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

94 different time scales under various degrees of temporal autocorrelation (Steele 1985, Halley
95 1996, Vasseur and Yodviz 2004, Furguson et al. 2016) and evolutionary processes can be
96 strongly dependent on both the temporal autocorrelation in selection and the average strength of
97 selection (Charlesworth 1993, Pieschl and Kirkpatrick 2012, Wardlaw and Agrawal 2012, Zhang
98 2012, Chevin et al. 2015, Cvijovic et al. 2015, Tufto 2015). In general, if changes in selection
99 occur rarely (very high temporal autocorrelation), then the probability of fixation for the
100 mutation is largely dependent on the selective environment in which it arose and can be
101 estimated from models assuming constant selection. If fluctuations are very frequent (very low
102 temporal autocorrelation), fixation probabilities can also be approximated by constant selection
103 models but using a time-averaged strength in selection (Mustonen and Lassig 2009, Peischl and
104 Kirkpatrick 2012, Cvijovic et al. 2015). However, in the intermediate range where selection and
105 environmental change operate on similar time scales, classic equations become inaccurate.
106 Within this range, Cvijovic et al. (2015) have shown in single locus models that selection
107 fluctuating in direction increases the probability of fixation for all mutations.
108 We examine selection that is always negative (or zero) but fluctuates in intensity (but not
109 direction) and in a system with strong linked selection. Results from single locus theory do not
110 extend simply to highly selfing populations where loci in the genome are tightly linked (Higgs
111 and Woodcock 1995, Charlesworth and Charlesworth 1993). Interference between deleterious
112 mutations leads to mutation accumulation in asexual and highly selfing populations (Muller
113 1932, Haigh 1978, Heller and Maynard Smith 1979, Charlesworth and Charlesworth 1993, 1997,
114 Gordo and Charlesworth 2000a,b; Kamran-Disfani and Agrawal 2014). However, little is known
115 about selective interference when some loci experience fluctuations in selection intensity. In a
116 related study, Wardlaw and Agrawal (2012) found that temporal autocorrelation in selection
117 intensity is an important determinant of Muller's ratchet in asexuals because the ratchet can turn
118 quickly during periods of relaxed selection. The current work extends their findings to selfing
119 populations with some recombination, explores more realistic environmental scenarios,
120 separately quantifies fixation rates for loci experiencing fluctuating versus constant selection
121 within the same genome, examines the extent to which the linked selection effects from
122 fluctuations can be accounted for through N_e, and considers selective interference involving
123 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

The simulation program was written in C++ and can be downloaded from https://github.com/EddieKHHo/SelfingMutAccum. 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

155 each experienced selection in response to separate ecological variables. For example, the first set
156 could represent loci that were only selected under dry conditions and the second set represents
157 those loci that were selected only when there was high herbivory. The two environmental
158 conditions (e.g., "dry" and "high herbivory") could occur independently or be positively or
159 negatively correlated. In the last extension on the model, we allowed for beneficial mutations to
160 occur at C-loci.
161
162
163 **Base model**
164 *Life cycle*
165 Each diploid individual carried 5000 fitness-affecting loci and three neutral quantitative loci on a
166 linear genome; neutral loci were used to estimate effective population size (discussed below). A
167 proportion, p_f, of fitness affecting loci were assigned to experience fluctuating selection and the
168 remaining proportion, 1-p_f, were assigned to experience constant selection; location assignment
169 of locus types are randomized for every simulation. Loci under constant selection (C-loci)
170 experienced a selection coefficient of s_c in all generations. Loci under fluctuating selection (F-
171 loci) experienced a strength of selection that changed over time $s_f(t)$, taking the value 0 or s_{max}.
172 The parameter f controlled the temporal autocorrelation in selection between consecutive
173 generations. With probability f selection automatically remained the same as the previous
174 generation. With probability, 1-f, $s_f(t)$ was randomly chosen to be 0 or s_{max} with probability (1- ϕ)
175 and ϕ, respectively; ϕ is the expected proportion of generations that $s_f(t) = s_{max}$. The correlation
176 in selection between time points separated by t generations is f^t. The expected run length that
177 $s_f(t)$ equals 0 and s_{max} is 1/ ((1-ϕ) (1-f)) and 1/ (ϕ (1-f)), respectively. The geometric average
178 strength of selection experienced by loci under fluctuating selection is $s_{avg} = 1-(1-s_{max})^{\phi}$. For all
179 simulations we chose s_{max} such that $s_{avg} = s_c$, so that loci under fluctuating selection experienced
180 the same geometric average selection strength as loci under constant selection (i.e., $s_c = s_{avg}$) to
181 provide the fairest comparison between these types of loci.
182
183 Populations started with N individuals where each individual received a number of
184 mutations sampled from a Poisson distribution with mean $U/(h\ s_{avg})$ where U is the genome-wide
185 per generation mutation rate and h = 0.25 is the dominance coefficient. Every generation, each of

186 N offspring was produced as follows. The fitness of parents at time t was calculated assuming
187 loci act multiplicatively as

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

188 where x_c and y_c represents the number of loci under constant selection that are homozygous and
189 heterozygous mutants and x_f and y_f represents the number of loci under fluctuating selection that
190 are homozygous and heterozygous mutants. Offspring were produced through sporophytic
191 selfing a proportion S of the time and through outcrossing a proportion 1-S of the time, on
192 average. Selfing rates were fixed and we focus on fully selfing populations ($S = 1$) and partially
193 selfing populations with a high rate of selfing ($S = 0.98$); see Table S1 for simulations with lower
194 selfing rates. A potential parent, i, was chosen randomly and their fitness relative to the
195 maximum in the population (W_i / W_{max}) was compared to a uniform random deviate (Uniform [0,
196 1]), where W_{max} is largest value of W_i in the population. The parent was kept if their relative
197 fitness surpasses the uniform random deviate, otherwise, the process was repeated until a suitable
198 parent is chosen. The offspring was produced via selfing if a uniform random deviate (Uniform
199 [0,1]) was smaller than the selfing rate, S. Otherwise, a second parent was chosen under the same
200 procedure as the first and the offspring is produced via outcrossing. Gametes were created
201 following recombination between homologous chromosomes of a parent with c crossover events
202 distributed randomly over the genome. c is a Poisson distributed random variable with a mean of
203 $M = 1$, where M represents the genomic map length. After combining two gametes from either
204 one or two parents, x mutations were randomly added to the genome of the offspring, where x is
205 a number sampled from a Poisson distribution with mean U. The locus to undergo mutation was
206 chosen randomly and the mutation event was ignored if the chosen locus was already a mutant.
207 Fixation occurs when all $2N$ alleles at a locus were mutant. When this occurs, we reset all alleles
208 back to wild type.
209
210 After a burn-in period of at least 20 000 generations, data was collected over at least 40
211 000 more generations; the burn-in and data collection period was much longer under low
212 mutations rates ($U < 1$). We calculated the per locus fixation rates for loci under constant and
213 fluctuating selection separately as the number of deleterious mutations that fixed at each type of
214 loci divided by the number of generations over which the data was collected and divided by the
215 number of loci for each type. Throughout, we compare per locus fixation rates in populations

216 that experienced fluctuating selection ($p_f > 0$) to the per locus fixation rates in populations that
217 did not experience fluctuating selection ($p_f = 0$, i.e., the "classic" model typical of most past
218 mutation accumulation models). More precisely, we calculated the *relative fixation rate* as the
219 per locus fixation rate in populations with $p_f > 0$ divided by the per locus fixation rate in
220 populations with $p_f = 0$ (holding all other parameters equal). Thus, the relative fixation rate for
221 loci under constant selection quantified the extent to which fixation rates were affected by
222 residing in genomes that experience fluctuating selection rather than residing in genomes that
223 only experience constant selection. The relative fixation rate for loci under fluctuating selection
224 quantified the direct effect of fluctuating selection as well as effect of residing in a genome
225 experiencing fluctuating selection. Each parameter set was replicated at least 20 times. For a
226 given parameter combination, relative fixation rates were calculated using the average rate over
227 all replicates divided by the average rate from the replicates of the corresponding classic model.
228
229 *Effective population size*
230 We estimated effective population size, N_e, using three linked quantitative neutral loci located at
231 one-quarter, one-half and three-quarters along the genome (following Keightley and Otto 2006,
232 Agrawal and Hartfield 2016). At reproduction, the value of each locus on each gamete was
233 perturbed by adding a random number drawn from a normal distribution with mean 0 and
234 variance 1. At steady state, the population wide variance at each locus gives an estimate of 2 N_e
235 (Lynch and Hill 1986). For each simulation, N_e was measured every 100 generations over at least
236 40 000 generations following a burn-in period of 20 000 generations; the burn-in and data
237 collection period was much longer under low mutations rates ($U < 1$). This burn-in period was
238 likely greater than 20 N_e generations given that estimates of N_e from all our simulations fell far
239 below 1000. We reported the genome-wide N_e averaged among all time points and among the
240 three neutral loci; N_e estimates are similar across all three loci. We confirmed that our estimates
241 of N_e matched theoretical expectations in fully selfing populations under background selection
242 when mutations fixed at negligible rates (Gordo et al. 2002). We also confirmed that N_e
243 measured 10 000, 20 000 or 30 000 generations after the burn-in period matched our reported
244 value (taken over 40 000 generations after the burn-in period).
245
246 **Simulations with exponentially distributed selection**

247 We extended the base model by allowing selection strength to vary over time following an
248 exponential distribution (as opposed to a bimodal distribution in the base model). Each
249 generation, consecutive environments remain the same with probability f. With probability, 1-f,
250 the selection strength is chosen from an exponential distribution with mean $1/s_{avg}$. Similar to the
251 base model, loci under constant and fluctuating selection experienced the same geometric
252 averaged selection strength. In particular, we focused on simulations with $s_{avg} = 0.025$ where the
253 geometric mean and the variance in selection strength under the exponential model was almost
254 identical to that of the conditionally neutral mutations with $s_{avg} = 0.025$ and $\phi = 0.5$ in the base
255 model.
256
257 **Simulations with two fluctuating ecological variables**
258 An unrealistic assumption of the base model is that all F-loci experience fluctuating selection in
259 exactly the same way. As the simplest way to relax this assumption, we extended the base model
260 by allowing for two equally sized sets (F1, F2) of loci under fluctuating selection and each set
261 responds to a different ecological variable. Each individual possess 5000 fitness affecting loci. A
262 proportion p_{f1} and p_{f2} of fitness affecting loci are assigned to experiencing fluctuating selection in
263 response to separate environmental factors and the remaining proportion, 1-p_{f1}-p_{f2}, are assigned
264 to experience constant selection; location assignment of loci is randomized every simulation. We
265 assume equally sized sets of fluctuating loci, $p_{f1} = p_{f2}$ (and $p_{f1} + p_{f2} = p_f$).
266 For simulations where mutations at loci under fluctuating selection are conditionally
267 neutral (as in the base model), we assume both groups experience fluctuations in selection of the
268 same magnitude (i.e., between 0 and s_{max}) and experience the same time average selection s_{avg}.
269 Each generation, consecutive environments remained the same with probability f. With
270 probability, 1-f, the environment was randomly selected among four possible states: $\{s_{f1}(t) = s_{max},$
271 $s_{f2}(t) = s_{max}\}$, $\{s_{f1}(t) = s_{max}, s_{f2}(t) = 0\}$, $\{s_{f1}(t) = 0, s_{f2}(t) = s_{max}\}$, $\{s_{f1}(t) = 0, s_{f2}(t) = 0\}$ with
272 probabilities $\phi^2 + d$, $\phi\,(1-\phi) - d$, $(1-\phi)\,\phi - d$, $(1-\phi)\,(1-\phi) + d$, respectively. Loci within F1 and F2
273 were expected to be under selection with strength s_{max} a proportion ϕ of the time, but are
274 otherwise neutral. The value of d must satisfy $d_{min} = \max\{-\phi^2, -(1-\phi)^2\}$ when $d < 0$ and $d_{max} =$
275 $(1-\phi)\,\phi$ when $d > 0$. The correlation in selective states between set F1 and F2, ρ, was determined
276 by the choice of d. We explore five different correlations in selective states between loci within
277 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 (ρ = 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 kth 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

309 the deleterious fixation rates of C- and F-loci in simulations *with* beneficial mutations ($U_b > 0$),
310 holding all parameters the same except for U_b. *Method 1*: We calculated the per locus deleterious
311 fixation rate in simulations with $U_b > 0$ divided by the per locus deleterious fixation rate in
312 populations with $U_b = 0$ for C-loci and F-loci separately (holding all other parameters equal;
313 Table S2). *Method 2*: We examined the number of additional deleterious fixations at C-loci and
314 F-loci per beneficial fixation. To do this we calculated the number of deleterious fixations at C-
315 loci (F-loci) for simulations with $U_b > 0$ minus the average number of deleterious fixations at C-
316 loci (F-loci) for simulations with $U_b = 0$ and divided this difference by the number of beneficial
317 fixations for the simulations with $U_b > 0$ (holding all other parameters equal; Table S3). Each
318 parameter set was replicated at least 20 times.
319
320 **Results**
321 In their model of obligately asexual populations, Wardlaw and Agrawal (2012) previously
322 showed that some degree of temporal autocorrelation in selection, $f > 0$, is required for
323 fluctuating selection to significantly elevate rates of mutation accumulation. We confirmed this
324 finding for partially selfing ($S = 0.98$) and obligately selfing ($S = 1$) populations (Figure S1, S2,
325 S10). Rather than further exploring the effects of f on fixation rates, we simply assume that
326 selection undergoes moderate degrees of temporal autocorrelation ($f = 0.95$, $\phi = 0.5$) such that
327 the correlation in selection between time points separated by 1, 10, and 100 generations is 0.95,
328 0.60, and 0.006, and the average run length between switches in the environment is ~40
329 generations. This regime elevates fixation rates at loci under fluctuating loci, but is moderate in
330 its effects; fixation rates can be much higher with higher values of f and lower values of ϕ
331 (Figure S1, S2, S10). We focus on exploring how loci under fluctuating selection in the genome
332 alter fixation rates at linked sites and genome wide. We also extend our model to test the
333 robustness of our results to different forms of fluctuating selection.
334
335 *Varying the composition of the genome, p_f*
336 We examine fixation rates in genomes where a fraction p_f of loci are under fluctuating selection
337 (F-loci) and the remaining loci are under constant selection (C-loci). Unless otherwise noted, we
338 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
339 partially and obligately selfing populations, per locus fixation rates within genomes experiencing

340 fluctuating selection ($p_f > 0$) are typically higher than fixation rates in the classic model ($p_f = 0$);
341 this is true for both C- and F-loci, but the effect is stronger for the latter (Figure 1, S2) The fact
342 that per locus fixation rates vary with p_f indicates that changing the density of F-loci in the
343 genome alters the effects of linked selection on both F- and C-loci. When less than half the
344 genome consists of F-loci ($p_f < 0.5$), increasing p_f generally elevates fixation rates at both C- and
345 F-loci and, thereby, the genome-wide average fixation rate. Curiously, at high mutation rates (U
346 $= 1$) and with partial selfing ($S = 0.98$), further increasing p_f reduces fixation rates for genomes
347 that contain more than 50% F-loci (Figure 1a). As it seems unlikely that a high fraction of loci
348 experience strong fluctuations in selection, the results for $p_f < 0.5$ are the most biologically
349 relevant. Therefore, in the relevant range, our results indicate that genomes experiencing more
350 loci under fluctuating selection fix deleterious mutations at faster rates.
351 When mutation rates are lower (so absolute fixation rates are lower), the effect of
352 fluctuating selection on the *relative* fixation rate is more dramatic for both C- and F-loci. For the
353 parameter sets examined in Fig. 1 for partial selfers, the inclusion of F-loci maximally increases
354 genome-wide fixation rate by a factor of 1.38 vs. 2.42 at the high and low mutation rate,
355 respectively (Fig. 1a, c). For obligate selfers, the maximal increase is 1.5 vs. 4.91 at the high and
356 low mutation rate, respectively (Fig 1b, d). This effect of mutation rate holds in simulations
357 using other values of autocorrelation f and frequency of selection ϕ (Figure S2). Although the
358 relative impact of fluctuating selection is larger at lower mutation rates, in the remaining sections
359 we focus on results from high mutation rates ($U = 1$) because simulations at lower mutation rates
360 require much longer runs to obtain accurate estimates of fixation rates.
361
362
363

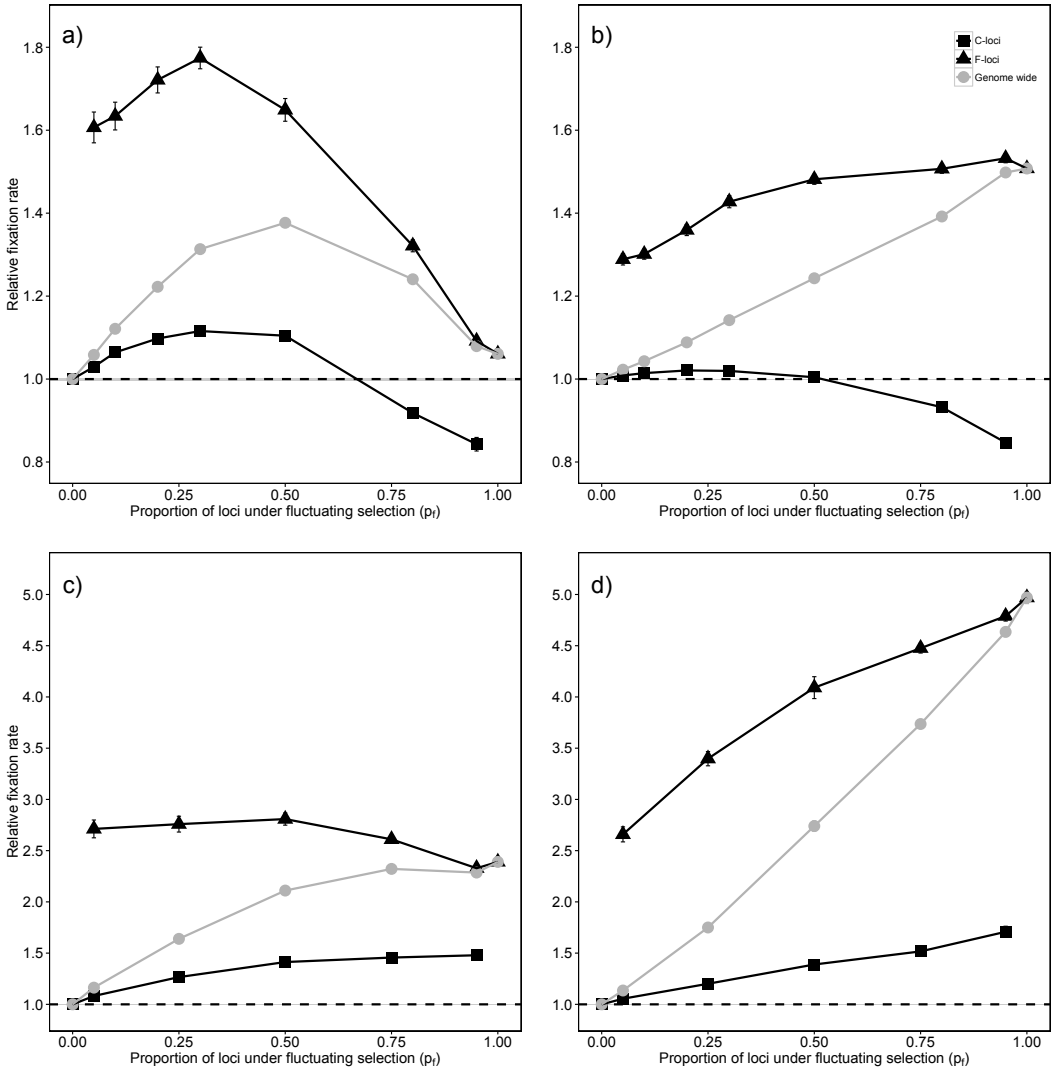

Figure 1. Relative fixation rates (mean ± SE for C- and F-loci in populations with varying genomic composition, p_f, under moderate temporal autocorrelation in selection (f = 0.95, ϕ = 0.5) with h = 0.25, s_{avg} = 0.025. Fixation rates under high mutation rates in (a) partially selfing populations (U = 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).

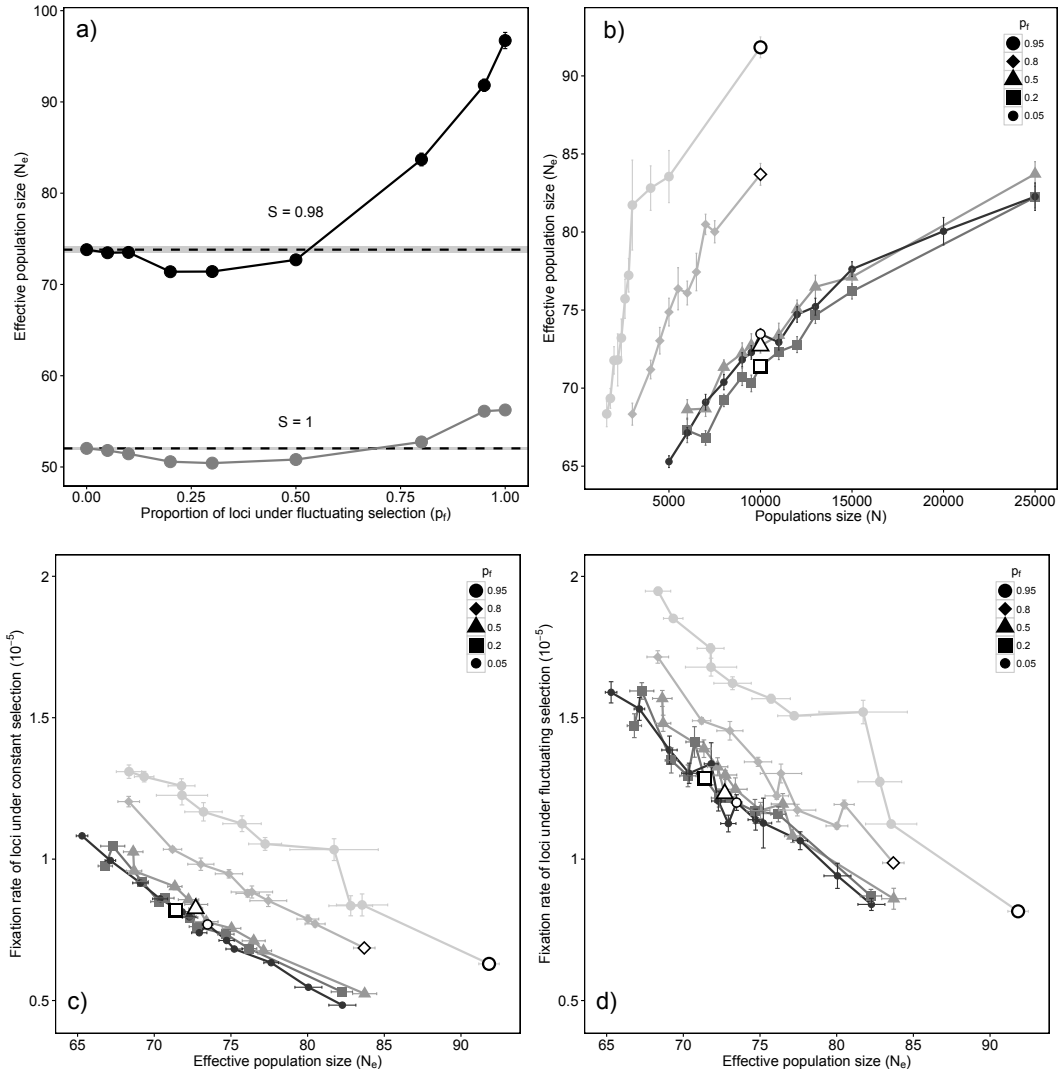

Figure 2. (a) Estimates of effective population size (mean ± SE) for genomes containing different densities of F-loci, p_f, in a population of $N = 10^4$. Shown are N_e values for partially selfing populations ($U = 1$, $S = 0.98$; black points) and obligately selfing populations ($U = 1$, $S = 1$, grey 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.

426 *Fixation bias at F-loci*

427 The fixation rate of deleterious mutations at F-loci is always greater than that for C-loci within
428 all mixed genomes (Figures 1, 2c, d). This bias is clearly stronger when selection fluctuates in
429 ways that directly increase fixation rates at F-loci (i.e., larger f and smaller ϕ; Figure S2).
430 Moreover, this bias is affected by the 'traditional' parameters affecting mutation accumulation
431 such as the strength of selection (s_{avg}), the mutation rate (U) and the population size (N). To
432 examine how this bias depends on aspects of selection, we performed simulations where half the
433 genome consist of F-loci (p_f = 0.5) at high mutations rates (U = 1) with an average selection
434 strength of $s_c = s_{avg}$ = {0.005, 0.025, 0.05}. The ratio of the fixation rate at F-loci to the fixation
435 rate at C-loci (within the same population) is consistently larger than unity for both partially and
436 obligately selfing populations (Figure 3a), indicating that mutations fix disproportionately
437 frequently at loci under fluctuating selection. This bias is stronger when selection is stronger on
438 average or when it is concentrated into shorter but more intense events (i.e., smaller values of ϕ;
439 Figure 3a). This bias in fixations at F-loci increases with the average selection strength as
440 stronger selection seems to reduced fixation rates at C-loci relatively more than at F-loci (Figure
441 3b).

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

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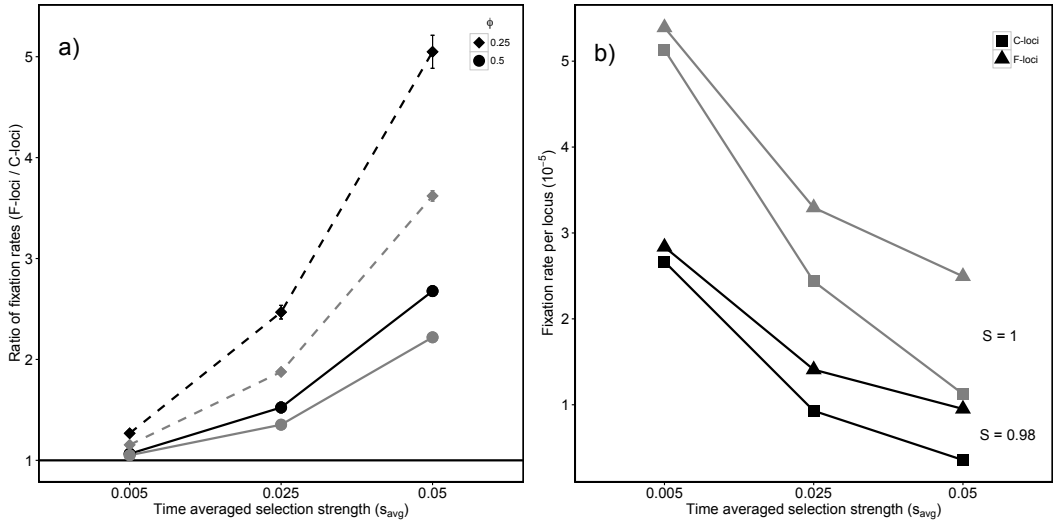

Figure 3. (a) Ratio of fixation rates at F-loci to fixation rates at C-loci (within the same population) for partially selfing (black; S = 0.98) and obligately selfing (grey; S = 1) populations when half of the genome consists of F-loci (p_f = 0.5); error bars are SE (often too small to be visible). When ϕ = 0.5, selection is non-neutral (s_{max}) half the time but when ϕ = 0.25, selection is non-neutral only a quarter of the time. To maintain s_{avg} = {0.005, 0.025, 0.05} when ϕ 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 ϕ = 0.5. Other parameters: $N = 10^4$, U = 1, h = 0.25, f = 0.95, $s_c = s_{avg}$.

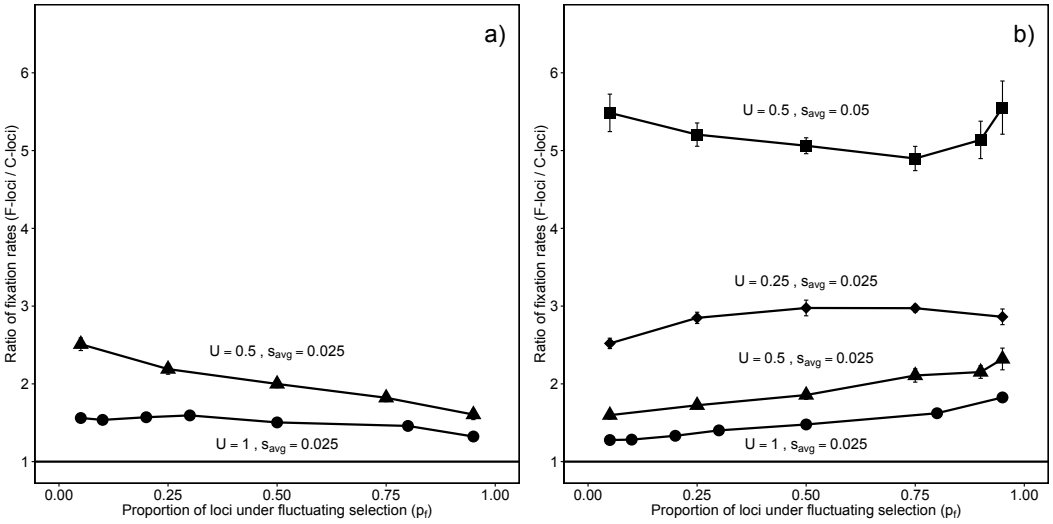

Figure 4. Ratio of fixation rates (mean ± SE) at F-loci to fixation rates at C-loci (within the same population) for populations with different proportions of F-loci, p_f for (a) partially selfing populations (S = 0.98) and (b) obligately selfing populations (S = 1). Changes to parameters that reduce 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, ϕ = 0.5.

473 *Exponentially distributed selection over time*

474 Above we assumed that mutations at F-loci are conditionally neutral, that is, selection fluctuates
475 bimodally over time between 0 and s_{max}. To examine whether our results are qualitatively robust
476 to different temporal distributions of fluctuating selection, we now consider simulations where
477 the selection strength for F-loci is randomly sampled from an exponential distribution when the
478 environment changes. The exponential distribution we use has a very similar mean and standard
479 deviation in selection strength compared to our base model of conditional neutrality model ($s_{avg} \cong$
480 0.025, standard deviation in $s_f(t) = 0.025$). Using $f = 0.95$, the correlation in selection between
481 time points separated by 1, 10, and 100 generations is 0.95, 0.60, and ~0, respectively. The
482 fixation rates for both C- and F-loci are slightly lower under the exponential model than the
483 bimodal model (Figure 5). Mutations fix disproportionately at F-loci under the exponential
484 selection model, but this bias is not quite as strong as observed in the bimodal selection model.
485 Both models are also similarly affected by changes in the mutation rate; we observe that
486 reducing mutations rates (from $U = 1$ to $U = 0.5$) increases relative fixation rates and the
487 proportion of mutations that fix at F-loci (Figure S8, S9). Though the bimodal and exponential
488 selection model represents qualitatively different forms of temporal heterogeneity in selection,
489 the effects of these models on fixation rates are reasonably similar. We speculate that any form
490 of fluctuations in selection intensity are likely to elevate fixation rates and fitness decline in
491 highly selfing populations as long as the variance in selection over time is sufficiently large and
492 there is some degree of temporal autocorrelation.
493
494

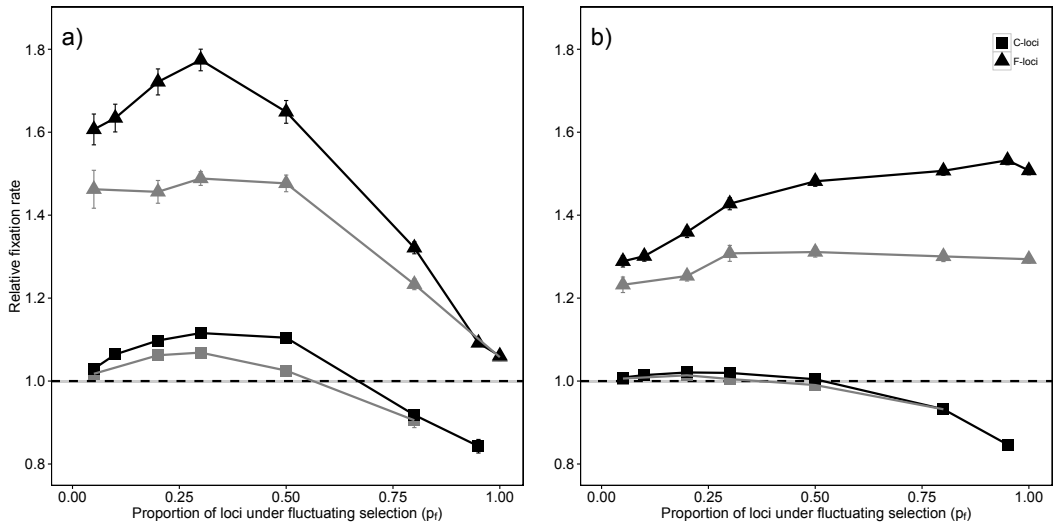

Figure 5. Relative fixation rates (mean ± SE) at C- and F-loci in populations with different genomic compositions, p_f, when selection strength is sampled from a bimodal (black) or exponential distribution (grey) over time in (a) partially selfing populations (S = 0.98) and (b) obligately selfing populations (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.

508 *Two components of fluctuating selection*

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

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

528

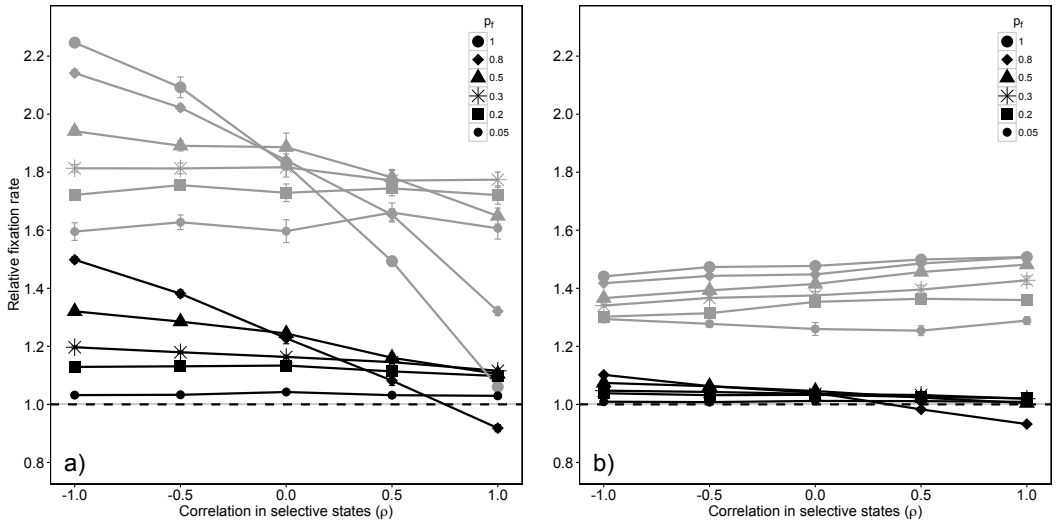

Figure 6. Relative fixation rates (mean ± SE) for C-loci (black) and F-loci (grey) in populations with different genomic compositions, p_f, for (a) partially selfing populations (S = 0.98) and (b) obligately selfing populations (S = 1). Loci under fluctuating selection are equally distributed between two sets of F-loci (F1, F2). The correlation in selective states between F1 and F2, ρ, 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, ϕ = 0.5, s_{avg} = 0.025, N = 10^4, U = 1, h = 0.25.

543 *Beneficial mutations*

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

550 The fixation probability of beneficial mutations was lower in simulations with F-loci ($p_f =$
551 0.2 than those without F-loci ($p_f = 0$) (Table S2) indicating that deleterious mutations at F-loci
552 cause more interference with beneficials than those at C-loci cause. Conversely, beneficials
553 increase the deleterious fixation rates at both C-loci and F-loci. We took two perspectives on this
554 change. The proportional increase in the deleterious fixation rate is similar for C- and F-loci
555 (Table S2). However, because the F-locus rate without beneficials is larger than the C-locus rate,
556 the similarity in "proportional increase" obscures the fact that the absolute increase in the
557 deleterious fixation rate caused by beneficials is larger for F-loci than C-loci. This is clearer from
558 our second perspective in which we calculate the average number of additional fixed deleterious
559 mutations per fixed beneficial (Table 1). In the simulations reported, each beneficial that fixes is
560 responsible for, on average, less than one additional deleterious fixation but these are not evenly
561 distributed between F- and C-loci. F-loci are only 25% as abundant as C-loci in these simulations
562 (i.e., $p_f = 0.2$), but the number of additional fixed deleterious F-alleles per fixed beneficial is
563 more than 25% the number of additional fixed deleterious C-alleles. From this we infer that F-
564 loci experience stronger interference from beneficials than do C-loci. Because we have explored
565 only a small set of parameters for simulations with beneficials, the conclusions above should be
566 regarded with greater caution.

567

568

569 **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)

570 Standard error for each statistic indicated within parentheses. '*# Gen.*' is the number of generations
571 examined for calculating the data. U, U_b are the genomic wide mutation rates of deleterious and beneficial
572 mutations, respectively. s_b is the fitness advantage of a beneficial mutation. '*Ben. prob. fix.*' is the fraction
573 of beneficial mutations that fixed per generation in the simulation assuming that the number of beneficial
574 mutations introduced per generation was $U_b * N$. '*Extra fix del per fix ben'* is the average number of extra
575 deleterious alleles fixed per beneficial fixed beyond what would have otherwise fixed. Other parameters:
576 $N = 10000$, $h = 0.25$, $s_{avg} = 0.025$, $h_b = 0.5$, $f = 0.95$, $\phi = 0.5$, $\rho = 1$. See Table S2 for further information
577 on these simulations.
578
579
580
581

582 **Discussion**

583 Highly selfing populations experience lower effective population sizes, N_e, and suffer from a
584 reduced efficacy of selection (reviewed in Hartfield 2014). As a consequence, highly selfing
585 populations can accumulate and permanently fix deleterious mutations at high rates leading to
586 rapid fitness decline and possibly to extinction (Heller and Maynard Smith 1979, Charlesworth
587 and Charlesworth 1993). It is generally accepted that environmental changes occur on many
588 different times scales (Steele 1985, Halley 1996, Vasseur and Yodviz 2004, Furguson et al.
589 2016) and can induce fluctuations in the intensity and direction of selection (Siepielski et al.
590 2009, 2017, Bell 2010) but the consequences for mutation accumulation in selfers is unstudied.

591 We found that loci experiencing selection that fluctuates in intensity, F-loci, fix deleterious
592 mutations at a higher rate than loci under constant selection, C-loci, experiencing the same time
593 averaged selection strength ($s_c = s_{avg}$). The extent to which F-loci contribute disproportionately to
594 total mutation accumulation depends on aspects of environmental heterogeneity. The bias is
595 stronger with increasing temporal autocorrelation in selection, f, and when selection is
596 concentrated into rarer but more intense episodes (low values of ϕ), holding s_{avg} constant
597 (Figures S1, S2, S10). This bias also becomes stronger with changes to "traditional" parameters
598 that lower the overall rate of mutation accumulation, i.e., higher values of s_{avg} or N as well as
599 lower values of U (Figures 3, 4, S5, S9). In sum, fluctuating selection can greatly increase the
600 rate of mutation accumulation in highly selfing populations and the loci under fluctuating
601 selection contribute disproportionately to mutational decline. These conclusions appear
602 qualitatively robust to the exact nature of fluctuations (bimodal vs. exponential distribution of
603 selection coefficients and positive, negative, or no temporal correlation in selection among loci
604 Figure 5, 6, S7, S8).
605
606 In our model, deleterious mutations fix as a consequence of selective interference
607 between tightly linked sites. Supporting this, simulations at higher rates of outcrossing ($S \leq 0.9$)
608 fix few to no mutations (Table S1). In contrast, obligately asexual organisms (Wardlaw and
609 Agrawal 2012) and pure selfers ($S = 1$) have high rates of mutation accumulation that are
610 strongly increased by fluctuating selection. These effects remain important even when low levels
611 of genetic mixing occur as with low levels of outcrossing ($S = 0.98$). It is well known that
612 interference between constantly selected loci can lead to the accumulation of deleterious
613 mutations in asexual and highly selfing populations, a process classically called Muller's ratchet
614 (Haigh 1978, Heller and Maynard Smith 1979, Charlesworth and Charleswroth 1997). However,
615 we do not have a robust understanding of selective interference between loci under fluctuating
616 selection and loci under constant selection. If F-loci are conditionally neutral (as in the base
617 model), mutations accumulate unimpeded by selection during periods of neutrality ($s_f(t) = 0$)
618 with an expected run length of $1/(\phi(1-f))$. Subsequently, when F-loci are under selection ($s_f(t) =$
619 s_{max}), selective interference likely prevents effective removal of deleterious mutations at
620 individual loci, increasing the probability of fixation.
621

622 In genomes containing a mix of F- and C-loci, F-loci have inherently high rates of
623 fixation but also tend to increase fixation rates at C-loci and other F-loci as illustrated in Figure 1
624 where increasing the fraction of loci under fluctuating selection increases the per-locus fixation
625 rates at both C- and F-loci (for $0 < p_f < 0.5$). This indicates that F-loci cause more selective
626 interference (with both C- and F-loci) than C-loci. Similarly, we found that deleterious mutations
627 at F-loci reduce fixation rates of beneficials more than deleterious mutations at C-loci do (Table
628 S2, S3). Previous work in asexual populations where selection is constant over time but varies
629 among loci shows that interference from strongly deleterious alleles increase fixation of more
630 weakly deleterious alleles (Gordo and Charlesworth 2001, Gordo et al. 2002, Soderberg and
631 Berg 2007). From this we can infer that strong interference caused by F-loci occurs during
632 periods when selection is strong, $s_f(t) = s_{max} > s_c$. However, during periods where $s_f(t) = 0$, F-loci
633 do not cause interference. In fact, compared to the classic model, interference experienced by C-
634 loci should be reduced relative to the classic model ($p_f = 0$) because C-loci are separated
635 physically by (temporarily) neutral F-loci when $s_f(t) = 0$. Given the observed net increase in
636 fixation rate at C-loci, we can infer that the increased interference when selection at F-loci is
637 strong ($s_f(t) = s_{max}$) outweighs the reduced interference when selection is weak ($s_f(t) = 0$).
638 Reciprocally, we observed that strongly selected beneficials under constant selection interfered
639 more strongly with deleterious alleles at F-loci than at C-loci (Table 1). From the perspective of
640 an F-locus, the sweeping beneficial causes very strong interference on an F-locus when the
641 selection at the F-locus is weak and weaker interference when direct selection on the F-locus is
642 strong. We can infer that the episodes of strong interference must outweigh periods of weak
643 interference because the total interference from beneficials experienced by F-loci is greater than
644 that experienced by C-loci.
645
646 In some, but not all cases, the effect of selective interference on fixation rates can be
647 interpreted as resulting from a reduction in the effective population size (Hill and Robertson
648 1966, McVean and Charlesworth 2000, Gordo and Charlesworth 2002, Good et al. 2014). We
649 observed that per-locus fixation rates varied as a function of p_f for both C- and F-loci (Fig. 1). In
650 the range where p_f was not too large ($0 < p_f \leq 0.5$), fixation rates for each locus type (F and C)
651 were nearly equal for different values of p_f when we adjusted N to obtain similar N_e values (Fig.
652 2). Because neutral diversity (used to infer N_e) and fixation rates are similarly affected by

653 selective interference within this range of p_f values, we conclude that selective interference in
654 this regime can be thought of as causing a reduction in N_e. Good et al. (2014) have shown that in
655 asexual populations experiencing strong selective interference the variance in fitness explains
656 neutral diversity much better than the N_e expected under deterministic mutation selection
657 balance. We examined whether the time-averaged variance in fitness could explain the variation
658 in fixation rates in relation to p_f better than our diversity-based estimates of N_e but found no
659 obvious improvement (Figure S4).
660
661 Neither N_e nor the variance in fitness can account for the variation in fixation rates when
662 considering populations where most loci undergo fluctuating selection ($p_f > 0.5$). Others have
663 noted that the effects of selective interference on fixation rates cannot always be captured
664 through N_e. A sweep of a strongly beneficial mutation differentially affects neutral diversity and
665 fixation probability at a linked weakly beneficial mutation (Stephan et al. 1992, Barton 1994).
666 Fluctuating selection may have some sweep-like properties because genotypes favoured during
667 the selective period ($s_f(t) = s_{max}$) can be different from those favoured during the neutral period
668 ($s_f(t) = 0$) due to the shift in the relative importance of F- and C-loci in determining fitness.
669 Recurrent sweeps of highly fit genotypes caused by changing selective environments may
670 represent another dynamical process that differentially affects fixation probability at selected loci
671 and genetic drift at linked neutral loci (Comeron et al. 2008).
672
673 We face several major questions when assessing the relevance of our model to natural
674 systems. To what extent does selection strength on individual genes depend on environmental
675 conditions? How many loci in the genome are under fluctuating selection? Do elevated rates of
676 mutation accumulation increase extinction rates? What happens if environmental changes alter
677 population size in addition to selection? Can we detect signatures of fluctuating selection in
678 molecular data?
679
680 In laboratory experiments, selection at individual loci has been shown to vary between
681 different abiotic and biotic environments for bacteria (Remold and Lenski 2001, Cooper et al.
682 2005), yeast (Hillenmeyer et al. 2008, Jasnos et al. 2008, Bell 2010b, Heitpas et al. 2013, Cao et
683 al. 2014), nematodes (Coolon et al. 2009) and fruit flies (Lewontin and Matsuo 1963, Wang et al.

684 2009, 2014, Young et al. 2009). In the largest study of this kind, Hillenmeyer et al. (2008) found
685 that 97% of gene deletions in *Saccharomyces cerevisiae* reduced fitness relative to the wild type
686 in at least one environment while having little to no effect in other environments. Although the
687 environmental conditions chosen for most experiments do not typically mimic those in nature,
688 studies find that selection at individual loci tend to differ in magnitude (rather than in direction)
689 between environments. We show that selection fluctuating only in magnitude increases fixation
690 rates of deleterious mutations, which may be relevant for many types of environmentally
691 sensitive loci. It remains a significant empirical challenge to parameterize models with selection
692 coefficients of naturally occurring mutations quantified across naturally occurring environments.
693
694 Another critical parameter for our model is the proportion of loci in the genome that
695 experiences fluctuating selection, p_f. Excluding mutations causing intrinsic lethality, we
696 speculate that almost every locus experiences temporal change in selection strength as it seems
697 implausible selection strength is exactly the same in all conditions. However, for many (or most)
698 loci, fluctuations in selection may be minor. Perhaps only a minority of mutations undergo
699 fluctuations in selection strength on the order of magnitude that we modeled and with at least a
700 moderate level of temporal autocorrelation. Although we are unaware of any empirical estimates
701 for p_f, we suspect that the results of simulations with values of $p_f < 0.5$ best represent reality.
702
703 Our study was motivated by the 'dead end' hypothesis for highly selfing species
704 (Stebbins 1957, Igic and Busch 2013) but we do not explicitly model extinction. Similar to
705 previous examinations of mutation accumulation in genomes with low rates of recombination
706 (Haigh 1978, Heller and Maynard Smith 1979, Stephan et al. 1993, Charlesworth and
707 Charleswroth 1997), we hypothesize that the fixation of deleterious mutations should lead to
708 fitness loss and eventual extinction. Models using realistic parameters to predict the expected
709 time to extinction under Muller's ratchet assume deleterious mutations directly reduce
710 population growth rates (Lynch and Gabriel 1993, Gabriel et al. 1993, Loewe 2006, Loewe and
711 Lamatsch 2008). However, it is not obvious how the deleterious fitness effects of mutations
712 directly affect ecological success and species persistence (Agrawal and Whitlock 2012). That
713 said, our model predicts that mutations accumulate disproportionately at loci experiencing
714 fluctuating selection. We might expect, for example, that populations are driven to extinction

715 when deleterious mutations fix at loci required for surviving rare but severe climatic events. The
716 chance of this occurring increases if those mutations hitchhike to high frequencies from linkage
717 to beneficial mutations. Predicting the impact of mutation accumulation on species persistence
718 requires more complex models that incorporate the functional role and ecological context of
719 genes and explicitly track population sizes to extinction.
720
721 Environmental changes that cause fluctuations in selection may also cause fluctuations in
722 population size (e.g. Bergland et al. 2014). Our goal in this study was to isolate the effects of
723 fluctuating selection on its own but population size changes may be important on their own
724 and/or interact with fluctuations in selection. The addition of fluctuating population size will
725 lower N_e, further elevating fixation rates (Ewens 1967). Moreover, fixation rates may depend on
726 the correlation between population size and selection strength but the nature of this relationship
727 is not obvious. Selective deaths may or may not simply substitute for ecological deaths. Stress,
728 which is sometimes defined as a reduction in absolute fitness or population growth (and thereby
729 population size), is a poor predictor of the average strength of selection on new mutations
730 (Martin and Lenormand 2006; Agrawal and Whitlock 2010). Since fluctuating population size
731 alone can increase extinction rates (Lande 1993, Foley 1994, Ovaskainen and Meerson 2010),
732 integrating how environmental changes affect ecological and evolutionary forces will be
733 important for predicting the persistence of highly selfing species.
734
735 Ideally, one would be able to test our prediction that F-loci fix disproportionately more
736 deleterious mutations than C-loci. Genes could be crudely classified as C- or F-loci depending on
737 whether their expression levels are stable or variable under different naturally occurring
738 environmental conditions (see examples of environmental sensitive expression in Sonna et al.
739 2002, Lopez-Maury et al. 2008, Lasky et al. 2014). Such a classification is far from perfect (e.g.,
740 selection can change even if expression does not). For a pair of selfing and outcrossing sister
741 species, the ratio of divergence at non-synonymous to synonymous sites could be compared
742 between genes with variable expression ($\omega_{v,selfing}$, $\omega_{v,outcross}$) and genes with stable expression
743 ($\omega_{s,selfing}$, $\omega_{s,outcross}$). Because high selfing is expected to disproportionately increase fixation rates
744 at loci under fluctuating selection, we may predict that $\omega_{v,selfing} - \omega_{v,outcross}$ would be greater than
745 $\omega_{s,selfing} - \omega_{s,outcross}$. Unfortunately, there are two major caveats with this approach. Differences in

746 the average strength of selection between the sets of genes with variable and stable expression
747 would confound the results. Furthermore, there is currently only weak evidence that selfing
748 species fix non-synonymous mutations at a higher rate than outcrossing species (Wright et al.
749 2011, Gioti et al. 2013, Burgarella et al. 2015). This may be due to the young age of selfing
750 species or that higher fixation rates of beneficial mutations raise the value of ω in outcrossers
751 more than selfers (Burgarella et al. 2015). The approach outlined above assumes selfing lineages
752 are old enough for differences in fixation rates to be apparent and, further, that beneficial
753 mutations do not make a substantial contribution to divergence in either selfers or outcrossers.
754
755 It is widely speculated that highly selfing species are short lived and prone to extinction.
756 This is supported by their rarity in nature (Igic and Kohn 2006) and higher estimates of
757 extinction rates in self-compatible species (Goldberg et al. 2009; see Johnson et al. 2012 for
758 similar results on asexuals). There is also genomic evidence that selection is less efficient in
759 selfing plants and animals (Wright et al. 2002, Cutter et al. 2006a,b, Slotte et al. 2013, Burgarella
760 et al. 2015). It is also generally accepted that environmental conditions change over time and can
761 alter selection. Fluctuations in the direction and intensity of selection may be important for long-
762 term evolutionary dynamics, but the effects on genomic inferences of selection are not well
763 understood (Huerta-Sanchez 2012, Gossmann et al. 2014). We find that selection fluctuating
764 only in intensity can elevate fixation rates of deleterious mutations. Our results may well
765 underestimate the relative importance given that we observe stronger effects under lower
766 mutation rates (Figure 1, S8, S9). Even if rare in the genome, loci experiencing fluctuating
767 selection make a disproportionately larger contribution to mutation accumulation and fitness
768 decline than loci under constant selection. Moreover, mutation accumulation at such loci may
769 make selfing species particularly susceptible to extinction to rare environmental conditions
770 where the functions of such loci are particularly important. Although detecting and measuring
771 the fitness impact of loci under fluctuating selection will be empirically challenging, our model
772 shows a plausible parameter space where fluctuating selection can strongly impact the
773 persistence of highly selfing species.
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