On the role of epistasis in adaptation

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Abstract

Although the role of epistasis in evolution has received considerable attention from experimentalists and theorists alike, it is unknown which aspects of adaptation are in fact sensitive to epistasis. Here, we address this question by comparing the evolutionary dynamics on all finite epistatic landscapes versus all finite non-epistatic landscapes, under weak mutation. We first analyze the fitness trajectory—that is, the time course of the expected fitness of a population. We show that for any epistatic fitness landscape and choice of starting genotype, there always exists a non-epistatic fitness landscape and starting genotype that produces the exact same fitness trajectory. Thus, surprisingly, the presence or absence of epistasis is irrelevant to the first-order dynamics of adaptation. On the other hand, we show that the time evolution of the variance in fitness across replicate populations can be sensitive to epistasis: some epistatic fitness landscapes produce variance trajectories that cannot be produced by any non-epistatic landscape. Likewise, the mean substitution trajectory—that is, the expected number of mutations that fix over time—is also sensitive to epistasis. These results on identifiability have direct implications for efforts to infer epistasis from the types of data often measured in experimental populations.

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1 Introduction

The ecological and physiological functioning of an organism typically relies on intricate interactions between a vast array of components. As a result, context dependence is the rule, rather than the exception, in biology. This basic principle is no doubt responsible for the exquisite co-evolution we observe between characters, both within and between individual organisms, and it is likely responsible for the diversity of forms and functions that continue to evolve. But, to paraphrase Fisher (1930, pg. vi), evolution and natural selection are not the same thing. Even if the idiosyncrasies of biological interaction play a major, or indeed, dominant role in determining the actual, detailed course of evolution, they need not play a dominant role in determining the rate or dynamics of adaption, that is, the pattern of fitness increase over time (Kryazhimskiy et al., 2014).

Context dependence arises in the study of adaptation because the fitness effect of a particular mutation may depend on the set of other mutations that are already present, a phenomenon known as epistasis (Whitlock et al., 1995; Wolf et al., 2000; Phillips, 2008). Epistasis for fitness is thought to play an important role in a number of evolutionary processes, ranging from the evolution of sex and recombination (de Visser and Elena, 2007) to molecular evolution under both stabilizing (Breen et al., 2012; McCandlish et al., 2013; Pollock et al., 2012) and directional (de Visser and Krug, 2014) selection. While epistatic interactions are clearly very important in shaping the specific evolutionary paths taken during adaptation (e.g. Weinreich et al., 2006; Blount et al., 2008; Bridgham et al., 2009; Lunzer et al., 2010; Bloom et al., 2010; Salverda et al., 2011; Kvitek and Sherlock, 2011; Tenaillo et al., 2012; Dickinson et al., 2013; Natarajan et al., 2013; Harms et al., 2013; Kryazhimskiy et al., 2014), the degree to which epistasis affects the gross features of adaptation remains a subject of debate (Crow, 2010; Hansen, 2013) and most widely-used used methods for detecting adaptive evolution from molecular data (e.g. Sawyer and Hartl, 1992; Goldman and Yang, 1994) assume that epistasis is absent.

One common strategy for investigating the role of epistasis in adaptation is to study some specific family of fitness landscapes that include the non-epistatic landscapes as a special case (e.g. NK landscapes, Kauffman 1993; the Rough Mount Fuji model, Aita et al. 2000; Franke et al. 2011; Szendro et al. 2013; or models where fitness decreases with the Hamming distance from the optimum genotype Kimura and Maruyama 1966; Kondrashov 1988 ). Differences between the adaptive dynamics on epistatic versus non-epistatic landscapes within such a family are then attributed to the presence of epistasis. However, there are two fundamental problems with this approach. First, the effects of epistasis per se are confounded with the effects of the specific form of epistasis assumed by the family of landscapes. Second, even if we perturb a non-epistatic landscape to create an epistatic landscape that exhibits some interesting behavior, this does not mean that we could not find a different non-epistatic landscape that exhibits the same behavior. Thus, while a colorful vocabulary for both the form (Kondrashov and Kondrashov, 2001; Weinreich et al., 2005; Phillips, 2008) and effect of epistasis has emerged, with
epistasis often said to “accelerate” or “decelerate” adaptation (Chou et al., 2011; Khan et al., 2011; Kryazhimskiy et al., 2011), the question remains as to whether the apparent effects of epistasis on adaptation are truly due to epistasis itself or whether the observed differences are actually artifacts of considering only a limited subset of fitness landscapes.

Here we take a different tack. Instead of considering any one family of fitness landscapes, we consider the class of all finite non-epistatic fitness landscapes and the class of all finite epistatic fitness landscapes. Assuming that mutation is weak, i.e. that each new mutation is lost or goes to fixation before the next new mutation enters the population (see McCandlish and Stoltzfus, 2014, for a review), we characterize the full space of evolutionary dynamics that are possible within each of these two classes of fitness landscapes. We then rigorously study the role of epistasis per se by identifying specific dynamical phenomena that can occur only when epistasis is present.

Our most important result concerns how the expected fitness of a population changes over time. In particular, we consider an ensemble of replicate populations that begin fixed for some genotype on a fitness landscape. We then ask, for any given time in the future, what will be the mean fitness across the ensemble of populations. The time evolution of this expected fitness is known as the “fitness trajectory” (Kryazhimskiy et al., 2009). We prove that, for any finite epistatic fitness landscape and choice of starting genotype, one can always construct a non-epistatic fitness landscape that produces the exact same fitness trajectory as the epistatic landscape produces.

Figure 1: A fitness landscape with reciprocal sign epistasis (A), and a non-epistatic fitness landscape (B) that both produce the exact same fitness trajectory (C). For the left and center panels, edges are mutations and points are genotypes. The height of a genotype indicates its scaled selection coefficient ($Ns$) relative to the initial (left-most) genotype. Edge thickness is proportional to mutation rate. The right panel shows expected fitness as a function of time (the fitness trajectory), where time is measured in terms of expected substitutions at a neutral locus with a unit mutation rate. Notice that the trajectory is quite complex; in particular, it decreases slightly at short times before increasing to its asymptotic value at long times.
To illustrate this general result in a specific case, the epistatic fitness landscape in Figure 1A and the non-epistatic fitness landscape in Figure 1B produce the same fitness trajectory (Figure 1C), for a population that starts at the left-most genotype. Note that the fitness landscape in Figure 1A is not merely epistatic, but in fact exhibits reciprocal sign epistasis (Weinreich et al., 2005), and the resulting fitness trajectory decreases slightly initially (due to deleterious fixations into the fitness valley) before increasing towards its asymptotic value as populations become more likely to have crossed the fitness valley. It may seem surprising that one can construct a non-epistatic landscape that produces the same, complex dynamics. And, yet, our main result says that constructing such a landscape is always possible – because the set of realizable fitness trajectories for epistatic landscapes is no more diverse than the set of fitness trajectories for non-epistatic landscapes.

Our main result shows that the presence or absence of epistasis has no effect on the range of dynamics of mean fitness – and, as a result, epistasis cannot possibly be inferred by empirical observations of fitness trajectories alone. Nonetheless, epistasis may affect more subtle features of the dynamics of adaptation. For instance, if we again consider an ensemble of replicate populations evolving from the same starting genotype, we can study the variance in fitness across these populations as a function of time. It turns out that there exist epistatic fitness landscapes whose “variance trajectory” has features that cannot be achieved on any non-epistatic landscape. In particular, a variance trajectory that is accelerating at short times can occur only on epistatic fitness landscapes. A similar result holds for the expected number of substitutions that accrue over time, i.e. the substitution trajectory (Kryazhimskiy et al., 2009): for any non-epistatic fitness landscape the expected substitution rate can never be less than half the equilibrium substitution rate, whereas some epistatic fitness landscapes violate this condition and are expected to produce a broader range of substitution rates.

These results provide basic insights into the consequences of epistasis on adaptation. But our results have important practical implications as well, concerning attempts to infer a fitness landscape from empirical observations of evolution in replicate laboratory populations (e.g., Lenski and Travisano, 1994; Wiser et al., 2013), an approach that has recently been the subject of some controversy (Frank, 2014; Good and Desai, 2014). We return to this problem of inferring epistasis from observed evolutionary dynamics in the Discussion.

2 Results

2.1 Population-genetic model

We consider the space of all possible fitness landscapes with a finite number of bi-allelic sites. We will write the forward mutation rate at site $l$ as $\mu_l$ and the back mutation rate as $\nu_l$. A
genotype is defined by the state at all of its sites, and the fitness of the $i$-th genotype is denoted $F(i)$. For simplicity, we will work in scaled Malthusian fitness (i.e. the fitness of a genotype is equal to the logarithm of the standard, Wrightean fitness times the population size) and we define the scaled selection coefficient genotype $j$ relative to genotype $i$ as $F(j) - F(i)$; our analyses are easy to adapt to other ways of measuring fitness.

Our main population-genetic assumption is that mutation is weak, i.e. that each new mutation is either fixed or lost before the next mutation enters the population. Because the time during which a mutation segregates in such a population is much shorter than the waiting time between new mutations, we neglect the time that a mutation segregates and simply model the population as monomorphic, jumping from genotype to genotype at the birth of each new mutation destined for fixation (Iwasa, 1988; Berg et al., 2004; Sella and Hirsh, 2005; McCandlish and Stoltzfus, 2014).

We use the standard model for a population evolving under weak mutation in continuous time. More formally, we model evolution as a continuous time Markov chain with rate matrix $Q$, where

$$Q(i, j) = \begin{cases} \frac{F(j) - F(i)}{1 - e^{-F(j) - F(i)}} Q_M(i, j) & \text{for } i \neq j \\ -\sum_{k \neq i} Q(i, k) & \text{for } i = j \end{cases}$$

and $Q_M$ is the mutational rate matrix, i.e. $Q_M(i, j)$ for $i \neq j$ is equal to $\mu_l > 0$ if genotype $j$ can arise from genotype $i$ by a forward mutation at site $l$, $\nu_l > 0$ if genotype $j$ can arise from genotype $i$ by a back mutation at site $l$, and 0 otherwise; the $Q_M(i, i)$ are chosen so that the row sums are zero. While for convenience the above expression is based on the classical approximation to the probability of fixation of a new mutation in the diffusion limit (Fisher, 1930; Wright, 1931), our results can easily be extended to hold exactly in the limit of weak mutation for a population of finite size $N$ evolving under a Moran process by using the appropriate exact expression for the probability of fixation (Moran, 1959).

We define a non-epistatic fitness landscape as one in which each site makes an additive contribution to fitness (recall that we are working in Malthusian fitness, so that this corresponds to a multiplicative landscape in Wrightean fitness). More formally, for a non-epistatic fitness landscape each site $l$ is associated with a value $S_l$ such that, for any ordered pair of genotypes $i$ and $j$ differing by a forward mutation at site $l$, we have $F(j) - F(i) = S_l$. Under this definition, the non-epistatic fitness landscapes are precisely those landscapes for which sites evolve independently of each other. This is because the forward substitution rate at site $l$ is always

$$\alpha_l = \frac{S_l}{1 - e^{-S_l}} \mu_l$$

and the corresponding backwards substitution rate is

$$\beta_l = \frac{-S_l}{1 - e^{-(-S_l)}} \nu_l.$$
Figure 2: The fitness trajectory shared by the two fitness landscapes in Figure 1 (black) together with each of the corresponding exponentially decaying deviations from the equilibrium expected fitness (gray) that can be combined together to compose the trajectory. There are two positive deviations from the equilibrium expected fitness that decay very rapidly, producing the small dip in fitness at short times, and a much more slowly decaying negative deviation from the equilibrium distribution that corresponds to crossing the fitness valley in the epistatic landscape, Figure 1A, or to having a substitution at the third site in the non-epistatic landscape, Figure 1B.

Thus, for a non-epistatic fitness landscape the evolutionary dynamics at site \( l \) depend only the state of site \( l \) and not on the states of the other sites.

### 2.2 Fitness trajectories

Suppose a population is initially fixed for some genotype \( i \), with fitness \( F(i) \), at time 0. At any time \( t \) in the future, the population has some probability of being fixed for each other genotype \( j \), with fitness \( F(j) \). We can therefore ask: What is the expected fitness of the population at time \( t \)? The course of the expected fitness over time is called the fitness trajectory, which we write as \( f(t) \). In the Appendix, we show that the fitness trajectory can always be written in the form:

\[
f(t) = f(\infty) + \sum_{k=2}^{n} c_k e^{-\lambda_k t}
\]

for some constants \( c_2, \ldots, c_n \), with \( \lambda_2, \ldots, \lambda_n > 0 \) and \( n \) denoting the number of genotypes in the fitness landscape. In other words, for an arbitrary fitness landscape, including all epistatic landscapes, the fitness trajectory can always be expressed as a sum of exponentially decaying deviations from the equilibrium mean fitness, \( f(\infty) \). Figure 2 illustrates this decomposition for the fitness trajectory shared by the two fitness landscapes in Figure 1.

Now, let us restrict our attention to non-epistatic landscapes, and consider what types of fitness trajectories can arise. Because fitness is additive over sites in such a landscape, we can
write the fitness trajectory as a sum over sites. In particular, using the standard solution for a two-state Markov chain, the fitness trajectory for a non-epistatic landscape is given by:

\[ f(t) = f(0) + \sum_l S_l \frac{\alpha_l}{\alpha_l + \beta_l} \left( 1 - e^{-(\alpha_l + \beta_l)t} \right) \]

where \( f(0) \) is the initial fitness and we assume (without loss of generality) that the population begins fixed for the first of the two states for each site at \( t = 0 \). Rewriting Equation 5 in the same form as Equation 4, we have:

\[ f(t) = \left( f(0) + \sum_l S_l \frac{\alpha_l}{\alpha_l + \beta_l} \right) + \sum_l -S_l \frac{\alpha_l}{\alpha_l + \beta_l} e^{-(\alpha_l + \beta_l)t}. \]

We now arrive at our main result. Comparing Equations 4 and 6, we see that given an arbitrary epistatic fitness landscape we can always construct a non-epistatic fitness landscape that will produce a fitness trajectory of the same shape, provided we can choose values \( S_l, \mu_l \) and \( \nu_l \) for each \( k \) such that

\[ c_k = -S_l \frac{\alpha_l}{\alpha_l + \beta_l} \]

and

\[ \lambda_k = \alpha_l + \beta_l. \]

Indeed, such choices can always be made. For instance, one solution is \( \alpha_l = \beta_l = \lambda_k / 2 \), \( S_l = -2c_k \) and \( \mu_l = \alpha_l \left( S_l / (1 - e^{-S_l}) \right)^{-1} \), \( \nu_l = \beta_l \left( -S_l / (1 - e^{-(S_l)}) \right)^{-1} \), but there are an infinite number of such solutions.

Thus far we have shown that given a fitness trajectory from an epistatic fitness landscape, we can construct a non-epistatic fitness landscape whose fitness trajectory has the same shape, i.e. one that differs from the target fitness trajectory by an additive constant. In order to match the fitness trajectory exactly, we need to be able to choose the term in parentheses in Equation 6 to be equal to \( f(\infty) \). But this, too, is always possible to do, because we can freely choose the initial fitness \( f(0) \) and the sum over sites has some definite value fixed by our previous choice of \( S_l, \mu_l \) and \( \nu_l \), i.e. we can always use the solution

\[ f(0) = f(\infty) - \sum_l S_l \frac{\alpha_l}{\alpha_l + \beta_l}. \]

To summarize, we have shown that given an arbitrary epistatic fitness landscape and choice of initial genotype, we can always construct a non-epistatic fitness landscape that will produce the exact same fitness trajectory. This means that the presence of epistasis does not expand the range of possible dynamics of expected fitness gains over time; and so the presence or
absence of epistasis per se does not determine the form of the fitness trajectory (accelerating or decelerating adaptation, etc.).

Let us take a moment to comment on the features of non-epistatic fitness landscapes that produce the same fitness trajectory as a focal epistatic landscape. First, such a non-epistatic fitness landscape will typically have many more genotypes than the epistatic landscape whose fitness trajectory it matches. For instance, given a fitness landscape with $L$ sites and $2^L$ genotypes, the corresponding non-epistatic fitness landscape with the same fitness trajectory produced by the method described above will have $2^L - 1$ sites and therefore $2^{2^L-1}$ genotypes. To give some intuition for why this is the case it is helpful to consider the number of parameters required to specify the fitness function $F$ for epistatic and non-epistatic landscapes. For an epistatic landscape there is one parameter per genotype, whereas for a non-epistatic fitness landscape there is essentially one parameter per site ($S_1, \ldots, S_L$). What the above result says is that the complexity of a fitness trajectory resulting from a landscape depends more on the number of parameters it takes to specify the landscape than on whether the landscape is epistatic or not. Small epistatic fitness landscapes and big non-epistatic fitness landscapes are capable of producing equally complex fitness trajectories.

Second, there is a technical point to mention about the role of weak mutation in the argument above. Given a non-epistatic fitness landscape that “spoofs” an epistatic fitness trajectory, one must consider whether the weak mutation assumption still holds for its mutation rates and selection coefficients. That is, the evolutionary dynamics might fall in the weak-mutation regime for an epistatic fitness landscape, but not for some non-epistatic fitness landscapes that would, otherwise, produce the same fitness trajectory. However, this complication does not arise when we work in the limit of weak mutation (i.e. let all mutation rates approach zero while keeping their ratios constant and rescaling time appropriately).

Finally, it is worth emphasizing that there are an infinite number of non-epistatic fitness landscapes than produce the same fitness trajectory as a given epistatic landscape. There is no unique or canonical non-epistatic landscape that corresponds to a given epistatic landscape and choice of initial genotype. This is true not only because there are multiple ways to satisfy Equations 7 and 8 (including the case of one-way mutation, if we extend our framework to allow $\nu_l = 0$ for all $l$) or because of the possibility of adding neutral sites (or, more generally, sets of sites whose fitness effects cancel each other out), but also because it is possible to have multiple sites corresponding to a single term in Equation 4. In particular, one can generalize Equation 7 to

$$c_k = -\sum_{l \in L_k} S_l \frac{\alpha_l}{\alpha_l + \beta_l}$$

where $L_k$ is the set of sites in the non-epistatic fitness landscape corresponding to the term $c_k e^{-\lambda_k t}$ in Equation 4, and $\alpha_l + \beta_l = \lambda_k$ for all $l \in L_k$. This flexibility means that one can control the higher moments of the time-dependent fitness distribution independently from the mean, i.e., from the fitness trajectory.
2.3 Variance trajectories

We have seen that any fitness trajectory produced by an epistatic fitness landscape can also be produced by a non-epistatic fitness landscape. However, the fitness trajectory captures only the central tendency of population fitness through time. If we initiate many replicate populations fixed for the same genotype, then it is likely that some populations will adapt more quickly than others, so that there will typically be variation in fitness across populations at any given time \( t > 0 \). Aside from the mean, discussed above, it is natural to ask whether the presence of epistasis affects the time evolution of the inter-population variation in fitness.

To make this idea more precise, let us consider the fitness of a population at time \( t \) as a random variable. The variance of this random variable viewed as a function of time is called the “variance trajectory”, \( v(t) \). In other words, the variance trajectory is the time evolution of the second central moment of the fitness distribution, across an ensemble of replicate populations.

We would like to know whether the set of variance trajectories that can be achieved by epistatic fitness landscapes is more diverse than the set that can be achieved by non-epistatic fitness landscapes. To answer this question, we will first use the standard solution for a two-state Markov chain to write down the variance trajectory for a single site:

\[
v(t) = \left( S \frac{\alpha}{\alpha + \beta} \right)^2 \left( \frac{\beta}{\alpha} (1 - e^{-(\alpha + \beta)t}) + (e^{-(\alpha + \beta)t} - e^{-2(\alpha + \beta)t}) \right),
\]

assuming without loss of generality that the population starts in the first state. It is easy to show that the first derivative of \( v(t) \) with respect to time is maximized at \( t = 0 \), since the derivatives of both \( 1 - e^{-t} \) and \( e^{-t} - e^{-2t} \) are maximized at \( t = 0 \). Thus, the rate that variance in fitness increases takes its maximum at \( t = 0 \), which makes sense, because at \( t = 0 \) the fitness of the alternative state is maximally different from the current mean, and the increase in the frequency of the alternative state is also maximized (because back-substitutions cannot occur at time \( t = 0 \)).

The variance trajectory of a non-epistatic fitness landscape is simply the sum of the variance trajectories across all sites (because variances can be summed when random variables are independent). Now, because the slope of the variance trajectory is maximized for each site at \( t = 0 \), it follows that the slope of the variance trajectory is maximized at \( t = 0 \) for any finite, non-epistatic fitness landscape. Furthermore, because the slope is maximized at \( t = 0 \), it follows that the second derivative of the fitness trajectory must be negative at \( t = 0 \). In other words, all non-epistatic fitness landscapes share a fundamental qualitative feature: their variance trajectories are concave at short times.

Are variance trajectories for all epistatic fitness landscapes also concave at short times? The answer is no. For instance, consider a two-site fitness landscape with genotypes ab, Ab, aB, and
Figure 3: Epistatic fitness landscapes can produce dynamics that differ from all non-epistatic landscapes. (A) We consider two-site landscapes with genotypes ab, Ab, aB, and AB, with the population initially fixed for ab. Assigning Ab and aB equal fitnesses, we let $C_1$ be the selection coefficient of Ab and aB relative to ab and let $C_2$ be the selection coefficient of AB relative to Ab and aB. We set the mutation rates to be $\mu_l = \nu_l = 1$ for both sites. (B) Properties of two-site fitness landscapes using the parameterization described in the previous panel. The dark gray region shows the set of landscapes whose variance trajectories are convex at $t = 0$. The light gray region shows the set of landscapes whose equilibrium substitution rates are greater than twice their initial substitution rates. The diagonal dashed line with positive slope shows the set of non-epistatic fitness landscapes, whereas the diagonal dashed line with negative slope shows the set of epistatic landscapes whose fitness dynamics cannot be distinguished from the non-epistatic case.

AB, and $\mu_l = \nu_l = 1$ for both sites, with a population initially fixed for genotype ab. Suppose the fitnesses of ab, Ab and aB are all equal but genotype AB has fitness advantage $S$ over the other three genotypes. The first derivative of the resulting variance trajectory at $t = 0$ (i.e., $v'(0)$) is zero, and the second derivative at $t = 0$ (i.e. $v''(0)$) is $S^3 / (1 - e^{-S})$, which is positive for $S \neq 0$. Thus, for any such landscape with $S \neq 0$, the variance trajectory is convex at short times – a feature that cannot be achieved by any non-epistatic landscape.

As illustrated by the example above, the range of possible dynamics for the variance trajectory is larger for epistatic landscapes than for non-epistatic landscapes. And so it may be possible to infer epistasis from the pattern of variance in fitness across populations, even though it is impossible to do so from the pattern of mean fitness alone.

To explore the range of epistatic landscapes that produce variance trajectories convex at short times, and which distinguishes them from all non-epistatic landscapes, we considered the
two-site landscapes described above, but allowed Ab and aB to have some selection coefficient relative to ab, and also allowed AB to have some other selection coefficient relative to Ab and aB (i.e. genotypes with equal hamming distances from ab are assigned equal fitnesses). The dark gray region in Figure 3 shows the subset of landscapes whose the variance trajectories are convex at short times. Although this region is primarily composed of landscapes with positive epistasis (above the line y=x), it is also possible to have convex variance trajectories for landscapes with negative epistasis (below the line y=x) when the selection coefficient of the first mutation is small.

We have seen that some epistatic fitness landscapes produce variance trajectories that cannot be achieved by any non-epistatic fitness landscapes. Thus, we have shown that one can sometimes tell that a fitness landscape is epistatic by observing its time-dependent fitness distribution. However, one might wonder whether given the time-dependent fitness distribution it is always possible to distinguish epistatic from non-epistatic fitness landscapes. The answer is no. As a counter-example, consider again the two-site case in which Ab and aB have equal fitness. If the selection coefficient of ab relative to Ab and aB is equal to the selection coefficient of AB relative to Ab and aB, then the entire fitness distribution can be matched by a single-site fitness landscape. These landscapes are illustrated by the dashed line with negative slope in Figure 3. Thus, there is no characteristic of the time-dependent fitness distribution that can be used to distinguish all epistatic landscapes from all non-epistatic fitness landscapes.

Another natural question, in light of our earlier results on fitness trajectories, concerns the relationship between the fitness trajectory and the variance trajectory, for a given landscape. Within the class of non-epistatic landscapes, it is easy to show that the variance and fitness trajectories can be modified essentially independently of each other. This is because the variance in fitness can be made arbitrarily small while preserving the fitness trajectory by replacing single sites of large fitness effect with many sites of small effects; on the other hand, the variance in fitness can be made arbitrarily large without altering the fitness trajectory by constructing pairs of sites whose site-specific fitness trajectories cancel each other out, but which still contribute to the time-evolution of the variance. As a result, considering the fitness and variance trajectories jointly is likely to provide little more information about the presence of epistasis than considering the variance trajectory alone.

2.4 Substitution trajectories

Changes in fitness during adaptation are the result of substitutions -- that is, mutations at individual sites that eventually reach fixation in the population. Therefore, aside from studying the expected fitness of a population, it is also interesting to consider the number of substitutions accumulate in a population over time. For instance, consider the time-evolution of the expected number of substitutions that have accumulated in the population by time $t$, what we
call the “substitution trajectory”, \( s(t) \) (Kryazhimskiy et al., 2009). Just as we did for fitness trajectories, we want to ask whether the set of possible substitution trajectories for epistatic fitness landscapes is more diverse than the set of possible substitution trajectories in the absence of epistasis.

To study the substitution trajectory, it is helpful to note that the derivative of the substitution trajectory is equal to the expected substitution rate at time \( t \), which we will write as \( q(t) \). That is, \( s'(t) = q(t) \). Because no substitutions have accumulated at time 0, this relation means that the substitution trajectory is fully specified by the time-dependent expected rates of substitution.

Consider the time-dependent rate of substitution at a single site. Assuming without loss of generality that the population begins in the first state, and using the standard solution for a two-state Markov chain, the expected substitution rate is given by:

\[
q(t) = \frac{2\alpha\beta}{\alpha + \beta} - \frac{\alpha}{\alpha + \beta} (\beta - \alpha)e^{-(\alpha + \beta)t},
\]

where the first term on the right-hand side is the equilibrium rate of substitution, \( q(\infty) \). The initial substitution rate is \( \alpha \), and so the ratio between the equilibrium substitution rate and the initial substitution rate is \( q(\infty)/q(0) = 2\beta/\alpha + \beta \) — a ratio than can never exceed 2, which is the value achieved in the limit as \( \beta/\alpha \to \infty \). Indeed, because the expected substitution rate approaches its equilibrium value monotonically, we also have a stronger result: the expected substitution rate can never be less than half of its equilibrium rate, i.e. \( q(\infty)/q(t) \leq 2 \).

For a non-epistatic landscape, the expected substitution rate is simply a sum of rates at each of its constituent sites. Using the inequality developed above, we thus have:

\[
\frac{q(\infty)}{q(t)} = \frac{\sum_l q_l(\infty)}{\sum_l q_l(t)} \leq \frac{\sum_l 2q_l(t)}{\sum_l q_l(t)} = 2,
\]

where \( q_l(t) \) is the expected substitution rate at the \( l \)-th site at time \( t \). In words, for any finite-state non-epistatic fitness landscape, the ratio between the equilibrium rate and the expected rate at any time can never exceed two.

For epistatic landscapes, by contrast, it is easy to see that this condition on the substitution rate can be violated. For instance, consider a fitness landscape with three or more sites in which all genotypes have the same fitness. Then, pick an initial genotype, and alter the fitnesses of its mutational neighbors such that these neighbors now have selection coefficient \( S \) relative to the initial genotypes, where we choose \( S \) to be negative. That is, consider a neutral plateau and modify it by constructing a fitness valley around the initial genotype. As the depth of this
valley increases (i.e. as \( S \) approaches \(-\infty\)), the initial substitution rate converges to 0, while the equilibrium substitution rate approaches some non-zero constant. This means that by choosing \( S \) to be sufficiently large and negative the ratio between the equilibrium substitution rate and the initial substitution rate can be made arbitrarily large and, in particular, larger than two. (While no adaptation occurs in this example – the mean equilibrium fitness is lower than the initial fitness – this defect is easy to correct by giving a fitness advantage to genotypes of distance two or more from the initial genotype). Thus, we conclude that the set of possible substitution trajectories is indeed enlarged by the presence of epistasis.

The light gray region in Figure 3 illustrates this fact, by indicating the set of two-site fitness landscapes whose ratios of equilibrium to initial expected substitution rates exceeds 2. Roughly speaking, this region corresponds to landscapes with a fitness valley, with population initialized on the fitter of the two peaks. Note that the light gray region does not extend all the way to the landscapes in which the two peaks have equal heights: these landscapes (dashed diagonal line with negative slope) have substitution trajectories of precisely the same form as a single site, and therefore the ratio of rates must be less than or equal to 2 along this line.

Why is the range of dynamics of mean fitness identical for epistatic and non-epistatic landscape, but not the range of dynamics of the mean number of substitutions? One way to understand these results is to notice that, because mutation can oppose or augment selection, two non-epistatic landscapes with different fitness functions and different mutation rates might still have the same evolutionary dynamics in genotype space (i.e. the same rate matrix, \( Q \)). As a consequence, for each non-epistatic matrix \( Q \) and choice of starting genotype, there is a large class of possible fitness trajectories, determined by the choices of the site-specific selection coefficients \( S_l \). In contrast, having specified the matrix \( Q \) and the initial genotype completely determines the substitution trajectory. The extra flexibility produced by choosing \( Q \) and the \( S_l \) independently allows non-epistatic fitness landscapes to produce fitness trajectories whose dynamics are as general as the time-evolution of the expectation of an arbitrary function defined on an arbitrary finite-state reversible Markov chain.

For completeness, we can also consider the ensemble variance in substitution rate as a function of time. The derivative of this trajectory is maximized at \( t = 0 \), just as the derivative of the variance in fitness is as well. While this criterion can be used to identify some fitness landscapes as epistatic, the time evolution of the variance in substitution rate is much more difficult to observe than the time evolution of the mean substitution rate or the time evolution of the fitness distribution, and so we will not discuss the matter further here.

### 2.5 Equilibrial dynamics

Although our main focus has been adaptation, it is also interesting to consider whether epistatic and non-epistatic landscapes differ in the range of dynamics they can produce at equi-
librium, i.e. in the limit of long times when all influence of the choice of initial genotype has been lost. We study the equilibrial dynamics by again considering an ensemble of replicate populations. However, instead of assuming that all of these populations are initially fixed at a single genotype, we assume that the initial genotype for each population is drawn from the equilibrium distribution, that is, the distribution that gives the probability of a population being fixed for any given genotype in the limit of long times.

An ensemble of populations that is initially distributed according to the equilibrium distribution will continue to be described by the equilibrium distribution at all future times. Hence the expected fitness, the variance for fitness, and indeed all moments of the fitness distribution are constant in time. Indeed, the equilibrium fitness distribution is determined solely by the fitnesses of the individual genotypes together with their equilibrium frequencies, and it is therefore independent of the structure of the fitness landscape in the sense that the structure of mutational adjacency is irrelevant (see McCandlish, 2011, pg. 1547). Because the equilibrium distribution remains constant in time and each genotype has its own substitution rate, substitutions likewise accumulate at a constant rate across the ensemble as a whole.

 Nonetheless, there are other features of the evolutionary process at equilibrium whose dynamics are not simply constant in time. In particular, while the fitness distribution remains constant at all times, there are correlations between the fitness of a population at one time and its fitness at another. Thus, we can consider the covariance in fitness between time 0 and time \( t \), where again the genotype at time \( 0 \) is drawn from the equilibrium distribution. Viewed as a function of \( t \), this covariance function is known as the equilibrium autocovariance for fitness, denoted \( a(t) \). In the Appendix, we show that the equilibrium autocovariance for an arbitrary epistatic fitness landscape has the form:

\[
a(t) = \sum_{k=2}^{n} d_k e^{-\lambda_k t},
\]

where \( d_2, d_3, \cdots \geq 0 \) and \( \lambda_2, \lambda_3, \ldots > 0 \).

For comparison, let us now consider the autocovariance for non-epistatic landscapes, considering first a landscape with a single site with selection coefficient \( S \). In this case the equilibrium autocovariance for fitness is given by

\[
a(t) = S^2 \frac{\alpha \beta}{(\alpha + \beta)^2} e^{-(\alpha + \beta)t}.
\]

The autocovariance of a sum of independent processes is the sum of the corresponding autocovariances, and the term \( S^2 \alpha \beta / (\alpha + \beta)^2 \) can assume any non-negative value even with a fixed value of \( \lambda_k = \alpha + \beta \). This implies that, given the equilibrium autocovariance function for an epistatic fitness landscape, one can always construct a non-epistatic landscape with an identical
equilibrium autocovariance function by assigning one site to correspond to each term in Equation 16. Thus, while the presence of epistasis increases the possible dynamics for the second moment of fitness for an adapting population, epistatic and non-epistatic fitness landscapes have the same range of possible dynamics for the equilibrium autocovariance in fitness.

3 Discussion

What are the effects of epistasis on adaptation? Here we have studied this question by identifying dynamical phenomena that can occur when epistasis is present but that cannot occur when epistasis is absent. We have considered the evolution of populations under weak mutation on arbitrary fitness landscapes defined on a finite number of bi-allelic sites. For each of several basic descriptors of adaptation—e.g. the expected fitness or number of substitutions accrued over time—we have asked whether the dynamics that are possible on epistatic fitness landscapes are more diverse than those possible on non-epistatic landscapes. The results are surprisingly heterogeneous.

The most basic and essential descriptor of adaptation is the fitness trajectory—that is, the expected pattern of fitness gains over time. In contrast to the received wisdom that the presence, and even specific form, of epistasis can alter the mean pattern of adaptation over time (e.g. Chou et al., 2011; Khan et al., 2011; Kryazhimskiy et al., 2011), we have shown that the set of possible fitness trajectories for epistatic fitness landscapes is no more diverse than for non-epistatic fitness landscapes. In particular, any fitness trajectory that can be achieved on an epistatic fitness landscape can also be achieved by an infinite number of non-epistatic fitness landscapes. Surely, therefore, epistasis should not be described as a factor that controls whether the rate of adaptation is expected to increase or decrease over time (that is, “accelerate” or “de-accelerate”), given that identical dynamics for the mean rate of adaptation can be produced in the absence of epistasis.

In contrast to the fitness trajectory, we have shown that that time evolution of the variance in fitness across populations can be sensitive to epistasis. Some epistatic fitness landscapes produce variance trajectories that are qualitatively different from those that can be achieved in the absence of epistasis. Likewise, the pattern of the expected number of substitutions accrued over time can also be qualitatively different on epistatic fitness landscapes than possible on non-epistatic landscapes. Thus, while the presence or absence of epistasis per se does not determine the time-evolution of the expected fitness gains, it does affect several other aspects of adaptation.

These results have implications for efforts to infer the presence of epistasis by experimentally observing the evolutionary dynamics in an ensemble of replicate populations (Lenski and Travisano, 1994; Wiser et al., 2013; Good and Desai, 2014). It is tempting to analyze data from such experiments by fitting a handful of simple epistatic and non-epistatic models and then
conclude whether epistasis is present or not based on whether the best-fit model is epistatic or not. However, as emphasized by Frank (2014), all we can really infer from such an experiment is the set of models consistent with the observed dynamics. Our results here show that, for populations under weak mutation, the shape of the fitness trajectory alone can never be used to infer the presence of epistasis, while the variance and substitution trajectories can in principle sometimes allow us to conclude that epistasis must be present.

Besides the problem of whether the presence of epistasis is identifiable from the quantities measured in such experiments, there are several other challenges that remain when attempting to detect epistasis from the gross features of adaptation in experimental populations. First, the results presented here pertain to knowledge of the exact trajectories. And so, despite our theoretical results on identifiability, it is unclear whether the variance and substitution trajectories can be used in practice to produce powerful tests for epistasis after accounting for measurement error and noise from random sampling. Second, fitting models of the form derived here to experimental data will be a difficult numerical and computational task. This is because the trajectories take the form of sums of an unknown number of exponential functions, and it is notoriously difficult to reliably fit models of this form (see, e.g. Holmström and Petersson, 2002, for a review). The combination of the numerical difficulties of fitting as well as various sources of noise suggests that we will not be able in practice to fit more than a few terms in these general expressions. (Nonetheless, it is not necessary to fit the full variance or substitution trajectories in order to apply our positive results on identifiability, which require only that one be able to measure the derivative of the trajectory at a few time points.) Finally, while our results here assume that mutation is weak, most experimental evolution involves large microbial populations within the regime of clonal interference (Gerrish and Lenski, 1998). Thus, it is possible that existing experimental fitness trajectories (e.g. Wiser et al., 2013) may contain information about the presence of epistasis. However, without a comparable demonstration that epistasis is indeed identifiable from the fitness trajectory for such populations, our negative results under weak mutation suggest extreme caution in using empirical fitness trajectories to argue for or against the hypothesis that epistasis is present.

Two recent theoretical studies have also analyzed the effects of epistasis on adaptation under weak mutation. Kryazhimskiy et al. (2009) considered the space of fitness landscapes in which the distribution of mutational effects on fitness (DFE) is solely a function of the current fitness of the population, and they concluded that it is possible to identify epistasis from the fitness trajectory. On the whole, the class of models studied by Kryazhimskiy et al. (2009) is much broader than the one considered here (since any finite-state fitness landscape can be arbitrarily well approximated in their framework so long as each genotype has a unique fitness), and it includes many models that are inconsistent with finite-site landscapes. In particular, Kryazhimskiy et al. (2009) considered a fitness landscape to be non-epistatic if its DFE is independent of the population’s fitness. Such a situation can never arise on a non-trivial finite-site landscape, because the DFE must be entirely negative at the fittest genotype and entirely positive at the least-fit genotype. Our results are thus complementary to those of Kryazhimskiy et al. (2009),
and we conclude that while the shape of the fitness trajectory may be informative in distinguishing between various models in their broader class, it is not informative for the narrower set of models corresponding to finite-site fitness landscapes. It is also worth noting that the analytical results presented by Kryazhimskiy et al. (2009) are approximations that hold only for a relatively limited subset of models within this broader class (see van Kampen, 2007, pp. 124–127), whereas the analytical results presented here are exact and apply to arbitrary fitness landscapes with a finite number of biallelic sites.

Good and Desai (2014) also investigated the effects of epistasis on the fitness and substitution trajectories, however some of their conclusions are qualitatively inconsistent with ours. Based on the same concept of epistasis as the one used here, Good and Desai (2014) claim (1) that non-epistatic landscapes can produce either an arbitrary fitness trajectory or an arbitrary substitution trajectory and (2) that choosing a landscape to match a given fitness trajectory completely determines the substitution trajectory and vice versa. Here, by contrast, we have shown that while a non-epistatic landscape can indeed produce an arbitrary fitness trajectory, a non-epistatic fitness landscape cannot produce an arbitrary substitution trajectory. Moreover, we have also shown that for a fixed substitution trajectory one can create non-epistatic fitness landscapes that produce an infinite number of possible fitness trajectories, and, conversely, it is easy to construct finite-site landscapes with different substitution trajectories but identical fitness trajectories.

There are many differences between our analysis and that of Good and Desai (2014) that could account for these discrepancies, including the fact that they model evolution at multiple sites using a continuous distribution of selection coefficients at each site and that they assume that selection is strong, so that deleterious and neutral substitutions are impossible. Importantly, the assumption of strong selection means that their analysis cannot accommodate even simple landscapes (such as that shown in Figure 1A) that involve valley crossing or non-monotonic fitness trajectories as shown in Figure 1C. The latter limitation is also shared by the analysis of (Kryazhimskiy et al., 2009), and it severely constrains the space of dynamics compared to those that occur without the assumption of strong selection (see also McCandlish et al. 2014).

While most of our results have focused on adaptive evolution, we also studied the nearly-neutral dynamics of a population evolving at equilibrium on time-invariant fitness landscape. In particular, we showed that the autocovariance function for fitness of such a population cannot be used to determine whether a fitness landscape is epistatic or not. This results is in contrast to the the autocovariance function of a completely random walk on the space of genotypes, whose characteristics have long been used to quantify the “ruggedness” of fitness landscapes (Weinberger, 1990, 1991; Stadler, 2003).

One potential limitation of our analysis is that we have considered fitness landscapes composed of only bi-allelic sites. This assumption does not, in fact, influence our results on the space of dynamics possible under epistatic landscapes. This is because, as shown in the Ap-
Appendix, our results for epistatic fitness landscapes hold for any time-independent, finite-state fitness landscape whose neutral mutational dynamics take the form of a reversible Markov chain (see, e.g. Sella and Hirsh, 2005; McCandlish, 2011). Thus, our results on epistatic dynamics apply also to models with more than two alleles per site (so long as the mutational dynamics within a site form a reversible Markov chain); and, indeed, they even apply when the genotypic space cannot be decomposed into individual sites. But our assumption of bi-allelic sites does influence our analysis of non-epistatic models, because our strategy for determining the behavior of such models has been to sum over the dynamics of independently evolving sites. The dynamics at a single site can be more complex when there are more than two alleles, and so the dynamics that are possible under multi-allelic finite-site non-epistatic fitness landscapes are more diverse than those described here for non-epistatic models with bi-allelic sites. Thus, all our negative results concerning whether epistatic models have more diverse dynamics than non-epistatic models (such as our main result on the fitness trajectory) continue to hold for multi-allelic models, but our positive results (such as our results on the variance trajectory) may no longer apply.

Another important limitation of our analysis is that we have considered the evolutionary dynamics only under weak mutation. This is because under weak mutation sites that do not interact epistatically evolve independently. However, for polymorphic populations with linked sites, even sites that do not interact epistatically have dynamics that are non-independent due to hitch-hiking and background selection. This non-independence makes it extremely difficult to provide a full treatment of even non-epistatic dynamics for finite, polymorphic populations. In the absence of analytical results, it is tempting to try to address the role of epistasis for finite polymorphic populations through simulation. However, the enormity of the space of possible fitness landscapes means that any such approach will be restricted to a tiny subset of fitness landscapes, and so it cannot definitively answer the types questions that we have addressed here.

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Appendix

We proceed in somewhat more generality than in the main text. Suppose that evolution under mutation alone proceeds as a reversible, continuous-time Markov chain on a finite state space with rate matrix (infinitesimal generator) $Q_{M}$ and equilibrium distribution $\pi_{M}$. If the scaled Malthusian fitness of genotype $i$ is given by $F(i)$, then evolution under weak mutation is a Markov chain with rate matrix $Q$ whose $i,j$-th entry is:

$$Q(i,j) = \begin{cases} 
\frac{F(j)-F(i)}{1-e^{-F(j)-F(i)}} Q_{M}(i,j) & \text{for } i \neq j \\
-\sum_{k \neq i} Q(i,k) & \text{for } i = j.
\end{cases}$$

(A1)

It is easy to verify that the equilibrium distribution of this chain is given by the vector $\pi$, where $\pi(i) \propto \pi_{M}(i) e^{F(i)}$, and that this equilibrium satisfies detailed balance, so that the chain defined by $Q$ is also reversible. Note that the more limited definition of $Q_{M}$ in the main text based on some finite number of bi-allelic sites with non-zero forward and reverse mutation rates necessarily results in a reversible Markov chain, since it is simply the rate matrix for a collection of independent two-state chains with non-zero transition rates, and any two-state continuous-time chain with non-zero transition rates is reversible.

Because the Markov chain defined by $Q$ is reversible, the definition of detailed balance implies that the matrix $D_{\frac{1}{2}}QD_{\frac{1}{2}}$ is symmetric, where $D_{x}$ is the diagonal matrix whose diagonal entries are given by the vector $x$. Because $Q$ is related by a similarity transform to a symmetric matrix, we know we can expand it in terms of its eigenvalues and eigenvectors as

$$-Q = \sum_{k=1}^{n} \lambda_{k} r_{k} l_{k}^{T}$$

(A2)

where $0 = \lambda_{1} < \lambda_{2} \leq \lambda_{3} \leq \ldots \leq \lambda_{n}$ are the eigenvalues of $-Q$; $l_{k}$ and $r_{k}$ are, respectively, the associated left and right eigenvectors; and the eigenvectors can be chosen so that $l_{k}^{T} D_{\pi}^{-1} l_{m} = 1 = r_{k}^{T} D_{\pi} r_{m}$ for $k = m$ and 0 otherwise, and $l_{k}(i) = \pi(i) r_{k}(i)$.

The transition probabilities for the Markov chain can then be written in terms of this expansion of $Q$. In particular, let $P_{t}$ be the matrix whose $i,j$-th element is the probability that a population that begins at time 0 fixed for genotype $i$ is fixed for genotype $j$ at time $t$. Then we can write:

$$P_{t}(i,j) = \sum_{k=1}^{n} e^{-\lambda_{k} t} r_{k}(i) l_{k}(j).$$

(A3)

As a result, for any function on the state space of the Markov chain, the expected value of that function at time $t$ for a population that begins fixed for genotype $i$ at time 0 is given by

$$\sum_{k=1}^{n} e^{-\lambda_{k} t} r_{k}(i) l_{k}^{T} g.$$  

(A4)
where $g(i)$ is the value of the function at genotype $i$. Equation 4 follows by choosing $g(i) = F(i)$ and noting that because the rows of $Q$ sum to zero, we must have $r_1 = 1$, where 1 is the vector of all 1s, for all $i$ and thus $l_1 = \pi$.

To study evolution at equilibrium, we again consider an ensemble of populations, but instead of assuming that all populations in the ensemble begin at some specified genotype, we let the initial genotype of each population be drawn from $\pi$, the equilibrium distribution of the Markov chain defined by $Q$. Using the definition of covariance, the covariance between the fitness of a population whose genotype is drawn from $\pi$ at time $0$ and its fitness at time $t$ is given by

$$a(t) = \sum_{i=1}^{n} \sum_{j=1}^{n} \pi(i) P_t(i,j) (F(i) - \pi^T F) (F(j) - \pi^T F).$$

(A5)

Defining the centered fitness vector $F' = F - (\pi^T F) 1$, we can rewrite this in matrix notation as

$$a(t) = (F')^T D \pi P_t F'.$$

(A6)

Using Equation A3, we can then expand $P_t$ in terms of its eigenvalues and eigenvectors and simplify to get

$$a(t) = \sum_{k=1}^{n} e^{-\lambda_k t} \left( (F')^T D \pi r_k \right) (l_k^T F').$$

(A7)

$$= \sum_{k=1}^{n} e^{-\lambda_k t} (l_k^T F')^2$$

(A8)

$$= \sum_{k=2}^{n} e^{-\lambda_k t} (l_k^T F')^2$$

(A9)

where the last line follows because by construction $l_1^T F' = \pi^T F' = 0$ and, for $k \geq 2$, $l_k^T 1 = l_k^T r_1 = 0$, so that for $k \geq 2$

$$l_k^T F' = l_k^T F - (\pi^T F) (l_k^T 1)$$

(A10)

$$= l_k^T F.$$ 

(A11)

Equation 16 in the main text then follows from Equation A9 by noting that $(l_k^T F)^2$ is non-negative.