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FIVE GENERATIONS OF ENFORCED SELFING AND OUTCROSSING IN *MIMULUS GUTTATUS*: INBREEDING DEPRESSION VARIATION AT THE POPULATION AND FAMILY LEVEL

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Abstract.—The focus of this study was to examine the consequences of five sequential generations of enforced selfing and outcrossing in two annual populations of the mixed-mating *Mimulus guttatus*. Our primary goal was to determine whether purging of deleterious recessive alleles occurs uniformly between populations and among families, and thus gain insights into the mode of gene action (dominance, overdominance, and/or epistasis) governing the expression of inbreeding depression at both the population and family levels across the life cycle.

Inbreeding depression was detected across the five-generation breeding program in both populations for germination success, total flower production and adult aboveground biomass. No inbreeding depression was detected for date of first flowering. The serial breeding program minimized selection and may have allowed the random fixation of deleterious recessive alleles. Thus, at the population level this experiment is consistent with (1) dominance with weak selection on partially deleterious recessive alleles; (2) random fixation of deleterious alleles; and (3) overdominance because we observed a steady state of performance following inbreeding and outcrossing in these two populations of *M. guttatus*. At the family level, however, significantly different maternal line responses (maternal line \times pollination treatment and maternal line \times pollination treatment \times generation) provide a mechanism for the invasion of a selfing variant into the population through any maternal line exhibiting purging of its genetic load. These family level differences in inbreeding depression across the five generations suggest that dominance rather than overdominance underlies the expression of inbreeding depression. No evidence of epistasis was detected at the population level. A significant interaction occurred, however, between the inbreeding coefficient, f^2 , and maternal families for all traits examined suggesting that epistasis could be playing a role in the expression of inbreeding depression among maternal lines.

Key words.—Dominance, family level variation, outcrossing, population level variation, purging, serial inbreeding.

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The phenomenon of inbreeding depression has received considerable attention during the past decade because of its potential predictive value for understanding the evolution of plant mating systems (e.g., Charlesworth and Charlesworth 1979, 1987, 1990; Lande and Schemske 1985; Schemske and Lande 1985; Campbell 1986; Holsinger 1988; Charlesworth et al. 1990, 1991; Uyenoyama and Waller 1991a,b,c; Lande et al. 1994). Fisher (1941) originally demonstrated that a selfing allele can invade an outcrossing population without inbreeding depression owing to its 50% transmission advantage compared to an outcrossing individual. This result assumes no pollen discounting, the loss of siring ability associated with an increased ability to self (e.g., Holsinger et al. 1984), in the system. This transmission bias has motivated the majority of further theoretical work (see references above) on mating system evolution and prompted empiricists to quantify the existence of inbreeding depression in both primarily selfing (e.g., Svensson 1988; Griffing 1989; Ågren and Schemske 1993; Johnston and Schoen 1995; Parker et al. 1995) and outcrossing species (e.g., Schemske 1983; Schoen 1983; Sakai et al. 1989; Carr 1990; Dudash 1990; Fenster 1991). Inbreeding depression has been found both theoretically (e.g., Lande and Schemske 1985) and empirically (e.g., Holtsford and Ellstrand 1990; and reviewed by Husband and Schemske 1996) to covary with selfing rate such that the highest levels of inbreeding depression occur in those populations or species with the lowest selfing rates. Recently, the relationship between population level estimates of inbreeding depression and family estimates within a pop-

ulation have been investigated to determine which level is more indicative of the potential for selfing to evolve in an outcrossing population (e.g., Uyenoyama et al. 1993). Our study examines the dynamic behavior of inbreeding depression across five generations in two populations of mixed-mating *Mimulus guttatus* at both the population and family level.

Holsinger (1988, 1991) and Uyenoyama and Waller (1991a,b,c) have demonstrated that population level estimates of inbreeding depression alone are insufficient to predict whether a selfing variant can invade a particular population. They showed that associations may develop between viability and mating system loci that are as important as inbreeding depression itself. The mechanisms for associations between mating system loci and viability loci are (1) identity disequilibrium (Weir and Cockerham 1973); and (2) the production of highly fit inbred progeny that are homozygous for wild-type alleles. These theoretical studies predict that inbreeding depression will be greater in an outcrossing species than a selfing species, but that inbreeding depression alone may not explain the evolution of mating systems. Their models indicate the importance of determining family level variation in inbreeding depression within a population because this alternative measure may better predict whether a selfing variant can invade an outcrossing population.

There exist two major nonmutually exclusive hypotheses to explain the genetic basis of inbreeding depression. The first is dominance, the expression of recessive or partially recessive alleles with deleterious effects (Wright 1977; Fal-

coner 1981; also referred to as partial dominance by Charlesworth and Charlesworth 1987). The second hypothesis is overdominance, the breakdown of heterozygote advantage at a single locus (Wright 1977) or many loci (Lande and Schemske 1985). The dominance hypothesis of inbreeding depression predicts that the amount of inbreeding depression *decreases* with increasing self-fertilization in the presence of selection. This occurs because inbreeding increases both homozygosity and the efficiency of selection in removing deleterious recessive alleles from the population. A population's genetic load is expected to be more difficult to purge if overdominance is responsible for the observed inbreeding depression (Lande and Schemske 1985; Charlesworth et al. 1990). In this case, the most fit genotypes are heterozygous, but when selfed, these genotypes continue to regenerate the less fit homozygous genotypes. The overdominance hypothesis predicts an initial increase in the amount of inbreeding depression expressed, but no further *increases* in inbreeding depression as long as the selection coefficients are asymmetrical (Charlesworth and Charlesworth 1987, 1990; Ziehe and Roberds 1989). Other factors, however, such as epistasis (e.g., Crow and Kimura 1970; Bulmer 1985; Lynch 1991), linkage (or pseudo-overdominance; e.g., Comstock and Robinson 1952; Wright 1977 and references therein), selection, and drift may all be influencing the genetic basis and subsequent expression of inbreeding depression. Epistasis can either enhance (reinforcing) or inhibit (diminishing) inbreeding depression as a function of increasing inbreeding coefficients (f) (Wright 1977), and is documented by the nonlinear decline in fitness because the expression of partially deleterious mutations are not independent (Crow and Kimura 1970). The presence of epistasis could impede the predictive value of inbreeding depression estimates in determining whether selfing can evolve by reducing the genetic load (Charlesworth et al. 1991).

Few multigenerational studies of controlled inbreeding in plants have been conducted, except for a number of studies involving maize (e.g., Hallauer and Miranda Fo 1985; Benson and Hallauer 1994). The large body of research on maize suggests that dominance is the primary mechanism responsible for inbreeding depression (but see Stuber et al. 1992). Benson and Hallauer (1994) observed an increase in performance of inbred lines in 13 plant and 16 ear traits following seven generations of selfing using a modified single-descent procedure (Good and Hallauer 1977) and reciprocal recurrent half-sib selection for alternative rates of inbreeding. Similarly, Darwin (1892) observed an unexpected increase in performance of progeny generated from five generations of selfing compared to outcrossed progeny in *Mimulus luteus*. The selfing advantage continued to increase through the seventh generation of selfing with the self progeny exhibiting an advantage in autofertility, being taller, flowering earlier, and producing more flowers, fruits, and seeds than outcross progeny. Barrett and Charlesworth (1991) demonstrated an initial decrease in flower production following one generation of selfing in an outcrossing aquatic, *Eichhornia paniculata*, but no further decline in subsequent generations of selfing. Following the fifth generation of selfing, inbred lines were randomly mated, resulting in progeny with enhanced flower production when compared to the parental outcrossed popula-

tion. They suggested that the plateau in inbreeding depression and the greater heterosis observed in the randomly mated inbred lines were consistent with the purging of partially recessive deleterious alleles from the population. McCall et al. (1994) examined the effects of serial inbreeding on fitness components in *Impatiens capensis* with inbreeding coefficients ranging from 0–0.875. Inbreeding depression was detected and the pattern of inbreeding depression across the breeding regime suggests either the rapid purging of deleterious recessive alleles or that diminishing epistasis was responsible for the observed pattern. Owing to high mortality throughout the experiment, McCall et al. (1994) were unable to quantify variation in maternal lines in response to the inbreeding regime.

The genus *Mimulus* of the Scrophulariaceae has recently become the focus of many studies aimed at understanding the processes responsible for the evolution of plant mating systems. Changes in mating systems are frequent within the genus (Ritland and Ritland 1989; Fenster and Ritland 1994), and polygenic inheritance of traits influencing the mating system has been demonstrated (Macnair and Cumbes 1989; Carr and Fenster 1994; Fenster and Ritland 1994; Fenster et al. 1995). The genetic architecture of mutational load has also come under investigation. Lethal recessive alleles that cause chlorophyll deficient mutants can be frequent in some populations (Kiang and Libby 1972; Willis 1992), and inbreeding depression has been demonstrated in a number of *Mimulus guttatus* populations (Dole and Ritland 1993; Willis 1993a; Carr and Dudash 1995, 1996). Willis (1993b) investigated the relationship between progeny performance and increasing inbreeding coefficient in two populations of outcrossing *M. guttatus*. The breeding design detected a decline in all fitness traits, except germination, and some evidence for the presence of reinforcing epistasis in pollen viability as relatedness increased. Fu and Ritland (1994) inferred the action of viability genes using a graphically based analysis of allozyme segregation patterns in *M. guttatus*. Eighteen of 24 linkage groups detected were consistent with either partial dominance, complete dominance, or underdominance while the remaining six linkage groups were consistent with recessivity or overdominance.

The primary focus of this study was to examine the consequences of five sequential generations of enforced selfing and outcrossing on inbreeding depression estimates at both the population and family level for two annual populations of the mixed-mating *M. guttatus*. We examined the traits germination success, date of first flowering, total flower production, and adult aboveground biomass. Specifically, we wanted to determine whether purging of genetic load occurs uniformly between populations and among families, and gain insights into whether dominance, overdominance, and/or epistasis underlie the expression of inbreeding depression in these traits. This paper represents one of two approaches that we have conducted to determine the genetic basis of inbreeding depression and complements an ongoing North Carolina 3 quantitative genetics study by Dudash and Carr in *M. guttatus* and the related selfing taxon, *M. micranthus*.

MATERIALS AND METHODS

Study Organism

Mimulus guttatus DC (Scrophulariaceae) is an annual to perennial hermaphroditic herb widely distributed in moist areas across western North America. It is fully self-compatible, and outcrossing rates measured for populations of this species vary between 0.25 and 1.00, averaging about 0.60 (Ritland and Ritland 1989; Ritland 1990; Dudash and Ritland 1991; Willis 1993a). Variation in outcrossing rates within and between species of *Mimulus* is associated with stigma-anther separation, with more highly selfing taxa and individuals exhibiting reduced stigma-anther separation (Ritland and Ritland 1989; Dole 1992; Carr and Fenster 1994).

Seed was collected in 1988 from two annual populations of *M. guttatus* (hereafter referred to as S and T) located approximately 10 km apart in Tuolumne County, California. Seed from 27 and 31 maternal families from the S and T populations, respectively, was used for this experiment. Dudash and Ritland (1991) have estimated the outcrossing rate for population T to be 0.70 (SE = 0.06), but no estimate is available for population S. All plants were raised in a pollinator-free greenhouse at the University of Maryland, College Park. The photoperiod was maintained at 18 h with sodium vapor lights as needed. Plants were grown in 46 cm² square, plastic pots filled with Progro[®] 300S mix. Pots were placed in trays filled with standing water with 20 pots per tray. No fertilizer was added during the course of the experiment.

Experimental Design

In the summer of 1990, 30 seeds from 27 and 31 field-collected maternal families of the S and T populations, respectively, were sown. One randomly selected seedling from each family was used as a seed parent for hand-pollinations. Pollen parents were chosen at random from within the seed parent's population until each plant had served once as a pollen donor. Cross-pollinations were not reciprocal. Self- and cross-pollinations were performed on different flowers at the same node to control for temporal and positional pollination effects on progeny performance. Corollas and stamens were removed following hand-pollinations to prevent any subsequent self-pollination (Dole 1990).

All subsequent generations descended from self seed from the previous generation. Outcross seeds were produced each generation using the same pairs of maternal lines as the first generation. As maternal lines were lost, new outcross pollen parents were randomly chosen from the surviving maternal lines. This crossing regime was continued through five generations of selfing and outcrossing over a 2.5-yr period. We maintained a total of 31 (S = 16 and T = 15) maternal families where both self and outcross seed from each family were successfully generated for a minimum of four generations. In the S population 15, 16, 16, 14, and 15 families were represented in 1, 2, 3, 4, and 5 generations of selfing and outcrossing, respectively. In the T population 15, 11, 15, 14, and 7 families were represented in 1, 2, 3, 4, and 5 generations of selfing and outcrossing, respectively. The number of families varied across generations because we

selected to include only those families for which both self and outcross seed was available for a given generation. Loss of maternal lines was primarily due to low pollen production and germination failure. Seed generated from each generation of selfing and outcrossing was stored in lab. The inbreeding depression estimates reported in this study are conservative because at the onset of this experiment only maternal lines that survived four of five generations of inbreeding and outcrossing were included, thus omitting maternal families where inbreeding produced more extreme effects.

Progeny performance from all five generations was measured simultaneously in the greenhouse. On September 17, 1992 a maximum of 30 seeds per maternal family per cross per generation were sown in individual pots. Each tray contained two families (10 pots each) randomized within a tray. The trays were rotated daily to minimize position effects on the greenhouse bench during the two-week germination period.

Germination was scored two weeks later and seedlings were transplanted and randomized on October 2 and 3, 1992. Seedlings from each generation within a maternal family were transplanted into self and outcross pairs within a pot. A maximum of 10 replicates for each pair of self and outcross seedlings were transplanted from each generation within each family.

Plants were grown in self-outcross pairs within a pot for two reasons. First, the competitive environment is thought to accentuate the effects of inbreeding depression by providing a more realistic environment (Darwin 1892; Schmitt and Ehrhardt 1990; Argyres and Schmitt 1992; Wolfe 1993; Carr and Dudash 1995; Latter and Mulley 1995; Parker et al. 1995). Second, twice as many plants could be grown in the same greenhouse space. In this design 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 the outcrosses improve as competitors across the five generations), but this was not observed. In a preliminary experiment we tested the performance of how well progeny from one (S1) and five (S5) generations of selfing grew with progeny from one (O1) and five (O5) generations of outcrossing (S1 vs. O1, S1 vs. O5, S5 vs. O1, S5 vs. O5). Regressing aboveground biomass on inbreeding coefficient demonstrated no significant difference in the slope (b) whether S1 or S5 competed with O1 or O5 ($b = -0.069 \pm 0.024$, $P < 0.0001$; $b = -0.071 \pm 0.022$, $P < 0.0001$, respectively). Furthermore Carr and Dudash (1995) found no difference in the performance of either self or outcross plants when grown with either self or outcross competitors. Therefore, the differences observed in performance among self progeny across the five generations reflects the effect of increasing homozygosity.

The replicate self-outcross pairs were randomized across 10 blocks such that each block contained all families across all generations. Each block consisted of seven trays with 20 pots per tray. The blocks were arranged on three benches in one greenhouse maintained at 21°C, and all plants were monitored daily for date of first flowering. The experiment ran for 95 d. Total flower production was censused immediately prior to biomass harvesting in January 1993. Plants were

harvested at soil level, dried at 50°C and weighed to the nearest 0.01 g.

Statistical Analyses

Population Level Inbreeding Depression.—The effect of serial inbreeding at the population level was examined by quantifying the pollination treatment \times generation interaction. Pollination treatment and generation were analyzed as fixed effects and population, maternal line, and two-way interactions between the fixed effects and population were analyzed as random effects. The random statement with the test option (SAS Institute 1989) was used to generate error mean squares for all main effects and Type III sums of squares were used in all analyses. Germination success was monitored as percent seeds germinated and arcsine square-root transformed to meet the assumptions of ANOVA. Maternal lines were not replicated for germination since all seed were sown into one pot per family per cross per generation, thus we are unable to examine the interaction between pollination treatment and maternal line.

Date of first flower, total flower production, and adult aboveground biomass were analyzed with the same mixed-model ANOVA. Flower production and aboveground biomass were log-transformed to meet assumptions of ANOVA. Pollination treatment and generation were treated as fixed effects, and population and maternal line within population were random effects. The pollination \times generation interaction was a fixed effect, but all other two- and three-way interactions were random. For date of first flower and flower production, population and interactions involving population were not significant and pooled into the error term.

Epistasis among partially deleterious recessive alleles can effect the shape of the pollination \times generation interaction depending on its magnitude and whether it is reinforcing or diminishing in its effect. If the effects of homozygous loci act in a multiplicative fashion, a linear relationship is expected between the log of fitness and the expected inbreeding coefficient (Crow 1970; Crow and Kimura 1970). Reinforcing epistasis can be detected if a significant nonlinear *negative* relationship occurs between fitness components and expected inbreeding coefficients. Significant nonlinear *positive* relationships can be due to either diminishing epistasis and/or purging of partially deleterious recessive alleles. Thus to examine epistasis, we calculated linear (f) and quadratic coefficients (f^2) of the quadratic regression of log-transformed fitness components, date of first flower, total flower production, and adult aboveground biomass on expected inbreeding coefficients ($f = 0, 0.5, 0.75, 0.875, 0.9375, 0.96875$) corresponding to the first generation of outcrossing, and 1, 2, 3, 4, and 5 generations of selfing, respectively. Family means for each fitness component were log-transformed and no zero values occurred in the data.

Family Level Inbreeding Depression.—Differences among maternal lines in inbreeding depression were detected by a significant maternal line by pollination treatment interaction and the three-way interaction with generation. A logarithmic transformation was necessary for total flower production and adult aboveground biomass to meet the assumptions of ANOVA. A logarithmic transformation was also used for date of

first flower to test whether the ratio between self and outcross progeny significantly varied among maternal lines as a function of pollination treatment (Johnston and Schoen 1994). ANOVA assumptions were met by both untransformed and transformed data for date of first flower.

To display the relationship between self and outcross progeny in each maternal line across the five generations, it was necessary to calculate a measure of relative performance. The relative performance of cross-types (RP) for date of first flower, total flower production, and adult aboveground biomass within each maternal line was calculated as

$$RP = \frac{(\text{outcross} - \text{self})}{\text{maximum}}, \quad (1)$$

where maximum = outcross when outcross \geq self and maximum = self when self $>$ outcross (Ågren and Schemske 1993). Relative performance of each maternal family was calculated for each generation. The maximum was used in the denominator of this estimate rather than the outcross value, which is used in the traditional estimation of inbreeding depression, to create a distribution of relative performance that was symmetrical and bounded by -1 and 1. Relative performance estimates are identical to traditional estimates of inbreeding depression when outcross progeny outperform self progeny. Unlike the traditional estimates of inbreeding depression, they can be summed in an unbiased fashion in the case where self progeny outperform outcross progeny.

Epistasis was examined at the family level within each population by performing a homogeneity of slopes ANOVA where maternal line was a class variable and f and f^2 were continuous variables (as defined above). Each analysis was performed on log-transformed fitness components: date of first flower, total flower production ($\log + 0.1$), and adult aboveground biomass. The relationship between f and the log of a fitness trait (x) may not be the same as the relationship of f and $\log(x + 1)$ of the same fitness trait (Willis 1993b). We chose to use a constant of 0.1 to minimize the bias introduced during the transformation of total flower production where individuals that never flowered were assigned a value of zero. This ANOVA approach allowed us to examine for main effects of maternal line, f , f^2 , and interactions between maternal family $\times f$, and maternal family $\times f^2$. Significant interaction terms indicate differences among maternal lines in their responses to increasing homozygosity.

RESULTS

Population Level Inbreeding Depression

Overall, self progeny germinated at a significantly lower level than outcross progeny, and maternal lines within populations significantly varied in overall germination ability as well (Table 1; Fig. 1). We detected a significant population \times generation interaction, resulting from overall low germination success in the fifth generation as well as a decrease in number of maternal lines from the loss of seven lines after the fourth generation in the T population.

Of those 2156 (84%) plants that flowered, serial generations of selfing did not significantly delay date of first flower, although generation and maternal line demonstrated significant main effects (Table 2). The average day to first flower

TABLE 1. Analysis of variance for the dependent variable "percent germination" (arcsine square-root transformed) contrasted between five generations of enforced selfing and outcrossing in two populations of *Mimulus guttatus*. Mean squares are based on Type III sums of squares. The model explains 28.9% of the variation in germination success.

Source	df	MS	F	P
Population	1	0.0436	0.261	0.6379
Maternal line within population	29	0.1132	1.948	0.0038
Pollination treatment	1	0.3515	174.208	0.0364
Generation	4	0.2629	1.554	0.3399
Population × pollination treatment	1	0.0019	0.033	0.8563
Population × generation	4	0.1692	2.911	0.0223
Pollination treatment × generation	4	0.1000	1.720	0.1463
Error	235	0.0581		

varied significantly among generations, ranging from 65–70 d following sowing, but the means showed no tendency to either increase or decrease across generations. Flower production was significantly reduced in the self progeny compared to outcross progeny consistently throughout the five-generation breeding program and we detected marginally significant main effects of maternal line and generation (Table 3; Fig. 2). However, there was no significant interaction be-

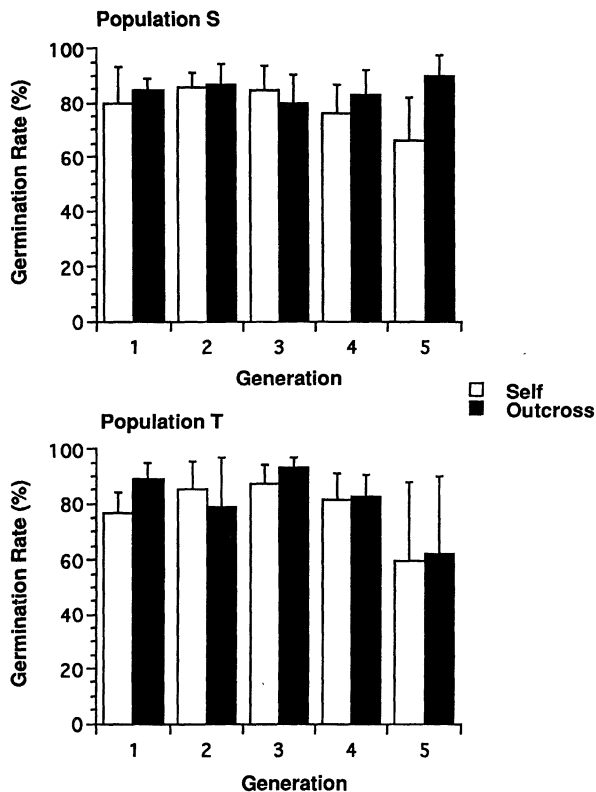


FIG. 1. Average germination rate of populations S and T across five generations of serial selfing and outcrossing in *Mimulus guttatus*. Means are back-transformed with error bars representing 95% confidence intervals.

TABLE 2. Analysis of variance for the dependent variable "date of first flower" contrasted between five generations of enforced selfing and outcrossing in two populations of *Mimulus guttatus*. Mean squares are based on Type III sums of squares. The model explains 56.8% of the variation in date of first flower. Nonsignificant effects of population and interactions involving population were pooled into the error term.

Source	df	MS	F	P
Block	9	426.75	3.934	0.0001
Maternal line	30	3203.22	2.776	0.0007
Pollination treatment	1	154.99	0.229	0.6352
Generation	4	1543.95	2.876	0.0260
Pollination treatment × generation	4	152.98	0.602	0.6621
Maternal line × poll. trmt.	30	811.30	2.989	0.0001
Maternal line × generation	103	656.48	2.247	0.0001
Maternal line × poll. trmt. × gen.	96	292.35	2.695	0.0001
Error	1749	108.47		

tween pollination treatment and generation across the five-generation breeding regime.

In contrast to date of first flower and total flower production, adult aboveground biomass did not exhibit significant main effects of either pollination treatment, generation, or maternal lines (Table 4). Despite a strong trend, the overall effect of pollination treatment was not significant owing to a marginally significant two-way interaction ($P = 0.085$) between pollination treatment and generation and a significant three-way interaction between population, generation, and pollination treatment ($P < 0.041$). The interaction was the result of a consistent level of inbreeding depression in adult aboveground biomass in the S population across generations compared to increasing levels of inbreeding depression across the five generations in the T population (Fig. 3). These significant interactions precluded us from pooling the nonsignificant population main effect into the error term (as done in the analyses of date of first flower and total flower production, see above).

TABLE 3. Analysis of variance for the dependent variable "flower number" (log-transformed) contrasted between five generations of enforced selfing and outcrossing in two populations of *Mimulus guttatus*. Mean squares are based on Type III sums of squares. The model explains 56.2% of the variation in flower number. Nonsignificant effects of population and interactions involving population were pooled into the error term.

Source	df	MS	F	P
Block	9	1.576	11.140	0.0001
Maternal line	30	3.466	1.758	0.0534
Pollination treatment	1	82.088	52.730	0.0001
Generation	4	1.563	2.311	0.0624
Pollination treatment × generation	4	0.425	0.915	0.4577
Maternal line × poll. trmt.	30	1.746	3.604	0.0001
Maternal line × generation	103	0.743	1.474	0.0252
Maternal line × poll. trmt. × gen.	103	0.504	3.564	0.0001
Error	2275	0.141		

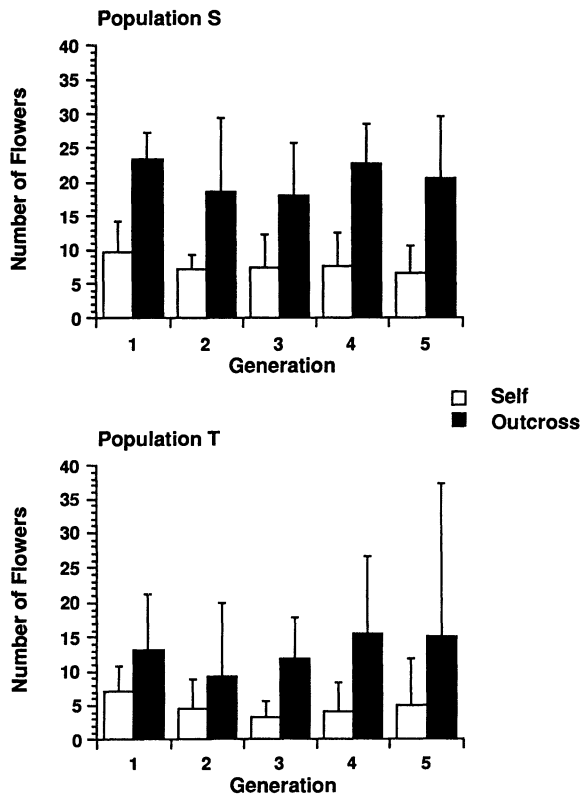


FIG. 2. Average total flower production of populations S and T across five generations of serial selfing and outcrossing in *Mimulus guttatus*. Means are back-transformed with error bars representing 95% confidence intervals.

The linear component of the quadratic regression analysis of fitness components on expected inbreeding coefficients demonstrated a significant overall decline in total flower production and biomass in the S population and for biomass in the T population (Table 5). None of the quadratic coefficients of the quadratic regression were significant. Thus significant inbreeding depression was detected in these analyses, concurrent with the ANOVAs presented above. Epistasis was not detectable for date of first flower, total flower production, or aboveground biomass in either population.

Family Level Inbreeding Depression

Inbreeding depression among maternal lines varied significantly in response to pollination treatment for date of first flower, total flower production, and aboveground biomass (Tables 2, 3, 4; significant interaction between maternal line and pollination treatment and a three-way interaction among maternal line \times pollination treatment \times generation). Date of first flower estimates of relative performance between the self and outcross maternal line sibships exhibited similar variation as in aboveground biomass and less variation than flower number (Fig. 4). Total flower production responses ranged from -0.7 to $+1.0$ with some maternal lines exhibiting patterns of increasing relative performance while others showed decreasing patterns, while other maternal families exhibited no pattern across the five-generation breeding program (Fig. 5).

TABLE 4. Analysis of variance for the dependent variable "adult aboveground biomass" (log-transformed) contrasted between five generations of enforced selfing and outcrossing in two populations of *Mimulus guttatus*. Mean squares are based on Type III sums of squares. The model explains 62.2% of the variation of adult aboveground biomass.

Source	df	MS	F	P
Block	9	0.0124	4.187	0.0001
Population	1	0.7185	2.778	0.3373
Maternal line within population	29	0.0280	1.445	0.1680
Pollination treatment	1	5.7057	22.559	0.1321
Generation	4	0.0103	6.022	0.6825
Population \times pollination treatment	1	0.2536	8.460	0.0187
Population \times generation	4	0.0170	0.842	0.5631
Pollination treatment \times generation	4	0.0911	4.576	0.0854
Maternal line w/i pop. \times poll. trmt.	29	0.0190	2.434	0.0006
Mat. line w/i population \times gen.	99	0.0085	1.051	0.4018
Population \times gen. \times poll. trmt.	4	0.0200	2.586	0.0411
Mat. line w/i pop. \times poll. trmt. \times gen.	99	0.0081	2.724	0.0001
Error	2280	0.0030		

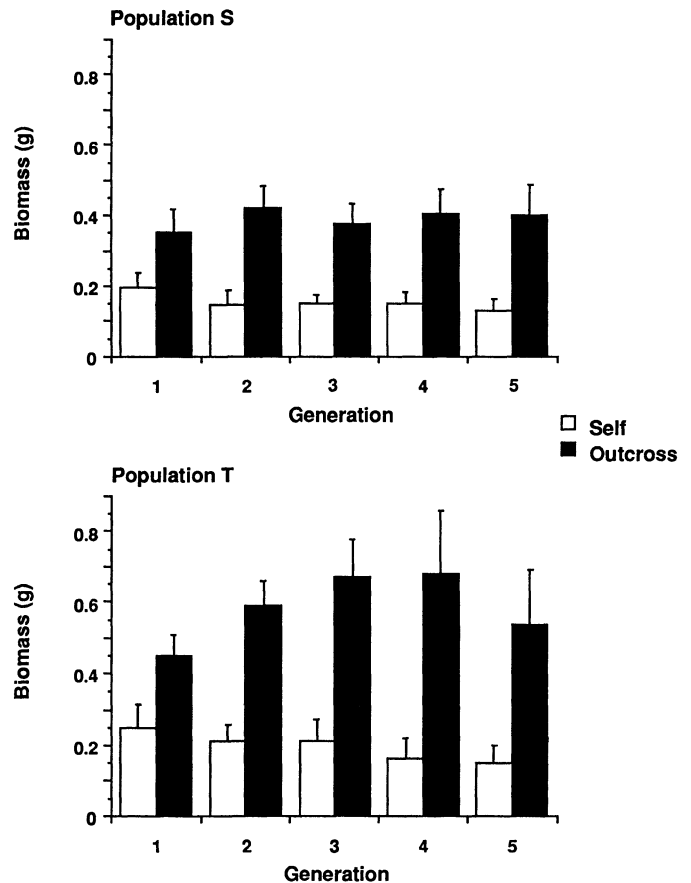


FIG. 3. Average adult aboveground biomass (g) of populations S and T across five generations of serial selfing and outcrossing in *Mimulus guttatus*. Means are back-transformed with error bars representing 95% confidence intervals.

TABLE 5. Linear (f) and quadratic (f^2) coefficients of the quadratic regression of fitness component on expected inbreeding coefficients ($f = 0.5, 0.75, 0.875, 0.9375, 0.96875$) as compared to a parental outbreeding population in two populations of *Mimulus guttatus*. Maternal line means were log-transformed with no zeros present.

Population	Linear coefficient \pm 1 SE	P	Quadratic coefficient \pm 1 SE	P
S				
Date of first flower	-0.005 ± 0.09	0.95	0.05 ± 0.09	0.53
Flower number	-0.95 ± 0.36	<0.009	0.50 ± 0.36	0.17
Biomass	-0.65 ± 0.23	<0.005	0.20 ± 0.23	0.38
T				
Date of first flower	-0.02 ± 0.10	0.86	0.03 ± 0.10	0.74
Flower number	-0.29 ± 0.42	0.49	-0.26 ± 0.42	0.54
Biomass	-0.53 ± 0.23	<0.02	0.03 ± 0.24	0.90

Significant interactions were found for date of first flower, total flower production, and adult aboveground biomass between all maternal line $\times f$ and maternal line $\times f^2$ effects within both populations. These significant interactions occurred because some families exhibited positive linear and quadratic coefficients, whereas others exhibited negative linear and quadratic coefficients. Also, although there was a strong directional trend, the magnitudes of these relationships varied among families. The linear coefficients observed for flower number in the S population found 15 of 16 families exhibiting negative coefficients. Additionally, aboveground

biomass in the T population found 13 of 15 families exhibiting negative coefficients indicating inbreeding depression in these traits. The quadratic coefficient for total flower production also demonstrated a strong directional trend with 12 of 16 families in the S population exhibiting a positive curvilinear slope suggesting either diminishing epistasis and/or purging of deleterious recessive alleles.

DISCUSSION

The consequences of five generations of inbreeding were measured for germination success, date of first flowering,

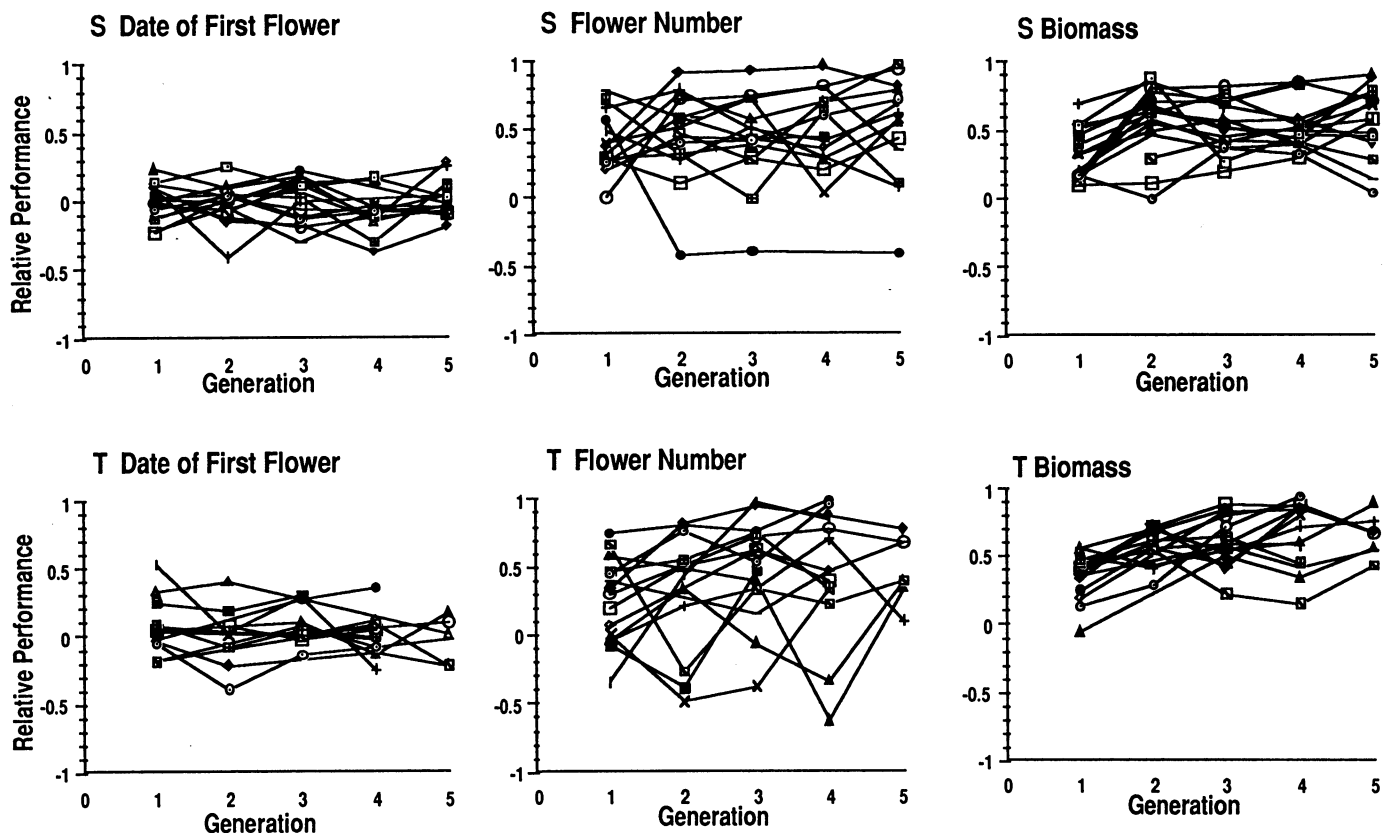


FIG. 4. Relative performance of S and T maternal line sibships across five generations of serial selfing and outcrossing in *Mimulus guttatus*. Each symbol within the S and T populations represents a maternal family for date of first flower, total flower production (flower number), and adult aboveground biomass (biomass). In the S population 15, 16, 16, 14, and 15 families were represented in 1, 2, 3, 4, and 5 generations of selfing and outcrossing, respectively. In the T population 15, 11, 15, 14, and 7 families were represented in 1, 2, 3, 4, and 5 generations of selfing and outcrossing, respectively.

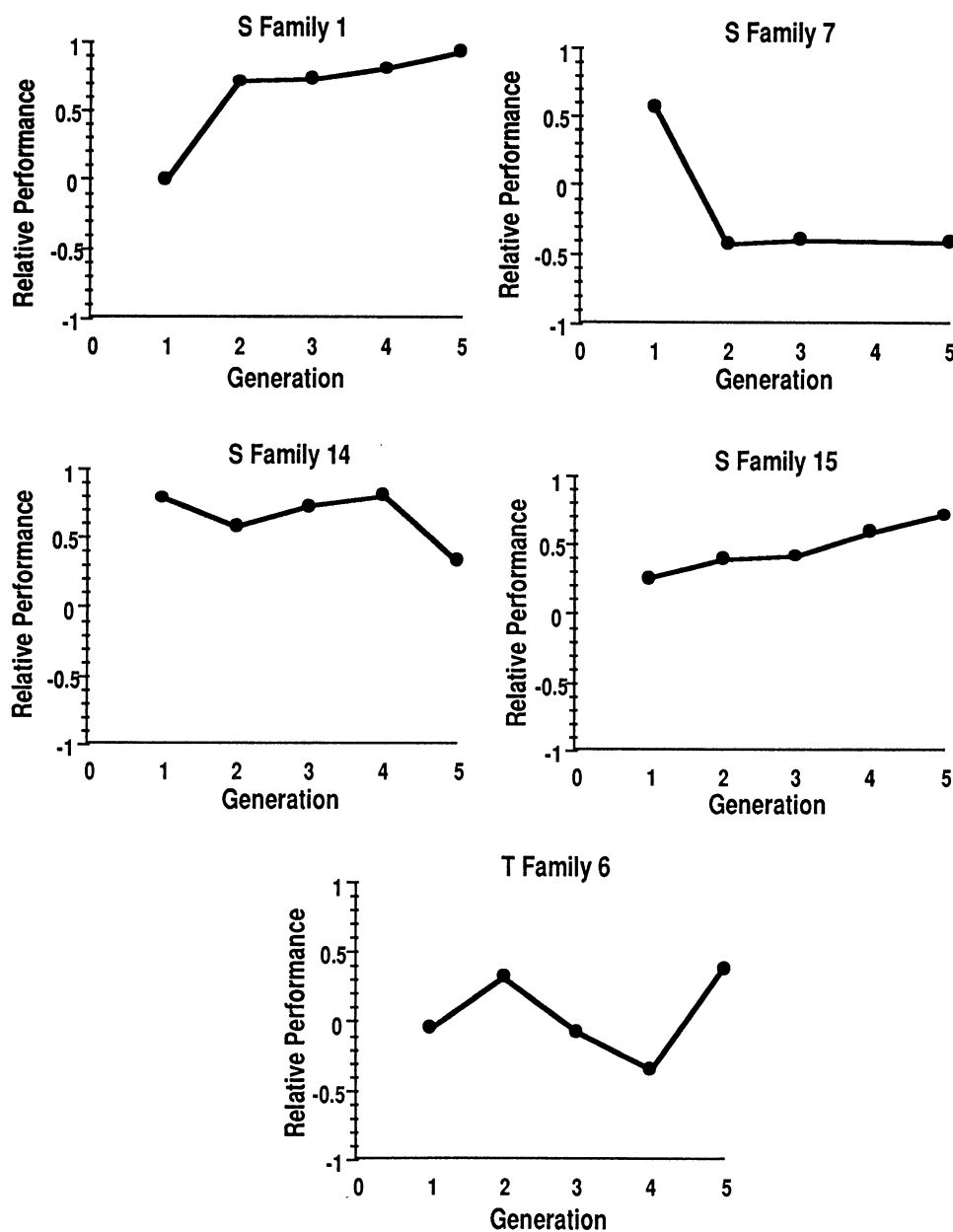


FIG. 5. Relative performance in total flower production of representative S and T maternal line sibships in total flower production from Figure 4. Inbreeding depression appears to be never purged (S Family 1), be rapidly purged (S Family 7), slowly decrease (S Family 14), slowly increase (S Family 15), and fluctuate demonstrating no discernible pattern (T Family 6) across the five-generation breeding program. Inbreeding depression increases with increasing relative performance (positive values indicate that outcross progeny are outperforming self progeny).

total flower production, and adult aboveground biomass. These characters, but especially germination success and total flower production, are expected to be highly correlated with individual fitness in these annual populations of *M. guttatus*. The consequences of serial inbreeding are dependent on the genetic basis of inbreeding depression, for example, dominance and/or overdominance. With a dominance mode we expect either purging (inbreeding with selection) or increased expression of inbreeding depression across the five-generation breeding program. In contrast, overdominance should lead to a relatively constant expression of inbreeding depression.

Below we discuss our findings in the context of mating system evolution.

Population Level Inbreeding Depression

Inbreeding depression at the population level was detected across the five-generation breeding program for germination, total flower production, and adult aboveground biomass. Removal of partial deleterious recessive alleles should increase the population mean in lifetime fecundity as wild-type alleles become fixed. However, our serial inbreeding and outcrossing

regime may have allowed for the random fixation of partially deleterious alleles within some maternal lines thus hindering our ability to detect purging of these alleles at the population level. Selection within an inbred line for purged genotypes can be balanced by drift causing random fixation of these deleterious recessive alleles. The population level analysis is congruent with purging of deleterious recessive alleles if weak selection is acting in the system, which may very well have occurred given our greenhouse breeding program where we were attempting to minimize selection. Overdominance, however, cannot be ruled out as an explanation for the differential progeny performance observed from serial selfing and outcrossing throughout the breeding program. Self progeny performed consistently less well than the outcross progeny for all traits, whereas the outcross progeny did not show strong evidence of improving, but remained relatively constant in their performance.

Our crossing design can be directly compared to Barrett and Charlesworth's (1991) study on the outcrossing Brazilian population of *Eichhornia paniculata*, where they detected a significant increase in mean flower production when they crossed among their fifth generation inbred lines. They attributed an increase in flower production to the reduction of the population's genetic load in the form of partially recessive mutations. The increase in biomass across the first four generations in the outcross progeny in the T population also supports the notion of population level purging of deleterious recessive alleles.

Evidence of population-wide purging of deleterious alleles has come from studies comparing populations of related species that differ in their selfing rates (e.g., Latta and Ritland 1994; Carr and Dudash 1996). In a recent review of the literature, Husband and Schemske (1996) observed the magnitude of inbreeding depression in selfing species to be less than outcrossing species and attributed this result to more efficient purging of genetic load in the selfing taxa. Latta and Ritland (1994) examined the role of prior inbreeding on current expression of inbreeding depression in 15 populations of four taxa of the *Mimulus* complex exhibiting varying selfing rates. They observed no immediate purging from one to two generations of selfing compared to within population crosses and suggested that partial dominance alone cannot explain the patterns of inbreeding depression observed in their study.

Family Level Inbreeding Depression

Modeling work by Holsinger (1988, 1991), Uyenoyama and Waller (1991a,b,c), and Uyenoyama et al. (1993) have demonstrated that population level estimates of inbreeding depression alone are insufficient to predict whether a selfing variant can invade a particular population. Secondly, they have advocated the importance of determining family level variation in inbreeding depression within a population because this alternative measure may better predict whether a selfing variant can invade an outcrossing population. Our study provides an empirical examination of both population and family estimates of inbreeding depression and allows insight to whether one level is more indicative of the potential for selfing to evolve in an outcrossing population.

The detection of maternal line improvement in relative performance across the five-generation breeding regime suggests that dominance through purging of partially deleterious recessive alleles underlies the expression of inbreeding depression. However, with overdominance one may also observe a decrease in inbreeding depression (or maternal line improvement) if heterozygosity levels decrease with serial inbreeding. This may be the situation in our experiment since we generated our maternal lines through single seed descent and we could be crossing between related individuals randomly fixed for similar partially deleterious recessive alleles. This condition could bias our comparison because we would be minimizing differences between the self and outcross progeny and observe relative improvement within a maternal line. With overdominance, no improvement of self progeny relative to outcross progeny within a maternal line would be observed if selection against homozygotes is sufficiently strong and/or symmetrical (e.g., Charlesworth and Charlesworth 1990). Either of these situations would prevent heterozygosity levels from decreasing with increased inbreeding, resulting in the maintenance of inbreeding depression within maternal lines.

Significant differences were observed among maternal lines in performance of self and outcross maternal sibships in date of first flower, total flower production, and adult aboveground biomass. Detection of this significant maternal line by pollination treatment interaction was independent of whether a main effect of pollination treatment and/or generation effect was detected as well. Significantly different maternal line responses (maternal line \times pollination treatment and maternal line \times pollination treatment \times generation) provide a mechanism for the invasion of a selfing variant into the population through any maternal line exhibiting purging of its genetic load and implicates dominance as the genetic basis of inbreeding depression. There are several examples of negative values of relative performance in maternal family sibships indicating that self progeny outperformed their outcross sibs (Figs. 4 and 5). The best maternal line of either the S and T populations following five generations of selfing exhibited an average adult aboveground biomass of 66% and 41% and an average flower production of 81% and 132% of the average outcrossed progeny performance across all five generations. Thus in the T population we have detected one instance where an inbred line is capable of outperforming the average performance of outcrossed progeny. These results suggest that if alleles increasing selfing become associated with the high performance inbred line then a selfing variant may invade the original outcrossing population. The best evidence of genetic load purging comes from maize where some inbred lines now outperform some hybrid varieties (Crow 1993).

Our empirical data support the theoretical work that places emphasis on the role of maternal line variation in the expression of inbreeding depression (see references cited above). We detected purging of genetic load in some maternal line sibships in both populations of *M. guttatus*, while other maternal line sibships showed no change or continued to decline across the five generations as well (Figs. 4 and 5). The improvement of some maternal lines in relative performance of self to outcross progeny across the five generations

suggests that dominance through purging of partially deleterious recessive alleles or overdominance through matings between outcrossed relatives randomly fixed for similar partially deleterious alleles underlies the expression of inbreeding depression. However, we also observed no improvement in relative performance of self progeny to outcross progeny within some maternal lines (Fig. 5), suggesting that selection against homozygotes is sufficiently strong and/or symmetrical to maintain inbreeding depression within these maternal lines.

Recent evidence for the presence of significant genetic variation among lineages in inbreeding depression within a population comes from work by Pray and Goodnight (1995) on the red flour beetle. They conducted four generations of full-sib mating, reaching an $f = 0.594$ and found significant variation in inbreeding depression among lineages for egg-adult viability, female and male relative fitness, and adult dry weight (lineage and inbreeding coefficient interaction). The similarity between our results and those of Pray and Goodnight is of interest, especially since the region of relatedness (f) in the flour beetle system is equal to only approximately one generation of selfing in *M. guttatus*. However, our results do differ from theirs in one significant way. Whereas more inbred sibs outperformed less inbred sibs in nine of 15 lineages for females and two of 15 lineages for males in *T. castaneum*, we only observed increased performance of self progeny compared to outcross progeny within a maternal sibship for one family in each population for the trait total flower production and none for biomass (Fig. 4). Other multigenerational plant studies of serial inbreeding have not explicitly examined maternal line variation. A number of single generation studies of inbreeding depression, however, have noted significant inbreeding depression variation among maternal lines (e.g., Schemske 1983; Schoen 1983; Sakai et al. 1989; Dudash 1990; Ågren and Schemske 1993).

The Role of Epistasis

The effects of varying degrees of inbreeding have also been studied by constructing progeny arrays that represent differing inbreeding coefficients ranging from fully outcrossing to varying degrees of relatedness including multiple generations of selfing. Researchers commonly perform quadratic regression analysis of fitness components on expected inbreeding coefficients (since actual coefficients are unknown at the outset) to examine overall fitness decline (f = linear coefficient) and the presence of epistasis (f^2 = nonlinear quadratic coefficient) (Crow and Kimura 1970). At the population level we detected no significant evidence of either reinforcing or diminishing epistasis in the fitness decline across the five generations of selfing for date of first flower, total flower production, or adult aboveground biomass (Table 5). At the family level, 12 of 16 maternal lines within population S exhibited a significant positive curvilinear response, either owing to diminishing epistasis and/or purging of deleterious recessive alleles, which slowed down the descent of total flower production across the five generations. In date of first flowering in both populations and total flower production in the T population, however, we observed both positive and

negative curvilinear responses as revealed by the highly significant maternal family $\times f^2$ interaction. Pray and Goodnight (1995) also observed no overall population effect of epistasis, but detected both positive and negative curvilinear responses among lineages as well. These results suggest that the effects of epistasis may not be evident unless one examines epistasis at the family level within a population.

Willis (1993b) detected inbreeding depression in two populations of *M. guttatus* ($f = 0, 0.25, 0.5, 0.75$) in probability of germination, flowering, number of flowers, and pollen viability, and found evidence for reinforcing epistasis in pollen viability only. McCall et al. (1994) examined inbreeding depression for two populations of *I. capensis* from the comparison of random outcrosses ($f = 0$) to one ($f = 0.5$) and three ($f = 0.875$) generations of selfing. Variation among life-history traits in the magnitude of inbreeding depression was found for seedling survival, emergence date, seed and final plant biomass. Using path analysis they demonstrated that progeny resulting from three generations of selfing actually outperformed progeny resulting from one generation of selfing and suggested that a rapid purging of partially recessive mutations and/or diminishing epistasis was responsible. High mortality in later life-history stages owing to pest and fungal infestations in the greenhouse may have biased against the detection of inbreeding depression (only 30% and 24% of the individuals survived until harvest in the Madison and Milwaukee populations, respectively). Neal's (1935) classic work on maize demonstrated a linear decline with little evidence for epistasis. In contrast, nonlinear declines in fitness, suggesting epistasis, have been observed in *Drosophila* (Mukai 1969) and dairy cattle, poultry, mice, and guinea pigs (reviewed by Geiger 1988).

Evolutionary Consequences for Mating System Evolution

This is one of the first attempts (see also Pray and Goodnight 1995) to compare population and family level purging of genetic load across serial generations of selfing and outcrossing to find whether these two levels of inbreeding depression have similar predictive value in examining the evolution of mating systems. At the population level, our data suggest that dominance is responsible for the observed patterns of inbreeding depression given that selection is weak against partially deleterious recessive alleles (which is a likely case), although we cannot rule out a role for drift and/or overdominance as well. Significant maternal line differences in inbreeding depression at the family level suggest that dominance, rather than overdominance, underlies the expression of inbreeding depression. The data presented here concur with other studies on *M. guttatus* demonstrating that the gene action governing inbreeding depression is not overdominance based (Fu and Ritland 1994; Dudash and Carr unpubl. data). We were unable to detect any evidence of reinforcing epistasis for inbreeding depression, but did detect some evidence of possible diminishing epistasis at the family level.

A dominance mode of inheritance for inbreeding depression provides a ready explanation for why inbreeding depression is usually lower in selfing taxa compared to outcrossing taxa (Husband and Schemske 1996). Deleterious recessive alleles can be more readily purged from the pop-

ulation by selection compared to the overdominance mode of inheritance (e.g., Charlesworth and Charlesworth 1987). The absence of reinforcing epistasis suggests that the rate of purging may be slower than if homozygous loci acted with negative reinforcement (Charlesworth et al. 1991).

If inbreeding depression levels within maternal lines and mating system are linked, they may be more relevant in predicting the evolution of mating systems within a population than overall population inbreeding depression and mating system estimates (e.g., Holsinger 1991). These data may in part be explained by variation among the maternal lines in their past history of inbreeding (e.g., Latta and Ritland 1994) or by random variation within populations (Schultz and Willis 1995). However, we have demonstrated elsewhere that family level variation in inbreeding depression within the S and T populations is not associated with family level differences in stigma-anther separation or autogamy rates (Carr et al., in press) suggesting that these linkage associations may be ephemeral at the within population level. Overall, our results suggest that where ecological conditions and natural selection favor selfing (e.g., Jain 1976), inbreeding depression can be reduced by the purging of deleterious recessive alleles from the population.

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