Mutation accumulation in selfing populations under fluctuating selection

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Abstract

Selfing species are prone to extinction, possibly because 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. We simulate selfing populations with genomes containing a mixture of loci experiencing constant selection and loci experiencing selection that fluctuates in strength (but not direction). Even when both types of loci experience the same average strength of selection, loci under fluctuating selection contribute disproportionately more to deleterious mutation accumulation. Moreover, the presence of loci experiencing fluctuating selection in the genome increases the deleterious fixation rate at loci 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 when selective environments are strongly autocorrelated over time and when selection is concentrated into rare bouts of strong selection. These results imply that loci under fluctuating selection are likely important drivers of extinction in selfing species.

Introduction

Self-fertilization has evolved many times independently in plants and animals (Goodwillie *et al*. 2005, Jarne and Auld 2006). 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. Under neutrality, N_e is reduced two-fold in obligately selfing populations because gametes are not sampled independently (Pollak 1987). In addition, 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 (Nordborg 1997, Roze 2016). 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 (Slotte *et al*. 2010, 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. 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

43 most likely to degrade or the types of environmental circumstances that might hasten genomic
44 degradation. Here we investigate how loci that experience fluctuations in intensity (but not
45 direction) affect deleterious mutation accumulation in highly selfing populations. As described
46 below, fluctuations in selection intensity affect fixation probabilities in both outcrossing and
47 selfing species but we focus on the latter because deleterious mutation accumulation is, in
48 general, expected to be much more prevalent in selfers.

49 There is abundant evidence that selection is environmentally sensitive in natural and in
50 laboratory experiments (Leimu and Fischer 2008, Bergland *et al*. 2014, Latta *et al*. 2015, Rutter
51 *et al*. 2017). Conceptually, selection can vary in direction and magnitude between environments
52 (Martin and Lenormand 2006), but empirically, selection varies mostly in magnitude. For
53 example, 'stressful' environments may alleviate or intensify the strength of selection, but rarely
54 reverses the direction of selection (Kishony and Leibler 2003, Agrawal and Whitlock 2010,
55 Kraemer *et al*. 2015). Hillenmeyer *et al*. (2008) found that 97% of all viable singe gene
56 knockouts in yeast reduce fitness in at least one environment while having little effect in other
57 environments after performing over 1000 chemical assays. This suggests that selection against
58 mutations can fluctuate in magnitude from neutrality to deleterious between environments (i.e.,
59 conditionally neutral mutations may be common). Other types of studies also indicate that
60 temporal changes in the environment create fluctuations in the strength of selection (Grant and
61 Grant 2002, Bell 2010, Siepielski *et al*. 2017). Despite this evidence, most mutation
62 accumulation models assume selection is constant. This likely underestimates deleterious
63 fixation rates if selection fluctuates temporally (Wardlaw and Agrawal 2012, Cvijovic *et al*.
64 2015).

65 Though classic single locus models for the fixation probability assume selection is
66 constant (Haldane 1927, Kimura 1962), fixation probabilities are known to be highly sensitive to
67 selection changing over time (Kimura and Ohta 1970, Gillespie 1978, Peischl and Kirkpatrick
68 2012). Often neglected are changes in selection that are stochastic and temporally autocorrelated,
69 especially for mutations that are on average deleterious. Environments can fluctuate over many
70 different time scales under various degrees of temporal autocorrelation (Halley 1996, Vasseur
71 and Yodviz 2004, Furguson *et al*. 2016) and evolutionary processes can be strongly dependent
72 on both the temporal autocorrelation in selection and the average strength of selection
73 (Charlesworth 1993, Pieschl and Kirkpatrick 2012, Wardlaw and Agrawal 2012). In general, if
74 changes in selection occur rarely (very high temporal autocorrelation), then the fixation
75 probability for the mutation is largely dependent on the selective environment in which it arose
76 and can be estimated from models assuming constant selection. If fluctuations are very frequent
77 (very low temporal autocorrelation), fixation probabilities can also be approximated by constant
78 selection models but using a time-averaged strength in selection. However, in the intermediate
79 range where selection and environmental change operate on similar time scales, classic equations
80 become inaccurate. Within this range, Cvijovic *et al*. (2015) have shown in single locus models
81 that selection fluctuating in direction increases the probability of fixation for all mutations.
82 We examine selection that is always negative (or zero) but fluctuates in intensity (but not
83 direction) in a system with strong linked selection due to selfing. Interference between
84 deleterious mutations leads to mutation accumulation in asexual and highly selfing populations
85 (Muller 1932, Haigh 1978, Charlesworth and Charlesworth 1993, 1997, Kamran-Disfani and
86 Agrawal 2014). However, little is known about selective interference when some loci experience
87 fluctuations in selection intensity. In a related study, Wardlaw and Agrawal (2012) found that

88 temporal autocorrelation in selection intensity is an important determinant of Muller's ratchet in
89 asexuals because the ratchet can turn quickly during periods of relaxed selection. The current
90 work extends their findings to selfing populations with some recombination, explores more
91 realistic environmental scenarios, separately quantifies fixation rates for loci experiencing
92 fluctuating versus constant selection within the same genome, examines the extent to which the
93 linked selection effects from fluctuations can be accounted for through N_e, and considers
94 selective interference involving beneficial mutations.

95 Our goal here is to investigate mutation accumulation in selfers under different models of
96 selection (rather than to contrast selfers and outcrossers). We find that genomes containing a
97 larger proportion of loci under fluctuating selection experience higher fixation rates genome
98 wide, both because of higher fixation rates at the fluctuating loci themselves but also through a
99 linked selection effect on other loci, including those under constant selection. These results
100 imply that loci under fluctuating selection contribute disproportionately to mutation
101 accumulation and fitness decline.

102

103 **Methods**

104 In most respects, the simulation follows standard assumptions so we only describe the important
105 features here but provide a detailed description in Supplementary File Part 2 (simulation code
106 available from https://github.com/EddieKHHo/SelfingMutAccum). The population consists of N
107 diploid hermaphrodites. Each genome consists of 5000 bi-allelic loci that affect fitness as well as
108 three neutral quantitative loci that are used to estimate effective population size, N_e. Loci are
109 arranged on a single linear chromosome that experiences, on average, $M = 1$ recombination event
110 per gamete. Fitness acts multiplicatively across loci and we assume deleterious alleles are

111 partially recessive, h = ¼. Reproduction occurs following standard assumptions assuming a
112 sporophitic selfing of S = 0.98. Each offspring receives, on average, U new deleterious
113 mutations. We confirmed the program produces the expected population genetic results under
114 constant selection (detailed in Supplementary File Part 3).
115
116 **Base model**
117 Our "base model" assumes individuals carry two types of loci that affect fitness. "C-loci"
118 experience a constant selection coefficient of s_c in all generations. "F-loci" experience a strength
119 of selection that changes over time, $s_f(t)$, taking the value 0 or s_{max}, i.e., mutations at F-loci are
120 conditionally neutral. A proportion, p_f, of the fitness affecting loci in the genome are F-loci and
121 the remainder are C-loci; location assignment of locus types within the genome was randomized
122 every simulation run. All F-loci experience the same selection in a given generation (0 or s_{max}) as
123 if they all are affected by the same environmental factor. The parameter f controls the temporal
124 autocorrelation in selection. With probability f selection remains the same as the previous
125 generation. With probability, 1-f, $s_f(t)$ is randomly chosen to be 0 or s_{max} with probability 1- ϕ
126 and ϕ, respectively; ϕ is the expected proportion of generations that $s_f(t) = s_{max}$. The geometric
127 average strength of selection experienced by loci under fluctuating selection is $\bar{s} = 1-(1-s_{max})^{\phi}$.
128 For all simulations we chose s_{max} such that $\bar{s} = s_c$, so that F-loci are expected to experience the
129 same geometric average selection strength as C-loci to provide the fairest comparison between
130 these types of loci.
131 After a burn-in period, we calculated the per locus fixation rates for C- and F-loci
132 separately as the number of fixations at each type of loci divided by the number of generations
133 over which the data was collected and divided by the number of loci for each type. Throughout,

134 we calculated the *relative fixation rate* as the fixation rate in populations with $p_f > 0$ divided by
135 the fixation rate in populations with $p_f = 0$ (holding all other parameters equal). The relative
136 fixation rate for C-loci quantifies the extent to which fixation rates are affected by residing in
137 genomes that experience fluctuating selection rather than residing in genomes that only
138 experience constant selection (i.e., the classic model). The relative fixation rate for F-loci
139 quantifies the direct effect of fluctuating selection as well as effect of residing in a genome
140 experiencing fluctuating selection.
141
142 **Simulations with exponentially distributed selection**
143 We modified the base model by allowing selection strength to vary over time following an
144 exponential distribution (as opposed to the discrete "on/off" version of conditional neutrality
145 assumed in the base model). Each generation, consecutive environments remain the same with
146 probability f. With probability, $1-f$, the selection strength is chosen from an exponential
147 distribution with mean $1/\bar{s}$. Similar to the base model, C- and F-loci experienced the $\bar{s}$. We
148 focused on simulations with $\bar{s} = 0.025$ where the geometric mean and the variance in selection
149 strength under the exponential model was almost identical to that of the conditionally neutral
150 mutations with $\bar{s} = 0.025$ and $\phi = 0.5$ in the base model.
151
152 **Simulations with two fluctuating ecological variables**
153 An unrealistic assumption of the base model is that all F-loci experience fluctuating selection in
154 exactly the same way. As the simplest way to relax this assumption, we allowed for two sets (F1,
155 F2) of loci under fluctuating selection and we imagine that each set responds to a different
156 ecological variable. These sets comprise p_{f1} and p_{f2} of the genome and the remaining proportion,

157 $1-p_{f1}-p_{f2}$, are C-loci. We assumed equally sized sets of fluctuating loci, $p_{f1} = p_{f2}$ (and $p_{f1} + p_{f2} =$

158 p_f).

159 If mutations at F-loci are assumed to be conditionally neutral (as in the base model), both

160 F-loci sets experience fluctuations in selection between 0 and s_{max} with the same $\bar{s}$. Each

161 generation, the environment remains in the same state with probability f. With probability, $1-f$,

162 the environment is randomly selected among four possible states: $\{s_{f1}(t) = s_{max}, s_{f2}(t) = s_{max}\}$,

163 $\{s_{f1}(t) = s_{max}, s_{f2}(t) = 0\}$, $\{s_{f1}(t) = 0, s_{f2}(t) = s_{max}\}$, $\{s_{f1}(t) = 0, s_{f2}(t) = 0\}$ with probabilities ϕ^2+d,

164 $\phi(1-\phi)-d$, $(1-\phi)\phi-d$, $(1-\phi)(1-\phi)+d$, respectively. The value of d determines correlation ρ in

165 selective states between sets F1 and F2. We also examined simulations with sets F1 and F2 in

166 which selection fluctuates over time following an exponential distribution; selection between sets

167 F1 and F2 was made to be either completely correlated, independent or negatively correlated ($\rho =$

168 1, 0, -0.645, respectively; detailed in Supplementary file Part 2).

169

170 **Simulations with beneficial mutations**

171 Lastly, we extended the base model by allowing beneficial mutations to occur at C-loci with a

172 genome wide rate of U_b, assuming $U_b << U$. We estimated the fixation probability for a new

173 beneficial mutation as the number of beneficial fixations divided by the expected number of

174 beneficials that were introduced (i.e., $N * U_b *$ number of generations).

175

176 **<u>Results</u>**

177

178 *Heuristic model*

179 We first outline predictions for the fixation probability of a new deleterious mutation occurring
180 at a locus experiencing temporal fluctuations in the intensity of negative selection, denoted as P_f.
181 These predictions are useful for interpreting the results of our multi-locus simulations. If
182 selection fluctuates temporally between two selection coefficients $s = \{0, s_{max}\}$ with equal
183 frequency ($\phi = 0.5$), we can make simple predictions for P_f in the limiting cases where selection
184 fluctuates very often or very rarely. In the first case, selection fluctuates so often that a new
185 mutation will experience many periods of neutrality and strong selection before fixation and thus
186 experiences its expected (geometric) average fitness. We predict that P_f would be similar to the
187 fixation probability under time constant selection with $s = \bar{s} = 1-(1-s_{max})^{0.5}$; we denote this
188 prediction as $P_{\bar{s}}$. In the second case, selection fluctuates so rarely that a new mutation is almost
189 certain to be lost or fixed before selection changes. The expected fixation probability is then $\bar{P}$ =
190 (0.5) $1/(2N)$ + (0.5) $P_{s_{max}}$, where $1/(2N)$ is the fixation probability for a neutral mutation ($s = 0$)
191 and $P_{s_{max}}$ is the fixation probability when $s = s_{max}$.

192 Given these two limiting cases, we make two predictions regarding P_f. Because the
193 fixation probability for a new deleterious mutation is a convex function of s (Fig. S1a; Kimura
194 1962), it follows that $\bar{P} > P_{\bar{s}}$ (via Jensen's inequality) and we predict P_f to lie within the
195 boundaries represented by the limiting cases of $\bar{P}$ and $P_{\bar{s}}$ (i.e., $\bar{P} \geq P_f \geq P_{\bar{s}}$; Fig. S1b, c). Second,
196 as $\bar{s}$ increases, $P_{\bar{s}}$ decays quickly and asymptotes at 0 while $\bar{P}$ decays more slowly and
197 asymptotes at $1/(4N)$ (Fig. S1b) so there is a larger proportional change in $P_{\bar{s}}$ than $\bar{P}$ with respect
198 to changes in $\bar{s}$ (Fig. S1e). This means that $P_{\bar{s}}$ is more sensitive to changes in s_{max} than $\bar{P}$; we
199 predict P_f to be intermediate in its sensitivity to changes in $\bar{s}$ (Fig. S1b, e). We see a similar
200 pattern when changing the population size, N. If we hold $\bar{s}$ constant, we observe that $P_{\bar{s}}$ is more
201 sensitive to changes in N compared to P_f and $\bar{P}$ (Fig. S1c, d, f).

202 The heuristic arguments above are based on single locus theory. Below we investigate
203 mutation accumulation in multi-locus models of a highly selfing species where selective
204 interference plays an important role in elevating fixation rates; nonetheless, we show that the
205 main ideas of the heuristic model apply. Within the context of our multi-locus simulations,
206 comparing the deleterious fixation rates of loci experiencing constant selection (C-loci) and loci
207 experiencing fluctuation selection (F-loci) is similar to comparing $P_{\bar{s}}$ and P_f, respectively
208 (assuming both loci experience the same $\bar{s}$). Thus, we predict fixation rates at F-loci to be higher
209 than that of C-loci (because $P_f > P_{\bar{s}}$). We also predict fixation rates at F-loci to be less sensitive
210 to changes in $\bar{s}$ and N_e than fixation rates at C-loci.
211
212 *Temporal autocorrelaition in selection*
213 Our heuristic model implies that the fixation probability of mutations should depend on
214 the temporal autocorrelation of selection, f, (i.e., $P_f \rightarrow \bar{P}$ as $f \rightarrow 1$ and $P_f \rightarrow P_{\bar{s}}$ as $f \rightarrow 0$). We
215 confirmed this in our multi-locus model of highly selfing populations (Fig. S3, S4); Wardlaw and
216 Agrawal (2012) found similar results for the deleterious fixation rate in obligately asexual
217 populations. Rather than further exploring the effects of f on fixation rates, we assume that
218 selection undergoes moderate degrees of temporal autocorrelation ($f = 0.95$, $\phi = 0.5$) such that
219 the correlation in selection between time points separated by 1, 10, and 100 generations is 0.95,
220 0.60, and 0.006, and the average run length between switches in the environment is ~40
221 generations. This regime elevates fixation rates at loci under fluctuating loci, but is moderate in
222 its effects; fixation rates can be much higher with higher values of f and lower values of ϕ (Fig.
223 S3, S4, S15). In the remaining sections, we focus on exploring how loci under fluctuating
224 selection in the genome alter fixation rates at linked sites and genome wide.

225

226 *Varying the composition of the genome, p_f*

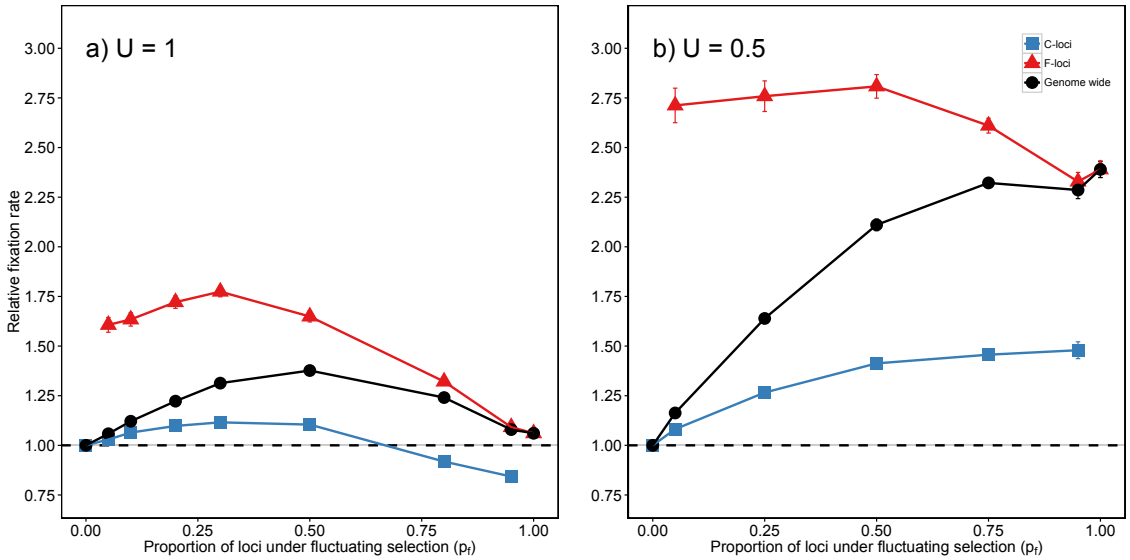
227 We examine fixation rates in genomes where a fraction p_f of loci are under fluctuating selection
228 (F-loci) and the remaining loci are under constant selection (C-loci). Unless otherwise noted, we
229 focus on simulations of highly selfing populations ($S = 0.98$) with $N = 10^4$, $\bar{s} = 0.025$, and $h =$
230 0.25 with $f = 0.95$ and $\phi = 0.5$; results for obligately selfing populations ($S = 1$) can be found in
231 Supplementary File Part 1. Per locus fixation rates within genomes experiencing fluctuating
232 selection ($p_f > 0$) are typically higher than fixation rates in the classic model ($p_f = 0$); this is true
233 for both C- and F-loci, but the effect is stronger for the latter (Fig. 1) The fact that per locus
234 fixation rates vary with p_f indicates that changing the density of F-loci in the genome alters the
235 effects of linked selection on both F- and C-loci. When less than half the genome consists of F-
236 loci ($p_f < 0.5$), increasing p_f generally elevates fixation rates at both C- and F-loci and, thereby,
237 the genome-wide average fixation rate. At high mutation rates ($U = 1$) further increasing p_f
238 reduces fixation rates for genomes that contain more than 50% F-loci (Fig. 1a). As it seems
239 unlikely that a high fraction of loci experience strong fluctuations in selection, the results for $p_f <$
240 0.5 are the most biologically relevant.

241 When mutation rates are lower (so absolute fixation rates are lower), the effect of
242 fluctuating selection on the *relative* fixation rate is more dramatic for both C- and F-loci. For the
243 parameter sets examined in Fig. 1, the inclusion of F-loci maximally increases genome-wide
244 fixation rate by a factor of 1.38 vs. 2.42 at the high and low mutation rate, respectively. This
245 effect of mutation rate holds in simulations using other values of autocorrelation f and frequency
246 of selection ϕ and in obligately selfing populations (Fig. S4, S5). Although the relative impact of
247 fluctuating selection is larger at lower mutation rates, in the remaining sections we focus on

248 results from high mutation rates ($U = 1$) because simulations at lower mutation rates require

249 much longer runs to obtain accurate estimates of fixation rates.

250



251

252 **Figure 1.** Relative fixation rates (mean ± SE) for C- (blue) and F-loci (red) in highly selfing

253 populations with varying genomic composition, p_f. Fixation rates under high mutation rates in (a)

254 $U = 1$ and low mutation rates (b) $U = 0.5$. Fixation rates are given relative to the fixation rate for

255 C-loci in the classic model ($p_f = 0$), which is represented by the horizontal dashed line at 1.0 (the

256 grey shading denotes SE). The absolute fixation rates in the classic model ($p_f = 0$) for panels a,b

257 are 7.47×10^{-6} and 8.74×10^{-7} per locus per generation respectively. Other parameters: $N = 10^4$,

258 $S = 0.98$, $f = 0.95$, $\phi = 0.5$, $h = 0.25$, $\bar{s} = 0.025$.

259

260 The density of F-loci alters the effective population size, N_e (Fig. 2a shows N_e for the

261 simulations represented by Fig. 1; Fig. S6 for obligately selfing populations). This might be the

262 reason for the variation in fixation rates in relation to p_f (Fig. 1a). If so, fixation rates should be

263 equal for simulations with different values of p_f if they experienced the same N_e. To test this, we

264 ran additional simulations changing the census population size, N, of the simulations to raise or

265 lower N_e for a given value of p_f. (We observe that N_e is not linearly related to N (Fig. 2b) as has

been observed previously in simulations of asexual populations accumulating deleterious mutations 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, fixation rates are similar across different values of p_f when N_e is similar, but only if p_f is not too high ($0 < p_f \leq 0.5$; Figs. 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.

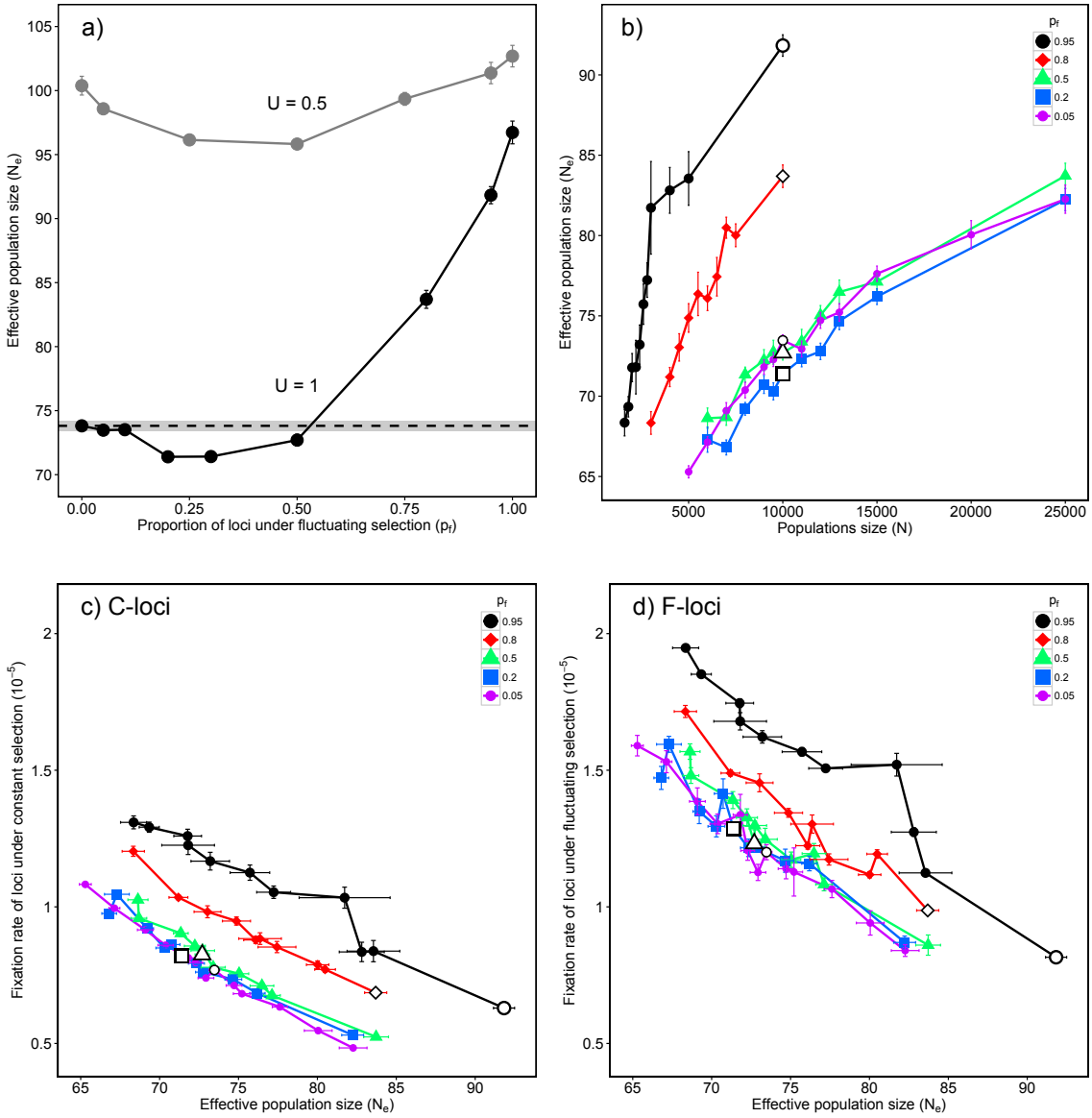


Figure 2. (a) Estimates of effective population size (mean ± SE) for genomes containing different densities of F-loci, p_f, in a population of $N = 10^4$. Shown are N_e values for highly selfing ($S = 0.98$) populations under high ($U = 1$) and lower ($U = 0.5$) mutation rates; these results are from the same simulations represented in Figs. 1a,b. The horizontal dashed line indicates the N_e (grey shading is SE) for the corresponding classic model ($p_f = 0$). (b) Estimates of N_e plotted against N ($U = 1$) 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 Fig. 1a). Fixation rates plotted against N_e (c) loci under constant selection (d) loci under fluctuating selection ($U = 1$).

294 *Fixation bias at F-loci*

295 As predicted from the heuristic model, the fixation rate of deleterious mutations at F-loci is
296 always greater than that for C-loci within all mixed genomes (Figs. 1, 2c, d). This bias is clearly
297 stronger when selection fluctuates in ways that directly increase fixation rates at F-loci (i.e.,
298 larger f and smaller ϕ; Figs. 3a, S4). Moreover, this bias is affected by the 'traditional'
299 parameters affecting mutation accumulation such as the strength of selection ($\bar{s}$), the mutation
300 rate (U) and the population size (N). To examine how this bias depends on aspects of selection,
301 we performed simulations where half the genome consist of F-loci ($p_f = 0.5$) with an average
302 selection strength of $s_c = \bar{s} = \{0.005, 0.025, 0.05\}$. The bias in fixations at F-loci increases with $\bar{s}$
303 as stronger selection reduces fixation rates at C-loci relatively more than at F-loci (Fig. 3b).
304 These results can be interpreted in light of the heuristic model that predicts F-loci will be less
305 sensitive than C-loci to increases in $\bar{s}$: mutations at F-loci can accumulate during episodes where
306 selection is weak regardless of how large s_{max} becomes in episodes when selection is strong.
307 Overall, conditions that slow down mutation accumulation, such as stronger $\bar{s}$ (Fig. 3), lower U
308 (Figs. 4, S4, S14b), larger N (Fig. S9), and less selfing (Fig. S10) reduce fixation rates at C-loci
309 more so than at F-loci, increasing the relative contribution of F-loci to total mutation
310 accumulation; see Fig. S8 for simulations of obligately selfing populations.

311

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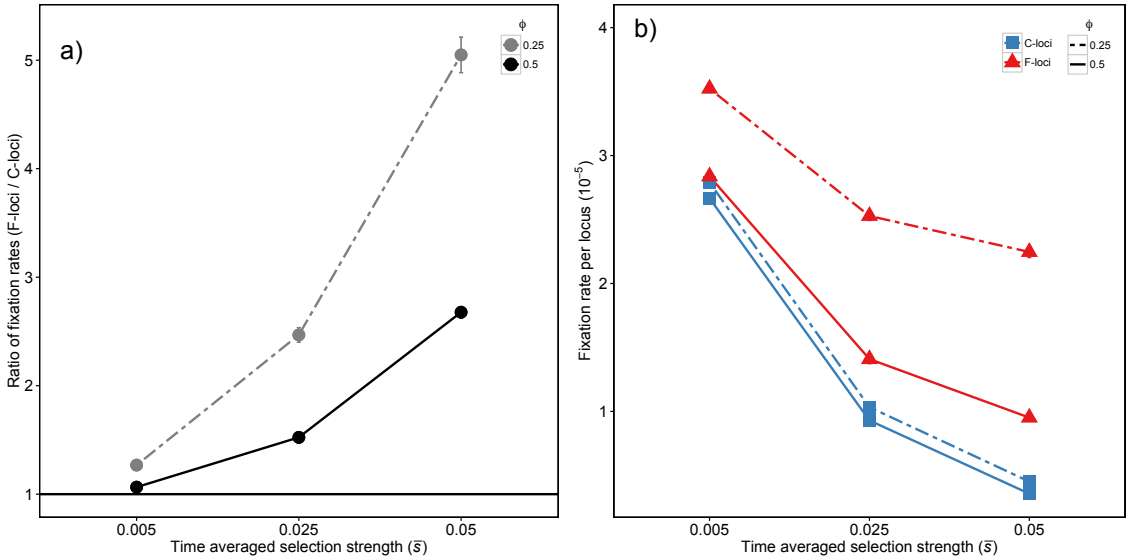

Figure 3. (a) Ratio of fixation rates at F-loci to fixation rates at C-loci when half of the genome consists of F-loci (p_f = 0.5); error bars are SE (often too small to be visible). When ϕ = 0.5 (solid black line), selection is non-neutral (s_{max}) half the time but when ϕ = 0.25 (double-dashed grey line), selection is non-neutral only a quarter of the time. To maintain $\bar{s}$ = {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 (red) and C-loci (blue) when ϕ = 0.5 (solid line) and when ϕ = 0.25 (double-dashed line). Other parameters: S = 0.98, N = 10^4, U = 1, h = 0.25, f = 0.95.

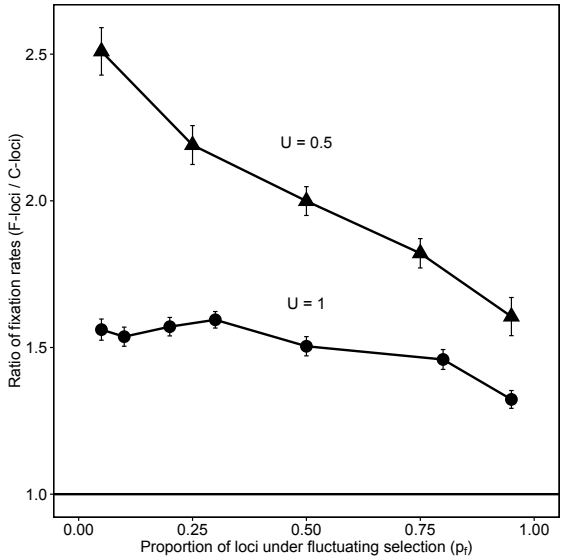

Figure 4. Ratio of fixation rates (mean ± SE) at F-loci to fixation rates at C-loci in populations with different proportions of F-loci under high (U = 1, circles) and low (U = 0.5, triangles) mutation rates. Other parameters: S = 0.98, N = 10^4, h = 0.25, f = 0.95, ϕ = 0.5.

Exponentially distributed selection over time

In the base model we assumed that mutations at F-loci are conditionally neutral with selection fluctuating discretely between 0 and s_{max}. As a test of whether the patterns above are robust to that assumption, we consider simulations where the selection strength at F-loci is randomly sampled from an exponential distribution when the environment changes. Both models have a similar mean and standard deviation in selection strength ($\bar{s} \cong 0.025$, standard deviation in $s_f(t)$ = 0.025). Using f = 0.95 in the exponential model (as in the base model), 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 discrete conditional neutrality model (Figs. 5, S12). Mutations fix disproportionately at F-loci under the exponential selection model, but this bias is not quite as strong as observed in the conditional neutrality model. As in the base model, reducing U (from U

= 1 to $U = 0.5$) under the exponential model increases relative fixation rates and the proportion of mutations that fix at F-loci (Figs. 5, S14). Though the base model and exponential model represent 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.

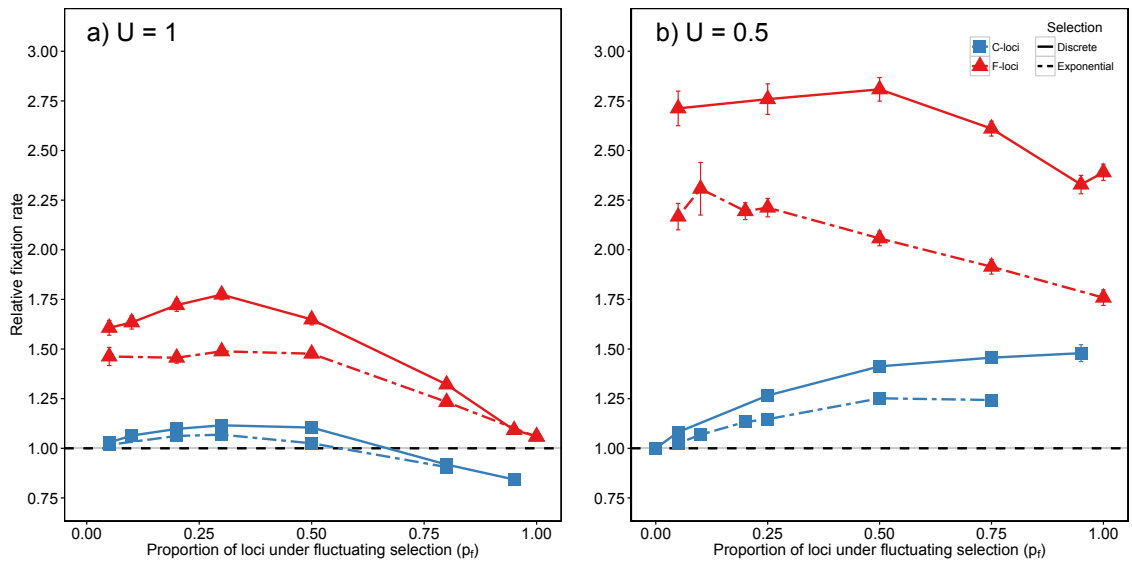

Figure 5. Relative fixation rates (mean ± SE) at C- (blue) and F-loci (red) in populations with different genomic compositions, p_f, when selection strength is modelled as discrete conditional neutrality (solid line) or following an exponential distribution (double-dashed line) over time under (a) high mutation rates (U = 1) and (b) low mutation rates (U = 0.5). Relative fixation rates under the conditional neutrality model are re-plotted from Figs. 1a,b. The geometric time average and the variance in selection strength and for the conditional neutrality and exponential selection model are approximately the same ($\bar{s}$ = 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: S = 0.98, f = 0.95, N = 10^4, h = 0.25.

360 *Two components of fluctuating selection*

361 In the previous sections, we assumed that all F-loci simultaneously respond to the same
362 environmental factor that fluctuates in time. As the simplest way to relax this assumption, we
363 allow for two equally sized sets of F-loci (F1, F2) that respond to two separate environmental
364 factors. The two environmental factors may fluctuate in perfect synchrony, causing a complete
365 correlation in selective states between loci within F1 and F2 ($\rho = 1$), which is equivalent to the
366 base model. Alternatively, the two environment factors fluctuate in anti-synchrony, causing a
367 negative correlation in selective states between F1 and F2 ($\rho = -1$). We perform simulations for a
368 range of different values of ρ, the correlation in selective states between set F1 and F2.

369 The environmental correlation ρ has little effect on the fixation rates of C- and F-loci
370 when the genome consists of mostly C-loci ($p_f \leq 0.5$); ρ is negatively related to fixation rate
371 when the genome consists of mostly F-loci, making our base model ($\rho = 1$) conservative in this
372 respect (Fig. 6a); obligately selfing populations are less insensitive to changes in ρ (Fig. S12).
373 We observe qualitatively similar effects of ρ when selection between the two groups is
374 exponentially distributed in time (Figs. 6b, S12, S13). In sum, under biologically realistic ranges
375 of p_f (i.e., $p_f < 0.5$), fixation rates do not appear to be strongly dependent on how fluctuations in
376 selection are correlated between two equally sized sets of F-loci.

377

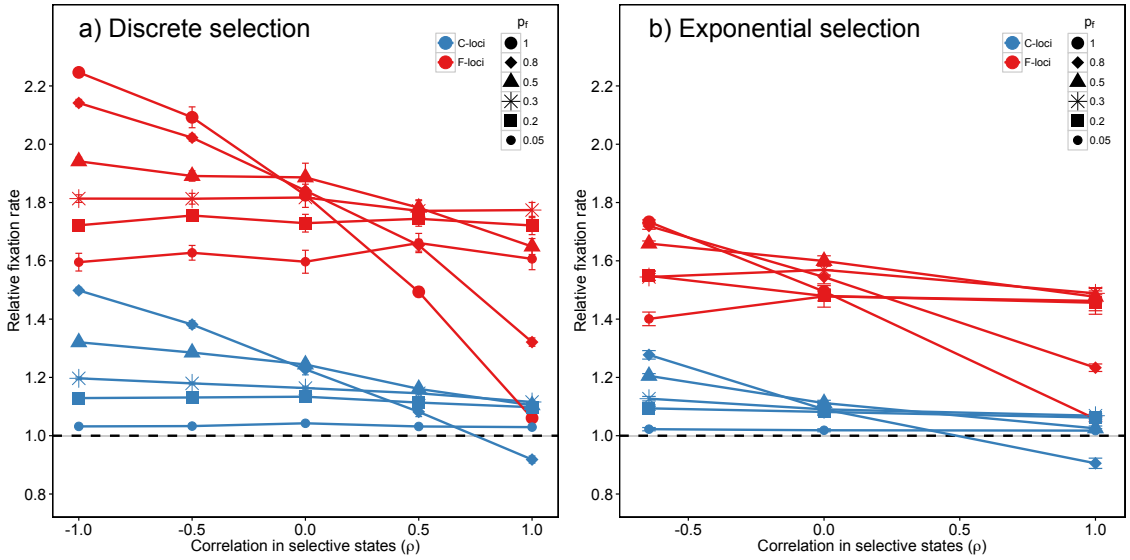

Figure 6. Relative fixation rates (mean ± SE) for C-loci (blue) and F-loci (red) as a function of the correlation in environmental variables responsible for fluctuating selection ρ. Loci under fluctuating selection are equally distributed between two sets of F-loci (F1, F2). The correlation in selective states between F1 and F2, ρ, can be positive or negative or zero. The F-loci (summing both F1 and F2) comprise a fraction p_f of the genome. (a) Selection under the model of discrete conditional neutrality; ρ = {1, 0.5, 0, -0.5, 1}. (b) Selection is exponentially distributed over time; ρ = {1, 0, -0.645}. 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); the actual fixation rates is 7.47 x 10^{-6}. Other parameters: S = 0.98, f = 0.95, ϕ = 0.5, $\bar{s}$ = 0.025, N = 10^4, U = 1, h = 0.25.

Beneficial mutations

We performed simulations in which beneficial mutations occurred at C-loci at much lower rates than deleterious mutations ($U_b \leq 0.001$) and had smaller, larger or equal fitness effects compared to deleterious mutations (beneficials: s_b = {0.01, 0.025, 0.05, 0.08}, h_b = 0.5; deleterious: $\bar{s}$ = 0.025, h = 0.025). F-loci only received deleterious mutations and experienced selection fluctuating discretely over time between 0 and s_{max} (f = 0.95, ϕ = 0.5).

397 The fixation probability of beneficial mutations was lower in simulations with F-loci (p_f =
398 0.2) than those without F-loci (p_f = 0) for all values of s_b (Table 1) indicating that deleterious
399 mutations at F-loci caused more interference with beneficials than those at C-loci. Beneficials
400 with smaller or similar effect size to deleterious mutations (i.e. s_b = 0.01, 0.025) had little effect
401 on the deleterious fixation rate. However, beneficials with larger fitness effects (s_b = 0.05, 0.08)
402 increased deleterious fixation rates at both C- and F-loci (Table 1). The deleterious fixation rate
403 of C-loci changed proportionately more with increases in the strength of beneficials (s_b)
404 compared to that of F-loci (Table 1), consistent with our heuristic model. Recall that comparing
405 fixation rates at F-loci and C-loci is similar to comparing P_f and $P_{\bar{s}}$, respectively, and $P_{\bar{s}}$ is
406 proportionally more sensitive to changes in N and s compared to P_f (Fig. S1e, f). Because an
407 increase in s_b causes a decrease in N_e (Table 1), we expect C-loci ($P_{\bar{s}}$) to be more sensitive to this
408 change than F-loci (P_f).
409 Because the F-locus rate without beneficials is larger than the C-locus rate, the fact that
410 the absolute increase in the deleterious fixation rate caused by beneficials is larger for F-loci than
411 C-loci is obscured by only looking at their "proportional increase". This is clearer by taking a
412 second perspective in which we calculate the average number of additional fixed deleterious
413 mutations per fixed beneficial (Table S3). When the beneficial effect size is larger than
414 deleterious (s_b = 0.05, 0.08), each beneficial that fixes is responsible for, on average, less than
415 one additional deleterious fixation but these are not evenly distributed between F- and C-loci. F-
416 loci are only 25% as abundant as C-loci (i.e., p_f = 0.2), but the number of additional fixed
417 deleterious F-alleles per fixed beneficial is more than 25% the number of additional fixed
418 deleterious C-alleles. From this perspective, F-loci experience stronger interference from

419 beneficials, in an absolute sense, than do C-loci even though C-loci experience a greater

420 proportional interference effect than F-loci (Table 1).

421

Table 1. Summary of simulations with beneficial mutations

U	U_b	s_b	p_f	N_e (SE)	Ben prob fix x 10^{-3} (SE)	Del fix rate relative to NO ben (SE)	
						C-loci	F-loci
0.5	0.0001	0.01	0	99.7	0.172	0.992	
				(0.35)	(0.004)	(0.006)	
			0.2	96.5	0.164	0.999	1.032
				(0.36)	(0.005)	(0.005)	(0.013)
		0.025	0	99.7	0.752	1.021	
				(0.37)	(0.011)	(0.009)	
			0.2	96.9	0.703	1.019	1.072
				(0.48)	(0.012)	(0.007)	(0.018)
		0.05	0	96.6	3.798	1.312	
				(0.25)	(0.015)	(0.008)	
			0.2	93.5	3.499	1.240	1.159
				(0.33)	(0.025)	(0.008)	(0.024)
		0.08	0	87.5	11.429	2.373	
				(0.34)	(0.040)	(0.011)	
			0.2	85.9	10.631	2.092	1.664
				(0.34)	(0.044)	(0.012)	(0.022)
1	0.001	0.01	0	73.8	0.121	0.994	
				(0.33)	(0.002)	(0.003)	
			0.2	71.2	0.119	0.980	1.039
				(0.24)	(0.002)	(0.003)	(0.018)
		0.025	0	72.8	0.384	1.016	
				(0.34)	(0.003)	(0.003)	
			0.2	71.0	0.356	1.005	1.008
				(0.33)	(0.004)	(0.003)	(0.011)
		0.05	0	71.0	1.531	1.199	
				(0.31)	(0.007)	(0.004)	
			0.2	67.7	1.405	1.152	1.129
				(0.31)	(0.007)	(0.004)	(0.018)
		0.08	0	62.5	3.916	1.774	
				(0.29)	(0.010)	(0.003)	
			0.2	60.9	3.672	1.650	1.478
				(0.28)	(0.008)	(0.004)	(0.012)

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 $U_b * N$. '*Del fix rate relative to NO ben'* is the fixation rate of C-loci and F-loci in simulations with beneficial mutations relative to the fixation rate of C-loci and F-loci in simulations without beneficial mutations (holding all other parameters constant). Other parameters: $S = 0.98$, $N = 10000$, $h = 0.25$, $\bar{s} = 0.025$, $h_b = 0.5$, $f = 0.95$, $\phi = 0.5$, $\rho = 1$. See Table S2, S3 for further information on these simulations.

432 **Discussion**

433 Because of the reduced efficacy of selection associated with high selfing rates, such populations
434 can accumulate and permanently fix deleterious mutations at high rates leading to rapid fitness
435 decline and possibly to extinction (Heller and Maynard Smith 1979, Charlesworth and
436 Charlesworth 1993). Environmental changes occur on many different times scales (Halley 1996,
437 Vasseur and Yodviz 2004, Furguson *et al*. 2016) and can induce fluctuations in the intensity and
438 direction of selection (Siepielski *et al*. 2017, Bell 2010) but the consequences for mutation
439 accumulation in selfers is unstudied. Loci experiencing selection that fluctuates in intensity, F-
440 loci, fix deleterious mutations at a higher rate than loci under constant selection, C-loci,
441 experiencing the same time averaged selection strength ($s_c = \bar{s}$). The extent to which F-loci
442 contribute disproportionately to total mutation accumulation depends on aspects of
443 environmental heterogeneity. The bias is stronger with increasing temporal autocorrelation in
444 selection, f, and when selection is concentrated into rarer but more intense episodes (low values
445 of ϕ), holding $\bar{s}$ constant (Fig. S3, S4, S14). This bias also becomes stronger with changes to
446 "traditional" parameters that lower the overall rate of mutation accumulation, i.e., higher values
447 of $\bar{s}$ or N as well as lower values of U (Fig. 3, 4, S8, S9). In sum, fluctuating selection can
448 increase the rate of mutation accumulation in highly selfing populations by several fold and the
449 loci under fluctuating selection contribute disproportionately to mutational decline. These
450 conclusions appear qualitatively robust to the exact nature of fluctuations (Fig. 5, 6, S12, S13).

451 In our model, deleterious mutations fix as a consequence of selective interference
452 between linked sites. Supporting this, simulations with the same parameter values but at higher
453 rates of outcrossing ($S \leq 0.9$) fix few to no mutations (Table S1). Obligately asexual organisms
454 (Wardlaw and Agrawal 2012) and obligate selfers ($S = 1$) have high rates of mutation

455 accumulation that are further increased by fluctuating selection. These effects remain important
456 even with low levels of outcrossing (S = 0.98) that permit some genetic mixing. Interference
457 between constantly selected loci can lead to the accumulation of deleterious mutations in asexual
458 and highly selfing populations, a process classically called Muller's ratchet (Haigh 1978, Heller
459 and Maynard Smith 1979). However, little attention has been given to understanding of selective
460 interference between loci under fluctuating selection and loci under constant selection.

461 In genomes containing a mix of F- and C-loci, F-loci have inherently higher rates of
462 fixation but also tend to increase fixation rates at C-loci and other F-loci as illustrated in Fig. 1
463 where increasing the fraction of F-loci in the genome increases the per-locus fixation rates at
464 both C- and F-loci (for $0 < p_f < 0.5$). This indicates that F-loci cause more selective interference
465 (with both C- and F-loci) than C-loci. Similarly, we found that deleterious mutations at F-loci
466 reduce fixation rates of beneficials more than deleterious mutations at C-loci do (Table 1, S2).
467 Previous work in asexual populations where selection is constant over time but varies among loci
468 shows that interference from strongly deleterious alleles increase fixation of more weakly
469 deleterious alleles (Gordo and Charlesworth 2001, Soderberg and Berg 2007). From this we can
470 infer that strong interference caused by F-loci occurs during periods when selection is strong,
471 $s_f(t) = s_{max} > s_c$. However, during periods where $s_f(t) = 0$, F-loci do not cause interference. Given
472 the observed net increase in fixation rate at C-loci, we infer that the increased interference when
473 selection at F-loci is strong ($s_f(t) = s_{max}$) outweighs the reduced interference when selection is
474 weak ($s_f(t) = 0$). On the other hand, we observed that strongly selected beneficials interfered
475 more strongly with deleterious alleles at C-loci than at F-loci in terms of the proportional change
476 in their fixation rates (Table 1). Viewing interference from the beneficials as a reduction in N_e,

477 the result above is consistent with the heuristic model prediction that fixation probabilities for F-
478 loci are less sensitive to N_e than for C-loci.

479 Indeed the effect of selective interference on fixation rates can be interpreted as resulting
480 from a reduction in the effective population size in some cases (Hill and Robertson 1966,
481 McVean and Charlesworth 2000, Gordo and Charlesworth 2002). We observed that per locus
482 fixation rates varied as a function of p_f for both C- and F-loci (Fig. 1). In the range where p_f was
483 not too large ($0 < p_f \leq 0.5$), fixation rates for each locus type (F and C) were nearly equal for
484 different values of p_f when we adjusted N to obtain similar N_e values (Fig. 2). Because neutral
485 diversity (used to infer N_e) and fixation rates are similarly affected by selective interference
486 within this range of p_f values, we conclude that selective interference in this regime can be
487 thought of as causing a reduction in N_e. Good *et al*. (2014) showed that in asexual populations
488 experiencing selective interference the variance in fitness explains neutral diversity much better
489 than the N_e expected under deterministic mutation-selection balance. However, the time-
490 averaged variance in fitness did not explain the variation in fixation rates in relation to p_f better
491 than our diversity-based estimates of N_e (Fig. S7).

492 Neither N_e nor the variance in fitness can account for the variation in fixation rates when
493 most loci undergo fluctuating selection ($p_f > 0.5$). Others have noted that the effects of selective
494 interference on fixation rates cannot always be captured through N_e. A sweep of a strongly
495 beneficial mutation differentially affects neutral diversity and fixation probability at a linked
496 weakly beneficial mutation (Stephan *et al*. 1992, Barton 1994). Fluctuating selection may have
497 some sweep-like properties because genotypes favoured during the selective period ($s_f(t) = s_{max}$)
498 can be different from those favoured during the neutral period ($s_f(t) = 0$) due to the shift in the
499 relative importance of F- and C-loci in determining fitness. Recurrent sweeps of highly fit

500 genotypes caused by changing selective environments may represent another dynamical process
501 that differentially affects fixation probability at selected loci and genetic drift at linked neutral
502 loci (Comeron *et al*. 2008).

503 There are several questions in assessing the relevance of our model to natural systems. As
504 highlighted in the Introduction, numerous lab studies show selection on mutations varies in
505 strength across the environmental conditions. However, we do not know the extent to which this
506 variation occurs in nature. Further, we have no estimate of the proportion of loci in the genome
507 that experiences fluctuating selection, p_f. Excluding mutations causing intrinsic lethality, we
508 speculate that almost every locus experiences temporal change in selection strength as it seems
509 implausible selection strength is exactly the same in all conditions. However, for many loci,
510 fluctuations in selection may be minor. Perhaps only a minority of mutations undergo
511 fluctuations in selection strength on the order of magnitude that we modeled and with at least a
512 moderate level of temporal autocorrelation.

513 Environmental changes that cause fluctuations in selection may also cause fluctuations in
514 population size (e.g., Bergland *et al*. 2014). Our main goal was to isolate the effects of
515 fluctuating selection on its own but population size changes may be important on their own
516 and/or interact with fluctuating selection. As described in Supplementary File Part 5, we briefly
517 explored the interaction between fluctuating N and s. As expected, simulations with only
518 fluctuating N fixed deleterious mutations at a rate similar to one with constant $N = N_h$, where N_h
519 represents the harmonic time average population size (Otto and Whitlock 1997). Fluctuations in s
520 alone increased deleterious fixation rates compared with one with constant $s = \bar{s}$. Keeping N_h
521 constant, the addition of fluctuating N into models of fluctuating s did not greatly change the
522 deleterious fixation rate of the latter regardless of the (positive and negative) correlations in the

523 fluctuations of *N* and *s*. In summary, fluctuations in *N* and *s* have different effects on deleterious
524 fixation rates; we cannot simply use fluctuations in the product *Ns* as a substitute for fluctuations
525 in each component alone.

526 Our study was motivated by the 'dead end' hypothesis for highly selfing species
527 (Stebbins 1957, Igic and Busch 2013) but we do not explicitly model extinction. Similar to
528 previous examinations of mutation accumulation in genomes with low rates of recombination
529 (Haigh 1978, Heller and Maynard Smith 1979, Charlesworth and Charlesworth 1997), we
530 hypothesize that deleterious fixations should lead to fitness loss and eventual extinction. Models
531 using realistic parameters to predict the expected time to extinction under Muller's ratchet
532 assume deleterious mutations directly reduce population growth rates (Gabriel *et al*. 1993,
533 Loewe 2006, Loewe and Lamatsch 2008). However, it is not obvious how the deleterious fitness
534 effects of mutations directly affect ecological success and species persistence (Agrawal and
535 Whitlock 2012). That said, our model predicts that mutations accumulate disproportionately at
536 loci experiencing fluctuating selection. We might expect that populations are driven to extinction
537 when deleterious mutations fix at loci required for surviving rare but severe climatic events.
538 Prior to extinction, the central prediction is that F-loci contribute disproportionately to mutation
539 accumulation. In reality, this would be challenging to test because it would be difficult to
540 categorize the genome into F- and C-loci. Even if one did so (perhaps based on plasticity in
541 expression), it would be extremely difficult to identify subsets of loci from each category that
542 have comparable average selection strengths.

543 We focused on the mutation accumulation in highly selfing populations but the dynamics
544 of deleterious mutations can affect the evolution of the selfing rate itself. In finite populations,
545 the build up of negative linkage disequilibrium (LD) at sites under negative selection can

546 increase deleterious fixation rates and favour the evolution of increased recombination and
547 outcrossing (Keightley and Otto 2006, Gordos and Campos 2008, Kamran-Disfani and Agrawal
548 2014). Kamran-Disfani and Agrawal (2014) found that selfing rates evolve to just below where
549 Muller's ratchet begins turning at high rates. Because the presence of F-loci in the genome
550 increases the deleterious fixation rate, it is natural to ask whether it also affects the evolution of
551 selfing. In Supplementary File Part 4, we briefly explored this by allowing selfing rates to evolve
552 in our model. We found that the equilibrium level of selfing was similar if F-loci are present (p_f =
553 0.25) or absent (p_f = 0), even though deleterious fixation rates are higher if F-loci are present.
554 Highly selfing species appear to be prone to extinction though we lack a detailed
555 understanding of how this occurs. Our work indicates fluctuations in the strength of purifying
556 selection can be an important contributor to this process. The results presented may
557 underestimate the relative importance given that we observe stronger effects under lower
558 mutation rates (Fig. 1, S13, S14). Even if rare in the genome, loci experiencing fluctuating
559 selection make a disproportionately larger contribution to mutation accumulation and fitness
560 decline than loci under constant selection. Moreover, mutation accumulation at such loci may
561 make selfing species particularly susceptible to extinction to rare environmental conditions
562 where the functions of such loci are particularly important. Although detecting and measuring
563 the fitness impact of loci under fluctuating selection will be empirically challenging, our results
564 demonstrate a plausible parameter space where fluctuating selection can strongly impact the
565 mutation accumulation in highly selfing species.
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