

# Inbreeding Load, Average Dominance and the Mutation Rate for Mildly Deleterious Alleles in *Mimulus guttatus*

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## ABSTRACT

The goal of this study is to provide information on the genetics of inbreeding depression in a primarily outcrossing population of *Mimulus guttatus*. Previous studies of this population indicate that there is tremendous inbreeding depression for nearly every fitness component and that almost all of this inbreeding depression is due to mildly deleterious alleles rather than recessive lethals or steriles. In this article I assayed the homozygous and heterozygous fitnesses of 184 highly inbred lines extracted from a natural population. Natural selection during the five generations of selfing involved in line formation essentially eliminated major deleterious alleles but was ineffective in purging alleles with minor fitness effects and did not appreciably diminish overall levels of inbreeding depression. Estimates of the average degree of dominance of these mildly deleterious alleles, obtained from the regression of heterozygous fitness on the sum of parental homozygous fitness, indicate that the detrimental alleles are partially recessive for most fitness traits, with  $\bar{h} \sim 0.15$  for cumulative measures of fitness. The inbreeding load,  $B$ , for total fitness is  $\sim 1.0$  in this experiment. These results are consistent with the hypothesis that spontaneous mildly deleterious mutations occur at a rate  $>0.1$  mutation per genome per generation.

THE offspring of consanguineous matings have reduced fitness in most outcrossing species of plants and animals (Darwin 1876; Wright 1977; Charlesworth and Charlesworth 1987). This inbreeding depression can prevent the evolution of mating systems such as self-fertilization (Darwin 1862, 1877; Lloyd 1979; Schemske 1983; Schoen 1983; Lande and Schemske 1985; Charlesworth and Charlesworth 1987, 1998). Whether it does so depends on the number of genes causing inbreeding depression and the nature of selection acting upon them. Knowledge of the genetic basis of inbreeding depression also can help explain the maintenance of genetic variation for fitness (Charlesworth and Hughes 1998), evaluate the chances of extinction for endangered or threatened species (Hedrick 1994; Lande 1994; Lynch *et al.* 1995; Charlesworth and Hughes 1998), and develop productive varieties of livestock and agricultural crops (Darwin 1868; Mather and Jinks 1982). While data on the genetics of inbreeding depression are scarce, there is increasing evidence from a few well studied plants and animals that inbreeding depression is due largely to deleterious recessive alleles rather than overdominant loci (Moll *et al.* 1964; Simmons and Crow 1977; Mather and Jinks 1982; Crow and Simmons 1983; Crow 1993; Jinks 1983; Sprague 1983; Charlesworth and Charlesworth 1987; Fu and Ritland

1994; Charlesworth and Hughes 1998; Dudash and Carr 1998). To evaluate the impact of deleterious alleles on evolution and extinction, we need to know (i) the relative contribution of major deleterious alleles like lethals and steriles to inbreeding depression, (ii) the degree of dominance of deleterious alleles, and whether severely harmful alleles are more recessive on average than more mildly deleterious alleles, and (iii) the rate at which deleterious alleles are introduced into a population by mutation.

The best data on the genetic properties of deleterious alleles in natural populations come from over 60 years of studies of inbreeding load in several species of *Drosophila* (reviewed by Lewontin 1974; Simmons and Crow 1977; Crow and Simmons 1983; Charlesworth and Hughes 1998). Several important conclusions can be made from these studies about the nature of genes underlying inbreeding depression in fruit flies. First, about half of the inbreeding depression for egg-to-adult viability is due to lethals or sublethals, and the remainder is due to a larger number of mildly deleterious alleles. While less extensive studies have been conducted on other fitness components, it is clear that both major and minor deleterious alleles contribute to the inbreeding load for traits like male and female fertility. Second, alleles of large effect, like lethals and sterility mutations, are almost completely recessive. In contrast, crosses between viable chromosomal homozygotes show that mildly deleterious alleles are only partially recessive. Third, viable chromosomal homozygotes with low values for one fitness component also tend to have low values

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for other fitness components, suggesting extensive pleiotropic effects of mildly deleterious alleles. Finally, studies of both spontaneous mutations and inbreeding load in natural populations suggest that the genomic mutation rate to mildly deleterious alleles far exceeds that to lethals, and may be on the order of one mildly deleterious mutation per genome per generation (but see Keightley 1994, 1996; Garcia-Dorado 1997). Unfortunately, such detailed studies of inbreeding depression have not been done on any other organism, so it is not clear to what extent the conclusions from *Drosophila* apply to other species.

In principle, similar analyses of inbreeding load can be conducted in any outcrossed species that can be bred in captivity by rapidly inbreeding lines initiated from a randomly mated ancestral population. The rapid formation of highly inbred lines is especially easy in plants that can be self-fertilized, because with selfing heterozygosity at neutral loci decreases by 50% each generation. Nearly complete homozygosity can be achieved in several generations. Because each selfed line has an effective population size of approximately one, natural selection during the process of inbreeding quickly eliminates lethals or steriles, while the dynamics of mildly deleterious alleles approximate effective neutrality. The performance of many of these lines can then be studied in the nearly homozygous state, or the lines can be crossed to study the performance of the heterozygotes. The use of inbred lines for the analysis of mildly deleterious alleles has advantages over the use of single-chromosome extractions in *Drosophila*, because inbred lines are asymptotically homozygous for all nuclear loci. Properties of the whole genome, such as total inbreeding load and other related quantities, must be estimated by extrapolation in chromosomal studies in *Drosophila*.

Here I report on an experiment that makes use of highly inbred lines to examine the nature of inbreeding load due to mildly deleterious alleles in a mainly outcrossing wild population of the common yellow monkeyflower, *Mimulus guttatus*. Previous studies of this population have demonstrated that there is substantial inbreeding depression for fitness components expressed at nearly every stage of the plant's annual life cycle (Willis 1993a,b, 1999b). Genetic analyses indicate that almost all of this inbreeding depression is due to deleterious alleles of minor effects rather than to alleles of large effect like lethals or steriles (Willis 1992, 1999a,b). In this study I quantify the inbreeding load due to these mildly deleterious alleles, determine the degree to which variation in fitness and its components is heritable, and estimate the average degree of dominance of the alleles causing inbreeding depression. Because the inbreeding load and average degree of dominance are estimated in a single experiment, I then estimate the genomic mutation rate to deleterious alleles under the hypothesis that the observed inbreeding

depression is solely due to deleterious alleles maintained by mutation-selection balance.

## MATERIALS AND METHODS

**Study species and population:** *M. guttatus* is a common, self-compatible wildflower native to western North America that exhibits tremendous differentiation among populations in terms of life history, floral and vegetative morphology, genetic variability, degree of self-fertilization, and inbreeding depression (Vickery 1978; Ritland and Ganders 1987a,b; Ritland and Ritland 1989; Ritland 1990; Dudash and Ritland 1991; Fenster and Ritland 1992; Dole and Ritland 1993; Willis 1993b; Carr and Fenster 1994; Carr and Dudash 1996; Galloway 1995; Awadalla and Ritland 1997; Kelly and Willis 1998). In this article I study an annual population of *M. guttatus* that is located on Iron Mountain in the Western Cascades of Oregon. This population consists of hundreds of thousands of individual plants and reproduces mainly through outcrossing via bee pollinators, although some self-fertilization does occur. The estimated rate of self-fertilization for this population ranged from 0.09 to 0.24 over 2 yr (Willis 1993b) and there is no evidence for biparental inbreeding (Willis 1993b; Sweigart *et al.* 1999). This population also exhibits substantial inbreeding depression in the field and greenhouse for most components of fitness and cumulative measures of lifetime gamete production as well as for other quantitative traits like flower morphology and flowering time (Willis 1992, 1993a,b, 1996, 1999a,b).

**Formation of inbred lines:** To study in detail the genetic properties of the alleles that cause inbreeding depression in this population, I formed from it a large number of highly inbred lines using a simple method described in Willis (1999b). I collected 530 unrelated maternal seed families from the wild Iron Mountain population and grew one seedling from each family to flowering in the greenhouse at the University of Oregon. All 530 plants were randomly outcrossed, using emasculated flowers, to produce 1200 full-sib families, with the condition that a plant served as a parent for no more than 5 full-sib families. Seed from each of the 1200 families was sown in the greenhouse on Sunshine soil-less potting mix with supplemental light from 230-watt sodium lamps (16-hr days), and one seedling per family was randomly chosen to serve as parent for the formation of 1200 inbred lines. The resulting 1200 plants were raised to flowering and self-fertilized to form the progeny for the next generation. This procedure of single seedling descent and self-fertilization was repeated for a total of five generations of selfing. During the inbreeding process lines were allowed to go extinct due to a number of genetic and environmental factors, including seed germination failure, plant mortality, and male or female sterility. After five generations of selfing, ~72% of the lines had been lost, leaving a total of 335 inbred lines that were largely purged of major deleterious alleles like recessive chlorophyll-deficient lethals and nuclear male sterility alleles (Willis 1999b). In another experiment, I compared the inbreeding depression in the ancestral population of full-sib families to that in an outcrossed population formed by randomly intercrossing the inbred lines and found that almost none of the original inbreeding depression had been eliminated by the purging process. The results strongly indicate only a minor contribution of alleles of large effect to inbreeding depression in the Iron Mountain population (Willis 1999b).

**Crossing design and estimation of the degree of homozygosity in the inbred lines:** At the start of this experiment, a random sample of 184 of the fifth generation inbred lines were selected for further genetic analysis. Seed from these lines were sown,

and a randomly selected seedling from each line was chosen to initiate this experiment. The seedlings were raised to flowering as described above and then were randomly paired. Each plant was self-fertilized to produce generation six inbred lines, and the paired plants were crossed to form 92 outbred lines. One plant from each pair was randomly chosen to serve as the maternal parent for the outcrossed progeny. Because some crosses were unsuccessful in producing seed due to culturing accidents, the actual number of inbred lines formed was 176, and the number of outbred lines formed was 88. A total of 84 pairs of parental plants successfully produced complete sets of the two sixth generation inbred lines and 1 outbred line per pair.

The six generations of self-fertilization should produce inbred lines that are homozygous at most loci. More specifically, the inbreeding coefficient,  $F$ , for the set of inbred lines after six generations of selfing is expected to be  $F = 1 - (1/2)^6 = 0.984$  for neutral alleles. To determine the actual extent of inbreeding in the set of inbred lines, we estimated the inbreeding coefficient by genotyping a single plant from 80 of the generation six inbred lines at two highly polymorphic microsatellite loci, AAT9 and AAT356. The expected heterozygosities of these markers in the Iron Mountain population are in excess of 0.8, so they are particularly suitable for estimating the reduction in heterozygosity due to selfing. These microsatellite markers and the methods of DNA extraction, PCR, and electrophoretic detection and sizing of PCR products are identical to those described elsewhere (Kelly and Willis 1998; Sweigart *et al.* 1999).

The inbreeding coefficient for the population of inbred lines was estimated for each marker as  $F = 1 - (H_{\text{obs}}/H_{\text{exp}})$ , where  $H_{\text{obs}}$  is the observed frequency of heterozygote and  $H_{\text{exp}}$  is the heterozygosity expected for that locus with random mating. The expected heterozygosity was calculated as  $H_{\text{exp}} = 1 - \sum p_i^2$ , where  $p_i$  is the observed frequency of the  $i$ th allele. Variances and standard errors of these estimates were obtained via bootstrapping by resampling individual plants with replacement. The bootstrap method was applied to each locus, with 1000 resamplings. A weighted average  $F$  and its standard error were calculated from the two single-locus estimates by using the two bootstrapped variances as the weights.

**Measurement of fitness in the inbred and outbred lines:** To compare measures of fitness between the inbred and outbred plants, I raised replicates from each line under common greenhouse conditions and measured fitness components expressed throughout the life cycle on all plants. Six seeds from a line were sown in 2.25-in. plastic pots filled with soil-less potting mix, with 5 replicate pots planted per line. A few families had <30 seeds, and in these cases all seeds were evenly divided among the 5 pots and the number of seeds planted in each pot was noted. A total of 1320 pots [(176 inbred lines + 88 outbred lines)  $\times$  5 pots per line] and  $\sim$ 7900 seeds were planted. Pots were placed in a completely randomized order on a single greenhouse bench under 230-W sodium lights (16-hr days) and were watered regularly without additional fertilizer to maintain soil moisture. The positions on the greenhouse bench of the flats containing the pots were rotated weekly. Pots were censused for all seedlings 14 days after planting, as germination had essentially ceased. One seedling per pot was randomly selected to be allowed to grow to maturity, and the rest of the seedlings were removed from the pot. Whenever seedlings failed to emerge in a given pot, a randomly selected seedling was transplanted, with minimal disruption of soil and roots, from another replicate pot for that line if possible. The fraction of seeds germinated for a given line was calculated as the total seedlings that emerged in all replicate pots divided by the total number of seeds planted.

The majority of seedlings began to flower within 4–5 wk of

planting, and many had started to senesce by 6 or 7 wk. Each plant that flowered within 8 wk of planting was scored for measures of male and female fertility per flower and for the number of flowers produced at the end of 8 wk. Plants that had not flowered within 8 wk or had died without flowering were scored as having produced zero flowers, and had unknown values of male and female fertility per flower. Therefore the fitness component flower number includes post-germination survival.

I estimated female fertility per flower on the first flower produced on each flowering plant as the number of seeds produced following hand-pollination with excess viable pollen. On the day that the first flower of each plant opened, excess pollen from a highly male-fertile inbred line (reference number IM62, inbreeding coefficient  $>0.98$ ) was applied to the receptive stigma. In this assay of female fertility, seed production should not be limited by viable pollen, so seed counts should be approximately equal to the number of viable ovules per flower. A uniform pollen source was used to pollinate all plants to ensure that differences in seed production were not due to variation in pollen quality. Variation in seed number could not have been influenced by self-incompatibility, because *M. guttatus* lacks such a system. The resulting outcrossed fruits were monitored until the seeds were ripe, and then the fruits were harvested and the seeds counted. These seed counts are referred to as the number of ovules per flower.

Male fertility per flower was estimated by counting viable and inviable pollen grains from the first two flowers produced on each flowering plant. On the day that a first or second flower opened, all four anthers were removed from the corolla and placed in an Eppendorf tube containing 60  $\mu$ l of aniline blue in lactophenol (Radford *et al.* 1974; Kearns and Inouye 1993). Anthers were lightly crushed with glass rods and forceps tips to release the pollen. A single Eppendorf tube was used for each plant, so that pollen from the first two flowers was combined. Aniline blue stains pollen grains with intact cytoplasm a dark blue, while aborted pollen grains lacking starchy cytoplasm stain a pale blue. Because it is possible for inviable pollen grains to have intact cytoplasm, the number of unstained pollen grains was taken to be a conservative estimate of pollen inviability. Aliquots from the Eppendorf tubes were placed in hemacytometers, and all the pollen grains in four separate regions of 0.2  $\mu$ l were counted under a compound microscope as being either viable or inviable. Because all pollen grains in a total volume of 0.8  $\mu$ l were counted, and the total volume in each sample was 60  $\mu$ l, the counts presented in this article represent  $\sim$ 1.3% of the total number of pollen grains produced in the first two flowers. Because these counts are proportional to the average number of pollen grains produced on the first two flowers, they are referred to as the number of pollen grains per flower. Three components of male fertility per flower were obtained from these data: the number of viable pollen grains per flower, the fraction of viable pollen grains, and the total number of pollen grains per flower.

In total six fitness components were measured in this study: the proportion of seeds that germinated, the number of flowers produced per seedling, the number of viable pollen grains per flower, the fraction of pollen grains that were viable, the total number of pollen grains per flower, and the number of ovules per flower. Cumulative measures of fitness were calculated in three ways. Cumulative measures estimating fitness through either male or female function are referred to as “germination to viable pollen” or “germination to ovules,” respectively. These were obtained for each line by multiplying the fraction of seed germinated in each line by the average of the total number of pollen grains (flower number  $\times$  viable

pollen grains per flower) or ovules (number of flowers  $\times$  number of ovules per flower) produced by each of five replicates per line.

The third estimate of fitness is a measure of total lifetime fitness through both male and female function. To combine male and female gamete production into a single measure of fitness, I first equalized the average outcrossed fitness through male and female function. This standardization of male and female gamete production implies that on average each outcrossed plant in the ancestral population successfully reproduces itself. This is reasonable, because the Iron Mountain population is roughly constant in population size from year to year and is primarily outcrossing. I divided each plant's total number of viable pollen by the mean total number of viable pollen for the outcrossed plants. I also divided each plant's total number of ovules by the mean total number of ovules of the outcrossed plants. To compute, for each line, a measure of cumulative fitness through lifetime gamete production, I simply summed each plant's standardized male and female gamete production and averaged these values across the five replicates for each line. I then multiplied this average total gamete production by the fraction of seeds that germinated in that line. This measure of total lifetime fitness is referred to as "germination to total gametes."

**Estimation of the inbreeding load:** To compare the average fitness of outbred and the inbred plants, I first calculated the means of each line for all the fitness components and the cumulative measures of fitness. All subsequent analysis of inbreeding load was done using these line means. The mean and standard error for each fitness trait was then estimated from the values of all inbred and outbred lines for which there were data. For statistical comparisons of the inbred and outbred line means, I averaged the mean fitnesses for the two inbred lines derived from a pair of parental inbred lines and compared it to the mean of the corresponding outbred line for that parental pair using *t*-tests. Only those parental pairs that had mean fitness estimates for the two inbred lines and the outbred line were included in the paired *t*-tests. Apparently there is little bias in excluding the several parental pairs that lacked complete inbred and outbred data, because the means for the two classes of lines included in the paired *t*-test were nearly identical to the means obtained from all the lines.

The reduction of fitness caused by inbreeding was estimated in two ways. The first was simply the average fitness of the inbred lines,  $\bar{w}_i$ , divided by the average fitness of the outbred lines,  $\bar{w}_o$ . This ratio,  $\bar{w}_i/\bar{w}_o$ , was calculated from all of the line means for each fitness component and for the cumulative measures of fitness. Uncertainty in both the numerator and the denominator causes the expected value of the ratio to not be equal to the ratio of the expected values, although the bias should be small, and there is no simple expression for the standard error of this ratio. For these reasons, the standard errors for the estimates of  $\bar{w}_i/\bar{w}_o$  were obtained by using a delete-one jackknife procedure. First, for each pair of parental plants, I calculated the mean fitness for the outbred line and the mean fitness of the two inbred lines as described above for the paired *t*-tests. For each run, the outbred and inbred means for a different parental pair were deleted from the data set, and a pseudo-value of ratio of inbred mean fitness to outbred mean fitness was recalculated. The standard deviation of the distribution of these pseudo-values was taken to be the standard error of the population estimate of inbred relative fitness,  $\bar{w}_i/\bar{w}_o$  (Sokal and Rohlf 1981).

The second measure of the effects of inbreeding on fitness is the inbreeding load, *B*, defined as minus the regression coefficient of  $\ln$  (mean fitness) on the inbreeding coefficient, *F* (Morton *et al.* 1956; Charlesworth and Charlesworth

1987; Lynch and Walsh 1998). Because inbreeding load should be calculated on a scale in which the mean outbred fitness is one, and in this study the six generation selfed lines were essentially completely inbred ( $F \approx 1$ ), I estimated the inbreeding load in this study for every component of fitness and cumulative measure of fitness as  $B = -\ln(\bar{w}_i/\bar{w}_o)$ . Standard errors for *B* were obtained by the delete-one jackknife procedure as described above.

**Genotypic variances for fitness in the inbred and outbred lines:** To determine the extent to which variation in the fitness traits is due to genotypic differences among the lines, I conducted one-way analyses of variance with line as the main effect. These ANOVAs were done separately for the outbred and inbred lines for all fitness components and cumulative measures of fitness. Because these ANOVAs require replicates within lines, some trait values were calculated differently for these analyses than as described above. For the ANOVAs, seed germination was calculated separately for each replicate pot. Cumulative measures of fitness were calculated separately for each replicate pot by multiplying its seed germination by its post-germination male, female, or total gamete production. The model II among line components of variance provided estimates of the genotypic variance,  $V_G$ , while the mean squares within lines provided estimates of the environmental variances,  $V_E$ . Genotypic coefficients of variation,  $CV_G$ , for the outbred and inbred lines were calculated as the genotypic standard deviation divided by the means of the outbred and inbred lines, respectively, multiplied by 100 (Houle 1992). Broad-sense heritabilities,  $H^2$ , were calculated as  $H^2 = V_G/V_P$ , where the phenotypic variance,  $V_P$ , is  $V_G + V_E$ .

**Estimation of the average degree of dominance:** The relationship between the homozygous and heterozygous fitnesses of inbred lines can provide information on the degree of dominance of deleterious alleles segregating in a natural population (Mukai *et al.* 1972; Mukai and Yamaguchi 1974). To understand why this is, consider the situation where fitness variation in a population is due to a number of diallelic autosomal loci, each with a wild-type allele and a deleterious allele. At the *i*th locus, the frequency of the deleterious allele is  $q_i$  (and  $p_i = 1 - q_i$ ) and the fitnesses of the wild-type homozygote, heterozygote, and deleterious homozygote are 1,  $1 - h_i s_i$ , and  $1 - s_i$ , respectively. The degree of dominance,  $h_i$ , is 0 if the deleterious allele is completely recessive and is 0.5 if the alleles are additive. Inbreeding depression at the *i*th locus will occur as long as  $h_i < 0.5$ . Many homozygous lines are extracted from this population without a change in the allele frequencies on average, and these lines are paired at random and crossed to produce heterozygous outbred lines. The regression coefficient, *b*, of the genotypic value of the outbred fitness on the sum of the genotypic values for the two parental inbred line fitnesses is

$$b = \frac{\sum_i p_i q_i s_i^2 [h_i + q_i(1 - 2h_i)]}{\sum_i p_i q_i s_i^2 (1 + 2q_i)}$$

If the deleterious alleles are rare, so that two inbred lines homozygous for the same deleterious allele essentially never are crossed to produce an outbred line ( $q^2 \approx 0$ ), then Mukai and colleagues showed that the regression coefficient is equal to a weighted average degree of dominance over loci,

$$\bar{h} \approx \frac{\sum_i p_i q_i s_i^2 h_i}{\sum_i p_i q_i s_i^2}$$

where the weights are proportional to the contribution that a locus makes to the genotypic variance in the collection of homozygous lines. This average degree of dominance also is interpreted to be equal to the harmonic mean degree of dominance of newly arising mutations weighted by the selec-

tive effect and mutation rate of the deleterious mutations (Simmons and Crow 1977, but see Caballero *et al.* 1997).

I estimated the average degree of dominance,  $\bar{h}$ , in this study by linear regression of the outbred line mean fitness on the sum of the parental inbred line mean fitnesses. Because the sums of the genotypic values for the parental inbred line fitnesses are estimated with some uncertainty, the regression coefficient is biased downward by the reliability ratio,  $k$ . I estimated this reliability ratio as  $k = V_G / (V_G + V_u)$ , where  $V_G$  is the estimate of the genotypic variance among inbred lines and  $V_u$  is the error variance for the individual estimates of the inbred line means (Caballero *et al.* 1997).  $V_u$  was estimated as  $V_E$  for the inbred lines divided by the effective number of replicates per line, taking into account the unbalanced nature of the data. To obtain an unbiased estimate of the regression coefficient, I simply divided the raw estimate of  $b$  by  $k$  (Caballero *et al.* 1997). Standard errors and 95% confidence limits of the unbiased regression coefficients were obtained as described in Sokal and Rohlf (1981, p. 473). Tests of whether the unbiased estimate of the regression coefficient was equal to zero were conducted with  $t$ -tests.

Researchers typically estimate  $\bar{h}$  by regression using raw values of fitness components (Hughes 1995). If the fitnesses of different loci combine multiplicatively, then it is more appropriate to estimate  $\bar{h}$  from regressions using ln-transformed line means (Deng 1998; Deng and Fu 1998). In this study I present results from both types of regressions to facilitate comparisons for other studies.

RESULTS

**Degree of homozygosity in the inbred lines:** The results from the microsatellite genotypic data for a subset of the generation six inbred lines are presented in Table 1. We observed the large numbers of alleles that are typical for both markers in the Iron Mountain popula-

TABLE 1

The estimated degree of inbreeding among the inbred lines obtained from genotypic data for two microsatellite loci

Locus	<i>N</i>	<i>n<sub>a</sub></i>	<i>H<sub>exp</sub></i>	<i>H<sub>obs</sub></i>	<i>F</i> (SE)
AAT9	65	18	0.927	0.015	0.983 (0.016)
AAT356	70	18	0.827	0.057	0.931 (0.029)
Average			0.866	0.029	0.971 (0.014)

Presented for each marker are the number of individuals genotyped,  $N$ , the number of alleles found,  $n_a$ , the heterozygosity expected with random mating,  $H_{exp}$ , the observed heterozygosity,  $H_{obs}$ , and the estimated inbreeding coefficient,  $F$ , with its standard error. The inbreeding coefficient expected after six generations of self-fertilization if  $F_{exp} = 1 - (1/2)^6 \approx 0.984$ .

tion. Because of this considerable allelic variation, the expected heterozygosity was very high for both markers: it was  $\sim 0.93$  for AAT9 and 0.83 for AAT356. These values are almost identical to those obtained from a sample of naturally occurring plants from this population (Sweigart *et al.* 1999). Despite the high expected heterozygosity, only 1 of 65 inbred lines was heterozygous for AAT9 and 4 of 70 were heterozygous for AAT356. The estimated inbreeding coefficients for the two loci were all large, and the variance-weighted average  $F$  for the two markers was 0.971, with a standard error of 0.014 (Table 1). Clearly this observed level of inbreeding is not significantly different from the inbreeding coefficient expected after six generations of self-fertilization, since  $F_{exp} = 1 - (1/2)^6 \approx 0.984$ .

TABLE 2

Means and standard errors of fitness components and cumulative measures of fitness for outcrossed and inbred lines

Trait	Type of line	No. of lines	Mean	SE	<i>t</i> -value
Proportion of germination	Outcrossed	88	0.76	0.02	4.54*
	Inbred	174	0.63	0.02	
Number of flowers	Outcrossed	87	9.57	0.41	5.59*
	Inbred	161	6.97	0.30	
Viable pollen per flower	Outcrossed	87	218.00	5.67	17.56*
	Inbred	165	111.67	4.64	
Fraction of viable pollen	Outcrossed	87	0.74	0.01	14.03*
	Inbred	165	0.50	0.01	
Pollen grains per flower	Outcrossed	87	287.08	5.35	13.12*
	Inbred	165	203.05	5.41	
Ovules per flower	Outcrossed	87	151.22	5.13	12.00*
	Inbred	164	84.55	3.22	
Germination to viable pollen	Outcrossed	87	1683.11	107.18	10.21*
	Inbred	161	567.20	45.40	
Germination to ovules	Outcrossed	87	1144.27	79.27	8.43*
	Inbred	160	466.51	37.20	
Germination to total gametes	Outcrossed	87	1.55	0.10	9.83*
	Inbred	160	0.58	0.04	

\*  $P < 0.0001$ .

TABLE 3

Estimates and standard errors of the ratio of mean inbred fitness to mean outbred fitness,  $\bar{w}_1/\bar{w}_0$ , and the inbreeding load,  $B = -\ln(\bar{w}_1/\bar{w}_0)$

Trait	$\bar{w}_1/\bar{w}_0$	SE	$B = -\ln(\bar{w}_1/\bar{w}_0)$	SE
Proportion of germination	0.831	0.033	0.185	0.040
Number of flowers	0.728	0.044	0.317	0.060
Viable pollen per flower	0.512	0.022	0.669	0.042
Fraction of viable pollen	0.676	0.020	0.391	0.029
Pollen grains per flower	0.707	0.020	0.346	0.028
Ovules per flower	0.559	0.026	0.581	0.047
Germination to viable pollen	0.337	0.032	1.088	0.094
Germination to ovules	0.408	0.039	0.897	0.096
Germination to total gametes	0.375	0.033	0.982	0.088

**Inbreeding load:** The means and standard errors of all fitness components and cumulative measures of fitness are presented in Table 2. Inbred lines had substantially lower mean values for all traits measured than the outbred lines, and this inbreeding depression was highly statistically significant for all traits. Two measures of the decline in fitness with inbreeding are presented in Table 3. The first, the mean fitness of inbred lines relative to the mean of outbred lines, ranged for fitness components from 0.83 for seed germination to 0.51 for the number of viable pollen grains per flower. The average relative inbred fitness for the four independent fitness components (germination, number of flowers, number of viable pollen per flower, and number of ovules per flower) is about 0.66. Inbred lines had even lower average cumulative measures of fitness relative to outbred lines: inbred lines had only ~33 to 40% of the cumulative fitness of outbred lines (Table 3). The second measure of inbreeding's effect on fitness, the inbreeding load,  $B$ , is also presented in Table 3. The inbreeding load for the fitness components ranges from ~0.19 for germination to 0.67 for viable pollen per flower, with

a mean for the four independent fitness components of 0.44. Inbreeding load was much larger for the cumulative measures of fitness, ranging from ~0.90 to 1.09 (Table 3).

**Genotypic variances:** The results for the one-way analyses of variance on the inbred lines are presented in Table 4, while those for the outbred lines are presented in Table 5. The variation among lines is highly significant for all fitness components and cumulative measures of fitness for both the inbred and outbred lines. Tables 4 and 5 also present the inbred and outbred genotypic components of variance,  $V_G$ , as well as the genotypic coefficients of variation,  $CV_G$ , and the broad-sense heritabilities,  $H^2$ . For the inbred lines, the  $CV_G$  for fitness components range from 28% for the number of pollen grains per flower to 46% for the number of viable pollen grains per flower, while those for the cumulative measures of fitness are between ~85–96% (Table 4). The values for the  $CV_G$  for inbred lines are all larger than those for the outbred lines, which range from 11 to 26% for the fitness components and from 44 to 49% for the cumulative measures of fitness. A similar pattern

TABLE 4

Genotypic components of variance and coefficients of variation for all fitness components and cumulative measures of fitness of the inbred lines

Trait	Among lines		Within lines		$P$	$V_G$	$CV_G$	$H^2$
	DF	MS	DF	MS				
Proportion of germination	175	0.311	669	0.072	<0.0001	0.050	35.2	0.410
Number of flowers	160	58.980	522	16.773	<0.0001	9.953	45.3	0.372
Viable pollen per flower	164	$1.66 \times 10^4$	620	$3.62 \times 10^3$	<0.0001	$2.73 \times 10^3$	46.8	0.430
Fraction of viable pollen	164	0.154	619	0.024	<0.0001	0.027	32.7	0.529
Pollen grains per flower	164	$2.21 \times 10^4$	620	$6.09 \times 10^3$	<0.0001	$3.36 \times 10^3$	28.5	0.355
Ovules per flower	163	$7.59 \times 10^3$	584	$2.16 \times 10^3$	<0.0001	$1.19 \times 10^3$	40.8	0.356
Germination to viable pollen	160	$1.48 \times 10^6$	451	$3.59 \times 10^5$	<0.0001	$2.96 \times 10^5$	95.9	0.452
Germination to ovules	159	$9.33 \times 10^5$	426	$3.21 \times 10^5$	<0.0001	$1.67 \times 10^5$	87.7	0.343
Germination to total gametes	159	1.229	422	0.339	<0.0001	0.245	85.3	0.420

TABLE 5  
Genotypic components of variance and coefficients of variation for all fitness components and cumulative measures of fitness of the outbred lines

Trait	Among lines		Within lines		<i>P</i>	$V_G$	$CV_G$	$H^2$
	DF	MS	DF	MS				
Proportion of germination	87	0.211	343	0.042	<0.0001	0.035	24.4	0.453
Number of flowers	86	55.323	273	29.524	<0.0001	6.239	26.1	0.175
Viable pollen per flower	86	$1.36 \times 10^4$	336	$5.89 \times 10^3$	<0.0001	$1.59 \times 10^3$	18.3	0.213
Fraction of viable pollen	86	0.067	336	0.016	<0.0001	0.010	13.5	0.385
Pollen grains per flower	86	$1.23 \times 10^4$	336	$7.26 \times 10^3$	0.0006	$1.03 \times 10^3$	11.2	0.124
Ovules per flower	86	$9.95 \times 10^3$	330	$4.03 \times 10^3$	<0.0001	$1.24 \times 10^3$	23.2	0.235
Germination to viable pollen	86	$4.25 \times 10^6$	263	$1.89 \times 10^6$	<0.0001	$5.85 \times 10^5$	45.5	0.236
Germination to ovules	86	$2.42 \times 10^6$	259	$1.20 \times 10^6$	<0.0001	$3.08 \times 10^5$	48.5	0.257
Germination to total gametes	86	3.492	257	1.623	<0.0001	0.473	44.4	0.226

of greater genotypic variance in the inbred lines than the outbred lines is apparent in the broad-sense heritabilities,  $H^2$ : the values for all but one trait are larger in the inbred lines.  $H^2$ 's for the inbred line fitness components range from 0.35 to 0.53, with values of 0.34 to 0.45 for the cumulative measures of fitness. Those for the outbred fitness components range from 0.12 to 0.45, while the  $H^2$ 's for the cumulative measures of fitness range from 0.22 to 0.26. Increases of genotypic variances are expected with inbreeding (Lynch and Walsh 1998). Clearly there is a substantial proportion of the phenotypic variance that is due to genotypic differences among both the inbred and outbred lines.

**Average degree of dominance:** To obtain unbiased estimates of the regression coefficient of outbred line fitness on the sum of the parental inbred line fitnesses, I first calculated the reliability ratio,  $k = V_G / (V_G + V_W)$ , as described above. This ratio was estimated from the inbred line ANOVA results presented in Table 4. In particular,  $k > 0.7$  for all traits, indicating that this experiment provided relatively reliable estimates of the parental fitnesses (Table 6). Because some replicates

within lines had values of zero for nearly every fitness component,  $k$  was not calculated separately for ln-transformed data. Comparisons of  $k$  obtained from subsets of the data that lacked zeroes indicated that  $k$ 's obtained from ln-transformed data were nearly identical to those obtained from raw fitness data (results not shown). For these reasons, the  $k$ 's presented above were used to correct the bias in the estimate of the regression coefficient for regressions with both untransformed fitness values and ln-transformed line mean fitness values.

The relationship between raw fitness for outbred lines and the sum of the raw fitnesses for their parental inbred lines is presented in Table 6. Most of the regression coefficients are significantly  $>0$ , except for that for the number of flowers, germination to ovules, and germination to total gametes. The significant estimates of the coefficients for the fitness components range from 0.109 for proportion germination to 0.329 for viable pollen per flower. For the cumulative measures of fitness, the coefficients range from 0.23 to 0.30. Regression coefficients based on raw fitness values, such as these, have traditionally been interpreted as a weighted average de-

TABLE 6  
The reliability ratio,  $k$ , and regressions of outbred line fitnesses on the sum of the parental inbred line fitnesses

Trait	$k$	Slope	SE	95% confidence limits	<i>t</i> -value
Proportion of germination	0.769	0.109	0.055	(0.0001, 0.217)	1.99*
Number of flowers	0.716	0.046	0.089	(-0.131, 0.223)	0.52
Viable pollen per flower	0.782	0.329	0.067	(0.196, 0.461)	4.94***
Fraction of viable pollen	0.844	0.166	0.053	(0.062, 0.271)	3.17**
Pollen grains per flower	0.724	0.240	0.050	(0.140, 0.341)	4.76***
Ovules per flower	0.716	0.303	0.086	(0.132, 0.474)	3.52***
Germination to viable pollen	0.758	0.301	0.148	(0.006, 0.597)	2.03*
Germination to ovules	0.656	0.230	0.132	(-0.033, 0.493)	1.74
Germination to total gametes	0.724	0.276	0.147	(-0.016, 0.567)	1.88

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

gree of dominance (Hughes 1995). If one were to similarly interpret the regressions of raw fitness in this study, one would conclude that the deleterious alleles causing inbreeding depression are partially recessive on average for nearly all traits.

Unfortunately, it is doubtful that the regression on raw fitness should be interpreted as the average degree of dominance. Inspection of the equation for  $\bar{h}$  presented above indicates that the regressions should be conducted on a scale in which the fitness effects of each locus combine additively. For example, if the fitnesses of different loci actually combine multiplicatively, then loci are additive on ln-scale and the regression should be conducted with ln-transformed data. Deng and Fu (Deng 1998; Deng and Fu 1998) have recently shown that if fitnesses are multiplicative, then estimates of  $\bar{h}$  in outcrossing populations using raw fitnesses are severely biased upward, sometimes considerably above the biologically plausible upper limit of 0.5, and they have large sampling variance. In contrast, estimates of  $\bar{h}$  using ln-transformed fitnesses are unbiased, with smaller variance.

In a previous study of this population, I showed that the relationship between fitness and inbreeding coefficient for traits such as seed germination and number of flowers was consistent with multiplicative fitnesses, while pollen viability showed significant synergistic epistasis (Willis 1993a). Data from similar experiments in other plant and animal species suggest that the decline in ln fitness is approximately linear with  $F$ , suggesting that locus-specific fitnesses are approximately multiplicative (reviewed in Charlesworth 1990; Willis 1993a). A caveat is that the power to detect deviations from linearity from such experiments is often quite low, and many of the studies seem to indicate that the decline in fitness with inbreeding is also roughly linear on the scale of raw fitness (Willis 1993a; Lynch and Walsh 1998). An additional reason for estimating  $\bar{h}$  using ln-transformed fitnesses is that the inbreeding load,  $B$ , is also estimated using ln-transformed fitnesses. Because  $B$  and  $\bar{h}$  can be used to estimate the genomic deleterious mutation rate (*i.e.*, Morton *et al.* 1956), they should be estimated on the same scale. Finally, the distributions of both the inbred and outbred line means for many of the traits are approximately normal on a ln-scale, while they are strongly skewed on the raw fitness scales (data not shown). This observation provides support, albeit weak and indirect, for the idea that the different genetic and environmental factors underlying these distributions are roughly additive on the ln-scale. For all of these reasons, in this study I equate  $\bar{h}$  with the regression coefficient obtained from regression using ln fitness.

Estimates of  $\bar{h}$  from regressions of ln(mean outbred fitness) on ln(sum of means for the parental inbred lines) are presented in Table 7. In contrast to the regression coefficients obtained from raw fitness data, most

of the estimates of  $\bar{h}$  are quite small and are roughly consistent among traits. Nonetheless, all estimates of  $\bar{h}$  are significantly  $>0$ , except for number of flowers, owing to the uniformly small standard errors of the estimates. The estimates of  $\bar{h}$  for the fitness components that are significantly  $>0$  range from 0.09 for viable pollen per flower to 0.21 for proportion seed germination. The average of the estimates of  $\bar{h}$  for the four independent fitness components (germination, number of flowers, viable pollen, and ovules per flower) is 0.13. Similar results are obtained for the cumulative measures of fitness, with estimates of  $\bar{h}$  ranging from 0.10 to 0.14. The upper 95% confidence limits on the estimates of  $\bar{h}$  for all but one trait, germination, are  $<0.26$ . These results strongly suggest that the deleterious alleles affecting the individual fitness components are quite recessive on the average.

## DISCUSSION

In the last 15 years, numerous studies have quantified the effects of inbreeding on fitness in natural populations of plants (reviewed by Husband and Schemske 1996). This sudden increase in the number of inbreeding depression studies in plants was prompted in large part by the role that inbreeding depression plays in theories of the evolution of self-fertilization and other plant mating systems (*e.g.*, Lloyd 1979; Charlesworth 1980; Lande and Schemske 1985; Campbell 1986; Charlesworth and Charlesworth 1987; D. Charlesworth *et al.* 1990; Holsinger 1988; Uyenoyama and Waller 1991a,b,c). It is clear from many of these models of plant mating system evolution that more than just the magnitude of inbreeding depression matters: the genetic basis of the inbreeding depression also can have profound effects on mating system evolution.

The purpose of the study described here is to provide a more complete picture of the genetics of inbreeding depression in a primarily outcrossing population of *M. guttatus*, in the hope that such information will provide insight into the evolution of self-fertilization in this species. Previous studies of this population indicate that there is tremendous inbreeding depression for nearly every fitness component, and that almost all of this inbreeding depression is due to mildly deleterious alleles (Willis 1992, 1999a,b). Alleles with large deleterious effects on fitness contribute relatively little to overall inbreeding depression. In this article I determined the degree to which the mildly deleterious alleles are recessive in their fitness effects. To do this I assayed the homozygous and heterozygous fitnesses of a large number of highly inbred lines extracted from a natural population. Major deleterious alleles were essentially eliminated from these lines, but because these lines were constructed by self-fertilization and single seedling descent, natural selection during the line formation was ineffective in purging alleles with minor fitness effects

TABLE 7  
Estimates of the average degree of dominance,  $\bar{h}$ , for alleles affecting fitness components and cumulative measures of fitness

Trait	$\bar{h}$	SE	95% limits	<i>t</i> -value
Proportion of germination	0.213	0.057	(0.100, 0.326)	3.74***
Number of flowers	0.057	0.058	(-0.058, 0.172)	0.98
Viable pollen per flower	0.087	0.024	(0.039, 0.135)	3.63***
Fraction of viable pollen	0.063	0.024	(0.015, 0.111)	2.63*
Pollen grains per flower	0.122	0.029	(0.064, 0.180)	4.21***
Ovules per flower	0.179	0.039	(0.101, 0.257)	4.58***
Germination to viable pollen	0.096	0.040	(0.016, 0.176)	2.41*
Germination to ovules	0.139	0.046	(0.048, 0.231)	3.02**
Germination to total gametes	0.135	0.047	(0.042, 0.229)	2.87**

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

(Willis 1999b). I then estimated the average degree of dominance of these mildly deleterious alleles from the regression of heterozygous fitness on the sum of parental homozygous fitness. I found that the detrimental alleles were partially recessive on the average for most traits measured, with  $\bar{h}$  near 0.1 for cumulative measures of fitness.

**The nature of inbreeding load in *Mimulus*:** The results from this experiment and others (Willis 1999a,b) indicate that partially recessive, mildly deleterious alleles are the primary cause of inbreeding depression in this population of *M. guttatus*. If overdominant loci were a major component of inbreeding depression in this population, then we would expect no relationship between homozygous and heterozygous fitness in an equilibrium population (Charlesworth and Hughes 1998). The fact that all but one of the estimates of  $\bar{h}$  are significantly greater than zero therefore is strong evidence that overdominance makes little if any contribution to inbreeding load in this population.

While it is clear that  $\bar{h} > 0$  for all traits except flower number, there is some possibility that the estimates of  $\bar{h}$  are biased downward. The regression method for estimating  $\bar{h}$  weights the dominance of alleles with more severe effects on fitness more heavily than those of mildly deleterious alleles, and it assumes no correlation between the dominance and selective effect of alleles (Simmons and Crow 1977; Caballero *et al.* 1997). If severely deleterious alleles are more recessive on average than less deleterious alleles, then  $\bar{h}$  will be underestimated. This potential problem is not unique to the regression method: it also applies to the variance component method of Comstock and Robinson (1952) for estimating the average dominance of alleles (Caballero *et al.* 1997). In this experiment major deleterious alleles like recessive lethals and steriles largely have been purged from the collection of inbred lines, so the bias should be reduced. Nonetheless, this purging was not 100% effective, especially for male sterility alleles (Wil-

lis 1999b), so a very small number of the lines used in this experiment may contain severely deleterious alleles.

I examined the possibility that such alleles biased the estimates of  $\bar{h}$  for each trait presented in Table 7 by excluding 10% of the inbred lines with lowest mean value for that trait from the regressions. These lines were especially likely to contain major deleterious alleles. I then reestimated  $\bar{h}$ , after correcting the regression coefficients by the reliability ratio,  $k$ , as described above. Typically, the sample sizes for the regressions were reduced by  $\sim 13$  families, because most of the low fitness lines happened to have been paired with higher fitness lines. The results from these regressions, labeled  $\bar{h}_{\text{excl}}$ , are presented in Table 8. The average degree of dominance remained essentially unchanged for the fitness components number of flowers, pollen grains per flower, ovules per flower, and for the cumulative measures of fitness germination to ovules and germination to total gametes. Surprisingly, the estimate of  $\bar{h}_{\text{excl}}$  for proportion germination actually decreased. The most interesting results, however, come from the traits that are strongly related to pollen viability. Estimates of  $\bar{h}_{\text{excl}}$  were more than double the corresponding estimates of  $\bar{h}$  for the number of viable pollen per flower ( $\bar{h}_{\text{excl}} = 0.21$ ), the fraction of viable pollen ( $\bar{h}_{\text{excl}} = 0.14$ ), and germination to viable pollen ( $\bar{h}_{\text{excl}} = 0.22$ ). These results suggest that the estimates of  $\bar{h}$  for these traits are biased by a few recessive alleles of large effect, and that mildly deleterious alleles have more intermediate degrees of dominance for these traits.

Recently there have been two other attempts to estimate the dominance of genes causing inbreeding depression in *M. guttatus*. In the first study, Fu and Ritland (1994) examined the segregation of several allozyme markers in selfed progeny of 31 heterozygous outbred plants. Genes heterozygous for alleles affecting early zygote and seedling viability that happened to be linked to the allozyme markers are expected to produce genotypic ratios in the progeny that deviate from Men-

TABLE 8

Estimates of the average degree of dominance,  $\bar{h}_{\text{excl}}$ , after excluding low fitness inbred lines from the regressions, and estimates of the deleterious genomic mutation rate,  $U$ , for alleles affecting fitness components and cumulative measures of fitness

Trait	$\bar{h}_{\text{excl}}$	SE	$U$ (using $\bar{h}$ )	$U$ (using $\bar{h}_{\text{excl}}$ )
Proportion of germination	0.074	0.071	0.069	0.016
Number of flowers	0.050	0.074	0.020	0.018
Viable pollen per flower	0.206	0.052	0.070	0.234
Fraction of viable pollen	0.139	0.068	0.028	0.075
Pollen grains per flower	0.120	0.061	0.056	0.055
Ovules per flower	0.193	0.066	0.162	0.183
Germination to viable pollen	0.219	0.064	0.129	0.424
Germination to ovules	0.105	0.067	0.173	0.119
Germination to total gametes	0.095	0.069	0.182	0.115

delian expectations. Fu and Ritland (1994) used this idea to estimate the dominance of linked viability alleles on the assumption of at most one heterozygous viability locus linked to a given marker in a given maternal plant. Because the approach involved single-marker analysis, rather than analysis of intervals between markers, they were not able to map the viability loci or provide estimates of their fitness effects. Instead, they used a graphical approach to determine which single locus genetic models were consistent with the segregation ratios. They found that while a few markers exhibited segregation patterns consistent with overdominance, the majority of markers showed patterns consistent with  $h > 0.5$ . Very few markers showed the pattern of partially recessive deleterious alleles ( $0 < h < 0.5$ ) expected for alleles causing inbreeding depression. This strange result is probably due to a combination of statistical uncertainty and the assumption of only one linked viability locus. This latter assumption is especially problematic, because linked deleterious partially recessive alleles can give estimates of  $h$  at a marker  $> 0.5$  if they are linked in repulsion (Sved 1972; Fu and Ritland 1994).

More recently Dudash and Carr (1998) published estimates of the average degree of dominance for several fitness components in two populations of *M. guttatus* and one population of the related selfer, *M. micranthus*. The investigators paired 20 inbred lines per population such that vigorous lines were paired with low vigor lines, crossed plants within each pair according to the North Carolina 3 breeding design to produce 10 sets of  $F_1$ 's,  $F_2$ 's, and backcross progeny, and estimated the additive and dominance components of variance averaged over the 10 sets. From the ratio of dominance variance to additive variance in such a design one can estimate an alternative measure of the average degree of dominance,  $\bar{a}$  (Comstock and Robinson 1952). Dudash and Carr (1998) were able to estimate the average degree of dominance in *M. guttatus* for four of the fitness components measured in this study (number of flowers,

ovules per flower, total pollen per flower, and fraction of viable pollen), as well as for above-ground biomass. They did not present estimates for cumulative measures of fitness. The results of their study were remarkably similar to the estimates of the average degree of dominance presented here: they found no evidence of overdominance, and most estimates of  $\bar{a}$  indicated that the deleterious alleles were partially recessive. Their estimates of  $\bar{a}$  can be converted into estimates of  $\bar{h}$ , because  $\bar{h} = (1 - \bar{a})/2$ . When one does this, their estimates of  $\bar{h}$ , averaged over their two *M. guttatus* populations, are  $\sim 0.12$  for number of flowers, 0.04 for fraction of viable pollen, 0.41 for pollen per flower (one population only), and 0.20 for ovules per flower. Only the value for pollen per flower is substantially different from the estimates reported here: all the rest are remarkably consistent (Tables 7 and 8). Unfortunately, the authors did not present data for either germination or cumulative measures of fitness.

There are two possible sources of bias in Dudash and Carr's (1998) results that were not mentioned in their article. First, it is not clear to what extent their intentional inclusion of low fitness lines biased their estimates of the average degree of dominance. As discussed above, such lines can lead to upwardly (downwardly) biased estimates of  $\bar{a}$  (or  $\bar{h}$ ) if they contain highly deleterious alleles that are more recessive on the average than mildly deleterious alleles (Simmons and Crow 1977; Caballero *et al.* 1997). Second, the authors apparently performed their analyses on raw fitness data. This is appropriate if the effects of different loci combine additively on that scale. However, if loci combine multiplicatively then estimates of  $\bar{a}$  (or  $\bar{h}$ ) should be conducted using ln-transformed data, as discussed above. The results presented here and elsewhere (Deng and Fu 1998) suggest that estimates of the average degree of dominance will be biased toward additivity for traits that decline linearly with  $F$  on a ln-scale. I emphasize that these potential problems are not unique to the Dudash and Carr

(1998) study; rather they are common to almost every other study, including the present one, that has ever reported on the average degree of dominance in a number of plant and animal species. Clearly, much more work needs to be done to assess the potential magnitude of these sources of bias and to develop alternative methods that avoid them.

The overall picture that emerges from this study and the others discussed above is that inbreeding load in populations of *M. guttatus* largely is due to mildly deleterious alleles, and that these alleles tend to be partially recessive. The estimates of the degree of dominance are remarkably similar to the results from studies of natural populations of *Drosophila* (reviewed by Lewontin 1974; Simmons and Crow 1977; Crow and Simmons 1983; Charlesworth and Hughes 1998) and two highly selfing *Amsinckia* species (Johnston and Schoen 1995). Charlesworth and Hughes (1998), for example, conclude that in *Drosophila melanogaster*, the average degree of dominance of alleles affecting a wide variety of fitness components on the second and third chromosomes is  $\sim 0.23$ . The average degree of dominance for some traits, like female fertility (Watanabe and Ohnishi 1975) and early male mating success (Hughes 1995), seems to be closer to  $\sim 0.1$ , while estimates for viability often tend to be closer to 0.3 or 0.4. It is hard to evaluate whether these differences among traits are biologically meaningful or are merely due to statistical uncertainty, because the standard errors tend to be large. Johnston and Schoen (1995) used naturally produced inbred lines from two populations from each of two self-fertilizing plant species to estimate  $\bar{h}$  for fitness components and cumulative fitness. While some estimates of  $\bar{h}$  were not significantly different from zero, the remaining estimates strongly indicate that deleterious alleles are partially recessive, with  $\bar{h}$  ranging from  $\sim 0.2$  to 0.4. While we clearly need more studies from diverse organisms, the results from the few studies published to date suggest that inbreeding depression may have a common genetic basis across widely divergent taxa.

**Indirect estimates of the genomic deleterious mutation rate in *Mimulus*:** If the alleles causing inbreeding depression in an outcrossing population are maintained by mutation-selection balance and fitnesses combine multiplicatively across loci, then the magnitude of inbreeding load in an equilibrium population is a simple function of the degree of dominance and the rate of mutation to deleterious alleles (Morton *et al.* 1956). Summing over  $i$  loci in the genome,  $B = \sum_i u_i (1/h_i - 2)$ , where  $u_i$  is the deleterious mutation rate at the  $i$ th locus. This equation suggests that we can use estimates of  $B$  and  $\bar{h}$  from outcrossing populations to obtain indirect estimates of the genomic deleterious mutation rate,  $U$ , given the assumptions listed above. This argument recently has been extended to the case of highly inbred populations (B. Charlesworth *et al.* 1990).

Here I use the estimates of  $B$  and  $\bar{h}$  obtained in this study to estimate the genomic deleterious mutation rate for the fitness components and cumulative measures of fitness in the Iron Mountain population of *M. guttatus*. Table 8 presents two sets of estimates of  $U$ : the first set is based on the average degree of dominance,  $\bar{h}$ , from the entire collection of inbred lines (Table 7), while the second is based on the average degree of dominance when low fitness lines are excluded,  $\bar{h}_{\text{excl}}$ . As expected, the two estimates of  $U$  for each trait are quite similar for most traits except for measures of male fertility, which are larger when  $\bar{h}_{\text{excl}}$  is used to calculate  $U$ . Focusing on the estimates based on  $\bar{h}_{\text{excl}}$ ,  $U$  for fitness components ranges from  $\sim 0.02$  for proportion germination and number of flowers to  $\sim 0.23$  for viable pollen per flower. The average  $U$  for the four nonoverlapping fitness components (germination, number of flowers, and viable pollen and ovule per flower) is  $\sim 0.11$ . Estimates of  $U$  are somewhat larger for the cumulative measures of fitness, ranging from  $\sim 0.12$  for germination to ovules or total gametes to 0.42 for germination to viable pollen. These results therefore suggest that deleterious mutation rates are quite high in *M. guttatus*, exceeding 0.1 deleterious mutations per genome per generation.

The indirect estimates of  $U$  presented in Table 8 are likely to be underestimates of the true genomic mutation rate for at least three reasons. First, the estimates of  $U$  are extremely sensitive to estimates of  $\bar{h}$ , such that small values of  $\bar{h}$  lead to small values of  $U$ . As pointed out above, our estimates of  $\bar{h}$  are likely to be underestimated if there is a positive correlation between the selective effect of a mutation and its degree of dominance. Second, the method used for estimating  $U$  assumes that the population is completely outcrossing. We know that the Iron Mountain population of *M. guttatus* is partially inbred: estimated selfing rates ranged from 0.09 in 1989 to 0.24 in 1990 (Willis 1993b), and the population inbreeding coefficient of seedlings in the wild is  $\sim 0.19$  (Sweigart *et al.* 1999). Theory indicates that for a given model of mutation and selection, partial inbreeding will reduce the inbreeding depression due to deleterious alleles over that found in an otherwise identical outcrossing population (*e.g.*, Lande and Schemske 1985; Charlesworth and Charlesworth 1987). For this reason we would expect the inbreeding load,  $B$ , to be greater than we observed here if the Iron Mountain population were completely outcrossing. Finally, the true genomic deleterious mutation rate could be larger than we estimated in this study if inbreeding load is more severe in the wild or under competitive conditions. Opportunities for competition among plants were minimal in this study, because seedlings were thinned to a single plant per pot after 14 days. It is well known that inbreeding depression can be greater in natural or stressful environments than under controlled greenhouse conditions (*e.g.*, Dudash 1990), although this was not the case for the effects of inbreeding on number

of flowers in the Iron Mountain population (Willis 1993b).

For all of these reasons, the data presented here suggest that the true  $U$  may lie somewhere between 0.1 and perhaps 1.0. This range of values is strikingly similar to direct estimates of the deleterious mutation rate in several species, including *D. melanogaster* (Mukai 1964; Mukai *et al.* 1972; Ohnishi 1977; but see Keightley 1994, 1996; Garcia-Dorado 1997), *Daphnia pulex* (Lynch *et al.* 1998), and *Arabidopsis thaliana* (Schultz *et al.* 1999), while estimates of  $U$  in *Caenorhabditis elegans* are much lower (Keightley and Caballero 1996). The indirect estimates of  $U$  in *M. guttatus* are also similar to other indirect estimates obtained for self-fertilizing plant species (B. Charlesworth *et al.* 1990, Charlesworth *et al.* 1994; Johnston and Schoen 1995). Taken together, all of these data suggest that the genomic deleterious mutation rate in many species of plants and animals is roughly on the order of 0.1 to 1.0 deleterious mutation per genome per generation (see also Lynch *et al.* 1999).

A critical assumption underlying the use of the Morton *et al.* (1956) equation outlined above to estimate  $U$  is that the inbreeding depression observed in a population is entirely due to mutational load and not to any alleles that are maintained by natural selection. If such alleles were present in the Iron Mountain population, than the inbreeding load due to deleterious mutations would be smaller than the estimates presented here. It is not clear how such alleles would affect estimates of  $\bar{h}$ , because the regression method assumes that deleterious alleles are rare. Overdominant loci at equilibrium would tend to bias  $\bar{h}$  downward, because the regression method yields  $h = 0$  for such loci (Mukai and Yamaguchi 1974; Charlesworth and Hughes 1998). There is no general relationship between the fitness of homozygotes and heterozygotes expected for alleles that are maintained by other forms of balancing selection, such as frequency-dependent selection, variable selection in space or time, or antagonistic pleiotropy (Charlesworth and Hughes 1998). Such alleles might be deleterious under the genetic or environmental conditions in which fitness is assayed, and yet they would not be rare. In this case we might expect these alleles to bias estimates of  $\bar{h}$  upward, because crosses between inbred lines could generate unfit homozygotes at that locus in the outbred lines. For these reasons, the estimates of  $U$  presented in Table 8 must be regarded as hypotheses that should ultimately be tested directly through mutation-accumulation studies. Nonetheless, the fact that these estimates of  $U$  are so similar to direct estimates of the genomic deleterious mutation rate in other organisms suggests that the inbreeding load in *M. guttatus* is largely due to deleterious, partially recessive alleles maintained by mutation-selection balance.

It should be pointed out that the potential problems outlined above apply equally to the indirect method for

estimating  $U$  in highly selfing species (B. Charlesworth *et al.* 1990). While overdominance *per se* is not expected to contribute to inbreeding depression in highly inbred populations (Kimura and Ohta 1971; Charlesworth and Charlesworth 1990), other forms of balancing selection can maintain variation and cause inbreeding depression in such populations. Moreover, indirect estimates of  $U$  from highly selfing populations may be difficult to interpret for another reason that has not received attention in the literature. Estimates of  $B$  and  $\bar{h}$  obtained from such populations may be biased if lines from different subpopulations or genetic neighborhoods are crossed, because heterosis can be caused by multiplicative fitness *in the absence of dominance* when there is linkage disequilibrium among the parental lines (Minvielle 1987; Schnell and Cockerham 1992). Extreme linkage disequilibrium is commonly observed in selfing populations (*e.g.*, Folz *et al.* 1982); however, it should be minimal in studies, like the one presented here, involving crosses between numerous inbred lines extracted from a large, randomly mated ancestral population.

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