**Mutation accumulation in selfing populations under fluctuating selection**

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**Abstract**

Selfing species tend to be prone to extinction and this may be because selfing reduces the efficacy of selection acting to establish beneficial mutations and remove deleterious mutations from the population. Past theory shows that highly selfing populations can suffer from a continuous accumulation of deleterious mutations, a process analogous to Muller's ratchet in asexual populations. However, current theory provides little insight into which types of genes are most likely to accumulate deleterious alleles and what environmental circumstances may accelerate genomic degradation. Here we investigate temporal changes in the environment that cause fluctuations in the strength of purifying selection acting on some genes. We simulate highly selfing populations with genomes containing a mixture of loci experiencing time constant selection and loci experiencing selection that fluctuates in strength over time (but not in direction). Even when both types of loci experience the same average strength of selection over time, loci under fluctuating selection contribute disproportionately more to deleterious mutation accumulation. Moreover, the presence of loci experiencing fluctuating selection in the genome increases the fixation rate of deleterious alleles at loci that are under constant selection; under most realistic scenarios this effect of linked selection can be attributed to a reduction in $N_e$. Fluctuating selection is particularly injurious to highly selfing populations when selective environments are strongly autocorrelated over time and when selection is concentrated into temporally rare environments in which selection is strong. From these results, we suggest that loci under fluctuating selection are likely to be important drivers of extinction in selfing species.

**Introduction**

Self-fertilization has evolved many times independently in plants and animals (Barrett 2002, Goodwillie et al. 2005, Jarne and Auld 2006). It is speculated that selfers evolve readily because they have a transmission advantage over outcrossers (Fisher 1941) and benefit from reproductive assurance when there is a lack of mates or pollinators (Lloyd 1979, Grossenbacher et al. 2017). Segregating recessive alleles result in high levels of inbreeding depression in

outcrossing species and can prevent the transition to self-fertilization (Lloyd 1979; Charlesworth 1980). However, low or moderate levels of selfing can lead to the purging of inbreeding depression, potentially allowing transitions to high levels of selfing (Lande and Schemske 1985). Despite frequent transitions from outcrossing to self-fertilization, highly selfing populations are relatively rare in nature and relegated at the tips of phylogenies (Stebbins 1957, Igic and Kohn 2006). Stebbins (1957) proposed that highly selfing species suffer from low rates of adaptation and are more prone to extinction; classically called the 'dead end' hypothesis.

Population genetics theory provides insights into why selfing is a dead end. Selfing reduces the effective population size, $N_e$, which diminishes the efficacy of selection. The reduction in $N_e$ occurs for two reasons. First, under neutrality, $N_e$ is reduced two-fold in obligately selfing populations because gametes are not sampled independently (Pollak 1987). Second, but perhaps more importantly, high homozygosity in selfing populations reduces the effectiveness of recombination and can further reduce $N_e$ due to linked selection effects (Golding and Strobeck 1979, Hudson and Kaplan 1995, Nordborg 1997, 2000). Due to linked selection, we expect genomes with low recombination rates to suffer from reduced rates of adaptation (Stebbins 1957, Hill and Robertson 1966, Barton 1995) and increased rates of deleterious mutation accumulation, which could lead to extinction (Muller 1932, van Valen 1975, Haigh 1978, Heller and Maynard Smith 1979, Nunney 1989, Charlesworth and Charlesworth 1993, 1997). Explicit contrasts of selfing and outcrossing species confirm lower rates of adaptation and higher rates of deleterious mutation accumulation in both theory (Glemin and Ronfort 2013; Kamran-Disfani and Agrawal 2014) and reality (Wright et al. 2002, Cutter 2006, Cutter et al. 2006, Slotte et al. 2010, Qui et al. 2011, Ness et al. 2012, Brandvain et al. 2013, Slotte et al. 2013, Arunkumar et al. 2014, Burgarella et al. 2015). Further, phylogenetic analyses infer higher extinction rates in self-compatible *Solanacaea* (Goldberg et al. 2009) relative to their self-compatible counterparts. (A similar pattern occurs between asexual and sexual *Onagraceae* (Johnson et al. 2012).) The disadvantage resulting from very strong linked selection can maintain low levels of outcrossing in species considered strong selfers (Kamran-Disfani and Agrawal 2014).

While both reduced rates of adaptation and higher rates of deleterious mutation accumulation may contribute to extinction, our focus here is on better understanding the latter. Though existing theory makes it clear that deleterious mutations accumulate more readily in

highly selfing populations, this literature does not aim to provide insight into the types of genes most likely to degrade or the types of environmental circumstances that might hasten genomic degradation. Here we investigate how loci that experience fluctuations in intensity (but not direction) affect deleterious mutation accumulation in highly selfing populations.

There is abundant evidence that selection is environmentally sensitive in natural and in laboratory experiments (Kassen 2002, Fry et al. 2002, Baer et al. 2006, Hedrick 2006, Leimu and Fischer 2008, Agrawal and Whitlock 2010, Bergland et al. 2014, Kraemer et al. 2015, Latta et al. 2015, Roles et al. 2016, Rutter et al. 2017). Conceptually, selection can vary in direction and magnitude between environments (Martin and Lenormand 2006), but empirically, selection varies mostly in magnitude. For example, 'stressful' environments may alleviate or intensify the strength of selection, but rarely reverses the direction of selection (Kishony and Leibler 2003, Agrawal and Whitlock 2010, Kraemer et al. 2015). In a comprehensive study on yeast, Hillenmeyer et al. (2008) found that 97% of all viable singe gene knockouts reduce fitness in at least one environment while having little to no effect in other environments after performing over 1000 chemical assays. This suggests that selection against mutations can fluctuate in magnitude from neutrality to deleterious between environments (i.e., conditionally neutral mutations may be common). Other types of studies also indicate that temporal changes in the environment create fluctuations in the strength of selection (Lynch 1987, Grant and Grant 2002, Bell 2010, MacColl 2011, Bergland et al. 2014, Siepielski et al. 2017). Despite this evidence, most mutation accumulation models assume selection is constant. This likely underestimates fixation rates of deleterious mutations if selection fluctuates temporally (Wardlaw and Agrawal 2012, Cvijovic et al. 2015).

Though classic single locus models for the fixation probability assume selection is constant (Haldane 1927, Kimura 1962), it is known that fixation probabilities can be highly sensitive to the different ways that selection may change over time (Kimura and Ohta 1970, Takahata et al. 1975, Gillespie 1978, Uecker and Hermisson 2011, Waxman 2011, Peischl and Kirkpatrick 2012, Cvijovic et al. 2015). Often neglected are changes in selection that are stochastic and temporally autocorrelated, especially with regards to fixations of mutations that are on average deleterious. It is well documented that environments can fluctuate over many

different time scales under various degrees of temporal autocorrelation (Steele 1985, Halley 1996, Vasseur and Yodviz 2004, Furguson et al. 2016) and evolutionary processes can be strongly dependent on both the temporal autocorrelation in selection and the average strength of selection (Charlesworth 1993, Pieschl and Kirkpatrick 2012, Wardlaw and Agrawal 2012, Zhang 2012, Chevin et al. 2015, Cvijovic et al. 2015, Tufto 2015). In general, if changes in selection occur rarely (very high temporal autocorrelation), then the probability of fixation for the mutation is largely dependent on the selective environment in which it arose and can be estimated from models assuming constant selection. If fluctuations are very frequent (very low temporal autocorrelation), fixation probabilities can also be approximated by constant selection models but using a time-averaged strength in selection (Mustonen and Lassig 2009, Peischl and Kirkpatrick 2012, Cvijovic et al. 2015). However, in the intermediate range where selection and environmental change operate on similar time scales, classic equations become inaccurate. Within this range, Cvijovic et al. (2015) have shown in single locus models that selection fluctuating in direction increases the probability of fixation for all mutations.

We examine selection that is always negative (or zero) but fluctuates in intensity (but not direction) and in a system with strong linked selection. Results from single locus theory do not extend simply to highly selfing populations where loci in the genome are tightly linked (Higgs and Woodcock 1995, Charlesworth and Charlesworth 1993). Interference between deleterious mutations leads to mutation accumulation in asexual and highly selfing populations (Muller 1932, Haigh 1978, Heller and Maynard Smith 1979, Charlesworth and Charlesworth 1993, 1997, Gordo and Charlesworth 2000a,b; Kamran-Disfani and Agrawal 2014). However, little is known about selective interference when some loci experience fluctuations in selection intensity. In a related study, Wardlaw and Agrawal (2012) found that temporal autocorrelation in selection intensity is an important determinant of Muller's ratchet in asexuals because the ratchet can turn quickly during periods of relaxed selection. The current work extends their findings to selfing populations with some recombination, explores more realistic environmental scenarios, separately quantifies fixation rates for loci experiencing fluctuating versus constant selection within the same genome, examines the extent to which the linked selection effects from fluctuations can be accounted for through $N_e$, and considers selective interference involving beneficial mutations.

We simulate highly selfing populations whose genomes contain both loci under constant selection and loci under selection that fluctuates in the intensity of negative selection. We examine how fluctuating selection affects the rate that deleterious mutations fix at loci experiencing fluctuation but also at linked loci experiencing constant selection. We find that genomes containing a larger proportion of loci under fluctuating selection experience higher fixation rates genome wide, both because of higher fixation rates at the fluctuating loci themselves but also through a linked selection effect on other loci, including those under constant selection. Fluctuating selection has relatively more dramatic effects for populations with lower mutations rates, stronger average selection, larger population sizes and higher outcrossing. This is because fixation rates of loci experiencing fluctuating selection are less sensitive to conditions that reduce fixation rates at loci experiencing constant selection. These results imply that loci under fluctuating selection contribute disproportionately to mutation accumulation and fitness decline in highly selfing populations.

## Methods

### Overview

Simulations were written in C++. Diploid populations of size *N* were simulated assuming a life cycle of selection, reproduction and mutation. Our "base model" assumes individuals carry two types of loci: those that experience constant negative selection on mutations (hereafter "constant loci" or C-loci) and loci that experience selection that fluctuates in intensity but not direction (hereafter "fluctuating loci" or F-loci) between neutrality and relatively strong negative selection, i.e., mutations are conditionally neutral. In the base model, all loci under fluctuating selection were affected identically by the environment so that these loci were all either selected or neutral in the same generations. We first examined the base model within populations that were either fully selfing or highly but not completely selfing (98% selfing; hereafter "partially selfing"). We then examined three extensions of the model. The first extension allowed selection to vary quantitatively over time. Specifically, the strength of selection against mutations occurring at loci under fluctuating selection was exponential distributed over time (rather than being "on" or "off" as in the base model). In the second extension the conditionally neutral loci were equally divided into two different sets of loci that each experienced selection in response to separate ecological variables. For example, the first set could represent loci that were only selected under dry

conditions and the second set represents those loci that were selected only when there was high herbivory. The two environmental conditions (e.g., "dry" and "high herbivory") could occur independently or be positively or negatively correlated. In the last extension on the model, we allowed for beneficial mutations to occur at C-loci.

**Base model**

*Life cycle*

Each diploid individual carried 5000 fitness-affecting loci and three neutral quantitative loci on a linear genome; neutral loci were used to estimate effective population size (discussed below). A proportion, $p_f$, of fitness affecting loci were assigned to experience fluctuating selection and the remaining proportion, $1-p_f$, were assigned to experience constant selection; location assignment of locus types are randomized for every simulation. Loci under constant selection (C-loci) experienced a selection coefficient of $s_c$ in all generations. Loci under fluctuating selection (F-loci) experienced a strength of selection that changed over time $s_f(t)$, taking the value 0 or $s_{max}$. The parameter $f$ controlled the temporal autocorrelation in selection between consecutive generations. With probability $f$ selection automatically remained the same as the previous generation. With probability, $1-f$, $s_f(t)$ was randomly chosen to be 0 or $s_{max}$ with probability $(1-\phi)$ and $\phi$, respectively; $\phi$ is the expected proportion of generations that $s_f(t) = s_{max}$ . The correlation in selection between time points separated by $t$ generations is $f^t$. The expected run length that $s_f(t)$ equals 0 and $s_{max}$ is $1/((1-\phi)(1-f))$ and $1/(\phi(1-f))$, respectively. The geometric average strength of selection experienced by loci under fluctuating selection is $s_{avg} = 1-(1-s_{max})^{\phi}$. For all simulations we chose $s_{max}$ such that $s_{avg} = s_c$, so that loci under fluctuating selection experienced the same geometric average selection strength as loci under constant selection (i.e., $s_c = s_{avg}$) to provide the fairest comparison between these types of loci.

Populations started with $N$ individuals where each individual received a number of mutations sampled from a Poisson distribution with mean $U/(h\, s_{avg})$ where $U$ is the genome-wide per generation mutation rate and $h = 0.25$ is the dominance coefficient. Every generation, each of $N$ offspring was produced as follows. The fitness of parents at time $t$ was calculated assuming loci act multiplicatively as

$$W_i(t) = (1 - s_c)^{x_c}(1 - h\, s_c)^{y_c}(1 - s_f(t))^{x_f}(1 - s_f(t))^{y_f}$$

where $x_c$ and $y_c$ represents the number of loci under constant selection that are homozygous and heterozygous mutants and $x_f$ and $y_f$ represents the number of loci under fluctuating selection that are homozygous and heterozygous mutants. Offspring were produced through sporophytic selfing a proportion $S$ of the time and through outcrossing a proportion 1-$S$ of the time, on average. Selfing rates were fixed and we focus on fully selfing populations ($S = 1$) and partially selfing populations with a high rate of selfing ($S = 0.98$); see Table S1 for simulations with lower selfing rates. A potential parent, $i$, was chosen randomly and their fitness relative to the maximum in the population ($W_i$ / $W_{max}$) was compared to a uniform random deviate (Uniform [0, 1]), where $W_{max}$ is largest value of $W_i$ in the population. The parent was kept if their relative fitness surpasses the uniform random deviate, otherwise, the process was repeated until a suitable parent is chosen. The offspring was produced via selfing if a uniform random deviate (Uniform [0,1]) was smaller than the selfing rate, $S$. Otherwise, a second parent was chosen under the same procedure as the first and the offspring is produced via outcrossing. Gametes were created following recombination between homologous chromosomes of a parent with $c$ crossover events distributed randomly over the genome. $c$ is a Poisson distributed random variable with a mean of $M = 1$, where $M$ represents the genomic map length. After combining two gametes from either one or two parents, $x$ mutations were randomly added to the genome of the offspring, where $x$ is a number sampled from a Poisson distribution with mean $U$. The locus to undergo mutation was chosen randomly and the mutation event was ignored if the chosen locus was already a mutant. Fixation occurs when all $2N$ alleles at a locus were mutant. When this occurs, we reset all alleles back to wild type.

After a burn-in period of at least 20 000 generations, data was collected over at least 40 000 more generations; the burn-in and data collection period was much longer under low mutations rates ($U < 1$). We calculated the per locus fixation rates for loci under constant and fluctuating selection separately as the number of deleterious mutations that fixed at each type of loci divided by the number of generations over which the data was collected and divided by the number of loci for each type. Throughout, we compare per locus fixation rates in populations that experienced fluctuating selection ($p_f > 0$) to the per locus fixation rates in populations that did not experience fluctuating selection ($p_f = 0$, i.e., the "classic" model typical of most past

mutation accumulation models). More precisely, we calculated the *relative fixation rate* as the per locus fixation rate in populations with $p_f > 0$ divided by the per locus fixation rate in populations with $p_f = 0$ (holding all other parameters equal). Thus, the relative fixation rate for loci under constant selection quantified the extent to which fixation rates were affected by residing in genomes that experience fluctuating selection rather than residing in genomes that only experience constant selection. The relative fixation rate for loci under fluctuating selection quantified the direct effect of fluctuating selection as well as effect of residing in a genome experiencing fluctuating selection. Each parameter set was replicated at least 20 times. For a given parameter combination, relative fixation rates were calculated using the average rate over all replicates divided by the average rate from the replicates of the corresponding classic model.

*Effective population size*

We estimated effective population size, $N_e$, using three linked quantitative neutral loci located at one-quarter, one-half and three-quarters along the genome (following Keightley and Otto 2006, Agrawal and Hartfield 2016). At reproduction, the value of each locus on each gamete was perturbed by adding a random number drawn from a normal distribution with mean 0 and variance 1. At steady state, the population wide variance at each locus gives an estimate of 2 $N_e$ (Lynch and Hill 1986). For each simulation, $N_e$ was measured every 100 generations over at least 40 000 generations following a burn-in period of 20 000 generations; the burn-in and data collection period was much longer under low mutations rates ($U < 1$). This burn-in period was likely greater than 20 $N_e$ generations given that estimates of $N_e$ from all our simulations fell far below 1000. We reported the genome-wide $N_e$ averaged among all time points and among the three neutral loci; $N_e$ estimates are similar across all three loci. We confirmed that our estimates of $N_e$ matched theoretical expectations in fully selfing populations under background selection when mutations fixed at negligible rates (Gordo et al. 2002). We also confirmed that $N_e$ measured 10 000, 20 000 or 30 000 generations after the burn-in period matched our reported value (taken over 40 000 generations after the burn-in period).

**Simulations with exponentially distributed selection**

We extended the base model by allowing selection strength to vary over time following an exponential distribution (as opposed to a bimodal distribution in the base model). Each

generation, consecutive environments remain the same with probability $f$. With probability, $1-f$, the selection strength is chosen from an exponential distribution with mean $1/s_{avg}$. Similar to the base model, loci under constant and fluctuating selection experienced the same geometric averaged selection strength. In particular, we focused on simulations with $s_{avg} = 0.025$ where the geometric mean and the variance in selection strength under the exponential model was almost identical to that of the conditionally neutral mutations with $s_{avg} = 0.025$ and $\phi = 0.5$ in the base model.

**Simulations with two fluctuating ecological variables**

An unrealistic assumption of the base model is that all F-loci experience fluctuating selection in exactly the same way. As the simplest way to relax this assumption, we extended the base model by allowing for two equally sized sets (F1, F2) of loci under fluctuating selection and each set responds to a different ecological variable. Each individual possess 5000 fitness affecting loci. A proportion $p_{f1}$ and $p_{f2}$ of fitness affecting loci are assigned to experiencing fluctuating selection in response to separate environmental factors and the remaining proportion, $1-p_{f1}-p_{f2}$, are assigned to experience constant selection; location assignment of loci is randomized every simulation. We assume equally sized sets of fluctuating loci, $p_{f1} = p_{f2}$ (and $p_{f1} + p_{f2} = p_f$).

For simulations where mutations at loci under fluctuating selection are conditionally neutral (as in the base model), we assume both groups experience fluctuations in selection of the same magnitude (i.e., between 0 and $s_{max}$) and experience the same time average selection $s_{avg}$. Each generation, consecutive environments remained the same with probability $f$. With probability, $1-f$, the environment was randomly selected among four possible states: $\{s_{f1}(t) = s_{max}, s_{f2}(t) = s_{max}\}$, $\{s_{f1}(t) = s_{max}, s_{f2}(t) = 0\}$, $\{s_{f1}(t) = 0, s_{f2}(t) = s_{max}\}$, $\{s_{f1}(t) = 0, s_{f2}(t) = 0\}$ with probabilities $\phi^2 + d$, $\phi\,(1-\phi) - d$, $(1-\phi)\,\phi - d$, $(1-\phi)\,(1-\phi) + d$, respectively. Loci within F1 and F2 were expected to be under selection with strength $s_{max}$ a proportion $\phi$ of the time, but are otherwise neutral. The value of $d$ must satisfy $d_{min} = \max\{-\phi^2, -(1-\phi)^2\}$ when $d < 0$ and $d_{max} = (1-\phi)\,\phi$ when $d > 0$. The correlation in selective states between set F1 and F2, $\rho$, was determined by the choice of $d$. We explore five different correlations in selective states between loci within set F1 and F2, $\rho = \{-1, -0.5, 0, 0.5, 1\}$. When $\rho = 1$, this model is the same as the base model.

For simulations where selection fluctuates over time following an exponential distribution, we modeled three cases that differ in the correlation of selective states between loci

within set F1 and F2. First, loci in set F1 and F2 may be completely correlated in selection ($\rho$ = 1), therefore $s_{f1}(t)$ equals $s_{f2}(t)$ and was drawn from the exponential distribution. Second, loci in the two sets may be uncorrelated in selection ($\rho = 0$), therefore $s_{f1}(t)$ and $s_{f2}(t)$ were independently drawn from an exponential distribution. Lastly, loci in the two sets may be negatively correlated in selection. $s_{f1}(t)$ is drawn from an exponential distribution, with a value falling on the $k^{th}$ quantile, and $s_{f2}(t)$ takes on the value that is the $(1\text{-}k)^{th}$ quantile of the exponential distribution; this represents the minimum correlation possible between two exponentially distributed random variables ($\rho = 1\text{-}\pi^2/6 \cong -0.645$; Bladt and Neilsen 2010).

**Simulations with beneficial mutations**

Lastly, we extended the base model by allowing beneficial mutations to occur at C-loci. During reproduction, $x$ beneficial mutations were randomly added to the C-loci of the gamete, where $x$ was a number sampled from a Poisson distribution with mean $U_b$ (the genome wide mutation rate of beneficial mutations); the beneficial rates examined were much lower than the deleterious rates ($U_b << U$). The locus to undergo mutation was chosen randomly among the C-loci. The mutation event was ignored if the chosen allele was already a beneficial mutant. If the chosen allele was a deleterious mutant, it was changed into a beneficial mutant. (A beneficial mutant was changed into a deleterious mutant if that allele was chosen to receive a deleterious mutation.) Fitness was calculated as previously described with an additional multiplicative factor of $(1 + s_b)^{x_b}(1 + h_b s_b)^{y_b}$ where $h_b$ and $s_b$ are the dominance and selective coefficient of beneficial mutations, respectively; $x_b$ and $y_b$ are the numbers of C-loci that are homozygous and heterozygous for the beneficial mutations, respectively. Beneficial fixation occurs when all $2N$ alleles at a locus were beneficial mutants; we reset all alleles back to wild type when this occurs.

Following the burn-in period, the per locus fixation rates for deleterious mutations was calculated as described above. We estimated the fixation probability for a new beneficial mutation as the number of beneficial mutations that fixed divided by the expected number of beneficial mutations that were introduced during the time interval over which the data was collected (i.e. $N * U_b *$ number of generations). In addition, we used two methods for comparing deleterious fixation rates at C- and F-loci in simulations *without* beneficial mutations ($U_b = 0$) to the deleterious fixation rates of C- and F-loci in simulations *with* beneficial mutations ($U_b > 0$), holding all parameters the same except for $U_b$. *Method 1*: We calculated the per locus deleterious

fixation rate in simulations with $U_b > 0$ divided by the per locus deleterious fixation rate in populations with $U_b = 0$ for C-loci and F-loci separately (holding all other parameters equal; Table S2). *Method 2*: We examined the number of additional deleterious fixations at C-loci and F-loci per beneficial fixation. To do this we calculated the number of deleterious fixations at C-loci (F-loci) for simulations with $U_b > 0$ minus the average number of deleterious fixations at C-loci (F-loci) for simulations with $U_b = 0$ and divided this difference by the number of beneficial fixations for the simulations with $U_b > 0$ (holding all other parameters equal; Table S3). Each parameter set was replicated at least 20 times.

## Results

In their model of obligately asexual populations, Wardlaw and Agrawal (2012) previously showed that some degree of temporal autocorrelation in selection, $f > 0$, is required for fluctuating selection to significantly elevate rates of mutation accumulation. We confirmed this finding for partially selfing ($S = 0.98$) and obligately selfing ($S = 1$) populations (Figure S1, S2, S10). Rather than further exploring the effects of $f$ on fixation rates, we simply assume that selection undergoes moderate degrees of temporal autocorrelation ($f = 0.95$, $\phi = 0.5$) such that the correlation in selection between time points separated by 1, 10, and 100 generations is 0.95, 0.60, and 0.006, and the average run length between switches in the environment is ~40 generations. This regime elevates fixation rates at loci under fluctuating loci, but is moderate in its effects; fixation rates can be much higher with higher values of $f$ and lower values of $\phi$ (Figure S1, S2, S10). We focus on exploring how loci under fluctuating selection in the genome alter fixation rates at linked sites and genome wide. We also extend our model to test the robustness of our results to different forms of fluctuating selection.

### *Varying the composition of the genome, $p_f$*

We examine fixation rates in genomes where a fraction $p_f$ of loci are under fluctuating selection (F-loci) and the remaining loci are under constant selection (C-loci). Unless otherwise noted, we focus on simulations with $N = 10^4$, $s_{avg} = 0.025$, and $h = 0.25$ with $f = 0.95$ and $\phi = 0.5$. For both partially and obligately selfing populations, per locus fixation rates within genomes experiencing fluctuating selection ($p_f > 0$) are typically higher than fixation rates in the classic model ($p_f = 0$); this is true for both C- and F-loci, but the effect is stronger for the latter (Figure 1, S2) The fact

that per locus fixation rates vary with $p_f$ indicates that changing the density of F-loci in the genome alters the effects of linked selection on both F- and C-loci. When less than half the genome consists of F-loci ($p_f < 0.5$), increasing $p_f$ generally elevates fixation rates at both C- and F-loci and, thereby, the genome-wide average fixation rate. Curiously, at high mutation rates ($U = 1$) and with partial selfing ($S = 0.98$), further increasing $p_f$ reduces fixation rates for genomes that contain more than 50% F-loci (Figure 1a). As it seems unlikely that a high fraction of loci experience strong fluctuations in selection, the results for $p_f < 0.5$ are the most biologically relevant. Therefore, in the relevant range, our results indicate that genomes experiencing more loci under fluctuating selection fix deleterious mutations at faster rates.

When mutation rates are lower (so absolute fixation rates are lower), the effect of fluctuating selection on the *relative* fixation rate is more dramatic for both C- and F-loci. For the parameter sets examined in Fig. 1 for partial selfers, the inclusion of F-loci maximally increases genome-wide fixation rate by a factor of 1.38 vs. 2.42 at the high and low mutation rate, respectively (Fig. 1a, c). For obligate selfers, the maximal increase is 1.5 vs. 4.91 at the high and low mutation rate, respectively (Fig 1b, d). This effect of mutation rate holds in simulations using other values of autocorrelation $f$ and frequency of selection $\phi$ (Figure S2). Although the relative impact of fluctuating selection is larger at lower mutation rates, in the remaining sections we focus on results from high mutation rates ($U = 1$) because simulations at lower mutation rates require much longer runs to obtain accurate estimates of fixation rates.

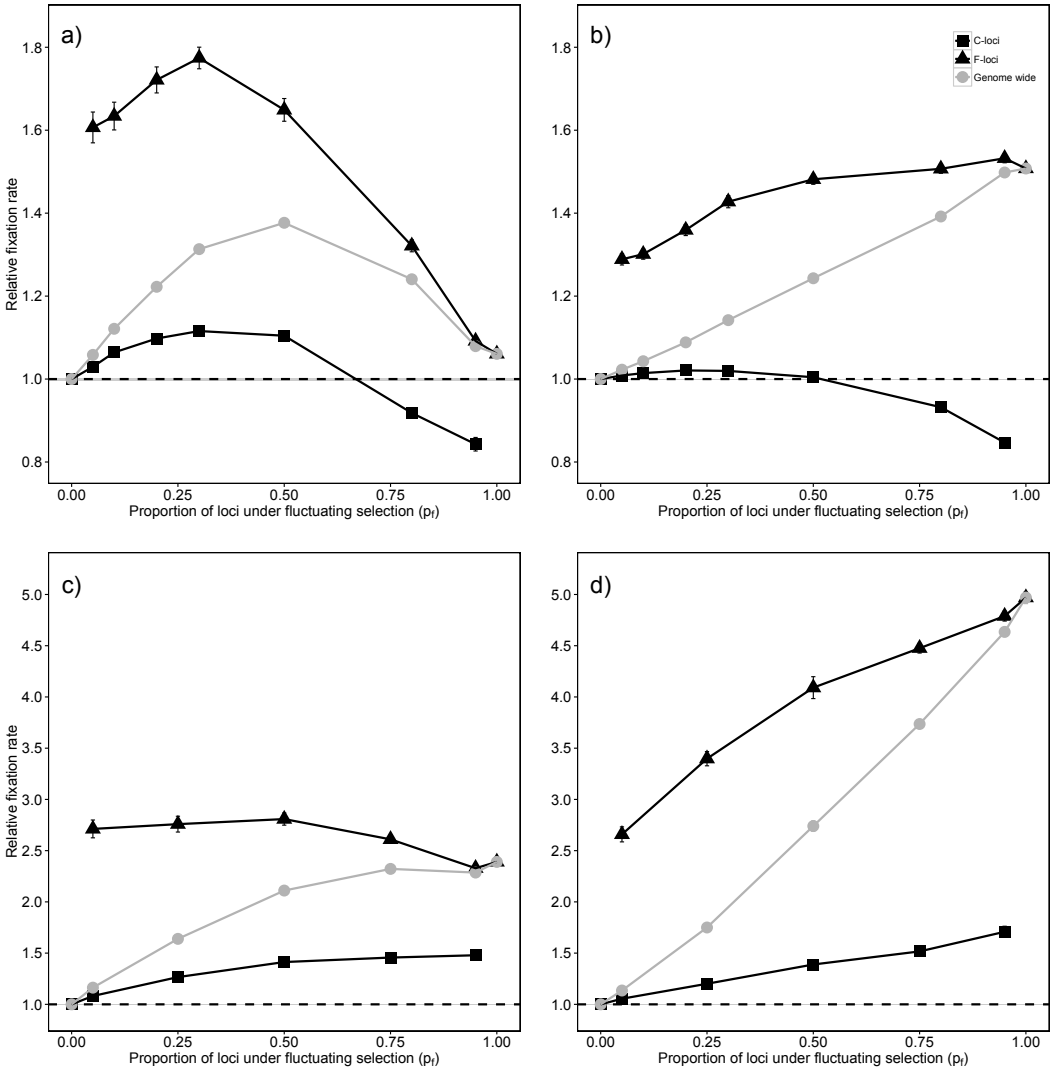


**Figure 1.** Relative fixation rates (mean ± SE for C- and F-loci in populations with varying genomic composition, $p_f$, under moderate temporal autocorrelation in selection ($f$ = 0.95, $\phi$ = 0.5) with $h$ = 0.25, $s_{avg}$ = 0.025. Fixation rates under high mutation rates in (a) partially selfing populations ($U$ = 1, $S$ = 0.98) and in (b) obligately selfing populations ($U$ = 1, $S$ = 1). Fixation rates under lower mutations rates in (c) partially selfing populations ($U$ = 0.5, $S$ = 0.98) and in (d) obligately selfing populations ($U$ = 0.25, $S$ = 1). Fixation rates are given relative to the fixation rate for C-loci in the classic model ($p_f$ = 0), which is represented by the horizontal dashed line at 1.0 (the grey shading denotes SE). The absolute fixation rates in the classic model ($p_f$ = 0) for panels a-d are 7.47 x $10^{-6}$, 2.33 x $10^{-5}$, 8.74 x $10^{-7}$ and 7.08 x $10^{-7}$ per locus per generation respectively.

The density of F-loci alters the effective population size, $N_e$ (see Figure 2a which shows $N_e$ for the simulations represented by Figures 1a-b; Figure S3 for simulations represented by Figures 1 c-d). We may suspect that variation in fixation rates in relation to $p_f$, as shown in Figure 1a, occurs because of changes in the strength of genetic drift (proportional to $1/N_e$). If so, fixation rates should be equal for simulations with different values of $p_f$ if they experienced the same $N_e$. To test this, we ran additional simulations changing the census population size, $N$, of the simulations to raise or lower $N_e$ for a given value of $p_f$. (We observe that $N_e$ is not linearly related to $N$ (Figure 2b) as has been observed previously in simulations of asexual populations accumulating deleterious mutation due to Muller's ratchet (Gordo *et al.* 2002).) Consistent with the idea that the relationship between $p_f$ and fixation rate results from the effect of $p_f$ on $N_e$, we observe fixation rates are similar across different values of $p_f$ when $N_e$ is similar, but only if $p_f$ is not too high ($0 < p_f \leq 0.5$; Figures 2c, d). However, when comparing simulations with similar estimates of $N_e$, very high values of $p_f$ (i.e., $p_f = 0.8, 0.95$) result in higher rates fixation rates than low or moderate values of $p_f$ (i.e., $p_f \leq 0.5$). This indicates that when $p_f$ is large (and the mutation rate is high), selective interference between C- and F-loci affects fixation rates in ways that cannot be captured by a genome-wide estimate of $N_e$ if a large fraction of the genome consists of F-loci.

Different methods of estimating of $N_e$ can yield different values when genomes experience selective interference at many loci (McVean and Charlesworth 2000, Comeron et al. 2008). Thus, we cannot reject the possibility that a different estimator of $N_e$ may explain the trends in fixation rates we observed even at high values of $p_f$. However, we suspect that a single genome wide summary statistic, such as $N_e$, cannot fully account for variation in fixation rates of both C- and F-loci because the two types of loci can qualitatively differ in their response to $p_f$ (Figure 1a). For example, we observe non-monotonic effect of $p_f$ on $N_e$ for obligately selfing populations (Figure 2a) but fixation rates at F-loci are monotonically increasing with $p_f$ while fixation rates at C-loci changes non-monotonically with $p_f$ (Figure 2b).

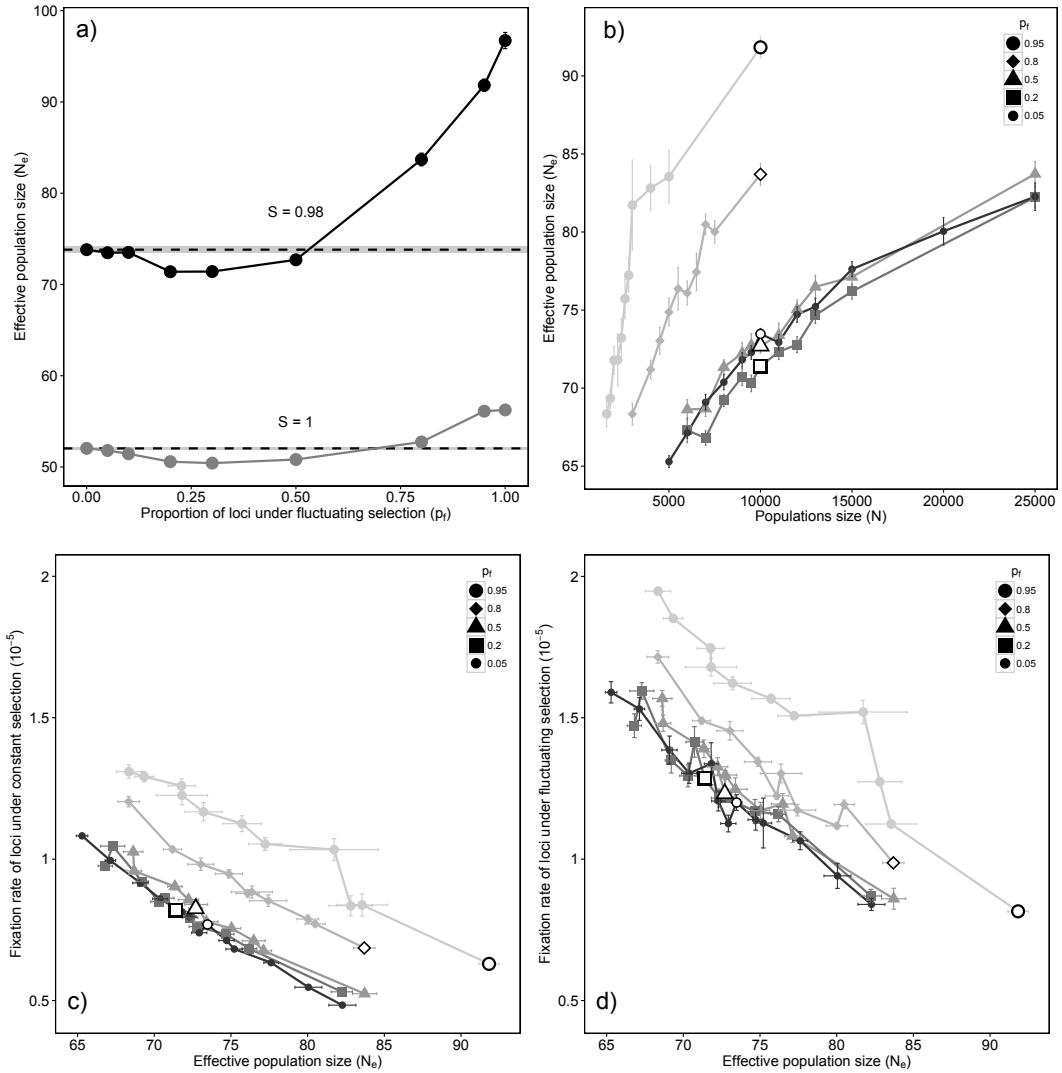


**Figure 2.** (a) Estimates of effective population size (mean ± SE) for genomes containing different densities of F-loci, $p_f$, in a population of $N = 10^4$. Shown are $N_e$ values for partially selfing populations ($U = 1$, $S = 0.98$; black points) and obligately selfing populations ($U = 1$, $S = 1$, grey points); these results are from the same simulations represented in Figures 1a-b. The horizontal dashed line indicates the $N_e$ (grey shading is SE) for the corresponding classic model ($p_f = 0$) under partially selfing (top) and obligate selfing (bottom). (b) Estimates of $N_e$ plotted against $N$ for partially selfing populations ($U = 1$, $S = 0.98$) for genomes with different values of $p_f$ as depicted in the legend. The open symbol indicates the $N_e$ when $N = 10^4$ (i.e., corresponding to simulations in Figure 1a). Fixation rates under partial selfing ($U = 1$, $S = 0.98$) plotted against $N_e$ (c) loci under constant selection (d) loci under fluctuating selection.

*Fixation bias at F-loci*

The fixation rate of deleterious mutations at F-loci is always greater than that for C-loci within all mixed genomes (Figures 1, 2c, d). This bias is clearly stronger when selection fluctuates in ways that directly increase fixation rates at F-loci (i.e., larger $f$ and smaller $\phi$; Figure S2). Moreover, this bias is affected by the 'traditional' parameters affecting mutation accumulation such as the strength of selection ($s_{avg}$), the mutation rate ($U$) and the population size ($N$). To examine how this bias depends on aspects of selection, we performed simulations where half the genome consist of F-loci ($p_f$ = 0.5) at high mutations rates ($U$ = 1) with an average selection strength of $s_c = s_{avg} = \{0.005, 0.025, 0.05\}$. The ratio of the fixation rate at F-loci to the fixation rate at C-loci (within the same population) is consistently larger than unity for both partially and obligately selfing populations (Figure 3a), indicating that mutations fix disproportionately frequently at loci under fluctuating selection. This bias is stronger when selection is stronger on average or when it is concentrated into shorter but more intense events (i.e., smaller values of $\phi$; Figure 3a). This bias in fixations at F-loci increases with the average selection strength as stronger selection seems to reduced fixation rates at C-loci relatively more than at F-loci (Figure 3b).

Reducing the mutation rate also increases the bias in fixations at F-loci for both partially selfing and obligately selfing populations (Figures 4, S2, S9). In obligately selfing populations, a reduction in $U$ in conjunction with an increase in $s_{avg}$ causes an even larger bias (Figure 4b). This bias seems reasonably insensitive to genome composition, $p_f$. The bias towards fixations at F-loci is also stronger with increasing population size (Figure S5) and increasing outcrossing rates (Figure S6). Overall, these results show that loci under fluctuating selection contribute disproportionately to the fixation of deleterious mutations and decline in fitness of highly selfing populations. Conditions that are expected to slow down mutation accumulation, such as lower $U$, stronger $s_{avg}$, larger $N$, and less selfing seem to reduce fixation rates at C-loci more so than at F-loci, making the relative contribution of F-loci to mutation accumulation even greater.

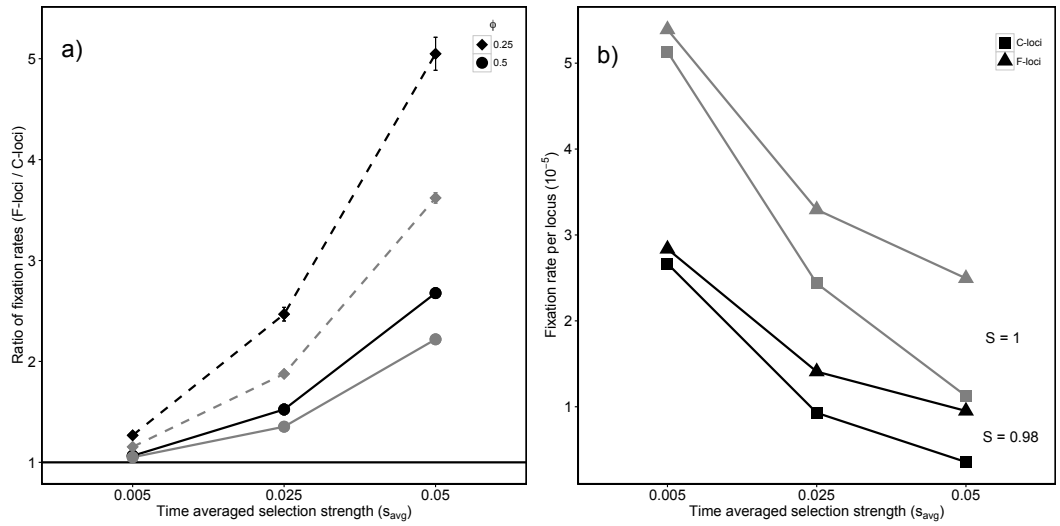


**Figure 3.** (a) Ratio of fixation rates at F-loci to fixation rates at C-loci (within the same population) for partially selfing (black; $S$ = 0.98) and obligately selfing (grey; $S$ = 1) populations when half of the genome consists of F-loci ($p_f$ = 0.5); error bars are SE (often too small to be visible). When $\phi$ = 0.5, selection is non-neutral ($s_{max}$) half the time but when $\phi$ = 0.25, selection is non-neutral only a quarter of the time. To maintain $s_{avg}$ = {0.005, 0.025, 0.05} when $\phi$ is reduced by half we approximately double the value of $s_{max}$ (Methods), therefore making selection concentrated into shorter but more intense events. (b) Fixation rates at F-loci and C-loci for partially selfing (black; $S$ = 0.98) and obligately selfing (grey; $S$ = 1) populations when $\phi$ = 0.5. Other parameters: $N = 10^4$, $U$ = 1, $h$ = 0.25, $f$ = 0.95, $s_c = s_{avg}$.

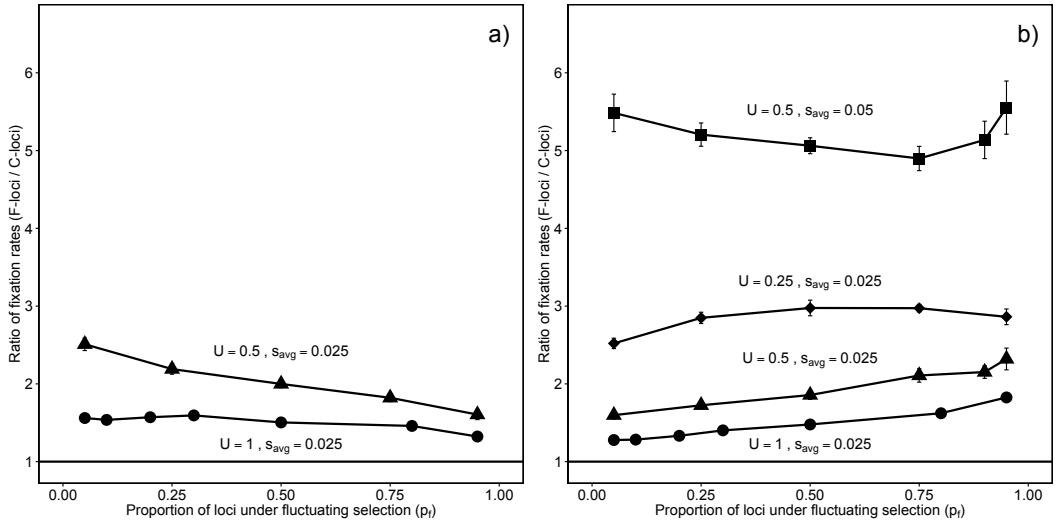


**Figure 4.** Ratio of fixation rates (mean ± SE) at F-loci to fixation rates at C-loci (within the same population) for populations with different proportions of F-loci, $p_f$ for (a) partially selfing populations ($S$ = 0.98) and (b) obligately selfing populations ($S$ = 1). Changes to parameters that reduce mutation accumulation (reduced $U$ or increased $s$) result in an increased bias of F-loci fixation over C-loci fixation. Other parameters: $N$ = 10000, $h$ = 0.25, $f$ = 0.95, $\phi$ = 0.5.

*Exponentially distributed selection over time*

Above we assumed that mutations at F-loci are conditionally neutral, that is, selection fluctuates bimodally over time between 0 and $s_{max}$. To examine whether our results are qualitatively robust to different temporal distributions of fluctuating selection, we now consider simulations where the selection strength for F-loci is randomly sampled from an exponential distribution when the environment changes. The exponential distribution we use has a very similar mean and standard deviation in selection strength compared to our base model of conditional neutrality model ($s_{avg} \cong$ 0.025, standard deviation in $s_f(t) = 0.025$). Using $f = 0.95$, the correlation in selection between time points separated by 1, 10, and 100 generations is 0.95, 0.60, and ~0, respectively. The fixation rates for both C- and F-loci are slightly lower under the exponential model than the bimodal model (Figure 5). Mutations fix disproportionately at F-loci under the exponential selection model, but this bias is not quite as strong as observed in the bimodal selection model. Both models are also similarly affected by changes in the mutation rate; we observe that reducing mutations rates (from $U = 1$ to $U = 0.5$) increases relative fixation rates and the proportion of mutations that fix at F-loci (Figure S8, S9). Though the bimodal and exponential selection model represents qualitatively different forms of temporal heterogeneity in selection, the effects of these models on fixation rates are reasonably similar. We speculate that any form of fluctuations in selection intensity are likely to elevate fixation rates and fitness decline in highly selfing populations as long as the variance in selection over time is sufficiently large and there is some degree of temporal autocorrelation.

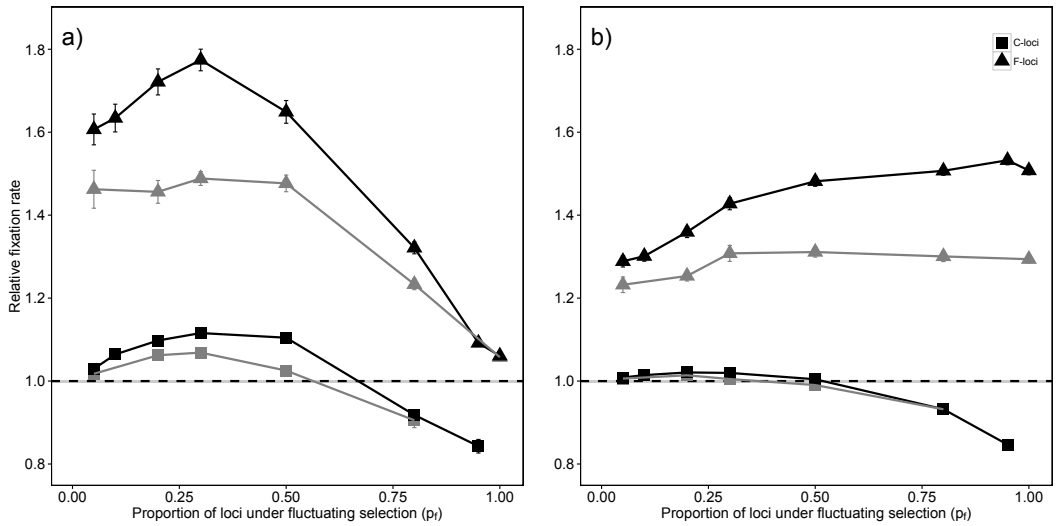


**Figure 5.** Relative fixation rates (mean ± SE) at C- and F-loci in populations with different genomic compositions, $p_f$, when selection strength is sampled from a bimodal (black) or exponential distribution (grey) over time in (a) partially selfing populations ($S$ = 0.98) and (b) obligately selfing populations ($S$ = 1). Relative fixation rates under the bimodal selection model are re-plotted from Figure 1a, b. The geometric time average and the variance in selection strength and for the bimodal and exponential selection model are approximately the same ($s_{avg}$ = 0.025, standard deviation in $s_f(t)$ = 0.025). Fixation rates are given relative to the fixation rate for C-loci in the classic model ($p_f$ = 0), which is represented by the horizontal dashed line at 1.0 (the grey shading denotes SE). Other parameters: $f$ = 0.95, $N$ = $10^4$, $U$ = 1, $h$ = 0.25.

*Two components of fluctuating selection*

In the previous results, we assume that all F-loci simultaneously respond to the same environmental factor that fluctuates in time. As the simplest way to relax this assumption, we allow for two equally sized sets of F-loci (F1, F2) that respond to two separate environmental factors. The two environmental factors can be correlated in any way. At one end of the spectrum, environmental factors fluctuate in perfect synchrony, causing a complete correlation in selective states between loci within F1 and F2 ($\rho = 1$), which is equivalent to the base model. At the other end, the two environment factors fluctuate in anti-synchrony, causing a negative correlation in selective states between F1 and F2 ($\rho = -1$). At this extreme, at any given time point, selection at one set is 0 while at the other set is $s_{max}$. We perform these simulations for a range of different values of $\rho$, the correlation in selective states between set F1 and F2.

For partially selfing populations, $\rho$ has little to no effect on the fixation rates of C- and F-loci when the genome consists of mostly C-loci ($p_f \leq 0.5$); $\rho$ is negatively related to fixation rate when the genome consists of mostly F-loci, making our base model ($\rho = 1$) conservative in this respect (Figure 6). Obligately selfing populations are largely insensitive to the correlation in selection between loci in F1 and F2 (Figure 6b). We observe qualitatively similar effects of $\rho$ when selection between the two groups is exponentially distributed in time under high and lower mutation rates (Figure S7, S8, S9). In sum, under biologically realistic ranges of $p_f$ (i.e., $p_f < 0.5$), fixation rates are not dependent on how fluctuations in selection are correlated between two equally sized sets of F-loci.

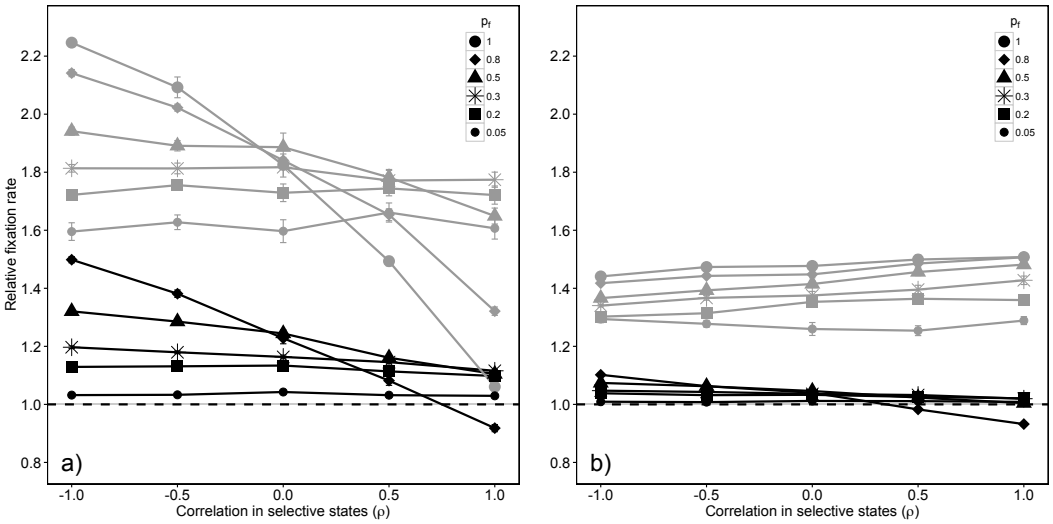


**Figure 6.** Relative fixation rates (mean ± SE) for C-loci (black) and F-loci (grey) in populations with different genomic compositions, $p_f$, for (a) partially selfing populations ($S$ = 0.98) and (b) obligately selfing populations ($S$ = 1). Loci under fluctuating selection are equally distributed between two sets of F-loci (F1, F2). The correlation in selective states between F1 and F2, $\rho$, can be positive or negative or zero. Fixation rates are shown for genomes with different frequencies of fluctuating loci, $p_f$. Fixation rates are given relative to the fixation rate for C-loci in the classic model ($p_f$ = 0), which is represented by the horizontal dashed line at 1.0 (the grey shading denotes SE). Other parameters: $f$ = 0.95, $\phi$ = 0.5, $s_{avg}$ = 0.025, $N$ = $10^4$, $U$ = 1, $h$ = 0.25.

*Beneficial mutations*

We performed a smaller set of simulations in which beneficial mutations occurred at C-loci (Table S2, S3). For these simulations, beneficial mutations rates were much lower than deleterious mutation rates ($U_b \leq 0.001$) and beneficial mutations were assumed to have larger effects than deleterious mutations ($s_b = 0.05$, $h_b = 0.5$ vs. $s_{avg} = 0.025$, $h = 0.025$). F-loci only received deleterious mutations and experienced selection fluctuating bimodally over time between 0 and $s_{max}$, with parameters $f = 0.95$ and $\phi = 0.5$.

The fixation probability of beneficial mutations was lower in simulations with F-loci ($p_f = 0.2$ than those without F-loci ($p_f = 0$) (Table S2) indicating that deleterious mutations at F-loci cause more interference with beneficials than those at C-loci cause. Conversely, beneficials increase the deleterious fixation rates at both C-loci and F-loci. We took two perspectives on this change. The proportional increase in the deleterious fixation rate is similar for C- and F-loci (Table S2). However, because the F-locus rate without beneficials is larger than the C-locus rate, the similarity in "proportional increase" obscures the fact that the absolute increase in the deleterious fixation rate caused by beneficials is larger for F-loci than C-loci. This is clearer from our second perspective in which we calculate the average number of additional fixed deleterious mutations per fixed beneficial (Table 1). In the simulations reported, each beneficial that fixes is responsible for, on average, less than one additional deleterious fixation but these are not evenly distributed between F- and C-loci. F-loci are only 25% as abundant as C-loci in these simulations (i.e., $p_f = 0.2$), but the number of additional fixed deleterious F-alleles per fixed beneficial is more than 25% the number of additional fixed deleterious C-alleles. From this we infer that F-loci experience stronger interference from beneficials than do C-loci. Because we have explored only a small set of parameters for simulations with beneficials, the conclusions above should be regarded with greater caution.

**Table 1.** Summary of simulations with beneficial mutations.

| Self rate | # Gen. | $U$ | $U_b$ | $s_b$ | $p_f$ | Ben. prob. fix. x $10^{-3}$ (SE) | Extra fix del per fix ben (SE) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | C-loci | F-loci |
| 0.98 | 120000 | 0.5 | 0.0001 | 0.05 | 0 | 3.80 (0.019) | 0.345 (0.023) | |
| | | | | | 0.2 | 3.50 (0.025) | 0.283 (0.015) | 0.122 (0.021) |
| | 50000 | 1 | 0.001 | 0.05 | 0 | 1.54 (0.011) | 0.492 (0.012) | |
| | | | | | 0.2 | 1.42 (0.007) | 0.372 (0.014) | 0.135 (0.032) |
| 1 | 120000 | 0.25 | 0.00005 | 0.05 | 0 | 2.41 (0.018) | 0.938 (0.028) | |
| | | | | | 0.2 | 2.28 (0.021) | 0.703 (0.035) | 0.225 (0.055) |
| | 50000 | 1 | 0.001 | 0.05 | 0 | 0.49 (0.006) | 0.757 (0.041) | |
| | | | | | 0.2 | 0.47 (0.005) | 0.608 (0.07) | 0.066 (0.043) |

Standard error for each statistic indicated within parentheses. '*# Gen.*' is the number of generations examined for calculating the data. $U$, $U_b$ are the genomic wide mutation rates of deleterious and beneficial mutations, respectively. $s_b$ is the fitness advantage of a beneficial mutation. '*Ben. prob. fix.*' is the fraction of beneficial mutations that fixed per generation in the simulation assuming that the number of beneficial mutations introduced per generation was $U_b * N$. '*Extra fix del per fix ben'* is the average number of extra deleterious alleles fixed per beneficial fixed beyond what would have otherwise fixed. Other parameters: $N = 10000$, $h = 0.25$, $s_{avg} = 0.025$, $h_b = 0.5$, $f = 0.95$, $\phi = 0.5$, $\rho = 1$. See Table S2 for further information on these simulations.

## Discussion

Highly selfing populations experience lower effective population sizes, $N_e$, and suffer from a reduced efficacy of selection (reviewed in Hartfield 2014). As a consequence, highly selfing populations can accumulate and permanently fix deleterious mutations at high rates leading to rapid fitness decline and possibly to extinction (Heller and Maynard Smith 1979, Charlesworth and Charlesworth 1993). It is generally accepted that environmental changes occur on many different times scales (Steele 1985, Halley 1996, Vasseur and Yodviz 2004, Furguson et al. 2016) and can induce fluctuations in the intensity and direction of selection (Siepielski et al. 2009, 2017, Bell 2010) but the consequences for mutation accumulation in selfers is unstudied.

We found that loci experiencing selection that fluctuates in intensity, F-loci, fix deleterious mutations at a higher rate than loci under constant selection, C-loci, experiencing the same time averaged selection strength ($s_c = s_{avg}$). The extent to which F-loci contribute disproportionately to total mutation accumulation depends on aspects of environmental heterogeneity. The bias is stronger with increasing temporal autocorrelation in selection, $f$, and when selection is concentrated into rarer but more intense episodes (low values of $\phi$), holding $s_{avg}$ constant (Figures S1, S2, S10). This bias also becomes stronger with changes to "traditional" parameters that lower the overall rate of mutation accumulation, i.e., higher values of $s_{avg}$ or $N$ as well as lower values of $U$ (Figures 3, 4, S5, S9). In sum, fluctuating selection can greatly increase the rate of mutation accumulation in highly selfing populations and the loci under fluctuating selection contribute disproportionately to mutational decline. These conclusions appear qualitatively robust to the exact nature of fluctuations (bimodal vs. exponential distribution of selection coefficients and positive, negative, or no temporal correlation in selection among loci Figure 5, 6, S7, S8).

In our model, deleterious mutations fix as a consequence of selective interference between tightly linked sites. Supporting this, simulations at higher rates of outcrossing ($S \leq 0.9$) fix few to no mutations (Table S1). In contrast, obligately asexual organisms (Wardlaw and Agrawal 2012) and pure selfers ($S = 1$) have high rates of mutation accumulation that are strongly increased by fluctuating selection. These effects remain important even when low levels of genetic mixing occur as with low levels of outcrossing ($S = 0.98$). It is well known that interference between constantly selected loci can lead to the accumulation of deleterious mutations in asexual and highly selfing populations, a process classically called Muller's ratchet (Haigh 1978, Heller and Maynard Smith 1979, Charlesworth and Charleswroth 1997). However, we do not have a robust understanding of selective interference between loci under fluctuating selection and loci under constant selection. If F-loci are conditionally neutral (as in the base model), mutations accumulate unimpeded by selection during periods of neutrality ($s_f(t) = 0$) with an expected run length of $1/(\phi(1-f))$. Subsequently, when F-loci are under selection ($s_f(t) = s_{max}$), selective interference likely prevents effective removal of deleterious mutations at individual loci, increasing the probability of fixation.

In genomes containing a mix of F- and C-loci, F-loci have inherently high rates of fixation but also tend to increase fixation rates at C-loci and other F-loci as illustrated in Figure 1 where increasing the fraction of loci under fluctuating selection increases the per-locus fixation rates at both C- and F-loci (for $0 < p_f < 0.5$). This indicates that F-loci cause more selective interference (with both C- and F-loci) than C-loci. Similarly, we found that deleterious mutations at F-loci reduce fixation rates of beneficials more than deleterious mutations at C-loci do (Table S2, S3). Previous work in asexual populations where selection is constant over time but varies among loci shows that interference from strongly deleterious alleles increase fixation of more weakly deleterious alleles (Gordo and Charlesworth 2001, Gordo et al. 2002, Soderberg and Berg 2007). From this we can infer that strong interference caused by F-loci occurs during periods when selection is strong, $s_f(t) = s_{max} > s_c$. However, during periods where $s_f(t) = 0$, F-loci do not cause interference. In fact, compared to the classic model, interference experienced by C-loci should be reduced relative to the classic model ($p_f = 0$) because C-loci are separated physically by (temporarily) neutral F-loci when $s_f(t) = 0$. Given the observed net increase in fixation rate at C-loci, we can infer that the increased interference when selection at F-loci is strong ($s_f(t) = s_{max}$) outweighs the reduced interference when selection is weak ($s_f(t) = 0$). Reciprocally, we observed that strongly selected beneficials under constant selection interfered more strongly with deleterious alleles at F-loci than at C-loci (Table 1). From the perspective of an F-locus, the sweeping beneficial causes very strong interference on an F-locus when the selection at the F-locus is weak and weaker interference when direct selection on the F-locus is strong. We can infer that the episodes of strong interference must outweigh periods of weak interference because the total interference from beneficials experienced by F-loci is greater than that experienced by C-loci.

In some, but not all cases, the effect of selective interference on fixation rates can be interpreted as resulting from a reduction in the effective population size (Hill and Robertson 1966, McVean and Charlesworth 2000, Gordo and Charlesworth 2002, Good et al. 2014). We observed that per-locus fixation rates varied as a function of $p_f$ for both C- and F-loci (Fig. 1). In the range where $p_f$ was not too large ($0 < p_f \leq 0.5$), fixation rates for each locus type (F and C) were nearly equal for different values of $p_f$ when we adjusted $N$ to obtain similar $N_e$ values (Fig. 2). Because neutral diversity (used to infer $N_e$) and fixation rates are similarly affected by

selective interference within this range of $p_f$ values, we conclude that selective interference in this regime can be thought of as causing a reduction in $N_e$. Good et al. (2014) have shown that in asexual populations experiencing strong selective interference the variance in fitness explains neutral diversity much better than the $N_e$ expected under deterministic mutation selection balance. We examined whether the time-averaged variance in fitness could explain the variation in fixation rates in relation to $p_f$ better than our diversity-based estimates of $N_e$ but found no obvious improvement (Figure S4).

Neither $N_e$ nor the variance in fitness can account for the variation in fixation rates when considering populations where most loci undergo fluctuating selection ($p_f > 0.5$). Others have noted that the effects of selective interference on fixation rates cannot always be captured through $N_e$. A sweep of a strongly beneficial mutation differentially affects neutral diversity and fixation probability at a linked weakly beneficial mutation (Stephan et al. 1992, Barton 1994). Fluctuating selection may have some sweep-like properties because genotypes favoured during the selective period ($s_f(t) = s_{max}$) can be different from those favoured during the neutral period ($s_f(t) = 0$) due to the shift in the relative importance of F- and C-loci in determining fitness. Recurrent sweeps of highly fit genotypes caused by changing selective environments may represent another dynamical process that differentially affects fixation probability at selected loci and genetic drift at linked neutral loci (Comeron et al. 2008).

We face several major questions when assessing the relevance of our model to natural systems. To what extent does selection strength on individual genes depend on environmental conditions? How many loci in the genome are under fluctuating selection? Do elevated rates of mutation accumulation increase extinction rates? What happens if environmental changes alter population size in addition to selection? Can we detect signatures of fluctuating selection in molecular data?

In laboratory experiments, selection at individual loci has been shown to vary between different abiotic and biotic environments for bacteria (Remold and Lenski 2001, Cooper et al. 2005), yeast (Hillenmeyer et al. 2008, Jasnos et al. 2008, Bell 2010b, Heitpas et al. 2013, Cao et al. 2014), nematodes (Coolon et al. 2009) and fruit flies (Lewontin and Matsuo 1963, Wang et al.

2009, 2014, Young et al. 2009). In the largest study of this kind, Hillenmeyer et al. (2008) found that 97% of gene deletions in *Saccharomyces cerevisiae* reduced fitness relative to the wild type in at least one environment while having little to no effect in other environments. Although the environmental conditions chosen for most experiments do not typically mimic those in nature, studies find that selection at individual loci tend to differ in magnitude (rather than in direction) between environments. We show that selection fluctuating only in magnitude increases fixation rates of deleterious mutations, which may be relevant for many types of environmentally sensitive loci. It remains a significant empirical challenge to parameterize models with selection coefficients of naturally occurring mutations quantified across naturally occurring environments.

Another critical parameter for our model is the proportion of loci in the genome that experiences fluctuating selection, $p_f$. Excluding mutations causing intrinsic lethality, we speculate that almost every locus experiences temporal change in selection strength as it seems implausible selection strength is exactly the same in all conditions. However, for many (or most) loci, fluctuations in selection may be minor. Perhaps only a minority of mutations undergo fluctuations in selection strength on the order of magnitude that we modeled and with at least a moderate level of temporal autocorrelation. Although we are unaware of any empirical estimates for $p_f$, we suspect that the results of simulations with values of $p_f < 0.5$ best represent reality.

Our study was motivated by the 'dead end' hypothesis for highly selfing species (Stebbins 1957, Igic and Busch 2013) but we do not explicitly model extinction. Similar to previous examinations of mutation accumulation in genomes with low rates of recombination (Haigh 1978, Heller and Maynard Smith 1979, Stephan et al. 1993, Charlesworth and Charleswroth 1997), we hypothesize that the fixation of deleterious mutations should lead to fitness loss and eventual extinction. Models using realistic parameters to predict the expected time to extinction under Muller's ratchet assume deleterious mutations directly reduce population growth rates (Lynch and Gabriel 1993, Gabriel et al. 1993, Loewe 2006, Loewe and Lamatsch 2008). However, it is not obvious how the deleterious fitness effects of mutations directly affect ecological success and species persistence (Agrawal and Whitlock 2012). That said, our model predicts that mutations accumulate disproportionately at loci experiencing fluctuating selection. We might expect, for example, that populations are driven to extinction

when deleterious mutations fix at loci required for surviving rare but severe climatic events. The chance of this occurring increases if those mutations hitchhike to high frequencies from linkage to beneficial mutations. Predicting the impact of mutation accumulation on species persistence requires more complex models that incorporate the functional role and ecological context of genes and explicitly track population sizes to extinction.

Environmental changes that cause fluctuations in selection may also cause fluctuations in population size (e.g. Bergland et al. 2014). Our goal in this study was to isolate the effects of fluctuating selection on its own but population size changes may be important on their own and/or interact with fluctuations in selection. The addition of fluctuating population size will lower $N_e$, further elevating fixation rates (Ewens 1967). Moreover, fixation rates may depend on the correlation between population size and selection strength but the nature of this relationship is not obvious. Selective deaths may or may not simply substitute for ecological deaths. Stress, which is sometimes defined as a reduction in absolute fitness or population growth (and thereby population size), is a poor predictor of the average strength of selection on new mutations (Martin and Lenormand 2006; Agrawal and Whitlock 2010). Since fluctuating population size alone can increase extinction rates (Lande 1993, Foley 1994, Ovaskainen and Meerson 2010), integrating how environmental changes affect ecological and evolutionary forces will be important for predicting the persistence of highly selfing species.

Ideally, one would be able to test our prediction that F-loci fix disproportionately more deleterious mutations than C-loci. Genes could be crudely classified as C- or F-loci depending on whether their expression levels are stable or variable under different naturally occurring environmental conditions (see examples of environmental sensitive expression in Sonna et al. 2002, Lopez-Maury et al. 2008, Lasky et al. 2014). Such a classification is far from perfect (e.g., selection can change even if expression does not). For a pair of selfing and outcrossing sister species, the ratio of divergence at non-synonymous to synonymous sites could be compared between genes with variable expression ($\omega_{v,selfing}$, $\omega_{v,outcross}$) and genes with stable expression ($\omega_{s,selfing}$, $\omega_{s,outcross}$). Because high selfing is expected to disproportionately increase fixation rates at loci under fluctuating selection, we may predict that $\omega_{v,selfing} - \omega_{v,outcross}$ would be greater than $\omega_{s,selfing} - \omega_{s,outcross}$. Unfortunately, there are two major caveats with this approach. Differences in

the average strength of selection between the sets of genes with variable and stable expression would confound the results. Furthermore, there is currently only weak evidence that selfing species fix non-synonymous mutations at a higher rate than outcrossing species (Wright et al. 2011, Gioti et al. 2013, Burgarella et al. 2015). This may be due to the young age of selfing species or that higher fixation rates of beneficial mutations raise the value of ω in outcrossers more than selfers (Burgarella et al. 2015). The approach outlined above assumes selfing lineages are old enough for differences in fixation rates to be apparent and, further, that beneficial mutations do not make a substantial contribution to divergence in either selfers or outcrossers.

It is widely speculated that highly selfing species are short lived and prone to extinction. This is supported by their rarity in nature (Igic and Kohn 2006) and higher estimates of extinction rates in self-compatible species (Goldberg et al. 2009; see Johnson et al. 2012 for similar results on asexuals). There is also genomic evidence that selection is less efficient in selfing plants and animals (Wright et al. 2002, Cutter et al. 2006a,b, Slotte et al. 2013, Burgarella et al. 2015). It is also generally accepted that environmental conditions change over time and can alter selection. Fluctuations in the direction and intensity of selection may be important for long-term evolutionary dynamics, but the effects on genomic inferences of selection are not well understood (Huerta-Sanchez 2012, Gossmann et al. 2014). We find that selection fluctuating only in intensity can elevate fixation rates of deleterious mutations. Our results may well underestimate the relative importance given that we observe stronger effects under lower mutation rates (Figure 1, S8, S9). Even if rare in the genome, loci experiencing fluctuating selection make a disproportionately larger contribution to mutation accumulation and fitness decline than loci under constant selection. Moreover, mutation accumulation at such loci may make selfing species particularly susceptible to extinction to rare environmental conditions where the functions of such loci are particularly important. Although detecting and measuring the fitness impact of loci under fluctuating selection will be empirically challenging, our model shows a plausible parameter space where fluctuating selection can strongly impact the persistence of highly selfing species.

## References


Agrawal, A.F. and Hartfield, M. 2016. Coalescence with background and balancing selection in systems with bi- and uniparental reproduction: contrasting partial asexuality and selfing. Genetics 202: 313-326.

Agrawal, A.F. and Whitlock, M.C. 2010. Environmental duress and epistasis: how does stress affect the strength of selection on new mutations? Trends Ecol. Evol. 25:450-458.

Agrawal, A.F., and Whitlock, M.C. 2012. Mutation load: the fitness of individuals in populations where deleterious alleles are abundant. Annu. Rev. Ecol. Evol. Syst. 43: 115-135.

Arunkumar, R., Ness, R. W., Wright, S. I. and Barrett, S. C. H. 2015 The evolution of selfing is accompanied by reduced efficacy of selection and purging of deleterious mutations. Genetics 199: 817-829.

Bachtrog, D. and Gordo, I. 2004. Adaptive evolution of asexual populations under Muller's ratchet. Evolution 7: 1403-1413.

Barrett, S.C.H. 2002. The evolution of plant sexual diversity. Nat. Rev. Genet. 3: 274-284.

Barton, N. H. 1995. A general model for the evolution of recombination. Genet. Res. Camb. 65: 123-144.

Bell, G. 2010a. Fluctuating selection: the perpetual renewal of adaptation in variable environments. Phil. Trans. R. Soc. B. 365: 87-97.

Bell, G. 2010b. Experimental genomics of fitness in yeast. P. R. Soc. B. 277: 1459-1467.

Bergland, A.O., Behrman, E.L., O'Brien, K.R., Schmidt, P.S. and Petrov, D.A. 2014. Genomic evidence of rapid and stable adaptive oscillations over seasonal time scales in *Drosophila*. PLoS Genet. 10: e1004775.

Bladt, M. and Nielsen, B.F. 2010. On the construction of bivariate exponential distributions with an arbitrary correlation coefficient. Stoch. Models 26: 295-308.

Brandvain, Y., Slotte, T., Hazzouri, K.M., Wright, S.I. and Coop, G. 2013. Genomic identification of founding haplotypes reveal the history of the selfing species *Capsella rubella*. PLoS Genet. 9: e1003754.

Burgarella, C., Gayral, P., Ballenghien, M. *et al*. 2015. Molecular evolution of freshwater snails with contrasting mating systems. Mol. Biol. Evol. 32: 2403-2416.

Cao, H., Butler, K., Hossain, M. and Lewis, J.D. 2014. Variation in the fitness effects of mutations with population density and size in *Escherichia coli*. PLoS One 9: e1059369.

Charlesworth, D. Morgan, M.T. and Charlesworth, B. 1993. Mutation accumulation in finite outbreeding and inbreeding populations. Genet. Res. Camb. 61: 39-56.

Charlesworth, B. 1980. The cost of sex in relation to mating systems. J. Theor. Biol. 84: 655-671.

Charlesworth, B. 1993. Directional selection and evolution of sex and recombination. Genet. Res. Camb. 61: 205-224.

Charlesworth, B. and Charlesworth, D. 1997. Rapid fixation of deleterious alleles can be caused by Muller's ratchet. Genet. Res. Camb. 70: 63-73.

Chevin, L., Visser, M.E., and Tufto, J. 2015. Estimating the variation, autocorrelation and environmental sensitivity of phenotypic selection. Evolution 69: 2319-2332.

Comeron, J.M., Williford, A. and Kliman, R.M. 2008. The Hill-Robertson effect: evolutionary consequences of weak selection and linkage in finite populations. Heredity 100: 19-31.

Coolon, J.D., Jones, K.L., Todd, T.C., Carr, B.C. and Herman, M.A. 2009. *Caenorhabditis elegans* genomic response to soil bacteria predict environmental-specific genetic effects on life history traits. PLoS Genet. 5: e1000503.

Cooper, T.F., Lenski, R.E. and Elena, S.F. 2005. Parasites and mutational load: an experimental test of a pluralistic theory for the evolution of sex. Proc. R. Soc. B. 272: 311-317.

Cutter, A.D. 2006. Nucleotide polymorphism and linkage disequilibrium in wild populations of the partial selfer *Caenorhabditis elegans*. Genetics 172: 171-184.

Cutter, A.D., Baird, S.E., Charlesworth, D. 2006. High nucleotide polymorphism and rapid decay of linkage disequilibrium in wild populations of *Caenorhabditis remanei*. Genetics 174: 901-913.

Cvijovic, I., Good, B.H., Jerison, E.R. and Desai, M.M. 2015. Fate of a mutation in a fluctuating environment. P. Nat. Acad. Sci. USA. 112: E5021-E5028.

Ewens, W.J. 1967. The probability of survival of a new mutant in a fluctuating environment. Heredity 22: 438-443.

Ferguson, J.M., Carvalho, F., Murillo-Garcia, O., Taper, M.L. and Ponciano, J.M. 2016. An updated perspective on the role of environmental autocorrelation in animal populations. Theor. Ecol. 9: 129-148.

Fisher, R.A. 1941. Average excess and average effect of a gene substitution. Ann. Eugenic 11: 53-63.

Foley, P. 1994. Predicting extinction times from environmental stochasticity and carrying capacity. Conserv, Biol. 8: 124-137.

Gabriel, W., Lynch, M. and Burger, R. 1993. Muller's ratchet and mutational meltdowns. Evolution 74: 1744-1757.

Gillespie, J.H. 1978. A general model to account for enzyme variation in natural populations. V. the SAS-CFF model. Thoer. Pop. Biol. 14: 1-45.

Gioti, A., Stajich, J.E. and Johannesson, H. 2013. *Neurospora* and the dead-end hypothesis: genomic consequences of selfing the model genus. Evolution 67: 3600-3616.

Glemin, S., and Ronfort, J. 2013. Adaptation and maladaptation in selfing and outcrossing species: new mutations versus standing variation. Evolution 67: 225-240.

Goldberg, E.E., Kohn, J.R., Lande, R., Robertson, K.A., Smith, S.A. and Igic, B. 2010. Species selection maintains self-incompatibility. Science 330: 493-495.

Golding, G.B. and Strobeck, C. 1979. Linkage disequilibrium in a finite population that is partially selfing. Genetics 94: 777-789.

Good, B.H., Walczak, A.M., Neher, R.A. and Desai, M.M. 2014. Genetic diversity in the interference selection limit. PLoS Genet. 10: e1004222.

Goodwillie, C., Kalisz, S. and Eckert, C. G. 2005. The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. Annu. Rev. Ecol. Evol. Syst. 36: 47-79.

Gordo, I. and Charlesworth B. 2000a. The degeneration of asexual haploid populations and the speed of Muller's ratchet. Genetics 154: 1379-1387.

Gordo, I. and Charlesworth B. 2000b. On the speed of Muller's ratchet. Genetics 156: 2137-2140.

Gordo, I. and Charlesworth, B. 2001. The speed of Muller's ratchet with background selection and the degeneration of Y chromosomes. Genet. Res. Camb. 78: 149-161.

Gordo, I., Navarro, A. and Charlesworth, B. 2002. Muller's ratchet and the pattern of variation at a neutral locus. Genetics 161: 835-848.

Gossman, T.I., Waxman, D. and Eyre-Walker, A. 2014. Fluctuating selection models and Mcdonald-Kreitman type analyses. PLoS One 9: e84540.

Grant, P.R. and Grant, B.R. 2002. Unpredictable evolution in a 30-year study of Darwin's finches. Science 296: 707-711.

Haigh, J. 1978. The accumulation of deleterious genes in a population-Muller's ratchet. Theor. Popul. Biol. 14: 251-267.

Haldane, J.B.S. 1927. A mathematical theory of natural and artificial selection Part V: selection and mutation. Proc. Camb. Philos. Soc. 23: 838-844.

Hartfield, M. 2016. Evolutionary genetic consequences of facultative sex and outcrossing. J. Biol. Evol. 29: 5-22.

Hedrick, P.W. 2006. Genetic polymorphism in heterogeneous environments: the age of genomics. Annu. Rev. Ecol. Evol. Syst. 37: 67-93.

Heller, R. and Maynard Smith, J. 1979. Does Muller's ratchet work with selfing? Genet. Res. Camb. 32: 289-293.

Hietpas, R.T., Bank, C., Jensen, J.D. and Bolon, D.N.A. 2013. Shifting fitness landscapes in response to altered environments. Evolution 67: 3512-3522.

Higgs, P.L. and Woodcock, G. 1995. The accumulation of mutations in asexual populations and the structure of genealogical trees in the presence of selection. J. Math. Biol. 33: 677-702.

Hill, W. G. and Robertson, A. 1966. The effects of linkage on limits to artificial selection. Genet. Res. Camb. 8: 269-294.

Hillenmeyer, M.E., Fung, E., Wildenhain, J., Pierce, S.E., Hoon, S., Lee, W., Proctor, M., St.Onge, R.P., Tyers, M., Koller, D. et al. 2008. The chemical genomic portrait of yeast: uncovering a phenotype for all genes. Science 320: 362-365.

Hudson, R.R. and Kaplan, N.L. 1995. Deleterious background selection with recombination. Genetics 141: 1605-1617.

Huerta-Sanchez, E., Durrett, R. and Bustamante, C.D. 2008. Population genetics of polymorphism and divergence under fluctuating selection. Genetics 178: 325-337.

Igic, B. and Busch, J.W. 2013. Is self-fertilization an evolutionary dead end? New Phytol. 198: 386-397.

Igic, B. and Kohn, J.R. 2006. The distribution of plant mating systems: study bias against obligately outcrossing species. Evolution 60: 1098-1103.

Jarne, P. and Auld, J.R. 2006. Animals mix it up too: the distribution of self-fertilization among hermaphroditic animals. Evolution 60: 1816-8124.

Jasnos, L., Tomala, K., Paczesniak, D. and Korona, R. 2008. Interactions between stressful environment and gene deletions alleviate the expected average loss of fitness in yeast. Genetics 178: 2105-2111.

Johnson, M.T.J., Fitzjohn, R.G., Smith, S.D., Rausher, M.D. and Otto, S.P. 2012. Loss of sexual recombination and segregation is associated with increased diversification in evening primroses. Evolution 65: 3230-3240.

Kamran-Disfani, A. and Agrawal, A.F. 2014. Selfing, adaptation and background selection in finite populations. J. Evol. Biol. 27:1360-1371.

Kassen, R. 2002. The experimental evolution of specialists, generalists, and the maintenance of diversity. J. Evol. Biol. 15: 173-190.

Kimura, M. 1962. On the probability of fixation of mutant genes in a population. Genetics 47: 713-719.

Kimura, M., and Ohta, T. 1969. Probability of fixation of a mutant gene in a finite population when selective advantage decreases with time. Genetics 65: 525-534.

Kishony, R. and Leibler, S. 2003. Environmental stress can alleviate the average deleterious effect of mutations. J. Biol. 2: 14.

Kraemer, S.A., Morgan, A.D., Ness, R.W., Keightley, P.D. and Colegrave, N. 2015. Fitness effects of new mutations in *Chlamydomonas reinhardtii* across two stress gradients. J. Evol. Biol. 29: 583-593.

Lande, R. 1993. Risks of population extinction from demographic and environmental stochasticity and random catastrophes. Am. Nat. 142: 911-927.

Lande, R. and Schemske, D.W. 1985. The evolution of self-fertilization and inbreeding depression in plants. I. genetic models. Evolution 39: 24-40.

Lasky, J.R., Marais, D.L.D., Lowry, D.B., Povolotskaya, I., McKay, J.K., Richards, J.M., Keitt, T.H. and Juenger, T.E. 2014. Natural variation in abiotic stress responsive gene expression and local adaptation to climate in *Arabidopsis thaliana*. Mol. Biol. Evol. 31: 2282-2296.

Latta IV, L.C., Peacock, M., Civitello, D.J., Dudycha, J.L., Meik, J.M. and Schaack, S. 2015. The phenotypic effects of spontaneous mutations in different environments. Am. Nat 185: 243-252.

Leimu, R. and Fischer, M. 2008. A meta-analysis of local adaptation in plants. PLoS One 3: e4010.

Lewontin, R.C. and Matsuo, Y. 1963. Interaction of genotypes determining viability in *Dosophila busckii*. Proc. Natl. Acad. Sci. 49: 270-278.

Lloyd, D.G. 1979. Some reproductive factors affecting the selection of self-fertilization in plants. Am. Nat. 113: 67-79.

Loewe, L. 2006. Quantifying the genomic decay paradox due to Muller's ratchet in human mitochondrial DNA. Genet. Res. Camb. 87: 133-159.

Loewe, L. and Lamatsch, D.K. 2008. Quantifying the threat of extinction from Muller's ratchet in the diploid Amazon molly (*Poecilia formosa*). BMC Evol. Biol. 8: 88.

Lopez-Maury, L., Marguerat, S. and Bahler, J. 2008. Tuning gene expression to changing environments: from rapid responses to evolutionary adaptation. Nat. Rev. Genet. 9: 583-593.

Lynch, M. and Gabriel, W. 1990. Mutation load and the survival of small populations. Evolution 44: 1725-1737.

Lynch, M. 1987. The consequences of fluctuating selection for isozyme polymorphisms in *Daphnia*. Genetics 115: 657-669.

Maccoll, A.D.C. 2011. The ecological causes of evolution. Trends Ecol. Evol. 26: 514-522.

Martin, L. and Lenormand, T. 2006. The fitness effect of mutations across environments: a survey in light of fitness landscape models. Evolution 60: 2413-2427.

McVean, G.A.T. and Charlesworth, B. 2000. The effects of Hill-Robertson interference between weakly selected mutations on patterns of molecular evolution and variation. Genetics 155: 929-944.

Muller, H.J. 1932. Some genetic aspects of sex. Am. Nat. 66: 118-138.

Mustonen, V. and Lassig, M. 2009. From fitness landscapes to seascapes: non-equilibrium dynamics of selection and adaptation. Trends Genet. 25: 111-119.

Ness, R. W., Siol., M., and Barrett, S. C. H. 2012. Genomic consequences of transitions from cross- to self-fertilization on the efficacy of selection in three independently derived selfing plants. BMC Genomics 13: 611.

Nordborg, M. 1997. Structured coalescent processes on different time scales. Genetics 146: 1501-1514.

Nordborg, M. 2000. Linkage disequilibrium, gene trees and selfing: an ancestral recombination graph with partial self-fertilization. Genetics 154: 923-929.

Nunney, L. 1989. The maintenance of sex by group selection. Evolution 43: 245-257.

Orr, A.H. The rate of adaptation in asexual. Genetics 155: 961-968.

Ovaskainen, O. and Meerson, B. 2010. Stochastic models of population extinction. Trends Ecol. Evol. 25: 643-652.

Peischl, S. and Kirkpatrick, M. 2012. Establishment of new mutations in changing environments. Genetics 191: 895-906.

Pollak, E. 1987. On the theory of partially inbreeding finite populations. I. partial selfing. Genetics 117: 353-360.

Qui, S., Zeng, K. Slotte, T., Wright, S. I. and Charlesworth, D. 2011. Reduced efficacy of natural selection on codon usage bias in selfing *Arabidopsis* and *Capsella* species. Genome Biol. Evol. 3: 868–880.

Remold, S.K. and Lenski, R.E. 2001. Contribution of individual random mutations to genotype-by-environmental interactions in *Escherichia coli*. P. Nat. Acad. Sci. USA 98: 11388-11393.

Roles, A.J., Rutter, M.T., Dworkin, I., Fenster, C.B. and Conner, J.K. 2016. Field measurements of genotype by environment interaction for fitness caused by spontaneous mutations in *Arabidopsis thaliana*. Evolution 70: 1039-1050.

Rutter, M.T., Wieckowski, Y.M., Murren, J. and Strand, A.E. 2017. Fitness effects of mutations: testing genetic redundancy in *Arabidopsis thaliana*. J. Evol. Biol. 30: 1124-1135.

Siepielski, A.M. Morrissey, M.B., Buoro, M., Carlson, S.M., Caruso, C.M., Clegg, S.M., Coulson, T., Dibattista, J., Gotanda, K.M., Francus, C.D. et al. 2017. Precipitation drives global variation in natural selection. Science 355: 959-962.

Siepielski, A.M., DiBattisa, J.D., Carlson, S.M. 2009. It's about time: the temporal dynamics of phenotypic selection in the wild. Ecol. Lett. 12: 1261-1276.

Slotte, T., Foxe, J. P., Hazzouri, K. M. and Wright, S. I. 2010. Genome-wide evidence for efficient positive and purifying selectionin *Capsella grandiflora*, a plant species with a large effective population size. Mol. Biol. Evol. 27: 1813-1821.

Soderberg, R.J. and Berg. O.G. 2007. Mutational interference and the progression of Muller's ratchet when mutations have a broad range of deleterious effects. Genetics 177: 971-986.

Sonna, L.A., Fijuta, J., Gaffin, S.L. and Lilly, C.M. 2002. Effects of hear and cold stress on mammalian gene expression. J. Appl. Phytol. 92: 1725-1742.

Stebbins, G. L. 1957. Self fertilization and population variability in the higher plants. Am. Nat. 91: 337-354.

Steele, J.H. 1985. A comparison of terrestrial and marine ecological systems. Nature 313: 335-358.

Steele, J.M. 1996. Ecology, evolution and the 1/f-noise. Trends Ecol. Evol. 11: 33-37.

Stephan W., Chao, L. and Smale, J.G. 1993. The advance of Muller's ratchet in a haploid asexual population: approximate solutions based on diffusion theory. Genet. Res. Camb. 61: 225-231.

Takahata, N., Ishii, K. and Matsuda, H. 1975. Effect of temporal fluctuation of selection coefficient on gene frequency in a population. P. Nat. Acad. Sci. USA 72: 4541-4545.

Tufto, J. 2015. Genetic evolution, plasticity, and bet-hedging as adaptive responses to temporally autocorrelated fluctuations in selection: a quantitative genetic model. Evolution 69: 2034-2049.

Uecker, H., and Hermisson, J. 2011. On the fixation process of a beneficial mutation in a variable environment. Genetics 188: 915-930.

van Valen, L. 1975. Group selection, sex and fossils. Evolution 29: 87-94.

Vasseur, D.A. and Yodvis, P. 2004. The color of environmental noise. Ecology 85: 1146-1152.

Wang, A.D., Sharp, N.P. and Agrawal, A.F. 2014. Sensitivity of the distribution of mutational fitness effects to environment, genetic background, and adaptedness: a case study with *Drosophila*. Evolution 68: 840-853.

Wang, A.D., Sharp, N.P., Spencer, C.C., Tedman-Aucoin, K. and Agrawal, A.F. 2009. Selection, epistasis, and parent-of-origin effects on deleterious mutations across environments in *Drosophila melanogaster*. Am. Nat. 174: 863-874.

Wardlaw, A.M., and Agrawal, A.F. 2012. Temporal variation in selection accelerates mutational decay by Muller's ratchet. Genetics 191: 907-916.

Waxman, D. 2011. A unified treatment of the probability of fixation when population size and the strength of selection change over time. Genetics 188: 907-913.

Wright, S.I., Kalisz, S. and Slotte, T. 2011. Evolutionary consequences of self-fertilization in plants. P. R. Soc. B. 280: 1-10.

Wright, S.I., Lauga, B. and Charlesworth, D. 2002. Rates and patterns of molecular evolution in inbred and outbred *Arabidopsis*. Mol. Biol. Evol. 19: 1407-1420.

Young, J.A., Yourth, C. and Agrawal, A.F. 2009. The effects of pathogen on selection against deleterious mutations in *Drosophila melanogaster*. J. Evol. Biol. 22: 2125-2129.

Zhang, X. 2012. Fisher's geometrical model of fitness landscape and variance in fitness within a changing environment. Evolution 66: 2350-2368.