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Adaptive Walks Toward a Moving Optimum

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ABSTRACT

We investigate how the dynamics and outcomes of adaptation by natural selection are affected by environmental stability by simulating adaptive walks in response to an environmental change of fixed magnitude but variable speed. Here we consider monomorphic lineages that adapt by the sequential fixation of beneficial mutations. This is modeled by selecting short RNA sequences for folding stability and secondary structure conservation at increasing temperatures. Using short RNA sequences allows us to describe adaptive outcomes in terms of genotype (sequence) and phenotype (secondary structure) and to follow the dynamics of fitness increase. We find that slower rates of environmental change affect the dynamics of adaptive walks by reducing the fitness effect of fixed beneficial mutations, as well as by increasing the range of time in which the substitutions of largest effect are likely to occur. In addition, adaptation to slower rates of environmental change results in fitter endpoints with fewer possible end phenotypes relative to lineages that adapt to a sudden change. This suggests that care should be taken when experiments using sudden environmental changes are used to make predictions about adaptive responses to gradual change.

FOLLOWING an environmental change, lineages adapt either by using standing genetic variation or by fixing novel beneficial mutations, depending in part on the timescale considered. Traditionally, adaptation is studied by considering the changes that take place in a population or lineage after it is suddenly placed in an environment to which it is poorly adapted. When this adaptation occurs by fixing sequential novel beneficial mutations, it is often described as an adaptive walk. The majority of experimental and theoretical studies of adaptation follow a change in phenotype in a novel constant environment (reviewed by Orr 2002; Elena and Lenski 2003). Some experimental studies also document adaptation to sequential environments (Travisano *et al.* 1995; Collins *et al.* 2006). However, few environmental changes outside of laboratories and natural disasters occur instantaneously, and few natural environments remain constant over the time needed to fix beneficial mutations. Because of this discrepancy between the stability of environments used to study adaptation and that of natural environments, there is a growing concern that changing environments should be taken into account in experiments and models of adaptation (Wilson *et al.* 2006).

Adaptive walks toward stationary optima have been described by both theory and experiments. Adaptation in a stable novel environment happens by first fixing beneficial mutations of large effect and then those of smaller effect, with adaptation following a "decreasing returns" scenario (Orr 1998). This has been shown to occur in large microbial populations (Gerrish 2001; Imhof and Schlötterer 2001). In addition, it has been suggested that the number of possible beneficial mutations decreases with the magnitude of effect of these mutations (Wichman *et al.* 1999; Riehle *et al.* 2001; Anderson *et al.* 2003). Models describing adaptation to a changing environment or with a changing optimum phenotype describe shorter-term processes, often with relatively weak selection, where selection acts primarily on standing variation or where few alleles are accessible by mutation (Pease *et al.* 1989; Lynch *et al.* 1991; Boulding and Hay 2001; Bello and Waxman 2006; Wilson *et al.* 2006). An extension of these models, where the effect of gradual increases in selection pressure influences the order of fixations, was recently reported (Kopp and Hermisson 2007). To the best of our knowledge there is no explicit study to date of how rates of environmental change systematically affect both the dynamics and the outcomes of adaptation by the fixation of novel beneficial mutations.

How rates of environmental change may affect adaptive outcomes is of interest in many medical and ecological problems where the end phenotypes themselves are of practical concern, such as the evolution of antibiotic resistance (Perron *et al.* 2005) and phytoplankton responses to rising CO_2 levels and temperature (Beardall *et al.* 1998; Collins and Bell 2004). Both of these cases involve large microbial populations that have the potential to adapt through the fixation of novel

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mutations, such that the end populations contain genotypes and phenotypes that are not present in contemporary populations.

Here we describe adaptive walks in an environment that changes in a constant direction, but at different rates. Although it is obvious that many natural environments vary periodically or stochastically, several aspects of natural environments change in a constant direction on average. Examples might be global levels of CO_2, mean global temperatures, and the levels of antibiotics experienced by pathogenic bacteria. One common feature of these environmental changes is that they occur slowly (over years) relative to the generation times of microbes (hours or days). Because of this, it is likely that, even if the total magnitude of environmental change is large, adjacent generations (or groups of generations, as in the cases of phytoplankton blooms or bacterial infections) of microbes probably experience the same mean environment, while very distant generations probably experience different mean environments.

We expect that the rate of environmental change should have a systematic effect on adaptive walks. This expectation can be explained qualitatively as follows: a well-adapted population is subjected to either a single sudden change in environment or a gradual environmental change. The gradual change can be considered to be a series of smaller step changes, which is reasonable if the environment changes slowly with respect to the generation time of the organism. The total magnitude of environmental change experienced over a given time is the same in both cases; only the rate differs. In the case of a sudden large change, fitness will substantially decrease once and be regained over time through a series of mutations of decreasing effect. In the case of the gradual change, fitness will repeatedly decrease by small amounts and be regained by mutations of small effect. A second possibility in the case of gradual change is that beneficial mutations of small effect may not fix rapidly enough, as selection pressure will be low, and so the fitness of the population may decrease over several "steps" before a mutation fixes, leading to fewer fixed mutations than in the first gradual change scenario. In terms of the size of mutations fixed, the initial mutation following an abrupt change causes a large increase in fitness, but in the case of gradual change (or small environmental change), the initial mutation causes a much smaller increase in fitness, assuming that it fixes at all. This explanation is a simplification and assumes the population to be moving toward the same adaptive peak(s) no matter how quickly the environment is changing; sudden change simply moves the population further away from the adaptive peak (produces a larger drop in fitness) than does a small change.

Here, we simulate adaptive walks in changing environments by modeling selection on short RNA molecules on the basis of their ability to fold stably while maintaining a secondary structure resembling that of their parent at high or increasing temperatures. This is not a model of RNA molecular evolution. Rather, RNA molecules are treated like individuals and are used because they have the convenient feature of having a definable sequence that results in a phenotype that can be quantified in terms of folding stability and secondary structure. Previous simulations have used changes in tRNA secondary structure as a toy model to examine how shape transitions may occur (Fontana and Schuster 1998) or to describe epistatic interactions (Wilke *et al.* 2003). We vary the rate of increase in temperature from a single step of 57° followed by 114 cycles of constant high temperature to 114 cycles where the temperature is raised half a degree per cycle. The single-step change is analogous to classical studies of adaptation where a population is suddenly placed in a novel stable environment. Several intermediate rates of change are also used. This simulation is based on the foundation laid out by Gillespie (1983, 1984, 1991) where adaptation of a well-adapted ancestor is modeled through the fixation of novel mutations and where each generation has access to all single point mutations (Gillespie 1984). This is intended to approximate adaptation in a large asexual population, and indeed most experiments describing adaptive walks use microbial or viral populations. However, the assumptions in this model may break down for extremely large population sizes or high mutation rates, such as in viral populations (Cuevas *et al.* 2002). We examine the dynamics and outcomes of adaptation in terms of fitness and phenotype. Since there is no *a priori* definition of the optimal phenotype, we can test how convergent or divergent the phenotypic outcomes of adaptation are. From the differences in the timing and magnitude of fixed beneficial mutations, we suggest how natural selection may systematically differ for sudden and slow rates of environmental change.

METHODS

Simulation: In this simulation, RNA molecules are treated like individuals under selection. Each individual can be described by two parameters: folding stability, which is measured as Gibb's free energy for the most stable structure that the molecule can fold into (ΔG), and secondary structure, which can be visualized by a structural dot plot. Differences between secondary structures can be quantified as a structural Hamming distance corresponding to the number of mismatches between structures, where each position is designated as either paired or unpaired. The simulation uses discrete asexual generations. At each step, all the single mutants of the starting sequence are created. This results in a mutation rate of 1/nucleotide/time step, which is reasonable for large microbial populations, where all single mutants may occur every generation, but where double mutants are very rare or absent. Secondary structure and ΔG are then determined for each mutant. The

change in fitness of the mutants (Δw) is then calculated on the basis of changes in stability and structure relative to their immediate predecessor (parent). Qualitatively, selection in this model acts on stability and similarity to parental phenotype. At each round of selection, a single winner is picked stochastically, where the chance that any given mutant replacing the parental sequence is $2s$. Single-mutant neighbors are sampled in random order. Mutants with a negative change in fitness or mutants that fail to fold (positive ΔG) have 0 probability of being the winner of the round of selection. All mutants that have the same stability as the parent and are structurally identical to the parent have same chance of fixing as does the parent of remaining fixed. If no beneficial mutation fixes in a given cycle, then the parent remains fixed or is replaced with a neutral mutant. Thus, the simulation allows for the fixation of either neutral or beneficial mutations. This winner of a cycle is then used as the "parent" sequence to create all single mutants in the next step. Note that this means that a single selective sweep may occur in each cycle, but is not obliged to. In theory, it is possible for only neutral changes to occur, or for the parental sequence to remain fixed, in this model. However, given the magnitude of environmental change and mutational supply, completely neutral walks where beneficial mutations fail to fix 114 times in a row are extremely unlikely. Each run of the simulation has 114 such steps (cycles 0–113), where each complete run of the simulation yields a single sequence, which we refer to as the "evolved" sequence. In all cases, the temperature is raised from 20° to 77°. The control simulation consists of 114 cycles at 20°.

The increase in fitness of each sequence as a function of temperature was defined as $\Delta w(T) = (\Delta G \text{ mutant} - \Delta G \text{ parent})/(\text{structural Hamming distance from parent} + 1)^4$. This is similar to a conventional selection coefficient. "Parent" is the sequence from which mutants were created at that step. The structural Hamming distance was calculated with the function "bp_distance" from the Vienna RNA Package. This is an arbitrary fitness function, which allows a fitness landscape with three axes (ΔG, phenotypic similarity to parent, and fitness) to be defined at any point in time and allows enough variation in fitness for selection to act without deterministically producing a single outcome or causing the population to go extinct. Structural Hamming distance is measured relative to the parent so that the topography of the fitness landscape may vary between environments. Since there is no *a priori* target structure, the optimal phenotypic solution(s) may change as the environment changes. Defining an optimal phenotype (structure) would result in a fitness landscape with a single peak or ridge and a single optimal phenotype or range of phenotypes no matter what the environment. However, large asexual populations tend to diverge as they adapt to different environments (see, for example, Travisano *et al.* 1995). In this study, we ask how rates of environmental change affect adaptive outcomes. If the phenotypic outcome of adaptive walks is deterministic or very strongly constrained to a certain range of endpoints, then, by definition, the rate of environmental change will not be able to affect the average phenotypic outcome. Here we have allowed the fitness landscape and optimal phenotype(s) to change as the environment changes. This also allows for the possibility that rugged adaptive landscapes may emerge and result in strong historical constraints on the outcomes of adaptation.

The function used to define fitness increases is arbitrary, but consistent for the entire study. In this study, the particular function used to define fitness is not important. Indeed, all that is needed is a definition of fitness with at least two dimensions in which both characters are correlated with fitness strongly enough to affect adaptation. Here the denominator is raised to the fourth power because the magnitude of change in ΔG is larger than the magnitude of changes in structure. We compensate for this difference in magnitude so that changes in structure may affect adaptation in this system.

We compare mutants to their parent rather than the ancestral sequence. Since mutants must displace their parent to fix, any selective advantage that they have must be relative to their parent, who is presumably present, rather than to some distant ancestor, who is absent. As a consequence of comparing mutant to parental rather than ancestral fitness, both transitive and nontransitive fitness increases are allowed in this model. This is a result of the Hamming distance not being a state function, even though ΔG is.

RNA secondary structure was predicted using the Vienna RNA Package, version 1.6.1 with the default setup, available at http://www.tbi.univie.ac.at/~ivo/RNA/. The starting sequence used was Acinetobacter sp. ADP1.trna5-ThrTGT, available from the Genomic tRNA Database at http://lowelab.ucsc.edu/GtRNAdb. Since this is not intended to be a model of tRNA molecular evolution, any random sequence that folds into a secondary structure and "melts" gradually as temperature rises and that was relatively stable in the starting environment could be used. However, most random sequences do not fulfill these criteria, so a real sequence with these properties was used.

The simulation was performed using five different rates of environmental change. The total increase in temperature was kept constant (57°). The different rates are referred to as follows:

Sudden: increase in temperature in a single step from 20° to 77°;
Intermediate 2: increase in temperature in the two first steps of the simulation, each of 28.5°;
Intermediate 10: increase in temperature in 10 first steps, each of 5.7°;
Intermediate 40: increase in temperature in 40 first steps, each of 1.425°;

Gradual: increase in temperature in 114 steps, each of 0.5°.

All simulations ran for 114 steps. For example, under Intermediate 2 conditions, the temperature would increase from 20° to 48.5° in the first step and from 48.5° to 77° in the second step, followed by 112 steps at 77°. The simulation was performed 3000 times for each condition.

Comparison of structures: The variability in final structures obtained following selection at different rates of environmental change were analyzed with a dot-plot style analysis using a program written by Ulrike Goebel (Max Planck Institute for Plant Breeding Research, Cologne, Germany). All of the final structures obtained after 114 rounds of selection were aligned and the results are graphically presented in a dot plot (Figure 5a). Each position in the dot plot shows the frequency (of 3000) that a pair forms between the two base pairs at those positions in the sequence. Such probabilities are graphically shown with solid dots of a size proportional to the probability itself. So, the number of solid dots in the *i*th row show how many different positions the nucleotide in position i can form a pair with. The number of different bindings is the number of different positions to which a given position is paired at the end of at least one replicate run. This was measured separately for each position in the evolved sequence. This measure is used as an indicator of the structure variability within the set.

Calculations and statistical tests: Relative fitnesses of endpoints in Figure 3 was defined as $W_{\text{relative}} = \Delta G/(\text{sum of Hamming distances over entire adaptive walk})$. Because fitness increases were not constrained to be transitive, the fitness of the endpoints was not measured relative to the ancestor. Instead, endpoints are compared to each other. Since ΔG is a state function, increases in stability will always be transitive, so the ΔG of endpoints can be compared directly without considering selection in the intermediate environments. We did not select on structural similarity to the ancestor, but rather on the ability to avoid large sudden changes in secondary structure over evolutionary time, so the measure of relative fitness is penalized for the amount of structural change that occurred over the entire adaptive walk. Note that this is different from the fitness measure (against ancestor) typically used in experimental evolution studies. W_{relative} ranks only endpoints and does not provide absolute values for fitnesses.

In all cases where multiple pairwise comparisons are made, a Bonferroni correction was used ($n = 14$). The corrected α-value is 0.003.

RESULTS

The dynamics of adaptive walks toward a stationary optimum for this model do not deviate from classical theoretical and experimental studies of adaptive walks.

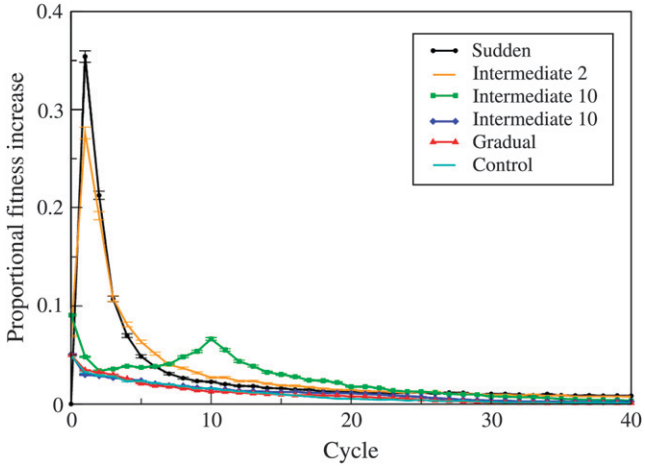

Figure 1.—Dynamics of adaptive walks at different rates of environmental change. Each line represents the mean ± SEM in the incremental increase in fitness (improvement over parent) at each step for 3000 independent simulations for a given rate of environmental change. The rates of environmental change are as follows: black—Sudden change (a single step, followed by 113 steps of stable environment); yellow—Intermediate 2 (two consecutive steps of equal size, followed by 112 steps of stable environment); green—Intermediate 10 (10 consecutive steps of equal size, followed by 104 steps of stable environment); violet—Intermediate 40 (40 consecutive steps of equal size, followed by 74 steps of stable environment); red—Gradual (114 consecutive steps of equal size); and light blue—Control (114 steps at 20°). In all cases, the magnitude of environmental change is the same, ranging from 20° to 77°.

The case of sudden environmental change in Figure 1 demonstrates that our simulation produces the expected results for adaptive walks toward a stationary optimum, where mutations of large effect fix early in the adaptive walk and are then followed by the fixation of mutations of decreasing effect. In our simulation for sudden environmental change, the largest single increase in fitness and the first step account for a large proportion of all adaptation. The average time of the largest step is also the same as in standard predictions, falling at about the second step. These dynamics agree with classic studies of adaptive walks (Orr 1998, 1999, 2002) and with those seen in experiments using large bacterial populations (Elena and Lenski 2003).

Dynamics of adaptive walks: Figure 1 shows how the rate of environmental change affects the dynamics of adaptive walks. This occurs by decreasing the magnitude of effects fixed and by increasing the range in time where mutations of largest effect are most likely to fix at slower rates of environmental change, as seen in Table 1. [With the exception of the two pairs "Gradual–Control" and Intermediate 10–Intermediate 2, all distributions differ from each other on the basis of a Kolmogorov–Smirnov test, and *P*-values range from 0.0005 to $<10^{-7}$. Kolmogorov–Smirnov statistics and *P*-values are in the appendix]. For very gradual environmental change, beneficial mutations are equally likely to fix at any point in

TABLE 1

Summary of adaptive dynamics

Rate of environmental change	Cycle of largest step	SD (cycle)	SEM (cycle)	Size of largest step	SD (size)	SEM (size)
Control	5.29	7.04	0.12	0.07	0.01	0.0002
Sudden	3.21	4.39	0.08	0.49	0.24	0.004
Gradual	3.21	7.50	0.13	0.11	0.27	0.0004
Intermediate 2	6.44	9.46	0.17	0.45	0.27	0.005
Intermediate 10	14.06	9.78	0.17	0.21	0.11	0.002
Intermediate 40	5.69	12.32	0.22	0.11	0.02	0.0004

time, and the dynamics of an adaptive walk at the slowest rate of environmental change used is not significantly different from the case of the control where the environment is being held constant. For intermediate rates of environmental change, there is a time when mutations of larger effect are more likely to fix, but this time is later and much more variable than for adaptive walks in a stable environment. At slower rates of environmental change, neutral steps are more likely to occur early on in adaptive walks. Representative individual adaptive walks are in supplemental Figure 1 at http://www.genetics.org/supplemental/.

Here, the dynamics of adaptive walks are presented in terms of fixation events rather than generations. The time needed for fixation is given by $(2/s)\ln(2N)$ (Kimura 1983), such that mutations of small effect fix more slowly. For at least the first 20 cycles of this simulation, where most of the differences in dynamics occur, differences in fitness are large enough that there is little difference between measuring time in fixation events or generations. Beyond 20 cycles, the fixation times in the Gradual treatment increase drastically, such that the differences in later dynamics shown here are conservative. Times needed for fixation events are shown in supplemental Figure 2 at http://www.genetics.org/supplemental/. Time in this simulation is defined in cycles, which correspond to a round of mutation after which a single selective sweep may occur, although this model also allows for the possibility that no selective sweep occurs following mutation.

Slower rates of environmental change also affect the distribution of effects of fixed beneficial mutations. The fitness effect of fixed beneficial mutations scales with the rate of environmental change such that mutations of larger effect fix when the environment changes more rapidly.

Figure 2 shows the total amount of adaptive change that occurs during an adaptive walk, measured as the sum of fixed effects. In this case, the faster the rate of change, the more total the adaptation that occurs. All treatments show more total adaptive change than does the control, and differences among all pairs of distributions are significant (all P-values $<10^{-7}$; Kolmogorov–Smirnov statistics and P-values are in the appendix). This shows that the large increases in fitness seen following a drastic change in environment result in more total adaptation, rather than the same total amount of adaptive change simply being divided up differently depending on the rate of environmental change. Since a large change in environment results in a greater drop in fitness, the total amount of adaptation that occurs may not correspond to the final fitness reached by the end of the adaptive walk. In all cases, the path length distribution is significantly different from the control simulation.

Note that when stability is selected for in secondary structure at any temperature, this results in an increase in the number of GC pairs. Adaptation in the control thus reflects the increase in stability possible once constraints other than structural constraints imposed by this simulation are lifted. Any adaptation in excess of that seen in the control reflects the contribution of the adaptation in response to the increase in temperature or the rate of this increase.

Outcomes of adaptive walks: Figure 3 shows how different rates of environmental change result in different

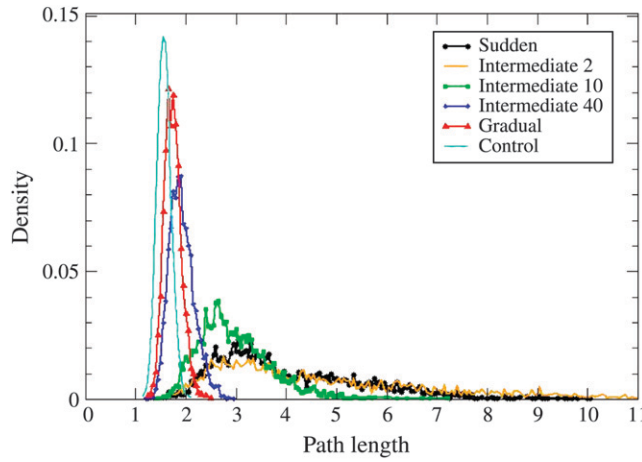

Figure 2.—Total amount of adaptation during an adaptive walk. Path length is measured as the sum of fitness increases(s) over the entire adaptive walk (114 steps). Each line is a density function for 3000 independent replicate simulations of a given rate of environmental change. The rates of environmental change are as follows: black—Sudden change; yellow—Intermediate 2; green—Intermediate 10; violet—Intermediate 40; red—Gradual; and light blue—Control. In all cases, the magnitude of environmental change is the same, ranging from 20° to 77°.

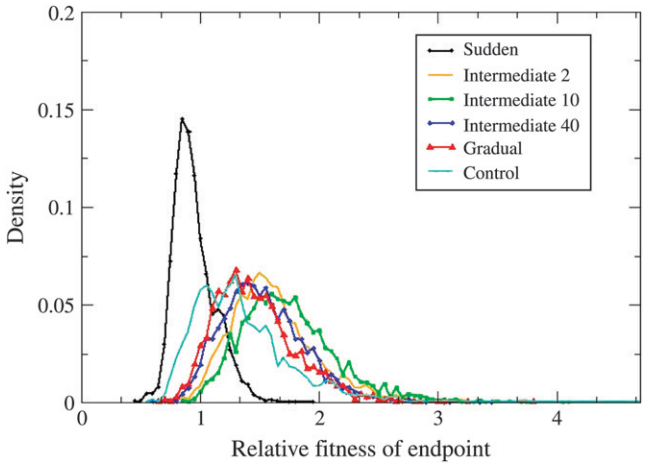

FIGURE 3.—Relative fitnesses of evolved lineages. Each line represents the density function of 3000 independent simulations for a given rate of environmental change. The rates of environmental change are as follows: black—Sudden change; yellow—Intermediate 2; green—Intermediate 10; violet—Intermediate 40; red—Gradual; light blue—Control. In all cases, the magnitude of environmental change is the same, ranging from 20° to 77°.

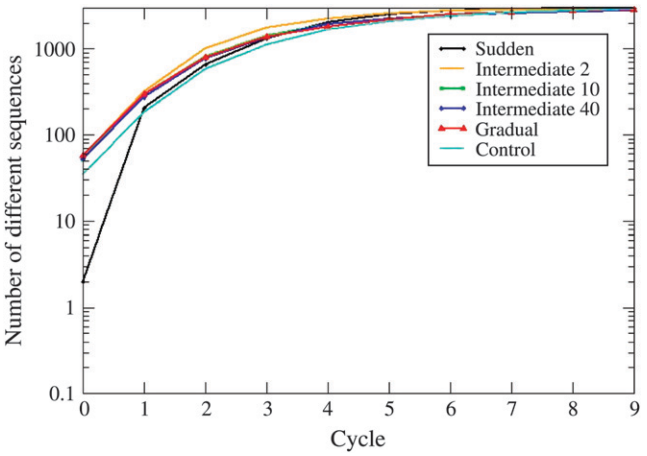

FIGURE 4.—Number of unique sequences present at a given cycle during an adaptive walk. Each line represents the total number of different sequences present during the first 10 cycles of an adaptive walk at a given rate of environmental change. The rates of environmental change are as follows: black—Sudden change; yellow—Intermediate 2; green—Intermediate 10; violet—Intermediate 40; red—Gradual; and light blue—Control. In all cases, the magnitude of environmental change is the same, ranging from 20° to 77°. In all cases, each line represents 3000 independent replicates. At the first step (cycle 0), the maximum number of different sequences of 3000 is 229 (3L + 1; this corresponds to all single point mutants plus the parent); after the first cycle, the maximum number of different sequences possible is 3000.

outcomes in terms of fitness, where slower rates result in fitter endpoints (differences among all pairs of distributions are significant; all *P*-values $<10^{-7}$. Kolmogorov–Smirnov statistics and *P*-values are in the APPENDIX). On average the slower rates of change resulted in evolved sequences with measures of relative fitness ~1.5 times higher than the evolved sequences produced by sudden change. In addition, the upper tail of the fitness distribution of the endpoints of adaptation in the slowly changing environment contains sequences with measures of relative fitness roughly twice as large as those of the evolved lineages produced in the static environment. However, the rank order of relative fitnesses of the evolved sequences does not correspond to the rank order of rates of environmental change. Rather, the evolved sequences with the highest relative fitness occur, on average, in the case where 10 steps of equal size are used for the temperature increase. Changes in the rate of gradual environmental change do not affect the range of relative fitness outcomes, at least for the range of rates investigated in this experiment. Since there is no significant difference in the ΔG values of the endpoints, differences in measures of relative fitness reflect mainly the number of structural changes that occurred during adaptation.

In Figure 4, we show that adaptation following a rapid environmental change results in less variable outcomes early in adaptation, measured as fewer unique sequences obtained across simulations over the first 10 cycles of selection (differences among all pairs of distributions are significant; all *P*-values $<10^{-7}$. Kolmogorov–Smirnov statistics and *P*-values are in the APPENDIX). This is most apparent during the first two cycles of selection. By cycle 10, the number of unique sequences is at the maximum in all cases and does not decrease for the remainder of the simulation.

Figure 5a shows dot plots of the variance in structure between replicates at the end of adaptation. By inspection, the endpoints of adaptive walks that occur at slower rates of environmental change show less variance in structure than do those following more rapid rates of environmental change. The number of possible structures increases with the rate of environmental change. This can be quantified as the number of unique base pairs formed at any given site in the sequence at the endpoints of adaptation. This is shown in Figure 5b. In this model, faster rates of environmental change result in a greater range of phenotypic outcomes (with the exception of the two pairs Gradual–Intermediate 40 and Sudden–Intermediate 2, all distributions differ from each other on the basis of a Kolmogorov–Smirnov test; *P*-values range from 0.0002 to $<10^{-7}$. Kolmogorov–Smirnov statistics and *P*-values are in the APPENDIX). The folding stability of the evolved sequences is not affected by the rate of environmental change, so that differences between evolved sequences are primarily due to differences in structure.

DISCUSSION

Our results show how the rate of environmental change alone can systematically affect the dynamics and outcomes

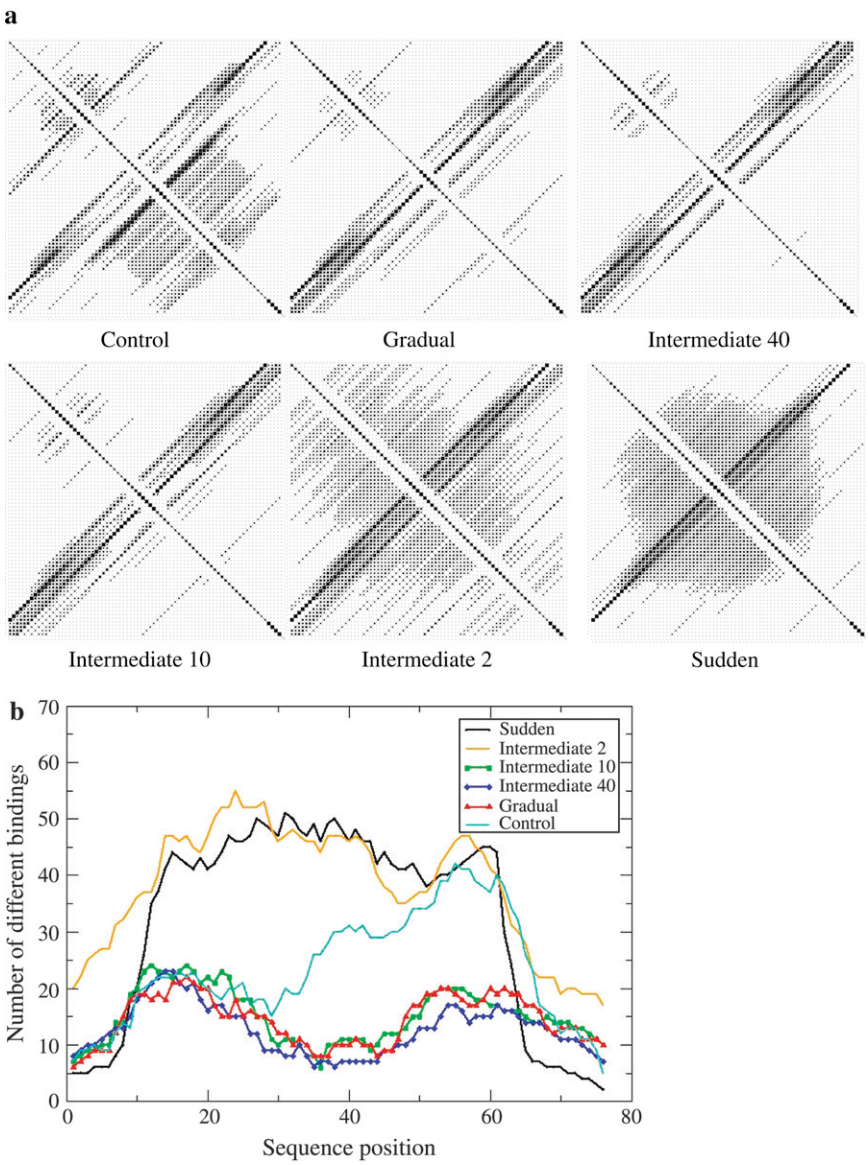

FIGURE 5.—(a) Dot plots of final structures. The secondary structures of all evolved sequences for a given rate of environmental change are aligned, and each position in the dot plot shows the frequency (of 3000) that a pair forms between the two bases at those positions in the sequence. The size of a dot is proportional to the probability that the two positions are paired. Dots along the diagonal (where a position aligns with itself) show the probability that the position remains unpaired. Because only point mutations are allowed during the simulation, the length of the sequence is fixed and structures are always aligned by sequence position. (b) The number of different bindings possible for a given position in the evolved sequence. Note that the value on the *y*-axis here is simply the number of dots present in any given column or row in the dot plots. The rates of environmental change are as follows: black—Sudden change; yellow—Intermediate 2; green—Intermediate 10; violet—Intermediate 40; red—Gradual; and light blue—Control.

of adaptive walks. Specifically, slower rates of environmental change decrease the magnitude of effects of fixed beneficial mutations, as well as increase variation in the time when mutations of larger effect are likely to fix. The increase in the variation in timing is partially attributable to more neutral or nearly neutral steps early on in the adaptive walks. These changes in dynamics are in good agreement with results from simulations of the evolution of a quantitative trait under stabilizing selection with a moving optimum. In a model where the optimal genotype was fixed but selection pressure varied over time, rates of adaptive evolution could be limited by rates of environmental change, and slower rates of change favored the earlier fixation of alleles of minor effect (KOPP and HERMISSON 2007). In our simulation, adaptation to sudden environmental change also results in lower maximum fitness and variance in fitness and a greater number of phenotypic outcomes than does adaptation to slower environmental changes of the same magnitude.

One of the most striking results of our simulation is that sudden change results in a lower possible maximum fitness at the end of the adaptive walk, even though more total adaptive change is needed to get there. This suggests that selection in the intermediate environments plays a large role in determining both the eventual outcome of adaptation and the amount of change that occurs on the way to that outcome. In the case of rapid environmental change, the lineage that does best in the final environment is the most fit. However, in the case of gradual environmental change, the evolved sequences with the highest measures of relative fitness are not necessarily those that do best in the final environment, but rather those that avoid doing poorly in any one of

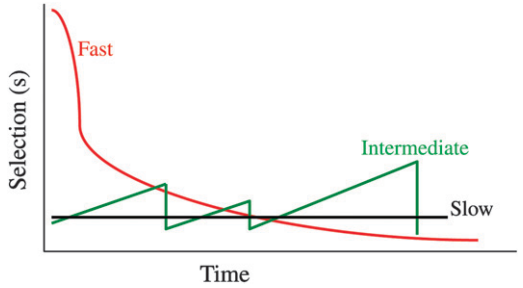

FIGURE 6.—Changes in selective coefficients over time at varying rates of environmental change.

the intermediate environments. This additional requirement may decrease the number of possible trajectories. In addition, the topology of the adaptive landscape itself may change in response to the environment and genotype at any given point in time, such that selection in intermediate environments may have a different magnitude and/or direction than it does in the final environment. This selection in intermediate environments may lead to higher historical constraints on adaptation. The environments used in this simulation are linear so that we select more strongly on the same characters (in this case, GC content and secondary structure) as the temperature increases. In this respect, the scenario here presents a conservative estimate of phenotypic and fitness differences that can be generated by selection in intermediate environments.

The dynamics and differences in dynamics produced at varying rates of environmental change can be explained intuitively in terms of selection coefficients expected following either large or small environmental changes (see Figure 6). At any given point in time, a lineage either can keep the same sequence or fix a mutant sequence. The probability that any given mutant sequence will fix at a given cycle is $\sim 2s$ (HALDANE 1927), where s is the selective coefficient measuring the fitness of the mutant sequence relative to the ancestral sequence that it would displace. Thus, the chance that some mutant fixes is the sum of $2s$ over all mutants. If s is very large, it is almost certain that the ancestral sequence will be displaced. However, if s is very small, there is a greater chance that the lineage will instead retain the same (ancestral) sequence, since most single point mutants will be less fit than the ancestor and any improvement over the ancestor is likely to be small. In other words, the drop in rank fitness of the ancestral sequence will be small if the magnitude of environmental change is small. If the environment changes drastically, the drop in rank fitness of the ancestral sequence will be larger. In this case, more mutants will have a higher rank fitness than the ancestor, so there is a greater chance that one of these will displace the ancestor. In addition, individual mutants will have a higher chance of fixation. This is what is almost always observed in adaptive walks toward stationary optima following a drastic change in environment (ELENA and LENSKI 2003). In contrast, selection coefficients will be small when the environment changes slowly as long as the ancestral sequence is well adapted to the ancestral environment. Environments tend to be autocorrelated in time, such that the best sequence at time zero is likely to be one of the best sequences at some point in the near future. If the environment continues to change, the rank fitness of the ancestral sequence will drop more and more over time, resulting in mutant sequences with higher and higher selective coefficients. In addition, the number of mutant sequences that are more fit than the ancestor will increase as the ancestral fitness drops. At some point, the chance of fixing a mutant sequence will be high. If the environment changes extremely slowly, the increase in selection coefficients of the mutants over time may not be rapid enough to tip the balance between the two processes, so that beneficial mutations are equally likely to fix at any point.

Several results of this study can be generalized. Qualitatively, phenotypes that are accessible only from mutations of large effect will be present in populations subjected to sudden environmental change, but will be absent in populations subjected to slower change. In our simulation, the large initial drop in fitness associated with the sudden change in environment pushes lineages very far from their ancestral adaptive peak and disperses them to different distant points on an adaptive landscape. Since there has been a sudden and large change in environment, the lineages suffer large drops in fitness. In our model, this corresponds to the structures melting. In this case, almost any change that produces an increase in stability will be beneficial, regardless of how dissimilar the structure is from the parental type, since all melted structures will have large and inevitable fitness costs with small differences between them, and fitness gains early in adaptation will be driven largely by gains in stability, and variance in structure between lineages will be nearly neutral. Once a reasonably stable structure is obtained, the cost of changing that structure will constrain a lineage to maintain that structure while increasing stability by increasing the number of GC pairs. On the other hand, when the environment changes gradually, lineages never experience a large drop in fitness. In this simulation, this corresponds to never "melting." In this case, adaptation is constrained by the cost of changing structure and is largely driven by increasing the number of GC pairs. The result of this is that the magnitude of environmental change at any given point in time affects whichever component of fitness is being acted upon more strongly by selection. The extent of this early divergence in phenotype may be an artifact of this model, specifically, the use of a very simple organism. As organismal complexity increases, constraints also increase (FISHER 1930; ORR 2000). Because of this, our model probably overestimates the

effect of sudden environmental change on early divergence. However, one would still expect a larger drop in fitness following a sudden environmental change than during a gradual one. This is important to adaptive outcomes when the end phenotype is caused primarily by a mutation of large effect. A population subjected to a slow change may reach the same or even higher fitness, but may be forced to do so using many mutations of smaller effect, resulting in a different phenotype. For example, bacterial populations readily evolve resistance to cationic antimicrobial peptides when the level of antimicrobial peptides is increased slowly, allowing time for the sequential substitution of several mutations (Perron *et al.* 2005), even though previous experiments showed that resistance was unlikely to evolve in response to sudden high doses of the peptides (Hancock 1997; Ge *et al.* 1999).

An extension of the above argument is that fixations of extremely large effect are likely to be advantageous in any genetic background even if the precise magnitude of the fitness increase varies (Lenski *et al.* 1998; Bull *et al.* 2000). Conversely, fixations of relatively small effect may show more extreme epistasis, where the sign of the effect may vary with genetic background (Elena and Lenski 2001). Increased epistasis would influence the future evolvability of the population (de Visser *et al.* 2003) as well as the phenotypes resulting from a single adaptive walk. Although a direct test of this is beyond the scope of this study, it seems quite likely that rates of environmental change should affect the amount of epistasis in adapted types.

A second aspect of this model, which suggests a direction for future work, is that the mutational supply is constant, since both the population size and the mutation rate are fixed. However, mutation rates themselves can be affected by rapid environmental change. For example, mutators often arise when bacterial populations are placed in a stressful environment (Taddei *et al.* 1995). Naively, one could expect that the mutational supply would be larger in populations subjected to sudden rates of environmental change if this proved stressful enough to increase mutation rate. Elevated mutation rates can speed up adaptation (Giraud *et al.* 2001), which would increase the magnitude of differences in adaptive dynamics among populations experiencing different rates of environmental change. In addition, mutation rates themselves can be adaptive (de Visser 2002), and different mutation rates may result from long-term selection under different rates of environmental change. Finally, mutation rates have been shown to affect the outcome of adaptation, where higher mutation rates favor "survival of the flattest," so that lineages with higher mutation rates tend to occupy lower and flatter peaks on adaptive landscapes than do lineages with lower mutation rates (Wilke *et al.* 2001).

Our results suggest that experiments designed to investigate phenotypic outcomes of adaptation in particular environments should take the rate of environmental change into account. One can argue that it is neither practical nor interesting to do microbial selection experiments using extremely slow rates of environmental change. After all, one of the advantages of using microbes in experimental evolution is that time can be accelerated. However, our results suggest that the differences in outcomes between a sudden change and any gradual change are much greater than differences between rates of gradual change. These differences seem largely attributable to the presence or absence of fixations of very large effect early in adaptive walks. This implies that the errors associated with speeding up an environmental change to some fast but non-instantaneous rate may often be acceptable, as long as mutations of extremely large effect that fix early in adaptive walks toward stationary optima remain inaccessible so that including even a few intermediate environments in a selection experiment or model may produce much more realistic results. In these cases, adaptive outcomes can still be interpreted meaningfully using the metaphor of an adaptive walk. This is because even though the magnitude of fixed effects and the timing of the fixations change, the outcomes seen are still the result of evolution dominated by relatively rapid selective sweeps.

The basic simplifying assumptions of adaptive walks, where a monomorphic population in which mutations arise *de novo* and fix rapidly from extremely low frequencies, are not violated here to a much greater degree than in the case of sudden environmental change. However, this may no longer be the case for very slow environmental change, represented by the Gradual scenario of this simulation, where rapid selective sweeps are rare for part of the time over which the environment changes. This can be seen by considering the average times needed for fixations of mutant sequences, which are longer on average in the Gradual scenario than in any of the other scenarios during the middle 70 cycles of the simulation. For Gradual change, the average pattern is for rapid fixations of mutants to occur at both ends of the simulation, while such rapid substitutions are unlikely in the middle of the simulation. Since mutations can continue over a long period of time in which no selective sweeps occur, populations experiencing a very gradual environmental change are likely have a large amount of standing genetic variance at some point in time. Note that this scenario is explained qualitatively in terms of selection coefficients by the Intermediate scenario in Figure 6. In the case of very gradual environmental change, the metaphor of an adaptive walk would need to explicitly incorporate the fixation of alleles segregating at relatively high frequencies in a population and the contribution of neutral evolution to evolutionary outcomes.

In this study we have shown how rates of environmental change alone can systematically alter the dynamics and outcomes of adaptive walks and have proposed how

natural selection may act to produce different outcomes in these cases. We have shown this using a simulation in a very simple system in what is presumably a relatively simple adaptive landscape. This is appropriate for large asexual populations under relatively strong selection and may be useful for examining microbial responses to directional changes, such as increases in global CO_2 levels, mean temperature, nutrient loading, and environmental levels of antibiotics. Recent studies have also shown that rates of environmental change are important to adaptive outcomes over shorter timescales and for smaller populations (Wilson *et al.* 2006). This suggests that caution should be used when interpreting laboratory studies meant to model much slower natural processes and that further studies in more realistic systems are needed so that the effect of environmental stability can be systematically taken into account in empirical and theoretical studies of adaptation.

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APPENDIX

Statistics

Rate of environmental change	Fast	Slow	Intermediate 10	Intermediate 40	Intermediate 2	Control	Statistic
				Dynamics (Figure 1)			
Fast		0.597	0.263	0.46	0.34	0.59	Kolmogorov–Smirnov value
Fast		$<10^{-7}$	0.0005	$<10^{-7}$	0.000002	$<10^{-7}$	*P*
Slow			0.403	0.37	0.44	0.14	Kolmogorov–Smirnov value
Slow			$<10^{-7}$	$<10^{-7}$	$<10^{-7}$	$<10^{-7}$	*P*
Intermediate 10					0.22	0.45	Kolmogorov–Smirnov value
Intermediate 10					0.037	$<10^{-7}$	*P*
Intermediate 40					0.43	0.32	Kolmogorov–Smirnov value
Intermediate 40					$<10^{-7}$	$<10^{-7}$	*P*
Intermediate 2						0.43	Kolmogorov–Smirnov value
Intermediate 2						$<10^{-7}$	*P*
				Path length (Figure 2)			
Fast		0.95	0.345	0.88	0.143	0.98	Kolmogorov–Smirnov value
Fast		$<10^{-7}$	$<10^{-7}$	$<10^{-7}$	$<10^{-7}$	$<10^{-7}$	*P*
Slow			0.88	0.4	0.94	0.41	Kolmogorov–Smirnov value
Slow			$<10^{-7}$	$<10^{-7}$	$<10^{-7}$	$<10^{-7}$	*P*
Intermediate 10				0.75	0.44	0.96	Kolmogorov–Smirnov value
Intermediate 10				$<10^{-7}$	$<10^{-7}$	$<10^{-7}$	*P*
Intermediate 40					0.88	0.64	Kolmogorov–Smirnov value
Intermediate 40					$<10^{-7}$	$<10^{-7}$	*P*
Intermediate 2						0.97	Kolmogorov–Smirnov value
Intermediate 2						$<10^{-7}$	*P*
				Final fitness (Figure 3)			
Fast		0.68	0.86	0.73	0.8	0.51	Kolmogorov–Smirnov value
Fast		$<10^{-7}$	$<10^{-7}$	$<10^{-7}$	$<10^{-7}$	$<10^{-7}$	*P*
Slow			0.31	0.1	0.2	0.18	Kolmogorov–Smirnov value
Slow			$<10^{-7}$	$<10^{-7}$	$<10^{-7}$	$<10^{-7}$	*P*
Intermediate 10				0.23	0.15	0.44	Kolmogorov–Smirnov value
Intermediate 10				$<10^{-7}$	$<10^{-7}$	$<10^{-7}$	*P*
Intermediate 40					0.11	0.26	Kolmogorov–Smirnov value
Intermediate 40					$<10^{-7}$	$<10^{-7}$	*P*
Intermediate 2						0.35	Kolmogorov–Smirnov value
Intermediate 2						$<10^{-7}$	*P*
				Unique sequences (Figure 4)			
Fast		0.91	0.93				Kolmogorov–Smirnov value
Fast		$<10^{-7}$	$<10^{-7}$	$<10^{-7}$	$<10^{-7}$	$<10^{-7}$	*P*
Slow			0.89	0.88	0.91	0.86	Kolmogorov–Smirnov value
Slow			$<10^{-7}$	$<10^{-7}$	$<10^{-7}$	$<10^{-7}$	*P*
Intermediate 10				0.89	0.9	0.9	Kolmogorov–Smirnov value
Intermediate 10				$<10^{-7}$	$<10^{-7}$	$<10^{-7}$	*P*
Intermediate 40					0.91	0.89	Kolmogorov–Smirnov value
Intermediate 40					$<10^{-7}$	$<10^{-7}$	*P*
Intermediate 2						0.92	Kolmogorov–Smirnov value
Intermediate 2						$<10^{-7}$	*P*
				Structure (Figure 5b)			
Fast		0.97	0.65	0.69	0.28	0.55	Kolmogorov–Smirnov value
Fast		$<10^{-7}$	$<10^{-7}$	$<10^{-7}$	0.005	$<10^{-7}$	*P*
Slow			0.38	0.22	0.88	0.5	Kolmogorov–Smirnov value
Slow			0.000019	0.04	$<10^{-7}$	$<10^{-7}$	*P*
Intermediate 10				0.47	0.75	0.34	Kolmogorov–Smirnov value
Intermediate 10				$<10^{-7}$	$<10^{-7}$	0.00018	*P*
Intermediate 40					0.88	0.57	Kolmogorov–Smirnov value
Intermediate 40					$<10^{-7}$	$<10^{-7}$	*P*
Intermediate 2						0.55	Kolmogorov–Smirnov value
Intermediate 2						$<10^{-7}$	*P*

All *P*-values are uncorrected.