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# INBREEDING DEPRESSION IN TWO SPECIES OF *MIMULUS* (SCROPHULARIACEAE) WITH CONTRASTING MATING SYSTEMS[1]

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We examined the effect of self- and cross-pollination on germination success, flowering probability, pollen and ovule production, survivorship, and adult aboveground biomass in two species of *Mimulus* with contrasting mating systems: the highly selfing *M. micranthus* and an outcrossing population of *M. guttatus*. Cross-pollinations were performed both within and between populations in order to examine the scale at which the genetic load is distributed. We found significant inbreeding depression in *M. guttatus* in four of the six traits, with the highest inbreeding depression observed in biomass (68% and 69% based on within- and between-population crosses, respectively) and lowest in ovule production (21% based on between-population crosses only). *M. micranthus* displayed significant inbreeding depression in only two of the six traits examined. Again, we observed the highest inbreeding depression in biomass (47–60% based on within- and between-population crosses, respectively), but both traits showing significant differences between self and outcross progeny expressed lower inbreeding depression than in *M. guttatus*. We detected no significant inbreeding depression for either pollen or ovule production in *M. micranthus*. An estimate of total inbreeding depression based on the multiplicative effects of all traits was also lower in *M. micanthus* th. n in *M. guttatus*. Our results are consistent with the expected purging of genetic load in populations with high selfing rates. The absence of inbreeding depression in *M. micranthus* pollen and ovule production, two traits with strong links to fitness in a selfing annual, further suggests the important role of directional selection in determining the population's genetic load. Comparison of cross-pollinations made within and between populations revealed little evidence of divergence of genetic load among the *M. micranthus* and *M. guttatus* populations examined.

**Key words**: inbreeding depression; mating systems; *Mimulus*; pollination; Scrophulariaceae.

A dynamic interaction exists between the mating system and the genetic load of a population. Inbreeding depression is considered to be a selective force that can prevent the spread of selfing variants through hermaphroditic plant populations (e.g., Maynard Smith, 1977; Charlesworth, 1980). However, the outcrossing rate of the population will have a profound effect on the equilibrium genetic load and hence inbreeding depression (Lande and Schemske, 1985; Campbell, 1986; Charlesworth and Charlesworth, 1987; Charlesworth, Morgan, and Charlesworth 1990; Lande, Schemske, and Schultz, 1994). If the genetic load is due primarily to deleterious recessive alleles, selection is efficient at "purging" these alleles from populations with high selfing rates because selfing increases homozygosity. Thus populations that have a high selfing rate are expected to have a lower genetic load and express less inbreeding depression than more outcrossing populations. The effects of selfing rates on the genetic load equilibrium will also be influenced by the genetic basis of inbreeding depression, including the number of contributing loci and the level of dominance of alleles at these loci. If alleles are only mildly deleterious and weakly recessive, higher genetic loads can be maintained, and increases in the selfing rate will have less of an effect on equilibrium levels of genetic load (Charlesworth, Charlesworth, and Morgan, 1990). Genetic load caused by alleles that interact in an overdominant manner rather than highly recessive alleles can be more persistent, and inbreeding depression can increase with an increase in selfing rate. Unless the fitness of homozygotes is highly symmetrical, variation at such loci eventually will be lost, however, and overdominant loci are not expected to make a large contribution to the genetic load of populations with a long history of inbreeding (Ziehe and Roberds, 1989; Charlesworth and Charlesworth, 1990). Comparisons of populations or closely related species that differ in their mating systems can provide valuable information for testing these expectations.

Charlesworth, Charlesworth and Morgan (1990) reviewed studies of five primarily selfing species and found that high levels of inbreeding depression were maintained in some species. For example, inbreeding depression (1 − self/outcross) for tiller number in *Avena fatua* was 58% (Imam and Allard, 1965). More recent studies of highly selfing taxa also have detected significant levels of inbreeding depression. Based on measurements of seed production, germination success, survival, and aboveground

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biomass, Ågren and Schemske (1993) estimated overall levels of inbreeding depression to be 22% and 42% in two selfing species of *Begonia,* and Parker, Nakamura, and Schemske (in press), found overall levels of inbreeding depression of 14% in a selfing species of *Epilobium.* Johnston and Schoen (1995) measured 17% inbreeding depression for total fitness in one selfing population of *Amsinckia.* However, inbreeding depression in highly selfing populations tends to be lower than in more outcrossing taxa (Husband and Schemske, in press), and examples exist of highly selfing species exhibiting no inbreeding depression (*Ludwigia peploides,* Flores 1990; *Eichhornia paniculata* from Jamaica, Barrett and Charlesworth 1991). Clearly more study of selfing populations is needed to evaluate the potential roles of mutation and, perhaps, overdominance in contributing to genetic load (Charlesworth, Charlesworth, and Morgan, 1990; Lande, Schemske, and Schultz, 1994).

The balance among selection, mutation, and genetic drift will affect the scale at which heterosis is demonstrated. Inbreeding depression revealed by a comparison of progeny produced with self-pollen to those produced with pollen donated from within the maternal parents' population demonstrates genetic load segregating within the population. Increased heterosis in crosses made between populations can reveal deleterious alleles that have been fixed by genetic drift (e.g., Dudash, 1990; Fenster, 1991; Waser and Price, 1994). Scale might be a more significant factor when measuring heterosis in highly selfing species where genetic variation tends to be partitioned to a much greater extent among populations rather than among individuals within populations (Loveless and Hamrick, 1984).

The genus *Mimulus* is a particularly good subject for studies of mating system evolution and inbreeding depression. Mating systems in the genus range from highly selfing to highly outcrossing (Ritland and Ritland, 1989). Phylogenetic data suggest that selfing has evolved from outcrossing populations more than once (Fenster and Ritland, 1994). Inbreeding depression has been documented in several populations of the mixed-mating *M. guttatus* with outcrossing rates ($t$) ranging from 0.66 to complete outcrossing (Willis, 1993a; Carr and Dudash, in press). Dole and Ritland (1993) have inferred inbreeding depression from multigenerational changes in the inbreeding coefficient and estimated that the relative fitness of self progeny of *M. guttatus* was only 19% that of outcross progeny, while fitness of self progeny in the more selfing *M. platycalyx* was 68% of outcross progeny. In a study of 15 populations representing four taxa in the *M. guttatus* species complex Latta and Ritland (1994) found that the magnitude of inbreeding depression was negatively correlated with the inbreeding coefficient for all characters measured, although the relationship was significant for plant height only.

The goal of this study was to examine the effect that mating systems have on the expression of inbreeding depression. We accomplished this by direct comparison of self and outcross progeny in two *Mimulus* taxa with contrasting mating systems. We measured inbreeding depression for a number of life history traits including germination success, probability of flowering, and adult aboveground biomass. We also quantified pollen and ovule production as measures of potential male and female function. Cross-pollinations were performed both within and between populations in order to examine the scale at which the genetic load is distributed.

## MATERIALS AND METHODS

***Study species***—*Mimulus guttatus* DC (Scrophulariaceae) is an annual to perennial herb widely distributed in western North America. The showy, yellow flowers are produced in pairs at each stem node, and greenhouse-raised plants produce an average of ≈50 flowers per plant (Carr and Dudash, personal observation). *Mimulus micranthus* Heller is strictly annual and is restricted to the coastal range of central California (Munz and Keck, 1959). It produces flowers that are 3–4 times smaller than those of *M. guttatus.* Both species are found in moist open habitats such as stream edges and ephemeral pools, and both are fully self-compatible. The two species are frequently sympatric.

These two species of *Mimulus* differ greatly in their mating systems. Outcrossing rates measured for populations of *M. guttatus* vary between $t = 0.25$ and 1.00, averaging ≈0.60 (Ritland and Ritland, 1989; Ritland, 1990; Dudash and Ritland, 1991; Willis, 1993a). *M. micranthus* is a highly selfing species with an outcrossing rate of only $t = 0.15$ (Ritland and Ritland, 1989). *M. micranthus* has other characteristics associated with selfing including a reduced pollen:ovule ratio (4.2–4.3 compared to 15.2–32.6 for *M. guttatus*; C. Fenster and D. Carr, University of Maryland, unpublished data) and the absence of herkogamy (Ritland and Ritland, 1989; Carr and Fenster, 1994).

Seed families of *M. guttatus* were collected from two annual populations: S ($N = 23$ families) and T (≈15 km from S, $N = 23$ families) both located in Tuolomne Co., California (Fig. 1). Population T consisted of hundreds of flowering individuals, while population S numbered in the thousands. Dudash and Ritland (1991) provided a single-generation estimate for the outcrossing rate for population T of $t = 0.74$. Seed families of *M. micranthus* were collected from three populations in California: 301 ($N = 11$ families, Lake Co.), and 305 ($N = 6$ families, Mendocino Co.), and 307 ($N = 30$ families, Mendocino Co.) (Fig. 1). Populations 301 and 305 were separated from one another by ≈50 km; each was separated from population 307 by ≈100 km.

***Mimulus guttatus crossing design***—All experimental 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 68-mm² plastic pots filled with Progro™ 300S soil. Trays were filled with standing water, and 20 pots were placed in each tray. No fertilizer was added during the course of the experiment.

We sowed field-collected seed from each family representing both populations of *M. guttatus.* One randomly selected seedling from each family was transplanted into its own pot for use in hand-pollinations. We performed two types of cross-pollinations: crosses between individuals of the same population (WITHIN crosses) and crosses between individuals of different populations (BETWEEN crosses). *Mimulus* produces two flowers at each stem node. Cross-pollinations were performed at separate nodes, and we self-pollinated the second flower at the same node as each cross-pollination. This enabled us to control for potential temporal pollination effects and positional effects within a maternal plant. In order to distinguish those self-pollinations paired with WITHIN crosses from those paired with BETWEEN crosses we hereafter use the notation SELFW and SELFB, respectively. Both cross- and self-pollinations were accomplished by rubbing dehiscent anthers from the appropriate pollen donor onto the stigma of the seed parent on the day of anthesis. We have not found it necessary to emasculate *M. guttatus* buds prior to hand-pollination, but corollas and stamens were removed following hand-pollinations to prevent any subsequent self-pollination (Dole, 1990). Ripe fruits were collected 2–3 wk after pollinations, ≈1 d prior to dehiscence.

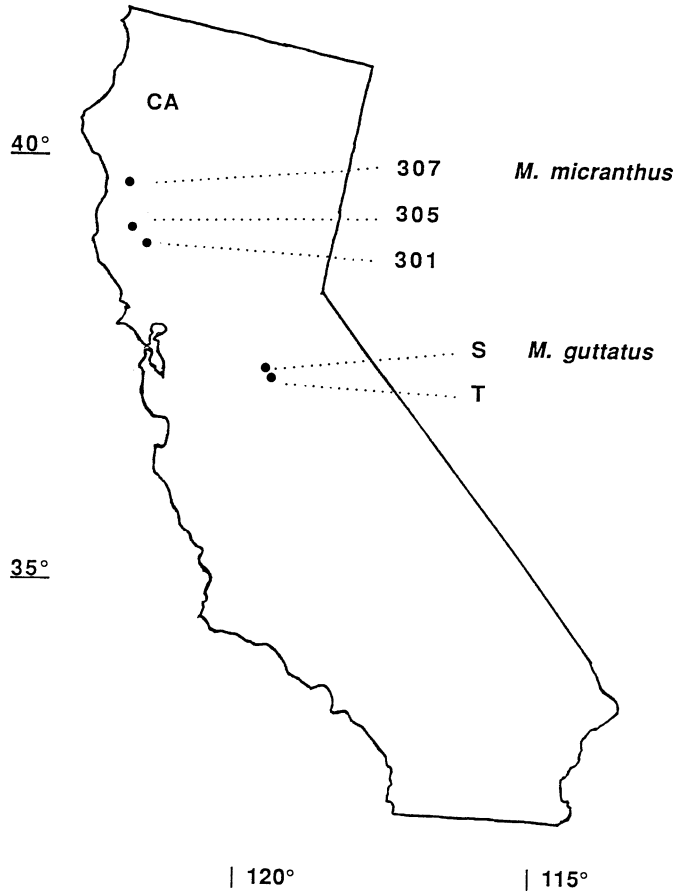


Fig. 1. Geographic locations of three *M. micranthus* and two *M. guttatus* populations in California.

Each of the 23 maternal families from population T served as a pollen recipient and pollen donor for one WITHIN cross. Pollen donors and recipients were paired at random, and WITHIN crosses were not reciprocal. Each maternal family from population T also served as a pollen recipient for one BETWEEN cross with a randomly selected family from population S serving as the pollen donor. Each maternal family from population S served as a pollen donor for only one BETWEEN cross. Plants from population S did not serve as pollen recipients. Thus our experimental material for *M. guttatus* consisted of 23 maternal families from population T. Each maternal family consisted of seeds from four pollination types: WITHIN, SELFW, BETWEEN, and SELFB.

***Mimulus micranthus crossing design***—Because of the difficulty in performing hand-pollinations on an autogamous selfer, it was necessary to have genetic markers in order to confirm paternity and rule out contamination in our controlled pollinations. Based on a previous study of allozyme variation (Fenster and Ritland, 1992), *Idh* 1, *Pgi,* and *Dia* 2 were known to be polymorphic in *M. micranthus,* and we genotyped one seedling from each field collected family using these three presumptive loci. Gel buffers and staining conditions were as in Ritland and Ganders (1987). We found that populations 301 and 305 were polymorphic at the *Dia* 2 and *Pgi* loci, respectively, but all families were homozygous at all three marker loci. Thirty percent of the field-collected families from population 307 were homozygous for fast alleles at both the *Pgi* and *Idh* 1 loci. All of these families had a floral morphology intermediate between typical *M. micranthus* and *M. guttatus,* presumably reflecting a hybrid ancestry. We excluded these nine families from the study. The remaining 21 families from population 307 had typical *M. micranthus* floral morphologies. They were monomorphic for all three loci but were fixed for a unique allele at the *Idh* locus, providing a marker for between-population crosses.

One seedling from each *M. micranthus* maternal family was transplanted into its own pot for use in the hand-pollinations. Because *M. micranthus* is an autogamous selfer, flowers selected to serve as pollen recipients were emasculated in bud 1 d prior to hand-pollinations. Again both self- and cross-pollinations were accomplished by rubbing dehiscent anthers from the pollen donor onto the stigma of the seed parent. We performed both WITHIN and BETWEEN outcrosses for *M. micranthus,* and, as with *M. guttatus,* we self-pollinated flowers at the same node as the cross-pollination in order to control for potential temporal and positional effects. We again use the designations SELFW and SELFB to distinguish self-pollinations paired with WITHIN and BETWEEN crosses.

For cross-pollinations involving *M. micranthus,* we selected pollen donors that differed from pollen recipients at one or more of our allozyme markers. This was necessary in order to ensure that our emasculations had been successful in preventing unintended self-pollinations. We sowed a sample of *M. micranthus* seeds from each BETWEEN and WITHIN pollination and randomly selected three seedlings for electrophoresis. We included in the study only those sibships in which all three seedlings were heterozygous at the marker loci. Screening only three progeny per cross could easily have missed contamination from self pollen if the contamination was at a relatively low frequency. For example, if 50% of the progeny had come from self pollen there would have been a 12.5% chance that this contamination would have been undetected in our electrophoretic screen. In reality however, test progeny were either 100% heterozygous at our marker locus or 100% homozygous, suggesting that contamination was likely an all or nothing phenomenon. After screening we were able to use four WITHIN crosses for population 301 and four WITHIN crosses from population 305. Because the maternal families that we used from population 307 exhibited no allozyme variation, no WITHIN crosses could be done. We were able to produce four, two, and 22 BETWEEN crosses for populations 301, 305, and 307, respectively.

***Offspring performance***—On 15 May 1992 30 seeds from all available cross types (WITHIN, SELFW, BETWEEN, and SELFB) of each maternal family from *M. guttatus* population T and *M. micranthus* populations 301, 305, and 307 were sown into individual pots. Two weeks later germination success, expressed as the proportion of emerged seedlings, was assessed.

The effects of pollination treatment on four adult characters, flowering probability, ovule production, pollen production, and adult aboveground biomass, were measured on plants grown in a competitive regime. Immediately after germination success was scored, pairs of seedlings were chosen randomly from each maternal family and transplanted into WITHIN-SELFW or BETWEEN-SELFB pairs. Each pair of seedlings was separated by ≈20–30 mm in a 68-mm$^2$ plastic pot. Each self-outcross pair was replicated twice. This resulted in a total of 92 pots for *M. guttatus* (23 maternal families × 2 types of self–outcross pair per family × 2 reps per outcross) and 72 pots for *M. micranthus* (23 BETWEEN pairs + 8 WITHIN pairs × 2 reps per pair). The pairwise competitive regime provides a more realistic environment in which to measure inbreeding depression than a noncompetitive situation (Darwin, 1876; Schmitt and Ehrhardt, 1990; Argyres and Schmitt, 1992; Wolfe, 1993; Carr and Dudash, 1995; Latter and Mulley, 1995; Parker, Nakamura, and Schemske, 1995) and has the added advantage of conserving greenhouse bench space. The plants were grown in two blocks with maternal families randomized within blocks.

We recorded the date of first flowering for each plant, and the effect of pollination treatment on pollen and ovule production was examined for each plant that flowered. Gynoecia were collected from the first two flowers produced, preserved in a 3:1 ethanol:glacial acetic acid solution, and stored in microcentrifuge tubes. Ovule production for each plant

TABLE 1. Performance estimates (and one standard error of means) of self and outcross progeny for six life history characters in *M. guttatus* and *M. micranthus*. The number of families (*N*) contributing to each self-outcross comparison is indicated for each character. Separate comparisons are made between within- (WITHIN) and between- (BETWEEN) population outcrosses and progeny from the appropriate self-pollinations (SELFW and SELFB, respectively). Separate estimates of inbreeding depression [(1 − self mean/outcross mean) × 100] are given for each type of outcross. Estimates of total inbreeding depression based on the multiplicative effects of all the characters is also provided. For each character, differences between self and outcross progeny that were significant at the $P < 0.05$, 0.01, and 0.001 levels are indicated by *, **, ***, respectively.

| Character | *N* | SELFW | Within | Inbreeding depression | *N* | SELFB | Between | Inbreeding depression |
|---|---|---|---|---|---|---|---|---|
| *M. guttatus* | | | | | | | | |
| Germination (%) | 23 | 48 (5.4) | 61 (4.8) | 21%* | 23 | 40 (5.4) | 56 (4.3) | 29%* |
| Flowering (%) | 23 | 82[a] | 73[a] | −12% | 23 | 57[a] | 80[a] | 29% |
| Ovule number | 6 | 195 (42) | 232 (43.6) | 16% | 17 | 207 (18) | 263 (22) | 21%* |
| Pollen number | — | — | — | — | 9 | 897 (132) | 1,244 (94) | 28%* |
| Survival (%) | 23 | 89[a] | 93[a] | 4% | 23 | 93[a] | 100[a] | 7% |
| Biomass (g) | 22 | 0.39 (0.07) | 1.22 (0.26) | 68%*** | 23 | 0.34 (0.04) | 1.09 (0.19) | 69%*** |
| Total | — | — | — | 82% | — | — | — | 91% |
| *M. micranthus* | | | | | | | | |
| Germination (%) | 8 | 38 (10) | 47 (8) | 19% | 28 | 40 (5) | 50 (4) | 19%* |
| Flowering (%) | 8 | 38[a] | 50[a] | 24% | 28 | 65[a] | 85[a] | 24% |
| Ovule number | 3 | 78 (4) | 51 (17) | −51% | 23 | 91 (12) | 93 (11) | 2% |
| Pollen number | — | — | — | — | 20 | 324 (62) | 304 (84) | −6% |
| Survival (%) | 8 | 81[a] | 100[a] | 19% | 28 | 98[a] | 94[a] | −4% |
| Biomass (g) | 8 | 0.20 (0.04) | 0.38 (0.05) | 47%** | 28 | 0.19 (0.03) | 0.48 (0.04) | 60%*** |
| Total | — | — | — | 62% | — | — | — | 73% |

[a] Standard errors for percentage taken over the entire sample cannot be calculated.

was determined by randomly dissecting one of the two symmetrical locules from the ovary from each of the two collected pistils. The ovules from each locule were spread onto a microscope slide, stained with lactophenol with 0.1% aniline blue, and counted under a dissecting microscope. The mean ovule count from the two locules was used for analysis, and we will refer to this count hereafter as "ovule number." Ovule number represents one-half the number of ovules per gynoecium.

Androecia were collected from the third and fourth flower buds prior to pollen dehiscence. The eight anthers from the two androecia were air-dried for 1 wk in an open microcentrifuge tube before storage. Pollen production was determined using an Elzone 280PC particle counter. Dehisced pollen from the eight anthers collected from each plant was suspended in 15 mL of 2% saline and assayed. A sample mean was calculated from three replicate 0.5-mL subsamples, and we will refer to this mean hereafter as "pollen number." Pollen number represents 1/15 of the total pollen grains per androecium.

After 3 mo of growth we assessed survivorship and harvested all surviving plants at soil level. Plants were dried at 50°C for 1 wk, and biomass was determined with a Mettler PM 4600 top-loading balance to the nearest 0.01 g. Adult aboveground biomass is significantly correlated with total flower number per plant in both *M. micranthus* and *M. guttatus* ($r = 0.586$ and 0.342, $P < 0.0001$, $N = 69$ and 2558, respectively; M. Dudash, D. Carr, C. Fenster, and B. Byer, University of Maryland, unpublished data).

Because inbreeding effects are cumulative across the life cycle, it is appropriate to examine inbreeding depression in a multiplicative fashion (e.g., Schemske and Pautler, 1984; Dudash, 1990; Fenster, 1991; Van Treuren et al., 1993). We included germination success, the probability of flowering, pollen and ovule number, survivorship, and adult aboveground biomass in this estimate of total inbreeding depression at the population level. We calculated the ratio of the mean from self-pollinations to the mean from outcross pollinations for each of the six characters. Total inbreeding depression was equal to one minus the product of these six ratios. Separate estimates of total inbreeding depression were made based on ratios from WITHIN and BETWEEN outcrosses and their appropriate self-pollinations.

***Statistical analysis***—All data analysis was performed with SAS Version 6 (SAS, 1989) on the University of Maryland IBM mainframe computer. We tested for the effect of pollination treatment on all response variables except probability of flowering and survivorship with analysis of variance using the SAS GLM procedure to estimate Type III sums of squares. For *M. micranthus,* no effect of population was detected for any of the fitness components examined, so we elected to pool maternal families across populations for all analyses. Variation among maternal families was treated as a random effect in all ANOVA models. Variation between or among pollination treatments was treated as a fixed effect. Because replication levels and experimental design imbalances differed among our response variables, deviations from this general model are described below. Sample sizes for specific comparisons are given in Table 1.

The dependent variable germination success was arcsine square-root transformed in order to meet the normality and homoscedasticity assumptions of ANOVA. Because few *M. micranthus* maternal families were represented by both WITHIN and BETWEEN crosses, we had to analyze the results from these two cross types separately for all dependent variables. Because all four cross types (WITHIN, SELFW, BETWEEN, and SELFB) were represented within each *M. guttatus* maternal family, we were able to examine these simultaneously with a single ANOVA for germination success. Multiple comparisons among pollination treatment means in *M. guttatus* were made with a Ryan-Einot-Gabriel-Welsch multiple range test as recommended by Day and Quinn (1989).

Because many plants did not flower, analyses of pollen and ovule number were too unbalanced for us to be able to consider all four cross types simultaneously in either species. Consequently separate ANOVAs were done to compare WITHIN to SELFW and BETWEEN to SELFB for each taxon. Ideally we would have preferred to make separate comparisons between the two competing plants grown in the same pot (as was done in the analysis of biomass below) but, to make use of as much information on progeny performance as possible, we pooled pollen and ovule data from the two blocks of the experiment within maternal families. Variation between blocks was not significant for either taxon in the analysis of biomass, and therefore pooling pollen and ovule data across blocks probably did not obscure treatment effects. For both *M. guttatus* and *M. micranthus,* we included only those families for which pollen or ovule counts were available from both self and outcross plants.

We performed analyses on family means for each pollination treatment. The dependent variable ovule number was log transformed in order to meet ANOVA assumptions. Pollen number did not require transformation.

In the analysis of adult aboveground biomass, the random effect of block was included in the ANOVA model for both *M. micranthus* and *M. guttatus*. Because biomass data were collected from replicate individuals we were able to test for a maternal family × pollination treatment interaction, but this was nonsignificant for both taxa, and the interaction mean square was pooled with the error in all analyses (this interaction could not be tested for any other character). All four pollination treatments were considered simultaneously for *M. guttatus* only. Again, multiple comparisons among pollination treatment means in *M. guttatus* were made with a Ryan-Einot-Gabriel-Welsch multiple range test. The dependent variable biomass was log transformed in the above analyses in order to meet ANOVA assumptions.

Date of first flower could not be transformed to meet ANOVA assumptions. Instead, flowering was treated as a binomial variable, and the probability of flowering was analyzed using a paired sign test. Survivorship was analyzed in the same manner. In both cases we performed separate tests for WITHIN and BETWEEN crosses for each species.

## RESULTS

*Mimulus guttatus* seed produced from self-pollinations germinated at a rate 21% and 29% lower than WITHIN and BETWEEN outcrosses, respectively (Table 1). There was a significant effect of pollination treatment in the ANOVA ($F_{3,64} = 3.94$, $P < 0.012$). A multiple comparison test revealed differences between both outcrosses and their respective self-pollinations, but the outcrosses did not differ from one another. *Mimulus micranthus* seed from SELFB crosses germinated at a rate 19% lower than seed generated from BETWEEN crosses ($F_{1,27} = 5.67$, $P = 0.024$; Table 1). An identical difference between self and outcross progeny was seen in the relative germination success of WITHIN and SELFW progeny, but this difference was not significant ($F_{1,7} = 0.917$, $P = 0.370$). There was significant variation among maternal families in germination success for both taxa. Significant maternal family effects were detected in the ANOVAs for all response variables. SELFW and SELFB progeny did not differ significantly in germination success or for any other character.

Not all plants in the experiment flowered before the termination of the study in late August. Although in *M. guttatus* SELFW plants had a greater tendency to flower than WITHIN plants (Table 1), in all other cases plants derived from outcrosses were more likely to flower than those derived from selfing (Table 1). However, paired sign tests revealed no significant differences between self and outcross progeny for both *M. micranthus* ($\chi^2 = 0.05$, df = 1, $P > 0.05$ based on WITHIN crosses; $\chi^2 = 1.52$, df = 1, $P > 0.05$ based on BETWEEN) and *M. guttatus* ($\chi^2 = 0.10$, df = 1, $P > 0.05$ based on WITHIN crosses; $\chi^2 = 3.27$, df = 1, $P > 0.05$ based on BETWEEN) with regard to the probability of flowering.

For *M. guttatus,* SELFW plants produced 16% fewer ovules than WITHIN plants, but this difference was not significant ($F_{1,5} = 2.02$, $P = 0.214$; Table 1). SELFB plants produced significantly fewer (21%) ovules than BETWEEN plants ($F_{1,16} = 5.23$, $P = 0.036$). Pollination treatment had no effect on ovule number in *M. micranthus* ($F_{1,2} = 4.51$, $P = 0.168$ based on a comparison of SELFW and WITHIN; $F_{1,22} = 0.04$, $P = 0.853$ based on a comparison of SELFB and BETWEEN; Table 1).

For *M. guttatus,* SELFB progeny produced 28% fewer pollen grains than BETWEEN plants ($F_{1,8} = 5.78$, $P = 0.043$; Table 1). The number of pollen grains produced by *M. micranthus* was not significantly affected by pollination treatment based on the analysis of BETWEEN progeny ($F_{1,19} = 0.03$, $P = 0.852$; Table 1). We were able to obtain pollen samples for both WITHIN and SELFW crosses from only one and two families from *M. micranthus* and *M. guttatus,* respectively, and therefore were unable to perform a meaningful analysis of these crosses for either taxon.

Survivorship was uniformly high across the experiment (Table 1). The differences between the WITHIN and BETWEEN treatments and their respective self treatments were not significant for either *M. guttatus* ($\chi^2 = 0.55$, df = 1, $P > 0.05$; $\chi^2 = 3.12$, df = 1, $P > 0.05$) or *M. micranthus* ($\chi^2 = 3.31$, df = 1, $P > 0.05$; $\chi^2 = 0.84$, df = 1, $P > 0.05$).

For both taxa we observed the highest inbreeding depression in the trait aboveground biomass (Table 1). The effect of pollination was significant in *M. guttatus* ($F_{3,146} = 23.62$, $P < 0.0001$). A multiple comparison test revealed significant differences between self and outcross progeny. SELFW plants were 68% smaller than WITHIN plants, and SELFB plants were 69% smaller than BETWEEN plants. WITHIN and BETWEEN outcrosses did not differ significantly from one another. *M. micranthus* SELFW plants were 47% smaller than WITHIN plants ($F_{1,19} = 8.81$, $P = 0.008$), and SELFB plants were 60% smaller than BETWEEN plants ($F_{1,76} = 31.37$, $P < 0.0001$).

Estimates of total inbreeding depression (Table 1) based on the multiplicative effects of all the above characters were ≈20% greater for *M. guttatus* than for *M. micranthus.* This was true for estimates based on both WITHIN and BETWEEN outcrosses. Because these estimates were based on population means, we were unable to provide estimates of standard errors, and differences between the taxa could not be compared statistically. In both taxa estimates based on WITHIN crosses are lower than estimates based on BETWEEN crosses, but in the case of *M. guttatus* this is due in large part to the fact that estimates of pollen production are not available for the WITHIN crosses.

## DISCUSSION

***Inbreeding depression and mating systems***—Inbreeding depression studies comparing populations or closely related taxa differing in their mating systems provide the opportunity to draw inferences about the long-term effects of selfing on the genetic load of populations. Significant inbreeding depression was observed for all characters except survival and the probability of flowering in the largely outcrossing ($t = 0.74$) *M. guttatus.* The magnitude of inbreeding depression was greatest for biomass (69%) and least for ovule production (21%). In an earlier study Carr and Dudash (1995) found comparable levels of inbreeding depression in these same characters in two populations of *M. guttatus,* although inbreeding depression for biomass was lower (24%–45%) than in the pres-

ent study. High levels of inbreeding depression in *M. guttatus* also have been measured by Willis (1993a, b) and by Dole and Ritland (1993).

Of the four traits with detectable inbreeding depression in *M. guttatus,* only two showed significant inbreeding depression in *M. micranthus.* Consistent with expectations (Lande and Schemske, 1985; Charlesworth and Charlesworth, 1987; Charlesworth, Morgan, and Charlesworth, 1990), levels of inbreeding depression, including estimates based on the multiplicative effects of all characters, were always lower in this highly selfing species than in the more outcrossing *M. guttatus.* This inbreeding depression pattern is similar to that observed in other *Mimulus* populations. Dole and Ritland (1993) estimated lower inbreeding depression in the highly selfing ($t$ = 0.16) *M. platycalyx* relative to *M. guttatus.* Latta and Ritland (1994) found weak support for a negative relationship between inbreeding depression and levels of prior inbreeding in their study of 15 *Mimulus* populations encompassing four taxa ranging in inbreeding coefficients from $F$ = 0.02 to 0.76. They suggest that the action selection and mutation together were insufficient in explaining the observed among-population variation in inbreeding depression.

***The role of selection***—Our observation of lower levels of inbreeding depression in the selfing *M. micranthus* is expected under the hypothesis that genetic load is due to the balance between selection and recurrent mutations to deleterious recessive alleles. The possibility that allelic variation at some loci acts in an overdominant manner cannot be excluded (Charlesworth and Charlesworth, 1990). Furthermore, lower levels of inbreeding depression could be simply a consequence of the reduction in levels of genetic variation in *M. micranthus* observed in both molecular surveys (Fenster and Ritland, 1992) and studies of quantitative traits (Carr and Fenster, 1994). Comparisons among the traits examined in our study, however, reveal patterns that suggest an important role of selection against deleterious alleles in determining the genetic load of the study populations.

As in *M. guttatus,* we observed the most severe inbreeding depression for *M. micranthus* in aboveground biomass (60%), but unlike *M. guttatus,* the selfing *M. micranthus* showed no inbreeding depression in either pollen or ovule number. Similarly, Fenster and Carr (University of Maryland, unpublished data) found no genetic variation for pollen number in two of the same *M. micranthus* populations (301 and 305) used in our study and significant broad-sense heritabilities for ovule number only in population 301. Both traits showed significant broad-sense heritability in the same two *M. guttatus* populations used in this study (C. Fenster and D. Carr, University of Maryland, unpublished data). The lack of both genetic variation and inbreeding depression for pollen number in a highly selfing species likely reflects the action of selection against the accumulation of mutational load that could interfere with pollen production and hence pollination assurance. To the best of our knowledge, ours is the only study to examine inbreeding depression in pollen number in a highly selfing species. Similarly, the lack of inbreeding depression (this study) and low levels of genetic variation for ovule number in *M. micranthus* (C. Fenster and D. Carr, University of Maryland, unpublished data) are also likely the product of strong purifying selection. Karoly (1994) also has found that ovule number was significantly reduced by selfing in the outcrossing *Lupinus nanus,* but no significant effect of selfing on ovule number was found in its highly selfing congener *L. bicolor.*

High levels of inbreeding depression in adult aboveground biomass in both *M. guttatus* and *M. micranthus* can be explained by the accumulation of mildly deleterious and weakly recessive alleles. Low levels of inbreeding depression in germination success likely reflect the elimination of lethal alleles (Husband and Schemske, in press). In their examination of stage specific expression of inbreeding depression Husband and Schemske (in press) noted that there is not a general pattern of increasing levels of inbreeding depression with the age of a plant. They note, however, that selfing species, in contrast to outcrossers, do demonstrate more inbreeding depression in later life history traits such as adult biomass or flower production than is manifested in earlier life history stages such as germination (Holtsford and Ellstrand, 1990; Karoly, 1991, 1994; Ågren and Schemske, 1993; Latta and Ritland, 1994; Johnston and Schoen, 1995; Husband and Schemske, in press; Parker, Nakamura, and Schemske, 1995). Husband and Schemske (in press) attribute the different inbreeding depression patterns in selfers and outcrossers to the enhanced opportunity for purging provided by the higher levels of homozygosity that occur with selfing mating systems. In light of this it was perhaps surprising that *M. guttatus* and *M. micranthus* showed such similar levels of inbreeding depression at the germination stage where the fitness consequences of early acting alleles would be most pronounced.

***Scale***—The frequencies of deleterious alleles will vary across populations owing to the random processes of mutation and genetic drift as well as differences in selection intensities. As a consequence the heterotic effects of crosses made between populations are often more dramatic than those produced by within-population outcrosses because recessive deleterious alleles unique to one population are masked by dominant alleles from the other population (e.g., Sobrevila, 1988; Dudash, 1990; Fenster, 1991; Van Treuren et al., 1993; Waser and Price, 1994). This may be especially true in a selfing species such as *M. micranthus* where most of the genetic variation appears to be partitioned between populations (Fenster and Ritland, 1992). *M. micranthus* biomass estimates from BETWEEN crosses were substantially higher than estimates from WITHIN crosses, but for most characters the relative differences between the means of SELFW and WITHIN crosses were usually very similar to the relative differences observed between SELFB and BETWEEN progeny. Our failure to find significant inbreeding depression for germination success based on WITHIN crosses despite finding significant differences between SELFB and BETWEEN crosses in germination success may be more a consequence of the smaller number of families representing the WITHIN crosses rather than a reflection of genetic load partitioning between populations.

Note that the observed differences between *M. mi-*

*cranthus* self and outcross progeny (especially SELFW and WITHIN crosses) could be overestimates. Cross-pollinations were not made at random. Instead, cross-pollinations were performed between individuals differing at electrophoretic markers. This likely resulted in matings between individuals that were less related than would be expected under the assumption of random mating, producing progeny that were more heterozygous than the random expectation. This could help explain the similarity of the results from WITHIN and BETWEEN outcrosses. However, the observation of inbreeding depression in biomass based on within-population crosses does indicate the presence of genetic load segregating within the populations of *M. micranthus.* The different electrophoretic genotypes within populations may represent independent founders of the population, each of which carried unique deleterious alleles.

In *M. guttatus,* the performance of WITHIN and BETWEEN progeny did not differ significantly with regard to germination success and adult aboveground biomass. For ovule production, the comparisons of WITHIN and BETWEEN outcrosses to their respective selfs were made with separate ANOVAs, and only the BETWEEN-SELFB comparison revealed a significant difference. BETWEEN crosses produced 12% more ovules than WITHIN crosses (Table 1). Due to small sample sizes the 95% confidence intervals overlap with the means, but there is reason to believe that this difference may be real. In an earlier study of *M. guttatus,* ovule number was the only character to show a significant different between WITHIN and BETWEEN crosses (Carr and Dudash, 1995). It appears that some deleterious alleles for ovule production are unique to some *M. guttatus* populations and have drifted to fairly high frequencies.

***Conclusions***—Inbreeding depression due to recessive alleles is expected to decrease over time as the selfing rate in a population increases. The few studies that have compared populations or closely related taxa that differ in their selfing rates generally have demonstrated this (Holtsford and Ellstrand, 1990; Barrett and Charlesworth, 1991; Dole and Ritland, 1993; Karoly, 1994; Parker, Nakamura, and Schemske, 1995; Husband and Schemske, in press; but see Latta and Ritland, 1994). Our study also supports these expectations, but, as in most of the studies cited previously, a substantial amount of inbreeding depression remains in these selfing populations. If recessive alleles are only mildly deleterious, a high equilibrium genetic load can be maintained in a population (Charlesworth, Morgan, and Charlesworth, 1990, 1991). Charlesworth, Charlesworth, and Morgan (1990) reviewed the available studies on inbreeding depression in selfing species and concluded that the observed levels were consistent with a selection–mutation model with a genomic mutation rate of 0.5. Our estimates of inbreeding depression in the selfing *M. micranthus* are within the range of those reported in the studies reviewed by Charlesworth, Charlesworth, and Morgan (1990). Although the role of overdominance in maintaining inbreeding depression cannot be completely ruled out, the absence of inbreeding depression in *M. micranthus* for traits very closely tied to fitness (pollen and ovule production) adds even greater weight to the hypothesis that purging via directional selection determines a population's genetic load.

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