Genetica **102/103:** 127–144, 1998.

The fate of competing beneficial mutations in an asexual population

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Key words: asexual population dynamics, beneficial mutations, fixation probability, clonal interference, substitution rate

Abstract

In sexual populations, beneficial mutations that occur in different lineages may be recombined into a single lineage. In asexual populations, however, clones that carry such alternative beneficial mutations compete with one another and, thereby, interfere with the expected progression of a given mutation to fixation. From theoretical exploration of such 'clonal interference', we have derived (1) a fixation probability for beneficial mutations, (2) an expected substitution rate, (3) an expected coefficient of selection for realized substitutions, (4) an expected rate of fitness increase, (5) the probability that a beneficial mutation transiently achieves polymorphic frequency ($\geq 1\%$), and (6) the probability that a beneficial mutation transiently achieves majority status. Based on (2) and (3), we were able to estimate the beneficial mutation rate and the distribution of mutational effects from changes in mean fitness in an evolving *E. coli* population.

Introduction

Asexual populations adapt to their environment by the occurrence and subsequent rise in frequency of beneficial mutations. Without recombination, a population must incorporate beneficial mutations in a sequential manner (Fisher, 1930; Muller, 1932, 1964; Crow & Kimura, 1965). The time required for fixation of a beneficial mutation may be considerable if the population is large; however, the mutation remains at low frequency for much of this time (Lenski et al., 1991). While the mutation is at low frequency, another beneficial mutation may arise on the ancestral background. If two such beneficial mutations occur in a sexual population, then the two novel genotypes can recombine to form a fitter double-mutant (assuming no negative gene interactions). In an asexual population, however, these two novel genotypes compete with one another. Such competition between beneficial mutations slows the spread of, and may even eliminate, the first mutation. Such 'clonal interference' between beneficial mutations has many important consequences for the dynamics of evolution in asexual populations.

The idea that progression of a beneficial mutation to fixation may be impeded by competing beneficial mutations was articulated by Muller (1932, 1964) in the context of discussions on the evolutionary advantage of sex. Almost in passing, a brief theoretical treatment was later given by Haigh (1978), in which he proposed a discrete-time model of competing beneficial mutations. Employing a different approach, we give a full theoretical treatment of the phenomenon of competing beneficial mutations and its consequences.

The body of this paper is presented in two main parts. In the first part, a probability of fixation is derived that incorporates the effect of competition between beneficial mutations, and some consequences of this derivation are then explored. The dynamics of fixation are such that a relatively simple derivation suffices. In the second part, the probability is derived that a beneficial mutation achieves a frequency greater than or equal to some specified frequency, f. From this, the probability that a beneficial mutation becomes transiently polymorphic ($0.01<f<1$) or transiently common ($0.5<f<1$) is derived. The derivations in the second part require treatment of the dynamics of a three-

genotype system; hence, the derivations are more complex than those of the first part.

Clonal interference and fixation

Clonal interference among beneficial mutations

Some definitions. We refer to the common progenitor of one or more mutants as the 'ancestor'; the ancestral genotype, which is haploid, is denoted by *ab*, and the number of carriers of the *ab* genotype present at time *t* is denoted by *x(t)*. A mutant that carries a beneficial mutation has genotype *Ab* and has *y(t)* carriers at time *t*. Another mutant that carries an alternative mutation, also beneficial, has genotype *aB* and has *z(t)* carriers at time *t*. When discussing a beneficial mutation that is followed by the appearance of one or more alternative mutations, the first beneficial mutation shall often be described in retrospect as the 'original mutation'. If the original mutation is followed by a superior mutation, then there is a significant probability that the original mutation will be eliminated. This phenomenon, whereby the fate of an original beneficial mutation is altered by the appearance of a superior alternative mutation, shall be called 'clonal interference'.

The expected number of interfering mutations. We derive here the expected number of alternative mutations that are superior to a beneficial mutation and hence interfere with the progress of that mutation to fixation. Assuming that the number of such interfering mutations is Poisson distributed, we determine the probability that no interfering mutation occurs by calculating the zero-class. This probability is an important factor in determining the likelihood of fixation of a beneficial mutation.

Suppose a population is homogeneous until the appearance of a beneficial mutation, at which time the population consists of two fitness variants: the ancestral genotype and the beneficial mutant. Let the beneficial mutant appear in the ancestral population at time $t = 0$. The beneficial mutant, being competitively superior to the ancestor, slowly displaces the latter until finally reaching fixation at some time, t_f. Therefore, the time interval during which the beneficial mutation is present but not yet fixed is $(0, t_f)$. The expected number of interfering mutations is simply the expected number of mutations occurring in the interval $(0, t_f)$ on the wildtype background that are competitively superior to the original beneficial mutation.

If total population size, *N*, is constant, then the dynamics of the two genotypes are logistic (see, for example, Crow & Kimura, 1970). Let *s* denote the difference in Malthusian parameters between ancestor *ab* and beneficial mutant *Ab*. We have chosen the letter *s* because, under logistic growth, a difference in Malthusian parameters is equivalent to a selection coefficient when the unit of time is generations. Let μ denote the beneficial mutation rate per capita, per generation. Define t_f as the time to virtual fixation of the mutant subpopulation, i.e., $y(t_f) = N\text{-}1$. The expected number of further beneficial mutations between the time of appearance of the original mutation and its fixation is

$$\mu \int_0^{t_f} x(t)dt = \frac{\mu}{s} N \ln N \, . \qquad (1)$$

Of these beneficial mutations produced, we now calculate the fraction that interfere with the growth of the original mutation. We assume the effects of beneficial mutations to be exponentially distributed (c.f. Kimura, 1970; see *Discussion*). Hence, the probability density for *s* is $\alpha\, e^{-\alpha s}$, where α characterizes the distribution of mutational effects and may be determined from empirical data (see *Estimation of parameters*).

In the first few generations of growth, a beneficial mutation may be lost due to random sampling events, or drift. We employ the general notation, $\pi(s)$, to denote the probability that a beneficial mutation is not lost by drift in these first few generations. (Although dependence on *s* alone is indicated by our notation, this probability may in some cases be a function of other parameters as well.) All further derivations employ the general notation, $\pi(s)$, whereas all computations implement the approximation, $\pi(s) \approx 4s$, which is derived in Appendix I for the special case of bacterial populations. We emphasize that our analytical results are general for asexual species; implementing these results, however, depends on first finding an appropriate function, $\pi(s)$, for the particular species under study. A viral species, for example, might be assumed to have a Poisson distribution of offspring, in which case $\pi(s) \approx 2s$ when population size is constant (Haldane, 1927). Expressions for probabilities of surviving drift in fluctuating populations are given in Otto and Whitlock (1997).

In light of the two preceding paragraphs, the probability that an arbitrarily chosen beneficial mutation (*i*) has a selection coefficient greater than *s* and (*ii*)

survives drift is

$$\int_{s}^{\infty} \pi(u)\alpha e^{-\alpha u} du.$$

All further derivations assume that $\pi(u)$ is linearly related to *u*, in which case this integral is equivalent to $e^{-\alpha s}\pi(s + \frac{1}{\alpha})$.

Because loss by drift occurs in the first few generations, whereas loss by clonal interference is more probable in later generations, we can make the simplifying assumption that these two processes are independent. Therefore, the expected number of mutations occurring in the interval (0, t_f) that are superior to a given beneficial mutation with selective advantage, *s*, and that survive the effects of drift is

$$\lambda(s, \alpha, \mu, N) = \frac{\mu}{s} N \ln(N) e^{-\alpha s}\pi(s + \frac{1}{\alpha}). \quad (2)$$

This is the expected number of interfering mutations.

Fixation probability of a beneficial mutation

If a beneficial mutation survives the first few generations and is not lost by drift, its fixation is still far from ensured. In fact, fixation of a beneficial mutation may be very unlikely as a consequence of the presence of, and competition with, alternative mutations.

A beneficial mutation is fixed only if *(i)* it survives drift, and *(ii)* no superior mutation appears and survives drift in the time interval required for fixation. Given selective advantage *s*, the probability that a beneficial mutation will not be lost by either drift or clonal interference is simply the product,

$$Pr\{fix|s, \alpha, \mu, N\} = \pi(s)e^{-\lambda(s,\alpha,\mu,N)}. \quad (3)$$

This probability is plotted against *s* in Figure 1. Finally, the probability density for the condition that a given beneficial mutation confers a selective advantage *s* is $\alpha\ e^{-\alpha s}$. The probability, therefore, that an arbitrarily chosen beneficial mutation will become fixed in a population is

$$Pr\{fix|\alpha, \mu, N\} = \alpha \int_{0}^{\infty} \pi(s)\ e^{-\lambda(s,\alpha,\mu,N)-\alpha s} ds. \quad (4)$$

In Figure 2, the fixation probability given by equation (4) is plotted for different combinations of α and *N*.

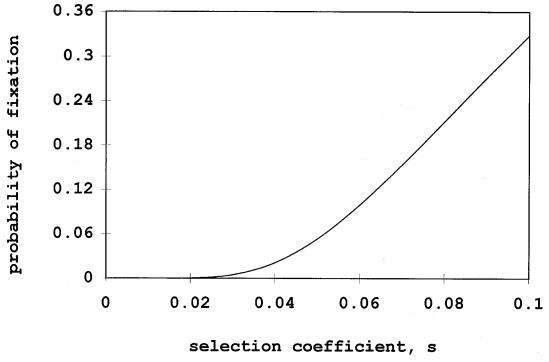

Figure 1. The probability that a given beneficial mutation with selection coefficient, *s*, achieves fixation. Equation (3) with α = 35, $\mu = 2.0 \times 10^{-9}$, and $N = 3.3 \times 10^{7}$.

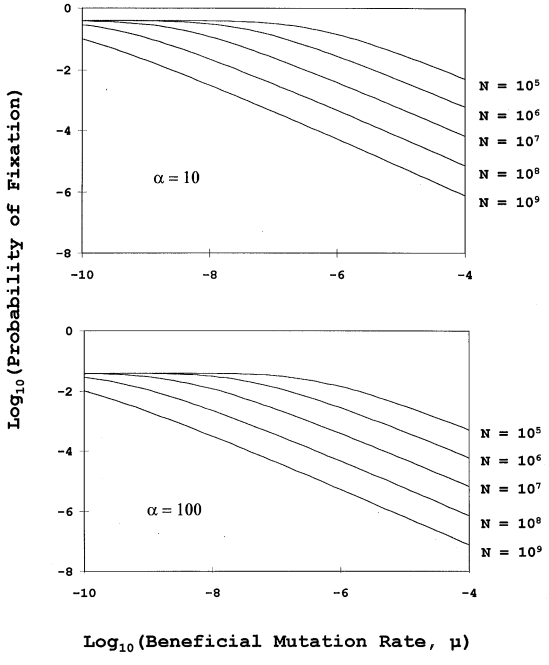

Figure 2. The probability of fixation of an arbitrarily chosen beneficial mutation is a decreasing function of both beneficial mutation rate, μ, and population size, *N* (Equation [4]). The exponential parameter, α, appears only to shift the curves down vertically without changing their shape.

Note the dramatic decrease in fixation probability with increasing beneficial mutation rate and with increasing *N*. The above calculations will become more informative and useful when the fixation probability is converted to an expected substitution rate of beneficial mutations in a population.

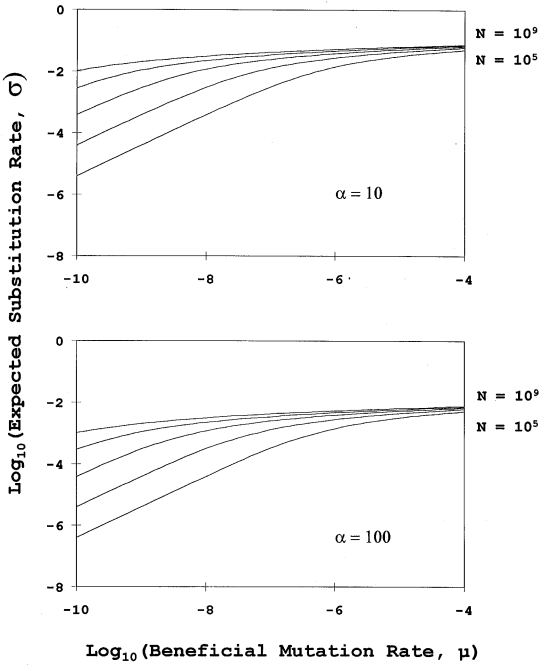

Figure 3. The substitution rate of a population is an increasing function of its beneficial mutation rate (Equation [5]). When the population size is large, however, a large change in beneficial mutation rate hardly affects the substitution rate.

The expected rate of substitution

We now make a simple, intuitive, but erroneous calculation in order to demonstrate that the results of this section may counter a seemingly reasonable train of thought. The total number of beneficial mutations produced per generation by a population is equal to the beneficial mutation rate times the number of individuals in the population, μN. Suppose that a fraction $4\overline{s}$ survive drift. If one made the assumption that a certain fixed fraction, β, of these beneficial mutations go to fixation, then the rate of substitution would be $4\overline{s}\beta\mu N$. Put differently, the rate of substitution might be presumed to be a linear function of either mutation rate or population size. In this section, however, we show that when clonal interference is taken into account and the population is large, mutation rate and population size have surprisingly small effects on substitution rate.

With the fixation probability given by equation (4), the expected substitution rate of beneficial mutations is given by

$$< \sigma(\alpha, \mu, N) >= \mu N Pr\{fix|\alpha, \mu, N\}, \quad (5)$$

where $< \cdot >$ denotes the expected value. As shown in Figure 3, a very large change in beneficial mutation rate (several orders of magnitude) has little effect on the substitution rate of the population, especially when the population is large. This constraint may be thought of as a 'law of diminishing returns,' where the investment is the number of beneficial mutations produced by a population and the returns are adaptive substitutions.

The expected selection coefficient of successful mutations

Figure 1 shows that there is some critical value of *s* below which the probability of fixation of a beneficial mutation is essentially zero. A beneficial mutation whose selective advantage is small is not likely to become fixed because it must compete with many superior mutations. On the other hand, a beneficial mutation whose advantage is large is less likely to be produced. Hence, there must be some intermediate selection coefficient that balances the fixation advantage of large *s* with the more frequent occurrence of small *s*. This balance corresponds to the expected selection coefficient of successful mutations.

Let $p(s) = K\pi(s)\ e^{-\lambda(s,\alpha,\mu,N)-\alpha s}$, where *K* is a normalizing constant such that $\int_0^\infty p(s)\ ds = 1$. Then *p(s)* is the probability density that a beneficial mutation of selective advantage *s* will be *(i)* produced and *(ii)* fixed. Therefore, the expected value for the selection coefficient of successful mutations is

$$< s(\alpha, \mu, N) > = \int_0^\infty s\ p(s)\ ds. \quad (6)$$

Figure 4 reveals that this expectation is essentially constant for $\mu N < 0.01$ and increases approximately linearly with the log of population size when $\mu N > 0.1$.

Effect of clonal interference on rate of fitness increase

At this point, sufficient information has been provided to determine how clonal interference between beneficial mutations affects the rate of adaptive evolution. Having derived *(i)* the rate at which substitutions occur and *(ii)* the expected selective advantage conferred by substitutions, we now calculate the expected rate of fitness increase simply as the product of *(i)* and *(ii)*:

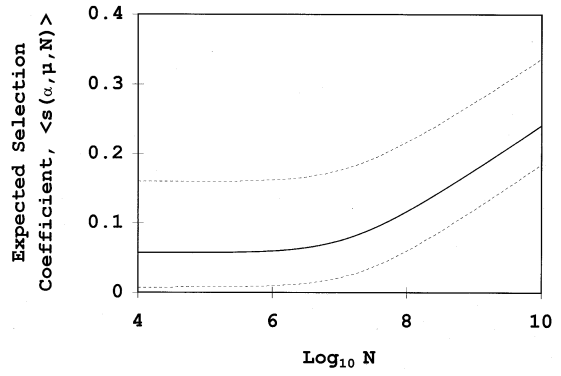

Figure 4. The expected selection coefficient of substitutions, $<s(\alpha, \mu, N)>$, is an increasing function of population size, *N*. Equation (6) with α = 35, and $\mu = 2.0 \times 10^{-9}$. The solid line indicates the expected value; the dashed lines indicate numerically determined 95% predictive confidence limits.

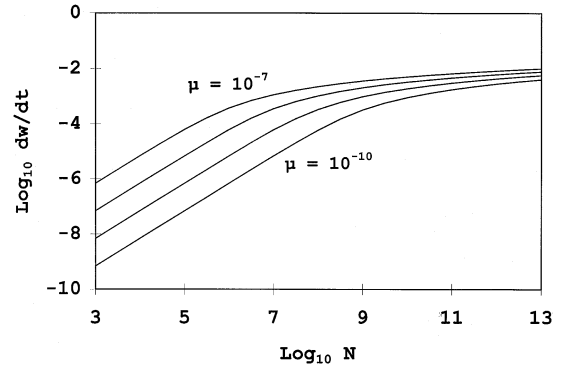

Figure 5. The rate of fitness improvement, $\frac{dw}{dt}$, is an increasing function of both population size, *N*, and beneficial mutation rate, μ. Equation (7) with α = 35. This rate of increase decelerates substantially, however, due to increased clonal interference when $\mu N > 0.1$ (i.e., when more than one beneficial mutation is produced on average every 10 generations).

$$\begin{aligned} \frac{dw}{dt} &= < \sigma(\alpha, \mu, N) > < s(\alpha, \mu, N) > \\ &= \alpha\ \mu\ N \int_0^\infty s\ \pi(s)\ e^{-\lambda(s,\alpha,\mu,N)-\alpha s}\ ds \end{aligned} \tag{7}$$

Equation (7) is plotted against $Log_{10}\ N$ in Figure 5 for different mutation rates. It appears that $\frac{dw}{dt}$ approaches a maximum value for increasing *N*. The same is true for μ. Indeed, that a maximum value exists can be shown mathematically. The implication is that there exists a sort of 'speed limit' for asexual evolution imposed by clonal interference.

Estimation of parameters: an empirical example

The previous developments show some characteristic consequences of clonal interference; yet, these developments remain at the level of sweeping generalities until we find the region of parameter space in which biological reality lies. We demonstrate here that the parameters α and μ may, in fact, be estimated empirically.

Equations (5) and (6) govern the expected rate of substitution and the expected selection coefficient of substitutions, respectively, both being functions of α, μ, and *N*. If *N* is known, then the resulting two equations contain two unknowns and are linearly independent:

$$\begin{aligned} < s(\alpha, \mu) > - s_{obs} &= 0 \\ < \sigma(\alpha, \mu) > - \sigma_{obs} &= 0 \end{aligned} \tag{8}$$

The parameters α and μ may, therefore, be determined from this pair of equations given observed values for the substitution rate, σ_{obs}, and the selection coefficient of substitutions, s_{obs}.

It is possible to obtain such values by tracking the fitness trajectory of an evolving population (Lenski et al., 1991; Lenski & Travisano, 1994). The average time between periodic selection events gives the reciprocal of the substitution rate estimate; the average fitness increase caused by periodic selection events gives an estimate for the selection coefficient of substitutions.

As an example, we estimate α and μ using the fitness trajectory observed for an evolving *Escherichia coli* population (Lenski et al., 1991; Lenski & Travisano, 1994). This example serves two purposes: *(i)* it demonstrates the estimation procedure, and *(ii)* it puts us in the 'biological ball-park' of parameter space. Lenski and colleagues serially propagated several *E. coli* populations for 10,000 generations of binary fission in a constant environment. (A particularly nice feature of working with bacteria is that samples of the evolving populations may be frozen and later 'resurrected' for comparison with samples from earlier or later times. In this way, one may track the evolution of populations over time by competing the evolved populations against the ancestor to estimate their relative fitness.) That calculation of generation number implies a discrete-time formulation of population growth, whereas the mathematics in this paper employ a continuous-time formulation. In the following estimation of parameters, we adjust the number of generations by a factor of *ln* 2 (≈ 0.693) to reflect this difference. During the first 2000 generations of binary fission

($\approx$ 1400 natural generations), they intensively assayed fitness for one population (Lenski & Travisano, 1994). The observed fitness trajectory was characteristically punctuated with sudden fitness increases followed by long periods of stasis. This general pattern is in accordance with the results of previous sections: that due to clonal interference, the substitution of a beneficial mutation is a rare, isolated event, and that the fitness increases due to substitutions are large. Based on three sudden fitness increases during $\sim$ 1400 natural generations (Lenski & Travisano, 1994), the average substitution rate is estimated as σ_{obs} = 0.002 substitutions per generation; the average fitness increase resulting from a substitution is s_{obs} = 0.1. The effective population size with respect to the substitution of beneficial mutations, and given the serial transfer regime, was determined to be 3.3×10^7 (Lenski et al., 1991).

We have estimated parameters α and μ from these data by finding the point of intersection between the solution curves of equations (8). The solution for this system of equations is $\alpha = 35$ and $\mu = 2.0 \times 10^{-9}$ beneficial mutations per replication. Given that the genomic mutation rate of *E. coli* is approximately 3×10^{-3} mutations per replication (Drake, 1991), one can infer that the proportion of mutations that are beneficial is roughly one in a million.

We emphasize that these estimates depend on *(i)* the assumption of an exponential distribution of beneficial mutational effects, and *(ii)* the assumption that α and μ remain constant even as mean fitness increases. The empirical fitness trajectories referred to in this section show a decreasing rate of increase, suggesting that assumption *(ii)* is false if the environment is constant. (See Assumptions of the models.)

Transiently common mutations

Clonal interference – a general model

Suppose that, while one beneficial mutation grows in number, a second beneficial mutation appears that is superior to the first. The population is now composed of three genotypes of interest: the ancestor and two competing beneficial mutations. If the first beneficial mutation is not close to fixation, then its growth is unaffected by the growth of the second, superior mutation until the latter has become sufficiently abundant to affect the mean fitness of the population noticeably. When the superior mutation attains sufficient number, the growth of the original mutation is retarded until, at some point, it reaches a maximum frequency and then begins to decline. We are interested in the probability that the frequency at which this maximum occurs is greater than or equal to some frequency, *f*. To determine the probability that any particular beneficial mutation achieves a frequency of at least *f*, we begin by computing the time, t_z, at which a superior mutation with selective advantage s_z must have appeared to insure that the original mutation achieves a maximum frequency of exactly *f*. Then we calculate the probability that no such superior mutation occurs in the interval $(0, t_z)$; this is the probability that the original mutation achieves a maximum frequency of at least *f*. (Note that t_z is itself a function of the selective advantage, s_z, of a given superior mutation.) To facilitate presentation of this development, we introduce the term 'candidate replication' to refer to any replication event which, if it were to produce a superior mutation, would prevent the original mutation from attaining frequency *f*.

Consider a three-genotype system with ancestor *x*, original beneficial mutant *y*, and alternative superior mutant *z*; the deterministic solution for the dynamics of such a system is derived in Appendix II. The time, t_{max}, at which beneficial mutation, *y*, reaches maximum number is a function of the time of occurrence, t_z, of an alternative mutation, *z*, which is superior to *y*, i.e., $t_{max} = t_{max}(t_z)$. The time, t_z, is that which satisfies $y(t_{max}(t_z)) = f N$, where $t_{max}(t_z)$ is such that $\frac{dy}{dt}|_{t_{max}(t_z)} = 0$. If the superior mutation, *z*, were to occur before time t_z, then the original mutation, *y*, would not achieve frequency *f*. We can, therefore, calculate the probability that no superior mutation occurs in the interval $(0, t_z)$ by determining the expected number of such mutations in this interval and assuming that they are Poisson distributed.

The first step in determining the expected number of superior mutations interfering with the original mutation is to calculate how many candidate replications take place, i.e., the number of ancestral replications in the interval (0, t_z), or $R = \int_0^{t_z} x(t)\,dt$. But t_z is a function of the selective advantage, s_z, of the superior mutation. *R* is closely approximated by evaluating $t_z(s_z)$ at the expected value for s_z conditional on it being greater than s_y, i.e., $\hat{t}_z = t_z(\langle s_z | s_z > s_y \rangle)$, where $\langle s_z | s_z > s_y \rangle = s_y + \frac{1}{\alpha}$ is the expected selection coefficient of a superior mutation.

The expected number of beneficial mutations in the interval (0, $\hat{t}_z$) is μR, where μ is the per-replication rate at which beneficial mutations are produced. Of these

μR beneficial mutations, only the fraction $e^{-\alpha s_y}$ will be competitively superior to *y*, the original beneficial mutation. And of these $\mu\, R\, e^{-\alpha s_y}$ superior mutations, only another fraction $\pi(s_y + \frac{1}{\alpha})$ will survive drift. Therefore, the expected number of beneficial mutations that occur in the interval (0, $\hat{t}_z$, that are superior to *y*, and that survive drift is $\psi = \mu R e^{-\alpha s_y} \pi(s_y + \frac{1}{\alpha})$. Because this expectation is a function of s_y but not s_z, we simplify our notation at this point by letting $s = s_y$. The analytical solution for *R*, the number of candidate replications, is derived in Appendix III. The resulting expected number of superior mutations that would prevent a given beneficial mutation from attaining frequency *f* is

$$\begin{array}{l} \psi(s, \alpha, \mu, N, f) = \\ \mu\, N \ln\,(N/\chi)\, e^{-\alpha s} \pi(s + \frac{1}{\alpha}), \end{array} \tag{9}$$

where

$$\chi = 1 + \frac{1}{\alpha s + 1}\left((\frac{1}{f} - 1)(\alpha s N)^{\frac{\alpha s}{\alpha s + 1}} - \alpha s \right).$$

Thus, the probability that a given beneficial mutation achieves a maximum frequency of at least *f* is

$$\pi(s)\; e^{-\psi(s, \alpha, \mu, N, f)}, \tag{10}$$

where the effect of drift is incorporated by π(s).

It is important to point out that equation (9) incorporates an approximation that is essentially an equality for $f < 0.95$, but which introduces significant error for $f > 0.99$. (See Appendix III for details.) A technical difficulty with equation (10) is that there is no guarantee that $\psi(s, \alpha, \mu, N, f)$ is non-negative, whereas a fundamental assumption of the Poisson process is that the Poisson parameter be non-negative. To remedy this problem, we impose the condition,

$$\psi(s, \alpha, \mu, N, f) = max\{\psi(s, \alpha, \mu, N, f), 0\}.$$

Otherwise, a negative Poisson parameter may arise if superior mutation *z* must appear *before* original beneficial mutation *y* to insure that the latter attains maximum frequency *f*, i.e., $\hat{t}_z$ is negative. In this case, the probability that a given beneficial mutation achieves a maximum frequency of at least *f* is equal to one, because an assumption of our analysis is that the superior mutation *z* does not appear before original mutation *y*. We have shown that this assumption does not introduce much error (see *Assumptions of the models*).

Probability of transiently polymorphic beneficial mutations

In this section, our objective is to determine with what probability one might expect a beneficial mutation to rise *temporarily* to polymorphic frequency. We define polymorphic frequency as any frequency greater than or equal to 0.01. In the *Clonal interference and fixation* section, we were only concerned with whether or not a beneficial mutation became fixed in a population, i.e., whether or not $f \geq \frac{N-1}{N}$. Now, we examine the probability that the frequency, *f*, of a beneficial mutation exceeds 0.01 yet never reaches $\frac{N-1}{N}$. This is the probability that a mutation will be transiently polymorphic.

Given that a beneficial mutation survives drift, the probability that it will achieve polymorphic frequency is $e^{-\psi(s, \alpha, \mu, N, 0.01)}$. Given that the same mutation achieves polymorphic frequency, the probability that it does not reach fixation is computed as the probability that at least one superior mutation appears in the interval ($\hat{t}_z, t_f$. The expected number of superior mutations appearing in this interval is:

$$\begin{array}{l} \gamma(s, \alpha, \mu, N, f) = \\ \frac{\mu}{s}\, N \ln\,(\chi) e^{-\alpha s}\; \pi\,(s + \frac{1}{\alpha}), \end{array} \tag{11}$$

where χ is as defined in equation (9). Therefore, given that a mutation with selective advantage, *s*, has achieved polymorphic frequency, the probability that it does not reach fixation is $1 - e^{-\lambda(s, \alpha, \mu, N, 0.01)}$. The probability that a mutation will be transiently polymorphic is the product of *(i)* the probability that the mutation survives drift, *(ii)* the probability that the mutation achieves polymorphic frequency given *(i)*, and *(iii)* the probability that the mutation does not reach fixation given *(ii)*. Therefore, the probability that any arbitrarily chosen beneficial mutation transiently achieves polymorphic frequency is

$$\begin{array}{l} Pr\{poly|\alpha, \mu, N\} = \\ \alpha \int\limits_0^\infty \pi(s)\; e^{-\psi(s, \alpha, \mu, N, 0.01) - \alpha s} \\ (1 - e^{-\gamma(s, \mu, \alpha, N, 0.01)})\; ds \end{array} \tag{12}$$

This equation is plotted in Figure 6. Given a certain population size, there is an intermediate value of the beneficial mutation rate at which the probability is greatest that an arbitrarily chosen beneficial mutation will transiently achieve polymorphic frequency. Likewise, given a certain beneficial mutation rate, there is an intermediate population size that maximizes the probability that an arbitrary beneficial mutation will be

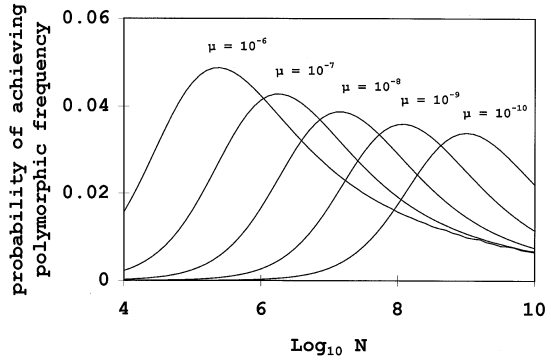

Figure 6. The probability that an arbitrarily chosen beneficial mutation transiently achieves polymorphic frequency is plotted against log population size for various beneficial mutation rates. Equation (12) with $\alpha = 35$. For a given beneficial mutation rate, there is an intermediate population size at which the probability of achieving polymorphic frequency is a maximum.

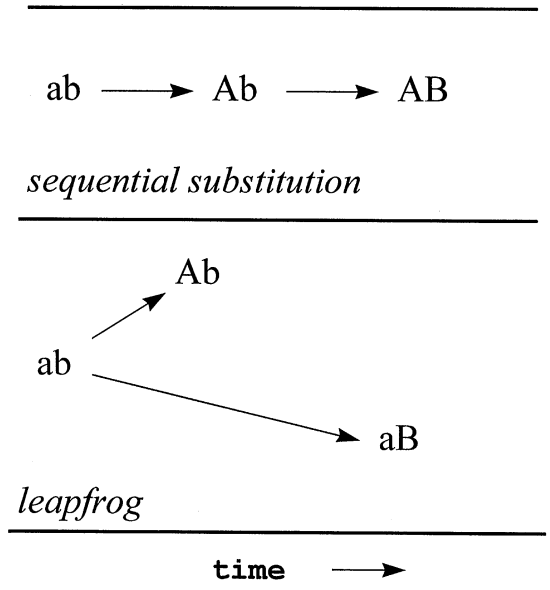

Figure 7. The leapfrog phenomenon illustrated phylogenetically. The phylogeny of majority genotypes is compared with that of sequential substitution.

transiently polymorphic. This result seems reasonable, because an increased recruitment rate of beneficial mutations, μN, increases the probability that a superior mutation occurs before a given beneficial mutation can reach polymorphic frequency (i.e., increases clonal interference). By lowering μN, on the other hand, one reduces the probability that a superior mutation occurs later, hence increasing the probability that a beneficial mutation, which has already achieved polymorphic frequency, will go to fixation (i.e., is not transient).

Given the parameters estimated previously for an evolving *E. coli* population ($\alpha = 35$, $\mu = 2.0 \times 10^{-9}$, $N = 3.3 \times 10^7$), the probability that an arbitrarily chosen beneficial mutation becomes transiently polymorphic is approximately 0.034. With $\mu N \approx 0.07$, a beneficial mutation would have occurred every 15 generations or so. Of these, about 1 in 30 would become transiently polymorphic. Hence, one would expect about three transient polymorphisms (f>0.01) in 1400 natural generations. This number is roughly comparable to the number of periodic selection events that were observed. This correspondence suggests that each beneficial mutation that went to fixation displaced not only its 'parent' genotype but also a 'sibling' genotype that had achieved some success.

Surprisingly, these estimates do not rely heavily on the assumption that beneficial mutations are exponentially distributed. Calculations based on an alternative rectangular distribution show that the probability that a beneficial mutation transiently achieves polymorphic frequency is approximately 0.05. Thus, by assuming a rectangular distribution, one might expect about four transient polymorphisms in 1400 natural generations. The fact that assuming such very different distributions results in less than a two-fold difference in estimates suggests that these results are fairly robust. The section *Assumptions of the models* gives a more complete discussion of this test of robustness.

Probability of transiently common mutations: the leapfrog

In a slight variation of the previous section, we will now examine the probability that a beneficial mutation achieves a frequency of 0.5 but is not fixed. We devote a separate section to this special case because of the strange dynamics it would present to an observer of a population in which it occurred. In this case, a mutant *Ab* reaches majority status before being supplanted by a superior mutant *aB*, where both mutants are derived directly from the same ancestor *ab*. At the genetic level, this appears as a 'leapfrog' episode in which *(i) Ab* replaces *ab* as the most common genotype and thereafter *aB* replaces *Ab* as the most common genotype, even though *(ii) aB* is more closely related to *ab* than to *Ab* (Figure 7). If one were to sample this population at times t_1, t_2, and t_3, as indicated in Figure 8, then one would observe that the sample from t_3 is more closely related at the genetic level to the sample taken at t_1 than to that taken at t_2.

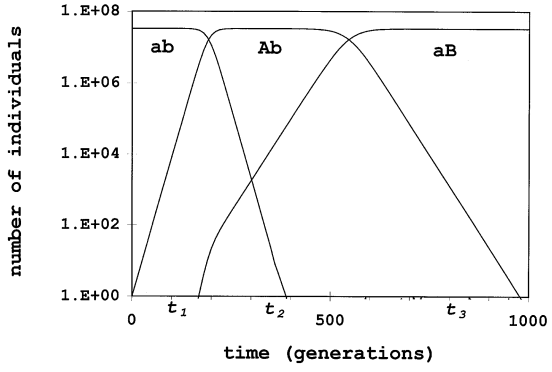

Figure 8. The leapfrog phenomenon illustrated dynamically. Genotype *ab* is displaced by mutant *Ab*, which is later displaced by alternative mutant *aB*. Equations (25) and (26) with s_y = 0.09, s_z = 0.13. Note that genotypes sampled at time t_3 are more closely related to those sampled at t_1 than to those sampled at t_2.

Following the derivation of equation (12), the probability that an arbitrarily chosen beneficial mutation transiently achieves a frequency of 0.5 or more is

$$Pr\{leapfrog|\alpha,\mu,N\} = \alpha \int_0^\infty \pi(s)\, e^{-\psi(s,\alpha,\mu,N,0.5)-\alpha s} (1 - e^{-\gamma(s,\mu,\alpha,N,0.5)})\, ds \quad (13)$$

Using the parameters previously estimated from an evolving *E. coli* population, and following the same logic as described at the end of the previous subsection, a beneficial mutation would occur every 15 generations or so. About one in every 55 of these mutations would be subject to the 'leapfrog' effect, which should thus occur every 800 generations or so. Therefore, it is quite possible that some of the three periodic selection events observed during the 1400 natural generations experiment were complicated by this effect. Whether empirical data would resolve the leapfrog as one or two periodic selection events would depend, in part, on how close in time the relevant genotypes became numerically dominant. If a leapfrog was resolved as two distinct periodic selection events, then the descendants after 1400 natural generations should differ from the founding ancestral genotype by fewer than the three beneficial mutations that would be expected under the presumption that each periodic selection event was caused by the sequential substitution of an additional mutation (Figure 8). Figure 9a shows a numerical simulation of the *E. coli* populations using the empirically estimated parameters. The resulting trajectory for mean fitness, shown in Figure 9b, illustrates that a single leapfrog episode may indeed give the appearance

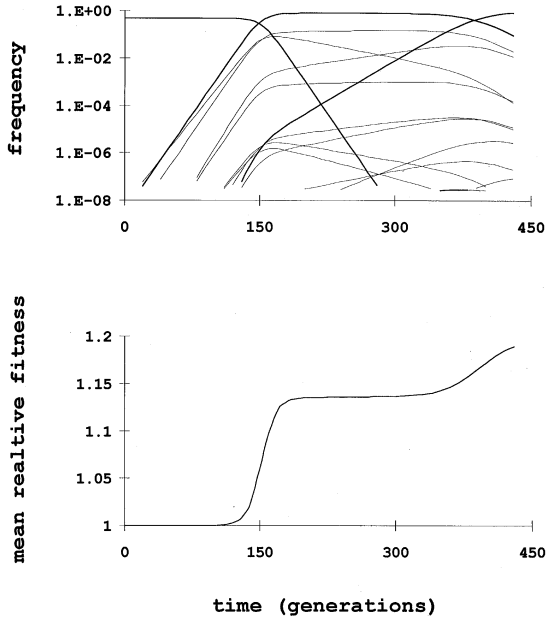

Figure 9. (a) A simulation of competition among numerous beneficial mutations. The heavy lines represent genotypes that achieve majority status. Note that a leapfrog event has occurred in this particular simulation. Selection coefficients were drawn at random from an exponential distribution. Parameters used are α = 35, $\mu = 2.0 \times 10^{-9}$, N = 3.3×10^7. (b) The mean fitness trajectory of the population simulated in panel (a). Note that the leapfrog phenomenon gives the appearance of two distinct periodic selection events.

of two periodic selection events. But rather than implying the successive fixation of two beneficial mutations, only a single substitution has actually occurred.

Discussion

Summary of results

Competition between clones that carry different beneficial mutations may be very important for the evolutionary dynamics of asexual populations. The prevalence of such 'clonal interference' among beneficial mutations increases dramatically with population size and with mutation rate. The following points summarize some of the most salient consequences of clonal interference:

1) The fixation probability of a given beneficial mutation is a decreasing function of both population size and mutation rate.
2) Substitutions appear as discrete, rare events, no matter how frequently beneficial mutations arise. If

a beneficial mutation is to overcome clonal interference and become fixed, then it must confer a substantial selective advantage. The advantage that is required for a reasonable probability of fixation is an increasing function of population size and mutation rate.

3) The rate of fitness increase is an increasing function of both population size and mutation rate, but it is only weakly dependent on these parameters when their product is not small.
4) Using observable trajectories for the mean fitness of evolving asexual populations, it is possible to estimate both the beneficial mutation rate and the distribution of beneficial mutational effects. We obtained such estimates for an evolving laboratory population of *Escherichia coli*.
5) Beneficial mutations that become transiently abundant, but which do not go to fixation, may be quite common in asexual populations.
6) Some of these transient polymorphisms may give rise to a 'leapfrog' effect, in which the majority genotype at some point in time is less closely related to the immediately preceding majority genotype than to an earlier genotype. Parameter estimates obtained for the evolving laboratory population of *E. coli* are consistent with this effect being an important feature of asexual dynamical systems.

Assumptions of the models

The models presented here assume that the general form of the distribution of beneficial mutational effects is that of an exponential distribution. Kimura (1979) employs the more general gamma distribution to describe the distribution of *deleterious* mutational effects. Elena et al. (1998) have shown that a compound gamma-rectangular distribution fits well to experimental data from transposon-induced mutations in *E. coli*. Intuitively, the exponential distribution seems a good choice for beneficial mutational effects, because it is reasonable to suppose that there are many more beneficial mutations of small effect than of large effect. Fisher (1930) reasoned that most mutations of large effect are deleterious as a geometrical consequence of the high dimensionality of fitness landscapes. He argued that the ratio of deleterious to beneficial mutations increases with mutational effect (i.e., phenotypic difference between mutant and non-mutant), because a large radius in phenotypic space is very likely to circumscribe potential improvements, whereas a small radius stands a better chance of being tangent to an improvement. That this effect increases with the dimensionality of the fitness landscape is an easily demonstrable fact of geometry. A convincing argument for the use of the exponential distribution in particular comes from extreme value theory (see Gillespie, 1991, p. 262). Suppose that M fitness alleles are present in a population such that $W_{[1]} > W_{[2]} > W_{[3]} > ... > W_{[M]}$ (where W denotes fitness). If the population is in dynamic equilibrium, then the fittest of these M alleles greatly outnumbers the other M-1 alleles, which are held at some low frequency by mutation-selection balance. A fitness mutation results in a genotype whose fitness is drawn at random from some unknown parent distribution. Now, imagine that a novel fitness mutation appears that is beneficial (i.e., fitter than the current fittest genotype). If we denote the fitness of this mutation by $W_{[0]}$, then $W_{[0]} > W_{[1]}$ and the selection coefficient of this novel mutation is $s = \frac{W_{[0]}}{W_{[1]}} - 1$. Gillespie (1991) shows that this s is exponentially distributed in the limit as $M \rightarrow \infty$, regardless of the shape of the unknown parent distribution. (The reason for this result has to do with the fact that $W_{[0]}$ and $W_{[1]}$ are the two largest fitnesses; they are extreme values of the parent distribution.) In other words, in the limit of infinite fitness alleles, the distribution of s is necessarily exponential.

To evaluate the sensitivity of our analysis to the assumption that selection coefficients are exponentially distributed, we replaced the exponential density with a rectangular density, i.e.,

$$p(s) = \begin{cases} 1/s_{max} & , \; s < s_{max} \\ 0 & , \; s > s_{max}. \end{cases}$$

See Appendix IV for results of these derivations. Following the logic employed in *Estimation of parameters*, we have estimated the parameters s_{max} and μ to be 0.12 and 5×10^{-10}, respectively, for an evolving laboratory population of *E. coli*. Note that the beneficial mutation rate estimate, μ, is of the same order of magnitude as was obtained assuming exponentially distributed selection coefficients. Figure 10 further shows that replacing the exponential with a rectangular density changes the resulting fixation probabilities only slightly. The slight discrepency when μN is very small, such that clonal interference is unimportant, arises because the average selection coefficient (and hence 4s) is slightly higher for the rectangular than for the exponential distribution. The probabilities of transient polymorphisms (either $f > 0.01$ or $f > 0.5$) are consistently higher when a rectangular density is assumed, although the discrepancies are small. In view

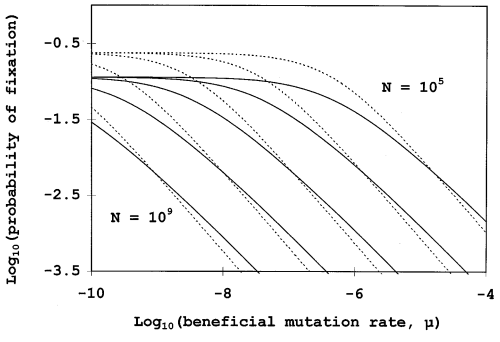

Figure 10. The probability of fixation of an arbitrarily chosen beneficial mutation is plotted against the beneficial mutation rate, μ, for various population sizes. The solid lines indicate probabilities assuming an exponential distribution of beneficial mutational effects (Eq. [4] with α = 35, as estimated from the *E. coli* populations). The dashed lines indicate probabilities that assume a rectangular distribution of beneficial mutational effects (Eq. [33] with s_{max} = 0.12, as estimated from the *E. coli* populations). The discrepancies resulting from the different distributional assumptions are small.

of these results, our analyses appear to be reasonably robust with respect to the form of the distribution of selection coefficients.

A second assumption of our analyses is that neither the beneficial mutation rate nor the distribution of selection coefficients changes over time. But in a constant environment, a population becomes better adapted with time, leaving progressively less room for further improvement. It is likely that a well-adapted population has *(i)* a lower overall rate of beneficial mutation, *(ii)* a smaller average effect of beneficial mutations, or both. Consequently, $\mu = \mu(w)$ may be a decreasing function of fitness, whereas $\alpha = \alpha(w)$ may increase with fitness. These parameters are therefore constant only when w is constant. This condition may be met in an environment that changes just fast enough to counter adaptation of a population.

A third assumption made in these models is that the progress of a given beneficial mutation is unaffected by the presence of inferior beneficial mutations. By definition, inferior beneficial mutations cannot themselves competitively exclude a given beneficial mutation. However, the selective advantage of a given beneficial mutation will be lower relative to these inferior beneficial mutations than relative to the ancestral genotype, and so inferior beneficial mutations may prolong the time that is required for fixation of a given beneficial mutation. As a consequence, there may be a longer interval in which a superior beneficial mutation could appear that would prevent fixation of the original beneficial mutation. To address this possible complication, the probabilistic models were made fully dynamic by considering all beneficial mutations since the most recent substitution. When the dust settled, the results were essentially unchanged from those that we have presented. For example, the fixation probability of an arbitrarily chosen beneficial mutation was changed by a factor of $\frac{\alpha}{\alpha+1}$ which is inconsequential because α is generally large.

An assumption made in estimating the parameters α and μ from observed fitness trajectories is that the sudden jumps observed in these trajectories are, in fact, fixation events. Based on the results of *The leapfrog*, however, this assumption is questionable; if a leapfrog event were to occur, then it would give the appearance of two such fixation events (Figure 9). Thus, of the three observed jumps in fitness during 1400 natural generations (Lenski & Travisano, 1994), for example, perhaps only two were actual fixations and one was the result of a leapfrog episode. If this were the case, then our estimates of α and μ would be incorrect. To evaluate the degree to which these estimates may be in error, we changed the assumption that observed fitness jumps represent fixations and assumed instead that these jumps represent beneficial mutations that achieved a frequency of $f > 0.5$. To that end, we employed the derivations of *Clonal interference – a general model*. This change of assumption did not appreciably affect the estimates (α = 29, $\mu = 1.6 \times 10^{-9}$), indicating that our initial assumption, at least in this case, did not introduce much error.

Model validation by simulation

A general result of the section, *Clonal interference and fixation*, is that trajectories of population mean fitness are characteristically punctuated, with sudden jumps followed by long periods of stasis, regardless of the mutation recruitment rate, μN. To assess this general prediction qualitatively, we simulated the occurrence of, and competition among, many different beneficial mutations whose selection coefficients were drawn at random from an exponential distribution. Figure 9 demonstrates that, despite fierce competition among numerous beneficial mutations (μN = 0.1), the population mean fitness is not appreciably affected until a fitness variant achieves high frequency. (These results also lend support to the assumption that mutations inferior to the currently dominant variant play a negligible role in clonal interference.)

To test the models quantitatively, we ran repeated simulations. The probabilistic predictions for *(i)* the probability of fixation, *(ii)* the expected fitness increase conferred by a substitution, *(iii)* the expected substitution rate, *(iv)* the probability of transiently achieving polymorphic frequency, all agreed well with a large number of fully stochastic simulations. We emphasize that these simulations allow for the more realistic situation in which a mutant may acquire further beneficial mutations at any time after its appearance.

Inclusion of the double mutant

To this point, we have emphasized competition between three genotypes, the progenitor (*ab*) and two mutants that carry different beneficial mutations (*Ab* and *aB*). However, a fourth genotype should eventually appear that has both beneficial mutations (*AB*). If the effects of the two beneficial mutations on fitness are additive, then the double mutant will eventually take over the population. A full treatment of the dynamics involving this fourth genotype is beyond the scope of this paper. For now, we address only one particular issue. If a leapfrog event is to be manifest, then genotypes *Ab* and *aB* must each achieve majority status before *AB* does; otherwise, the dynamics will appear as a sequential substitution (Figure 7). The probability of occurrence of the leapfrog must, therefore, incorporate the probability that sequential substitution does not occur. We have conservatively estimated this probability as

$$exp\left\{-\frac{\pi(s)\mu}{e}\int_0^{2t_z} y(t)\,dt\right\};$$

this factor was incorporated into the integrand of equation (13) and found to have a negligible effect (probabilities were reduced by no more than five percent for a wide range of parameters). Therefore, we neglected this factor in our earlier developments in order to keep things as simple as possible.

Implications for the evolution of reproductive strategies

Muller (1964) briefly alludes to the concept of clonal interference while making a case for the evolutionary advantage of sex. Muller argued that adaptive evolution of asexual populations is inefficient, because the fraction of beneficial mutations that are lost due to competition with alternative beneficial mutations may be substantial in a large population. Recombination would remedy such inefficiency, which suggested an evolutionary advantage for sex. This argument was restated and explored analytically by Crow and Kimura (1965), to which Maynard Smith (1968) responded by pointing out that Muller's original argument relied on the erroneous assumption that mutations were unique events, such that each could occur only once. In a counter-example, Maynard Smith demonstrated that models of sexual and asexual systems yielded the same rate of adaptive evolution when mutations were treated as recurrent events. For a nice summary of this controversy and further developments on this topic, see Felsenstein (1974, 1988). Much recent work has focused on how fixation probabilities are affected by variance in fitness at background loci and the degree of linkage to these loci (Barton, 1993, 1995; Keightley, 1991; Pamilo, Nei & Li, 1987; Peck, Barreau & Heath, 1997). Barton (1994) derived the conditional probability of fixation of a beneficial mutation given that a single substitution occurs or that substitutions occur at a given rate. He explored the dynamics of this probability under varying degrees of recombination. We believe that the models presented here may contribute to understanding the evolution of sex by giving an explicit expression for the unconditional probability of fixation of a beneficial mutation, in the limit as recombination rate goes to zero.

Another part of a population's reproductive strategy, namely its mutation rate, may also be affected by the clonal interference phenomenon. Much work has been done to determine whether and how natural selection may adjust mutation rates. A high mutation rate may confer an evolutionary advantage, for example, if it increases the rate of substitution of beneficial alleles. This advantage, however, must overcome the disadvantage of a parallel increase in deleterious mutations. Leigh (1970) demonstrated theoretically that elevated mutation rates can evolve in asexual populations that experience oscillating selection on some locus. Since then, much work has supported the notion that evolutionary elevation of mutation rates is at least possible, and perhaps likely, in changing environments (Gillespie, 1981; Ishii et al., 1989). In light of the developments presented in this paper, however, it seems that the strength of selection to elevate mutation rates (above some minimal value set by the physiological cost of fidelity) may be smaller than the established theory would indicate, especially when populations are large. As we have shown, an increase in mutation rate hardly changes the rate of adaptation of large popula-

tions because of clonal interference (Figure 5). To gain an appreciable increase in the rate of adaptation for a large population would, therefore, require a disproportionate increase in mutation rate. Such a large increase in mutation rate, however, would undoubtedly have a detrimental effect due to the greatly increased production of deleterious alleles. Consequently, it seems reasonable to suggest that selection for elevated mutation rates should be weak in large populations.

Implications for the general nature of adaptive evolution

Three especially interesting consequences of the results obtained here concern the general nature of adaptive evolution in asexual populations. The first is that one should expect the trajectory for mean fitness of any asexual population to be punctuated with short bursts of rapid, significant increase followed by long periods of stasis, regardless of the size of the population or its mutation rate. This result contradicts the intuitive, but erroneous, view that discrete bouts of periodic selection (in which individual mutations sweep to fixation) should overlap, thus giving the appearance of continuity, when the mutation recruitment rate, μN, is sufficiently high. A second intriguing implication is that there exists a 'speed limit' on the rate of adaptive evolution in asexual populations. As shown in Figure 5, the rate of improvement in a population's mean fitness decelerates with increasing μ and N. This result reflects intensified clonal interference as well as the longer time required for selection to proceed to fixation in large populations. A third important consequence is closely related to the second: the rate of adaptive evolution is clearly not always limited by mutation rate. In fact, because of clonal interference, the rate of adaptive evolution is only weakly dependent on mutation rate and population size unless μN is small ($\mu N < 0.1$ for $\alpha = 35$).

Evidence for transiently common beneficial mutations in microbial populations

One of the intriguing consequences of asexuality is that beneficial mutations may become quite common temporarily but eventually go extinct as superior mutations arise (Figures 8 and 9). In principle, it should be possible to find evidence for this effect in natural populations of asexual organisms. A complication arises, however, in that a beneficial mutation may also become transiently common, but then disappear, if the environment changes so that the mutation is no longer favored.

For example, Holmes et al. (1992) followed the molecular evolution of a population of the human immunodeficiency virus (HIV) within a single infected patient, and their data show several instances of transiently common mutations. In particular, they monitored changes in the RNA sequence encoding the third hypervariable loop of gp120 (V3) throughout the asymptomatic phase of infection (7 years) of a single hemophiliac patient. All 12 viral sequences that were obtained immediately after infection were identical, and these were denoted as sequence A. In year three, a set of related sequences, denoted C1 through C5, were numerically dominant (11 of 15), but in year seven they and their descendants comprised only a small fraction of the population (2 of 13). By contrast, sequence E1 was present only as a small minority (1 of 15) after three years, but after seven years it and its descendants were numerically dominant (10 of 13). Within the E1 clade, a subset of derived sequences, denoted E2 through E5, became numerically dominant after five years (19 of 23). However, neither they nor their descendants were represented by even a single sequence in years six (0 of 15) and seven (0 of 13). Thus, it is evident that certain mutations became transiently common, only to decline subsequently in frequency. Moreover, the data show the 'leapfrog' effect in which the majority type at one point in time is *not* descended from the majority type that immediately preceded it. Holmes et al. (1992, p. 4838) recognized the importance of these dynamics when they said that changes in the viral population, 'instead of being the sequential replacement of one antigenically distinct variant by another, may involve a complex interaction between the different, and competing, evolutionary lineages present in the plasma.'

The one caveat to our interpretation of these data, however, is that the host immune system responds to the viruses, and so the HIV population is evolving in a changing environment. Thus, for example, sequences E2 through E5 may not have been driven extinct solely by an intrinsically superior mutation, but instead they may have become selectively disadvantaged after they were targeted by the immune system. This scenario is supported by the fact that the V3 loop is a principal target of the immune system. But even with the added complication of the changing immune environment, asexuality can have important dynamical consequences for HIV and other pathogens. In particular, the 'leapfrog' effect necessarily increases the genetic

distance between successive majority types (Figures 7 and 8), and so it may actually facilitate a pathogen's evasion of the host immune response.

An unambiguous demonstration of the 'leapfrog' effect will require data from an asexual organism living in a constant environment. To that end, we are now using molecular methods to determine the phylogeny of clones sampled over time from an experimental population of *E. coli*, as it evolved for thousands of generations in a defined laboratory environment (Lenski et al., 1991; Lenski & Travisano, 1994; Elena et al., 1996). If the 'leapfrog' phenomenon is important, then we expect to see a clade become numerically dominant, only to be driven extinct by the emergence of another, even more successful clade that is derived from its ancestral base (rather than from the formerly dominant clade).

A suggestion for further research

Clonal interference is not the only dynamic that inhibits the progression of beneficial mutations to fixation in an asexual population. A similar inhibition may be caused by Muller's ratchet (Muller, 1964; Haigh, 1978), in which deleterious mutations will tend to accumulate in small asexual populations. As shown by Manning and Thompson (1984) and by Peck (1994), the fate of a beneficial mutation is determined as much by the selective disadvantage of any deleterious mutations with which it is linked as by its own selective advantage. In asexual organisms, the entire genome in which a beneficial mutation occurs will remain linked to that mutation and will hitchhike to fixation if that is the fate of the mutation. Therefore, a beneficial mutation that spreads to fixation presents a severe population bottleneck in which only a single genome is sampled, thus exacerbating the effect of Muller's ratchet. Consequently, a beneficial mutation may only be considered advantageous if its benefit more than compensates for the drastic reduction in effective population size caused by its fixation and the associated acceleration of Muller's ratchet.

Haigh (1978) modeled the effect of deleterious mutations on population fitness; Manning and Thompson (1984) and Peck (1994) modeled the effect of deleterious mutations on the fate of beneficial mutations; and the models presented here provide a quantitative account of how beneficial mutations affect one another. A logical next step would be to integrate these models into a single theoretical treatment of mutation in asexual populations. Such a synthesis would be a valuable contribution toward a general understanding of evolutionary dynamics in asexual systems.

Acknowledgments

This manuscript was greatly improved by interactions with P. Sniegowski. Special thanks to V. Mandrekar, A. Sorto, and A. White for help with various aspects of the mathematical development. Thanks also to F. Adler, S. Elena, W. Ewens, J. Mongold, J. Peck, D. Rozen, and an anonymous reviewer for comments and discussion. This work was supported by National Science Foundation grant DEB-9421237 to R.E.L.

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Appendix I: Probability of surviving drift

In the first few generations of growth, a beneficial mutation may be lost by random sampling events, or drift. Haldane (1927) derived the probability of surviving drift for a single beneficial mutation. His derivation made use of a result from the theory of branching processes, which states that probability of extinction (i.e., *not* surviving drift) is obtained by solving the equation $f(\phi) = \phi$, where $f(\phi)$ is the probability-generating function for number of offspring (see Ewens, 1969, p. 79). A simple assumption for multicellular, sexual organisms is that this function generates a Poisson distribution, in which case the probability of survival of a beneficial mutation approximates *2s*. Our analyses, however, are based on the fundamental assumption of no recombination. We may further restrict our analysis to a particular kind of asexual organism, namely asexual bacteria. Bacteria reproduce by binary fission, and so we derive the generating function as follows. Our assumption of a constant population size (see below) implies a sampling event every generation. Thus, a bacterium that divides before sampling will leave zero, one, or two offspring after sampling. In the case of bacteria, therefore, the probability-generating function for number of offspring is

$$f(\phi) = (1 - c/2)^2 + c(1 - c/2)\phi + (c/2)^2\phi^2, \tag{14}$$

where *c* is the expected number of offspring after division and sampling. Thus, the probabilities of passing zero, one, and two offspring to the next generation are, respectively, $(1 - c/2)^2, c(1 - c/2)$, and $(c/2)^2$. The selective advantage of the mutant is $s = \ln c$ by definition, or approximately $s \approx c - 1$ when *s* is small. Let $\pi(s)$ denote the probability that a beneficial mutant survives drift. Then, by substituting 1 + *s* for *c* in (14) and solving the equation $f(1 - \pi(s)) = 1 - \pi(s)$, we obtain $\pi(s) = \frac{4s}{(1+s)^2}$, which is approximately 4*s* for small *s*. All derivations in this paper employ the general notation, $\pi(s)$, whereas all computations implement the approximation, $\pi(s) \approx 4s$.

Appendix II: *n*-genotype logistic system with mutation

General solution

Logistic dynamics of an *n*-genotype system are modeled by assuming that *(i)* total population size is constant, i.e.,

$$\sum_{i=1}^{n} x_i = N,$$

where x_i is number of individuals of genotype *i*, and *(ii)* the differences in Malthusian parameters are constant:

$$m_i - m_1 = s_i, \quad i = 2, ..., n, \tag{15}$$

where

$$m_i = \frac{1}{x_i}\frac{dx_i}{dt}, \text{and}$$
$$m_1 = \frac{1}{x_1}\frac{dx_1}{dt} = \left(\sum_{i=2}^{n} x_i - N\right)^{-1}\left(\sum_{i=2}^{n}\frac{dx_i}{dt}\right).$$

Equation (15) may, therefore, be rewritten as:

$$\frac{1}{x_i}\frac{dx_i}{dt} + \left(N - \sum_{j=2}^{n} x_j\right)^{-1} \left(\sum_{j=2}^{n} \frac{dx_j}{dt}\right) = s_i, \quad i = 2, 3, ..., n. \tag{16}$$

This system of *n*-1 equations can be rearranged as follows:

$$\frac{dx_i}{dt} = x_i \left(s_i - \frac{1}{N}\sum_{j=2}^{n} s_j x_j\right), \tag{17}$$

where *i* = 2,3,...,*n*. Although this system of equations is non-linear, its symmetry makes an analytical solution possible. The key to its solution is the transformation $X_i = \ln x_i - s_i t$. The system of equations now becomes:

$$\frac{dX_i}{dt} = -\frac{1}{N}\sum_{j=2}^{n} s_j \, e^{X_j + s_j t}, \tag{18}$$

where *i* = 2,3,...,*n*. Thus, the time derivatives of all transformed variables are equal:

$$\frac{dX_i}{dt} - \frac{dX_j}{dt} = 0, \tag{19}$$

where *i,j* = 2,3,...,*n*. Integration of (19) yields $X_i - X_j = k_{ij}$, and k_{ij} is a constant of integration that is determined from initial conditions:

$$k_{ij} = X_i(0) - X_j(0) = \ln x_i(0) - \ln x_j(0), \tag{20}$$

where *i,j* = 2,3,...,*n*. Thus, the system of equations is uncoupled by substituting X_j from (18) with X_i - k_{ij}, which yields:

$$\frac{dX_i}{dt} = -\frac{1}{N}\sum_{j=2}^{n} s_j \, e^{X_i - k_{ij} + s_j t}, \tag{21}$$

where *i* = 2,3,...,*n*. From solution and subsequent back-transformation of equation (21), the analytical solution of an *n*-genotype logistic system is obtained:

$$x_i(t) = x_i(0) e^{s_i t} \left(1 + \frac{1}{N}\sum_{j=2}^{n} x_j(0)\,(e^{s_j t} - 1)\right)^{-1}, \tag{22}$$

where *i* = 2,3,...,*n*, and

$$x_1(t) = N - \sum_{j=2}^{n} x_j(t). \tag{23}$$

Application of boundary conditions due to mutation

If genotype *i* appears by mutation at time τ_i, then boundary conditions are $x_i(\tau_i = 1$. From these, the initial conditions are determined; they are

$$\mathbf{X}_0 = \mathbf{R}^{-1}\mathbf{N}, \tag{24}$$

where $\mathbf{X}_0$ is a vector whose elements are $x_i(0), i = 2, 3, ..., n$, $\mathbf{R}$ is an *n-1* × *n-1* matrix whose elements are

$$r_{ij} = \begin{cases} N\, e^{s_i \tau_i} + 1, & i = j \\ 1 - e^{s_j \tau_i}, & i \neq j, \end{cases}$$

i,j = 2,3,...,*n*, and ***N*** is a vector whose *n-1* elements are the constant *N*.

Notation for the 3-genotype case

The developments in this appendix use a more general notation than is used in the rest of the paper, where x_1 is simply denoted by *x*, x_2 is denoted by *y*, and x_3 is denoted by *z*. This 3-genotype case has the particular solution:

$$\begin{aligned} y(t) &= e^{s_y t}\left[\frac{1}{y(0)} + \frac{1}{N}\left(e^{s_y t} - 1 + \frac{z(0)}{y(0)}(e^{s_z t} - 1)\right)\right]^{-1} \\ z(t) &= e^{s_z t}\left[\frac{1}{z(0)} + \frac{1}{N}\left(e^{s_z t} - 1 + \frac{y(0)}{z(0)}(e^{s_y t} - 1)\right)\right]^{-1}. \end{aligned} \tag{25}$$

The initial conditions are determined from the boundary conditions, *y*(0) = 1 and *z*(t_z) = 1; they are

$$\begin{aligned} y(0) &= 1 \\ z(0) &= \frac{e^{s_y t_z} + N}{N\, e^{s_z t_z} + 1} \end{aligned} \tag{26}$$

Appendix III: Expected number of candidate replications

Here we derive the expected number of replications that may generate superior mutations that prevent a given beneficial mutation from attaining some frequency, *f*.

We have called these *candidate replications*, denoted by *R*, in the subsection, *Clonal interference – a general model*. The crucial step in the derivation of *R* is finding an expression for the time, t_z, at which a superior mutation must appear if the original mutation is to attain a maximum frequency of exactly *f*.

The time, t_{max}, at which *y* reaches a maximum number is determined from $\frac{dy}{dt}|t_{max}(t_z) = 0$; it is

$$t_{max}(t_z) = \frac{1}{s_z} \ln\left(\frac{s_y}{s_z - s_y}\frac{N^2}{e^{(s_y - s_z)t_z} + N\, e^{-s_z t_z}}\right). \quad (27)$$

When $e^{(s_y - s_z)t_z} < Ne^{-s_z t_z}$ (i.e., when $e^{s_y t_z} < N$), equation (27) is well approximated by omitting the term $e^{(s_y - s_z)t_z}$ from the denominator, resulting in the approximation

$$t_{max}(t_z) = \frac{1}{s_z} \ln\left(\frac{Ns_y}{s_z - s_y}\right) + t_z. \quad (28)$$

The constraints under which this approximation works well are discussed later.

We now calculate the time t_z at which superior mutation *z* must appear if *y* is to achieve a maximum of exactly *fN*. The solution to $y(t_{max}(t_z)) = fN$ is

$$\mathrm{t_z} = \frac{1}{\mathrm{s_y}} \ln\left(\frac{\mathrm{N}\left(1 + \frac{\mathrm{s_y}}{\mathrm{s_z - s_y}}\right)}{\left(\frac{1}{f} - 1\right)\left(\frac{\mathrm{Ns_y}}{\mathrm{s_z - s_y}}\right)^{\mathrm{s_y/s_z}} - \frac{\mathrm{s_y}}{\mathrm{s_z - sy}}}\right). \quad (29)$$

Next, we use the fact that the expected number of candidate replications, *R*, given s_y and $s_z > s_y$, is well approximated by evaluating *R* at $s_z = \langle s_z | s_z > s_y \rangle = s_y + \frac{1}{\alpha}$, to derive the expected number of candidate replications:

$$R \simeq \int_0^{\hat{t}_z} x(t)dt = \frac{N}{s}\ln\left(N\left[1 + \left(\frac{1}{1+\alpha s}\right)\left((\frac{1}{f} - 1)(\alpha s N)^{\frac{\alpha s}{\alpha s + 1}} - \alpha s\right)\right]^{-1}\right) \quad (30)$$

where $\hat{t}_z$ is simply t_z evaluated at $s_z = s_y + \frac{1}{\alpha}$.

The approximation made in equation (28) is, for our purposes, essentially an equality when $e^{s_y t_z} < N$. If we combine this condition with equation (29), then the approximation works well only when the frequency *f* meets the following condition:

$$f < \left[\frac{2}{N}\left(\frac{Ns_y}{s_z - s_y}\right)^{1 - \frac{s_y}{s_z}} + 1\right]^{-1}. \quad (31)$$

If we let $s_z = \langle s_z | s_z > s_y \rangle = s_y + \frac{1}{\alpha}$, and if we simplify the notation so that $s = s_y$, then the above condition becomes

$$f < \left[\frac{2}{N}(\alpha s N)^{\frac{1}{1+\alpha s}} + 1\right]^{-1}. \quad (32)$$

This upper bound on *f* reaches a minimum value when $\frac{\partial f}{\partial s} = 0$, so that an overall bound below which the approximation works well is obtained by solving for the value of *s* that satisfies $\ln(\alpha s N) = 1 + \frac{1}{\alpha s}$ and using that value in equation (32). In general, the approximation is valid when $f < 0.95$ provided that *N* is greater than 10^4. For the purposes of this paper, the approximation is essentially an equality because we are concerned only with the cases $f = 0.01$ and $f = 0.5$, for which the approximation works extremely well. We compute fixation probabilities, i.e., the boundary case $f > \frac{N-1}{N}$, using the simpler derivations in *Clonal interference and fixation*.

Appendix IV: Functions employing the rectangular distribution

We present here the results only of the derivations in which a rectangular distribution replaces the exponential distribution of beneficial mutational effects. The probability of fixation of an arbitrarily chosen beneficial mutation is:

$$Pr\{fix|s_{max}, \mu, N\} = \frac{1}{s_{max}} \int_0^{s_{max}} \pi(s) e^{-\lambda_R(s, s_{max}, \mu, N)} ds, \quad (33)$$

where

$$\lambda_R(s, s_{max}, \mu, N) = \frac{\mu}{s} N \ln N \pi\left(\frac{s^{2max} - s^2}{2s_{max}}\right)$$

(assuming that π (*u*) is approximately linear). The expected rate of substitution of beneficial mutations is:

$$< \sigma_R(s_{max}, \mu, N) >= \mu N Pr\{fix|s_{max}, \mu, N\}. \quad (34)$$

The expected selection coefficient of successful mutations is:

$$< s_R(s_{max}, \mu, N) >= \frac{\int_0^{s_{max}} s\pi(s)e^{-\lambda_R(s, s_{max}, \mu, N)ds}}{\int_0^{s_{max}} \pi(s)e^{-\lambda_R(s, s_{max}, \mu, N)ds}}, \quad (35)$$

where $\lambda_R(s, s_{max}, \mu, N)$ is as defined above for equation (33). The expected number of superior mutations in the interval (0, $\hat{t}_z$) is:

$$\psi_R(s, s_{max}, \mu, N, f) = \frac{\mu}{s} N \ln\left(\frac{N}{\chi_R}\right) \pi\left(\frac{s^{2max} - s^2}{2s_{max}}\right). \quad (36)$$

The expected number of superior mutations in the interval ($\hat{t}_z, t_f$) is:

$$\gamma_R(s, s_{max}, \mu, N, f) = \frac{\mu}{s} N \ln(\chi_R)\pi\left(\frac{s^{2max} - s^2}{2s_{max}}\right), \quad (37)$$

where

$$\chi_R = 1 + \frac{1}{s_{max} + s} \left[(s_{max} - s)(\tfrac{1}{f} - 1)\left(\frac{2sN}{s_{max} - s}\right)^{\frac{2s}{s_{max}+s}} - 2s\right].$$

The probability that an arbitrarily chosen beneficial mutation transiently achieves polymorphic frequency ($f > 0.01$) is:

$$Pr\{poly|s_{max}, \mu, N\} = \frac{1}{s_{max}} \int_0^{s_{max}} \pi(s) e^{-\psi_R(s, s_{max}, \mu, N, 0.01)} (1 - e^{-\gamma_R(s, s_{max}, \mu, N, 0.01)})\, ds \quad (38)$$

Finally, the probability that an arbitrarily chosen beneficial mutation transiently achieves majority status is obtained by replacing 0.01 in equation (38) with 0.5.