Modeling the genetic footprint of fluctuating balancing selection: From the local to the genomic scale

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Running head: Footprint of fluctuating balancing selection

Abstract

Natural selection not only affects the actual loci under selection but also leaves "footprints" in patterns of genetic variation in linked genetic regions. This offers exciting opportunities for inferring selection and for understanding the processes shaping levels of genetic variation in natural populations. Here we develop analytical approximations based on coalescent theory to characterize the genetic footprint of a complex, but potentially common type of natural selection: balancing selection with seasonally fluctuating allele frequencies. We show that seasonal allele frequency fluctuations can have important (and partly unexpected) consequences for the genetic footprint of balancing selection. As also confirmed by stochastic simulations, fluctuating balancing selection generally leads to an increase in genetic diversity close to the selected site, the effect of balancing selection, but reduces diversity further away from the selected site, which is a consequence of the allele-frequency fluctuations effectively producing recurrent bottlenecks of allelic backgrounds. This negative effect usually outweighs the positive effect when averaging diversity levels across the entire chromosome. Strong fluctuating balancing selection even induces a loss of genetic variation in unlinked regions, e.g. on different chromosomes. If many loci in the genome are simultaneously under fluctuating balancing selection this could lead to substantial genome-wide reductions in genetic diversity. This may be the case, even if allele-frequency fluctuations are so small that individual footprints are hard to detect. Thus, together with genetic drift, selective sweeps and background selection, fluctuating selection could be one of the major forces shaping levels of genetic diversity in natural populations.

Keywords

Seasonal selection, structured coalescent, genetic diversity, temporally variable selection.

Introduction

One of the key insights of modern population genetics is that natural selection at one locus in the genome can influence patterns of genetic variation at other loci, in particular at closely linked sites (Barton 2000; Cutter and Payseur 2013; Maynard Smith and Haigh 1974). Characterizing these genetic footprints of selection is important for two main reasons. Expected patterns of genetic diversity derived from models can be used to scan genomes for regions under selection or to infer the type of selection acting in a candidate region (Gillespie 1997; Vitti *et al.* 2013). Moreover, cumulatively, the effects of linked selection at many loci contribute to shaping levels of genome-wide variation at neutral loci but also other selected loci (Corbett-Detig *et al.* 2015; Huang *et al.* 2014; Leffler *et al.* 2012). Ultimately, genetic footprints therefore also affect a population's ability to adapt to a changing environment.

The genetic footprints of positive selection (hard and soft selective sweeps) and purifying selection (background selection) have been characterized in detail in numerous studies (for recent reviews see Charlesworth and Jensen 2021; Hermisson and Pennings 2017). Likewise, footprints of balancing selection, where two alleles are maintained at (roughly) constant frequency, are well-understood (e.g. Charlesworth 2006; Fijarczyk and Babik 2015; Gao *et al.* 2015). However, other typical modes of selection in natural populations have received much less attention. One of them is temporally fluctuating selection. Many natural populations experience bouts of strong selection with frequent, more or less regular changes in the direction of selection (Bell 2010; Moorcroft *et al.* 1996; Nicolaus *et al.* 2016; Siepielski *et al.* 2009; Thurman and Barrett 2016). At the genetic level, such fluctuating selection can cause rapid changes in allele frequencies over short time scales (Garcia-Elfring *et al.* 2021; Rudman *et al.* 2022). For example, Bergland *et al.* (2014) found large seasonal allele-frequency fluctuations at hundreds of loci across the genome in a temperate *Drosophila melanogaster* population. Recently, large allele-frequency fluctuations due to fluctuating temperatues have also been found in the non-biting midge *Chironomus riparius* (Pfenninger *et al.* 2022).

Persistent fluctuating selection at a locus can only be observed if polymorphism is maintained. Several plausible mechanisms for the long-term maintenance of genetic variation due to selection exist. In particular, fluctuating selection itself can maintain genetic polymorphism under appropriate conditions (e.g. Gillespie 1973, 1978; Haldane and Jayakar 1963; Wittmann *et al.* 2017; Yi and Dean 2013). Alternatively, it could act in conjunction with other forces maintaining variation, such as negative frequency-dependent selection. In Bergland et al.'s *Drosophila* study, many of the fluctuating polymorphisms found appear to be long-term sta-

ble and are shared with other *Drosophila* populations around the world or even with the sister species *Drosophila simulans*. Also in *Chironomus riparius*, some of the loci under fluctuating selection exhibited signals of long-term balancing selection (Pfenninger *et al.* 2022). In this paper, we will focus on this scenario of "fluctuating balancing selection", where the alleles of a stable polymorphism exhibit temporal fluctuations and explore the corresponding genetic footprint.

Fluctuating balancing selection is essentially a combination of classical balancing selection (maintenance of selected alleles at constant frequencies) and strong directional selection, leading to rapid changes in allele frequencies as in a selective sweep. Well-known results for these limiting scenarios can give us first clues to the footprint of fluctuating balancing selection. Classical balancing selection leads to an increase in diversity at closely linked neutral sites (Charlesworth 2006). This is because close to the selected site where the recombination rate between neutral and selected locus is small, there is little exchange between the two allelic "subpopulations". They become differentiated from each other, and samples including chromosomes from different subpopulations harbor substantial diversity. By contrast, rapid changes in allele frequencies, as in a selective sweep, tend to reduce genetic diversity at linked neutral sites (Maynard Smith and Haigh 1974; Stephan 2019), where alleles can hitchhike to high frequencies or to fixation. More generally, diversity is reduced by hitchhiking when effects of selection in the genomic background lead to an increased offspring number variance at the focal locus (Barton 2000). This also holds if the strength and direction of linked selection fluctuates with time.

These opposite effects of balancing selection and recurrent sweeps on linked diversity lead to the question whether (or when) fluctuating balancing selection has a positive or negative effect on levels of linked neutral variation. Interestingly, numerical and simulation results by Gillespie (1997) and Taylor (2013) for uncorrelated random environmental fluctuations already suggest that fluctuating balancing selection can in some cases increase diversity levels close to the selected site but decrease diversity levels further away from the selected site. However, so far there is a lack of analytical results and the case of seasonally or more generally cyclically fluctuating selection has not been explored in depth. Thus it is not well understood yet how the shape and magnitude of these footprints depends on the parameters of fluctuating selection, such as the strength and dominance of selection and the period of the fluctuation. Furthermore, it is not clear whether the positive or negative effects of fluctuating balancing selection on diversity levels will dominate on the scale of chromosomes and genomes. Here we will investigate these questions in the context of strong selection, where large allele frequency changes happen on a fast timescale (e.g. a few generations), much faster than the timescale of genetic drift, and we will focus on seasonal, i.e. cyclical cases.

Table 1 Fitness values $w_{WW,i}, w_{WS,i}$, and $w_{SS,i}$ for the three genotypes in winter and summer. The index i denotes the generation of the year (starting at 0).

	$w_{WW,i}$	$w_{WS,i}$	$w_{SS,i}$
Winter ($0 \leq i < g_w$)	$1 + s_w$	$1 + h_w s_w$	1
Summer ($g_w \leq i < g_{total}$)	1	$1 + h_s s_s$	$1 + s_s$

Methods

Fluctuating balancing selection and allele-frequency trajectories at the selected locus

As our standard model, we consider a diploid population of size N living in a seasonal environment. Generations are discrete, and every year there are g_w generations of winter followed by g_s generations of summer, with $g_{total} = g_w + g_s$. We initially assume that selection acts at a single locus with two alleles, which we will call the *winter allele* W, and the *summer allele* S. We assume that selection follows a yearly cycle such that the fitness of a genotype (WW, WS, or SS) depends on the generation of the year $i = t \bmod g_{total}$ (Table 1), where t is time in generations and mod is the modulo operator. The winter allele is favored in winter with selection coefficient s_w and dominance coefficient h_w, and the summer allele in summer with selection coefficient s_s and dominance coefficient h_s. While we focus on symmetric scenarios with $s_w = s_s = s$, $h_w = h_s = h$, and $g_w = g_s = g$, we will also explore the role of asymmetry. In addition, we will later relax the assumption of cyclical selection to consider random switches between seasons.

Stable polymorphism in this model occurs if there is marginal overdominance (Haldane and Jayakar 1963), that is, if heterozygotes have a higher geometric mean fitness than either homozygote, so that each allele can invade when rare. If $s_w = s_s = s$ and $h_w = h_w = h$, polymorphism is stable if $(1 + hs)^2 > 1 + s$ or equivalently $h > (\sqrt{1+s} - 1)/s$. In other words, the currently beneficial allele needs to be sufficiently dominant. Note that $h = 0.5$ fulfills this condition. If $h \neq 0.5$, dominance switches between seasons. Coexistence in this model is a special case of the "segregation lift" mechanism for the maintenance of multi-locus polymorphism under cyclically fluctuating selection (Wittmann *et al.* 2017). As mentioned above, multiple other mechanisms could also give rise to fluctuating balancing selection. For example, seasonally fluctuating selection coefficients could act in conjunction with some form of rare-type advantage, e.g. due to specialization on different resources or differences in the interactions with other species. As discussed below, some details of our results will depend on the specific selection model, but most results only depend on the allele-frequency trajectories.

We assume the following life cycle: Each generation t starts with a gamete pool, at which point also the allele frequency p_t is quantified. Second, each allele mutates into the respective

other allele with a probability u per allele copy per generation. Third, there is random union of gametes to form diploid individuals. Finally, there is reproduction according to current fitness values. If we let the population size go to infinity while keeping all selection and dominance parameters constant, the dynamics become deterministic, and we can compute the allele frequency of the winter allele in generation $t+1$, p_{t+1}, from the allele frequency in generation t, p_t, in two steps accounting for mutation,

$$p'_t = (1-u) \cdot p_t + u \cdot (1-p_t), \tag{1}$$

and selection,

$$p_{t+1} = \frac{p_t'^2 \cdot w_{WW,t \bmod g_{total}} \cdot + p'_t(1-p'_t) \cdot w_{WS,t \bmod g_{total}}}{\bar{w}_t}, \tag{2}$$

where $\bar{w}_t = p_t'^2 w_{WW,t \bmod g_{total}} + 2 \cdot p'_t(1-p'_t) \cdot w_{WS,t \bmod g_{total}} + (1-p'_t)^2 w_{SS,t \bmod g_{total}}$ is the average fitness of diploid adults in generation t. Note that because we assume random union of gametes, the population is in Hardy-Weinberg equilibrium before reproduction.

In this study, we assume that the same selection dynamics have been acting for a long time. We thus need to characterize the long-term behavior of (1) and (2). In situations where polymorphism is maintained, we obtain, independently of the initial conditions, a cyclical allele-frequency trajectory described by winter-allele frequencies π_i for $0 \leq i < g_{total}$ such that $p_t = \pi_{t \bmod g_{total}}$. In symmetric cases, we show in Appendix 1 that

$$\frac{\pi_i}{1-\pi_i} \approx \rho^{i-g/2} \text{ for } 0 \leq i \leq g \tag{3}$$

with $\rho := \frac{4+3s}{4+s}$, and thus

$$\pi_i \approx \begin{cases} \frac{1}{1+\rho^{i-g/2}} & \text{for } 0 \leq i \leq g \\ 1 - \frac{1}{1+\rho^{(i-g)-g/2}} = \frac{1}{1+\rho^{-i+3/2 \cdot g}} & \text{for } g \leq i \leq 2g. \end{cases} \tag{4}$$

Note that $\pi_0 = \pi_{2g}$ and that both expressions give the same result for $i = g$.

Overall, we find excellent agreement between this analytic approach and stochastic simulations with $N = 10,000$ (Fig. 1, see below for details of the stochastic simulation methods). Relevant summary statistics of the allele-frequency trajectories are shown in Fig. 2. The magnitude of fluctuations increases both with increasing number of generations per season (Figs. 1 and 2 A) and with increasing selection coefficient (Fig. 2 B), but is barely affected by changes in the dominance coefficient and the mutation rate (Fig. 2 C, D).

Although we focus mainly on seasonal (periodic) fluctuations, we also present some results for an uncorrelated random environment, where selection in each generation is of type W or S independently and with equal probability in every generation (see Table 1). Since autocor-

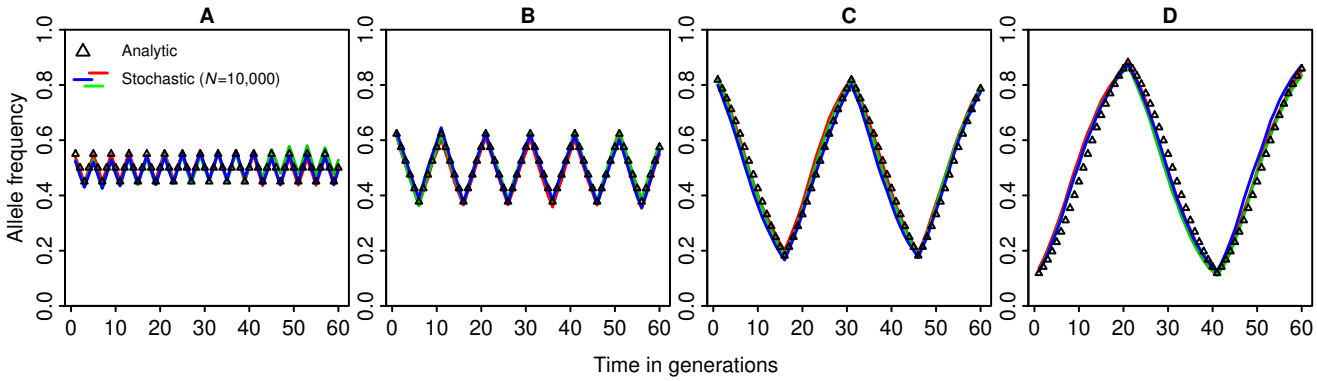

Figure 1 Comparison of deterministic allele-frequency trajectories as determined analytically using (4) with three replicate stochastic simulations (conducted via the Python package simuPOP, see below for details) shown in different colors, though overlapping to a large extent. The panels differ in season length, g. A) $g = 2$, B) $g = 5$, C) $g = 15$, and D) $g = 20$. Other parameters: $s = 0.5, h = 0.6, u = 10^{-6}$.

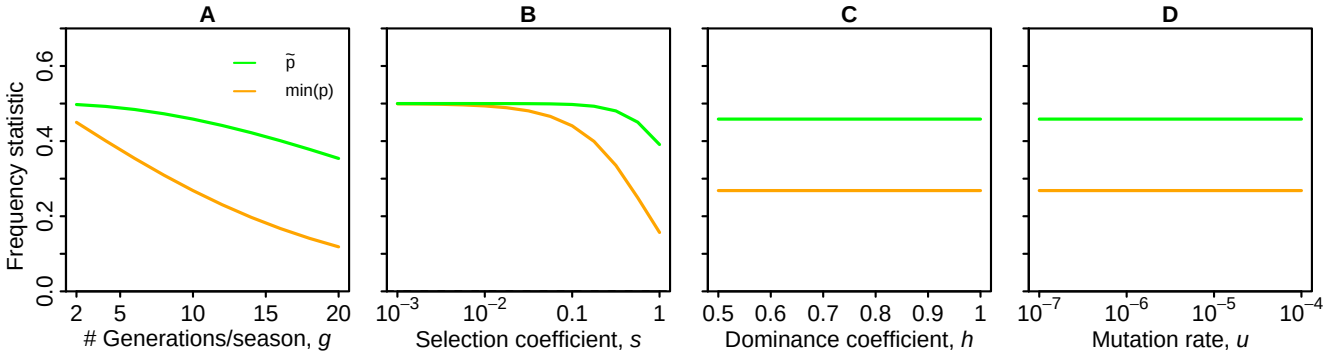

Figure 2 Statistics of cyclical allele frequency trajectories calculated using the analytic approximation in (4) (lines). $\tilde{p}$ is the harmonic mean allele frequency that is important for the quantification of diversity below. $\min(p)$ is the minimum winter allele frequency in a cycle and thus a measure for the magnitude of fluctuations. Other parameters: $N = 10,000, u = 10^{-6}, g = 10, s = 0.5, h = 0.6$.

relation is irrelevant for the stability of polymorphism by marginal overdominance (Gillespie 1973), the conditions for stable polymorphism are the same as in the seasonal model.

Quantifying expected diversity levels

We now turn our attention to the linked variation at either side of the selected locus, and consider a biallelic neutral locus at recombination distance r (with recombination probability $0 \leq r \leq 0.5$). Symmetric mutation at this locus changes its allelic state with probability u per generation. Our quantity of interest is the expected heterozygosity, defined as the probability that two randomly chosen chromosomes have different alleles at the site of interest. With symmetric biallelic mutation, this requires an odd number of mutations on the ancestral lineages connecting the two sampled chromosomes with their common ancestor.

Fluctuating balancing selection affects linked diversity in two main ways, which we will address in two separate steps in our analytical approximation. First, balancing selection (fluctuating or not) that maintains two alleles at the selected locus for a long time, structures the population into two parts according to the allelic state: winter and summer. Ancestral lines of descent from different parts of this genetically structured population can only coalesce after a recombination event along these lines unites them in the same background. This effect is most conveniently described by a structured coalescent model. Second, rapid allele frequency changes due to fluctuating selection increase the offspring variance not only at the selected site, but also at linked (and even unlinked) neutral sites due to the hitchhiking effect (Hill and Robertson 1966; Robertson 1961). Following Barton (2000), this effect can be captured by an appropriate rescaling of the effective population size.

Analytical approximation step 1: structured coalescent

In the first step of our approximation, we use a version of the classical structured coalescent model (Hudson and Kaplan 1988; Kaplan *et al.* 1988) to derive the expected time to the most recent common ancestor (coalescence) for samples of size two from a neutral locus at recombination distance r from the selected site. Each lineage at the neutral locus can be either in a winter or summer background, meaning that the chromosome carries the winter or the summer allele, respectively, at the selected locus. We trace the ancestry of two sampled lineages backward in time and keep track of their genetic background. Formally, for a sample of size two, this leads to a Markov chain with four states: state (2,0) with both lineages in the W (winter allele) background, state (0,2) with both lineages in the S (summer allele) background, state (1,1) with one lineage in each background, and finally the absorbing state (*) where both lineages have coalesced. Coalescence is only possible if both lineages are in the same background. Lineages can switch to the other background either due to a recombination between neutral and selected site or due to a mutation at the selected site.

Given an allele frequency p for a focal allele at the selected locus, lineages within this background coalesce at rate $1/(2Np)$, they recombine into the other background with rate $r(1-p)$ and they mutate into the other background with rate $u(1-p)/p$. In the standard model of balancing selection, allele frequencies are constant over time. In our model, however, allele frequencies and thus transition probabilities vary cyclically over time. This would make our Markov chain time-inhomogeneous and analytically intractable. In the first step of our approximation, we therefore assume that N is sufficiently large and that u and r are small such that coalescence, mutation, and recombination probabilities are small per generation and even per year, and the probability of multiple events per year is negligible (we relax this assumption in the second step of our approximation below). We can then approximate the ancestral process by a homogeneous continuous-time Markov process whose rates are given by the respective average transition probabilities over the cyclic allele-frequency trajectory as given by the frequencies π_i. The coalescence rate per generation in the new process is

$$c := \frac{1}{2g} \sum_{t=0}^{2g-1} \frac{1}{2N\pi_t} = \frac{1}{2N\tilde{p}}, \tag{5}$$

where $\tilde{p}$ is the harmonic mean allele frequency over the cycle. Here we focus on symmetric situations, such that both alleles have the same harmonic mean frequency, i.e. the harmonic mean of π_t is equal to the harmonic mean of $1-\pi_t$ (see Appendix 6 for asymmetric cases). Next, we consider the transition rate between backgrounds due to mutation:

$$m_1 := u \cdot \frac{1}{2g} \sum_{t=0}^{2g-1} \frac{1-\pi_t}{\pi_t} = u \cdot \frac{1}{2g} \sum_{t=0}^{2g-1} \frac{\pi_t}{1-\pi_t}. \tag{6}$$

Again, the middle equality holds because of symmetry, so that the transition rate is the same in both directions. m_1 can be expressed in terms of the harmonic mean:

$$m_1 = u \cdot \frac{1}{2g} \sum_{t=0}^{2g-1} \left(\frac{1}{\pi_t} - 1 \right) = u \cdot \left(\frac{1}{\tilde{p}} - 1 \right). \tag{7}$$

Finally, we consider the transition rate between backgrounds due to recombination:

$$m_2 := r \cdot \frac{1}{2g} \sum_{t=0}^{2g-1} 1 - \pi_t = r \cdot \frac{1}{2g} \sum_{t=0}^{2g-1} \pi_t =: r \cdot \bar{p}, \tag{8}$$

where $\bar{p}$ is the arithmetic mean frequency along the cycle, i.e. 0.5 in symmetric cases. Let $m = m_1 + m_2$ be the total rate at which lineages switch to the respective other background due to mutation or recombination.

We now use these transition rates to compute the expected time to coalescence for a

sample of two lineages via first-step analysis:

$$\mathbf{E}[T_{(2,0)}] = \mathbf{E}[T_{(0,2)}] = \frac{1}{c+2m} + \frac{2m}{c+2m} \cdot \mathbf{E}[T_{(1,1)}] \tag{9}$$

and

$$\mathbf{E}[T_{(1,1)}] = \frac{1}{2m} + \mathbf{E}[T_{(2,0)}]. \tag{10}$$

Solving the recursion, we have

$$\mathbf{E}[T_{(2,0)}] = \mathbf{E}[T_{(0,2)}] = \frac{2}{c} = 4N\tilde{p} \tag{11}$$

and

$$\mathbf{E}[T_{(1,1)}] = \frac{1}{2m} + \frac{2}{c} = \frac{1}{r + 2u\left(\frac{1}{\tilde{p}} - 1\right)} + 4N\tilde{p}. \tag{12}$$

In summary, for symmetric selection scenarios our analytical approximation depends just on population size, mutation rate, recombination rate, and the harmonic mean allele frequency over the cycle, which can be computed from the analytical approximation for the cyclical allele-frequency trajectory in (4) as

$$\tilde{p} \approx \frac{2g}{\sum_{i=1}^{g} \frac{1}{\pi_i} + \sum_{i=1}^{g} \frac{1}{1-\pi_i}} = \frac{2g}{2g + \sum_{i=1}^{g} \rho^{i-g/2} + \sum_{i=1}^{g} \rho^{g/2-i}} \tag{13}$$

$$= \frac{2g}{2g + \rho^{g/2} + \rho^{-g/2} + 2\sum_{i=1}^{g-1} \rho^{i-g/2}}. \tag{14}$$

$$= \frac{2g(\rho - 1)}{2g \cdot (\rho - 1) + (\rho^{g/2} - \rho^{-g/2}) \cdot (\rho + 1)}, \tag{15}$$

where we have used the fact that the sum in (14) is a finite geometric sum after factoring out $\rho^{-g/2}$. Note that the harmonic mean allele frequency does not depend on the ordering of allele frequencies in a cycle and can take the same value also for cases with different cycle lengths.

Finally, given the expected coalescence time for sample configuration (i, j), we approximate the corresponding expected heterozygosity as

$$\mathbf{E}[H_{(i,j)}] \approx \frac{1}{2}\left(1 - \frac{1}{1 + 4u \cdot \mathbf{E}[T_{(i,j)}]}\right), \tag{16}$$

which takes into account back-mutations (see derivation in Appendix 2). Since sampling in our model happens before selection, we can assume Hardy-Weinberg equilibrium, and the expected heterozygosity in a random sample at time t is

$$\mathbf{E}[H_t] \approx p_t^2 \cdot \mathbf{E}[H_{(2,0)}] + 2p_t(1 - p_t)\mathbf{E}[H_{(1,1)}] + (1 - p_t)^2 \cdot \mathbf{E}[H_{(0,2)}]. \tag{17}$$

The baseline heterozygosity under neutrality to which heterozygosities are compared in all three approaches, is given by substituting $2N$, the expected coalescence time under neutrality, for $\mathbf{E}[T_{(i,j)}]$ in (16).

If mutation rate is very small compared to recombination rate, we can further approximate (17). Using a first-order Taylor approximation around $u = 0$, we obtain

$$\mathbf{E}[H_{(2,0)}] = \mathbf{E}[H_{(0,2)}] \approx 8N\tilde{p}u \tag{18}$$

and

$$\mathbf{E}[H_{(1,1)}] \approx 2u \cdot \left(\frac{1}{r} + 4N\tilde{p}\right) \tag{19}$$

and thus

$$\mathbf{E}[H_t] \approx 8N\tilde{p}u + 4p_t(1-p_t)\frac{1}{r}. \tag{20}$$

The % change in diversity relative to the neutral expectation for small u ($4Nu$) is then given by

$$100 \cdot \left(2\tilde{p} + p_t(1-p_t)\frac{1}{N \cdot r \cdot u} - 1\right). \tag{21}$$

Interestingly, this just depends on the compound parameter $N \cdot r$. The underlying biological reason is that the decrease of the diversity close to the selected site only depends on the relative size of two time scales: the coalescence time scale ($\sim\ N$) and the recombination time scale $\sim\ (1/r)$. With large $N \cdot r$, recombination is fast enough that it mixes backgrounds before coalescence typically occurs and the genetic structure does not play a large role any more.

Equation (17) (or for small mutation rate (20)) complete the first step of our analytical approximation, which is expected to be accurate as long as recombination is rare on the timescale of the fluctuation period (or season).

Analytic approximation step 2: Hitchhiking

In our approximation so far, we have assumed that all ancestral lineages experience an averaged environment of summer and winter generations on their course back in time to the first non-trivial genealogical event, with can be mutation, recombination, or coalescence. For seasonal fluctuations and realistic population sizes and mutation rates, this is generally true for mutation and coalescence. With loose linkage and recombination probabilities up to 0.5, however, lineages will typically switch backgrounds multiple times per cycle. On these short timescales, random associations of lineages at the neutral locus with either the W- or S-allele do not average out, but contribute to an increased offspring number variance among lineages, or to a decreased variance-effective population size.

The second step of our approximation accounts for these hitchhiking effect of fluctuat-

ing selection with loose linkage. Instead of extending the structured coalescent to a time-inhomogeneous Markov process, we use a method by Barton (2000) to derive the effect of the fluctuations. To this end, we start from Eq. (A6) in Barton (2000), which states that the hitchhiking effect of fluctuating selection decreases the variance-effective population sizes by a factor F, i.e. $N_e = N/F$, with

$$F = 1 + \mathbf{E}\left[\frac{1}{p_{\tau'}(1-p_{\tau'})}\left(\int_{\tau'}^{\infty}\frac{dp_\tau}{d\tau}e^{-r(\tau-\tau')}d\tau\right)^2\right], \tag{22}$$

where the expectation is over the time course of p'_τ. Note that we use here τ' as a lower integration boundary because the 0 in Barton (2000) appears to be a typo. Discretizing this equation to match our discrete-time model leads to

$$F = 1 + \mathbf{E}\left[\frac{1}{p_{\tau'}(1-p_{\tau'})}\left(\sum_{\tau=\tau'}^{\infty}(p_{\tau+1}-p_\tau)e^{-r(\tau-\tau')}\right)^2\right]. \tag{23}$$

For the uncorrelated selection scenario we use this equation directly, but with an upper summation boundary of $\tau' + 500$. For cyclical selection, we can further simplify:

$$F = 1 + \frac{1}{g_{total}}\cdot\sum_{i=0}^{g_{total}-1}\frac{1}{\pi_i\cdot(1-\pi_i)}\cdot\left(\underbrace{\sum_{\tau=0}^{\infty}\Delta_{(i+\tau)\bmod g_{total}}\cdot e^{-r\cdot\tau}}_{=:S_i}\right)^2, \tag{24}$$

where

$$\Delta_i = \begin{cases}\pi_{i+1}-\pi_i & \text{for } i < g_{total}-1\\ \pi_0-\pi_{g_{total}-1} & \text{for } i = g_{total}-1\end{cases} \tag{25}$$

are the allele-frequency increments from generation to generation in the cycle.

To evaluate the infinite sum, we split up the terms according to position in the cycle and then use one geometric series per point in the cycle:

$$S_i = \sum_{j=0}^{g_{total}-1}\sum_{c=0}^{\infty}\Delta_{(i+j)\bmod g_{total}}\cdot e^{-r\cdot(c\cdot g_{total}+j)} \tag{26}$$

$$= \sum_{j=0}^{g_{total}-1}\Delta_{(i+j)\bmod g_{total}}\cdot e^{-r\cdot j}\sum_{c=0}^{\infty}\left(e^{-r\cdot g_{total}}\right)^c \tag{27}$$

$$= \sum_{j=0}^{g_{total}-1}\Delta_{(i+j)\bmod g_{total}}\cdot\frac{e^{-r\cdot j}}{1-e^{-r\cdot g_{total}}}. \tag{28}$$

In Appendix 3 we show that F converges to $1/(2\tilde{p})$ as the recombination rate between

neutral and selected locus approaches zero. We can thus complete our analytical approximation by substituting $1/(2F)$ for $\tilde{p}$ in (11) and (12). In summary, this extended approximation reduces to the one from our first step for small recombination distances, but captures the phenomenon that lineages can switch background multiple times per season for large recombination distances. Also note that unlike the first analytical approximation, the ordering of allele-frequencies in a cycle and the cycle length can now have an effect via F.

Stochastic individual-based simulations

To check our analytical results, we ran individual-based simulations using the Python library simuPOP version 1.1.7 (Peng and Kimmel 2005). Because of computational constraints, we did not simulate entire chromosomes of several millions to billions of sites. Instead, we simulated L sites (e.g. 40,000) linked to the selected locus and placed them strategically so that we obtain more data close to the selected site where patterns of variation are expected to change on a smaller scale than far away from the selected locus. Specifically, we set a minimum recombination probability $r_{min} = 10^{-8}$ and a maximum recombination probability $r_{max} = 0.49$ with the selected site and placed the ith simulated site at a recombination distance of $r_{min} \cdot \exp\left((i-1) \cdot \frac{\ln(r_{max})-\ln(r_{min})}{L-1}\right) = r_{min}^{1-(i-1)/(L-1)} \cdot r_{max}^{(i-1)/(L-1)}$ from the selected site. That is, the sites were evenly spaced on a logarithmic scale. To explore the genome-wide effects of multiple loci under fluctuating balancing selection, we will also extend our simulation approach to multiple loci under selection, as described below.

To initialize a simulation run, we drew from a simulated distribution of allele counts under neutrality (see Appendix 4 for details). This was also done for the selected loci. That is, the start of the simulation can also be seen as the onset of selection. Unless stated otherwise, all simulations ran for $15 \cdot N$ generations. Samples of 1,000 individuals (or the entire population if it was below 1,000) were taken roughly every $0.5 \cdot N$ generations, with the precise sampling interval fine-tuned such that sampling always happens after the first generation of a year. Unless stated otherwise, we only used the last five sampling points (at roughly $13N, 13.5N, \ldots, 15N$ generations) as a basis for our results. Our measure of diversity at site i is the heterozygosity $2 \cdot p_i \cdot (1 - p_i)$, where p_i is the sample allele frequency at site i. Heterozygosities at all 40,000 linked sites (i.e. excluding the selected sites) were put into 25 bins of equal size (i.e. the 1600 sites closest to the selected site, the next closest 1,600 sites), and then averaged. The average change in heterozygosity relative to the neutral expectation is plotted as a function of recombination distance, whose summary value for the respective bin is obtained by averaging at the logarithmic scale and then taking the exponential.

Results

Local footprint

The local footprint of fluctuating balancing selection strongly depends on the magnitude of fluctuations (Fig. 3, see Fig. 1 for the corresponding allele-frequency trajectories). For small allele-frequency fluctuations around 0.5 (Fig. 3 A), the genetic footprint looks like the one expected for constant balancing selection or population structure, with a region of increased diversity close to the selected site and a return to neutral levels with increasing recombination distance from the selected site. This is also predicted by our analytical approximations. In the limit of very small fluctuations around 0.5, $\tilde{p} = \bar{p} = 0.5$ and $\mathbf{E}[T_{(2,0)}] = \mathbf{E}[T_{(0,2)}] = 2N$, which is equal to the expected coalescence time under neutrality, whereas $\mathbf{E}[T_{(1,1)}] > 2N$. Thus expected heterozygosity in samples from different backgrounds or in random samples is larger than under neutrality, but is predicted to approach neutral levels with increasing recombination distance from the selected site (see (12)). Close to the selected site, the analytical approximations predict slightly higher expected diversity levels compared to the stochastic simulations and this discrepancy increases in regions even closer to the selected site (Fig. A3). The reason for this discrepancy appears to be that the stochastic simulations did not run for sufficiently many generations to allow diversity to build up to the high equilibrium levels expected very close to the selected site.

As allele-frequency fluctuations increase in magnitude (for example with increasing number of generations per season in Fig. 3 B, C, D), a characteristic footprint of fluctuating balancing selection emerges. Close to the selected site, there is still an increase in diversity relative to neutral expectations, as expected also for constant balancing selection. But now with increasing recombination distance from the selected site, eventually diversity levels fall below neutral expectations. Qualitatively, this can be seen directly in (11) and (12). If selection is fluctuating, the harmonic mean allele frequency, $\tilde{p}$, is smaller than 0.5 and thus the expected coalescence time for two lineages from the same background will be below the neutral baseline of $2N$. In other words, allelic backgrounds experience regular bottlenecks, i.e. times of low frequency during which pairs of lineages currently in the background have an increased coalescence rate (Fig. 4). The second consequence of an increasing magnitude of fluctuations is that transitions between backgrounds due to mutations at the selected locus (allelic turnover) increase with increasing magnitude of fluctuations and thus decreasing $\tilde{p}$ ((12), Fig. 4), which has a negative effect on diversity according to analytic approximation 1. Note that far away from the selected locus an increase in transition rate between backgrounds can have an additional positive effect because it allows lineages to escape shrinking backgrounds, an effect that is only captured in the second step of our analytical approximation.

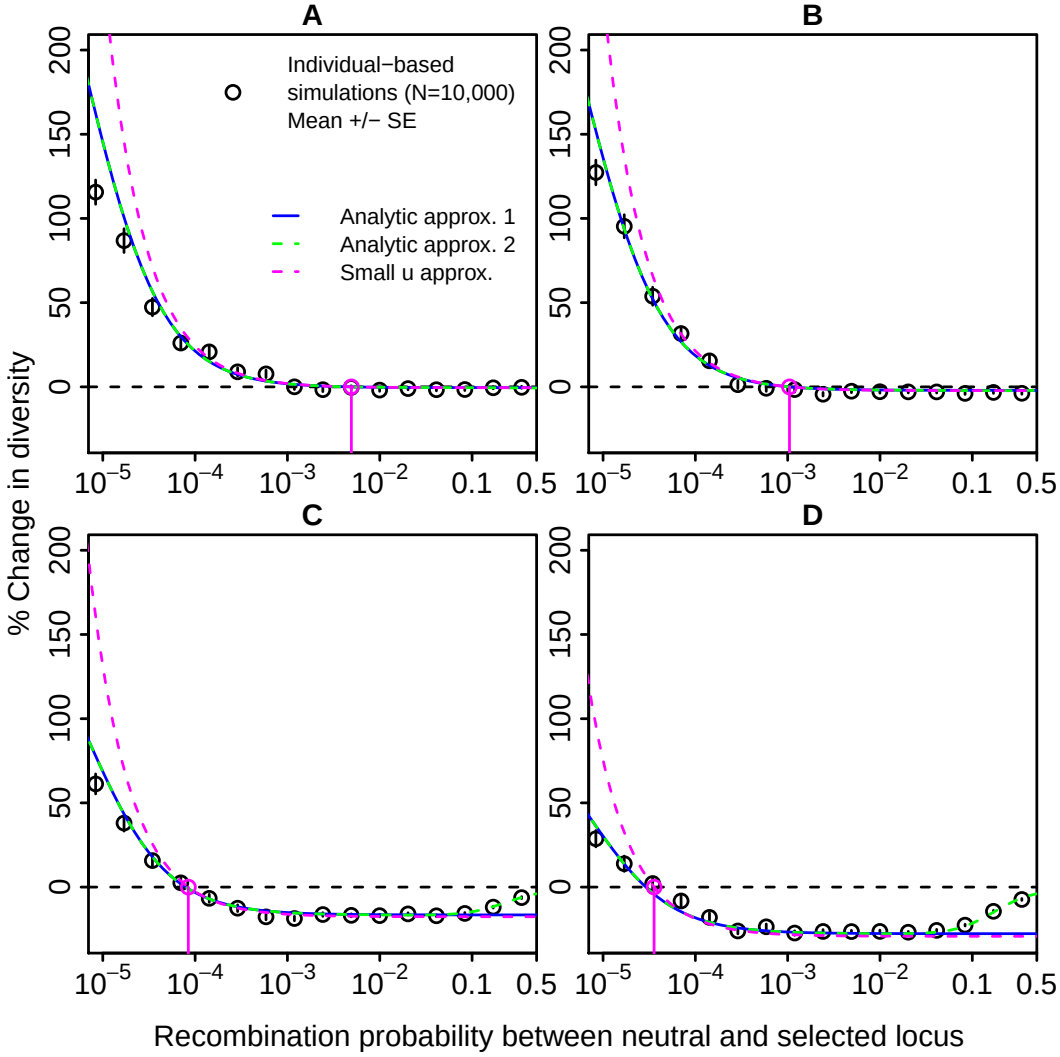

Figure 3 Genetic footprints of fluctuating balancing selection for various season lengths, g. A) $g = 2$, B) $g = 5$, C) $g = 15$, and D) $g = 20$. The y-axis represents the relative change in diversity compared to the neutral case. Points are averages over 100 replicates with five sampling points each. See Fig. 1 for the corresponding allele-frequency trajectories. The pink vertical line and circle indicate the recombination distance at which expected heterozygosity falls below neutral levels as estimated by (29). A zoom into the region of large recombination rates is shown in Fig. A2 and an extension to lower recombination rates is shown in Fig. A3.

For samples from different backgrounds, whether diversity is above or below the neutral baseline depends on the distance from the selected locus. If u is sufficiently small, the first term in (12) will be sufficiently large close to the selected site (for small r) such that $\mathbf{E}[T_{(1,1)}]$ will be larger than $2N$. As we move further away from the selected site (increasing r), $\mathbf{E}[T_{(1,1)}]$ will decrease and approach $4N\tilde{p}$. Diversity levels in a random sample will behave qualitatively similar to diversity levels in samples from different backgrounds. The small mutation rate approximation (20) does not give a good fit close to the selected site where u is not small relative to r, but it can be used to derive an approximation for the recombination rate r^* where heterozygosity levels fall below the small mutation neutral expectation of $4Nu$:

$$r^* = \frac{p_t(1-p_t)}{N(1-2\tilde{p})}, \tag{29}$$

which is shown as a pink vertical line and circle in (Fig. 3). Thus the recombination rate at which diversity falls below neutral levels decreases with increasing N. With increasing magnitude of fluctuations, $\tilde{p}$ decreases and the intersection point moves closer to the selected locus.

From the point where diversity levels cross the neutral expectation, they further decrease and eventually stay roughly constant at a diversity valley over a range of recombination distances. This diversity level is well predicted by all analytic approximations and is given by $H_{min} = [1 - 1/(16uN\tilde{p})]/2$. That is, it just depends on the harmonic mean allele frequency. The larger the allele-frequency fluctuations, the smaller is $\tilde{p}$ and the deeper is the diversity valley.

When recombination distance to the selected locus further increases and approaches 0.5, eventually diversity levels leave the "valley" and start recovering (see Fig. 3 C,D). This is predicted by the second analytical approximation and supported by the individual-based simulations. However, the first analytical approximation suggests that diversity levels stay constant at the valley bottom even as recombination distances become very large. This mismatch arises because analytical approximation 1 does not account for the fact that with larger r lineages are increasingly able to "track" allele frequencies through multiple background switches per year. This suggests that at high recombination distances lineages can escape shrinking backgrounds quickly enough to not be forced together through a bottleneck, especially for a large season length, g . However, diversity levels in the individual-based simulations and analytical approximation 2 do not fully return to neutral levels even at a recombination probability of 0.5 (see Fig. 3 D, Fig. 5). The consequences of this will be explored below when we look at the genome-wide effect of fluctuating balancing selection.

In summary, the whole range of recombination rates can be divided into two regimes. At small and intermediate recombination rates, diversity levels do not depend on the order of allele frequencies in the cycle and also not on the length of the cycle given a constant

harmonic mean allele frequency. This is where our first analytic approximation fits. The second regime holds at high recombination rates where such order effects matter. These are taken into account by the second analytical approximation, which therefore also fits at high recombination rates.

The various ways in which the model parameters influence genetic diversity are qualitatively summarized in Figs. 4 and 5. Detailed results analogous to Fig. 3 for s, h, N, and u are shown in Figs. A4–A7.

Individual-based simulations with different selection coefficients, s, indicate that for small s, diversity levels are close to neutral expectations at all recombination distances (Fig. A4). As s increases, diversity close to the selected site increases while the decrease further away remains negligible. However, when s is further increased and fluctuations become ever larger (see Fig. 2 B), eventually diversity close to the selected site starts to decrease and the diversity valley further away becomes deeper. Since our analytical approaches are based on the assumption of deterministically cycling allele frequencies, they require that selection be strong relative to genetic drift and thus perform poorly at small selection coefficients (Fig. A4).

Similarly, at small population sizes, N, where genetic drift is strong, the genetic footprint of fluctuating balancing selection is weak and the analytical approximations do not fit well. For larger N, approximations and individual-based simulations agree well. While the relative change in diversity far away from the selected site is unaffected by N, the size of the region of increased diversity around the selected site should be inversely proportional to population size, as mentioned above ((29), see also Fig. 5).

The most striking effect of increasing the mutation rate u is that both the increase in diversity close to the selected site and the decrease further away decrease in magnitude (Figs. 5, A6). This is the case because in our biallelic and symmetric mutation model, heterozygosities start to saturate at high mutation rates and are then less sensitive to fluctuating balancing selection (see Appendix 2).

The dominance coefficient, h, has a negligible effect on the genetic footprint of balancing selection (Figs. A7). This is not surprising since h does not even appear in the analytical approximations. Recall, however, that all of our analyses are based on the assumption that the dominance coefficient is large enough for polymorphism to be long-term stable.

Due to the allele-frequency fluctuations, the expected footprint of a random sample also depends on the time in the seasonal cycle in which the sample is taken (Fig. A8). Towards the middle of a season where allele frequencies are more intermediate, there is a stronger increase in diversity close to the selected site. However, the long-range effects are not noticeably affected by the seasonal sampling point.

So far, we have focused on diversity levels long after the onset of cyclical selection pressures. The temporal emergence of these footprints after the onset of fluctuating balancing selection

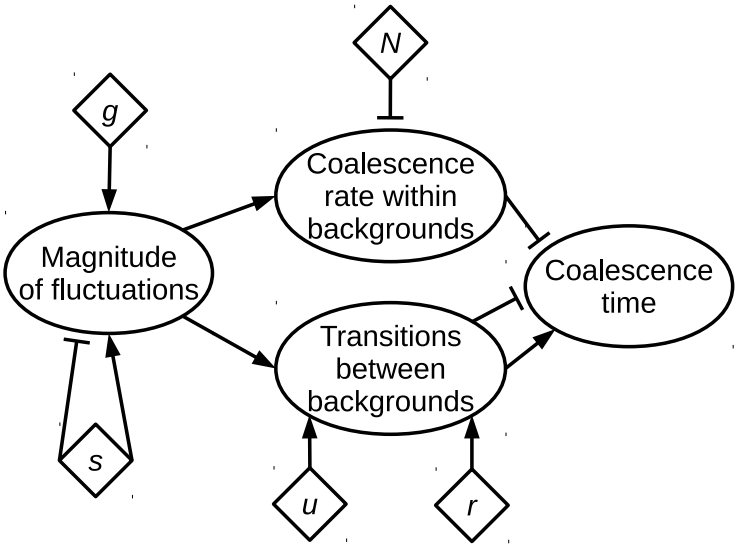

Figure 4 Flowchart illustrating the major effects of the various model parameters (diamonds) on expected coalescence times for a random sample of two lineages. Arrows with arrowheads denote a positive effect (e.g. an increase in season length, g, leads to an increase in the magnitude of fluctuations) whereas arrows with a bar indicate a negative effect (e.g. an increase in population size, N, leads to a decrease in coalescence rates within backgrounds). An increase in selection coefficient, s, makes fluctuations around 0.5 more determinstic and at small s this decreases the magnitude of fluctuations, whereas at large s a further increase increases the magnitude of fluctuations. Similarly, an increase in the transition rate between backgrounds decreases coalescence times as long as it is small, but at large values where multiple transitions per cycle occur it increases coalescence times.

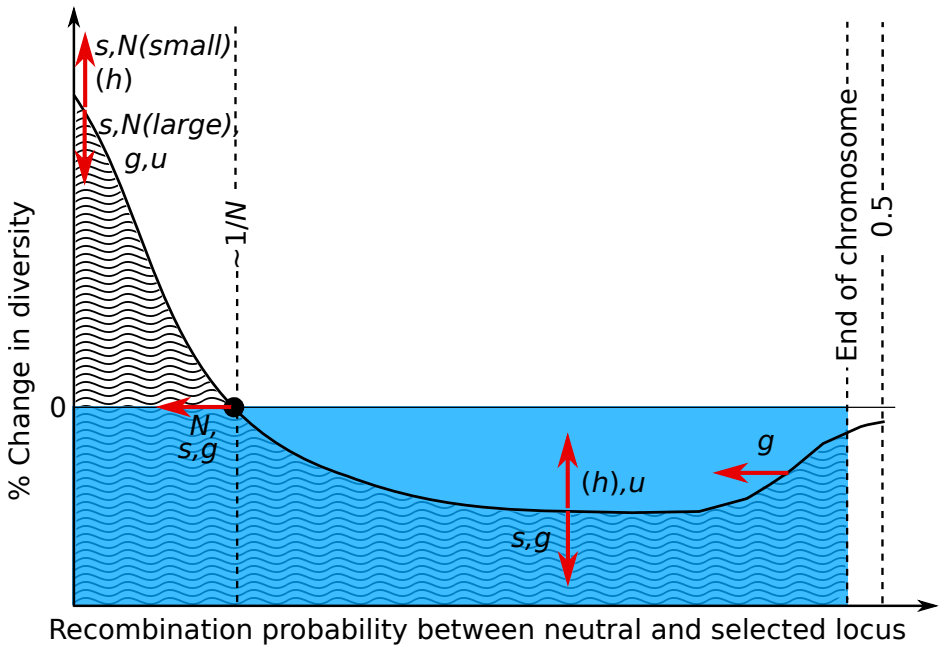

Figure 5 Cartoon illustrating how the various model parameters influence the shape of the genetic footprint of fluctuating balancing selection (area with wave pattern). The blue area corresponds to neutral diversity levels. The red arrows indicate the direction in which the shape of the genetic footprint will change when the respective model parameters are increased. The effect of h is minimal.

is shown in Fig. A9. The negative effect on diversity develops first, especially close to the selected site. Over time, backgrounds become differentiated and diversity close to the selected site builds up and starts to exceed neutral levels. At the same time, also the diversity reduction further away from the selected site intensifies and approaches the final levels.

While we focus on symmetric scenarios, we also simulated asymmetric scenarios and extended the first analytical approximation accordingly (see Appendix 6). The results suggest that with increasing asymmetry the reduction in diversity away from the selected site becomes weaker (Fig. A10). In asymmetric scenarios, there is a substantial reduction in diversity for samples from the rare background close to the selected site, whereas samples from the overall more common background exhibit either a slight increase or a slight decrease in diversity.

A model version with random switches between selection parameters and thus irregular allele frequency fluctuations gives a qualitatively similar local genetic footprint as the model with seasonally fluctuating selection (Fig. 6). Again, we compare the results from individual-based simulations to two analytic approximations. However, in this case, the approximations should rather be called semi-analytic because we based them on allele-frequency trajectories obtained from iterating the deterministic model equations (1) and (2) for 100,000 generations with random environments. From these trajectories, we then calculated the frequency statistics for (11) and (12). We also used the trajectories to evaluate (23). The first semi-analytic approximation again well predicts minimum diversity levels along the chromosome, but not the recovery at high recombination distances. The semi-analytic approximation 2 can also predict this recovery. Compared to our results for the seasonal selection model, it appears that diversity levels recover from this valley bottom at smaller recombination rates. The reason may be that the largest fluctuations in the random-selection model occur over a larger time scale than the typical yearly cycle, thus giving lineages more time to escape from shrinking genetic backgrounds.

Chromosome-wide effect

Given that fluctuating balancing selection can lead to increases in diversity close to the selected site but decreases further away (see Figs. 3 and 5), we also quantify the net effect across the entire chromosome. Additional parameters in this analysis are r_0, the per-generation recombination probability between adjacent base pairs, and r_{max}, the recombination probability between the selected locus and the end of the chromosome. We assume that recombination occurs uniformly along the chromosome.

We quantify the net chromosomal effect in two ways. First, we run individual-based simulations with 40,000 sites between recombination distances $r_0 = 10^{-6}$ and $r_{max} = 0.49$, with the same base-pair distance between all pairs of adjacent sites. We then average heterozygosity levels across this entire chromosome, including the selected site. Second, we use analytic

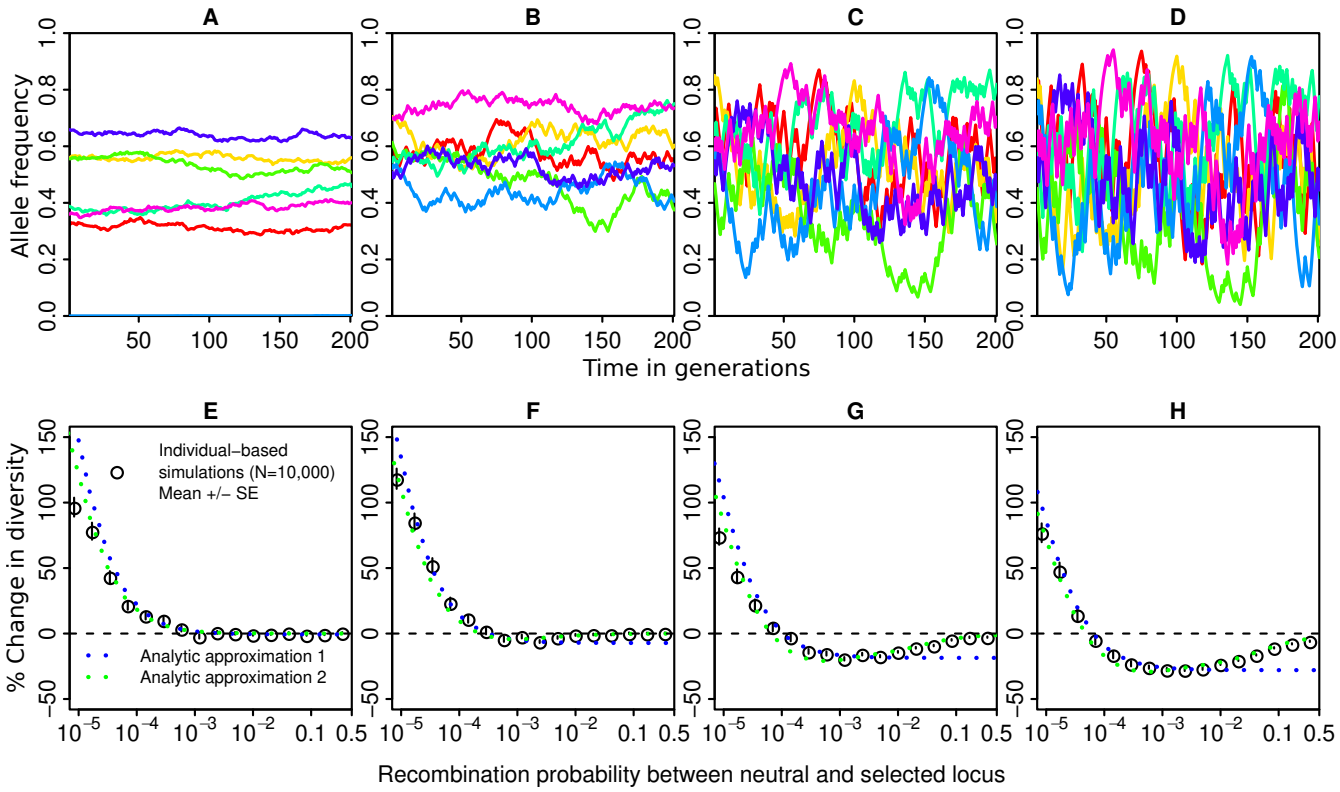

Figure 6 Results for the alternative scenario with uncorrelated selection across generations. The upper row shows seven replicate allele-frequency trajectories with A) $s = 0.01$, B) $s = 0.1$, C) $s = 0.5$, D) $s = 1$. The lower row depicts the corresponding genetic footprints. The simulation results are averages over 100 replicates. Other parameters: $h = 0.6, N = 10,000, u = 10^{-6}$.

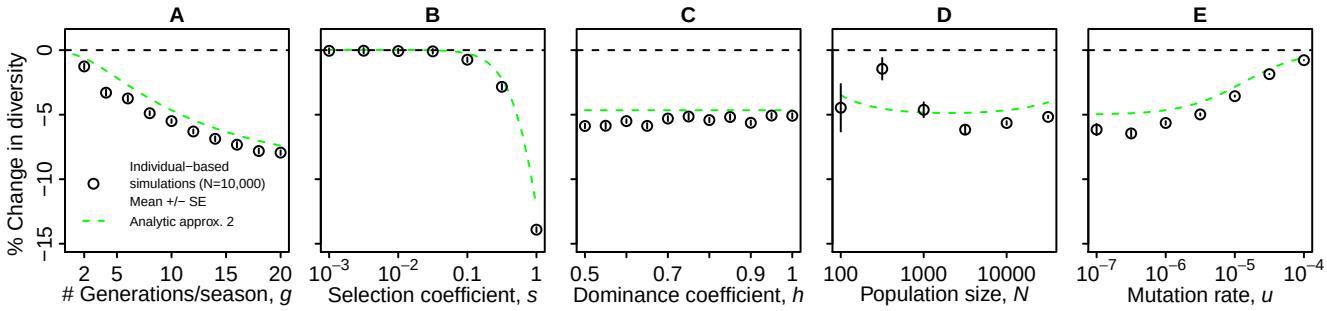

Figure 7 Comparison of analytical approximation 2 and stochastic simulations for the net effect of fluctuating balancing selection. Default parameter values: $r_0 = 10^{-6}, r_{max} = 0.49, N_e = 10,000, u = 10^{-6}, s = 0.5, h = 0.6, g = 10$. Averages over 40 replicates per data point with the last 10 sampling points taken into account. Vertical bars indicate standard errors. See Fig. A11 for the development of the net effect over time.

approximation 2 to quantify the average heterozygosity for 1,000 recombination probabilities, r, between r_0 and r_{max}, evenly spaced on a logarithmic scale. Haldane's map then gives the corresponding distances in base pairs, l, to the selected site as

$$l = -\frac{1}{2 \cdot r_0} \cdot \ln(1 - 2r). \tag{30}$$

We then used the trapezoid rule to average over the footprint. For both approaches, we again set the resulting average heterozygosity in relation to the expected heterozygosity under neutrality. Since analytic approximation 1 breaks down for very high recombination rates, we did not attempt to use it to quantify the net chromosomal effect.

Overall, we find good qualitative agreement between stochastic simulations and analytic approximation 2 (Fig. 7). Both suggest that the net chromosomal effects of fluctuating balancing selection are predominantly negative. The reduction in average diversity levels along the chromosome is particularly strong with long seasons, large selection coefficients, intermediate to large population sizes, and small mutation rates. The dominance coefficient again has a minor effect. Note that at small selection coefficients, the error bars for the stochastic simulation results overlap with zero, so we cannot distinguish whether the average effect is weakly positive or weakly negative. The analytic approximation predicts a very slight increase in diversity beyond neutral levels. Note, however, that this is based on the assumption that selection is strong relative to genetic drift such that deterministic and cyclic allele-frequency trajectories are a good approximation to the actual allele-frequency trajectories, an assumption that is certainly violated for small s. Overall, the chromosome-wide reduction in diversity in the individual-based simulations is slightly larger than predicted by analytic approximation 2.

As with the local genetic footprint, the net chromosomal effect of fluctuating balancing

selection intensifies over time, but with our parameter combinations already saturated after about $3.5N$ generations (Fig. A11).

Genome-wide effect of fluctuating balancing selection

In the previous sections, we have learnt that fluctuating balancing selection at a focal locus can have an impact on remote regions on the same chromosome, and that even for a recombination probability of 0.5 per generation, there is still a slight reduction in diversity. Given these far-reaching effects and given that many loci in the genome may be under strong fluctuating selection (e.g. on the order of hundreds in *Drosophila*, Bergland *et al.* 2014), the question is how genome-wide diversity levels will be affected by the joint action of fluctuating balancing selection at multiple loci. To address this question, we randomly placed n_l selected loci on a roughly *Drosophila*-sized genome with three chromosomes of 50 Mb each. We then used our individual-based simulation approach to simulate patterns of genetic diversity on the three chromosomes (see Appendix 8 for detailed methods). For simplicity, we focus on scenarios where all loci have the same parameters (s, h, g, and u) and we assume that an individual's fitness is the product over the fitness values for individual loci as given by Table 1. As a comparison, we also ran simulations assuming that all selected loci are unlinked from each other (i.e. on different chromosomes) and unlinked from the focal neutral region.

We ran simulations with various selection coefficients, s, and various numbers of selected loci, n_l (Fig. 8). For sufficiently large selection coefficients and numbers of selected loci, we observed substantial genome-wide reductions in diversity. For the largest selection coefficient in this genome-wide simulation study ($s = 0.1$), allele-frequencies change on average by about 0.12 over one season and genome-wide diversity is reduced by about 30 %. Thus the selection coefficients used here and the associated allele-frequency fluctuations are still relatively small (see Fig. A13) compared to those in Fig. 1 C and D underlying the pronounced local reductions in diversity in Fig. 3 C and D. As with the net chromosomal effect, the genome-wide effect took some time to build up, but was fully formed after about $3.5N$ generations (Fig. A14). Additional simulations where the selection coefficients were drawn from an exponential distribution rather than being the same for all loci, yielded very similar results (Fig. A15).

For the genome-wide footprint of balancing selection, it mattered little whether the 100 loci were distributed across three chromosomes or were each on a different chromosome, i.e. unlinked (Fig. 8 A). Thus, most of the genome-wide effect in this multi-locus model appears to be due to the effects of fluctuating balancing selection on diversity at unlinked loci (at $r = 0.5$). In other words, the average region in the genome might be more affected by the cumulative long-distance effects of all loci in the genome that are under fluctuating balancing selection than by the short- or intermediate-distance effects of the nearest locus under selection. However, there was a discrepancy in cases with a relatively small number of

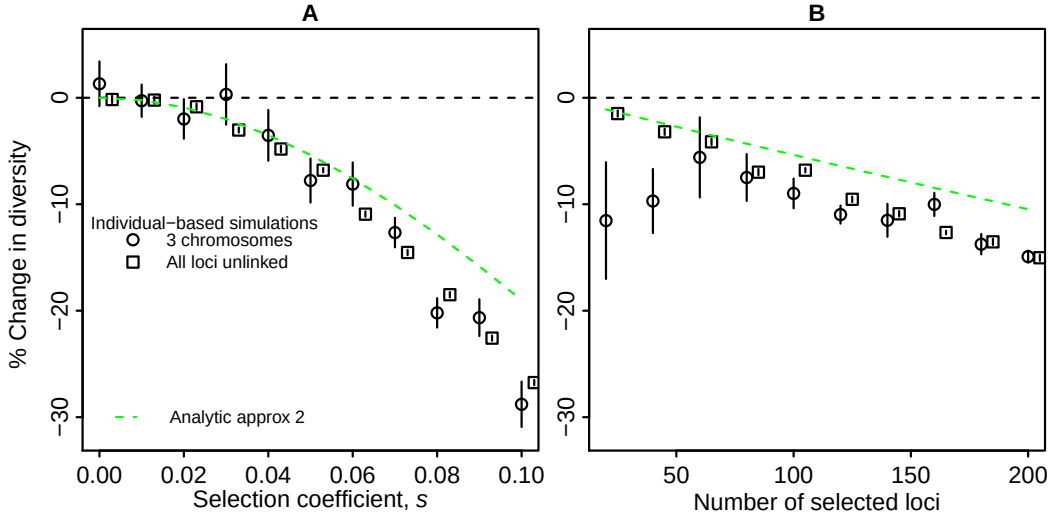

Figure 8 Relative change in genome-wide diversity due to fluctuating balancing selection at A) 100 randomly placed loci as a function of the selection coefficient, or B) different numbers of selected loci with $s = 0.05$. Each panel shows results for simulations where loci are randomly distributed over three chromosomes (circles, mean over 10 replicates ± standard error) and for corresponding simulations with all loci unlinked from each other and from the focal neutral region (squares, mean over 20 replicates ± standard error, slightly shifted to the right to avoid overlap). Other parameters: $r_0 = 10^{-6}, N_e = 10,000, u = 10^{-6}, h = 0.6, g = 10$. See Figs. A13 and A14 for information on the corresponding allele-frequency fluctuations and on the development of the footprint over time.

loci (Fig. 8 B).

We also compare the simulation results to a multi-locus extension of our analytical approximation 2. Here we assumed a recombination probability 0.5 for each locus and assumed that every locus under selection multiplies the effective population size by the same factor. That is, we computed F from (24) for $r = 0.5$ and then used (16) to obtain

$$\mathbf{E}[H] = \frac{1}{2}\left(1 - \frac{1}{1 + 4u \cdot 2N/F^{n_l}}\right). \tag{31}$$

This approximation predicts the qualitative shape of the curve, but again somewhat underestimates the reduction in diversity due to fluctuating balancing selection.

Discussion

Our results indicate that fluctuating balancing selection has a positive effect on levels of genetic diversity close to the selected site, but a negative effect on diversity levels further away. Although the positive effect is often larger in magnitude, the negative effect occurs over a much

larger scale such that the net effect of fluctuating balancing selection on chromosome-wide diversity is generally negative. Moreover, if several loci are under such fluctuating balancing selection, genome-wide diversity can be reduced substantially even if fluctuations and footprints at individual loci are weak.

With the help of simulations and analytical approximations based on the structured coalescent and hitchhiking theory, we disentangled how the genetic footprint of fluctuating balancing selection is shaped by the various model parameters (see Fig. 4 and 5). We found that the depth of the "diversity valley" is determined by the harmonic mean allele frequency and thus by the magnitude of fluctuations, which mostly depends on selection strength per generation and the number of generations per season. Intuitively, with large fluctuations, lineages in the same background are forced through recurrent bottlenecks, which reduces diversity. With increasing magnitude of fluctuations, the region of increased diversity around the selected site also becomes smaller. For a clear footprint of fluctuating balancing selection to emerge, the population needs to be sufficiently large and selection needs to be sufficiently strong. The dominance coefficient hardly influences the footprint of fluctuating balancing selection, but in our selection model a sufficiently large dominance coefficient is required to generate fluctuating balancing selection in the first place. With increasing mutation rate, the footprint becomes overall flatter with both the positive and the negative effect of fluctuating balancing selection decreasing in magnitude, but this is somewhat specific to our biallelic symmetric mutation model. In reality, there will be four bases available at each site and mutation rates are often much smaller such that diversity levels may be far away from saturation.

When simulating the chromosome-wide and genome-wide impact of fluctuating balancing selection, we chose a population size $N = 10,000$, mutation rate $u = 10^{-6}$, and a recombination rate between adjacent sites $r_0 = 10^{-6}$. However, in the analytical approximations, the expected coalescent times relative to neutral expectations (in particular $\mathbf{E}[T_{(1,1)}]/(2N)$) only depend on Nr and Nu (see also Fig. A16). This suggests that our results could approximate the results with a more realistic but computationally less feasible parameter combination $N = 10^6$ and $u = r_0 = 10^{-8}$.

We developed two analytical approximations based on the structured coalescent to capture the genetic footprint of fluctuating balancing selection. In the first approximation, diversity levels just depend on the harmonic mean allele frequency over a cycle. This approximation worked very well at small or medium distance from the selected locus. But since it is based on the assumption that all transition rates are small per generation, it breaks down at large recombination rates where transitions become frequent. With our second analytical approximation, we were able to overcome this by replacing the harmonic mean by a quantity $1/(2F)$ derived based on results from Barton (2000), which converges to the harmonic mean for small recombination rates but can additionally capture the effects of multiple transitions per cycle for large recombination rates.

For the local footprint of fluctuating balancing selection, we found excellent agreement between this second analytical approximation and individual-based simulations. Only very close to the selected site the analytical approach predicted slightly higher diversity levels than the individual-based simulation. One reason is that the assumption of a deterministic cycle could cause the structured-coalescent approaches to overestimate diversity levels. This is particularly important at small selection coefficients or small population sizes (see Fig. A4 and A5) where genetic drift is relatively strong and allele-frequency fluctuations have a large stochastic component. An additional discrepancy between approaches close to the selected site can arise if diversity levels have not fully equilibrated by the end of the simulation. The large diversity levels close to the selected site are due to very large coalescence time and our simulation might not have been long enough to cover the tail end of the distribution, thus leading to a slight underestimation of diversity levels close to the selected site. This is consistent with the fact that diversity levels were still increasing in the final phase of the simulation close to the selected site in Fig. A9. Because of both of these effects, the diversity levels very close to the selected site should be between the predictions of the individual-based model and those of the analytical approximations.

Although the fit between analytic approximation 2 and individual-based simulations for the local footprint appears almost perfect, the approximation slightly underestimates the chromosome-wide and genome-wide reduction in diversity due to fluctuating balancing selection. For the net chromosome-wide effect of a single locus under selection, this mismatch appears to be due to a slight discrepancy in the predicted diversity levels at very large recombination distances. One possible cause for these discrepancies is that the analytic approaches do not take into account that even within a background there is variance in reproductive success due to fitness differences between homozygotes and heterozygotes. With multiple loci under fluctuating balancing selection, as assumed in our analyses of the genome-wide effect, there could additionally be interference effects between loci that are not captured in our numerical and analytical models.

Here we have studied the genetic footprints of fluctuating balancing selection generated by a specific selection scenario based on marginal overdominance. However, there are many other scenarios that could lead to both stable polymorphism and allele-frequency fluctuations, for example temporal heterogeneity with a storage effect (see e.g. Chesson and Warner 1981; Gulisija *et al.* 2016; Reinhold 2000) or relative nonlinearity (e.g. Armstrong and McGehee 1980; Chesson 2000). Also, in many cases with fluctuating selection, another force might be responsible for maintaining polymorphism in the long run, e.g. spatial heterogeneity or sexually antagonistic selection. Thus, a natural question is to what extent the patterns observed depend on the underlying selection model and not just on the allele-frequency trajectories or the magnitude of fluctuations. Note that our analytical approximations depend solely on the allele-frequency trajectories but still the second approximation fits well over the entire range

of recombination distances. This suggests that the genetic footprint of fluctuating balancing selection is relatively independent of the underlying selection scenario and mostly determined by allele-frequency trajectories. However, models that make very different assumptions, and for example additionally have spatial structure, might give rise to different footprints. In the future, it would be nice to extend our model to characterise the footprints of balancing selection arising from various combinations of spatially and temporally heterogeneous selection (Svardal *et al.* 2015).

Qualitatively, the genetic footprints of fluctuating balancing selecting selection are the same also without periodicity, i.e. when selection pressures switch randomly from generation to generation (see Fig. 6). However, the exact shape of recovery of diversity at large recombination rates is somewhat different from the case of seasonal selection, supposedly because of a difference in time scale of the fluctuations. However, the general conclusion that diversity levels can be substantially reduced genome-wide appears robust. This is also in accordance with results by Taylor (2013) who found long-range reductions in coalescent times at loci loosely linked to a locus under weak fluctuating selection without temporal autocorrelation and by Gillespie (1997) who also found a long-range reduction in diversity in simulations of some parameter combinations of the SAS-CFF (stochastic additive scale - concave fitness function) model of temporally variable selection. A relevant empirical study is the evolve-and-resequence experiment by Huang *et al.* (2014). Here, the authors compared *Drosophila melanogaster* populations evolving in four different environments: a constant salt-enriched or cadmium-enriched environment, a spatially heterogeneous, and a temporally variable environment. Although the study could not directly identify the actual targets of selection, they used proxies to classify sites as closely linked or further away from likely targets of selection. They found that for closely linked sites, populations in the spatially heterogeneous environments had the highest diversity, followed by the temporally variable environment, with the constant environments having the lowest diversity. Interestingly, for sites further away, populations in the spatially heterogeneous environment still had the highest diversity, but now the constant environments were intermediate and the temporally variable environments had the lowest levels of genetic diversity, which is consistent with our predictions.

As mentioned in the Introduction, one of the motivations for characterizing the expected footprints of various types of selection is to be able to scan genomes for specific types of selection. Our results suggest that only in cases of large allele-frequency fluctuations will there be a clear genetic footprint of fluctuating balancing selection with a detectable diversity peak around the selected site and a diversity valley further away. In this study we have focused on expected diversity levels. Given that the valley in expected diversity is often quite shallow, it might be hard to detect in real samples with substantial noise. Although the peak close to the selected site might be more detectable, it provides no clues for distinguishing fluctuating and constant balancing selection. Going beyond diversity levels, future work will need to see

whether the site-frequency spectrum (SFS) or haplotype patterns can provide additional clues for the detection of fluctuating balancing selection. Results by Huerta-Sanchez *et al.* (2008) suggest that fluctuating selection can under some conditions leave signatures in the SFS that can be distinguished from other types of selection. Alternatively, recent developments in detecting selection from data sets with multiple sampling points (Buffalo and Coop 2020) could be fruitful also for detecting fluctuating balancing selection.

Even if fluctuations are small and local footprints might be hard to detect, the net effect on the chromosome and, if multiple loci are under fluctuating balancing selection, on the genome can be substantial. It has been known for a long time that genome-wide levels of supposedly neutral genetic variation do not correlate well with census population sizes, contrary to what one would expect under neutral evolution (Leffler *et al.* 2012; Lewontin 1974; Maynard Smith and Haigh 1974). Levels of genetic diversity in large populations are often much smaller than predicted based on census sizes. There is increasing evidence that the effects of linked selection at many loci in the genome are responsible for this pattern (Corbett-Detig *et al.* 2015; Elyashiv *et al.* 2016). So far, the main modes of selection that are thought to reduce diversity levels disproportionately in large populations are selective sweeps and background selection, i.e. the loss of variation due to the elimination of linked deleterious mutation (Charlesworth and Jensen 2021; Elyashiv *et al.* 2016). Our results now suggest that fluctuating balancing selection could be another mode of selection playing an important role in shaping levels of genome-wide genetic variation.

It is currently still unclear whether the reduction in diversity at loosely linked or unlinked loci can be captured in terms of a single effective population size. The effects of strong purifying selection in a large population can at least be approximated by a reduction in effective population size and this approximation improves if the effective population size is made time-dependent (Nicolaisen and Desai 2012, 2013). Research on related complex forms of selection (Charlesworth and Jensen 2021; Taylor 2013), however, suggests that it might not always be possible to capture the effects of selection in terms of a single effective population size, but more work is needed to explore this in depth for fluctuating balancing selection.

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Online Appendix

Appendix 1 Analytical approximation of allele-frequency trajectories

Focusing on symmetric scenarios and neglecting the effect of mutation on allele frequencies, we can state a recursion for the ratio of the two allele frequencies. For $i < g$, the winter allele is favored and we have

$$\begin{aligned}\frac{\pi_{i+1}}{1-\pi_{i+1}} &= \frac{\pi_i^2 \cdot (1+s) + \pi_i \cdot (1-\pi_i) \cdot (1+hs)}{\pi_i \cdot (1-\pi_i) \cdot (1+hs) + (1-\pi_i)^2} \\ &= \frac{\pi_i}{1-\pi_i} \cdot \frac{1+\pi_i \cdot s + (1-\pi_i) \cdot hs}{1+\pi_i \cdot hs} \approx \rho \cdot \frac{\pi_i}{1-\pi_i}, \end{aligned} \tag{A1}$$

where $\rho := \frac{4+3s}{4+s}$. This approximation is based on the assumption that both h and π_i are close to 0.5. Thus, $\pi/(1-\pi)$ grows by a factor ρ every generation. We could also keep h as a parameter in the growth factor rather than setting it to 0.5, but it turned out that this does not add much accuracy.

Solving this geometric growth model, the allele frequencies during winter ($i \leq g$) are

$$\frac{\pi_i}{1-\pi_i} \approx \frac{\pi_0}{1-\pi_0} \cdot \rho^i, \tag{A2}$$

where π_0 is the frequency of the winter allele at the beginning of the year, i.e. its minimum allele frequency along the cycle. To achieve symmetry, the two alleles must exchange their allele frequencies after g generations such that we have

$$\frac{1-\pi_0}{\pi_0} = \frac{\pi_0}{1-\pi_0} \cdot \rho^g \Leftrightarrow \frac{\pi_0}{1-\pi_0} = \rho^{-g/2} \tag{A3}$$

and thus

$$\frac{\pi_i}{1-\pi_i} \approx \rho^{i-g/2}. \tag{A4}$$

Hence the frequencies along the cycle are

$$\pi_i \approx \begin{cases} \frac{1}{1+\rho^{i-g/2}} & \text{for } 0 \leq i \leq g \\ 1 - \frac{1}{1+\rho^{(i-g)-g/2}} = \frac{1}{1+\rho^{-i+3/2\cdot g}} & \text{for } g \leq i \leq 2g. \end{cases} \tag{A5}$$

The above approximation is based on an assumption of constant, in particular frequency-independent, fitness effects over the course of one season. In symmetric cases, the gains in frequency over one season balance exactly the losses in the other season. In asymmetric situations these gains and losses are not generally balanced. Coexistence of alleles in those cases is only possible via negative frequency-dependent selection due to marginal overdominance. Thus an approximation based on constant (frequency-independent) fitness cannot work. Thus, for asymmetric cases we use the numerically-determined cyclic allele-frequency trajectories as a basis.

Appendix 2 Expected heterozygosity

Since we consider a symmetric, biallelic mutation model, there can be back-mutations, which becomes important if the product of expected coalescence time and mutation rate is large. Two sampled lineages with coalescence time T will carry different alleles if and only if there is an odd number of mutations along the branches of length $2T$ in their genealogy. The number of mutations is Poisson-distributed with parameter $2uT$. Thus, the probability that the two

sampled lineages carry the same allele is

$$
\begin{aligned}
P(\text{same}|T) &= e^{-2uT} \sum_{n=0}^{\infty} \frac{(2uT)^{2n}}{(2n)!} \\
&= e^{-2uT} \cdot \frac{1}{2} \cdot \left(\sum_{n=0}^{\infty} \frac{(2uT)^n}{n!} + \sum_{n=0}^{\infty} \frac{(-2uT)^n}{n!} \right) \\
&= \frac{1}{2} \cdot e^{-2uT} \cdot \left(e^{2uT} + e^{-2uT} \right) = \frac{1 + e^{-4uT}}{2}. \qquad \text{(A6)}
\end{aligned}
$$

Assuming that coalescence time is approximately exponentially distributed with parameter λ, we obtain:

$$
\begin{aligned}
P(\text{same}) &\approx \int_0^{\infty} \lambda \cdot e^{-\lambda t} \cdot \frac{1 + e^{-4ut}}{2} dt \\
&= \frac{1}{2} \left(1 + \lambda \cdot \int_0^{\infty} e^{-(\lambda + 4u)t} dt \right) \\
&= \frac{1}{2} \left(1 + \frac{\lambda}{\lambda + 4u} \right). \qquad \text{(A7)}
\end{aligned}
$$

Then for sample configuration (i, j), we use $\lambda = 1/\mathbf{E}[T_{(i,j)}]$. Defining the expected heterozygosity $H = 1 - P(\text{same})$, i.e. as the probability that the two lineages carry different alleles, we obtain

$$
\mathbf{E}[H_{(i,j)}] \approx \frac{1}{2} \left(1 - \frac{1}{1 + 4u \cdot \mathbf{E}[T_{(i,j)}]} \right). \qquad \text{(A8)}
$$

That is, as the product of mutation rate and expected coalescence time increases, heterozygosity increases, but with diminishing returns and a saturation at 0.5 in our biallelic model (see Fig. A1). Using this formula instead of the classical $2u\mathbf{E}[T]/(1 + 2u\mathbf{E}[T])$ improved the fit between simulations and analytical results. Under neutrality, $\mathbf{E}[T_{(i,j)}] = 2N$ and (A8) coincides with the result in Ewens (2004), p. 97.

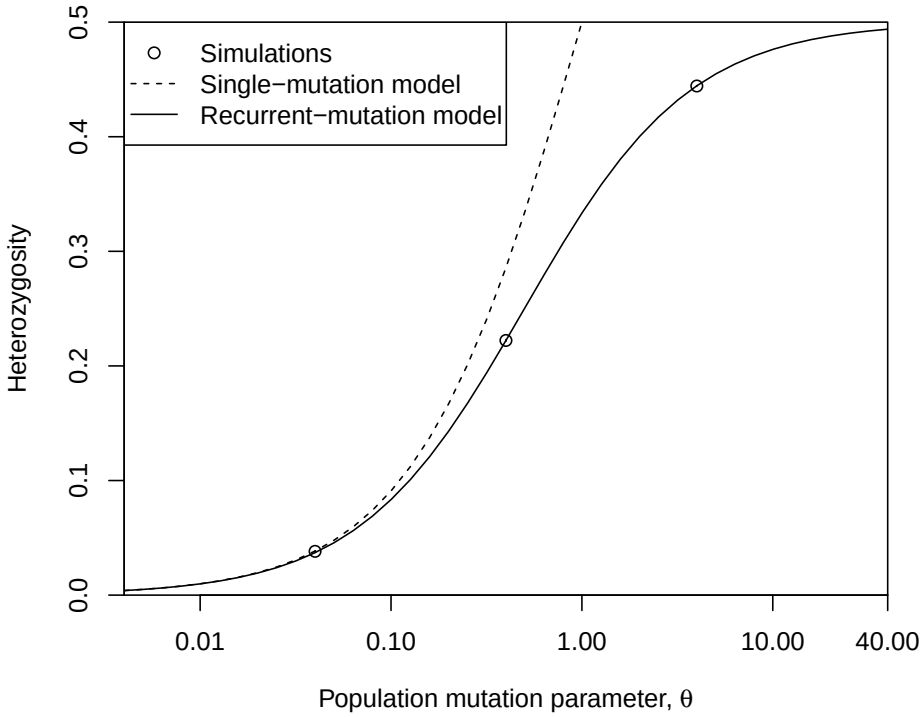

Figure A1 Expected heterozygosity under neutrality as a function of the population mutation parameter, $\theta = 4Nu$. Simulation results are for $N = 10,000$ and each point represents the mean over 16 replicates. The corresponding standard errors are too small to be visible. The single-mutation model uses the standard formula $(2 \cdot u \cdot \mathbf{E}[T])/(1+2 \cdot u \cdot \mathbf{E}[T])$ whereas the multi-mutation model is based on (16). Both use $\mathbf{E}[T] = 2N$.

Appendix 3 Convergence of analytic approximation 2 for small recombination rates

To understand how this approximation connects to the analytical approximation in (11) and (12), we derive the limit of F as the recombination rate r goes to zero. Using the shorthand

π'_i for $\pi_{i \bmod g_{total}}$, we have for S_i (28) for small r:

$$
\begin{aligned}
S_i &= \frac{\sum_{j=0}^{g_{total}-1}(\pi'_{i+j+1} - \pi'_{i+j})e^{-r\cdot j}}{1 - e^{-r\cdot g_{total}}} && \text{(A9)}\\
&\approx \frac{\sum_{j=0}^{g_{total}-1}(\pi'_{i+j+1} - \pi'_{i+j})(1 - r\cdot j)}{1 - (1 - r\cdot g_{total})} && \text{(A10)}\\
&= -\frac{\sum_{j=0}^{g_{total}-1}(\pi'_{i+j+1} - \pi'_{i+j})r\cdot j}{r\cdot g_{total}} && \text{(A11)}\\
&= -\frac{-\pi'_{i+1} - \pi'_{i+2} - \pi'_{i+3} - \cdots - \pi'_{i+g_{total}-1} + (g_{total}-1)\pi'_{i+g_{total}}}{g_{total}} && \text{(A12)}\\
&= -\frac{-\pi'_{i+1} - \pi'_{i+2} - \pi'_{i+3} - \cdots - \pi'_{i+g_{total}-1} - \pi'_{i+g_{total}} + g_{total}\cdot\pi'_{i+g_{total}}}{g_{total}} && \text{(A13)}\\
&= \frac{(\sum_{j=0}^{g_{total}-1}\pi_j) - g_{total}\pi_i}{g_{total}} && \text{(A14)}\\
&= \bar{p} - \pi_i && \text{(A15)}\\
&= -(\overline{1-p} - (1-\pi_i)) && \text{(A16)}
\end{aligned}
$$

Inserting this in (24) gives:

$$
\begin{aligned}
F &\approx 1 + \sum_{i=0}^{g_{total}-1}(\bar{p} - \pi_i)^2\frac{\frac{1}{\pi_i} + \frac{1}{1-\pi_i}}{g_{total}} && \text{(A17)}\\
&= 1 + \frac{1}{g_{total}}\left(\sum_{i=0}^{g_{total}-1}\frac{(\bar{p}-\pi_i)^2}{\pi_i} + \sum_{i=0}^{g_{total}-1}\frac{(\overline{1-p} - (1-\pi_i))^2}{1-\pi_i}\right) && \text{(A18)}\\
&= 1 + \frac{1}{g_{total}}\left(\sum_{i=0}^{g_{total}-1}\frac{\bar{p}^2 - 2\pi_i\cdot\bar{p} + \pi_i^2}{\pi_i} + \frac{(\overline{1-p})^2 - 2\cdot\overline{1-p}\cdot(1-\pi_i) + (1-\pi)^2}{1-\pi_i}\right) && \text{(A19)}\\
&= 1 + \frac{1}{g_{total}}\left(\sum_{i=0}^{g_{total}-1}\frac{\bar{p}^2}{\pi_i}\right) - 2\bar{p} + \bar{p} + \frac{1}{g_{total}}\left(\sum_{i=0}^{g_{total}-1}\frac{(\overline{1-p})^2}{1-\pi_i}\right) - 2(1-\bar{p}) + 1 - \bar{p} && \text{(A20)}\\
&= \frac{\bar{p}^2}{\tilde{p}} + \frac{(1-\bar{p})^2}{\widetilde{1-p}}, && \text{(A21)}
\end{aligned}
$$

where $\tilde{}$ denotes the harmonic mean over a cycle.

In symmetric cases, $\bar{p} = 0.5$ and $\tilde{p} = \widetilde{1-p}$ and we have for small r

$$F \approx \frac{1}{2\tilde{p}} \tag{A22}$$

and

$$\tilde{p} \approx \frac{1}{2F}. \tag{A23}$$

Appendix 4 Initialization of individual-based simulations

Under neutrality, the number of copies M of an arbitrarily chosen allele evolves according to the following model:

$$M_{t+1} \sim \text{Binomial}\left(2N, (1-u) \cdot \frac{M_t}{2N} + u \cdot \left(1 - \frac{M_t}{2N}\right)\right), \tag{A24}$$

where N is the population size and u is the mutation probability. To initialize the initial allele frequencies in the population, we start at allele frequency 0.5 and iterate (A24) for $0.1N \cdot (L + 10)$ generations, where L is the number of sites that we want to simulate. Every $0.1N$ generations, we take the current allele frequency (which can be 0 or 1) to initialize the frequency at one of the sites (throwing away the first 10 to allow for some burn-in. We randomly shuffle the vector of allele frequencies. simuPOP is then randomly assigning genotypes to individuals in the initial populations such that these allele frequencies are respected.

Appendix 5 Additional results for the local effect

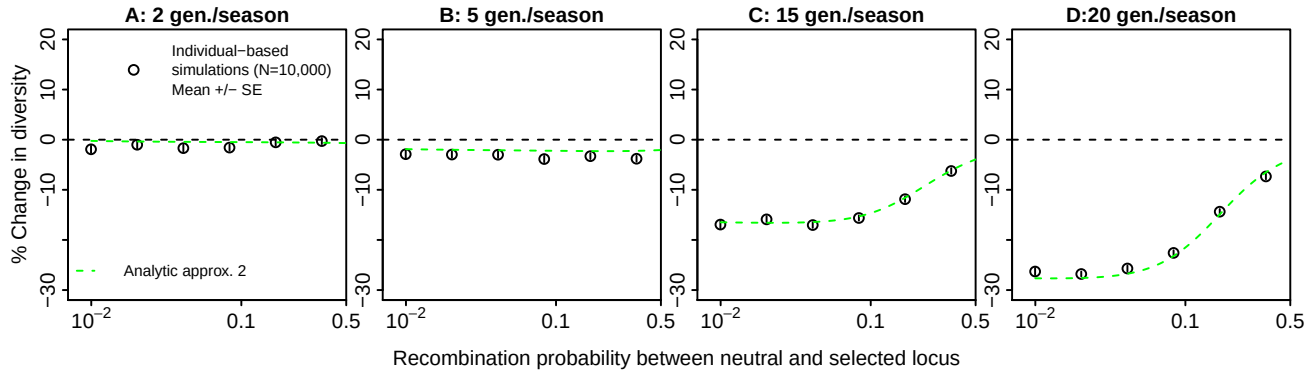

Figure A2 Zoom into the region of large recombination rate in Fig. 3.

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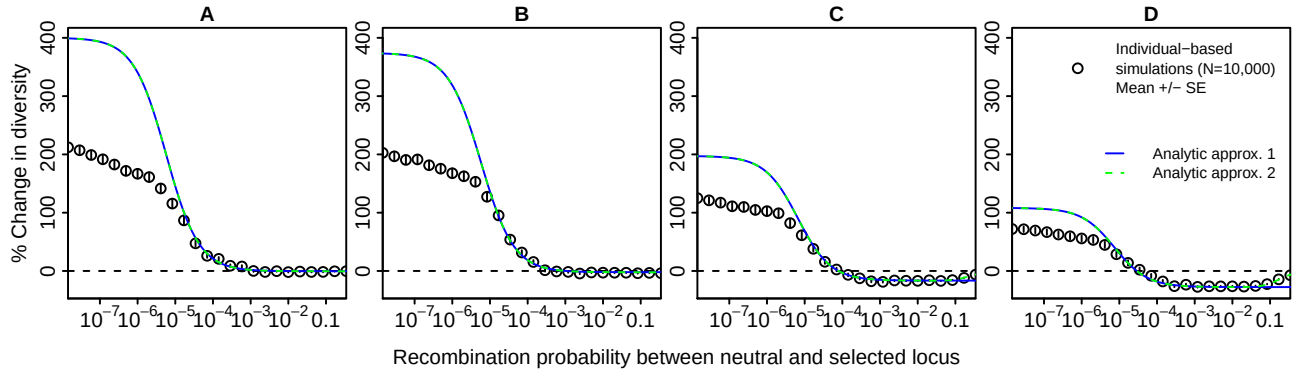

Figure A3 Genetic footprint of fluctuating balancing selection for various season length, g, as in Fig. 3, but now including the region very close to the selected site with a low recombination probability between neutral and selected locus. A) $g = 2$, B) $g = 5$, C) $g = 15$, and D) $g = 20$. The y-axis represents the relative change in diversity compared to the neutral case. Points are averages over 100 replicates with five sampling points each.

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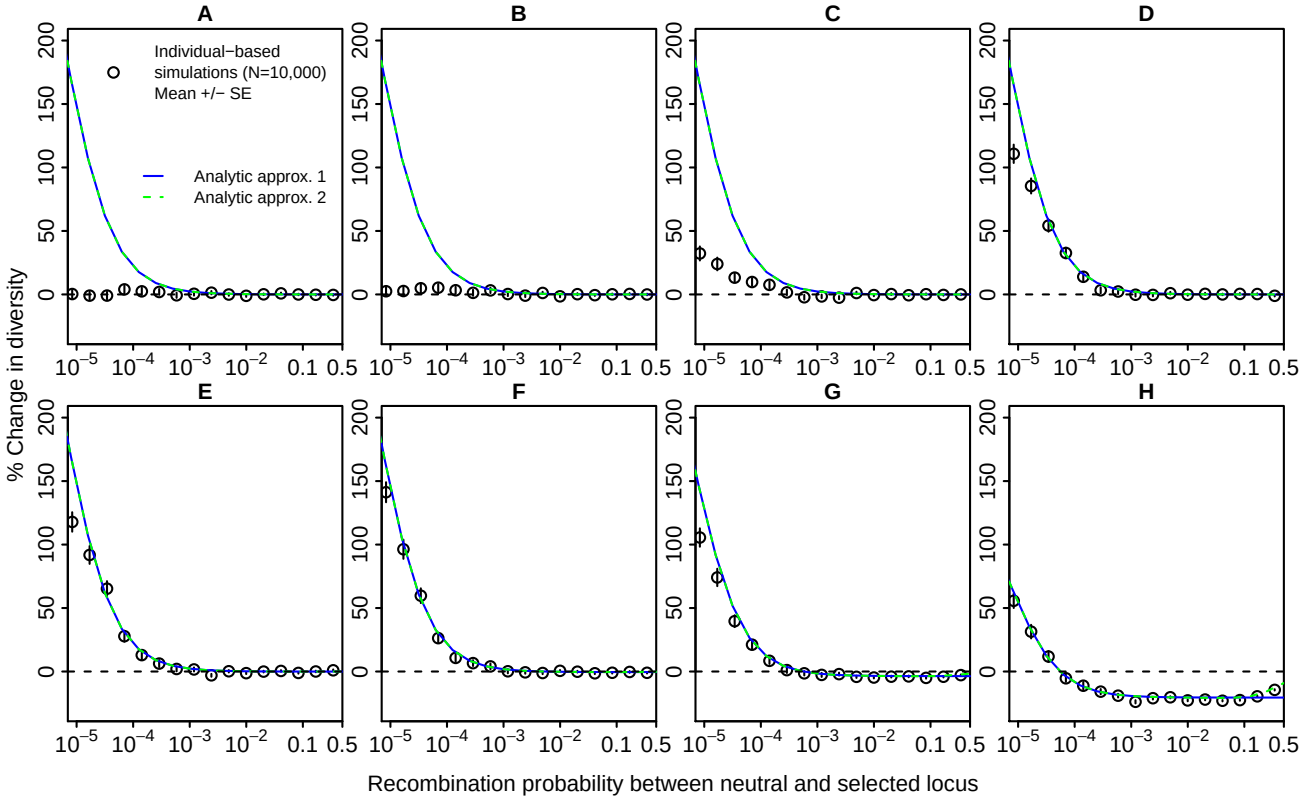

Figure A4 Genetic footprints of fluctuating balancing selection for various selection coefficients, s. In A) $s = 0$, and the values of s in B) to H) are $10^{-3}, 10^{-2.5}, 10^{-2}, 10^{-1.5}, 10^{-1}, 10^{-0.5}, 1$. The y-axis represents the relative change in diversity compared to the neutral case. Points are averages over 100 replicates with five sampling points each. Note that the stochastic simulations in A) are truly neutral, whereas the analytical approximations with $s = 0$ erroneously assumes constant allele frequencies of 0.5, i.e. constant balancing selection.

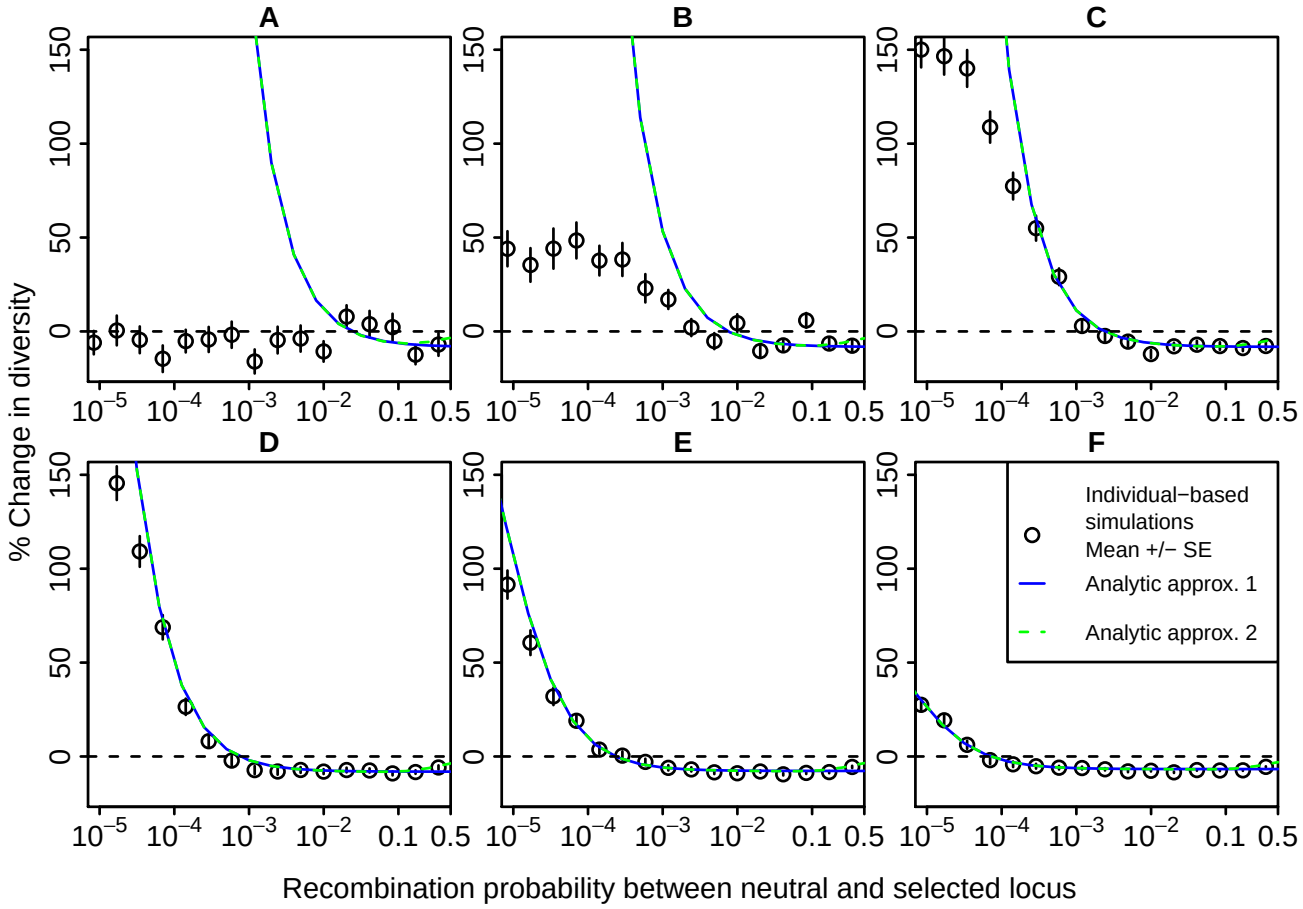

Figure A5 Genetic footprints of fluctuating balancing selection for various population sizes, N. A) $N = 100$, B) $N = 316 = \lfloor 10^{2.5} \rfloor$, C) $N = 1,000$, D) $N = 3,162 = \lfloor 10^{3.5} \rfloor$, E) $N = 10,000$, F) $N = 31,623 = \lfloor 10^{4.5} \rfloor$, where the "floor" function $\lfloor x \rfloor$ gives the largest integer smaller or equal to x. The y-axis represents the relative change in diversity compared to the neutral case. Points are averages over 100 replicates with five sampling points each.

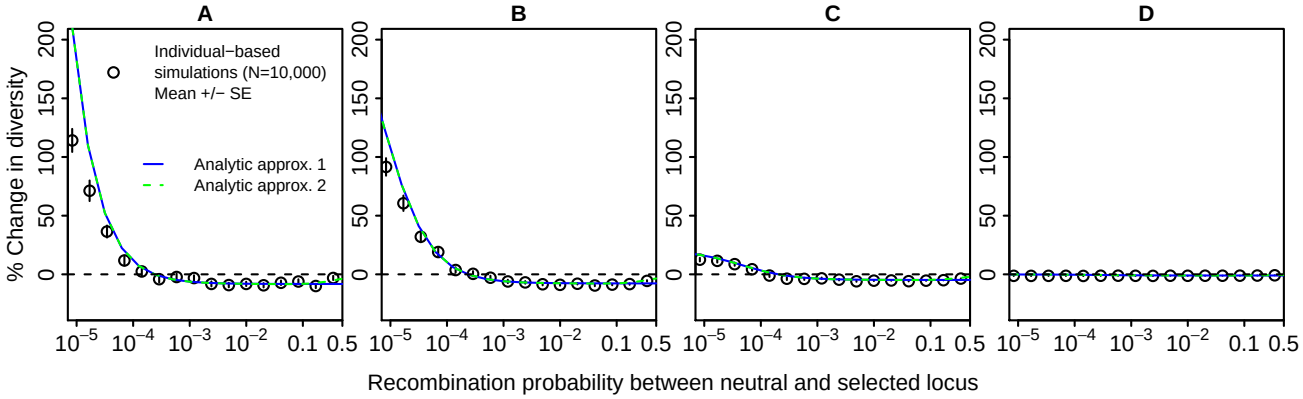

Figure A6 Genetic footprints of fluctuating balancing selection for various mutation rates, u. A) $u = 10^{-7}$, B) $u = 10^{-6}$, $u = 10^{-5}$, $u = 10^{-4}$. The y-axis represents the relative change in diversity compared to the neutral case. Points are averages over 100 replicates with five sampling points each.

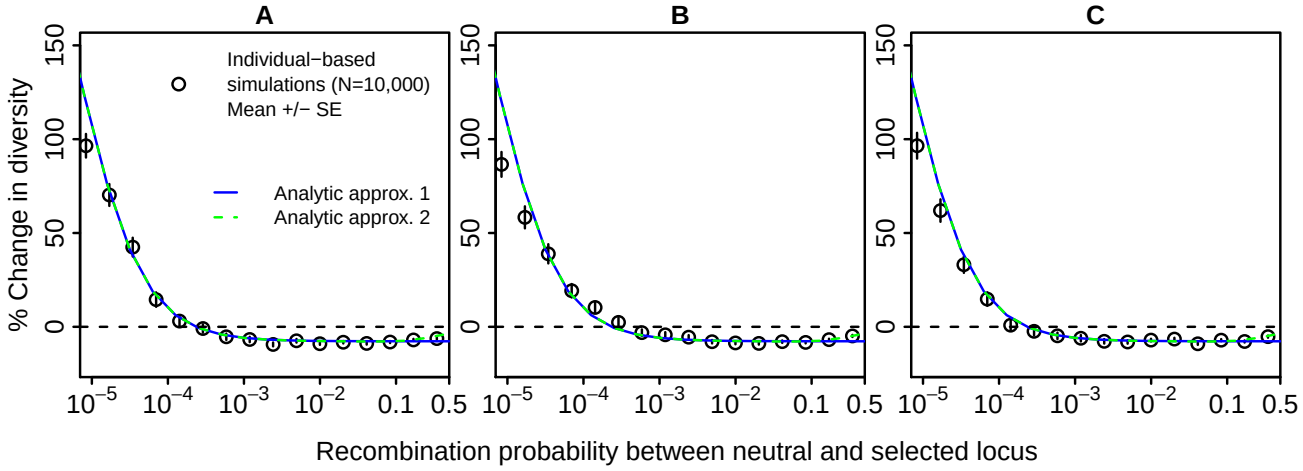

Figure A7 Genetic footprints of fluctuating balancing selection for various dominance coefficients, h. A) $h = 0.5$, B) $h = 0.75$, and C) $h = 1$. The y-axis represents the relative change in diversity compared to the neutral case. Points are averages over 100 replicates with five sampling points each.

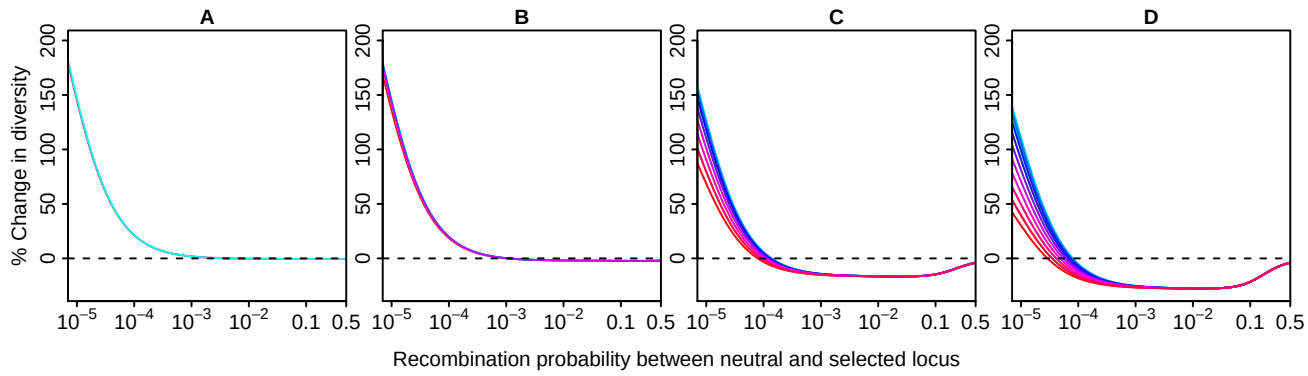

Figure A8 The expected local footprint of selection changes depending on time generation of the seasonal cycle in which the sample is taken. Curves based on analytic approximation 2. Red curves correspond to sampling times when allele frequencies are rather extreme whereas shades of blue represent sampling points where allele frequencies are more intermediate.

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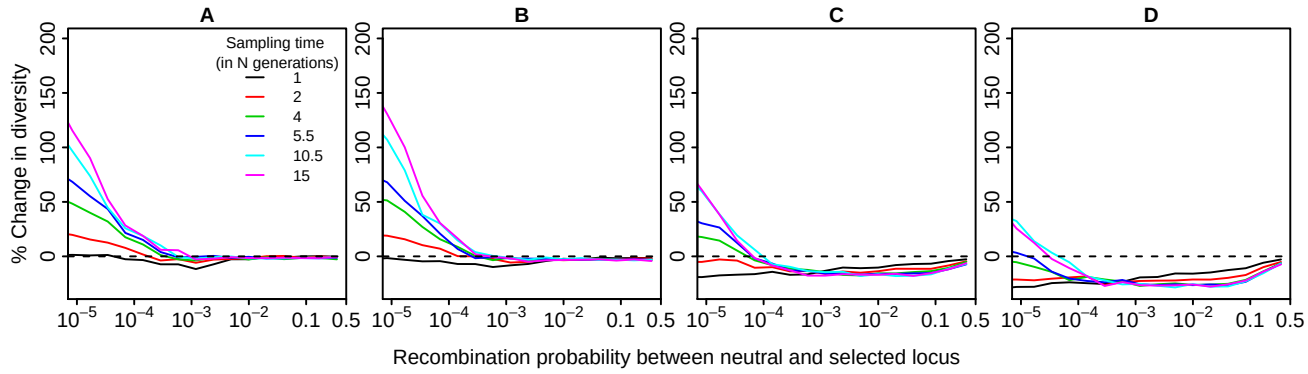

Figure A9 Development of genetic footprints over time for the scenarios in Fig. 3, i.e. with A) $g = 2$, B) $g = 5$, C) $g = 15$, and D) $g = 20$. The curves represent the average over 100 replicates of relative change in diversity compared to the neutral case at different sampling times. Sample i is taken $(i + 1)/2 \cdot N$ generations after the onset of the seasonal selection pressure.

Appendix 6 Asymmetric scenarios

We assume that

$$(1 + h_s s_s)^{g_s} \cdot (1 + h_w s_w)^{g_w} > \max\left((1 + s_s)^{g_s}, (1 + s_w)^{g_w}\right) \tag{A25}$$

such that there is marginal overdominance and thus protected polymorphism. Since our analytical approximation for allele-frequency trajectories (Appendix 1) does not work for asymmetric scenarios, we determine the cyclical allele frequency trajectories numerically by iterating (1) and (2).

The structured coalescent Markov process in asymmetric cases is determined by four parameters: 1) the coalescent rate within the winter-allele background $c_w = \frac{1}{2N\tilde{p}_w}$, where $\tilde{p}_w$ is the harmonic mean frequency of the winter allele, 2) the coalescent rate within the summer-allele background $c_s = \frac{1}{2N\tilde{p}_s}$, where $\tilde{p}_s$ is the harmonic mean frequency of the summer allele, 3) the rate at which lineages move from the winter background to the summer background $m_w = u \cdot (1/\tilde{p}_w - 1) + r \cdot (1 - \bar{p})$, where $\bar{p}$ is the arithmetic mean winter allele frequency, and 4) the rate at which lineages move from the summer background to the winter background $m_s = u \cdot (1/\tilde{p}_s - 1) + r \cdot \bar{p}$.

With these transition rates, the recursions for the expected coalescence times for the three possible starting configurations are

$$\mathbf{E}[T_{(2,0)}] = \frac{1}{c_w + 2m_w} + \frac{2m_w}{c_w + 2m_w} \cdot \mathbf{E}[T_{(1,1)}], \tag{A26}$$

$$\mathbf{E}[T_{(0,2)}] = \frac{1}{c_s + 2m_s} + \frac{2m_s}{c_s + 2m_s} \cdot \mathbf{E}[T_{(1,1)}], \tag{A27}$$

and

$$\mathbf{E}[T_{(1,1)}] = \frac{1}{m_s + m_w} + \frac{m_s}{m_s + m_w}\mathbf{E}[T_{(2,0)}] + \frac{m_w}{m_s + m_w}\mathbf{E}[T_{(0,2)}]. \tag{A28}$$

Solving for $\mathbf{E}[T_{(2,0)}], \mathbf{E}[T_{(0,2)}]$, and $\mathbf{E}[T_{(1,1)}]$ yields

$$\mathbf{E}[T_{(2,0)}] = \frac{1}{\xi}\left(2(m_s + m_w)^2 + c_s \cdot (m_s + 3m_w)\right), \tag{A29}$$

$$\mathbf{E}[T_{(0,2)}] = \frac{1}{\xi}\left(2(m_s + m_w)^2 + c_w \cdot (m_w + 3m_s)\right), \tag{A30}$$

and

$$\mathbf{E}[T_{(1,1)}] = \frac{1}{\xi}\left(2(m_s + m_w)^2 + c_w \cdot (2m_s + m_w) + c_s(c_w + m_s + 2m_w)\right), \tag{A31}$$

where $\xi = 2c_w m_s^2 + 2c_s m_w^2 + c_s c_w \cdot (m_s + m_w)$. The expected coalescence times are then transformed to relative changes in heterozygosity as explained above for the symmetric case. Fig. A10 shows genetic footprints of fluctuating selection for different degrees of asymmetry. Note that here we only went to the first step of our analytic approximation. Extending the second analytical approximation to asymmetric cases is an interesting direction for the future.

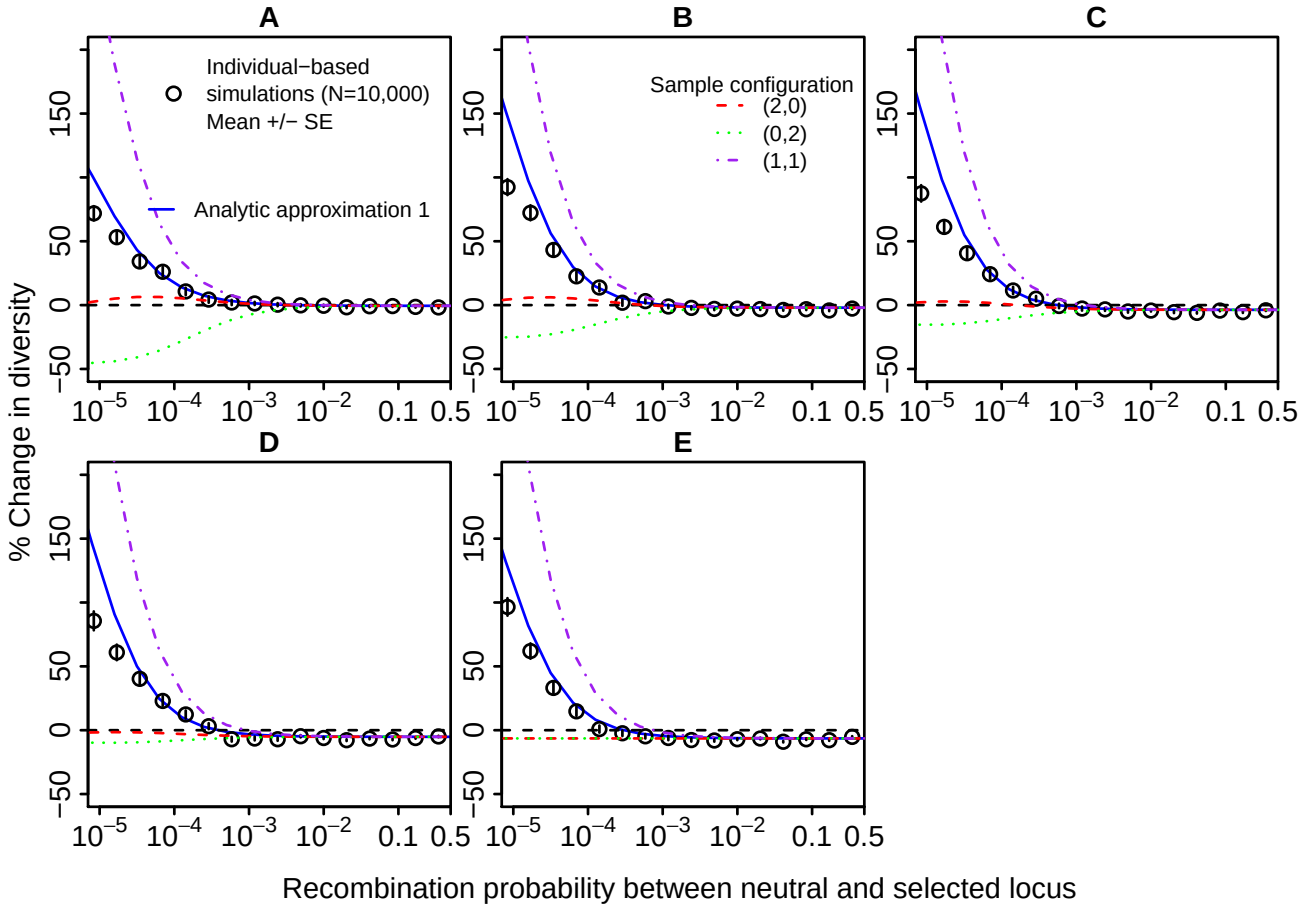

Figure A10 Genetic footprint of fluctuating balancing selection with different degrees of asymmetry in the number of generations per season. The number of winter generations is $g_w = 10$ while the number of summer generations g_s is 2 in (A), 4 in (B), 6 in (C), 8 in (D) and 10 in (E). Points are averages over 100 replicates with five sampling points each. Other parameters: $s_s = s_w = 0.5, h_s = h_w = 1, N = 10,000, u = 10^{-6}$.

Appendix 7 Additional results for the chromosome-wide effect

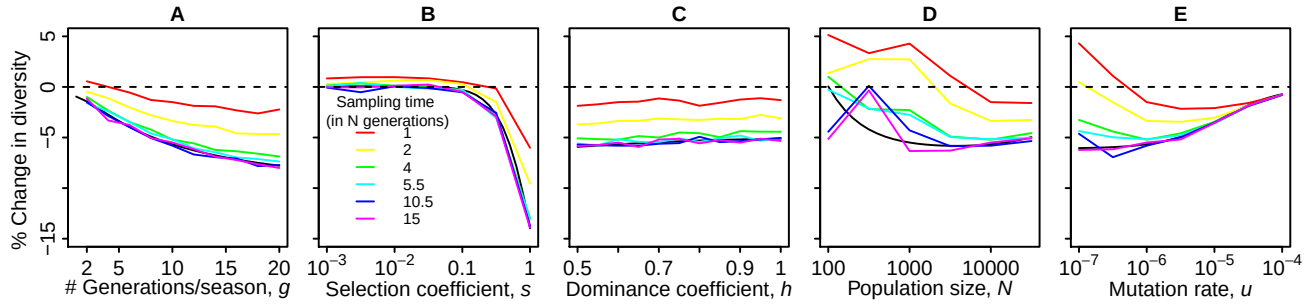

Figure A11 Development over time of the net effect of fluctuating balancing selection on chromosome-wide diversity in the individual-based simulations. Samples were taken every 0.5 N generations, with the first sample 0.5 N generations after the onset of fluctuating balancing selection. Default parameter values: $r_0 = 10^{-6}, r_{max} = 0.49, N_e = 10,000, u = 10^{-6}, s = 0.5, h = 0.6, g = 10$. 40 replicates per data point. The black solid line represents the long-term net effect based on the numeric structured coalescent approach. Note that the increase in diversity in early samples with small population sizes and small mutation rates appears to be an artifact because the burn-in time underlying the initial allele-frequency distribution (see Appendix 4) was a bit too short for these parameter combinations.

Appendix 8 Quantifying the effects of multiple randomly-placed selected loci on genome-wide diversity

The n_l selected loci are placed randomly on the chromosomes, that is, the number of selected loci on each chromosome is drawn from a multinomial distribution, and then for each of the three chromosomes the positions of the respective numbers of loci under selection are drawn uniformly without replacement.

In addition to the selected loci, we simulated a number of neutral sites on each chromosome. To capture the rapidly-changing diversity landscape around the selected site without having to simulate too many sites, we selected the positions of the neutral sites according to the following scheme: We chose a minimum and maximum recombination probability r_{min} and r_{max} and set up a vector of recombination probabilities to a selected locus with L_r elements such that the ith element is

$$r_i = \exp\left(\ln(r_{min}) + i \cdot \frac{\ln(r_{max}) - \ln(r_{min})}{L_r - 1}\right) \tag{A32}$$

for $i \in \{0, 1, \ldots, L_r - 1\}$. That is, we choose the recombination probabilities evenly spaced on a logarithmic scale. In all simulations shown, we use $L_r = 500$.

For each selected locus, we simulate neutral loci at distances given by r_i to the left and right, but only up to the midpoint of the stretch to the next selected locus. We place an additional neutral locus at the midpoint and then switch to the recombination vector for the next locus. We proceed analogously at the ends of the chromosome.

Let ρ_i be the recombination probability between the $i-1$th and the ith of these sites. To compute ρ_i, we notice that a recombination event between the selected locus and locus i occurs if there is a recombination event between the selected locus and locus $i-1$ or a recombination event between locus $i-1$ and locus i, but not both. Thus

$$r_i = r_{i-1} \cdot (1 - \rho_i) + (1 - r_i) \cdot \rho_i. \tag{A33}$$

Solving for ρ_i gives:

$$\rho_i = \frac{r_i - r_{i-1}}{1 - 2 \cdot r_{i-1}} \tag{A34}$$

for $i \in \{1, 2, \ldots, L_r - 1\}$. We set r_{min} to r_0 the assumed recombination probability between adjacent sites per generation. An example recombination map with just four loci is shown in Fig. A12. We again used Haldane's map (30) and the trapezoid rule as described above for the net chromosomal effect to obtain average diversity levels across the chromosome. For further details of the implementation, please refer to the python and R scripts in the supplement.

As a comparison, we ran simulations with all selected loci on different chromosomes and a focal neutral region, also unlinked, with 40,000 sites evenly spaced on a chromosome with

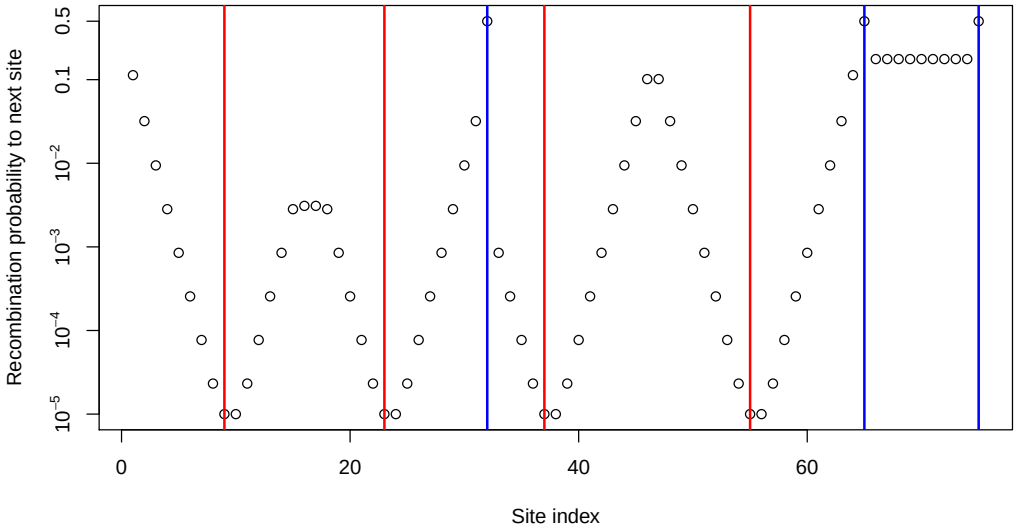

Figure A12 Example for a chromosome map with four selected loci thrown onto three chromosomes. Blue vertical lines represent the end of a chromosome. Red vertical lines represent loci under selection. For each site, the point represents the recombination probability to the next site to the right.

a recombination probability 0.49 between its two extremes.

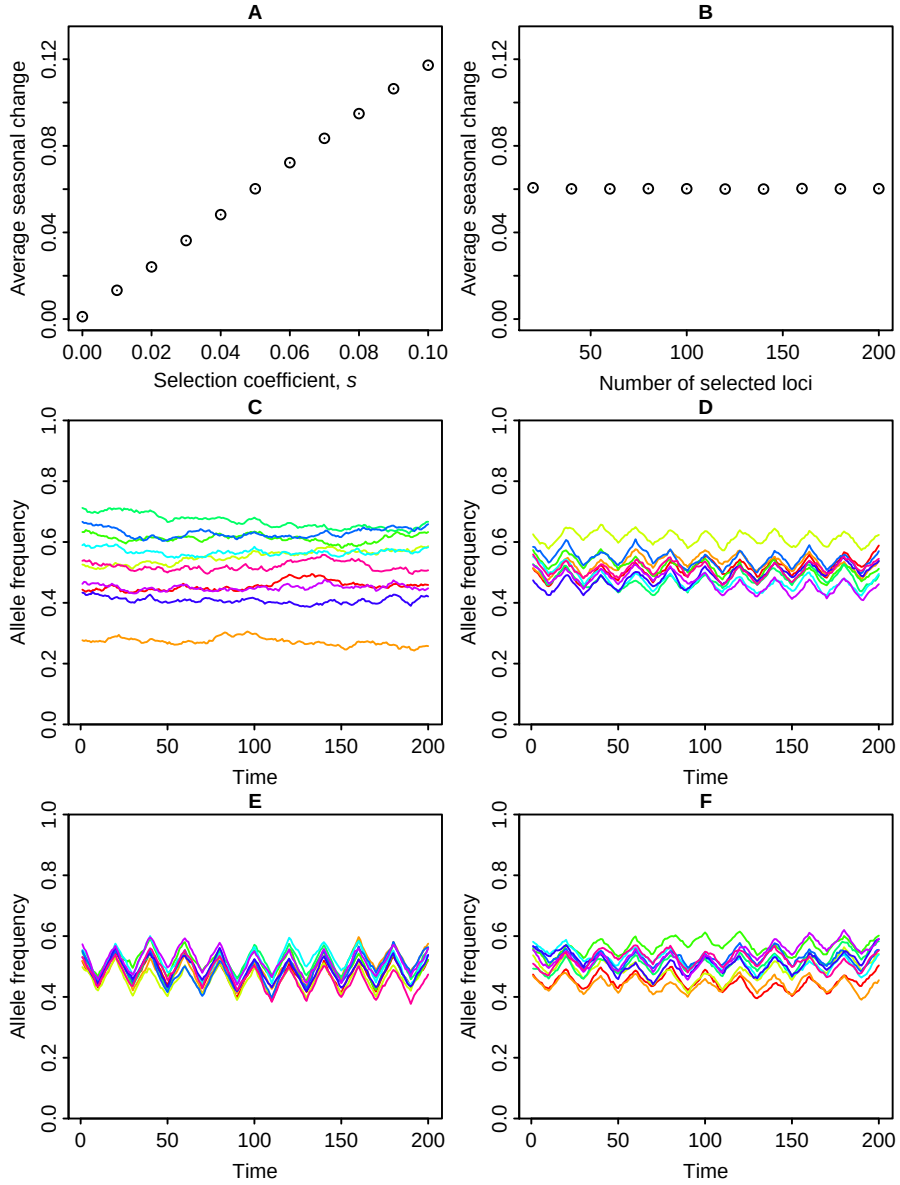

Figure A13 Allele-frequency dynamics corresponding to the 3-chromosome scenario in Fig. 8. Average absolute value of the allele-frequency change over one season as a function of (A) the selection coefficient, and (B) the number of selected loci, and example allele-frequency trajectories for 10 randomly selected loci (C–F). In (A, C, and E), there are 100 loci under selection. In (C), $s = 0.02$, and in (E), $s = 0.1$. In (B, D, and F), $s = 0.05$. The total number of selected loci is 40 in (D) and 200 in (F).

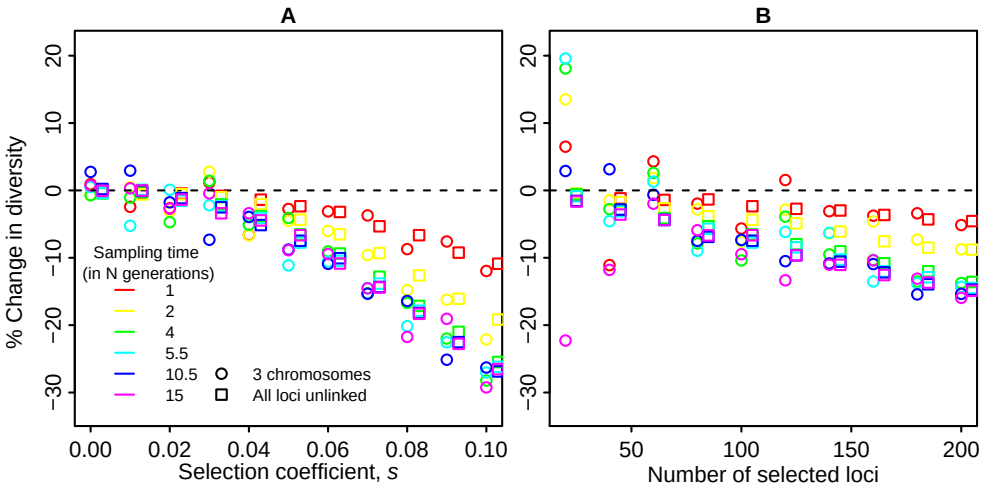

Figure A14 Temporal development of the impact of fluctuating balancing selection on genome-wide diversity in the scenarios shown in Fig. 8. A) 100 randomly placed loci with different selection coefficients. B) Different numbers of selected loci with $s = 0.05$. Each panel shows results for simulations where loci are randomly distributed over three chromosomes (circles, mean over 10 replicates) and for corresponding simulations with all loci unlinked from each other and from the focal neutral region (squares, mean over 20 replicates, slightly shifted to the right to avoid overlap). Other parameters: $r_0 = 10^{-6}, N_e = 10,000, u = 10^{-6}, h = 0.6, g = 10$.

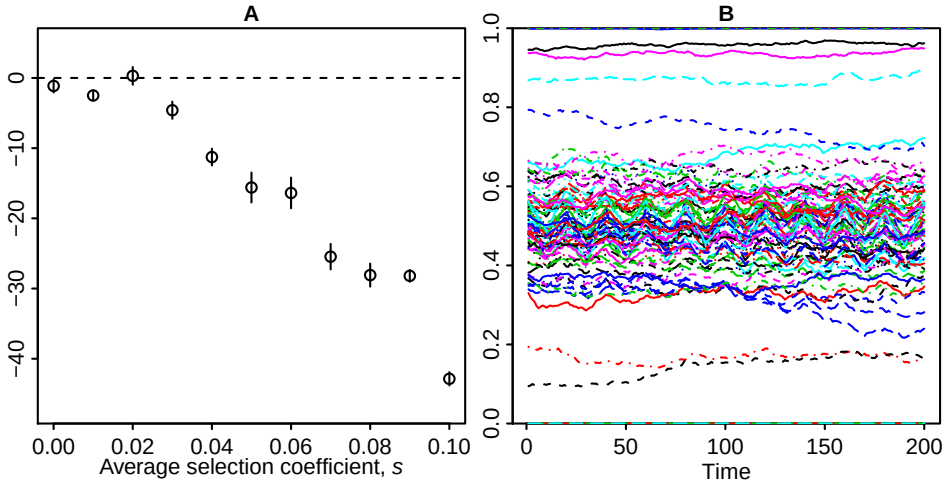

Figure A15 Fluctuating balancing selection at 100 randomly placed loci with exponentially distributed selection coefficients (with $s_w = s_s$). A) Relative change in genome-wide diversity (mean $\pm$ standard error over 10 replicates) as a function of the average selection coefficient. Allele-frequency fluctuations at the 100 loci over the final 200 generations in one of the replicates. Other parameters: $r_0 = 10^{-6}, N_e = 10,000, u = 10^{-6}, h = 0.6, g = 10$.

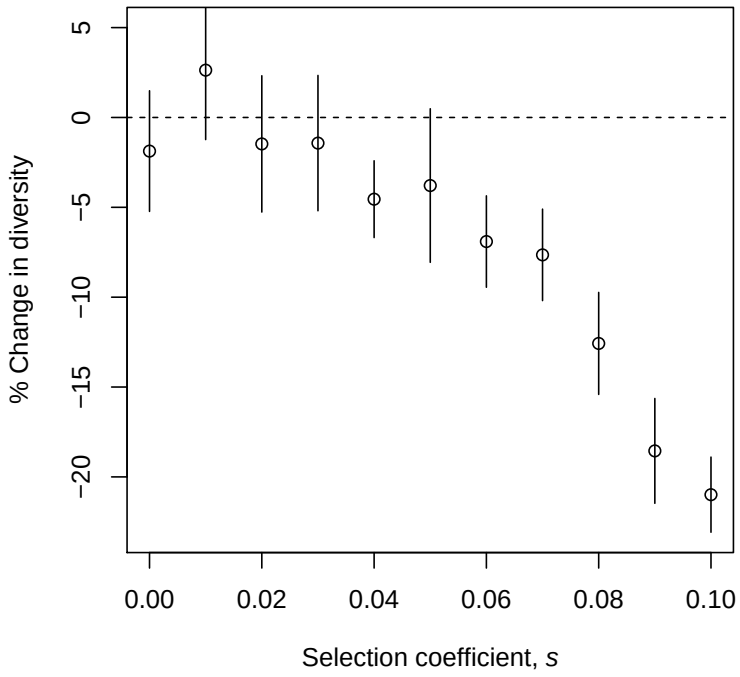

Figure A16 Relative change in genome-wide diversity (mean ± standard error over 10 replicates) due to fluctuating balancing selection at 100 randomly placed loci as a function of the selection coefficient. Compare to Fig. 8 to assess the quality of the scaling relationship. Other parameters: $r_0 = 10^{-5}, N_e = 1,000, u = 10^{-5}, h = 0.6, g = 10$.