Stochastic tunneling across fitness valleys can give rise to a logarithmic long-term fitness trajectory

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Adaptation, where a population evolves increasing fitness in a fixed environment, is typically thought of as a hill-climbing process on a fitness landscape. With a finite genome, such a process eventually leads the population to a fitness peak, at which point fitness can no longer increase through individual beneficial mutations. Instead, the ruggedness of typical landscapes due to epistasis between genes or DNA sites suggests that the accumulation of multiple mutations (via a process known as stochastic tunneling) can allow a population to continue increasing in fitness. However, it is not clear how such a phenomenon would affect long-term fitness evolution. By using a spin-glass type model for the function that takes into account microscopic epistasis, we find that hopping between metastable states can mechanistically and robustly give rise to a slow, logarithmic average fitness trajectory.

RESULTS
The model
We consider a genome as a finite sequence of \(L\) sites, where each site represents a DNA base or a gene and each site can take two possible discrete states, \(s_i = \pm 1\) and \(i = 1, 2, \ldots, L\) (Fig. 1A). The fitness, here equivalent to the exponential growth rate of a cell, depends on the states of all sites and is given by

\[
F = \sum_{i=1}^{L} h_i s_i + \sum_{i<j} J_{ij} s_i s_j + F_{\text{offset}}
\]

where the first term sums over the independent contributions to fitness \(h_i \sim \mathcal{N}(0, \sigma_h^2)\) of individual sites (Fig. 1A), the second term takes into account microscopic epistasis in the form of pairwise interactions between sites [which can be considered as the lowest-order expansion in the interaction strength \((13)\)], and \(F_{\text{offset}}\) is a constant whose value is chosen such that the fitness of the initial strain is 1. This is a convenient choice because we are interested in the trajectory of the relative fitness (i.e., fitness relative to the wild-type strain).

The strengths of the pairwise interactions are captured by the symmetric interaction matrix \(J\) (Fig. 1A) and are randomly drawn from the following distribution

\[
P(J) = \rho \mathcal{N}(0, \sigma_J^2) + (1 - \rho) \delta(J)
\]

where \(\rho\) is the average fraction of sites each site interacts with and hence determines the sparsity of the matrix. This allows the presence of frustrated interactions (Fig. 1B), which gives rise to a rugged fitness landscape (Fig. 1, C and D). We set \(\sigma_h = (1 - \beta) \Delta\) and \(\sigma_J = \beta \Delta / (\sqrt{\rho L})\), where \(\beta\) controls the relative contribution of the field and interaction terms (for fixed \(\rho\) and \(L\)), while \(\Delta\) affects the fitness effects of mutations. In our analysis, \(\Delta\) is chosen such that the fitness effects of fixed mutations

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Kauffman’s NK model in the limit of including orders up to $K$ (17–19), which is the number of genes each gene interacts with, and in our model is determined by $p$. However, to keep things computationally tractable even for large $p$, we will not include these higher-order interactions in our analysis. It is important to note that although we only consider pairwise interactions, each site can interact with a large number of other sites. This is in contrast to the NK model with $K = 2$. Similar spin-glass–type models have also been used to study other systems such as the conformational states of proteins (20, 21) and networks of neurons (22, 23). Although our model assumes that the fitness effects of independent mutations add up, mapping to the other commonly used multiplicative null model (24) gives similar trajectories (section SA).

Dynamics of mutation accumulation
We consider a population of fixed size $N$ in a continuous culture such that growth occurs in a constant environment, and hence, the fitness landscape does not change over time. Our results also hold for batch cultures subject to the standard dilution protocol (section SF).

We model the dynamics using the Moran process, where, at each time step, a random cell is chosen to leave the population and a random cell is chosen to divide. We assume that mutations occur at a constant rate $\mu$ per cell per division and that all sites mutate with equal probability. Whenever a mutation occurs, its fitness effect $s = \frac{4\Delta}{7}$ is obtained using Eq. 1. To model the dynamics of how these mutations accumulate in a large population within this process, we work in the strong-selection, weak-mutation (SSWM) regime $N\mu \ll 1$, where the time between successful mutations emerging is much longer than the time taken for a successful mutation to fix. This allows us to assume that if a mutation fixes, it immediately takes over the whole population. The population can then be thought of as performing an adaptive walk through a multidimensional genotypic space (Fig. 1C). Within this model, the fixation probability of a single mutant is known to be given by (25)

$$p_f(s) = \frac{1 - \frac{1}{1 + \frac{s}{s_{\text{eff}}}}}{1 - \frac{1}{1 + \frac{s}{s_{\text{eff}}}}}$$ (3)

Taking $|s| \ll 1$ and the large population limit, all deleterious and neutral mutations will eventually go extinct, while $p_f \approx s$ for $s \gg 1/N$.

During the time taken for deleterious/neutral mutants to go extinct, it might be possible for them to gain a second mutation such that the net fitness effect $s_{\text{eff}}$ of the double mutant is positive. This double mutant then has a chance of fixing in the population. When the system is at a local fitness maximum, this mechanism of gaining multiple mutations is the only way the fitness can continue to increase (Fig. 1, C and D).

Hence, we investigated the stability of the local peaks to multiple mutations and found that, for a small $p$, many of the fitness maxima in our model are unstable to double mutations (section SC).

We therefore take into account the probability $p_d$ that a single mutant with fitness effect $s_1 \leq 0$ gains a second mutation and eventually fixes, which in the large $N$ limit and for $|s_1| \ll 1$ is given by (26)

$$p_d = \frac{s_1 + \sqrt{s_1^2 + 4\mu s_{\text{eff}}}}{2}$$
$$= \left\{ \begin{array}{ll}
\sqrt{\mu s_{\text{eff}}}, & \text{for } |s_1| \ll 2\sqrt{\mu s_{\text{eff}}} \\
\frac{s_{\text{eff}}}{s_1}, & \text{for } 2\sqrt{\mu s_{\text{eff}}} \ll |s_1| \end{array} \right.$$ (4)
where \( \langle s_{\text{eff}} \rangle = \langle \max (s_{\text{eff}}, 0) \rangle \ll 1 \), with the averaging carried out over all \( L \) possible second mutation sites. Intuitively, \( s_1 \ll 2\sqrt{\mu \langle s_{\text{eff}} \rangle} \) is the limit where the first mutant is effectively neutral and has a chance of surviving long enough (number of divisions before going extinct \( n_d > 1/\sqrt{\mu \langle s_{\text{eff}} \rangle} \)) such that the probability of a successful double mutant emerging is of order 1. In the opposite limit, the first mutant is so deleterious that it would not survive for that long. Instead, \( n_d \) is at most \( \sim 1/|s_1| \) and \( p_d \) is the probability that a successful double mutant emerges during this period (12).

This implies that if a population is large enough such that \( N \gg 1/\sqrt{\mu \langle s_{\text{eff}} \rangle} \), the probability of stochastic tunneling outweighs the probability of the first deleterious mutant fixing on its own even when the first mutant is nearly neutral (first limit in Eq. 4). This condition can be satisfied together with the SSWM assumption (\( N \ll 1/\mu \)) if \( \mu \) is sufficiently small \( \mu \ll \langle s_{\text{eff}} \rangle \). For typical \( \langle s_{\text{eff}} \rangle \) of a few percent, this can be easily satisfied. For the SSWM assumption to hold, a large population already implies that the mutation rate must be very small: \( \mu \ll 1/N \). Nevertheless, we still expect stochastic tunneling to dominate the fixation of deleterious mutations at large population sizes because for any given \( N \psi \), \( p_t \) (Eq. 3) decreases with \( N \) faster than \( p_d \) (Eq. 4) for \( s < 0 \).

To take into account the number of possible deleterious mutations, which is large for a long genome and can be larger than the number of possible beneficial double mutants, we constructed a typical quenched fitness landscape and, for any given state, calculated the relative probabilities of the next successful mutation event falling into one of three possible categories: (A) beneficial mutation fixing, (B) deleterious mutation fixing on its own, and (C) stochastic tunneling via a double mutant. Within our regime of interest, as long as the number of available beneficial mutations, which we refer to as the rank of the state, is positive, the next successful event must be of type A (Fig. 1E). At an MS (rank = 0), for any given \( L \), the probability of C substantially outweighs that of B for sufficiently large \( N \) (Fig. 1F).

Because the fraction of first mutants in the population is always small, the double mutants can be assumed to be competing against the original population once it emerges. Therefore, given that a first deleterious mutant has produced a successful double mutant, the relative probabilities of the second mutation site are proportional to their fixation probabilities \( p_t (s_{\text{eff}}) \).

**Effective simulation model**

Putting the above elements together, our effective simulation model is as follows: From any given state \( \bar{s} \), we randomly draw a mutation site, calculate its fitness effect \( s \), and accept this mutation with probability \( p_t (s) \). If \( \bar{s} \) is a fitness peak, we allow a successful double mutant to emerge with probability \( p_d \) (Eq. 4), which is obtained by considering all possible \( L \) second mutation sites and calculating the effective selection coefficients of their corresponding double mutants \( s_{\text{eff}} \). If a successful double mutant emerges, we then draw the second mutation site randomly with weights proportional to their fixation probabilities \( p_t (s_{\text{eff}}) \). For convenience, within our simulations, time is measured in terms of the number of mutational attempts (because at each time step we mutate a site and ask if the mutation successfully fixes). Although \( N \) does not explicitly enter in the simulations, we discuss in section SB the range of \( N \) for which our assumptions and results hold.

**Hill-climbing regime: Relaxation toward a fitness peak**

We first consider the regime where the initial state is far from a fitness peak and adaptation brings the population closer to a fitness peak.

If there is no epistasis (\( J = 0 \)), there is a single well-defined peak in the fitness landscape. As mutations accumulate, the number of available beneficial mutations left, which we refer to as the rank of the system, decreases monotonically until the system eventually reaches a fitness plateau (Fig. 2A). In this case, the average fitness trajectory is uniquely defined by the distribution \( p(h) \) of \( h \) and for \( p(h) \) that follows a normal distribution, \( F_{\text{max}} - F(t) \sim \frac{1}{\sqrt{2\pi}} \) (Fig. 2B) (27).

In the presence of epistasis (\( J > 0 \)), a mutation at a specific site can be beneficial or deleterious depending on the states of other sites. This is known as sign epistasis because the sign of the fitness effect of a mutation changes with the accumulation of other mutations and is responsible for a rugged fitness landscape. The rank of the system is therefore no longer guaranteed to decrease monotonically with time. By varying the relative contribution of the field and interaction terms (Eq. 1), we found that while the trajectory still follows a power law (Fig. 2, A and B), the relaxation to the first encountered local fitness maximum slows down as the relative strength of the interaction term increases (section D). Here, as in the case when \( J = 0 \), the exact form of the fitness trajectory is also sensitive to the chosen form of \( p(h) \) and \( p(J) \) but eventually reaches a fitness plateau.

**Hopping between MSs**

With multiple local fitness maxima, the only way for fitness to continue increasing from any of these maxima is to first gain a deleterious mutation (that does not fix by itself) followed by one or more beneficial ones (Fig. 1D). In our model, these fitness maxima are MSs that are stable to single mutations but are unstable to double mutations. Once the system escapes from an MS via a double mutant, it may enter a state with rank > 0, which is now able to gain one or more single beneficial mutations before again entering another MS, and the process repeats itself. This dynamics results in a scenario where the system spends a long time in MSs but transits between MSs relatively quickly. The phenomenon that fitness goes through periods of stasis followed by rapid jumps is known as “epochal dynamics” and has also been observed and studied in other models, such as nearly neutral holey fitness landscapes, where the epochal nature comes about from degeneracies in the genotype-to-fitness mapping (28–30).

To obtain the average fitness trajectory, instead of repeating the simulations multiple times (which would be computationally expensive...
because the escape events from MSs are rare and the space of possible trajectories is large), we constructed a Markov chain by mapping out all the possible states that a state can go to and by calculating the corresponding transition rates (Fig. 3A). We start the system in a randomly chosen MS (by randomly drawing a genotypic state and successively flipping sites chosen from all possible beneficial mutation sites with equal probability), assuming that the cells are already relatively close to a local fitness peak. Because double-mutation transitions are typically much slower than single-mutation transitions (Fig. 3B), we only include double-mutation transitions out of MSs, i.e., when there are no possible single-mutation transitions out of a state. The transition probability of going to a state via a single mutation at site $i$ with effect $\tilde{s}_i$ is then $1/p_i$ ($s = s^{(i)}$), while the probability $p_{ij}$ of going to a double mutant state via mutations at sites $i$ and $j$ with net effect $\tilde{s}_i + \tilde{s}_j$ is given by

$$p_{ij} = \frac{1}{L} \left( p_d(s_i = s^{(i)}, s^{(j)}) = \langle \tilde{s}^{(i)} \rangle \right) \left( \frac{p_i(s = s^{(i)})}{\sum_k p_i(s = s^{(k)})} \right) + i \leftrightarrow j \quad (5)$$

where the first term is the probability of first gaining a mutation at $i$ followed by $j$, and vice versa for the second term. The fixation probability of a mutant $p_F(s)$ and the probability of a depressive mutant $p_d(s_i, s^{(j)})$ (Eq. 4) are as defined previously, and $\langle \tilde{s}^{(i)} \rangle = \sum_k \min(\tilde{s}^{(i)}_k, 0)/L$. The probability of staying in the same state after a mutation event is then $1 - \sum p_i - \sum_{i,j} p_{ij}$. By propagating the dynamics using this matrix (Methods), we find that the average fitness increases approximately logarithmically with time (Fig. 3C).

Because the time taken to escape from an MS is much longer than the time spent between MSs, the dynamics is governed by the hopping between these MSs. This is reminiscent of Bouchaud’s trap model, where these MSs can be considered as “traps” with long trapping times $\tau$ (31), which is the average time spent in the state before transiting to another state. We also find that the average trapping time $\langle \tau \rangle$ increases with time (Fig. 3D), supporting the concept that the system becomes traps in deeper and deeper states that are harder to get out of. This is also why our model exhibits aging—the dependence on the system properties on the time from the start of the experiment—one of the hallmarks of glassy systems (section SE).

In the trap model, one assumes that the time taken to overcome energy barriers follows the Arrhenius law (31). Here, although we did not a priori assume any relationship between the trapping time $\tau$ and the fitness of a state, we find that, on average, $\tau$ increases exponentially with $F$ (Fig. 3E). Because $t \sim \tau$ and $F \sim \log(\tau)$, the logarithmic fitness trajectory $F \sim \log(t)$ naturally emerges.

There are two factors that determine $\langle \tau \rangle$: (i) the number of escape paths $n_p$ (i.e., number of possible double mutations that increases $F$) and (ii) the average escape times through one of those escape paths $\langle \lambda \rangle$. More specifically

$$\langle \tau \rangle = \frac{1}{n_p(\lambda)} \quad (6)$$

While $\lambda$ depends on the fitness difference between two states and is a representative of the energy barrier between states, $n_p$ governs the strength of the entropic barrier, which refers to the low probability of a favorable set of mutations occurring. We find that the exponential increase in $\tau$ with $F$ is predominantly due to the exponential decrease in $n_p$ with $F$ (Fig. 3F) (section SE). This could be related to the exponential decrease in the number of local minima with energy in typical
has been shown that the number of steps to a local maximum of the fitness trajectory is governed by entropic factors. However, it has an exponentially decaying tail, the number of available beneficial mutations in the genome and the distribution of their site on a genome. We find that such a model captures realistic evolution are.

The dynamics of adaptation depends on both the fraction of available beneficial mutations in the genome and the distribution of their fitness effects. While it is common in other models to make assumptions about the beneficial mutation rate and the specific form for the fitness effect distribution, including its variation with current fitness (“macroscopic epistasis”), these aspects of the dynamics, such as the decrease in the beneficial mutation rate, emerge naturally from the microscopic model presented here.

If the number of beneficial mutations is depleted over time, as it occurs in our model, the system eventually reaches a local fitness maximum. In this case, although adaptation is commonly thought of as a hill-climbing process that only involves the accumulation of beneficial mutations, the escape from MSs via multiple mutations is crucial for the continued increase in fitness over many generations and can robustly give rise to a logarithmic fitness trajectory.

The slow fitness trajectory that emerges from hopping between MSs arises due to the ruggedness of the fitness landscape. This is reminiscent of glassy dynamics, where the existence of multiple local energy minima has also been the cause of slow relaxations in many other models of glassy systems such as spin glasses, structural glasses, electron glasses, polymers, and granular materials. However, the exact dynamics for the fitness increase in our model is different from that in these other scenarios and thus provides an alternative mechanism for how slow relaxations could arise in a real system.

A logarithmic fitness trajectory has also been known to emerge on an uncorrelated, House-of-Cards fitness landscape, where mutant fitness values are assumed to be drawn from the same distribution regardless of the fitness of the current state. If this distribution has an exponentially decaying tail, the number of available beneficial mutations will also decrease exponentially with fitness. This has a similar flavor to the dynamics observed in our model in that the slowness of the fitness trajectory is governed by entropic factors. However, it has been shown that the number of steps to a local maximum on an uncorrelated landscape is very short \( N_{\text{steps}} \sim \log(L) \). In contrast, the fitness landscape studied here is highly correlated (fig. S1B), and hence, both \( N_{\text{steps}} \) and the number of steps between successive MSs \( d \) increase linearly with \( L \). The presence of long-range correlations shows that the fitness values of successive MSs are still highly correlated. If each genotype was allowed to jump beyond the correlation length of the landscape by gaining many \( \sim O(L) \) mutations in one step, the dynamics would effectively correspond to that on an uncorrelated landscape. Studies of this “long-jump” adaptation have been carried out for the evolution of NK Boolean networks, in which a logarithmic fitness trajectory has been observed (44). However, here, we found that even when considering realistic dynamics for the accumulation of single individual mutations, as long as one takes into account the phenomenon of stochastic tunneling, a slow, logarithmic average fitness trajectory can still arise on such a highly correlated landscape.

Our model can potentially be extended to account for different forms of the interaction matrix. We have so far assumed that each site interacts with a random subset of other sites, but there might be some other structure in the interaction network between genes that may affect the dynamics. For example, genes might be connected in such a way where beneficial mutations always change potential deleterious mutations into beneficial ones. This could maintain or increase the rank of the system even if it is far from an MS.

Together, our theoretical study demonstrates the utility of a microscopic model in providing a mechanistic understanding of the evolutionary dynamics and in allowing us to probe details of the system that might not be accessible in a macroscopic model. Using this approach, we explored the consequences of epistasis on fitness trajectories, both in the hill-climbing regime and after the population reaches a fitness peak, and found that hopping between MSs via stochastic tunneling can robustly give rise to a logarithmic trajectory.

### METHODS

#### Obtaining average fitness trajectories

We obtained the probability distribution \( \mathcal{P}_k \) of all states in the Markov chain at time \( k \) using \( \mathcal{P}_k = \mathcal{M} \mathcal{P}_{k-1} \), where \( \mathcal{M} \) is the probability of going from state \( j \) to state \( i \) in one time step (i.e., after each mutation event). The elements of this transition matrix are determined as follows:

1. From a non-MS, the transition probability \( p_{ij} \) of going to a state via a single mutation at site \( i \) with effect \( s^{(i)} > 0 \) is given by

\[
p_{ij} = \frac{1}{L} \max(s^{(i)}, 0)
\]

2. From an MS, the probability \( p_{ij} \) of going to a double mutant state via mutations at sites \( i \) and \( j \) with net effect \( s^{(ij)} \) is given by

\[
p_{ij} = \frac{1}{L} \left( p_{\text{double}}(s_1 = s^{(i)}, s_{\text{eff}} = s^{(ij)}), \sum_{k=1}^{L} p_{ij}(s = s^{(j)}) \right)
\]

where the first term is the probability of first gaining a mutation at \( i \) followed by \( j \) and vice versa for the second term, the fixation probability of a mutant \( p_{ij}(s = \max(s_{\text{eff}}), \langle s_{\text{eff}} \rangle = \sum_k \max(s^{(jk)}, 0)/L, and the probability of a deleterious single mutant becoming a successful double mutant \( p_{\text{double}}(s_1, s_{\text{eff}}) = \frac{s_{\text{eff}}}{2} \).


3) The probability of staying in the same state after a mutation event is $1 – \sum p_i – \sum p_j$.

With this transition matrix, the average fitness $F_k$ at time $k$ can then be found from $F_k = P_k f$, with $f$ being the vector of fitness values of all states.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/5/7/eaav3842/DC1

REFERENCES AND NOTES


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