Dynamic Mutation–Selection Balance as an Evolutionary Attractor

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ABSTRACT The vast majority of mutations are deleterious and are eliminated by purifying selection. Yet in finite asexual populations, purifying selection cannot completely prevent the accumulation of deleterious mutations due to Muller's ratchet: once lost by stochastic drift, the most-fit class of genotypes is lost forever. If deleterious mutations are weakly selected, Muller's ratchet can lead to a rapid degradation of population fitness. Evidently, the long-term stability of an asexual population requires an influx of beneficial mutations that continuously compensate for the accumulation of the weakly deleterious ones. Hence any stable evolutionary state of a population in a static environment must involve a dynamic mutation–selection balance, where accumulation of deleterious mutations is on average offset by the influx of beneficial mutations. We argue that such a state can exist for any population size N and mutation rate U and calculate the fraction of beneficial mutations, ε, that maintains the balanced state. We find that a surprisingly low ε suffices to achieve stability, even in small populations in the face of high mutation rates and weak selection, maintaining a well-adapted population in spite of Muller's ratchet. This may explain the maintenance of mitochondria and other asexual genomes.

PURIFYING selection maintains well-adapted genotypes in the face of deleterious mutations (Haigh 1978). Yet in asexual populations, random genetic drift in the most-fit class of individuals will occasionally lead to its irreversible extinction, a process known as Muller's ratchet (Muller 1964; Felsenstein 1974). The repetitive action of the ratchet leads to the accumulation of deleterious mutations, despite the action of purifying selection. This ratchet effect has been extensively analyzed (Gessler 1995; Charlesworth and Charlesworth 1997; Gordo and Charlesworth 2000a,b; Stephan and Kim 2002; Jain 2008) and has been observed in experiments (Chao 1990; Duarte *et al.* 1992; Andersson and Hughes 1996; Zeyl *et al.* 2001) and in nature (Rice 1994; Lynch 1996; Howe and Denver 2008). In small populations when deleterious mutation rates are high or selection pressures are weak, the ratchet can proceed quickly, causing rapid degradation of asexual genomes (Gabriel and Burger 1993; Lynch *et al.* 1993, 1995). Hence Muller's ratchet has been described as a central problem for the maintenance of asexual populations such as mitochondria (Loewe 2006). Avoiding this mutational catastrophe is thought to be a major benefit of sex and recombination (see Barton and Charlesworth 1998 and De Visser and Elena 2007 for reviews).

However, new studies indicate that natural and laboratory asexual populations do not always melt down as predicted. For example, Silander *et al.* (2007) recently showed that even very small laboratory populations of phage with high mutation rates tend toward fitness plateaus. In addition, a recent comparison of human, chimpanzee, and rhesus Y chromosomes demonstrated that after an initial period of degradation following the halt of recombination, gene loss in the human Y chromosome effectively stopped (Hughes *et al.* 2012). Finally, theoretical work by Loewe (2006) has revealed several "genomic decay paradoxes" (*i.e.*, species that persist despite predicted unsustainable genomic decay), including human mitochondria.

These results argue for a reexamination of the assumptions behind the classic ratchet model. In the absence of

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recombination (Bell 1998) or epistasis (Kondrashov 1994), the only forces that can check the deterioration of fitness due to the accumulation of deleterious mutations are back and compensatory mutations (Schultz and Lynch 1997). These mutations are typically assumed to be rare and are either neglected in models of the ratchet or assumed to only slightly slow its rate (Haigh 1978; Kondrashov 1995; Lande 1998; Bachtrog and Gordo 2004). However, as most recently discussed by Charlesworth (2012), it is natural to expect that these back and compensatory mutations become more common as a population declines in fitness. At a minimum, back mutations that revert deleterious point mutations will cause the proportion of available beneficial mutations to increase linearly with the accumulation of these deleterious point mutations. Recent experimental work suggests that the pool of available compensatory mutations in fact increases even faster than this (Silander *et al.* 2007; Barrick *et al.* 2010). Hence, based on experimental evidence it is reasonable to assume that the probability that a random mutation is beneficial increases with decreasing absolute fitness (Escarmis *et al.* 1999; Estes and Lynch 2003; Poon and Chao 2005; Poon *et al.* 2005; Schoustra *et al.* 2009). Equivalently, but perhaps more intuitively, one expects the probability that a random mutation is beneficial to decrease with increasing fitness.

Provided that beneficial (back and compensatory) mutation rates do increase as fitness declines, Muller's ratchet will eventually come to a halt. Once the fraction of beneficial mutations is high enough to counter the ratchet, the population will remain in a stable dynamic equilibrium state, as illustrated in Figure 1. Qualitatively, we can see that the state is stable from the following argument: let the critical fraction of beneficial mutations needed to precisely counter the ratchet and maintain the equilibrium be ε_c. In a poorly adapted population, a higher fraction of mutations will be beneficial, $\varepsilon > \varepsilon_c$. This excess of beneficial mutations will push the population toward higher fitness. At the same time, adaptation will deplete the available pool of beneficial mutations, until ε_c is reached, as shown in Figure 1. Conversely, in an "overadapted" population, we expect $\varepsilon < \varepsilon_c$, so that deleterious mutations dominate, reducing population fitness until ε_c is recovered. Thus we expect the dynamic mutation–selection balance point to be a stable evolutionary "attractor." Furthermore, since back and compensatory mutations are selectively favored, they can balance deleterious mutations even while they are relatively rare, thereby maintaining a well-adapted population. This mechanism may be responsible for the fitness plateaus observed in the natural and laboratory populations mentioned above. Moreover, we expect these "treadmill" dynamics, in which continual fixation of deleterious mutations due to the ratchet is exactly offset by fixation of back and compensatory mutations, to be the generic null state of an asexual population under purifying selection. Characterizing this state is an important step toward predicting the patterns of genetic diversity these populations maintain.

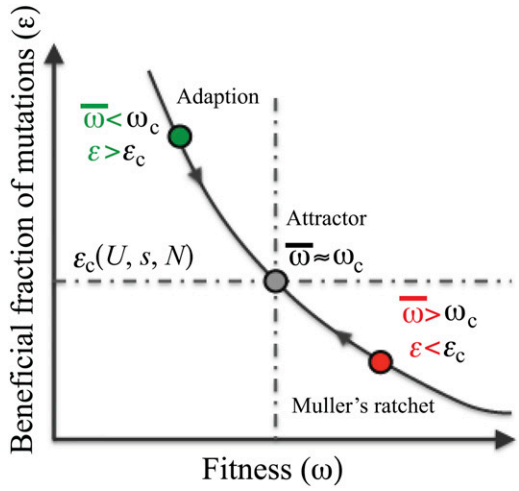

Figure 1 The relationship between the fraction of mutations that are beneficial, ε, and the absolute fitness of the population, ω. A poorly adapted population (green) with $\bar{\omega} < \omega_c$ and $\varepsilon < \varepsilon_c$ will adapt toward the dynamic equilibrium "attractor" state (gray) with higher fitness and lower ε. Conversely, an "overadapted" population (red) with $\bar{\omega} > \omega_c$ and $\varepsilon < \varepsilon_c$ will decline in fitness toward the dynamic equilibrium state due to Muller's ratchet.

Several earlier studies have considered aspects of these treadmill dynamics in which both beneficial and deleterious mutations accumulate, including simulations by Wagner and Gabriel (1990), Antezana and Hudson (1997), and Schultz and Lynch (1997); experimental work by Silander *et al.* (2007); and a combination of simulations and analytical work by Rouzine *et al.* (2003, 2008) and Manrubia *et al.* (2003). Theoretical work by Poon and Otto (2000) also considered compensatory mutations using Fisher's geometrical model, and analytical work by Lande (1998) described alternate fixation of deleterious and beneficial mutations in small sexual populations. These studies have all shown that finite populations settle to fitness plateaus in the presence of purifying selection. However, the nature of this dynamic equilibrium state remains poorly understood: the critical fraction of beneficial mutations ε_c, the mean fitness of the population at equilibrium, and the distribution about that mean all have yet to be characterized.

In this article, we present a detailed analytical description of the nature of the dynamic equilibrium mutation–selection balance. Crucially, we decouple the problem of the global stability of the dynamic balance state (which depends on how the rate of compensatory mutations changes with absolute fitness) from the properties of the dynamic balance state itself. We accomplish this by treating the probability that a mutation is beneficial as an independent parameter ε. The dependence of ε on absolute fitness becomes important for the evolutionary dynamics of the population away from the equilibrium point, but not at the fixed point itself. This allows us to identify the dynamic balance condition $\varepsilon = \varepsilon_c$ and determine how it depends on population size, mutation rate, and strength of selection, independent of any assumptions regarding the dependence of ε on absolute fitness.

We show that even a very modest rate of compensatory mutations is sufficient to forestall Muller's ratchet. Even for remarkably high mutation rates and small population sizes, dynamic balance can be maintained with most mutations being deleterious. Muller's ratchet notwithstanding, selection enables rare beneficial mutations to compensate for more frequent deleterious mutations and to maintain a well-adapted population.

Materials and Methods

Simulation methods

Our simulations are done using a custom-written Python code available on request. We implement a discrete-time Wright–Fisher model where the population is represented by a vector n_k with elements corresponding to the number of individuals in fitness class k. Each generation consists of separate selection and mutation steps. To implement selection, the vector n_k is multiplied by $e^{s(k-\bar{k})-\alpha}$ to obtain a vector $\tilde{n}_k$ containing the expected number of offspring in class k. Here $(k-\bar{k})$ is the fitness of class k relative to the population mean, and $\alpha = (\sum n_k - N)/N$ maintains an approximately constant population size around N.

Our model is simplified by using beneficial and deleterious mutation with effect $\pm s$ and mutation rate $U\varepsilon$ and $U(1-\varepsilon)$, respectively. To implement mutation, we calculate the probability $P(i, j)$ of a genome being hit by i beneficial and j deleterious mutations, which are Poisson distributed. This mutation matrix is then applied to $\tilde{n}_k$; *i.e.*, the parts of $\tilde{n}_k$ are moved up or down according to the net number of mutations they accrued in this time step. Having constructed the expected number of individuals in fitness class k after selection and mutation, we draw a population sample from each class from a Poisson distribution. If necessary, the population is recentered in the discrete vector n_k. This prevents occupied classes from running off the grid due to accumulated increase or decrease in their absolute fitness.

The above process is repeated for a specified number of generations. The speed of adaptation, v (*i.e.*, the rate of change of population-averaged fitness), and other features of the dynamics are measured after an equilibration time to remove transient effects from the initial conditions. In the parameter regimes studied, we found that 10^4 generations were generally sufficient for establishing a steady "traveling wave" with velocity v dependent on ε and other parameters.

To solve for $\varepsilon_c(U, N, s)$, we rerun the simulation while iteratively adjusting ε to get v as close to zero as possible. Since v is a fluctuating quantity, ε_c can be determined only with limited accuracy, but this can be improved by increasing the number of generations for each run.

We have also run simulations in which ε increases with decreasing fitness (*i.e.*, assuming $\varepsilon_k = \varepsilon_0 - ck$, where c is a small constant) to demonstrate that the population indeed evolves toward the values of k such that the nose of the distribution is at the appropriate ε_c for local dynamical balance.

Model

Our analysis is based on the standard discrete-generation Wright–Fisher model with population size fixed at N. For the bulk of this article, we assume that beneficial and deleterious mutations increase or decrease fitness by the same constant amount s (where $s > 0$ by convention). In a section below, we use simulations and bounding analysis to show that similar behavior occurs when beneficial and deleterious mutations have different fitness effects. In our simple model with a single s, each individual can be described by its number, k, of deleterious mutations relative to the perfectly adapted state. We define $\bar{k}$ to be the mean number of deleterious mutations per individual, so that an individual with k deleterious mutations has fitness $s(\bar{k}-k)$ relative to the mean, as shown in Figure 2. The population can be characterized by the number of individuals n_k in each of these fitness classes. We define U to be the total mutation rate and ε to be the fraction of mutations that are beneficial, so that the beneficial mutation rate is $U_b = U\varepsilon$ and the deleterious mutation rate is $U_d = U(1-\varepsilon)$.

Although mutations and genetic drift are stochastic processes, in the bulk of the distribution—where fitness classes contain many individuals—the population dynamics are well captured by a deterministic approximation. On average, the number of individuals in fitness class k evolves as

$$\frac{dn_k}{dt} = \underbrace{s(\bar{k}-k)n_k}_{\text{I}} - \underbrace{Un_k}_{\text{II}} + \underbrace{U_d n_{k-1}}_{\text{III}} + \underbrace{U_b n_{k+1}}_{\text{IV}}. \qquad (1)$$

The terms on the right-hand side of this equation describe the different processes acting on fitness class k: (I) the effect of selection relative to the mean fitness, (II) mutational load, (III) deleterious mutations from more-fit individuals, and (IV) beneficial mutations from less-fit individuals. We assume that the selective effect of a single mutation is small, $s \ll 1$.

Although we expect that the probability a mutation is beneficial will depend on the absolute fitness and hence on k, as discussed in the Introduction, we assume ε to be an independent constant parameter. The neglect of the k dependence of ε is a reasonable approximation because at any given time there will be only a relatively narrow range of k present in the population. The k dependence of ε becomes important away from the dynamic-balance point corresponding to the stationary solution of Equation 1, since on longer timescales $\bar{k}$ and hence the population mean fitness begin to increase or decrease significantly. This dependence—assuming that ε increases with decreasing absolute fitness—ensures the global stability of the dynamic balance point, making it an evolutionary attractor. Yet our approximation that ε is locally constant allows us to accurately compute the ε_c, and corresponding distribution n_k, at which the population stays on average at a constant fitness. We have tested this approximation against simulations that introduce k dependence of ε and verified that our characterization

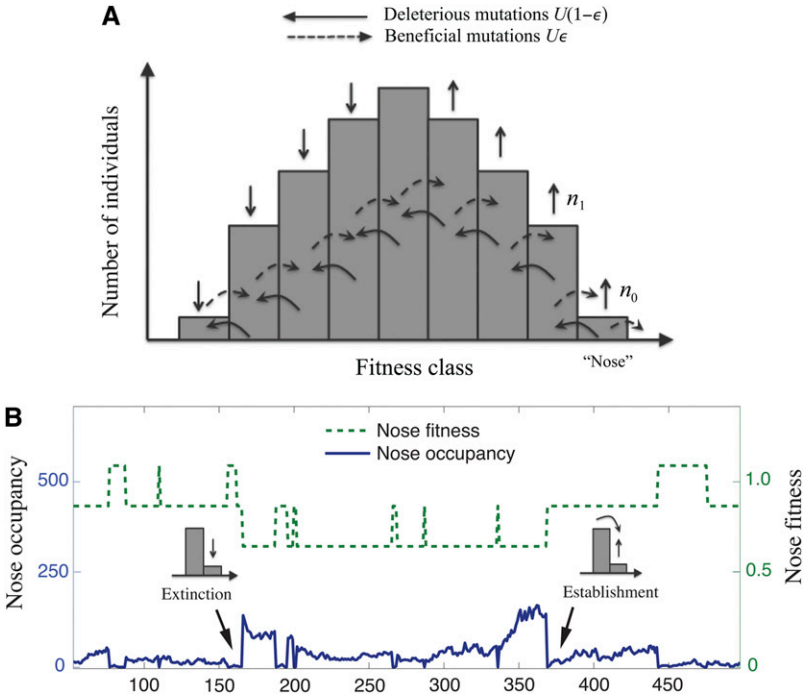

Figure 2 (A) Schematic illustration of the fitness distribution within a population. We refer to the most-fit class as the "nose" of the distribution. (B) A typical realization of the stochastic dynamics at the nose. In the event of extinction of the nose, n_1 becomes the new nose and relative nose fitness decreases by s. Conversely, when a more-fit nose is established, the relative nose fitness increases by s.

of the dynamic equilibrium remains accurate; these simulations are described in supporting information, File S1.

We also note that by defining fitness relative to the population mean in Equation 1, as is standard in Wright–Fisher dynamics, we neglect the possible dependence of population size on absolute fitness, an effect that could potentially lead to mutational meltdown. We return to this possibility in the *Discussion*.

Analysis

In steady state, the fitness distribution n_k stays constant on average: $dn_k/dt = 0$ for all k. Solving Equation 1 for the steady state in the case of $\varepsilon = 0$ leads to the familiar mutation–selection balance (Haigh 1978) with a Poisson distribution of fitness,

$$n_k = \frac{Ne^{-\lambda}}{k!}\lambda^k, \tag{2}$$

where $k = 0$ corresponds to the most-fit class. Here we have defined

$$\lambda = \frac{U}{s}. \tag{3}$$

This dimensionless ratio is a key parameter that appears often in the analysis below.

The deterministic approximation (Equation 1) corresponds to an infinite population where Muller's ratchet (*i.e.*, the stochastic extinction of the fittest genotype) does not operate. In the absence of such stochastic extinction, any beneficial mutation from the fittest class would move the distribution toward higher fitness, in conflict with the steady-state assumption. Hence, in infinite populations, steady state can be achieved only with $\varepsilon_c = 0$. Conversely, in a finite population where the fittest class can be lost due to genetic drift, Muller's ratchet will eventually lead to a decrease in fitness if no beneficial mutations are available. Thus we must have $\varepsilon_c > 0$ to be in the dynamic steady state.

A correct description of the dynamic equilibrium state therefore requires a suitable treatment of genetic drift. These stochastic effects are particularly important in the most-fit edge of the distribution, which we call the "nose," where the number of individuals with a particular fitness is small. Our analysis is based on matching a stochastic treatment of the nose with a deterministic description of the bulk of the fitness distribution (as governed by Equation 1 with $\varepsilon > 0$). The stochastic dynamics of the nose are determined by two competing processes: the random extinction of the most-fit class *vs.* the establishment of a new more-fit class due to a beneficial mutation. At stationarity, the rates of these two processes have to be equal; this condition determines the number of individuals in the nose class. We match this stochastic condition with the number of individuals in the nose class calculated based on the deterministic distribution. This determines the critical fraction of beneficial mutations ε_c as a function of the population parameters.

The Shape of the Fitness Distribution

We begin with a deterministic analysis of the shape of the fitness distribution at steady state. Since only relative fitnesses matter, it is convenient to set the origin of k such that the mean of the fitness distribution is $\bar{k} = \lambda$. In File S1, we solve for the steady state of Equation 1, using Fourier analysis. We find

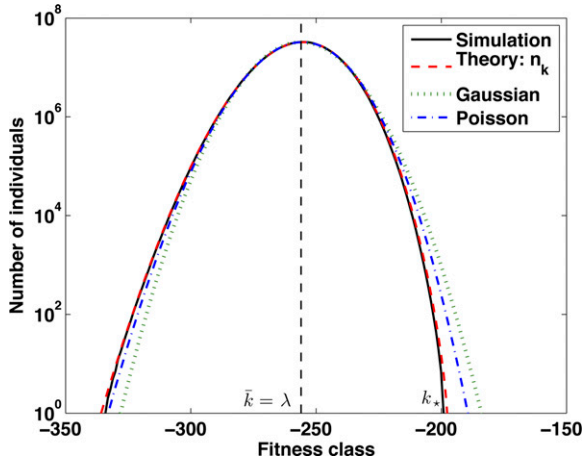

Figure 3 Comparison of the fitness distribution obtained in simulations with the analytic result Equation 4 and a Gaussian with equal mean and variance. The simulation result is the median fitness distribution observed over 10^6 generations with parameters $\lambda = 256$, $Ns = 1.25 \times 10^6$, and $N = 10^9$.

$$n_k = Ne^{-\lambda(1-2\varepsilon)+k\,\log\sqrt{(1-\varepsilon)/\varepsilon}} J_k(2\alpha), \tag{4}$$

where J_k denotes the Bessel function of order k and we have defined $\alpha \equiv \lambda\sqrt{\varepsilon(1-\varepsilon)}$. A comparison of this solution to simulation results is shown in Figure 3. For k in the vicinity of $\bar{k}$, this distribution is approximately Gaussian with variance $\sigma_k^2 = \lambda(1-2\varepsilon)$. While the low-fitness tail of the distribution decays less rapidly than a Gaussian, the high-fitness side decays more rapidly than a Gaussian.

It is important to note that in the deterministic limit there is no true stationary state for arbitrary $\varepsilon > 0$; this manifests itself in the loss of positivity of the solution given by Equation 4 for high-fitness classes above the position of the nose class. While the position of the nose, which we call $k_\star$, requires a more careful treatment, Equation 4 gives a very accurate description of the *bulk* of the fitness distribution (*i.e.*, n_k for $k > k_\star$).

To determine the position of the nose, $k_\star$, we observe that if classes with $k < k_\star$ carry no individuals, fitness class $k_\star$ does not receive an influx of mutations from the more-fit class $k_\star - 1$. That is, term III in Equation 1 is absent, yielding $(\bar{k} - k_\star - \lambda)n_{k_\star} + \lambda\varepsilon n_{k_\star+1} = 0$. This, along with Equation 4, gives the following equation for $k_\star$:

$$k_\star J_{k_\star}(2\alpha) = \alpha\, J_{k_\star+1}(2\alpha). \tag{5}$$

This must in general be solved numerically. However, in File S1 we show that

$$k_\star \approx \begin{cases} \lambda^2\varepsilon & \text{for } \varepsilon\lambda^2 \ll 1 \\ 2\lambda\sqrt{\varepsilon(1-\varepsilon)} & \text{for } \varepsilon\lambda^2 \gg 1. \end{cases} \tag{6}$$

To gain some intuition into the significance of $k_\star$, note that $k_\star = 0$ in an infinite population with $\varepsilon_c = 0$. In this case, the fitness distribution is the familiar Poisson distribution and the fittest class contains a fraction $e^{-\lambda}$ of all individuals. In a finite population, $Ne^{-\lambda} < 1$ for sufficiently large $\lambda = U/s$, which implies that at steady state the mutation-free genotype is typically absent and therefore $k_\star > 0$. This adjustment of $k_\star$ for finite population size is reflected in Equation 6, which states that the most-fit class gets closer to the population mean as ε increases.

The Stochastic Matching Condition

We seek to determine the $\varepsilon = \varepsilon_c$ at which the distribution is stationary for a given finite N. Since the most-fit class is populated by a comparatively small number of individuals, fluctuations due to genetic drift may change its occupancy significantly. In particular, the population at the nose can go extinct, in which case the next fitness class becomes the new nose. Alternatively, a lucky beneficial mutation can cause the nose to advance by one class. Stationarity can therefore be achieved only in an average sense: the rate of advancing the nose has to equal the rate of extinction of the nose.

Since these two processes depend sensitively on the number of individuals in the nose, requiring the equality of extinction and establishment rates determines the average size of the population of the nose, $n_{k_\star}$, as a function of U, s, and ε. We match this $n_{k_\star}$ to the deterministic solution determined above to find $\varepsilon_c(N, s, U)$.

The nature of the dynamics at the nose depends qualitatively on $n_{k_\star}$. If $n_{k_\star}$ is large enough that its dynamics are dominated by selection, it is rarely lost and Muller's ratchet is slow. Conversely, if $n_{k_\star}$ is small, the class turns over neutrally and is easily lost and reseeded. We begin by considering the slow-ratchet regime, where $n_{k_\star}$ is relatively large, and then turn to the opposite fast-ratchet regime.

Slow-ratchet regime

In the regime where $n_{k_\star}s > 1$, the fittest class is only rarely lost due to drift. Note that this regime corresponds to $\varepsilon\lambda^2 < 1$, which implies $n_{k_\star} \approx Ne^{-\lambda}$ and $k_\star \approx 0$. In this regime the mean extinction rate for the nose, r_-, is due to rare large fluctuations that overcome the "restoring force" due to selection trying to preserve mutation–selection balance. Estimates of this extinction rate have been obtained via diffusion theory (Haigh 1978; Gordo and Charlesworth 2000a; Stephan and Kim 2002; Jain 2008; Neher and Shraiman 2012) and have the form (see File S1) $r_- \approx e^{-\gamma s n_{k_\star}}\gamma s\sqrt{\gamma s n_{k_\star}/\pi}$, where γ is a "phenomenological" parameter characterizing the effective strength of selection on the fittest class, introduced by Haigh (1978) and used in Gordo and Charlesworth (2000a), Stephan and Kim (2002), and Jain (2008) (see below).

To impose our stochastic condition, we must also calculate the rate at which a new more-fit class establishes, r_+. This is given by the product of the rate at which new beneficial mutants are generated and the probability they establish, $r_+ = n_{k_\star}U_b P_{\text{est}}$. Since $k_\star \approx 0$ in this regime, we have $P_{\text{est}} \approx 2s$. Equating the two rates yields the stochastic condition

$$\varepsilon_c = \frac{\gamma^2}{2U/s} \frac{e^{-\gamma s N e^{-\lambda}}}{\sqrt{\pi \gamma s N e^{\lambda}}}. \quad (7)$$

This condition suggests that ε_c in the slow-ratchet regime is a function of a combination of population parameters: $\lambda\varepsilon_c(U, N, s)/\gamma^2 \sim f(\gamma s N e^{-\lambda})$. We find that contrary to earlier studies (Haigh 1978; Gordo and Charlesworth 2000a), constant γ is not consistent with simulations. Instead, as shown in Figure 4A, the simulation data in the presented range are well explained by $\gamma = 1/\sqrt{\lambda}$, which for values of λ considered, is a good approximation to the more general expression found in Neher and Shraiman 2012. This empirical dependence is, however, consistent with the approximation $\gamma \approx 0.6$ used by Gordo and Charlesworth (2000a), within the range of parameters $(0.4 \lesssim 1/\sqrt{\lambda} \lesssim 0.8)$ addressed by their study. Since we consider a much broader range of parameters $(0.1 \lesssim 1/\sqrt{\lambda} \lesssim 1)$, accounting for the dependence of γ on U/s becomes important. Our analytical solution Equation 7, shown as a dotted line in Figure 4A, is in good agreement with the simulation data across this wide range of parameters.

In this regime, ε_c depends exponentially on $Nse^{-\lambda}$ and hence rapidly approaches zero as λ becomes small, approaching the infinite population limit where Muller's ratchet does not operate.

Fast-ratchet regime

For larger mutation rate, smaller population size, or weaker selection, the occupancy of the most-fit class decreases, thereby increasing its rate of extinction. Consequently, a higher rate of beneficial mutations (larger ε) is required to match the extinction rate. The resulting rapid turnover of the population at the nose leads to the failure of the quasi-static approximation we used in the slow-ratchet regime.

As the occupancy of the nose decreases, in particular when $n_{k_\star} s \lesssim 1$, the dynamics of the fittest class are governed by drift. The rate of extinction, r_-, can therefore be estimated from neutral diffusion: $r_- \approx 1/(2n_{k_\star})$. The rate at which a new more-fit class is established is given by the same formula as before, $r_+ = \varepsilon U n_{k_\star} P_{\text{est}}$. However, P_{est} now refers to the probability that a new mutant lineage reaches $1/2(k_\star + 1)s$ individuals. At this point, the lineage crosses over from stochastic to deterministic dynamics, entering the domain described by the deterministic solution Equation 4. Thus $P_{\text{est}} = 2(k_\star + 1)s$. Equating $r_- = r_+$ yields the stochastic condition

$$n_{k_\star} \approx \frac{1}{\sqrt{4\varepsilon U s k_\star}}. \quad (8)$$

This condition, together with the solution Equation 4 for the distribution, allows us to determine $\varepsilon_c(N, s, U)$. As shown in Figure 4, our solution Equation 8 is in excellent agreement with simulations for most of the data. However, our solution overestimates ε_c for populations with small population size and small λ that still satisfy $n_{k_\star} s \lesssim 1$. A better description in this regime will require a more careful analysis of fluctuations beyond the nose.

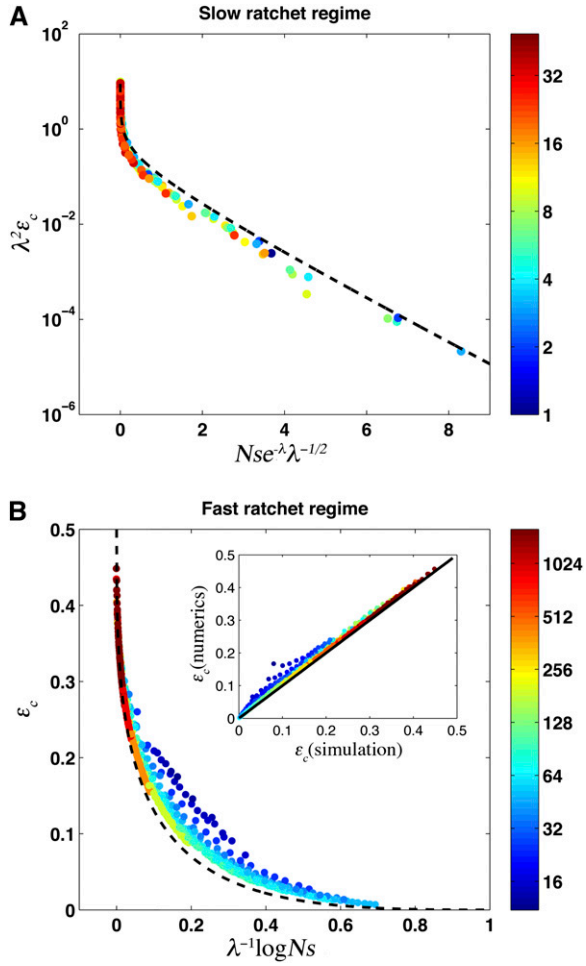

Figure 4 The critical proportion of beneficial mutations, ε_c, across both slow- and fast-ratchet regimes for many combinations of U, s, and N for the two regimes. Simulation results are shown as points, with color indicating λ. (A) Slow-ratchet regime. We show $\varepsilon_c\lambda^2$ as a function of $(1/\sqrt{\lambda})sNe^{-\lambda}$ for $\varepsilon_c\lambda^2 < 1$. The dashed black line is the analytic solution given by Equation 7. (B) Fast-ratchet regime. Note that ε_c is a function of $\lambda^{-1}\log Ns$ and even in small populations approaches $\varepsilon_c \approx 1/2$ only for very large λ. The dashed black line shows the asymptotic solution ε_c in the large population size limit $(\varepsilon_c\lambda^2 \gg 1)$, Equation 9. The inset compares the numerical solution from Equation 8 to simulation data.

Large population size limit

Large populations can maintain a well-adapted genome with $\varepsilon_c \ll 1/2$ even for moderately large λ. In the limit of large populations and large U/s, with the limit taken so that $\varepsilon_c\lambda^2 \gg 1$ while $\varepsilon_c \ll 1/2$, the matching condition reduces to $\sqrt{\varepsilon_c}\log\varepsilon_c = \lambda^{-1}\log(Nse^{-\lambda(1-2\varepsilon_c)})$. This equation has the approximate solution

$$\varepsilon_c \approx \frac{z^2}{\log^2[z/\log(z^{-1})]}, \quad 2z \equiv 1 - \frac{\log Ns}{\lambda}. \quad (9)$$

This result can be made more precise through iteration (see File S1 for derivations and comparisons to simulation data).

Note here that ε_c depends only very weakly on Ns and on λ, in contrast to the slow-ratchet regime where ε_c declines exponentially with $Nse^{-\lambda}$.

High mutation rate or weak purifying selection

In this limit, the dynamic equilibrium tends toward the state where beneficial and deleterious mutations are equally frequent, $\varepsilon = 1/2$. This corresponds to a population that can no longer maintain a well-adapted state. In File S1, we derive an approximate expression for ε_c in the fast-ratchet regime in the limit of large λ:

$$\varepsilon_c \approx \frac{1}{2} - \left(\frac{3}{4\lambda}\log Ns\right)^{1/3}. \tag{10}$$

From this expression we can immediately determine the point at which the population can no longer maintain a well-adapted state, as defined by a maximum fraction of beneficial mutations, ε_{max}. To have $\varepsilon_c < \varepsilon_{max}$, we require

$$\frac{\lambda}{\log Ns} \leq \frac{3}{4}\left[\frac{1}{0.5-\varepsilon_{max}}\right]^3, \tag{11}$$

valid for $\varepsilon_{max} \gtrsim 0.2$. Note that for $\varepsilon_{max} = 1/4$, the right-hand side (RHS) of this expression is 48; for $\varepsilon_{max} = 1/3$, the RHS is 162. Thus even small populations with $Ns \gtrsim 1$ can maintain relatively well-adapted genomes in the face of high mutation rates and weak selection (*i.e.*, when $U \gg s$ so that $\lambda \gg 1$).

Different fitness effects for beneficial and deleterious mutations

Our analysis to this point has assumed that both beneficial and deleterious mutations have the same fitness effect s. However, compensatory mutations often only partially compensate for the cost of a deleterious mutation. Hence beneficial mutations may tend to have a smaller effect on fitness than deleterious mutations. The opposite case, where beneficial mutations have larger effects than deleterious mutations, may also be relevant, as weak-effect deleterious mutations accumulate more rapidly and for some effect distributions may dominate. We now consider the effects of these differences in the fitness effects of beneficial and deleterious mutations. We denote the effect of a deleterious mutation by s_d and the effect of a beneficial mutation by s_b, where by convention both s_d and s_b are positive.

When $s_d \neq s_b$, there still exists a critical value of $\varepsilon = \varepsilon_c$ corresponding to a dynamic equilibrium state, which (provided as always that ε increases as fitness decreases) is globally stable. However, the shape of the fitness distribution in this equilibrium will be different from that calculated above, and the critical ε_c will also change. Fortunately, our analysis of the single-s model places bounds on this new critical ε_c that applies for $s_d \neq s_b$. To see this, we first define the ε_c that corresponds to a particular single-s model (as calculated above) to be $\varepsilon_c(s)$. Now consider the case $s_b < s_d$. The critical ε_c for this more complex situation must be less than $\varepsilon_c(s_b)$, because stronger-effect deleterious mutations can only decrease ε_c (stronger selection against deleterious mutations cannot speed their accumulation). On the other hand, the critical ε_c must also be greater than $\varepsilon_c(s_d)$, because weaker-effect beneficial mutations can only increase ε_c. A similar argument leads to analogous bounds for the case $s_b > s_d$.

These simple bounding arguments imply that the critical ε_c is at most $\varepsilon_c(s_b)$ or $\varepsilon_c(s_d)$, whichever is greater. Since we have seen that ε_c remains small even for remarkably small s and large U, this can still correspond to a relatively well-adapted state. Provided that deleterious mutations and the corresponding compensatory mutations do not have widely differing fitness effects, these bounding arguments also constrain ε_c to a narrow range. Thus although the quantitative details of the stable equilibrium state change whenever $s_b \neq s_d$, the main qualitative conclusions of our single-s analysis still apply.

To provide a more precise analysis of how ε_c depends on both s_b and s_d, we use simulations to compare ε_c for $s_b = s_d/\zeta$ with $\zeta = 0.5$, 1, 2 and show that in the slow-ratchet regime $\varepsilon_c(\zeta) \approx \zeta^2\varepsilon_c(1)$, demonstrated by the collapse of simulation data for different parameter values onto the same curve, as shown in Figure 5. This behavior in the slow-ratchet regime is quite intuitive, as the rate of forward motion of the nose increases as s_b^2. Here one factor of s_b arises from the establishment probability and the other from the size of the forward step due to each established beneficial mutation. Hence if $s_b \to s_b/\zeta$, the rate of beneficial mutations necessary to maintain dynamic balance must increase by ζ^2. Remarkably, this simple scaling approximation holds well into the crossover to the rapid-ratchet regime.

We finally note that of course in general not all beneficial mutations will have the same fitness effect s_b nor will all deleterious mutations have the same cost s_d. Instead, the effect of any individual mutation will be drawn from some distribution of beneficial or deleterious fitness effects. The values of s_b and s_d then represent some weighted average effect of a beneficial or deleterious mutation. A full analysis of this more complex situation is beyond the scope of this article, but we return to this issue, qualitatively, in the *Discussion* below.

Discussion

In infinite populations it has long been recognized that the balance between mutational pressure and purifying selection leads to a fitness equilibrium (Eigen 1971; Haigh 1978). Our analysis demonstrates that such an equilibrium also exists in a finite population, despite the action of genetic drift and Muller's ratchet. Much of the previous work on these finite populations has focused primarily on the rate of Muller's ratchet in the absence of beneficial mutations and has generally assumed that back and compensatory mutations are rare enough to be neglected (Gordo and

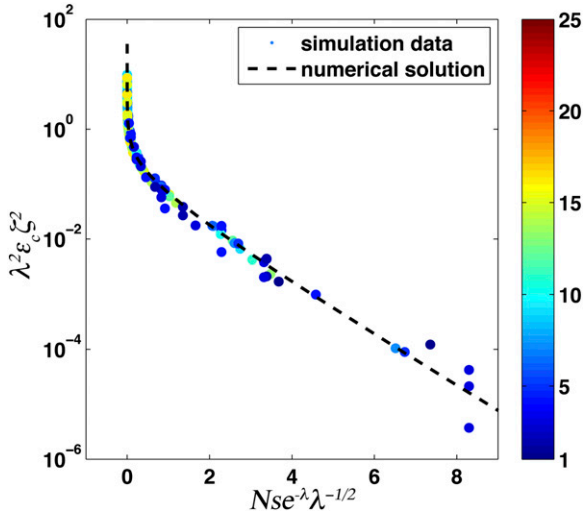

Figure 5 The critical fraction of beneficial mutations, ε_c, in the slow-ratchet regime for $s_b = s_d/\zeta$ with $\zeta = 0.5$, 1, 2 and many combinations of U, s, and N. As in Figure 4, simulation results are shown as points, with color indicating λ. The dashed black line is the analytic solution given by Equation 7.

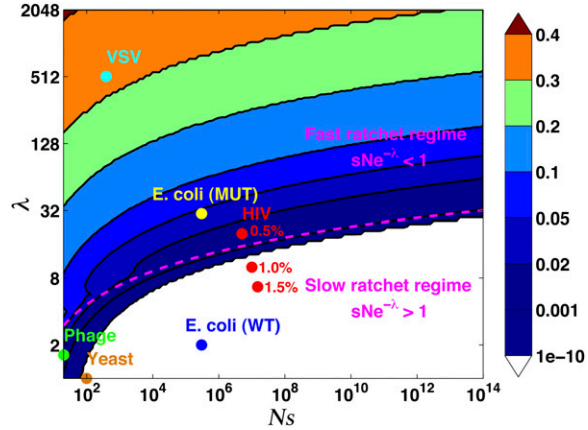

Figure 6 The fraction of beneficial mutations ε_c necessary to maintain the dynamic mutation–selection balance in a population with parameters Ns and $\lambda = U/s$. The dashed line separates the slow- and fast-ratchet regimes. Experiments with various model organisms with different population parameters are represented as points.

Charlesworth 2000a,b; Stephan and Kim 2002; Bachtrog and Gordo 2004; Etheridge *et al*. 2009). However, both intuition and experimental data (Silander *et al*., 2007) strongly suggest that beneficial mutations become more common as a population accumulates deleterious mutations. Silander *et al.* (2007) also provided a simple analysis of the possible dynamic balance, but it did not extend beyond the low mutation rate limit in which mutant alleles fix or go extinct within an otherwise clonal population. The dynamics of diverse populations subject to the combined effect of both deleterious and beneficial mutations have remained understudied (for notable exceptions see Rouzine *et al.* 2003, 2008 and Pfaffelhuber *et al.* 2011).

We have shown that for any population size, mutation rate, and selection pressure, there exists a proportion of beneficial mutations, ε_c, which balances the accumulation of deleterious mutations. Because beneficial mutations are favored by natural selection, a small-fraction beneficial mutation can suffice to maintain stability, $\varepsilon_c \ll 1$. We have demonstrated this by explicitly calculating ε_c as a function of population size, mutation rate, and the strength of selection. There are two qualitatively different regimes for ε_c, as shown in Figure 6. In the slow-ratchet regime, selection stabilizes the fittest subset of the population at the nose of the distribution, so ε_c is small and depends exponentially on the population parameters Ns and λ. On the other hand, in the fast-ratchet regime, ε_c must be larger to balance rapid accumulation of deleterious alleles, and it depends more weakly on the population parameters Ns and λ. The boundary of the two regimes is approximately given by $Nse^{-\lambda} = 1$.

We note that Rouzine *et al.* (2003, 2008) studied population dynamics under the combined action of purifying and positive selection in a model similar to ours, finding both simulation and analytical evidence for the existence of the dynamic balance state. However, their analysis focuses on the rate of the ratchet (with $\varepsilon \ll \varepsilon_c$) or the rate of adaptation (with $\varepsilon \gg \varepsilon_c$) and does not apply as $\varepsilon \to \varepsilon_c$. Our work, by contrast, focuses exclusively on the dynamic equilibrium state and the critical ε_c required to maintain it.

Our analysis treats ε as an independent parameter, even though we expect it to depend on absolute fitness ω. As discussed above, we expect $\varepsilon(\omega)$ to decrease with increasing ω. In this case the steady state corresponding to dynamic balance at $\varepsilon_c(N, \lambda)$ is stable and is a global attractor point. For $\varepsilon > \varepsilon_c$ mean fitness increases ($d\omega/dt > 0$), while for $\varepsilon < \varepsilon_c$ mean fitness decreases ($d\omega/dt < 0$), which for $d\varepsilon(\omega)/d\omega < 0$ guarantees local stability of the $\varepsilon = \varepsilon_c$ equilibrium. While our presentation focused on the properties of dynamic equilibrium, our approach also defines the actual rate of change of $d\omega/dt = r_+ - r_-$ as a function of instantaneous ε and N (note that, as in Rouzine *et al.* 2008, in general $r_\pm$ themselves become dependent on this "velocity"). This quantitative understanding of differential dynamics, *i.e.*, the rate of change of mean fitness conditional on a given value of ε, can be integrated for any given $\varepsilon(\omega)$ defining the corresponding evolutionary trajectory as a function of time. This consideration can be readily generalized (by allowing N to change along ω) to include possible reduction in the size of the population that may follow reduction of absolute fitness. In particular, it is possible that under some conditions the population will collapse—*i.e.*, "mutational meltdown" will occur—before the stable dynamic equilibrium is reached (Lynch *et al.* 1993) (see File S1 for more extended discussion). The possibility of such a meltdown instability depends critically on precisely how ε depends on ω and hence on details of the ecology of the specific system. However, given any specific set of assumptions about $\varepsilon(\omega)$, this effect can be analyzed quantitatively in terms of the dynamics formulated above.

Our analytic results for ε_c in the dynamic equilibrium were made possible by the explicit calculation of the shape of the fitness distribution, given in Equation 4 and illustrated in Figure 3. With increasing λ and decreasing N this distribution deviates from the Poisson distribution for the classic mutation–selection balance (Haigh 1978), most notably reducing the fitness of the top class, $k_\star$, relative to the mean. Examining the properties of the dynamic mutation–selection equilibrium over the full parameter range, shown in Figure 6, has revealed a strong asymmetry between beneficial and deleterious mutations. For example, for a small population $Ns \sim 10^3$ with high mutation rate $\lambda \sim 8$, just 2% beneficial mutations are enough to counteract the effect of deleterious mutations. This indicates that purifying selection is remarkably effective even for conditions where Muller's ratchet would proceed extremely quickly in the absence of back and compensatory mutations.

Populations with $\varepsilon > \varepsilon_c$ should adapt, while populations with $\varepsilon < \varepsilon_c$ should decline in fitness. Experimental evolution of model organisms in controlled laboratory environments appears to be consistent with this expectation. In particular, Silander *et al.* (2007) showed that bacteriophage φ*X*174 converged to a population-size–dependent fitness plateau, as our model would predict. For their population parameters ($s \sim 0.08$, $U \sim 0.13$) our theory predicts ε_c ranging from 2% to 0.2% for their experiments (in which N ranged from 100 to 200), consistent with the beneficial mutation rates they infer. Their experiments with lower population sizes ($N \lesssim 30$) are in the fast-ratchet regime, and estimating ε_c requires further analysis of fluctuations beyond the nose.

Other experiments with vesicular stomatitis virus found that large populations adapt while small populations melt, also consistent with our analysis (Moya *et al.* 2000). Similar results have been observed in yeast (Desai *et al.* 2007; Lang *et al.* 2011). In the long-term evolution experiments of Lenski and collaborators in *Escherichia coli*, parameters are such that we expect the critical ε_c to be very small, $\varepsilon_c \ll 10^{-10}$, consistent with their observation of continuous adaptation even after tens of thousands of generations. On the other hand, mutator strains of *E. coli* (Trindade *et al.* 2010) and yeast (Desai *et al.* 2007) sometimes show decrease in fitness, which could be used to further test the model. In Figure 6, we show approximate estimates for the parameter regimes in which each of these experimental systems lie. For example, population parameters for human immunodeficiency virus seem to place it close to the boundary between the slow- and fast-ratchet regimes (Figure 6), so that its evolutionary dynamics depend sensitively on the strength of selection.

While a global "phase diagram" of population dynamics is highly intriguing, our present attempt to construct it highlights the challenge of doing so. First, our analysis has assumed that all mutations have the same selective effect s. In general, however, mutations have a range of selective effects. Mutations with a particular characteristic strength may tend to dominate the dynamics, if more strongly selected mutations are quickly eliminated by selection while more weakly selected mutations are effectively neutral given interference from selection on the other mutations. Thus, our λ parameter must be regarded as an effective parameter corresponding to mutations with effect strength that dominate the balance. The possibility of defining such an effective λ in the model of Muller's ratchet with deleterious effects of different size has been discussed by Soderberg and Berg (2007) on the basis of numerical simulations. Yet the general description of interference within an arbitrary distribution of (deleterious and beneficial) mutational effects that would provide a precise definition of effective λ_{eff} remains an open problem and an important avenue for future work. Second, our analysis has focused on asexual populations and neglected recombination. Since even weak recombination has the potential to significantly slow Muller's ratchet, it would be interesting to generalize our dynamic balance to include its effects (Bell 1998; Gordo and Campos 2008). Increasing recombination rates would presumably allow the population to maintain better-adapted genotypes in this steady state. Last but not least, better experimental characterization of mutation effect distributions for different organisms will be needed to fully achieve the synthesis attempted in Figure 6.

The dynamic balance state has important implications for patterns of molecular evolution. Recent analysis of the effects of purifying selection on the structure of genealogies has suggested that Muller's ratchet plays a crucial role in determining the structure of genetic variation in asexual populations or on short distance scales in the genomes of sexual organisms (Gordo *et al.* 2002; Seger *et al.* 2010). However, these expectations must be revised if populations exist instead in the dynamic state in which both beneficial and deleterious mutations fix, without any continuous net degradation or growth of fitness. This means that even though no change in fitness occurs, signatures of both positive and negative selection are likely to be found in patterns of molecular evolution, as has been suggested by earlier studies (Hartl and Taubes 1996; Antezana and Hudson 1997). We argue that this state is the natural null expectation for the effects of mutations and purifying selection on patterns of genetic variation; efforts to look for positive selection that represent "true" adaptation should look for deviations from this situation.

Finally, we note that although the dynamic balance we have analyzed is stable, a population will typically fluctuate around this steady state. A few beneficial mutations may become established at the nose by chance, leading to a temporary increase in mean fitness that is later balanced by a reduction in ε at this higher fitness, restoring the population to the equilibrium state. Conversely, a few clicks of Muller's ratchet will occasionally lead to temporary reductions in population fitness before the corresponding increase in ε restores the steady state. These fluctuations around the dynamic balance may be important to the clonal structure of

the population and hence are likely to play a key role in patterns of molecular evolution and in understanding the effects of recombination.

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Literature Cited

Andersson, D., and D. Hughes, 1996 Muller's ratchet decreases fitness of a dna-based microbe. Proc. Natl. Acad. Sci. USA 93: 906–907.

Antezana, M., and R. Hudson, 1997 Point-mutations, the ratchet, and the initial success of eukaryotic sex: a simulation study. Evol. Theory Rev. 11: 209–235.

Bachtrog, D., and I. Gordo, 2004 Adaptive evolution of asexual populations under Muller's ratchet. Evol. Int. J. Org. Evol. 58: 1403–1413.

Barrick, J., M. Kauth, C. Strelioff, and R. Lenski, 2010 Escherichia coli rpob mutants have increased evolvability in proportion to their fitness defects. Mol. Biol. Evol. 27: 1338–1347.

Barton, N., and B. Charlesworth, 1998 Why sex and recombination? Science 281: 1986–1990.

Bell, G., 1998 Recombination and the immortality of the germ line. J. Evol. Biol. 1: 67–82.

Chao, L., 1990 Fitness of RNA virus decreased by Muller's ratchet. Nature 348: 454–455.

Charlesworth, B., 2012 The effects of deleterious mutations on evolution at linked sites. Genetics 190: 5–22.

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

De Visser, J., and S. Elena, 2007 The evolution of sex: empirical insights into the roles of epistasis and drift. Nat. Rev. Genet. 8: 139–149.

Desai, M. M., D. S. Fisher, and A. W. Murray, 2007 The speed of evolution and maintenance of variation in asexual populations. Curr. Biol. 17: 385–394.

Duarte, E., D. Clarke, A. Moya, E. Domingo, and J. Holland, 1992 Rapid fitness loss in mammalian RNA virus clones due to Muller's ratchet. Proc. Natl. Acad. Sci. USA 89: 6015–6019.

Eigen, M., 1971 Self organization of matter and the evolution of biological macromolecules. Naturwissenschaften 58: 465–523.

Escarmis, C., M. Davila, and E. Domingo, 1999 Multiple molecular pathways for fitness recovery of an RNA virus debilitated by operation of Muller's ratchet. J. Mol. Biol. 285: 495–505.

Estes, S., and M. Lynch, 2003 Rapid fitness recovery in mutationally degraded lines of Caenorhabditis elegans. Evolution 57: 1022–1030.

Etheridge, A., P. Pfaffelhuber, and A. Wakolbinger, 2009 How often does the ratchet click? Facts, heuristics, asymptotics, pp. 365–390 in *Trends in Stochastic Analysis*, edited by P. M. Jochen Blath and M. Scheutzow. Cambridge University Press, Cambridge, UK.

Felsenstein, J., 1974 The evolutionary advantage of recombination. Genetics 78: 157–159.

Gabriel, W., and R. Burger, 1993 Muller's ratchet and mutational meltdowns. Evolution 47: 1744–1757.

Gessler, D., 1995 The constraints of finite size in asexual populations and the rate of the ratchet. Genet. Res. 49: 135–146.

Gordo, I., and P. Campos, 2008 Sex and deleterious mutations. Genetics 179: 621–626.

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

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

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

Haigh, J., 1978 The accumulation of deleterious genes in a population. Theor. Popul. Biol. 14: 251–267.

Hartl, D., and C. Taubes, 1996 Compensatory nearly neutral mutations: selection without adaptation. J. Theor. Biol. 182: 303–309.

Howe, D., and D. Denver, 2008 Muller's ratchet and compensatory mutation in Caenorhabditis briggsae mitochondrial genome evolution. BMC Evol. Biol. 8: 62.

Hughes, J. F., H. Skaletsky, L. G. Brown, T. Pyntikova, T. Graves *et al.*, 2012 Strict evolutionary conservation followed rapid gene loss on human and rhesus Y chromosomes. Nature 483: 82–86.

Jain, K., 2008 Loss of least-loaded class in asexual populations due to drift and epistasis. Genetics 179(4): 2125–2134.

Kondrashov, A., 1994 Muller's ratchet under epistatic selection. Proc. Natl. Acad. Sci. USA 136: 1469–1473.

Kondrashov, A., 1995 Contamination of the genome by very slightly deleterious mutations: Why have we not died 100 times over? J. Theor. Biol. 175: 583–594.

Lande, R., 1998 Risk of population extinction from fixation of deleterious and reverse mutations. Genetica 102/103: 21–27.

Lang, G. I., D. Botstein, and M. M. Desai, 2011 Genetic variation and the fate of beneficial mutations in asexual populations. Genetics 188: 647–661.

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

Lynch, M., 1996 Mutation accumulation in transfer RNAs: molecular evidence for Muller's ratchet in mitochondrial genomes. Mol. Biol. Evol. 13: 209–220.

Lynch, M., R. Burger, D. Butcher, and W. Gabriel, 1993 The mutational meltdown in asexual populations. J. Hered. 84: 339–344.

Lynch, M., J. Conery, and R. Burger, 1995 Mutation accumulation and the extinction of small populations. Am. Nat. 146: 489–518.

Manrubia, S. C., E. Lazaro, and J. Perez-Mercader, 2003 Fitness distributions in exponentially growing asexual populations. Phys. Rev. Lett. 90(18): 188102.

Moya, A., S. F. Elena, A. Bracho, R. Miralles, and E. Barrio, 2000 The evolution of RNA viruses: a population genetics view. Proc. Natl. Acad. Sci. USA 97: 6967–6973.

Muller, H., 1964 The relation of recombination to mutational advance. Mutat. Res. 1: 2–9.

Neher, R. A., and B. I. Shraiman, 2012 Fluctuations of fitness distributions and the rate of Muller's ratchet. Genetics 191: 1309–1319.

Pfaffelhuber, P., P. R. Staab, and A. Wakolbinger, 2011 Muller's ratchet with compensatory mutations. Ann. Appl. Probab. (in press).

Poon, A., and L. Chao, 2005 The rate of compensatory mutation in the DNA bacteriophage ϕx174. Genetics 170: 989–999.
Poon, A., and S. Otto, 2000 Compensating for our load of mutations: freezing the meltdown of small populations. Evolution 54: 1467–1479.
Poon, A., B. Davis, and L. Chao, 2005 The coupon collector and the suppressor mutation: estimating the number of compensatory mutations by maximum likelihood. Genetics 170: 1323–1332.
Rice, W., 1994 Degeneration of a nonrecombining chromosome. Science 263: 230–232.
Rouzine, I., J. Wakeley, and J. Coffin, 2003 The solitary wave of asexual evolution. Proc. Natl. Acad. Sci. USA 100: 587–592.
Rouzine, I., E. Brunet, and C. Wilke, 2008 The traveling-wave approach to asexual evolution: Muller's ratchet and speed of adaptation. Theor. Popul. Biol. 73: 24–46.
Schoustra, S., T. Bataillon, D. Gifford, and R. Kassen, 2009 The properties of adaptive walks in evolving populations of fungus. PLoS Biol. 7: e1000250.
Schultz, S., and M. Lynch, 1997 Deleterious mutation and extinction: the role of variable mutational effects, synergistic epistasis, beneficial mutations, and degree of outcrossing. Evolution 51: 1363–1371.
Seger, J., W. A. Smith, J. J. Perry, J. Hunn, Z. A. Kaliszewska *et al.*, 2010 Gene genealogies strongly distorted by weakly interfering mutations in constant environments. Genetics 184: 529–545.
Silander, O., O. Tenaillon, and L. Chao, 2007 Understanding the evolutionary fate of finite populations: the dynamics of mutational effects. PLoS Biol. 5: e94.
Soderberg, R., and O. Berg, 2007 Mutational interference and the progression of Muller's ratchet when mutations have a broad range of deleterious effects. Genetics 177: 971–986.
Stephan, W., and Y. Kim, 2002, *Recent Applications of Diffusion Theory to Population Genetics*. Oxford University Press, Oxford, pp. 72–93.
Trindade, S., L. Perfeito, and I. Gordo, 2010 Rate and effects of spontaneous mutations that affect fitness in mutator Escherichia coli. Philos. Trans. R. Soc. B Biol. Sci. 365: 1177–1186.
Wagner, G., and W. Gabriel, 1990 Quantitative variation in finite parthenogenetic populations: What stops Muller's ratchet in the absence of recombination? Evolution 44: 715–731.
Zeyl, C., M. Mizesko, and J. de Visser, 2001 Mutational meltdown in laboratory yeast populations. Evolution 55: 909–917.

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Dynamic Mutation–Selection Balance as an Evolutionary Attractor

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Rate of extinction of the fittest genotype in the slow ratchet regime. Stochastic fluctuations of the fittest class can approximately be described by a diffusion equation:

$$\partial_t p(x,t) = -\partial_x \left[D_1(x)p(x,t)\right] + \partial_x^2 \left[D_2(x)p(x,t)\right] \tag{1}$$

where $p(x)$ is the probability distribution of the nose occupancy, $x = N^{-1}n_{k_\star}$ being defined as the fraction of the population in the "top bin" $k_\star$. Diffusive "drift" is represented by $D_2(x) = x(1-x)/2N$ and effect of selection acting in the top bin is represented by $D_1(x) = \hat{s}x(1 - x/x_\star)$, where $x_\star = e^{-U/s}$ is the equilibrium nose occupancy, , where $\hat{s} = \gamma s$ and γ is a "phenomenological" parameter introduced by Haigh (1978). Both $x = 1$ (fixation) and $x = 0$ (extinction) are absorbing boundary conditions (Jain, 2008; Stephan and Kim, 2002). The density function for fixation/extinction time $\varphi(t;x)$ satisfies the backward Kolmogorov equation

$$\partial_t \varphi(t;x) = D_1(x)\partial_x \varphi(t;x) + D_2(x)\partial_x^2 \varphi(t;x) \tag{2}$$

where t is the time interval between initial state x and fixation or extinction. The mean time to fixation/extinction starting at $x = y$ at $t = 0$ is given by $\bar{t}(y) = \int_0^\infty t\varphi(t;y)dt$, which satisfies

$$-1 = D_1(y)\partial_y \bar{t}(y) + D_2(y)\partial_y^2 \bar{t}(y). \tag{3}$$

Using the integrating factor $\varphi(x) = e^{\int_0^x dz \frac{D_1(z)}{D_2(z)}}$, yields for the mean time to extinction of the fittest class:

$$\bar{t}(x_\star) = \int_0^{x_\star} dy \frac{1}{\varphi(y)} \int_y^1 d\zeta \frac{\varphi(\zeta)}{D_2(\zeta)}, \tag{4}$$

where

$$\varphi(x) = (1-\zeta)^{\frac{2N\hat{s}(1-x_\star)}{x_\star}} e^{\frac{2N\hat{s}\zeta}{x_\star}}, \tag{5}$$

The second integral in the limit $Ns \gg 1$ can be approximated as

$$2N \int_y^1 d\zeta \frac{(1-\zeta)^{\frac{2N\hat{s}(1-x_\star)}{x_\star}} e^{\frac{2N\hat{s}\zeta}{x_\star}}}{\zeta(1-\zeta)} \approx 2N \int_y^1 d\zeta \frac{e^{2N\hat{s}x_\star\left(\frac{2\zeta}{x_\star} - \frac{\zeta^2}{x_\star^2}\right)}}{\zeta} \tag{6}$$

and for small x, $\varphi(x)$ approximates to

$$\varphi(x) \approx e^{2N\hat{s}x_\star\left(\frac{2\zeta}{x_\star} - \frac{\zeta^2}{x_\star^2}\right)} \tag{7}$$

Using the above approximations and $\alpha = N\hat{s}x_\star, n_\star = Nx_\star$ and changing the variables, $\eta = y/x_\star - 1, z = \zeta/x_\star - 1$ yields:

$$\bar{t}(x_\star) \approx 2n_{k_\star} \int_{-1}^{0} d\eta e^{\alpha\eta^2} \int_\eta^{1/\star - 1} dz \frac{e^{-\alpha z^2}}{1+z} \tag{8}$$

For $\alpha \gg 1$ the integral the second integral can be readily approximated yielding

$$\bar{t}(x_\star) \approx 2n_{k_\star} \sqrt{\frac{\pi}{4\alpha}} \int_{-1}^{0} d\eta e^{\alpha\eta^2} \left[erf(\sqrt{\alpha}\beta) - erf(\sqrt{\alpha}\eta)\right] \tag{9}$$

where $\beta = \frac{1}{k_\star} - 1$. Using $\eta^2 = 1 - \theta^2/\alpha$ allows the evaluation of the integral in the $\alpha \gg 1$ limit and yields

$$\bar{t}(x_\star) \approx n_{k_\star} \sqrt{\pi} \alpha^{-3/2} e^{\alpha}. \tag{10}$$

Assuming an exponential distribution for time of extinction of the fittest class, the rate of extinction is given by $r_- = 1/\bar{t}(x_\star)$. Replacing $\alpha = \gamma s n_{k_\star}$ yields:

$$r_- \approx e^{-\gamma s n_{k_\star}} \gamma s \sqrt{\gamma s n_{k_\star}/\pi} \tag{11}$$

which we use to arrive at Eq. (11) of the main text.

This standard calculation of extinction probability has glossed over the non-trivial "many-body" element of the problem: the fact that the strength of selection acting on the fittest genotype is defined relative to the population mean, which depends on the deviations from the steady state for all other, less-fit, genotypes. The latter in turn depends on the history of the dynamics in the top fitness "bin". As in all of the previous work, we deal with this problem here by introducing a phenomenological parameter γ, known as the "Haigh factor" (Gordo and Charlesworth, 2000a,b; Haigh, 1978; Stephan and Kim, 2002). However, unlike earlier work we shall not assume this factor to be a constant and determine its λ-dependence numerically (see discussion of the slow ratchet regime in the main text). An analytic method for describing the effect of history dependence on the fluctuations of the top bin goes beyond the scope of the present work and will be described in a separate publication (Neher and Shraiman, submitted).

Asymptotic expressions for ϵ_c The matching condition used to determine ϵ_c in the fast-ratchet regime can be rewritten as follows.

$$\frac{1}{\sigma\sqrt{\epsilon k_\star}} = N e^{-\lambda(1-2\lambda)+\frac{k_\star}{2}\log\frac{1-\epsilon}{\epsilon}} J_{k_\star}(2\alpha). \tag{12}$$

In the limit of $\alpha \gg 1$, the zero of the Bessel function is approximately at $k_\star + 1 \approx \alpha$ and the Bessel function at $k_\star$ evaluates to roughly $\sim k_\star^{2/3}$ (Abramowitz 9.3.33 and 9.1.27). Hence we have

$$\lambda(1-2\epsilon) - \lambda\sqrt{\epsilon(1-\epsilon)}\log\frac{1-\epsilon}{\epsilon} = \log Ns \tag{13}$$

where we have neglected powers to the $\frac{1}{6}$ and $\mathcal{O}(1)$ factors inside the logarithm. In the limit $\epsilon \ll 1$ (but $\lambda^2\epsilon \gg 1$), the matching simplifies to

$$1 - 2\epsilon + \sqrt{\epsilon}\log\epsilon = \lambda^{-1}\log Ns \tag{14}$$

which simplifies further to

$$\sqrt{\epsilon}\log\epsilon \approx \lambda^{-1}\log(Ns) - 1 = -2z \tag{15}$$

where we defined z for convenience. This can be solved for ϵ:

$$\epsilon_c = \frac{z^2}{W(-z)^2} \approx \frac{z^2}{(\log(z) - \log(-\log(z)))^2} \tag{16}$$

where $W(x)$ is the -1 branch of Lambert's W-function, i.e. the solutions of $W(x)e^{W(x)} = x$. The linear correction in Eq. 14 can be incorporated iteratively.

$$\epsilon_{i+1} = \frac{(z+\epsilon_i)^2}{W(-z+\epsilon_i)^2}. \tag{17}$$

This iteration converges for small C and ϵ. At larger C, the branch of the $W(x)$ function is lost. The result ϵ_2 obtained after the first iteration, starting with Eq. 16, is also shown in Fig. S1 as dashed red line.

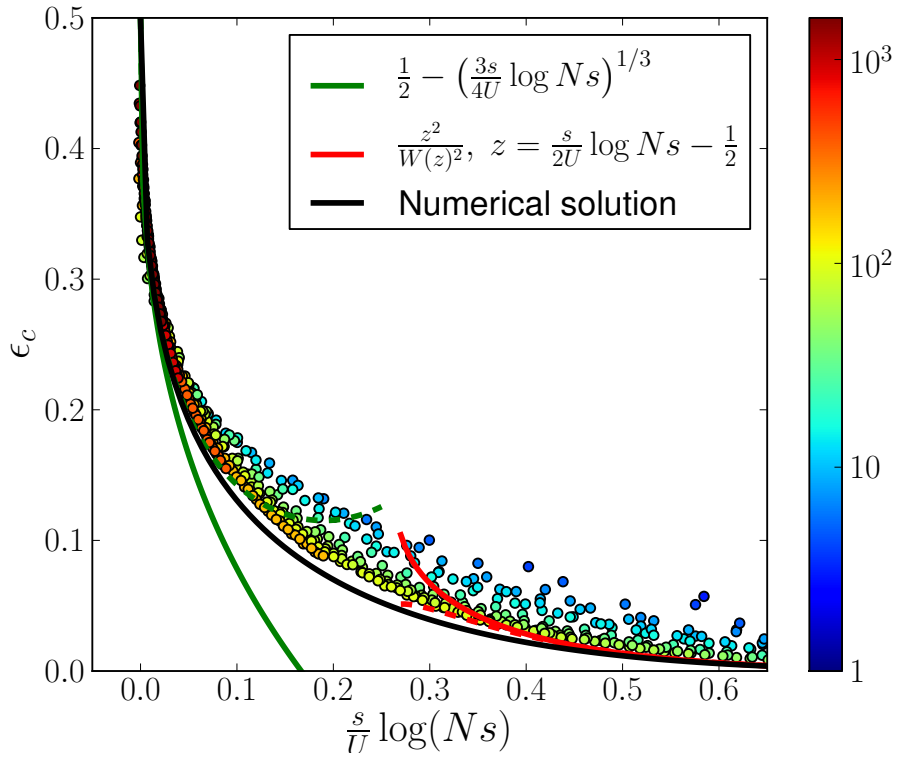

Figure S1. Dynamic mutation-selection balance. The figure shows ϵ_c for many combinations of U, s and N as a function of $\frac{s}{U}\log Ns$, while the color codes for $\epsilon_c U^2 s^{-2}$. If $\epsilon_c U^2 s^{-2} \gg 1$, ϵ_c is solely a function of $\frac{s}{U}\log Ns$ and is well described by the numerical solution of Eq. 6 in Main text, shown as a black line. The asymptotic approximations for large ϵ_c (Eq. 19) and small ϵ_c (Eq. 16) are shown as green and red lines, respectively. The dashed lines correspond to the more accurate version mentioned in the main text.

The other limit that is amenable to analytic calculations is the limit $\epsilon \to 1/2$. To this end, we define $\delta = \frac{1}{2} - \epsilon$ and expand the right hand side of Eq. 14

$$1 - 2\epsilon - \sqrt{\epsilon(1-\epsilon)}\log\frac{1-\epsilon}{\epsilon} = \frac{4}{3}\delta^3 + \frac{44}{15}\delta^5 + \mathcal{O}(\delta^7) = \lambda^{-1}\log Ns. \tag{18}$$

From this, we find

$$\epsilon \approx \frac{1}{2} - \left(\frac{3}{4\lambda}\log(Ns)\right)^{1/3}. \tag{19}$$

This expression is compared to simulation results in Fig. S1. The term proportional to δ^5 can again be included by iteration and the result is shown as the dashed green line in Fig. S1.

Convergence to the dynamic mutation-selection balance state. In the main text we discuss the possibility that ϵ itself depends on the absolute fitness. As the simplest model of this dependence here we consider the case of $\epsilon(k) = k/L$ which may be thought of as a "genome" of length L, that for

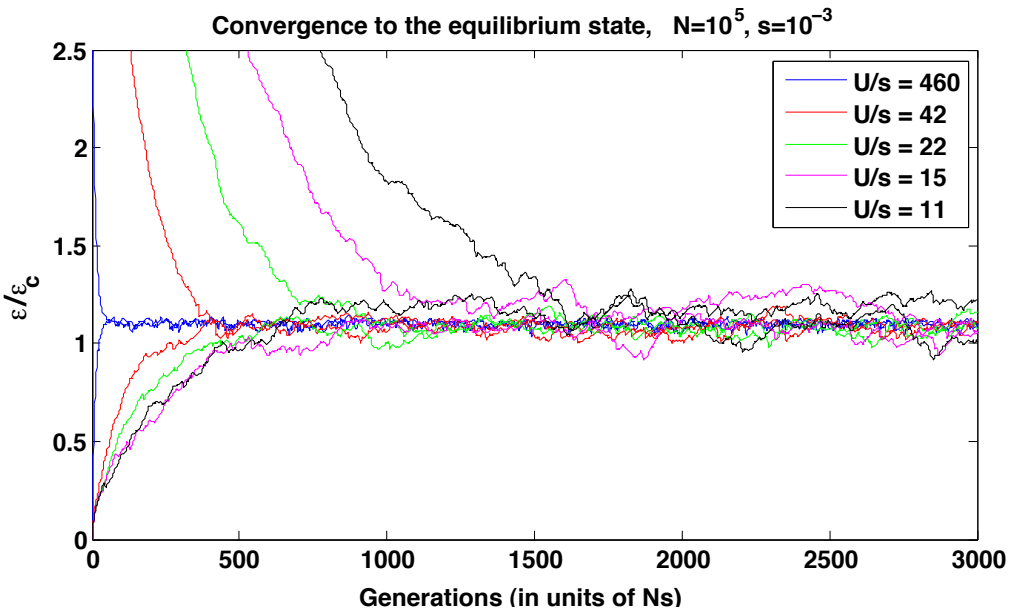

Figure S2. Evolution of the beneficial mutation fraction in the model with $\epsilon(k) = k/L$. The figure shows time evolution of ϵ towards e_c for populations which start above or below the absolute fitness (i.e. average number of mutations $\bar{k}$ per genome) corresponding to the dynamic balance state which is realized when $\epsilon(k)$ at the nose of the distribution is at ϵ_c. The rate of convergence depends on the population parameters Ns, λ. Fluctuations around ϵ_c arise from the stochastic nature of the dynamic balance state.

$k = 0$ cannot be improved in fitness, but allows for a k/L fraction of compensatory mutations, once $k > 0$. Dependence of beneficial mutation fraction on absolute fitness is parameterized by L here set to 10^3, so that differences in ϵ within any population are relatively small as $\lambda/L << 1$. Figure S2 presents the result of numerical simulations for this model, for different values of λ and different population sizes (see Methods section of the main text for the description of the numerical method). We ran simulations starting with populations in the high $(k - 0)$ and low $(k = L)$ fitness states and observed the dynamics of population averaged fitness $\bar{k}$ decrease or increase with time eventually approaching a statistically steady state. This asymptotic state is characterized by average ϵ close to ϵ_c appropriate for the given population parameters. This convergence is evident in Figure S2 which displays the time dependence of population averaged ϵ normalized to $\epsilon_c(N, \lambda)$. Asymptotic value of the $\epsilon/\epsilon_c(N, \lambda)$ deviates slightly from unity because of the residual variation of ϵ within the population in the equilibrium state. The rate of evolution towards dynamic balance slows down as the population approaches its ϵ_c, which is expected as the $v \to 0$ as $\epsilon \to \epsilon_c$.

Mutational Meltdown. While the focus of our analysis has been on understanding the properties of the dynamic balance state, we have in the main text of the paper also discussed the global condition for its stability: the monotonic increase in the beneficial mutation rate (i.e. ϵ) with decreasing absolute fitness. That discussion however assumed constant population size, which may not be a good assumption once the absolute fitness falls below certain threshold. Declining absolute population fitness can lead to a reduction in population size, speeding Muller's ratchet and leading to further declines in absolute fitness

and eventual extinction, a process is known as "mutational meltdown." We now consider the conditions under which the dynamic equilibrium state allows a population to avoid this mutational meltdown.

To address this question, we must first specify the relationship between absolute fitness and changes in population size. This depends on the details of the population dynamics, and a variety of different models are plausible. Here we use the framework proposed by Lynch *et al.* (1993), in which the average number of offspring of an individual is Rw, where R is the average fecundity of a mutation-free individual and w is its absolute fitness. In our model an individual with ℓ deleterious mutations has $w = (1-s)^{\ell}$. The population then melts down when the average individual produces less than one offspring, which occurs when

$$R(1-s)^{\bar{\ell}} < 1. \tag{20}$$

To connect this condition to our analysis of the dynamic equilibrium state, we next need to specify the relationship between ϵ and absolute fitness. This depends on the specific model of back and compensatory mutations. Here we assume that ϵ increases linearly with the number of deleterious mutations,

$$\epsilon = \frac{c\ell}{L}, \tag{21}$$

where c represents the average number of back or compensatory mutations available per deleterious mutation and L represents the total number of potentially deleterious sites.

Given these assumptions, our dynamic equilibrium state must satisfy

$$\epsilon_c < \frac{c}{L}\frac{\ln R}{\ln(1-s)^{-1}} \approx \frac{c \ln R}{sL} \tag{22}$$

in order to avoid mutational meltdown. We can simply apply our analysis as described above to compute ϵ_c; if the resulting value is greater than this critical value given the relevant values of R, s, and L, the dynamic equilibrium will not be stable due to reductions in population size, and meltdown will occur. We note however that since ϵ_c is bounded above by $\frac{1}{2}$, meltdown can only occur for sufficiently strong selection, $sL > 2 \ln R$. At the same time, stronger selection tends to reduce ϵ_c. Thus meltdown will typically be a concern only for a specific range of intermediate selection strengths.

The assumptions we made about population dynamics and the linear relationship between ϵ and absolute fitness are of course arbitrary. Recent work by Silander *et al.* (2007) has argued that in fact ϵ increases faster than linearly with ℓ. In this case, mutational meltdown will be less likely than we have suggested above (i.e. larger ϵ_c is required for meltdown). The alternative scenario where ϵ increases less than linearly with ℓ is also possible in principle, in which case mutational meltdown becomes more probable, though this scenario seems implausible given that back-mutation rates should increase linearly with ℓ.

References

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

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

Haigh, J., 1978 The accumulation of deleterious genes in a population. Theor. Popul. Biol. **14**: 251–26.

Jain, K., 2008 Loss of least-loaded class in asexual populations due to drift and epistasis. Genetics **179(4)**: 2125–34.

Lynch, M., R. Burger, D. Butcher, and W. Gabriel, 1993 The mutational meltdown in asexual populations. J. Hered. **84**: 339–344.

Silander, O., O. Tenaillon, and L. Chao, 2007 Understanding the evolutionary fate of finite populations: the dynamics of mutational effects. PLoS Biol. **5**: e94.

Stephan, W., and Y. Kim, 2002 *Recent Applications of Diffusion Theory to Population Genetics*. Oxford University Press, Oxford, UK, 72–93.