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## MUTATIONAL MELTDOWNS IN SEXUAL POPULATIONS

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**Abstract.**—Although it is widely acknowledged that the gradual accumulation of mildly deleterious mutations is an important source of extinction for asexual populations, it is generally assumed that this process is of little relevance to sexual species. Here we present results, based on computer simulations and supported by analytical approximations, that indicate that mutation accumulation in small, random-mating monoecious populations can lead to mean extinction times less than a few hundred to a few thousand generations. Unlike the situation in obligate asexuals in which the mean time to extinction ( $\bar{t}_e$ ) increases more slowly than linearly with the population carrying capacity ( $K$ ),  $\bar{t}_e$  increases approximately exponentially with  $K$  in outcrossing sexual populations. The mean time to extinction for obligately selfing populations is shown to be equivalent to that for asexual populations of the same size, but with half the mutation rate and twice the mutational effect; this suggests that obligate selfing, like obligate asexuality, is inviable as a long-term reproductive strategy. Under all mating systems, the mean time to extinction increases relatively slowly with the logarithm of fecundity, and mutations with intermediate effects (similar to those observed empirically) cause the greatest risk of extinction. Because our analyses ignore sources of demographic and environmental stochasticity, which have synergistic effects that exacerbate the accumulation of deleterious mutations, our results should yield liberal upper bounds to the mean time to extinction caused by mutational degradation. Thus, deleterious mutation accumulation cannot be ruled out generally as a significant source of extinction vulnerability in small sexual populations or as a selective force influencing mating-system evolution.

**Key words.**—Extinction, mutation accumulation, obligate selfing, random mating, sexual species.

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It is now generally accepted that mildly deleterious mutations arise at a substantial rate in most higher organisms, probably as frequently as one per gamete (Crow and Simmons 1983; Bell 1988a; Kondrashov 1988; Charlesworth et al. 1990; Houle et al. 1992). Faced with this biological fact, theoreticians have wrestled with the problem of how populations can withstand this constant onslaught of mutations without experiencing a cumulative loss in individual fitness (Haldane 1937; Kimura et al. 1963; Crow and Kimura 1979; Kondrashov 1988; Charlesworth 1990; Charlesworth et al. 1992, 1993).

It has long been thought that sexual reproduction is critical (Fisher 1930; Muller 1964). Assuming back mutations are rare, a parent experiencing no recombination (or segregation) can never produce an offspring with fewer deleterious mutations than it carries itself. Because there is always a chance that the best class of individuals in a population will not reproduce in some generation, or will produce only progeny with new mutations, that class must be lost eventually, as must the previously second-best class, and so on. Many theoretical studies have considered the rate at which deleterious mutations accumulate in obligately asexual populations via Muller's ratchet (Felsenstein 1974; Haigh 1978; Maynard Smith 1978; Pamilo et al. 1987; Charlesworth 1990; Charlesworth et al. 1993; Stephan et al. 1993; Higgs 1994), and it is now clear that this phenomenon imposes a serious risk of extinction for such populations. Although many additional ecological and evolutionary factors can contribute to extinction, it is difficult to see how a non-recombining population, even one of relatively large size, could ever withstand the cumulative effects of Muller's ratchet for more than a few thousand generations (Bell 1988a,b; Lynch and Gabriel 1990; Melzer and Koeslag 1991; Gabriel et al. 1993; Lynch et al. 1993).

Recombination slows down the ratchet through the production of progeny chromosomes that incorporate the best portions of two parental chromosomes. In diploid populations, segregation has a similar effect at individual loci when two heterozygous parents produce mutation-free genotypes. However, the question remains as to whether sexual reproduction is sufficient to prevent a gradual decline in fitness due to recurrent mutation. Sexual reproduction alone does not eliminate the chance fixation of mildly deleterious mutations by genetic drift, nor does it prevent segregation of recurrent deleterious mutations at multiple loci. However, the probability of fixation of a deleterious mutation does become diminishingly small in large populations (Kimura 1962), and for populations with effective sizes greater than a few hundred individuals, segregating deleterious mutations reduce mean fitness by a factor no greater than  $e^{-\mu}$ , where  $\mu$  is the deleterious mutation rate per individual summed over all loci (Kimura et al. 1963). Thus, large sexual populations with a high enough reproductive rate to offset this segregational load can be expected to be quite resistant to deleterious mutation accumulation, perhaps indefinitely.

Charlesworth et al. (1993, p. 39) recently suggested that "Even with inbreeding, sexual populations larger than 100 will probably rarely experience mutation accumulation to the point that their survival is endangered." In their computer-simulation study, deleterious mutations affected only the relative fitness of genotypes within the population, having no influence on population size even when mean fitness (relative to the optimal genotype) declined dramatically. The population size was kept constant by allowing adults to produce more and more progeny as the mutation load for viability increased. Such reproductive compensation seems unlikely in ecological settings with limited resources, and even if the optimal genotype is defined by other ecological factors, de-

viations from the optimum are likely to have eventual repercussions for population size (Clarke 1973). Thus, although the study of Charlesworth et al. (1993) has provided useful insight into the effects of the mating system and of recombination frequency on the rate of mutation accumulation, it leaves open questions about the role of mutation in the extinction process.

Focusing on the situation in which the reproductive capacity of individuals is fixed and using single-locus diffusion theory to predict the rate of loss of absolute fitness, Lande (1994) addressed this issue explicitly. He suggests that even for populations with effective sizes on the order of 1000, the risk of extinction due to fixation of deleterious mutations can be comparable to that due to environmental stochasticity, the factor that had previously been thought to be the dominant source of extinction for most populations (Goodman 1987; Lande 1993, 1994). A similar conclusion was reached earlier by Gabriel et al. (1991), although Lande (1994) focused attention on the situation in which mutations have variable deleterious effects.

Although Lande's (1994) conclusions depart significantly from those of Charlesworth et al. (1993), there are several reasons why his results may actually predict times to extinction that are too high. First, it is now well established that the probability of fixation of a deleterious mutation is increased when it occurs on a background containing other segregating loci (Robertson 1961; Hill and Robertson 1966; Felsenstein 1974, 1988; Birky and Walsh 1988; Charlesworth et al. 1993). The essence of the Hill-Robertson effect is that background genetic variance reduces the efficiency of selection on individual loci. This occurs both through the stochastic development of linkage disequilibrium and through a reduction in the effective population size caused by variance in fitness among parental genotypes. Single-locus diffusion theory assumes such interference to be of negligible importance, but with genomic mutation rates on the order of one per individual, such background variation will always be present. A second potential limitation of Lande's (1994) approximation is that it ignores stochastic fluctuations in population size. Such fluctuations, which can be driven by the mutation-accumulation process itself as well as by extrinsic sources of demographic and environmental stochasticity, cause the long-term effective population size to be less than the arithmetic mean, thereby enhancing the rate of fixation of deleterious genes. Third, Lande (1994) considered only the mutational load due to mutations that have gone to fixation, ignoring the pool of segregating mutations.

Thus, although it appears that deleterious mutations are a more important contributor to extinction risk than previously believed, there is still uncertainty as to how large and how fecund sexual populations need to be to gain immunity from such a threat. To evaluate this problem, we have performed extensive computer simulations similar to those of Charlesworth et al. (1993) but allowing for the absolute effects of mutations on individual viability, which are necessary for ultimate extinction. The observed times to extinction in these simulations will be compared with the predictions of some analytical approximations that we develop below.

## COMPUTER SIMULATIONS

We studied the influence of viability mutations on the mean extinction time for randomly mating monoecious populations growing in discrete generations. A simple form of density dependence followed juvenile production and viability selection each generation. Letting  $R$  be the reproductive rate per surviving adult and  $N(t)$  be the number of reproducing adults at time  $t$ , the number of progeny produced before selection was  $RN(t)$ . The gametes required to produce these progeny were drawn randomly, with free recombination and allowing random selfing, from the  $N(t)$  parents. Hence, the expected distribution of family sizes prior to selection was Poisson. The progeny were then subjected to viability selection, with the probability of survival to maturity being determined by the fitness function  $W(n_1, n_2) = (1 - 2hs)^{n_1}(1 - 2s)^{n_2}$ , where  $2s$  is the fractional reduction in viability caused by a homozygous mutation,  $h$  is a measure of dominance (with  $h = 0.5$  denoting additivity within loci), and  $n_1$  and  $n_2$  are the numbers of loci in the individual that are heterozygous and homozygous for deleterious mutations. The potential reproductive adults were identified by drawing uniformly distributed random numbers in the interval 0 to 1 for each of the juveniles and imposing mortality whenever this was greater than their respective  $W(n_1, n_2)$ . If, after selection, the number of potential adults exceeded  $K$ , the carrying capacity of the environment, the population was reduced to size  $K$  by genotype-independent culling.

All simulations were initiated with mutation-free genotypes. Newborns incurred new mutations, prior to selection, following a Poisson distribution with expectation  $\mu$ . All new mutations were assumed to arise at loci that were not currently segregating in the population and were assumed to have the constant properties  $s$  and  $h$ . The fitness function  $W(n_1, n_2)$  allows for dominance but assumes that the effects of mutations at different loci are independent.

Using the life-cycle sequence described above (mutation, selection, density dependence, and reproduction), we also examined the consequences of obligate self-fertilization, and for comparative purposes, obligate (ameiotic) asexuality. In the latter case, the fitness function is simply  $W(n) = (1 - 2hs)^n$ , where  $n$  is the number of mutant alleles in an individual, because the likelihood of homozygous mutants arising in the absence of segregation (by parallel mutation) is extremely small.

In addition, we considered the situation in which sexes were separate and matings were monogamous. This type of reproductive system introduced demographic stochasticity that was not present in the cases of random-mating monoecy, self-fertilization, or obligate asexuality. Because density dependence was applied without respect to sex, the sex ratio of the population at the time of reproduction was typically unequal to 1:1. When that is the case, the number of mating pairs, which is defined by the rarest sex, is  $\leq K/2$ .

To estimate mean extinction times for a given set of the parameters  $R$ ,  $K$ ,  $\mu$ ,  $h$ , and  $s$ , we always simulated at least 256 extinction events, and in most cases 1024 or more simulations were performed. All analyses were performed on a parallel-processing computer, which enabled us to simulate at least 64 populations simultaneously.

## ANALYTICAL APPROXIMATIONS

In this section, we derive some theory for the accumulation of deleterious mutations in a randomly mating monoecious population under some necessarily simplifying assumptions. In particular, we assume that random genetic drift is the only stochastic process. The mean number of new mutations incurred by newborns is assumed to be exactly  $\mu$  per generation, global linkage equilibrium is assumed to exist at all times, and stochastic fluctuations in population size caused by viability selection are ignored. Otherwise, the life cycle, the starting conditions, and the fitness function are identical to those described above. These assumptions enable us to use results from single-locus theory to predict the dynamics of mean multilocus fitness.

As noted by Lynch et al. (1993), when a population initially consists of mutation-free individuals, the process of mutation accumulation consists of three phases, provided the reproductive capacity of the population is reasonably large. During phase 1, mutations accumulate relatively rapidly due to the fact that the genetic variance in fitness, and consequently the response to selection, is low. Soon, however, the rate of mutation accumulation begins to slow as a stochastic balance arises between the forces of mutation, drift, and selection. This marks the beginning of phase 2, during which the number of mutations entering the population each generation is balanced by the number being fixed or lost. These conditions result in an approximately constant number of segregating loci in the population and in an approximately constant rate of fixation of mutant alleles. Despite the gradual decline in viability during the first two phases, the number of reproductive adults remains very close to  $K$ , until the stochastic nature of selection starts to cause occasional declines below  $K$  as  $\bar{W}_S$  begins to approach 1 (at which point the population can just replace itself).

When  $\bar{W}_S = 1$ , phase 3 is entered, and subsequent increases in the mutation load lead to cumulative reductions in population size. This, in turn, causes a progressive increase in the rate of mutation accumulation as genetic drift begins to overwhelm selection, until extinction occurs when no offspring survive to maturity. We refer to the phase 3 synergism between population decline, genetic drift, and mutation accumulation, and the resultant rapid decline to extinction, as a mutational meltdown (Lynch and Gabriel, 1990).

*Diffusion Theory*

The simplest mathematical route to approximating the mean time to extinction due to mutation accumulation involves diffusion approximations for the behavior of mutant alleles (Felsenstein 1974, 1988; Lande 1994). This approach has some limitations in that it assumes a constant population size, and it does not lend itself readily to the computation of the lengths of phases 1 and 3. However, because these two phases are usually only a few percent of the length of phase 2 (Lynch et al. 1993), a theory for the length of phase 2 has the potential to approximate the mean time to extinction closely. During this phase, the mean viability of individuals can be viewed as the product of two contributions—the first, due to segregating mutations, stochastically fluctuates around

a constant mean; whereas the second, due to fixations, increases continuously.

To obtain the expected segregational load during phase 2, we note that  $\mu K$  mutations enter the population each generation. Letting  $\bar{t}_a$  be the mean time to absorption (loss or fixation) of a mutant allele, the rate of conversion of polymorphic to monomorphic loci is  $1/\bar{t}_a$ . Hence, the equilibrium number of segregating loci is  $\mu K \bar{t}_a$ . During its sojourn through the population, a mutant allele causes a total (cumulative) load  $L$ . Dividing this by the time to absorption yields the average load per generation, and recalling the multiplicative fitness function, the expected segregational fitness is

$$\bar{W}_S = \left(1 - \frac{L}{\bar{t}_a}\right)^{\mu K \bar{t}_a}. \quad (1)$$

For the special case of additivity of mutant effects,

$$L = \frac{1}{K} + O\left(\frac{1}{sN_e^2}\right), \quad (2)$$

and

$$\begin{aligned} \bar{t}_a &= 2 + 2(1 + s)E_1(2s) + \frac{1}{2N_e s} + O\left(\frac{1}{s^2 N_e^2}\right) \\ &\approx 2[1 - \gamma + 2s(1 - \gamma) - \ln(2s)], \end{aligned} \quad (3)$$

for  $N_e s$  large and  $s$  small,

where  $E_1$  denotes the exponential integral (Abramowitz and Stegun 1972),  $\gamma = 0.577$  is Euler's constant,  $N_e$  is the effective population size (defined below), and  $O(x)$  denotes a residual term of order  $x$ . Derivations of these expressions are given in the Appendix. These results imply that  $\bar{t}_a$  tends to a constant, and  $\bar{W}_S \rightarrow e^{-\mu}$  as  $K \rightarrow \infty$ , in agreement with the predictions of infinite population theory (Haldane 1937; Crow 1970; Kondrashov and Crow 1988; Bürger and Hofbauer 1994). However, numerical evaluation of the exact expressions for  $L$  and  $\bar{t}_a$  indicates that this asymptotic approximation requires that  $Ks$  be on the order of 5 or greater (see also Kimura et al. 1963). Moreover, the approach to  $e^{-\mu}$  is not monotonic with  $K$ . The relationship of  $\bar{W}_S$  and  $K$  is u-shaped, with  $\bar{W}_S$  taking on a minimum value at an intermediate  $K$ .

With recessive mutations, computation of the expected segregational load requires the numerical integration of equations given in the Appendix. For complete recessives,  $\bar{W}_S \rightarrow e^{-\mu/2}$  as  $K \rightarrow \infty$ , also a well known result (Haldane 1937; Kimura et al. 1963; Crow 1970).

The loss in fitness caused by fixation of deleterious mutations can be obtained by noting that each new mutation eventually causes an average fitness reduction of  $(1 - 2su_F)$ , where  $u_F$  is the probability of fixation of a mutation that initially occurs as a single copy in a population of effective size  $N_e$  and absolute size  $K$ . For the special case of mutations with additive effects, we use

$$u_F = \frac{e^{2N_e s/[K(1-s)]} - 1}{e^{4N_e s/(1-s)} - 1}. \quad (4)$$

This expression differs slightly from the commonly used expression of Crow and Kimura (1970) in that they employ  $s$  rather than  $s/(1-s)$  in the exponents. Our modification yields

much more accurate estimates of  $u_F$  (Appendix). Because  $\mu K$  new mutations appear each generation, the expected viability reduction due to fixation each generation during phase 2 is

$$\bar{W}_F = (1 - 2su_F)^{\mu K} \quad (5)$$

To complete the above expressions, we require an estimate of the effective size of a population under selection. Robertson (1961) noted that  $N_e$  is depressed below  $K$  in the presence of selection, due to variance among parents in the number of successful gametes produced. He showed that

$$N_e \approx \frac{K + C_W^2}{1 + C_W^2}, \quad (6)$$

where  $C_W$  is the coefficient of variation of individual contributions to the gamete pool caused by segregating mutations. We obtain an approximation of  $C_W$  by assuming additivity of mutational effects within loci and free recombination. In that case, the number of segregating mutations will be approximately Poisson distributed among individuals, and provided  $K$  and  $R$  are large, the mean and variance in the segregational fitness can be shown to be approximately  $\bar{W}_S = e^{-\bar{n}s}$  and  $\sigma^2(W_S) = e^{-\bar{n}s(2-s)} - \bar{W}_S^2$ , where  $\bar{n}$  is the mean number of mutations (at segregating loci) per individual. Because only a finite number of individuals reproduce, the true variance in fitness among individuals is actually  $[1 - (1/K)]\sigma^2(W_S)$ , and sampling a finite number of gametes per adult inflates the realized variance by the factor  $[1 + (1/2R)]$ . Thus, the squared coefficient of variation is  $C_W^2 \approx \lambda(\bar{W}_S^{-s} - 1)$ , where  $\lambda = [1 - (1/K)][1 + (1/2R)]$ , and the estimate of  $\bar{W}_S$  is obtained by solving equation (1). For large  $K$  and  $R$ ,  $\bar{W}_S \approx e^{-\mu}$ , and  $N_e \approx Ke^{-\mu s}$ , which is not greatly different from  $K$  provided the rate of input of mutational damage  $\mu s \ll 1$ . This approximation also appears to work well with small  $R$  and  $K$  (data not shown), and we use it in all of the following diffusion approximations.

Assuming that the length of phase 1 is short relative to the fixation time, the mean fitness at the beginning of phase 2 is approximately  $\bar{W}_S$  (see Lynch et al. 1993, for justification of this assumption). The length of phase 2 can then be calculated with the relationship  $\bar{W}_S W_F^T R = 1$ , where the solution  $T_2$  is the time required for the average realized fecundity of selected adults to decline from  $\bar{W}_S R$  to one. Taking logarithms and rearranging,

$$T_2 = -\frac{\ln R + \ln \bar{W}_S}{\ln \bar{W}_F}. \quad (7)$$

This expression is similar in form to the equation for "the mean time to reach genetic inviability" derived by Lande (1994), except for our specification of the load caused by segregating mutations and of the influence of such mutations on  $N_e$ .

#### Transition-Probability Theory

Because the accuracy of the diffusion approximations for the probabilities of fixation and times to absorption for deleterious mutations is poorly understood, we evaluated the adequacy of the single-locus model by use of a more exact transition-probability approach. Following Ewens (1979, p. 19), for a population of  $K$  individuals, the transition proba-

bility from a state of having  $i$  mutant alleles at a locus in generation  $t - 1$  to a state of having  $j$  mutant alleles in generation  $t$  is

$$P_{ji} = \binom{2K}{j} \eta_i^j (1 - \eta_i)^{2K-j}, \quad (8)$$

where

$$\eta_i = \frac{(1 - 2s)i^2 + (1 - 2hs)(2K - i)i}{(1 - 2s)i^2 + 2(1 - 2hs)(2K - i)i + (2K - i)^2}. \quad (9)$$

There are  $2K + 1$  possible states of the population, ranging from complete loss ( $i = 0$ ) to complete fixation ( $i = 2K$ ) of the mutant allele. Letting  $\mathbf{P}$  be the  $(2K + 1) \times (2K + 1)$  matrix of transition probabilities, and  $\mathbf{x} = (0, 1, 0, 0, \dots, 0)'$  be the  $(2K + 1) \times 1$  column vector describing the initial distribution of a mutation (with certainty, it is initially in a single copy), then the probability distribution for the number of copies of the allele after  $n$  generations is given by the vector  $\mathbf{p}_n = \mathbf{P}^n \mathbf{x}$ . To account for the reduction in effective population size due to selection (above) and the consequent reduction in the effectiveness of selection, we use  $s_e = se^{-\mu s}$  in place of  $s$  in equation (9).

For a randomly mating population in which the frequency of the mutant allele is  $i/2K$ , the single-locus fitness is  $w(i) = 1 - (is/K)[2h + (1 - 2h)(i/2K)]$ . Thus, the expected single-locus fitness after  $n$  generations is

$$\bar{w}_n = \sum_{i=0}^{2N} p_n(i)w(i), \quad (10)$$

where  $p_n(i)$  is the  $i$ th element of  $\mathbf{p}_n$ . Initially,  $\bar{w}_0 = 1 - (hs/K)$ , and as  $n \rightarrow \infty$ ,  $\bar{w}_n$  increases monotonically and converges to  $(1 - 2su_F)$ . Because  $\mu K$  new mutations arise each generation, and these are assumed to influence fitness multiplicatively and to have independent dynamics, the expected fitness resulting from the cohort of mutations arising  $n$  generations in the past is  $\bar{w}_n^{\mu K}$ . The expected mean viability after  $t$  generations is the product of these cohort-specific fitnesses,

$$\bar{W}(t) = \prod_{n=0}^t \bar{w}_n^{\mu K}. \quad (11)$$

This expression includes the effects of both segregating and fixed mutations, and to the extent that single-locus theory can approximate the multilocus situation, it should apply throughout phases 1 and 2. Thus, we estimated the combined length of phases 1 and 2 ( $T_1 + T_2$ ) by iterating equation (11) on a computer until  $\bar{W}(t)$  was reduced to  $1/R$ .

To obtain an estimate of the length of phase 3 ( $T_3$ ), we assumed that the segregational load remains approximately constant during the period of population decline, such that all subsequent loss of fitness is due to additional fixation of mutant alleles. A numerical estimate of  $T_3$  was obtained by iterating the recursion

$$N_{t+1} = N_t \prod_{n=0}^t [1 - 2su_F(N_e, t)]^{\mu N_t}, \quad (12)$$

until  $N_{t+1} < 1$ , starting with  $N_0 = K$ , and defining  $u_F(N_e, t)$  each generation to account for the reduction in  $N_e$  (in accordance with eq. [6]). This expression assumes that the fix-

ation probability of a mutant allele is determined only by the size of the population in which it originates, which because  $N$  is declining, must be an underestimate. For this reason, and because equation (12) allows noninteger  $N$ , does not incorporate the substantial stochasticity in  $N$  that occurs during phase 3 and does not incorporate the expected increase in  $\bar{W}_S$  as  $N_e$  declines, it should overestimate  $T_3$ .

The diffusion approximation assumes that  $s$  is of order  $1/K$ , and in principle, when that condition is met, the results from the diffusion theory should converge on those of the transition-probability approach as  $K$  becomes large, and  $s$  becomes small. However, we cannot expect the agreement to be perfect, because the estimated time to extinction using the diffusion theory does not include phase 1. It should be noted also that neither analytical approach is expected to work very well if the fecundity is very low. Because  $e^{-\mu}$  (or lower) is the expected fitness due to segregating mutations in phase 2, if  $Re^{-\mu} < 1$ , the population size begins to decline before phase 2 is entered. This invalidates the computation of  $T_2$  by the diffusion theory and also violates the starting conditions assumed in the computation of  $T_3$ .

RESULTS

*Randomly Mating, Monoecious Populations.*—Most of the simulations that we have performed for this mating system assume a genomic mutation rate of 1, additivity of mutational effects within loci, and  $s = 0.025$ , conditions that closely approximate empirical observations in *Drosophila* (Crow and Simmons 1983; Houle et al. 1992). For these mutational properties, for a given population size, the mean time to extinction ( $\bar{t}_e$ ) increases with the logarithm of  $R$ , provided  $R > 10$  (fig. 1a). This observation is in good agreement with the scaling predicted by the diffusion theory, equation (7). As a first approximation, provided  $R \geq 10$ ,  $\bar{t}_e$  increases exponentially with the population size (fig. 1a), also in qualitative agreement with the scaling predicted by the diffusion theory. For populations with 64 or fewer individuals, the simulations predict extinction times that are less than a few thousand generations unless the fecundity is very high. These are clearly upper limits to the mean time to extinction, because no sources of demographic or environmental stochasticity imperil the populations in our simulations for monoecy.

For very low fecundities, the scaling of  $\bar{t}_e$  with  $K$  becomes much more gradual, and with  $R = 2$ ,  $\bar{t}_e$  is hardly influenced by  $K$  (fig. 1a). With a fecundity this low, there seems to be no way for even large populations to escape the meltdown

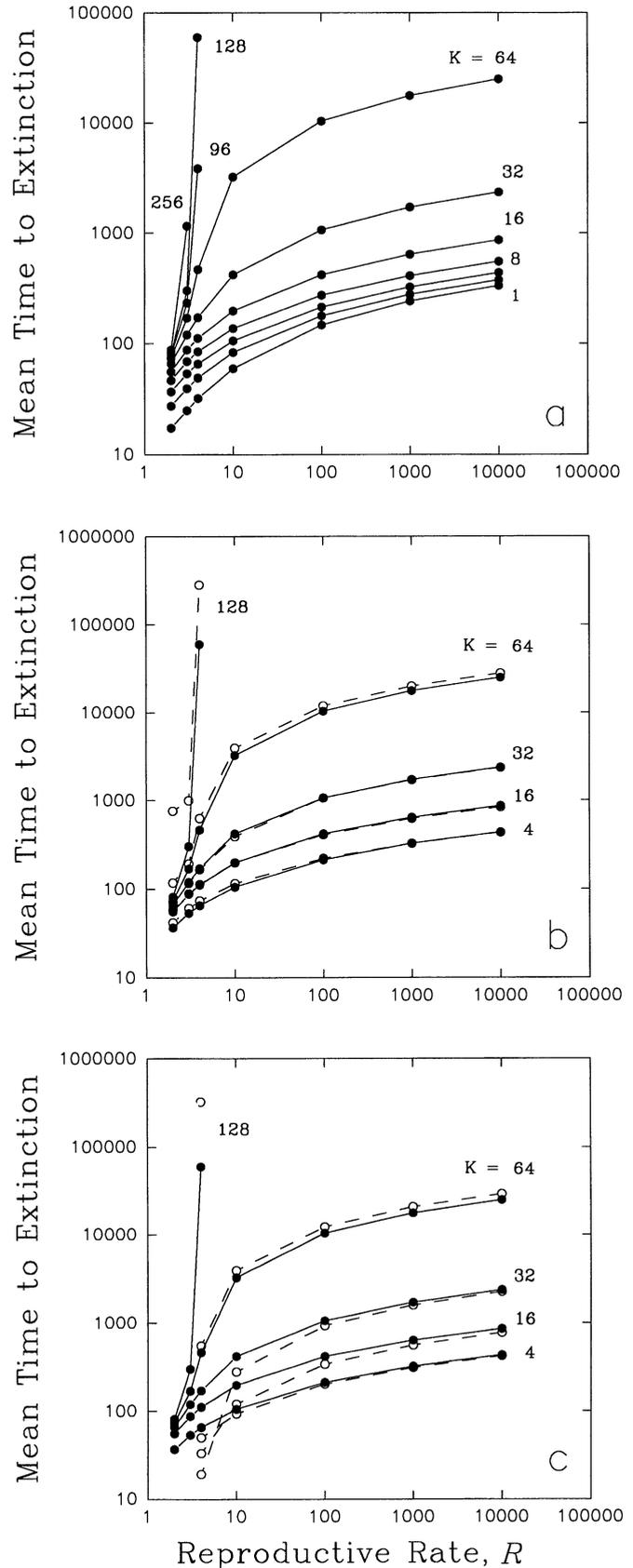


FIG. 1. a. The relationship between mean time to extinction  $\bar{t}_e$  (in generations), population carrying capacity  $K$ , and reproductive rate  $R$  for randomly mating, monoecious populations obtained by computer simulation. The genomic mutation rate is  $\mu = 1$ , the selection coefficient is  $s = 0.025$ , and the mutations are assumed to be additive in their effects within loci. The two unlabeled lines are for  $K = 2$  and 4. b. Dashed lines give the predictions from the single-locus transition-matrix approximation described in the text (the sum of predicted lengths of phases 1, 2, and 3). c. Dashed lines give the predictions from the diffusion approximation described in the text (the length of phase 2 only). Note that the diffusion approximations for  $R = 2$  and 3 are highly inaccurate and are not shown.

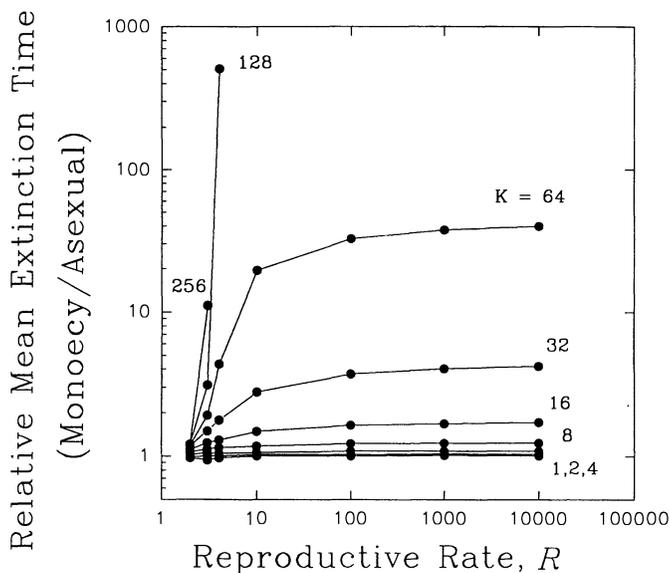


FIG. 2. The data in figure 1a divided by the mean times to extinction for obligately asexual populations with the same mutational properties.

for very long. The reason for this is that with large  $K$ , the segregational fitness stabilizes at approximately  $\bar{W}_S = e^{-\mu}$  (or lower), which is equal to 0.37 when  $\mu = 1$ . Thus, with  $R = 2$ , even with no fixations, a population cannot be expected to do better than a realized fecundity of  $\bar{W}_S R = 0.74$ . This is less than the replacement rate of one necessary to maintain a stable population size. With a slight increase in  $R$  to 3, the realized fecundity in the absence of fixations approaches 1.11 as  $K$  becomes large, and large populations begin to show an increased ability to resist the meltdown (fig. 1a).

The scaling of the mean time to extinction with the logarithm of  $R$  has been noted before for asexual populations (Lynch et al. 1993) and can be understood intuitively by noting that populations experience a steady fractional decline in fitness during the prolonged phase 2, whereas entry into the final (and rapid) phase 3 does not begin until mean viability has declined to  $1/R$ . However, contrary to the situation in sexual populations, the scaling of  $\bar{t}_e$  with  $K$  is less than linear in asexual populations (Lynch et al. 1993). For  $4Ks < 1$ , selection is ineffective at eliminating deleterious mutations under any breeding system, and both asexual and sexual populations have similar mean extinction times (fig. 2). However, when  $4Ks > 1$ , the dynamics of mutant alleles are strongly influenced by selection, and recombinational and segregational repair result in a very large increase in the longevity of sexual populations relative to asexual ones with the same mutational properties (fig. 2).

For high reproductive rates ( $R \geq 100$ ), the mean times to extinction predicted by the transition-probability approach (fig. 1b) and the diffusion approximation (fig. 1c) converge on similar values, and provided the population size is not very large ( $K \leq 64$ ), these predictions are close to those obtained in the simulations. However, when population sizes increase beyond this level, both analytical approaches begin to greatly overestimate the mean time to extinction. For lower

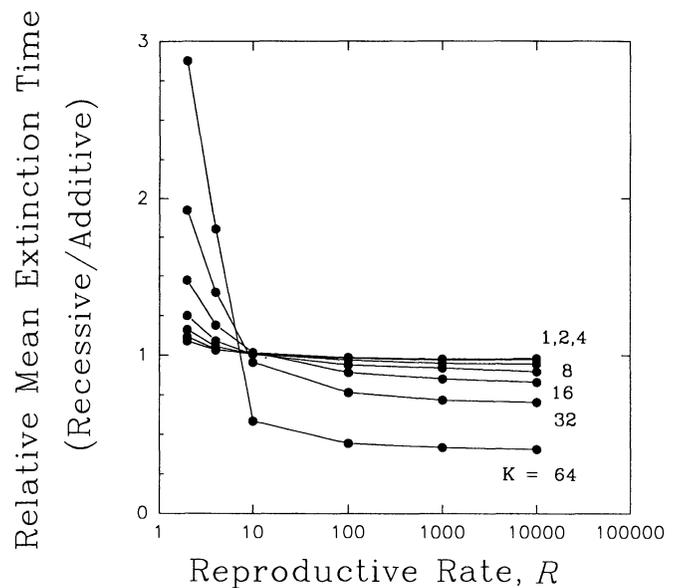


FIG. 3. Mean times to extinction in randomly mating, monoecious populations caused by recessive ( $h = 0$ ) mutations, for  $\mu = 1$  and  $s = 0.025$ . The data are divided by those presented in figure 1a, for mutations with additive effects, to give the relative increase in the mean time to extinction caused by recessivity of mutational effects.

reproductive rates, only the transition-probability approach yields reasonable results.

Recessivity of mutational effects does not have a large influence on the results outlined above. For large populations with high fecundity, most of the risk of extinction is posed by fixation of mutations rather than by the segregational variance, and consequently, recessivity of mutations reduces the longevity of populations, due to the higher fixation probability of recessive mutations (fig. 3). This effect is diminished at small  $K$  where both additive and recessive mutations behave as though they are effectively neutral. The effect of nonadditivity is reversed at lower reproductive rates, where extinction times are low. Under these conditions, a larger fraction of the mutations are still segregating at the time of extinction, and the segregation load is lower with recessive mutations.

Table 1 shows the relationship between the mean extinction time, mutation rate, and selection coefficient. The mean time to extinction declines nearly exponentially with increasing genomic mutation rate when  $s$  is small and much more rapidly with high  $s$ . The relationship between  $\bar{t}_e$  and  $s$  is u-shaped, that is, there is an intermediate value of  $s$  that minimizes the extinction time. This critical value of  $s$  increases with increasing mutation rates.

*Obligate Self-Fertilization.*—Using single-locus diffusion theory, Caballero and Hill (1992) and Charlesworth (1992) recently produced results that suggest that the probability of fixation of a deleterious mutation in an obligately selfing population is independent of the degree of dominance and identical to that expected for a mutation with additive effects in a random-mating population of the same absolute size. Using their results, for mutations with additive effects, the theory developed above would predict that the mean extinc-

TABLE 1. Mean extinction times for randomly mating, monoecious populations as a function of the genomic mutation rate  $\mu$  and the selection coefficient  $s$ . The mutations are assumed to have additive effects ( $h = 0.5$ ), and the population carrying capacity and reproductive rate are  $K = 64$  and  $R = 10$ , respectively. For each set of parameter values ( $\mu, s$ ), the mean time to extinction is given above its coefficient of variation (in parentheses). At least 512 simulations were performed for each set of parameter values.

$\mu$	Selection coefficient, $s$					
	0.00312	0.00625	0.0125	0.0250	0.0500	0.1000
0.250	4479 (0.05)	3488 (0.08)	4860 (0.11)	30,399 (0.17)	—	—
0.500	2252 (0.05)	1690 (0.08)	2148 (0.11)	11,936 (0.18)	—	—
1.000	1106 (0.05)	785 (0.08)	818 (0.13)	3198 (0.22)	—	—
2.000	535 (0.05)	335 (0.07)	232 (0.11)	182 (0.22)	234 (0.55)	611 (0.96)
4.000	255 (0.05)	143 (0.06)	81 (0.06)	46 (0.08)	26 (0.09)	16 (0.11)

tion time would be the same for both breeding systems. However, this is clearly not the case. For  $4Ks < 1$ , both breeding systems yield equivalent mean extinction times, as expected under effective neutrality (fig. 4a,b). For  $4Ks > 1$ , except for very low fecundity organisms, there is a dramatic reduction in the mean extinction times of obligately selfing populations relative to those for randomly mating monoecious populations. As in the case of obligate asexuality, the mean extinction time of obligate selfers increases more slowly than linearly with the logarithm of population carrying capacity.

The increased risk of extinction under obligate selfing is a consequence of a rate of fixation of mutant alleles that is much higher than in random mating populations (fig. 5). This enhancement of the fixation rate is caused by an extreme form of the Hill-Robertson effect—the high amount of repulsion linkage disequilibrium, and the consequent low amount of variance in mutation load among lineages, that develops in populations of obligate selfers. Thus, for self-fertilizing populations, single-locus diffusion theory yields grossly inaccurate predictions for the fixation probabilities of deleterious genes segregating on a polygenic background under selection. Charlesworth et al. (1993) have shown this to be true for partially as well as for obligately self-fertilizing populations.

Although we have been unable to develop an analytical theory for the mean time to extinction under obligate selfing, a simple analogy with clonal populations provides substantial insight. When a new mutation arises in a selfing individual, within a very few generations all descendants of that individual are homozygous. With weak selection on the mutant allele, the rate of segregation is much greater than the rate at which selection changes the allele frequency. Thus, in obligate selfers, segregation acts as a repair mechanism that effectively reduces the mutation rate by one-half. However, because the mutant allele rapidly becomes restricted to the homozygous state, obligate selfing effectively doubles the intensity of selection associated with the mutant allele. These arguments suggest that with respect to the decline in fitness due to mutation accumulation, obligately selfing populations with mutation rate  $\mu$  and selection coefficient  $s$  should be

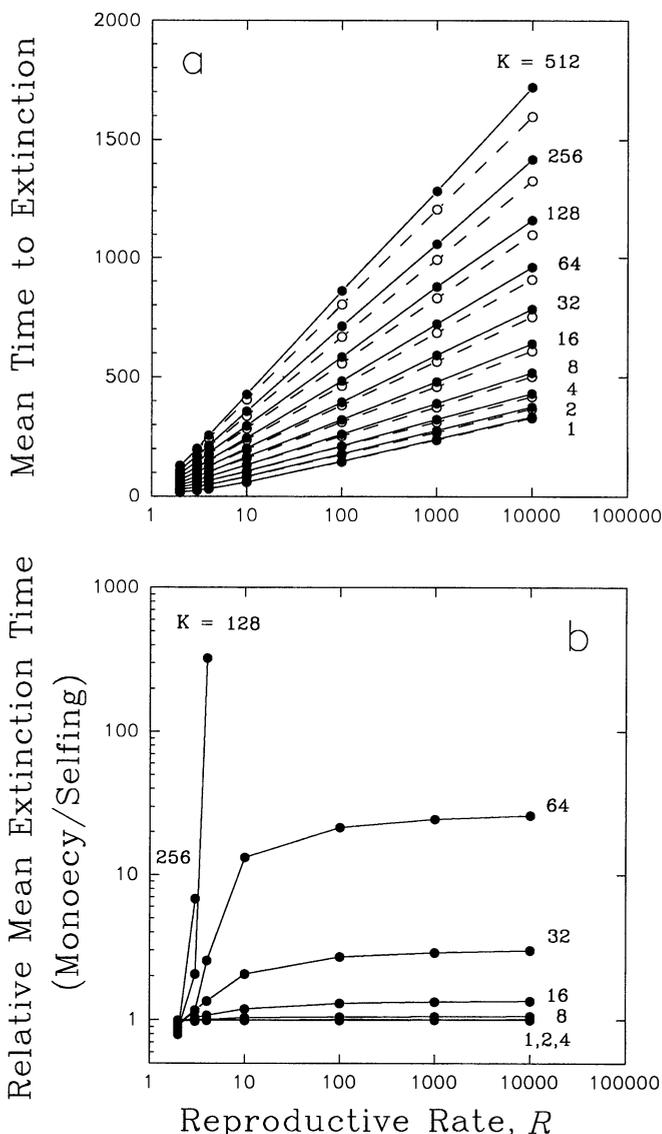


FIG. 4. a. The relationship between mean time to extinction  $\bar{t}_e$  (in generations), population carrying capacity  $K$ , and reproductive rate  $R$  for obligately self-fertilizing populations. The genomic mutation rate is  $\mu = 1$ , and the selection coefficient is  $s = 0.025$ . The mutations are assumed to be additive in their effects within loci (solid lines) or completely recessive (dashed lines). b. The data in figure 1a are divided by the data in figure 4a for mutations with additive effects, to give proportional increase in mean times to extinction caused by random mating.

roughly equivalent to clonal populations with mutation rate  $\mu/2$  and selection coefficient  $2s$ .

The results in table 2 show that this supposition holds up remarkably well for a broad range of  $\mu$  and  $s$ . Except when selection is very strong (stronger than is realistic for average spontaneous mutations), the mean and variance of extinction time is essentially the same for an obligately selfing population and a clonal population with half the mutation rate but twice the mutational effect. Although we have not explored it extensively, this logic should also apply to mutations with nonadditive effects within loci, except that the equivalence

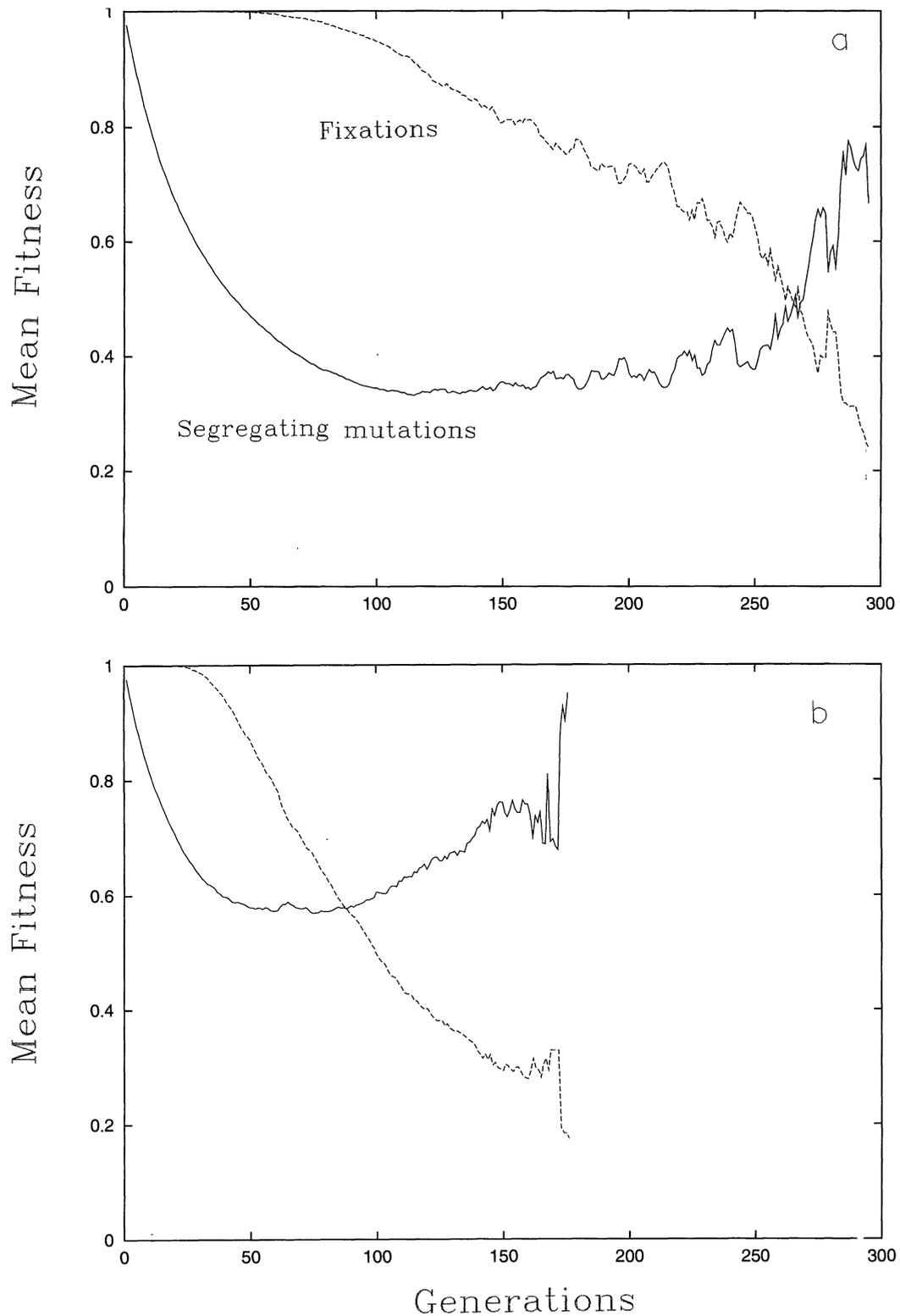


FIG. 5. a. The decline in fitness due to segregating (solid line) and fixed (dashed line) mutations,  $\bar{W}_S$  and  $\bar{W}_F$ , respectively, for randomly mating, monoecious populations with carrying capacity  $K = 64$  and reproductive rate  $R = 10$ . Results are averaged over 256 replicate simulations, with genomic mutation rate  $\mu = 1$  and selection coefficient  $s = 0.025$ , assuming additivity of mutational effects within loci. Mean population fitness is approximately equal to the product  $\bar{W}_S \bar{W}_F$ . The data become more noisy as time progresses because, due to extinctions, fewer populations contribute to the mean fitness estimates. b. Data for obligately self-fertilizing populations with the same population and mutation parameters.

TABLE 2. Comparison of mean extinction times for clonal populations with those for obligately selfing populations, with the latter having twice the mutation rate  $\mu$  but half the selection coefficient  $s$  of the former. The mutations are assumed to have additive effects,  $h = 0.5$ , and the population carrying capacity and reproductive rate are  $K = 64$ , and  $R = 10$ , respectively. For each set of parameter values ( $\mu, s$ ), the mean time to extinction is given above its coefficient of variation (in parentheses) for the clonal populations. The values for the obligately self-fertilizing populations are divided by the appropriate clonal values (in the matching cell in the top of the table), such that a value of 1.00 implies identity of the corresponding values. For each set of parameter values, at least 512 simulations were performed.

$\mu$	Selection coefficient, $s$				
	0.0125	0.0250	0.0500	0.1000	0.2000
<b>Obligately asexual populations</b>					
0.125	2570 (0.06)	1810 (0.08)	1686 (0.12)	3351 (0.18)	805,960 (0.35)
0.250	1214 (0.06)	796 (0.08)	618 (0.12)	719 (0.16)	7511 (0.34)
0.500	576 (0.06)	361 (0.08)	251 (0.11)	214 (0.15)	408 (0.28)
1.000	280 (0.06)	168 (0.07)	108 (0.10)	79 (0.13)	78 (0.22)
2.000	139 (0.05)	81 (0.06)	50 (0.09)	33 (0.11)	25 (0.18)
<b>Obligately selfing populations</b>					
0.250	1.00 (0.97)	1.01 (1.01)	1.00 (1.02)	1.00 (1.02)	0.87 (0.98)
0.500	1.00 (0.98)	1.01 (1.08)	0.99 (0.95)	1.00 (1.09)	0.88 (0.93)
1.000	0.99 (1.00)	0.99 (0.99)	0.99 (1.00)	0.97 (1.05)	0.92 (0.99)
2.000	0.99 (0.96)	0.99 (1.03)	0.98 (0.92)	0.94 (1.06)	0.90 (0.97)
4.000	0.98 (0.92)	0.99 (1.03)	0.96 (0.92)	0.93 (0.97)	0.83 (1.00)

would be obtained by using a selection coefficient of  $s$  in selfers and  $s/h$  in asexuals. For mutations with additive effects,  $h = 0.5$ , this yields the result described above. For completely recessive mutations,  $h = 0$ , and no amount of mutation can drive obligately asexual populations to extinction. For obligately selfing populations, the mean time to extinction is not greatly influenced by nonadditivity of mutational effects because any mutations that survive in the population for more than a few generations are almost always in the homozygous state (fig. 4a).

**Monogamy, Separate Sexes.**—For populations with separate sexes, the mean time to extinction is approximately  $2^{K-1}$  generations in the ideal situation in which there is no selection, no environmental stochasticity, and the only source of demographic stochasticity is random fluctuation in the sex ratio (Gabriel and Bürger 1992). For populations with  $K = 4, 8,$  and  $16$  individuals, this expression predicts mean extinction times of 8, 128, and 32,768 generations, respectively; thus, operating as a single factor, sex-ratio fluctuation jeopardizes only the survival of very small dioecious populations. However, by causing the typical number of reproductive pairs in a population to be less than  $K/2$ , random variation in the sex ratio has synergistic effects on the fixation probabilities of mutant alleles that can greatly enhance the vulnerability of a population to a mutational meltdown. This effect is quite

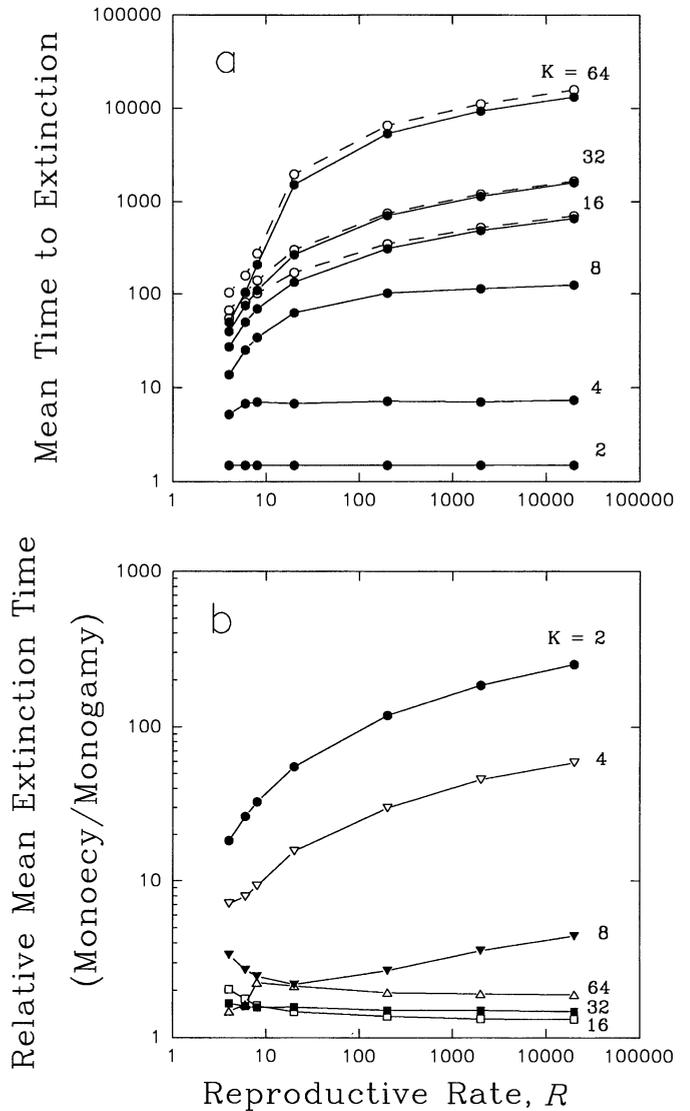


FIG. 6. a. The relationship between mean time to extinction  $\bar{t}_e$  (in generations), population carrying capacity  $K$ , and reproductive rate  $R$  for monogamous populations with separate sexes. The genomic mutation rate is  $\mu = 1$ , the selection coefficient is  $s = 0.025$ , and the mutations are assumed to be additive in their effects within loci. Solid lines give simulation results; dashed lines are results from the transition-probability approach. b. The data in figure 1A are divided by the data in figure 6A, to give proportional increase in mean time to extinction caused by monoecy. Note that in this figure, the values of  $R$  for the monogamous populations are double those for the monoecious populations, such that for the same number of reproductive adults, both types of breeding systems have the same total offspring production.

pronounced in populations with small  $K$  and small  $R$ , because these are the conditions that insure the greatest deviations of the sex ratio from 1:1, and even with large  $K$  and  $R$ , the mean time to extinction under monogamy is approximately half that expected for a randomly mating, monoecious population (fig. 6a,b). These results are quite conservative, because our simulations assume that each monogamous female produces the same expected number of offspring as two monoecious individuals.

We obtain the expected effective population size under monogamy by letting  $p_i$  be the probability that  $i$  of  $K$  surviving adults are female. Then, because the number of breeding adults ( $N_a$ ) is twice the number of the rarest sex,

$$\bar{N}_a = 2[0 \cdot p_0 + 1 \cdot p_1 + \dots + \frac{K}{2} \cdot p_{K/2} + \left(\frac{K}{2} - 1\right) \cdot p_{(K/2)-1} + \dots + 0 \cdot p_K]. \quad (13A)$$

Because  $N_a$  is binomially distributed, it can be approximated reasonably well by treating the number of females as a normally distributed variable with mean  $K/2$  and variance  $K/4$ , which leads to

$$\bar{N}_a \approx K[1 - \sqrt{2/(\pi K)}]. \quad (13B)$$

Because the sex ratio fluctuates from generation to generation, the long-term effective population size is described more accurately by the harmonic mean, which using the normal approximation, can be shown to be

$$N_H \approx \bar{N}_a(1 - C_N^2), \quad (14A)$$

where  $C_N^2$  is the squared coefficient of variation of  $N_a$ ,

$$C_N^2 = \frac{K[1 - (2/\pi)]}{\bar{N}_a^2}. \quad (14B)$$

To account for the reduction in the effectiveness of selection caused by both sex-ratio fluctuations and variance in fitness, we substituted the effective selection coefficient  $s_e = s(N_H/K)e^{-\mu s}$  for  $s$  in the transition probability given by equation (9). The predicted times to extinction, given as dashed lines in figure 6A, are in reasonable accord with the simulation results provided  $8 < K < 64$ .

#### DISCUSSION

Although several ecological and genetical assumptions underlying our models might appear to be somewhat unrealistic, violations of most of them are likely to yield substantially lower times to extinction. For example, because our monoecious, obligately selfing, and obligately asexual populations experienced no form of intrinsic demographic or environmental stochasticity and were assumed to be mutation-free initially, they could not become extinct in the absence of mutation accumulation. The monogamous populations that we simulated did have a minor form of demographic stochasticity, random fluctuation in the sex ratio, which can cause extinction in very small populations (Gabriel and Bürger 1992), but as in all of our simulations,  $K$  and  $R$  were assumed to be temporally stable. The form of density dependence that we applied to all breeding systems is extremely generous, allowing populations to return to the carrying capacity each generation until the mean viability has declined below the potential replacement rate of individuals ( $1/R$ ).

Even in the absence of genetic factors, demographic and environmental forms of stochasticity that influence  $K$  and/or  $R$  pose a significant risk of extinction for populations of several hundreds or fewer individuals (Leigh 1981; Goodman 1987; Lande 1988, 1993). However, when operating on a background of recurrent deleterious mutation, environmen-

TABLE 3. Mean extinction times and their coefficients of variation (in parentheses) for randomly mating, monoecious populations as a function of temporal variation in the carrying capacity  $K$ . For each set of simulations, the carrying capacity was assumed to be normally distributed with an expected mean of 64 individuals and standard deviation  $\sigma_K$ . In all cases, the reproductive rate  $R = 10$ , the genomic mutation rate  $\mu = 1$ , the selection coefficient  $s = 0.025$ , and the mutations are assumed to have additive effects. A new carrying capacity was drawn each generation, and in the rare event that the drawn number was less than 1, it was assumed that  $K = 1$ . For each set of parameter values, 512 simulations were performed.

$\sigma_K$	$\bar{t}_e$	CV ( $t_e$ )
0	3198	0.22
2	3200	0.22
4	3163	0.22
8	2858	0.23
16	1845	0.25

tally and/or demographically induced fluctuations in population size can also have strong synergistic effects on the process of mutation accumulation, substantially magnifying the vulnerability to extinction (Gabriel et al. 1991; Gabriel and Bürger 1994). Because the fixation and survival probabilities for deleterious mutations increase approximately exponentially with decreasing population size, temporary bottlenecks in population size can result in significant increases in the mutation load. When such periods last long enough for fixations to occur, populations suffer from an irreversible decline in fitness, making them further vulnerable to future stochastic events and eventually to entry into the mutational meltdown phase. Thus, it seems certain that stochastic variation in  $K$  and/or  $R$  driven by nongenetic effects will reduce the mean time to extinction induced by mutational degradation. This contention is strongly supported by the substantial reduction in extinction times for monogamous dioecious populations relative to random-mating monoecious populations (fig. 6), due to demographic stochasticity and the resultant reduction in  $N_e$ . It is also supported by limited simulations we have performed to evaluate the consequences of randomly fluctuating  $K$  (table 3).

Our assumption that all segregating mutations are freely recombining may not be too unreasonable for small populations of organisms with a large number of chromosomes. However, as Charlesworth et al. (1993) have shown with extensive simulations, restricted recombination enhances the rate of fitness decline, by increasing both the fixation rate and the segregational load. Thus, our assumption of free recombination also must lead to an overestimation of the mean time to extinction. Our results for obligate asexuals show the dramatic decline in extinction time that occurs in the complete absence of recombination and segregation. Recombinational repair plays only a small role in prolonging the longevity of obligately selfing lineages since mutant alleles rapidly go to homozygosity in this case (Charlesworth et al. 1993). Thus, the increase in population viability for obligate selfers relative to obligate asexuals is due almost entirely to segregational repair.

Our results appear to provide compelling evidence that obligate self-fertilization is inviable as a long-term reproductive strategy, at least insofar as mutation accumulation,

relative to other purely ecological forces, is an important risk of extinction of breeding system variants. This conclusion is contrary to Charlesworth's (1992) argument that self-fertilization has only a minor effect on the rate of fixation of deleterious mutations, although it is consistent with subsequent simulation results of Charlesworth et al. (1993). Using infinite population theory, Lande and Schemske (1985) have argued that predominant selfing and predominant outcrossing are alternative stable states of the mating system in plants. We did not examine the consequences of partial selfing. However, the results of Charlesworth et al. (1993) suggest that roughly 10% outcrossing can cause a substantial reduction in the rate of fixation of deleterious alleles; thus, a small amount of outcrossing may be sufficient to reduce the likelihood of a mutational meltdown to a negligible level. A test of these ideas will ultimately require empirical data on the phylogenetic distribution of breeding systems. For example, comparative molecular data indicate that most parthenogenetic animals are recent derivatives of their sexual ancestors and hence are in good accord with the prediction that mutation accumulation causes the extinction of most obligately asexual lineages within a few thousand generations (Lynch and Gabriel 1990; Lynch et al. 1993). Similar information on the molecular distance between highly selfing species and their sexual ancestors is necessary to test the hypothesis that a high degree of self-fertilization is ultimately an evolutionary dead end.

Our simulation results have revealed that, except in a limited range of the parameter space, analytical approaches based on the expected dynamics of single-locus systems provide inaccurate estimates of the mean time to extinction. Nevertheless, the theory does approximate the correct scaling of  $\bar{t}_e$  with  $K$  and  $R$ , and for certain situations in which  $\bar{t}_e$  is fewer than 1000 or so generations (in particular, monoecious populations, with  $R > 10$  and  $K \leq 64$ ), the theory makes predictions that are quantitatively informative. Thus, single-locus theory may provide a useful guide for understanding situations in which the risk to extinction from mutation accumulation is substantial on time scales of 1000 or fewer generations, as in conservation biology. That the theory performs most poorly in large populations, in which large numbers of loci are expected to be segregating, suggests that interference to fixation posed by a polygenic background (the Hill-Robertson effect), rather than stochasticity associated with small population size, is responsible for the overestimation of the mean extinction times.

Our earlier work on the mutational meltdown in asexual populations indicated that, despite the stochasticity of the mutation-accumulation process, the coefficients of variation (CV) of extinction time due to mutation accumulation are quite low, typically less than 0.1 (Lynch and Gabriel 1990; Gabriel et al. 1993; Lynch et al. 1993). The results given above indicate that this is also true for obligate selfers (table 2). Under monoecy, the CV of extinction time tends to be higher, more on the order of 0.1 to 0.2, decreasing with increasing fecundity (table 4). Under monogamy, it can be substantially higher, approaching 1.0 (data not shown). Lande (1994) predicted the CV for monoecious populations to be approximately  $(2s/\ln R)$ . This does not quite agree with our simulation results, which show a dependence of the CV on

TABLE 4. Coefficients of variation (CV) of extinction times for randomly mating, monoecious populations as a function of the reproductive rate  $R$  and the population carrying capacity  $K$ . The genomic mutation rate is  $\mu = 1.0$ , the selection coefficient is  $s = 0.025$ , and mutations are assumed to have additive effects ( $h = 0.5$ ). For each set of parameter values, at least 512 simulations were performed.

$K$	Reproductive rate, $R$					
	2	4	10	100	1000	10,000
1	0.34	0.31	0.25	0.14	0.11	0.09
2	0.33	0.25	0.20	0.13	0.10	0.08
4	0.26	0.21	0.17	0.12	0.09	0.08
8	0.21	0.19	0.15	0.10	0.08	0.07
16	0.19	0.18	0.15	0.11	0.09	0.08
32	0.17	0.22	0.18	0.12	0.10	0.08
64	0.16	0.42	0.23	0.12	0.10	0.09
128	0.14	0.63	—	—	—	—

$K$ , and in any event, exact agreement should not be expected since Lande (1994) really considered only the CV of the length of phase 2. Nevertheless, Lande's result does give a reasonable first-order approximation for situations in which the mean extinction time is fewer than 1000 generations, predicting CV = 0.147, 0.104, 0.085, and 0.074 for  $s = 0.025$ , and  $R = 10, 100, 1000$ , and 10,000 respectively (compare results in table 4).

It is now well established that mutations with small, but intermediate, deleterious effects cause the most cumulative damage to populations (Kimura et al. 1963; Gabriel et al. 1993; Charlesworth et al. 1993). Mutations with very large effects are eliminated efficiently by selection and have essentially no chance of fixation, whereas neutral mutations have no influence on individual fitness. The diffusion theory developed by Lande (1994) suggests that the value of  $s$  that minimizes the time to extinction, that is, that does the most damage, is  $s^* \approx 0.4/N_e$ . This inverse scaling with the effective population size arises because, although the damage per fixed mutation (a linear function of  $s$ ) is independent of population size, the probability of fixation declines with increasing  $N_e$ . Although this qualitative scaling of  $s^*$  with  $N_e$  appears to be generally valid, the prediction that  $s^*$  is quantitatively equivalent to  $0.4/N_e$  does not hold up well, at least not in situations in which the diffusion approximation yields poor predictions of the mean extinction time. Again, part of the problem may be that Lande (1994) confined his attention to the rate of fitness decline in phase 2. For both asexual and selfing populations (table 2), as well as for monoecious populations (table 1), the true value of  $s$  that minimizes the mean extinction time increases with increasing mutation rate. The reason for this seems to be that high mutation rates, through the production of substantial background variation, reduce the efficiency by which selection can eliminate specific deleterious mutations (Gabriel et al. 1993).

Because we have restricted our attention to the situation in which mutations have constant individual effects, we are unable to shed much additional light on the consequences of variable selection coefficients, which is still an area of debate. Noting the u-shaped relationship between mean extinction time and  $s$ , we argued and supported with simulations using an approximately normal distribution of  $s$ , that variation in

the spectrum of mutational effects increases the mean time to extinction (Lynch and Gabriel 1990; Lynch et al. 1993). However, when the distribution of mutational effects is more skewed, depending on the mean effect, the mean extinction time can be decreased by the variance of effects (Lande 1994). There is some indirect empirical support that deleterious mutations with small effects do, in fact, have an extremely skewed distribution that may be approximated crudely with a negative exponential (Gregory 1965; Edwards et al. 1987; Mackay et al. 1992; Santiago et al. 1992). Extrapolating from diffusion theory, Lande (1994) showed that in this case, for populations with  $K > 50$ , the mean extinction time can be reduced by several orders of magnitude relative to the expectation with constant  $s$ . This effect occurs because an exponential distribution places a large fraction of mutations in the range of effective neutrality, thereby enhancing their rate of fixation. Unfortunately, the range of population sizes for which the effect is most pronounced is precisely the range in which the diffusion theory performs most poorly; thus, we cannot yet be certain of the general validity of Lande's result. However, because a continuous distribution of mutational effects is certainly more realistic biologically than the assumption of a fixed effect, the existing theoretical results clearly highlight the need for empirical estimates of the form of this distribution.

Indeed, one of the main messages of our analyses is the importance of further empirical information on the genomic deleterious mutation rate as well as on the distribution of  $s$ . Credible arguments have been made that  $\mu$  may actually be greater than 1 (Kondrashov 1988), in which case the mean extinction times that we have reported may be vast overestimates of the true situation (table 1). However, the mutations we have modeled were assumed to be unconditionally deleterious. If most deleterious mutations are only conditionally so, because of genotype  $\times$  environment interaction in fitness or because compensatory mutations are more common than generally believed, our estimates of  $\bar{t}_e$  could be too low. All of the mutation-accumulation results with *Drosophila* are consistent with the idea that the vast majority of mutations are deleterious. However, in a large sexual population, a low frequency of beneficial, compensatory, or back mutation may be adequate to prevent the eventual entry into a mutational meltdown.

Roughly speaking, immunity to the meltdown requires a large enough population size that during the period in which we predict a high probability of extinction (due purely to unconditionally deleterious mutations), there are enough opportunities (individuals  $\times$  generations) with effective enough selection to correct the damage. Certainly, for situations in which our theory predicts mean extinction times on the order of  $10^5$  or more generations, the possibility of such rescue seems quite high, and our quantitative results should not be taken seriously. However, given that our predicted extinction times are almost certainly overestimates, perhaps by an order of magnitude or more, it seems safe to assume that mutational degradation is a significant risk of extinction for situations in which we predict  $\bar{t}_e < 10^4$ . Given that the effective sizes of populations are typically severalfold smaller than their actual sizes, it appears that sexual populations as large as  $10^3$  individuals and asexual and obligately selfing populations

perhaps as large as  $10^5$  individuals are potential victims of the mutational meltdown.

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#### LITERATURE CITED

- Abramowitz, M., and I. A. Stegun, eds. 1972. Handbook of mathematical functions. Dovers-Publs., Inc. New York.
- Bell, G. 1988a. Sex and death in the protozoa. Cambridge University Press, New York.
- . 1988b. Recombination and the immortality of the germ line. *Journal of Evolutionary Biology* 1:67–82.
- Birky, C. W., Jr., and J. B. Walsh. 1988. Effects of linkage on rates of molecular evolution. *Proceedings of the National Academy of Sciences, USA* 85:6414–6418.
- Burger, R., and W. J. Ewens. 1995. Fixation probabilities of additive alleles in diploid populations. *Journal of Mathematical Biology* 33:557–575.
- Bürger, R., and J. Hofbauer. 1994. Mutation load and quantitative genetic traits. *Journal of Mathematical Biology* 32:193–218.
- Caballero, A., and W. G. Hill. 1992. Effects of partial inbreeding on fixation rates and variation of mutant genes. *Genetics* 131:493–507.
- Charlesworth, B. 1990. Mutation-selection balance and the evolutionary advantage of sex and recombination. *Genetical Research* 55:199–221.
- . 1992. Evolutionary rates in partially self-fertilizing species. *American Naturalist* 140:126–148.
- Charlesworth, B., D. Charlesworth, and M. T. Morgan. 1990. Genetic loads and estimates of mutation rates in highly inbred plant populations. *Nature* 347:380–382.
- Charlesworth, D., M. T. Morgan, and B. Charlesworth. 1992. The effect of linkage and population size on inbreeding depression due to mutational load. 59:49–61.
- Charlesworth, D., M. T. Morgan, and B. Charlesworth. 1993. Mutation accumulation in finite outbreeding and inbreeding populations. *Genetical Research* 61:39–56.
- Clarke, B. 1973. The effect of mutation on population size. *Nature* 242:196–197.
- Crow, J. F. 1970. Genetic loads and the cost of natural selection. Pp. 128–177 in K.-I. Kojima, ed. *Mathematical models in population genetics*. Springer, Berlin.
- Crow, J. F., and M. Kimura. 1970. An introduction to population genetics theory. Harper and Row, New York.
- . 1979. Efficiency of truncation selection. *Proceedings of the National Academy of Sciences, USA* 76:396–399.
- Crow, J. F., and M. J. Simmons. 1983. The mutation load in *Drosophila*. Pp. 1–35. in M. Ashburner, et al., eds. *The genetics and biology of Drosophila*, Vol. 3c. Academic Press, New York.
- Edwards, M. D., C. W. Stuber, and J. F. Wendel. 1987. Molecular-marker-facilitated investigations of quantitative-trait loci in

- maize. I. Numbers, genomic distribution, and types of gene action. *Genetics* 116:113–125.
- Ewens, W. J. 1972. Concepts of substitutional load in finite populations. *Theoretical Population Biology* 3:153–161.
- . 1979. *Mathematical population genetics*. Springer, New York.
- Felsenstein, J. 1974. The evolutionary advantage of recombination. *Genetics* 78:737–756.
- . 1988. Sex and the evolution of recombination. Pp. 74–86 in R. E. Michod and B. R. Levin, ed. *The evolution of sex*. Sinauer, Sunderland, MSS.
- Fisher, R. A. 1930. *The genetical theory of natural selection*. Clarendon, Oxford, U.K.
- Gabriel, W., and R. Bürger. 1992. Survival of small populations under demographic stochasticity. *Theoretical Population Biology* 41:44–71.
- . 1994. Extinction risk by mutational meltdown: synergistic effects between population regulation and genetic drift. Pp. 69–84 in V. Loeschke et al., eds. *Conservation genetics*. Birkhäuser, Basel, Switzerland.
- Gabriel, W., R. Bürger, and M. Lynch. 1991. Population extinction by mutational load and demographic stochasticity. Pp. 49–59 in A. Seitz and V. Loeschke, eds. *Species conservation: a population-biological approach*. Birkhäuser, Basel.
- Gabriel, W., M. Lynch, and R. Bürger. 1993. Muller's ratchet and mutational meltdowns. *Evolution* 47:1744–1757.
- Goodman, D. 1987. Consideration of stochastic demography in the design and management of biological reserves. *Natural Resources Modelling* 1:205–234.
- Gregory, W. C. 1965. Mutation frequency, magnitude of change, and the probability of improvement in adaptation. *Radiation Botany* 5 (Suppl.): 429–441.
- Haigh, J. 1978. The accumulation of deleterious genes in a population. *Theoretical Population Biology* 14:251–267.
- Haldane, J. B. S. 1937. The effect of variation on fitness. *American Naturalist* 71:337–349.
- Higgs, P. G. 1994. Error thresholds and stationary mutant distributions in multi-locus diploid genetics models. *Genetical Research* 63:63–78.
- Hill, W. G., and A. Robertson. 1966. The effect of linkage on limits to artificial selection. *Genetical Research* 8:269–294.
- Houle, D. 1989. The maintenance of polygenic variation in finite populations. *Evolution* 43:1767–1780.
- Houle, D., D. K. Hoffmaster, S. Assimakopoulos, and B. Charlesworth. 1992. The genomic mutation rate for fitness in *Drosophila*. *Nature* 359:58–60.
- Kimura, M. 1962. On the probability of fixation of mutant genes in a population. *Genetics* 47:713–719.
- Kimura, M., and T. Maruyama. 1966. The mutational load with epistatic gene interactions in fitness. *Genetics* 54:1337–1351.
- Kimura, M., T. Maruyama, and J. F. Crow. 1963. The mutation load in small populations. *Genetics* 48:1303–1312.
- Kimura, M., and T. Ohta. 1969. The average number of generations until fixation of a mutant gene in a finite population. *Genetics* 61:763–771.
- Kondrashov, A. S. 1988. Deleterious mutations and the evolution of sexual reproduction. *Nature* 334:435–440.
- Kondrashov, A. S., and J. F. Crow. 1988. King's formula for the mutation load with epistasis. *Genetics* 120:853–856.
- Lande, R. 1988. Genetics and demography in biological conservation. *Science* 241:1455–1460.
- . 1993. Risks of population extinction from demographic and environmental stochasticity, and random catastrophes. *American Naturalist* 142:911–927.
- . 1994. Risk of population extinction from new deleterious mutations. *Evolution* 48:1460–1469.
- Lande, R., and D. W. Schemske. 1985. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution* 39:24–40.
- Leigh, E. G., Jr. 1981. The average lifetime of a population in a varying environment. *Theoretical Population Biology* 90:213–239.
- Lynch, M., R. Bürger, D. Butcher, and W. Gabriel. 1993. The mutational meltdown in asexual populations. *Journal of Heredity* 84:339–344.
- Lynch, M., and W. Gabriel. 1990. Mutation load and the survival of small populations. *Evolution* 44:1725–1737.
- Mackay, T. F. C., R. F. Lyman, and M. S. Jackson. 1992. Effects of *P* element insertions on quantitative traits in *Drosophila melanogaster*. *Genetics* 130:315–332.
- Maynard Smith, J. 1978. *The evolution of sex*. Cambridge University Press, Cambridge.
- Melzer, A. L., and J. H. Koeslag. 1991. Mutations do not accumulate in asexual isolates capable of growth and extinction—Muller's ratchet reexamined. *Evolution* 45:649–655.
- Muller, H. J. 1964. The relation of recombination to mutational advance. *Mutation Research* 1:2–9.
- Nei, M. 1971. Extinction time of deleterious mutant genes in large populations. *Theoretical Population Biology* 2:419–425.
- Pamilo, P., M. Nei, and W.-H. Li. 1987. Accumulation of mutations in sexual and asexual populations. *Genetical Research* 49:135–146.
- Robertson, A. 1961. Inbreeding in artificial selection programmes. *Genetical Research* 2:189–194.
- Santiago, E., J. Albornoz, A. Dominguez, M. A. Toro, and C. Lopez-Fanjul. 1992. The distribution of effects of spontaneous mutations on quantitative traits and fitness in *Drosophila melanogaster*. *Genetics* 132:771–781.
- Stephan, W., L. Chao, and J. G. Smale. 1993. The advance of Muller's ratchet in a haploid asexual population: Approximate solutions based on diffusion theory. *Genetical Res.* 61:225–231.

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#### APPENDIX

Here we use single-locus diffusion theory to derive some of the expressions in the main text, notably equations (2) and (3). Implicit in all of these results is the assumption that, under free recombination, the one-dimensional theory adequately describes the diffusion dynamics at individual loci, that is, that mutations at different loci have no influence on each others' dynamics. All of the general results may be found in Ewens (1979, chaps. 4, 5), and will be used without further reference. We use the classical one-locus Wright-Fisher model of a monoeucious population of size  $K$  with two alleles:  $A_1$  the deleterious mutant, and  $A_2$  the wild type. The fitnesses of the genotypes  $A_1A_1$ ,  $A_1A_2$ , and  $A_2A_2$  are, respectively,  $1 - 2s$ ,  $1 - 2hs$ , and  $1$ . The frequency of the  $A_1$  allele is initially  $x_0 = 1/2K$ , and more generally, is denoted by  $x = i/2K$ . In accordance with the general simulation model, initially exactly one  $A_1$  allele is present, and no further mutation occurs at the locus as long as the locus is polymorphic.

*Fixation Probability.*—Setting  $\alpha = -4N_e s$ , and defining the function

$$\psi(y) = \exp\{-2\alpha hy - \alpha(1 - 2h)y^2\}, \quad (A1)$$

the fixation probability of the mutant allele is

$$u_F = \frac{\int_0^{x_0} \psi(y) dy}{\int_0^1 \psi(y) dy}, \quad (A2)$$

which, for  $h = 0.5$ , simplifies to

$$u_F = \frac{e^{4N_e s x_0} - 1}{e^{4N_e s} - 1}. \quad (A3)$$

For other values of  $h$ , no simple formula exists.

*Cumulative Load per Mutant Allele.*—The load caused by a mutant whose current frequency is  $x$  is  $\ell(x) = 1 - w(x) = 2sx[2h + (1 - 2h)x]$ , where  $w(x)$  is the mean fitness at the locus. Therefore, the total (cumulative) load is

$$L = \int_0^1 \ell(x)t(x) dx, \quad (A4)$$

where  $t(x)$  is the expected time the mutation spends at frequency  $x$ ,

$$t(x) = \frac{4N_e(1 - u_F)}{x(1 - x)\psi(x)} \int_0^x \psi(y) dy, \quad 0 \leq x \leq x_0, \tag{A5}$$

$$t(x) = \frac{4N_e u_F}{x(1 - x)\psi(x)} \int_x^1 \psi(y) dy, \quad x_0 \leq x \leq 1.$$

For  $h = 0.5$ ,  $\ell(x) = 2sx$ , and equation (A4) reduces to

$$L = 2(1 - u_F) \int_0^{x_0} \frac{1 - e^{-4N_e s x}}{1 - x} dx + 2u_F \int_{x_0}^1 \frac{e^{4N_e s(1-x)} - 1}{1 - x} dx \tag{A6}$$

(compare Ewens 1972).

To obtain a simpler expression for the cumulative load, the integrals in equation (A6) can be expressed by the exponential integral Ei (see Abramowitz and Stegun 1972, chap. 5), such that for mutations with additive effects,

$$L = 2(1 - u_F)\{-e^{-4N_e s}[\text{Ei}(4N_e s) - \text{Ei}(4N_e s(1 - x_0))] - \ln(1 - x_0)\} + 2u_F\{\text{Ei}(4N_e s(1 - x_0)) - \ln(4N_e s(1 - x_0)) - \gamma\}, \tag{A7}$$

where  $\gamma = 0.5772$  is Euler's constant. The exponential integral has the following series expansion

$$\text{Ei}(x) = \frac{e^x}{x} \left[ 1 + \frac{1}{x} + O\left(\frac{1}{x^2}\right) \right]. \tag{A8}$$

Using this, equation (A3), and omitting all terms of order  $e^{-4N_e s} \ln(K)$  and smaller, equation (A7) becomes

$$L = -2e^{-4N_e s} \text{Ei}(4N_e s) + 2e^{-4N_e s(1-x_0)} \text{Ei}[4N_e s(1 - x_0)] - 2 \ln(1 - x_0) + O[e^{-4N_e s} \ln(K)].$$

Application of equation (A8) and a series expansion of  $\ln(1 - x_0) = \ln[1 - (1/2K)]$  yields

$$L = 2 \left( -\frac{1}{4N_e s} + \frac{1}{4N_e s(1 - x_0)} + \frac{1}{2K} \right) = \frac{1}{K} + O\left(\frac{1}{sN_e^2}\right), \tag{A9}$$

which is equation (2) in the main text.

*Time to Absorption.*—The mean absorption time, that is, the mean time until a new mutant allele is either lost or fixed, is

$$\bar{t}_a = \int_0^1 t(x) dx, \tag{A10}$$

where  $t(x)$  has been defined above. In the case of additivity, equation (A10) reduces to

$$\bar{t}_a = \frac{1 - u_F}{s} \int_0^{x_0} \frac{1 - e^{-4N_e s x}}{x(1 - x)} dx + \frac{u_F}{s} \int_{x_0}^1 \frac{e^{4N_e s(1-x)} - 1}{x(1 - x)} dx, \tag{A11}$$

with  $u_F$  defined as in (A3).

Equation (A11) can be simplified by applying the decomposition  $1/[x(1 - x)] = (1/x) + [1/(1 - x)]$  to the integrals, and using equations (A6) and (A9) for the integrals involving  $1/(1 - x)$ ,

$$\bar{t}_a = \frac{1}{2Ks} + O\left(\frac{1}{N_e^2 s^2}\right) + \frac{1 - u_F}{s} \int_0^{x_0} \frac{1 - e^{-4N_e s x}}{x} dx + \frac{u_F}{s} \int_{x_0}^1 \frac{e^{4N_e s(1-x)} - 1}{x} dx. \tag{A12}$$

The first of these integrals is computed to be  $\text{Ei}(4N_e s x_0) - \ln(4N_e s x_0) - \gamma$ , and the second is  $e^{4N_e s}[-E_1(4N_e s) + E_1(4N_e s x_0)] - \ln(4N_e s) + \ln(4N_e s x_0)$ , where  $E_1$  is the exponential integral of order one (see Abramowitz and Stegun 1972, chap. 5). Using  $[\text{Ei}(x) - \ln(x) - \gamma]/x = 1 + O(x)$  with  $x = 4N_e s x_0$ , and  $N_e x_0 = O(1)$ , we obtain that the first integral is  $4N_e s x_0 + O(s^2)$ . For the second integral, we use

$$E_1(x) = \frac{e^{-x}}{x} \left[ 1 - \frac{1}{x} + O\left(\frac{1}{x^2}\right) \right], \tag{A13}$$

and obtain finally

$$\bar{t}_a = \frac{1}{2Ks} + 2\frac{N_e}{K} + 2\frac{N_e}{K} \left( 1 + \frac{N_e}{K} \right) E_1\left( 2\frac{N_e}{K} \right) + O\left(\frac{1}{s^2 N_e^2}\right) + O(s). \tag{A14}$$

A similar formula for the average time until a deleterious mutant is lost was derived by Nei (1971).

*Computation of the Segregational Fitness.*—The segregational fitness can be evaluated numerically by use of equation (1) with  $L$  and  $\bar{t}_a$  defined by equations (A6) and (A11) respectively. With increasing  $K$ ,  $\bar{W}_s$  declines from a value near 1 for  $K = 1$  to a value below  $e^{-\mu}$  and then converges to  $e^{-\mu}$  from below. The deviation from  $e^{-\mu}$  is always less than 5% when  $N_e s \geq 5$ .

Simulations also show that the diffusion approximation does not work particularly well for deleterious mutants, unless  $s$  is extremely small. The fixation probability, defined by equation (A3), always overestimates the “true” fixation probability obtained from the transition matrix  $P$  (see the main text), and this overestimate becomes substantial for  $Ks > 1$ . For example, if  $s = 0.025$  and  $N_e = K = 128$ , the true fixation probability is  $1.06 \times 10^{-5}$ , whereas equation (A3) predicts  $1.42 \times 10^{-5}$ . For larger  $K$  or  $s$ , equation (A3) may be off by several orders of magnitude. Interestingly, it turns out (Bürger and Ewens 1995.) that an extremely accurate approximation for the fixation probability is obtained if, in equation (A3),  $s$  is substituted by  $s/(1 - s)$ . For example, for the same  $s$  and  $K$  as above, this gives a fixation probability of  $1.05 \times 10^{-5}$ .

For recessive mutants ( $h = 0$ ), the general formulas for  $u_F$ ,  $\bar{t}_a$ , and  $L$  cannot be simplified, and numerical integration is required.