Estimation of Deleterious-Mutation Parameters in Natural Populations

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ABSTRACT

The rate and average effects of spontaneous deleterious mutations are important determinants of the evolution of breeding systems and of the vulnerability of small populations to extinction. Nevertheless, few attempts have been made to estimate the properties of such mutations, and those studies that have been performed have been extremely labor intensive, relying on long-term, laboratory mutation-accumulation experiments. We present an alternative to the latter approach. For populations in which the genetic variance for fitness is a consequence of selection-mutation balance, the mean fitness and genetic variance of fitness in outbred and inbred generations can be expressed as simple functions of the genomic mutation rate, average homozygous effect and average dominance coefficient of new mutations. Using empirical estimates for the mean and genetic variance of fitness, these expressions can then be solved to obtain joint estimates of the deleterious-mutation parameters. We employ computer simulations to evaluate the degree of bias of the estimators and present some general recommendations on the application of the technique. Our procedures provide some hope for obtaining estimates of the properties of deleterious mutations from a wide phylogenetic range of species as well as a mechanism for testing the validity of alternative models for the maintenance of genetic variance for fitness.

The rate at which deleterious mutations arise spontaneously and the distribution of their effects are important determinants of the evolutionary advantage of alternative breeding systems and of the evolution of recombination rates (Pamilo et al. 1987; Kondrashov 1988; Charlesworth et al. 1992, 1993). The accumulation of genetic load due to segregating and fixed mutations also plays a significant role in the extinction of small populations (Lynch et al. 1993, 1995a, b). Unfortunately, we know very little about the properties of deleterious mutations, except in Drosophila melanogaster (Mukai 1979; Crow and Simmons 1983; Houle et al. 1992; Keightley 1994), and even for this species, the data have been very hard to come by, requiring large and long-term mutation-accumulation experiments that rely on special chromosomal constructs.

An alternative, less labor-intensive, approach to characterizing the deleterious mutation process was suggested by Morton et al. (1956). For populations in selection-mutation balance, they showed how the genomic deleterious mutation rate (U) can be estimated from the observed inbreeding depression in a normally outcrossing population provided an estimate of the average degree of dominance (h) is available. Their approach seems to have gone largely unnoticed by empiricists until Charlesworth et al. (1990) showed how the same logic can be used to derive an estimator for U that can be applied to highly selfing populations. In this case, it is the enhancement in fitness observed upon outcrossing selfed lines that is related directly to U, again provided that information on the average degree of dominance of new mutations is available. The combined results of Morton et al. (1956) and Charlesworth et al. (1990) raise the possibility that estimates of U may be obtained from relatively simple assays of natural populations. Johnston and Schoen (1995) recently used the approach of Charlesworth et al. (1990) to obtain estimates of U from the annual plant Amsinckia that are in line with earlier estimates from Drosophila derived from mutation-accumulation experiments. Similar results were achieved with Leavenworthia (Charlesworth et al. 1994). Throughout this paper, we will refer to the general approach to estimating U from observations on inbreeding depression or outbreeding enhancement in equilibrium populations as the Morton-Charlesworth technique.

Several issues need to be resolved before the generality of this method can be established. First, the need for an estimate of the average degree of dominance is not trivial. In principle, rough estimates of h can be obtained for segregating mutations from a comparison of the fitness of inbred parental lines and their outcrossed progeny using methods described by Comstock and Robinson (1948), Hayman (1954), and Mukai et al. (1972). However, the acquisition of unbiased estimates of h by the methods of Comstock and Robinson (1948) and Hayman (1954) requires that all segregating alleles have frequency 0.5, and with all of the methods, the estimate of h is based on a weighting of the individual alleles by their unobserved homozygous effects. In addition, since the time that deleterious
alleles segregate before being eliminated by selection is expected to decline with the increasing degree of dominance, the average degree of dominance in the pool of segregating alleles in an equilibrium population does not accurately reflect $h$ for newly arisen mutations. Under selection-mutation balance, the arithmetic mean $h$ of segregating mutations is expected to be close to the harmonic mean $h$ of new mutations (Morton et al. 1994).

Second, the Morton-Charlesworth estimators assume that the effects of all mutations have constant and independent effects on fitness. This is unlikely to ever be true, raising the question as to whether variance in the effects of mutations and/or epistatic interactions cause significant bias in estimates of $U$. Such bias is likely to exist since the Morton-Charlesworth estimators are nonlinear functions of both the inbreeding depression and the degree of dominance.

Third, although the average homozygous effect of mutations ($\bar{\delta}$) has very little influence on the mutation load in large populations resulting from segregating mutations (Haldane 1937; Burger and Hofbauer 1994), it is a critical determinant of the role of deleterious mutations in extinction through the accumulation of fixed mutations (Lande 1994; Lynch et al. 1995a). Thus, a technique that yields estimates of $\bar{\delta}$ as well as $U$ would be of considerable practical value.

In this paper, we extend the ideas of Morton et al. (1956) and Charlesworth et al. (1990) in several ways. First, we show that when observations are available for the genetic variance in fitness as well as for the mean fitness in inbred and outcrossed generations, estimates of the mean mutational effects, $\bar{x}$ and $\bar{h}$, can be obtained jointly with an estimate of $U$. Second, we consider the bias in parameter estimates that is induced by variation in mutational effects and by epistatic interactions between mutations. Third, through computer simulations, we evaluate the power of our proposed approaches for estimating the mutational parameters.

**THEORY**

Throughout this paper we assume that the population to be assayed has been effectively infinite for a long enough time that all loci are in selection-mutation balance for segregating polymorphisms. For any locus, this requires that the product of the effective population size and the selection coefficient of the deleterious allele exceeds five or so (Kimura et al. 1963; Lynch et al. 1995b). Initially, we consider the situation envisioned by Morton et al. (1956)—populations that are assumed to have been randomly mating historically. We first analyze the simple situation in which all mutations are assumed to have constant effects, showing how expressions for the expected mean and variance of fitness can be used to derive joint estimators for $U$, $s$, and $h$. We then demonstrate how variance in mutational effects can complicate things. Finally, we return to the situation considered by Charlesworth et al. (1990), extending their results to derive joint estimators for the mutational parameters in populations of obligate selfers. Our derivations assume that deleterious mutations at different loci have independent effects on individual fitness. The significance of this assumption will be evaluated later in the paper.

**Random-mating populations: Mutations with fixed effects:** Assume that there are $N$ equivalent, freely recombining loci per genome, each of which can be characterized by three genotypic states: $AA$, $Aa$, and $aa$, where $a$ denotes the deleterious allele. The relative fitnesses of the three genotypes are $1$, $1 - hs$, and $1 - s$, respectively, where $h$ is a measure of dominance ($h = 0.5$ denoting additivity). So long as the deleterious allele is not completely recessive and the back-mutation rate is negligible, the frequency of mutant homozygotes is small relative to that of heterozygotes (Crow and Kimura 1970). Under these conditions, letting $u$ denote the mutation rate of the normal allele $A$ to the deleterious allele $a$, the expected frequency of the $a$ allele under selection-mutation balance is very close to $u/(hs)$. Letting $U = 2Nu$ be the genomic deleterious mutation rate, this implies that the mean number of deleterious alleles per individual is $\bar{n} = U/(hs)$.

Assuming that the frequency of mutant homozygotes is negligible, that the number of mutant alleles per individual follows the Poisson distribution $p(n) = \pi^n e^{-\pi}/n!$, and that the different loci have independent (multiplicative) effects on fitness (i.e., that there is no epistasis), we obtain the well-known result

$$W_0 = W_{\text{max}} \sum_{n=0}^{\infty} (1 - hs)^n p(n)$$

$$= W_{\text{max}} \exp(-hs\bar{n}) = W_{\text{max}} \exp(-U) \quad (1a)$$

(Haldane 1937; Kimura et al. 1963; Burger and Hofbauer 1994), where $W_{\text{max}}$ is the expected fitness of a mutation-free individual in an experimental setting. Thus, the mean relative fitness in a randomly mating population ($W_0$) depends only on the genomic deleterious mutation rate, not on the effects of the individual mutations. The genetic variance of fitness is given by

$$\sigma^2_W(O) = W_{\text{max}}^2 \sum_{n=0}^{\infty} \frac{(1 - hs)^n p(n)}{1 - (1 - hs)^n} - W_0^2$$

$$= W_0^2 \{\exp(Uh\bar{s}) - 1\}. \quad (1b)$$

Suppose now that the members of the outcrossed population are self-fertilized with multiple selfed progeny being produced by each family. For each parental locus that is heterozygous, the inbred progeny are expected to be heterozygous or homozygous for the deleterious allele with probabilities $1/4$ and $1/4$, respectively. Thus, under the assumption of free recombination, the mean fitness and the genetic variance in fitness among the selfed families are
Deleterious-Mutation Parameters

\[ W_0 = W_{max} \sum_{n=0}^{\infty} \left( \frac{1}{4} + \frac{1-h_s}{2} + \frac{1-s}{4} \right)^n \mu(n) \]

\[ = W_{max} \exp \left( -\frac{U(h+0.5)}{2h} \right) \]  
(1c)

\[ \sigma_w^2(S) = W_{max}^2 \sum_{n=0}^{\infty} \left( \frac{1}{4} + \frac{1-h_s}{2} + \frac{1-s}{4} \right)^2 \mu(n) - W_0^2 \]

\[ = W_0^2 \exp \left( \frac{U_s(1+2h)^2}{16h} \right) - 1 \]  
(1d)

There are three potentially informative composite functions of the genotypic means and variances:

\[ x = \ln \left( \frac{\sigma_w^2(O)}{W_0^2} + 1 \right), \]  
(2a)

\[ y = \ln \left( \frac{W_0}{W_0} \right), \]  
(2b)

\[ z = \ln \left( \frac{\sigma_w^2(S)}{W_0^2} + 1 \right). \]  
(2c)

Note that \( y \) is simply the natural logarithm of the ratio of fitnesses observed in selfed and outcrossed generations, whereas the fractions in \( x \) and \( z \), respectively, are the squared coefficients of genetic variation of fitness in the random-mating population and of mean fitness of selfed families. From Equations 1, a–d, the expected values of \( x \), \( y \), and \( z \), in terms of the mutational parameters are, respectively,

\[ E(x) = Uhs, \]  
(3a)

\[ E(y) = \frac{U(2h-1)}{4h}, \]  
(3b)

\[ E(z) = \frac{U_s(1+2h)^2}{16h}. \]  
(3c)

Rearranging and letting a circumflex (\(^\hat{\ }\)) denote an estimate, we obtain potential estimators for the mutational parameters in terms of the observable moments of fitness in the outcrossed and selfed generations,

\[ \hat{h} = \frac{1}{4\sqrt{\hat{k}} - 2}, \]  
(4a)

\[ \hat{U} = \frac{4\hat{h}\hat{s}}{2h - 1}, \]  
(4b)

\[ \hat{\xi} = \frac{\hat{\xi}}{\hat{U}h}. \]  
(4c)

Estimators for other useful composite functions of the mutational parameters are:

1) the mean number of deleterious mutations per genome,

\[ \hat{n} = \frac{\hat{U}}{\hat{h}s}, \]  
(5a)

2) the rate of introduction of genetic variance in fitness by mutation per generation,

\[ \hat{V}_M = \frac{\hat{U}(\hat{h}s)^2}{8}. \]  
(5b)

3) the decline in fitness due to a single generation of mutations,

\[ \hat{L}_M = \xi = \frac{\hat{U}\hat{s}}{\hat{h}}, \text{ and} \]  
(5c)

4) the long-term rate of decline in fitness (due to fixations) that would result if natural selection against the recurrent deleterious mutations were rendered ineffective (as in an inbred line maintained by single-seed descent),

\[ \hat{R}_M = \frac{\hat{U}^2}{2}. \]  
(5d)

Mutations with variable effects: The preceding expressions were derived under the assumption that deleterious mutations have constant effects within and among loci, a situation that is unlikely to ever be very realistic biologically. With variable mutational effects, the expressions for the means and genetic variances of fitness become,

\[ W_0 = W_{max} \exp (-U), \]  
(6a)

\[ \sigma_w^2(O) = W_{max} \exp \left( \frac{U}{\hat{h}s} \right) - 1, \]  
(6b)

\[ W_S = W_{max} \exp \left[ -\left( \frac{U}{4}\right) \left( 2 + \left( \frac{1}{\hat{h}} \right) \right) \right], \]  
(6c)

\[ \sigma_w^2(S) = W_{max} \exp \left( \frac{U}{4} \left( \frac{h_s + s}{h} \right) - 1 \right). \]  
(6d)

where quantities with overlines denote arithmetic mean properties of new mutations and \( \hat{h} \) is the harmonic mean dominance coefficient of new mutations.

Failure to account for the variance in mutational effects causes Equations (4 a–c) to yield biased estimates of the mutation parameters. Estimators that take this variation into account are

\[ \hat{h} \approx \frac{1}{8\sqrt{\hat{\xi}} - 2} \left( \frac{C_h^2}{1 + C_{\alpha,h}} + \frac{2 - C_h^2}{1 + C_{\alpha,h}} \right), \]  
(7a)

\[ \hat{U} \approx \frac{4h\hat{s}}{2h - 1 - C_h^2}, \]  
(7b)

\[ \hat{\xi} = \frac{\hat{\xi}}{\hat{U}h}. \]  
(7c)

where \( C_h^2 = \sigma_h^2/h^2 \) is the squared coefficient of variation of dominance coefficients, and \( C_{\alpha,h} = \sigma_{\alpha,h}/(\hat{h}s) \) is the coefficient of covariation between \( s \) and \( h \). Both of these coefficients refer to the properties of newly arisen mutations (as opposed to the pool of segregating mutations), as do the estimators of the mean effects \( \hat{h} \) and \( \hat{\xi} \).
To gain some quantitative insight into the magnitude of bias that might exist for the estimators given by Equations 4, a–c, we require information on \( C_{a,h} \) and \( C_b \). Approximate values of these terms may be obtained by considering the following. The available data suggest that the distribution of homozygous effects is roughly exponential with density distribution

\[
p(s) = \frac{1}{\bar{s}} \exp\left(-\frac{s}{\bar{s}}\right) \quad (8a)
\]

(GREGORY 1965; MACKAY et al. 1992; KEIGHTLEY 1994), although more extreme distributions are also compatible with the data. Little information exists on the distribution of dominance coefficients, but biochemical arguments suggest that an inverse relationship exists between the homozygous effect of a deleterious mutation and its degree of dominance, genes with large effects tending to be nearly recessive (KACSER and BURNS 1981). The few available data are consistent with this idea. Results from mutation-accumulation experiments with Drosophila melanogaster suggest that the dominance coefficient associated with deleterious mutations ranges from near zero for lethals to near 0.5 for mutations with very mildly deleterious effects (CROW and SIMMONS 1981). For mildly deleterious mutations, the average value of \( \bar{s} \) over several mutation-accumulation studies is \( \sim 0.03 \) if one assumes a negative exponential distribution, while the average dominance coefficient is approximately \( h = 0.36 \) (LYNCH et al. 1995b). Thus, as a rough approximation to the inverse relationship between \( h \) and \( s \), we consider the function

\[
h = \frac{e^{-\alpha s}}{2}, \quad (8b)
\]

With \( \alpha = 13 \), this function yields \( h = 0.36 \) when \( s = 0.03, h \rightarrow 0.5 \) as \( s \rightarrow 0 \), and \( h \rightarrow 0.0 \) as \( s \rightarrow 1.0 \), all in rough accordance with the data. True mutational spectra may be such that the degree of dominance of individual mutations is broadly scattered around such a function (CABALLERO and KEIGHTLEY 1994).

Integrating over the distribution of \( s \) defined by Equation 8a, with the function defined by Equation 8b, it can be shown that

\[
C_{a,h} = -\frac{\alpha \bar{s}}{\alpha \bar{s} + 1}, \quad (9a)
\]

\[
C_b^2 = \frac{(\alpha \bar{s})^2}{2\alpha \bar{s} + 1}. \quad (9b)
\]

Thus, with \( \alpha = 13 \) and \( \bar{s} = 0.03, C_{a,h} \approx -0.28 \) and \( C_b^2 \approx 0.085 \). To the extent that these estimates are reliable, referring to Equation 7, a–c, Equation 4a may underestimate the average degree of dominance by \( \sim 50\% \). Equation 4b may underestimate the genomic mutation rate by \( \sim 45\% \), and Equation 4c may overestimate the average homozygous effect by \( \sim 75\% \).

**Self-fertilizing populations:** All of the procedures just outlined can be extended to naturally self-fertilizing populations that are forced to outcross. Here we assume that the natural population reproduces by obligate selfing, in which case the equilibrium frequency of a deleterious allele under selection-mutation balance is about \( u/s \) (CROW and KIMURA 1970). Under obligate selfing, the frequency of heterozygotes is negligible, so this is also the frequency of individuals that are \( aa \) homozygous at a locus. Using the same procedures that led to Equations 6, a and b, the mean fitness and the genetic variance in fitness in the selfing population are found to be

\[
W_\bar{s} \approx W_{max} \exp\left(-U/2\right), \quad (10a)
\]

\[
\sigma_{W_{\bar{s}}}^2(S) \approx W_{max}^2 \left[ \exp\left(U\bar{s}/2\right) - 1 \right] \quad (10b)
\]

regardless of whether \( s \) and \( h \) are constant or variable.

Now consider the situation in which random pairs of normally selfing individuals are forced to outcross. Let \( \bar{\pi} \) be the expected number of loci that are homozygous for deleterious alleles within individuals in the selfing population. Assuming that the equilibrium allele frequencies are very small, then there will be essentially no overlap in the sets of deleterious loci carried in two random individuals (CHARLESWORTH et al. 1990, 1992).

Thus, the number of heterozygous loci in outcrossed progeny can be assumed to be a Poisson variable with expectation \( 2\bar{\pi} \), and the number of loci that are homozygous for segregating deleterious alleles can be assumed to be 0. The mean fitness in outcrossed progeny and the genetic variance in fitness among outcrossed families are then

\[
W_o \approx W_{max} \exp\left(-U\bar{h}\right) \quad (10c)
\]

\[
\sigma_{W_o}^2(O) \approx W_{max}^2 \left[ \exp\left(U\bar{h}^2\bar{s}\right) - 1 \right] \quad (10d)
\]

Equation 10, a and c, forms the basis of the estimator for \( U \) derived by CHARLESWORTH et al. (1990).

To obtain estimators for the mutational parameters, we again utilize the intermediate terms \( x, y, \) and \( z \) defined in Equation 2, a–c. The expectations of these are now

\[
E(x) \approx U\bar{h}^2 \bar{z}(1 + C_b^2 + 2C_{a,h}), \quad (11a)
\]

\[
E(y) \approx U(\bar{h} - 0.5), \quad (11b)
\]

\[
E(z) \approx U\bar{s}/2, \quad (11c)
\]

rearrangement of which leads to approximate estimators for the mutational parameters,

\[
\hat{h} = \sqrt{\frac{\bar{s}}{2\bar{z}(1 + C_b^2 + 2C_{a,h})}}, \quad (12a)
\]

\[
\hat{U} = \frac{\bar{y}}{\bar{h} - 0.5}, \quad (12b)
\]

\[
\hat{\bar{s}} = \frac{2\bar{z}}{\bar{U}}. \quad (12c)
\]
Equation 12b is equivalent in form to the estimator derived by Charlesworth et al. (1990).

SIMULATION RESULTS

A number of approximations have been made in the derivation of the preceding equations, e.g., dropping terms of order $s^2$ and $(hs)^2$ in the case of random-mating populations. To evaluate the ability of Equations 4 a–c, and 12 a–c, to generate accurate estimates of the mutational parameters $U$, $s$, and $h$ in the face of these approximations, we first performed computer simulations under the assumption of constant mutational effects. Here we assumed that a selection-mutation balance had been reached in the parental population such that, in the case of random mating, the number of mutations per individual (all in the heterozygous state) was Poisson distributed with expectation $\bar{n} = U/(hs)$. In the case of obligate selfing, the number of loci homzygous for mutations in each individual was assumed to be Poisson distributed with expectation $\bar{n} = U/(2s)$.

To evaluate the power of the estimation procedures in the case of random-mating populations, for each set of parameters, a variable $K$ individuals were sampled from the parental generation, and from each of these, $M$ selfed progeny were produced. In these simulations, the fitness of every individual was assumed to be known without error, defined by the multiplicative function used in the preceding derivations,

$$W(n) = W_{\text{max}} \prod_{i=1}^{n} (1 - h_i s_i).$$

where $n$ is the number of mutation-bearing loci within an individual, obtained by random sampling from the Poisson distribution defined above, and $s_i$ and $h_i$ are the effects associated with the $i$th mutation. The fitness of each selfed offspring was obtained by allowing the $n$ heterozygous loci of the parent to segregate randomly into the $AA$, $Aa$, and $aa$ classes with respective probabilities $0.25$, $0.5$, and $0.25$. Letting $i = 1, \ldots, n_w$ and $j = 1, \ldots, n_h$ be the number of heterozygous and homozygous loci containing mutations in an offspring obtained by selfing (obtained by random segregation), then

$$W(n_w, n_h) = W_{\text{max}} \prod_{i=1}^{n_w} (1 - h_i s_i) \prod_{j=1}^{n_h} (1 - h_j s_j).$$

Estimates of $\sigma^2_{W}(S)$, the variance in fitness among selfed families, were obtained by one-way analysis of variance (ANOVA) of the simulated data with parental clones as main effects and selfed progeny within each family as random effects. For each set of parameters $(U, s, h, K, M)$, we performed 100 simulations. We arbitrarily let $W_{\text{max}} = 1$ throughout, as the value of $W_{\text{max}}$ does not influence the mutation-parameter estimates.

For selfing populations, we again assumed that the parental fitnesses were known without error, in this case defined by

$$W(n) = W_{\text{max}} \prod_{i=1}^{n} (1 - s_i).$$

The numbers of mutation-bearing loci ($n$) for $K$ such individuals were obtained as random Poisson variables (defined above), and then each parent was mated with another random parent (not in the original set of $K$) to produce a total of $K$ progeny (one per family) with expected fitness

$$W(n_w, n_h) = W_{\text{max}} \prod_{i=1}^{n_w} (1 - h_i s_i) \prod_{j=1}^{n_h} (1 - h_j s_j),$$

where $i = 1, \ldots, n_w$ and $j = 1, \ldots, n_h$ are the numbers of homozygous mutant loci in the two parents. Estimates of $\sigma^2_{W}(O)$, the variance in fitness among outcrossed families, were obtained directly from the variance among offspring fitness (under the assumption that different parental lines are fixed for different mutations, all $F_1$ progeny from a specific outcross have identical fitness, so analysis of variance is unnecessary in the estimation of $\sigma^2_{W}(O)$ in this set of simulations).

Two conclusions emerge under the ideal conditions in which mutational effects are constant and genotypic fitnesses of parents and offspring are known without error (Table 1). First, application of the model to both obligately selfing and outcrossing populations yields essentially unbiased parameter estimates for $s$, $h$, and $U$. Second, the sampling error of parameter estimates is generally higher when the analysis is applied to selfing populations, despite the fact that in our simulations for random mating populations the mean fitnesses of selfed families were subject to some sampling error due to Mendelian segregation.

To evaluate the extent to which bias in parameter estimates is introduced by variable mutational effects, we evaluated the situation in which new mutations have an exponential distribution of $s$ defined by Equation 8a and a dominance coefficient defined by Equation 8b with $a = 13$. For selfing populations, if the variance and covariance of mutational effects is ignored (by setting $C_a = C_s = 0$ in Equation 12a), Equation 12 a–c, yields estimates of $\bar{r}$ that are upwardly biased and estimates of $h$ and $U$ that are downwardly biased (Table 2). However, the product $US$, which is only a function of the genetic coefficient of variation of fitness in the obligate selfing parental generation (Equation 11c), is nearly unbiased. Note that for the range of parameter values given in the table, which encompass the few available empirical data, the bias causes the average estimated values to deviate from the parametric values by $\approx 50\%$. If, on the other hand, known values of $C_a$ and $C_s$ are applied to Equation 12a, much of the bias in the parameter estimates is removed (Table 2). Thus, Equation 12, a–c, provides reasonably good estimates of the mu-
tation parameters, particularly if some information on the distribution of effects is available.

For outcrossing populations with variable mutational effects, Equation 4, a–c, also causes bias in the estimates of \( s, h, \) and \( U \) (Table 2), with the estimated product \( U \) again being nearly unbiased. For small \( s \), the bias is only slightly more extreme than for the same parameters in an obligately selfing population, and in terms of statistical power, this seems to be offset by much lower standard deviations of the estimates derived from outcrossing populations. However, for \( s \) as high as 0.05 (probably an extreme case biologically), the bias becomes quite large, in some cases approaching a factor of 10. Again, application of Equation 7, a–c, with the known values of \( G^2 \) and \( G_{n,h} \), removes essentially all the bias when \( s \) is small, and the vast majority of it when \( s \) is larger.

Evidence that bias in the estimators for outcrossing populations is partly a consequence of rare mutations with lethal or nearly lethal effects derives from additional simulations that we performed. These simulations were identical in all respects to those just discussed but with an additional low genomic mutation rate to lethals (defined as having \( s = 1.0 \) and \( h = 0.02 \)) (Table 3). The presence of rare lethals (in the simulations shown, an expected number of 0.75 per individual) causes the estimates of \( s \) to inflate by a factor of approximately five and of \( U \) and \( \bar{h} \) to decrease by factors of nearly three and two, respectively. In practical applications of our proposed technique, this type of problem can perhaps be minimized by eliminating individuals that are homozygous for lethals from the final analysis. By dropping inviable selfed progeny from our analyses, we do indeed recover estimates of the mutational parameters that are much closer to their parametric values (Table 3).

In the simulations discussed above, we assumed that the genotypic fitnesses of parents and progeny are known without error. In reality, this would require that each individual be clonally replicated and assayed a very large number of times. In Table 4, we consider the effects of finite sample size on the sampling error of parameter estimates. We examined situations in which the broad-sense heritabilities (\( H^2 \)) of fitness were 0.20 and 0.40 in the parental generation. The environmental variance (including individual sampling error) for fitness was then defined as \( \sigma^2 = (1 - H^2) \sigma^2_{e} / H^2 \), where \( \sigma^2_{e} \) is the genetic variance of fitness defined by Equation (1b) in the case of outcrossing populations and by Equation (10b) in the case of obligately selfing populations. Individual fitnesses were then determined by their genotypic properties as described above, plus a random environmental deviation drawn from a normal distribution with 0 mean and variance \( \sigma^2_{e} \). Estimates of

### Table 1

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<th>( \bar{s} )</th>
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For each set of the parameters \( s, h, \) and \( U \), 100 simulations were performed. Each simulation is based on 200 parents for which the genotypic fitnesses were assumed to be known without error. For selfing populations, 20% random outcrossed progeny were evaluated per simulation (one for each of the original 200 parents, randomly outcrossed) and their fitnesses were assumed to be known without error. For outcrossing populations, 40 selfed progeny, whose genotypes were determined by random segregation, were evaluated per parent, and the variance of fitness among selfed families was estimated by one-way analysis of variance. All mutations were assumed to have constant effects \( s \) and \( h \). Values for \( \bar{s}, \bar{h}, \) and \( \bar{U} \) are means ± SD.
forming one-way ANOVAs on the simulated data, and outcrossed progeny from unique parent pairs with
components of variance. Type) yielded average estimates of the mutational pa-
genetic variances for fitness were obtained by performing one-way ANOVAs on the simulated data, and extracting the among-parent or among-family components of variance.
For selfing populations, experiments involving samples sizes of 10,000 individuals (100 parents and 100 outcrossed progeny from unique parent pairs with 50 cloned replicates of each parental and progeny genotype) yielded average estimates of the mutational parameters that were fairly close to their true values (Ta-

<table>
<thead>
<tr>
<th>( \tau )</th>
<th>( \bar{h} )</th>
<th>( U )</th>
<th>( \bar{s} )</th>
<th>( \bar{h} )</th>
<th>( \bar{U} )</th>
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<td></td>
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<tr>
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<td>0.44</td>
<td>1.50</td>
<td>0.0175 ± 0.0020</td>
<td>0.40 ± 0.01</td>
<td>0.89 ± 0.07</td>
</tr>
<tr>
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<td>0.0172 ± 0.0018</td>
<td>0.40 ± 0.01</td>
<td>0.30 ± 0.03</td>
</tr>
<tr>
<td>0.0500</td>
<td>0.30</td>
<td>1.50</td>
<td>0.0726 ± 0.0037</td>
<td>0.22 ± 0.01</td>
<td>1.05 ± 0.02</td>
</tr>
<tr>
<td>0.0500</td>
<td>0.30</td>
<td>0.50</td>
<td>0.0705 ± 0.0030</td>
<td>0.34 ± 0.01</td>
<td>0.36 ± 0.01</td>
</tr>
<tr>
<td>Outcrossing populations</td>
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<td></td>
<td></td>
<td></td>
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<td>0.39 ± 0.001</td>
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<td>0.0185 ± 0.0003</td>
<td>0.43 ± 0.001</td>
<td>1.34 ± 0.022</td>
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<tr>
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<td>0.1255 ± 0.0022</td>
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<td>0.90 ± 0.011</td>
</tr>
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<td>0.50</td>
<td>0.1517 ± 0.0025</td>
<td>0.11 ± 0.001</td>
<td>0.28 ± 0.002</td>
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</tbody>
</table>

The simulation conditions are as described in the legend to Table 1, except that 1000 parents were sampled per run, the mutational effect \( s \) was assumed to be a random variable following an exponential distribution, Equation 8a, and the dominance coefficient \( h \) for each mutation was defined by Equation 8b with \( \alpha = 13 \). We used a discrete version of the exponential distribution by dividing the entire range of \( s \) (0–1) into 200 classes of width 0.005. Each parental individual in a simulation was then randomly assigned a number of mutations from each of these classes by drawing from a Poisson distribution with expectation \( \lambda U \), where \( \lambda \) is the density of the mutational distribution in the \( i \)th class. For each set of parameters, we give two sets of estimates: the estimates in the first row are obtained under the assumption that \( C_{1} = C_{2} = 0 \), applying Equation 12, \( \alpha c \), with selfing populations and Equation 7, \( \alpha c \), with outcrossing populations; the estimates in the second row are obtained with the same equations, but after the known values of \( C_{1} \) and \( C_{2} \) are applied.

<table>
<thead>
<tr>
<th>( \tau )</th>
<th>( \bar{h} )</th>
<th>( U )</th>
<th>( \bar{s} )</th>
<th>( \bar{h} )</th>
<th>( \bar{U} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>No lethals in parents; with inviable selfs included</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0300</td>
<td>0.360</td>
<td>1.50</td>
<td>0.0537 ± 0.0027</td>
<td>0.24 ± 0.005</td>
<td>0.86 ± 0.03</td>
</tr>
<tr>
<td>An average of 0.75 lethals (( h = 0.02 )) per parent; with inviable selfs included</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0380</td>
<td>0.359</td>
<td>1.51</td>
<td>0.2575 ± 0.0158</td>
<td>0.12 ± 0.006</td>
<td>0.39 ± 0.02</td>
</tr>
<tr>
<td>Lethals present as above, but inviable first-generation selfed progeny excluded from analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0380</td>
<td>0.359</td>
<td>1.51</td>
<td>0.0509 ± 0.0062</td>
<td>0.27 ± 0.004</td>
<td>0.91 ± 0.02</td>
</tr>
</tbody>
</table>

The simulation conditions are as described in the legends to Tables 1 and 2 with 200 parents being sampled per assay. The parameter estimates were derived by use of Equation 4, \( \alpha c \). In all cases, there is an underlying distribution of mildly deleterious mutational effects, obtained as described in the legend to Table 2 with \( \tau = 0.0300 \) and total genomic mutation rate to these mutations of \( U = 1.5 \). For simulations in which lethals are present, these were assumed to enter the genome at a rate of 0.015 per individual per generation in a Poisson fashion and to have dominance coefficient \( h = 0.02 \).
Moreover, the standard deviations of estimates from independent simulations were fairly small—on the order of 10–20% of the estimated values. The situation is still quite good with only 50 parents and 50 outcrossed progeny, even when the number of clonal replicates is reduced to 20. However, when the number of genotypes is reduced beyond this point, the standard errors of the parameter estimates become a bit high for practical purposes.

The same total sampling effort (10,000 vs. 5000 vs. 2000 total individuals) was evaluated for outcrossing populations by considering the situation in which 100, 50, and 20 parental genotypes were clonally replicated 20 times, with each parent yielding 80 selfed progeny (not clonally replicated) (Table 4). The degree of bias and sampling error of the outcrossing estimates for $T$, $\hat{h}$, and $U$ are comparable with the situation with obligately selfing populations, and a substantial reduction in the number of selfed progeny per parent does not greatly elevate the sampling error.

Finally, we note that the theory that we have developed in this paper assumes that the deleterious effects of mutations at different loci act in a multiplicative manner to determine individual fitness. The detection of general epistatic effects among mutant alleles is a very difficult empirical problem and little convincing information exists on the subject. To evaluate the potential consequences of epistasis on the mutation-parameter estimates derived from our model, we considered the epistatic fitness model described by Charlesworth (1990),

\[
W(n) = \exp \left( -an - \frac{\beta h^2}{2} \right)
\]

where in an outcrossing population $\alpha = hs$, and $n = n_1 + (n_2/h)$ is the effective number of heterozygous mutations per individual. The parameter $\beta$ provides a measure of the synergistic effects of deleterious alleles. With $\beta = 0$, the model reduces to one of multiplicative effects, and with $\beta > 0$, the effects of deleterious alleles are synergistic, i.e., as more deleterious alleles are added to the genome, the decline in fitness per additional deleterious allele increases. The ratio $\beta h/(2a\alpha)$ provides a measure of the relative contribution of synergistic effects to mean fitness.

We implemented this model by assuming mutations with constant properties ($s$ and $h$). Under selection-mutation balance, in random-mating populations with free recombination, $n$ is approximately normally distributed with the mean and variance being functions of $U$, $s$, $h$, and $\beta$, defined by Equations 3 and 11 in Charlesworth (1990). For outcrossing populations, we again assumed that all deleterious mutations exist in the heterozygous state before inbreeding, so that the $n$ drawn for a parental individual is the number of heterozygous loci in the individual. We then assumed free recombination in the production of selfed progeny, with the effective number of heterozygous loci per progeny being defined as noted above. The means and variances of fitness for the two generations were then computed, and our estimators, Equations 4, a–c, which assume no epistasis, were applied to the data.

### Table 4

<table>
<thead>
<tr>
<th>P</th>
<th>O</th>
<th>R</th>
<th>$s$</th>
<th>$\hat{h}$</th>
<th>$U$</th>
<th>$s$</th>
<th>$\hat{h}$</th>
<th>$U$</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>100</td>
<td>50</td>
<td>0.046±0.010</td>
<td>0.29±0.02</td>
<td>1.02±0.13</td>
<td>0.048±0.010</td>
<td>0.28±0.02</td>
<td>0.99±0.12</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
<td>50</td>
<td>0.047±0.016</td>
<td>0.29±0.04</td>
<td>1.02±0.22</td>
<td>0.048±0.016</td>
<td>0.28±0.04</td>
<td>0.99±0.19</td>
</tr>
<tr>
<td>20</td>
<td>20</td>
<td>50</td>
<td>0.047±0.023</td>
<td>0.29±0.04</td>
<td>1.03±0.38</td>
<td>0.048±0.023</td>
<td>0.29±0.06</td>
<td>1.07±0.39</td>
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<tr>
<td>100</td>
<td>100</td>
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<td>0.047±0.014</td>
<td>0.29±0.03</td>
<td>1.03±0.21</td>
<td>0.048±0.012</td>
<td>0.28±0.03</td>
<td>0.99±0.15</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
<td>20</td>
<td>0.047±0.016</td>
<td>0.29±0.04</td>
<td>1.03±0.22</td>
<td>0.048±0.015</td>
<td>0.28±0.03</td>
<td>0.99±0.16</td>
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<tr>
<td>20</td>
<td>20</td>
<td>20</td>
<td>0.052±0.023</td>
<td>0.28±0.05</td>
<td>1.10±0.56</td>
<td>0.052±0.024</td>
<td>0.27±0.05</td>
<td>0.99±0.32</td>
</tr>
</tbody>
</table>

$P$, $O$, and $R$ denote, respectively, the number of parental genotypes, the number of progeny per parent, and the number of cloned replicates per parental genotype assayed. In all cases, there is an underlying distribution of mildly deleterious mutational effects, obtained as described in the legend to Table 2 with $r = 0.030$, $h = 0.36$, and $U = 1.5$. In the case of outcrossing populations only, lethals were assumed to be Poisson distributed with expectation of 0.75 per individual and with dominance coefficient $h = 0.02$. In all analyses, the expected fitness of mutation-free individuals is $W_{max} = 10$. The estimates are uncorrected for bias introduced by variation in mutational effects.
whereas $s$ tends to be overestimated. When the ratio of the contribution of epistatic effects to fitness is evaluated in this paper is that large populations in selection—mutation equilibrium have distributions of fitness that can be expressed as relatively simple functions of the rate and effects of recurrent deleterious mutations. The parameters.

generate wildly unrealistic estimates of the single-locus genomic mutation rate tend to be underestimated, relative to that expected in the absence of epistasis, whereas $s$ tends to be overestimated. When the ratio $\beta \pi / (2 \alpha)$ approaches one, so that synergistic epistasis approximately halves the average fitness of individuals relative to that expected in the absence of epistasis, the bias becomes more substantial. Even with strong epistasis, the estimates of $h$ are only altered slightly, and the estimates of $U$ are not downwardly biased by more than $\sim 40\%$, although the estimates of $s$ can be too high by a factor as large as six. Qualitatively similar results were obtained with selfing populations, although $U$ tended to be underestimated to a greater degree and $s$ overestimated to a lesser degree. These results suggest that epistasis must be quite strong for our technique to generate wildly unrealistic estimates of the single-locus parameters.

In Table 5, we consider the bias in parameter estimates that result from the application of our model to outcrossing populations when synergistic epistasis exists among the effects of deleterious alleles. In general, the biases in estimates of $U$, $s$, and $h$ are quite small provided the contribution of epistatic effects to fitness is on the order of $\leq 10\%$. The dominance coefficient and the genomic mutation rate tend to be underestimated, whereas $s$ tends to be overestimated. When the ratio $\beta \pi / (2 \alpha)$ approaches one, so that synergistic epistasis approximately halves the average fitness of individuals relative to that expected in the absence of epistasis, the bias becomes more substantial. Even with strong epistasis, the estimates of $h$ are only altered slightly, and the estimates of $U$ are not downwardly biased by more than $\sim 40\%$, although the estimates of $s$ can be too high by a factor as large as six. Qualitatively similar results were obtained with selfing populations, although $U$ tended to be underestimated to a greater degree and $s$ overestimated to a lesser degree. These results suggest that epistasis must be quite strong for our technique to generate wildly unrealistic estimates of the single-locus parameters.

**DISCUSSION**

As with the Morton-Charlesworth method, the general philosophy underlying the procedures developed in this paper is that large populations in selection-mutation equilibrium have distributions of fitness that can be expressed as relatively simple functions of the rate and effects of recurrent deleterious mutations. The validity of this approach is compromised with historically small populations, since the equilibrium frequency of deleterious alleles will then also be modified by random genetic drift to an extent depending on the relative values of the product $N_s$. Previous work (Kimura et al. 1963; Lynch et al. 1995a,b) has shown that the equilibrium expressions for fitness that we have applied provide excellent approximations for segregating mutations with selective effects ($s$) greater than $1 / N_s$, where $N_s$ is the effective population size, and still rather good approximations for $s$ a tenth of this value. Thus, roughly speaking, if the population under investigation has had an annual effective population size in excess of $N_s$ for a time span (in generations) at least a few times $N_s$ (which is long enough for an equilibrium situation to develop), one can be reasonably confident that the entire pool of mutations for which $s > 1 / N_s$ contributes to the parameter estimates derived with our technique.

In any population, there is a pool of very mildly deleterious but effectively neutral mutations [those with $s < 1 / (4N_s)$]. By exhibiting transient polymorphisms, such mutations contribute to the standing fitness properties of populations to a degree that becomes diminishingly small with increasing population size. However, it seems unlikely that the segregating pool of effectively neutral mutations will greatly influence the mutation-parameter estimates obtained with our technique. Assuming that mutations with very small effects on fitness are approximately additive ($h \approx \frac{1}{2}$), then the contribution of the effectively neutral pool of deleterious mutations to the genetic variance in fitness in a randomly mating population is approximately $N_s U_s E(s^2) / 2$, where $U_s$ is

<table>
<thead>
<tr>
<th>$s$</th>
<th>$h$</th>
<th>$\beta/\alpha$</th>
<th>$\beta \pi / (2 \alpha)$</th>
<th>$s$</th>
<th>$h$</th>
<th>$U$</th>
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<td>1.050</td>
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For each set of the parameters $s$, $h$, $U$, and $\beta$, 100 simulations were performed. Each simulation is based on 200 parents for which the genotypic fitnesses were assumed to be known without error. For outcrossing populations, $40$ selfed progeny, whose genotypes were determined by random segregation, were evaluated per parent, and the variance of fitness among selfed families was estimated by one-way analysis of variance. All mutations were assumed to have constant effects relative to that expected in the absence of epistasis, the bias becomes more substantial. Even with strong epistasis, the estimates of $h$ are only altered slightly, and the estimates of $U$ are not downwardly biased by more than $\sim 40\%$, although the estimates of $s$ can be too high by a factor as large as six. Qualitatively similar results were obtained with selfing populations, although $U$ tended to be underestimated to a greater degree and $s$ overestimated to a lesser degree. These results suggest that epistasis must be quite strong for our technique to generate wildly unrealistic estimates of the single-locus parameters.

**DISCUSSION**

As with the Morton-Charlesworth method, the general philosophy underlying the procedures developed in this paper is that large populations in selection-mutation equilibrium have distributions of fitness that can be expressed as relatively simple functions of the rate and effects of recurrent deleterious mutations. The.

\[
\beta \pi / (2 \alpha) = 1.5 
\]

\[
E(s^2) / 2 
\]
the genomic mutation rate to effectively neutral mutations and $E(s^2)$ is the mean squared homozygous effect of such mutations (LYNCH and HILL 1986). Even letting $E(s^2)$ be as large as $1/(16N_s^2)$, this implies that the genetic variance caused by effectively neutral mutations is less than $U_s/(32N_s)$. Since $U_s$ declines with increasing $N_s$ and cannot exceed the total genomic mutation rate (itself unlikely to exceed ten or so; KONDRAHSHOV 1995), this implies that effectively neutral mutations contribute negligibly to the genetic variance of fitness unless the long-term effective population size is so small that the application of our technique would be of questionable validity anyhow. Thus, the effectively neutral pool of mutations should have negligible influence on the estimable parameters $x$ and $z$ given by Equation 3, $a$ and $c$, and if very mildly deleterious mutations are additive in their effects, they will have no influence on $y$. As these are the observable quantities used in the estimation of $U$, $\bar{s}$, and $\bar{h}$, estimates of the latter parameters appear to be uninfluenced by the presence of effectively neutral mutations.

The extent to which our technique can yield unbiased estimates of the mutational parameters depends on the degree to which the fitness model employed in our derivations yields a reasonable depiction of the fitness properties of natural populations. As with the MORTON-CHARLESWORTH method, we have assumed that the relationship between fitness and numbers of mutation-bearing loci is multiplicative, i.e., that epistatic effects of mutations are of negligible significance. Our computer simulations show that synergistic epistasis causes a directional bias in the estimates of single-locus properties. However, unless epistasis is quite strong, the magnitude of the bias is not large, particularly for estimates of $\bar{h}$ and $U$.

We have developed the estimation theory in this paper under the assumption that selection-mutation balance is the primary mechanism responsible for the maintenance of genetic variation for fitness. There are, of course, alternative hypotheses for the maintenance of genetic variation for fitness, although no strong case has yet emerged for their generality. For example, functional overdominance or overdominance induced by fluctuating selection can, in principle, maintain polymorphisms for fitness-related characters. The evidence for functional overdominance is not very compelling, most cited examples being compatible with associative overdominance, an artifact of linked deleterious recessive genes (HOULE 1989, 1994). Data that directly bear on the issue of the maintenance of variation by fluctuating selection are essentially nonexistent. However, the fact that the mean persistence times of new mutations for fitness are typically shorter than 100 generations (D. HOULE, B. MORIKAWA, and M. LYNCH, unpublished data) argues against any type of balancing selection being a prominent mechanism for the maintenance of genetic variance for fitness and is quite consistent with the selection-mutation balance model as well as with empirical estimates of $\bar{s}$. For situations in which balancing-selection mechanisms are responsible for the maintenance of a large fraction of the standing variation for fitness, then the use of our model, as well as the MORTON-CHARLESWORTH estimators, is unjustified.

In principle, the approach that we have taken could be used to test the validity of the selection-mutation balance model. There are two possible approaches. First, for species in which estimates of $U$, $\bar{s}$, and $\bar{h}$ can be procured in controlled mutation-accumulation experiments, the values of $x$, $y$, and $z$ under selection-mutation balance can be predicted and then compared with those observed in natural populations. We recently used this approach to show that inbreeding depression for egg-to-adult viability in Drosophila is compatible with lab-derived estimates of $U$ and $\bar{h}$ (LYNCH et al. 1995b). However, because of the laborious nature of mutation-accumulation experiments, this is a difficult test to implement for most species. A second approach is to consider additional observable genetic properties of a study population. For the approach we have outlined, the three unknowns ($U$, $\bar{s}$, and $\bar{h}$) are estimated from functions of three observable properties ($x$, $y$, and $z$). So the validity of the model cannot be evaluated. However, estimates of other population properties are possible with our experimental design, e.g., the covariance of parents and offspring, and for outcrossing populations, the variance among selfed genotypes within parental lineages, each of which can also be expressed in terms of $U$, $\bar{s}$, and $\bar{h}$. With these additional observations (and degrees of freedom), the parameter estimates that give the best fit to the entire data set could be extracted by a maximum-likelihood procedure. The fit of the selection-mutation balance model could then be evaluated by standard procedures and compared with the fit of alternative models, such as those involving overdominance (BARTON 1990).

Assuming for the remainder of the discussion that selection-mutation balance is indeed responsible for most of the genetic variance for fitness in natural populations, there are two potential advantages to our proposed approaches to estimating deleterious-mutation parameters. First, unlike laborious laboratory mutation-accumulation experiments, which require initial lines that are genetically pure followed by many generations of careful maintenance, our approach uses individuals drawn randomly from natural populations and requires only two generations of husbandry in the laboratory (just long enough to produce clonal replicates and sexual progeny of parental individuals). Second, unlike the MORTON-CHARLESWORTH technique, which only yields estimates of the genomic mutation rate $U$, our approach generates joint estimates of $U$, the mean selection coefficient $\bar{s}$, and the mean dominance coefficient $\bar{h}$, each of which has relevance in various areas of evolutionary theory.
Although our estimators are in some cases biased, this is also true of all previously proposed estimators of mutation parameters, including the BATeman-Mukai technique used in mutation-accumulation experiments (Mukai 1979; Lynch 1994) as well as those of Morton et al. (1956) and Charlesworth et al. (1990). Knowledge of the direction of bias, as presented above, is informative as it allows one to evaluate whether empirical estimates are likely to be lower or upper bounds to the parameters being estimated. For example, it is generally assumed that the estimators used in mutation-accumulation experiments provide lower-bound estimates for $U$ and upper-bound estimates for $S$ (Mukai 1979), although as estimators, the "bounds" determined in such studies are themselves subject to error (Lynch 1994).

The bias in all existing estimators seems largely to be a consequence of the variation of mutational effects, although as we and Charlesworth et al. (1990) have shown, epistasis can also cause problems. We have shown that if one is willing to make certain assumptions about the distribution of mutational effects, it is possible to remove a substantial amount (although not all) of the bias. When variation in mutational effects and epistasis is ignored, our technique tends to lead to upwardly biased estimates of $S$, and downwardly biased estimates of $h$ and $U$. This is the same direction of bias as occurs in estimates of these parameters in laboratory mutation-accumulation experiments (Mukai 1979; Lynch 1994). Our simulations suggest that most of the bias arises from the presence of rare deleterious mutations with large, but recessive, effects. Such mutations only rarely attain high frequencies in obligately selfing populations, and as a consequence, the problem of bias appears to be substantially diminished (essentially to negligible levels) with obligately selfing populations. The problem also appears to be greatly reduced with outcrossing populations when progeny genotypes with zero fitness are excluded from the analysis.

Certain informative composite estimators that we present appear to be essentially unbiased, even with variable mutational effects, provided epistasis is not strong, e.g., the product $U_S$. Half this quantity equals the rate of decline in fitness that a small population would experience if random genetic drift totally overwhelmed the power of selection against new deleterious mutations. Thus, $U_S$ is of particular relevance to issues concerning the extinction of small populations (Lynch et al. 1993, 1995a,b). Our results show that $U_S$ is estimated quite well by the quantity $\ln [ C_W(S) + 1 ]$, where $C_W(S)$ is the squared coefficient of genetic variation of fitness among selfed families. This expression applies to either the selfed progeny in an experiment starting with an outcrossing population or to the parents directly derived from an obligately selfing population.

As with the Bateman-Mukai technique for the analysis of mutation-accumulation lines, the power of our technique derives from the information that resides in the genetic variance of fitness in parental and offspring generations (beyond that which resides in mean fitnesses). Although it is well known that accurate estimates of variance components require more sampling effort than do accurate estimates of means, the requisite estimates are achievable, provided that parental genotypes can be replicated clonally. Such replication provides the basis for cleanly separating the total genetic variance for fitness from the environmental component by statistical analysis such as ANOVA. Cloning in the progeny generation is not necessary, as the measure of interest is simply the variance in fitness among families derived from independent parents. This also can be extracted from the observed mean squares in a one-way ANOVA.

Maternal environmental effects are often a significant source of phenotypic variation among families. Where this is likely to be the case, the approach of Lynch (1985) can be used to ensure that maternal-effects variance does not contribute to the among-family component of variance. This is accomplished by taking the clonal replicates of the parental generation through a generation before assay, deriving from each of them a single (second generation) clonal descendant. Maternal effects then contribute to the within-family component of variance, and with an additional generation of clonal propagation, grand-maternal effects can be ruled out as well.

Ideally, in any practical application of our method, the performances of both parental and progeny genotypes should be assayed jointly in the same randomized design to avoid the possibility of intergenerational changes in the mean and/or variance of fitness due to environmental effects. The cloning of parental lines insures that this can be done. The necessity of clonal propagation does, of course, limit the utility of our technique to certain taxonomic groups. However, a wide variety of plants, fungi, and invertebrates are capable of both sexual reproduction and clonal expansion. Unless this subset of organisms has unusual mutational properties, results from them should be extrapolatable to related species that cannot be cloned easily.

The equations that we have presented apply to populations that consist of self-compatible hermaphrodites, a mating system that occurs commonly in plants, but less frequently in animals. In principle, our technique for outcrossing populations can be extended to species with separate sexes. In this case, the inbred generation would need to consist of families created by full-sib mating. The expressions that we presented above for the outbred parental generation still apply. Equation 1, c and d, would be replaced by

$$\bar{W}_N \approx \bar{W}_{max} \exp \left( - \frac{U(3h + 0.5)}{4h} \right), \quad (14a)$$

$$\sigma^2_W(\bar{W}) \approx \bar{W}_N^2 \left[ \exp \left( \frac{3Us}{4} \right) - 1 \right], \quad (14b)$$
where $W_{FS}$ is the mean fitness of progeny obtained by mating full sibs and $\sigma^2_{W}/(FS)$ is the variance in fitness among independent full-sib families. Approximate estimators for $U$, $\gamma$, and $\bar{F}$ are obtainable by solving Equations 1, a and b, and 14, a and b, in the manner described above.

Although the focus of this paper has been on the construction of reasonably unbiased estimators for mutational parameters, in any practical application, there is a need to procure standard errors and / or confidence intervals for the estimates as well. A reasonable approach is to apply a bootstrap procedure at the level of parental genotypes.

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LITERATURE CITED


