



A universal scaling law determines time reversibility and steady state of substitutions under selection

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ABSTRACT

Monomorphic loci evolve through a series of substitutions on a fitness landscape. Understanding how mutation, selection, and genetic drift drive this process, and uncovering the structure of the fitness landscape from genomic data are two major goals of evolutionary theory. Population genetics models of the substitution process have traditionally focused on the weak-selection regime, which is accurately described by diffusion theory. Predictions in this regime can be considered universal in the sense that many population models exhibit equivalent behavior in the diffusion limit. However, a growing number of experimental studies suggest that strong selection plays a key role in some systems, and thus there is a need to understand universal properties of models without *a priori* assumptions about selection strength. Here we study time reversibility in a general substitution model of a monomorphic haploid population. We show that for *any* time-reversible population model, such as the Moran process, substitution rates obey an exact scaling law. For several other irreversible models, such as the simple Wright–Fisher process and its extensions, the scaling law is accurate up to selection strengths that are well outside the diffusion regime. Time reversibility gives rise to a power-law expression for the steady-state distribution of populations on an arbitrary fitness landscape. The steady-state behavior is dominated by weak selection and is thus adequately described by the diffusion approximation, which guarantees universality of the steady-state formula and its applicability to the problem of reconstructing fitness landscapes from DNA or protein sequence data.

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1. Introduction

A key goal of evolutionary theory is to determine the role of natural selection in the evolution of genotypes, and to infer information about selection strength from the growing abundance of genomic data. Theoretical work on these issues takes many different forms, both because of the inherent differences among biological systems and because different simplifying assumptions are necessary for the sake of mathematical tractability. One common approximation is to consider unlinked loci in the monomorphic limit, valid for neutral evolution once sufficiently low mutation rates and effective population sizes ensure that genetic drift dominates (Crow and Kimura, 1970). Even larger populations or those with greater mutation rates can be nearly monomorphic if selection is significant.

If at any given time the population is dominated by a single genotype at the locus of interest, to a good approximation such a

population evolves as a single entity on a fitness landscape (Wright, 1932) over genotype space, assuming that the evolutionary success of a genotype can be distilled into a fitness value. The movement of the entire population from one genotype to another is known as the substitution process, where each substitution event consists of a single mutation arising and then fixing instantaneously (Kimura, 1983). This picture greatly simplifies the theory, especially because it permits fixation events to be analyzed using two-allele models of population genetics (Crow and Kimura, 1970). Moreover, it is believed that many higher eukaryotes (Kimura, 1983) and some microorganisms contain loci that can be adequately described as monomorphic (Ochman and Selander, 1984; Wick et al., 2002; Dos Vultos et al., 2008; Achtman, 2008). As a result, this approach has been followed in settings as diverse as the evolution of transcription factor (TF) binding sites in yeast (Lässig, 2007; Mustonen et al., 2008), viral protein evolution (Bloom et al., 2007; Bloom and Glassman, 2009), and codon usage bias (e.g., McVean and Vieira, 2001; Yang and Nielsen, 2008). These theoretical and computational studies complement recent experimental work that has begun to reconstruct empirical fitness landscapes directly (Weinreich et al., 2006; Poelwijk et al., 2007).

Much theoretical work in population genetics has focused on gradual models of adaptation in which evolutionary change

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proceeds through selection of alleles with very small fitness advantage (Orr, 2005). The idea of the extremely slow rate of phenotypic evolution was proposed by Darwin (1859) and subsequently made popular by Fisher (1958) in the context of the infinitesimal model. In more recent decades, experimental evidence like the molecular clock and high levels of sequence variation in some proteins suggested that genetic drift, and not selection, was the key evolutionary driving force. This led to the neutral and nearly neutral theories of molecular evolution (Kimura, 1983; Ohta and Tachida, 1990; Ohta, 1992).

From the theoretical perspective, a key motivation for weak-selection models is their universality: many specific models are equivalent in the weak-selection, or diffusion, regime. This equivalence is observed for the simple Wright–Fisher (Wright, 1931; Fisher, 1958) and Moran (Moran, 1958) models, which share a diffusion limit with a variety of more elaborate models under the appropriate mapping of parameters (e.g., Ewens, 1967; Maruyama, 1970; Otto and Whitlock, 1997; Möhle, 2001; Möhle and Sagitov, 2001; Whitlock, 2003; Wakeley, 2005). Even though the simple Wright–Fisher model is undoubtedly a gross simplification of natural populations, this universality has driven the use of its diffusion limit (Kimura, 1955, 1962), and more generally, the use of exchangeable models (Cannings, 1974) as plausible effective theories in a wide variety of applications.

However, there is mounting experimental evidence that stronger selection may be common in nature. Strongly deleterious mutations have long been known to exist, although they are typically eliminated by selection so efficiently that they play little role in evolutionary dynamics (Kimura, 1983). Mutations with strong selective advantage, on the other hand, may routinely occur in organisms faced with novel environments or environmental stresses such as high temperature (Wichman et al., 1999; Bull et al., 2000; Holder and Bull, 2001; Barrett et al., 2006b), with early steps in adaptation typically exhibiting larger fitness gains than later ones. Furthermore, several QTL-mapping experiments have demonstrated that adaptive evolution frequently involves relatively few genetic changes with large fitness effects (reviewed in Orr, 2001, 2005; Eyre-Walker and Keightley, 2007). Using approaches developed in the weak-selection limit to predict the dynamics of strongly beneficial mutations (such as fixation times and the probability of fixation) may lead to significant errors (Morjan and Rieseberg, 2004; Whitlock, 2003; Barrett et al., 2006a).

Models attempting to include a wider range of selection strengths are often deterministic (Eigen et al., 1989; Bürger, 2000) and therefore exclude populations with non-negligible genetic drift, while stochastic theories typically demonstrate model-dependent behavior when selection becomes too strong (Proulx, 2000; Shpak, 2007; Parsons et al., 2010), which limits their application to natural systems. Thus there is a need to study universal properties of classes of stochastic models in which no *a priori* assumptions about the strength of selection are made.

In this paper we investigate such properties, focusing on time reversibility (i.e., detailed balance) and the steady state of the substitution process. We restrict ourselves to asexual haploids for simplicity, which includes many populations of single-cell organisms (Ochman and Selander, 1984; Wick et al., 2002; Dos Vultos et al., 2008; Achtman, 2008). For *any* time-reversible population model, such as the Moran process, we show that the substitution rates obey a simple scaling law. This result is exact in the monomorphic limit and requires no diffusion or weak-selection approximation. For irreversible models, we find that the scaling law is an accurate approximation for sufficiently weak selection, and in fact may hold for a large range of selection strengths beyond the classical diffusion limit, as we show for the simple Wright–Fisher model and its extensions. Since this scaling behavior is equivalent to time reversibility, this contradicts the belief that selection should break reversibility (McVean and Vieira, 2001).

The scaling law also gives rise to a power-law formula for the steady-state distribution, which is exact for any reversible model. This generalizes the work of Sella and Hirsh (2005), who obtain this result in the special case of the Moran model. Moreover, we find that strong selection plays little role in steady state, which is dominated by genetic drift and weak selection. Since evolutionary behavior in this regime is known to be universal through established results based on the diffusion approximation, the steady-state formula is accurate within a sizable range of selection strengths for a large class of population models, including many irreversible ones. The wide range of applicability of the time-reversibility condition greatly simplifies computational studies of evolutionary dynamics in biological systems, such as probabilistic phylogenetic inference (Yang, 2006). Finally, the simple power-law form of the steady-state distribution allows inference of fitness landscapes from genomic data in systems for which the steady state is believed to be a good approximation, such as TF binding sites in yeast (Mustonen et al., 2008).

2. Substitution model for monomorphic populations

We consider the evolution of a single locus in the monomorphic limit, where the mutation rate is sufficiently low that the vast majority of single mutations either fix or become extinct before a second mutation on the locus arises (Kimura, 1983). Thus we can describe evolution of this locus as a series of substitution events in which the entire population switches from genotype σ to genotype σ' . Since the time scale for fixation or extinction of a mutant (during which the population is actually polymorphic) is very short compared to the time scales of interest, we approximate these events as instantaneous. For a locus of length L and single-site mutation rate μ , Champagnat (2006) and Champagnat et al. (2006) have shown that the condition necessary to guarantee a monomorphic population is $\mu \leq 1/(LN \log N)$ for a population of size N . However, if most mutations introduce significant selective effects, the fixation or extinction of mutants will occur more rapidly, weakening the condition on μ . For beneficial mutations of selective advantage s (where $1 \ll Ns \ll N$), Desai and Fisher (2007) have shown that the monomorphic condition becomes $\mu \leq 1/(LN \log(Ns))$.

We will assume that the locus of interest is unlinked to the rest of the genome (linkage equilibrium) by frequent recombination with rate ρ , which satisfies $\rho \gg N\mu L$ (Mustonen and Lässig, 2010); here, recombination also includes homologous DNA transfer such as that observed in bacteria. Therefore we can consider the evolution of the locus independently from the rest of the genome. We assume that the locus is short enough that recombination does not occur within the locus itself. In general, we are interested in loci with $<10^3$ nucleotides, which easily meet these conditions. Such loci include short regulatory sequences of nucleotides such as TF binding sites, and coding regions. Viruses or loci with mutation or recombination hotspots are outside the scope of this model. Note that while the locus of interest is unlinked to other genomic sites, there may be epistasis among the nucleotides or amino acids constituting the locus itself.

Let σ and σ' be two genotypes (i.e., sequences of L nucleotides or amino acids) at the locus of interest. The substitution rate from σ to σ' can be approximated by the rate of producing a single mutant times the probability that the mutation fixes (Kimura and Ohta, 1971; Kimura, 1983):

$$W(\sigma'|\sigma) \approx N\mu(\sigma'|\sigma) \cdot \phi(\sigma'|\sigma), \quad (1)$$

where N is an effective population size, $\mu(\sigma'|\sigma)$ is the nucleotide or amino acid mutation rate from σ to σ' , and $\phi(\sigma'|\sigma)$ is the probability that a single σ' mutant fixes in a population of wild-type σ . We will assume that μ is nonzero only for genotypes σ and σ' differing by a single nucleotide or amino acid.

Given an ensemble of populations evolving with these rates, we can define $\pi(\sigma, t)$ to be the probability that a population is monomorphic at the locus with genotype σ at time t . This probability evolves over time via the master equation

$$\frac{d}{dt}\pi(\sigma', t) = \sum_{\sigma \in \mathcal{S}} [W(\sigma'|\sigma)\pi(\sigma, t) - W(\sigma|\sigma')\pi(\sigma', t)], \quad (2)$$

where \mathcal{S} is the set of all possible genotypes at the locus of interest. This Markov process is finite and irreducible, since there is a nonzero probability of reaching any genotype from any other genotype in finite time. Hence it has a unique steady-state distribution $\tilde{\pi}(\sigma)$ (Allen, 2011) satisfying

$$\sum_{\sigma \in \mathcal{S}} [W(\sigma'|\sigma)\tilde{\pi}(\sigma) - W(\sigma|\sigma')\tilde{\pi}(\sigma')] = 0. \quad (3)$$

The form of this steady-state distribution depends on the underlying population genetics model that gives the fixation probability ϕ .

The monomorphic limit permits us to consider two-allele population models without mutation. First, we consider Wright–Fisher and Moran models that describe populations of fixed size N , with the selective value of each genotype σ specified by a single parameter $f(\sigma)$ which we refer to as the genotype's fitness. Next, we extend our treatment to a model in which population size varies periodically with time, and finally consider more general models proposed by Gillespie (1974) which allow for different variances in offspring number.

An important consequence of fixed size N is that only relative fitnesses matter. Relative fitness can be an arithmetic difference or a ratio, depending on the parameterization. These are equivalent under a simple exponential mapping. Note that the model is then symmetric under either a shift or rescaling of all fitnesses, a symmetry which is convenient to maintain at all stages of an approximation. For instance, in the Wright–Fisher or Moran models, it is typical to incorporate fitness as a multiplicative weight in the transition probabilities, in which case all observable quantities depend only on the ratio of the wild-type to mutant fitness. In particular, the probability that a single σ' mutant fixes in a population of wild-type σ must only depend on $r = f(\sigma')/f(\sigma)$ and, implicitly, on the population size N : $\phi(\sigma'|\sigma) \equiv \phi(r)$.

3. The scaling law and steady state

Since substitution rates depend on the fixation probability $\phi(r)$, we aim to use arguments from population genetics to study time reversibility (or simply “reversibility”), which in turn determines the form of the steady state. Time reversibility is equivalent to detailed balance, a sufficient but not necessary condition for steady state:

$$W(\sigma'|\sigma)\tilde{\pi}(\sigma) = W(\sigma|\sigma')\tilde{\pi}(\sigma'), \quad (4)$$

where $\tilde{\pi}(\sigma)$ denotes the steady-state distribution. The left- and right-hand sides of this equation are the steady-state probability currents $\sigma \rightarrow \sigma'$ and $\sigma' \rightarrow \sigma$, respectively. Eq. (4) means that these currents are exactly balanced for each pair of genotypes σ and σ' , and hence there are no net currents, consistent with the notion that it is impossible to distinguish the forward and backward flow of time in steady state.

Throughout this paper, we will assume that neutral evolution – when all genotypes are selectively neutral relative to each other – is reversible. In the neutral model, the fixation probability $\phi(\sigma'|\sigma) = 1/N$ for all σ and σ' , and hence Eq. (1) shows that the neutral substitution rates are just the mutation rates (Kimura, 1983): $W(\sigma'|\sigma) = \mu(\sigma'|\sigma)$. Let the steady-state distribution of

the neutral substitution process be $\tilde{\pi}_0(\sigma)$. Then reversibility of the neutral model is expressed by

$$\mu(\sigma'|\sigma)\tilde{\pi}_0(\sigma) = \mu(\sigma|\sigma')\tilde{\pi}_0(\sigma'). \quad (5)$$

Many popular neutral models are reversible (see Yang, 2006, for a summary), although this condition is not guaranteed. This issue will be explored further in Section 5.

We now consider the reversibility of the substitution rates under selection, $N\mu(\sigma'|\sigma)\phi(r)$. Let us first define the function

$$\psi(r) \equiv \frac{\phi(r)}{\phi(1/r)}. \quad (6)$$

Hence the ratio of the forward and backward substitution rates between σ and σ' is

$$\frac{W(\sigma'|\sigma)}{W(\sigma|\sigma')} = \frac{\mu(\sigma'|\sigma)}{\mu(\sigma|\sigma')} \cdot \frac{\phi\left(\frac{f(\sigma')}{f(\sigma)}\right)}{\phi\left(\frac{f(\sigma)}{f(\sigma')}\right)} = \frac{\tilde{\pi}_0(\sigma')}{\tilde{\pi}_0(\sigma)} \cdot \psi\left(\frac{f(\sigma')}{f(\sigma)}\right), \quad (7)$$

where we have invoked the reversibility of the neutral rates (Eq. (5)). Studying the properties of the ψ function is the main focus of this paper: it will determine the existence of reversibility under selection and the form of the steady-state distribution. We will investigate both its general properties and its form for specific models.

We will first assume that the substitution rates $W(\sigma'|\sigma)$ under selection are reversible, which we will show completely constrains the form of ψ and the steady state under selection $\tilde{\pi}(\sigma)$. In this case, $W(\sigma'|\sigma)\tilde{\pi}(\sigma) = W(\sigma|\sigma')\tilde{\pi}(\sigma')$, and hence

$$\frac{\tilde{\pi}(\sigma')}{\tilde{\pi}(\sigma)} = \frac{W(\sigma'|\sigma)}{W(\sigma|\sigma')} = \frac{\tilde{\pi}_0(\sigma')}{\tilde{\pi}_0(\sigma)} \cdot \psi\left(\frac{f(\sigma')}{f(\sigma)}\right). \quad (8)$$

It follows that

$$\psi\left(\frac{f(\sigma'')}{f(\sigma')}\right) \cdot \psi\left(\frac{f(\sigma')}{f(\sigma)}\right) = \psi\left(\frac{f(\sigma'')}{f(\sigma)}\right), \quad (9)$$

that is, ψ generally satisfies $\psi(r_1)\psi(r_2) = \psi(r_1r_2)$. Therefore $\psi(r)$ must be a simple power law:

$$\psi(r) = r^\nu, \quad (10)$$

for some constant ν (Roberts, 1979). The constant ν can only depend on the population size N , since this is the only other parameter in our population model. We will refer to Eq. (10) as the scaling law for ψ . Using the definition of $\psi(r)$ (Eq. (6)), one can show that

$$\nu = \frac{2\phi'(1)}{\phi(1)} = 2N\phi'(1), \quad (11)$$

where $\phi'(1) = d\phi(r)/dr|_{r=1}$ and $\phi(1) = 1/N$ is the neutral fixation probability.

Now rewriting Eq. (8) with our explicit form of ψ ,

$$\frac{\tilde{\pi}(\sigma')}{\tilde{\pi}(\sigma)} = \frac{\tilde{\pi}_0(\sigma')}{\tilde{\pi}_0(\sigma)} \left(\frac{f(\sigma')}{f(\sigma)}\right)^\nu, \quad (12)$$

we can deduce the steady state:

$$\tilde{\pi}(\sigma) = \frac{1}{Z}\tilde{\pi}_0(\sigma)(f(\sigma))^\nu, \quad (13)$$

where Z is a normalization constant. Note that Eq. (13) can be rewritten in the form of a Boltzmann distribution, with energy replaced by the negative logarithm of fitness:

$$\tilde{\pi}(\sigma) = \frac{1}{Z}\tilde{\pi}_0(\sigma)e^{\nu \log f(\sigma)}. \quad (14)$$

The Boltzmann form in Eq. (14) suggests a straightforward analogy with statistical mechanics (Iwasa, 1988; Sella and Hirsh, 2005). One may think of the evolutionary model defined by Eqs. (1) and (2) as describing an ensemble of monomorphic populations taking random walks on a fitness landscape. The ensemble of walkers eventually reaches steady state in genotype space, which is given by Eq. (13) or (14). Populations will be driven toward the peaks of the landscape by selection, which manifests itself as the f^ν factor in the steady state; this effect becomes exponentially stronger as ν increases. This is analogous to energy minimization in statistical mechanics. However, as in statistical mechanics, we also expect the entropy of states to affect the steady-state distribution, since typically there are few states with optimal or near-optimal fitness and many states with low fitness. This density of states is given by the neutral distribution $\tilde{\pi}_0$. The corresponding entropy (defined as $-\log \tilde{\pi}_0$) competes with selection the same way energy and entropy compete in statistical mechanics: selection favors high fitness states while entropy favors low fitness states since there are usually many more of them. These competing forces reach some balance in the form of a “free fitness” function that is maximized in the steady state, as explored in Iwasa (1988) and Sella and Hirsh (2005).

This steady-state formula was derived in the special case of the Moran model by Sella and Hirsh (2005). We generalize this earlier result by showing that any reversible substitution process leads to the power law for ψ and the steady-state formula of Eq. (13). Note that this conclusion, obtained in the monomorphic limit, requires no additional assumptions, such as the weak-selection diffusion approximation.

Next, we show that the power law implies reversibility. We now assume Eq. (10) without assuming reversibility. Then

$$\frac{W(\sigma'|\sigma)}{W(\sigma|\sigma')} = \frac{\tilde{\pi}_0(\sigma')}{\tilde{\pi}_0(\sigma)} \left(\frac{f(\sigma')}{f(\sigma)} \right)^\nu. \quad (15)$$

We can combine this with the steady-state condition (Eq. (3)) to show that

$$\begin{aligned} 0 &= \sum_{\sigma \in \mathcal{S}} [W(\sigma'|\sigma)\tilde{\pi}(\sigma) - W(\sigma|\sigma')\tilde{\pi}(\sigma')] \\ &= \sum_{\sigma \in \mathcal{S}} W(\sigma|\sigma') \left[\frac{\tilde{\pi}_0(\sigma')}{\tilde{\pi}_0(\sigma)} \left(\frac{f(\sigma')}{f(\sigma)} \right)^\nu \tilde{\pi}(\sigma) - \tilde{\pi}(\sigma') \right]. \end{aligned} \quad (16)$$

Clearly the distribution in Eq. (13) satisfies this condition, so it must be the unique steady state. The reversibility condition (Eq. (4)) is satisfied as well, and thus the power law implies reversibility.

Therefore, time reversibility and the scaling behavior of ψ are mathematically equivalent, and both lead to the steady-state formula of Eq. (13). We will refer to these collective results as the *scaling law* of the substitution process. This means that we can concentrate our attention on determining the form of ψ , since its scaling behavior tells us the extent to which reversibility and Eq. (13) hold. Obviously not all models are reversible, so the scaling law will not hold exactly in those cases. However, we demonstrate below that the scaling behavior of ψ is at least an approximate feature of a large class of models, and therefore reversibility and the steady-state formula (Eq. (13)) provide a good approximation within a sizable range of selection strengths.

Since it will be more convenient to describe the scaling behavior of ψ on logarithmic scales, we expand $\log \psi(r)$ in a power series in $\log r$ around the neutral limit ($\log r = 0$):

$$\begin{aligned} \log \psi(r) &= \sum_{j=0}^{\infty} \frac{c_{2j+1}}{(2j+1)!} (\log r)^{2j+1} \\ &= c_1(\log r) \left[1 + \frac{1}{c_1} \sum_{j=1}^{\infty} \frac{c_{2j+1}}{(2j+1)!} (\log r)^{2j} \right], \end{aligned} \quad (17)$$

where

$$c_i = \left(\frac{d^i}{d(\log r)^i} \log \psi(r) \right) \Big|_{r=1}. \quad (18)$$

Note that $\log \psi(r)$ is an odd function in $\log r$, and hence there are only odd powers in the expansion. Since $c_1 = 2\phi'(1)/\phi(1) = \nu$, we can write

$$\log \psi(r) = \nu(\log r) \left[1 + \frac{1}{\nu} \sum_{j=1}^{\infty} \frac{c_{2j+1}}{(2j+1)!} (\log r)^{2j} \right]. \quad (19)$$

The scaling behavior of ψ is captured by the first-order term in this expansion. As long as ν is nonzero, there will always be some neighborhood of selection strengths around the neutral limit, $r = 1$, in which the scaling law holds. We give an argument that $\nu \neq 0$ in Appendix A. The argument relies on the universal nature of the diffusion approximation to a population model. That is, discrete population models can be approximated by a continuous diffusion equation, and it is known that a large class of population models are equivalent under this approximation (e.g., Ewens, 1967; Maruyama, 1970; Otto and Whitlock, 1997; Möhle, 2001; Möhle and Sagitov, 2001; Whitlock, 2003; Wakeley, 2005). The diffusion approximation is valid for weak-selection strengths: $r - 1 = s \sim \mathcal{O}(N^{-1})$ (Ewens, 2004). Since the scaling behavior of ψ appears in the diffusion regime, it is shared by a large class of models.

The diffusion argument in Appendix A also gives us insight into the interpretation of $\nu = 2N\phi'(1)$: it suggests that $\phi'(1) \sim \mathcal{O}(N^0)$ and therefore $\nu \sim \mathcal{O}(N)$. Thus we can interpret ν as a “scaling” effective population size that is of the same order as the census population size for fixed-size models or the variance effective population size for more general models. This is sensible in light of the Boltzmann form of the steady state (Eq. (14)), which suggests that $1/\nu$ plays the role of temperature, i.e., the scale of stochastic fluctuations.

There is a range of selection strengths in which the scaling law is approximately valid. Specifically, we wish to find the range of fitness ratios r , which we will denote as (r_0^{-1}, r_0) with $r_0 > 1$, such that

$$\nu(1 \mp \epsilon) \log r < \log \psi(r) < \nu(1 \pm \epsilon) \log r, \quad (20)$$

where the upper signs are valid for $r > 1$, the lower signs are valid for $r < 1$, and $\epsilon > 0$ is a small number that we choose to control the accuracy of the power law approximation. This range is determined by the next coefficient in the expansion of Eq. (19),

$$\frac{c_3}{6\nu} = \frac{1}{12\nu} (\nu^3 - 3\nu^2 + 2\nu - 6N(\nu - 2)\phi''(1) + 4N\phi^{(3)}(1)), \quad (21)$$

where we have evaluated the derivative of $\log \psi(r)$ in terms of $\phi(r)$ and substituted $\phi(1) = 1/N$ and $\nu = 2N\phi'(1)$. For small ϵ ,

$$\frac{|c_3|}{6\nu} (\log r_0)^2 = \epsilon \longrightarrow r_0 = \exp \left(\sqrt{\frac{6\nu\epsilon}{|c_3|}} \right). \quad (22)$$

For any particular model, we need only compute ν and c_3 to obtain the range of selection strengths (r_0^{-1}, r_0) for which the scaling law is a good approximation.

Even outside of this range, however, deviations from the power law likely lead to negligible errors in estimating the probabilities of extremely unfit genotypes. This is a situation encountered when the monomorphic population is in steady state on the fitness landscape, with the majority of time spent in locally optimal high-fitness states from which many strongly deleterious but no strongly beneficial substitutions can be made. Specifically, assume that the range of fitness ratios for which the scaling-law approximation is valid, computed from Eq. (22), is (r_0^{-1}, r_0) .

Suppose that genotype σ_1 has fitness f_1 and genotype σ_2 has fitness less than f_1/r_0 ($r_0 > 1$), and also assume that they are separated by a single mutation. By construction, the substitution from σ_1 to σ_2 is outside the range for which the power law is a valid approximation. Now suppose that there is a third genotype σ_3 (also separated by a single mutation from σ_1) with fitness of exactly f_1/r_0 , so that its probability is given by Eq. (13). Since ψ must be monotonically increasing, the probability of the unfit σ_2 is bounded from above by the probability of σ_3 :

$$\bar{\pi}(\sigma_2) < \frac{1}{2} \bar{\pi}_0(\sigma_3) r_0^{-\nu} f_1^\nu. \quad (23)$$

Then the ratio of $\bar{\pi}(\sigma_2)$ to $\bar{\pi}(\sigma_1)$ has an upper bound as well:

$$\frac{\bar{\pi}(\sigma_2)}{\bar{\pi}(\sigma_1)} < \frac{\bar{\pi}_0(\sigma_3) r_0^{-\nu} f_1^\nu}{\bar{\pi}_0(\sigma_1) f_1^\nu} \simeq r_0^{-\nu}, \quad (24)$$

where the last relation holds because the neutral probabilities $\bar{\pi}_0(\sigma_1)$ and $\bar{\pi}_0(\sigma_3)$ are of the same order of magnitude (under the reasonable assumption that mutation rates within the locus are all of the same order). Since ν is proportional to the population size, the maximum fitness ratio r_0 in the scaling region need not be very large to generate an enormous suppression of the unfit genotype in steady state. Thus inaccuracies in the probabilities of unfit genotypes caused by deviations from the scaling law will be negligible for all practical purposes.

Furthermore, we can explicitly show that the selection strengths of the dominant substitutions in steady state are precisely those described by the diffusion approximation. In steady state, it is sufficient to consider genotypes that have relative probabilities, with respect to the most fit genotype, of at least $\delta > 0$. Then the relevant fitness ratios r are constrained by $r^{-\nu} > \delta$ or $r < \delta^{-1/\nu}$. Since $\nu \sim \mathcal{O}(N)$, we expand in powers of $1/\nu$ to obtain

$$r < 1 - \frac{1}{\nu} \log \delta + \mathcal{O}(\nu^{-2}). \quad (25)$$

In terms of $s = r - 1$, this implies $s \sim \mathcal{O}(\nu^{-1}) \sim \mathcal{O}(N^{-1})$, which is the selection strength for which the diffusion approximation is valid (Ewens, 2004). Therefore the steady state of substitutions is adequately described by the diffusion approximation and thus by the scaling law (Eqs. (10) and (13)). As a result, only the optimal genotype and slightly less fit neighboring states have non-negligible probabilities in steady state.

The steady-state distribution of Eq. (13) was previously derived for the special cases of the Moran process by Sella and Hirsh (2005) and for the diffusion limit of the Wright–Fisher model by Sella and Hirsh (2005), Lässig (2007), and Li (1987), among others. Indeed, some form of this formula can even be found in Wright (1931). We have generalized these results by showing that the steady-state formula holds exactly for any reversible model, not just the Moran process, without requiring any diffusion approximation. For irreversible models, we have shown how this result arises as an approximation, and determined its range of validity. Surprisingly, weak selection dominates steady-state behavior in a wide class of population models, justifying application of the steady-state formula to systems which may include mutations with large fitness effects.

4. Specific population models

We now verify the general results of the previous section for specific models, computing the scaling effective population size ν and the range of selection strengths for which the scaling law is a good approximation.

4.1. The Moran model

Consider a haploid population of fixed size N with two alleles, A and B , and let n denote the number of B alleles. The single time-step transition probabilities of the Moran model are then (Moran, 1958; Ewens, 2004)

$$\begin{aligned} \Pi(n+1|n) &= \frac{f_B}{\bar{f}} \frac{n}{N} \left(1 - \frac{n}{N}\right) \\ \Pi(n-1|n) &= \frac{f_A}{\bar{f}} \frac{n}{N} \left(1 - \frac{n}{N}\right) \end{aligned} \quad (26)$$

$$\Pi(n|n) = 1 - \Pi(n+1|n) - \Pi(n-1|n),$$

where f_A, f_B are fitnesses of alleles A and B and $\bar{f} = (n/N)f_B + (1 - n/N)f_A$ is the average fitness. In this case the probability of fixing a single mutant is (Ewens, 2004)

$$\phi(r) = \frac{1 - r^{-1}}{1 - r^{-N}}, \quad (27)$$

where $r = f_B/f_A$. A straightforward calculation shows that $\psi(r) = \phi(r)/\phi(1/r) = r^{N-1}$ (Sella and Hirsh, 2005). Hence $\nu = N - 1$ for Moran, and the scaling law holds exactly if the neutral substitution rates are reversible (Fig. 1A).

4.2. The Wright–Fisher model

Next we define the simple Wright–Fisher model for a haploid population of fixed size N with two alleles A and B of fitness f_A and f_B , respectively (Wright, 1931; Fisher, 1958). Given that there are n alleles of type B in the current generation, the probability of having n' B alleles in the next generation is (Rouzine et al., 2001; Ewens, 2004)

$$\Pi(n'|n) = \binom{N}{n'} q^{n'} (1-q)^{N-n'}, \quad \text{where } q \equiv \frac{n f_B}{N \bar{f}}. \quad (28)$$

Unlike the Moran model, the Wright–Fisher model is ill-suited to exact treatment, and hence the traditional approach to it has been the diffusion approximation. The diffusion theory yields many results in the neutral and weak-selection regimes (Kimura, 1955, 1957, 1962), such as the formula for the fixation probability:

$$\phi(r) = \frac{1 - e^{2(1-r)}}{1 - e^{2N(1-r)}}, \quad (29)$$

where $r = f_B/f_A$. However, there are two problems with the classical diffusion approach. The first is that the moment functions $M(x, r)$ and $V(x, r)$ are typically expanded to the lowest order in $r - 1$ for the weak-selection regime (as in Appendix A), and so all subsequent calculations, including those leading to the fixation probability in Eq. (29), are not strictly valid for selection strengths beyond $s = r - 1 \sim \mathcal{O}(N^{-1})$. This expansion in selection strength, however, is not necessary, as it is possible to carry out the diffusion approximation using the exact moments derived from Eq. (28). This approach yields accurate results in the polymorphic limit, but fails to give an accurate formula for the fixation probability. This is due to the inherent breakdown of diffusion when the underlying discrete nature of the model becomes important, which is especially pronounced when selection effects are strong.

Since the diffusion approach is unsuitable to describe fixation outside of a fairly narrow range of selection strengths, we take a more accurate but numerical approach: computing fixation probabilities directly from the discrete Markov chain defined in Eq. (28) (Appendix B). The end result is an efficient numerical procedure for accurate computation of the fixation probability, and hence the ψ function, for any N and r . Fig. 2 compares a

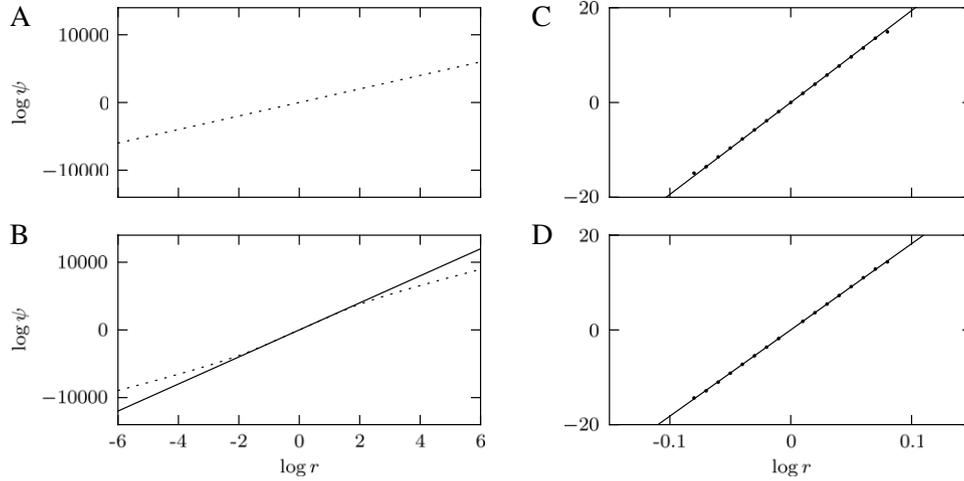


Fig. 1. Plot of $\log \psi(r)$ as a function of $\log r$ for several population models. The scaling law appears as the straight line $\log \psi(r) = \nu \log r$. (A) The Moran model with $N = 1000$. Here the scaling law is exact with $\nu = N - 1$. (B) The simple Wright–Fisher model for $N = 1000$, calculated using the numerical procedure from Appendix B. The numerical calculation is the dashed line and the scaling-law prediction is the solid line. Here the scaling law is not exact but holds as a good approximation for a large range of selection strengths. The scaling effective population size is $\nu = 2(N - 1)$. (C) A modified Wright–Fisher model with population size N that varies sinusoidally as in Eq. (33), with $N_0 = 100$, $\alpha = 20$ and $T = 20$ generations. Simulation results are shown as dots (with each dot an average over 10^6 independent runs), and the scaling law as a solid line. The scaling law is an accurate approximation with $\nu = 2(N_e - 1)$, where $N_e = \sqrt{N_0^2 - \alpha^2}$ is the harmonic mean of the census population sizes. Because explicit simulations are required (as opposed to the numerical procedure used for the simple Wright–Fisher model), poor statistics on deleterious fixations and beneficial extinctions restricts us to considering smaller population sizes and range of selection strengths. (D) A model based on those in Gillespie (1975), where the mutant and wild-type may have different variances in offspring number in addition to different means. Here fitness is defined as $\mu - \sigma^2/N$, where μ is the average number of offspring and σ^2 is the variance. As in (C), we use $N = 100$ for numerical reasons. The scaling law is deduced by a linear fit.

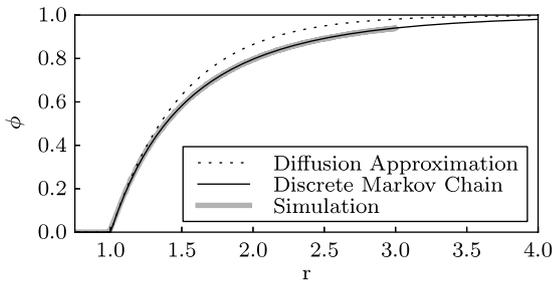


Fig. 2. Plot of $\phi(r)$, the probability that a single mutant fixes as a function of its fitness ratio with the wild-type. For $N = 1000$, we compare an explicit simulation of the Wright–Fisher model with our discrete Markov chain approach (Eq. (B.8)) and Kimura’s diffusion approximation (Eq. (29)). The explicit simulation data is averaged over 10^6 independent runs. The agreement between the discrete Markov chain and the simulation is excellent, in contrast with the noticeable disagreement between the simulation and the diffusion approximation at larger selection strengths.

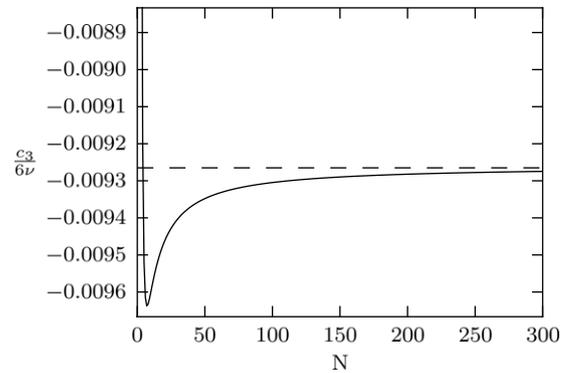


Fig. 3. Plot of $c_3/6\nu$ as a function of N for the simple Wright–Fisher model, obtained numerically from $\phi(r)$ using the procedure described in Appendix B. For realistic N values it rapidly converges to the constant ≈ -0.0093 . This small value means that the scaling-law approximation is valid for a large range of selection strengths, and its N -independence means that this range does not shrink as N grows, contrary to the prediction of diffusion theory.

simulation of $\phi(r)$ with this numerical approach along with the diffusion approximation (Eq. (29)). The numerical calculation and the simulation match very well for all selection strengths, but there is noticeable disagreement with the diffusion result beyond the weak-selection regime.

Now we consider the expansion of $\psi(r)$ for the simple Wright–Fisher model. We know from diffusion theory that $\nu = 2N\phi'(1) = 2(N - 1)$ (Kimura, 1962). Hence the expansion of $\psi(r)$ has the form

$$\log \psi(r) = 2(N - 1) \log r + \mathcal{O}((\log r)^3). \quad (30)$$

Thus the power law and the steady state in Eq. (13) hold approximately with $\nu = 2(N - 1)$. As Appendix B shows, the form of the exact fixation probability is too complex to be useful for analytical calculations, such as computing c_3 in Eq. (21) to determine the range of selection strengths for which the power-law approximation is approximately valid. However, we can numerically compute this next-order coefficient for a range of N using the method in Appendix B to obtain derivatives of fixation probabilities for Eq. (21). Fig. 3 shows, remarkably, that the

next-order correction is independent of N for large N . Indeed, as N increases to realistic values, the next-order coefficient rapidly converges to a small value of

$$\frac{c_3}{6\nu} \approx -0.0093. \quad (31)$$

Its smallness means that the scaling law is valid for a large range of selection strengths in the simple Wright–Fisher model. Indeed, for deviations from the power law of at most 5%, we set $\epsilon = 0.05$ in Eq. (22) and find that the fitness ratio r is constrained to be between 0.098 and 10.2. This corresponds to a selection coefficient s between -0.9 and 9.2 , well beyond the typical weak-selection limits of $\pm\mathcal{O}(N^{-1})$. A numerical calculation of ψ confirms this large scaling region (Fig. 1B). Indeed, using the argument leading to Eq. (24), unfit genotypes that might exhibit deviations from the scaling law will be suppressed by at least a factor of $r_0^{-\nu}$, where (r_0^{-1}, r_0) is the range of fitness ratios for which the scaling law approximately holds. If we let $r_0 \approx 10.2$, even a very conservative

$N = 200$ means that these unfit genotypes are suppressed by more than 10^{-402} relative to the most fit genotype.

The N -independence of $c_3/6\nu$ means that the size of the scaling region does not change with N . The standard diffusion approach implies a degeneracy of N and s : $Ns \sim \mathcal{O}(1)$, so that as N increases, the range of selection strengths that are considered weak shrinks. This is not intrinsic to the Wright–Fisher model, but is merely an emergent property in the diffusion limit (Wakeley, 2005). Our result, however, shows that the scaling law is valid well beyond diffusion. In contrast, $c_3/6\nu$ calculated using Kimura’s diffusion approximation (Eq. (29)) is given by:

$$\frac{c_3}{6\nu} = -\frac{1}{6}N. \quad (32)$$

Since this coefficient grows with N , the scaling region for r shrinks as N increases. This is consistent with the selection-drift degeneracy predicted by diffusion, but it is clearly misleading in light of our analysis of the full Wright–Fisher model, since it would erroneously imply that the scaling law and reversibility hold for an extremely small range of selection strengths. This provides an example of the danger posed by extrapolating diffusion results to arbitrary regions of parameter space: the universality of the scaling law is much stronger than diffusion could predict. While this turns out to be unimportant for steady state, which is dominated by weak selection, the fact that reversibility approximately holds in systems with strong selection affects dynamical properties as well.

4.3. Other models

Models that share the diffusion limit with the Moran and Wright–Fisher models will also share the scaling law. This encompasses a wide class of exchangeable models (Cannings, 1974; Möhle, 2001; Möhle and Sagitov, 2001). For instance, many generalizations of the Wright–Fisher model with varying N are known to have properties equivalent to the simple Wright–Fisher model with some effective population size N_e (Ewens, 1967; Otto and Whitlock, 1997; Sjödin et al., 2005). Other generalizations, such as incorporating the effects of subdivided populations, also lead to equivalencies (Maruyama, 1970; Whitlock, 2003).

As an example we consider the case when N varies periodically. For periods of oscillation smaller than fixation times, it is known that the Wright–Fisher diffusion results carry over with an effective population size N_e equal to the harmonic mean of the census population sizes (Ewens, 1967; Otto and Whitlock, 1997). Let the transition probabilities be of the Wright–Fisher form (Eq. (28)), with N changing over time according to

$$N(t) = N_0 + \alpha \sin\left(\frac{2\pi t}{T}\right), \quad (33)$$

where N_0 is the average size and T is the period of oscillation. The harmonic mean can be shown to be $N_e = \sqrt{N_0^2 - \alpha^2}$. In Fig. 1C, we use explicit simulations to compute $\psi(r)$, and we indeed find scaling behavior with $\nu = 2(N_e - 1)$. This slope, predicted through mapping to the simple Wright–Fisher model, is also obtained by a linear fit to the explicit simulation. Thus the scaling law still holds. For this model we do not have a computational technique for fixation probabilities like the one used for the simple Wright–Fisher model Appendix B, and explicit simulations prevent accurate statistics on fixation of very deleterious and extinction of very beneficial mutations, limiting us to a smaller range of selection strengths. Nevertheless, deviations beyond this smaller range can still be shown to be negligible in steady state. As Fig. 1C shows, the scaling region extends to at least $r_0 \approx 1.08$. Therefore any unfit genotypes leading to deviations must be suppressed by at least a factor of $r_0^{-\nu}$: even for $N_e = 200$, this is a suppression of 10^{-14} .

Other models beyond the paradigms of exchangeable and Wright–Fisher-type models may also demonstrate the scaling behavior. For instance, whereas Wright–Fisher and Moran models typically incorporate selective advantage as a difference in the mean number of offspring between allele types, Gillespie proposed to incorporate stochasticity at the level of selection by allowing for different variances in offspring number (Gillespie, 1974, 1975, 1977). In these models fitness is characterized by $\mu - \sigma^2/N$, where μ is the mean and σ^2 is the variance of the offspring number for a given allele. Other authors have extended models of this type to describe spatial variation, age structure, and demographic stochasticity, which may be important for small populations or populations subdivided into small demes (Proulx, 2000; Shpak, 2007; Parsons et al., 2010).

Here we simulate a model described in Gillespie (1975). Consider a haploid population of two allele types, A and B . Each generation, every individual i produces a number of offspring $1 + X_i$, where X_i is a binomially-distributed random variable. This variable has mean μ_A and variance σ_A^2 if i is of type A , or μ_B and σ_B^2 if i is of type B . Adding 1 to X_i simply guarantees that there are at least N total offspring. These offspring are then culled by sampling without replacement until there is a new generation of exactly N alleles. We simulate this process to obtain the ψ function (Fig. 1D). Fitness ratios r are defined using the fitness definition $f_i = \mu_i - \sigma_i^2/N$. For each i , X_A or X_B is generated from the binomial distribution $B(n, p_A)$ or $B(n, p_B)$, respectively, where $n = 10$ and p_A and p_B are given by the desired fitness ratio r ($p_A + p_B = 1$). By repeating the simulation for several population sizes, we observe that ν is proportional to N (for each N , ν is obtained by a linear fit, one of which is shown in Fig. 1D).

5. Discussion

5.1. Universality

The notion of universality has been key to the success of population genetics. The remarkable fact that many population models with varying degrees of complexity share the same diffusion limit when selection is weak has proven to be a strong justification of their use as effective phenomenological theories (Wakeley, 2005; Parsons et al., 2010). However, in light of the growing body of evidence that strong or at least intermediate selection may be important in some systems, it is desirable to pursue models that make no *a priori* assumptions about the strength of selection, and in particular, to find universal properties of such models. Our study shows that strong-selection effects are negligible in the steady state of the substitution process, so that the universality of the diffusion limit gives rise to a universal scaling law (Eq. (10)) which determines the steady-state distribution (Eq. (13)). Furthermore, the scaling law is proven to hold exactly for any reversible process (such as the Moran model), and holds approximately within a sizable range of selection strengths even for irreversible models. In some cases such as the simple Wright–Fisher model, this range is so large that deviations from it are not practically important. This finding significantly generalizes previous work of Sella and Hirsh (2005), Lässig (2007), Li (1987), and others.

5.2. Theoretical significance of time reversibility

The existence of reversibility in the weak-selection limit is not surprising in light of diffusion theory. Indeed, diffusion models are essentially always reversible (Watterson, 1977; Levikson, 1977; Ewens, 2004), and diffusion is known to adequately capture weak-selection behavior (Kurtz, 1981). The fact that reversibility is

broken by some models and not others when selection is strong is also clear. The Moran process, for instance, is well-known to be exactly reversible in all regimes, as are all models with tridiagonal transition matrices (Ewens, 2004). The Wright–Fisher model is not exactly reversible, and indeed we see that reversibility becomes significantly broken beyond a certain selection strength. In general, we find that the scaling behavior of the ψ function (Eq. (6)) indicates the extent to which a model is time reversible.

But besides being a technical convenience, what is the deeper significance of reversibility? In modern studies of population genetics and evolution, reversibility plays a crucial role in linking the prospective and retrospective paradigms (Ewens, 1990). Traditional population models are prospective; the interest is in calculating future properties given the current ones. However, more recent approaches, especially due to the emergence of large-scale molecular data, have led to the wide use of the retrospective paradigm, which looks backward in time from the present. This is the essence of coalescent theory and phylogenetics (Kingman, 1982; Yang, 2006). Time reversibility links the prospective and retrospective paradigms and thus has been exploited, for instance, in studies of age properties (Watterson, 1976, 1977; Ewens, 2004) and in phylogenetic methods (Yang, 2006).

An additional consequence of reversibility is the nonexistence of net probability currents in steady state, as guaranteed by Eq. (4). That is, reversible Markov models will have no net probability currents through any cycle of states, since such a current would distinguish between forward and backward directions in time. What does this mean for evolutionary models? Consider, for instance, a monomorphic substitution model with three alleles, A , B , and C , in order of decreasing fitness. If the substitution process is irreversible, there would be a net current around the loop $C \rightarrow B \rightarrow A \rightarrow C$. The net currents $C \rightarrow B$ and $B \rightarrow A$ flow from less fit to more fit alleles, but to complete the cycle, there is also a current $A \rightarrow C$ from a more fit allele to a less fit allele. This current must exist in *any* irreversible substitution model with selection, a strange consequence of evolutionary irreversibility.

5.3. Applications

Models of monomorphic populations evolving through successive substitutions on a fitness landscape have important applications to molecular data, since loci in many asexual populations are believed to be well-approximated as monomorphic (Ochman and Selander, 1984; Wick et al., 2002; Dos Vultos et al., 2008; Achtman, 2008). In particular, population genetics-based approaches allow for inference of biologically meaningful parameters, such as selection coefficients, as opposed to merely inferring overall substitution rates (McVean and Vieira, 2001). A precise form of the steady-state distribution is important in these applications, since it can be used to weigh ancestral nodes in phylogenetic inference calculations.

Several recent studies of codon usage bias have employed population genetics-based models of substitution with selection (e.g., Li, 1987; Bulmer, 1991; McVean and Charlesworth, 1999; McVean and Vieira, 1999, 2001; Nielsen et al., 2007; Yang and Nielsen, 2008). Results for the steady-state distribution using the standard Wright–Fisher diffusion approximation (Eq. (29)) for individual codons have been reported that are consistent with Eq. (13) in the limit of weak selection. However, there is growing experimental evidence that big-benefit single mutations may occur more often than previously thought. Studies on bacteriophages adjusting to new environmental conditions reported fitness ratios of nearly 4 (Wichman et al., 1999; Bull et al., 2000; Holder and Bull, 2001; Barrett et al., 2006b), clearly beyond the diffusion regime. Thus, it is necessary to understand the role of these mutations in steady state

and whether the steady-state distribution predicted from weak-selection must be modified in such systems. Our theoretical framework has enabled us to show that mutations with large fitness ratios are negligible in steady state.

Throughout this work we have assumed reversibility of the underlying mutation process. Reversible models are much more suitable to analytic and computational treatment, and thus reversibility is a key feature of many widely-used nucleotide and amino acid mutation models (e.g., Jukes and Cantor, 1969; Kimura, 1980; Tamura and Nei, 1993; Felsenstein, 1981; Yang, 2006; Felsenstein, 2011). Moreover, Rodríguez et al. (1990) have shown that it is not even possible to make self-consistent estimates of substitution rates from pairwise sequence alignments without assuming reversibility, although some work has been done to treat this type of molecular data with irreversible models (e.g., Barry and Hartigan, 1987). Nevertheless, mutation rates are determined by complex biochemical factors (such as replication and error-correcting machinery), so there is no obvious reason to believe that reversibility must hold.

Our approach can be used to describe arbitrary fitness landscapes for the locus under consideration, including those with a fitness function that depends on the state of the entire DNA or protein sequence at the locus. Standard models of sequence evolution typically assume that all nucleotides or amino acids evolve independently of each other (Yang, 2006). This approximation excludes correlations among sites within a locus and the corresponding epistatic effects, whose importance is being increasingly emphasized (DePristo et al., 2005; Bershtein et al., 2006; Weinreich et al., 2006; Poelwijk et al., 2007).

One application of particular interest is the ability to infer an arbitrary fitness landscape from sequence data under the assumption of steady state. Indeed, Eq. (13) can be inverted to obtain the fitness function in terms of the neutral distribution and the steady-state distribution under selection (Lässig, 2007; Mustonen et al., 2008):

$$\log \left(\frac{\tilde{\pi}(\sigma)}{\tilde{\pi}_0(\sigma)} \right) = \nu \log f(\sigma) - \log Z. \quad (34)$$

Here the left-hand side depends only on genotype distributions that can, in principle, be obtained from sequence data. Since the scaling effective population size ν and normalization Z are unknown in real systems, Eq. (34) gives logarithmic fitness up to an overall scaling and shift.

The application of Eq. (34) requires an ensemble of loci that have reached evolutionary steady state. To assess this assumption, we estimate the time required to reach steady state in our substitution model. As discussed in Section 2, the monomorphic limit requires $\mu \leq 1/(LN \log N)$ for neutral evolution (Champagnat, 2006; Champagnat et al., 2006). Assuming that deleterious substitutions do not affect equilibration towards steady state (due to exponential suppression of their substitution rates), equilibration times will be dominated by neutral evolution. Eq. (1) then implies that the neutral substitution rate is equal to the mutation rate.

For sequences consisting of L nucleotides, we can model the locus genotype space as the vertices of a hypercube in $2L$ dimensions, since two bits encode a single nucleotide. A random walk on a hypercube of dimension d with standard connectivity reaches steady state on the order of $d \log d$ steps (Levin et al., 2009). However, since the nucleotide sequence space hypercube is more connected, we may take $2L \log(2L)$ as an upper bound on the required number of steps. Combining this with the minimum average time to make a single neutral substitution step, $LN \log N$, we estimate that evolutionary steady state will be reached on the order of

$$(LN \log N) \times (2L \log(2L)) \text{ generations.} \quad (35)$$

For small genomic loci ($L = \mathcal{O}(10)$ nucleotides) in microbial organisms with generation times of approximately 10^{-4} years, an effective population size $N \sim 10^6$ yields an estimated time to reach steady state of about a million years, a reasonable value on evolutionary timescales. Moreover, the presence of selection, the additional connectivity of genotype space compared to a standard hypercube, and a smaller effective population size N will further shorten this timescale.

Moreover, the genotype space may be projected onto a lower-dimensional subspace. Previous work has described models of TF binding site evolution in *S. cerevisiae* in which the distribution of binding sites has been projected onto free energies of TF-DNA binding (Berg and Lässig, 2003; Berg et al., 2004; Lässig, 2007; Mustonen et al., 2008). The steady state is expected to be reached more quickly in the one-dimensional energy space than in the high-dimensional genotype space (Mustonen et al., 2008). Mustonen et al. (2008) also find that energy distributions of binding sites for the same TF in different yeast species are remarkably similar despite significantly different divergence times, suggesting that these distributions have indeed reached evolutionary steady state.

This previous work, however, has relied purely on the diffusion approximation of the Wright–Fisher model. Such an approximation is not obviously valid in this application, since strong-selection effects are expected from binding site biophysics: single base pair mutations may be sufficient to completely inhibit TF binding (Sarai and Takeda, 1989; Lehming et al., 1990), potentially causing misregulation of an essential gene. We have demonstrated in this work that strong selection does not affect the steady state. The universality of the steady-state distribution then justifies application of Eq. (34) to genomic data such as collections of TF binding sites. Current work is in progress to apply these results to evolution of regulatory sites in yeast, exploring the biophysical origins of the underlying fitness landscapes.

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Appendix A. The scaling law in the weak-selection limit

Here we present an argument that the leading-order behavior of $\psi(r)$ is always a power law in the diffusion limit. Since $\nu = 2N\phi'(1)$, this is equivalent to showing that $\phi'(1) \neq 0$, which means that the fixation probability must be locally linear around the neutral limit $r = 1$. The fixation probability in the diffusion approximation is given by Kimura (1962):

$$\phi(r) = \frac{\int_0^{1/N} dx G(x, r)}{\int_0^1 dx G(x, r)}, \quad (\text{A.1})$$

$$G(x, r) = \exp\left(-2 \int_0^x dy \frac{M(y, r)}{V(y, r)}\right),$$

where $M(x, r)$ and $V(x, r)$ are the first two moments of the change in mutant fraction x per unit time. Define expansions of the moments:

$$\begin{aligned} M(x, r) &= M_0(x) + (r-1)M_1(x) + \mathcal{O}((r-1)^2) \\ V(x, r) &= V_0(x) + (r-1)V_1(x) + \mathcal{O}((r-1)^2). \end{aligned} \quad (\text{A.2})$$

Since evolution under pure drift ($r = 1$) is unbiased, the mean change in mutant fraction without selection is zero: $M_0(x) = 0$. Substituting these expansions into Eq. (A.1) and expanding to lowest order in $r - 1$, we obtain

$$\begin{aligned} \phi(r) &= \frac{1}{N} + 2(r-1) \left(\frac{1}{N} \int_0^1 dx \int_0^x dy \frac{M_1(y)}{V_0(y)} \right. \\ &\quad \left. - \int_0^{1/N} dx \int_0^x dy \frac{M_1(y)}{V_0(y)} \right) + \mathcal{O}((r-1)^2). \end{aligned} \quad (\text{A.3})$$

Therefore

$$\phi'(1) = 2 \left(\frac{1}{N} \int_0^1 dx \int_0^x dy \frac{M_1(y)}{V_0(y)} - \int_0^{1/N} dx \int_0^x dy \frac{M_1(y)}{V_0(y)} \right), \quad (\text{A.4})$$

where $\phi'(1) = d\phi(r)/dr|_{r=1}$. Note that $V_1(x)$ does not appear—the correction to the second moment by weak selection does not affect the fixation probability expanded to the lowest order. Thus, barring some coincidental cancelation of terms in Eq. (A.4), $\phi'(1)$ should be nonzero as long as $M_1(x)$ is nonzero.

To argue that $M_1(x) \neq 0$, we invoke an operational definition of selection strength. Experimental measurements of selection strength are often made by inferring it as the exponential growth rate of a small mutant sub-population, at least for microorganisms (Lenski and Elena, 2003), so we require that the population model show this behavior. If X is the random variable denoting the fraction of mutants in the population, its deterministic equation is

$$\frac{d}{dt} \mathbb{E}[X] = \mathbb{E}[M(X, r)], \quad (\text{A.5})$$

where $\mathbb{E}[\cdot]$ is the expected value operator. In the limit of weak selection ($r \sim 1$) and small mutant fraction ($X \ll 1$),

$$\frac{d}{dt} \mathbb{E}[X] \approx (r-1) \mathbb{E}[M_1(X)] \propto (r-1) \mathbb{E}[X], \quad (\text{A.6})$$

assuming that $M_1(x)$ is linear in x to the lowest order. This yields exponential growth at a rate proportional to the selection strength $s = r - 1$. Therefore $M_1(x)$ should be nonzero and hence $\phi'(1)$ is nonzero, establishing the power-law behavior of $\psi(r)$ in the limit of weak selection.

Eq. (A.4) suggests an interpretation of ν . Under the appropriate rescaling of time units, the pure drift $V_0(x)$ is proportional to $1/N$ and $M_1(x)$ is independent of N . For example, this is true in the Wright–Fisher model with generations as the time unit, and it also holds in the Moran model with the single birth/death time scaled by a factor of N . Then Eq. (A.4) implies that $\phi'(1) \sim \mathcal{O}(N^0)$, and therefore $\nu \sim \mathcal{O}(N)$. This observation can be generalized to a broader class of models in which $V_0(x)$ is proportional to $1/N_e$, where N_e is the variance effective population size (Cannings, 1974; Ewens, 2004).

Appendix B. Exact Wright–Fisher fixation probability from discrete Markov chain

Studying discrete Markov chain properties of the Wright–Fisher model is not new (Ewens, 2004). However, previous work has typically focused on explicit results using spectral theory, with particular emphasis placed on neutral evolution. In contrast, we will obtain an implicit result suitable for numerical application. These results will allow investigation of the dynamics of the model under large selection effects that are beyond the scope of diffusion theory.

We can represent the transition probabilities $\Pi(n'|n)$ from Eq. (28) as elements of an $(N+1) \times (N+1)$ matrix \mathbf{P} . We will adopt the convention in which the final state n' is the row index and the initial state n is the column index. Transition probabilities between different states at different time steps are given by the matrix elements of powers of \mathbf{P} . That is, the probability of transitioning

from n to n' in m generations is given by $(\mathbf{P}^m)_{n',n}$. Therefore the probability of fixation by generation m from initial state n is given by $(\mathbf{P}^m)_{N,n}$, and the probability of fixing a single mutant in the infinite time limit is given by

$$\lim_{m \rightarrow \infty} (\mathbf{P}^m)_{N,1} = \phi(r). \tag{B.1}$$

This limit can be conveniently expressed by permuting the states to group the transient states ($n = 1, \dots, N-1$) together and the absorbing states ($n = 0, N$) together. Define elements of the $(N-1) \times (N-1)$ submatrix $\mathbf{A}_{ij} = \Pi(i|j)$ for $i, j = 1, \dots, N-1$; this matrix describes transitions between transient states only. Next, define elements of the $2 \times (N-1)$ submatrix $\mathbf{B}_{\alpha i} = \Pi(\alpha|i)$ for $\alpha = 0, N$ and $i = 1, \dots, N-1$; this matrix describes single-generation transitions from transient states to absorbing states. Now we permute the indices to put \mathbf{P} in the canonical form (Kemeny and Snell, 1960):

$$\mathbf{P} = \begin{bmatrix} \mathbf{A} & \mathbf{0} \\ \mathbf{B} & \mathbf{1}_2 \end{bmatrix}, \tag{B.2}$$

where $\mathbf{0}$ is the $(N-1) \times 2$ zero matrix and $\mathbf{1}_k$ is a $k \times k$ identity matrix. We can now easily compute the infinite time limit:

$$\begin{aligned} \lim_{m \rightarrow \infty} \mathbf{P}^m &= \lim_{m \rightarrow \infty} \begin{bmatrix} \mathbf{A} & \mathbf{0} \\ \mathbf{B} & \mathbf{1}_2 \end{bmatrix}^m \\ &= \lim_{m \rightarrow \infty} \begin{bmatrix} \mathbf{A}^m & \mathbf{0} \\ \mathbf{B}(\mathbf{1}_{N-1} + \mathbf{A} + \dots + \mathbf{A}^{m-1}) & \mathbf{1}_2 \end{bmatrix} \\ &= \begin{bmatrix} \mathbf{0} & \mathbf{0} \\ \mathbf{B}(\mathbf{1}_{N-1} - \mathbf{A})^{-1} & \mathbf{1}_2 \end{bmatrix}, \end{aligned} \tag{B.3}$$

since $\mathbf{A}^m \rightarrow \mathbf{0}$ as $m \rightarrow \infty$ and

$$(\mathbf{1}_{N-1} - \mathbf{A})^{-1} = \sum_{j=0}^{\infty} \mathbf{A}^j. \tag{B.4}$$

The fixation probability of a single mutant is given by the element of the matrix $\mathbf{B}(\mathbf{1}_{N-1} - \mathbf{A})^{-1}$ in the second row (corresponding to the final state $n = N$) and the first column (corresponding to the initial state $n = 1$):

$$\phi(r) = (\mathbf{B}(\mathbf{1}_{N-1} - \mathbf{A})^{-1})_{2,1}. \tag{B.5}$$

Alternatively, this expression can be expanded in powers of \mathbf{A} :

$$\phi(r) = \mathbf{B}_{2,1} + \sum_{i=1}^{N-1} \mathbf{B}_{2,i} \mathbf{A}_{i,1} + \sum_{i,j=1}^{N-1} \mathbf{B}_{2,i} \mathbf{A}_{i,j} \mathbf{A}_{j,1} + \dots \tag{B.6}$$

Each term in the expansion represents the probability of fixing in a certain finite number of generations: the first term is the probability of fixing in exactly one generation, the second term is the probability of fixing in exactly two generations, etc.

For small population sizes N , Eq. (B.5) can be evaluated explicitly:

N	$\phi(r)$	(B.7)
2	$\frac{r^2}{1+r^2}$	
3	$\frac{r^3(8r^3+48r^2+6r+1)}{8r^6+48r^5+6r^4+65r^3+6r^2+48r+8}$	
⋮	⋮	
N	$\frac{r^N a_N(r)}{b_N(r)}$	

Empirically we observe that $a_N(r)$ is a degree $N(N-2)$ polynomial and $b_N(r)$ is a degree $N(N-1)$ polynomial. Note that $b_N(r)$ appears to be palindromic: $b_N(r) = r^{N(N-1)} b_N(1/r)$. Unfortunately, the

polynomials in these exact expressions grow increasingly intractable with N , making a numerical computation of $\phi(r)$ the only option. Eq. (B.5) can be rewritten as

$$(\mathbf{1}_{N-1} - \mathbf{A})^T \mathbf{u}^T = \mathbf{B}^T, \tag{B.8}$$

where \mathbf{u} is the $2 \times (N-1)$ matrix of fixation and extinction probabilities from all initial mutant fractions. The resulting system of linear equations can be efficiently solved to find \mathbf{u} for the arbitrary fitness ratio r . The solution agrees extremely well with explicit simulations (Fig. 2).

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