1 The Behavior of Molecular Measures of Natural
2 Selection after a Change in Population Size

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ABSTRACT

A common model to describe natural selection at the molecular level is the nearly neutral theory, which emphasizes the importance of mutations with slightly deleterious fitness effects as they have a chance to get fixed due to genetic drift. Since genetic drift is stronger in smaller than in larger populations, a negative relationship between molecular measures of selection and population size is expected within the nearly neutral theory. Originally, this hypothesis was formulated under equilibrium conditions. A change in population size, however, pushes the selection-drift balance off equilibrium leading to alterations in the efficacy of selection. To investigate the nonequilibrium behavior, we relate measures of natural selection and genetic drift to each other, considering both, measures of micro- and macroevolution. Specifically, we use a Poisson random field framework to model π_N/π_S and ω as time-dependent measures of selection and assess genetic drift by an effective population size. This analysis reveals a clear deviation from the expected equilibrium selection-drift balance during nonequilibrium periods. Moreover, we find that microevolutionary measures quickly react to a change in population size and reflect a recent change well, at the same time as they quickly lose the knowledge about it. Macroevolutionary measures, on the other hand, react more slowly to a change in population size but instead capture the influence of ancient changes longer. We therefore conclude that it is important to be aware of the different behaviors of micro- and macroevolutionary measures when making inference in empirical studies, in particular when comparing results between studies.

INTRODUCTION

Among the key driving factors of evolution are mutations, selection, and genetic drift. The analysis of the interplay between them therefore provides valuable understanding on a population's ability to evolve and adapt. Population genetics theory predicts that the strength of genetic drift is weaker in larger populations than in smaller populations, due to the stochastic nature of reproduction (Wright 1931; Kimura 1964). This results in a positive correlation between the efficacy of selection and population size, and hence deleterious mutations are more likely to go extinct due to purifying selection if population size is large. This relationship can for example explain why asexual (Lynch *et al.* 1993; Lumley *et al.* 2015), small, or endangered populations (Lynch *et al.* 1995; Lachapelle *et al.* 2017) have a higher risk to go extinct or suffer from genetic diseases (Karlsson *et al.* 2014; Seppälä 2015).

The relationship between the efficacy of selection and population size also provides the foundation for the nearly neutral theory of molecular evolution (Ohta 1973, 1976, 1992). In this framework, typically the distribution of fitness effects (DFE) of new mutations is weighted towards purifying selection: most mutations are deleterious, of which a nonnegligible amount is slightly deleterious, and only a small proportion of mutations is advantageous. While strongly deleterious mutations rarely segregate in the population or get fixed, slightly deleterious mutants contribute significantly to segregating polymorphisms and can eventually get fixed due to genetic drift. The fact that only a small fraction of mutants is under positive selection makes the nearly neutral theory predict a negative correlation between molecular measures of natural selection and population size. The validity of this hypothesis can be tested

67 by comparing measures of selection and genetic drift, and has been supported
68 across a wide range of taxa (e.g. Hughes 2008; Elyashiv *et al.* 2010; Figuet
69 *et al.* 2016; Chen *et al.* 2017; Bolívar *et al.* 2019).

70 The traditional approach to assessing genetic drift is to apply some con-
71 cept of an effective population size, N_{eff} . The concept of N_{eff} is used to com-
72 pare a given (non-ideal) population with a simpler idealized reference model,
73 such as the ideal Wright-Fisher model, with respect to a particular property.
74 This leads to different definitions of N_{eff} , e.g. inbreeding and variance (Wright
75 1931; Crow and Kimura 1970) or eigenvalue effective size (Ewens 1979). All
76 approaches predict N_{eff} under different circumstances as for example certain
77 spatial and temporal scales and demographic scenarios. Most often it is not
78 evident whether underlying assumptions of the various models are met and
79 how accurate resulting estimates of N_{eff} are when assumptions are violated.
80 For this, the spatial and temporal scales of different estimates of N_{eff} have
81 to be interpreted (Wang *et al.* 2016) to draw the right conclusions. These
82 issues are discussed frequently in the literature (Ewens 1969; Sjödin *et al.*
83 2004; Waples 2005; Wang *et al.* 2016; Galtier and Rousselle 2020) confirming
84 the need for more clarification in this field.

85 To detect evidence of selection in genome data, different approaches and
86 methods have been developed. A common feature of them is to contrast
87 neutral reference and test data. Under the assumption that synonymous
88 mutations are neutral, the contrast between synonymous and nonsynony-
89 mous mutations in protein-coding sequences provides a relevant approach.
90 Measures of natural selection at the microevolutionary timescale, which are
91 based on polymorphisms, are designed to identify selective events within a

92 species and give a *snapshot* of the current state. A popular representative is
 93 the ratio of nonsynonymous and synonymous diversity, π_N/π_S (Nei and Li
 94 1979). Other measures look at interspecific differences in form of fixations
 95 that arise through events which happened in a lineage since a speciation
 96 event. Those measures are *accumulative* and give insight into the macroevo-
 97 lution of a population or species. A measure that belongs to this group is the
 98 ratio of the nonsynonymous and synonymous fixation rate, ω , commonly as-
 99 sessed by the ratio of nonsynonymous and synonymous sequence divergence
 100 (Goldman and Yang 1994; Muse and Gaut 1994).

101 The nearly neutral theory predicts a negative correlation between N_{eff}
 102 and the measures π_N/π_S and ω : the smaller N_{eff} the larger the ratios, i.e. the
 103 less efficient selection. However, the prediction is based on the equilibrium
 104 assumption, where the effect of genetic drift on segregating sites balances
 105 the efficacy of selection in a stable manner implying a constant evolutionary
 106 rate. Yet there are many factors that can cause a nonequilibrium such as
 107 a change in population size, population structure or migration, a change
 108 in recombination rate, or environmental variation (Brandvain and Wright
 109 2016). These events disturb the selection-drift balance.

110 Several empirical studies (Lu *et al.* 2006; Campos *et al.* 2014; Hollister
 111 *et al.* 2014; Renaut and Rieseberg 2015; Leroy *et al.* 2020; Galtier and Rous-
 112 selle 2020) provide evidence that the efficacy of natural selection is limited in
 113 nonequilibrium conditions. In a meta-analysis, Brandvain and Wright (2016)
 114 compare predictions of classical theory with results from a large number of
 115 data analyses, which stresses the need of care for nonequilibrium conditions
 116 when testing for differences in selection efficacy between species. To enable

such care to be taken, theoretical models are a critical tool to provide conceptual understanding of the interplay between selection and genetic drift and the extent of nonequilibrium periods. Given the mathematical challenges in modeling natural selection in nonequilibrium scenarios, such studies are still rare. [Gordo and Dionisio \(2005\)](#), for example, develop a nonequilibrium model to estimate parameters of deleterious mutations, which can be applied to quantify the mutation rate to deleterious alleles. Alternatively, simulations can be used to provide observational insight into the nonequilibrium behavior of measures of evolution. [Rousselle *et al.* \(2018\)](#) investigate the rate of adaptive molecular evolution in fluctuating population size and find that this violation of the equilibrium assumption can lead to a severely biased inference based on current methodology.

In this study we investigate the effect of a change in population size on micro- and macroevolutionary measures of selection in an otherwise ideal population. While being aware of other factors that lead to a nonequilibrium, concentrating on this isolated aspect provides conceptual understanding that is straight forward to interpreted. For this purpose, we first introduce a mathematical model and state and derive relevant quantities needed to model measures of selection in nonequilibrium. Among these, an important statistic is the allele frequency spectrum (AFS) from which other measures such as nucleotide diversity can be derived. Several different approaches exist to model the AFS in nonequilibrium ([Evans *et al.* 2007](#); [Živković and Stephan 2011](#); [Živković *et al.* 2015](#); [Kaj and Mugal 2016](#)). We here build on the Poisson random field framework approach as in [Kaj and Mugal \(2016\)](#). With the help of this framework, the above addressed measures, π_N/π_S and ω ,

are formulated as functions of time, $(\pi_N/\pi_S)(t)$ and $\omega(t)$. Equipped with the analytical model, we discuss the following questions. First, how does a change in population size affect micro- and macroevolutionary measures of natural selection, $(\pi_N/\pi_S)(t)$ and $\omega(t)$? Second, does the prediction of the nearly neutral theory also hold in a nonequilibrium? To this end we investigate different choices of N_{eff} as measures of genetic drift. Finally, we discuss the relevance of micro- and macroevolutionary measures in the study of natural selection.

MODELS AND METHODS

Model formulation

Let N represent the population size of a haploid population—which under the assumption of additive fitness effects in a diploid organism, is equivalent to a diploid population of size $N/2$. We consider the evolution of a population that undergoes an instantaneous change in population size at a single point in time t^* from constant size N to constant size κN , where κ is a positive parameter. In other words, the population size over time is a step function N_κ such that $N_\kappa(t) = N$, $t < t^*$, and $N_\kappa(t) = \kappa N$, $t \geq t^*$. Throughout this work we use N as reference size and apply an evolutionary timescale where t units of time correspond to $[Nt]$ generations. Each individual is characterized by L independent sites, which corresponds to the assumption of free recombination between sites. Random mutations arrive independently and uniformly over individuals on mono-allelic sites with population mutation intensity θ per generation in the reference population. Hence, as long as $t \leq t^*$, the mutation intensity per time unit is θN . Consequently, for $t \geq t^*$,

the mutation intensity is $\kappa\theta$ per generation and $\kappa\theta N$ per time unit. Each mutation is assigned a population selection intensity γ .

We use the Wright-Fisher model with selection (Fisher 1930; Wright 1931) for two alleles segregating at one site to model reproduction and then study the population dynamics of the collection of all L independent sites. In the limit as L tends to infinity and N is large but fixed, the Poisson random field approximation (Sawyer and Hartl 1992; Kaj and Mugal 2016) applies. This means that the number of new mutations over all mono-allelic sites is approximately Poisson distributed with mean θN_κ per time unit. The derived allele frequencies in a polymorphic site starting at time s evolve as independent Wright-Fisher diffusion processes with selection, that is, solutions of the stochastic differential equation

$$d\xi_t^s = \gamma \xi_t^s (1 - \xi_t^s) dt + \sqrt{\frac{1}{N_\kappa(t)/N}} \xi_t^s (1 - \xi_t^s) dB_t, \quad t \geq s, \quad (1)$$

with initial value $\xi_s^s = y \in (0, 1)$, where B_t is a standard Brownian motion. Here, $N_\kappa(t)/N = 1$ whenever $t \leq t^*$ and $N_\kappa(t)/N = \kappa$ for $t > t^*$. We denote such a Markov process by $(\xi_t^s)_{t \geq s}$ or simply $(\xi_t)_{t \geq 0}$ when the initial time is $s = 0$. Furthermore, let $\mathbb{P}_y^{\gamma, \kappa}$ and $\mathbb{E}_y^{\gamma, \kappa}$ be the law and expectation of processes $(\xi_t)_t$ that start in y , have selective pressure γ , and evolve in a population of size κN . Let τ_1 be the time to fixation of the derived allele. The fixation probability is given by (Kimura 1962)

$$q_{\gamma, \kappa}(y) = \mathbb{P}_y^{\gamma, \kappa}(\tau_1 < \infty) = \frac{1 - e^{-2\gamma\kappa y}}{1 - e^{-2\gamma\kappa}}, \quad \gamma \neq 0, \quad q_{0, \kappa}(y) = y. \quad (2)$$

As $N \rightarrow \infty$, the scaled fixation rate emerges as

$$Nq_{\gamma, \kappa}(1/N) \rightarrow \omega_{\gamma, \kappa} = \frac{2\gamma\kappa}{1 - e^{-2\gamma\kappa}}, \quad \gamma \neq 0, \quad \omega_{0, \kappa} = 1. \quad (3)$$

Figure 1: Polymorphisms and fixations in $[0, t]$. Vertical lines as for example at t^* represent a snapshot of the population dynamics and the distribution of allele frequencies at that specific point in time are summarized in the AFS. Polymorphisms in grey go to extinction or still segregate at t . Polymorphisms leading to a substitution are colored: fixations before t^* (blue), fixations after t^* emerging from segregating polymorphisms at t^* (orange), and fixations of mutations that arose after the change in population size (green).

186 This means, in an equilibrium population of size κN , the fixation rate ratio
 187 of a class of selected (with selective pressure γ) and neutral mutations in the
 188 limit equals $\omega_{\gamma, \kappa}$.

189 Returning to the Poisson random field setting, the allele frequencies are
 190 represented by Poisson points (s, ξ^s) on the collection of sites according to
 191 the Poisson distribution with intensity θN_κ : once such a mutation event
 192 takes place at a certain time s , a path $(\xi_t^s)_{t \geq s}$ is initialized at frequency
 193 $1/N_\kappa(s)$. We fix $t^* > 0$ and represent the state of the Poisson random field
 194 at time t as a random measure $\mathcal{X}_t^{N_\kappa}(dy)$ on $(0, 1]$. For $t \leq 0$, however, we will
 195 ignore fixations but start from polymorphic frequencies on $(0, 1)$ at $t = 0$.
 196 A visualization of the setup is presented in Fig. 1. It is known that the
 197 aggregate of all mutations from the infinite past in the ancestral population

198 together build up a Poisson measure in steady state. Let

$$\psi_{\gamma,\kappa}(y) = \frac{1 - e^{-2\gamma\kappa(1-y)}}{\gamma y(1-y)}, \quad \gamma \neq 0, \quad \psi_{0,\kappa}(y) = \frac{2\kappa}{y}. \quad (4)$$

199 Now the relevant initial distribution at $t = 0$ for our model, that is $\mathcal{X}_0^{N_\kappa}(dy)$,
 200 is a Poisson measure with intensity measure $\omega_{\gamma,1} \psi_{\gamma,1}(y) dy$ on $(0, 1)$. The
 201 initial distribution at $t = 0$ plus the arrival of new mutations during $(0, t]$
 202 together preserve the Poisson distribution which is invariant as long as the
 203 population size does not change, i.e. for $0 < t \leq t^*$. To account for fixations
 204 during $[0, t]$ we also include the singular contribution at $y = 1$, $\mathcal{X}_t^{N_\kappa}(\{1\})$,
 205 $t \geq 0$, which is a Poisson counting process with time-inhomogeneous intensity.

206 Formally, we construct our model $\mathcal{X}_t^{N_\kappa}$ using stochastic Poisson integrals.
 207 The detailed presentation and most of the technical aspects are deferred to
 208 the appendix sections. For $0 < t \leq t^*$,

$$\begin{aligned} \langle \mathcal{X}_t^{N_\kappa}, f \rangle &= \int_0^1 \int_{\mathcal{D}} f(\xi_t^{0,y}) \mathcal{M}_\gamma(dy, d\xi^{0,y}) \\ &\quad + \int_0^t \int_0^1 \int_{\mathcal{D}} f(\xi_t^{s,y}) \mathcal{N}_{N_\kappa}(ds, dy, d\xi^{s,y}), \end{aligned} \quad (5)$$

209 for suitable functions $f \in \tilde{\mathcal{F}}$, specified in Appendix A, satisfying sufficient
 210 conditions for these integrals to be well defined. In particular, $f(0) = 0$.
 211 The class $\mathcal{D}$ is the path space for the diffusion processes $t \mapsto \xi_t$, consisting of
 212 functions $g: \mathbb{R} \rightarrow [0, 1]$ which are right continuous and have left limits. More-
 213 over, $\mathcal{M}_\gamma(dy, d\xi^{0,y})$ is a Poisson random measure on $[0, 1] \times \mathcal{D}$ with intensity
 214 $m_{\gamma,1}(dy, d\xi^{0,y}) = \theta \omega_{\gamma,1} \psi_{\gamma,1}(y) dy \mathbb{P}_y^{\gamma,1}(d\xi^{0,y})$ and $\mathcal{N}_{N_\kappa}(ds, dy, d\xi^{s,y})$ is a Poisson
 215 random measure on $\mathbb{R} \times [0, 1] \times \mathcal{D}$ with intensity measure $n_{N_\kappa}(ds, dy, d\xi^{s,y}) =$
 216 $\theta N_\kappa(s) ds \delta_{1/N_\kappa(s)}(dy) \mathbb{P}_y^{\gamma, N_\kappa/N}(d\xi^{s,y})$. The first term in Eq. (5) represents the
 217 family of ancestral allele frequencies with initial values at $t = 0$ given by

the Poisson measure $\mathcal{X}_0^{N_\kappa}(dy)$, $0 < y < 1$. The second term contains additional allele frequencies sparked off by mutations during $(0, t]$. Similarly, the collection of paths at some time $t > t^*$ consists of two contributions,

$$\begin{aligned} \langle \mathcal{X}_t^{N_\kappa}, f \rangle &= \int_0^1 \int_{\mathcal{D}} f(\xi_t^{t^*, y}) \mathcal{M}_{\gamma, \kappa}^{\mathcal{X}_{t^*}}(dy, d\xi^{t^*, y}) \\ &\quad + \int_{t^*}^t \int_0^1 \int_{\mathcal{D}} f(\xi_t^{s, y}) \mathcal{N}_{N_\kappa}(ds, dy, d\xi^{s, y}). \end{aligned} \quad (6)$$

Here, conditional on $\mathcal{X}_{t^*}$, $\mathcal{M}_{\gamma, \kappa}^{\mathcal{X}_{t^*}}(dy, d\xi^{t^*, y})$ is a Poisson random measure on $(0, 1] \times \mathcal{D}$ with intensity $\mathcal{X}_{t^*}^{N_\kappa}(dy) \mathbb{P}_y^{\gamma, \kappa}(d\xi^{t^*, y})$. This term represents the fate of the allele frequencies extending beyond t^* of all alleles, polymorphic or fixed, which were present at t^* . The additional term again covers new mutations taking place subsequent to the change in population size.

The nonequilibrium AFS

The AFS accounts for the collection of all derived allele frequencies across sites at a fixed point in time. The spectrum of allele frequencies y , $0 < y < 1$, represents the average intensity of attained frequency values at t , $\xi_t^s = y$ for some $s \leq t$, compare Fig. 1. In our approach we also include alleles which have reached fixation during $[0, t]$. As a reference case we begin with the equilibrium AFS, which arises as the scaled limit of expected values for the case of a fixed size population, say κN . Then,

$$\lim_{N \rightarrow \infty} \mathbb{E} \langle \mathcal{X}_t^{\kappa N}, f \rangle = \theta \omega_{\gamma, \kappa} f(1) t + \theta \int_0^1 f(y) \omega_{\gamma, \kappa} \psi_{\gamma, \kappa}(y) dy, \quad (7)$$

see Lemma 3 iii) in Appendix A. In particular, taking $\kappa = 1$, the limiting expected value of relation (5) is given by this linear function, as long as $t \leq t^*$. The linear term in t represents the effect of constant rate fixations and the

integral term independent of time represents the steady-state spectrum of polymorphic frequencies.

In order to analyze the nonequilibrium AFS caused by applying population size N_κ , we derive the limiting expected values of each term in Eq. (6), see Theorem 1, Appendix B. The limiting expected contribution from the ancestral component in Eq. (6), mutations which occurred prior to t^* , equals

$$\begin{aligned} \lim_{N \rightarrow \infty} \mathbb{E} \left[\int_0^1 \int_{\mathcal{D}} f(\xi_t^{t^*, y}) \mathcal{M}_{\gamma, \kappa}^{\mathcal{X}_{t^*}}(dy, d\xi^{t^*, y}) \right] \\ = \theta \omega_{\gamma, 1} f(1) t^* + \theta \int_0^1 \mathbb{E}_y^{\gamma, \kappa}[f(\xi_t^{t^*})] \omega_{\gamma, 1} \psi_{\gamma, 1}(y) dy. \end{aligned} \quad (8)$$

compare Eq. (B.1) in Theorem 1. Similarly, the limiting expected value of the latter contribution in Eq. (6) yields the nonstationary build-up AFS (Kaj and Mugal 2016, Theorem 1), that arises from a completely mono-allelic population. In Lemma 3 i) together with Theorem 1 we show

$$\begin{aligned} \lim_{N \rightarrow \infty} \mathbb{E} \left[\int_{t^*}^t \int_0^1 \int_{\mathcal{D}} f(\xi_t^{s, y}) \mathcal{N}_{N_\kappa}(ds, dy, d\xi^{s, y}) \right] \\ = \theta \omega_{\gamma, \kappa} f(1) (t - t^*) + \theta \int_0^1 (f(y) - \mathbb{E}_y^{\gamma, \kappa}[f(\xi_t^{t^*})]) \omega_{\gamma, \kappa} \psi_{\gamma, \kappa}(y) dy. \end{aligned} \quad (9)$$

While in these representations we do not see directly a spectrum of frequencies y with explicit weights affecting $f(y)$, we do see indirectly the time-dependence effect due to the nonequilibrium framework. By adding up both contributions we obtain our main result regarding the nonequilibrium AFS,

252 as

$$\begin{aligned} & \lim_{N \rightarrow \infty} \mathbb{E} \langle \mathcal{X}_t^{N_\kappa}, f \rangle \\ &= \theta f(1) \{ \omega_{\gamma,1} t^* + \omega_{\gamma,\kappa} (t - t^*) \} + \theta \int_0^1 f(y) \omega_{\gamma,\kappa} \psi_{\gamma,\kappa}(y) dy \\ &+ \theta \int_0^1 \mathbb{E}_y^{\gamma,\kappa} f(\xi_t^{t^*}) (\omega_{\gamma,1} \psi_{\gamma,1}(y) - \omega_{\gamma,\kappa} \psi_{\gamma,\kappa}(y)) dy, \end{aligned} \quad (10)$$

253 see Theorem 1 in Appendix B for a detailed derivation.

254 We make two remarks: for $f \in \tilde{\mathcal{F}}$ with the additional property $f(1) = 0$,
255 the time-dependent AFS provides a bridge between the “marginal” limit
256 spectra

$$\mathbb{E} \langle \mathcal{X}_{t^*}^{N_\kappa}, f \rangle \rightarrow \theta \int_0^1 f(y) \omega_{\gamma,1} \psi_{\gamma,1}(y) dy, \quad \mathbb{E} \langle \mathcal{X}_\infty^{N_\kappa}, f \rangle \rightarrow \theta \int_0^1 f(y) \omega_{\gamma,\kappa} \psi_{\gamma,\kappa}(y) dy.$$

257 For the case of neutral evolution, $\gamma = 0$, we have $\omega_{0,1} \psi_{0,1}(y) - \omega_{0,\kappa} \psi_{0,\kappa}(y) =$
258 $2(1 - \kappa)/y$ and hence, for $f \in \tilde{\mathcal{F}}$, $t \geq t^*$, and $N \rightarrow \infty$,

$$\mathbb{E} \langle \mathcal{X}_t^{N_\kappa}, f \rangle \rightarrow 2\theta(1 - \kappa) \int_0^1 y^{-1} \mathbb{E}_y^{0,\kappa} [f(\xi_t^{t^*})] dy + 2\theta\kappa \int_0^1 y^{-1} f(y) dy + \theta f(1)t.$$

259 Application of selected functions f to the nonequilibrium AFS in Eq. (10)
260 allows to retrieve expressions for relevant summary statistics, such as nu-
261 cleotide diversity and the fixation rate.

262 The ratio of nucleotide diversity during nonequilibrium

263 We derive and investigate the ratio of nucleotide diversity, π_N/π_S (Nei and
264 Li 1979), in a population undergoing a change in population size according to
265 N_κ . Nucleotide diversity measures the number of pairwise differences, which
266 entails integrating the specific function $f_{\text{pw}}(y) = 2y(1 - y)$ with respect to the
267 AFS. As a reference case we observe that during equilibrium in a population

controlled by a size parameter κ and a fixed selection coefficient $\gamma \neq 0$, we have by Eq. (7) with $f_{\text{pw}}(1) = 0$,

$$\pi_N^{\gamma, \kappa} = \theta \int_0^1 2y(1-y) \omega_{\gamma, \kappa} \psi_{\gamma, \kappa}(y) dy = 4\theta \kappa \left(\frac{1}{1 - e^{-2\gamma\kappa}} - \frac{1}{2\gamma\kappa} \right). \quad (11)$$

More generally, by applying Eq. (10), we obtain the time-dependent non-synonymous nucleotide diversity measure $\pi_N^{\gamma, \kappa}(t) = \lim_{N \rightarrow \infty} \mathbb{E} \langle \mathcal{X}_t^{N_\kappa}, f_{\text{pw}} \rangle$ in nonequilibrium.

In order to allow for variation in selection across sites for the non-synonymous diversity, we integrate the previous expressions over a distribution of fitness effects (DFE). We will denote the random variable generating the γ -values by $\mathcal{V}$ and assume it has a continuous density function $h_{\mathcal{V}}$. Because of the presumed rarity or negligibility of advantageous mutations at the genome-wide level within the nearly neutral theory, we focus on weak and strong purifying selection following Eyre-Walker *et al.* (2006); Loewe and Charlesworth (2006); Galtier and Rousselle (2020). A common choice of DFE in this scenario is the negative gamma distribution. The density function is

$$h_{\mathcal{V}}(v) = \frac{(-v)^{a-1} e^{v/b}}{b^a \Gamma(a)}, \quad v \leq 0, \quad (12)$$

with shape parameter $a > 0$, scale parameter $b > 0$, and mean $-ab$. Integration of the expression in Eq. (11) and $\pi_N^{\gamma, \kappa}(t)$ over this density yields an averaged diversity measure π_N^{κ} . Taken together it holds

$$\pi_N^{\kappa}(t) = \begin{cases} \mathbb{E} \pi_N^{\mathcal{V}, 1}, & t \leq t^*, \\ \mathbb{E} \pi_N^{\mathcal{V}, \kappa} + 2\theta \mathbb{E} \int_0^1 \mathbb{E}_y^{\mathcal{V}, \kappa} [\xi_t^{t^*} (1 - \xi_t^{t^*})] \\ \quad \times (\omega_{\mathcal{V}, 1} \psi_{\mathcal{V}, 1}(y) - \omega_{\mathcal{V}, \kappa} \psi_{\mathcal{V}, \kappa}(y)) dy, & t > t^*, \end{cases} \quad (13)$$

see Appendix C for details. The expectations in the above expression are used to indicate integration over the DFE. We observe that $\pi_N^\kappa(t)$ approaches a new equilibrium, $\pi_N^\kappa(t) \rightarrow \mathbb{E}\pi_N^{\mathcal{V},\kappa}$ as $t \rightarrow \infty$, since $\xi_t^{t^*} \in \{0, 1\}$ for $t \rightarrow \infty$. For the case of neutral evolution, $\gamma = 0$, the result simplifies considerably and we obtain the synonymous diversity as

$$\pi_S^\kappa(t) = \begin{cases} 2\theta, & t \leq t^*, \\ 2\theta\kappa + 2\theta(1 - \kappa)e^{-(t-t^*)/\kappa}, & t > t^*, \end{cases} \quad (14)$$

with $\pi_S^\kappa(t) \rightarrow 2\theta\kappa$ as $t \rightarrow \infty$. The ratio of nonsynonymous and synonymous diversity, which we denote $(\pi_N/\pi_S)^\kappa(t) := \pi_N^\kappa(t)/\pi_S^\kappa(t)$, is also time dependent and determined by Eqs. (13) and (14). The ratio in equilibrium will be denoted $(\pi_N/\pi_S)^\kappa$.

The fixation rate ratio during nonequilibrium

The nonsynonymous to synonymous fixation rate ratio of selected and neutral mutations (Goldman and Yang 1994; Muse and Gaut 1994), $\omega^\kappa(t)$, is determined by the ratio of the number of nonsynonymous and synonymous fixations in a finite interval. We therefore consider the number of fixations in the population during $[0, t]$ with a change in population size as before given by N_κ . To account for fixations in the random field setting, we wish to count all Poisson points (s, ξ^s) such that $\xi_t^s = 1$. In other words, we evaluate the indicator function $f_{\text{fix}}(y) := \mathbb{1}_{\{1\}}(y)$ at the nonequilibrium AFS, Eq. (10). Hence, the number of fixations in the population during $[0, t]$ with a change in population size given by N_κ is $Z^{\gamma,\kappa}(t) := \lim_{N \rightarrow \infty} \mathbb{E} \langle \mathcal{X}_t^{N_\kappa}, f_{\text{fix}} \rangle$. The decomposition of $\lim_{N \rightarrow \infty} \mathbb{E} \langle \mathcal{X}_t^{N_\kappa}, f_{\text{fix}} \rangle$ into the ancestral contribution in Eq. (8) and the buildup in Eq. (9) allows for matching the different categories

of fixations in Fig. 1 with the corresponding analytic representation: the first term in Eq. (8) reflects fixations (in blue) appearing during $[0, t^*]$, whereas the second part corresponds to fixations (in orange) during $[t^*, t]$ for which the mutation happened before t^* . The buildup component in Eq. (9) accounts for fixations (in green) during $[t^*, t]$ for which the mutation occurred after t^* .

To obtain an explicit representation of $Z^{\gamma, \kappa}(t)$, we note that $f_{\text{fix}}(1) = 1$ and that the expectation operator applied to $f_{\text{fix}}(y)$ can be rewritten in terms of the fixation time distribution,

$$\mathbb{E}_y^{\gamma, \kappa}[f_{\text{fix}}(\xi_t)] = \mathbb{E}_y^{\gamma, \kappa}[\mathbb{1}_{\{1\}}(\xi_t)] = \mathbb{P}_y^{\gamma, \kappa}(\xi_t = 1) = \mathbb{P}_y^{\gamma, \kappa}(\tau_1 \leq t). \quad (15)$$

Thus,

$$Z^{\gamma, \kappa}(t) = \begin{cases} \theta \omega_{\gamma, 1} t, & t \leq t^*, \\ \theta \{\omega_{\gamma, 1} t^* + \omega_{\gamma, \kappa}(t - t^*)\} \\ \quad + \theta \int_0^1 \mathbb{P}_y^{\gamma, \kappa}(\tau_1 \leq t - t^*)(\omega_{\gamma, 1} \psi_{\gamma, 1}(y) - \omega_{\gamma, \kappa} \psi_{\gamma, \kappa}(y)) dy, & t > t^*, \end{cases}$$

compare Appendix D for technical details. Fixations that originate from nonsynonymous mutations are averaged over the DFE in Eq. (12); for synonymous fixations γ is set to zero. Finally, the fixation rate ratio in nonequilibrium is defined as

$$\omega^{\kappa}(t) = \frac{\mathbb{E}Z^{\mathcal{V}, \kappa}(t)}{Z^{0, \kappa}(t)}.$$

The nonequilibrium quantity $\omega^{\kappa}(t)$ is consistent with the equilibrium fixation rate ratio $\omega_{\gamma, \kappa}$ stated in Eq. (3), since $\omega^{\kappa}(t) = \mathbb{E}\omega_{\mathcal{V}, 1}$ for $t \leq t^*$ and $\omega^{\kappa}(t) \rightarrow \mathbb{E}\omega_{\mathcal{V}, \kappa}$ for $t \rightarrow \infty$.

We note that measures modeled in this study are population functionals—in contrast to sample functionals which can be viewed as estimators of pop-

ulation functionals. In case of $(\pi_N/\pi_S)^\kappa(t)$ the population and sample functional are equal. When using nonsynonymous over synonymous sequence divergence as estimate for the fixation rate ratio $\omega^\kappa(t)$, sample functional and population functional are not equal but converge for $t \rightarrow \infty$ (Wolf *et al.* 2009; Mugal *et al.* 2014, 2020).

Stochastic simulations

For performing stochastic simulation of paths $(\xi_t)_t$, we apply the discrete Wright-Fisher model with selection to a population of size N . It suffices to simulate paths for the reference population, since a polymorphism in a population of size κN evolves as in the reference population but with time scaled by κ . Paths are simulated over a maximum of n generations using the binomial Wright-Fisher sampling with selection. The selection coefficient in the discrete setting is obtained from the relation $s = \gamma/N$.

For the distribution of the time to fixation, $\mathbb{P}_y^{\gamma,\kappa}(\tau_1 \leq t)$, we generate m_{τ_1} paths for each tuple (y, γ) . If the derived allele does not get fixed, the time to fixation is set to infinity. Otherwise the fixation time is set to the generation it got fixed. Finally, the distribution function of the time to fixation on the evolutionary timescale is obtained by scaling generations with N . For the expected value $\mathbb{E}_y^{\gamma,\kappa}[f(\xi_t^{t^*})]$ in the nonequilibrium AFS we simulate and average over m_ξ paths for each triplet $(y, \gamma, (t - t^*)/\kappa)$.

Parameters that we used are $N = 1000$, $n = 20.000$, $s \in [-1, 0]$ or equivalently $\gamma \in [-1000, 0]$, $m_{\tau_1} = 100.000$ (if $\gamma = 0$) and $m_{\tau_1} = 10.000$ (if $\gamma < 0$), respectively, $m_\xi = 1000$.

349 Data availability statement

350 Code used to implement the stochastic simulations can be found on [Git-](#)
351 [Lab](#).

352 RESULTS AND DISCUSSION

353 Measures of natural selection in a nonequilibrium population

354 We study the behavior of measures of natural selection as functions of
355 time after a change in population size. Figure 2A shows the behavior of
356 $(\pi_N/\pi_S)^\kappa(t)$ for $\kappa \in \{0.1, 0.25, 0.5, 1, 2, 4\}$. The time it takes to reach the
357 new equilibrium depends on the direction of change in population size: the
358 new equilibrium is reached quickly in case of a reduction ($\kappa < 1$), whereas
359 for an increase in size ($\kappa > 1$) it takes longer. Also the extent of change de-
360 termines how fast $(\pi_N/\pi_S)^\kappa(t)$ attains the new equilibrium value. The more
361 the population size is reduced, the faster $(\pi_N/\pi_S)^\kappa(t)$ reaches its new equilib-
362 rium value; the more a population increases in size, the longer it takes. Also,
363 given a DFE restricted to deleterious mutations, $(\pi_N/\pi_S)^\kappa(t)$ is negatively
364 correlated with population size as predicted by the nearly neutral theory of
365 molecular evolution.

366 The behavior of $\omega^\kappa(t)$ after a change in population size is depicted in
367 Fig. 2B and is similar to the behavior of $(\pi_N/\pi_S)^\kappa(t)$. The ratio decreases
368 for $\kappa > 1$, which means that less deleterious nonsynonymous mutations went
369 to fixation—in accordance with observations about selection acting more effi-
370 ciently in larger populations. However, $\omega^\kappa(t)$ is an accumulative measure over
371 the time interval $[0, t]$, while $(\pi_N/\pi_S)^\kappa(t)$ reflects a *snapshot* of the strength

of selection at time t . As a consequence, $\omega^\kappa(t)$ takes longer to reach its new equilibrium than $(\pi_N/\pi_S)^\kappa(t)$.

Another model to capture the impact of a change in population size on $\omega^\kappa(t)$ can be achieved by the weighted sum of the ancestral and the new equilibrium value (Eyre-Walker 2002). In our notation this reads $\omega_w^\kappa(t) := [t^*\omega^1 + (t - t^*)\omega^\kappa]/t$ for equilibrium values ω^1 and ω^κ , respectively. This means, the approach based on the weighted sum does not explicitly model nonstationarity. To visualize the difference between $\omega^\kappa(t)$ and $\omega_w^\kappa(t)$, the weighted fixation rate ratios are included as dashed lines in Fig. 2B. The nonequilibrium model derived in this study shows that $\omega^\kappa(t)$ takes longer to react to the change in population size and to reach the new equilibrium value compared to the approach of weighting the equilibrium values. This illustrates that ignoring the nonequilibrium phase leads to an underestimation of the time until the new equilibrium is reached.

Note that Fig. 2B shows the fixation rate ratio for a change in population size at time $t^* = 1$. Changes at other time points can change the behavior severely. A change at $t^* = 0$ for example, would lead to $\omega_w^\kappa(t) = \omega^\kappa$ without reflecting any influence of the ancestral population. On the other hand, if the change in population size happens more recently in time, the contribution of the ancient population size becomes more pronounced (see Appendix Fig. E.6).

Proxies of effective population size as measures of genetic drift

In order to evaluate the efficacy of selection during nonequilibrium we relate the above-introduced measures of selection to estimates of the effective population size. Since there are various ways to define N_{eff} , it is funda-

Figure 2: Measures of selection for different values of κ as functions of time. Panel A: the ratio of nonsynonymous and synonymous diversity $(\pi_N/\pi_S)^\kappa(t)$. Panel B: the fixation rate ratio $\omega^\kappa(t)$. For comparison, colored, dashed curves represent the weighted fixation rate ratio $\omega_w^\kappa(t)$. Vertical, dashed lines indicate the time $t^* = 1$. Parameters: $\theta = 1$, and $a = 0.15$ and $ab = 2500$ for the DFE.

mental to discuss the differences and to assess which of the definitions are relevant to relate to $\omega^\kappa(t)$ and $(\pi_N/\pi_S)^\kappa(t)$ in our modeling approach. The most commonly considered concepts of N_{eff} among others are variance and inbreeding (Wright 1931, 1938; Crow 1954; Crow and Kimura 1970), coalescent (Lynch and Conery 2003), and eigenvalue effective population size (Ewens 1969, 1979, 1982). The properties, that these concepts aim to model, are the variance in allele frequencies over time due to random genetic drift, the average inbreeding coefficient, the rate of coalescence of neutral alleles, and the leading non-unit eigenvalue of the allele frequency transition matrix.

We here focus on the harmonic mean effective population size over $[0, t]$ (Wright 1938; Karlin 1968) and the pairwise synonymous nucleotide diversity (Lynch and Conery 2003; Wakeley and Sargsyan 2008; Ellegren and Galtier

2016). The harmonic mean effective size over $[0, t]$ is a representative of
variance effective population size and defined as the average of genetic drift
over the time interval $[0, t]$ with $t^* \in [0, t]$,

$$N_{\text{eff}}^h(t) := \left(\frac{1}{t} \int_0^t \frac{1}{N_{\kappa}(s)} ds \right)^{-1} = \frac{\kappa N t}{t^*(\kappa - 1) + t},$$

with $N_{\text{eff}}^h(t^*) = N$, and $N_{\text{eff}}^h(t) \rightarrow \kappa N$ for t large. This means the ancestral
population size N loses its influence on $N_{\text{eff}}^h(t)$ the further in the past the
change took place. Overall the quantity is mainly controlled by the smaller
population size (Wright 1938). If κ is constant over $[0, t]$, then $N_{\text{eff}}^h(t) = \kappa N$.
Also, in view of the genetic drift term in Eq. (1)—the variance term of the
SDE—the parameter κ at time t multiplied by N can be seen as variance
effective population size at time t .

The scaled pairwise synonymous diversity, $N_{\text{eff}}^{\pi}(t) := \pi_S^{\kappa}(t)/(2\mu)$, where
 $\mu := \theta/N$ is the mutation rate per generation and individual, is an estimate
of effective population size based on nucleotide variation, often also perceived
as coalescent effective population size (Lynch and Conery 2003; Wakeley and
Sargsyan 2008). We note that the definition of coalescent effective population
size is not consistent in literature (Sjödín *et al.* 2004). We here follow the
definition of Lynch and Conery (2003); Wakeley and Sargsyan (2008).

With the two measures, $N_{\text{eff}}^h(t)$ and $N_{\text{eff}}^{\pi}(t)$, of effective population size
at hand, we investigate and compare how a change in population size is
reflected in each of them. We consider two scenarios, an ancient change
at $t^* = 1$ and a recent change at $t^* = 18$. For this purpose, $N_{\text{eff}}^h(t)$ is
plotted against $N_{\text{eff}}^{\pi}(t)$ as parametric curve of time, $t \geq t^*$, for an ancient
(Fig. 3A) and recent (Fig. 3B and Appendix Fig. E.7) change in population
size. The nonequilibrium behavior is compared to the equilibrium values, i.e.

433 $N_{\text{eff}}^h(t) = N_{\text{eff}}^\pi(t) = \kappa N$, as functions of κ . To explain Fig. 3 more explicitly,
 434 starting from a common initial equilibrium value at time t^* indicated as
 435 black cross on the dashed equilibrium line, the relationship between $N_{\text{eff}}^h(t)$
 436 and $N_{\text{eff}}^\pi(t)$ is shown for different values of κ for $t^* < t < 20$ as colored curves.

437 For an ancestral change, it seems that both proxies mirror the change in
 438 size to a large degree. However, $N_{\text{eff}}^h(t)$ reaches the new equilibrium value
 439 more slowly compared to $N_{\text{eff}}^\pi(t)$. This holds in particular for $\kappa > 1$, leading
 440 to the presumption that the more a population increases, the slower the new
 441 equilibrium is reached and vice versa. For a recent change in population
 442 size, the difference between the two estimates is much more striking and we
 443 observe that there is hardly any correlation between them. The proxy $N_{\text{eff}}^\pi(t)$
 444 reacts quickly to a change in population size, as expected for a measure
 445 relevant at the microevolutionary timescale. The harmonic mean effective
 446 population size, $N_{\text{eff}}^h(t)$, reacts more slowly and its variation is very narrow
 447 in comparison to $N_{\text{eff}}^\pi(t)$. This suggests that $N_{\text{eff}}^\pi(t)$ reflects a recent change in
 448 population size better than $N_{\text{eff}}^h(t)$. Considering the analytical definitions of
 449 the two estimates, one can additionally state that changes which happened
 450 before time zero, i.e. $t^* \leq 0$, will not at all be captured by $N_{\text{eff}}^h(t)$ but poten-
 451 tially by $N_{\text{eff}}^\pi(t)$ given t is small enough such that the AFS has not reached
 452 its new equilibrium.

453 Relationship between effective population size and measures of nat- 454 ural selection

455 We investigate the selection-drift relationship after a change in population
 456 size and compare it to the equilibrium behavior. Therefore, we relate the
 457 two estimates $N_{\text{eff}}^h(t)$ and $N_{\text{eff}}^\pi(t)$ to the fixation rate ratio, $\omega^\kappa(t)$, and the

Figure 3: The effective population size based on nucleotide variation, $N_{\text{eff}}^{\pi}(t)$, versus the harmonic mean effective population size, $N_{\text{eff}}^h(t)$, as parametric curves of time, $t^* \leq t \leq 20$, for different values of κ . Panel A shows an *ancient* change in population size at $t^* = 1$, panel B a *recent* change at $t^* = 18$. Black, dashed lines show the expected behavior in equilibrium populations. Black crosses at $N_{\text{eff}}^h(t^*) = N_{\text{eff}}^{\pi}(t) = 1000$ mark the time of change in population size, t^* . Colored crosses indicate the expected equilibrium value for each κ . Parameters $\theta = 1$ and $N = 1000$.

ratio of nucleotide diversity, $(\pi_N/\pi_S)^{\kappa}(t)$, as parametric curves of time after an ancient ($t^* = 1$, Fig. 4) and recent ($t^* = 18$, Fig. 5) change in size. To evaluate the discrepancy between the nonequilibrium behavior and the expected equilibrium balance between genetic drift and selection, we indicate ω^{κ} and $(\pi_N/\pi_S)^{\kappa}$ as functions of κ .

First, we consider an ancient change in population size (Fig. 4). This reveals a clear deviation from the equilibrium prediction of the nearly neutral theory for a relevant time period after the change in population size. For an increase in population size, $\omega^{\kappa}(t)$ is larger than expected in equilibrium, implying that more nonsynonymous and hence deleterious mutations get fixed during the nonequilibrium phase than during equilibrium, see

469 Figs. 4A and 4B. This implies that the efficacy of selection is limited during
 470 the nonequilibrium period. The reverse is true for a decrease in size. More-
 471 over, the discrepancy between nonequilibrium and equilibrium conditions is
 472 larger, the stronger the change in population size. In addition, the deviation
 473 from the selection-drift relationship appears more pronounced when compar-
 474 ing $\omega^\kappa(t)$ and $N_{\text{eff}}^\pi(t)$ than $\omega^\kappa(t)$ and $N_{\text{eff}}^h(t)$. For $(\pi_N/\pi_S)^\kappa(t)$ the deviation
 475 from equilibrium is less striking—even when relating to $N_{\text{eff}}^h(t)$ in Fig. 4C.
 476 But still, there exists a deviation which notably is the other way around
 477 than for $\omega^\kappa(t)$. While for $\kappa < 1$ the measure $\omega^\kappa(t)$ falls below the equilib-
 478 rium expectation, the measure $(\pi_N/\pi_S)^\kappa(t)$ for a given N_{eff} is larger than in
 479 equilibrium. The reverse holds for $\kappa > 1$.

480 For a recent change in population size in Fig. 5, when using the fixa-
 481 tion rate ratio $\omega^\kappa(t)$ as measure of selection, the selection-drift relationship
 482 becomes very weak (see Figs. 5A and 5B). The lack of a clear relationship
 483 between $\omega^\kappa(t)$ and N_{eff} can be explained by the slow reaction of $\omega^\kappa(t)$ on
 484 the change in population size. Since also $N_{\text{eff}}^h(t)$ reacts slow on the change
 485 in population size, $N_{\text{eff}}^h(t)$ and $\omega^\kappa(t)$ are both close to the ancestral value,
 486 and the lack of the selection-drift relationship is less apparent. However, it
 487 becomes clearly visible when using $N_{\text{eff}}^\pi(t)$ as estimate of genetic drift.

488 The measures $(\pi_N/\pi_S)^\kappa(t)$ and $N_{\text{eff}}^\pi(t)$ react much faster on a change in
 489 population size, which is especially eye catching in the comparison of Figs. 5A
 490 and 5D. Figure 5D shows that there is hardly any deviation from the expected
 491 equilibrium relationship since $(\pi_N/\pi_S)^\kappa(t)$ and $N_{\text{eff}}^\pi(t)$ both react quickly to
 492 a change in population size and are hence close to their equilibrium expec-
 493 tation. This means comparison of microevolutionary measures of natural

Figure 4: Measures of selection versus proxies of N_{eff} as parametric curves of time, $1 \leq t \leq 20$, after an *ancient* change in population size at $t^* = 1$ for different values of κ . Panels A and C: genetic drift estimated by the harmonic mean effective population size, $N_{\text{eff}}^h(t)$. Panels B and D: genetic drift estimated by $N_{\text{eff}}^\pi(t)$. Black, dashed lines show the expected relation in equilibrium populations. Black crosses at $N_{\text{eff}}^h(t^*) = N_{\text{eff}}^\pi(t) = 1000$ indicate the time of change in population size, t^* . Colored crosses indicate the expected equilibrium value for each κ . Parameters $a = 0.15$ and $ab = 2500$ in the DFE, $N = 1000$, and $\theta = 1$.

Figure 5: Measures of selection versus proxies of N_{eff} as parametric curves of time, $18 \leq t \leq 20$, after a recent change in population size at $t^* = 18$ for different values of κ . Panels A and C: genetic drift estimated by the harmonic mean effective population size, $N_{\text{eff}}^h(t)$. Panels B and D: genetic drift estimated by $\pi_S^\kappa(t)$. Black, dashed lines show the expected relation in equilibrium populations. Black crosses at $N_{\text{eff}}^h(t^*) = N_{\text{eff}}^\pi(t^*) = 1000$ indicate the time of change in population size, t^* . Colored crosses indicate the expected equilibrium value for each κ . Parameters $a = 0.15$ and $ab = 2500$ in the DFE, $N = 1000$, and $\theta = 1$.

494 selection and genetic drift recover the equilibrium selection-drift relationship
 495 quickly. In contrast, comparison of macroevolutionary measures of natural
 496 selection and genetic drift (Fig. 5A) appear not applicable to capture recent
 497 changes in population size. If a microevolutionary measure is compared with
 498 a macroevolutionary measure after a recent change in population size, this
 499 can essentially lead to a lack of a selection-drift relationship (Fig. 5B), or
 500 stronger observed variation in natural selection than observed variation in
 501 genetic drift (Fig. 5C), dependent on the choice of combination.

502 Overall, we can recognize that the prediction of the nearly neutral theory,
 503 i.e. a negative correlation between measures of selection and population size,
 504 holds even after a change in population size. But the strength of relation-
 505 ship is clearly influenced dependent on what measures of selection and N_{eff}
 506 are chosen. The analysis and discussion of the various approaches to N_{eff}
 507 confirm that it is not trivial to choose an appropriate estimate that reflects
 508 the population behavior. Our results suggest that $N_{\text{eff}}^{\pi}(t)$ and $(\pi_N/\pi_S)^{\kappa}(t)$
 509 as microevolutionary measures reflect a recent change in population size well
 510 but quickly loose the knowledge about it. In contrast, the macroevolutionary
 511 measures $N_{\text{eff}}^h(t)$ and $\omega^{\kappa}(t)$ need more time to react to the change but are
 512 longer influenced by the ancestral population size. We can further conclude
 513 that, in order to investigate the selection-drift relationship, it seems advis-
 514 able to correlate microevolutionary measures of N_{eff} with microevolutionary
 515 measures of selection in case of a recent change in population size. For an
 516 ancient change, microevolutionary measures of N_{eff} should be correlated with
 517 microevolutionary measures of selection and macroevolutionary measures of
 518 N_{eff} with macroevolutionary measures of selection. Moreover, it could be of

interest to investigate and compare the selection-drift relationship of micro- and macroevolutionary measures. If the observed relationships show a different behavior, this could be indicative of a nonequilibrium condition.

Obviously, data availability is an important prerequisite for such an analysis. Microevolutionary measures of genetic drift and natural selection can be directly computed based on population re-sequencing data (Muyle *et al.* 2020). On the other hand, in practice $N_{\text{eff}}^h(t)$ as a macroevolutionary measure of N_{eff} is difficult to assess based on genomic data. Instead, life-history traits, such as body mass or propagule size, are commonly used as proxies for long-term N_{eff} (e.g. Waples 2016; Tabak *et al.* 2018; Kutschera *et al.* 2020). The lack of a distantly related reference species can further hinder the computation of macroevolutionary measures of natural selection (Mugal *et al.* 2020; Muyle *et al.* 2020). Such limitations are often unavoidable, but our results highlight that it is important to be aware of the measures used in different studies when making comparisons among studies. When comparing the relationship between the efficacy of selection and the effects of genetic drift among different studies, as for example asexually and sexually reproducing species, or domesticated versus wild species, it could therefore be advisable to take the nature of measures of effective population size and natural selection into account (Brandvain and Wright 2016).

Conclusion

Addressing our initial question of how a change in population size affects $(\pi_N/\pi_S)^\kappa(t)$ and $\omega^\kappa(t)$, both measures show a similar behavior but differ in how quickly they react to the change. For a population decrease, both ratios increase and vice versa, but $(\pi_N/\pi_S)^\kappa(t)$ as a microevolutionary measure re-

acts much faster to the change than the macroevolutionary measure $\omega^\kappa(t)$.
 Second, we asked if the selection-drift relationship predicted by the nearly
 neutral theory also holds in a nonequilibrium. For this purpose, we com-
 pared the relationship between measures of genetic drift and selection after
 the change in population size with the expected equilibrium balance. Our
 analytical results reveal that the expected negative relationship between pop-
 ulation size and measures of selection, such as $(\pi_N/\pi_S)^\kappa(t)$ and $\omega^\kappa(t)$, is still
 valid, but we observe a clear deviation from the expected equilibrium balance
 during nonequilibrium periods. Moreover, we conclude that since measures
 of micro- and macroevolution show different behaviors, it is important to
 be aware of this fact when making inference and comparing results among
 studies—different observations could arise from using measures at different
 evolutionary timescales.

We built our model of a nonequilibrium scenario on several simplifying
 assumptions of which one is the focus on a single change in population size.
 The advantage of such narrow focus is that interpretations can be done more
 easily and straightforward. On the other hand, the framework we presented
 here is only directly comparable to a limited number of empirical scenarios,
 such as (but not limited to) reductions in effective population size due to
 isolation of island and mainland populations (Woolfit and Bromham 2005;
 Wang *et al.* 2014; Leroy *et al.* 2020). In addition, it might also be relevant to
 study periodic changes in population size as for example done by Rousselle
et al. (2018) using simulations. Our analytical modeling approach, especially
 the derivation of a nonequilibrium AFS in the Poisson random field frame-
 work, may be extended to such situations. Periodic changes in population

size would entail a sequence $t_k^* = kT$, for $k = 0, 1, \dots$, of time points of period T and a sequence $\kappa_0, \kappa_1, \dots$ of size parameters making up a new step function N_κ . For $t \in [t_{n-1}^*, t_n^*)$, the more general nonequilibrium AFS extending Eq. (10) is

$$\begin{aligned} & \lim_{N \rightarrow \infty} \mathbb{E} \langle \mathcal{X}_t^{N_\kappa}, f \rangle \\ &= \theta \int_0^1 f(y) \omega_{\gamma, \kappa_0} \psi_{\gamma, \kappa_0}(y) dy + \theta f(1) \int_0^t \sum_{k=1}^n \omega_{\gamma, \kappa_{k-1}} 1_{\{t_{k-1}^* < s \leq t_k^*\}} ds \\ &+ \theta \sum_{k=1}^n \int_0^1 (f(y) - \mathbb{E}_y^{\gamma, \kappa_{k-1}} f(\xi_{t_k^* \wedge t}^{t_{k-1}^*})) (\omega_{\gamma, \kappa_k} \psi_{\gamma, \kappa_k}(y) - \omega_{\gamma, \kappa_{k-1}} \psi_{\gamma, \kappa_{k-1}}(y)) dy. \end{aligned}$$

In fact, this AFS is not restricted to periodically changing environments, and hence may be used to develop the further case of allowing a prescribed, continuously varying, deterministic, scaled population size, such that $N(t)/N \rightarrow \kappa(t)$.

Apart from change in population size, there are other mechanisms that can cause a demographic nonequilibrium and affect the selection-drift balance. Examples of such mechanisms are population structure or migration. That the latter scenario can impact inference from genomic data is exemplified by the inference of estimates of gene flow in nonequilibrium conditions (e.g. [Austin et al. 2004](#); [Pinho et al. 2008](#)). It could therefore be interesting to extend the description of the nonequilibrium AFS in order to incorporate migration and investigate its effect on the selection-drift relationship. Another evolutionary force that could impact the selection-drift relationship is recombination ([Hill and Robertson 1966](#); [Hollister et al. 2014](#); [Campos et al. 2014](#)). Our analytical model relies on the assumption of free recombination between sites, i.e. no linkage between sites. We show in [Mugal et al. \(2020\)](#)

that measures of natural selection are affected by recombination, but that evolutionary trends of measures of selection are robust to the assumption of free recombination at least in equilibrium. However, evolutionary changes in the recombination landscape cause a deviation from equilibrium and could be a relevant topic for the future.

Finally, we restricted our analysis to purifying selection based on the argument that positive selection can be neglected due to the rarity of advantageous mutants on a genome-wide scale. This argument is still under debate (Kern and Hahn 2018; Jensen *et al.* 2018), and several empirical studies argue for a more significant role of positive selection at the genome-wide scale, (e.g. Fay *et al.* 2002; Begun *et al.* 2007; Lefebure and Stanhope 2009; Bazykin and Kondrashov 2012; Williamson *et al.* 2014). Looking at the role of positive selection could in particular be of interest when investigating the behavior of specific genes that are candidates for episodic positive selection (Schmid and Tautz 1997; Zheng *et al.* 2004; Nevado *et al.* 2019), for example immune genes (Enard *et al.* 2016) and genes involved in reproduction (Singh and Kulathinal 2000; Swanson and Vacquier 2002), rather than looking at the genome-wide average. The flexibility of our model allows for an extension to model advantageous mutations by considering for example a mixed DFE (Mugal *et al.* 2020). However, change in population size might push the population closer or further away from its fitness optimum dependent on the direction of the change, which would induce a change in the DFE. Such model extension would require a different setting, such as a mutation-selection-drift framework (Halpern and Bruno 1998; Spielman and Wilke 2015; Jones *et al.* 2016), which is a non-trivial extension.

614 To conclude, some of the model assumptions constitute simplifying as-
 615 sumptions that lead to a narrow focus. The crucial advantage of such a nar-
 616 row focus is that exact analytical solutions can be derived and that valuable
 617 conceptual understanding can be gained based on straightforward interpreta-
 618 tions. The framework we presented here is also flexible and allows for various
 619 modifications, which gives the opportunity to investigate different extensions
 620 in some future work.

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627 References

- 628 Austin, J. D., S. C. Loughheed, and P. T. Boag, 2004 Controlling for the
 629 Effects of History and Nonequilibrium Conditions in Gene Flow Estimates
 630 in Northern Bullfrog (*Rana catesbeiana*) Populations. *Genetics* **168**: 1491–
 631 1506.
- 632 Bazykin, G. A. and A. S. Kondrashov, 2012 Major role of positive selection in
 633 the evolution of conservative segments of *Drosophila* proteins. *Proceedings*
 634 *of the Royal Society B: Biological Sciences* **279**: 3409–3417.

635 Begun, D. J., A. K. Holloway, K. Stevens, L. W. Hillier, Y.-P. Poh, *et al.*,
636 2007 Population Genomics: Whole-Genome Analysis of Polymorphism and
637 Divergence in *Drosophila simulans*. PLoS Biology **5**: e310.

638 Bolívar, P., L. Guéguen, L. Duret, H. Ellegren, and C. F. Mugal, 2019 GC-
639 biased gene conversion conceals the prediction of the nearly neutral theory
640 in avian genomes. Genome Biology **20**: 1–13.

641 Brandvain, Y. and S. I. Wright, 2016 The Limits of Natural Selection in a
642 Nonequilibrium World. Trends in Genetics **32**: 201–210.

643 Campos, J. L., D. L. Halligan, P. R. Haddrill, and B. Charlesworth, 2014
644 The Relation between Recombination Rate and Patterns of Molecular Evo-
645 lution and Variation in *Drosophila melanogaster*. Molecular Biology and
646 Evolution **31**: 1010–1028.

647 Chen, J., S. Glémin, and M. Lascoux, 2017 Genetic Diversity and the Efficacy
648 of Purifying Selection across Plant and Animal Species. Molecular Biology
649 and Evolution **34**: 1417–1428.

650 Crow, J. F., 1954 Breeding structure of populations. II. Effective population
651 number. Statistics and Mathematics in Biology, Iowa State College Press,
652 Ames, Iowa pp. 543–556.

653 Crow, J. F. and M. Kimura, 1970 *An Introduction to Population Genetics*
654 *Theory*. Harper and Row, New York.

655 Ellegren, H. and N. Galtier, 2016 Determinants of genetic diversity. Nature
656 Reviews Genetics **17**: 422–433.

657 Elyashiv, E., K. Bullaughey, S. Sattath, Y. Rinott, M. Przeworski, *et al.*,
658 2010 Shifts in the intensity of purifying selection: An analysis of genome-
659 wide polymorphism data from two closely related yeast species. Genome

660 Research **20**: 1558–1573.

661 Enard, D., L. Cai, C. Gwennap, and D. A. Petrov, 2016 Viruses are a domi-
662 nant driver of protein adaptation in mammals. *eLife* **5**.

663 Evans, S. N., Y. Shvets, and M. Slatkin, 2007 Non-equilibrium theory of the
664 allele frequency spectrum. *Theoretical Population Biology* **71**: 109–119.

665 Ewens, W., 1982 On the concept of the effective population size. *Theoretical*
666 *Population Biology* **21**: 373–378.

667 Ewens, W. J., 1969 *Population Genetics*. Methuen, London.

668 Ewens, W. J., 1979 *Mathematical Population Genetics*. Springer, New York.

669 Eyre-Walker, A., 2002 Changing Effective Population Size and the
670 McDonald-Kreitman Test. *Genetics* **162**: 2017–2024.

671 Eyre-Walker, A., M. Woolfit, and T. Phelps, 2006 The Distribution of Fitness
672 Effects of New Deleterious Amino Acid Mutations in Humans. *Genetics*
673 **173**: 891–900.

674 Fay, J. C., G. J. Wyckoff, and C.-I. Wu, 2002 Testing the neutral theory
675 of molecular evolution with genomic data from *Drosophila*. *Nature* **415**:
676 1024–1026.

677 Figuet, E., B. Nabholz, M. Bonneau, E. Mas Carrio, K. Nadachowska-
678 Brzyska, *et al.*, 2016 Life History Traits, Protein Evolution, and the Nearly
679 Neutral Theory in Amniotes. *Molecular Biology and Evolution* **33**: 1517–
680 1527.

681 Fisher, R. A., 1930 *The genetical theory of natural selection*. The Clarendon
682 Press.

683 Galtier, N. and M. Rousselle, 2020 How Much Does N_e Vary Among Species?
684 *Genetics* **216**: 559–572.

685 Goldman, N. and Z. Yang, 1994 A Codon-based Model of Nucleotide Sub-
686 stitution for Protein-coding DNA Sequences. *Molecular Biology and Evo-*
687 *lution* **11**: 725–736.

688 Gordo, I. and F. Dionisio, 2005 Nonequilibrium model for estimating param-
689 eters of deleterious mutations. *Physical Review E* **71**: 031907.

690 Halpern, A. L. and W. J. Bruno, 1998 Evolutionary distances for protein-
691 coding sequences: modeling site-specific residue frequencies. *Molecular Bi-*
692 *ology and Evolution* **15**: 910–917.

693 Hill, W. G. and A. Robertson, 1966 The effect of linkage on limits to artificial
694 selection. *Genetical Research* **8**: 269–294.

695 Hollister, J. D., S. Greiner, W. Wang, J. Wang, Y. Zhang, *et al.*, 2014 Recur-
696 rent Loss of Sex Is Associated with Accumulation of Deleterious Mutations
697 in *Oenothera*. *Molecular Biology and Evolution* **32**: 896–905.

698 Hughes, A. L., 2008 Near-Neutrality: the Leading Edge of the Neutral Theory
699 of Molecular Evolution. *Annals of the New York Academy of Sciences*
700 **1133**: 162–179.

701 Jensen, J. D., B. A. Payseur, W. Stephan, C. F. Aquadro, M. Lynch, *et al.*,
702 2018 The importance of the Neutral Theory in 1968 and 50 years on: A
703 response to Kern and Hahn 2018. *Evolution* **73**: 111–114.

704 Jones, C. T., N. Youssef, E. Susko, and J. P. Bielawski, 2016 Shifting Balance
705 on a Static Mutation–Selection Landscape: A Novel Scenario of Positive
706 Selection. *Molecular Biology and Evolution* **34**: 391–407.

707 Kaj, I. and C. F. Mugal, 2016 The non-equilibrium allele frequency spectrum
708 in a Poisson random field framework. *Theoretical Population Biology* **111**:
709 51–64.

710 Karlin, S., 1968 Rates of approach to homozygosity for finite stochastic mod-
711 els with variable population size. *The American Naturalist* **102**: 443–455.

712 Karlsson, E. K., D. P. Kwiatkowski, and P. C. Sabeti, 2014 Natural selection
713 and infectious disease in human populations. *Nature Reviews Genetics* **15**:
714 379–393.

715 Kern, A. D. and M. W. Hahn, 2018 The Neutral Theory in Light of Natural
716 Selection. *Molecular Biology and Evolution* **35**: 1366–1371.

717 Kimura, M., 1962 On the probability of fixation of mutant genes in a popu-
718 lation. *Genetics* **47**: 713.

719 Kimura, M., 1964 Diffusion models in population genetics. *Journal of Applied*
720 *Probability* **1**: 177–232.

721 Kutschera, V. E., J. W. Poelstra, F. Botero-Castro, N. Dussex, N. J. Gem-
722 mell, *et al.*, 2020 Purifying selection in corvids is less efficient on islands.
723 *Molecular biology and evolution* **37**: 469–474.

724 Lachapelle, J., N. Colegrave, and G. Bell, 2017 The effect of selection history
725 on extinction risk during severe environmental change. *Journal of Evolu-*
726 *tionary Biology* **30**: 1872–1883.

727 Lefebure, T. and M. J. Stanhope, 2009 Pervasive, genome-wide positive selec-
728 tion leading to functional divergence in the bacterial genus *Campylobacter*.
729 *Genome Research* **19**: 1224–1232.

730 Leroy, T., M. Rousselle, M.-K. Tilak, A. Caizergues, C. Scornavacca, *et al.*,
731 2020 Endemic island songbirds as windows into evolution in small effective
732 population sizes. *bioRxiv* .

733 Loewe, L. and B. Charlesworth, 2006 Inferring the distribution of mutational
734 effects on fitness in *Drosophila*. *Biology Letters* **2**: 426–430.

735 Lu, J., T. Tang, H. Tang, J. Huang, S. Shi, *et al.*, 2006 The accumulation of
736 deleterious mutations in rice genomes: a hypothesis on the cost of domes-
737 tication. *Trends in Genetics* **22**: 126–131.

738 Lumley, A. J., L. Michalczyk, J. J. Kitson, L. G. Spurgin, C. A. Morrison,
739 *et al.*, 2015 Sexual selection protects against extinction. *Nature* **522**: 470–
740 473.

741 Lynch, M., R. Bürger, D. Butcher, and W. Gabriel, 1993 The Mutational
742 Meltdown in Asexual Populations. *Journal of Heredity* **84**: 339–344.

743 Lynch, M., J. Conery, and R. Burger, 1995 Mutation accumulation and the
744 extinction of small populations. *The American Naturalist* **146**: 489–518.

745 Lynch, M. and J. S. Conery, 2003 The Origins of Genome Complexity. *Science*
746 **302**: 1401–1404.

747 Mugal, C. F., V. E. Kutschera, F. Botero-Castro, J. B. Wolf, and I. Kaj,
748 2020 Polymorphism Data Assist Estimation of the Nonsynonymous over
749 Synonymous Fixation Rate Ratio ω for Closely Related Species. *Molecular*
750 *Biology and Evolution* **37**: 260–279.

751 Mugal, C. F., J. B. Wolf, and I. Kaj, 2014 Why Time Matters: Codon
752 Evolution and the Temporal Dynamics of dN/dS . *Molecular Biology and*
753 *Evolution* **31**: 212–31.

754 Muse, S. V. and B. S. Gaut, 1994 A Likelihood Approach for Comparing Syn-
755 onymous and Nonsynonymous Nucleotide Substitution Rates, with Appli-
756 cation to the Chloroplast Genome. *Molecular Biology and Evolution* **11**:
757 715–724.

758 Muyle, A., H. Martin, N. Zemp, M. Mollion, S. Gallina, *et al.*, 2020 Dioecy
759 is associated with high genetic diversity and adaptation rates in the plant

760 genus *silene*. Molecular Biology and Evolution .

761 Nei, M. and W.-H. Li, 1979 Mathematical model for studying genetic vari-
762 ation in terms of restriction endonucleases. Proceedings of the National
763 Academy of Sciences **76**: 5269–5273.

764 Nevado, B., E. L. Wong, O. G. Osborne, and D. A. Filatov, 2019 Adaptive
765 Evolution Is Common in Rapid Evolutionary Radiations. Current Biology
766 **29**: 3081–3086.e5.

767 Ohta, T., 1973 Slightly Deleterious Mutant Substitutions in Evolution. Na-
768 ture **246**: 96–98.

769 Ohta, T., 1976 Role of Very Slightly Deleterious Mutations in Molecular
770 Evolution and Polymorphism. Theoretical Population Biology **10**: 254–
771 275.

772 Ohta, T., 1992 The nearly neutral theory of molecular evolution. Annual
773 Review of Ecology and Systematics **23**: 263–286.

774 Pinho, C., D. J. Harris, and N. Ferrand, 2008 Non-equilibrium estimates of
775 gene flow inferred from nuclear genealogies suggest that Iberian and North
776 African wall lizards (*Podarcis* spp.) are an assemblage of incipient species.
777 BMC Evolutionary Biology **8**.

778 Renaut, S. and L. H. Rieseberg, 2015 The Accumulation of Deleterious Mu-
779 tations as a Consequence of Domestication and Improvement in Sunflowers
780 and Other Compositae Crops. Molecular Biology and Evolution **32**: 2273–
781 2283.

782 Rousselle, M., M. Mollion, B. Nabholz, T. Bataillon, and N. Galtier, 2018
783 Overestimation of the adaptive substitution rate in fluctuating popula-
784 tions. Biology letters **14**: 20180055.

785 Sawyer, S. A. and D. L. Hartl, 1992 Population Genetics of Polymorphism
786 and Divergence. *Genetics* **132**: 1161–1176.

787 Schmid, K. J. and D. Tautz, 1997 A screen for fast evolving genes from
788 *Drosophila*. *Proceedings of the National Academy of Sciences* **94**: 9746–
789 9750.

790 Seppälä, O., 2015 Natural selection on quantitative immune defence traits:
791 a comparison between theory and data. *Journal of Evolutionary Biology*
792 **28**: 1–9.

793 Singh, R. S. and R. J. Kulathinal, 2000 Sex gene pool evolution and specia-
794 tion: A new paradigm. *Genes & Genetic Systems* **75**: 119–130.

795 Sjödin, P., I. Kaj, S. Krone, M. Lascoux, and M. Nordborg, 2004 On the
796 Meaning and Existence of an Effective Population Size. *Genetics* **169**:
797 1061–1070.

798 Spielman, S. J. and C. O. Wilke, 2015 The Relationship between dN/dS and
799 Scaled Selection Coefficients. *Molecular Biology and Evolution* **32**: 1097–
800 1108.

801 Swanson, W. J. and V. D. Vacquier, 2002 The rapid evolution of reproductive
802 proteins. *Nature Reviews Genetics* **3**: 137–144.

803 Tabak, M. A., C. T. Webb, and R. S. Miller, 2018 Propagule size and struc-
804 ture, life history, and environmental conditions affect establishment success
805 of an invasive species. *Scientific Reports* **8**.

806 Wakeley, J. and O. Sargsyan, 2008 Extensions of the Coalescent Effective
807 Population Size. *Genetics* **181**: 341–345.

808 Wang, J., E. Santiago, and A. Caballero, 2016 Prediction and estimation of
809 effective population size. *Heredity* **117**: 193–206.

810 Wang, S., W. Zhu, X. Gao, X. Li, S. Yan, *et al.*, 2014 Population size and
811 time since island isolation determine genetic diversity loss in insular frog
812 populations. *Molecular Ecology* **23**: 637–648.

813 Waples, R. S., 2005 Genetic estimates of contemporary effective population
814 size: to what time periods do the estimates apply? *Molecular Evolution*
815 **14**: 3335–3352.

816 Waples, R. S., 2016 Life-history traits and effective population size in species
817 with overlapping generations revisited: the importance of adult mortality.
818 *Heredity* **117**: 241–250.

819 Williamson, R. J., E. B. Josephs, A. E. Platts, K. M. Hazzouri, A. Haudry,
820 *et al.*, 2014 Evidence for Widespread Positive and Negative Selection in
821 Coding and Conserved Noncoding Regions of *Capsella grandiflora*. *PLoS*
822 *Genetics* **10**: e1004622.

823 Wolf, J. B., A. Künstner, K. Nam, M. Jakobsson, and H. Ellegren, 2009 Non-
824 linear Dynamics of Nonsynonymous (d_N) and Synonymous (d_S) Substitu-
825 tion Rates Affects Inference of Selection. *Genome Biology and Evolution*
826 **1**: 308–319.

827 Woolfit, M. and L. Bromham, 2005 Population size and molecular evolution
828 on islands. *Proceedings of the Royal Society B: Biological Sciences* **272**:
829 2277–2282.

830 Wright, S., 1931 Evolution in mendelian populations. *Genetics* **16**: 97–159.

831 Wright, S., 1938 Size of population and breeding structure in relation to
832 evolution. *Science* **87**: 430–431.

833 Zheng, Y., R. J. Roberts, and S. Kasif, 2004 Identification of genes with
834 fast-evolving regions in microbial genomes. *Nucleic Acids Research* **32**:

- 835 6347–6357.
- 836 Živković, D., M. Steinrücken, Y. S. Song, and W. Stephan, 2015 Transi-
837 tion Densities and Sample Frequency Spectra of Diffusion Processes with
838 Selection and Variable Population Size. *Genetics* **200**: 601–617.
- 839 Živković, D. and W. Stephan, 2011 Analytical results on the neutral non-
840 equilibrium allele frequency spectrum based on diffusion theory. *Theoret-*
841 *ical Population Biology* **79**: 184–191.

A. THE AFS FOR THE EQUILIBRIUM CASE

We begin with the reference case $\kappa = 1$ of fixed population size and consider the Wright-Fisher diffusion $\xi^s = (\xi_t^s)_{t \geq s}$, $\xi_s^s = y$, with law $\mathbb{P}_y^\gamma(d\xi^s)$. To emphasize the initial value we may write $(\xi_t^{s,y})_{t \geq s}$. The associated expectation operator $T_{t-s}^\gamma f(y) = \mathbb{E}_y^\gamma[f(\xi_t^s)]$, $t \geq s$, satisfies the semigroup property $T_s^\gamma(T_{t-s}^\gamma f)(y) = T_t f(y)$, and the diffusion infinitesimal generator is the differential operator

$$Q_\gamma f(x) = \gamma x(1-x) f'(x) + \frac{1}{2} x(1-x) f''(x), \quad f \in \mathcal{D},$$

for a suitable domain $\mathcal{D}$ of twice differentiable functions on the unit interval. We denote by $\mathcal{F}$ the class of real-valued bounded functions on $[0, 1]$ with $f(0) = 0$, satisfying $\int y^{-1} |f(y)| dy < \infty$, and put

$$\tilde{\mathcal{F}} = \{f \in \mathcal{F} \cap \mathcal{D} : Q_\gamma f \in \mathcal{F}\}.$$

Similarly, $\mathcal{F}_0$ and $\tilde{\mathcal{F}}_0$ denote the further restricted classes of functions which, in addition, satisfy $f(1) = 0$. It is convenient to derive the following property first for the restricted class of functions vanishing at both boundary points, case i), and then make the necessary observations for handling the larger class of functions, as case ii).

Lemma 1. *For each $t \geq 0$,*

- i) *if $f \in \tilde{\mathcal{F}}_0$ then $T_t^\gamma f \in \mathcal{F}_0$; and*
- ii) *if $f \in \tilde{\mathcal{F}}$ then $T_t^\gamma f \in \mathcal{F}$.*

Proof. For statement i) let $f \in \tilde{\mathcal{F}}_0$. Clearly, $T_t^\gamma f$ is bounded with $T_t^\gamma f(0) = T_t^\gamma f(1) = 0$. We need to show $\int_0^1 x^{-1} |T_t^\gamma f|(x) dx < \infty$.

862 As a consequence of Itô's formula we have the basic relation

$$T_t^\gamma f(x) = f(x) + \mathbb{E}_x^\gamma \left[\int_0^t Q_\gamma f(\xi_r) dr \right],$$

863 and so

$$|T_t^\gamma f(x) - f(x)| \leq \mathbb{E}_x^\gamma \left[\int_0^t |Q_\gamma f(\xi_r)| dr \right].$$

864 Let τ be the absorption time of (ξ_t) . Then, since $Q_\gamma f \in \mathcal{F}_0$ by assumption,

$$\mathbb{E}_x^\gamma \left[\int_0^t |Q_\gamma f(\xi_r)| dr \right] = \mathbb{E}_x^\gamma \left[\int_0^{t \wedge \tau} |Q_\gamma f(\xi_r)| dr \right] \leq \mathbb{E}_x^\gamma \left[\int_0^\tau |Q_\gamma f(\xi_r)| dr \right]$$

865 so that

$$|T_t^\gamma f(x) - f(x)| \leq \mathbb{E}_x^\gamma \left[\int_0^\tau |Q_\gamma f(\xi_r)| dr \right] = \int_0^1 |Q_\gamma f(y)| G_\gamma(x, y) dy,$$

866 where $G_\gamma(x, y)$ is the Green function of the Wright-Fisher diffusion. Hence,

$$\begin{aligned} \int_0^1 x^{-1} |T_t^\gamma f(x) - f(x)| dx &\leq \int_0^1 \int_0^1 |Q_\gamma f(y)| x^{-1} G_\gamma(x, y) dy dx \\ &\leq \sup_{x, y} |x^{-1} G_\gamma(x, y) y| \int_0^1 y^{-1} |Q_\gamma f(y)| dy \end{aligned}$$

867 and therefore, uniformly in t ,

$$\begin{aligned} \int_0^1 x^{-1} |T_t^\gamma f(x)| dx &\leq \int_0^1 x^{-1} |f(x)| dx \\ &\quad + \sup_{x, y} |x^{-1} G_\gamma(x, y) y| \int_0^1 y^{-1} |Q_\gamma f(y)| dy. \end{aligned} \tag{A.1}$$

868 As the two integrals on the right hand side are finite by assumption, it

869 remains to show that

$$x^{-1} G_\gamma(x, y) y = \mathbb{1}_{\{x < y\}} x^{-1} q_\gamma(x) \psi_\gamma(y) y + \mathbb{1}_{\{x > y\}} x^{-1} (1 - q_\gamma(x)) \tilde{\psi}_\gamma(y) y$$

is bounded on the unit square $0 \leq x, y \leq 1$. Here, $q_\gamma(x)$ is defined in Eq. (2), $\psi_\gamma(y)$ in Eq. (4), and

$$\tilde{\psi}_\gamma(y) = \frac{e^{2\gamma y} - 1}{\gamma y(1 - y)}, \quad \gamma \neq 0, \quad \tilde{\psi}_0(y) = \frac{2}{1 - y}.$$

For $\gamma = 0$,

$$x^{-1}G_0(x, y)y = \mathbb{1}_{\{x < y\}}2 + \mathbb{1}_{\{x > y\}}\frac{1 - x}{x}\frac{2y}{1 - y} \leq 2.$$

For $\gamma \neq 0$, the nonnegative function $x^{-1}G_\gamma(x, y)y$ has the representation and upper bound

$$\begin{aligned} & \frac{4\gamma}{1 - e^{-2\gamma}} \left\{ \mathbb{1}_{\{x < y\}} \frac{1 - e^{-2\gamma x}}{2\gamma x} \frac{1 - e^{-2\gamma(1-y)}}{2\gamma(1 - y)} + \mathbb{1}_{\{x > y\}} \frac{e^{-2\gamma x} - e^{-2\gamma}}{2\gamma x} \frac{e^{2\gamma y} - 1}{2\gamma(1 - y)} \right\} \\ & \leq \frac{4\gamma}{1 - e^{-2\gamma}} \left\{ \mathbb{1}_{\{x < y\}} \frac{1 - e^{-2\gamma x}}{2\gamma x} \frac{1 - e^{-2\gamma(1-y)}}{2\gamma(1 - y)} + \mathbb{1}_{\{x > y\}} \frac{e^{-2\gamma x} - e^{-2\gamma}}{2\gamma(1 - x)} \frac{e^{2\gamma y} - 1}{2\gamma y} \right\}. \end{aligned}$$

From here using straightforward estimates one obtains, for example,

$$\sup_{0 \leq x, y \leq 1} x^{-1}G_\gamma(x, y)y \leq \frac{e^{2|\gamma|} - 1}{|\gamma|},$$

which concludes the proof that $T_t^\gamma f$ belongs to $\mathcal{F}_0$.

Turning to statement ii), by using Kaj and Mugal (2016, Lemma 1, Eq. (30)), we now have

$$T_t^\gamma f(x) = f(x) + \mathbb{E}_x^\gamma \left[\int_0^{t \wedge \tau} (Q_\gamma f(\xi_r) - Q_\gamma f(1)q_\gamma(\xi_r)) dr \right] + Q_\gamma f(1)q_\gamma(x)t.$$

Since $q_\gamma(1) = 1$, the integrand function $y \mapsto Q_\gamma f(y) - Q_\gamma f(1)q_\gamma(y)$ belongs to $\mathcal{F}_0$. Hence we can proceed as above and obtain as replacement of Eq. (A.1),

$$\begin{aligned} \int_0^1 x^{-1}|T_t^\gamma f(x)| dx & \leq \int_0^1 x^{-1}|f(x)| dx + Q_\gamma f(1)t \int_0^1 x^{-1}q_\gamma(x) dx \\ & \quad + \sup_{x, y} |x^{-1}G_\gamma(x, y)y| \int_0^1 y^{-1}|Q_\gamma f(y) - Q_\gamma f(1)q_\gamma(y)| dy, \end{aligned}$$

where the supremum is the same factor already treated under i). The observation

$$\int_0^1 x^{-1} q_\gamma(x) dx < \infty,$$

completes the proof. $\square$

The stationary AFS arises in the Poisson random field approach as a limiting intensity measure of a random Poisson measure which adds up the allele contributions from all mutations in the infinite past. Formally, let $\mathcal{N}_N(ds, dy, d\xi^s)$ be a Poisson measure on $\mathbb{R} \times [0, 1] \times \mathcal{D}$ with intensity $N ds \delta_{1/N}(dy) \mathbb{P}_y^\gamma(d\xi^s)$, and let $\mathcal{N}_\gamma(dy)$ be a Poisson random measure on $[0, 1]$ with intensity measure $\omega_\gamma \psi_\gamma(y) dy$. Note that for simplicity we assume $\theta = 1$ throughout Appendix A and Appendix B. For $f \in \mathcal{F}_0$, we have for fixed t as $N \rightarrow \infty$ the convergence in distribution

$$\int_{-\infty}^t \int \int_{\mathcal{D}} f(\xi_t^{s,y}) \mathcal{N}_N(ds, dy, d\xi^{s,y}) \Rightarrow \int_0^1 f(y) \mathcal{N}_\gamma(dy)$$

and the associated convergence of expected values

$$\mathbb{E} \left[\int_{-\infty}^t \int \int_{\mathcal{D}} f(\xi_t^{s,y}) \mathcal{N}_N(ds, dy, d\xi^{s,y}) \right] \rightarrow \omega_\gamma \int_0^1 f(y) \psi_\gamma(y) dy$$

(Kaj and Mugal 2016, Section 2.4.). Each Poisson point in $\mathcal{N}_N$ represents the allele frequency of a mutation starting at time s at a fraction $1/N$ of the population. Taking all mutations with $s \leq t$ together and considering the resulting spectrum of polymorphic sites at t , we obtain $\mathcal{N}_\gamma$.

Next, we decompose the stationary Poisson measure $\mathcal{N}_\gamma$ at a fixed time $t \geq 0$, in two independent contributions by splitting up the mutation times, separating $s \leq 0$ and $0 < s \leq t$, so that

$$\mathbb{E} \left[\left\{ \int_{-\infty}^0 + \int_0^t \right\} \int \int_{\mathcal{D}} f(\xi_t^{s,y}) \mathcal{N}_N(ds, dy, d\xi^{s,y}) \right] \rightarrow \omega_\gamma \int_0^1 f(y) \psi_\gamma(y) dy. \quad (\text{A.2})$$

900 The limit of the first term in the decomposition is obtained in the following
 901 lemma, relation ii). The limit of the second term follows immediately, rela-
 902 tion iii). Let $\mathcal{M}_\gamma(dy, d\xi^{0,y})$ be a Poisson random measure on $[0, 1] \times \mathcal{D}$ with
 903 intensity measure $m_\gamma(dy, d\xi^{0,y}) = \omega_\gamma \psi_\gamma(y) dy \mathbb{P}_y^\gamma(d\xi^0)$. The Poisson points
 904 $(y, \xi^{0,y})$ are paths with initial state $\xi_0 = y$ according to the stationary inten-
 905 sity $\omega_\gamma \psi_\gamma(y) dy$ and evolve as Wright-Fisher diffusions $(\xi_t^{0,y})_{t \geq 0}$.

906 **Lemma 2.** For $f \in \tilde{\mathcal{F}}_0$ we have as $N \rightarrow \infty$ the convergence in distribution,

$$\text{i)} \quad \int_{-\infty}^0 \int \int_{\mathcal{D}} f(\xi_t^{s,y}) \mathcal{N}_N(ds, dy, d\xi^{s,y}) \Rightarrow \int_{[0,1] \times \mathcal{D}} f(\xi_t^{0,y}) \mathcal{M}_\gamma(dy, d\xi^{0,y})$$

and convergence of expected values,

$$\text{ii)} \quad \mathbb{E} \left[\int_{-\infty}^0 \int \int_{\mathcal{D}} f(\xi_t^{s,y}) \mathcal{N}_N(ds, dy, d\xi^{s,y}) \right] \rightarrow \int_0^1 T_t^\gamma f(y) \omega_\gamma \psi_\gamma(y) dy,$$

and

$$\text{iii)} \quad \mathbb{E} \left[\int_0^t \int \int_{\mathcal{D}} f(\xi_t^{s,y}) \mathcal{N}_N(ds, dy, d\xi^{s,y}) \right] \rightarrow \int_0^1 (f(y) - T_t^\gamma f(y)) \omega_\gamma \psi_\gamma(y) dy.$$

907 *Proof.* The logarithmic moment generating functional of the N -dependent
 908 Poisson integral equals

$$\ln \mathbb{E} \left[\exp \left\{ \int_{-\infty}^0 \int \int_{\mathcal{D}} f(\xi_t^{s,y}) \mathcal{N}_N(ds, dy, d\xi^{s,y}) \right\} \right] = N \int_{-\infty}^0 \mathbb{E}_{1/N}^\gamma [e^{f(\xi_t^s)} - 1] ds. \quad (\text{A.3})$$

909 Here, conditioning on $\{\xi_0^s\}_{s < 0}$,

$$\begin{aligned} \int_{-\infty}^0 \mathbb{E}_x^\gamma [e^{f(\xi_t^s)} - 1] ds &= \int_{-\infty}^0 \mathbb{E}_x^\gamma \mathbb{E}_{\xi_0^s}^\gamma [e^{f(\xi_t^0)} - 1] ds \\ &= \mathbb{E}_x^\gamma \left[\int_{-\infty}^0 T_t(e^f - 1)(\xi_0^s) ds \right] = \mathbb{E}_x^\gamma \left[\int_0^\infty T_t(e^f - 1)(\xi_r) dr \right]. \end{aligned}$$

910 Since $f \in \tilde{\mathcal{F}}_0$ we have $e^f - 1 \in \tilde{\mathcal{F}}_0$. By Lemma 1 i), $T_t(e^f - 1) \in \mathcal{F}_0$, $t \geq 0$.

Thus, the rightmost expression in Eq. (A.3) equals

$$N\mathbb{E}_{1/N}^{\gamma}\left[\int_0^{\tau}T_t^{\gamma}(e^f-1)(\xi_r)dr\right]=N\int_0^1G_{\gamma}(1/N,y)T_t^{\gamma}(e^f-1)(y)dy.$$

Therefore, as $N \rightarrow \infty$,

$$\begin{aligned} N\int_{-\infty}^0\mathbb{E}_{1/N}^{\gamma}[e^{f(\xi_t^s)}-1]ds &\rightarrow \int_0^1T_t^{\gamma}(e^f-1)(y)\omega_{\gamma}\psi_{\gamma}(y)dy \\ &= \ln\mathbb{E}\left[\exp\left\{\int_{[0,1]\times\mathcal{D}}f(\xi_t^{0,y})\mathcal{M}_{\gamma}(dy,d\xi^{0,y})\right\}\right], \end{aligned}$$

which shows convergence of the marginal distributions, for a fixed t . The convergence of the finite-dimensional distributions can be established as in Kaj and Mugal (2016, Proposition 2). Statement ii), which is

$$N\int_{-\infty}^0\mathbb{E}_{1/N}^{\gamma}[f(\xi_t^s)]ds \rightarrow \int_0^1T_t^{\gamma}f(y)\omega_{\gamma}\psi_{\gamma}(y)dy,$$

follows as above, now using that $T_t^{\gamma}f \in \mathcal{F}_0$. Then we obtain iii) by subtracting ii) from Eq. (A.2). $\square$

In view of Lemma 2 i), from now on we consider the allele frequency model given by the Poisson integral $\langle \mathcal{X}_t^N, f \rangle$, $t \geq 0$, defined by

$$\langle \mathcal{X}_t^N, f \rangle = \int_{[0,1]\times\mathcal{D}}f(\xi_t^{0,y})\mathcal{M}_{\gamma}(dy,d\xi^{0,y}) + \int_0^t\int\int_{\mathcal{D}}f(\xi_t^{s,y})\mathcal{N}_N(ds,dy,d\xi^{s,y}).$$

Here, the site frequency paths enter the system either at $t = 0$ according to a Poisson measure with intensity $\omega_{\gamma}\psi_{\gamma}(y)dy$, $0 \leq y \leq 1$, or at constant rate Nds , $s \geq 0$, with initial frequency $1/N$. In particular,

$$\langle \mathcal{X}_0^N, f \rangle = \int_0^1f(y)\mathcal{N}_{\gamma}(dy)$$

is independent of N and we write $\mathcal{X}_0$. This setting allows us to analyze the effect of allele fixations, by dropping the restriction $f(1) = 0$ required for

925 $\tilde{\mathcal{F}}_0$. The next result shows that fixations build up at an asymptotically linear
926 rate over time with the deviation from linearity controlled by

$$T_t^\gamma f(x) = \mathbb{P}_x^\gamma(\tau_1 \leq t)f(1) + \mathbb{E}_x^\gamma[f(\xi_t), \tau > t] \rightarrow \frac{1 - e^{-2\gamma x}}{1 - e^{-2\gamma}} f(1), \quad t \rightarrow \infty,$$

927 where τ_1 is fixation time and τ absorption time.

928 **Lemma 3.** *For each $f \in \tilde{\mathcal{F}}$ as $N \rightarrow \infty$*

- i) $\mathbb{E} \left[\int_0^t \int \int_{\mathcal{D}} f(\xi_t^{s,y}) \mathcal{N}_N(ds, dy, d\xi^{s,y}) \right]$
 $\rightarrow \int_0^1 (f(y) - T_t^\gamma f(y)) \omega_\gamma \psi_\gamma(y) dy + f(1) \omega_\gamma t,$
- ii) $\mathbb{E}_{\mathcal{X}_0} \langle \mathcal{X}_t^N, f \rangle \rightarrow \langle \mathcal{X}_0, T_t^\gamma f \rangle + \int_0^1 (f(y) - T_t^\gamma f(y)) \omega_\gamma \psi_\gamma(y) dy + f(1) \omega_\gamma t,$
- iii) $\mathbb{E} \langle \mathcal{X}_t^N, f \rangle \rightarrow \int_0^1 f(y) \omega_\gamma \psi_\gamma(y) dy + f(1) \omega_\gamma t.$

929 *Proof.* We begin with the basic representation of the expected value given
930 by

$$\begin{aligned} & \mathbb{E} \left[\int_0^t \int \int_{\mathcal{D}} f(\xi_t^{s,y}) \mathcal{N}_N(ds, dy, d\xi^{s,y}) \right] \\ &= N\mathbb{E}_{1/N}^\gamma \left[\int_0^t f(\xi_t^s) ds \right] = N\mathbb{E}_{1/N}^\gamma \left[\int_0^t f(\xi_u^0) du \right]. \end{aligned}$$

931 and apply the rewriting

$$\begin{aligned} N\mathbb{E}_{1/N}^\gamma \left[\int_0^t f(\xi_u) du \right] &= N\mathbb{E}_{1/N}^\gamma \left[\int_0^{t \wedge \tau} (f(\xi_u) - f(1)q_\gamma(\xi_u)) du \right] \\ &\quad + f(1) Nq_\gamma(1/N) t, \end{aligned}$$

932 see [Kaj and Mugal \(2016, Section 6.3, Eq. \(30\)\)](#). Here, $Nq_\gamma(1/N) \rightarrow \omega_\gamma$,
933 $N \rightarrow \infty$. Moreover, the function g defined by $g(y) = f(y) - f(1)q_\gamma(y)$

934 belongs to $\tilde{\mathcal{F}}_0$ and hence, by iii) in Lemma 2,

$$\begin{aligned} N\mathbb{E}_{1/N}^\gamma \left[\int_0^{t \wedge \tau} g(\xi_u) du \right] &= N\mathbb{E}_{1/N}^\gamma \left[\int_0^t g(\xi_u) du \right] \\ &\rightarrow \int_0^1 (g(y) - T_t^\gamma g(y)) \omega_\gamma \psi_\gamma(y) dy, \end{aligned}$$

935 where

$$g(y) - T_t^\gamma g(y) = f(y) - T_t^\gamma f(y) + f(1)(T_t^\gamma q_\gamma(y) - q_\gamma(y)).$$

936 It is a well-known property of the Wright-Fisher diffusion, derived using

937 Itô's formula, that $q_\gamma(\xi_t)$ is a P_x^γ -martingale, in particular $E_y^\gamma[q_\gamma(\xi_t)] = q_\gamma(y)$.

938 Thus, $g(y) - T_t^\gamma g(y) = f(y) - T_t^\gamma f(y)$, and so

$$N\mathbb{E}_{1/N}^\gamma \left[\int_0^t f(\xi_u) du \right] \rightarrow \int_0^1 (f(y) - T_t^\gamma f(y)) \omega_\gamma \psi_\gamma(y) dy + f(1) \omega_\gamma t,$$

939 as required to show i). Statements ii) and iii) follow by observing

$$\mathbb{E}_{\mathcal{X}_0} \left[\int_{[0,1] \times \mathcal{D}} f(\xi_t^{0,y}) \mathcal{M}_\gamma(dy, d\xi_t^{0,y}) \right] = \int_0^1 T_t^\gamma f(y) \mathcal{N}_\gamma(dy) = \langle \mathcal{X}_0, T_t^\gamma f \rangle$$

940 and

$$\mathbb{E} \int_0^1 T_t^\gamma f(y) \mathcal{N}_\gamma(dy) = \int_0^1 T_t^\gamma f(y) \omega_\gamma \psi_\gamma(y) dy.$$

941

□

942 B. THE AFS DURING NONEQUILIBRIUM AFTER A 943 CHANGE IN POPULATION SIZE

944 The results in Appendix A extend to the (γ, κ) Wright-Fisher model with
945 infinitesimal generator

$$Q_{\gamma,\kappa} f(x) = \gamma x(1-x) f'(x) + \frac{1}{2\kappa} x(1-x) f''(x), \quad f \in \mathcal{D},$$

by invoking the associated expectation operator $T_t^{\gamma,\kappa} f(x) = \mathbb{E}_x^{\gamma,\kappa}[f(\xi_t)]$, the scaled fixation probability $\omega_{\gamma,\kappa}$, and the intensity function $\psi_{\gamma,\kappa}(y)$, introduced in Eqs. (3) and (4). We are then in position to derive the AFS during nonequilibrium enforced by population size N_κ , that is, we run the system with $\kappa = 1$ up to a time point $t^* \geq 0$ and apply $\kappa \neq 1$ from thereon. The modified Poisson random measure, denoted $\mathcal{N}_{N_\kappa}(ds, dy, d\xi^{s,y})$, applies Poisson points for which the dynamics of the paths $(\xi_t^s)_{t \geq s}$ change with the current size of the population.

Theorem 1. For $f \in \tilde{\mathcal{F}}$ the nonequilibrium AFS at time $t \geq t^*$ after a change in population size at t^* is given by

$$\lim_{N \rightarrow \infty} \mathbb{E} \langle \mathcal{X}_t^{N_\kappa}, f \rangle = f(1) \{ \omega_{\gamma,1} t^* + \omega_{\gamma,\kappa} (t - t^*) \} + \int_0^1 T_{t-t^*}^{\gamma,\kappa} f(y) (\omega_{\gamma,1} \psi_{\gamma,1}(y) - \omega_{\gamma,\kappa} \psi_{\gamma,\kappa}(y)) dy + \int_0^1 f(y) \omega_{\gamma,\kappa} \psi_{\gamma,\kappa}(y) dy.$$

Proof. The allele frequencies originating from mutations before t^* form a stationary AFS with Poisson intensity $\omega_{\gamma,1} \psi_{\gamma,1}(y) dy$ at time t^* , compare Lemma 2 i) for $t = 0$. Hence,

$$\int_{-\infty}^{t^*} \int_{\mathcal{D}} \int_{\mathcal{D}} f(\xi_{t^*}^{s,y}) \mathcal{N}_{N_\kappa}(ds, dy, d\xi^{s,y}) \Rightarrow \int_0^1 f(y) \mathcal{N}_\gamma(dy).$$

Conditioning on $\mathcal{X}_{t^*}^{N_\kappa} = \mathcal{N}_\gamma$, by Lemma 3 ii) (with t^* replacing $t = 0$ as initial time),

$$\mathbb{E}_{\mathcal{X}_{t^*}^{N_\kappa}} \langle \mathcal{X}_t^{N_\kappa}, f \rangle \rightarrow \langle \mathcal{N}_\gamma, T_{t-t^*}^{\gamma,\kappa} f \rangle + \int_0^1 (f(y) - T_{t-t^*}^{\gamma,\kappa} f(y)) \omega_{\gamma,\kappa} \psi_{\gamma,\kappa}(y) dy + f(1) \omega_{\gamma,\kappa} (t - t^*).$$

By Lemma 3 iii),

$$\mathbb{E} \langle \mathcal{N}_\gamma, T_{t-t^*}^{\gamma,\kappa} f \rangle \rightarrow \int_0^1 T_{t-t^*}^{\gamma,\kappa} f(y) \omega_{\gamma,1} \psi_{\gamma,1}(y) dy + T_{t-t^*}^{\gamma,\kappa} f(1) \omega_{\gamma,1} t^*. \quad (\text{B.1})$$

962 Since $T_t^{\gamma,\kappa} f(1) = f(1)$ for each t , it follows that

$$\begin{aligned} \mathbb{E}\langle \mathcal{X}_t^{N_\kappa}, f \rangle &\rightarrow \int_0^1 T_{t-t^*}^{\gamma,\kappa} f(y) \omega_{\gamma,1} \psi_{\gamma,1}(y) dy + f(1) \omega_{\gamma,1} t^* \\ &+ \int_0^1 (f(y) - T_{t-t^*}^{\gamma,\kappa} f(y)) \omega_{\gamma,\kappa} \psi_{\gamma,\kappa}(y) dy + f(1) \omega_{\gamma,\kappa} (t - t^*), \end{aligned}$$

963 which is the desired relation and hence completes the proof. $\square$

964 C. NUCLEOTIDE DIVERSITY

965 For computing nucleotide diversity, we apply the AFS to the function
966 $f_{\text{pw}}(y) = 2y(1 - y)$ and note that $f_{\text{pw}}(1) = 0$. For the neutral case it holds
967 in equilibrium according to Eq. (7)

$$\pi_S^\kappa = \theta \int_0^1 2y(1 - y) \omega_{0,\kappa} \psi_{0,\kappa}(y) dy = 4\theta\kappa \int_0^1 (1 - y) dy = 2\theta\kappa,$$

968 since $\omega_{0,\kappa} = 1$ and $\psi_{0,\kappa} = 2\kappa/y$. The synonymous diversity during nonequi-
969 librium is obtained by applying the AFS in Eq. (10) to $f_{\text{pw}}(y)$,

$$\begin{aligned} \pi_S^\kappa(t) &= \theta \int_0^1 \mathbb{E}_y^{0,\kappa}[2\xi_t^{t^*}(1 - \xi_t^{t^*})] \left(\frac{2}{y} - \frac{2\kappa}{y}\right) dy + \theta \int_0^1 2y(1 - y) \frac{2\kappa}{y} dy \\ &= 2\theta(1 - \kappa)e^{-(t-t^*)/\kappa} + 2\theta\kappa, \end{aligned}$$

970 where we used Itô's formula to compute $\mathbb{E}_y^{0,\kappa}[\xi_t^{t^*}(1 - \xi_t^{t^*})] = e^{-(t-t^*)/\kappa} y(1 - y)$.

971 It holds $\pi_S^\kappa(t^*) = 2\theta$ and $\pi_S^\kappa(t) = \pi_S^\kappa = 2\theta\kappa$ in the limit $t \rightarrow \infty$.

972 For nonsynonymous nucleotide differences we proceed similarly but use
973 the DFE in Eq. (12) to allow for variation in selection. The stationary case
974 follows by integration of Eq. (11) over the DFE,

$$\mathbb{E}\pi_N^{\mathcal{V},\kappa} = 4\theta\kappa \int_{-\infty}^0 \left(\frac{1}{1 - e^{-2v\kappa}} - \frac{1}{2v\kappa} \right) h_{\mathcal{V}}(v) dv.$$

975 This integral is well-defined, which can be seen by looking at the Taylor
 976 expansion of the integrand around zero and noting that the moments of the
 977 gamma distribution are finite. For the behavior of π_N in nonequilibrium we
 978 apply the nonstationary AFS stated in Eq. (10) to $f_{\text{pw}}(y)$, i.e.

$$\begin{aligned}\pi_N^\kappa(t) &= \theta \int_{-\infty}^0 \int_0^1 \mathbb{E}_y^{v,\kappa}[2\xi_t^{t^*}(1 - \xi_t^{t^*})](\omega_{v,1}\psi_{v,1}(y) - \omega_{v,\kappa}\psi_{v,\kappa}(y))h_{\mathcal{V}}(v) dy dv \\ &\quad + \theta \int_{-\infty}^0 \int_0^1 2y(1 - y)\omega_{v,\kappa}\psi_{v,\kappa}(y)h_{\mathcal{V}}(v) dy dv \\ &= 2\theta\mathbb{E} \int_0^1 \mathbb{E}_y^{\mathcal{V},\kappa}[\xi_t^{t^*}(1 - \xi_t^{t^*})](\omega_{\mathcal{V},1}\psi_{\mathcal{V},1}(y) - \omega_{\mathcal{V},\kappa}\psi_{\mathcal{V},\kappa}(y)) dy + \mathbb{E}\pi_N^{\mathcal{V},\kappa}.\end{aligned}$$

979 Since $\xi_t^{t^*} \in \{0, 1\}$ for $t \rightarrow \infty$, it holds $\mathbb{E}_y^{\mathcal{V},\kappa}[\xi_t^{t^*}(1 - \xi_t^{t^*})] = 0$ in the limit
 980 $t \rightarrow \infty$, implying $\pi_N^\kappa(t) \rightarrow \mathbb{E}\pi_N^{\mathcal{V},\kappa}$. We can further explicitly write

$$\begin{aligned}\omega_{\gamma,1}\psi_{\gamma,1}(y) - \omega_{\gamma,\kappa}\psi_{\gamma,\kappa}(y) \\ = \frac{2}{y(1 - y)} \left(\frac{1 - e^{-2\gamma(1-y)}}{1 - e^{-2\gamma}} - \frac{\kappa(1 - e^{-2\gamma\kappa(1-y)})}{1 - e^{-2\gamma\kappa}} \right)\end{aligned}\tag{C.1}$$

981 for $\gamma \neq 0$, and $\psi_{0,1}(y) - \psi_{0,\kappa}(y) = 2/y - 2\kappa/y = 2(1 - \kappa)/y$.

982 D. THE NUMBER OF FIXATIONS

983 The number of fixations before the change in population size, i.e. $Z^{\gamma,\kappa}(t)$
 984 for $t \leq t^*$, is obtained by applying the equilibrium AFS in Eq. (7) to $f_{\text{fix}}(y)$,

$$Z^{\gamma,\kappa}(t) = \theta \omega_{\gamma,1} f_{\text{fix}}(1) t + \theta \int_0^1 f_{\text{fix}}(y) \omega_{\gamma,1} \psi_{\gamma,1}(y) dy = \theta \omega_{\gamma,1} t.$$

985 The integral term vanishes and $\kappa = 1$, since for $t \leq t^*$ the population size is
 986 $N_\kappa = N$.

987 For $t > t^*$, we apply the nonequilibrium AFS in Eq. (10),

$$\begin{aligned} Z^{\gamma,\kappa}(t) &= \theta\{\omega_{\gamma,1} t^* + \omega_{\gamma,\kappa}(t - t^*)\} \\ &\quad + \theta \int_0^1 \mathbb{E}_y^{\gamma,\kappa} f_{\text{fix}}(\xi_t^{t^*}) (\omega_{\gamma,1} \psi_{\gamma,1}(y) - \omega_{\gamma,\kappa} \psi_{\gamma,\kappa}(y)) dy \\ &\quad + \theta \int_0^1 f_{\text{fix}}(y) \omega_{\gamma,\kappa} \psi_{\gamma,\kappa}(y) dy. \end{aligned}$$

988 Since the last term vanishes and $\mathbb{E}_y^{\gamma,\kappa} f_{\text{fix}}(\xi_t^{t^*}) = \mathbb{P}_y^{\gamma,\kappa}(\tau_1 \leq t - t^*)$, as we have
989 shown in Eq. (15), it follows

$$\begin{aligned} Z^{\gamma,\kappa}(t) &= \theta\{\omega_{\gamma,1} t^* + \omega_{\gamma,\kappa}(t - t^*)\} \\ &\quad + \theta \int_0^1 \mathbb{P}_y^{\gamma,\kappa}(\tau_1 \leq t - t^*) (\omega_{\gamma,1} \psi_{\gamma,1}(y) - \omega_{\gamma,\kappa} \psi_{\gamma,\kappa}(y)) dy. \end{aligned}$$

990 The explicit representation for $\omega_{\gamma,1} \psi_{\gamma,1}(y) - \omega_{\gamma,\kappa} \psi_{\gamma,\kappa}(y)$ is given in Eq. (C.1).

991

E. SUPPLEMENTARY FIGURES

Figure E.6: The fixation rate ratio $\omega^\kappa(t)$ for different values of κ as functions of time. Panel A: change in population size at time $t^* = 10$. Panel B: change in population size at time $t^* = 18$. For comparison, colored, dashed curves represent the weighted fixation rate ratio $\omega_w^\kappa(t)$. Vertical, dashed lines indicate the time t^* . Parameters: $\theta = 1$, and $a = 0.15$ and $ab = 2500$ for the DFE.

Figure E.7: The effective population size based on nucleotide variation, $N_{\text{eff}}^{\pi}(t)$, versus the harmonic mean effective population size, $N_{\text{eff}}^h(t)$, as parametric curves of time after a *recent* change in population size, $t^* = 18 \leq t \leq 20$, for different values of κ . Black, dashed lines show the expected behavior in equilibrium populations. Black crosses at $N_{\text{eff}}^h(t^*) = N_{\text{eff}}^{\pi}(t^*) = 1000$ mark the time of change in population size, t^* . Colored crosses indicate the expected equilibrium value for each κ . Parameters $\theta = 1$ and $N = 1000$.