/\rho)^{N_B}. \tag{3}$$

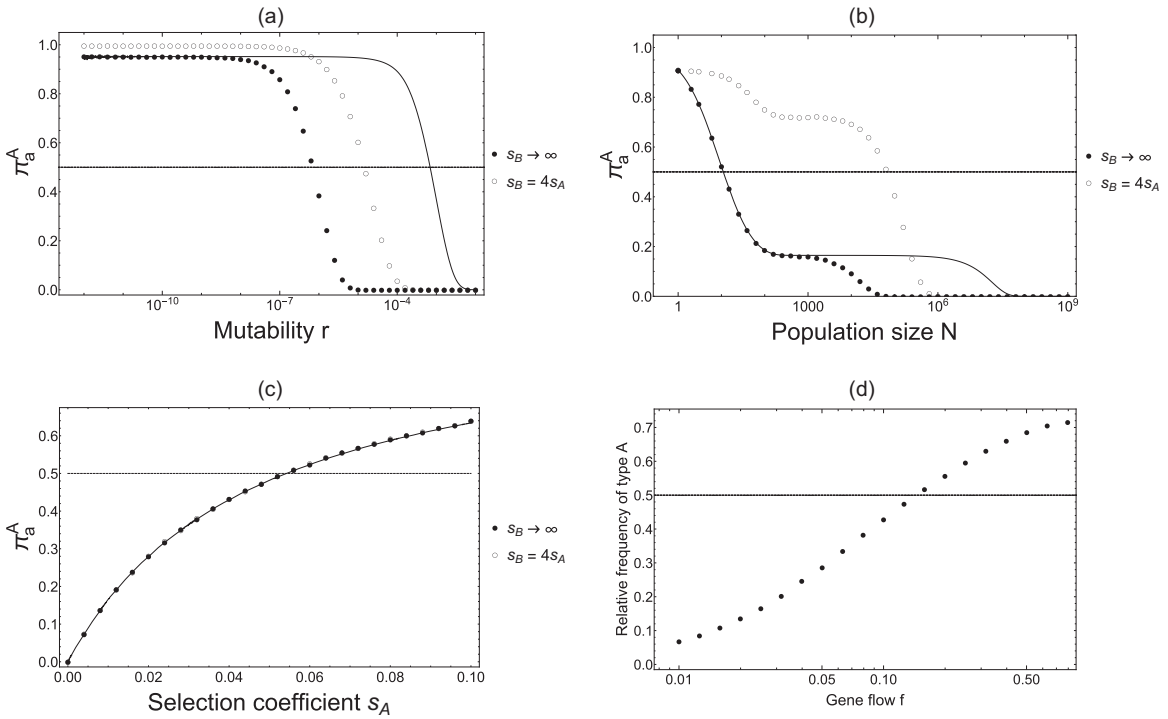

FIG. 2. Dependence of the prevalence of type A on the mutability r, the population size N, the fitness (in terms of the selection coefficient s_A) and the gene flow f. The disks and circles represent results from simulations. The solid disks (•) represent an infinite fitness for type B and the empty circles (○) represent a selection coefficient of B four times higher than type A. The continuous line (—) represents the analytical approximation, Eq. (5). In panel (d), "relative frequency" means the frequency of A relative to both mutants in the stationary state. The selection coefficients s_A and s_B are given by $s_A = w_A - 1$ and $s_B = w_B - 1$. Parameters are (a) $N = 10^6$, $s_A = 0.01$, $\rho = 1000$; (b) $r = 1 \times 10^{-6}$, $s_A = 0.01$, $\rho = 10$; (c) $N = 10^6$, $r = 1 \times 10^{-12}$, $\rho = 10$; and (d) $N = 100$, $\rho = 1.2$, $w_A = 1.01$, $w_B = 1.04$.

When type B has an infinite fitness, type A will fix only if type B does not arise, that is, $N_B = 0$. In the presence of selection only, the probability of fixation of N_A individuals in a population with $N - N_A$ wild individuals is given by the classic result [30,35]

$$\Pi_A(N_A) = \frac{1 - e^{-2N_A s_A}}{1 - e^{-2N s_A}} = \frac{1 - \omega_A^{N_A}}{1 - \omega_A^N}, \tag{4}$$

where $w_A = 1 + s_A$ (s_A is called the selection coefficient) and $\omega_A = e^{-2s_A}$.

Thus, the probability of fixation of A can be approximated as the product of the probability of a configuration $(N_W, N_A, 0)$ and the probability of fixation departing from that configuration, summed over all configurations, that is,

$$\begin{aligned}\pi_f^A &= \sum_{N_A=1}^{N} \frac{N!}{N_W!N_A!}(1 - r - r/\rho)^{N_W} r^{N_A} \frac{1 - \omega_A^{N_A}}{1 - \omega_A^N} \\ &= \frac{\left[1 - \frac{(\rho+1)r}{\rho}\right]^N}{\omega_A^N - 1}\left\{\left[1 - \frac{\rho r \omega_A}{\rho(r-1) + r}\right]^N \right. \\ &\quad \left. - \left[\frac{r - \rho}{\rho(r-1) + r}\right]^N\right\}. \end{aligned} \tag{5}$$

For a very small mutability r, the term with $N_A = 1$ dominates the sum, and the expression reduces to

$$\pi_f^A = \theta_A \frac{1 - \omega_A}{1 - \omega_A^N},$$

where $\theta_A = Nr$ is the mutation supply of type A in a wildtype population, and the second factor is the probability of a single mutant A to fix. The restriction to $r \ll 1/N$ indicates that the absorption of type A is strongly affected by the mutants arising in the first generations, that is, by the initial mutation supply.

Similarly, the probability of fixation of B is given by the probability that at least one mutant B arises ($N_B > 0$) and does not depend on the presence of type A, that is,

$$\begin{aligned}\pi_f^B &= \sum_{N_A=0}^{N-1} \sum_{N_B=1}^{N-N_A} \frac{N!}{N_W!N_A!N_B!}(1 - r - r/\rho)^{N_W} r^{N_A} (r/\rho)^{N_B} \\ &= \left[1 - \frac{(\rho+1)r}{\rho}\right]^N \left\{\left(\frac{1}{1-r}\right)^N \left[\frac{\rho(r-1)}{\rho(r-1) + r}\right]^N \right. \\ &\quad \left. - \left[\frac{r - \rho}{\rho(r-1) + r}\right]^N\right\}. \end{aligned}$$

Again, for a very small mutability r, this formula simplifies to

$$\pi_f^B = \theta_B,$$

where $\theta_B = \theta_A/\rho$ is the mutation supply of type B in a wildtype population. Comparison with the small-r limit of the

absorption probability of type A, shows that the mutation supply is a determinant factor. However, for type B, it is as if there were a fixation probability term equal to unity multiplying the mutation supply. In other words, the small-r approximation shows that although type A has a small fixation probability, this can be compensated by a greater mutation supply.

The expressions of the absorption probabilities are plotted as solid lines in Figs. 2(a)–2(c). They work well in the low-mutability regime, but at high mutability they overestimate π_a^A. This is explained by the more important role of recurrent mutations at intermediate mutabilities, facilitating absorption by B. Naturally, the higher the fitness of B, the stronger the effect. For sufficiently high mutabilities, the continuous curve coincides with the solid points: In this case essentially all mutants appear at the first generation of the fixation process.

The assumption used to derive the analytical approximation sheds light on the mechanism driving absorption. For example, the good agreement of the approximation for small r indicates that the first generation of mutants is determinant for the fixation success of type A. Considering mutational supply, that is, the product of population size times total mutation rate, we see that with a small mutation supply ($Nr \ll 1$) the dynamics is well described as "one mutant at a time." By contrast, the disagreement between the approximation and the simulation at high levels of mutation supply indicates the crucial role of recurrent mutations, which occur only because there are enough wild types that can mutate, even after the first generation of mutants. The plateau observed in Fig. 2(b) suggests that recurrent mutations have no role in the absorption process. However, at some point, for sufficiently large population sizes, recurring mutations are responsible for the abrupt decrease of π_a^A.

B. Fixation probability and fixation times

The route to absorption is not always direct. Mutants can arise and go extinct before they spread. The fixation probability of the wild type is a measure of the likelihood of the mutations to disappear before they can fix. The fixation time of type A or B is the number of generations between the occurrence of the first mutation of that type and its fixation. It is also possible to define the fixation time of the wild type as the number of generations between the occurrence of the first mutation, either A or B, and the fixation of W. Figure 3 shows the dependence of the fixation probability and fixation times on the mutation rate.

At low mutation rates, the wild type fixes more than at other mutabilities because of its numerical superiority over the rare mutant. Although the fixation probability of the mutants seems to be low for lower mutation rates, notice that, in the end, no wild type remains. Because the fixation time of W measures the time to return to the homogeneous state after it was modified by a single mutant, one expects its fixation time to be very small compared to the other mutants, as shown in Fig. 3. Indeed, fixation of the wild type can be seen as a renewal process [36] in which the population can return to the all-W state after the first mutation. The probability that the first mutation is of type A or type B is equal to $r_A/(r_B + r_A)$ or $r_B/(r_B + r_A)$, respectively. Thus, in the rare mutation regime, the fixation probability of the wild type is

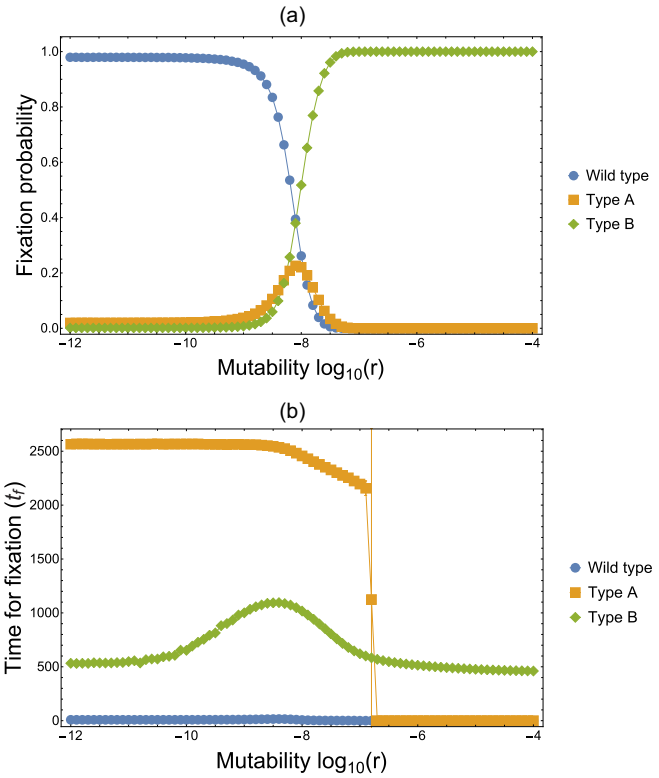

FIG. 3. Fixation probability of each type (a) and the fixation time (b) as a function of mutability. The abrupt fall of the fixation time of type A and its standard deviation is due to the finite sample size. Parameters are $N = 10^7$, $r = 1 \times 10^{-9}$, $s_A = .01$, $s_B = 0.056$, and $\rho = 100$.

given by

$$\pi_f^W = \frac{\rho}{\rho+1}\frac{1-\left(\frac{1}{\omega_A}\right)^{N-1}}{1-\left(\frac{1}{\omega_A}\right)^{N}} + \frac{1}{\rho+1}\frac{1-\left(\frac{1}{\omega_B}\right)^{N-1}}{1-\left(\frac{1}{\omega_B}\right)^{N}}.$$

Notice that if N is large, then $\pi_f^W \approx 1$, that is, most of the time the mutations are lost and the population returns to the homogenous wild state.

Figure 3 shows that in the regime of low mutation rates, although type A fixes more often than B, type A takes more time to fix. Interestingly, the fixation time for the mutant A is reduced as mutability is increased, although competition from B is also enhanced. The fixation time of mutant B shows a peak at mutation rate close to the value at which the probability of fixation of mutant A is higher than other variants. The fixation time increases because there are more individuals of type A to compete with. As mutability is further increased, the number of both mutants increase, and fluctuations no longer favor a greater competitivity of type A, so that the fixation time of B decreases.

To have a better picture of the mechanisms of fixation of each type, we analyze a population composed of only the wild type and N_e initial mutants of the same type with effective selection coefficient s_e, without any further mutation in the evolutionary process. Then, we can ask what are the effective values N_e and s_e that yield the same fixation probability and fixation times as the full model. To calculate these effective values, we need two equations. The first is Eq. (4); for the

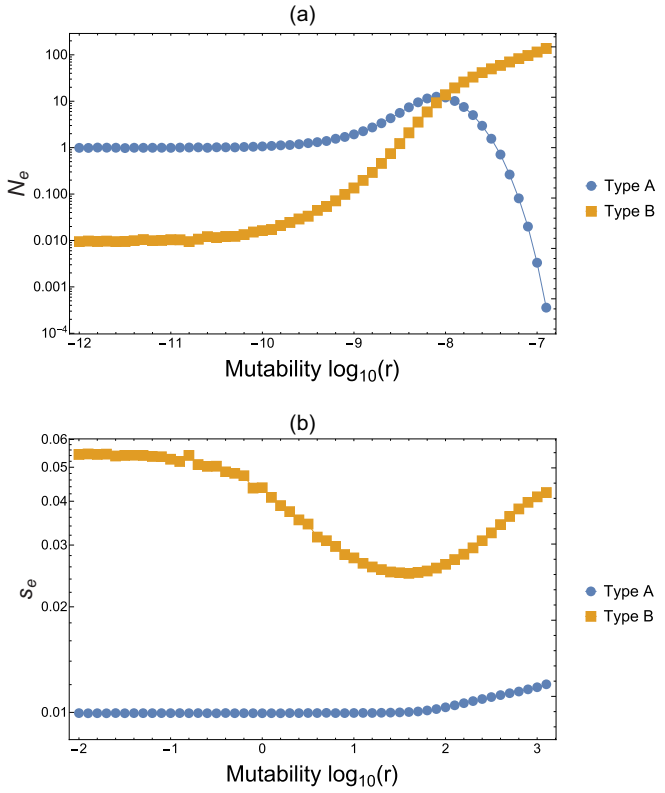

FIG. 4. Effective size and selection coefficients. The two graphs, (a) and (b), must be read in pairs: For each r, the values of N_e and s_e correspond to the solution of a system composed with the wild type and N_e mutants of a single type that yields the same fixation probability and fixation time as the full model. The original data come from simulation. Parameters are $N = 10^7$, $s_A = 0.01$, $s_B = 0.056$, and $\rho = 100$.

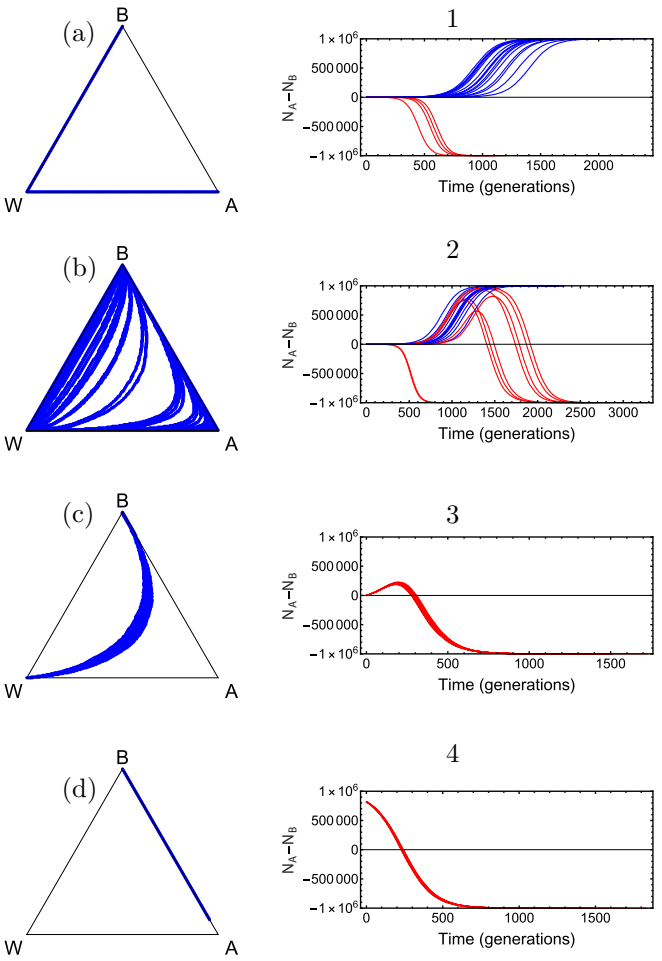

FIG. 5. Typical simulated histories of population composition, ending in absorption by A or B. The left panels [(a)–(d)] show the evolution on the simplex $N_W + N_A + N_B = N$, with the vertices coresponding to single-species states and edges to two-species states. The right panels (1–4) show the difference $N_A - N_B$, (regardless the value of N_W) versus time. Histories ending in absorption by A (B) are shown in blue (red). Rows correspond to mutabilities (from top) $r = 10^{-11}$, $r = 10^{-7}$, $r = 10^{-3}$, and $r = .91$. Parameters are $N = 10^6$, $s_A = .01$, $s_B = 0.02$, and $\rho = 10$.

second we can use the fixation time in the diffusive limit [37] for our conditions (high $\alpha = 2Ns$), given by,

$$\tau = \int_q^1 \frac{[1 - e^{-2(1-q)Ns}](1 - e^{-2qNs})}{(1-q)qs(1 - e^{-2Ns})} dq, \qquad (6)$$

where q is the initial frequency of the mutant type. For our $\alpha = 10^5$ the integrand between 0 and p is essentially null. The initial number of mutants is then Nq. The numerical results shown in Fig. 3 are used as input to Eqs. (4) and (6). The resulting system of equations is solved using the integration algorithm QAGS (quadrature routine, adaptive integrand, general function, singularities can be more readily integrated) and the gls_multiroot_fsolver algorithm, both from the GNU Scientific Library.

Figure 4 shows the effective subpopulation size of mutants and the effective selection coefficient for both types of mutants. The greater the effective number of mutants, the more readily fixation occurs in the equivalent population. At small mutabilities, the initial effective number of mutants is close to the probability of having one mutation in the first step of the full model, $\rho/(\rho + 1)$ and $1/(\rho + 1)$ for types A and B, respectively. This result corroborates the regime of "one mutant at a time." As the mutability value increases, the same qualitative behavior found in Fig. 3 is observed, with a peak of N_e near the r value associated with the peak in the fixation probability. In contrast to the large variation in the effective size, the effective selection is roughly constant for type A and has small variations for type B. In the diffusive limit, the expected change of the number of mutants in one step is proportional to the selection coefficient and the variance of that change is inversely proportional to the size. Thus, for type B, the variance decreases steadily with the increase of mutability, while its mean step has a minimum close to where competition has a maximum. So, at this mutability, type B varies in small steps while the steps of type A vary in a less-regular manner. This may be the reason for the relatively higher competitiveness type B faces in the vicinity of this mutability value.

The temporal evolution of the abundances of types A and B, shown in Fig. 5, exhibits several interesting features. At low mutation rates, there is no A-B coexistence at any point in the evolution, but as we leave the rare-mutation

regime, coexistence does occur. Interestingly, the evolution in cases featuring fixation of type A is always monotonic, whereas those in which type B fixes can be nonmonotonic. The reason for nonmonotonicity in the latter case is simple: Since type B is fitter, it can recover after a delayed emergence. If the mutation rate is high, then type B easily dominates.

Finally, the evolution predicted by the approximation used in the previous subsection, where its is supposed that all mutations take place at once, is shown in the lowest row of Fig. 5. The trajectories occur mostly at the segment AB (except for the vertex all-W) beginning around $N_A = N\rho/(1+\rho)$ and $N_B = N - N_A$, depending on the parameters, sometimes shifted toward vertex all-A or all-B. For such parameters, the fate of fixation is almost exclusively to B.

C. Rare mutations

In the regime in which mutations occur more slowly than selection, fixation takes place before the next mutation appears. This is the "strong selection-weak mutation" (SSWM) regime, as defined by Gillespie (1983) [38]. In this regime, the analysis is greatly simplified and provides analytical support for the simulations in the limit of low mutation supply.

To determine the absorption probabilities we note that, in the SSWM regime, only one mutant coexists with the wild type. Thus, the next equation furnishes the fixation probabilities of both A and B, that is,

$$\pi_f^i = \frac{1-\omega_i}{1-\omega_i^N}. \tag{7}$$

The absorption probability of type i is then given by

$$\pi_a^i = \frac{r_i \pi_f^i}{r_A \pi_f^A + r_B \pi_f^B}, \tag{8}$$

which, after substitution, becomes

$$\pi_a^A = \frac{1}{1 + \frac{1}{\rho}\frac{1-\omega_B}{1-\omega_B^N}\frac{1-\omega_A^N}{1-\omega_A}}, \tag{9}$$

where $\omega_A = e^{-2s_A}$ and $\omega_B = e^{-2s_B}$.

To check this formula, the case $\omega_A = \omega_B$ is helpful. In this case there is no competitive advantage between the types and the absorption probability should depend only on the relative mutation rates. Indeed, Eq. (9) simplifies to

$$\pi_a^A = \frac{\rho}{1+\rho}. \tag{10}$$

In our initial problem, type A is less fit than type B, but arises through mutation more often than type B, so that fixation of A remains possible. To quantify the prevalence of A over B, we determine the condition to have $\pi_a^A > 1/2$. Rearranging the terms in Eq. (9), we find

$$\rho > \frac{1-\omega_B}{1-\omega_B^N}\frac{1-\omega_A^N}{1-\omega_A}. \tag{11}$$

Since we are analyzing adaptive mutations, we have that $0 < \omega_B < \omega_A < 1$. In the regime $N \to \infty$, the condition simplifies to

$$\rho \gtrsim \frac{1-\omega_B}{1-\omega_A}. \tag{12}$$

Because $\omega_B < \omega_A$, it follows that ρ must be always greater than 1. For weak selection, $e^{s_A} \approx w_A = 1 + s_A$ and $w_B = e^{s_B} \approx 1 + s_B$, and Eq. (12) can be further simplified to

$$\rho \gtrsim s_B/s_A. \tag{13}$$

The condition in Eq. (11), or the simplification in Eq. (13), shows that, if type A is to have an evolutionary advantage over the fitter type, then the mutation rate to type A should be higher than that to type B at least as much as determined by Eq. (11). Notice that, the greater the fitness advantage of B, the greater should be ρ, as shown in Fig. 6.

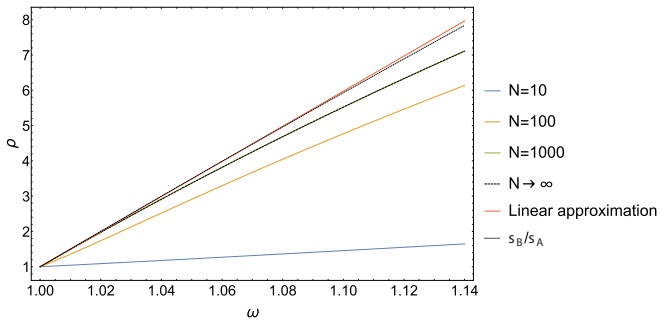

FIG. 6. Threshold for ρ above which type A prevails in the regime of rare mutations from Eq. (11) for $N = 10$ (blue), $N = 100$ (yellow), and $N = 1000$ (green). The limit $N \to \infty$, Eq. (12) (black, dashed) and the approximation of Eq. (13) (red) are also shown. Notice that the $N \to \infty$ approximation fits the $N = 1000$ data well. The greater the advantage of B over A or the larger the population size, the larger the ratio ρ required for A to prevail.

D. Frequent mutations

In the regime of frequent mutation, all mutations take place nearly at once. Thus, the population is composed of types A and B only and the equivalent of Eq. (4) (replacing w_W, which is equal to 1, by w_B) is

$$\pi_f^A = \frac{1-\omega^{N_A}}{1-\omega^N},$$

where $\omega = \omega_A/\omega_B$. The probability of having N_A mutants in the first generation is given by the binomial distribution $B(N, \frac{\rho}{1+\rho})$. Thus, the probability of absorption of A is

$$\Pi_a^A = \sum_{N_A=1}^{N} B\left(N, \frac{\rho}{1+\rho}\right)\frac{1-\omega^{N_A}}{1-\omega^N} = \frac{1-\left(\frac{\rho\,\omega+1}{\rho+1}\right)^N}{1-\omega^N}. \tag{14}$$

This equation also simplifies to Eq. (10) if $s_A = s_B$, which is again a consistent result.

The condition for $\pi_a^A > 1/2$ is

$$\rho > \frac{T-1}{\omega - T}, \tag{15}$$

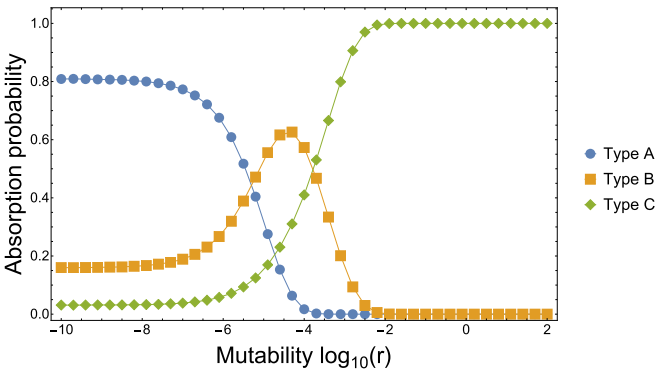

FIG. 7. System with three adaptive mutants, with increasing fitness and decreasing mutation rate. Parameters are $N = 10^6$, $s_A = .01$, $s_B = 0.02$, $s_C = 0.04$, and $\rho = 10$ from A to B and from B to C.

where

$$T = \left[\frac{1}{2}(\omega^N + 1)\right]^{1/N} = \omega\left(1 - \frac{\log 2}{N} + \frac{1}{N\omega^N} - \frac{\log 2}{N^2 w^N} + \cdots\right).$$

For $N \gg 1$, because $\omega > 1$, T is well approximated by the leading terms,

$$T \approx \omega\left(1 - \frac{\log 2}{N}\right),$$

and Eq. (15) becomes

$$\rho > N\frac{(\omega - 1)}{\omega \log 2}. \tag{16}$$

Thus, the greater the size or the greater the selective advantage of B over A ($\omega = e^{2(s_B - s_A)}$), the harder it is for type A to prevail. It is still possible that type A prevail, but the mutation rate to this type must be very high.

E. A further result

The results obtained in our model with two adaptive mutants are also observed in systems that have three adaptive mutants, as shown in Fig. 7. In this last model all three mutants are generated by mutation from the W type. The selection coefficients of types A, B, and C are $s_A = 0.01$, $s_B = 0.02$, and $s_C = 0.04$. Mutation rates are $r_A = r$, $r_B = r_A/\rho$, and $r_C = r_B/\rho = r_A/\rho^2$, with $\rho = 10$. Population size is $N = 10^6$. Sampling of new individuals is performed according to

$$p_W^{t+1} \propto N_W^t(1 - r_A - r_B - r_C),$$
$$p_A^{t+1} \propto w_A N_A^t + N_W^t r_A,$$
$$p_B^{t+1} \propto w_B N_B^t + N_W^t r_B,$$
$$p_C^{t+1} \propto w_C N_C^t + N_W^t r_C,$$

The overall qualitative behavior persists: As we increase the mutability, mutants with decreasing mutation rates and increasing fitness prevail.

For small r, the most frequent mutant is absorbed more often because the effectiveness of fixation itself, given that at least one mutant already exists, is similar among the types. So the mutation rate alone decides which type absorbs. As we increase the mutation rate, we favor the coexistence of types A and B while the mutation rate is too low for type C to appear. Because type B is fitter than A, type B wins the competition in this regime. Finally, with higher mutabilities, type C coexists with the other types and wins the competition since it is the fittest.

IV. CONCLUSIONS

The traditional understanding since the modern synthesis of the role of mutations is that in the most typical cases, the majority of the variability of a population, after some rounds of mutation, is already available and the role of mutations is merely to shift the frequency of preexisting variants [2–4]. However, Yampolsky and Stoltzfus (2001) [6] showed that, under competition between mutation rate and selection, mutation bias can change the prevailing allele. We present a thorough examination of the conditions for which bias in mutation changes the prevailing type.

Our analysis employs a Wright-Fisher model starting with a wildtype population which can mutate one-way to two other mutants, A and B, with B fitter than A and the mutation rate to A higher than that to B. We verify that a sufficiently strong mutation bias can change the type that would prevail if selection were the only mechanism in operation. We observe this under varying mutability, drift (created by fluctuation in small populations), selective advantage and gene flow parameters.

Our results imply that the conditions under which the fate of evolution changes are much broader than commonly acknowledged. A focus on the effect of mutability shows that distinct kinetics are possible, but that there are two basic regimes: one with rare mutations and the other with frequent mutations. In the rare regime, the less-fit type can prevail, whereas in the frequent mutation regime this is nearly impossible.

In addition, we show that increasing mutability may determine the order which mutant among three. In addition, we show that increasing mutability may determine the order which mutant among three is more likely to prevail.

We expect that this work will underpin explanations for the dynamics in more complex fitness landscapes, revealing why one path prevails over others. This leads to questions related to the predictability and repeatability of evolutionary processes [39–44]. In future work we plan to investigate the fate of evolution in simple, completely smooth fitness landscapes while varying population size, fitness, and mutability. This should yield further insights to understanding the kinetics and evolutionary fate in realistic and experimental landscapes [45–51].

ACKNOWLEDGMENTS

A.A.S. was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES); L.W. was supported by CNPq; L.B.K. was supported by CNPq, Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP); R.D. acknowledges support from CNPq, Brazil, through Project No. 303766/2016-6.

[1] B. Shorrocks, *A Origem da Diversidade* (Edusp, São Paulo, 1978).

[2] J. Haldane, The part played by recurrent mutation in evolution, Am. Nat. **67**, 5 (1933).

[3] J. Maynard-Smith, R. Burian, S. Kauffman, P. Alberch, J. Campbell, B. Goodwin, R. Lande, D. Raup, and L. Wolpert, Developmental constraints and evolution: A perspective from the mountain lake conference on development and evolution, Q. Rev. Biol. **60**, 265 (1985).

[4] J. Bull, M. Badgett, H. Wichman, J. Huelsenbeck, D. Hillis, A. Gulati, C. Ho, and I. Molineux, Exceptional convergent evolution in a virus, Genetics **147**, 1497 (1997).

[5] D. Futuyma, *Biologia Evolutiva* (SBG: CNPq, 1992).

[6] L. Yampolsky and A. Stoltzfus, Bias in the introduction of variation as an orienting factor in evolution, Evol. Dev. **3**, 73 (2001).

[7] A. Stoltzfus and D. M. McCandlish, Mutational biases influence parallel adaptation, Mol. Biol. Evol. **34**, 2163 (2017).

[8] A. Stoltzfus, Mutation-biased adaptation in a protein nk model, Mol. Biol. Evol. **23**, 1852 (2006).

[9] J. F. Storz, C. Natarajan, A. V. Signore, C. C. Witt, D. M. McCandlish, and A. Stoltzfus, The role of mutation bias in adaptive molecular evolution: insights from convergent changes in protein function, Philos. Trans. R. Soc. London B **374**, 20180238 (2019).

[10] K. Jain, J. Krug, and S.-C. Park, Evolutionary advantage of small populations on complex fitness landscapes, Evolution **65**, 1945 (2011).

[11] A. Stoltzfus and R. W. Norris, On the causes of evolutionary transition: Transversion bias, Mol. Biol. Evol. **33**, 595 (2016).

[12] P. Higgs, Frequency distributions in population genetics parallel those in statistical physics, Phys. Rev. E **51**, 95 (1995).

[13] L. Peliti, Introduction to the statistical theory of darwinian evolution (1997), arXiv:cond-mat/9712027.

[14] B. Drossel, Biological evolution and statistical physics, Adv. Phys. **50**, 209 (2001).

[15] N. H. Barton and J. B. Coe, On the application of statistical physics to evolutionary biology, J. Theor. Biol. **259**, 317 (2009).

[16] H. P. de Vladar and N. H. Barton, The contribution of statistical physics to evolutionary biology, Trends Ecol. Evol. **26**, 424 (2011).

[17] H. Flyvbjerg and B. Lautrup, Evolution in a rugged fitness landscape, Phys. Rev. A **46**, 6714 (1992).

[18] L. S. Tsimring, H. Levine, and D. A. Kessler, Rna Virus Evolution Via a Fitness-Space Model, Phys. Rev. Lett. **76**, 4440 (1996).

[19] E. Baake, M. Baake, and H. Wagner, Ising Quantum Chain is Equivalent to a Model of Biological Evolution, Phys. Rev. Lett. **78**, 559 (1997).

[20] S. Galluccio, Exact solution of the quasispecies model in a sharply peaked fitness landscape, Phys. Rev. E **56**, 4526 (1997).

[21] M. Nilsson and N. Snoad, Error Thresholds for Quasispecies on Dynamic Fitness Landscapes, Phys. Rev. Lett. **84**, 191 (2000).

[22] D. B. Saakian, E. Muñoz, C.-K. Hu, and M. W. Deem, Quasispecies theory for multiple-peak fitness landscapes, Phys. Rev. E **73**, 041913 (2006).

[23] L. Tan, S. Serene, H. X. Chao, and J. Gore, Hidden Randomness Between Fitness Landscapes Limits Reverse Evolution, Phys. Rev. Lett. **106**, 198102 (2011).

[24] T. Brotto, G. Bunin, and J. Kurchan, A model with darwinian dynamics on a rugged landscape, J. Stat. Phys. **166**, 1065 (2017).

[25] Alfred Ajay Aureate R. and Vaibhav Madhok, Typicality in quasispecies evolution in high dimensions, Phys. Rev. E **100**, 042407 (2019).

[26] D. B. Saakian, T. Yakushkina, and E. V. Koonin, Allele fixation probability in a Moran model with fluctuating fitness landscapes, Phys. Rev. E **99**, 022407 (2019).

[27] B. Ottino-Löffler and M. Kardar, Population extinction on a random fitness seascape, Phys. Rev. E **102**, 052106 (2020).

[28] P. Gerrish and R. Lenski, The fate of competing beneficial mutations in an asexual population, Genetica **102-3**, 127 (1998).

[29] R. Miralles, P. Gerrish, A. Moya, and S. Elena, Clonal interference and the evolution of rna viruses, Science **285**, 1745 (1999).

[30] W. Ewens, *Mathematical Population Genetics 1: Theoretical Introduction*, Interdisciplinary Applied Mathematics (Springer, New York, 2004).

[31] T. Ohta, Slightly deleterious mutant substitutions in evolution, Nature (London) **246**, 96 (1973).

[32] T. Ohta, The nearly neutral theory of molecular evolution, Annu. Rev. Ecol. Evol. Syst. **23**, 263 (1992).

[33] T. Ohta and J. H. Gillespie, Development of neutral and nearly neutral theories, Theor. Pop. Biol. **49**, 128 (1996).

[34] M. Galassi *et al.*, GNU scientific library reference manual.

[35] J. Gillespie, *Population Genetics: A Concise Guide*, Population Genetics (Johns Hopkins University Press, Baltimore, MD, USA, 2004).

[36] N. Van Kampen, *Stochastic Processes in Physics and Chemistry*, North-Holland Personal Library (Elsevier Science, Amsterdam, The Netherlands, 2011).

[37] B. Charlesworth, How long does it take to fix a favorable mutation, and why should we care?, Am. Nat. **195**, 753 (2020).

[38] J. H. Gillespie, Some properties of finite populations experiencing strong selection and weak mutation, Am. Nat. **121**, 691 (1983).

[39] J. Meyer, D. Dobias, J. Weitz, J. Barrick, R. Quick, and R. Lenski, Repeatability and contingency in the evolution of a key innovation in phage lambda, Science (New York, N.Y.) **335**, 428 (2012).

[40] M. K. Burke, G. Liti, and A. D. Long, Standing genetic variation drives repeatable experimental evolution in outcrossing populations of saccharomyces cerevisiae, Mol. Biol. Evol. **31**, 3228 (2014).

[41] J. Lachapelle, J. Reid, and N. Colegrave, Repeatability of adaptation in experimental populations of different sizes, Proc. R. Soc. B **282**, 20143033 (2015).

[42] B. Bauer and C. S. Gokhale, Repeatability of evolution on epistatic landscapes, Sci. Rep. **5**, 9607 (2015).

[43] T. van Dijk, S. Hwang, J. Krug, J. A. de Visser, and M. Zwart, Mutation supply and the repeatability of selection for antibiotic resistance, Phys. Biol. **14**, 055005 (2017).

[44] D. Nichol, J. Rutter, C. Bryant, A. Hujer, S. Lek, M. Adams, P. Jeavons, A. Anderson, R. Bonomo, and J. Scott, Antibiotic collateral sensitivity is contingent on the repeatability of evolution, Nat. Commun. **10**, 334 (2019).

[45] M. Lunzer, S. Milter, R. Felsheim, and A. Dean, The biochemical architecture of an ancient adaptive landscape, Science **310**, 499 (2005).

[46] C. P. Goulart, M. Mahmudi, K. A. Crona, S. D. Jacobs, M. Kallmann, B. G. Hall, D. C. Greene, and M. Barlow, Designing antibiotic cycling strategies by determining and understanding local adaptive landscapes, PLoS One **8**, e56040 (2013).

[47] P. M. Mira, J. C. Meza, A. Nandipati, and M. Barlow, Adaptive landscapes of resistance genes change as antibiotic concentrations change, Mol. Biol. Evol. **32**, 2707 (2015).

[48] A. C. Palmer, E. Toprak, M. Baym, S. Kim, A. Veres, S. Bershtein, and R. Kishony, Delayed commitment to evolutionary fate in antibiotic resistance fitness landscapes, Nat. Commun. **6**, 7385 (2015).

[49] J. V. Rodrigues, S. Bershtein, A. Li, E. R. Lozovsky, D. L. Hartl, and E. I. Shakhnovich, Biophysical principles predict fitness landscapes of drug resistance, Proc. Natl. Acad. Sci. USA **113**, E1470 (2016).

[50] Y. T. Tamer, I. K. Gaszek, H. Abdizadeh, T. A. Batur, K. A. Reynolds, A. R. Atilgan, C. Atilgan, and E. Toprak, High-order epistasis in catalytic power of dihydrofolate reductase gives rise to a rugged fitness landscape in the presence of trimethoprim selection, Mol. Biol. Evol. **36**, 1533 (2019).

[51] M. Knopp and D. Andersson, Predictable phenotypes of antibiotic resistance mutations, mBio **9**, e00770 (2018).