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## THE EFFECTS OF FIVE GENERATIONS OF ENFORCED SELFING ON POTENTIAL MALE AND FEMALE FUNCTION IN *MIMULUS GUTTATUS*

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**Abstract.**—In prior work we detected no significant inbreeding depression for pollen and ovule production in the highly selfing *Mimulus micranthus*, but both characters showed high inbreeding depression in the mixed-mating *M. guttatus*. The goal of this study was to determine if the genetic load for these traits in *M. guttatus* could be purged in a program of enforced selfing. These characters should have been under much stronger selection in our artificial breeding program than previously reported characters such as biomass and total flower production because, for example, plants unable to produce viable pollen could not contribute to future generations. Purging of genetic load was investigated at the level of both the population and the individual maternal line within two populations of *M. guttatus*. Mean ovule number, pollen number, and pollen viability declined significantly as plants became more inbred. The mean performance of outcross progeny generated from crosses between pairs of maternal inbred lines always exceeded that of self progeny and was fairly constant for each trait through all five generations. The consistent performance of outcross progeny and the universally negative relationships between performance and degree of inbreeding are interpreted as evidence for the weakness of selection relative to the quick fixation of deleterious alleles due to drift during the inbreeding process. The selective removal (purging) of deleterious alleles from our population would have been revealed by an increase in performance of outcross progeny or an attenuation of the effects of increasing homozygosity. The relationships between the mean of each of these traits and the expected inbreeding coefficient were linear, but one population displayed a significant negative curvilinear relationship between the log of male fertility (a function of pollen number and viability) and the inbreeding coefficient. The generally linear form of the responses to inbreeding were taken as evidence consistent with an additive model of gene action, but the negative curvilinear relationship between male fertility and the inbreeding coefficient suggested reinforcing epistasis. Within both populations there was significant genetic variation among maternal lineages for the response to inbreeding in all traits. Although all inbred lineages declined at least somewhat in performance, several maternal lines maintained levels of performance just below outcross means even after four or five generations of selfing. We suggest that selection among maternal lines will have a greater effect than selecting within lines in lowering the genetic load of populations.

**Key words.**—Dominance, epistasis, inbreeding depression, mating-system evolution, *Mimulus*, overdominance, ovule, pollen, purging, serial inbreeding.

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Theoretical (e.g., Maynard Smith 1971; Lloyd 1979; Charlesworth and Charlesworth 1979, 1987, 1990; Lande and Schemske 1985; Campbell 1986) and empirical (e.g., see Charlesworth and Charlesworth 1987) studies have illustrated the potentially important role of inbreeding depression ( $\delta = 1 - \text{self/outcross}$ ) in preventing the spread of selfing variants through plant populations. Because the magnitude of inbreeding depression is a function of allele frequencies, it is not a population constant and is free to evolve. The mating system of a population will determine the levels of heterozygosity and hence the expression of the population's genetic load. If inbreeding depression is due to deleterious recessive or partially recessive alleles, expression of these alleles is enhanced by the increased homozygosity resulting from selfing. Consequently, selection is more efficient at removing or purging these alleles from an inbreeding population relative to a random mating population. This produces the expectation of lower inbreeding depression in highly selfing populations relative to populations with higher outcrossing rates (e.g., Lande and Schemske 1985; D. Charlesworth et al. 1990; B. Charlesworth et al. 1991). In this study we increase the level of inbreeding within two populations of the mixed-mating *Mim-*

*ulus guttatus* through five generations of enforced selfing in the greenhouse. We test for purging of genetic load for important qualitative and quantitative components of potential male and female reproductive success.

Previous studies of *M. guttatus* have demonstrated inbreeding depression for pollen viability (Willis 1993a) and for pollen and ovule production (Carr and Dudash 1995). Inbreeding depression for any of these characters would present a formidable barrier for the evolution of selfing unless the inbreeding depression can be purged. In the highly selfing *M. micranthus*, a derivative of *M. guttatus* (Fenster and Ritland 1992), no significant inbreeding depression for pollen and ovule production was observed (Carr and Dudash 1996). The comparison of levels of inbreeding depression in these two closely related taxa suggests that selection against deleterious alleles affecting male and female function has been intense. In contrast to pollen and ovule characters, the level of inbreeding depression for biomass in *M. micranthus* was more similar to that seen in *M. guttatus* (Carr and Dudash 1996), suggesting much weaker selection on this character.

The relatively high levels of inbreeding depression for biomass in the selfing *M. micranthus* are concordant with the persistence of even greater inbreeding depression for this trait in highly inbred lines of *M. guttatus* created in the greenhouse. Dudash et al. (1997) examined the effects of serial inbreeding on aboveground biomass as well as germination

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rate, date of first flower, and flower production in *M. guttatus* and found little evidence of a populationwide purging of deleterious alleles. Similarly Pray and Goodnight (1995) found no evidence of populationwide purging in serial brother-sister matings in the flour beetle, *Tribolium*. Both Dudash et al. (1997) and Pray and Goodnight (1995) attributed the lack of purging to the weakness of selection relative to the quick fixation of deleterious alleles due to drift during the inbreeding process. However, purging has been suggested by Barrett and Charlesworth (1991) based on their crosses among experimentally inbred lines of *Eichhornia paniculata*. They observed significantly higher flower production in these progeny relative to the parental generation from which their inbred lines were derived. They interpreted this increase in flower production as evidence of the removal of partially recessive deleterious alleles from their experimental population during their serial inbreeding regime.

Although evidence of purging as a populationwide phenomenon was not detected by either Dudash et al. (1997) and Pray and Goodnight (1995), they did find significant differences among maternal lines in response to the inbreeding program. In both studies, some inbred lines actually increased in performance as their expected inbreeding coefficient increased. Variation among lines in inbreeding depression could be important in mating system evolution. Holsinger (1988) and Uyenoyama and Waller (1991a,b,c) predict that variation in genetic load should become associated with variation in mating systems. If inbreeding depression is due to deleterious recessive alleles, high fitness genotypes (i.e., those homozygous for many wild-type alleles) will become associated with high selfing genotypes. This association can allow selfing variants to invade an outcrossing population even if population-level inbreeding depression is high enough to counteract the transmission advantage inherent to selfing (Fisher 1941). However, Schultz and Willis (1995) have questioned this result, suggesting that variation among family lines within a population in the occurrence of mutations can promote differences in inbreeding depression independent of a family's history of inbreeding.

Studies of serial inbreeding have also been used to detect the presence of epistasis (e.g., Mukai 1969; Willis 1993a). If loci have independent multiplicative effects on fitness (i.e., fitness is reduced by the product of the effects of homozygous loci), the relationship between the log of fitness and the inbreeding coefficient (Wright 1977) is expected to be linear (Crow and Kimura 1970). Epistatic interactions among loci would cause either a concave upward deviation (diminishing epistasis) or downward deviation (reinforcing epistasis). Epistasis could alter the rate at which deleterious alleles are removed from a population, therefore changing the dynamics of mating system evolution (B. Charlesworth et al. 1991).

The goal of this study was to examine the effects of five generations of enforced selfing on pollen production, pollen viability, and ovule production, traits that appear to be under strong selection in natural populations of *Mimulus*. These characters should be under much stronger selection in an artificial breeding program than previously reported characters such as biomass and total flower production (Dudash et al. 1997). For example, plants unable to produce viable pollen could not contribute through male function to future

generations in our selfed lines. We tested for purging and the presence of epistasis at both the population level and at the level of maternal families within populations. To our knowledge, this is the first study to examine the effects of long-term inbreeding on these qualitative and quantitative components of male and female reproductive success in a natural system.

## MATERIALS AND METHODS

### *Study Organism*

*Mimulus guttatus* (Scrophulariaceae) is an annual-to-perennial, hermaphroditic herb occupying moist habitats of western North America. Populations of *M. guttatus* range in mating system from complete outcrossing ( $t = 1.0$ ) to  $t = 0.25$ , averaging about 0.60 (Ritland and Ritland 1989; Ritland 1990; Dudash and Ritland 1991; Willis 1993b). Seed for the establishment of our greenhouse lineages were collected in 1988 from two annual populations (hereafter referred to as S and T) located approximately 10 km apart in Tuolumne County, California. Dudash and Ritland (1991) estimated the outcrossing rate for population T to be 0.70 (SE = 0.06), but no estimate is available for population S. Single ripe fruits were collected from 27 and 31, respectively, plants along linear transects in populations S and T.

### *Breeding Design*

In the summer of 1990 we sowed 30 seeds from each field-collected fruit into 76-cm square plastic pots. Two weeks later we transplanted one randomly selected seedling from each sow into its own pot for use in our hand-pollinations. Each plant served as a seed parent for one cross-pollination. We randomly chose pollen donors for cross-pollinations from within the same population as the recipient. Cross-pollinations were not reciprocal. *Mimulus guttatus* produces a pair of flowers at each stem node, and we self-pollinated the second flower at the same node as the cross-pollination. This pairing controlled for variation within a maternal plant. We removed corollas and stamens after hand-pollinations to prevent any subsequent self-pollination (Dole 1990).

All subsequent generations descended from a single self seed from the previous generation. We also produced outcross seed each generation as described above. We attempted to keep the specific line pairings used for the production of outcross seed constant throughout the breeding program to control for specific combining abilities (Falconer 1981). As maternal families were lost during the course of the breeding program, we randomly chose new outcross pollen parents from the surviving lines. We continued this breeding program for five generations over a 2.5-year period. In this study we included 16 and 15 maternal families from populations S and T, respectively. We included only families for which we had both self and outcross seed for at least four of the five generations. Generations one through five were represented by 15, 16, 16, 14, and 15 maternal families in population S and 15, 11, 15, 14, and seven families in population T.

Loss of maternal families was due primarily to poor pollen production. Within a family, if a plant failed to produce sufficient pollen to act as either a pollen donor for a cross-

pollination or its self-pollination, we substituted a sibling until we were able to perform satisfactory hand-pollinations. This should have exerted positive selection for pollen production within families. When all available siblings within a maternal family failed to produce adequate pollen for hand-pollinations, the family was lost. This should have exerted positive selection among families for pollen production. Our sample of maternal families is therefore biased toward the best pollen producers from our experimental populations. The loss of inbred lines occurred primarily in the fourth and fifth generations of inbreeding.

#### *Experimental Design*

We measured performance of progeny from all five generations simultaneously. We sowed a maximum of 30 seeds per maternal family per pollination treatment per generation. After two weeks we transplanted a maximum of five randomly chosen seedlings from each pollination  $\times$  generation  $\times$  maternal family combination. We transplanted two seedlings into each pot, with each pot containing one self and one outcross seedling of the same generation from a single maternal family. Pairing seedlings likely provided a more realistic environment, and the competitive environment may have accentuated the effects of inbreeding depression (Schmitt and Ehrhardt 1990; Argyres and Schmitt 1992; Wolfe 1993; Carr and Dudash 1995; Latter and Mulley 1995; Parker et al. 1995). It is possible that the relative performance of self-outcross pairs could have been influenced by changes in the intensity of competition across the generations (e.g., if outcross progeny improve as competitors), but this is not the case here (for details, see Dudash et al. 1997).

We randomized the five replicate self-outcross pairs across five blocks such that each block contained all families across all generations. Each block consisted of seven trays with 20 pots per tray. Plants grew in soil-saturated conditions without fertilizer. Sodium vapor lights extended the photoperiod to 18:6 L:D. To obtain an ovule sample from each plant, we preserved the pistil from the first open flower in 3:1 ethanol:glacial acetic acid. We removed the ovules from one of the two symmetrical locules from each pistil, stained them with 1% aniline blue in lactophenol (all ovules stained positively), and counted them under a dissecting microscope. This count represents one-half the number of ovules per pistil, and we will refer to this as ovule number.

We obtained samples for measuring pollen production by collecting anthers from both buds at the second node of every flowering plant. These samples were air-dried for one week and stored in sealed microcentrifuge tubes. We used an Elzone 280PC particle counter to quantify the number of pollen grains in our samples. A sample mean was calculated from three replicate subsamples. The sample mean is equivalent to 1/15 of the total pollen production per flower, and we will refer to this as pollen number.

To obtain pollen samples for a pollen viability assay, we placed the anthers from one open flower at the third stem node into a microcentrifuge tube containing 0.1% aniline blue in lactophenol (Radford et al. 1974). The number of samples collected daily prohibited the use of assays that needed to be processed daily as well (e.g., fluorochromatic reaction test:

Heslop-Harrison et al. 1984). We assayed pollen stainability for each individual by averaging two replicate samples of 100 randomly selected pollen grains on hemacytometer grids under a compound microscope. This assay stains the cytoplasm of normal pollen grains dark blue and they were classified as "viable" and lightly stained pollen grains were categorized as "nonviable" (e.g., Mayer 1991; Willis 1993a). We utilized a DIFFCOUNT counter that allowed us to keep a running total of the two categories of pollen grains and beeped when we had scored 100 pollen grains. Mayer (1991) has shown a good relationship between the aniline blue technique and a vital stain, malate dehydrogenase, that requires rapid processing. We will be referring to pollen stainability as pollen viability throughout this study. For each plant in the experiment we also estimated a composite trait that incorporates both pollen production and pollen viability and will refer to this as male fertility by multiplying mean pollen number by the mean proportion of viable pollen grains for each individual in the study.

#### *Statistical Analysis*

Using the GLM procedure in SAS (SAS Institute 1989), we ran two types of statistical analyses for all dependent variables. First, to test for changes in inbreeding depression across the five generations we used an ANOVA with block, population, and line nested within population as random main effects. Generation, pollination treatment, and their two-way interaction were entered as fixed effects. The model included two-way and three-way interactions involving population and line within population as random effects. In those cases where there were no significant effects involving population, we pooled these mean squares into the error term (Sokal and Rohlf 1981). Ovule number, pollen number, and male fertility were log-transformed to meet ANOVA assumptions. The proportion of viable pollen grains was arcsine-transformed. Error mean squares were derived using the SAS "random" statement with its "test" option.

Our second type of analysis tested for the presence of epistasis. If loci affect a trait in a multiplicative fashion, we expect a linear relationship between the expected inbreeding coefficient ( $f$ ) and the log of the trait. A deviation from linearity indicates epistasis. We log-transformed trait means for each family and performed polynomial regressions to test for deviations from linearity at the population level. If the second order term ( $f^2$ ) did not explain a significant portion of the variation, we reduced the model to include only the first-order regression term ( $f$ ). Each population was analyzed separately. We also tested for homogeneity of slopes among maternal families using ANOVA. Block and maternal line were analyzed as random main effects. The expected inbreeding coefficient and its square were entered as regression effects. Heterogeneity of slopes would be revealed by significant interactions between maternal line and either  $f$  or  $f^2$ .

## RESULTS

### *Changes in Inbreeding Depression*

The effect of pollination treatment was highly significant for ovule number (Table 1). This effect was only marginally

TABLE 1. ANOVA for the effects of five generations of inbreeding and outcrossing on log-transformed ovule number in *Mimulus guttatus*.

Source	df	MS	F	P
Block	4	0.015	0.835	0.5040
Maternal line	30	0.101	1.315	0.2342
Generation	4	0.162	4.710	0.0014
Pollination treatment	1	5.528	82.245	0.0001
Line × generation	99	0.038	1.067	0.3804
Line × poll. trmt.	30	0.077	2.242	0.0017
Generation × poll. trmt.	4	0.074	2.173	0.0780
Line × generation × poll. trmt.	84	0.036	2.052	0.0001
Error	661	0.017		

significant for pollen number (Table 2), but this was due to the fact that a significant population × generation × pollination treatment interaction prevented a strong test of this main effect (there was only a single degree of freedom in the error term). The effect of pollination was highly significant for both pollen viability (Table 3), and male fertility (Table 4). In all four cases outcross progeny outperformed self progeny (Fig. 1).

Ovule number showed a significant generation effect but no interaction between pollination treatment and generation (Table 1), declining steadily from generation 1 to 5. Inbreeding depression was fairly consistent across the generations, varying from 24% to 33% in population S and 25% to 37% in population T with no consistent pattern of change.

Pollen number, viability, and male fertility showed either a significant generation × pollination treatment or population × generation × pollination treatment interaction (Tables 2–4). The pattern was similar for all three traits. Outcross progeny performed at a fairly consistent level each generation. Self progeny showed a gradual decline in performance across the five generations. As a result the relative difference between self and outcross, that is, inbreeding depression, generally became greater each generation. For pollen number, the change was more extreme in population T where inbreeding depression ( $1 - \text{self/outcross}$ ) increased from 39% in generation 1 to 80% in generation 5. Inbreeding depression

TABLE 2. ANOVA for the effects of five generations of selfing and outcrossing on log-transformed pollen number in *Mimulus guttatus*.

Source	df	MS	F	P
Block	4	0.113	1.686	0.1514
Population	1	1.279	4.196	0.3976
Maternal line w/i population	29	0.394	2.061	0.0399
Generation	4	0.588	19.872	0.0074
Pollination treatment	1	33.435	92.199	0.0658
Population × generation	4	0.030	0.120	0.9673
Population × poll. trmt.	1	0.364	1.098	0.3318
Line w/i pop. × generation	94	0.092	0.905	0.6813
Line w/i pop × poll. trmt.	29	0.200	2.013	0.0067
Generation × poll. trmt.	4	0.614	2.407	0.2090
Pop. × gen. × poll. trmt.	4	0.253	2.629	0.0387
Line w/i pop × gen. × poll. trmt.	79	0.102	1.531	0.0031
Error	723	0.067		

TABLE 3. ANOVA for the effects of five generations of selfing and outcrossing on arcsine-transformed pollen viability in *Mimulus guttatus*.

Source	df	MS	F	P
Block	4	0.048	1.042	0.3847
Maternal line	30	0.774	2.552	0.0083
Generation	4	1.097	9.527	0.0001
Pollination treatment	1	20.155	75.275	0.0001
Line × generation	99	0.128	1.007	0.4886
Line × poll. trmt.	30	0.305	2.468	0.0006
Generation × poll. trmt.	4	0.661	5.486	0.0005
Line × generation × poll. trmt.	82	0.130	2.789	0.0001
Error	619	0.046		

for pollen viability increased over fourfold in both populations.

ANOVAs on all four dependent variables showed significant maternal line × pollination treatment interactions and maternal line × generation × pollination interactions (Tables 1–4). The two-way interaction indicates that lines varied significantly in levels of inbreeding depression. The three-way interaction indicates that changes in inbreeding depression varied among maternal families.

#### Epistasis

The quadratic terms ( $f^2$ ) were not significant in our polynomial regressions of log-transformed ovule number and both pollen number and viability. In the simple regressions, the linear coefficients demonstrated significant declines with increasing homozygosity (Fig. 2, Table 5). In the analysis of male fertility in population S, the quadratic term again was not significant, but in the linear regression, male fertility declined significantly with increasing homozygosity (Fig. 2). In population T, however, there was a significant negative curvilinear relationship between male fertility and expected inbreeding coefficient.

All homogeneity of slopes ANOVAs revealed significant interactions between maternal line and  $f$  and maternal line and  $f^2$ , indicating genetic variation for the response to inbreeding (Fig. 3). For all four traits, the majority of maternal lines exhibited negative slopes, but in both populations there were always a few maternal lines with positive quadratic

TABLE 4. ANOVA for the effects of five generations of selfing and outcrossing on log-transformed male fertility in *Mimulus guttatus*.

Source	df	MS	F	P
Block	4	0.062	0.368	0.8316
Maternal line	30	1.935	2.233	0.0312
Generation	4	2.971	8.230	0.0001
Pollination treatment	1	92.840	108.932	0.0001
Line × generation	98	0.395	0.792	0.8636
Line × poll. trmt.	30	0.968	2.017	0.0068
Generation × poll. trmt.	4	2.546	5.478	0.0006
Line × generation × poll. trmt.	77	0.504	2.990	0.0001
Error	601	0.169		

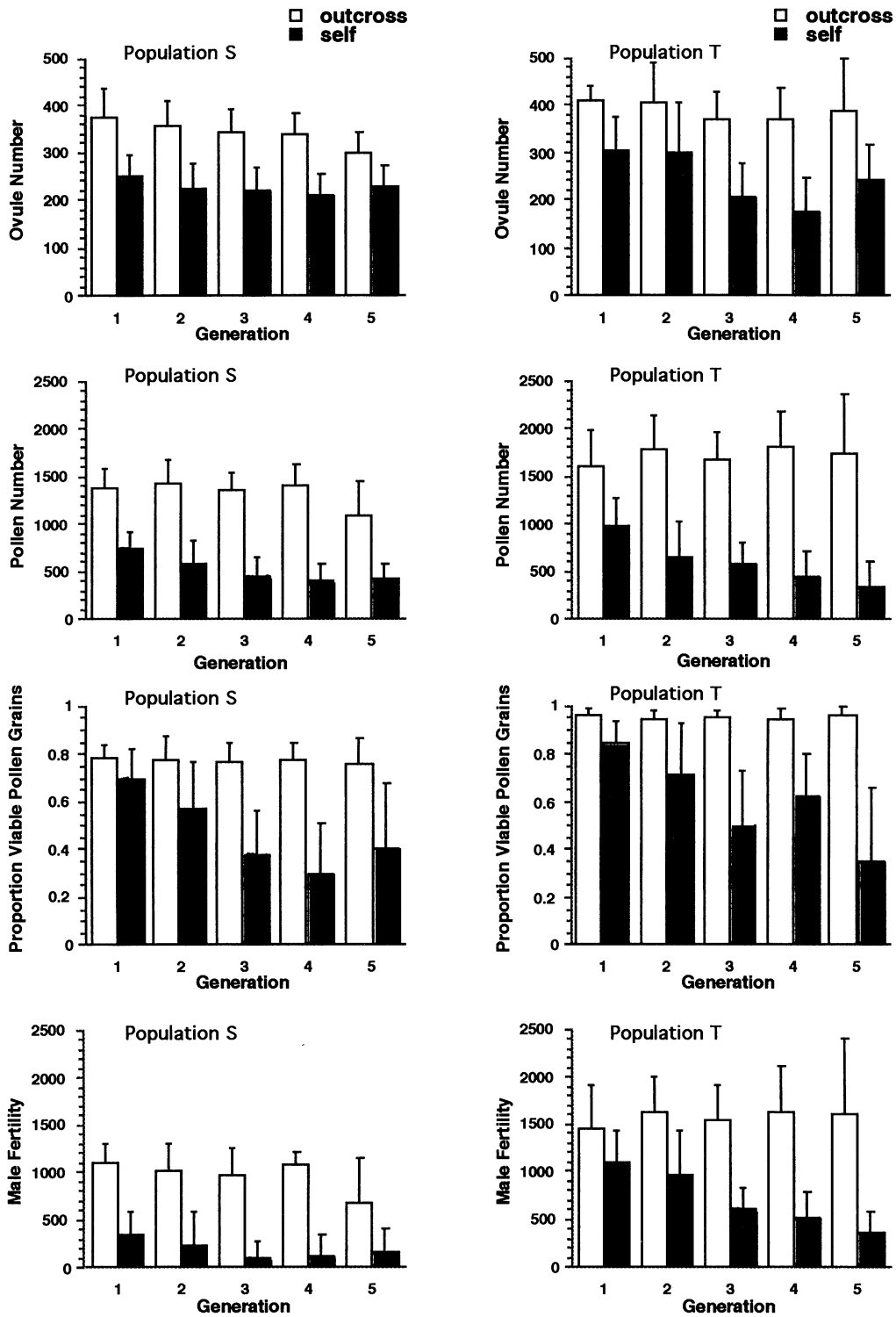


FIG. 1. The effects of five sequential generations of selfing and outcrossing on ovule and pollen traits in two populations of *Mimulus guttatus*. Means are back-transformed and error bars indicate the upper 95% confidence limits.

terms, indicating improved or at least moderated performance. Performance of the most inbred progeny (fourth or fifth generations) was uncorrelated to performance in the initial generation of selfing for all traits except pollen viability in population T, which showed a negative correlation ( $r_s =$

$-0.58$ ;  $P = 0.0375$ ). Despite the observation that some of the most inbred progeny performed fairly well, within-maternal line means for each trait expressed in the most inbred progeny (fourth or fifth generation) always fell below the means from outcross progeny in those generations. Mean

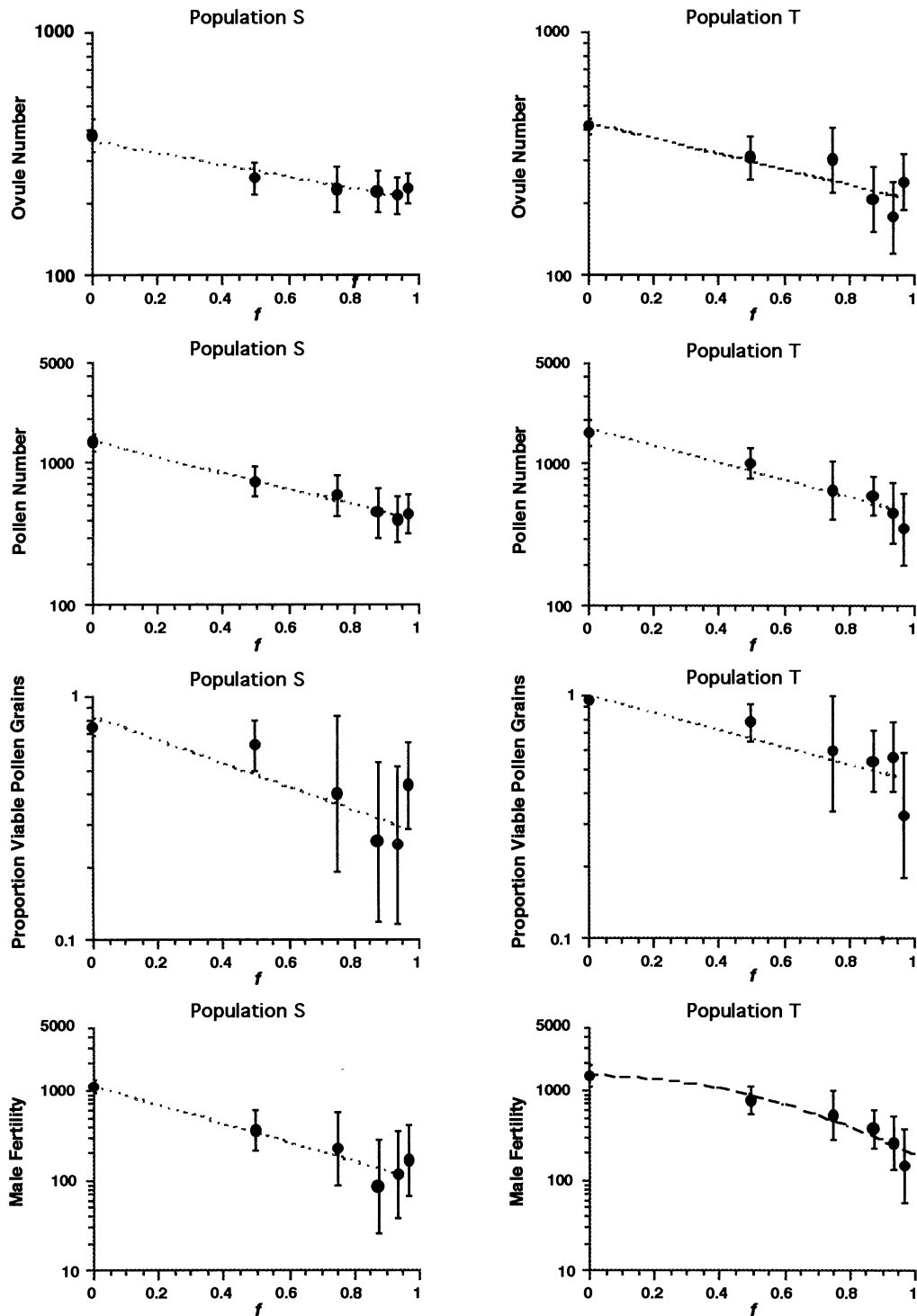


FIG. 2. Regressions of fitness traits against expected inbreeding coefficients ( $f$ ) in two populations of *Mimulus guttatus*. Analyses are based on family means for each trait. Slopes and their statistical significances are listed in Table 5. Error bars indicate 95% confidence intervals.

pollen number and pollen viability in the most inbred progeny were positively correlated (population S:  $r_s = 0.52$ ,  $P = 0.0491$ ; population T:  $r_s = 0.63$ ,  $P = 0.0159$ ). However, neither trait was correlated with ovule production in either population (population S:  $r_s = 0.20$ ,  $P = 0.4829$ ; population T:  $r_s = 0.19$ ,  $P = 0.5126$ ).

## DISCUSSION

### *Population Response to Inbreeding*

This study examined quantitative and qualitative aspects of potential male and female reproductive success. Our observations on the effects of five generations of selfing in

TABLE 5. Regression coefficients for ovule and pollen traits versus the expected inbreeding coefficient ( $f$ ) based on family means from two populations of *Mimulus guttatus* (NS =  $P > 0.05$ , \* =  $P < 0.05$ , \*\* =  $P < 0.01$ , \*\*\* =  $P < 0.001$ ). Intercepts were back-transformed from a log scale. If quadratic coefficients were non-significant, regressions were recomputed using only the linear terms.

Population	Trait	Intercept	Linear coefficient	Quadratic coefficient
S	Ovules	357	-0.22***	NS
	Pollen	1392	-0.55***	NS
	Pollen viability	0.82	-0.49***	NS
	Male fertility	1116	-1.05***	NS
T	Ovules	423	-0.31***	NS
	Pollen	1731	-0.60***	NS
	Pollen viability	1.01	-0.31***	NS
	Male fertility	1420	0.04 NS	-0.94*

greenhouse populations of *M. guttatus* reveal an overall decline in ovule number and two components of male fertility. This result and the consistent performance of outcross progeny generated throughout the course of the breeding program provide little evidence for the selective removal of recessive deleterious alleles from the populations. If purging had been occurring, outcross performance would have been expected to improve from generation 1 to 5 as partially recessive deleterious alleles were removed from the maternal lines, and the decline in performance of self progeny should have attenuated.

The opportunity for selection to remove alleles with negative effects on ovule and especially pollen production was much greater in our present study than it was for biomass and flower production in our previous study of these same populations (Dudash et al. 1997). We imposed no direct selection on biomass and flower number throughout the course of the inbreeding program, but because individuals that were poor pollen producers did not contribute to subsequent generations, we exerted strong selection against alleles that interfered with components of male fertility. Our observation that both pollen and ovule production declined with the expected inbreeding coefficient in a manner similar to that observed for flower number and biomass by Dudash et al. (1997) suggests that in this breeding design the effects of drift aided by this extreme form of inbreeding were overwhelming the effects of selection in both cases. A similar conclusion was reached by Pray and Goodnight (1995) from their observations of an overall decline in seven life-history traits of increasingly inbred populations of the flour beetle *Tribolium castaneum*. In all of these studies an effort was made to maintain as many lines as possible. In nature, selection may have the opportunity to play a larger role both within and among lines.

The interpretation of the results up to this point has assumed that the genetic load is due to recessive mutations, but the observed declines in performance are consistent with a segregational load from overdominant loci as well. Models of inbreeding with overdominance predict an initial increase in inbreeding depression with an increase in the rate of selfing (Lande and Schemske 1985; Charlesworth and Charlesworth 1990). Data on the genetic basis of inbreeding depression in

natural plant populations are few, but those available are more suggestive of a mutational load than a segregational load (Charlesworth and Charlesworth 1987; but see Bush et al. 1987; Bush and Smouse 1991), and this may be true of *Mimulus* in particular. Using a North Carolina 3 breeding design with the same *M. guttatus* populations as this study, Dudash and Carr (unpubl. data) demonstrated that alleles responsible for inbreeding depression in biomass, flower production, ovule number, and pollen viability are, on average, recessive or partially recessive. However, data on pollen number shows evidence of overdominance in population T.

#### Genetic Variation for Inbreeding Depression

Here and elsewhere *M. guttatus* has been shown to exhibit inbreeding depression for ovule number, pollen number (Carr and Dudash 1995, 1996), and pollen viability (Willis 1993a) after one generation of selfing. However, its closely related, highly selfing congener, *M. micranthus*, exhibited no inbreeding depression for pollen and ovule number (Carr and Dudash 1996). The enhanced expression of deleterious recessive alleles in the more highly selfing taxon and their subsequent elimination by natural selection were inferred to be responsible for the difference in inbreeding depression between *M. guttatus* and *M. micranthus*. Similar suggestions have been made to explain lower levels of inbreeding depression in more selfing populations of *Mimulus* in particular (Dole and Ritland 1993; Latta and Ritland 1994) and in selfing plants in general (Husband and Schemske 1996). Assuming these inferences drawn from comparisons of populations differing in their degree of inbreeding are valid, how can these observations be reconciled with the lack of evidence of purging from our greenhouse studies?

Although deleterious alleles persisted in our experimental populations and mean pollen and ovule production declined as these alleles became more homozygous, not all maternal families were affected by the inbreeding program quantitatively in the same way. After five generations of inbreeding, ovule number, pollen number, and pollen viability in every maternal line was lower than the means for each character in the fifth generation outcross plants. Variation among the self lines, however, was substantial, and some lineages approached the outcross means for some characters. Although our results do not provide evidence of purging on a populationwide scale, some maternal lineages manifest less genetic load than others. Similar levels of variation in response to long-term inbreeding have been reported for biomass and flower production in *M. guttatus* (Dudash et al. 1997) and for fitness traits in *Tribolium* (Pray and Goodnight 1995). Variation among families has also been observed after only one generation of selfing in a diversity of plant species (Schemske 1983; Schoen 1983; Sakai et al. 1989; Dudash 1990; Ågren and Schemske 1993). These differences in response to inbreeding may be important sources of variation in populations undergoing a change in mating system (Holsinger 1988, 1991; Uyenoyama and Waller 1990a,b,c). Selection among maternal lineages could maintain a population of plants with both a high selfing rate and a high mean fertility. Falconer (1981) points out that several studies have observed little or no response to artificial selection applied within lines of mice



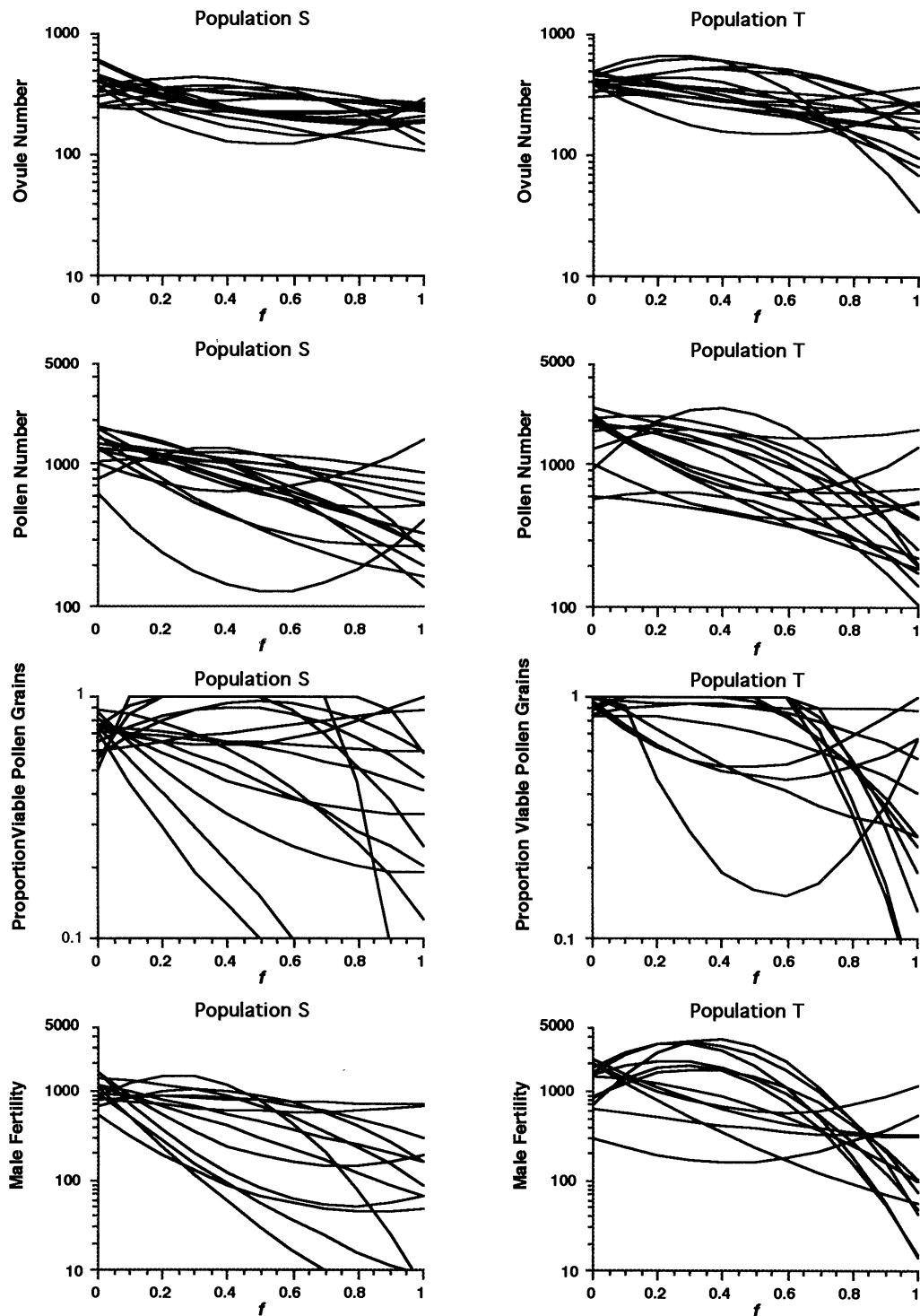


FIG. 3. Regressions of fitness traits from each individual maternal line against expected inbreeding coefficients ( $f$ ) for two populations of *Mimulus guttatus*. All regression lines were calculated from the full quadratic regression equation. For pollen viability, predicted proportions exceeding 1.0 were set to zero for these figures.

during inbreeding programs. However, selection among lines can almost completely eliminate inbreeding depression from these laboratory populations, suggesting that most of the genetic variation for fitness is at this level.

Maintaining high mean fitness in a population undergoing

a shift toward a higher selfing rate is made more difficult if a maternal lineage does not perform equally well across all components of fitness. For instance Carr and Dudash (1995) found that inbreeding depression estimates for pollen and ovule number within individual *M. guttatus* maternal families

were independent of one another, suggesting that many independent loci contribute to inbreeding depression. Similarly, Fenster and Carr (1997) found no genetic correlation between pollen and ovule number in two species of *Mimulus*. In our current study we found that pollen number and pollen viability in the most inbred plants (i.e., generations 4 or 5) were correlated, but ovule number was not correlated with either pollen character. Selection and drift may therefore act independently on these two components of reproductive success in this hermaphroditic species. Consequently maternal lines with high male fertility may not have high female fertility, and the production of an elite line with high all-round fitness may be rare. Large initial population sizes or high female fecundity may be essential if a population is going to maintain relatively high fitness with a shift toward a high selfing rate.

The origin of the observed variation in inbreeding depression is unknown. Theoretical work by Holsinger (1988) and Uyenoyama and Waller (1990a,c) predict that low levels of genetic load will become associated with mating system characters that promote selfing. Our selfing lineages were initiated from field-collected seed of unknown parentage. *Mimulus guttatus* has a mixed mating system in all populations thus far examined (Ritland and Ritland 1989; Ritland 1990; Dudash and Ritland 1991; Willis 1993b), and it is likely that some of our maternal families had already experienced inbreeding prior to being incorporated into our greenhouse studies. This may have resulted in maternal families that started with less genetic load than others. However, the performance of the most inbred plants (generations 4 and 5) in our experiment was uncorrelated to their performance after just one generation of selfing, suggesting that previous inbreeding cannot account for all of the observed variation. An alternative explanation proposed by Shultz and Willis (1995) is that, if mutations occur in a Poisson manner, variation among families within a population in their number of mutations will produce more variation in inbreeding depression than prior history of inbreeding.

#### *Additive versus Nonadditive Gene Action*

Significant deviations from linearity in the relationship between quantitative fitness traits and Wright's inbreeding coefficient can indicate selection against deleterious alleles or the presence of epistatic gene action (Dobzhansky 1970; Falconer 1981). The only population-level regression showing significant curvilinearity was our estimate of overall male fertility in population T. Because this curvilinearity is negative, it cannot be produced by selective removal of deleterious alleles (B. Charlesworth et al. 1991) and therefore is presumably the result of reinforcing epistasis (Crow and Kimura 1970). The addition of homozygous loci has an increasingly deleterious effect as these plants become more inbred. Willis (1993a) detected significant curvilinearity for pollen viability in *M. guttatus* over a range of inbreeding coefficients from 0.0 to 0.75. The presence of this type of epistasis can favor stable mating systems with selfing rates less than unity (B. Charlesworth et al. 1991) because the effects of new mutations introduced into inbred lines will

tend to be large, lowering the fitness of the most extremely inbred.

For the most part, though, the population level analyses are consistent with a model of multiplicative gene action (i.e., additive on a log scale). These linear responses have been reported for other fitness traits in these same *M. guttatus* populations (Dudash et al. 1997) and in other studies (Mukai 1969; Geiger 1988; Pray and Goodnight 1995). These linear responses cannot completely rule out reinforcing epistasis, however. The linear regression predicted by the model of multiplicative gene action assumes that alleles are fixing at random. If deleterious alleles are being selectively removed, the observation of negative quadratic regression terms could be obscured (Lynch 1988; B. Charlesworth et al. 1991). The fact that the loss of maternal lines during the inbreeding process was almost always due to problems with male fertility likely had the effect of selectively eliminating the most extremely negative linear and quadratic effects.

Although our population level analyses did not reveal much evidence for epistasis, variation among lineages was large. In both populations, there are examples of lineages displaying strongly negative quadratic terms and even some with positive quadratic terms. This is true for each of the fitness traits that we examined, and is similar to the findings of Dudash et al. (1997) for these same *Mimulus* populations and by Pray and Goodnight (1995) for *Tribolium*. The genetic interpretations of this variation is difficult. It could represent variation among maternal lineages in the chance fixation of deleterious alleles. It could also represent variation in the response to increasing homozygosity caused by lineage-specific genetic backgrounds; some maternal lineages may be better buffered against the effects of high levels of homozygosity than others. The fact that some maternal lines show positive quadratic terms, and hence do improve somewhat in their most inbred generations, adds further support for the general importance of deleterious recessive alleles to inbreeding depression because lines are not expected to improve with greater inbreeding under the overdominance hypothesis (e.g., Lande and Schemske 1985; Charlesworth and Charlesworth 1990). No matter what the cause, this variation again highlights the importance of among-lineage selection relative to within-lineage selection in overcoming the effects of inbreeding depression in highly inbred populations.

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