

SLOWLY SWITCHING BETWEEN ENVIRONMENTS FACILITATES REVERSE EVOLUTION IN SMALL POPULATIONS

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Natural populations must constantly adapt to ever-changing environmental conditions. A particularly interesting question is whether such adaptations can be reversed by returning the population to an ancestral environment. Such evolutionary reversals have been observed in both natural and laboratory populations. However, the factors that determine the reversibility of evolution are still under debate. The time scales of environmental change vary over a wide range, but little is known about how the rate of environmental change influences the reversibility of evolution. Here, we demonstrate computationally that slowly switching between environments increases the reversibility of evolution for small populations that are subject to only modest clonal interference. For small populations, slow switching reduces the mean number of mutations acquired in a new environment and also increases the probability of reverse evolution at each of these “genetic distances.” As the population size increases, slow switching no longer reduces the genetic distance, thus decreasing the evolutionary reversibility. We confirm this effect using both a phenomenological model of clonal interference and also a Wright–Fisher stochastic simulation that incorporates genetic diversity. Our results suggest that the rate of environmental change is a key determinant of the reversibility of evolution, and provides testable hypotheses for experimental evolution.

KEY WORDS: Adaptation, epistasis, fitness, models/simulations, population genetics, selection–natural.

Natural populations adapt to novel environments by evolving new functions or structures. A natural question is whether such adaptations can be reversed by returning the population to its ancestral environment. Indeed, as early as the 1890s, Louis Dollo put forth the now classic “law,” which argues that complex adaptations are never fully reversible (Gould 1970; Bull and Charnov 1985; Porter and Crandall 2003).

Contrary to Dollo’s hypothesis, evolutionary reversals have been observed both in the wild and in the laboratory. A famous example is the observation of color changes in the melanic peppered moth (Clarke et al. 1985). In response to the soot released during the Industrial Revolution, peppered moths in Britain evolved a darker coloration. When air pollution was gradually diminished, the population restored its ancestral pale form. This natural observation of reversible evolution was followed by recent phylogenetic discoveries in insects (Whiting et al. 2003), gastropods

(Collin and Cipriani 2003), amphibians (Wiens 2011; Chippindale et al. 2004), and reptiles (Kohlsdorf and Wagner 2006; Lynch and Wagner 2010). Reversible evolution was also realized in laboratory evolution of *Drosophila* (Teotonio and Rose 2000), virus-resistant *E. coli* strains (Lenski 1988), and both DNA and RNA viruses (Burch and Chao 1999; Crill et al. 2000). These experimental results led to different conclusions regarding the extent of reverse evolution (partial or complete reversals) and its mechanism (standing genetic variation, direct reversals, or compensatory mutations), leaving the factors that determine the reversibility of evolution largely unknown.

In the studies described above, the effect of the drastically different rates at which environmental fluctuations occur was not explored. In the peppered moth example (Clarke et al. 1985), environmental change (air pollution) and the corresponding adaptations (color change) occurred over decades. In the laboratory

examples, environments were switched instantly, whereas the subsequent adaptations took years (Teotonio and Rose 2000), months (Lenski 1988), or days (Burch and Chao 1999; Crill et al. 2000).

The rate of environmental change may have a substantial influence on the reversibility of evolution. Although the rate of environmental change is known to be exceedingly important to the evolutionary dynamics of populations, little is known about its effect on reverse evolution. As an analogy, the reversibility of a thermodynamic process is maximized when it occurs at an infinitely slow rate. For example, the expansion and compression of a gas is only perfectly reversible if the process is done infinitely slowly. Otherwise, the gas will have increased in temperature over the course of the cycle, leading to an irreversible process. Whether a similar conclusion holds for biological evolution is currently unknown. In this article, we quantify the reversibility of evolution by simulating both sudden and slow switching between environments. We find that slow switching facilitates reverse evolution for small populations where clonal interference is not extensive.

Models

CONSTRUCTION OF THE MODEL SYSTEM

A biological organism can be characterized by its set of all possible genotypes. We consider an asexual haploid genome with n mutational sites, each with two alternative alleles (0 and 1). Each genotype can be represented by a bit-string of length n , yielding 2^n possible genotypes. For example, in our previous study on a bacterial antibiotic resistance gene, five point mutations jointly contribute to the resistance to a specific drug (Tan et al. 2011). If we represent the absence or presence of each mutation by a bit 0 or 1, each possible genotype can be written as 00000 (without any mutations), 00001 (with only the fifth mutation) . . . to 11111 (with all mutations). In this study, there were then $2^5 = 32$ genotypes in total.

We consider two different environments, which we label “ancestral” and “new.” Each genotype has a fitness value corresponding to each of the two environments. The resulting fitness landscapes are hypercubic graphs with each genotype connected to n adjacent genotypes via single mutations (Kauffman and Weinberger 1989). In the above example considering five mutations, the genotype 00000 is directly connected to five neighbors: 00001, 00010, 00100, 01000, and 10000.

QUANTIFICATION OF EVOLUTIONARY REVERSIBILITY

Starting from any genotype on the fitness landscape in the ancestral environment (such as a , b in Fig. 1A), a population will evolve to one of the local maxima of fitness (such as a' , b'). This local maximum can be denoted as the “ancestral genotype.” If a

new environment is imposed (Fig. 1B), adaptations (blue arrows) will in general change the genotype of the population into a new local maximum of fitness (such as $a' \rightarrow a''$, or $b' \rightarrow b''$). An example would be the dark (melanic) form of peppered moths in the polluted environment.

Evolving the population again in the ancestral environment, previous adaptations to the new environment may be reversed (Fig. 1C). Such reverse evolution is likely if the genetic change in the new environment was sufficiently small that the population stays near its ancestral genotype (such as $a' \rightarrow a'' \rightarrow a'$). In contrast, if the genetic change is significant, the population is likely to end at a local maximum different from the ancestral genotype (such as $b' \rightarrow b'' \rightarrow a'$).

We define the evolutionary reversibility of the system to be the probability of reversible evolution considering all possible starting genotypes (a , b , and so on). On each pair of fitness landscapes (ancestral and new), 2^n simulations were done with the population starting at each of the 2^n possible genotypes in the ancestral environment. For each of these simulations, the population was first allowed to adapt to a local maximum in the ancestral environment before the environmental switching was imposed. The reversibility of this landscape pair was recorded as the fraction of simulations that ended with reverse evolution to the ancestral genotype before the environment was switched. Each reported value and its SD were obtained from 1000 randomly generated pairs of fitness landscapes.

The above definition is for genotypic reverse evolution, because the ancestral genotype must be exactly restored. Such direct reversal in genotype has been shown to be the primary cause of reverse evolution in a virus (Crill et al. 2000). Another type of reverse evolution, “fitness recovery,” can be defined as restoring a fitness value that is equal to or higher than the ancestral fitness, regardless of the final genotype. For example, in one study, phage-resistant *E. coli* strains restored a higher fitness than their ancestral form when evolved again in the phage-free environment, and at the end of this process retained resistance (Lenski 1988, although it is unclear whether the ancestral form was actually a local maximum of fitness). This phenomenon suggests the possibility of achieving a better local maximum, or even the global maximum, by switching between different environments. A computational study has indeed demonstrated that a fluctuating environment can in some cases accelerate adaptation (Kashtan et al. 2007).

MODELING EPISTASIS

Gaining a mutation (substituting 0 with 1 or vice versa at any of the n mutational sites) will generally cause a change in fitness. The simplest way to model the effect of multiple mutations would be to sum their individual effects. Fitness landscapes with this property are called additive landscapes. However, in reality, the effect of a particular mutation often depends upon the presence of other

mutations in the genome. Such interactions between mutations are called epistasis.

We start with additive fitness landscapes, in which each mutation always changes the overall fitness by +1 (beneficial) in the ancestral environment, and by -1 (deleterious) in the new environment. We also consider alternative methods in Figure S8, with either the same effect (+1 in both environment) or uncorrelated effects (randomly choosing +1 or -1, independently in each environment). To simulate epistasis, we impose independent Gaussian noise (with zero mean, and variance σ^2) on the fitness of each genotype. The noise terms are also independent between the two environments. This method has been previously studied as the “rough Mt. Fuji type” in evolutionary molecular engineering (Aita and Husimi 2000).

SWITCHING BETWEEN ENVIRONMENTS

Under sudden switching, the fitness of each genotype is immediately changed into the new value, and the population is then allowed to evolve until a local maximum is reached. This corresponds to the instant environmental change implemented in several laboratory evolution experiments (Lenski 1988; Burch and Chao 1999; Crill et al. 2000; Teotonio and Rose 2000). More generally, this limit applies to any situation where the environmental changes occur much faster than the adaptation time of the population. Under slow switching, the fitness values are always infinitely slowly modified (linearly from one landscape to the other, with a rate proportional to the fitness difference between the two environments). The population will always stay at a local maximum, until one or more of its adjacent genotypes gradually become more fit. This corresponds to adaptation times that are short compared to the time scale of environmental change.

INDIVIDUAL-BASED STOCHASTIC SIMULATION

To fully investigate the effect of genetic diversity in larger populations, we used Wright–Fisher simulations with constant population size N . After each discrete generation, each offspring inherits a parent’s genotype with probabilities proportional to the parent’s fitness, and acquires a mutation with probability μ (Fog 2008).

To assess the reversibility of evolution, we define the ancestral and the final genotypes as the dominant genotype in the population after each round of adaptation. With our parameter choice, the dominant genotype typically occupies more than 99.9% of the population, and can thus be unambiguously identified. The population starts with a random genotype on a maximally epistatic landscape, constructed according to the house-of-cards model (Jain et al. 2011): The fitness value of each genotype was assigned as $(1 + s \cdot Y)$, where s is the typical selective advantage. Y is an exponential random variable with mean value 1, and was drawn independently for each genotype in each environment.

Results

The evolutionary dynamics of a population is determined by three crucial parameters: population size (N), mutation rate (μ , the number of mutations per generation per genome), and the selective advantage of the mutation (s , fractional change in fitness). If $|Ns| \gg 1$, a deleterious mutation ($s < 0$) is extremely unlikely to reach fixation. If there are beneficial mutations available to the population, then neutral mutations ($s = 0$) are also unlikely to reach fixation in reasonably sized populations ($N \gg 1$). Therefore, we begin by assuming that only beneficial mutations can reach fixation in the population. We denote μ_b and s_b as the mutation rate and the selective advantage of beneficial mutations ($s_b > 0$).

When there are multiple possible beneficial mutations from a given genotype, the population size will influence their relative probabilities of fixation. Such a difference in relative probabilities can be thought of as different search strategies on the fitness landscape (choosing different paths in the sequence space). As the population size increases, more mutants tend to appear and compete at the same time. This phenomenon is called clonal interference (Gerrish and Lenski 1998; Hegreness et al. 2006). Under such circumstances, mutations with higher selective advantages are comparatively favored. This effect has been experimentally demonstrated in an RNA virus (Burch and Chao 1999; Miralles et al. 1999) and in *Drosophila* populations (Weber 1990).

EVOLUTIONARY BEHAVIOR OF SMALL POPULATIONS

In this article, we mainly consider small populations, where there is little clonal interference between multiple mutants in a population. In this case, a new mutant will either take over the population (reach fixation) or go extinct, and in either case the fate of this mutant will be resolved before the next mutant enters the population. If the typical selective advantage of a beneficial mutation is s_b , the number of interfering mutations can be proved to be $\pi(s_b) \mu_b N \ln(Ns_b)/s_b$, where $\pi(s_b)$ is the probability of fixation assuming no clonal interference (Desai et al. 2007). In asexual populations, $\pi(s_b)$ is generally proportional to s_b for $s_b \ll 1$, and approaches 1 for $s_b \gg 1$ (Ewens 2004). Clonal interference becomes negligible when there are very few interfering mutations. This requirement sets a limit for small population sizes: $\pi(s_b) \mu_b N \ln(Ns_b)/s_b \ll 1$. For example, in bacterial populations, previous work estimated $\mu_b = 10^{-5}$ and $s_b = 0.01$ for *E. coli* (Perfeito et al. 2007). Therefore, small populations (negligible clonal interference) correspond to $N \ll 10^5$ in this organism, which can be obtained experimentally with bottlenecks that reduce the effective population size. Many animal populations will also be in the small population regime, but the recombination caused by sexual reproduction requires an analysis beyond the scope of this article.

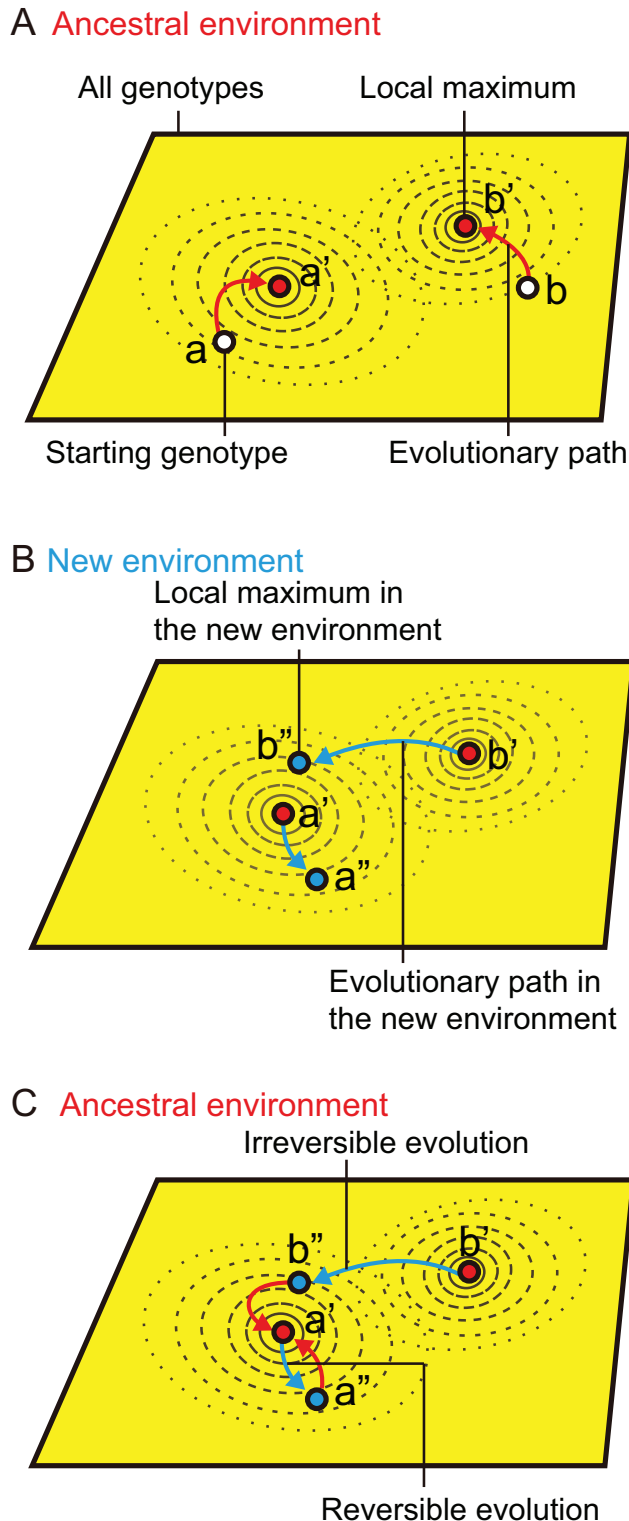


Figure 1. Quantification of evolutionary reversibility. (A) A population of organisms evolves on a fitness landscape consisting of all the possible genotypes (yellow box). In the ancestral environment, the population evolves from its starting genotype to a local maximum of fitness (such as $a \rightarrow a'$, or $b \rightarrow b'$). The concentric circles around each local maximum represent its attractive effect on evolutionary paths from nearby genotypes. (B) After a population

In small populations where clonal interference is negligible, the relative probability of fixation is dominated by the probability of surviving stochastic extinction, namely $\pi(s_b)$ (Gerrish and Lenski 1998). When there are multiple possible beneficial mutations, the probability for each one to eventually reach fixation will be the same if $s_b \gg 1$, or proportional to s_b if $s_b \ll 1$ (assuming in both cases $|Ns| \gg 1$). Therefore, small populations under strong selection ($s_b \gg 1$) can be characterized by a random walker on fitness landscapes: The population will only fix beneficial mutations, but each mutation is equally likely to fix. However, our core conclusions also apply to the case of $s_b \ll 1$ (assuming $|Ns| \gg 1$).

SUDDEN SWITCHING: NONMONOTONIC EFFECT OF EPISTASIS ON EVOLUTIONARY REVERSIBILITY

Here, we concentrate on small populations under strong selection ($s_b \gg 1$ and $|Ns| \gg 1$), which can be characterized by random walkers on a fitness landscape. In the absence of epistasis, evolutionary reversibility is perfect because there is a single peak in the fitness landscapes for both the ancestral and new environments. Evolutionary paths taken by the population might be random, but the global maximum of fitness is always reached.

Increasing the extent of epistasis (represented by the variance of the nonadditive part of fitness, σ^2) initially decreases reversibility, because the emergence of multiple local maxima presents the possibility for a population to become “stuck” (Fig. 2A). This is reasonable because epistasis adds to the ruggedness of the landscape, blocking return paths toward the ancestral genotype. This is consistent with previous studies that suggested epistatic interactions as a primary reason for the irreversibility of evolution (Bull and Charnov 1985; Porter and Crandall 2003; Zufall and Rausher 2004).

Interestingly, we find that evolutionary reversibility is minimized for moderately rugged landscapes ($\sigma \sim 1$). Purely random landscapes with maximal epistasis ($\sigma \gg 1$) have the largest number of local maxima, but nevertheless do not lead to a

Figure 1. Continued. evolves to a local maximum in the ancestral environment (the “ancestral genotype”), a new environment is imposed. Adaptations will in general change the genotype of the population to a new local maximum of fitness (such as $a' \rightarrow a''$, or $b' \rightarrow b''$). (C) Reverse evolution is likely only if the genetic change in the new environment was sufficiently small that the population stays near its ancestral genotype (such as $a' \rightarrow a'' \rightarrow a'$). Otherwise, the population is likely to end up at a different local maximum (such as $b' \rightarrow b'' \rightarrow a'$). We define the evolutionary reversibility of the system to be the probability of reversible evolution considering all possible starting genotypes (a , b , and so on).

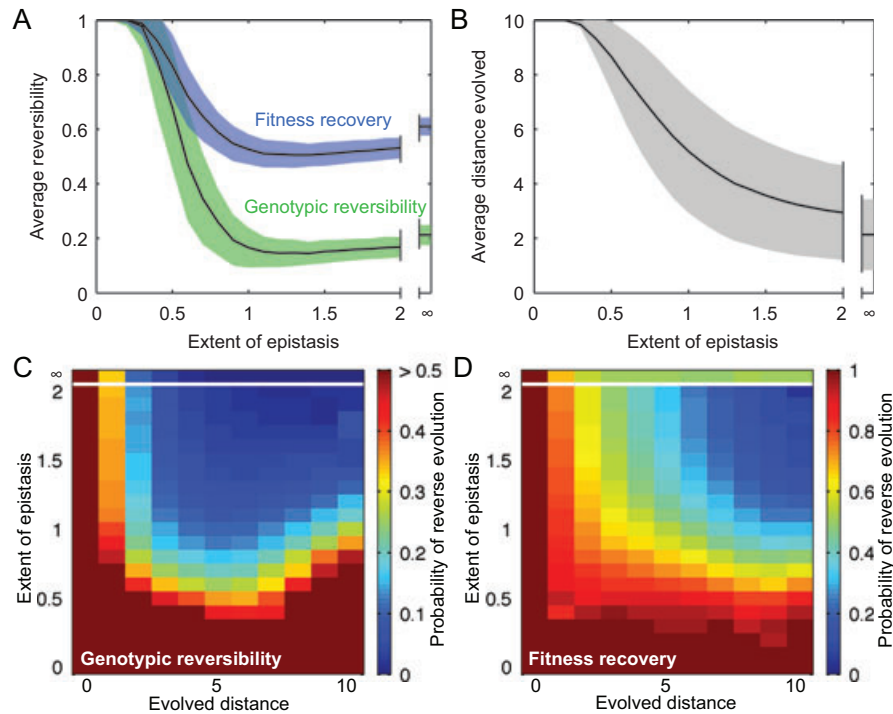


Figure 2. Nonmonotonic effect of epistasis on the reversibility of evolution under sudden switching between environments. (A) Increasing the extent of epistasis initially decreases the average level of reversibility. Interestingly, evolutionary reversibility is minimized for moderately rugged landscapes ($\sigma \sim 1$). (B) Adding epistasis blocks some previously allowed evolutionary paths, sharply reducing the genetic distance evolved (the number of acquired mutations). Shaded areas are SDs of 1000 samples. SEs are smaller than the curve width. (C) Genotypic reverse evolution is generally less likely when the genetic distance increases. At each distance, epistasis decreases the probability of genotypic reverse evolution. (D) The probability of fitness recovery is also decreased by epistasis, but partially recovers at maximal epistasis. In conclusion, the nonmonotonic reversibility curves in (A) reflect the competition between two opposing effects of epistasis: decreasing the genetic change in the new environment (which generally increases reversibility), and decreasing the probability of reverse evolution at each given distance. The fitness landscapes considered in this figure have a total of $n = 10$ possible mutations. A few regions are exceedingly rare, and their values are replaced by theoretical estimates.

minimum in reversibility. This nonmonotonic behavior of reversibility with epistasis is true for both genotypic reversibility and fitness recovery (Fig. 2A).

To explain this nonmonotonic change in reversibility, we first investigated the extent of genetic change caused by adaptations to the new environment (measured by the number of acquired mutations) (Fig. 2B). As epistasis increases, previously allowed evolutionary paths may be blocked, sharply reducing the typical number of mutations acquired in the new environment (namely, the genetic distance) from n to $\approx \ln n$ (Fig. S1).

Next, we studied the probability of reverse evolution at each given genetic distance that was evolved in the new environment (Fig. 2C, D). Genotypic reverse evolution is generally less likely when the genetic distance increases. We have observed such a decline in an experimental study on bacterial resistance to antibiotics (Tan et al. 2011). The decline of reversibility with the genetic distance (the complexity of adaptations) suggested a molecular, probabilistic form of Dollo's Law (Dawkins 1996). At each distance, epistasis decreases the probability of genotypic reversibil-

ity. This is reasonable because epistasis adds to the ruggedness of the landscape, blocking return paths to the ancestral genotype.

The nonmonotonic reversibility curves reflect the competition between two opposing effects of epistasis: decreasing the genetic change in the new environment (which generally increases reversibility), and decreasing the probability of reverse evolution at each given distance. The situation is more complicated for fitness recovery; but the overall nonmonotonic behavior is very similar.

SLOW SWITCHING: INCREASED REVERSIBILITY FOR SMALL POPULATIONS

The average reversibility of evolution under slow switching was simulated with different magnitudes of epistasis (Fig. 3). Slowly switching between environments increases the average reversibility for a wide range of fitness landscapes (except for a very slight decrease in fitness recovery for $\sigma < 0.8$). The increase in reversibility is especially significant for landscapes with extensive epistasis.

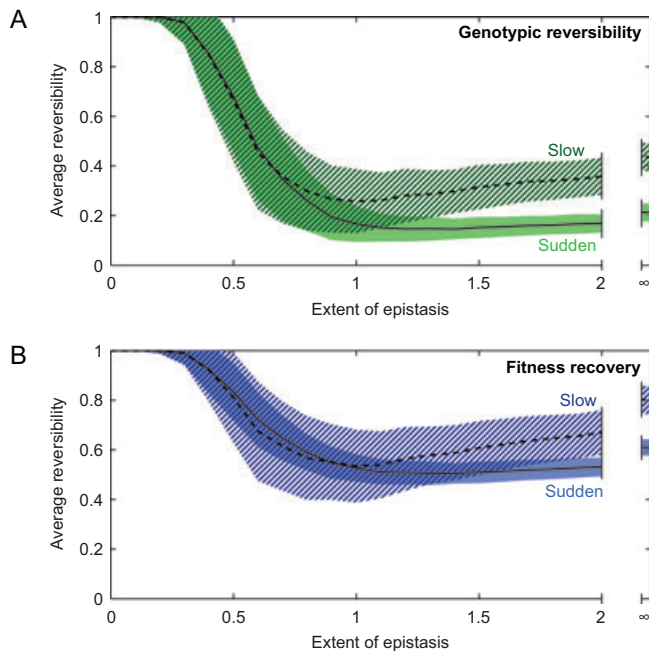


Figure 3. Slowly switching between environments increases the average reversibility for small populations under strong selection. Such populations are subject to only negligible clonal interference, and can be characterized by a random walker on fitness landscapes. The increase in reversibility is especially significant for landscapes with extensive epistasis. Shaded areas are SDs of 1000 fitness landscape pairs (total number of possible mutations is $n = 10$). SEs are smaller than the curve width.

The mechanism of this effect can be understood by dissecting reverse evolution according to different evolved distances in the new environment, similar to our analysis of sudden switching (Fig. 2). Slow switching generally decreases the number of mutations acquired in the new environment, and at the same time, increases the probability of reverse evolution at each given distance (Figs. S3, S5). Both effects contribute to the increase in reversibility.

Slow switching decreases the mean number of mutations acquired in the new environment because it influences the effective “strategy” in which the population searches for evolutionary paths on a fitness landscape. For example, a population that is initially at a local maximum in the ancestral environment will typically not be at a local maximum when transferred suddenly to the new environment. In this case, the population will randomly acquire and fix one of the possible beneficial mutations. However, if the environment is changed slowly then the population will acquire the mutation that first becomes beneficial. This mutation will tend to be one of the more fit mutations in the new environment, meaning that slow switching biases the evolutionary path toward the more fit mutations at each step. This bias explains the smaller number of mutations acquired in the new environment, as the population will find a

fitness peak more rapidly if it follows a path of steeper increase in fitness.

Our observation that slow switching increases the probability of reverse evolution at each genetic distance suggests that there is a sense in which the population is being “guided” back to its original state. For example, if a population evolves only one step at each instance during a slow switching, its behavior is in a sense “guided” by the gradual change in environment: It acquires a certain mutation at a certain intermediate environment. When the environmental change is played exactly backwards, the population can follow the backward path (being “guided” back) to the ancestral genotype, leading to perfect reversibility regardless of the genetic distance. Although this is not guaranteed for the case of gaining two or more consecutive mutations in a given intermediate environment, the above example illustrates how slow switching helps to guide a population back to its ancestral genotype.

The increase in reversibility with slow switching does not depend upon the total number of mutations n in the fitness landscape. We simulated reverse evolution from $n = 1$ (two possible genotypes) to $n = 20$ (about 1 million possible genotypes). On fitness landscapes with maximal epistasis ($\sigma = \infty$), slow switching always leads to an increase in reversibility (Fig. 4). This effect is more significant for larger fitness landscapes. For large n , our analytical estimates suggest that genotypic reversibility decays as $1/n$ under sudden switching, and decays slower than $\ln n/n$ under slow switching (Fig. S5).

SLOW SWITCHING: MULTIPLE ROUNDS OF SWITCHING

Natural populations sometimes experience multiple rounds of switching between the ancestral and the new environments. Such repetitive environmental fluctuations can significantly influence the evolutionary behavior of populations (Bergstrom et al. 2004; Kashtan et al. 2007). We simulated reverse evolution under 20 rounds of switching between a fixed pair of environments. In each simulation, the two environments were generated independently with maximal epistasis ($\sigma = \infty$). The simulation procedure is similar to previous sections (Fig. 1), but with multiple iterations of the two steps in Figure 1B, C. The “ancestral genotype” in each round is defined as the genotype at the end of the previous round.

We find that slow switching increases reversibility under all rounds of switching that were simulated (Fig. S9A, B). As the number of rounds increases, both genetic reversibility and fitness recovery monotonically increase and approach 1 (perfectly reversible), but at a faster rate under slow switching.

Under sudden switching, the average genetic distance evolved in the new environment gradually decays after multiple rounds of switching (Fig. S9C). After 20 rounds, the average

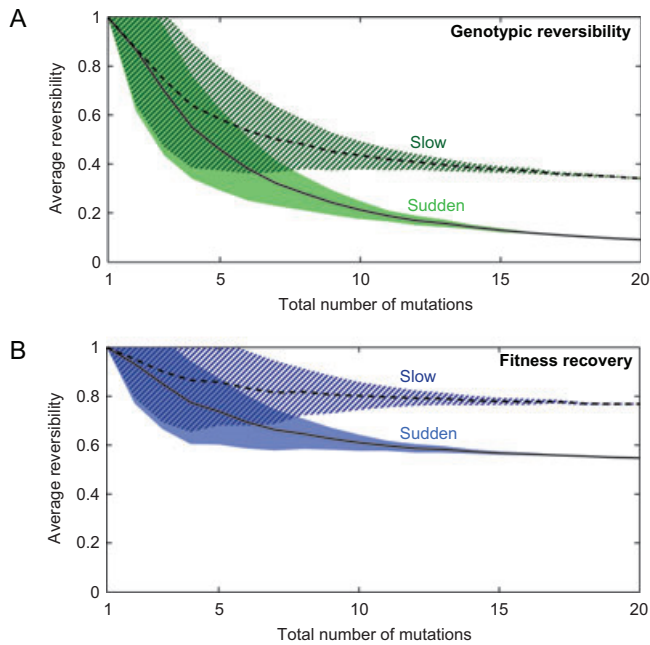


Figure 4. Slowly switching between environments increases the reversibility of evolution regardless of the total number of mutations n . This effect is more significant for larger fitness landscapes, and applies for both genotypic reversibility (A) and fitness recovery (B). For large n , our analytical estimates suggest that genotypic reversibility decays as $1/n$ under sudden switching, and decays slower than $\ln n/n$ under slow switching (Figure S5). Shaded areas are SDs of 1000 samples. SEs are smaller than the curve width. Fitness landscapes were generated with maximal epistasis ($\sigma = \infty$).

distance has fallen below 0.4, suggesting that the population tends to find and stay at a mutual local maximum of the two fitness landscapes after multiple periods of environmental fluctuations—a “generalist” strategy in the two environments. As a result, the reversibility of evolution increases, but the average fitness acquired in the ancestral environment is compromised: The average fitness in the ancestral environment moderately decays after each round except for a slight increase in the first round, whereas the fitness in the new environment always steadily increases (Fig. S9E). This effect is observed with both uniformly and normally distributed fitness values used to construct the landscapes.

In contrast, the average distance evolved under slow switching achieves a steady value of 1.2 within the first three rounds (Fig. S9C) with an almost perfect genetic reversibility (Fig. S9A). Therefore, instead of staying at a mutual local maximum, the majority of simulated populations oscillate between a pair of genotypes separated by a distance of one or two mutations—a “specialist” strategy in each environment. Interestingly, the average fitness in the ancestral environment is significantly improved after the first round of slow switching, but additional rounds do not further increase the fitness (Fig. S9E). Slow switching

also increases the probability of reaching the global maximum by a factor of 1.6 compared to evolution in only one environment (Fig. S9D), consistent with a recent computational study that shows an enhanced ability of populations to reach a specific goal in temporally fluctuating environments (Kashtan et al. 2007).

In conclusion, slowly switching between environments facilitates reverse evolution even after multiple rounds of switching. In particular, the genetic reversibility becomes almost perfect after two to three rounds of slow switching. We also confirmed this effect in small populations with small-effect mutations ($s_b \ll 1$, but still “strong” in the sense that $Ns_b \gg 1$), which search on a fitness landscape as a biased random walker (Fig. S10).

EVOLUTION OF LARGER POPULATIONS: SLOW SWITCHING NO LONGER FACILITATES REVERSE EVOLUTION

When the population size gets larger, clonal interference becomes significant. In the classic description of clonal interference, mutations still reach fixation one at a time, but the more advantageous mutations will be comparatively favored (Gerrish and Lenski 1998). In the extreme case where the number of interfering mutations, $\pi(s_b) \mu_b N \ln(Ns_b)/s_b$ is very large, the most advantageous available mutation will always win the competition. With a simple phenomenological model, we simulated the effect of classic clonal interference by tuning the bias of a random walker on fitness landscapes according to the number of interfering mutations (Fig. S11, Rokyta et al. 2006). We found that slow switching no longer facilitates reverse evolution in very large populations (Fig. S12).

Our phenomenological model has certain limitations. It only reflects the classic description of clonal interference and treats a population as a homogeneous group of the same genotype. This model thus neglects the role of genetic diversity within a large population and does not allow for the fixation of deleterious mutations. Therefore, we conducted individual-based Wright–Fisher simulations to confirm our conclusions from the phenomenological model.

Reverse evolution was simulated on 10-dimensional fitness landscapes with maximal epistasis (the house-of-cards model) and the experimental beneficial mutation rate in *E. coli*: $\mu = 10^{-5}$ (Perfeito et al. 2007). Note that the condition for extensive clonal interference, $\pi(s) \mu N \ln(Ns_b)/s \ll 1$, depends only weakly on the value of s if $s \ll 1$ ($\pi(s) \approx 2s$ in the Wright–Fisher model). To facilitate simulation speed, we chose a relatively large $s = 0.1$, which still roughly falls into the category of small-effect mutations. The population experienced either a sudden switching between two environments (consisting of three stages: evolution in the ancestral environment, in the new one, and in the ancestral one again), or a slow switching that linearly changes fitness

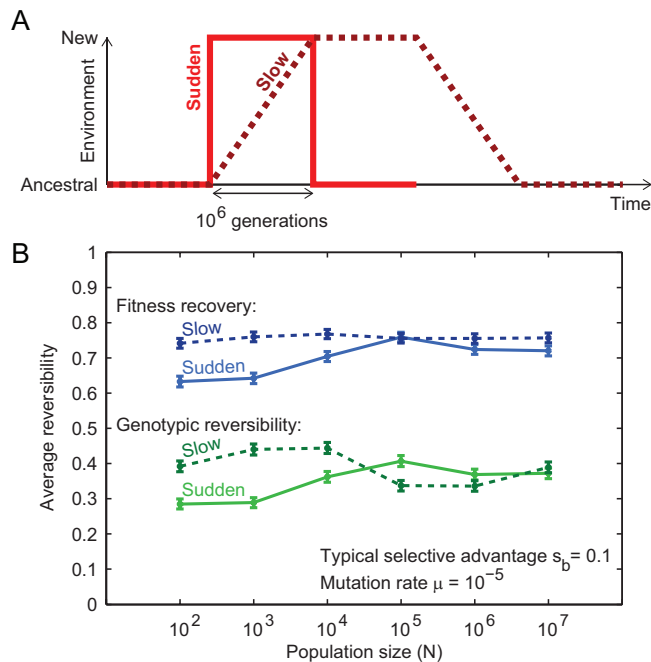


Figure 5. Individual-based Wright–Fisher simulations show that slow switching only facilitates reverse evolution in small populations. (A) A schematic illustration of our individual-based simulation. Fitness landscapes were generated independently in the two environments with maximum epistasis (the house-of-cards model with typical selective advantage $s = 0.1$). The mutation rate was set to the experimental value of *E. coli*: $\mu = 10^{-5}$. The time interval between environmental switching and the duration of a slow switching (both 10^6 generations) is much longer than the longest typical time of reaching the first local maximum (10^5 generations in the smallest populations). Fitness values were linearly modified in each generation of the slow switching. (B) Average levels of evolutionary reversibility for different population sizes. Slow switching only significantly increases reversibility for small populations, where clonal interference is negligible or moderate (the number of competing mutations is moderate). This is consistent with observations under our phenomenological model. Error bars are binomial errors of 1000 simulations.

values during a long period (two additional stages for linear environmental transition) (Fig. 5A).

We found that slow switching only significantly enhances evolutionary reversibility for small populations ($N \leq 10^4$, Fig. 5B), where clonal interference is negligible or moderate. The increase in reversibility starts to vanish between $N = 10^4$ and 10^5 , agreeing well with predictions from our phenomenological model (Figs. S11, S12).

Although our primary conclusions from the phenomenological model still hold, the inclusion of genetic diversity has substantially altered the evolutionary behavior of the population. In particular, the presence of genetic diversity allows a population to escape from one local maximum into another fitter local maxi-

mum (Weinreich and Chao 2005). In our simulations, the evolved distance increases monotonically with the population size for $N \geq 10^4$ (Fig. S13), contrary to our phenomenological model. The largest simulated populations, $N = 10^7$, evolved an average distance 2.72 from a random genotype when initially adapting to the ancestral environments, much longer than the average distance 1.72 expected by the simple phenomenological model (Orr 2003). Moreover, 30% of the ending genotypes are inaccessible from the starting genotype by any walker that only fixes beneficial mutations.

Discussion

In this article, we quantitatively studied the effect of slowly switching between environments on the reversibility of evolution. This effect is more complicated than in thermodynamic systems, where reversibility is always maximized at an infinitely slow rate. In small populations where clonal interference is negligible, slow switching enhances the reversibility of evolution. This significant increase is observed even in populations with a moderate level of clonal interference. Perhaps surprisingly, the underlying mechanism is a reduced number of mutations acquired in a new environment, and an elevated probability of reverse evolution at each of these genetic distances. In contrast, slow switching no longer increases reversibility in very large populations, where clonal interference is extensive.

Our conclusions are robust against different sizes of the fitness landscapes (Fig. 4), and against different methods of constructing them (Figs. S7, S8). Although this article primarily focuses on the “rough Mt. Fuji type” of epistatic landscapes, we have also tested another widely used method, the Kauffman NK model (Kauffman and Weinberger 1989; Hordijk and Kauffman 2005). In fact, the two models share two limiting cases: If $\sigma = 0$, the resulting landscapes are purely additive ($K = 0$ in the NK model); If $\sigma = \infty$, epistasis is maximal and the landscapes are purely random ($K = N-1$ in the NK model, also called Derrida landscapes [Derrida 1981]). Our conclusions are valid for all intermediate values of K in the NK model, and with both uniformly and normally distributed fitness values used to construct the fitness landscape (Fig. S7).

Beyond the topic of reversibility, we also simulated multiple rounds of environmental switching to explore how fluctuating environments could affect the dynamics of evolving populations (Figs. S9, S10). Consistent with previous studies (Bergstrom et al. 2004; Kashtan et al. 2007), we found that fluctuating environments have a substantial impact on evolution. Furthermore, our simulations show that the sign of this effect depends crucially on whether the environment varies slowly or suddenly. Populations tend to locate a mutual local maximum of the two environments and remain there under sudden switching, whereas they

oscillate between two different genotypes under slow switching. Only in a slowly varying environment does the population acquire a higher fitness value and a higher probability of reaching the global optimum than simply evolving in one invariant environment (Figs. S9, S10).

We modeled large populations by two approaches: a phenomenological model that treats a population as a random walker with a bias toward more advantageous mutations (Figs. S11, S12), and an individual-based simulation that includes genetic diversity within a population (Fig. 5). Our phenomenological model only reflects the classic viewpoint on clonal interference, in which mutations reach fixation one at a time (Gerrish and Lenski 1998). Under this assumption, a population can be represented by a random walker that takes one beneficial mutation at each step. However, in the presence of genetic diversity, a mutant lineage can acquire a second mutation before its eventual fixation or extinction. This additional effect allows a large population to gain multiple beneficial mutations at once (Desai et al. 2007) or to escape from one local maximum to a fitter local maximum (Weinreich and Chao 2005).

Although both approaches agreed on the effect of slow environmental changes, the increased genetic diversity in large populations has a profound effect on evolutionary reversibility. On one hand, a longer evolved distance is less likely to be reverted, impairing reverse evolution. On the other hand, the presence of genetic diversity allows the population to retain previous genotypes in small quantities, potentially facilitating reverse evolution. Furthermore, as the population size approaches infinity, the global maximum of fitness will almost always be reached, resulting in perfect reversibility regardless of sudden or slow switching. For $N = \infty$ where evolution is deterministic, the global maximum of an n -dimensional landscape will appear in the population after no later than n generations, with a fraction of $\sim \mu^n$. Afterwards, this global maximum will rapidly sweep the population in $-\ln(\mu^n)/s$ generations ($\approx 10^3$ with our parameter choice).

Our current computational models have several limitations. We assumed for sudden switching that the time between environmental changes is sufficient for the population to always reach a local maximum. Sudden switching with shorter intervals would be interesting but is beyond the scope of this article. We also assumed that the fitness of each genotype changed linearly during environmental switching. This is a reasonable assumption, but biological systems can sometimes be highly nonlinear. For example, an antibiotic may have little effect on bacteria before reaching a certain concentration threshold (the “minimum inhibitory concentration”). In such circumstances, the difference between slow and sudden switching may be diminished.

Despite these limitations, our study offers important quantitative insight into reverse evolution under fluctuating environ-

ments. Previous research has extensively studied reverse evolution both theoretically and experimentally. However, these studies were almost exclusively conducted assuming instant environmental changes. In contrast, we concentrate in this article on the rate of environmental change, which has not been recognized as a factor that could substantially influence reverse evolution. Our computational model has provided a robust prediction that can be tested with natural observations and experimental evolution. In particular, our results suggest that the evolution of a species should yield significantly different outcomes (reversibility, fitness, and genetic distance) under different rates of environmental change. Indeed, two species experiencing the same environmental fluctuations may respond very differently because the environmental switching may be sudden or slow with respect to their own rates of adaptation. Laboratory evolution experiments could employ a scheme similar to our individual-based simulation to test our prediction that slowly switching between environments typically increases the reversibility of evolution.

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Supporting Information

The following supporting information is available for this article:

Figure S1. Comparison between simulated walk length distribution walk distance distribution and the analytical estimates by Flyvbjerg and Lautrup (1992) for a random walker.

Figure S2. Comparison between simulated walk distance distribution of a greedy walker and two analytical estimates.

Figure S3. Simulated walk distance distribution for a random walker under sudden or slow switching on purely random landscapes.

Figure S4. Simulated walk distance distribution for a greedy walker under sudden or slow switching on purely random landscapes.

Figure S5. Simulated probability of reverse evolution at each genetic distance for a random walker on purely random landscapes.

Figure S6. Simulated probability of reverse evolution at each genetic distance for a greedy walker on purely random landscapes.

Figure S7. Similar to Figure 3 in the main text, but the fitness landscapes were constructed using the Kauffman NK model (Kauffman and Weinberger 1989), with either uniform or normal distributions for the underlying fitness values.

Figure S8. Similar to Figure 3 in the main text, but the initial additive (nonepistatic) landscapes were constructed differently.

Figure S9. In small populations with large-effect mutations ($s_b \gg 1$), slowly switching between environments facilitates reverse evolution even after multiple rounds of switching.

Figure S10. Similar to Figure S9, but with small-effect mutations ($s_b \ll 1$).

Figure S11. We propose a phenomenological model to simulate the effect of clonal interference in large populations, and use individual-based simulations to confirm the rough consistency of this phenomenological model on a simple fitness landscape.

Figure S12. Under our phenomenological model of clonal interference slowly switching between environments no longer facilitates reverse evolution for very large populations that are subject to extensive clonal interference.

Figure S13. Average distance evolved in the new environment for individual-based Wright–Fisher simulations.

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

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