*The Annals of Applied Probability*
2010, Vol. 20, No. 5, 1663–1696
DOI: 10.1214/09-AAP668


# A TIME-DEPENDENT POISSON RANDOM FIELD MODEL FOR POLYMORPHISM WITHIN AND BETWEEN TWO RELATED BIOLOGICAL SPECIES

BY AMEI AMEI[1] AND STANLEY SAWYER[2]

*University of Nevada, Las Vegas and Washington University in St. Louis*

We derive a Poisson random field model for population site polymorphisms differences within and between two species that share a relatively recent common ancestor. The model can be either equilibrium or time inhomogeneous. We first consider a random field of Markov chains that describes the fate of a set of individual mutations. This field is approximated by a Poisson random field from which we can make inferences about the amounts of mutation and selection that have occurred in the history of observed aligned DNA sequences.

**1. Introduction.** A traditional goal of population genetics is to understand the relationship between Darwinian selection and evolution. A particular problem is to estimate the distribution of the fitness of genes that become fixed in a natural population as a way of determining whether evolution is going "uphill" or "downhill" in that population (both are possible; [7, 18, 19, 29, 30]).

One approach is to use the numbers of site polymorphisms between and within a pair of closely related species at a genetic locus (McDonald and Kreitman [31]; see also [4, 9, 12, 15, 18, 19, 24, 25, 42]). Sawyer and Hartl [38] developed a Poisson random field (PRF) model for these counts that can be used to estimate the amounts of selection that are occurring at individual loci ([2, 6, 20, 30, 39]; see also [48]). Multilocus extensions of the model can be used for large numbers of different loci [3, 5, 7, 40, 41, 47]. The models assume a high level of recombination between nucleotides and are well suited for the analysis of polymorphism and divergence at multiple loci distributed across a genome [7]. Other authors have extended the basic PRF model to more general biological settings [8, 21, 41, 45–47] and have used numerical simulations to study alternative models and the effects of deviations from model assumptions [1, 5, 6, 49]. Williamson et al. [47] used a time-dependent PRF model to estimate the time since a hypothetical sudden change in human population size. The purpose of this paper is to provide a rigorous derivation of a time-inhomogeneous PRF model by approximating discrete time Markov

Received March 2009; revised October 2009.
[1]Supported by Washington University Dissertation Fellowship and NSF Grant DMS-01-07420.
[2]Supported in part by NSF Grant DMS-01-07420.
*AMS 2000 subject classifications.* Primary 60J70, 92D10, 92D20; secondary 92D15, 60F99.
*Key words and phrases.* Poisson random field, DNA sequences, diffusion approximation, population genetics.

chains by diffusion processes and, so that the model can be applied to data, to derive the corresponding sampling formulas for aligned DNA sequences.

First, a brief introduction to genetics and population genetics. The genetic material or DNA of most living creatures is located in one or more chromosomes. A chromosome can be thought of as a long string of letters from the alphabet A, C, G, T, where each letter corresponds to a specific nucleotide. Most higher plants and animals are *diploid*, which means that their DNA is arranged in pairs of chromosomes. Each of these paired chromosomes typically has double-stranded DNA, amounting to four DNA strands for each chromosome pair. The nucleotides on the two DNA strands of each chromosome are mirrored as a way of providing redundant information. Creatures with nonpaired chromosomes (such as bacteria and viruses) are called *haploid*. A *gene* or *genetic locus* is a relatively-short segment of a chromosome that affects a particular trait or set of traits. *Random mating* for a population of $M$ diploid individuals, along with Mendel's laws for the offspring of two diploid individuals, can be statistically approximated by random sampling with replacement of $N = 2M$ haploid individuals to form the next generation. That is, random mating of $M$ diploid individuals is approximated by random mating of their $N = 2M$ haploid genomes.

Proteins are built from peptides, which are strings of amino acids. Peptide strings are created by reading a sequence of *codons*, which are consecutive triples of nucleotides, from one or more subsegments of a gene. One amino acid is added to the peptide for each codon of DNA. (The amino acids and codons have no chemical similarity to one another.) There are around twenty different amino acids as opposed to $4^3 = 64$ possible codons on one DNA strand, so that the mapping of codons to amino acids cannot be one to one. About half of amino acids are encoded by four different codons that vary in the third codon position. Most of the remaining amino acids are encoded by two different codons. A mutation at one of the three sites in a codon position is called a *silent* mutation if the resulting new codon encodes the same amino acid. A site mutation that results in a different amino acid is called a *replacement* mutation. Most mutations at first and second position sites in codons are replacement, while mutations at the third position can be either silent or replacement. The majority of replacement mutations severely damage the gene product and may be lethal to the host, but a substantial number have mild to moderate effects and can be advantageous.

A DNA site is *polymorphic* in a population if there is more than one nucleotide at that site. If there is only one nucleotide at that site in a population, the site is *monomorphic* and the population is *fixed* at that nucleotide.

The *fitness* of a gene is defined as the expected relative number of surviving offspring (genes) in the next generation that are descendants of that gene, assuming constant lifetimes for individuals. Suppose that we have $N$ haploid individuals that are of one of two types $A$ or $a$ at a particular site or gene, where $a$ is the mutant type and $A$ is the pre-existing or "wild type." Assume that $a$ has the fitness coefficient $\omega_N(a) = 1 + \sigma_N$ relative to the pre-existing $A$ with $\omega_N(A) = 1$. If $\sigma_N$ is scaled as

$\sigma_N \sim \gamma/N$ for a large haploid population size $N$, then $\gamma$ is the *selection coefficient* of the mutant. The mutation is favorable if $\gamma > 0$, detrimental if $\gamma < 0$ and neutral if $\gamma = 0$.

**2. Main results and discussion.** We first derive two results that lead to Poisson random fields (see below) for the distribution of site polymorphisms in a limiting infinitely large random-mating population.

The population results cannot be directly applied to data, both because we cannot sample the entire population and also because the results imply that there are an infinitely large number of site polymorphisms in each gene, most of which have very small population frequencies (see below). However, we can use the population results to derive the distribution of sample statistics for aligned DNA sequences arising from two related but not too distantly related species. The sample statistics will turn out to be independent Poisson with means depending on population parameters for mutation and selection rates. These, in turn, lead to likelihood methods for the estimation of mutation, selection and divergence time parameters.

The proofs of the population results are deferred to Sections 4–8. Mathematically, the key steps in the proofs of both of the main population results depend on Trotter's theorem for the dual process of a diffusion process (Section 6). The sampling formulas are discussed in Sections 2.2 and 2.3 with the details in Section 3.

2.1. *Population formulas.* The first result describes the limiting distribution of the population proportions of mutant nucleotides at polymorphic sites at a single genetic locus in a large population. Under the assumptions of Section 1, this distribution is a Poisson random field (PRF) [28] on $(0, 1)$, where each point is a population frequency ratio at a different site (see Theorem 2.1 below). The second result (Theorem 2.2) describes the expected number of polymorphic sites that have become fixed in the population at the mutant nucleotide since time 0. This random variable is also Poisson and is independent of the PRF derived in Theorem 2.1.

Silent and replacement polymorphic sites are modeled separately and provide different information (see below).

We begin with a model for the frequency of a mutant nucleotide at a single site in a finite population of haploid size $N$. Assume that mutation is sufficiently rare at the site level that repeat mutations at the same site can be neglected. We use Moran's second model [32] for which, at each step, an individual is randomly chosen from the population with equal probabilities and replaced by a copy of a second individual (perhaps the same as the first) chosen with probability proportional to the fitness. Then the number $X_k$ of individuals in the population that have the mutant nucleotide at a particular site is a Markov chain on $S_N = \{0, 1, 2, \ldots, N\}$ with

transition function

$$p_N(i,i+1) = \frac{(1+\sigma_N)i/N(1-i/N)}{1+\sigma_N i/N},$$
$$p_N(i,i-1) = \frac{i/N(1-i/N)}{1+\sigma_N i/N}, \tag{2.1}$$
$$p_N(i,i) = 1 - p_N(i,i-1) - p_N(i,i+1)$$

for $0 < i < N$ with $p_N(0,0) = p_N(N,N) = 1$, where $1+\sigma_N$ is the fitness of the mutant nucleotide $a$ with respect to $A$. A generation corresponds to $N$ time steps. The states 0 and $N$ are traps corresponding to the loss of all mutant ($a$-type) or original ($A$-type) nucleotides, respectively. We write $X_k^N$ instead of $X_k$ when we want to emphasize the size of the state space $N$.

We assume that, at time 0, there are $M_0$ different sites that are population polymorphic with both mutant and nonmutant nucleotides. There are mutations at $M_r$ new sites at times $r = 1, 2, \ldots$. The $M_0$ and $M_r$ are independent Poisson with $E(M_r) = \mu_N$ and typically $E(M_r) \ll E(M_0)$ $(r \geq 1)$. All mutations are assumed to occur at new sites. Given $M_0$ and $M_r$, the numbers of mutant nucleotides at these sites at times $k \geq 0$ in the future (for the initial polymorphic sites) and at times $k \geq r$ (for the new mutations) are given by independent Markov chains $X_{1,a,k}$ $(1 \leq a \leq M_0)$ and $X_{2,b,r,k}$ $(1 \leq b \leq M_r)$, each of which has the transition function (2.1). By assumption, $X_{2,b,r,r} = 1$ for $r \geq 1$ and $0 \leq X_{1,a,k}, X_{2,b,r,k} \leq N$.

The number of sites at which there are $j$ mutant nucleotides at time $k$ $(1 \leq j \leq N, k \geq 0)$ is

$$N_k(j) = \#\{a : X_{1,a,k} = j\} + \#\{(b,r) : X_{2,b,r,k} = j\}, \tag{2.2}$$

where # means cardinality, $1 \leq a \leq M_0$, $1 \leq b \leq M_r$ and $1 \leq r \leq k$. Thus

$$\sum_{j=1}^{N} f\left(\frac{j}{N}\right) N_k(j) = \sum_{a=1}^{M_0} f\left(\frac{X_{1,a,k}}{N}\right) + \sum_{r=1}^{k} \sum_{b=1}^{M_r} f\left(\frac{X_{2,b,r,k}}{N}\right) \tag{2.3}$$

for functions $f(x)$ on $[0,1]$. The expected value of $N_k(j)$ in (2.2) is

$$E(N_k(j)) = \sum_{i=1}^{N-1} \omega_i^N p_N^k(i,j) + \mu_N \sum_{r=1}^{k} p_N^{k-r}(1,j), \tag{2.4}$$

where $\omega_i^N = E(N_0(i))$ and $p_N^k(i,j)$ is the $k$th matrix power of $p_N(i,j)$. The random variables $N_k(j)$ are somewhat unintuitive in that each variable depends on information from many different polymorphic sites, rather than one, but turn out to have good statistical properties.

We assume that the random variables $M_r$ and $N_0(i)$ are independent and Poisson distributed. Then, by a slight extension of what is called Bartlett's theorem [28], this implies that, for each fixed $k > 0$, the counts $N_k(i)$ $(i = 1, 2, \ldots)$

are independent Poisson and thus define a Poisson random field (PRF) on $I_N = \{1/N, 2/N, \ldots, 1\}$. If the mean measures defined by (2.4) for $y = j/N$ and $k = k_N$ converge weakly as $N \to \infty$ on compact subsets of $(0, 1]$, then $N_{k_N}(i)$ for $x = i/N$ converge weakly in the same sense to a PRF on $(0, 1]$. The points of the limiting PRF will be a countably-infinite set of diffusion processes on $(0, 1]$ each fixed at a particular time $t$.

Each of the Markov chains $X_{1,a,k}$, $X_{2,b,r,k}$ can be approximated for large $N$ by a diffusion process $X_t$ on $(0, 1)$ with time scaled as $t \sim k/N^2$ (Section 4). The diffusion process $X_t$ has traps at the endpoints 0, 1, similarly to the Markov chains. Specifically, $X_t$ is the diffusion process on $(0, 1)$ generated by the differential operator

$$L_x = x(1-x)\frac{d^2}{dx^2} + \gamma x(1-x)\frac{d}{dx}, \tag{2.5}$$

where $\gamma = \lim_{N\to\infty} N\sigma_N$ for $\sigma_N$ in (2.1). We write $L_x$ in the Feller form

$$L_x = \frac{d}{m(dx)}\frac{d}{s(dx)}, \tag{2.6}$$

where $m(dx) = m'(x)\,dx$ and $s(dx) = s'(x)\,dx$ for

$$s(x) = \frac{1 - e^{-\gamma x}}{\gamma} \quad \text{and} \quad m(dx) = \frac{e^{\gamma x}}{x(1-x)}\,dx. \tag{2.7}$$

The function $s(x)$ and measure $m(dx)$ are called the *scale* and *speed measure* of $X_t$, respectively. The diffusion process $X_t$ has a smooth symmetric transition density $p(t, x, y) = p(t, y, x)$ with respect to $m(dx)$ such that

$$\begin{aligned} E_x(f(X_t)) = Q_t f(x) &= E\big(f(X_t) \mid X_0 = x\big) \\ &= \int_0^1 p(t, x, y) f(y) m(dy) \end{aligned} \tag{2.8}$$

for $f \in C[0, 1]$ with $f(0) = f(1) = 0$.

Fix $t > 0$ and assume that $\sigma_N, \mu_N, k_N$ are scaled so that

$$N\sigma_N \to \gamma, \qquad N\mu_N \to \theta \quad \text{and} \quad k_N/N^2 \to t \qquad \text{as } N \to \infty. \tag{2.9}$$

As mentioned earlier, each step in the Moran model (2.1) puts one individual at risk, so that each individual is put at risk on the average every $N$ time steps. Thus $N\sigma_N \to \gamma$ and $k_N \sim tN^2$ mean that selection at intensity $\gamma$ is applied to each individual in the diffusion time scale $t$.

The relation $N\mu_N \to \theta$ implies that the rate of arrival of new mutations in the diffusion time scale is $(N^2)\mu_N \sim N\theta \to \infty$. However, the new mutant Markov chains begin at $X_{2,b,r,r} = 1$ which corresponds to states $1/N \to 0$ for the approximation diffusion. The limiting processes $X_t$ have a trap at $x = 0$, so that one might expect that only $O(1/N)$ of the new mutant Markov chains survive the first few

generations. This would suggest that only of order $O((1/N)(N^2\mu_N)) = O(\theta)$ of the new mutants would survive, so that the rate $N\mu_N \to \theta$ in (2.9) is not paradoxical.

The equilibrium distribution of the limiting PRF of diffusion processes is

$$\mu_{\theta,\gamma}(dx) = \theta\frac{s(1)-s(x)}{s(1)-s(0)}m(dx) = \frac{s(1)-s(x)}{x(1-x)}\frac{\theta e^{\gamma x}}{s(1)}\,dx \tag{2.10}$$

([38, 48]; see also Corollary 2.1 below). In particular, the equilibrium mean density has a $1/x$ singularity at $x = 0$ in a large population but is bounded at $x = 1$. This means that, in the limit, there are an infinite number of sites that have polymorphic mutant alleles with population proportions $p_i > 0$. By properties of Poisson random fields,

$$E\Bigg(\sum_i p_i\Bigg) = \int_0^1 x\mu_{\theta,\gamma}(dx) = \frac{\theta}{s(1)}\int_0^1 \frac{s(1)-s(x)}{1-x}e^{\gamma x}\,dx < \infty.$$

Thus the vast majority of the $p_i$ are too small for the mutant nucleotide to show up in finite samples. However, this does show that we need to allow for initial limiting PRF mean measures that are not normalizable at zero.

Specifically, for the initial distribution of polymorphic sites, we assume that there exists a Borel measure $\nu(dx)$ on $(0, 1)$ such that $\int_0^1 x\nu(dx) < \infty$ and

$$\lim_{N\to\infty}\sum_{j=1}^{N-1} g\Big(\frac{j}{N}\Big)\frac{j}{N}\omega_j^N = \int_0^1 g(y)y\nu(dy) \tag{2.11}$$

for all $g \in C[0, 1]$ for $\omega_j^N = E(N_0(j))$ in (2.4). Since $s'(0) = 1$ in (2.7), this is equivalent to

$$\lim_{N\to\infty}\sum_{j=1}^{N-1} g\Big(\frac{j}{N}\Big)s\Big(\frac{j}{N}\Big)\omega_j^N = \int_0^1 g(y)s(y)\nu(dy). \tag{2.12}$$

Our first result describes the limiting PRF on $(0, 1)$ which in turn describes the population distribution of polymorphic mutant sites. The proof is deferred to Section 7.

THEOREM 2.1. *Assume that $\sigma_N, \theta_N, k_N$ satisfies (2.9) and that $N_k(i)$ defined in (2.2) satisfies (2.11) for $\omega_j^N = E(N_0(j))$. Then for $Q_t f(x)$ in (2.8)*

$$\begin{aligned}\lim_{N\to\infty} E&\Bigg(\sum_{i=1}^{N-1} f\Big(\frac{i}{N}\Big)N_{k_N}(i)\Bigg)\\ &= \int_0^1 Q_t f(x)\nu(dx) + \theta\int_0^1 \frac{s(1)-s(x)}{s(1)-s(0)}\big(f(x) - Q_t f(x)\big)m(dx)\end{aligned} \tag{2.13}$$

*for any $f \in C[0, 1]$ with $f(0) = f(1) = 0$ such that $g(x) = f(x)/x$ for $x > 0$ extends to a continuous function on $[0, 1]$.*

The limiting PRF mean density in (2.13) is $g(t,\theta,\gamma,y)m(dy)$ where

$$g(t,\theta,\gamma,y) = \int_0^1 p(t,x,y)\nu(dx) + \theta\frac{s(1)-s(y)}{s(1)-s(0)} - \theta\int_0^1 \frac{s(1)-s(x)}{s(1)-s(0)}p(t,x,y)m(dx). \tag{2.14}$$

The first terms on the right in (2.13) and (2.14) are transient terms that are due to the initial (or "legacy") polymorphisms at time $t=0$. The remaining terms are due to new mutations that were introduced at times $t>0$.

If $\nu(dx)$ in (2.11) and (2.14) is the measure $\mu_{\theta,\gamma}(dx)$ in (2.10), then the right-hand side of (2.13) is identically $\int_0^1 f(x)\mu_{\theta,\gamma}(dx)$, so that $\mu_{\theta,\gamma}(dx)$ is an equilibrium measure. The following corollary shows that $\mu_{\theta,\gamma}(dx)$ is an asymptotic measure as well (Section 7.3).

COROLLARY 2.1. *Let $G_t(f)$ be the right-hand side of (2.13) for any function $f(x)$ satisfying the conditions of Theorem 2.1. Then*

$$\lim_{t\to\infty} G_t(f) = \theta\int_0^1 \frac{s(1)-s(x)}{s(1)-s(0)}f(x)m(dx) = \int_0^1 f(x)\mu_{\theta,\gamma}(dx). \tag{2.15}$$

Theorem 2.1 implicitly assumes that all new replacement mutants at a given genetic locus have the same selection coefficient $\gamma$. The model can be extended to allow within-locus random distributions of selective effects of new replacement mutants, for example, Gaussian [3, 40, 41], exponential [1] or gamma [5].

The second main population result describes the limiting expected number of mutant sites that have become fixed in the population at the mutant nucleotide by time $t>0$ (i.e., such that the nonmutant or "wild type" nucleotide has been lost). The limit can be expressed in terms of the hitting times $T_a = \min\{t : X_t = a\}$ for the limiting diffusion process $X_t$ and for its dual process $\widetilde{X}_t$, but can also be expressed in terms of the transition density of $X_t$. The proof is deferred to Section 8.

THEOREM 2.2. *Under the conditions of Theorem 2.1, the asymptotic expected number of mutant sites that have become fixed in the population at the mutant nucleotide by time $t$ is*

$$\begin{aligned}&\lim_{N\to\infty} E(N_{k_N}(N))\\ &= \lim_{N\to\infty}\left(\sum_{i=1}^{N-1}\omega_i^N p_N^{k_N}(i,N) + \mu_N\sum_{r=1}^{k_N} p_N^{k_N-r}(1,N)\right)\\ &= \int_0^1 P_x(T_1\le t)\nu(dx) + \frac{\theta}{s(1)}\int_0^t \widetilde{P}_0(T_1\le u)\,du,\end{aligned} \tag{2.16}$$

*where $\widetilde{P}$ in (2.16) refers to the diffusion process $X_t$ conditioned on $T_1 < T_0$, which has an entrance boundary at $x=0$ (Section 6).*

The right-hand side of (2.16) can also be written (Section 8.2)

$$\frac{1}{s(1)}\Bigg(\int_0^1 s(x)v(dx) - \int_0^1\int_0^1 p(t,x,y)s(y)m(dy)v(dx) + \theta t - \theta\int_0^t\int_0^1 q(u,0+,y)s(y)^2 m(dy)\,du\Bigg), \tag{2.17}$$

where $q(t,x,y)$ is the transition density of the dual-process diffusion (Section 6).

2.2. *McDonald–Kreitman tables*. Theorems 2.1 and 2.2 give the distribution of polymorphic sites and fixations of mutant nucleotides in a large population but are not directly applicable to samples from a finite number of individuals.

Suppose that we have random samples from two species that are related but not extremely distantly related [31], for example, the *Drosophila* species *melanogaster* and *simulans* [7, 40, 41] or humans and chimpanzees [33]. Specifically, assume that we have a DNA alignment of $m+n$ sequenced genes from the same genetic locus in two species, of which $m$ are randomly chosen from one species and $n$ from the second species. The sample can be viewed as an $(m+n)\times L$ matrix, where $L$ is the number of nucleotide sites in the alignment and the matrix elements are chosen from the four letters A, C, G, T, which stand for the four nucleotides in DNA. If mutation is sufficiently rare so that it never occurs more than once at the same site, then there will be at most two nucleotides at any one site in the population and hence at most two letters in any one column of the matrix.

Given the joint alignment, we say that a site is a *fixed difference* if it is monomorphic in the sample within each species but polymorphic in the two species (i.e., monomorphic within each species, but at different nucleotides). The site is *polymorphic* if it is polymorphic within either or both of the two species.

Let $K_s$, $K_r$ be the number of silent and replacement fixed differences in the joint alignment, and $V_s$, $V_r$ the number of (within-species) polymorphisms at silent and replacement sites. These counts can be arranged in the $2\times 2$ contingency table

$$\begin{array}{c} \\ S \\ R \end{array}\begin{array}{c} \begin{array}{cc} D & P \end{array} \\ \begin{bmatrix} K_s & V_s \\ K_r & V_r \end{bmatrix} \end{array}, \tag{2.18}$$

where the column headings $D$, $P$ refer to fixed differences and polymorphisms and the row headings $S$, $R$ to silent and replacement mutations, respectively. The table (2.18) is called a McDonald–Kreitman table [31] and also a DPRS table due to the row and column headings in (2.18).

A statistically significant excess in the lower-left corner ($K_r$) of the table suggests that one or both species has seen significant positive or directional selection at that gene since the two species diverged. In contrast, a statistically significant deficit of $K_r$ suggests that, instead, most new replacement mutations have been subject to strong negative selection. If the sites are assumed independent, one can

apply traditional $2 \times 2$ contingency table tests to infer an excess or deficit of replacement fixed differences [12, 31].

We say that a polymorphic site in the joint alignment is a *legacy polymorphism* if it is the descendent of a site that was polymorphic in the common ancestral species at the time of divergence. In contrast, a *new polymorphism* is a polymorphic site that was caused by a mutation since the time of divergence in one of the two daughter species.

An important difference between the two types of polymorphisms is that legacy polymorphisms can lead to shared polymorphisms, which are sites that are polymorphic in both samples. In contrast, if mutations are sufficiently rare so that they never occur more than once at the same site, then new polymorphisms can cause sample polymorphisms in only one daughter species. Another difference is that legacy polymorphisms begin at mutant population frequencies strictly between 0 and 1, while new polymorphisms begin at mutant population frequency 0 (or $1/N$ for the Markov chain). Thus legacy polymorphisms are more likely to have multiple copies of both the mutant and original nucleotides for relatively recent divergence times.

An alternative to the $2 \times 2$ table in (2.18), that may have more accuracy in estimating parameters for recently diverged populations, is

$$\begin{array}{c} \\ S \\ R \end{array} \begin{array}{c} \begin{array}{ccc} D & O & H \end{array} \\ \begin{bmatrix} K_s & O_s & H_s \\ K_r & O_r & H_r \end{bmatrix} \end{array}, \tag{2.19}$$

where $O_s$, $O_r$ are numbers of sites in the joint sample that are polymorphic in only one sample and $H_s$, $H_r$ the numbers of sites that are polymorphic in both samples. Thus $V_s = O_s + H_s$ and $V_r = O_r + H_r$. A disadvantage of the DOHRS table (2.19) is that rare events in which multiple mutations occur at the same site may lead to a pair of new polymorphisms being misclassified as a legacy polymorphism. On the other hand, the more usual practice of counting sites that are polymorphic in both samples as two polymorphic sites may misclassify a single legacy polymorphism. (See [43] for other examples of higher-dimensional McDonald–Kreitman tables.)

2.3. *The distribution of sampling statistics.* For simplicity, we assume that the effective population sizes $N_e$, the mutation rates $\theta$ and the selection coefficients $\gamma$ are the same in the two daughter populations. It then follows from Theorem 3.1 in Section 3.3 that, under the assumptions of Theorem 2.1, the counts $K_s$, $V_s$, $K_r$, $V_r$ in (2.18) and $K_s$, $O_s$, $H_s$, $K_r$, $O_r$, $H_r$ in (2.19) are independent Poisson with means depending on $\beta_r = (t, \theta_r, \gamma)$ for replacement sites and $\beta_s = (t, \theta_s)$ at silent sites. If the effective population sizes for the two daughter species are different, the resulting formulas in Section 3 are easy to modify.

Let $Z_a$ $(1 \le a \le 4)$ be the counts in (for example) (2.18) in some order. Let $m_a = m_a(\beta) = E(Z_a)$ be the corresponding formulas in Section 3.3 for

$\beta = (\beta_r, \beta_s)$. Then the likelihood of (2.18) can be written

$$L(\beta, Z) = \prod_{a=1}^{4} \left( \exp(-m_a(\beta)) \frac{1}{Z_a!} m_a(\beta)^{Z_a} \right) \tag{2.20}$$

or

$$\log L(\beta, Z) = C(Z) - \sum_{a=1}^{4} m_a(\beta) + \sum_{a=1}^{4} Z_a \log m_a(\beta). \tag{2.21}$$

While in principle the parameters $\beta_r, \beta_s$ can be estimated by maximizing the log likelihood (2.21), normally only a few loci in a few species are sufficiently polymorphic to allow $\beta$ to be estimated from data from a single locus. More typically, likelihoods of the form (2.20) are combined over many loci to form a single likelihood. The resulting expression can either be maximized to estimate the model parameters [5, 25, 47] or else analyzed by Bayesian methods [1, 3, 7, 40, 41].

Although the mutation rate per site per generation is normally assumed to be the same for sites in the same genetic locus, which would suggest $\theta_r > 2\theta_s$, most replacement mutations are either lethal to their host or else are severely deleterious. This means that most replacement mutations are immediately lost on the diffusion time scale. This can show up in the model as a censored replacement mutation rate with $\theta_r/\theta_s < 1$ and sometimes $\theta_r/\theta_s \ll 1$. In particular, there is no simple relation between $\theta_s$ and $\theta_r$ in (2.20).

Simulations have shown that methods based on (2.20) for multilocus data are relatively robust to violations of basic model assumptions, such as lack of local independence of polymorphic sites [1, 5, 6, 49].

2.4. *Final comments.* These results generalize sampling formulas in Sawyer and Hartl [38] who made the approximation that the two species populations are individually at equilibrium. Numerical simulations have shown that these lead to biased estimates of the population divergence time [1], particularly when the divergence time is small, but that estimates of the mutation and selection parameters are relatively unbiased. Nonequilibrium results can also be applied to situations where a population has been subject in the past to an abrupt change in selection parameters or population size (see, e.g., [47]).

The sampling formulas in Section 3 for the Poisson means in (2.18) and (2.19) are more complex than the equilibrium results [38], but likelihoods based on these means can be analyzed numerically as in the time homogeneous case. Terms involving diffusion transition densities (Section 3) can be estimated by the Crank–Nicholson method [34, 47]. As in [41], Gauss–Legendre quadrature can be used for integrals over a finite range. Generalizations for random distributions of selection coefficients within loci have been handled by Gauss–Hermite quadrature for within-locus normal variation [3, 41] and Gauss–Laguerre quadrature for within-locus exponential variation [1]. The behavior of the models described in this paper on simulated data, and applications to biological DNA sequence data, will be discussed in future publications.

**3. Sampling formulas for aligned DNA sequences.** Since the two species in Section 2.2 are assumed to be relatively closely related on an evolutionary time scale, it is natural to assume that they have the same mutation and selection rates at each genetic locus and also the same average generation times. For simplicity, we also assume that the effective population sizes of the two daughter species are the same. This means that the scaled mutation rates $\theta_s, \theta_r$ and selection coefficients $\gamma$ are the same at each genetic locus, and the scaled time $t$ since the divergence of the two species from their most recent common ancestral species is also the same [see (2.9)]. As mentioned earlier, the formulas below can be easily modified if these assumptions are violated.

As in Section 2.2, assume that we have a DNA alignment of $m+n$ sequenced genes from the same genetic locus in two species, of which $m$ are randomly chosen from one species and $n$ from the second species. Each of the sample statistics $K_s, V_s, K_r, V_r$ in (2.18) and $K_s, O_s, H_s, K_r, O_r, H_r$ in (2.19) are counts of sites of two types, legacy and new polymorphisms, that lead to different formulas for the expected counts.

3.1. *Legacy polymorphisms.* It follows from Section 7.1 that the distribution of polymorphic site frequencies $p_i$ in a single daughter population at time $t>0$ that are derived from ancestral legacy polymorphic sites is a Poisson random field with mean density

$$f_L(\beta, y) = \int_0^1 p(t, x, y)\nu(dx), \tag{3.1}$$

where $\beta = \beta_s = (t, \theta_s, 0)$ for silent mutations and $\beta = \beta_r = (t, \theta_r, \gamma)$ for replacement mutations. It follows similarly from Section 8 that the number of population mutant fixations in a single daughter population by time $t>0$ that are derived from legacy polymorphic sites is Poisson with mean

$$G_L(\beta) = \int_0^1 P_x(T_1 \le t)\nu(dx).$$

Assume that a particular legacy polymorphic site has population frequency $x$ $(0<x<1)$ for the mutant nucleotide at time $t=0$. For a sample of size $n$ from a daughter population at time $t>0$, let $I(x,n)$ be the probability that this site is monomorphic in the sample at the wild-type (nonmutant) nucleotide, $J(x,n)$ that it is polymorphic in the sample and $K(x,n)$ the probability that it is monomorphic at the mutant nucleotide. Then we have the following lemma.

LEMMA 3.1. *For $I(x,n)$, $J(x,n)$ and $K(x,n)$ defined above,*

$$\begin{aligned} I(x,n) &= P_x(T_0 \le t) + \int_0^1 p(t,x,y)(1-y)^n m(dy), \\ J(x,n) &= \int_0^1 p(t,x,y)\bigl(1-y^n-(1-y)^n\bigr)m(dy), \end{aligned} \tag{3.2}$$

$$K(x,n) = P_x(T_1 \le t) + \int_0^1 p(t,x,y)y^n m(dy)$$

*for* $T_a = \min\{a : X_t = a\}$ *as in Section 2.*

The first terms in the formulas for $I$ and $K$ are due to population fixations. The remaining terms are due to sampling from polymorphic sites. Note that $I + J + K = 1$ in Lemma 3.1 and that $I$, $J$ and $K$ depend implicitly on $\beta = (t, \theta, \gamma)$ through both $p(t,x,y)$ and $m(dy)$.

PROOF OF LEMMA 3.1. For a legacy polymorphism of initial mutant frequency $x$, condition on its population frequency $y$ at time $t > 0$. □

Given a sample of size $m + n$ from the two populations at time $t > 0$, let $L_1$, $L_2$, $L_3$ be the random numbers of legacy polymorphism sites that are fixed differences in the sample ($L_1$), polymorphic in only one of the two samples ($L_2$) or polymorphic in both samples ($L_3$). Then we have the following lemma.

LEMMA 3.2. *The random variables* $L_1, L_2, L_3$ *defined above are independent Poisson with means*

$$\begin{aligned}
E(L_1) = C_1(\beta) &= \int_0^1 \big(I(x,m)K(x,n) + I(x,n)K(x,m)\big)\nu(dx),\\
E(L_2) = C_2(\beta) &= \int_0^1 \big[J(x,m)\big(I(x,n) + K(x,n)\big)\\
&\qquad + J(x,n)\big(I(x,m) + K(x,m)\big)\big]\nu(dx) \qquad (3.3)\\
&= \int_0^1 \big(J(x,m) + J(x,n) - 2J(x,m)J(x,n)\big)\nu(dx),\\
E(L_3) = C_3(\beta) &= \int_0^1 J(x,m)J(x,n)\nu(dx).
\end{aligned}$$

PROOF. Consider the random mapping $x \to z = \{1,2,3\}$ corresponding to random sampling at time $t > 0$ for these three outcomes. Then Lemma 3.2 follows from Lemma 3.1 and Bartlett's theorem [28]. □

In particular, it follows from Lemma 3.2 that the expected number of polymorphic sites at time $t > 0$ due to legacy polymorphisms is

$$E(L_2 + L_3) = \int_0^1 \big(J(x,m) + J(x,n) - J(x,m)J(x,n)\big)\nu(dx).$$

The third term in the integrand above corrects for double counting at shared polymorphisms.

3.2. *Polymorphisms from new mutations.* It follows from Section 7.2 that the contribution of new polymorphisms to site polymorphisms at time $t > 0$ in a single

population is a Poisson random field with density

$$f_N(\beta, y) = \frac{\theta}{s(1)}\Big(s(1) - s(y) - \int_0^1 \big(s(1) - s(x)\big)p(t, x, y)m(dx)\Big). \tag{3.4}$$

It follows similarly from Section 8 that the number of mutant fixations in a single daughter population due to new polymorphisms is a Poisson random variable with mean

$$G_N(\beta) = \frac{\theta}{s(1)} \int_0^t \widetilde{P}_0(T_1 \le u)\, du. \tag{3.5}$$

In particular, the limiting PRF mean density for population site polymorphic frequencies in a single population is $f(\beta, y) = f_L(\beta, y) + f_N(\beta, y)$ as in (2.14), and the limiting expected number of mutant fixed differences is $G(\beta) = G_L(\beta) + G_N(\beta)$ as in (2.16).

For a sample of size $n$ from a single population at time $t > 0$, let $Z_k$ $(0 \le k \le n)$ be the number of new polymorphic sites that have $k$ mutant nucleotides. Then we have the following lemma.

LEMMA 3.3. *The random variables $Z_k$ defined above are independent Poisson-distributed random variables with means*

$$E(Z_k) = F_N(\beta, n, k) = \int_0^1 f_N(\beta, y) \binom{n}{k} y^k (1 - y)^{n-k} m(dy) \tag{3.6}$$

*for $1 \le k \le n$ and $f_N(\beta, y)$ in (3.4).*

PROOF. Consider the random mapping $y \to K = \{0, 1, \ldots, n\}$ defined by binomial sampling with parameters $y$ and $n$ at each polymorphic site. The range of the mapping is a Poisson random field by Bartlett's theorem [28]. Thus the random variables $Z_k$ for $1 \le k \le n$ are independent Poisson with the means in (3.2). Note that Bartlett's theorem applies here even though $Z_0 = \infty$ a.s. and $E(Z_0) = \infty$. □

Since the random variables $Z_k$ in Lemma 3.3 are independent, the expected number of polymorphic sites at time $t > 0$ due to new polymorphisms is Poisson with mean

$$E_N(\beta, n) = \sum_{k=1}^{n-1} F_N(\beta, n, k) = \int_0^1 f_N(\beta, y)\big(1 - y^n - (1 - y)^n\big)m(dy). \tag{3.7}$$

The expected number of sites due to new polymorphisms that are monomorphic in a sample of size $n$ at the mutant nucleotide is (i) the expected number of sites that have fixed in the population at the mutant nucleotide by time $t$ plus (ii) the expected number of sites that are not fixed in the population but are monomorphic at the mutant nucleotide in a sample, which is

$$D_N(\beta, n) = G_N(\beta) + F_N(\beta, n, n) \tag{3.8}$$

for $G_N(\beta)$ in (3.5).

3.3. *Sampling formulas in DPRS and DOHRS tables.* We have now completed the proof of the following result.

THEOREM 3.1. *Assume that the two species have the same effective population size $N_e$ and the same scaled parameter values $\beta_r = (t, \theta_r, \gamma)$ and $\beta_s = (t, \theta_s, 0)$. Then the counts $K_s, O_s, H_s, K_r, O_r, H_r$ in the table (2.19) in Section 2 are independent and Poisson distributed with means*

$$\begin{aligned}
E(K_s) &= C_1(\beta_s) + D_N(\beta_s, m) + D_N(\beta_s, n),\\
E(O_s) &= C_2(\beta_s) + E_N(\beta_s, m) + E_N(\beta_s, n),\\
E(H_s) &= C_3(\beta_s),\\
E(K_r) &= C_1(\beta_r) + D_N(\beta_r, m) + D_N(\beta_r, n),\\
E(O_r) &= C_2(\beta_r) + E_N(\beta_r, m) + E_N(\beta_r, n),\\
E(H_r) &= C_3(\beta_r)
\end{aligned} \tag{3.9}$$

*for $C_i(\beta)$ in (3.3), $E_N(\beta, n)$ in (3.7) and $D_N(\beta)$ in (3.8).*

COROLLARY 3.1. *The counts $K_s, V_s, K_r, V_r$ in the $2 \times 2$ table (2.18) are independent Poisson with means $E(V_r) = E(H_r) + E(O_r)$ and $E(V_s) = E(H_s) + E(O_s)$ in (3.9).*

**4. Diffusion operators and diffusion approximations.** This section describes the diffusion approximation [14, 18, 44] of a single Markov chain $X_{1,a,k}$ ($k \geq 0$) or $X_{2,b,r,k}$ ($k \geq r \geq 1$) in Section 2, which are assumed to have the same transition function $p_N(i, j)$ in (2.1). A major purpose of this section is to show that the transition density $p(t, x, y)$ of the limiting diffusion process and its first partial derivative $(\partial/\partial s(x))p(t, x, y)$ are smooth for $t > 0$ and $0 \leq x, y \leq 1$. (The latter will be used in Section 8.2.)

Each of the Markov chains $X_{1,a,k}, X_{2,b,r,k}$ will be approximated by a diffusion process $X_t$ for continuous $t$ scaled in terms of $N^2$ steps of the Markov chain, so that $t \sim k/N^2$. The limiting diffusion process $X_t$ is determined by the differential operator

$$L_x = x(1-x)\frac{d^2}{dx^2} + \gamma x(1-x)\frac{d}{dx}, \tag{4.1}$$

where $\gamma = \lim_{N\to\infty} N\sigma_N$ for $\sigma_N$ in (2.1). We write the operator $L_x$ in Feller form $L_x = (d/m(dx))(d/s(dx))$ where

$$s(x) = \frac{1 - e^{-\gamma x}}{\gamma} \quad \text{and} \quad m(dx) = \frac{e^{\gamma x}}{x(1-x)}\,dx \tag{4.2}$$

are called the *scale* and *speed measure* of $L_x$, respectively [14, 17, 22, 23, 37]. At silent sites, and in general if $\gamma = 0$, the scale and speed measure are $s(x) = x$ and $m(dx) = dx/(x(1-x))$.

4.1. *Green's functions and transition densities.* Define

$$g(x,y)=\frac{(s(1)-s(x\vee y))(s(x\wedge y)-s(0))}{s(1)-s(0)}, \tag{4.3}$$

where $x\vee y=\max\{x,y\}$ and $x\wedge y=\min\{x,y\}$. Set

$$B_{01}=\{f\in C[0,1]\colon f(0)=f(1)=0\}, \tag{4.4}$$

where $C[0,1]$ is the class of continuous functions on $0\le x\le 1$. Then, for any $f\in C[0,1]$, $h(x)=\int_0^1 g(x,y)f(y)m(dy)$ is the unique solution $h(x)\in C^2(0,1)\cap B_{01}$ such that $L_xh(x)=-f(x)$ for $0<x<1$. (For $s(x),m(dx)$ in (4.2), this does *not* imply $h\in C^2[0,1]$, nor even that $h\in C^1[0,1]$.)

Since $s(x)$ is increasing, $g(x,y)\le\min\{g(x,x),g(y,y)\}$ by (4.3). Hence by (4.3) and (4.2)

$$k(x)=\int_0^1 g(x,y)^2m(dy)\le g(x,x)\int_0^1 g(y,y)m(dy)<\infty \tag{4.5}$$

and $\int_0^1 k(x)m(dx)\le(\int_0^1 g(x,x)m(dx))^2<\infty$. Thus

$$\int_0^1\int_0^1 g(x,y)^2m(dx)m(dy)<\infty$$

and $g(x,y)$ is a Hilbert–Schmidt kernel on $L^2(I,m)$ [36]. This implies that there exists a complete orthonormal system of functions $\alpha_n(x)$ in $L^2(I,m)$ such that

$$\int_0^1 g(x,y)\alpha_n(y)m(dy)=\beta_n\alpha_n(x),\qquad \int_0^1\alpha_n(y)^2m(dy)=1, \tag{4.6}$$

where $\sum_{n=1}^\infty\beta_n^2<\infty$ and $\alpha_n(0)=\alpha_n(1)=0$. By the same arguments as after (4.4), $\beta_n\ne 0$ and the integral equation in (4.6) is equivalent to

$$L_x\alpha_n(x)=-\lambda_n\alpha_n(x),\qquad \lambda_n=1/\beta_n,\qquad \alpha_n\in C^2(0,1)\cap B_{01}. \tag{4.7}$$

Since $\alpha_n(0)=\alpha_n(1)=0$, $\lambda_n>0$ by (4.1) and $\sum_{n=1}^\infty 1/\lambda_n^2<\infty$. In particular, we can assume $0<\lambda_1\le\lambda_n\uparrow\infty$.

By Cauchy's inequality in (4.6) and by (4.5) and (4.3)

$$|\alpha_n(x)|\le\lambda_n\sqrt{k(x)}\le C_1\lambda_n\sqrt{x(1-x)}\le C_1\lambda_n.$$

Since $g(x,y)\le g(x,x)$ and $\int_0^1\sqrt{y(1-y)}m(dy)<\infty$, (4.6) implies

$$|\alpha_n(x)|\le C_2\lambda_n^2 g(x,x)\le C_3\lambda_n^2 x(1-x). \tag{4.8}$$

Since $g(x,y)/(x(1-x))$ is bounded and continuous by (4.3), it follows from (4.6) and (4.8) that $\alpha_n(x)/(x(1-x))$ extend to continuous functions on $[0,1]$. If $\gamma=0$, the functions $\alpha_n(x)$ are polynomials related to Jacobi polynomials [27]. If $\gamma\ne 0$, they are entire functions that are never polynomials.

By Mercer's theorem [36],

$$g(x,y)=\sum_{n=1}^{\infty}\frac{\alpha_n(x)\alpha_n(y)}{\lambda_n} \tag{4.9}$$

converges absolutely and uniformly for $0\le x,y\le 1$. The series

$$p(t,x,y)=\sum_{n=1}^{\infty}e^{-\lambda_n t}\alpha_n(x)\alpha_n(y) \tag{4.10}$$

converges uniformly for $0\le x,y\le 1$ and $t\ge a>0$ since $\sum_{n=1}^{\infty}1/\lambda_n^2<\infty$. Thus

$$\int_a^{\infty}p(u,x,y)\,du=\sum_{n=1}^{\infty}e^{-\lambda_n a}\frac{\alpha_n(x)\alpha_n(y)}{\lambda_n} \tag{4.11}$$

converges absolutely and uniformly for $a\ge 0$. In particular

$$g(x,y)=\int_0^{\infty}p(t,x,y)\,dt \tag{4.12}$$

with uniform convergence for $0\le x,y\le 1$.

It follows from (4.10) and (4.7) that

$$\begin{aligned}&p(t+s,x,y)=\int_0^1 p(t,x,z)p(s,z,y)m(dz)\quad\text{and}\\ &(\partial/\partial t)p(t,x,y)=L_x p(t,x,y),\qquad t>0,\,0<x,y<1.\end{aligned} \tag{4.13}$$

Choose $f\in B_{01}$ with $f(x)=0$ for $0\le x\le c$ and $1-c\le x\le 1$ for some $c>0$. Let $u(t,x)=\int_0^1 p(t,x,y)h(y)m(dy)$ for $h(x)=\int_0^1 g(x,y)f(y)m(dy)$. Then by (4.11) and (4.12)

$$u(t,x)=\sum_{n=1}^{\infty}\frac{e^{-\lambda_n t}}{\lambda_n}\alpha_n(x)c_n\qquad\text{for } c_n=\int_0^1 f(y)\alpha_n(y)m(dy),$$

where the series converges uniformly for $0\le x\le 1$ and $0\le t<\infty$ by (4.11). Thus $u(t,x)\in C([0,\infty)\times[0,1])$ with

$$\begin{aligned}(\partial/\partial t)u(t,x)&=L_x u(t,x),\qquad t>0,\,0<x<1,\\ u(t,0)&=u(t,1)=0,\qquad u(0,x)=h(x).\end{aligned}$$

It follows from maximum principles for parabolic partial differential equations [35] that

$$p(t,x,y)\ge 0,\qquad \int_0^1 p(t,x,z)m(dz)\le 1 \tag{4.14}$$

for $0<x,y<1$. If $u(x,t)=Q_t h(x)=\int p(t,x,y)h(y)m(dy)$, then

$$\lim_{t\to 0}Q_t f(x)=f(x) \tag{4.15}$$

uniformly for $0 \le x \le 1$ for a dense set of $f \in C^2(0,1) \cap B_{01}$, and hence for all $f \in B_{01}$ by standard arguments.

4.2. *Diffusion processes and semigroups.* It follows from the relations (4.13)–(4.15) that there exists a diffusion process $X_t$ with continuous sample paths with $0 \le X_t \le 1$ such that

$$Q_t f(x) = E_x(f(X_t)) = \int_0^1 p(t,x,y) f(y) m(dy) \tag{4.16}$$

for $f \in C[0,1]$ with $f(0) = f(1) = 0$ [11, 13, 16, 22, 37]. The process $X_t$ satisfies $0 < X_t < 1$ up to the time that it is trapped at one of the endpoints $0, 1$, which happens eventually with probability one. The relation

$$|Q_t f(x)| \le C_4 e^{-\lambda_1 t} \|f\|, \qquad \|f\| = \sup_{0 \le y \le 1} |f(y)| \tag{4.17}$$

from (4.10) and (4.8) gives the rate at which $X_t$ is trapped at the endpoints. The exit point for $X_t$ is given by the scale function

$$P_x(T_1 < T_0) = \frac{s(x) - s(0)}{s(1) - s(0)} = \frac{s(x)}{s(1)}, \tag{4.18}$$

where $T_a = \min\{s : X_s = a\}$ [16, 22].

It follows from (4.8)–(4.15) that $Q_t : B_{01} \to B_{01}$ for all $t > 0$ for $B_{01}$ in (4.4) and that $\{Q_t\}$ is a strongly continuous semigroup of linear operators on $B_{01}$.

The *infinitesimal generator* [10, 36, 44] of a semigroup of linear operators $Q_t$ on a Banach space $B$ is the linear operator $A$ defined by $Ah = f$ on the linear subspace

$$\mathcal{D}(A) = \left\{ h \in B : \lim_{t \to 0} \|(1/t)(Q_t h - h) - f\| = 0 \text{ for some } f \in B \right\},$$

where $\|f\|$ is the norm in the Banach space. If $Q_t$ is strongly continuous, then $\mathcal{D}(A)$ is dense in $B$ and

$$\|Q_t f\| \le M e^{Kt} \|f\| \qquad \text{all } f \in B \tag{4.19}$$

for some real $K$. If $K < 0$ as in (4.17), $\mathcal{D}(A)$ is the range of the resolvent operator $R_0 f = \int_0^\infty Q_t f \, dt$ on $B$ with $-AR_0 = I$. This implies $Ah = -f$ if $h = R_0 f$ [10, 36, 44]. By (4.12)

$$R_0 f(x) = \int_0^\infty Q_t f(x) \, dt = \int_0^1 g(x,y) f(y) m(dy) \tag{4.20}$$

is the Green's operator defined by $g(x,y)$.

A *core* of a strongly-continuous semigroup $Q_t$ with $K < 0$ is a subset $\mathcal{C} \subseteq \mathcal{D}(A)$ such that $B_c = A(\mathcal{C})$ is dense in $B$. Since $R_0$ is one–one on $B$, this is equivalent to specifying a dense subset $B_c \subseteq B$ and setting $\mathcal{C} = R_0(B_c)$.

4.3. *Diffusion approximations and Trotter's theorem.* Let $X_k^N$ be the Markov chain defined by the Moran model (2.1). Define $Y_j^N = X_j^N/N$, so that $0 \le Y_j^N \le 1$. It follows from standard arguments and (2.1) that

THEOREM 4.1. *Let $i_N$ be integers such that $0 \le i_N \le N$ and such that $x_N = i_N/N \to x$ for some $x$, $0 \le x \le 1$. Then for any $\delta > 0$*

$$\begin{aligned} &\lim_{N\to\infty} N^2 E_{i_N}(Y_1^N - x_N) = \gamma x(1-x), \\ &\lim_{N\to\infty} N^2 E_{i_N}\big((Y_1^N - x_N)^2\big) = 2x(1-x), \\ &\lim_{N\to\infty} N^2 E_{i_N}(|Y_1^N - x_N|^{2+\delta}) = 0. \end{aligned} \tag{4.21}$$

Since the functions on the right-hand side of (4.21) are continuous, convergence in (4.21) is equivalent to uniform convergence in $x$ for $i_N = [Nx]$. By Taylor's theorem

$$\begin{aligned} &\lim_{N\to\infty} N^2 E_{i_N}\big(h(Y_1^N) - h(x_N)\big) \\ &\qquad = L_x h(x) = x(1-x)h''(x) + \gamma x(1-x)h'(x) \end{aligned} \tag{4.22}$$

uniformly for $0 \le x \le 1$ for any $h \in C^2[0,1]$. Then by Trotter's theorem [44]:

THEOREM 4.2. *For $Y_j^N$ as above, $i_N = [Nx]$, and the diffusion process $X_t$ in (4.16),*

$$\lim_{N\to\infty} E_{i_N}\big(f\big(Y_{[N^2t]}^N\big)\big) = E_x(f(X_t)) = Q_t f(x) \tag{4.23}$$

*uniformly for $0 \le x \le 1$ for any $f \in C[0,1]$ with $f(0) = f(1) = 0$. The convergence is also uniform for $0 \le t \le T$ for any $T > 0$.*

We cannot apply (4.22) for $h \in C^2[0,1]$ directly for Trotter's theorem, since in this case there exist $h \in \mathcal{D}(A)$ with $h \notin C^1[0,1]$, let alone $C^2[0,1]$. However, it is sufficient to verify (4.22) for all $h$ in a core for $A$ [44]. If $\mathcal{C} = R_0(B_c)$ where $B_c$ is the set of all function $f \in B_{01}$ such that $f(x) = 0$ for $0 \le x \le a$ and $1-a \le x \le 1$ for some $a > 0$, then $\mathcal{C}$ is such a core.

The result (4.23) also holds for $f \in C[0,1]$ without the conditions $f(0) = f(1) = 0$ with an appropriate modification of the definition of $Q_t f(x)$. See Corollary 5.1 in Section 5 below.

Thus, after suitable rescaling, the Markov chains $\{X_{1,a,k}, X_{2,b,r,k}\}$ in Section 2 converge in distribution to diffusion processes $\{X_t\}$ with infinitesimal generator (2.5) and scale and speed measure (2.7) in Section 2.

**5. Exit probabilities for Markov chains.** Let $X_k^N$ be the Moran-model Markov chain defined by (2.1). Recall that $N\sigma_N \to \gamma$ as $N \to \infty$ by (2.9). Then we have the following lemma.

LEMMA 5.1. *Let $i, m$ be integers such that $1 \le i \le m \le N$. Then*

$$P_i(T_m^N < T_0^N) = \frac{1-(1+\sigma_N)^{-i}}{1-(1+\sigma_N)^{-m}} \tag{5.1}$$

*with the right-hand side replaced by $i/m$ if $\sigma_N = 0$.*

PROOF. By (2.1), $p_N(i, i+1)/p_N(i, i-1) = 1+\sigma_N$ for $0 < i < N$. Since we can ignore "wait states" with $X_{k+1}^N = X_k^N$, (5.1) follows from the classical Gambler's Ruin problem (see, e.g., [23], pages 50, 92–94, and Moran [32]). (See Lemma 8.2 for a second proof.) □

As a consequence of Lemma 5.1:

LEMMA 5.2. *Let $i_N$ be integers such that $0 \le i_N \le N$ and $i_N/N \to x$ for some $x$, $0 \le x \le 1$. Then*

$$\lim_{N\to\infty} P_{i_N}(T_N^N < T_0^N) = P_x(T_1 < T_0) = \frac{s(x)-s(0)}{s(1)-s(0)} = \frac{s(x)}{s(1)} \tag{5.2}$$

*for $T_0, T_1$ in (4.18) and $s(x)$ in (4.2). If $i_N = i_N(x) = [Nx]$, the convergence in (5.2) is uniform in $x$ for $0 \le x \le 1$.*

PROOF. If $\sigma_N \neq 0$ and $\gamma \neq 0$, it follows from (5.1) that

$$P_{i_N}(T_N^N < T_0^N) = \frac{1-(1+N\sigma_N/N)^{-i_N}}{1-(1+N\sigma_N/N)^{-N}} \to \frac{1-e^{-\gamma x}}{1-e^{-\gamma}} \tag{5.3}$$

as $N \to \infty$. The proof is similar if $\gamma = 0$. □

Lemma 5.2 can be used to extend Theorem 4.2 to all $f \in C[0,1]$:

COROLLARY 5.1. *Assume $f \in C[0,1]$. Let $X_k^N$ and set $Y_k^N = X_k^N/N$ as in Theorem 4.1. Set $i_N = [Nx]$. Then*

$$\lim_{N\to\infty} E_{i_N}\big(f\big(Y_{[N^2t]}^N\big)\big) = E_x(f(X_t)) = \overline{Q}_t f(x) \tag{5.4}$$

*uniformly for $0 \le x \le 1$, where*

$$\overline{Q}_t f(x) = f(0)P_x(T_0 \le t) + \int_0^1 p(t,x,y)f(y)m(dy) + f(1)P_x(T_1 \le t). \tag{5.5}$$

PROOF. Any $f \in C[0,1]$ can be written

$$f(x) = g(x) + f(0)\frac{s(1)-s(x)}{s(1)-s(0)} + f(1)\frac{s(x)-s(0)}{s(1)-s(0)},$$

where $g(0) = g(1) = 0$, so that $g \in B_{01}$. Since (5.4) holds for $g \in B_{01}$ by Theorem 4.2, it only remains to prove (5.4) for $f(x) = s(x)$. However,

$$h_N(i_N) = P_{i_N}(T_N^N < T_0^N) = E_{i_N}(h(X_1^N)) = E_{i_N}\big(h_N\big(X_{[N^2t]}^N\big)\big) \tag{5.6}$$

for all $t > 0$. Lemma 5.2 applied to both sides of (5.6) implies (5.4) for $f(x) = s(x)$. □

A stronger result than Lemma 5.2 is the "local limit theorem."

LEMMA 5.3. *Let $i_N$ be integers with $1 \le i_N \le N$ and set $x_N = i_N/N$. Then*

$$\lim_{N\to\infty} \frac{P_{i_N}(T_N^N < T_0^N)}{s(x_N)/s(1)} = 1. \tag{5.7}$$

*Similarly, if $0 \le i_N \le N-1$ and $x_N = i_N/N$, then*

$$\lim_{N\to\infty} \frac{1 - P_{i_N}(T_N^N < T_0^N)}{(s(1)-s(x_N))/s(1)} = 1. \tag{5.8}$$

The most important cases of Lemma 5.3 are when $x_N \to 0$ or $x_N \to 1$. If $i_N = i_N(x) = \min\{[Nx]+1, 1\}$, then (5.7) holds uniformly in $x$ for $0 \le x \le 1$. Similarly, (5.8) holds uniformly in $x$ if $i_N = i_N(x) = [Nx]$.

PROOF OF LEMMA 5.3. The ratio in (5.7) can be written

$$\begin{aligned}&\left(\frac{1-(1+\sigma_N)^{-i_N}}{1-e^{-\gamma x_N}}\right)\left(\frac{1-e^{-\gamma}}{1-(1+\sigma_N)^{-N}}\right)\\ &\quad= \left(\frac{\int_0^{x_N} e^{-yN\log(1+\sigma_N)}\,dy}{\int_0^{x_N} e^{-y\gamma}\,dy}\right)\left(\frac{\int_0^1 e^{-y\gamma}\,dy}{\int_0^1 e^{-yN\log(1+\sigma_N)}\,dy}\right),\end{aligned} \tag{5.9}$$

where the first line of (5.9) holds for $\gamma \ne 0$ and the second line for all $\gamma$. Since $N\sigma_N \to \gamma$, then $N\log(1+\sigma_N) \to \gamma$ and the ratios converge uniformly in $x_N$. Thus (5.7) follows from (5.9). Similarly,

$$P_x(T_0 < T_1) = \frac{s(1)-s(x)}{s(1)-s(0)} = \frac{e^{-\gamma x}-e^{-\gamma}}{1-e^{-\gamma}} = \frac{e^{\gamma(1-x)}-1}{e^{\gamma}-1}$$

with a corresponding relation for $h_N(i)$. Then the ratio in (5.8) can be written

$$\left(\frac{\int_0^{1-x_N} e^{yN\log(1+\sigma_N)}\,dy}{\int_0^{1-x_N} e^{y\gamma}\,dy}\right)\left(\frac{\int_0^1 e^{y\gamma}\,dy}{\int_0^1 e^{yN\log(1+\sigma_N)}\,dy}\right)$$

with a similar conclusion. □

**6. Dual Markov chains and dual diffusion processes.** Define $h_N(i) = P_i(T_N^N < T_0^N)$ as in (5.1) where, as before, $P_i$ means conditional on $X_0^N = i$. For $p_N(i,j) = P_i(X_1^N = j)$ in (2.1) and $1 \le i \le N$, define

$$q_N(i,j) = P_i(X_1^N = j \mid T_1^N < T_0^N) = \frac{1}{h_N(i)} p_N(i,j) h_N(j). \tag{6.1}$$

Since $\sum_{j=1}^N q_N(i,j) = 1$ for $1 \le i \le N$, $q_N(i,j)$ defines a Markov chain $\{\widetilde{X}_k^N\}$ on $S_N = \{1, 2, \ldots, N\}$ that never attains $\widetilde{X}_k^N = 0$ and has $N$ as an absorbing boundary. Similarly

$$E_i(\Phi(\widetilde{X}_1^N, \ldots, \widetilde{X}_k^N)) = E_i\big(\Phi(X_1^N, \ldots, X_k^N) \mid T_1 < T_0\big)$$

for all functions $\Phi(j_1, \ldots, j_k)$ on $(S_N)^k$. The chain $\widetilde{X}_k^N$ [or $q_N(i,j)$] can be called an *h-process* of $X_k^N$ [26].

6.1. *The limiting dual diffusion process.* We use the formula for $h_N(i)$ in Lemma 5.1 to find a diffusion approximation for $\{\widetilde{X}_k^N\}$. Define $\widetilde{Y}_j^N = \widetilde{X}_j^N / N$, so that $0 \le \widetilde{Y}_j^N \le 1$. The analog of Theorem 4.1 is:

THEOREM 6.1. *Let $i_N$ be integers such that $1 \le i_N \le N$ and $x_N = i_N/N \to x$ where $0 \le x \le 1$. Then, for any $\delta > 0$,*

$$\begin{aligned}
&\lim_{N\to\infty} N^2 \widetilde{E}_{i_N}(\widetilde{Y}_1^N - x_N \mid T_N^N < T_0^N) = b(x) = \gamma x(1-x)\frac{1+e^{-\gamma x}}{1-e^{-\gamma x}},\\
&\lim_{N\to\infty} N^2 \widetilde{E}_{i_N}\big((\widetilde{Y}_1^N - x_N)^2 \mid T_N^N < T_0^N\big) = 2x(1-x),\\
&\lim_{N\to\infty} N^2 \widetilde{E}_{i_N}(|\widetilde{Y}_1^N - x_N|^{2+\delta} \mid T_N^N < T_0^N) = 0,
\end{aligned} \tag{6.2}$$

*where $b(x) = 2$ if $x = 0$. If $i_N = i_N(x) = \min\{[Nx] + 1, 1\}$, the convergence is uniform in $x$.*

PROOF. By (2.1) and Lemma 5.1, writing $j = j_N = i_N$ for ease of notation,

$$\begin{aligned}
&N^2 \widetilde{E}_j(Y_1^N - x_N \mid T_N^N < T_0^N)\\
&\quad = \frac{Nj/N(1-j/N)}{1+\sigma_N j/N}\left[\frac{(1+\sigma_N)(1-(1+\sigma_N)^{-j-1}) - (1-(1+\sigma_N)^{-j+1})}{1-(1+\sigma_N)^{-j}}\right]\\
&\quad = \frac{j/N(1-j/N)N}{1+\sigma_N j/N}\left[\frac{\sigma_N + \sigma_N(1+\sigma_N)^{-j}}{1-(1+\sigma_N)^{-j}}\right]\\
&\quad \to b(x) = \gamma x(1-x)\frac{1+e^{-\gamma x}}{1-e^{-\gamma x}}.
\end{aligned}$$

Similarly

$$\begin{aligned}
&N^2\widetilde{E}_j\big((Y_1^N - x_N)^2 \mid T_N^N < T_0^N\big)\\
&\quad= \frac{j/N(1-j/N)}{1+\sigma_N j/N}\left[\frac{(1+\sigma_N)(1-(1+\sigma_N)^{-j-1}) + (1-(1+\sigma_N)^{-j+1})}{1-(1+\sigma_N)^{-j}}\right]\\
&\quad= \frac{j/N(1-j/N)}{1+\sigma_N j/N}\left[\frac{2+\sigma_N - (2+\sigma_N)(1+\sigma_N)^{-j}}{1-(1+\sigma_N)^{-j}}\right]\\
&\quad\to 2x(1-x)
\end{aligned}$$

and

$$\begin{aligned}
&N^2\widetilde{E}_j(|Y_1^N - x_N|^{2+\delta} \mid |T_N^N < T_0^N)\\
&\qquad= \frac{1}{N^\delta} N^2 E_{j/N}\big((Y_1^N - x_N)^2 \mid T_N^N < T_0^N\big) \to 0.
\end{aligned}$$

□

As in Section 4, Theorem 6.1 implies, by Taylor's theorem, that

$$\begin{aligned}
&\lim_{N\to\infty} N^2\widetilde{E}_j\big(h(\widetilde{Y}_1^N) - h(x_N) \mid T_N^N < T_0^N\big)\\
&\qquad= \widetilde{L}_x h(x) = x(1-x)h''(x) + b(x)h'(x)
\end{aligned} \tag{6.3}$$

uniformly for $0 \le x \le 1$ for any $h \in C^2[0,1]$ for $b(x)$ defined in (6.2).

As suggested by $q_N(i,j) = h_N(i)^{-1} p_N(i,h) h_N(j)$ in (6.1), the operator $\widetilde{L}_x$ in (6.3) satisfies

$$\widetilde{L}_x h(x) = \frac{1}{s(x)} L_x(sh)(x), \qquad 0 < x < 1.$$

The operator $\widetilde{L}$ can be written in Feller form as

$$\widetilde{L}_x h(x) = \frac{d}{\widetilde{m}(dx)} \frac{d}{\widetilde{s}(dx)} h(x) \tag{6.4}$$

for scale and speed measure

$$\widetilde{s}(x) = -\frac{1}{s(x)} \quad \text{and} \quad \widetilde{m}(dx) = s(x)^2 m(dx) \tag{6.5}$$

for $s(x)$ and $m(dx)$ in Section 4.

Let $\widetilde{X}_t$ be the diffusion process in $(0,1)$ generated by $\widetilde{L}_x$. Since

$$\lim_{x\to 0} \widetilde{s}(x) = -\infty \quad \text{and} \quad \int_0^{1/2} |\widetilde{s}(x)| \widetilde{m}(dx) < \infty$$

the boundary point 0 is an *entrance boundary* for $\widetilde{L}_x$ or $\widetilde{X}_t$ [16, 22, 23]. Since 0 is an entrance boundary, $\widetilde{P}_x(\widetilde{T}_0 < \infty) = 0$ for $x > 0$ where $\widetilde{T}_a = \min\{t \ge 0 : \widetilde{X}_t = a\}$ (i.e., 0 is inaccessible for $\widetilde{X}_t$), but

$$\lim_{\substack{x>0\\ x\to 0}} \widetilde{E}_x(f(\widetilde{X}_t)) = \widetilde{E}_0(f(\widetilde{X}_t))$$

exists for all $f \in C[0,1]$ with a probability distribution $\widetilde{P}_0(\widetilde{X}_t \in dx)$ on $(0,1]$ for $t > 0$. Thus $\widetilde{P}_0(\widetilde{X}_t > 0) = 1$ for any $t > 0$ and, given $\widetilde{X}_0 = 0$, $\widetilde{X}_t$ leaves 0 immediately [22].

The Green function for (6.4) and (6.5) is

$$\begin{aligned}\widetilde{g}(x,y) &= \lim_{\varepsilon\to 0} \frac{(\widetilde{s}(1)-\widetilde{s}(x\vee y))(\widetilde{s}(x\wedge y)-\widetilde{s}(\varepsilon))}{\widetilde{s}(1)-\widetilde{s}(\varepsilon)} \\ &= \widetilde{s}(1)-\widetilde{s}(x\vee y) = \frac{1}{s(x\vee y)} - \frac{1}{s(1)} \\ &= \frac{(s(1)-s(x\vee y)(s(x\wedge y)-s(0))}{s(1)s(x\vee y)s(x\wedge y)} = \frac{g(x,y)}{s(x)s(y)}\end{aligned} \tag{6.6}$$

by (4.3). Since $\iint \widetilde{g}(x,y)^2 \widetilde{m}(dx)\widetilde{m}(dy) = \iint g(x,y)^2 m(dx)m(dy) < \infty$, the kernel $\widetilde{g}(x,y)$ is Hilbert–Schmidt with respect to $\widetilde{m}(dx)$. The eigenfunction equation

$$\int_0^1 \widetilde{g}(x,y)\widetilde{\alpha}(y)\widetilde{m}(dy) = \frac{1}{s(x)}\int_0^1 g(x,y)s(y)\widetilde{\alpha}(y)m(dy) = \beta\widetilde{\alpha}(x)$$

is the same as (4.6) for $\alpha(x) = s(x)\widetilde{\alpha}(x)$, so that we can take $\widetilde{\alpha}_n(x) = \alpha_n(x)/s(x)$ with the same eigenvalues $\lambda_n$. Define

$$B_1 = \{f \in C[0,1] : f(1) = 0\} \tag{6.7}$$

and

$$\begin{aligned}q(t,x,y) &= \sum_{n=1}^{\infty} e^{-\lambda_n t}\widetilde{\alpha}_n(x)\widetilde{\alpha}_n(y) = \frac{p(t,x,y)}{s(x)s(y)}, \\ \widetilde{Q}_t f(x) &= \widetilde{E}_x(f(\widetilde{X}_t)) = \int_0^1 q(t,x,y)f(y)\widetilde{m}(dy), \\ \widetilde{g}(x,y) &= \int_0^{\infty} q(t,x,y)\,dt = \frac{g(x,y)}{s(x)s(y)}.\end{aligned} \tag{6.8}$$

For $f \in B_1$, by (6.8) and (4.8),

$$|\widetilde{Q}_t f(x)| \le C_5 e^{-\lambda_1 t}\|f\|, \qquad \|f\| = \sup_{0\le y\le 1}|f(y)|. \tag{6.9}$$

Since $\widetilde{\alpha}_n(x) = \alpha_n(x)/s(x) \in B_1$ by the discussion in Section 4, the operators

$$\widetilde{Q}_t f(x) = \frac{1}{s(x)}\int_0^1 p(t,x,y)s(y)f(y)m(dy) = \frac{1}{s(x)}Q_t(sf)(x) \tag{6.10}$$

preserve $B_1$. The principal result of this section is presented in Section 6.2.

6.2. *Trotter's theorem for the dual process.* Let $\widetilde{X}_k^N$ and $q_N(i,j)$ be for the dual Markov chain in (6.1) and set $\widetilde{Y}_k^N = \widetilde{X}_k^N/N$.

As in Section 4, the relation (6.3) defining $\widetilde{L}h$ with uniform convergence for $h \in C^2[0,1]$ does not cover all $h \in \mathcal{D}(\widetilde{A}) = \widetilde{R}_0(B_1)$ since $\mathcal{D}(\widetilde{A})$ contains functions $h \notin C^1[0,1]$. However, if $B_c$ is the set of all $f \in B_1$ such that $f(x) = b$ for $0 \le x \le a$ and $f(x) = 0$ for $1-a \le x \le 1$ for constants $a, b$ with $a > 0$, then (6.3) holds for all $h$ in the core $\mathcal{C} = \widetilde{R}(B_c)$. Then by Trotter's theorem [44]:

THEOREM 6.2. *Let $i_N$ be integers such that $1 \le i_N \le N$ and $i_N/N \to x$ for some $x$, $0 \le x \le 1$. Then*

$$\lim_{N\to\infty} \widetilde{E}_{i_N}\big(f\big(\widetilde{Y}^N_{[N^2t]}\big)\big) = \widetilde{E}_x(f(\widetilde{X}_t)) = \widetilde{Q}_t f(x) \tag{6.11}$$

*for any $f \in C[0,1]$ with $f(1) = 0$. If $i_N = i_N(x) = \min\{[Nx]+1, 1\}$, the convergence is uniform in $x$, and is also uniform in $t$ for $0 \le t \le T$ for any $T > 0$.*

A weaker version of (6.11) could be obtained from Theorem 4.2 directly: By (6.1)

$$\begin{aligned}
\widetilde{E}_{i_N}(f(\widetilde{Y}_k^N)) &= \sum_{j=1}^{N-1} q_N^k(i_N, j) f\left(\frac{j}{N}\right) \\
&= \frac{1}{h_N(i_N)} \sum_{j=1}^{N-1} p_N^k(i_N, j) h_N(j) f\left(\frac{j}{N}\right) \qquad (6.12) \\
&= \frac{s(x)}{h_N(i_N)} \frac{1}{s(x)} \sum_{j=1}^{N-1} p_N^k(i_N, j) \left[\frac{h_N(j)}{s(j/N)} s\left(\frac{j}{N}\right) f\left(\frac{j}{N}\right)\right],
\end{aligned}$$

where $1 \le i_N \le N$ and $i_N/N \to x > 0$. Now $g(x) = s(x)f(x) \in B_{01}$ if $f \in B_1$, and $\lim_{N\to\infty} h_N(i)/s(i/N) = s(1)$ uniformly for $1 \le i \le N$ by Lemma 5.3. Hence by Theorem 4.2

$$\begin{aligned}
\lim_{N\to\infty} \widetilde{E}_{i_N}\big(f\big(\widetilde{Y}^N_{[N^2t]}\big)\big) &= \frac{1}{s(x)} Q_t(sf)(x) \\
&= \frac{1}{s(x)} \int_0^1 p(t,x,y) s(y) f(y) m(dy) \\
&= \widetilde{Q}_t f(x)
\end{aligned}$$

uniformly for $s(x) \ge a$ for any $a > 0$. However, this argument does not extend to uniform convergence for $0 \le x \le 1$ nor to $x = 0$.

**7. The limiting distribution of polymorphic sites.** The purpose of this section is to prove Theorem 2.1 and Corollary 2.1 in Section 2. By (2.3), the expected value of the left-hand side of (2.13) is

$$E\left(\sum_{i=1}^{N-1} f\left(\frac{i}{N}\right)N_{k_N}(i)\right) = E\left(\sum_{a=1}^{M_0} f\left(\frac{X^N_{1,a,k_N}}{N}\right) + \sum_{r=1}^{k_N}\sum_{b=1}^{M_r} f\left(\frac{X^N_{2,b,r,k_N}}{N}\right)\right). \tag{7.1}$$

7.1. *The first term in* (7.1) (*legacy polymorphisms*). The expected value of the sum in (7.1) that corresponds to legacy polymorphisms is

$$\begin{aligned} E\left(\sum_{a=1}^{M_0} f\left(\frac{X^N_{1,a,k_N}}{N}\right)\right) &= \sum_{i=1}^{N-1} E(N_0(i)) \sum_{j=1}^{N-1} p_N^{k_N}(i,j) f\left(\frac{j}{N}\right) \\ &= \sum_{i=1}^{N-1} \omega_i^N \sum_{j=1}^{N-1} p_N^{k_N}(i,j) f\left(\frac{j}{N}\right) \\ &= \sum_{i=1}^{N-1} \omega_i^N Q^N_{k_N} f(i). \end{aligned}$$

By (6.1)

$$\begin{aligned} \widetilde{Q}^N_k f(i) &= \sum_{j=1}^{N-1} q_N^k(i,j) f\left(\frac{j}{N}\right) \\ &= \frac{1}{h_N(i)} \sum_{j=1}^{N-1} p_N^k(i,j) h_N(j) f\left(\frac{j}{N}\right) \\ &= \frac{1}{h_N(i)} Q^N_k(h_N f)(i), \end{aligned} \tag{7.2}$$

where $(h_N f)(x) = h_N([Nx]) f(x)$. Thus

$$\begin{aligned} \sum_{i=1}^{N-1} \omega_i^N Q^N_k f(i) &= \sum_{i=1}^{N-1} h_N(i) \widetilde{Q}^N_k\left(\frac{f}{h_N}\right)(i)\omega_i^N \\ &= \sum_{i=1}^{N-1} \left(\frac{h_N(i)}{s(i/N)}\right) \widetilde{Q}^N_k\left(\frac{f}{h_N}\right)(i) s\left(\frac{i}{N}\right)\omega_i^N. \end{aligned} \tag{7.3}$$

By Lemma 5.3, $\lim_{N\to\infty} h_N(i_N)/s(i_N/N) = 1/s(1)$ uniformly in $x$ for $i_N = i_N(x) = [Nx]$, and we can write

$$\frac{f(i/N)}{h_N(i)} = g(i/N) - g(i/N)\left(1 - \frac{s(i/N)/s(1)}{h_N(i)}\right) \tag{7.4}$$

for $g(y) = s(1)f(y)/s(y)$. By the assumptions of Theorem 2.1, $g(y)$ extends to a continuous function on $C[0, 1]$ with $g(1) = 0$, so by Theorem 6.2

$$\lim_{N\to\infty} \widetilde{Q}^N_{[N^2t]} g([Nx]) = \widetilde{Q}_t g(x) \tag{7.5}$$

uniformly for $0 \le x \le 1$ and $0 \le t \le T$ for any $T > 0$. By (2.12)

$$\lim_{N\to\infty} \sum_{i=1}^{N-1} g\left(\frac{i}{N}\right) s\left(\frac{i}{N}\right) \omega_i^N = \int_0^1 g(y)s(y)\nu(dy). \tag{7.6}$$

Thus by (7.3) and (7.5)

$$\begin{aligned} \lim_{N\to\infty} \sum_{i=0}^{N-1} \omega_i^N Q_k^N f(i) &= \int_0^1 \widetilde{Q}_t\big(s(1)f/s\big)(y)\frac{s(y)}{s(1)}\nu(dy) \\ &= \int_0^1 Q_t f(y)\nu(dy). \end{aligned} \tag{7.7}$$

This completes the proof of Theorem 2.1 for the legacy terms in (7.1).

7.2. *The second term in* (7.1) (*new mutations*). The expected value of the double sum in (7.1), which corresponds to new mutations, is

$$\begin{aligned} E\left(\sum_{r=1}^{k_N}\sum_{b=1}^{M_r} f\left(\frac{X^N_{2,b,r,k_N}}{N}\right)\right) &= \sum_{r=1}^{k_N} E(M_r)E_1\left(f\left(\frac{X^N_{k_N-r}}{N}\right)\right) \\ &= \mu_N \sum_{r=1}^{k_N} Q^N_{k_N-r} f(1) \\ &= \mu_N \sum_{r=0}^{k_N-1} Q^N_r f(1), \end{aligned} \tag{7.8}$$

where $Q^N_r f(1) = \sum_{j=1}^{N-1} p_N(1, j) f(j/N)$. As in (7.2)

$$\begin{aligned} \mu_N \sum_{r=0}^{k_N-1} Q^N_r f(1) &= \mu_N h_N(1) \sum_{r=0}^{k_N-1} \widetilde{Q}^N_r (f/h_N)(1) \\ &= (N\mu_N)(Nh_N(1)) \int_0^{k_N/N^2} \widetilde{Q}^N_{[N^2u]}(f/h_N)(1)\,du, \end{aligned} \tag{7.9}$$

where $(f/h_N)(y) = f(y)/h_N([Ny]+1)$.

As $N \to \infty$, $N\mu_N \to \theta$ and $k_N/N^2 \to t < \infty$ by (2.9) and, since $s'(0) = 1$, $Nh_N(1) \to 1/s(1)$ by Lemma 5.3. Thus by (7.9) and (7.4) and (7.5)

$$\lim_{N\to\infty} \mu_N \sum_{r=0}^{k_N-1} Q^N_r f(1) = \frac{\theta}{s(1)} \int_0^t \widetilde{Q}_u g(0)\,du, \tag{7.10}$$

where $g(y) = s(1) f(y)/s(y)$. By (6.6) and (6.9), the Green operator

$$\begin{aligned}\widetilde{G} f(x) &= \int_0^\infty \widetilde{Q}_t f(x)\, dt = \int_0^1 \widetilde{g}(x, y) f(y) \widetilde{m}(dy) \\ &= \int_0^1 \Big( \frac{1}{s(x \vee y)} - \frac{1}{s(1)} \Big) f(y) \widetilde{m}(dy)\end{aligned}$$

is bounded in the supremum norm, and

$$\begin{aligned}&\frac{\theta}{s(1)} \int_0^t \widetilde{Q}_u g(0)\, du \\ &\quad = \frac{\theta}{s(1)} \Big( \int_0^\infty \widetilde{Q}_u g(0)\, du - \int_t^\infty \widetilde{Q}_u g(0)\, du \Big) \\ &\quad = \frac{\theta}{s(1)} \int_0^\infty \widetilde{Q}_u (g - \widetilde{Q}_t g)(0)\, du \\ &\quad = \frac{\theta}{s(1)} \int_0^1 \Big( \frac{1}{s(y)} - \frac{1}{s(1)} \Big) (g(y) - \widetilde{Q}_t g(y)) \widetilde{m}(dy) \\ &\quad = \frac{\theta}{s(1)} \int_0^1 \frac{s(1) - s(y)}{s(1) s(y)} (g(y) - \widetilde{Q}_t g(y)) s(y)^2 m(dy).\end{aligned}$$

Since $\widetilde{Q}_t g(y) = (1/s(y)) Q_t(sg)$ by (6.10) and $g(y) = s(1) f(y)/s(y)$, we conclude $g(y) - \widetilde{Q}_t g(y) = (s(1)/s(y))(f(y) - Q_t f(y))$ and hence

$$\frac{\theta}{s(1)} \int_0^t \widetilde{Q}_u g(0)\, du = \theta \int_0^1 \frac{s(1) - s(y)}{s(1) - s(0)} (f(y) - Q_t f(y)) m(dy).$$

This is the second term on the right-hand side of (2.13) and completes the proof of Theorem 2.1.

7.3. *Proof of Corollary* 2.1. The first term $\int_0^1 Q_t f(y) \nu(dy)$ in the last line in (2.13) equals

$$\int_0^1 \widetilde{Q}_t (f/s)(y) s(y) \nu(dy) \tag{7.11}$$

as in (7.7) or (6.10), where $f(y)/s(y)$ is bounded and $\int_0^1 s(y) \nu(dy) < \infty$. Thus the integral in (7.11) is $O(e^{-\lambda_1 t})$ by (6.9) and converges to zero as $t \to \infty$. By the same argument applied with $(s(1) - s(x)) m(dx)$ in place of $\nu(dx)$,

$$\int_0^1 \frac{s(1) - s(x)}{s(1) - s(0)} Q_t f(x) m(dx) = O(e^{-\lambda_1 t})$$

as $t \to \infty$ as well. This completes the proof of Corollary 2.1.

## 8. The limiting numbers of fixations.

8.1. *Proof of Theorem 2.2.* By Bartlett's theorem [28], the number of processes $\{X_{1,a,k}\}$ and $\{X_{2,b,r,k}\}$ that have been trapped at state $N$ by time $k$ is Poisson with mean

$$E(N_k(N)) = \sum_{i=1}^{N-1} \omega_i^N p_N^k(i,N) + \mu_N \sum_{r=0}^{k-1} p_N^r(1,N). \tag{8.1}$$

The first term on the right-hand side of (8.1) corresponds to legacy polymorphisms and the second to new polymorphisms. The proof of Theorem 2.2 for both terms depends on:

LEMMA 8.1. *Let $i_N$ be integers such that $1 \le i_N \le N$ and $i_N/N \to x$ for some $x$ with $0 \le x \le 1$. Then*

$$\lim_{N\to\infty} P_{i_N}\left(\frac{1}{N^2} T_N^N \le t \;\middle|\; T_N^N < T_0^N\right) = P_x(T_1 \le t \mid T_1 < T_0). \tag{8.2}$$

We first show how Lemma 8.1 implies Theorem 2.2.

PROOF OF THEOREM 2.2 GIVEN LEMMA 8.1. The first sum in (8.1) can be written

$$\sum_{i=1}^{N-1} \omega_i^N p_N^k(i,N) = \sum_{i=1}^{N-1} \frac{p_N^k(i,N)}{s(i/N)} s\left(\frac{i}{N}\right)\omega_i^N. \tag{8.3}$$

Let $i = i_N$ be integers with $1 \le i_N \le N$ and $i/N \to x$, and assume $k/N^2 \to t$ as in Section 2. Then

$$\begin{aligned}
\frac{p_N^k(i,N)}{s(i/N)} &= \frac{h_N(i)}{s(i/N)} P_i(X_k^N = N \mid T_N^N < T_0^N) \\
&= \left(\frac{h_N(i)}{s(i/N)}\right) P_i\left(\frac{1}{N^2} T_N^N \le \frac{k}{N^2} \;\middle|\; T_N^N < T_0^N\right) \\
&\to \frac{1}{s(1)} P_x(T_1 \le t \mid T_1 < T_0)
\end{aligned}$$

by Lemma 5.3 and (8.2), with uniform convergence for $0 \le x \le 1$ if $i_N = i_N(x) = \min\{[Nx]+1, 1\}$. Thus by (8.3) and (2.12)

$$\begin{aligned}
\lim_{N\to\infty} \sum_{i=1}^{N-1} \omega_i^N p_N^k(i,N) &= \frac{1}{s(1)} \int_0^1 P_x(T_1 \le t \mid T_1 < T_0) s(x) \nu(dx) \\
&= \int_0^1 P_x(T_1 \le t \mid T_1 < T_0) P_x(T_1 < T_0) \nu(dx) \qquad (8.4) \\
&= \int_0^1 P_x(T_1 \le t) \nu(dx).
\end{aligned}$$

The second term on the right-hand side of (8.1) is

$$\begin{aligned}
&\mu_N \sum_{r=0}^{k-1} p_N^r(1,N) \\
&\quad = \mu_N h_N(1) \sum_{r=0}^{k-1} P_1(T_N^N \le r \mid T_N^N < T_0^N) \\
&\quad = (N\mu_N)(Nh_N(1)) \int_0^{k/N^2} P_1\Big(\frac{1}{N^2} T_N^N \le \frac{[N^2 u]}{N^2} \,\Big|\, T_N^N < T_0^N\Big)\, du \\
&\quad \to \frac{\theta}{s(1)} \int_0^t P_0(T_1 \le u \mid T_1 < T_0)\, du
\end{aligned} \tag{8.5}$$

by Lemma 8.1, since $N\mu_N \to \theta$ and $Nh_N(1) \to 1/s(1)$ as in (7.9). Combining (8.4) and (8.5) completes the proof of Theorem 2.2. □

PROOF OF LEMMA 8.1. Assume $1 \le i_N \le N$ and $i_N/N \to x$ for $0 \le x \le 1$ as before. Then by Theorem 6.2

$$\lim_{N\to\infty} \widetilde{E}_{i_N}\big(f\big(Y^N_{[N^2 t]}\big)\big) = \widetilde{E}_x(f(X_t)),$$

if $f \in C[0,1]$, $0 \le f(x) \le 1$, and $f(1) = 0$. Since $N$ is a trap, this implies

$$\widetilde{P}_x(T_1 > t) \le \liminf_{N\to\infty} \widetilde{P}_{i_N}\Big(\frac{1}{N^2} T_N^N > t\Big) \tag{8.6}$$

and

$$\widetilde{E}_x(T_1) \le \liminf_{N\to\infty} \widetilde{E}_{i_N}\Big(\frac{1}{N^2} T_N^N\Big)$$

by two applications of Fatou's theorem. If we are able to prove

$$\widetilde{E}_x(T_1) = \lim_{N\to\infty} \widetilde{E}_{i_N}\Big(\frac{1}{N^2} T_N^N\Big) < \infty, \tag{8.7}$$

then a standard compactness argument for weak convergence would imply equality in (8.6) with lim inf replaced by lim, which would prove Lemma 8.1. Hence it is sufficient to prove (8.7).

Consider an arbitrary birth-and-death Markov chain $X_n$ on the state space $S = \{0, 1, \dots, N\}$ with absorbing endpoints. As in the Moran model in (2.1), assume that the transition function can be written

$$p_{ij} = \begin{cases} q_i : j = i+1, \\ r_i : j = i, \\ p_i : j = i-1, \end{cases} \tag{8.8}$$

where $p_{00} = p_{NN} = 1$, $p_i + r_i + q_i = 1$, and $p_i, q_i > 0$ for $1 \le i < N$ (see, e.g., [23], pages 50, 92–94). Then we have the following lemma.

LEMMA 8.2. *Let* $g_{ij} = \sum_{n=0}^{\infty} p_{ij}^{(n)}$ *where* $p^{(n)}$ *are the matrix powers of* $p$ *in* (8.8). *Then*

$$h_i = P_i(T_N < T_0) = \left(\sum_{j=1}^{i-1} \alpha_j\right) \Big/ \left(\sum_{j=1}^{N-1} \alpha_j\right) = A_i/A_N,$$
$$g_{ij} = A_N \frac{h_{i\wedge j}(1 - h_{i\vee j})}{\alpha_j q_j} \tag{8.9}$$

*for* $1 \le i, j \le N-1$, $i \wedge j = \min\{i, j\}$ *and* $i \vee j = \max\{i, j\}$. *Here*

$$\alpha_j = \prod_{k=1}^{j} (p_k/q_k) \quad \textit{and} \quad A_i = \sum_{j=0}^{i-1} \alpha_j, \qquad (1 \le i \le N). \tag{8.10}$$

PROOF. The probabilities $h_i = P_i(T_N < T_0)$ satisfy the recurrence $h_i = E_i(h_{X_1}) = p_i h_{i-1} + r_i h_i + q_i h_{i+1}$ for $0 < i < N$. This implies $h_{i+1} - h_i = (p_i/q_i)(h_i - h_{i-1})$ and hence $h_{i+1} - h_i = \alpha_i(h_1 - h_0)$. By summation, $h_i - h_0 = A_i(h_1 - h_0)$. The conditions $h_0 = 0$ and $h_N = 1$ imply $h_1 = 1/A_N$ and hence $h_i = A_i/A_N$.

The Green matrix $g_{ij}$ satisfies the recurrence

$$\sum_{j=0}^{N} p_{ir} g_{r,j} = p_i g_{i-1,j} + r_i g_{ij} + q_i g_{i+1,j} = g_{ij} - \delta_{ij}$$

and thus $g_{i+1,j} - g_{ij} = (p_i/q_i)(g_{i,j} - g_{i-1,j}) - \delta_{ij}/q_j$. The boundary conditions $g_{0j} = g_{Nj} = 0$ for $0 < j < N$ and arguments similar to those for $h_i$ lead to the formula for $g_{ij}$. □

For the Moran model (2.1), $p_k/q_k = 1/(1+\sigma_N)$, $\alpha_j = (1+\sigma_N)^{-j}$, and $A_i = \frac{1+\sigma_N}{\sigma_N}(1 - (1+\sigma_N)^{-i})$. Thus by (8.9)

$$h_N(i) = P_i(T_N^N < T_0^N) = \frac{A_i}{A_N} = \frac{1 - (1+\sigma_N)^{-i}}{1 - (1+\sigma_N)^{-N}}, \tag{8.11}$$

which gives a second derivation of Lemma 5.1.

If $f(i) = 1$ for $0 < i < N$ and $f(0) = f(N) = 0$, then

$$E_i(T_N^N) = E_i\left(\sum_{k=0}^{T_N^N - 1} f(X_k)\right) = \sum_{k=0}^{\infty} E_i(f(X_k))$$
$$= \sum_{j=1}^{N-1} g_N(i, j), \tag{8.12}$$

where $g_N(i,j) = \sum_{k=0}^{\infty} p_N^k(i,j)$. Then by (8.12) for the dual Markov chain in (6.1) and Lemma 8.2

$$\begin{aligned}
\widetilde{E}_i\Big(\frac{1}{N^2}T_N^N\Big) &= \frac{1}{N^2}\sum_{j=1}^{N-1}\widetilde{g}_N(i,j) = \frac{1}{Nh_N(i)}\frac{1}{N}\sum_{j=1}^{N-1} g_N(i,j)h_N(j) \\
&= \frac{A_N}{Nh_N(i)}\frac{1}{N}\sum_{j=1}^{N-1}\big(h_N(i\wedge j)\big(1-h_N(i\vee j)\big)\big)\frac{h_N(j)}{\alpha_j q_j} \\
&= \frac{1-(1+\sigma_N)^{-N}}{\sigma_N N h_N(i)} \\
&\quad\times\frac{1}{N}\sum_{j=1}^{N-1}\Bigg(\Big(1+\sigma_N\frac{j}{N}\Big)\big(h_N(i\wedge j)\big(1-h_N(i\vee j)\big)\big) \\
&\qquad\qquad\times h_N(j)\frac{(1+\sigma_N)^j}{j/N(1-j/N)}\Bigg)
\end{aligned} \tag{8.13}$$

by (2.1) and (8.9), since $\alpha_j = (1+\sigma_N)^{-j}$. By Lemma 5.3

$$\lim_{N\to\infty}\frac{h_N(i)(1-h_N(j))}{s(i/N)(s(1)-s(j/N))} = \frac{1}{s(1)^2}$$

uniformly for $1 \le i, j \le N-1$. Since $\sigma_N \sim \gamma/N$ by (2.9), the terms in the sum in (8.13) are uniformly bounded. Then by Lemma 5.3 again, with $i/N \to x$ and $j/N \to y$ in (8.13),

$$\begin{aligned}
&\lim_{N\to\infty}\widetilde{E}_i\Big(\frac{1}{N^2}T_N^N\Big) \\
&\quad= \Big(\frac{1-e^{-\gamma}}{\gamma}\Big)\frac{1}{s(x)}\int_0^1\frac{s(x\wedge y)(1-s(x\vee y))}{s(1)^2}s(y)\frac{e^{\gamma y}}{y(1-y)}\,dy \\
&\quad= \frac{1}{s(x)}\int_0^1 g(x,y)s(y)m(dy) = \int_0^1\widetilde{g}(x,y)\widetilde{m}(dy) = \widetilde{E}_x(T_1)
\end{aligned} \tag{8.14}$$

for $g(x,y)$ in (4.3), $m(dx)$ in (4.2), $\widetilde{g}(x,y)$ in (6.8) and $\widetilde{m}(dx)$ in (6.5). This completes the proof of (8.7) and hence of Lemma 8.1 and Theorem 2.2. □

8.2. *Proof of* (2.17) *after Theorem* 2.2. For fixed $t > 0$

$$\begin{aligned}
\widetilde{P}_x(T_1 \le t) &= 1 - \widetilde{P}_x(T_1 > t) = 1 - \int_0^1 q(t,x,y)\widetilde{m}(dy) \\
&= 1 - \int_0^1\frac{p(t,x,y)}{s(x)}s(y)m(dy)
\end{aligned} \tag{8.15}$$

by (6.8). Thus

$$\begin{aligned} P_x(T_1 \le t) &= P_x(T_1 < T_0) P_x(T_1 \le t \mid T_1 < T_0) \\ &= \big(s(x)/s(1)\big) \widetilde{P}_x(T_1 \le t) \\ &= \frac{1}{s(1)} \bigg( s(x) - \int_0^1 p(t, x, y) s(y) m(dy) \bigg). \end{aligned}$$

By (4.3), (4.8) and (4.6)

$$\left| \frac{d\alpha_n(x)}{ds(x)} \right| \le \lambda_n \int_0^1 |\alpha_n(y)| m(dy) \le C_3 \lambda_n^3$$

and

$$\frac{\partial}{\partial s(x)} p(t, x, y) = \sum_{n=1}^{\infty} e^{-\lambda_n t} \frac{d\alpha_n(x)}{ds(x)} \alpha_n(y) \tag{8.16}$$

converges uniformly for $t \ge a > 0$ and $0 \le x, y \le 1$. Thus by (8.15)

$$\begin{aligned} \widetilde{P}_0(T_1 \le t) &= 1 - \lim_{a \to 0} \int_0^1 \frac{p(t, a, y)}{s(a)} s(y) m(dy) \\ &= 1 - \int_0^1 \frac{\partial}{\partial s(x)} p(t, 0+, y) s(y) m(dy) \qquad (8.17) \\ &= 1 - \int_0^1 q(t, 0, y) s(y)^2 m(dy). \end{aligned}$$

## REFERENCES


[1] ABEL, H. J. (2009). The role of positive selection in molecular evolution: Alternative models for within-locus selective effects. Ph.D. thesis, Washington Univ. in St. Louis.

[2] AKASHI, H. (1999). Inferring the fitness effects of DNA mutations from polymorphism and divergence data: Statistical power to detect directional selection under stationarity and free recombination. *Genetics* **151** 221–238.

[3] BAINES, J. F., SAWYER, S. A., HARTL, D. L. and PARSCH, J. (2008). Effects of X-linkage and sex-biased gene expression on the rate of adaptive protein evolution in Drosophila. *Mol. Biol. Evol.* **25** 1639–1650.

[4] BIERNE, N. and EYRE-WALKER, A. (2004). The genomic rate of adaptive amino acid substitution in *Drosophila*. *Mol. Biol. Evol.* **21** 1350–1360.

[5] BOYKO, A. R., WILLIAMSON, S. H., INDAP, A. R., DEGENHARDT, J. D., HERNANDEZ, R. D. ET AL. (2008). Assessing the evolutionary impact of amino acid mutations in the human genome. *PLoS Genetics* **4** e1000083.

[6] BUSTAMANTE, C. D., WAKELEY, J., SAWYER, S. A. and HARTL, D. L. (2001). Directional selection and the site-frequency spectrum. *Genetics* **159** 1779–1788.

[7] BUSTAMANTE, C. D., NIELSEN, R., SAWYER, S. A., PURUGGANAN, M. D., OLSEN, K. M. and HARTL, D. L. (2002). The cost of inbreeding: Fixation of deleterious genes in Arabidopsis. *Nature* **416** 531–534.

[8] BUSTAMANTE, C. D., NIELSEN, R. and HARTL, D. L. (2003). Maximum likelihood and Bayesian methods for estimating the distribution of selective effects among classes of mutations using DNA polymorphism data. *Theory Popul. Biol.* **63** 91–103.

[9] CAICEDO, A. L., WILLIAMSON, S. H., HERNANDEZ, R. D., BOYKO, A., FLEDEL-ALON, A. ET AL. (2007). Genome-wide patterns of nucleotide polymorphism in domesticated rice. *PLoS Genetics* **3** e163.

[10] DUNFORD, C. and SCHWARTZ, J. (1958). *Linear Operators. Part I: General Theory*. Interscience, New York. MR0117523

[11] DYNKIN, E. B. (2006). *Theory of Markov Processes*. Dover, Mineola, NY. MR2305744

[12] DURRETT, R. (2002). *Probability Models for DNA Sequence Evolution*. Springer, New York. MR1903526

[13] ETHIER, S. N. and KURTZ, T. G. (1986). *Markov Processes: Characterization and Convergence*. Wiley, New York. MR838085

[14] EWENS, W. J. (2004). *Mathematical Population Genetics*, 2nd ed. Springer, New York.

[15] EYRE-WALKER, A. and KEIGHTLEY, P. D. (2007). The distribution of fitness effects of new mutations. *Nat. Rev. Genet.* **8** 610–618.

[16] FELLER, W. (1952). The parabolic differential equations and the associated semi-groups of transformations. *Ann. of Math.* (2) **55** 468–519. MR0047886

[17] FELLER, W. (1955). On second order differential operators. *Ann. of Math.* (2) **61** 90–105. MR0068082

[18] HARTL, D. L. (2000). *A Primer of Population Genetics*, 3rd ed. Sinauer, Sunderland, MA.

[19] HARTL, D. L. and CLARK, A. (2007). *Principles of Population Genetics*, 4th ed. Sinauer, Sunderland, MA.

[20] HARTL, D. L., MORIYAMA, E. N. and SAWYER, S. A. (1994). Selection intensity for codon bias. *Genetics* **138** 227–234.

[21] HUERTA-SANCHEZ, E., DURRETT, R. and BUSTAMANTE, C. D. (2008). Population genetics of polymorphism and divergence under fluctuating selection. *Genetics* **178** 325–337.

[22] ITÔ, K. and MCKEAN, H. P. JR. (1965). *Diffusion Processes and Their Sample Paths*. Academic Press, New York. MR0199891

[23] KARLIN, S. and TAYLOR, H. M. (1981). *A Second Course in Stochastic Processes*. Academic Press, New York. MR611513

[24] KEIGHTLEY, P. D. (1994). The distribution of mutation effects on viability in Drosophila melanogaster. *Genetics* **138** 1315–1322.

[25] KEIGHTLEY, P. D. and EYRE-WALKER, A. (2007). Joint inference of the distribution of fitness effects of deleterious mutations and population demography based on nucleotide polymorphism frequencies. *Genetics* **177** 2251–2261.

[26] KEMENY, J. G., SNELL, J. L. and KNAPP, A. W. (1966). *Denumerable Markov Chains*. Van Nostrand, New York. MR0207042

[27] KIMURA, M. (1955). Solution of a process of random genetic drift with a continuous model. *Proc. Natl. Acad. Sci. USA* **41** 144–150.

[28] KINGMAN, J. F. C. (1993). *Poisson Processes. Oxford Studies in Probability* **3**. Oxford Univ. Press, New York. MR1207584

[29] LEWONTIN, R. C. (1974). *The Genetic Basis of Evolutionary Change*. Columbia Univ. Press, New York.

[30] LI, W. H. (1997). *Molecular Evolution*. Sinauer, Sunderland, MA.

[31] MCDONALD, J. H. and KREITMAN, M. (1991). Adaptive protein evolution at the *Adh* locus in *Drosophila*. *Nature* **351** 652–654.

[32] MORAN, P. A. P. (1959). The survival of a mutant gene under selection. *J. Aust. Math. Soc.* **1** 121–126. MR0105747

[33] NIELSEN, R., BUSTAMANTE, C., CLARK, A. G., GLANOWSKI, S., SACKTON, T. B. ET AL. (2005). A scan for positively selected genes in the genomes of humans and chimpanzees. *PLoS Biology* **3** e170.

[34] PRESS, W. H., TEUKOLSKY, S. A., VETTERLING, W. T. and FLANNERY, B. P. (2007). *Numerical Recipes*: *The Art of Scientific Computing*, 3rd ed. Cambridge Univ. Press, Cambridge. MR2371990

[35] PROTTER, M. H. and WEINBERGER, H. F. (1967). *Maximum Principles in Differential Equations*. Prentice Hall, Englewood Cliffs, NJ. MR0219861

[36] RIESZ, F. and SZ. NAGY, B. (1955). *Functional Analysis*. Frederick Ungar Publishing, New York. MR0071727

[37] SAWYER, S. (1974). A Fatou theorem for the general one-dimensional parabolic equation. *Indiana Univ. Math. J.* **24** 451–498. MR0350880

[38] SAWYER, S. A. and HARTL, D. L. (1992). Population genetics of polymorphism and divergence. *Genetics* **132** 1161–1176.

[39] SAWYER, S. A. (1994). Inferring selection and mutation from DNA sequences: The McDonald–Kreitman test revisited. In *Non-Neutral Evolution*: *Theories and Molecular Data* (G. B. Golding, ed.) 77–87. Chapman and Hall, New York.

[40] SAWYER, S. A., KULATHINAL, R. J., BUSTAMANTE, C. D. and HARTL, D. L. (2003). Bayesian analysis suggests that most amino acid replacements in Drosophila are driven by positive selection. *J. Mol. Evol.* **57** S154–S164.

[41] SAWYER, S. A., PARSCH, J., ZHANG, Z. and HARTL, D. L. (2007). Prevalence of positive selection among nearly neutral amino acid replacements in Drosophila. *Proc. Natl. Acad. Sci. USA* **104** 6504–6510.

[42] SMITH, N. G. C. and EYRE-WALKER, A. (2002). Adaptive protein evolution in *Drosophila*. *Nature* **415** 1022–1024.

[43] TEMPLETON, A. R. (1996). Contingency tests of neutrality using intra/interspecific gene trees: The rejection of neutrality for the evolution of the mitochondrial cytochrome oxidase II gene in the hominoid primates. *Genetics* **144** 1263–1270.

[44] TROTTER, H. F. (1958). Approximation of semi-groups of operators. *Pacific J. Math.* **8** 887–919. MR0103420

[45] WAKELEY, J. (2003). Polymorphism and divergence for island-model species. *Genetics* **163** 411–420.

[46] WILLIAMSON, S., ALON, A. F. and BUSTAMANTE, C. D. (2004). Population genetics of polymorphism and divergence for diploid selection models with arbitrary dominance. *Genetics* **168** 463–475.

[47] WILLIAMSON, S., HERNANDEZ, R., ALON, A. F., ZHU, L., NIELSEN, R. and BUSTAMANTE, C. D. (2005). Simultaneous inference of selection and population growth from patterns of variation in the human genome. *Proc. Natl. Acad. Sci. USA* **102** 7882–7887.

[48] WRIGHT, S. (1938). The distribution of gene frequencies under irreversible mutation. *Proc. Natl. Acad. Sci. USA* **24** 253–259.

[49] ZHU, L. and BUSTAMANTE, C. D. (2005). A composite-likelihood approach for detecting directional selection from DNA sequence data. *Genetics* **170** 1411–1421.

DEPARTMENT OF MATHEMATICAL SCIENCES
UNIVERSITY OF NEVADA, LAS VEGAS
LAS VEGAS, NEVADA 89154
USA
E-MAIL: amei.amei@unlv.edu

DEPARTMENT OF MATHEMATICS
WASHINGTON UNIVERSITY IN ST. LOUIS
ST. LOUIS, MISSOURI 63130
USA
E-MAIL: sawyer@math.wustl.edu