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Adaptation in Sexuals *vs.* Asexuals: Clonal Interference and the Fisher-Muller Model

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ABSTRACT

Fisher and Muller's theory that recombination speeds adaptation by eliminating competition among beneficial mutations has proved a popular explanation for the advantage of sex. Recent theoretical studies have attempted to quantify the speed of adaptation under the Fisher-Muller model, partly in an attempt to understand the role of "clonal interference" in microbial experimental evolution. We reexamine adaptation in sexuals *vs.* asexuals, using a model of DNA sequence evolution. In this model, a modest number of sites can mutate to beneficial alleles and the fitness effects of these mutations are unequal. We study (1) transition probabilities to different beneficial mutations; (2) waiting times to the first and the last substitutions of beneficial mutations; and (3) trajectories of mean fitness through time. We find that some of these statistics are surprisingly similar between sexuals and asexuals. These results highlight the importance of the choice of substitution model in assessing the Fisher-Muller advantage of sex.

POPULATIONS adapt to new environments by the substitution of beneficial mutations. The rate of adaptation thus depends on how often mutations having given favorable effects arise and how quickly these mutations increase in frequency. Evolutionary biologists have spent a good deal of time attempting to identify factors that speed or slow adaptation. One of the most intensively studied of these factors is sexual *vs.* asexual reproduction. As Fisher (1930) and Muller (1932) first pointed out, two segregating beneficial mutations can be fixed simultaneously in an asexual population only if one arises on a chromosome that already carries the other; otherwise, beneficial mutations must be fixed sequentially, as only one nonrecombining chromosome can sweep through a population at a time. In a sexual population, on the other hand, beneficial mutations avoid such competition: with recombination, beneficial mutations that arise on different chromosomes can be brought together onto the same chromosome, allowing the simultaneous substitution of both mutations. It seems likely, therefore, that sexual populations would incorporate beneficial mutations faster than asexual ones, allowing more rapid increases in fitness. This simple idea, commonly referred to as the Fisher-Muller advantage of sex, has proved a popular explanation for the ubiquity of sexual reproduction (see Maynard Smith 1978 and Otto and Lenormand 2002 for reviews).

The Fisher-Muller theory is not, however, without problems. If the rate of mutation is sufficiently high, chromosomes carrying multiple beneficial mutations appear even in asexual populations. Similarly, in infinitely large populations, all combinations of beneficial mutations appear at their expected frequencies, and recombination confers no advantage. Put differently, infinite populations show no linkage disequilibrium (LD), and recombination cannot, therefore, effect any change in a population's genetic composition. Consequently, there is no advantage to sex in an infinite population (assuming no epistatic fitness interaction between loci) (Maynard Smith 1968; Eshel and Feldman 1970).

But since real populations are finite and because beneficial mutations are rare, it is important to determine if the Fisher-Muller effect yields any advantage of sex given realistic population sizes (N) and mutation rates (μ). To this end, Crow and Kimura (1965) modeled a finite population that experiences recurrent mutation to beneficial alleles. They concluded that the Fisher-Muller advantage of sex is large: sexual populations incorporate new beneficial alleles much faster than asexual populations. Unfortunately, though, the calculations supporting this conclusion ignored the effect of genetic drift on rare beneficial mutations and thus overestimated the advantage of sex. Later work, which took into account genetic drift, showed that the advantage of sex is considerably smaller, although still substantial (Maynard Smith 1971; Felsenstein 1974). Other studies have demonstrated an advantage of sex by showing that the fixation probability of a beneficial mutation is reduced in an asexual population as a result of competition among beneficial mutations ("clonal interference"; Hill and Robertson 1966; Barton

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1995; Gerrish and Lenski 1998; Orr 2000; Kim and Stephan 2003).

Hill and Robertson (1966) provided an important insight into the Fisher-Muller advantage of sex. Using computer simulations, they showed that a finite population initially in linkage equilibrium for beneficial alleles develops negative LD—individuals carrying both beneficial alleles are less frequent than expected—unless recombination acts to break such nonrandom associations. This bias toward negative LD grows stronger with greater genetic drift. Theory would thus seem to predict that the advantage of sex will be greatest in populations of intermediate size: large populations experience negligible genetic drift, while small populations rarely segregate simultaneously for multiple beneficial mutations. Recent studies, involving either computer simulations (Otto and Barton 2001; Iles *et al.* 2003) or experimental evolution of bacteriophage (Poon and Chao 2004), have confirmed this prediction.

In the studies of the Fisher-Muller model mentioned above, less attention was paid to the effect of the substitution model on the advantage of sex. The importance of the substitution model is revealed by different predictions about the effect of population size in different studies. For example, Maynard Smith (1968) predicts no advantage of sex in a population of infinite size. However, in the model of clonal interference in Gerrish and Lenski (1998) and Wilke (2004), the fixation probability of a beneficial mutation decreases monotonically with increasing N, leading to an ever-increasing advantage of sex. This discrepancy can be explained by the fact that Maynard Smith (1968) used a two-locus model in which all possible combinations of alleles are generated by recurrent mutation in a large population, while Gerrish and Lenski (1998) and Wilke (2004) assumed that each new mutation occurs at a new site. In the latter case, the number of possible combinations of alleles increases exponentially with population size, making it impossible to attain linkage equilibrium by mutation (for more on this, see the discussion). Therefore, a model of adaptive substitution with an unlimited supply of beneficial mutations (the "shift" model; Gillespie 2001) ensures an advantage to sex in arbitrarily large populations. It might, however, be biologically unrealistic to assume a genome having an infinite number of sites at which beneficial mutations can occur at a given time.

Here, we study the adaptation of sexuals *vs.* asexuals in a particular DNA sequence-based model of evolution. This work is an extension of Gillespie's (1984, 1991) "mutational landscape" model. In particular, we study a large but finite population that experiences a sudden change in environment. At that moment, a small number of sites (loci) become mutable to beneficial alleles; mutation to these alleles is recurrent. Because there is no reason to believe that different beneficial mutations will enjoy the same selective advantage—and good reason to think that they will not, *e.g.*, see Orr (2003a) who suggests that fitness effects among new beneficial mutations might often be approximately exponentially distributed—we allow different selection coefficients, s, among beneficial mutations.

While previous analyses of the mutational landscape model have assumed that the product $N\mu$ is small enough that beneficial mutations have independent fates (Gillespie 1983, 1984, 1991; Orr 2002, 2003a,b), we relax this assumption here. In particular, we allow arbitrary population sizes and mutation rates and so explore the effects of large $N\mu$ on adaptation. When $N\mu$ is large, beneficial mutations do not enjoy independent fates and clonal interference may be important. In summary, our model differs from most previous studies of sex in that we allow arbitrary $N\mu$ and, more importantly, consider a *limited* number of beneficial mutations that have *different* fitness effects. We ask how previous conclusions about the Fisher-Muller advantage of sex do or do not change under these assumptions.

We calculate several statistics that characterize the genetics of adaptation. In each case, we contrast our findings for sexual and asexual populations. First, we calculate transition probabilities to different beneficial mutations; *e.g.*, how often do populations fix the *best* available beneficial mutation? Second, we calculate waiting times to fixation of the first and the last beneficial mutations. Third, we study the trajectory of mean fitness through time. We find that the above statistics are often similar between sexual and asexual populations. This finding suggests that the magnitude of the Fisher-Muller advantage of sex depends on the model of adaptive evolution considered. Finally, we reconsider the effect of population size on adaptation in sexuals *vs.* asexuals.

MODEL AND SIMULATION METHODS

Gillespie's (1984, 1991) mutational landscape model considers adaptation at a DNA sequence (a gene or small genome) that is L base pairs long. Here we simplify the model by considering only two allelic states at each site. The population is made up of N haploid individuals fixed for a single wild-type sequence. We assume that the present wild-type sequence was, until recently, the fittest allele available. The population is fixed for the wild-type sequence (we ignore the segregation of rare deleterious sequences at mutation-selection balance). Following an environmental change, one or a few of the L "one-mutant" neighboring sequences (those that differ from wild type at a single site) become more fit than the wild type. Mutation occurs with probability μ per site per generation. Assume that, of the $L + 1$ relevant sequences (L one-mutant sequences plus wild type), the wild type is the ith fittest, where i is small ($1 < i \ll L$). Equivalently, $i - 1$ beneficial mutations are available. All $i - 1$ of these mutations are assumed to be definitely beneficial

(selection coefficients, $s \gg 1/N$). The beneficial mutation with the greatest selective advantage is denoted allele 1 and that with the next greatest advantage allele 2 and so on. Thus $s_1 \geq s_2 \geq \cdots \geq s_{i-1}$.

Although each of the $i-1$ beneficial alleles may be accidentally lost each time it appears, mutation is recurrent and one allele will ultimately be fixed. At that time, one step in an adaptive walk is complete, and the process repeats itself. Unlike previous work (Gillespie 1983, 1984, 1991; Orr 2002, 2003a,b), we consider a smooth fitness landscape in which the fitness effects of different mutations combine multiplicatively. The next step in adaptation thus proceeds with the same set of beneficial mutations minus the one just substituted.

Although our approach is largely analytic, we use two fully stochastic computational methods to study the above process. In both, the population evolves in discrete generations following the Wright-Fisher model of reproduction. Our first method uses recurrence equations to speed computer simulations. Considering the case of $i = 3$ (that is, two loci produce beneficial mutations), we simulate the changes of four haplotype frequencies through time (x_1, x_2, x_3, and x_4 representing frequencies of b_1b_2, B_1b_2, b_1B_2, and B_1B_2, where B_1 is beneficial allele 1 and B_2 is beneficial allele 2). The system starts with $x_1 = 1$, and $x_2 = x_3 = x_4 = 0$. Each generation, the haplotype frequencies are transformed by the following deterministic forces (in the order of events):

i. Selection:

$$y_i = \frac{w_i}{\bar{w}} x_i \quad (w_1 = 1,\ w_2 = 1 + s_1,\ w_3 = 1 + s_2,\ w_4 = (1 + s_1)(1 + s_2),\ \bar{w} = \sum_{i=1}^{4} w_i x_i). \tag{1}$$

ii. Mutation:

$$z_1 = (1-2\mu)y_1 + \mu y_2 + \mu y_3, \quad z_2 = \mu y_1 + (1-2\mu)y_2 + \mu y_4,$$
$$z_3 = \mu y_1 + (1-2\mu)y_3 + \mu y_4, \quad z_4 = \mu y_2 + \mu y_3 + (1-2\mu)y_4. \tag{2}$$

iii. Recombination,

$$v_1 = z_1 - rD, \quad v_2 = z_2 + rD,$$
$$v_3 = z_3 + rD, \quad v_4 = z_4 - rD \qquad (D = z_1 z_4 - z_2 z_3), \tag{3}$$

where r is the recombination rate. We focus only on the extreme cases of $r = 0$ or $r = 0.5$. The haplotype frequencies at the next generation are obtained by simulating a multinomial sampling of N individuals proportional to $v_1, \ldots, v_4$ (Kim and Stephan 2000; Otto and Barton 2001). Genetic drift is, therefore, taken fully into account.

Our second method involves exact computer simulations of the Wright-Fisher reproduction of N chromosomes. After mutation, two chromosomes are randomly chosen and recombination occurs. The fitness of this "zygote" is evaluated and pseudorandom numbers are used to determine whether to pass one of its "gametes" to the next generation. Fitness effects of beneficial mutations in the same locus or in different loci combine multiplicatively. These two methods yield identical results for $i = 3$. We mainly use the first method for $i = 3$ and use the second method for $i > 3$.

RESULTS

Transition probabilities: One of the most fundamental statistics characterizing adaptation is the "size" of a step in an adaptive walk: does a population move, at the next substitution, to the best mutant allele available, or to the next best, and so on? To answer this question, we calculate the transition probability, P_{ij}, from the current wild-type sequence of fitness rank i to a beneficial mutant sequence of fitness rank j (since $i-1$ different beneficial mutations are available, $j = 1, 2, \ldots, i-1$). Under strong-selection-weak-mutation (SSWM) assumptions, in which beneficial mutations are rare and have independent fates, Gillespie (1983, 1984, 1991) showed that $P_{ij} = s_j/(s_1 + \cdots + s_{i-1})$, where s_j is the selective advantage of jth fittest allele.

Here, because we are interested in large populations or those with high mutation rates, we would like to find the transition probability P_{ij} when beneficial mutations are common enough that they do *not* have independent fates. While each substitution was assumed to be instantaneous in Gillespie's classic calculation, the time spent by an allele on its way to fixation ("transit" time) can no longer be ignored when $N\mu$ is large, as this time becomes longer relative to the waiting time for the next successful beneficial mutation. For the simple case in which the current wild type has rank $i = 3$ and two beneficial mutations compete for fixation, we derive transition probabilities analytically. We study two cases: arbitrary $N\mu$ with sexual organisms (free recombination) and arbitrary $N\mu$ with asexual organisms (no recombination).

We make several assumptions to simplify our derivations. We first consider the situation in which less than one beneficial mutation (on average) arises each generation that survives loss while rare ($2N\mu s < 1$; $N\mu$ can still be large with small s). Put differently, new beneficial alleles appear and are lost for several generations before the appearance of a "successful" beneficial mutation. In this case, we must account for the fact that mutations that become fixed tend to have experienced especially rapid stochastic increases in frequency when still very "young." These beneficial mutations quickly reach the threshold frequency at which natural selection dominates drift and allele frequencies change nearly deterministically. Among mutations going to fixation, this early trajectory of allele frequency is elevated by a factor

$1/(2s)$ relative to the exponential increase from $1/N$, if weak selection ($s \ll 1$) is assumed (Maynard Smith 1971; Barton 1998). Thus, throughout our analysis, we model the trajectory of a beneficial mutation by a deterministic increase from $1/(2Ns)$ to $1 - 1/(2Ns)$. We assume $\mu \ll s$, thus ignoring the contribution of recurrent mutations to this deterministic increase. More formally, the frequency at time t of allele i that enters the population at time z is given by

$$X_i(z,\, t) = \frac{1}{1 + (2Ns_i - 1)\exp(-s_i(t - z))}, \qquad (4)$$

conditional on fixation. Thus, the transit time for allele i is $\tau_i = (2/s_i)\ln(2Ns_i)$ generations. It should be noted that we have ignored the variance of this transit time.

Let T_1 (T_2) be the number of generations until allele 1 (allele 2) is fixed, including the stochastic time until first appearance and the deterministic time until fixation, τ_1 (τ_2). Using a continuous-time approximation, the transition probability from the wild-type allele ($i = 3$) to the best available allele ($j = 1$) is

$$P_{31} = P[T_2 > T_1] \approx \int_0^\infty P[T_2 > t | T_1 = t]P[T_1 = t]dt. \quad (5)$$

In sexuals ($r \gg s$), new mutations independently suffer a probability of loss of about $1 - 2s$. Consequently

$$P[T_2 > t | T_1 = t] = P[T_2 > t] \approx \begin{cases} 1 & (t < \tau_2) \\ \prod_{i=1}^{t-\tau_2}(1 - 2s_2)^{N\mu} \approx \exp(-2N\mu s_2(t - \tau_2)) & \\ & (t \geq \tau_2). \end{cases} \qquad (6)$$

Similarly,

$$P[T_1 = t] \approx 2N\mu s_1 \exp(-2N\mu s_1(t - \tau_1)) \quad (t > \tau_1). \quad (7)$$

Then,

$$P_{31} \approx 1 - \frac{s_2}{s_1 + s_2}\exp\left\{-4N\mu\left(\frac{s_1}{s_2}\ln(2Ns_2) - \ln(2Ns_1)\right)\right\} \qquad (8)$$

and $P_{32} = 1 - P_{31}$. We have thus obtained an approximation to the transition probability on the mutational landscape for arbitrary values of $N\mu$ in sexuals. As expected, as $N\mu \to \infty$, P_{31} approaches 1: given an unlimited supply of beneficial mutants, the fittest beneficial mutant always goes to fixation first. Also as expected, as $N\mu \to 0$, transition probabilities collapse to Gillespie's (1983, 1991) SSWM solution as beneficial mutations now enjoy independent fates.

Next, we obtain transition probabilities in asexuals (no recombination). Because $s_1 > s_2$, it seems reasonable to assume that substitution of allele 2 has little influence on that of allele 1, while substitution of allele 1 does affect that of allele 2. This is the same assumption made by Gerrish and Lenski (1998) and Kim and Stephan (2003). In this case, we can retain Equation 7 and obtain transition probabilities by a modification of Equation 6. Let B_1 (B_2) be the copy of allele 1 (allele 2) that survives initial genetic drift and increases in frequency by selection. Without recombination, B_2 may reach fixation before B_1 only when B_1 arises on a chromosome that already carries B_2 (including the case when B_2 is already fixed). The probability of this event is given by the frequency of B_2, X_2, when B_1 arises (Gerrish and Lenski 1998). Thus, the probability that B_2 fixes after B_1 is given by

$$P[T_2 > t | T_1 = t] \approx \prod_{i=1}^{t-\tau_2}(1 - 2s_2X_2(i,\, t - \tau_1))^{N\mu} \approx \exp\left\{-2N\mu s_2 \int_0^{t-\tau_2} X_2(z,\, t - \tau_1)dz\right\}, \qquad (9)$$

where $X_2(z, t)$ is the frequency at time t of allele B_2 that enters the population at time z. To obtain the probability of fixing allele 1 first under zero recombination, we replace Equation 6 with Equation 9 in the above solution for P_{31}. It should be noted that this approximation assumes constant transit time for B_2, τ_2, regardless of any interference effect. However, τ_2 may become smaller if B_1 occurs on a chromosome carrying B_2. This will reduce $P[T_2 > t | T_1 = t]$ and thus P_{31}. We examine the effect of ignoring this factor in the simulations below.

Figure 1 shows predicted and simulated values of P_{31} under both free and zero recombination for various combinations of s_1 and s_2. As expected under clonal interference, P_{31} increases with an increasing supply of new beneficial mutations (Gerrish and Lenski 1998; Rozen *et al.* 2002). Surprisingly, however, our approximations yield almost identical values for P_{31} in asexuals *and* sexuals when s_1 is considerably larger than s_2 ($s_1 \geq 2s_2$; Figure 1, A and B). This reflects the fact that $X_2(.\ ,\ .)$ in Equation 9 becomes close to one if s_1 is at least twice as great as s_2. In an asexual population in which B_2 starts to increase first, late-arising B_1 may go to fixation before B_2 if B_1 enters in the repulsion phase with B_2 and thus displaces B_2 in the population. This interference occurs with high probability when B_2 is rare, *i.e.*, soon after its appearance. Surprisingly, this is effectively the same condition for late-arising B_1 to outcompete B_2 in a sexual population; *i.e.*, it must enter the population when B_2 is still in an early stage of its fixation. The net result is that transition probabilities in sexuals are nearly indistinguishable from those in asexuals.

If, however, selection coefficients for two beneficial mutations are similar (and thus transit times are similar), clonal interference in asexuals has a greater effect and asexuals are more likely than sexuals to fix the best allele (Figure 1C). Surprisingly, when $s_1 = 0.06$ and $s_2 = 0.02$ (the expected ratio of effects for the two fittest mutations under extreme value theory; Orr 2002), P_{31} in

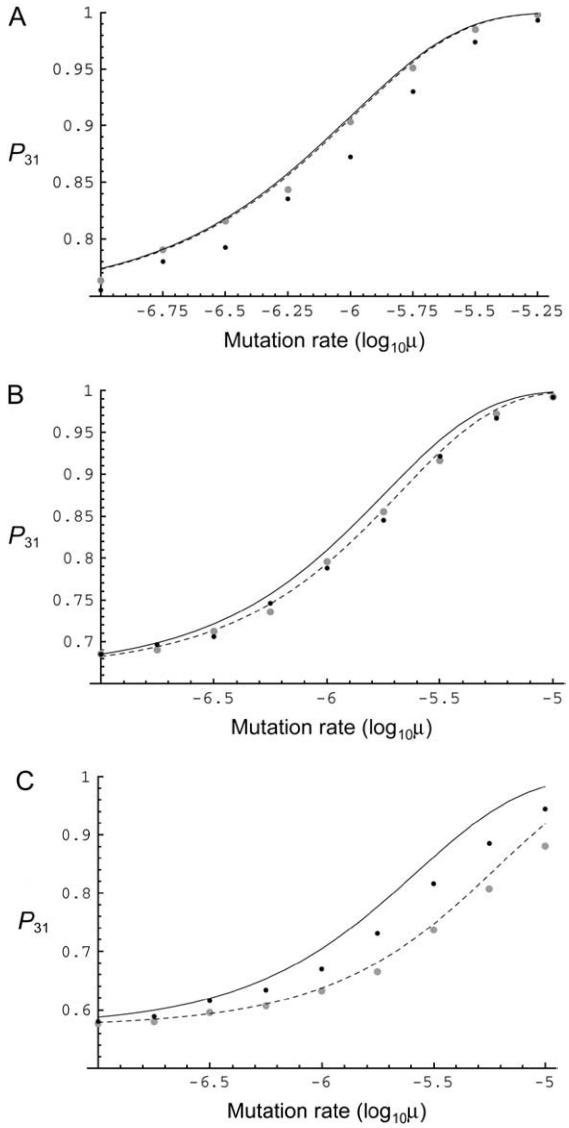

FIGURE 1.—Transition probability to the fittest allele for $i = 3$. The solid curve shows P_{31} for zero recombination as a function of mutation rate given by Equations 5 and 9. The dashed curve is that for free recombination, given by Equation 8. Solid (shaded) circles represent simulation results for an asexual (sexual) population. Simulation results were averaged over 5000 replicates. $N = 2 \times 10^4$. (A) $s_1 = 0.06$, $s_2 = 0.02$; (B) $s_1 = 0.04$, $s_2 = 0.02$; and (C) $s_1 = 0.04$, $s_2 = 0.03$.

asexuals is actually smaller than that in sexuals in our simulations. This may reflect a shortened transit time for B_2 conditional on the occurrence of B_1 on its background, as explained above.

Fixation times: A sensible measure of the speed of adaptation is the waiting time until fixation of beneficial alleles. Here we derive approximate solutions to the waiting times until the substitution of the first and second beneficial mutations (again assuming $i = 3$). We begin by considering a sexual population (free recombination). The time to the first fixation, $T_{(1)} = \min(T_1, T_2)$, satisfies

$$P[T_{(1)} < t] = F(t) = 1 - P[T_1 > t]P[T_2 > t].$$

Here, $P[T_i > t] \approx \prod_{k=1}^{t-\tau_i}(1 - 2s_i)^{N\mu} \approx \exp(-2N\mu s_i(t - \tau_i))$ for $t > \tau_i$ and 1 for $0 < t \le \tau_i$. Then,

$$F(t) = \begin{cases} 0 & (t < \tau_1) \\ 1 - \exp(-2N\mu s_1(t - \tau_1)) & (\tau_1 \le t < \tau_2) \\ 1 - \exp(-2N\mu\{s_1(t - \tau_1) + s_2(t - \tau_2)\}) & (t \ge \tau_2) \end{cases}. \quad (10)$$

The mean waiting time until the first substitution in the sexual population is therefore

$$E[T_{(1),\text{sex}}] = \int_0^\infty t dF(t) \approx \tau_1 + \frac{1}{\lambda_1} - \left(\frac{1}{\lambda_1} - \frac{1}{\lambda_1 + \lambda_2}\right)\exp(-\lambda_1(\tau_2 - \tau_1)), \quad (11)$$

where $\lambda_i = 2N\mu s_i$ is the number of new copies of allele i at each generation that survive loss while rare. As expected, as $N\mu \to \infty$, $\mathrm{E}[T_{(1),\text{sex}}]$ approaches $\tau_1 + 1/\lambda_1$, the expected waiting time to the fixation of allele 1. And as $N\mu \to 0$, we recover the expected waiting time under SSWM assumptions, $\mathrm{E}[T_{(1),\text{sex}}] \approx 1/(\lambda_1 + \lambda_2)$ (GILLESPIE 1991).

The expected waiting time to the second substitution, $T_{(2)} = \max(T_1, T_2)$, is obtained from $P[T_{(2)} < t] = P[T_1 < t]P[T_2 < t]$. By arguments similar to those used above,

$$E[T_{(2),\text{sex}}] \approx \tau_2 + \frac{1}{\lambda_2} + \left(\frac{1}{\lambda_1} - \frac{1}{\lambda_1 + \lambda_2}\right)\exp(-\lambda_1(\tau_2 - \tau_1)). \quad (12)$$

As $N\mu \to \infty$, this approaches $\tau_2 + 1/\lambda_2$, the expected waiting time to fixation of allele 2.

It is much more difficult to calculate waiting times in asexuals. Following GERRISH and LENSKI (1998) and KIM and STEPHAN (2003), we assume asymmetric interference: s_1 is much larger than s_2 and thus allele 1 affects the fate of allele 2, while allele 2 barely affects the fate of allele 1. Then,

$$P[T_{(1)} < t] = 1 - \int_t^\infty P[T_2 > t | T_1 = y]P[T_1 = y]dy, \quad (13)$$

where

$$P[T_2 > t | T_1 = y] \approx \begin{cases} 1 & (\tau_1 \le t < \tau_2) \\ \exp\{-2N\mu s_2 \int_0^{t-\tau_2} X_2(z, y - \tau_1)dz\} & (t > \tau_2) \end{cases}$$

and $X_2(z, y - \tau_1)$ is effectively the probability that a B_2 mutation entering the population at time z survives the interfering effect of a B_1 mutation that enters the population at time $y - \tau_1$ ($> z$), because this B_1 should land

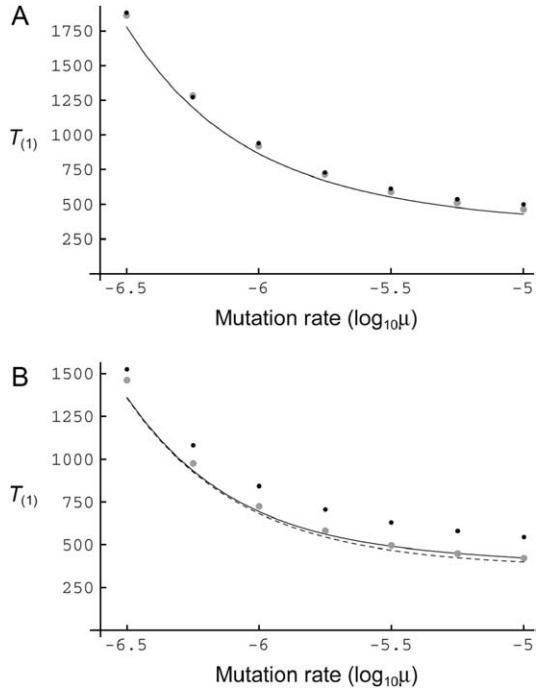

FIGURE 2.—Waiting time until the first fixation of a beneficial mutation with increasing mutation rate ($i = 3$). The solid (dashed) curve, representing an asexual (sexual) population, is produced by Equation 14 (Equation 11). Solid (shaded) circles represent simulation results for no (free) recombination. Simulation results were averaged over 5000 replicates. $N = 2 \times 10^4$. (A) $s_1 = 0.04$, $s_2 = 0.02$; (B) $s_1 = s_2 = 0.04$.

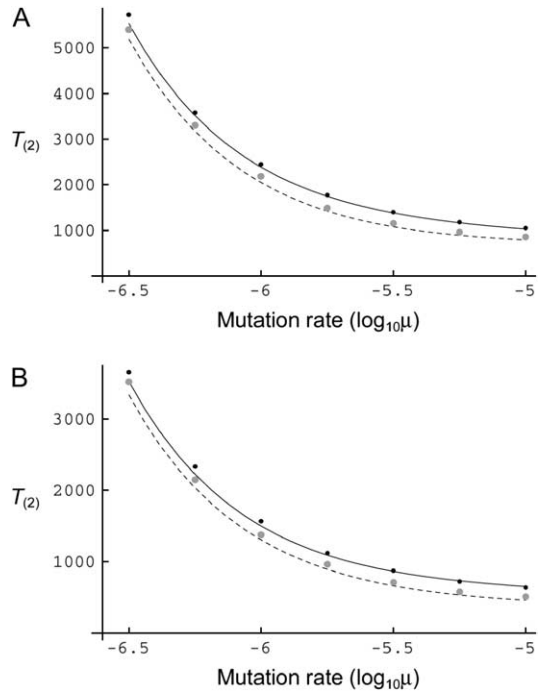

FIGURE 3.—Waiting time until the second fixation of a beneficial mutation ($i = 3$). The solid (dashed) curve, representing an asexual (sexual) population, is produced by Equation 16 (Equation 12). Solid (shaded) circles represent simulation results for no (free) recombination. Simulation results were averaged over 5000 replicates. $N = 2 \times 10^4$. (A) $s_1 = 0.04$, $s_2 = 0.02$; (B) $s_1 = s_2 = 0.04$.

on a B_2 chromosome. The expected time to the first substitution in an asexual population is

$$E[T_{(1),\text{asex}}] \approx \int_0^\infty (1 - P[T_{(1)} < t])\,dt, \qquad (14)$$

where $P[T_{(1)} < t]$ is given by Equation 13.

The waiting time to the second fixation, $T_{(2)}$, in the asexual population is expected to be more affected by clonal interference. From our asymmetric interference assumption,

$$P[T_{(2)} < t] = \int_0^t P[T_2 < t | T_1 = y] P[T_1 = y]\,dy. \qquad (15)$$

Calculation of $P[T_2 < t | T_1 = z]$ under no recombination is more complex than our other calculations and is presented in the APPENDIX. Using the solution given there,

$$E[T_{(2),\text{asex}}] \approx \int_0^\infty (1 - P[T_{(2)} < t])\,dt. \qquad (16)$$

The above solution can be calculated numerically.

Figures 2 and 3 show our analytic approximations and simulation results for $T_{(1)}$ and $T_{(2)}$ under free *vs.* no recombination. $T_{(1)}$ is almost identical between sexual and asexual populations when $s_1 = 0.04$ and $s_2 = 0.02$ (Figure 2A) both in theory and in simulations. This suggests that clonal interference has little effect on $T_{(1)}$ when s_1 is much larger than s_2. However, when $s_1 = s_2$ (Figure 2B), $T_{(1)}$ in asexuals is larger than that in sexuals. Our approximation (Equation 14) fails to predict this increase, presumably because our asymmetric interference assumption is violated. Competition of two linked beneficial alleles may increase their transit times, due to the reduced efficacy of selection (HILL and ROBERTSON 1966); this effect is especially strong when competing beneficial mutations have equal fitness effects (BARTON 1995; KIM and STEPHAN 2003).

$T_{(2)}$ is clearly larger in asexuals than in sexuals (Figure 3). This is not surprising, as the fixation probability of allele 2 is substantially reduced under clonal interference. Interestingly, our approximation for $T_{(2)}$ agrees well with the simulation results even for $s_1 = s_2$, a case that violates our asymmetric interference assumption.

Mean fitness trajectory: The fact that transition probabilities and waiting times to fixation are nearly the same in sexuals and asexuals, at least when $s_1 \gg s_2$, raises the intriguing possibility that rates of adaptation in sexuals and asexuals may also be similar, even given clonal interference. To test this, we tracked change in mean fitness through time in our computer simulations. Figure 4 shows trajectories of $\bar{w}$ in sexuals and asexuals for various s_1 and s_2. As expected, the gap between sexual and asexual populations increases as s_2 nears s_1. But

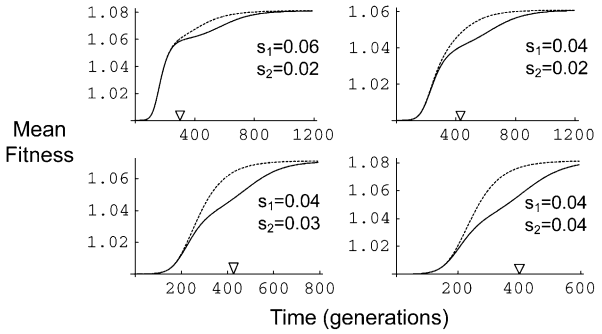

FIGURE 4.—Mean fitness trajectories for sexual (dashed curve) and asexual (solid curve) populations ($i = 3$). The expected times of the first fixation ($E[T_{(1),\text{sex}}]$) are marked by a triangle on the *x*-axis. Results are based on 5000 replicates of simulation. $N = 2 \times 10^4$, $\mu = 10^{-5}$.

when $s_1/s_2 = 3$ (the mean extreme-value expectation), the trajectories are nearly identical until the expected time of the first fixation ($E[T_{(1),\text{sex}}]$), after which the fitness of sexuals increases faster than that of asexuals. The point at which the sexual and asexual trajectories begin to diverge gets earlier relative to $E[T_{(1),\text{sex}}]$ as s_1/s_2 becomes smaller.

Multiloci simulations ($i > 3$): Using computer simulations, we asked whether our results for $i = 3$ generalize to the situation in which more than two beneficial alleles are available. Simulation results for $i = 5$ are given in Table 1. Two fitness schemes were used, with fitness effects of the four beneficial mutations given by their expected extreme value spacing ($s_1 - s_2 = \Delta$, $s_2 - s_3 = \Delta/2$, $s_3 - s_4 = \Delta/3$, $s_4 = \Delta/4$, with $\Delta = 0.02$; ORR 2002) or by equal spacings with small absolute differences ($s_1 - s_2 = s_2 - s_3 = s_3 - s_4 = 0.005$). The mutation rate chosen was high enough to shift transition probabilities from SSWM expectations. Under both fitness schemes, transition probabilities are nearly identical between sexuals and asexuals. Thus, clonal interference appears to cause even smaller differences in transition probabilities between sexuals and asexuals when more beneficial mutations are available to a wild type.

TABLE 1

Transition probabilities and waiting times ($i = 5$, $N = 10^4$, $\mu = 10^{-5}$)

r^a	P_{51}	P_{52}	P_{53}	P_{54}	$T_{(1)}$	$T_{(4)}$
i. $s_1 = 0.04167$, $s_2 = 0.02167$, $s_3 = 0.01167$, $s_4 = 0.005$						
0	0.930	0.065	0.005	0	425.5	3927
0.5	0.942	0.056	0.002	0	397.5	2790
ii. $s_1 = 0.05$, $s_2 = 0.045$, $s_3 = 0.04$, $s_4 = 0.035$						
0	0.488	0.288	0.152	0.073	396.8	950.1
0.5	0.489	0.297	0.160	0.055	290.9	560.3

Simulation results are based on 5000 replicates for each parameter set.

[a] Recombination fraction between loci.

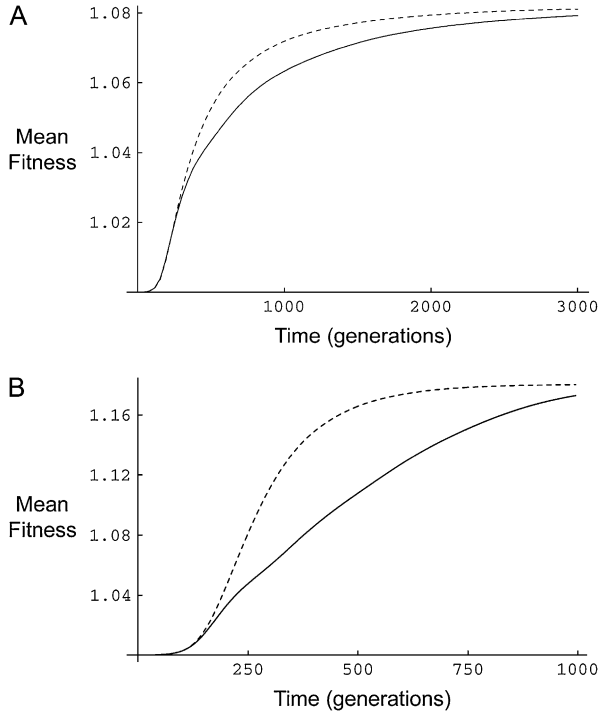

FIGURE 5.—Mean fitness trajectories for sexual (dashed curve) and asexual (solid curve) populations ($i = 5$). Results are based on 5000 replicates of simulation. $N = 10^4$, $\mu = 10^{-5}$. (A) $s_1 = 0.04167$, $s_2 = 0.02167$, $s_3 = 0.01167$, $s_4 = 0.005$; (B) $s_1 = 0.05$, $s_2 = 0.045$, $s_3 = 0.04$, $s_4 = 0.035$.

The waiting times to the first and last substitutions, however, are larger in asexual than in sexual populations given more beneficial mutations. As expected, this difference is less pronounced given larger differences in fitnesses (extreme value spacings) than given small differences (equal spacings). Similarly, mean fitness trajectories in sexuals and asexuals are most similar when beneficial mutations have very different fitness effects (Figure 5). Qualitatively, then, our main conclusions from the $i = 3$ case remain valid in the $i = 5$ case.

We would also expect clonal interference's effect on the rate of adaptation to increase as the number of competing beneficial mutations increases. To test this, we simulated adaptation with equal *s* among beneficial mutations, the condition under which fitness increase is most affected by clonal interference; this scenario corresponds to the one considered in many previous studies of sex (CROW and KIMURA 1965; MAYNARD SMITH 1971). The time to fixation of the last beneficial mutation provides a convenient measure of the speed of adaptation. Table 2 confirms our expectations: $T_{(l),\text{asex}}/T_{(l),\text{sex}}$ increases with the number of beneficial mutations, *l*. This ratio is, however, much smaller than that predicted by MAYNARD SMITH (1971), who argued that $T_{(l),\text{asex}}/T_{(l),\text{sex}} \approx l$.

Effect of population size: So far, we have considered only two population sizes ($N = 10^4$ or 2×10^4). Previous studies have suggested that population size itself should

TABLE 2

Waiting times in multilocus simulations ($N = 10^4$, $\mu = 10^{-5}$, $s = 0.025$)

$i-1$	$T_{(1),\text{asex}}$	$T_{(1),\text{sex}}$	$T_{(i-1),\text{asex}}$	$T_{(i-1),\text{sex}}$
2	689.3	537.7	955.3	753.6
4	674.9	480.0	1439	880.2
6	639.5	458.0	1829	960.5
8	622.7	445.1	2147	1015
10	607.2	437.2	2474	1062
12	594.2	428.4	2737	1081

Simulation results are based on 1000 replicates for each parameter set.

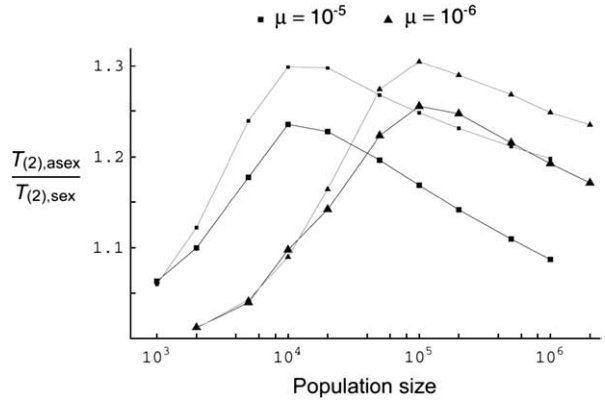

FIGURE 6.—Slower adaptation in asexuals relative to sexuals, measured by $T_{(2),\text{asex}}/T_{(2),\text{sex}}$, with varying population size. Large triangles and squares represent simulation results for $\mu = 10^{-6}$ and 10^{-5}, respectively. Small triangles and squares are theoretical predictions given by Equations 12 and 16.

have a large effect on adaptation in sexuals *vs.* asexuals. As noted earlier, sex should confer no advantage in either infinite (MAYNARD SMITH 1968; ESHEL and FELDMAN 1970) or small populations; instead, sex should have the greatest effect in populations of intermediate size (OTTO and BARTON 2001). We asked whether this pattern holds in our model ($i = 3$).

In an infinite population (with no genetic drift), the trajectory of mean fitness with free recombination should be described by

$$\bar{w} = (1 + s_1 p_1)(1 + s_2 p_2), \tag{17}$$

where

$$\frac{dp_i}{dt} \approx s_i p_i (1 - p_i) + \mu(1 - 2p_i) \quad (p_i = 0 \text{ at } t = 0) \text{ for } i = 1, 2. \tag{18}$$

The numerical solution of Equation 17 is indistinguishable from $\bar{w}$ obtained by iteration of Equations 1–3 with $r = 0$. Thus, the rates of adaptation in sexuals and asexuals are identical when populations are infinitely large, as expected. This reflects the fact that recombination lessens the effect of clonal interference only when there is negative LD between beneficial alleles. Here, populations begin with no linkage disequilibrium ($D = x_1x_4 - x_2x_3 = 0$; $x_1 = 1$, $x_2 = x_3 = x_4 = 0$) and selection and mutation preserve D [Equations 1 and 2 are rearranged to $y_1y_4 - y_2y_3 \approx (1 + s_1)(1 + s_2)(x_1x_4 - x_2x_3)$ and $z_1z_4 - z_2z_3 \approx (1 - 4\mu)(y_1y_4 - y_2y_3)$, respectively]. LD thus remains zero in an infinitely large asexual population and recombination has no effect.

This suggests that multinomial sampling of individuals (*i.e.*, genetic drift) is the only step at which differences between sexual and asexual populations can arise. HILL and ROBERTSON (1966) showed that LD among beneficial alleles becomes progressively more negative in a finite population due to genetic drift. This effect can be easily understood in our model ($i = 3$). Immediately after an environmental change, mutation and selection create a nearly infinite pool of chromosomes in linkage equilibrium. However, the expected frequency of chromosomes carrying two beneficial alleles is low initially, so double mutants are likely to be sampled only much later, after the frequencies of the two beneficial mutations have increased substantially. Thus, negative LD between beneficial mutations builds up during this period. This argument implies that adaptation depends critically on the product of population size and mutation rate, as this product determines just how often double mutants are sampled. Previous work on the effect of N (HILL and ROBERTSON 1966; OTTO and BARTON 2001) focused on the buildup of LD in the absence of new mutations. As our model allows recurrent mutation, we examined the effect of N and μ on $T_{(2)}$ in sexuals *vs.* asexuals. We used $s_1 = 0.04$, $s_2 = 0.02$, $\mu = 10^{-6}$ or 10^{-5} and studied many values of N.

As expected, asexuals suffer the greatest increases in $T_{(2)}$ relative to sexuals [$T_{(2),\text{asex}}/T_{(2),\text{sex}}$] at intermediate N (Figure 6). Although our Equations 12 and 16 overestimate $T_{(2),\text{asex}}/T_{(2),\text{sex}}$, they correctly predict the values of N that maximize this ratio. We also plotted the mean population fitness trajectories for various N in Figure 7 ($\mu = 10^{-5}$). As expected, the rates of adaptation for sexual and asexual populations become more similar as N increases.

We next explored the effect of varying mutation rate. As Figure 6 shows, the value of N that maximizes $T_{(2),\text{asex}}/T_{(2),\text{sex}}$ decreases as μ increases. Thus, as predicted, the difference in the rate of adaptation between sexuals and asexuals depends critically on the product $N\mu$, not on N alone.

DISCUSSION

We have studied three aspects of adaptation in sexuals *vs.* asexuals: (i) transition probabilities to various beneficial mutations; (ii) waiting times to the first, second, and subsequent substitutions; and (iii) trajectories of mean fitness through time. Our results show that these statistics are often similar in sexuals and asexuals,

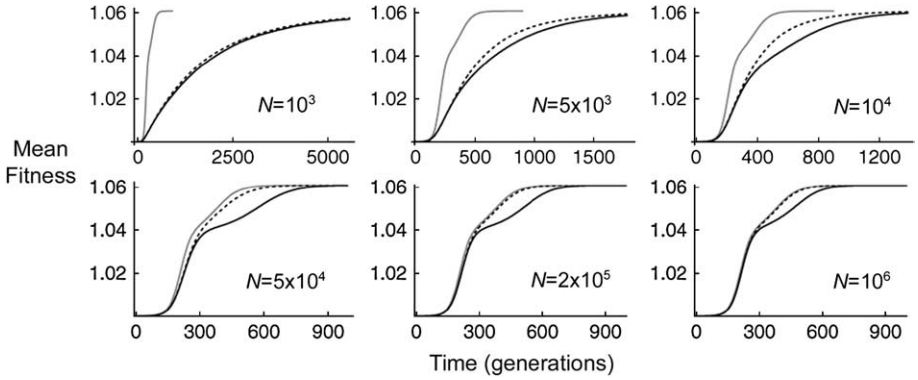

FIGURE 7.—Mean fitness trajectories for sexual (dashed curves) and asexual (solid curves) populations ($i = 3$) with $s_1 = 0.04$, $s_2 = 0.02$, $\mu = 10^{-5}$, and various N. The numerical solution of Equations 17 and 18 (trajectory of an infinite population) is given by the shaded curve. Note that this deterministic curve stays the same in each part.

particularly when a small number of beneficial mutations is available and selection coefficients among these mutations are dissimilar. We believe that this finding requires some reassessment of the generality of the Fisher-Muller theory of the advantage of sex and, especially, of recent theories of clonal interference.

It is widely believed, for instance, that clonal interference between beneficial mutations will cause the preferential fixation of large-effect mutations. Both GERRISH and LENSKI (1998) and ROZEN *et al.* (2002) suggested that, in an asexual population experiencing many mutations, beneficial mutations of large effect have higher probabilities of fixation than that expected with no clonal interference, as large-effect mutations are more likely to survive both genetic drift and clonal interference. MIRALLES *et al.* (1999) and ROZEN *et al.* (2002) tested this prediction experimentally using RNA virus and *Escherichia coli*, respectively. In these experiments, populations were placed in novel environments and serially transferred with various bottleneck sizes. For each replicate, the first sweep of a beneficial mutation was identified using genetic markers. The strain presumed to contain the beneficial mutation was then extracted and its fitness gain over the ancestral strain was measured. As expected from previous ideas about clonal interference, MIRALLES *et al.* (1999) found greater fitness increases in larger populations. Our results, however, suggest that this correlation should occur even without clonal interference: Equation 8 shows that the transition probability to the fittest allele increases with population size *even in sexuals*. The reason is that beneficial mutations with different-sized fitness effects have different transit times: beneficial mutations of large effect typically sweep through populations faster than those of smaller effect. Beneficial mutations of larger effect thus typically "out race" those of smaller effect, sweeping to fixation first even in sexuals. This effect of transit times increases with $N\mu$.

Our results also suggest that clonal interference may not have a large effect on the speed of adaptation. For example, when the selection coefficients distinguishing various beneficial mutations are very different (as suggested by extreme value theory; GILLESPIE 1984, ORR 2003a), the waiting time to fixation of the first favorable substitution is usually similar between sexuals and asexuals. (The waiting time to subsequent substitutions is typically longer in asexuals, although not as long as suggested by much classical theory, *e.g.*, MAYNARD SMITH 1971.) Finally, our results show that, although mean fitness typically increases faster in sexuals than in asexuals, the difference is small when beneficial mutations have very different effects (see Figure 4).

Why do our results contradict several popular intuitions about clonal interference? We believe the answer is that, in our model, a modest number of beneficial mutations are available to a population at any moment in time and mutation to these alleles is recurrent. Put conversely, ignoring recurrent mutation to a finite number of alleles might exaggerate the effect of clonal interference and the advantage of sex. Recent theoretical work on clonal interference (GERRISH and LENSKI 1998; WILKE 2004) assumed a model in which the rate of mutation to beneficial alleles is constant over time and each new mutation represents a new sequence (*i.e.*, mutation occurs at a new site). Given such an infinite supply of new beneficial mutations, the rate of substitution of beneficial alleles in a haploid sexual population is about $2N\mu s$, where μ is the beneficial mutation per genome per generation. The substitution rate thus increases linearly with population size. Under this model, GERRISH and LENSKI (1998) showed that the fixation probability of a beneficial mutation decreases by a factor e^{-I}, where I (the expected number of interfering mutations) is proportional to $(N\mu)\log N$ (Equation 2 of their article). On the basis of this result, WILKE (2004) argued that the rate of adaptive substitution in an asexual population reaches a limit on the order of s, even if population size increases indefinitely. Thus, adaptation in sexuals becomes infinitely faster than that in asexuals as $N \to \infty$.

The above conclusions may not hold, however, if a finite number of beneficial mutations are available. If beneficial mutations can occur only at l sites, the rate of substitution in sexuals in a period of time cannot exceed l even if $N \to \infty$. Fitness in sexuals thus cannot grow indefinitely faster than that in asexuals. Similarly, the

expected number of unique interfering mutations, I, cannot grow indefinitely as $N \to \infty$; instead, the number of different interfering mutations cannot exceed $l - 1$. Consequently, clonal interference should have a smaller effect on fixation probabilities and thus on the rate of adaptation, when adaptation involves a finite number of recurrent mutations. We also note that the finiteness of the number of beneficial alleles (and thus of the number of combinations of them) ensures convergence of the rates of adaptation in sexual and asexual populations as $N\mu \to \infty$, since chromosomes carrying all combinations of alleles are produced by mutation.

It is entirely possible, of course, that our assumption of recurrent mutation to a finite number of beneficial alleles represents one extreme end of possible adaptive substitution models. For the reasons provided above, this extreme may yield the minimal possible advantage to sex. But it is unclear, to us at least, if this model is less realistic than the opposite extreme model: one that allows unique beneficial mutations to appear at an infinite number of sites, a model that likely yields a maximal advantage to sex. After all, real adaptation must occur in a space of DNA sequences in which the number of possible beneficial changes is finite and, presumably, often small.

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APPENDIX

To calculate $P[T_2 < t | T_1 = z]$ in Equation 15, we need to consider two cases: B_1 might prevent the fixation of B_2 when B_1 enters the population either earlier or later than B_2. If earlier, B_2 can go to fixation only if it occurs on a chromosome that already carries B_1. This event happens with probability X_1, the allele frequency of B_1. If B_1 enters later, the fixation of B_2 happens only when B_1 occurs on a chromosome carrying B_2. Let $J(z, t)$ be the probability that B_2 that enters at time z eventually goes to fixation despite the interfering allele B_1 that enters at time t. If $t < z$, $J(z, t) = X_1(t, z)$. If $t > z$, $J(z, t) = X_2(z, t)$. Equation 14 is solved using

$$P[T_2 < t | T_1 = z] \approx 1 - \exp\left\{-2N\mu s_2 \int_0^{t-\tau_2} J(y, z-\tau_1)\, dy\right\} \quad (t \geq \tau_2).$$