Temporal Variation in Selection Accelerates Mutational Decay by Muller's Ratchet

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ABSTRACT Asexual species accumulate deleterious mutations through an irreversible process known as Muller's ratchet. Attempts to quantify the rate of the ratchet have ignored the role of temporal environmental heterogeneity even though it is common in nature and has the potential to affect overall ratchet rate. Here we examine Muller's ratchet in the context of conditional neutrality (*i.e.*, mutations that are deleterious in some environmental conditions but neutral in others) as well as more subtle changes in the strength (but not sign) of selection. We find that temporal variation increases the rate of the ratchet (mutation accumulation) and the rate of fitness decline over that of populations experiencing constant selection of equivalent average strength. Temporal autocorrelation magnifies the effects of temporal heterogeneity and can allow the ratchet to operate at large population sizes in which it would be halted under constant selection. Classic studies of Muller's ratchet show that the rate of fitness decline is maximized when selection is of a low but intermediate strength. This relationship changes quantitatively with all forms of temporal heterogeneity studied and changes qualitatively when there is temporal autocorrelation in selection. In particular, the rate of fitness decline can increase indefinitely with the strength of selection with some forms of temporal heterogeneity. Our finding that temporal autocorrelation in selection dramatically increases ratchet rate and rate of fitness decline may help to explain the paucity of asexual taxa.

ASEXUAL species are doomed to irreversibly accumulate deleterious mutations that can lead to a decline in overall fitness and eventually extinction (Gabriel *et al.* 1993; Lynch *et al.* 1993). In finite populations, the small number of individuals with the fewest deleterious mutations may fail to contribute mutation-free progeny to the next generation due to drift or mutation. The perpetual loss of the "least-loaded class" by chance events, irreversible in the absence of recombination and back mutation, is a process known as Muller's ratchet (Muller 1964; Felsenstein 1974). Quantifying the operation of the ratchet can help explain a variety of phenomena such as the extinction of asexual species (Loewe and Cutter 2008; Loewe and Lamatsch 2008), the degeneration of nonrecombining DNA like that of human mitochondria (Loewe 2006) or the Y chromosome (Gordo and Charlesworth 2001; Engelstadter 2008), and the evolutionary maintenance of sex (Howard and Lively 1994; Gordo and Campos 2008).

Haigh was the first to develop an explicit mathematical model of Muller's ratchet (Haigh 1978). Haigh's model and subsequent variations aimed at estimating the rate of loss of the least-loaded class make simplifying assumptions about the nature of selection acting on a population. Of particular relevance here, most studies have considered the environment to be unchanging such that the strength of selection acting on accumulating mutations does not vary with time (Haigh 1978; Butcher 1995; Gessler 1995; Gordo and Charlesworth 2000; Soderberg and Berg 2007).

Besides being common and well documented in nature (see Siepielski *et al.* 2009 and references therein), the importance of considering temporal environmental variation is highlighted in a recent study of the budding yeast *Saccharomyces cerevisae* (Hillenmeyer *et al.* 2008). Previous work suggested that ~60% of genes in the yeast genome had no detectable effect on growth rate under standard conditions (Winzeler *et al.* 1999). However, when growth rate was assayed in 1144 chemical environments, Hillenmeyer *et al.* (2008) found that 97% of gene deletions caused measurable growth deficiencies in one or more environments. Although there are potential issues with respect to measurement error, these results taken at face value suggest that deleterious mutations with little or no effect in one environment could

have stronger effects in a different environment. Environmental heterogeneity may therefore lead to fluctuations in the strength of selection acting on deleterious mutations.

To understand the effects of temporal variation in selection, it is helpful to first consider the well-studied case of the ratchet under constant selection. A major determinant of the ratchet rate R (defined here as the rate of loss of the least-loaded class) is the expected equilibrium size of the least-loaded class, $n_0 = Ne^{-U/s}$, where N is the population size, U is the genome-wide mutation rate, and s is the strength of selection against deleterious mutations (Haigh 1978, but see Gessler 1995). After the loss of the least-loaded class, the next class becomes the most fit and its size approaches a value close to n_0. The rest of the population reestablishes mutation–selection balance before the new least-loaded class is then lost (Stephan *et al.* 1993). For small n_0 the least-loaded class is easily lost by genetic drift and the ratchet rate is high. Thus, ratchet rate increases with mutation rate and decreases with increasing population size and increasing selection strength (Haigh 1978; Gordo *et al.* 2002; Soderberg and Berg 2007). Because n_0 is a function of s, we expect the speed of the ratchet to vary if the strength of selection varies over time. What will be its net rate, averaging over periods where selection is weak and strong? Can we understand the average rate of the ratchet simply by using the average strength of selection? In other classic population genetic contexts, the effects of temporal heterogeneity can be captured simply by using the geometric mean fitnesses of different genotypes (Nagylaki 1975; Dempster 1955). Is the rate of the ratchet with temporal variation the same as that with constant selection of equivalent strength (based on geometric mean fitness) or are other aspects of the temporal distribution of selection important?

The effect of environmental heterogeneity on the ratchet rate may depend on the scale of temporal variation in selection. In nature, changes in selection occur over days (*e.g.*, Blanckenhorn *et al.* 1999), seasons (*e.g.*, Hendry *et al.* 2003), years (reviewed in Siepielski *et al.* 2009), and decades (Grant and Grant 2002). That is, selection may change rapidly or slowly across generations, depending on the life span of the organism relative to the timescale of the environmental change. The scale of variation can be captured by the degree of temporal autocorrelation in selection, which describes the dependency of the selection coefficient in one generation on that of previous generations. Holding the total amount of variation constant, a low degree of temporal autocorrelation represents a rapidly fluctuating environment while a high degree of temporal autocorrelation represents a slowly fluctuating one. The degree of temporal autocorrelation is likely to be important in determining the overall ratchet rate because mutations can accumulate rapidly if selection is weak for longer continuous time periods (*i.e.*, a slowly fluctuating environment).

As the ratchet "clicks" (*i.e.*, as the least-loaded class is lost), population mean fitness declines, which can lead to mutational meltdown and ultimately the extinction of asex-

ual populations (Gabriel *et al.* 1993; Lynch *et al.* 1993). The rate of fitness decline depends on the rate of the ratchet and the strength of selection. Under constant selection, the rate of fitness decline is maximized at intermediate s because if s is very small, the ratchet clicks quickly but with minimal fitness consequences, whereas if s is large, the ratchet clicks too slowly to cause substantial fitness loss (Felsenstein 1974; Gabriel *et al.* 1993; Butcher 1995; Gordo and Campos 2008). In fluctuating environments, the effects of the ratchet may be more severe if mutations that rapidly accumulate during benign periods are strongly selected under harsher environments.

Here we use simulations to investigate the effects of temporal heterogeneity in selection on the rate of the ratchet and the decline in fitness. We explore several forms of temporal heterogeneity and compare these results to those expected under constant selection of the same average strength. For the most part, especially with temporal autocorrelation, temporal variation gives different results than the constant selection case. Many of these differences can be interpreted from an understanding of the processes underlying the classic ratchet model. In many cases, we find that asexual populations and nonrecombining genomes in heterogeneous environments may be speeding toward their eventual demise faster than classically predicted.

Methods

Overview

The model involves N asexual haploid individuals, each characterized by the number of deleterious mutations it carries. An individual's prospective fitness is determined by the number of mutations it carries and the strength of selection acting in that generation. An individual's realized fitness is expected to be proportional to its prospective fitness but is subject to stochasticity (representing genetic drift). Below we describe how selection changes across generations and provide more details about the simulations.

Varying selection

Unless otherwise stated, we assume selection within a generation is the same across all loci. To simulate temporal fluctuations in the environment we examined scenarios in which selection varied between s_{min} and s_{max} (*i.e.*, $s[t] \in \{s_{min}, s_{max}\}$). Temporal autocorrelation in selection is quantified by the correlation in environmental state between consecutive generations, f. Each generation, selection automatically remains the same as it was in the previous generation with probability f. With probability $1 - f$ the selection coefficient is chosen at random, equaling s_{max} with probability ϕ and s_{min} with probability $(1 - \phi)$. The value ϕ therefore represents the expected average fraction of generations where selection is strong (s_{max}); we use $\phi = 0.5$ unless otherwise stated. Three levels of temporal autocorrelation were examined: (i) no temporal autocorrelation, $f = 0$; (ii) low temporal

autocorrelation, $f = 0.8$; and (iii) high temporal autocorrelation, $f = 0.95$. Higher values of f result in longer runs of consecutive generations with the same selection strength. The no, low, and high temporal autocorrelation levels used here correspond to average run lengths of 2.0, 10.2, and 41.0 generations, respectively, when $\phi = 0.5$.

Simulation methods

Simulations, written in C (available upon request), were used to model the accumulation of mutations in asexual haploid populations. Individuals were described by the number of mutations in their genome. The genome was initially assumed to be in mutation–selection balance and thus created from a Poisson distribution with mean $\theta = U/\bar{s}$, where U is the genome-wide mutation rate and $\bar{s}$ the expected geometric time-averaged selection coefficient. When s varies over time, $\bar{s} = 1 - (1 - s_{\max})^{\phi}(1 - s_{\min})^{1-\phi}$. For each generation, an individual was randomly selected from the population and its fitness value was calculated. Fitness was assumed to be the multiplicative effect of all mutations in the genome, i.e., $(1 - s[t])^k$, where $s[t]$ is the selection coefficient in generation t and k is the number of mutations in the genome at that time. The fitness value was compared with a uniform random variate and, if greater, the individual became a parent. The parent then produced a single clonal offspring with an additional x new mutations added to its genome, where x was a random number from a Poisson distribution with mean U. The process was repeated with replacement until N new individuals were created. Simulations were carried out with a genome-wide mutation rate of $U = 0.5$ at three population sizes, $N = 500$, $N = 5000$, and $N = 50,000$.

The simplest model we investigate is one of conditional neutrality: selection changes between $s_{\min} = 0$ and $s_{\max}$, with selection expected to be strong half of the time ($\phi = 0.5$). We then investigate two variations of this model. First, we consider other cases of conditional neutrality. To examine the effects of varying the frequency of selection, we vary ϕ between 0 and 1 but keep $\bar{s}$ fixed across the range of ϕ by setting $s_{\max} = 1 - ((1 - \bar{s})/(1 - s_{\min})^{1-\phi})^{1/\phi}$. One end of this range thereby represents cases where selection is rare but strong ($\phi \rightarrow 0$) and the other end represents constant but comparatively mild selection ($\phi = 1$).

The second variation of the model we explore is quantitative variation in selection ($0 < s_{\min} < s_{\max}$) rather than conditional neutrality ($0 = s_{\min} < s_{\max}$). This could be explored in a number of ways but we chose to study the case where the strengths of selection across environments were proportional to one another, i.e., $s_{\min} = a \times s_{\max}$, where $0 < a < 1$.

For each simulation, the number of mutations in the least-loaded class (the group of individuals with the fewest deleterious mutations) was tracked for 2000 generations. Ratchet rate for each treatment was determined from the average of 100 replicates. To measure the rate of fitness decline, cumulative geometric mean fitness (CGMF) of the
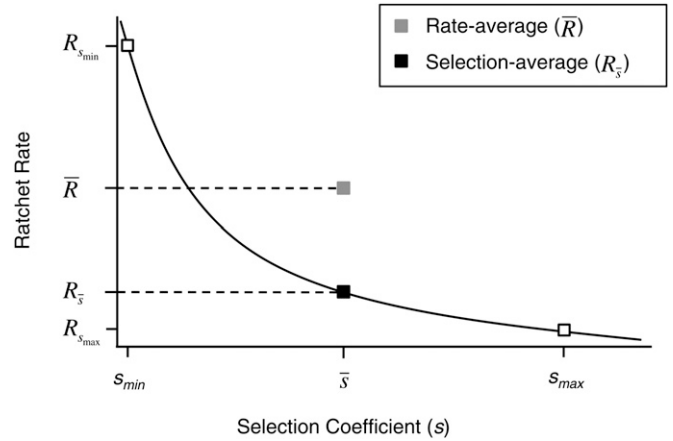

Figure 1 Ratchet rate as a function of selection strength. The solid curve shows a qualitative depiction of the rate of the ratchet as a function of s assuming no temporal variation in selection. If selection varies temporally such that $s = s_{\max}$ for a fraction ϕ of generations and $s = s_{\min}$ for the remainder $(1 - \phi)$, overall ratchet rate might be predicted by the rate average, $\bar{R} = \phi R_{s_{\max}} + (1 - \phi)R_{s_{\min}}$, or the selection average $(R_{\bar{s}})$, the rate of the ratchet if selection were held constant at $\bar{s}$, the geometric time average of $s_{\min}$ and $s_{\max}$.

least-loaded class was calculated at each generation using $\log(\text{CGMF}) = (1/t)\sum_{i=1}^{t} W_i$, where W_i is the fitness value of the least-loaded class in generation i. The rate of decline was estimated as the slope of the regression of the log-transformed CGMF data for 100 replicates.

Results

We first outline some "benchmark" predictions that are helpful in interpreting the simulation results. If the strength of selection varies temporally between two selection coefficients, $s_{\min}$ and $s_{\max}$, there are two alternative "null" predictions for the overall ratchet rate R (Figure 1). The first is that the realized R is equal to the rate of the ratchet if selection were held constant at $\bar{s}$, the geometric time average of $s_{\min}$ and $s_{\max}$. We refer to this as the "selection-average" prediction $(R_{\bar{s}})$. Alternatively, the realized R may simply equal the average of the ratchet rates at $s_{\min}$ and $s_{\max}$, i.e., the "rate average," $\bar{R} = \phi R_{s_{\max}} + (1 - \phi)R_{s_{\min}}$, where R_s is the rate under constant selection pressure s and ϕ is the fraction of generations where $s = s_{\max}$. It is always the case that $R_{\bar{s}} < \bar{R}$ because of the nonlinear relationship between s and R_s (Figure 1). The rate-average and selection-average predictions represent an upper and a lower bound, respectively, on overall ratchet rate for populations experiencing temporally fluctuating selection (Figure 1). Simulation runs were compared to the selection-average and rate-average predictions computed from simulation results of constant selection at $s_{\min}$, $s_{\max}$, and $\bar{s}$.

Conditional neutrality

We begin by considering the case of conditional neutrality ($0 = s_{\min} < s_{\max}$). With temporal variation in selection, the
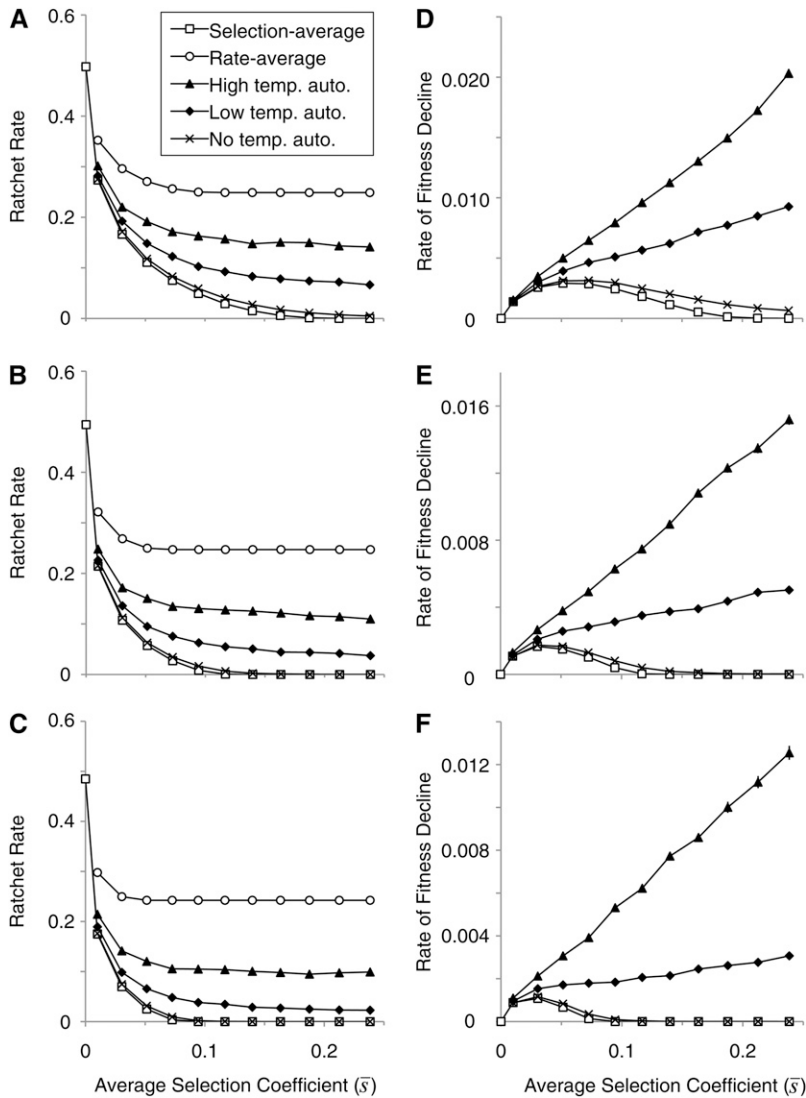
Figure 2 Effects of temporal heterogeneity on ratchet rate and fitness decline. (A–C) Average per generation rate of loss of the least-loaded class (ratchet rate) $\pm$ SE for a population of (A) 500, (B) 5000, and (C) 50,000. Simulation results are shown for three types of temporal heterogeneity (no, low, and high autocorrelation) as well as for the constant selection-average ($R_{\bar{s}}$) and rate-average ($\bar{R}$) predictions. (D–F) Mean rate of fitness decline of the least-loaded class $\pm$ SE in a population of (D) 500, (E) 5000, and (F) 50,000. Simulation results are shown for three types of temporal heterogeneity (no, low, and high autocorrelation) as well as for the constant selection-average scenario. In most cases SE bars are too small to see.

realized ratchet rate falls between the values predicted by $R_{\bar{s}}$ and $\bar{R}$. The realized ratchet rate is close to the selection-average prediction when there is temporal variation (but no autocorrelation) in selection and increases toward the rate-average prediction with increasing temporal autocorrelation (Figure 2, A–C).

To understand these results, it is useful to think about the underlying distribution of mutations in the population. The shape of this distribution determines the number of individuals in the least-loaded class and, thus, plays a key role in determining ratchet rate. Recall that the loss of the least-loaded class is followed by the reestablishment of mutation–selection balance (Stephan *et al.* 1993). The population must transition between the two mutation–selection balance equilibrium distributions (that of s_{min} and s_{max}) when the strength of selection changes. [For $s_{min} = 0$, the distribution is not shaped by mutation–selection balance but rather by mutation–drift balance. The mean of this distribution increases at the mutation rate but it maintains its characteristic shape (Fox and Wolf 2006)]. Consider the case where

selection rapidly oscillates between s_{min} and s_{max}. It is likely that the mutational distribution never obtains the shape predicted by either selection coefficient. Because selection changes rapidly, we can think of individual mutations experiencing the average selection so that the mutational distribution should be that expected under $\bar{s}$ and the ratchet proceeds at the corresponding rate, $R_{\bar{s}}$. At the other extreme, we can consider the limit of very strong temporal autocorrelation in which the population experiences one long period with $s = s_{min}$, followed by another long period with $s = s_{max}$. Relatively little time is spent transitioning between the distribution expected under s_{min} and that expected under s_{max}. Mutations accumulate at the rate $R_{s_{min}}$ during the first period and at $R_{s_{max}}$ during the second period so the average rate is equal to the rate average, $\bar{R}$. At more moderate levels of temporal autocorrelation, such as those considered here, it is reasonable to assume that a proportionally greater fraction of time is spent transitioning between the respective equilibrium distributions. Although it is difficult to find analytical approximations for the ratchet rate during these
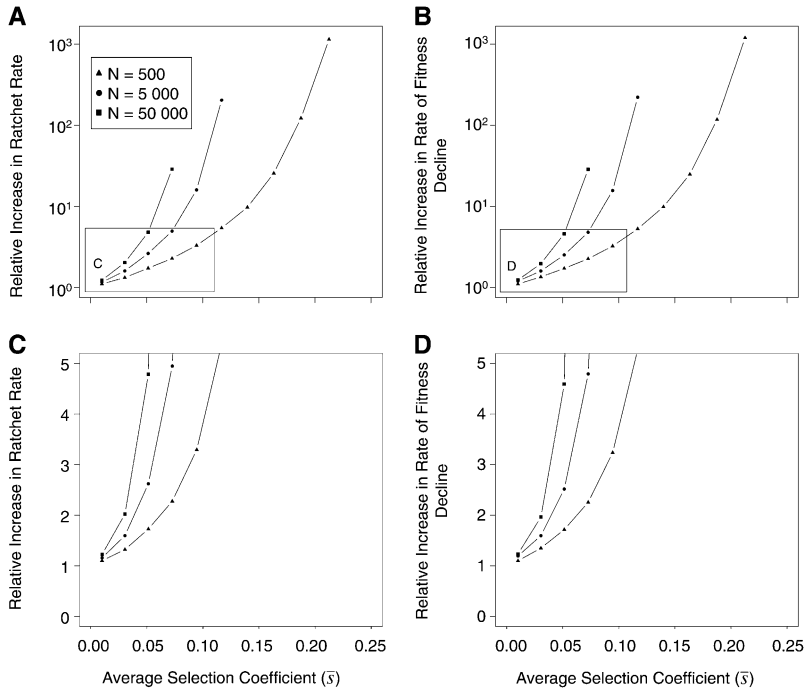
Figure 3 Effect of temporal heterogeneity relative to constant selection of equivalent strength. (A and B) Relative increase in (A) ratchet rate and (B) rate of fitness decline over that of populations experiencing the corresponding constant time-averaged selection coefficient. Relative increases are infinite for values of $\bar{s}$ beyond those shown because the ratchet rate is zero under constant selection. Data shown are for the high temporal autocorrelation selection regime only. For clarity, boxed regions of A and B are shown magnified in C and D, respectively.

transition periods, the logic outlined above provides a qualitative understanding of why the realized ratchet rate falls between the boundary conditions set by $R_{\bar{s}}$ and $\bar{R}$.

Even when there is no temporal autocorrelation, the realized ratchet rate is close to, but greater than, the selection-average prediction $R_{\bar{s}}$. In this case, the absolute difference in rates is small such that the two values appear almost indistinguishable on the scale shown in Figure 2, but the proportional differences can be reasonably large as $R_{\bar{s}}$ approaches zero. For example, with $N = 5000$ and $\bar{s} = 0.1168$, $R_{\bar{s}} = 6.25 \times 10^{-4}$, whereas the rate with temporal heterogeneity with no autocorrelation is $R = 6.51 \times 10^{-3}$, meaning that the ratchet rate is 10.41 times faster in the heterogeneous case.

Within each level of autocorrelation, the ratchet rate decreases with increasing selection strength before reaching a plateau at large $\bar{s}$ (Figure 2, A–C). Under constant selection, this plateau always occurs at a rate of zero ($R_{\bar{s}} = 0$). With temporal variation the plateau can occur at considerably higher rates, especially when there is temporal autocorrelation in selection. The nonzero rate occurs because we are considering conditionally neutral mutations so that, during periods when selection is relaxed, mutations can accumulate at the neutral rate regardless of how strong selection is during the periods when $s = s_{\max}$. The overall rate of fitness decline, however, may not level off at large $\bar{s}$, depending on the type of temporal heterogeneity (Figure 2, D–F).

As expected from past studies (Gabriel *et al.* 1993; Butcher 1995; Gordo and Campos 2008) under constant selection, the rate of decline of fitness, measured here as log(CGMF), is greatest at intermediate values of $\bar{s}$ and is approximately zero for very strong and very weak selection (Figure 2, D–F). Qualitatively similar results are observed

for the case of temporal heterogeneity when there is no autocorrelation in selection. In contrast, under temporal autocorrelation, the rate of fitness decline continues to increase with the strength of selection (Figure 2, D–F) even though the ratchet rate plateaus (Figure 2, A–C), as described above. Mutations are accumulating at the same rate across the plateau in Figure 2, A–C, but their effect on fitness when selection is operating increases in severity with $s_{\max}$, thus resulting in an increased rate of fitness decline.

Above we have described the effects of temporal heterogeneity in absolute terms. It is also worth considering these effects as the proportional change from the traditional scenario of constant selection of equivalent average strength. Relative to the ratchet with constant selection, the effects of temporal heterogeneity on ratchet rate and the decline in fitness increased with the strength of selection (Figure 3), reaching infinity as the ratchet rate grinds to a halt with increased s under constant selection. Importantly, these effects of temporal heterogeneity are more dramatic in large populations where the relative increase in ratchet rate and fitness decline over that of $\bar{s}$ reaches infinity at lower selection coefficients (Figure 3).

Varying the frequency of selection

For the conditionally neutral case, we varied ϕ, the frequency of generations in which mutations were nonneutral, but adjusted $s_{\max}$ accordingly to hold average selection constant at $\bar{s} = 0.0513$ (Figure 4). Consequently, in the context of Figure 4, temporal heterogeneity is maximized at low values of ϕ and minimized as ϕ approaches 1. Low values of ϕ correspond to situations where mutations are usually neutral but, in those generations where mutations are selected against, selection is strong (*e.g.*, during extreme
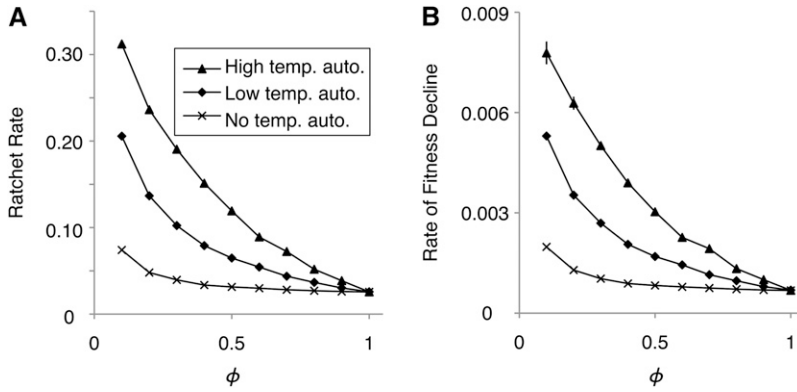
Figure 4 Effects of varying the frequency of selection on ratchet rate and fitness decline. Shown are (A) average per generation rate of loss of the least-loaded class (ratchet rate) $\pm$ SE and (B) mean rate of fitness decline of the least loaded class $\pm$ SE for a population of 50,000 experiencing selection varying between $s_{min} = 0$ and s_{max} such that the geometric time-averaged selection coefficient $\bar{s} = 0.0513$ for all values of ϕ. When $\phi = 1$, $s_{max} = \bar{s}$. Simulation results are shown for three types of temporal heterogeneity (no, low, and high temporal autocorrelation). In most cases SE bars are too small to see.

weather events). In contrast, high values of ϕ correspond to situations where mutations are selected in most generations at a more moderate level. For $\phi = 1$, mutations experience constant selection with $s = 0.0513$ every generation. As shown in Figure 4, ratchet rate and the rate of fitness decline decrease with increasing ϕ. In other words, the effects of the ratchet decline as the degree of temporal heterogeneity declines. Autocorrelation increases the effects of the ratchet for all values of ϕ but is particularly dramatic when temporal variation is high (*i.e.*, ϕ is low). This occurs because when selection is rare (low ϕ), there are reasonably long periods where $s = s_{min} = 0$ (even without temporal autocorrelation) during which mutations can accumulate. Adding temporal autocorrelation exaggerates this effect.

Quantitative variation in selection

A less extreme form of heterogeneity than conditional neutrality occurs when there is quantitative variation in selection ($0 < s_{min} < s_{max}$). Relative to the case of conditional neutrality with equivalent $\bar{s}$, we expect quantitative variation in selection to be more like constant selection for the simple reason that there is a smaller difference in selection strength between "harsh" and "benign" environments (*i.e.*, less heterogeneity). For comparison with results shown in Figure 2C, we use $\phi = 0.5$ and $N = 50,000$. When selection varies quantitatively, the patterns we observed for conditional neutrality (Figure 2C) weaken as the difference between s_{min} and s_{max} declines, as illustrated in Figure 5, A–C, showing $s_{min} = 0.01 \times s_{max}$, $0.05 \times s_{max}$, and $0.25 \times s_{max}$, respectively. Nonetheless, it is clear that even for this more subtle form of heterogeneity, the effects of Muller's ratchet are more severe than expected under constant selection of equivalent strength.

There is an important qualitative difference in the results between conditional neutrality and quantitative variation as we have modeled it. Unlike with conditional neutrality (Figure 2), with quantitative variation in selection the ratchet rate does not plateau above zero as $\bar{s}$ increases, but rather continues decreasing (Figure 5, A–C). This occurs because both s_{min} and s_{max} increase with $\bar{s}$ in the model we have used for quantitative variation in selection (in which s_{min} is proportional to s_{max}); consequently, mutations accu-

mulate at a slower rate as $\bar{s}$ increases. The ratchet can be brought to a complete halt if s_{min} is sufficiently large (as illustrated in Figure 5C). As a result of these effects on the ratchet rate, the decline in fitness may increase indefinitely with $\bar{s}$ (Figure 5D) or may be maximized at an intermediate $\bar{s}$ (Figure 5F), depending on whether s_{min} becomes sufficiently large relative to N and U to halt the ratchet. If we had modeled quantitative variation by fixing s_{min} (*e.g.*, $s_{min} = 0.001$), then we could have obtained results for these population sizes more similar to those for the conditional neutrality case in which the ratchet does not halt with increasing $\bar{s}$.

With conditional neutrality, the effects of autocorrelated temporal heterogeneity are exaggerated in larger populations (Figures 2 and 3). This is because the effect of population size on ratchet rate is different for constant selection and conditional neutrality. With constant selection, the ratchet slows as population size increases. However, with autocorrelated temporal heterogeneity, the reduction in net ratchet rate with increased N is lessened because the rate of accumulation during periods when selection is absent ($s = s_{min} = 0$) is unaffected by population size. This is not the case with quantitative variation in selection ($s_{min} > 0$), as the ratchet rate will always decline for any nonzero s as N increases. Nonetheless, we still observe considerable differences between autocorrelated temporal heterogeneity and constant selection at large population sizes provided s_{min} and s_{max} are not too similar (Figure 5).

Relaxing genome assumptions

For simplicity, we assumed that selection acts uniformly across the genome; *i.e.*, all genes are equally affected by the changing environment. In reality, fluctuations in the environment are more likely to induce strong selection on only a fraction of the genes in the genome. Thus, we also examined a model in which a fraction, F, of accumulating deleterious mutations experienced selection fluctuating between s_{min} and s_{max}, while the remaining fraction ($1 - F$) experienced a constant selection coefficient $s = \bar{s}$ (Figure 6). The former category of mutations can be thought of as being under selection only in particular circumstances, *e.g.*, years with extremely cold winters. The simulation model is similar
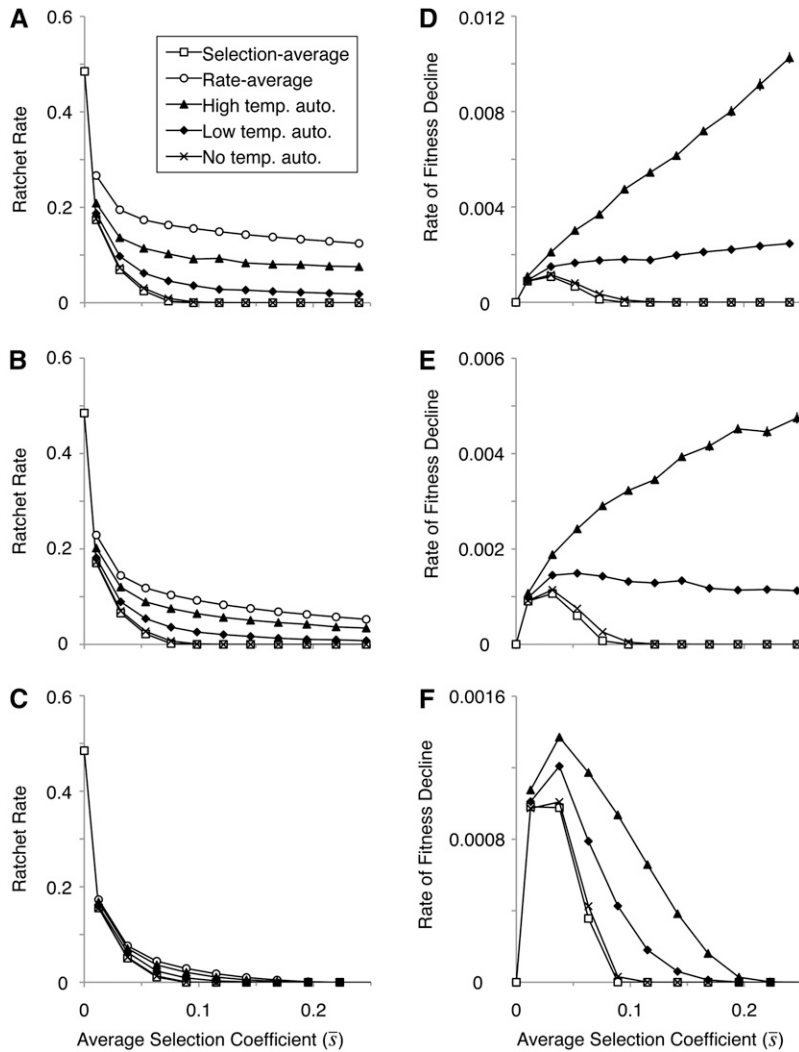
Figure 5 Effects of temporal heterogeneity with quantitative variation in selection on ratchet rate and fitness decline. (A–C) Average per generation rate of loss of the least-loaded class (ratchet rate) $\pm$ SE for a population of 50,000 experiencing selection varying between s_{max} and (A) $s_{min} = 0.01 \times s_{max}$, (B) $s_{min} = 0.05 \times s_{max}$, and (C) $s_{min} = 0.25 \times s_{max}$. Simulation results are shown for three types of temporal heterogeneity (no, low, and high autocorrelation) as well as for the constant selection-average (R_s) and rate-average ($\bar{R}$) predictions. (D–F) Mean rate of fitness decline of the least-loaded class $\pm$ SE for a population of 50,000 experiencing selection varying between s_{max} and (D) $s_{min} = 0.01*s_{max}$, (E) $s_{min} = 0.05 \times s_{max}$, and (F) $s_{min} = 0.25 \times s_{max}$. Simulation results are shown for three types of temporal heterogeneity (no, low, and high autocorrelation) as well as for the constant selection-average scenario. In most cases SE bars are too small to see.

to the one for the uniform genome except that members of the least-loaded class can have differing numbers of mutations in each genome component. Consequently, not every member of the class has the same fitness value and the fittest member can change from generation to generation (regardless of whether or not there is mutation accumulation) if the strength of selection against the fluctuating genome component changes.

Results were qualitatively similar to those where selection is uniform across the genome. Ratchet rate and fitness decline increased with the fraction of the genome under fluctuating selection (Figure 6). More precisely, ratchet rate can be accurately predicted ($r^2 = 0.996$) by calculating the weighted average $R = F*R_{fluctuating} + (1 - F)*R_{constant}$, where $R_{fluctuating}$ is the rate if 100% of the genome experiences fluctuating selection and $R_{constant}$ is the rate if 100% of the genome experiences constant selection. Fitness decline can be predicted analogously ($r^2 = 0.994$).

We also considered situations where different components of the genome experienced temporal variation in selection independently of one another or in a negatively

correlated fashion (out of phase) and found qualitatively similar results (not shown) to those when selection fluctuates uniformly across the genome. In sum, there is no reason to believe that the types of effects reported here do not apply to more realistic scenarios when scaled appropriately for the amount of variation and temporal autocorrelation.

Discussion

In principle, the accumulation of mutations through Muller's ratchet can lead to the decay of nonrecombining chromosomes and the extinction of asexual species. Estimating the ratchet rate and the rate of fitness decline can provide some insight into how fast asexual populations are speeding toward their demise. The accuracy of these estimates, however, depends on simplifying assumptions that are not always realistic about the nature of mutation and selection. Largely overlooked in the Muller's ratchet literature, temporal heterogeneity in selection is common in nature and has the potential to cause serious consequences for nonrecombining genomes. Indeed, we found that temporal variation
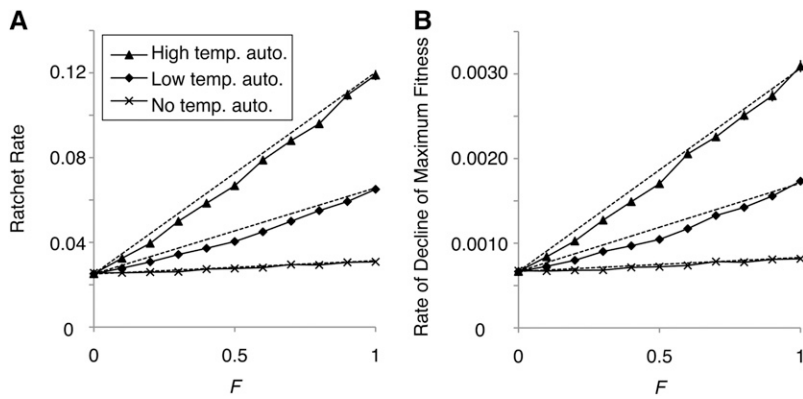
Figure 6 Effects of varying the fraction of the genome experiencing fluctuating selection on ratchet rate and fitness decline. Shown are (A) average per generation rate of loss of the least-loaded class (ratchet rate) $\pm$ SE and (B) mean rate of fitness decline of the fittest individual in the least-loaded class $\pm$ SE for a population of 50,000 experiencing selection varying between $s_{min} = 0$ and $s_{max} = 0.1$ on a fraction, F, of its genome. The remainder of the genome experiences constant selection $\bar{s} = 0.0513$ such that the geometric time-averaged selection coefficient across the genome is also $\bar{s} = 0.0513$. Simulation results are shown for three types of temporal heterogeneity (no, low, and high temporal autocorrelation) with predictions for overall ratchet rate based on $R = F \times R_{fluctuating} + (1 - F) \times R_{constant}$ shown by the corresponding dashed line. Predictions for rate of fitness decline are calculated analogously. In most cases SE bars are too small to see.

in selection, especially when there is some autocorrelation, dramatically increases the ratchet rate and fitness decline over those of populations experiencing constant selection of equivalent average strength.

Ratchet rate under temporal variation was bounded by the selection-average and rate-average predictions ($R_{\bar{s}}$ and $\bar{R}$, respectively), increasing toward the latter when temporal autocorrelation was stronger (Figures 2, A–C, and 5, A–C). We observed reasonably dramatic effects of temporal heterogeneity even without long periods of time between environmental changes; in Figures 2 and 5, "low" and "high" levels of autocorrelation correspond to average runs of consecutive generations with the same selection strength of 10.2 and 41.0 generations, respectively. Thus, with relatively little temporal autocorrelation, the corresponding increases in ratchet rate can have severe fitness consequences for asexual populations.

In the classic ratchet model, the rate of fitness decline peaks at intermediate values of s and decreases as increasing selection strength slows the rate of mutation accumulation (Gabriel *et al.* 1993; Butcher 1995; Gordo and Campos 2008). While ratchet rate slows down with increasing average selection strength (Figures 2, A–C, and 5, A–C), fitness decline under temporal autocorrelation in selection does not always slow down with increasing $\bar{s}$ (Figures 2, D–F, and 5, D and E). In fact, fitness decline can increase indefinitely under conditional neutrality (Figure 2, D–F). Conditionally neutral mutations can accumulate when selection is relaxed, regardless of their fitness effects during those periods when mutations are subject to selection. Consequently, fitness decline continues increasing with s_{max} (Figure 2, D–F).

Increases in ratchet rate and fitness decline with temporal heterogeneity are observed across a range of population sizes. Although the consequences of Muller's ratchet are often considered to be pertinent only to small populations, temporal heterogeneity in selection, particularly when there is some autocorrelation, amplifies the effects of the ratchet in larger populations (see Figures 2 and 3). Most notably, under conditional neutrality, temporal autocorrelation allows the ratchet to operate at any finite size because the least-loaded class is lost deterministically through mutation

when selection is "off". Thus, large populations of nonrecombining genomes may be declining in fitness faster than previously thought.

Elevated rates of fitness decline can have dire consequences for asexual populations by increasing the advantage of sex or the probability of extinction. Gordo and Campos (2008) investigated the spread of a recombination modifier allele through asexual populations accumulating unconditionally deleterious mutations by Muller's ratchet. They found that the probability of fixation of the modifier allele was maximized at intermediate values of s, where mean fitness decline was maximized in the corresponding asexual population. Following this logic, we might expect temporal autocorrelation in selection to increase the advantage of sex because we found that rates of fitness decline for asexuals are increased by temporal autocorrelation. However, in the case of conditional neutrality, there will be no advantage to sex during those generations when selection is off. We speculate that the critical issue is the average reduction in the genetic variance in fitness due to linkage disequilibrium. The reduction in variance will be zero during the neutral phases (because both the expected and the realized variance in fitness will be zero) but the variance in fitness is likely to be strongly reduced during the selective phases because of negative disequilibrium that arises from the interaction of selection and drift acting on the mutations that accumulated during the neutral phases. In future work, we will explicitly examine the consequences of temporal variation on modifiers of recombination.

Given the prevalence of temporal heterogeneity in the wild (see Siepielski *et al.* 2009 and references therein), future studies should incorporate fluctuating selection into estimates of mean extinction times. Furthermore, these estimates should also consider the mutational meltdown in asexual populations (Lynch *et al.* 1993). Briefly, the loss of fitness due to accumulating deleterious mutations leads to an eventual reduction in population size, which further increases the chance of mutation accumulation. This positive feedback loop accelerates asexual populations toward extinction (Lynch *et al.* 1993). On the basis of our results, temporal heterogeneity, and in particular temporal

autocorrelation in selection, would reduce the time to mutational meltdown in small and large populations.

The role of varying selection in mutational meltdowns has been studied in one particular context. Specifically, Howard and Lively (1994), and others since (Howard and Lively 1998; Park *et al.* 2010), investigated the competitive ability of sexual *vs.* asexual populations of hosts in the presence of coevolving parasites and deleterious mutations. By focusing on the asexuals, we can compare their model with ours. Howard and Lively (1994) assume most of the genome experiences constant selection. However, a small number of loci (those involved in parasite resistance) experience a very strong form of temporally fluctuating selection in which alleles change from being selectively favored to disfavored in repeated cycles. Because selection on these loci fluctuates in sign and can be much stronger than on "regular" genes, mutations can rapidly accumulate by Muller's ratchet in susceptible genotypes when they are driven to low numbers by parasites. Later in the coevolutionary cycle, when those previously susceptible genotypes become resistant to common parasites, genotypes loaded with numerous unconditionally deleterious alleles of small effect may be favored over genomes with fewer deleterious alleles because of parasite-mediated selection. Thus, the strong and fluctuating selection on resistance loci can drive the accumulation of deleterious alleles in the rest of the genome. In our model, when selection is weak or off, deleterious alleles accumulate by mutation pressure rather than because they are linked to genes favored in the changed environment. Had we allowed for reversals in the sign of selection, we would expect even more dramatic effects of temporal heterogeneity.

While environmental heterogeneity is common, it remains a major empirical challenge to determine to what extent selection varies substantially in strength or direction across environments. Although changes in direction may be limited to a small subset of genes, changes in magnitude are likely to be quite common (Kishony and Leibler 2003; Jasnos *et al.* 2008; Wang *et al.* 2009). Environmental variation may therefore have damaging effects on asexual populations beyond those expected under constant selection.

Acknowledgments

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Communicating editor: L. M. Wahl