

## INBREEDING ALTERS RESISTANCE TO INSECT HERBIVORY AND HOST PLANT QUALITY IN *MIMULUS GUTTATUS* (SCROPHULARIACEAE)

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**Abstract.**—Previous studies have demonstrated genetic variation for resistance to insect herbivores and host plant quality. The effect of plant mating system, an important determinant of the distribution of genetic variation, on host plant characteristics has received almost no attention. This study used a controlled greenhouse experiment to examine the effect of self- and cross-pollination in *Mimulus guttatus* (Scrophulariaceae) on resistance to and host plant quality for the xylem-feeding spittlebug *Philaenus spumarius* (Homoptera: Cercopidae). Spittlebugs were found to have a negative effect on two important fitness components in *M. guttatus*, flower production and aboveground biomass. One of two *M. guttatus* populations examined showed a significant interaction between the pollination and herbivore treatments. In this case, the detrimental effects of herbivores on biomass and flower production were much more pronounced in inbred (self) plants. The presence of spittlebug nymphs increased inbreeding depression by as much as three times. Pollination treatments also had significant effects on important components of herbivore fitness, but these effects were in opposite directions in our two host plant populations. Spittlebug nymphs maturing on self plants emerged as significantly larger adults in one of our host plant populations, indicating that inbreeding increased host plant quality. In our second host plant population, spittlebug nymphs took significantly longer to develop to adulthood on self plants, indicating that inbreeding decreased host plant quality. Taken together these results suggest that the degree of inbreeding in host plant populations can have important and perhaps complex effects on the dynamics of plant-herbivore interactions and on mating-system evolution in the host.

**Key words.**—Herbivory, host plant quality, inbreeding, mating system, *Mimulus guttatus*, *Philaenus spumarius*, resistance.

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By reducing heterozygosity within individuals, inbreeding alters the distribution of genetic variation, thus increasing the expression of recessive alleles and reducing the contribution of overdominance (Falconer 1981). These genetic changes are usually accompanied by a fitness loss referred to as ‘inbreeding depression’ (Charlesworth and Charlesworth 1987; Husband and Schemske 1996). Inbreeding, in particular selfing, is widespread in plants, with more than half of plants surveyed by Barrett and Eckert (1990) exhibiting selfing rates in excess of 20%. Inbreeding depression, however, is a potent evolutionary force that likely serves as a major impediment to the evolution of self-pollination in plants (e.g., Maynard Smith 1971; Nagylaki 1976; Lloyd 1979; Charlesworth and Charlesworth 1979, 1987, 1990; Lande and Schemske 1985; Campbell 1986; Charlesworth et al. 1990, 1991). Because levels of inbreeding increase with a decrease in effective population size, inbreeding depression may also significantly increase the risk of extinction for small populations (Fenster and Dudash 1994; Frankham 1995a,b; Newman and Pilson 1997; Saccheri et al. 1998).

Insect herbivores can dramatically impact their host plants and, as a result, are often one of the most important environmental variables that affect plant fitness (Louda et al. 1989; Marquis 1992). Variation in damage due to insect herbivory among individuals of the same plant species in the same location appears to be universal (Marquis 1992). Although many environmental factors can affect the impact of insect herbivores on their host plants (Brown et al. 1984; Bernays and Lewis 1986; Collinge and Louda 1988), intraspecific variation in herbivore damage is often genetically

based (Marquis 1984, 1990; Berenbaum et al. 1986; Simms and Rausher 1987, 1989; Kennedy and Barbour 1992).

Host plant variation also affects the insects that consume them (Price 1997; Speight et al. 1999). Herbivore fitness is often positively correlated with total nitrogen and amino acid concentrations of host plants (Feeny 1970; Mattson 1980; Olmstead et al. 1997). Fitness components of herbivorous insects are often negatively correlated with increasing levels of plant allelochemicals (Harborne 1982, 1988; Rosenthal and Berenbaum 1991; Speight et al. 1999) and with morphological features such as trichomes (Gange 1995; Lambert et al. 1995) or the toughness of leaves and stems (Raupp 1985; Stevenson et al. 1993; Eigenbrode et al. 1995). As with resistance, intraspecific variation in host plant quality is often genetically based (Whitham 1983; Larsson et al. 1986; McCrea and Abrahamson 1987; Karban 1992).

Because host plant resistance to insect herbivores and host plant quality are both critically important to the interaction between host and herbivore, genetic variation affecting these traits influences plant-herbivore dynamics (Fritz and Simms 1992; Hunter and Price 1992; Karban 1992). The effect of processes that control the distribution and expression of genetic variation will be of similar importance. The effect of inbreeding in plants on their interactions with natural enemies has received almost no theoretical or empirical attention. In the only study explicitly examining the effect of inbreeding on resistance to herbivory in natural plant populations, Núñez-Farfán et al. (1996) found no effect of mating system (selfing and outcrossing) on leaf damage caused by a chrysomelid beetle and a grasshopper in an herbaceous annual, *Datura stramonium*. It should be emphasized, however, that

this study failed to detect any inbreeding depression for any postgermination trait irrespective of herbivore damage in this highly selfing species. Studies of host species with higher natural outcrossing rates—and typically higher inbreeding depression (Husband and Schemske 1996)—seem warranted.

Two lines of evidence suggest that inbreeding may be generally important in determining levels of resistance and host plant quality. First, inbreeding in plants produces broad, detrimental, systemic changes in plant phenotypes (Charlesworth and Charlesworth 1987). Many of these gross phenotypic changes are almost certainly accompanied by smaller changes in some of the numerous physiological and morphological traits that affect the preference and performance of insect herbivores (see reviews in Price 1997; Speight et al. 1999). A second line of evidence comes from studies of crop breeding. Surveys of the agricultural literature reveal a wide variety of genetic mechanisms involved in resistance to herbivores, including dominance and partial dominance (Smith 1989; Kennedy and Barbour 1992). Under these types of genetic architecture, resistance would be expected to decline under imposed inbreeding in genetically variable populations. Reduced resistance, in fact, has been observed in the selection of inbred lines of several crops (Kennedy and Barbour 1992).

In this study, we examine the effect of inbreeding on host plant resistance to herbivory and host plant quality. Here we define host plant resistance as the qualities possessed by some individuals or genotypes that result in less damage than a susceptible individual or genotype of the same plant species when attacked by an insect herbivore. This would include antibiosis (the negative effects of chemical and morphological plant defenses on the biology of an insect attempting to use that plant as a host) and host plant tolerance (sensu Smith 1989; Kennedy and Barbour 1992). We define host plant quality as attributes possessed by some individuals or genotypes of a plant species that affect the performance of insect herbivores. By necessity this definition may include some aspects of antibiosis resistance (Smith 1989). We evaluate resistance and host plant quality by measuring important fitness components for the host plant, *Mimulus guttatus*, and herbivore, *Philaenus spumarius*. Specific questions addressed are: (1) Does *P. spumarius* affect flower production and aboveground biomass differently in self and outcross *M. guttatus* progeny? and (2) Do *P. spumarius* nymphs maturing on self and outcross hosts differ in their maturation time or adult body size?

## MATERIALS AND METHODS

### *Study Species*

*Mimulus guttatus* DC (Scrophulariaceae) ranges throughout western North America from Mexico to Alaska, occupying a variety of moist, open habitats. Estimates of outcrossing rates from different populations vary from about 75% selfing ( $t = 0.25$ ) to complete outcrossing ( $t = 1.0$ ), averaging  $t \approx 0.60$  (Ritland and Ritland 1989; Ritland 1990; Dudash and Ritland 1991; Willis 1993a). It typically produces a pair of large (20–30 mm in width), perfect, yellow, zygomorphic flowers at each flowering node and may produce as many as 100 or more flowers during a season. Populations in habitats

that are moist year-round are commonly perennial, but in seasonally dry areas populations tend to be annual (Dole 1992).

Meadow spittlebugs, *P. spumarius* (Homoptera: Cercopidae), are perhaps the most polyphagous of all insect herbivores, with over 500 host plant species recorded (Weaver and King 1954; Hamilton 1982; Owen 1988). Meadow spittlebugs are univoltine. Females lay their eggs on plant tissue or in the soil in the fall, eggs overwinter, and first-instar nymphs hatch in the spring. In coastal California, meadow spittlebugs were the most abundant herbivore on *M. guttatus*. Infested plants typically hosted one or two spittlebug nymphs. Meadow spittlebugs initially attack during the rosette stage in early spring, and nymphs develop on the plants throughout spring and into early summer (D. E. Carr and M. D. Eubanks, unpubl. data). Once a feeding site is established, nymphs feed on xylem with their strawlike mouthparts and generate the spittle for which they are named (Severin 1950; Wiegert 1964; Horsfield 1978). The spittle mass covers their bodies and primarily protects nymphs from desiccation (Weaver and King 1954; McEvoy 1986). Nymphs rarely move to a new plant once they settle and usually complete development (five nymphal instars) on a single plant.

Phytochemical traits such as xylem amino acid concentration (Horsfield 1977) and physical traits of the host plant such as stem tissue hardness, stem width, axil angle, and trichome density affect *P. spumarius* performance (McEvoy 1986). Variation in *P. spumarius* performance can be accounted for, in part, by host plant genotype (Cronin and Abrahamson 1999). *Philaenus spumarius* nymphs often severely damage their host plants after only a few weeks of feeding. Nymphal feeding can reduce stem length, stem weight, overall plant biomass, specific leaf area, growth rate, photosynthetic rate, number of lateral stems, and fruit and seed production and can delay plant maturity (Parman and Wilson 1982; Zajac and Wilson 1984; Meyer and Whitlow 1992; Meyer 1993; Meyer and Root 1993; Cronin and Abrahamson 1999).

Ripe *M. guttatus* fruits were collected from 15 plants in each of two annual populations in June 1997. Population M5 came from Santa Clara County, CA (37°17'N, 122°09'W), and population M13 came from Napa County, CA (38°33'N, 122°22'W). Outcrossing rates have not been estimated for either population. We collected second- and third-instar spittlebug nymphs for this experiment in a *M. guttatus* population at the Point Reyes National Seashore in Marin County, CA (38°07'N, 122°56'W) in April 2000. Insects were collected from *M. guttatus* and other hosts in the immediate area. This site was selected to ensure that the herbivores and hosts in this experiment were naive to one another.

### *Experimental Design*

Field-collected seed from each *M. guttatus* maternal family from populations M5 and M13 was sown in a pollinator-free greenhouse at the Blandy Experimental Farm (BEF) in Boyce, Virginia, in the fall of 1998. One individual grown from each of these families served as the maternal plant for the production of both self and outcross seed. We emasculated all flowers in bud prior to hand-pollination (both self-

and cross-pollinations). To produce outcross seed, we randomly paired individuals from the same population, applying pollen directly from the anthers of the pollen donor to the receptive stigma of the recipient. Each family served only once as a pollen donor for outcross seed. Self seed was produced by applying pollen from another flower on the same plant to the receptive stigma of a newly opened, previously emasculated flower (geitonogamous pollination).

To examine the effects of inbreeding on the interaction between host plant and herbivore, we sowed 40 seeds per pollination treatment (outcrossing and selfing) per maternal family on 12 March 2000 into 72-mm square plastic pots and Wetsel (Harrisonburg, VA) Professional Growing Media III. Pots were arranged 20 to a tray and were bottom-watered. Seeds were allowed to germinate under natural light. On 30 March we transplanted 10 randomly selected seedlings per pollination treatment per maternal family into individual 72-mm pots. Two seedlings (one randomly designated as a control plant, the other designated to receive a spittlebug nymph) per pollination treatment per maternal family were placed in each of five blocks (greenhouse bench) for a total of 120 plants per block (2 populations  $\times$  15 maternal families  $\times$  2 pollination treatments  $\times$  2 herbivore treatments) and 600 plants in total. Seedlings were completely randomized within each block. Pots were arranged 20 to a tray and were bottom-watered throughout the experiment. Sodium vapor lights (one per bench) extended the photoperiod to 18 h.

On 13–14 April 2000 we collected second- and third-instar *P. spumarius* nymphs from Point Reyes National Seashore, California. Spittlebugs were packed in Ziploc bags and were provided with fresh plant material for transport to BEF. On 16 April we applied one randomly selected spittlebug (a typical number for infested plants) to each of the 300 designated plants. At this time, all plants were in a large rosette stage, just prior to bolting. Most nymphs immediately established feeding sites on the plants. On 17–18 April any nymph that failed to establish a feeding site was replaced with a new nymph. Twelve plants (10 of them self) were repeatedly rejected by nymphs, apparently because the plants were extremely small. This forced us to omit these plants from the study. This may have slightly biased our findings toward not finding herbivore effects because the loss of plants from the herbivore treatment was nonrandom.

We monitored the experiment on a daily basis, and as adults emerged from their spittle masses they were captured, placed in microcentrifuge tubes, and immediately frozen. We recorded the date of emergence and calculated a development time based on the difference between the emergence date and the date each spittlebug was placed on the experimental plant. Adult spittlebugs emerged from 27 April through 10 May. Once the last spittlebug adult was collected (10 May), we dried each insect to a constant weight at 50°C for 18 h. We weighed spittlebugs to the nearest 0.01 mg on a Cahn Instruments (Cerritos, CA) C-31 microbalance.

On 10 May, immediately after the emergence of the last spittlebug adult, we censused the number of flowers on each *M. guttatus*. Plants had not finished flowering at this time, but we wanted to examine the life stages that were most immediately impacted by the spittlebugs. Each plant was then harvested at ground level, placed in a paper bag, and dried

to a constant weight at 50°C. We weighed the entire above-ground portion of the plant to the nearest 0.01 g using an Ohaus model TS400S balance. In natural populations of *M. guttatus*, biomass and flower production are highly correlated ( $r = 0.89$ ; Galloway 1995), and biomass is highly correlated with total seed production ( $r = 0.68$ – $0.80$ ; Fenster and Ritland 1994).

### Statistical Analysis

Preliminary analyses of plant flower production and biomass revealed highly significant three-way interactions among population, pollination treatment, and herbivore treatment. Separate analyses for each population are reported for ease of display and interpretation. The responses of flower number and aboveground biomass were both analyzed by mixed-model three-way factorial ANOVA using SAS (ver. 6.12, SAS Institute, Cary, NC) Proc Mixed. The full model included a block effect and three main-effects: pollination treatment (fixed), herbivore treatment (fixed), and maternal plant family (random) plus the two- and three-way interactions among the main effects. The full model was tested hierarchically beginning with the highest order (three-way) interaction. The significance of the random effects (and interactions with random effects) were tested by running models with and without the highest order term and calculating likelihood-ratio statistics (Littell et al. 1996). Nonsignificant interactions were pooled into the error before testing the next random effect in the hierarchy. Hypotheses regarding fixed effects were tested with the  $F$ -ratios generated by Proc Mixed. Flower number was square-root transformed to better meet ANOVA assumptions.

The effect of host plant inbreeding on adult spittlebug mass and emergence time was analyzed for both populations combined using SAS Proc Mixed. Population and pollination treatment were analyzed as fixed effects. Block and family nested within population were analyzed as random effects. The model also included the interactions between family and pollination treatment, and random effects were tested hierarchically as before. Log-transformation was used for spittlebug mass. All means reported are least squares means (backtransformed, if necessary).

Inbreeding depression for each family in the experiment was calculated following the methods of Dudash et al. (1997). Means and variances of these family-based estimates were calculated for each population and compared with  $t$ -tests.

## RESULTS

### Resistance to Herbivory

*Philaenus spumarius* feeding usually arrested shoot elongation in *M. guttatus*. Spittlebugs had a significant negative effect on flower production in population M5 (Table 1; Fig. 1). Selfing also significantly reduced flower number, but the interaction between the herbivore and pollination treatments was not significant (Table 1). Inbreeding depression calculated from family-based estimates was 12% in control plants and 17% in plants with spittlebugs, and was not significantly different based on a  $t$ -test ( $t = 0.39$ ,  $df = 28$ ,  $P = 0.39$ ). A similar pattern was seen in aboveground biomass in popu-

TABLE 1. Treatment effects and variance components (for random effects) for flower production and aboveground biomass in population M5.

Source	Flower production		Aboveground biomass	
	Var comp	$\chi^2$ or $F$	Var comp	$\chi^2$ or $F$
Block	13.10	17.65***	0.0023	41.25***
Pollination	—	11.51***	—	9.55**
Herbivore	—	4.45*	—	27.06***
Family	3.94	8.92**	0.0013	13.10***
Pollination $\times$ Herbivore	—	0.12	—	0.5355
Pollination $\times$ Family	1.50	0.44	0.0006	0.94
Herbivore $\times$ Family	0.00	0.00	0.0000	0.00
Poll. $\times$ Herb. $\times$ Fam.	0.00	0.00	0.0004	0.24
Error	51.96	—	0.0132	—

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

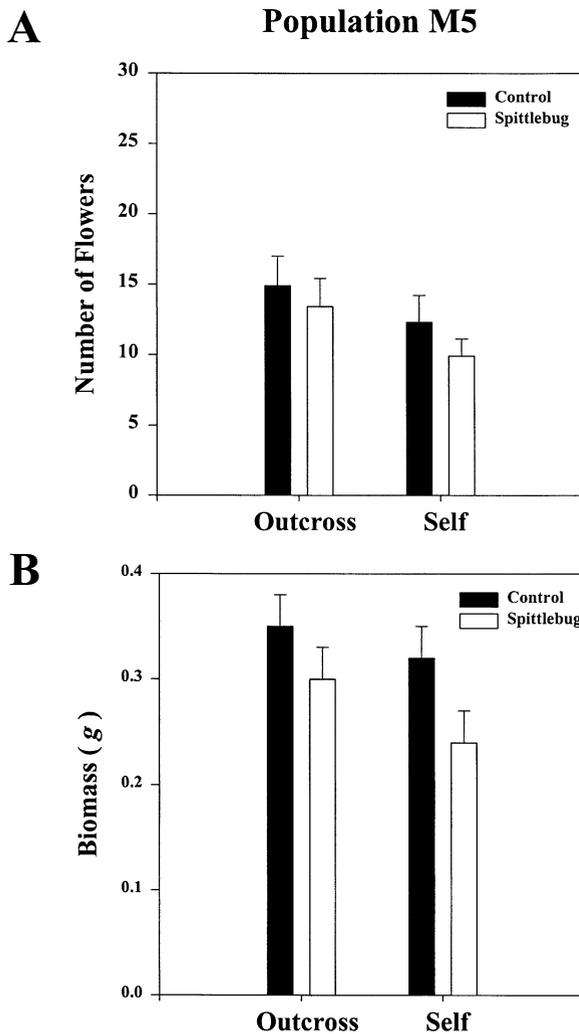


FIG. 1. The effects of pollination and herbivore treatments on (A) flower production and (B) aboveground biomass in *Mimulus guttatus* population M5. Error bars represent 95% confidence intervals.

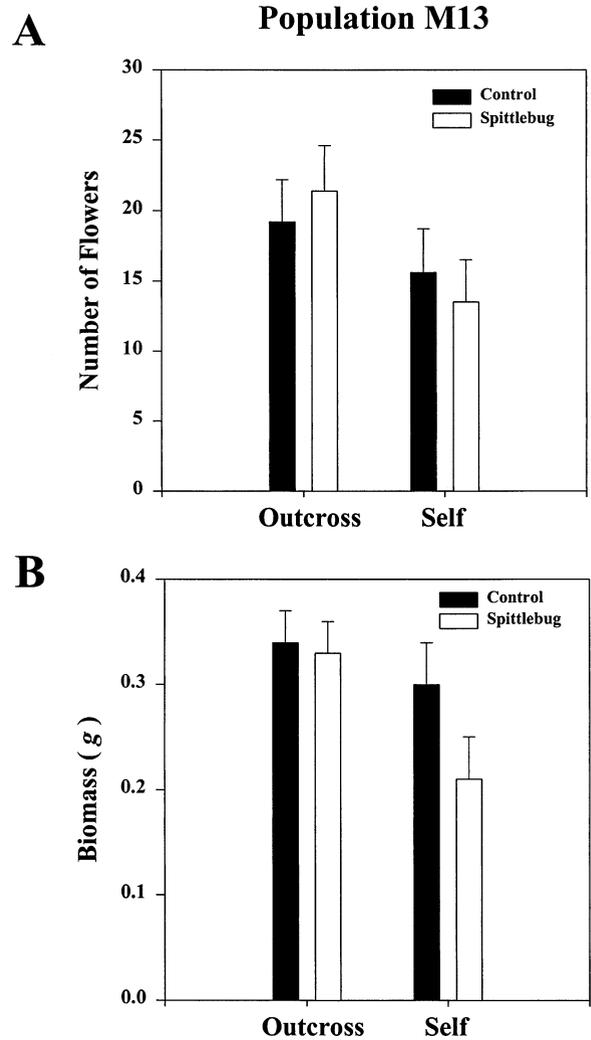


FIG. 2. The effects of pollination and herbivore treatments on (A) flower production and (B) aboveground biomass in *Mimulus guttatus* population M13. Error bars represent 95% confidence intervals.

lation M5 (Fig. 1). Herbivores and selfing significantly reduced *M. guttatus* biomass, but again there was no interaction between herbivore and pollination treatments (Table 1). Inbreeding depression was 2% for control plants and 18% for plants with spittlebugs ( $t = 1.28$ ,  $df = 28$ ,  $P = 0.11$ ).

In population M13 selfing significantly reduced flower number, but herbivores had no overall significant effect on flower production (Fig. 2; Table 2). There was, however, a significant interaction between herbivore and pollination treatments (Table 2). Whereas outcross plants with herbivores actually produced slightly more flowers than control plants, herbivores on self plants reduced flower production by 13% relative to controls. Inbreeding depression nearly doubled from 18% in control plants to 35% in plants with spittlebugs ( $t = 2.40$ ,  $df = 28$ ,  $P = 0.012$ ). These effects were more dramatic when aboveground biomass was examined (Fig. 2). Both herbivores and selfing significantly reduced biomass in population M13, and again there was a significant interaction between herbivore and pollination treatments (Table 2). There was little difference between outcross plants with and

TABLE 2. Variance components (for random effects) and treatment effects for flower production and aboveground biomass in population M13.

Source	Flower production		Aboveground biomass	
	Var comp	$\chi^2$ or $F$	Var comp	$\chi^2$ or $F$
Block	11.30	8.00**	0.0012	27.29***
Pollination	—	30.58***	—	25.00***
Herbivore	—	0.14	—	9.23**
Family	4.58	4.23*	0.0014	8.33**
Pollination $\times$ Herbivore	—	4.05*	—	5.46*
Pollination $\times$ Family	0.00	0.00	0.0003	0.11
Herbivore $\times$ Family	1.86	0.22	0.0004	0.18
Poll. $\times$ Herb. $\times$ Fam.	0.00	0.00	0.0000	0.00
Error	87.51	—	0.0182	—

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

without herbivores, but herbivores reduced biomass in self plants by 30% relative to controls. Inbreeding depression tripled from 11% in control plants to 36% in plants with spittlebugs ( $t = 3.10$ ,  $df = 28$ ,  $P = 0.002$ ).

In population M5 there was significant variation among *M. guttatus* maternal families in both flower production (Table 1) and aboveground biomass (Table 1), but there were no significant interactions between or among family and either of the two fixed effects. Maternal families in *M. guttatus* population M13 also varied significantly in flower production (Table 2) and biomass (Table 2), but again there were no significant interactions between or among family and the two fixed effects.

#### Host Plant Quality

*Philaenus spumarius* nymphs began molting into adults as early as 27 April, with the last adult emerging on 10 May. There was a host population  $\times$  pollination treatment interaction for adult spittlebug body mass (Table 3). An orthogonal contrast demonstrated that adult spittlebugs that matured on self plants were 16% larger than spittlebugs maturing on outcross plants in population M5 ( $t = 1.96$ ,  $df = 235$ ,  $P = 0.05$ ; Fig. 3A). In population M13 an orthogonal contrast found no difference in the size of adult spittlebugs maturing on self and outcross plants ( $t = 1.00$ ,  $df = 235$ ,  $P = 0.32$ ; Fig. 4A).

There was a marginally significant ( $P = 0.09$ ) pollination treatment effect for spittlebug development time (Table 3). An orthogonal contrast revealed no difference in maturation time for nymphs developing on self or outcross M5 host plants ( $t = 0.09$ ,  $df = 231$ ,  $P = 0.93$ ; Fig. 3B), but spittlebugs took 10% longer to reach adulthood when maturing on inbred plants ( $t = 2.22$ ,  $df = 231$ ,  $P = 0.03$ ; Fig. 4B) in host population M13.

Adult body size and development time did not vary significantly among host plant families, and there were no significant interactions between host plant families and pollination treatment for either spittlebug development time or adult body size (Table 3).

TABLE 3. Variance components (for random effects) and treatment effects for development time and log-transformed adult body mass for spittlebugs feeding on M5 and M13 host plants.

Source	Adult body mass		Development time	
	Var comp	$\chi^2$ or $F$	Var comp	$\chi^2$ or $F$
Block	0.0000	0.00	0.00	0.00
Population	—	1.47	—	0.68
Pollination	—	0.43	—	2.78†
Family(Pop.)	0.0001	0.28	0.42	0.99
Population $\times$ Pollination	—	4.33*	—	2.38
Pollination $\times$ Family(Pop.)	0.0002	0.08	0.00	0.00
Error	0.0090	—	10.15	—

†  $P < 0.10$ , \*  $P < 0.05$ .

## DISCUSSION

### Inbreeding and Herbivory

Inbreeding in *M. guttatus* altered its resistance to herbivory and its quality as a host plant for the spittlebug *P. spumarius*, but effects differed between host plant populations. In host population M13, spittlebugs impacted self plants much more

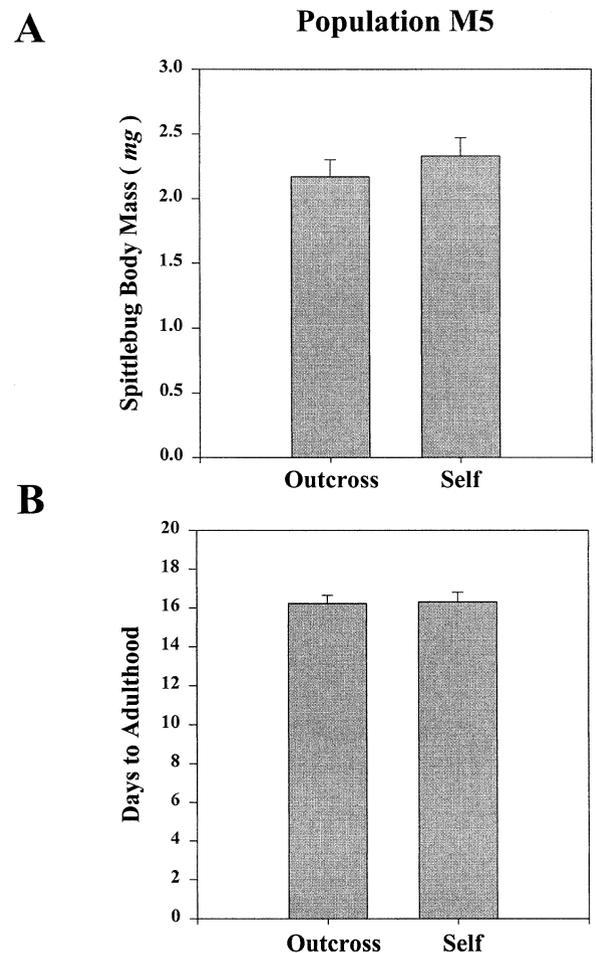


FIG. 3. The effects of pollination treatment in *Mimulus guttatus* population M5 on adult *Philaenus spumarius* (A) adult body mass and (B) maturation time. Error bars represent 95% confidence intervals.

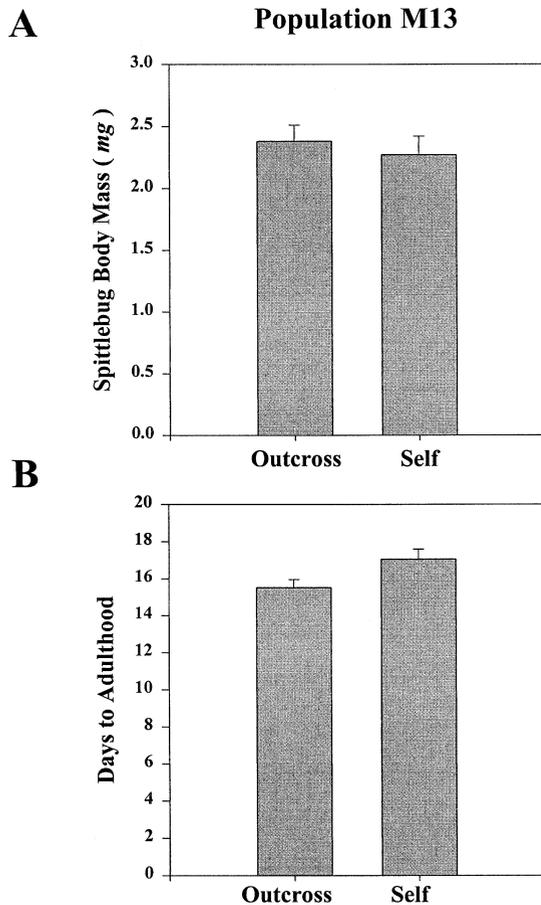


FIG. 4. The effects of pollination treatment in *Mimulus guttatus* population M13 on *Philaenus spumarius* (A) adult body mass and (B) maturation time. Error bars represent 95% confidence intervals.

severely than outcross plants, suggesting that inbreeding decreased tolerance to herbivory. Spittlebug nymphs took longer to reach maturity on self plants in this host population, suggesting that inbreeding decreased host plant quality. Decreases in both tolerance and host plant quality in M13 suggest that inbreeding is affecting resistance traits as well as other plant traits important for nymphal development. In host population M5, inbreeding did not alter the impact of spittlebugs, but nymphs developing on self plants matured at a larger size, suggesting that inbreeding improved host plant quality in this plant population. Inbreeding in population M5 may therefore decrease antibiosis without altering tolerance. The differences between populations might be explained, in part, by the much lower level of overall inbreeding depression observed in M5. Differences in inbreeding depression are commonly associated with differences in inbreeding history (Husband and Schemske 1996).

To the best of our knowledge, this is the first study demonstrating the effect of inbreeding on components of host plant resistance and host plant quality in plants taken from natural populations. Núñez-Farfán et al. (1996) found no effect of inbreeding on resistance in the highly selfing *D. stramonium*. Outcross progeny of the perennial *Erigeron glaucus* supported fewer thrips (*Apterothrips apteris*) than self progeny only if the self progeny were derived from a parent that

already supported high number of thrips (presumably a low resistance genotype) and the cross-pollination sire supported few thrips (presumably a high resistance genotype; Strauss and Karban 1994). In a plant-pathogen system, Ouborg et al. (2000) found that active resistance of the highly outcrossing perennial *Silene alba* to the fungus *Microbotryum violaceum* was altered by inbreeding. The effect of inbreeding was variable among lines and among populations, in some cases increasing resistance, in others decreasing resistance.

#### *Implications for Plant-Herbivore Interactions*

Variation in host plant defense characters and nutritional quality can influence the size of herbivore populations (Hunter and Price 1992; Karban 1992; Underwood and Rausher 2000). Because inbreeding influences the expression of genetic variation, it can become an important component of the plant-herbivore dynamic. Results from our study suggest that as levels of inbreeding rise in the host population, resistance to herbivores may decline. In *M. guttatus* population M13 this is accompanied by a decline in host plant quality. Under these circumstances, a small inbreeding host plant population may represent a habitat patch of declining size and quality from the perspective of a herbivore. In population M5, resistance did not change with inbreeding, but host plant quality increased. Under these circumstances, an inbreeding host population could become a herbivore source.

A number of studies have tested for broad-sense genetic variation within natural populations of host plants to determine the potential of hosts to respond to the selective pressures caused by insect herbivores (e.g., Marquis 1984, 1990; Berenbaum et al. 1986; Simms and Rausher 1987, 1989; Fornoni and Núñez-Farfán 2000). The inbreeding effects observed here for both resistance and host plant quality traits provide evidence that genetic variation due to nonadditive allelic interactions (dominance or overdominance) must exist within families (Falconer 1981; Lynch and Walsh 1998). Quantifying the relative contributions of additive and non-additive genetic variation, which are not distinguished in estimates of broad-sense heritability, will be essential to fully evaluate the response of host populations to their herbivores because nonadditive variation can greatly affect the response to selection in partially self-fertilizing species such as *M. guttatus* (Kelly 1999a,b).

#### *Implications for Plant Mating System Evolution*

Inbreeding depression is the primary obstacle to the evolution of self-fertilization in most models of mating-system evolution (e.g., Charlesworth and Charlesworth 1979, 1987, 1990; Lande and Schemske 1985; but see Holsinger 1988; Uyenoyama and Waller 1991a,b,c). Estimates of inbreeding depression in plants commonly increase in magnitude with increased environmental stress (Dudash 1990; Roff 1997). Our data suggest that herbivory can greatly increase the level of inbreeding depression. Estimates of inbreeding depression taken from plants with spittlebug nymphs were 1.5 to 3.0 times greater than estimates from control plants. Interestingly, estimates of inbreeding depression for natural populations of *M. guttatus* based on changes in inbreeding coefficients (Dole and Ritland 1993) are much higher ( $\delta = 81\%$ ) than

estimates from greenhouse studies (e.g., Willis 1993a,b; Carr and Dudash 1995).

### Conclusions

This study demonstrates that inbreeding, one of the fundamental processes controlling the distribution of genetic variation in plant populations, can interact with insect herbivory, an ecological interaction of fundamental importance to both host and herbivore fitness. The nature of this interaction appears complex and may vary across populations. This interaction could potentially affect population dynamics of host and herbivore populations, the evolution of resistance in host plant populations, and plant mating system evolution.

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