

The Lower Bound to the Evolution of Mutation Rates

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Abstract

Despite substantial attention from theoreticians, the evolutionary mechanisms that drive intra- and interspecific variation in the mutation rate remain unclear. It has often been argued that mutation rates associated with the major replicative polymerases have been driven down to their physiological limits, defined as the point at which further enhancement in replication fidelity incurs a cost in terms of reproductive output, but no evidence in support of this argument has emerged for cellular organisms. Here, it is suggested that the lower barrier to mutation rate evolution may ultimately be defined not by molecular limitations but by the power of random genetic drift. As the mutation rate is reduced to a very low level, a point will eventually be reached at which the small advantage of any further reduction is overwhelmed by the power of drift. This hypothesis is consistent with a number of observations, including the inverse relationship between the per-site mutation rate and genome size in microbes, the negative scaling between the per-site mutation rate and effective population size in eukaryotes, and the elevated error rates associated with less frequently deployed polymerases and repair pathways.

Key words: antimutator, genome evolution, molecular evolution, mutation rate, mutator, random genetic drift.

Introduction

Although evolutionary biologists have explained many attributes of biodiversity in terms of extrinsic adaptive challenges, much less is known about the intrinsic mechanisms that define the tempo and mode of evolutionary change itself. The origins of the substantial divergence in error rates associated with different polymerases, nucleotide pool sanitizers, and repair enzymes within and among species remain especially unclear. Developing such an understanding is critical to establishing a general theory for the constraints on evolutionary processes in divergent phylogenetic lineages.

Without genetic variation, there can be no evolution, so a nonzero mutation rate is essential for adaptive progress in the face of a changing environment. However, because the vast majority of mutations with effects on fitness are deleterious, special conditions are required for positive selection to promote increases in the mutation rate. Although both theoretical work and empirical work demonstrate that strong mutator alleles can sometimes rise to high frequencies by hitchhiking with linked beneficial mutations in asexual populations (Sniegowski et al. 1997; Taddei et al. 1997; Oliver et al. 2000; Tenaillon et al. 2001; Hall and Henderson-

Begg 2006), such a mechanism has limited explanatory power for mutation rates in sexual species. This is because almost all mutations in recombining genomes will be either unlinked or loosely linked to the locus responsible for their production and hence remain in statistical association with a mutator allele for only two to a few generations. These general considerations have led to a common view, dating to Sturtevant (1937), that natural selection will typically drive mutation rates to the minimum possible level (Kimura 1967; Dawson 1999; Baer et al. 2007). This lower limit has usually been thought to be rendered nonzero by fitness costs associated with excessive investment in replication fidelity, for example, a reduction in reproductive rates resulting from slow genome replication. Because a structural trade-off exists between the rate of polymerization and the investment in proofreading (Bessman et al. 1974; Loh et al. 2007, 2010; Tian et al. 2008), such costs must exist at some level. However, there is no direct evidence that time constraints on genome replication are substantial enough to influence the mutation rate, except perhaps in viruses (Furió et al. 2005, 2007). In prokaryotes, for example, genome replication constitutes just a small fraction of the overall energy budget

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(Ingraham et al. 1983), rates of DNA replication are more than 10-fold faster than those for messenger RNA elongation (Cox 2004), cells often experience nested genome replication events before dividing (Casjens et al. 1998), and there is no discernible association between growth rate and genome size (Vieira-Silva et al. 2010). Yet, the fact that prokaryotes with reduced mutation rates can be obtained experimentally (Tröbner and Piechocki 1984; Fijalkowska et al. 1993; Schaaper 1998; Galán et al. 2007; Loh et al. 2007) suggests that the accuracy of the replication machinery is less than what is achievable at the molecular level, although arguments have been made that pleiotropic constraints often result in declines in some types of fidelity in response to increases in others (Drake 1993; Bebenek et al. 2005).

An additional but unexplored barrier to mutation rate reduction is the reduced efficiency of selection for weakly advantageous antimutator alleles. As the mutation rate is driven to lower and lower levels by selection, a point must eventually be reached where the advantage of any further increase in replication fidelity is smaller than the power of random genetic drift (Lynch 2008, 2010). The goal here is to evaluate the extent to which such an intrinsic barrier can provide an adequate explanation for the patterns of mutation rates known to have evolved in natural populations. To analyze this problem, the situation involving a single mutation rate modifier in an effectively infinite asexual population (where drift plays a negligible role relative to selection) will first be explored, with attention then being expanded to the effects of multiple mutator states, finite population size, and recombination. Throughout, it is assumed that the only selective pressure on the mutation rate is the statistical association of excess deleterious mutations with mutator alleles. The expectations of the resultant theory are then shown to be consistent with empirical observations on the replication machinery and associated error rates in various lineages.

Results

An Effectively Infinite Asexual Population

Consider a modifier allele **m** that magnifies the genome-wide mutation rate to deleterious alleles by an amount ΔU . Individuals are assumed to be diploid, but provided the selective disadvantage of mutator heterozygotes is much greater than the rate of origin of mutator alleles, mutator homozygotes are expected to be extremely rare, and the population can be effectively treated as containing two classes of individuals, **MM** and **Mm**. Genotype **MM** is converted to **Mm** at rate 2μ , where μ is the mutation rate per **M** gene copy, whereas reversion of the latter to **MM** occurs at rate v . The dynamics of the frequency of **Mm** individuals can be written as

$$p'_{\mathbf{Mm}} = [p_{\mathbf{Mm}}(1 - \tilde{s}_d)(1 - v) + 2\mu p_{\mathbf{MM}}]/\bar{W}, \quad (1)$$

where \tilde{s}_d is the selective disadvantage of **Mm** individuals and \bar{W} the mean population fitness relative to an **MM** fitness of 1.0. The equilibrium frequency of the mutator genotype is then $\tilde{p}_{\mathbf{Mm}} \simeq 2\mu/(\tilde{s}_d + v + 2\mu)$. This expression and all the remaining results of this section also apply to a haploid asexual population if μ is substituted for 2μ .

The central remaining issue is the magnitude of the selective disadvantage \tilde{s}_d resulting from the accumulation of excess deleterious mutations on **Mm** relative to **MM** backgrounds. Because new **Mm** individuals are derived recurrently from relatively mutation-free **MM** genotypes, whereas their descendants stochastically accumulate new deleterious mutations, the pool of **Mm** individuals will be heterogeneous with respect to fitness. Letting U be the genome-wide deleterious mutation rate in the **MM** background, deleterious mutations arise at rate $U + \Delta U$ within **Mm** individuals, and we assume that each mutation arises at a unique site (ensuring that all mutations remain heterozygous in a diploid species). With all mutations assumed to have a fixed deleterious effect s , the fitness function is defined to be $W(n) = (1 - s)^n$, where n is the number of deleterious mutations in an individual.

To obtain a time-averaged measure of the selective disadvantage of mutator individuals, an estimate of the average excess number of deleterious mutations residing on **Mm** backgrounds is required. With new mutations arising randomly, under the multiplicative fitness model, the number of excess mutations arising follows a Poisson distribution with the mean cumulative number being fractionally reduced by $(1 - s)$ by selection and increased by ΔU by mutation each generation (Haigh 1978). Assuming these features are closely approximated from the time of appearance of a new **Mm** genotype, the average buildup of excess mutations is then described by

$$\bar{n}(t) = (\Delta U/s)[1 - (1 - s)^t]. \quad (2)$$

Relative to the situation in **MM** individuals, which at equilibrium carry an average U/s deleterious mutations, the mean selective disadvantage of a mutator genotype t generations after introduction is

$$s_d(t) = 1 - (1 - s)^{\bar{n}(t)}, \quad (3)$$

so that the average selective disadvantage over the expected life span of an **Mm** sublineage is:

$$\tilde{s}_d \simeq \frac{\sum_{k=1}^{\infty} s_d(k) \cdot \prod_{i=1}^k [1 - s_d(i)]}{\sum_{k=1}^{\infty} \prod_{i=1}^k [1 - s_d(i)]}. \quad (4)$$

This expression follows from the fact that an expected fraction $[1 - s_d(i)]$ of the descendants of a newly arisen

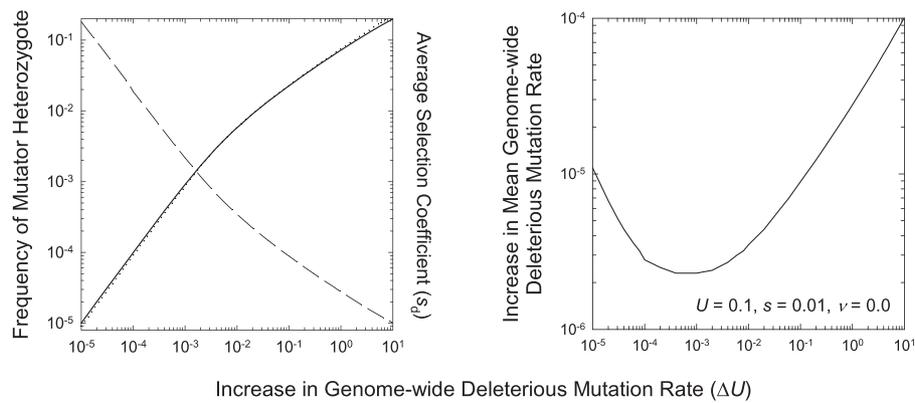


FIG. 1.—Properties of a mutator allele **m** in an infinite asexual population, assuming a mutation rate to mutator genotype **Mm** of $2\mu=10^{-6}$, and a negligible back mutation rate. Results were obtained by a set of recursion equations that tracked the distributions of deleterious mutation numbers within **MM** and **Mm** individuals, until the population achieved mutation–selection equilibrium. Left: Equilibrium frequency of the **Mm** mutator heterozygotes (dashed line) and average selective disadvantage of the **Mm** genotype at equilibrium (solid line obtained from recursions; dotted line from eq. (4)). Right: Population average mutation rate at equilibrium.

cohort of **Mm** individuals survives the i th generation, with the cumulative product giving the survivorship of the cohort through time. Further simplification is achieved by approximating powers of $(1-s)$ as exponentials,

$$\tilde{s}_d \simeq 1 - e^{-\Delta U} \left(\frac{\sum_{k=1}^{\infty} e^{-(\Delta U/s)[ks+(1-s)^{k+1}]}}{\sum_{k=1}^{\infty} e^{-(\Delta U/s)[ks+(1-s)^k]}} \right). \quad (5)$$

Although this expression ignores the variation in n within mutator and nonmutator genotypes, it yields a very close approximation to the average selection coefficient determined from the mean genotypic fitnesses of a fully characterized infinite population iterated to equilibrium (fig. 1). For $\Delta U \ll s$, which is likely to be the usual case given that $\bar{s} \simeq 0.001$ to 0.01 (Lynch and Walsh 1998), a newly arisen sublineage of mutators generally achieves its excess equilibrium mutation load, $\Delta U/s$, before being purged from the population, and $\tilde{s}_d \simeq 1 - (1-s)^{\Delta U/s} \simeq \Delta U$; that is, the selective disadvantage of the mutator is independent of the effects of the mutations it produces. However, highly aggressive mutator alleles are relatively rapidly eliminated (as a consequence of their substantial indirect mutation load; Johnson 1999a), resulting in $\tilde{s}_d < 1 - (1-s)^{\Delta U/s}$.

At mutation–selection equilibrium, the increase in the mean genome-wide deleterious mutation rate resulting from the recurrent production of the mutator allele is $\Delta \bar{U} = \Delta U \tilde{p}_{Mm}$. Assuming a low level of mutator allele reversion, ($v \ll \mu, \tilde{s}_d$), provided $\Delta U \ll s$, $\Delta \bar{U} \simeq 1/[(1/2\mu) + (1/\Delta U)]$, that is, half the harmonic mean of the diploid mutation rate to mutator alleles and the elevation in the genome-wide deleterious mutation rate per such mutation, which approaches 2μ if $\Delta U \gg 2\mu$. An evaluation of a more extensive model with an array of mutator classes (differing by a constant multiplicative factor) demonstrates that this

expression still closely approximates the inflation of \bar{U} if ΔU is taken to be the mutation rate difference between the two least mutagenic classes, which is where nearly all of the population resides. Thus, weak mutator alleles generally result in only a small increase in the population average mutation rate, which is independent of the effects of the induced deleterious mutations.

Finite Asexual Populations

Although the preceding results imply that the mutation rate will evolve to the minimum possible level in an infinite population, they are best viewed as providing a conceptual basis for determining the influence that finite population size has on the minimum mutation rate achievable by selection (ignoring, for the time being, any counterselection that might be associated with a cost to replication fidelity). Because \tilde{s}_d represents the absolute magnitude of selection operating against a cohort of mutator alleles in an infinite population, the capacity of natural selection to further reduce a prevailing genome-wide mutation rate will be limited unless there are accessible antimutator alleles with \tilde{s}_d larger than the power of drift, defined as $\sim 1/N_a$ in asexuals, where N_a is the asexual effective population size. Although the degree to which mutations operating on highly refined replication/repair loci can produce alleles with $\Delta U > 1/N_a$ is uncertain, it is clear that ΔU cannot exceed U itself.

To evaluate the extent to which a population evolves to such a barrier, stochastic computer simulations were performed on clonal populations in which the genome-wide deleterious mutation rate was subject to modification through an unbounded range of alternative states, with the mutation rates (U) of adjacent allelic classes differing by a constant factor, $1 + \lambda$. This has the effect of $\Delta U = \lambda U$ becoming progressively smaller as U declines, which must occur with

diminishing room for improvement. New deleterious mutations arose in the various mutation rate backgrounds in a Poisson fashion, and genotypic fitnesses were entirely a function of the number of deleterious mutations carried, using the multiplicative fitness function noted above. Each mutation rate class was subject to stepwise mutational conversion to the two immediately adjacent classes, with the overall rate of such conversion being proportional to the class-specific U , and a fraction f_d of such events being in the direction of higher U . Drift was imposed by multinomial sampling of the pool of expected genotype frequencies following each generation of selection and mutation, as in the classical Wright–Fisher discrete-generation model.

Because mutations that either increase or decrease the mutation rate are allowed for in this model, regardless of the starting conditions, the population average mutation rate (\bar{U}) gradually converges to a quasi-steady-state level dictated by the effective population size (fig. 2). If the population is initiated at a high genomic mutation rate, antimutators with a substantial selective advantage are produced relatively quickly, and \bar{U} rapidly declines toward the quasi-equilibrium. The latter state is attained when the mean mutation rate has become so low that the production of antimutators with an associated ΔU greater than $1/N_a$ is no longer possible. At this point, slight further reductions in \bar{U} may still result from the fortuitous increase of a very weak antimutator by drift, but subsequent increases in \bar{U} will also occur as weak (and effectively neutral) mutator alleles arise.

The time to converge on the quasi-equilibrium from above is especially prolonged in populations of large size because of the progressively reduced rate of production of antimutators as the population approaches a lower and lower mutation rate state. In contrast, if the population is initiated at a very low mutation rate, the mean mutation rate increases toward the quasi-equilibrium but does so slowly because the rate of production of mutator genotypes is low. This gradual rise of the average mutation rate is not a reflection of selection for an optimal mutation rate, as all mutations are deleterious, but a passive outcome of an upward mutational bias toward the production of mutator versus antimutator alleles ($f_d > 0.5$).

As anticipated from the theoretical results presented above, the quasi-equilibrium \bar{U} is essentially independent of the effects of mutations on fitness (s), depending only on the effects of mutator/antimutators on the mutation rate itself, ΔU (fig. 2). Moreover, \bar{U} is driven to lower levels in populations with larger size because of the greater efficiency of selection.

The Temporal Scale of Mutation Rate Evolution

Although the argument laid out above provides a heuristic basis for understanding the limits to what selection can accomplish with replication fidelity, it is of interest to have a more quantitative picture of the rate at which the drift

barrier is approached, and the degree to which the mutation rate itself is subject to drift via the fixation of sufficiently mild antimutator/mutator alleles in the vicinity of the quasi-equilibrium. With large numbers of potential mutation rate states but an unknown distribution of effects in real organisms, a complete understanding of these issues is not yet possible. However, motivated by the observation that populations generally reside in a nearly monomorphic state with rare and relatively rapid excursions to adjacent states (fig. 2), some insight can be gained by considering the rates of transition between adjacent pure states via the fixation of derived alleles. To achieve quantitative expressions for the waiting times for such transitions, it is useful to subdivide the process into two phases: 1) the arrival time of newly derived antimutator/mutator alleles and 2) the time for such alleles to progress to fixation.

A key to understanding the transition process in an asexual population is the fact that newly derived mutator/antimutator genotypes are only likely to go to fixation if they arise in the most fit background, as all other fitness classes are destined to eventual loss, assuming the population is large enough to avoid progressive mutational deterioration. The latter condition requires that the ratio of the power of selection to the power of drift, sN_a , be much greater than one (Gordo and Charlesworth 2000). Provided this condition is fulfilled, a population will approach a selection–mutation balance in which the fraction of individuals contained within the best (deleterious mutation free) class is $e^{-U/s}$ (Haigh 1978), so the long-term effective population size is approximately $N_a = Ne^{-U/s}$, where N is the actual number of adults. If $U \ll s$ at the mutation rate barrier, as it is in figure 2, then $e^{-U/s} \simeq 1$, and the effective population size is near the expectation based on the effective number of adults ($N_a \simeq N$). However, if U at the barrier is such that $e^{-U/s} \ll 1$, the relevant drift barrier ($1/N_a$) will be much greater than $1/N$. In the following, it is assumed that $N_a \gg 1$, for if this is not the case, the highest fitness classes will be progressively lost by Muller’s ratchet, eventually leading to population extinction via mutational meltdown (Lynch and Gabriel 1990; Lynch et al. 1993).

Letting μ be the mutation rate from the current mutation rate to a derived antimutator genotype, a population arrives at a state containing one or more such alleles with evolutionary potential at rate $\varphi_a \simeq 1 - e^{-2N_a\mu}$, which is just the rate of origin of new sustainable alleles ($2N_a\mu$) for $2N_a\mu \ll 1$, and approaches one for $2N_a\mu \gg 1$. Given such a starting point, the probability of fixation of the derived antimutator allele in an asexual population is:

$$\varphi_f \simeq \frac{1 - e^{-2N_a s^* p_0}}{1 - e^{-2N_a s^*}} \quad (6)$$

This is the standard diffusion approximation for the fixation probability for an advantageous mutation, with two

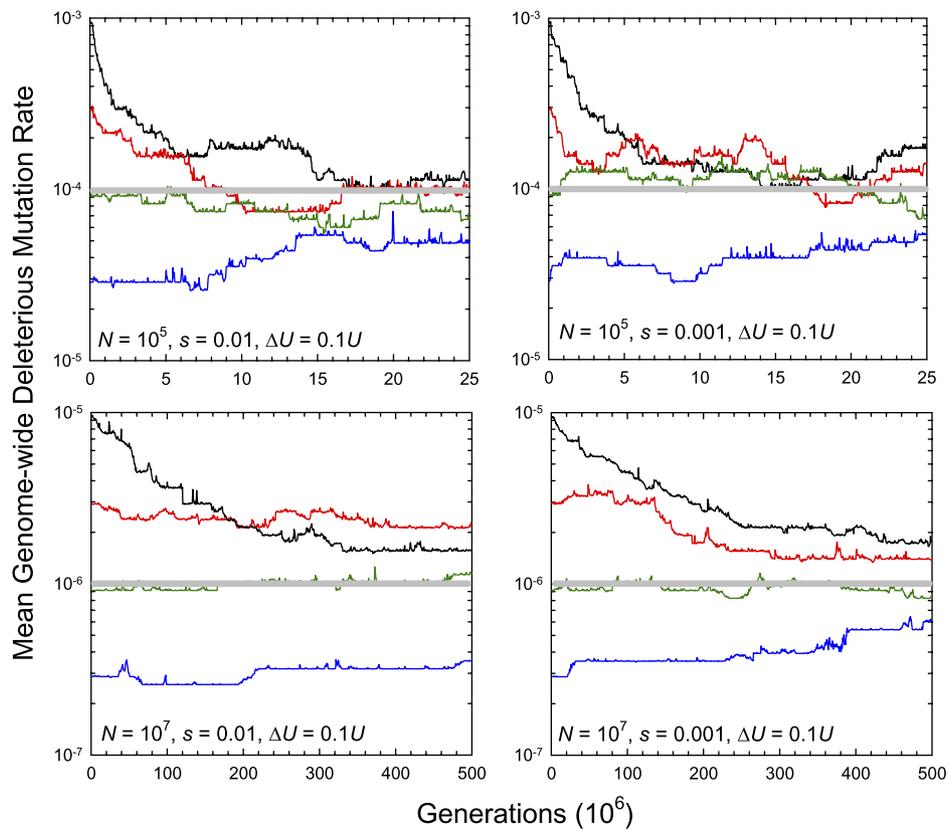


FIG. 2.—Sample evolutionary trajectories for the average genome-wide deleterious mutation rate (\bar{U}) in finite asexual populations with different monomorphic starting conditions. Results are shown for two different population sizes and two different selection coefficients against deleterious mutations, with 48 mutation rate classes differing by a factor of 1.111 between adjacent classes and with the mutation rate to mutator/antimutator genotypes being equal to 0.02 times the total genome-wide mutation rate to deleterious alleles at fitness loci. Mutations to antimutators were relatively rare (10% of the total; $f_d=0.1$). Note that the quasi-steady-state predictions for \bar{U} (given by the horizontal gray lines) are simply the points at which $\Delta U=0.1U$ is equal to $1/N$.

modifications: $s^* = \Delta U + 2\mu$ (where ΔU is positive for an antimutator), and $p_0 = (1/N_a) + [2\mu(N_a - 1)/N_a] \simeq (1/N_a) + 2\mu$. The modified selection coefficient (s^*) includes the rate of production of derived alleles 2μ , as this might be nontrivial (relative to ΔU) near the drift barrier. The modified starting frequency of the derived allele (p_0) allows for the fact that when $2N_a\mu > 1$, more than a single copy of the derived genotype can appear per generation. The mean time to arrive at a state at which the derived antimutator is destined to fixation is then:

$$\bar{t}_a \simeq 1/(\varphi_a \varphi_f). \tag{7}$$

To obtain the additional time to fixation, we employ the following reasoning. Newly derived alleles with small influences on the mutation rate will be subject to relatively strong stochastic processes in their early phase of establishment. However, once a weak antimutator allele has arisen to sufficiently high frequency, here denoted as p^* , it can be promoted by selection in an effectively deterministic fashion. From a modification of the standard fixation equation for a beneficial allele, the frequency to which

a beneficial mutation must rise in order to have a 0.9 probability of fixation is:

$$p^* \simeq - \frac{\ln[1 - 0.9(1 - e^{-2N_a s^*})]}{2N_a s^*}. \tag{8}$$

The mean time to fixation of an antimutator is then approximated by subdividing the dynamics into an early phase of drift until the derived allele first reaches frequency p^* , and a subsequent phase of deterministic selection. Modifying from Kimura and Ohta (1969), the mean time to drift to frequency p^* from starting frequency p_0 , conditional on eventual fixation, is:

$$\bar{t}_d \simeq 4N \left\{ p^* - 1 - \left(\frac{1 - p_0}{p_0} \right) \ln(1 - p_0) \right\}. \tag{9}$$

The subsequent deterministic time is approximated by assuming that the antimutator simply expands exponentially to frequency 1.0 once it has reached the critical frequency,

$$\bar{t}_s \simeq -\ln(p^*)/s^*. \quad (10)$$

The average number of generations required for a transition from a monomorphic ancestral state to fixation of a derived antimutator allele is then:

$$\bar{t}_{\text{tot}} \simeq \bar{t}_a + \bar{t}_d + \bar{t}_s. \quad (11)$$

The results for the arrival time for advantageous antimutator alleles (\bar{t}_a) are readily extended to deleterious mutator alleles by allowing ΔU be negative in the latter case. The total time to fixation of a mutator (conditional on fixation, despite its disadvantage) can be assumed to be equal to that for an antimutator with the same absolute selective advantage, based on prior work (Nei and Roychoudhury 1973; Maruyama 1974; Taylor et al. 2006).

The sets of expressions for both mutators and antimutators yield good approximations to the mean transition times obtained by computer simulations of the stochastic process, provided $sN_a > 1$ (fig. 3). (When $sN_a < 1$, the effective population size is actually elevated relative to $Ne^{-U/s}$ because mutator/antimutator alleles in multiple classes of individuals have the potential for fixation when the permanent maintenance of the best class by selection is no longer guaranteed.) To obtain a fuller understanding of the dynamics of mutation rate evolution, the preceding formulations might be applied in a transition matrix approach for the various mutation rate classes, although in the absence of explicit information on the rates of origin of mutator/antimutator alleles, the issue is not pursued here. Nevertheless, the preceding results help clarify two key issues with respect to the constraints on mutation rate evolution.

First, the lower bound on the mutation rate depends not just on the magnitude of ΔU that is accomplishable for antimutator alleles but also on the net rate of mutational production of such alleles. The latter is treated here as μ under the assumption that the antimutator undergoes no back mutation while en route to fixation, although μ should be viewed more generally as the difference between forward and backward mutation rates to such an allele. Provided $|\Delta U| \gg 2\mu$, both mutators and antimutators are potentially driven by indirect selection on the associated deleterious mutation load. Such selection is ineffective if $|\Delta U| \ll (1/N_a)$ (below the drift barrier), but otherwise the fixation of mutator alleles is strongly impeded, the more so in very large populations. In contrast, when $|\Delta U| \ll 2\mu$, both mutators and antimutators behave in an effectively neutral fashion (regardless of population size), being driven only by directional mutation pressure.

Second, although the success or failure of a mutator/antimutator depends on the magnitude of ΔU , rather than on the specific value of U for the derived allele, the absolute value of U in the ancestral genotype does influence the fates

of derived alleles through its influence on the number of individuals (N_a) in the restricted class with evolutionary potential. Although smaller N_a prolongs the arrival times of potentially fixable mutator/antimutator alleles, it also reduces the time to drift to fixation. The latter effect tends to dominate, so that populations with small N_a (either because of small N or large U) undergo more frequent transitions between mutation rate classes (fig. 3).

Sexual Populations

As first pointed out by Kimura (1967), the magnitude of indirect selection opposing mutator alleles is greatly diminished in sexual populations because the statistical associations with induced deleterious mutations are rapidly removed by recombination. As loci are no longer inherited as a unit in recombining species, it is more appropriate to evaluate mutational properties on a per-locus basis. Considering a modifier allele \mathbf{m} that magnifies the mutation rate to deleterious alleles by an amount Δu at a locus directly influencing fitness, Kimura (1967) showed that in an infinite population, an equilibrium will rapidly be reached where the input of new deleterious alleles is balanced by their disassociation from \mathbf{m} by recombination, at which point the selective disadvantage of the mutator allele is approximately $s\Delta u/(s+r-sr)$, where s is the heterozygous effect of a deleterious mutation and r the recombination rate between the two (fitness and fidelity) loci. To obtain the total selection coefficient against the mutator, \tilde{s}_d , this expression must be summed over all loci affecting fitness. For the case of no recombination ($r=0$), the result from the preceding section, $\tilde{s}_d \simeq \Delta U_h$, is recovered (where U_h is now the haploid genome-wide deleterious mutation rate), whereas with free recombination ($r=0.5$), $\tilde{s}_d \simeq 2\bar{s}\Delta U_h/(1+\bar{s})$, which is closely approximated by $2\bar{s}\Delta U_h$ when $\bar{s} \ll 1$ (the usual situation; Lynch and Walsh 1998).

This result, which has been obtained in a number of different ways (Kondrashov 1995; Dawson 1999; Johnson 1999a), shows that free recombination reduces the magnitude of indirect selection experienced by a mutator allele by a factor of $\sim 1/\bar{s}$. This is because the deleterious mutation load associated with a mutator quickly reaches a mutation–recombination equilibrium rather than building up to the much larger mutation–selection equilibrium. With free recombination, a mildly deleterious mutation becomes disassociated from the mutator in just two generations on average. A mutator allele will then have an elevated probability of being associated with a deleterious mutation of $2\Delta u$, the overall indirect fitness reduction being $2\bar{s}\Delta u/(1+\bar{s})$, with the denominator accounting for the slight reduction in frequency of associated deleterious alleles resulting from selection.

Because some mutations will be linked with the mutator locus, $2\bar{s}\Delta U_h$ will be a slight underestimate of the selective disadvantage of a mutator in a sexual population. However,

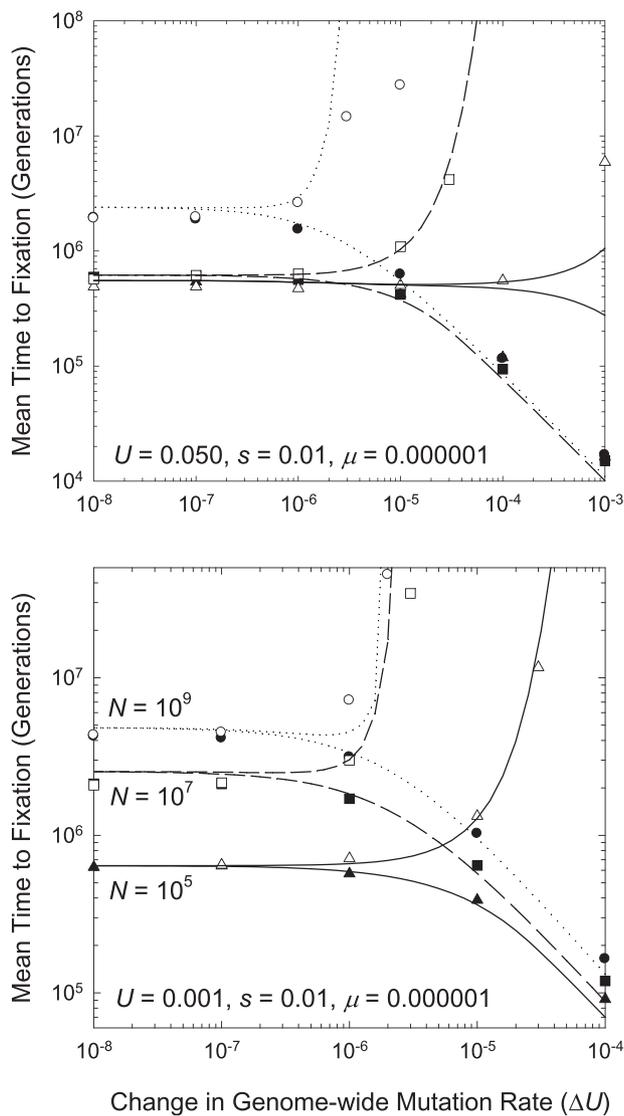


FIG. 3.—Mean times (in generations) for transitions from one fixed mutation rate state to another, given for three asexual population sizes. Upwardly bowed curves refer to derived mutators, and downward curves to antimutators. Data points are the averages of 500 stochastic simulations, whereas the curved lines are the expectations based on the theory in the text.

given that most organisms contain five or more chromosomes, the vast majority of mutations induced by a mutator allele will generally arise on different chromosomes (a fraction $1 - n^{-1}$ with n chromosomes of equal length) or distant from the mutator when on the same chromosome. More precise expressions that account for finite chromosome numbers and integrate over positions of linked mutations show that \tilde{s}_d is generally no more than $4\bar{s}\Delta U_h$ (Johnson 1999b; Lynch 2008). Because the excess equilibrium load of a mutator allele is established within just a few generations of its appearance in a sexual population, these expressions provide a very close approximation to the

time-averaged selective disadvantage of a segregating mutator allele at all but enormous (and unrealistic) ΔU_h .

Recalling $\Delta U = 2\Delta U_h$ as the diploid genome-wide increase in the deleterious mutation rate, and noting that the power of drift in a diploid sexual population is $1/(2N_e)$, where N_e is the effective population size, the expected drift barrier to the downward evolution of the mutation rate in a sexual species is $\Delta U < 1/(2N_e s)$, in contrast to $1/N_a$ for an asexual population. As in figure 2, this prediction is well supported by simulations of a replication fidelity locus with an array of alleles differing in the mutation rate by a constant factor (and acting in an additive fashion to define the genotypic mutation rates), with deleterious mutations assumed to arise in a freely segregating background.

Discussion

Although the focus here has been primarily on deleterious mutation accumulation, the net forces in favor of a newly arisen antimutator allele in an asexual population can be viewed as the sum $s^* \approx \Delta U + (\mu - \nu) + s_p$, where ΔU is the reduction in the genome-wide deleterious mutation rate, $(\mu - \nu)$ the net mutation pressure in the direction of antimutator alleles, and s_p the pleiotropic selective effect of the antimutator allele (independent of the reduced mutation load). When this summed quantity is smaller than the power of drift, the ability of natural selection to further reduce the mutation rate will be strongly diminished. In the context of an antimutator allele, ΔU is positive by definition, whereas $(\mu - \nu)$ is likely to be negative (assuming it is more difficult to produce antimutator alleles than disrupt them). Under the assumption that there is a physiological cost to high replication fidelity, s_p would be negative. However, conditions in which s_p is positive can be envisioned, for example, in multicellular organisms, where the rate of germline replication is unlikely to be a limiting factor in reproduction, a reduced somatic mutation rate may be highly advantageous (Lynch 2008).

As the mutation rate is pushed to a lower and lower level by selection, the effects of further molecular refinements of the replication/repair apparatus must become progressively diminished, and ΔU will necessarily become smaller, $(\mu - \nu)$ likely more negative, and s_p smaller (and potentially negative) as well, eventually leading to the inability of selection to diminish the mutation rate any further in the face of random genetic drift. Although the ways in which these three components vary with U and their relative quantitative contributions are unknown, and may differ among phylogenetic lineages, this overall theoretical construct yields qualitative predictions that have the potential to explain several previously disconnected observations.

First, consider Drake's (1991) contention that an inverse relationship exists between the mutation rate per nucleotide site and genome size in microbes, such that the genome-

wide mutation rate remains constant across taxa. This original conjecture was based on rather limited data, but a more recent analysis (Lynch 2010) continues to support the general pattern for bacteriophage and prokaryotes, although because of the limited range of bacterial genome sizes, the overall pattern remains highly dependent on the inclusion of bacteriophage in the analysis. Can Drake's contention can be explained on theoretical grounds? Imagine a series of mutation rate states such that U_0 is the minimum possible rate, and with multiplicative increases among states such that $U_i = (1 + \lambda)U_{i-1}$, or $\Delta U = \lambda U_{i-1}$. The theory implies that the minimum selectable genome-wide deleterious mutation rate satisfies $\Delta U + (\mu - \nu) + s_p = 1/N_a$ (for an asexual population), or in other words,

$$U_{\min} \simeq \frac{1}{\lambda} \left(\frac{1}{N_a} - (\mu - \nu + s_p) \right), \quad (12a)$$

where the composite term $(\mu - \nu + s_p)$ is the implied value of the sum at the boundary that satisfies U_{\min} . Thus, the theory predicts an approximate inverse scaling between the total genome-wide deleterious mutation rate and the effective size of an asexual population. Noting that the minimum mutation rate per nucleotide site (u_{\min}) is equal to $U_{\min}/(\phi_d G)$, where ϕ_d is the fraction of mutations with deleterious effects and G the number of nucleotide sites per genome, the lower bound to the per-site mutation rate is predicted to be:

$$\log(u_{\min}) = \log \left[\frac{1}{\phi_d \lambda} \left(\frac{1}{N_a} - (\mu - \nu + s_p) \right) \right] - \log(G). \quad (12b)$$

Thus, for a group of organisms with comparable effective population sizes, the theory predicts an inverse relationship between the mutation rate per nucleotide site (u_{\min}) and total genome size (G), with species-specific U_{\min} being distributed around the regression to a degree that depends on variation in the composite parameter in large brackets. Although the latter quantity must be subject to variation, it is plausible that the degree of variation among microbes is small relative to that in G . For example, with few exceptions, microbial genomes are about 95% coding DNA, so ϕ_d is likely to be roughly constant. In addition, microbial populations clearly vary in absolute population sizes (N), but a case can be made that once N exceeds a very large size (as it does in microbes), the effective population size is no longer limited by absolute numbers but by the physical structure of the genome, that is, by the selective interference that results from linked sites on chromosomes (Lynch 2007, 2010). Possibly, most microbes are near this limit.

In contrast, eukaryotic effective population sizes vary by a few orders of magnitude, with multicellular species typically having much smaller N_e (Lynch 2007, 2010). The existing data on such species suggest that u (per generation)

scales with roughly the -0.6 power of N_e , although the true scaling could be as extreme as -1.0 (Lynch 2010). Thus, keeping in mind that it is unclear whether the composite term $(\mu - \nu + s_p)$ contributes substantially to the overall pressure on the mutation rate in eukaryotes, it can at least be stated that the theory is qualitatively consistent with the negative scaling between u and N_e of cellular species. (For sexual species, the right side of equation (12a) is only modified by a factor of $1/s$, which does not alter the predicted negative scaling between u and N_e .)

Second, most genomes contain two or more "error-prone" polymerases, often used in replication across bulky lesions in the DNA and often elicited in periods of cellular stress. In vitro studies indicate that the error rates of these enzymes are typically 10- to 10,000-fold higher than those for polymerases involved in genome replication (fig. 4). (In vivo error rates could be lower than those summarized in figure 4, although it is unlikely that the qualitative pattern would be altered.) Because it is unlikely that such polymerases are molecularly constrained to be so inaccurate, many investigators have argued that natural selection has promoted stress-induced mutation as a strategy to facilitate adaptive evolution during challenging periods (Radman et al. 2000; Rosenberg 2001; Tenaillon et al. 2001; Earl and Deem 2004; Foster 2007; Galhardo et al. 2007). However, with the vast majority of mutations being deleterious, it is unclear that there is any net long-term advantage for elevated levels of stress-induced mutation, and the preceding results provide an alternative explanation that eliminates the need to invoke such an argument.

As implied by equation (12b), the error rate of a polymerase is expected to scale inversely with the number of nucleotide transactions engaged in per generation. Thus, there is no reason to invoke selection for evolvability to explain the error-prone nature of the polymerases involved in stress-induced mutagenesis. Rather, such a pattern is expected to be a natural outcome of the reduction in the efficiency of selection operating on infrequently invoked enzymes. This argument does not deny the critical role of error-prone polymerases in the elimination of DNA damage, nor does it deny the possibility that induced mutagenesis occasionally plays a role in survival/adaptation in extreme times. The hypothesis that mutation rates should naturally evolve to higher levels with enzymes involved in fewer replication events is also consistent with the fact that polymerases involved in the replacement of replication initiation primers have higher error rates than those involved in bulk polymerization (fig. 4) and that the primases that lay down the RNA primers initially involved in replication but subsequently replaced are extraordinarily error prone (Zhang and Grosse 1990; Sheaff and Kuchta 1994; Kuchta and Stengel 2010).

Third, for the reasons just noted, the error rates of pathways downstream in the stages of genome replication are expected to be elevated relative to those for the initial

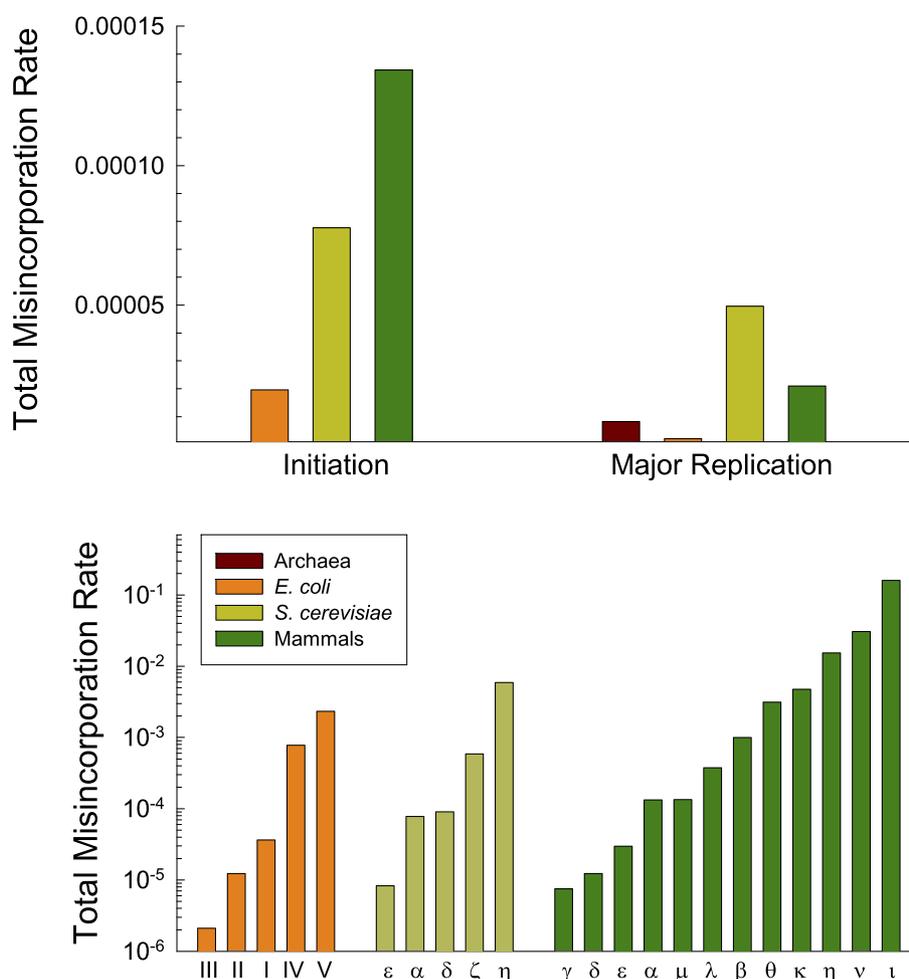


FIG. 4.—In vitro estimates of rates of base misincorporation by polymerases in four species groups (given as averages from multiple estimates from independent studies in [Supplementary Material](#) online). Rates are averaged over all nucleotide contexts, and for the error-prone polymerases, some sites are replicated at fidelity rates considerably lower (and others considerably higher) than the average. Note that for *Escherichia coli*, Pol I is used to replace the small RNA primers that initiate replication, and Pol III is the major replicative polymerase. For eukaryotes, Pol α is used to extend the RNA primers to a DNA length sufficient for Pol δ to take over, and Pols δ and ϵ are the major replicative polymerases (one for the leading and the other for the lagging strand). Data are limited for archaeal polymerases.

polymerization machinery, as a smaller number of nucleotide sites will remain to be serviced. Thus, it is worth noting that the in vitro proofreading error rates associated with the major replication polymerases in *Escherichia coli* and *Saccharomyces cerevisiae*, the only species for which data exist, are much higher than those in the initial polymerization step (Bebenek et al. 1990; Cai et al. 1995; Bloom et al. 1997; Shimizu et al. 2002; Hashimota et al. 2003; Shcherbakova et al. 2003; Fortune et al. 2005; Nick McElhinny et al. 2007; Pursell et al. 2007; McCulloch et al. 2009). Observations on the still further downstream mismatch repair (MMR) pathway are also consistent with theoretical expectations. In *E. coli*, the in vitro error rate of the major replicative polymerase is approximately 10^{-6} per base incorporation (fig. 4), whereas the in vivo error rate associated with MMR in this and most other species of eubacteria is in

the range of 0.01 to 0.05 (e.g., Schaaper and Dunn 1998; Prudhomme et al. 1991; Schaaper 1993; Fujii et al. 1999; Oliver et al. 2000; Richardson and Stojilkovic 2001; Rossolillo and Albertini 2001; Young and Ornston 2001; Mérimo et al. 2002; Shaver and Sniegowski 2003; Prunier and Leclercq 2005). (Here, the MMR error rate is simply defined as the fraction of errors emerging after polymerase action that are not eliminated by MMR.) Similarly, in the yeast *S. cerevisiae*, the in vitro error rate of the major replicative polymerase is approximately 5×10^{-5} (fig. 4), whereas that for MMR is approximately 0.025 (Prolla et al. 1994; Johnson et al. 1996; Marsischky et al. 1996; Sia et al. 1997; Harrington and Kolodner 2007); and in mammals, the respective rates are approximately 10^{-5} (fig. 4) and 0.05 (Bhattacharyya et al. 1995; Glaab and Tindall 1997; Tindall et al. 1998; Umar et al. 1998; Baross-Francis et al. 2001; Xu et al. 2001; Zhang

et al. 2002; Dobrovolsky et al. 2003; Hegan et al. 2006). Thus, for the limited systems for which data are available, error rates associated with MMR are three to four orders of magnitude greater than those associated with the initial stages of polymerization. Qualitatively, such a reduction is consistent with the theory in the sense that during replication, the MMR pathway operates at only the 10^{-6} to 10^{-5} fraction of genomic sites that emerge with errors following the initial stages of polymerization, while also engaging in other repair processes in nonreplicating DNA.

Fourth, the theory predicts that evolved mutation rates should be elevated in recombining species relative to asexual taxa with the same effective population sizes, by a factor equal to approximately the inverse of the average selective disadvantage of a new mutation, which is typically in the range of 0.001–0.01 (Lynch and Walsh 1998). More generally, if a species switches from obligate outcrossing to obligate asexual reproduction, selection is expected to reduce the mutation rate if $N_a > 2N_e\bar{s}$, which suggests that truly asexual species with very large population sizes will likely harbor particularly accurate replication systems. In the absence of accurate information on N_a/N_e for any such lineages, it is currently difficult to address this matter in a confident way. It might be argued that the reduced rate of mutation in prokaryotes relative to eukaryotes is qualitatively consistent with this hypothesis, as prokaryotes are commonly viewed as being asexual. However, indirect evidence suggests a comparable amount of recombination in prokaryotes and eukaryotes when scaled to the mutation rate (Lynch 2007).

It has been suggested that a bacterial endosymbiont inhabiting aphids, and thought to have $N_a/N_e \ll 1$ and essentially no recombination, has a mutation rate approximately $10\times$ that in free-living bacterial species (Moran et al. 2009). As the two derived life history changes are expected to have conflicting effects on the mutation rate, the theory suggests that the reduction in N_e has had a greater effect than the reduction in the recombination rate, although this interpretation is clouded by the fact that an absence of recombination is expected to induce a reduction in N_e via hitchhiking effects. A related point concerns the argument that increased sensitivity to amino acid-altering mutations in thermophilic bacteria results in the evolution of a reduced base-substitution mutation rate (Friedman et al. 2004). As noted above, in nonrecombining species, the magnitude of selection on the mutation rate is independent of the fitness effects of mutations, which only become a factor when the recombination rate is much greater than the average deleterious effect of mutations. Thus, the validity of the interpretation of Friedman et al. (2004) depends on the degree to which the genomes of thermophilic bacteria are inherited in a clonal fashion.

Finally, it should be emphasized that the theory yields predictions on how the per-generation mutation rate is expected to evolve in response to deleterious mutation

load. Although large multicellular organisms exhibit substantially higher per-generation germline mutation rates than do single-celled eukaryotes (Lynch 2010), consistent with the theory, the former also experience multiple germline cell divisions per generation. As the per-generation mutation rate is a consequence of the net accumulation of mutations throughout germline development, the theory predicts that the mutation rate per germline cell division will scale inversely with the number of such divisions per generation. Although the evolution of multicellularity results in a reduction in N_e , which is predicted to encourage an increase in the mutation rate per generation, if the number of germline cell divisions per generation is large enough, some multicellular species may actually evolve lower mutation rates per germline cell division than the per-generation rates observed in microbes. This seems to be the case for several well-studied model species, including humans, which exhibit mutation rates per germline cell division that are comparable with or lower than mutation rates in *E. coli* and yeast (Lynch 2010). The results in figure 4 suggest that such low rates may be achieved by a reduction in intracellular activities (e.g., metabolism) leading to premutations rather than by an increase in the accuracy of the replication/repair machinery.

Supplementary Material

Supplementary material is available at *Genome Biology and Evolution* online (<http://www.gbe.oxfordjournals.org/>).

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