# The Relationship Between Mating-System Characters and Inbreeding Depression in Mimulus guttatus

David E. Carr; Charles B. Fenster; Michele R. Dudash

*Evolution*, Vol. 51, No. 2 (Apr., 1997), 363-372.

*Evolution*, 51(2), 1997, pp. 363–372

# THE RELATIONSHIP BETWEEN MATING-SYSTEM CHARACTERS AND INBREEDING DEPRESSION IN *MIMULUS GUTTATUS*

DAVID E. CARR,[1,2] CHARLES B. FENSTER,[1] AND MICHELE R. DUDASH[1,3]
[1]*Department of Plant Biology, University of Maryland, College Park, Maryland 20742-5815*
[3]*Maryland Agriculture Experiment Station, University of Maryland, College Park, Maryland 20742-5815*

*Abstract.*—Several recent theoretical considerations of mating-system evolution predict within-population covariation between levels of inbreeding depression and genetically controlled mating-system characters. If inbreeding depression is caused by deleterious recessive alleles, families with characters that promote self-pollination should show lower levels of inbreeding depression than families with characters that promote outcrossing. The converse is expected if inbreeding depression is due to overdominant allelic interactions. Whether these associations between mating-system and viability loci evolve will have important consequences for mating-system evolution. The evolution of selfing within the genus *Mimulus* is associated with a reduction in stigma-anther separation (i.e., a loss of herkogamy) and high autogamous seed set. In this study we compared families from two *M. guttatus* populations that differed genetically in their degree of stigma-anther separation. In one of these populations we also compared families that differed genetically in the degree to which they autogamously set seed in a pollinator-free greenhouse. Dams often differed significantly in levels of inbreeding depression for aboveground biomass and flower production, but variation in inbreeding depression was never explained by herkogamy class or autogamy class. Several factors might account for why families with traits associated with selfing did not show lower inbreeding depression, and these are discussed. Our study also demonstrated significant variation among self progeny from a given female likely due to differences in pollination date and position of fruit maturation. The detection of significant dam × sire interactions suggests biparental inbreeding or differences in combining ability for specific pairs of parents.

*Key words.*—Combining ability, identity disequilibrium, inbreeding depression, maternal effects, mating system, *Mimulus*.

Received February 6, 1996. Accepted December 6, 1996.

Within a plant population, genotypes capable of self-pollination enjoy several potential advantages over outcrossing genotypes. If there is no reduction in their ability to serve as pollen donors in cross-pollinations, selfing variants have a 50% transmission advantage over strictly outcrossing genotypes (Fisher 1941). However, if self progeny are less than half as fit as outcross progeny due to inbreeding depression, the transmission bias is nullified and the spread of alleles that increase the selfing rate is prevented (e.g., Maynard Smith 1971; Charlesworth and Charlesworth 1979, 1987, 1990; Lloyd 1979; Lande and Schemske 1985). Most empirical studies of mating-system evolution have focused on average levels of inbreeding depression within populations, and many have found that inbreeding depression often exceeds the 50% threshold required to prevent the invasion of selfing variants into outcrossing populations (e.g., Dudash 1990; Fenster 1991a; Dole and Ritland 1993). Only recently have studies begun to focus on genetic variation for inbreeding depression within populations (e.g., Pray and Goodnight 1995; Dudash et al., in press). If the progeny of individuals that have higher selfing rates express lower inbreeding depression than progeny from lineages with a history of outcrossing, the average level of inbreeding depression in the population may not be relevant to the evolutionary fate of mating-system alleles (Holsinger 1988, 1991; Uyenoyama and Waller 1991a,b). In this study we test for associations between inbreeding depression and traits known to affect selfing rates in the genus *Mimulus*.

Although the mean fitness of offspring produced from selfing may be lower than that of offspring produced through outcrossing, the fitness variation among sibs derived from self-pollination can be greater than the variation among full sibs derived from outcrossing due to the increased frequency of homozygous genotypes (Uyenoyama and Waller 1991a,b). If inbreeding depression is due to partially recessive deleterious alleles, offspring from selfing genotypes will include a higher number of homozygous recessive genotypes, which tend to have lower fitness than the population average. However, these same families also will include a high frequency of homozygous dominant genotypes, which can exhibit higher than average fitness. The high-fitness, homozygous offspring that also have inherited the alleles that caused their parent to self-pollinate can perpetuate their high-fitness genotypes, producing offspring that are homozygous for even more wild-type alleles. As selection removes the least-fit genotypes from the selfing lineages, a disequilibrium is generated such that alleles that increase the selfing rate become associated with alleles conveying high fitness. This disequilibrium between mating-system and fitness alleles will allow selfing alleles to increase in frequency even if the inbreeding depression in the population started at a level greater than 50% (Holsinger 1991; Uyenoyama and Waller 1991a,b).

If inbreeding depression is due to overdominant allelic interactions, the highest fitness offspring will come from parents that produce the most heterozygous genotypes (Holsinger 1988; Uyenoyama and Waller 1991c). Because selfing lineages lose heterozygosity each generation, offspring fitness will decline continuously with increasing homozygosity. The increased heterozygosity resulting from outcrossing to an unrelated individual will restore heterozygosity and high fitness in the offspring. Consequently, outcrossing genotypes

---

[2] Present address: Department of Biology, Dickinson College, P.O. Box 1773, Carlisle, Pennsylvania 17013. E-mail: carrd@dickinson.edu.

can be maintained in populations with inbreeding depression well below 50%.

High selfing rates have evolved multiple times in the genus *Mimulus* (Ritland 1989; Fenster and Ritland 1992, 1994b) and are consistently associated with a suite of floral characters, including the absence of herkogamy (the spatial separation between stigma and anthers) and the ability to set seed autogamously (Ritland and Ritland 1989; Dole 1992; Carr and Fenster 1994). Both stigma-anther separation and autogamy rates have been demonstrated to be heritable traits within *M. guttatus* populations (Carr and Fenster 1994; Robertson et al. 1994). If narrow stigma-anther separation and high autogamy rates increase rates of self-pollination in natural populations of *M. guttatus*, then families with reduced herkogamy and increased ability to set seed autogamously may represent lineages with a history of inbreeding. If this is so, then these families may also show different levels of inbreeding depression than families with wider stigma-anther separation and lesser ability to set seed autogamously, which would presumably have a history of outcrossing.

Species (Dole and Ritland 1993; Carr and Dudash 1996) and populations (Latta and Ritland 1993) of *Mimulus* that have higher selfing rates have lower inbreeding depression than those that are more outcrossing, a pattern common in flowering plants (Husband and Schemske 1996). What is not known is whether these current associations were initially responsible for driving the evolution of selfing in their ancestral populations (Holsinger 1991; Uyenoyama and Waller 1991a,b). In this study, we test for associations between levels of inbreeding depression and floral characters that appear to affect rates of self-pollination, a necessary precondition if linkage disequilibrium is to drive mating-system evolution. In two populations of *M. guttatus* we compare levels of inbreeding depression in families that differ in their degree of herkogamy. In one of these two populations, we also compare levels of inbreeding depression in families that differ in their autogamous seed-set in a pollinator-free environment.

## Materials and Methods

*Study Species.*—*Mimulus guttatus* DC (Scrophulariaceae) is an annual to perennial herb widely distributed in western North America, where it occupies moist habitats such as stream edges and ephemeral pools. *Mimulus guttatus* populations vary widely in their estimated outcrossing rates ($0.25 \leq t \leq 1.00$, averaging about 0.60; Ritland and Ritland 1989; Ritland 1990; Dudash and Ritland 1991; Willis 1993). Low outcrossing rates in *Mimulus* taxa (Ritland and Ritland 1989) and in *M. guttatus* populations (Dole 1992) are associated with narrow stigma-anther separation and high autogamous seed set. In a previous study (Carr and Fenster 1994), we observed a weak negative correlation between autogamous seed set in the greenhouse and stigma-anther separation in one population of *M. guttatus*. However, in a field study using experimental arrays of *M. ringens*, stigma-anther separation showed a strong negative correlation with individual selfing rates (Karron et al., in press).

We collected seed families of *M. guttatus* from two annual populations in Tuolomne County, California: S ($n = 23$ families) and T ($\approx$ 15 km from S, $n = 23$ families). 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 $t = 0.74$. No estimate is available for Population S.

*Culture Conditions.*—All of the experiments described below took place in the greenhouses at the University of Maryland, College Park. We used 68-mm plastic pots and 300S Progro soil without fertilizer for all phases of the experiment. Pots were arranged 20 to a tray, and the plants grew in water-saturated conditions. Seeds germinated under natural light, but we used sodium vapor lights, as needed, to create an 18L:6D photoperiod after transplanting.

*Family Selection.*—We sowed 30 seeds from each field-collected maternal family from populations S and T and transplanted seedlings into separate pots two weeks later. Each maternal family was paired at random with another family from within the same population and crossed in a reciprocal fashion, generating 25 and 23 full-sib families from populations S and T, respectively. Using these same $F_1$ families, Carr and Fenster (1994) observed significant broad-sense heritability of mating-system characters in both populations. In population T, broad-sense heritabilities of stigma-anther separation and autogamous-seed set were 0.72 and 0.45, respectively, with family means for stigma-anther separation ranging from 1.03–2.85 mm and autogamous seed set ranging from 0.1–65.2 seeds per fruit (full seed-set in our greenhouse populations is approximately 500–1000). The broad-sense heritability for stigma-anther separation in population S was 0.56 with family means ranging from 1.57–3.67 mm.

We selected maternal families for this study based on the rank of their family mean for either stigma-anther separation or autogamous seed set (from Carr and Fenster 1994). We selected families from the extreme ends of the distributions of mating-system characters to create as much contrast as possible between two mating-system classes. We presumed that the families with the narrowest stigma anther separation or the highest autogamous seed set represented families with mating-system characters that promote selfing. In contrast, families with wide stigma-anther separation or low autogamy rate presumably represent families with mating-system characters that promote outcrossing. For population S, we selected seven families from both the extreme low and high ends of the stigma-anther separation distribution. Similarly we selected maternal families from population T on the basis of their ranks in the stigma-anther separation and autogamy distributions, eight families from each end of each distribution. Because the correlation between herkogamy and autogamy rate based on family means was weak (Carr and Fenster 1994), only six families were common to both the herkogamy and autogamy comparisons.

*Experimental Crosses.*—We sowed 30 seeds from each selected family and two weeks later transplanted a single seedling from each family to serve as a maternal parent (dam) for the experimental crosses. To estimate inbreeding depression for each maternal parent, we created sets of self and outcross progenies. Within each population, three plants served as pollen donors (sires) for cross-pollinations to control for potential dam × sire interactions that could otherwise confound a study of variation in inbreeding depression. These three sires were selected from across the distributions of both stigma-anther separation and autogamous seed set (population T only), representing phenotypes from the middle of the distribution as well as the high and low ends. We applied pollen from the three sires onto the stigmas of three separate flowers at three randomly selected stem nodes on each dam. *Mimulus* flowers are produced in pairs at each node, and we self-pollinated the alternate flower at each node. Pairing self and outcross pollinations allowed us to control for variation in maternal effects within a dam. Each maternal family therefore consisted of a total of three paired sets of seeds: three separate sets of outcross seed sired by three different males each paired with a set of self seed.

*Measurement of Inbreeding Depression.*—On 23 September 1993, we sowed 30 seeds from the three paired sets from each maternal family in population T into separate pots. We treated each self-outcross pair as an experimental unit by always placing their pots side-by-side. We arranged our experimental units on the greenhouse bench in a completely random fashion with respect to maternal family and mating-system class. Two weeks after sowing, we recorded the number of seedlings germinating in each pot. The following day, we transplanted 10 seedlings from every set into separate pots. Pots containing self plants again were placed adjacent to the pot containing their paired outcross, and the pair was treated as an experimental unit. We randomized our experimental units within each of 10 blocks. After eight weeks we counted the total number of flowers produced by each plant and harvested them by cutting the stems at ground level. We dried the plants to a constant weight and measured the biomass to the nearest 0.01 g. On 2 December 1993 we sowed the seed from population S and repeated the above procedure, harvesting the plants on 10 February 1994 when the plants reached a growth stage similar to that of population T when it was harvested.

*Analysis.*—We created a measure of relative performance (RP) for each experimental unit (a self-outcross pair) following the method proposed by Ågren and Schemske (1993). In this measurement,

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

Positive values of RP indicate that the performance of outcross plants exceeds that of selfs (inbreeding depression), and negative values indicate that the performance of self plants exceeds that of outcross plants (outbreeding depression). This measure is bounded by $+1$ and $-1$ and can be summed in an unbiased fashion. For positive values, RP is identical to the traditional measure of inbreeding depression,

$$\delta = 1 - (\text{self/outcross}), \quad (2)$$

but the traditional measure is bounded by $+1$ and $-\infty$ and is weighted toward outbreeding depression when summed if negative values occur. This latter property of $\delta$ could give misleading results in our study of family-level variation in inbreeding depression, thus we elected to use RP rather than $\delta$ as the dependent variable in all of the analyses described below.

We analyzed each population separately using SAS version 6.08 (1989). To examine the effects of mating-system class on RP for germination success, our GLM model included mating-system class as a fixed effect with dam nested within class as a random effect. Variation among sires used to estimate RP was analyzed as a random effect (treating sire as a fixed effect did not change the conclusions of any analysis). The SAS RANDOM statement with its TEST option was used to generate the appropriate error mean squares for hypothesis tests. The GLM models used to analyze both RP for flower number and RP for aboveground biomass included a random block effect, the fixed effect of mating-system class, variation due to the random effects of dams within mating-system classes, variation due to the random effects of sires, the random interaction between sire and mating-system class, and the random interaction between dam and sire.

In cases where we detected significant dam × sire interactions, we ran two additional analyses. Because RP is a composite trait based on separate measurements taken on a self plant and its paired outcross plant, the dam × sire interactions could be accounted for by interactive variation in either or both of these measurements. First, to examine whether the dam × sire interaction in the original analysis was due, at least in part, to specific interactions with the sire we ran a factorial ANOVA that included block, dam, and sire as random main effects and a sire × dam interaction term. The dependent variables for these analyses were log-transformed, using only those plants produced through outcrossing. The interactions observed in the RP analyses could also have been produced by consistent differences among self offspring produced from the three separate self-pollinations performed within a maternal plant. To test whether this was an important source of variation in this experiment we conducted a nested ANOVA in all cases where the RP analysis indicated a significant sire × dam interaction. In these models block and dam were main effects, and variation among self-pollinations was nested within dams. The dependent variables for these analyses were log-transformed, using only those plants produced by self-pollination.

An alternative way to test for differences in inbreeding depression among families and between mating-system classes is to follow the recommendations of Johnston and Schoen (1994). If dependent variables are log-transformed, significant interactions between pollination treatment (self or outcross) and family (or, in our case, mating-system class) would indicate differences among families (or between mating-system classes) in the relative performance of self and outcross progeny. We elected not to include these analyses for two reasons. First, within a family, progeny were not randomized with respect to pollination treatment since self progeny were always placed adjacent to their outcross half-sibs. Second, this analysis does not allow us to incorporate a sire × family interaction term into the model. Even without this important term in the model, however, the results from the approach recommended by Johnston and Schoen (1994) did not differ from the reported analyses with respect to differences between mating-system classes.

## RESULTS

*Herkogamy Classes.*—Very low levels of inbreeding depression were found for germination success, with a 7.8% reduction in self performance calculated across families. The

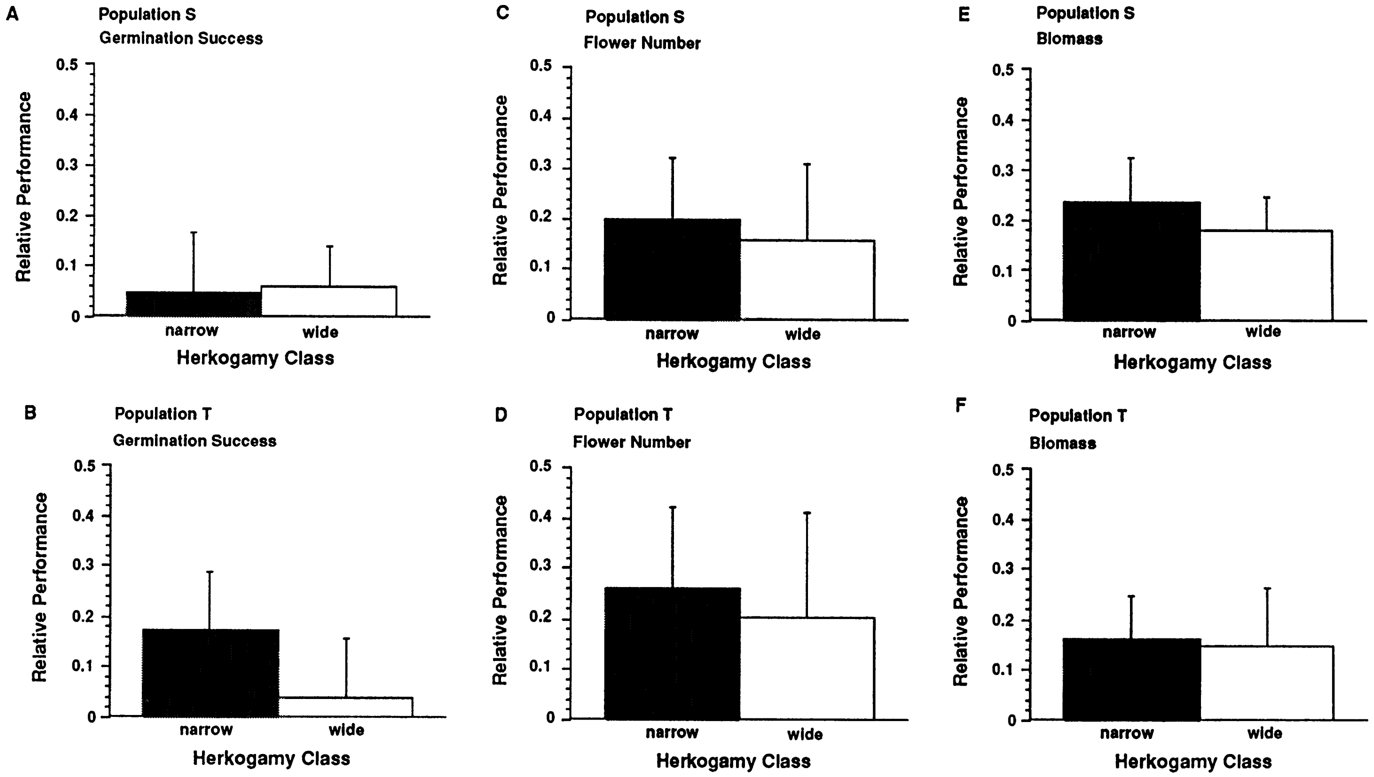

FIG. 1. Herkogamy class comparisons: relative performance of self and outcross progeny in *Mimulus guttatus* populations S and T with respect to germination success (A, B), flower number (C, D), and aboveground biomass (E, F). Error bars indicate 95% confidence intervals.

95% confidence intervals of our estimates of relative performance (RP) of self and outcross progeny overlapped with zero (no inbreeding depression) for both herkogamy classes in population S and for the narrow herkogamy class in population T (Fig. 1A,B). The ANOVA revealed no significant effect of herkogamy class in either population (population S: $F_{1,12} = 0.056$, $P = 0.818$; population T: $F_{1,14} = 3.409$, $P = 0.085$), suggesting that RP was equivalent for both narrow and wide stigma-anther separation groups.

Both herkogamy classes exhibited significant inbreeding depression for flower number in population S and for the narrow class in population T, with a mean reduction across all families of 20% (Fig. 1C,D). The 95% confidence interval for the estimate of RP in the wide class of population T barely overlapped zero, with a lower limit of $-0.01$. The two herkogamy classes did not differ significantly in RP (Table 1). Population T displayed a significant dam × sire interaction. This could not have obscured a herkogamy class effect because there was not a significant class × sire interaction, indicating that our sires, on average, produced consistent estimates of inbreeding depression across herkogamy classes. There were strong sire main effects in both populations, but these were only marginally significant ($0.07 > P > 0.05$) because of the small denominator degrees of freedom.

In populations S and T, both herkogamy classes exhibited significant inbreeding depression for aboveground biomass, with a mean reduction across all families of 18% (Fig. 1E,F). Again, herkogamy classes did not differ significantly in RP in either population (Table 1). There was a significant dam × sire interaction in population T, but again the herkogamy class × sire interaction was not significant.

*Autogamy Classes.*—Our comparisons of autogamy classes in population T provided much the same pattern as manifested by the herkogamy class comparisons described above. We found significant inbreeding depression for all three traits, with reductions in self performance ranging from 11% for germination success to 30% for flower number (Fig. 2), but our estimates of RP never differed significantly between autogamy classes (Table 2). There was a significant dam × sire interaction for flower number and biomass. In both cases there was not a significant autogamy class × sire interaction, suggesting that these dam × sire interactions were not obscuring differences between classes.

*Variation among Dams.*—Despite the fact that none of the analyses detected significant differences between mating-system classes, several of the analyses did reveal significant variation among dams in the relative performance of their self and outcross offspring. Dam effects were highly significant for RP in flower production in population S (Table 1) and in flower production and biomass in the analysis of the autogamy families from population T (Table 2). Dam effects were marginally significant for RP in biomass for populations S and T in the herkogamy class comparisons (Table 1). Ranking family means for RP in these analyses revealed distributions that were independent of the herkogamy or autogamy class to which the family belonged (Fig. 3).

*Dam × Sire Interactions.*—In all cases where the analyses on RP described above revealed significant dam × sire in-

TABLE 1. Herkogamy class comparisons. ANOVA results for relative performance (RP) of self and outcross progeny with respect to flower number and aboveground biomass for (A) population S; and (B) population T of *Mimulus guttatus.*

| (A) Population S | Flower number RP | | | | Biomass RP | | | |
|---|---|---|---|---|---|---|---|---|
| Source | df | MS | *F* | *P* | df | MS | *F* | *P* |
| Block | 9 | 0.1062 | 0.760 | 0.653 | 9 | 0.1005 | 0.973 | 0.462 |
| Herkogamy class | 1 | 0.1931 | 0.372 | 0.561 | 1 | 0.3407 | 1.342 | 0.310 |
| Dam w/i herkogamy class | 12 | 0.6384 | 3.432 | 0.005 | 12 | 0.1977 | 2.014 | 0.069 |
| Sire | 2 | 1.1604 | 15.553 | 0.061 | 2 | 0.7361 | 4.708 | 0.175 |
| Herk. class × sire | 2 | 0.0746 | 0.403 | 0.673 | 2 | 0.1563 | 1.591 | 0.223 |
| Dam × sire | 24 | 0.1864 | 1.339 | 0.138 | 24 | 0.0981 | 0.950 | 0.534 |
| Error | 340 | 0.1397 | | | 329 | 0.1033 | | |

| (B) Population T | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Source | df | MS | *F* | *P* | df | MS | *F* | *P* |
| Block | 9 | 0.1349 | 0.537 | 0.848 | 8 | 0.1738 | 2.194 | 0.027 |
| Herkogamy class | 1 | 0.9338 | 0.951 | 0.384 | 1 | 0.0022 | 0.004 | 0.954 |
| Dam w/i herkogamy class | 13 | 1.2635 | 1.799 | 0.103 | 14 | 0.3605 | 2.059 | 0.054 |
| Sire | 2 | 4.4225 | 10.231 | 0.092 | 2 | 0.3930 | 0.988 | 0.503 |
| Herk. class × sire | 2 | 0.4338 | 0.617 | 0.548 | 2 | 0.3974 | 2.268 | 0.124 |
| Dam × sire | 24 | 0.7024 | 2.796 | 0.001 | 26 | 0.1754 | 2.215 | 0.001 |
| Error | 368 | 0.2512 | | | 347 | 0.0792 | | |

teractions, a factorial ANOVA of log-transformed flower number or biomass of outcross progeny demonstrated significant sire × dam interactions (Table 3). These interactions were greatest in the analysis of log-transformed biomass where the dam × sire interaction variance component was 1.5 and 2.4 times greater than the among dam variance component in the analyses of the herkogamy and autogamy analyses, respectively.

In all cases where the RP analysis indicated a significant sire × dam interaction, a nested ANOVA of log-transformed flower number or biomass of self progeny demonstrated that within a dam, progeny produced from different fruits varied significantly (Table 4). This indicates that the effect of the maternal environment varied as a function of the pollination date and the location of fruit maturation on the maternal plant. The among-self-pollination variance component accounted for 16% and 13% of the total environmental variation in the herkogamy and autogamy analyses, respectively.

## DISCUSSION

*Associations.*—Models of mating-system evolution suggest that if inbreeding depression is due to deleterious recessive alleles, positive associations should rapidly develop between alleles conveying high fitness and alleles increasing the selfing rate (Holsinger 1988, 1991; Uyenoyama and Waller

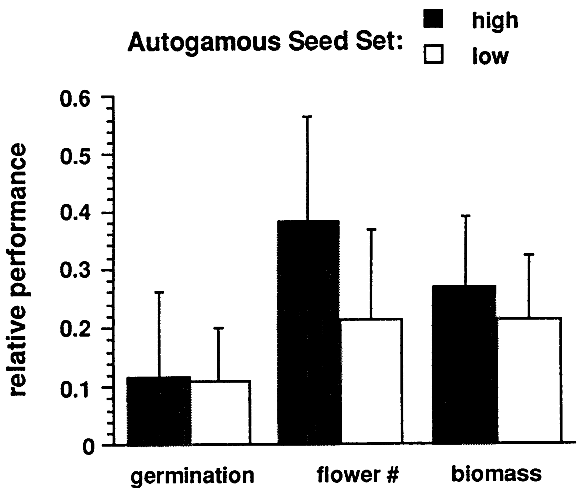

FIG. 2. Autogamy class comparisons for *Mimulus guttatus* population T: relative performance of self and outcross progeny with respect to germination success, flower number, and aboveground biomass. Error bars indicate 95% confidence intervals.

TABLE 2. Autogamy class comparisons for *Mimulus guttatus* population T. ANOVA results for relative performance (RP) of self and outcross progeny with respect to (A) germination success; (B) aboveground biomass; and (C) flower number.

| (A) Germination success | | | | |
|---|---|---|---|---|
| Source | df | MS | *F* | *P* |
| Autogamy class | 1 | 0.0019 | 0.025 | 0.876 |
| Dam w/i autogamy class | 16 | 0.0746 | 0.841 | 0.635 |
| Sire | 2 | 0.0641 | 0.723 | 0.493 |
| Error | 33 | 0.0888 | | |

| (B) Aboveground biomass | | | | |
|---|---|---|---|---|
| Source | df | MS | *F* | *P* |
| Block | 8 | 0.1867 | 2.313 | 0.020 |
| Autogamy class | 1 | 0.3180 | 0.826 | 0.396 |
| Dam w/i autogamy class | 16 | 0.5933 | 2.841 | 0.006 |
| Sire | 2 | 0.1936 | 50.480 | 0.023 |
| Aut. class × sire | 2 | 0.0039 | 0.019 | 0.981 |
| Dam × sire | 31 | 0.2093 | 2.594 | 0.001 |
| Error | 398 | 0.0807 | | |

| (C) Flower number | | | | |
|---|---|---|---|---|
| Source | df | MS | *F* | *P* |
| Block | 9 | 0.1608 | 0.575 | 0.818 |
| Autogamy class | 1 | 3.4255 | 2.049 | 0.199 |
| Dam w/i autogamy class | 16 | 1.4590 | 2.694 | 0.009 |
| Sire | 2 | 5.4063 | 7.151 | 0.123 |
| Aut. class × sire | 2 | 0.7560 | 1.396 | 0.263 |
| Dam × sire | 31 | 0.5421 | 1.939 | 0.002 |
| Error | 451 | 0.2795 | | |

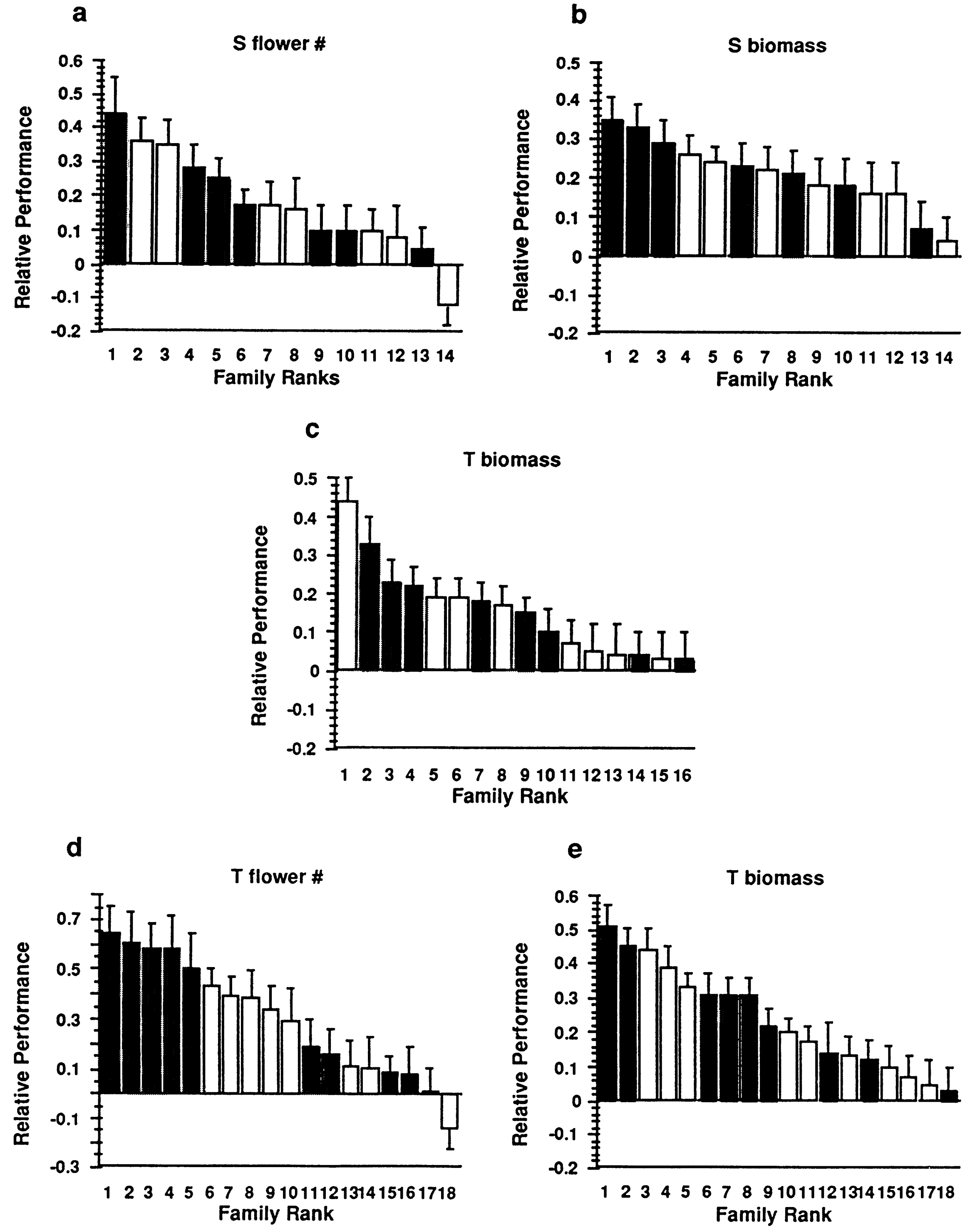

FIG. 3. Variation among *Mimulus guttatus* dams in levels of relative performance (RP). Solid bars indicate those families displaying mating-system characters that suggest a history of selfing (narrow stigma-anther separation or high levels of autogamous seed set) and open bars indicate those families displaying characters that suggest a history of outcrossing (wide stigma-anther separation or low levels of autogamous seed set). Error bars indicate one standard error of the mean. (a) RP for flower number in the population S herkogamy class comparisons; (b) RP for biomass in the population S biomass herkogamy class comparisons; (c) RP for biomass in the population T herkogamy class comparisons; (d) RP for flower number in the population T autogamy class comparisons; and (e) RP for biomass in the population T autogamy class comparisons.

TABLE 3. Factorial ANOVAs of log-transformed flower number and biomass. (A) Analyses of outcross progeny from *Mimulus guttatus* population T families used in the herkogamy class comparisons. (B) Analysis of outcross progeny from *M. guttatus* population T families used in the autogamy class comparisons.

(A) Herkogamy classes

| | Flower number | | | | Biomass | | | |
|---|---|---|---|---|---|---|---|---|
| Source | df | MS | *F* | *P* | df | MS | *F* | *P* |
| Block | 9 | 0.264 | 1.69 | 0.089 | 8 | 0.034 | 6.94 | 0.001 |
| Dam | 15 | 0.895 | 2.61 | 0.014 | 15 | 0.014 | 1.64 | 0.127 |
| Sire | 2 | 5.672 | 16.54 | 0.001 | 2 | 0.004 | 0.43 | 0.654 |
| Dam × sire | 28 | 0.343 | 2.20 | 0.001 | 28 | 0.009 | 1.74 | 0.013 |
| Error | 399 | 0.156 | | | 355 | 0.005 | | |

(B) Autogamy classes

| | Flower number | | | | Biomass | | | |
|---|---|---|---|---|---|---|---|---|
| Source | df | MS | *F* | *P* | df | MS | *F* | *P* |
| Block | 9 | 0.244 | 1.30 | 0.234 | 8 | 0.028 | 6.21 | 0.001 |
| Dam | 17 | 1.001 | 3.19 | 0.002 | 17 | 0.021 | 1.81 | 0.072 |
| Sire | 2 | 7.635 | 24.33 | 0.001 | 2 | 0.022 | 1.95 | 0.158 |
| Dam × sire | 33 | 0.314 | 1.67 | 0.012 | 33 | 0.011 | 2.50 | 0.001 |
| Error | 462 | 0.187 | | | 408 | 0.005 | | |

1991a,b). Within the genus *Mimulus*, higher rates of selfing are associated with narrow stigma-anther separation and high rates of autogamy. This appears to be true whether one considers variation among *Mimulus* taxa (Ritland and Ritland 1989), among populations within the species *M. guttatus* (Dole 1992), or among individuals within *M. guttatus* populations (Carr and Fenster 1994; Karron et al., in press). Several studies also have shown that variation in stigma-anther separation and autogamous seed set in *M. guttatus* has a strong genetic component (MacNair and Cumbes 1989; Carr and Fenster 1994; Fenster and Ritland 1994a). For these reasons, we felt that stigma-anther separation and autogamous seed set were ideal characters for testing the expectations of the models put forth by Holsinger (1988, 1991) and later Uyenoyama and Waller (1991a,b). The results from our study of two *M. guttatus* populations conflict with the expectation of lower inbreeding depression evolving associations with mating-system characters. Families differing in either their degree of herkogamy or autogamy did not differ significantly in their levels of inbreeding depression and the trends in both populations were in the opposite direction of the above predictions (i.e., families with traits associated with selfing tended to have higher levels of inbreeding depression).

There are a number of possible explanations for the disagreement between a priori expectations and our empirical results. First our assumption about the genetic basis of inbreeding depression could be incorrect. Inbreeding depression can also be caused by the segregation of alleles that act in an overdominant manner (Falconer 1981). Holsinger (1988) and Uyenoyama and Waller (1991c) have modeled mating-system evolution under this assumption as well. In this case, high-fitness genotypes should become associated with alleles that promote outcrossing and consequently maximum levels of heterozygosity. High levels of genetic load should always be present. Our experimental results would seem to support these models, but other lines of evidence strongly support the general importance of recessive alleles in the expression of inbreeding depression in *Mimulus*. Fu and Ritland (1994) found little evidence for overdominance in *M. guttatus* based on a study of allozyme segregation pat-

TABLE 4. Nested ANOVAs of log-transformed flower number and biomass. (A) Analyses of self progeny from *Mimulus guttatus* population T families used in the herkogamy class comparisons. (B) Analysis of self progeny from *M. guttatus* population T families used in the autogamy class comparisons.

(A) Herkogamy classes

| | Flower number | | | | Biomass | | | |
|---|---|---|---|---|---|---|---|---|
| Source | df | MS | *F* | *P* | df | MS | *F* | *P* |
| Block | 9 | 0.411 | 1.89 | 0.052 | 8 | 0.043 | 7.61 | 0.001 |
| Dam | 15 | 3.369 | 5.33 | 0.001 | 15 | 0.046 | 4.18 | 0.001 |
| Fruit w/i dam | 30 | 0.632 | 2.91 | 0.001 | 30 | 0.011 | 1.98 | 0.002 |
| Error | 398 | 0.217 | | | 351 | 0.006 | | |

(B) Autogamy classes

| | Flower number | | | | Biomass | | | |
|---|---|---|---|---|---|---|---|---|
| Source | df | MS | *F* | *P* | df | MS | *F* | *P* |
| Block | 9 | 0.230 | 1.04 | 0.410 | 8 | 0.048 | 7.36 | 0.001 |
| Dam | 17 | 6.020 | 12.56 | 0.001 | 17 | 0.076 | 8.16 | 0.001 |
| Fruit w/i dam | 35 | 0.480 | 2.16 | 0.001 | 35 | 0.009 | 1.43 | 0.057 |
| Error | 456 | 0.222 | | | 405 | 0.006 | | |

terns. Using a North Carolina 3 breeding design Dudash and Carr (unpubl. data) estimated average levels of dominance for biomass and flower production in these same *M. guttatus* populations, and all estimates suggest that inbreeding depression is due to partially recessive alleles.

Our system likely violated other important genetic assumptions of the Holsinger (1988, 1991) and Uyenoyama and Waller (1991a,b) models. All of these models assume only a single locus affecting mating system. Minimal estimates of the number of factors involved in the differentiation of stigma-anther separation in *Mimulus* indicate polygenic inheritance of this character (Fenster and Ritland 1994a). The large number of loci involved in altering the selfing rate in *Mimulus* and the large environmental contribution governing their expression (Carr and Fenster 1994) may produce only small differences in selfing rates between genotypes. Based on their model of the coevolution of mating systems and inbreeding depression, Schultz and Willis (1995) concluded that associations between mating system and viability loci may not be detectable when mating system modifiers have only small quantitative effects on selfing rates. Similarly, Latta and Ritland (1993) have demonstrated the mode of inheritance of mating-system determinants has a large effect on equilibrium predictions, with multilocus inheritance promoting more favorable conditions for mixed mating. Clearly the theory needs to be expanded to include multilocus determination of selfing rates as well as multilocus fitness determinants.

Our comparisons rest on the assumption that our measurements of herkogamy and autogamous seed set in the greenhouse reflect differences in actual selfing rates in the field. Many environmental factors influence selfing rates, however. For example, although Dole (1990) demonstrated that corolla shedding contributes to autogamous seed set in greenhouse populations of *M. guttatus*, there is little evidence of autogamous selfing in the field (Dudash and Ritland 1991; Leclerc-Potvin and Ritland 1994). Most selfing in *M. guttatus* appears to be mediated by pollinators, by either the facilitated movement of pollen from anthers to stigma within the same flower or movement between flowers (geitonogamy) of the same individual (Leclerc-Potvin and Ritland 1994). If there were only small differences in the history of selfing between the family lines contributing to either our narrow and wide stigma-anther-separation classes or our high and low autogamous seed-set classes, associations between levels of inbreeding depression and mating-system characters may not have been detectable.

Our experimental design itself may have obscured any associations between mating-system characters and inbreeding depression. The families selected for this experiment, regardless of their mating-system phenotype, were all the result of crosses made between plants grown from field-collected seed. Thus, all of the families that we tested had an immediate history of outcrossing. This single outcrossing event may have increased the heterozygosity of any line that had a history of selfing in their natural populations and may have decreased the linkage disequilibrium of unlinked mating-system modifier loci by 50% (Crow and Kimura 1970). Such a cross would also tend to equalize the genetic load among families. However, the detection of significant RP differences among maternal families of selfed progeny suggests that initial differences in genetic load among genotypes were preserved at least in part. Because stigma-anther separation shows significant narrow-sense heritability ($h^2 = 0.38$ in population S and $h^2 = 0.56$ in population T; Carr and Fenster 1994), families with narrow stigma-anther separation used in this experiment came from parents with narrow stigma-anther separation. The alleles contributing to the inbreeding depression observed in this study must have been present in the field-collected plants along with the alleles for narrow stigma-anther separation. Thus, our conclusion that associations between mating system and fitness loci are difficult to develop and maintain still appears to be supported.

Estimates of inbreeding depression made in the greenhouse are often conservative when compared to field-based studies (e.g., Dudash 1990). If the level of inbreeding depression in our greenhouse study is substantially lower than what would be observed in the field, then differences among families may have been obscured. However, in companion studies of these same *M. guttatus* populations we observed comparable levels of inbreeding depression for germination rate, biomass, and flower number when both self and outcross plants were grown in the same pot (Carr and Dudash 1996; Dudash et al. 1997). This experimental design presumably allowed competitive interactions between plants, which may have mimicked field conditions more closely.

Finally, even if the expected associations between viability and mating system loci develop, their detection may be beyond the power of reasonably sized empirical studies (Schultz and Willis 1995). More among-family variation in inbreeding depression is produced by a Poisson distribution of mutations than is produced by variation in inbreeding history over a range of biologically realistic conditions. Schultz and Willis (1995) demonstrate that even variation at viability loci with purely additive effects can cause families to vary in the relative fitness of self and outcross families. Because the distribution of alleles at these loci is unaffected by inbreeding history, variation at these loci will further obscure differences among mating-system classes in measured inbreeding depression. Schultz and Willis (1995) go on to suggest that families with a history of inbreeding may actually show greater inbreeding depression in some cases. Our observations appear to support their theoretical predictions because although families varied significantly in their levels of inbreeding depression, the variation was unrelated to differences in mating-system characters.

*Sire × Dam Interactions.*—The results of our study highlight two potential problems that need to be addressed in the design of experiments intended to examine variation in inbreeding depression among families. The first of these is a potential interaction between sires and dams that could arise from nonadditive genetic effects. Because we crossed each dam with three sires, we were able to test for this interaction, and it proved to be significant in several instances. Because herkogamy class × sire and autogamy class × sire interactions were never significant, this interaction was due to specific dam × sire combinations rather than combinations of dams and sires with like phenotypes and similar histories of inbreeding. Biparental inbreeding has been documented to commonly occur in *M. guttatus* (Dudash and Ritland 1991;

Leclerc-Potvin and Ritland 1994), suggesting that there is enough local genetic structure in the population to allow this phenomenon in the field. Our sample of genotypes from these populations may have included families with recent shared ancestry, producing some consanguineous matings from our hand-pollinations in the greenhouse. Such biparental inbreeding may be the origin of the interaction variation. The goal of our study was to document variation among dams in their genetic load and test whether these differences were accounted for by differences in mating-system characters. If we had used only one sire, variation due to dam × sire interactions would have been included in the dam and, potentially, mating-system class terms in the ANOVA models. We would have overestimated the extent of dam main effects and potentially the effects of mating-system class. Because we did use multiple sires we were able to test dam and mating-system class terms against error terms that included interaction variance components. However, we may have inflated the dam × sire interaction because of our choice of sires from the extremes of the mating-system classes.

*Within-Plant Effects.*—A second potential problem in attempting to quantify inbreeding depression was revealed by significant differences among sets of self progeny from the same dam. These differences cannot have a genetic basis and instead represent variation due to special environment (Falconer 1981) caused by temporal and positional effects on the allocation of resources to developing seeds and fruit (Diggle 1995). Strong maternal and within-plant environmental effects have been observed often for early life-history traits, for example, seed size and early juvenile growth (Roach and Wulff 1987; Fenster 1991b), and our results indicate that these effects may be expressed in later life-history traits as well. These strong environmental effects may influence the evolution of genetic load in the population by either exacerbating or attenuating the consequences of selfing depending on whether or not self-pollinations occur randomly with regard to these environmental factors (Naylor 1964; Kirkpatrick and Lande 1989). Experimental designs comparing genotypes from the same dam (e.g., self vs. outcross genotypes) need to control for this source of variation. In our experiment we performed self- and cross-pollinations at the same node so that variation due to the position of fruit and timing of the pollination would not contribute to differences between self and outcross progeny.

*Evolutionary Consequences.*—A negative relationship between selfing rate and magnitude of inbreeding depression is supported by comparisons across populations and species. Husband and Schemske (1996) reviewed studies that compared closely related taxa or populations that differed in their mating system and found that populations characterized as selfing had significantly lower inbreeding depression relative to populations characterized as outcrossing. The genus *Mimulus* is no exception. Dole and Ritland (1993) and Carr and Dudash (1996) found this relationship to be true based on comparisons of *M. guttatus* versus the selfing *M. platycalyx*, and *M. guttatus* versus the selfing *M. micranthus*. Latta and Ritland (1994) examined inbreeding depression for five traits in 15 populations of four *Mimulus* taxa. Inbreeding depression in all traits showed negative relationships with selfing rates, although the relationship was significant for only one trait. These studies all demonstrate covariation between mating system and inbreeding depression, but this may not have been driven by a disequilibrium between mating-system characters and fitness loci in ancestral populations.

Given the polygenic inheritance of mating-system characters in *Mimulus*, the environmental effects on mating-system, and the likelihood that differences in selfing rates among genotypes are probably small, the probability of an initial development of disequilibrium between mating-system and viability loci may be low. If so, a more plausible explanation is that self-pollination was initially favored by ecologically based factors and viability selection among self-pollinating lines followed. Strong selection for reduced stigma-anther separation has been documented in a *M. guttatus* population that was likely pollinator limited (Fenster and Ritland 1994b). Selection for more rapid floral development also may have favored selfing in *Mimulus* (MacNair and Cumbes 1989; Dole 1992; Fenster et al. 1995) and in other taxa where selfers flower in marginal or rapidly drying habitats (Jain 1976).

## ACKNOWLEDGMENTS

B. Beyers, C. Christiansen, J. Denkevitz, L. Galloway, K. Ganjianpour, C. Hardy, R. Irausquin, C. Jennings, N. Kalu, M. Kehoe, J. Lee, K. Oplinger, A. Parker, A. Rodhe, S. Ruhren, M. Sanchez, and H. Williams provided invaluable assistance in the greenhouse and laboratory. M. Cimino, S.-M. Chang, L. Galloway, C. Goodwillie, K. Holsinger, and E. Nagy provided valuable comments on earlier versions of this manuscript. J. Cronin provided assistance with statistical analysis. An NSERC grant through K. Ritland of the University of Toronto sponsored MRD's collection of the parental seed for this study. Computer time for this project was supported in full through the facilities of the Computer Science Center at the University of Maryland. Funding for this study was provided by a grant from the Maryland Agriculture Experiment Station to MRD and by National Science Foundation grant DEB9220906 to MRD. This is scientific article number A-7855, contribution number 9185, from the Maryland Agriculture Experiment Station.

## LITERATURE CITED

ÅGREN, J., AND D. W. SCHEMSKE. 1993. Outcrossing rate and inbreeding depression in two annual monoecious herbs, *Begonia hirsuta* and *B. semiovata*. Evolution 47:125–135.

CARR, D. E., AND M. R. DUDASH. 1996. Inbreeding depression in two species of *Mimulus* (Scrophulariaceae) with contrasting mating systems. Am. J. Bot. 83:586–593.

CARR, D. E., AND C. B. FENSTER. 1994. Levels of genetic variation and covariation for *Mimulus* (Scrophulariaceae) floral traits. Heredity 72:606–618.

CHARLESWORTH, D., AND B. CHARLESWORTH. 1979. The evolutionary genetics of sexual systems on flowering plants. Proc. R. Soc. Lond. B Biol. Sci. 205: 513-530.

———. 1987. Inbreeding depression and its evolutionary consequences. Annu. Rev. Ecol. Syst. 18:237–268.

———. 1990. Inbreeding depression with heterozygote advantage and its effect on selection for modifiers changing the outcrossing rate. Evolution 44:870–888.

CROW, J. F., AND M. KIMURA. 1970. An introduction to population genetics theory. Harper and Row, New York.

DIGGLE, P. K. 1995. Architectural effects and the interpretation of patterns of fruit and seed development. Annu. Rev. Ecol. Syst. 26:531–552.

DOLE, J. A. 1990. Role of corolla abscission in delayed self-pollination of *Mimulus guttatus*. Am. J. Bot. 77:1505–1507.

———. 1992. Reproductive assurance mechanisms in three taxa of the *Mimulus guttatus* complex (Scrophulariaceae). Am. J. Bot. 79:650–659.

DOLE, J. A., AND K. RITLAND. 1993. Inbreeding depression in two *Mimulus* taxa measured by multigenerational changes in the inbreeding coefficient. Evolution 47:361–373.

DUDASH, M. R. 1990. Relative fitness of selfed and outcrossed progeny in a self-compatible, protandrous species, *Sabatia angularis* L. (Gentianaceae): A comparison in three environments. Evolution 44:1129-1139.

DUDASH, M. R., AND K. RITLAND. 1991. Multiple paternity and self-fertilization in relation to floral age in *Mimulus guttatus* (Scrophulariaceae). Am. J. Bot. 78:1746–1753.

DUDASH, M. R., D. E. CARR, AND C. B. FENSTER. 1997. Five generations of enforced selfing and outcrossing in *Mimulus guttatus*: Inbreeding depression variation at the population and family level. Evolution 51:54–65.

FALCONER, D. S. 1981. Introduction to quantitative genetics. 2d ed. Longman Press, London.

FENSTER, C. B. 1991a. Gene flow in *Chamaecrista fasciculata*. II. Gene establishment. Evolution 45:410–422.

———. 1991b. Effect of male pollen donor and female seed parent on allocation of resources to developing seeds and fruit in *Chamaecrista fasciculata* (Leguminosae) Am. J. Bot. 78:13–23.

FENSTER, C. B., AND K. RITLAND. 1992. Chloroplast DNA and isozyme diversity in two *Mimulus* species (Scrophulariaceae) with contrasting mating systems. Am. J. Bot. 79:1440-1447.

———. 1994a. The quantitative genetics of mating system divergence in the yellow monkeyflower species complex. Heredity 73:422–435.

———. 1994b. Evidence for natural selection on plant mating systems in *Mimulus* (Scrophulariaceae). Int. J. Plant Sci. 155: 588–596.

FENSTER, C. B., P. K. DIGGLE, S. C. H. BARRETT, AND K. RITLAND. 1995. The genetics of floral development differentiating two species of *Mimulus* (Scrophulariaceae). Heredity 74:258–266.

FISHER, R. A. 1941. Average excess and average effect of a gene substitution. Ann. Eugenics 11:53–63.

FU, Y.-B., AND K. RITLAND. 1994. Evidence for the partial dominance of viability genes contributing to inbreeding depression in *Mimulus guttatus*. Genetics 136:323–331.

HOLSINGER, K. E. 1988. Inbreeding depression doesn't matter: The genetic basis of mating system evolution. Evolution 42:1234–1244.

———. 1991. Inbreeding depression and the evolution of plant mating systems. Trends Ecol. Evol. 6:307–308.

HUSBAND, B. C., AND D. W. SCHEMSKE. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. Evolution 50:54–70.

JAIN, S. K. 1976. The evolution of inbreeding in plants. Annu. Rev. Ecol. Syst. 7:469–495.

JOHNSTON, M. O., AND D. J. SCHOEN. 1994. On the measurement of inbreeding depression. Evolution 48:1735–1741.

KARRON, J. D., R. T. JACKSON, N. N. THUMSER, AND S. L. SCHLICHT. In press. Outcrossing rates of individual *Mimulus ringens* genets are correlated with anther-stigma separation. Heredity.

KIRKPATRICK, M., AND R. LANDE. 1989. The evolution of maternal characters. Evolution 43:485–503.

LANDE, R., AND D. W. SCHEMSKE. 1985. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. Evolution 39:24–40

LATTA, R., AND K. RITLAND. 1993. Models for the evolution of selfing under alternative modes of inheritance. Heredity 71:1–10.

———. 1994. The relationship between inbreeding depression and prior inbreeding among populations of four *Mimulus* taxa. Evolution 48:806–817.

LECLERC-POTVIN, C., AND K. RITLAND. 1994. Modes of self-fertilization in *Mimulus guttatus* (Scrophulariaceae): A field experiment. Am. J. Bot. 1:199–205.

LLOYD, D. G. 1979. Some reproductive factors affecting the selection of self-fertilization in plants. Am. Nat. 113:67–79.

MAYNARD SMITH, J. 1971. The origin and maintenance of sex. Pp. 163–175 *in* G. Williams, ed. Group selection. Aldine-Atherton, Chicago.

MACNAIR, M. R., AND Q. L. CUMBES. 1989. The genetic architecture of interspecific variation in *Mimulus*. Genetics 122:211–222.

NAYLOR, A. 1964. Natural selection through maternal influence. Heredity 19:509–511.

PRAY, L. A., AND C. J. GOODNIGHT. 1995. Genetic variation in inbreeding depression in the red flour beetle *Tribolium castaneum*. Evolution 49:179–188.

RITLAND, K. 1989. Genetic differentiation, diversity and inbreeding in the mountain monkeyflower *Mimulus caespitosus* of the Washington Cascades. Can. J. Bot. 67:2017–2024

———. 1990. Inferences about inbreeding depression based on changes of the inbreeding coefficient. Evolution 44:1230–1241.

RITLAND, K., AND C. RITLAND. 1989. Variation of sex allocation among eight taxa of *Mimulus guttatus* species complex (Scrophulariaceae). Am. J. Bot. 76:1731–1739.

ROACH, D. A., AND R. D. WULFF. 1987. Maternal effects in plants. Annu. Rev. Ecol. Syst. 18:209–236.

ROBERTSON, A. W., A. DIAZ, AND M. R. MACNAIR. 1994. The quantitative genetics of floral characters in *Mimulus guttatus*. Heredity 72:200–311.

SAS INSTITUTE. 1989. SAS/STAT user's guide. Vers. 6. Vol. 2. SAS Institute, Inc., Cary, NC.

SCHULTZ, S. T., AND J. H. WILLIS. 1995. Individual variation in inbreeding depression: The roles of inbreeding history and variation. Genetics 141:1209–1223.

UYENOYAMA, M. K., AND D. M. WALLER. 1991a. Coevolution of self-fertilization and inbreeding depression I. Mutation-selection balance at one and two loci. Theor. Popul. Biol. 40:14–46.

———. 1991b. Coevolution of self-fertilization and inbreeding depression III. Homozygous lethal mutations at multiple loci. Theor. Popul. Biol. 40:173–210.

———. 1991c. Coevolution of self-fertilization and inbreeding depression II. Symmetric overdominance in viability. Theor. Popul. Biol. 40:47–77.

WILLIS, J. H. 1993. Partial self-fertilization and inbreeding depression in two populations of *Mimulus guttatus*. Heredity 71: 145–154.

Corresponding Editor: V. Sork