Beneficial Mutations, Hitchhiking and the Evolution of Mutation Rates in Sexual Populations

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ABSTRACT

Natural selection acts in three ways on heritable variation for mutation rates. A modifier allele that increases the mutation rate is (i) disfavored due to association with deleterious mutations, but is also favored due to (ii) association with beneficial mutations and (iii) the reduced costs of lower fidelity replication. When a unique beneficial mutation arises and sweeps to fixation, genetic hitchhiking may cause a substantial change in the frequency of a modifier of mutation rate. In previous studies of the evolution of mutation rates in sexual populations, this effect has been underestimated. This article models the long-term effect of a series of such hitchhiking events and determines the resulting strength of indirect selection on the modifier. This is compared to the indirect selection due to deleterious mutations, when both types of mutations are randomly scattered over a given genetic map. Relative to an asexual population, increased levels of recombination reduce the effects of beneficial mutations more rapidly than those of deleterious mutations. However, the role of beneficial mutations in determining the evolutionarily stable mutation rate may still be significant if the function describing the cost of high-fidelity replication has a shallow gradient.

THE evolution of the genetic system has been the subject of much theoretical research, ever since Fisher (1928) first studied the evolution of dominance. More recent studies have employed population genetic models that include modifier loci with alleles that modify the values of various genetic parameters. Examples include recombination rate (reviewed by Otto and Michalakis 1998), sex ratio (Charnov 1982), transposition rate (Charlesworth and Langley 1986), or deleterious mutation rate (Kondrashov 1995). A modifier allele may be subject to direct selection and also to indirect selection due to linkage disequilibrium with other loci that are under selection (see Ewens 1979, p. 195). Because there is heritable variation for mutation rates, they are subject to alteration through the action of natural selection (Sturtevant 1937). This article examines indirect selection acting on a modifier of mutation rates, through its association with both beneficial and deleterious mutations.

When a new beneficial mutation arises, it may be lost by genetic drift, or it may rise in frequency and become fixed. In either of these cases, the genetic background in which the beneficial mutation arose remains associated with it until separated by recombination. If the beneficial mutation is fixed, then other alleles initially associated with it will rise in frequency, and in an asexual population will also become fixed. This phenomenon

was first observed in bacteria and termed *periodic selection* (Atwood *et al.* 1951; Dykhuizen 1990). In a continuous culture of bacteria, recurrent mutation causes rare neutral markers to increase linearly in frequency. Periodically, beneficial mutations sweeping to fixation cause clonal replacements: sudden decreases in the frequency of rare alleles not initially associated with the mutations. The more general term genetic hitchhiking (Maynard Smith and Haigh 1974) describes this process in both asexual and sexual populations. This is important in the evolution of mutation rates, because a modifier that increases the mutation rate is more likely to increase in frequency by hitchhiking on beneficial mutations. Linkage disequilibrium is generated when the beneficial mutation arises, and so the frequency of the modifier changes by indirect selection (Sniegowski *et al.* 1997; Taddei *et al.* 1997).

A second form of indirect selection acts on a modifier of the mutation rate, because a greater number of deleterious mutations arise in the higher mutation rate modifier background. In an asexual population, the net effect of these two forces is to move the mutation rate toward a stable equilibrium value that is also the value that maximizes the population mean fitness (Kimura 1967). This result is reproduced below. In this article, I study whether the genetic hitchhiking of a modifier allele affecting the mutation rate can be important in a sexually reproducing population, when both beneficial and deleterious mutations are modeled.

The indirect selection resulting from beneficial mutations on a modifier of mutation rate has been studied

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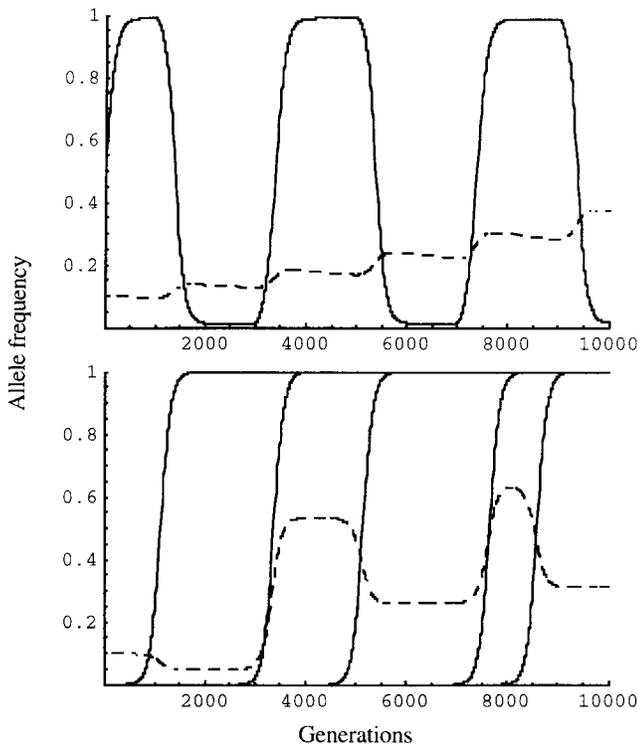
Figure 1.—Numerical results for (top) a deterministic model like that studied by Leigh (1973) and (bottom) typical results for the stochastic model studied here. To show substantial effects on the frequency of the modifier (dashed line), all mutations are beneficial ($K = 0.0005$, $s_b = 0.01$) and tightly linked ($r = 0.001$) to a mutator doubling the mutation rate ($U = 0.001$, $\Delta U = 0.001$). In the deterministic model, there is symmetric mutation between two alternately favored alleles (solid line) at a selected locus, wheras in the stochastic model five beneficial mutations (solid lines) sweep through the population at random times. The calculations were made with identical parameters (plus $4N_e s_b = 1000$ for the stochastic model). It can be seen that, in the stochastic model, the effects on the modifier frequency are greater in magnitude, but cause it to either increase or decrease in frequency, depending on in which background the unique beneficial mutation arises.

before in sexual populations (Leigh 1973; Gillespie 1981b; Ishii *et al.* 1989). Leigh (1973) concluded that the effect of beneficial mutations on the evolution of mutation rates was negligible in sexual populations. In contrast, both Gillespie (1981b) and Ishii *et al.* (1989) concluded that changing environments could favor increases in the mutation rate. These conclusions differ because only Leigh's (1973) model included a large class of unconditionally deleterious mutations.

All of these previous studies have used a model of a changing environment, in which there is a fixed set of alleles at a single locus. The selection coefficients change over time, in either a random (Gillespie 1981b) or a periodic manner (Leigh 1973; Ishii *et al.* 1989). All of the alleles are maintained at nonzero frequency by recurrent mutation. It has been suggested (Maynard Smith 1978, p. 192; see also Figure 1) that studies of such a model may underestimate the effect of beneficial

mutations on the evolution of mutation rates, because when a selected allele starts to increase in frequency it will be in only weak linkage disequilibrium with the modifier. Therefore, this study models a succession of initially unique beneficial mutations arising in a stochastic manner, so that there is much stronger linkage disequilibrium between the new allele and the modifier background in which it arises.

The population genetic model that is used to study the fate of a modifier of mutation rate is described below. It is a multi-locus model, but the analysis is made tractable by treating only the simplest case of a single rare modifier of small effect. Linkage disequilibrium between sets of loci at which mutations occur can then be ignored, and only the two-way linkage disequilibrium between each mutable locus and the modifier needs to be considered. There are four main parts to the analysis, as follows: (i) the effect of many deleterious mutations scattered over a given genetic map is determined; (ii) the expectation of the change in allele frequency at the modifier locus is found for a single beneficial mutation sweeping through the population; (iii) this is used to find the long-term average fitness of the modifier allele for a series of beneficial mutations sweeping through the population. These results are presented in terms of a parameter that describes the average effect of hitchhiking events, and (iv) this parameter is estimated for a sexual population with beneficial mutations scattered over a given genetic map.

The main new results obtained in this article are expressions for the indirect selection coefficient acting at the modifier locus, caused by (i) deleterious mutations scattered over a genetic map and (ii) beneficial mutations sweeping through the population. The expressions are appropriate for a rare modifier, with a small effect on the mutation rate. The effect of beneficial mutations on the evolutionarily stable mutation rate toward which the population evolves is then discussed in the context of a "cost" function that describes the direct effect on fitness associated with a difference in mutation rate. Previously, such cost functions have only been included in models in which mutations are unconditionally deleterious (Kondrashov 1995; Dawson 1998).

MODEL AND ANALYSIS

The notations used are summarized in Table 1.

The modifier of mutation rate: There is a randomly mating population of $2N$ haploid individuals. The population is polymorphic at a modifier locus that affects the genome-wide mutation rate. The deleterious mutation rate per genome, per generation, is U in genomes containing the Q allele and $U + \Delta U$ in genomes containing the P allele, which is rare. The mean mutation rate is $\bar{U} = U + p\Delta U$, where q and p are the frequencies of the two alleles. The beneficial mutation rate is proportional to the deleterious mutation rate. This haploid

TABLE 1

Frequently used notations

Symbol	Usage
b, B	Alleles at a selected locus; b is a new beneficial mutation
$E(\ )$	Expectation of a random variable
h	Parameter describing the effect of a hitchhiking event
K	Rate at which beneficial mutations sweep through the population
M	Map length of each chromosome, in morgans
$2N$	Number of haploid genotypes in the population
n	Chromosome number
Q, P	Alleles at a modifier locus affecting the rate of mutation
q, p	Frequencies of the Q and P alleles
r	Recombination probability between P/Q and b/B loci
s_b	Selection coefficient for a beneficial mutation
s_d	Selection coefficient for a deleterious mutation; each deleterious mutation multiplies fitness by the same factor $(1 - s_d)$
U	Deleterious mutation rate, per haploid genome, in the Q (or QQ) genetic background
ΔU	Amount by which a single P allele increases the mutation rate
$\hat{U}$	ESS mutation rate
$\overline{U}$	Mean mutation rate
W	Fitness of the P allele relative to the Q allele
W_b	Component of W caused by beneficial mutations
W_c	Component of W caused by direct selection
W_d	Component of W caused by deleterious mutations
w	Fitness of an individual with mutation rate U, carrying the B allele and no deleterious mutations
z	Map distance

model can be easily generalized to randomly mating diploids, because the P allele is rare and so PP homozygotes are vanishingly rare; it should be noted, however, that the definition of U remains as per haploid genome.

The fitness of genomes carrying the P allele, relative to genomes carrying the Q allele, is written W. For a modifier of small effect, W is close to unity, and so ln W is approximately the effective net selection coefficient favoring the P allele. The notation of fitness is used to avoid confusion with the selection coefficients for beneficial and deleterious mutations (s_b and s_d, see below). The term fitness is used to describe the effect of beneficial mutations, even though they will cause p to increase and decrease in a stochastic manner. I am considering a long-term limit expectation of the change in p, such that

$$E(p(t)) = W^t\, p(0) \quad \text{for large } t,$$

where time, t, is measured in generations, and $E(\ )$ stands for the expectation of a random variable. Because the main interest is determining the conditions under which P will spread (*i.e.*, when $W > 1$), rather than an exact description of the dynamics at the modifier locus, this definition of fitness is compatible with the restriction that p is small.

If evolutionary forces are weak then, to a good approximation, we have

$$\ln W \approx \ln W_d + \ln W_b + \ln W_c,$$

assuming that the indirect effects of deleterious mutations (W_d) and of beneficial mutations (W_b), and the direct effects on fitness (W_c or cost), act multiplicatively. This approximation holds only for a modifier of small effect because of second-order interactions between these effects. For example, the fixation probability of a beneficial mutation is reduced in the higher mutation rate background, because of its association with a greater number of deleterious mutations (Charlesworth 1994; Peck 1994; Barton 1995).

Deleterious mutations: The occurrence of deleterious mutations is assumed to be adequately described by a deterministic process. The net effect can then be represented as constant indirect selection at the modifier locus, which for a modifier of small effect will be proportional to ΔU. The precise relationship can be determined for any particular model of deleterious mutation.

For example, consider a model (Kimura and Maruyama 1966) that takes the limiting case of an infinite number of unlinked loci segregating for infinitesimally rare alleles. Selection occurs before mutation, both in the haploid phase of the life cycle. In the case where each deleterious mutation has an equal, multiplicative, effect on fitness of $(1 - s_d)$, an exact expression for the reduction in log fitness experienced by a rare neutral modifier was derived by Dawson (1999),

$$\ln W_d = -\Delta U \frac{s_d}{1 + s_d}.$$

A similar but approximate result, for small ΔU, was obtained by Kondrashov (1995).

In a large population (*i.e.*, $2Ns_d > 1$) with no recombination, any individual carrying more than the minimum number of deleterious mutations ultimately leaves no descendants (Fisher 1930, p. 136), and so

$$\ln W_d = -\Delta U.$$

This result was also obtained from deterministic analyses of population genetic models incorporating modifier loci (Kimura 1967; Leigh 1973).

Here, I use a result derived by Leigh (1973) for a two-locus model with arbitrary linkage to estimate ln W_d for deleterious mutations randomly scattered over a genetic map of n chromosomes, each of length M morgans. By analyzing a model in which both mutation

and selection are deterministic processes, Leigh (1973) obtained an equation for the strength of indirect selection on a modifier, which increases the mutation rate at a single linked locus by $\Delta\mu$. His analysis of a continuous-time model assumes that the linkage disequilibrium between the modifier and the selected locus changes rapidly relative to the allele frequency of the modifier. This quasi-linkage equilibrium approach is appropriate for a modifier of small effect and yields

$$\frac{dp}{dt} \approx -p(1-p)\Delta\mu\frac{s_d}{s_d + r} \ .$$

A similar result has been derived by Kimura (1967). A more general result for a deterministic multi-locus model has been derived by K. J. Dawson (unpublished results). Dawson's analysis further demonstrates that, if there is no epistasis in log fitness between deleterious mutations, then linkage disequilibrium between them is only generated because a modifier segregates in the population. The linkage disequilibrium is of order $(\Delta U)^2$; when ΔU is small, the individual effects on the modifier therefore combine multiplicatively, to a good approximation.

Now consider deleterious mutations scattered randomly over a genome of n chromosomes, each of length M morgans. A deleterious mutation is unlinked to the modifier with probability $(n-1)/n$, and otherwise the map distance, z, between it, and a modifier in the middle of a chromosome is a random variable with a uniform distribution on $[0, M/2]$. This gives

$\ln W_d \approx -\Delta U$

$$\times \left(\frac{2}{nM}\int_{z=0}^{M/2}\frac{s_d}{s_d + r(z)}\,dz + \frac{(n-1)}{n}\frac{s_d}{s_d + \frac{1}{2}}\right) \text{(1a)}$$

$$\approx -\Delta U\,2s_d\left\{1 + \frac{\ln(1/2s_d)}{nM}\right\}, \tag{1b}$$

where $r(z)$ is the recombination probability obtained from z by using Haldane's (1919) mapping function, $r(z) = \frac{1}{2}(1 - e^{-2z})$. The quantity contained in braces in Equation 1b describes the increase over the free linkage ($nM \to \infty$) case. Equation 1b is obtained from Equation 1a in the limiting case where $s_d \ll 1$ and $M \gg 1$ and is surprisingly accurate for almost all plausible values of these parameters. The approximation is least accurate when $n = 1$, but as long as $s_d < 0.1$, the error is <2% for $M > 2$, and <11% for $M > 1$. The error is reduced for larger n; it is roughly halved for $n = 4$. Note that, in the case of free recombination, this result differs by a factor of two from Dawson's (1999) analysis of the infinitesimally rare alleles model, where mutation occurs after selection, and hence each deleterious mutation has a 50% chance of being separated from the modifier by recombination before selection acts on it.

Beneficial mutations: In this model, I consider only a single beneficial mutation to be segregating at any one time. However, as is seen below, in sexual populations only beneficial mutations that are tightly linked to the modifier locus and that are destined to be fixed have any role to play in the evolution of mutation rates, and so this is only a weak restriction on the total rate of beneficial mutations. Because the effect at the modifier locus depends on whether the beneficial mutation arises in the Q or the P background, which is a single random event, it is necessary to study the long-term dynamics over the course of many beneficial mutations, each sweeping through the population in turn. The approach is to calculate the expectation of the effect of a single beneficial mutation, and then to combine the individual effects to estimate the net effect.

Each beneficial mutation that is destined to be fixed is assumed to arise at a point in time such that it does not interfere with other beneficial mutations sweeping through the population. This allele, b, confers a selective advantage s_b compared with the alternative allele B. It is assumed that stochastic effects are important only when b is rare (*i.e.*, $2Ns_b \gg 1$). The probability of recombination between this locus and the modifier locus is r. For each beneficial mutation that arises, r is a random variable, and so the effect of many beneficial mutations can be found by taking the expectation of the effect of a single beneficial mutation over a distribution of values of r.

The rate of occurrence, in the whole population, of beneficial mutations that are destined to be fixed, is K per generation. K may implicitly be a function of $2N$ and $\overline{U}$ and may vary through time, depending on the model of adaptive evolution. If, for example, adaptation is limited by the rate of environmental change (as assumed by Kaplan *et al.* 1989), then K would be independent of both $2N$ and $\overline{U}$. Note that even if the delay between an environmental change and the ensuing beneficial mutations arising is a function of $2N$ and $\overline{U}$, the overall rate of beneficial mutations remains independent of these parameters. The opposite extreme is a model of adaptation where there are very many loci at which beneficial mutations could potentially arise, so K would be proportional to both $2N$ and $\overline{U}$. A model intermediate between these two extremes seems most likely to be realistic.

The hitchhiking effect is simply represented by the parameter h, which is the fraction by which the frequency of the allele *not* initially associated with the beneficial mutation is multiplied, as a net effect of the entire selective sweep. If, for example, b arises in the P background, then

$$h = \frac{\text{frequency of Q (as Qb) after b is fixed}}{\text{frequency of Q (as QB) before b arises}} \ .$$

Previous work has concentrated on the effect of hitchhiking on neutral diversity. For a totally asexual population, $h = 0$. For sexual populations, the hitchhiking effect was first studied in by Maynard Smith and Haigh (1974), who derived an approximate expression for h. However, their analysis ignored stochastic fluctuations

in the frequency of the b allele while it is rare. Taking this into account and conditioning on the ultimate fixation of b, Barton (1998) has found an exact expression for h in terms of gamma functions,

$$1 - h = (4N_e s_b)^{-r/s_b}\Gamma(1 + r/s_b)\Gamma(1 - r/s_b)\Gamma(1 + 2r/s_b) \tag{2}$$

for $r/s_b < 1$ and $4N_e s_b > 1$. The dependence on N_e, the effective population size, arises because this is conditional on the fixation of b, which has probability $2s_b$ (N_e/N). In sexual populations, the hitchhiking effect decreases with increasing population size, because of the greater number of generations (and hence recombination events) between a beneficial mutation arising and sweeping to fixation.

In the model studied here, the modifier allele is not neutral. However, the direct selection ($\ln W_c$) and indirect selection due to deleterious mutations ($\ln W_d$) are assumed to be weak relative to the selection acting on the beneficial mutation (s_b), and so the result for a neutral modifier should be a sufficiently accurate approximation.

Effect of a single beneficial mutation: In this part of the analysis, q and p denote the modifier allele frequencies at the moment the beneficial allele b arises. I derive an expression for the expectation of p', the frequency of the P allele after the b allele has swept to high frequency. Because the rate of beneficial mutation in each modifier background is proportional to the deleterious mutation rate, the probability of b arising in the Q background is $qU/\overline{U}$, and in the P background is $p(U + \Delta U)/\overline{U}$. In the former case, $p' = hp$, and in the latter case $p' = (1 - q') = (1 - hq)$. Because h is a random variable, independent of which background the mutation arises on,

$$E\left(\frac{p'}{p}\right) = \frac{qU}{\overline{U}}E(h) + \frac{p(U + \Delta U)}{\overline{U}}\frac{(1 - E(h)\,q)}{p}$$

$$= \left(1 + \frac{\Delta U}{\overline{U}}E(1 - h)(1 - p)\right). \tag{3}$$

Net effect of a succession of beneficial mutations: Consider a series of x beneficial mutations arising at rate K over a total time t. I make use of the fact that the expectation of the product of independent random variables is the product of the expectations. While p is small, $E(p'/p)$ is independent of p, and hence of the outcome of previous events. In this case

$$t \ln W_b = \ln\left(E\left(\frac{p'}{p}\right)^x\right).$$

Because $x \to Kt$ as $t \to \infty$, using (3) we obtain

$$\ln W_b = K \ln\left(1 + \frac{\Delta U}{\overline{U}}E(1 - h)\right)$$

$$\approx K\frac{\Delta U}{\overline{U}}E(1 - h). \tag{4}$$

For asexual populations ($h = 0$), Equation 4 is identi-

cal to a result derived by Leigh (1973). Although the linkage disequilibrium is much stronger in the model analyzed here, when the consequently larger effects are averaged over the different genetic backgrounds, the net effect is the same as in Leigh's model.

For sexual populations, Leigh (1973) tabulated values of $(p' - p)$ for a range of r/s_b found by approximate solution of similar equations to those used to study hitchhiking (Maynard Smith and Haigh 1974), but assuming deterministic mutation and hence weaker linkage disequilibrium. The result obtained here is much simpler and clearly shows the relationship between the indirect selection at the mutator locus and the mean magnitude of hitchhiking events in the population in question.

Expectation of the hitchhiking effect: The results obtained above depend on the expectation of $(1 - h)$. For no recombination, this is equal to one, and hitchhiking events have maximum effect on the frequency of the modifier. For a sexual population, $E(1 - h)$ can be estimated by assuming that the beneficial mutations that arise are randomly scattered over n chromosomes, each M morgans long. Only a small fraction of these mutations are likely to have any effect, because $(1 - h)$ is insignificant unless $r < s_b$. Unless the selective advantage of the b allele is very large, r is small enough for it to be reasonable to directly equate r with map distance rather than use Haldane's (1919) mapping function (see Nordborg *et al.* 1996).

When Equation 2 is averaged over a distribution of r, the gamma functions in Equation 2 can be ignored to a good approximation if $N_e s_b$ is large. This is because when $r/s_b \ll 1$, the gamma functions are all approximately one, and when r/s_b is larger, $(4N_e s_b)^{-r/s_b}$ becomes very small. In the calculation that follows, the error in making this approximation is <3% when $N_e s_b > 10^3$, and <15% when $N_e s_b > 10^2$.

In the same way as for deleterious mutations, the probability that the modifier and a beneficial mutation are on the same chromosome is $1/n$. When the map distance between the two is chosen from a uniform distribution on $[0, M/2]$, the probability that $r < s_b$ is simply $2s_b/M$. In this case, r is uniformly distributed on the interval $[0, s_b]$, and the expectation of $(1 - h)$ according to Equation 2 without the gamma functions is given by

$$E(1 - h \mid r < s_b)$$

$$\approx \frac{1}{s_b}\int_{r=0}^{s_b}(4N_e s_b)^{-r/s_b}\,dr = \frac{1 - (4N_e s_b)^{-1}}{\ln(4N_e s_b)}$$

and therefore, for beneficial mutations scattered randomly over the entire genetic map and large $N_e s_b$

$$E(1 - h) \approx \frac{2s_b}{nM\ln(4N_e s_b)}. \tag{5}$$

Direct selection on the modifier: The log-fitness of the P allele relative to the Q allele is a function of both

U and ΔU. The component of this, due to differences in the direct fitness effects of the Q and P alleles, is ln W_c, which is also a function of both U and ΔU. Let $w(U)$ be the fitness of an individual with mutation rate U, carrying the B allele and no deleterious mutations. Assume that there is no epistasis between the modifier alleles and any fitness-affecting mutations. Then, for a modifier of small effect, ln W_c is linear in ΔU, as follows:

$$\ln W_c(U, \Delta U) = \ln w(U + \Delta U) - \ln w(U)$$

$$\approx \frac{d \ln w(U)}{dU} \Delta U \quad \text{for small } \Delta U.$$

Although it is widely believed that increasing the fidelity of DNA replication is costly (Sturtevant 1937; Leigh 1973; Kirkwood *et al.* 1986; Kondrashov 1995), very little is known about the nature of such a cost. Here I assume that the direct selection results only from increasing costs of higher-fidelity replication or mutation repair. This cost approaches infinity for perfect fidelity (Kirkwood *et al.* 1986, p. 5), and therefore fitness w is zero for $U = 0$. If the general form of the cost is as shown in Figure 2, then it would be reasonable to assume that $w(U)$ asymptotically approaches some maximum as U increases. In this case, the derivative of the fitness function, $d \ln w(U)/dU$, is a strictly positive, monotonically decreasing function of U. This is important in determining the existence and uniqueness of an evolutionarily stable mutation rate (ESS; see below and Figure 3). It appears that it is not possible to make such a statement if the effect of the modifier is considered in relative ($\Delta U/U$) rather than absolute (ΔU) terms.

Asexual populations: Although the model described here is a reasonable one with which to study the evolution of mutation rates in sexual populations, it is inap-propriate for asexual populations. In a totally asexual population each beneficial mutation will cause a complete clonal replacement, and hence the restriction that p should remain small would be violated. Hypermutators (modifiers) increasing the rate of certain mutations by factors of up to a thousand have been found at low frequency in natural populations of the bacteria *Escherichia coli* and *Salmonella enterica* (LeClerc *et al.* 1996). The rate of mutation at modifier loci themselves would be increased in a mutator phenotype, and hence a mutator allele coupled to a beneficial mutation stands an appreciable chance of back-mutation once at high frequency. This can result in ultimate fixation of a genotype combining the low mutation rate modifier with the beneficial mutation (Taddei *et al.* 1997). In other words, clonal replacement need not occur, and the modifier that "caused" the beneficial mutation is not fixed, so $h \neq 0$. Microorganisms maintained in continuous culture show population turnovers that are too rapid to be explained by sequential fixation of unique beneficial mutations (Dykhuizen 1990). A fundamentally different model such as the one studied by Taddei *et al.* (1997) is clearly more appropriate. However, this would not allow easy comparison with results from the model used here for sexual populations. Therefore the treatment of asexual populations in this article is better regarded as a limiting case for sexual populations, as recombination rates approach zero.

The evolutionarily stable mutation rate: An ESS (see Maynard Smith 1982), $\hat{U}$, is defined here such that, given suitable genetic variation, natural selection will always move U toward $\hat{U}$. In the preceding sections, I derived an expression for ln W as a function of U and ΔU. Because the modifier is of small effect, this expression is linear in ΔU, and so we need consider only $d \ln W/d\Delta U$. If this derivative is positive then modifiers increasing the rate of mutation are favored, and if it is negative
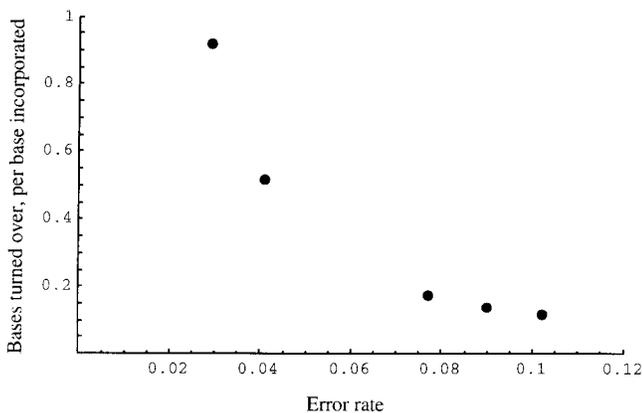

Figure 2.—Data obtained by Bessman *et al.* (1974) for polymerase extracted from bacteriophage T4 strains characterized as antimutator (left two points), wild type (central point), or mutator (right two points). The assay was made in equal concentrations of adenine triphosphate and its analogue, 2-aminopurine triphosphate. A base is turned over if it is temporarily polymerized into the DNA chain and then excised again as a monophosphate. This is costly in terms of time and energy.
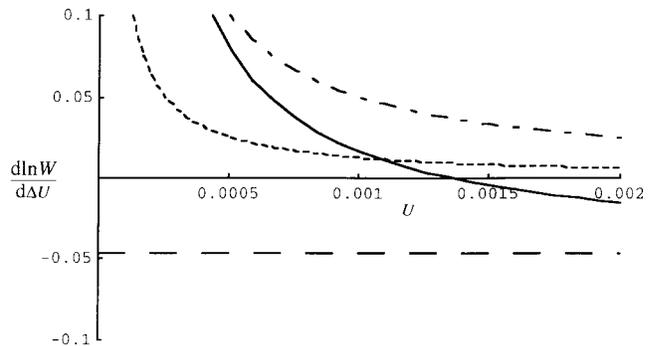

Figure 3.—The ESS is the value of U where $d \ln W/d\Delta U$ (solid line) passes through the U-axis. The functions from the three contributing effects act additively, $d \ln W_b/d\Delta U$ (dotted line) from beneficial mutations, $d \ln W_d/d\Delta U$ (dashed line) from deleterious mutations, and $d \ln W_c/d\Delta U$ (dot-dashed line) from the direct fitness effect of the modifier. $K = 0.01$, $s_b = s_d = 0.01$, $nM = 3$, $2N_e = 10^4$. A cost function of appropriate shape was invented for illustrative purposes.

then modifiers decreasing the rate of mutation are favored. At the ESS it will be zero and all modifiers (of small effect) are selectively neutral. A graph of $d \ln W/d\Delta U$ against U will therefore cross the U-axis, with a negative gradient, at the ESS.

If the slope of this graph is instead positive at the point it crosses the U-axis, then all modifiers of small effect are still selectively neutral, so it is an evolutionary equilibrium. However, populations even a small distance away from this equilibrium will not move toward it, and hence it is not an ESS.

Because the components of $\ln W$ combine additively, they can be differentiated individually, and a necessary condition for the ESS can be written

$$\frac{d \ln W}{d\Delta U} = \frac{d \ln W_d}{d\Delta U} + \frac{d \ln W_b}{d\Delta U} + \frac{d \ln W_c}{d\Delta U} = 0 \; \Leftarrow U = \hat{U}.$$

(6a)

This is shown graphically in Figure 3. It is also useful to determine the ESS for the nonbiological case where there is no direct selection acting on the modifier, which I call the "neutral" ESS, $\hat{U}_{\text{neutral}}$. A necessary condition for this is simply

$$\frac{d \ln W}{d\Delta U} = \frac{d \ln W_d}{d\Delta U} + \frac{d \ln W_b}{d\Delta U} = 0 \; \Leftarrow U = \hat{U}_{\text{neutral}}. \quad (6b)$$

A general relationship between the indirect selection pressures due to beneficial and deleterious mutations: The result derived in this section relies only on the general form of the equations derived above and should therefore be robust to many of the specific assumptions made in this article (constant s_d and s_b, rare modifier). It requires only that K does not depend on $\overline{U}$, $i.e.$, that adaptation is not mutation limited. Equation 1, in agreement with other analyses (Kimura 1967; Leigh 1973; Kondrashov 1995; Dawson 1999), states that the indirect selection on a modifier due to deleterious mutations is proportional to the absolute change in the mutation rate caused by that modifier, ΔU. This is likely to be true for (at least) all cases where deleterious mutations are modeled as a deterministic process, because the number of extra deleterious mutations associated with a mutator allele will vary with ΔU. Then, using D to represent a function of any of the model parameters except U and ΔU, we can write

$$\frac{d \ln W_d}{d\Delta U} = D. \quad (7a)$$

Equations 4 and 5 state that the indirect selection on a modifier caused by beneficial mutations is proportional to the relative change in the mutation rate caused by that modifier, $\Delta U/\overline{U}$. This is likely to be true for any model where beneficial mutations arise as a stochastic process with low fixed rate. This is because, given that a beneficial mutation arises, its subsequent effect on the dynamics at the modifier locus depends only on the

probability that it arose in the modifier background, which depends only on $\Delta U/\overline{U}$ (see Equation 3). Using B to represent a function of any of the model parameters except U and ΔU, we can write

$$\frac{d \ln W_b}{d\Delta U} = B \frac{1}{\overline{U}}. \quad (7b)$$

In all models where these two conditions (7a and 7b) are satisfied, it is possible to write an exact expression for the indirect selection caused by both beneficial and deleterious mutations combined, as a fraction of the indirect selection caused by deleterious mutations alone, as follows. In terms of B and D, the condition for the neutral ESS (6b) is

$$B \frac{1}{\hat{U}_{\text{neutral}}} + D = 0.$$

Multiplying all the terms by $\hat{U}_{\text{neutral}}/\overline{U}$ gives

$$B \frac{1}{\overline{U}} + \frac{\hat{U}_{\text{neutral}}}{\overline{U}} D = 0.$$

Referring back to the definitions of B and D in Equations 7a and 7b gives the general result

$$\frac{d \ln W_b}{d\Delta U} + \frac{d \ln W_d}{d\Delta U} = \left\{ 1 - \frac{\hat{U}_{\text{neutral}}}{\overline{U}} \right\} \frac{d \ln W_d}{d\Delta U}. \quad (7c)$$

Equation 7c is true for all values of $\overline{U}$ over which K remains constant. It describes the indirect selection on a modifier caused by both deleterious and beneficial mutations (for some value of $\overline{U}$), in terms of the indirect selection caused by deleterious mutations alone (at that $\overline{U}$). The term in braces depends only on $\overline{U}$ relative to the neutral ESS, $\hat{U}_{\text{neutral}}$. This equation summarizes indirect selection on a weak modifier of mutation rates. If $\overline{U} = \hat{U}_{\text{neutral}}$, there is no net indirect selection. As $\overline{U}$ increases, the effect of beneficial mutations vanishes. As $\overline{U}$ approaches zero, the effect of beneficial mutations becomes increasingly important, although the restriction of constant K cannot hold when this limit is reached.

DISCUSSION

The relative effects of beneficial and deleterious mutations: All other things being equal, both beneficial and deleterious mutations have greater effects on the modifier in an asexual population than in a sexual population. It is therefore instructive to determine the relative magnitudes of the two effects for each case. This can be achieved by determining the neutral ESS, $\hat{U}_{\text{neutral}}$, as described above. Some other treatments of the evolution of mutation rates have also considered neutral modifiers, and so it is interesting to compare their results with those obtained here. Substituting Equations 1b, 4, and 5 into 6b, and solving, gives the neutral ESS for a sexual population (assuming $nM > 1$ and $N_e s_b > 10^2$),

$$\hat{U}_{\text{neutral}} \approx K \frac{s_{\text{b}}}{s_{\text{d}}} \frac{1}{\ln(4N_{\text{e}}s_{\text{b}}) \ (nM + \ln(1/2s_{\text{d}}))} \ , \quad (8a)$$

and for an asexual population,

$$\hat{U}_{\text{neutral}} = K \ . \quad (8b)$$

A unique $\hat{U}_{\text{neutral}}$ always exists if K is a constant. The result for the asexual population (8b) was derived previously (Kimura 1967; Leigh 1973), and, furthermore, is the mutation rate that maximizes the population mean fitness or minimizes the genetic load (Kimura 1967).

In the general case where K may be any chosen function of U, it is still possible to determine $\hat{U}_{\text{neutral}}$. For asexuals, for example, it is simply the mutation rate that satisfies Equation 8b, $U = K(U)$. In general such a $\hat{U}_{\text{neutral}}$ will exist, but not for the simplest example, where K is proportional to U for all U. In this case Equations 8a and 8b take the general form $U = cU$ for some constant c. Depending on whether c is greater or less than one, the indirect selection will always act to increase or decrease the mutation rate, respectively.

If $\hat{U}_{\text{neutral}}$ exists and if $U < \hat{U}_{\text{neutral}}$, then, in the absence of a cost, modifiers increasing the rate of mutation would be favored, because the effect of beneficial mutations outweighs the effect of deleterious mutations. Alternatively, if $U > \hat{U}_{\text{neutral}}$ then the effect of deleterious mutations predominates, and modifiers decreasing the rate of mutation are favored. It can be seen from Equations 8a and 8b that if $s_{\text{b}} = s_{\text{d}}$, $\hat{U}_{\text{neutral}}$ is always smaller in sexual than in asexual populations. Suppose $s_{\text{d}} = 0.01$ (estimated for *E. coli* by Kibota and Lynch 1996). Then under the restrictions used in deriving (8a), that $nM > 1$ and $N_{\text{e}}s_{\text{b}} > 10^{2}$, the following upper bound for the case $s_{\text{b}} = s_{\text{d}}$ is obtained:

$$\hat{U}_{\text{neutral}} \text{ (sexual)} < 0.034 \ \hat{U}_{\text{neutral}} \text{ (asexual)}.$$

It is possible to find a wide range of biologically reasonable sets of parameters (such as large nM) for which $\hat{U}_{\text{neutral}}$ is several orders of magnitude smaller in sexual than in asexual populations. Only when $s_{\text{b}} \gg s_{\text{d}}$ is it possible for the neutral ESS to be greater in sexual than in asexual populations.

To make any further consideration of this result, it is necessary to consider the available data on U and K. Although many more mutations are deleterious than are beneficial, the relationship between the two is not immediately apparent because U is a rate per individual, whereas K is a rate per population, conditional on ultimate fixation of the beneficial mutations.

The rate of beneficial mutations: It is clear that K, the rate of beneficial mutations sweeping through a population, is an important parameter. However, it is difficult to estimate, and is likely to vary greatly across different groups of organisms. One approach (as taken by Maynard Smith and Haigh 1974) is to determine an upper bound by assuming that, at most, all nonsynonymous nucleotide substitutions were caused by selec-

tion. Most of the available data of this sort are for mammals. Nonsynonymous substitution rates in 363 protein-coding genes, obtained from comparisons between mouse and rat, are listed by Wolfe and Sharp (1993). By assuming the divergence to be 10 mya (Catzeflis *et al.* 1992), and assuming that an estimate of 6 mo per generation for wild populations of mice (H. C. Hauffe, unpublished results) is representative, and crudely extrapolating the data to 10^{5} genes, an estimate of $K < 0.03$ is obtained.

It is also possible to make an estimate from the frequency of periodic selection events in asexual populations. Paquin and Adams (1983) observed clonal replacements for populations of the yeast *Saccharomyces cerevisiae* in glucose-limited chemostats to occur at a reasonably uniform rate corresponding to about $K \approx 0.025$. For *E. coli* in batch culture, Lenski *et al.* (1991) observed step-like increases in fitness to occur at a slightly declining rate over 2000 generations, with mean $K \approx 0.002$. Because these microorganisms were in novel environments, these could be considered upper bounds for these particular organisms, corresponding to bouts of adaptive evolution.

The rate of deleterious mutations: The rate of deleterious mutations per genome is an important genetic parameter in many areas of evolutionary biology. The field of mutation rate estimation is comprehensively reviewed by Drake *et al.* (1998). Data from mutation accumulation experiments give a lower bound for U, because deleterious mutations of small effect are likely to remain undetected, some experiments have studied only components of fitness, and the usual method of analysis assumes that all mutations are of equal effect. In *E. coli* an estimate of $U > 0.0002$ was obtained by Kibota and Lynch (1996). Estimates for *Drosophila melanogaster* are $U > 0.35$ (Mukai 1964), $U > 0.42$ (Mukai *et al.* 1972), and $U > 0.15$ (Ohnishi 1977) per haploid genome. Using a different method, which avoids a potential problem of long-term increases in fitness in the control lines, but assuming a specific form of distribution of mutational affects, Garcia-Dorado (1997) obtained a much lower estimate of $U > 0.025$ per haploid genome. Indirect estimates for other eukaryotes are mostly in the range $0.1 < U < 1$ (Drake *et al.* 1998). In the nematode *Caenorhabditis elegans*, Keightley and Caballero (1997) have estimated $U > 0.0026$ per haploid genome, using a maximum-likelihood analysis and assuming a gamma distribution of mutational effects. What constitutes a representative value for U remains a contentious issue (for example, Peck and Eyre-Walker 1997; Drake *et al.* 1998).

An upper bound for U can also be deduced, because it must certainly be less than the total genomic mutation rate. In a range of DNA-based microbes with wide variation in genome size (bacteriophages, *E. coli*, *S. cerevisiae*, and *Neurospora crassa*), this figure is remarkably constant, with mean 0.0034 (Drake *et al.* 1998). This implies

wide variation in the per-nucleotide rate. In higher eukaryotes, the "effective" rate per sexual generation ranges from 0.14 in Drosophila to 1.6 in humans (Drake *et al.* 1998), but these are extrapolated from data for only a few loci and are probably underestimates because the "effective" rate includes only mutations with conspicuous effects.

Theory applied to the data: In sexual populations of higher eukaryotes, there is extensive data showing that $U \gg K$. The theory presented above suggests that the net effect of beneficial and deleterious mutations would be to favor reductions in the mutation rate. It can be seen from Equation 7c that because $U \gg \hat{U}_{neutral}$ in sexual populations, the term in braces is close to one, and so the combined indirect selection caused by both deleterious and beneficial mutations is very similar to the indirect selection caused by deleterious mutations alone. Assuming the populations are near equilibrium, this indirect selection pressure must be balanced by direct selection on the modifier, to which attention is turned below.

In microbes, the data suggest that in novel or fluctuating environments or during a bout of adaptive evolution, K might exceed U. For totally asexual populations, modifiers increasing the rate of mutation would then be favored (Kimura 1967; Leigh 1970, 1973). Because the results for sexual populations obtained here are only appropriate if recombination levels exceed an average of one crossover per generation ($nM > 1$), no statement about the reduction in $\hat{U}_{neutral}$ caused by limited recombination in predominantly asexual microbes can be made. This question would be better answered in the context of a more realistic model for microbes, including, for example, modifiers of large effect (hypermutators).

Are beneficial mutations important in sexual populations? This article has validated the belief that in sexual populations, the combined effect of beneficial and deleterious mutations is to favor a decreased rate of mutation (Leigh 1973), and that the indirect selection resulting from beneficial mutations is small or negligible compared to that resulting from deleterious mutations. However, this does not necessarily mean that removing the beneficial mutation effect altogether would result in only a small change in the ESS. In the absence of any information about the cost function, a general argument is presented to explain why this is so.

Consider two models, identical except for the presence or absence of beneficial mutations. Figure 4 shows the ESS determined in each case. By reflecting the graphs describing indirect selection caused by deleterious (or deleterious and beneficial) mutations about the U axis, the ESS is determined by the intercept with the graph describing the cost. In this example a cost function of suitable shape has been invented, such that the difference to the ESS made by including beneficial mutations in the model is large, to emphasize the follow-

ing point. Even if the combined indirect selection caused by beneficial and deleterious mutations is very similar to the indirect selection caused by deleterious mutations alone, the effect of beneficial mutations in determining the ESS may be substantial if the cost function has shallow gradient in the region around the ESS.

Note that a shallow gradient on a graph of $d \ln W_c/d\Delta U$ against U is not inconsistent with a large cost, but requires only that the cost change slowly over the mutation rate U. It is equivalent to a low curvature on a plot of fitness against mutation rate (a low $d^2 \ln w/dU^2$; see model and analysis). Because very little is known about the nature of such a function, it would seem unreasonable to state that the role of beneficial mutations in determining the ESS is negligible. On the contrary, it seems that, especially in metazoa, the time and energy devoted to high-fidelity replication of germ-line cell DNA would have a very slight effect on the fitness of the organism as a whole. The effect would be more substantial (and hence the fitness function more curved) if the somatic mutation rate shares a genetic basis with the germ-line mutation rate.

An obvious corollary is that small changes in the indirect selection caused by deleterious mutations alone would equally be expected to produce substantial changes in the ESS mutation rate. This would perhaps be an easier experimental approach to follow. There are two pieces of experimental evidence supporting this idea.

First, by exposing populations of *D. melanogaster* to various levels of X rays for long periods of time, Nöthel (1987) was able to cause large heritable changes in the rate of X-ray-induced mutation. However, this hardly constitutes small changes in the selection pressure: at the lowest level of exposure, the control population experienced a >50% rate of dominant lethals, which
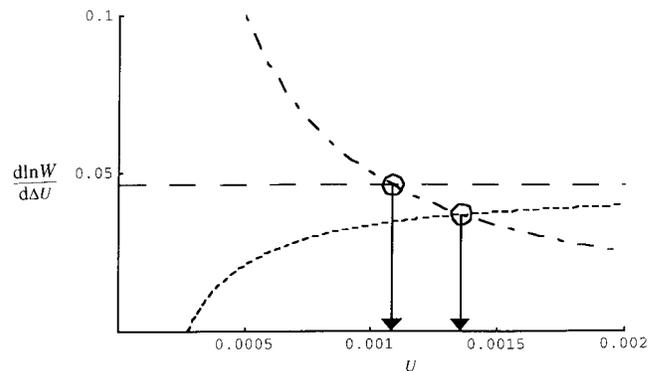

Figure 4.—The slope of the cost function determines the change in the ESS caused by beneficial mutations. The ESS without beneficial mutations is the left-hand arrow, determined by the intercept between $d \ln W_c/d\Delta U$ (dot-dashed line) and $-d \ln W_d/d\Delta U$ (dashed line). The ESS with beneficial mutations is the right-hand arrow, determined by the intercept between $d \ln W_c/d\Delta U$ (dot-dashed line) and $-d \ln(W_d + W_b)/d\Delta U$ (dotted line).

fell to ~30% in lines exposed to this for long periods of time. Additionally, it is not clear that the spontaneous mutation rate changed in the course of this experiment.

Second, McVean and Hurst (1997) have shown theoretically that the indirect selection caused by deleterious mutations is stronger for a modifier controlling the mutation rate on an X chromosome than on an autosome. They examined rates of nucleotide substitution for 238 autosomal and 33 X-linked genes in mouse and rat, and found that the rate of synonymous substitution was significantly lower for the X-linked genes, as predicted.

Limitations of the model: The model studied here, in which initially unique mutations sweep through the population, is not the only model under which an increase in the mutation rate is favored. Models in which the environment fluctuates randomly (Gillespie 1981b) or periodically (Leigh 1973; Ishii *et al.* 1989) were discussed above. In a static environment, heterozygote advantage may cause a modifier increasing the mutation rate to be favored in finite populations (Gillespie 1981a), selfing populations (Holsinger and Feldman 1983), or where selection acts on fecundity (Holsinger *et al.* 1986). All of these models, with the exception of that of Leigh (1973), do not include the large class of unconditionally deleterious mutations, and therefore the approach of determining the ESS mutation rate demonstrates only the qualitative fact that modifiers increasing the rate of mutation can be favored. Where indirect selection coefficients are estimated (Gillespie 1981a,b), they are proportional to the absolute change in mutation rate (ΔU) rather than the relative change in mutation rate ($\Delta U/U$). Therefore they would contribute a constant positive term to $d \ln W/d\Delta U$. Because the number of loci at which there is a fluctuating or overdominant selection regime is much less than the number of loci at which unconditionally deleterious mutations can arise, this term would be overwhelmed by the constant negative term caused by deleterious mutations. In contrast, the effect of hitchhiking with beneficial mutations studied here depends only on the relative change in mutation rate, and hence its contribution to $d \ln W/d\Delta U$ becomes asymptotically more important as U approaches zero. The number of loci at which the beneficial mutations arise is accounted for by the parameter K, which can in principle be estimated and compared to U.

The analysis presented here was restricted to the case where the selective effects of both deleterious and beneficial mutations (s_d and s_b) are constant, because it appears that the results would depend not only on the means of the distributions but on the higher moments, and so an analysis would have had to assume specific forms for the distributions. Such an approach was not followed further as it seemed unlikely to yield further insights. Note, however, that the general relationship described by Equation 7c remains valid for any distributions of s_d and s_b.

The present work was restricted to panmictic populations. The effects of breeding system on the evolution of mutation rates is an interesting area for theoretical research. There is an increasing quantity of data on nucleotide substitution rates for selfing and outcrossing plant species, which could be used to determine the importance of beneficial mutations in the evolution of mutation rates. If mutation rates are determined by the balance between cost and deleterious mutations alone, then mutation rates would be lower in a selfing (asexual) than an outcrossing (sexual) species (Dawson 1998). Alternatively, if beneficial mutations have a significant role, then mutation rates could be higher in a selfing than an outcrossing species.

The estimation of indirect selection caused by deleterious mutations assumes that all deleterious mutations stay close to their deterministic mutation-selection equilibria frequencies. This would not be appropriate for slightly deleterious mutations (for which $2Ns_d < 1$), which may make up a substantial proportion of the total mutational load (Ohta 1973; for a more recent perspective see Ohta and Gillespie 1996). In this case, both beneficial and deleterious mutations would have to be modeled as stochastic processes.

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