THE EFFECTS OF HERBIVORY ON PLANT MATING SYSTEMS

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THE EFFECTS OF HERBIVORY ON PLANT MATING SYSTEMS

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Joel Tindle, son of Daryl and Margaret Tindle, was born April 14, 1976, in St. Cloud, Minnesota. He graduated from Monticello High School in Monticello, Minnesota in 1994. He attended St. Cloud State University in St. Cloud, Minnesota and graduated with a Bachelor of Science degree in Biology in May, 2002. After graduation, Joel worked as a Field Biologist for Rocky Mountain College on the Little Missouri National Grassland in North Dakota for one summer. He moved to Auburn in July of 2003, and entered the graduate program in the Department of Entomology and Plant Pathology at Auburn University.

THESIS ABSTRACT

THE EFFECTS OF HERBIVORY ON PLANT MATING SYSTEMS

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Inbreeding is common in many plant species. Inbreeding reduces heterozygosity and increases the expression of recessive deleterious alleles that cause inbreeding depression. The level or intensity of inbreeding depression plays an important role in the evolution of plant mating systems. Furthermore, high levels of inbreeding depression increase the probability of extinction for small, isolated populations. Consequently, many studies have quantified inbreeding depression in order to predict the evolutionary trajectory of plant populations or to estimate the probability of extinction of small populations of threatened plants. The vast majority of these studies have been conducted under controlled conditions. Inbreeding depression, however, is not static and can vary with environmental conditions. Thus, by altering the magnitude of inbreeding depression, environmental and ecological interactions could alter the rate and direction of

mating system evolution or dramatically increase the threat of extinction of small plant populations. An important ecological interaction that affects plant fitness is herbivory. Because inbreeding alters genetic variation, it is likely that inbred offspring will have pronounced physiological and morphological changes, making them more susceptible to herbivory and increasing inbreeding depression. This study examined the effects of herbivory on inbreeding depression.

Compromised defense in inbred plants may serve as the mechanism whereby herbivory increases inbreeding depression. In the first chapter of my thesis, I tested the hypotheses that herbivory by a xylem feeder, the sunflower spittlebug, *Clastoptera xanthocephala* (Cercopidae) affects the expression of inbreeding depression in the yellow monkeyflower, *Mimulus guttatus*. In addition, I tested the hypothesis that changes in a morphological trait in inbred plants (trichomes) are responsible for changes in the interaction between spittlebugs and inbred plants. My results suggest that herbivory increases inbreeding depression by reducing plant tolerance of herbivory, but this effect varied among plant genotypes and among plant populations. Inbreeding, however, did not affect resistance to sunflower spittlebugs.

Thus, herbivory can increase inbreeding depression and potentially alter the evolutionary dynamics of plant populations and the persistence of plant populations. The results of this and other studies, however, suggest that the effect of herbivory on inbreeding depression varies among plant genotypes and populations. The goal of my second chapter was to explain variation in the effects of selfing on plant-herbivore interactions. I hypothesized that variation in the effect of selfing on plant defense may be explained by the mating history of plant populations. Plant populations with a history of

outcrossing are often dramatically affected by selfing and exhibit high levels of inbreeding depression. Conversely, plant populations with a long history of selfing are typically not strongly affected by selfing and exhibit low levels of inbreeding depression.

To test this hypothesis, I correlated the level of inbreeding depression due to herbivory with flower size. I used flower size as an estimate of mating history because plants with smaller flowers are far less attractive to pollinators, significantly more likely to inbreed, and typically come from lineages with a long history of inbreeding. Deleterious alleles are much more likely to have been purged within these lineages resulting in reduced inbreeding depression. The results of my experiment strongly supported this hypothesis. I found that as flower size increased, so did the effects of herbivory on inbreeding depression. Corolla width was significantly correlated with inbreeding depression due to herbivory.

This study adds to a growing body of work that suggests that environmental and ecological interactions can alter the expression of inbreeding depression and, consequently, alter the rate and direction of mating system evolution and the persistence of plant populations. The results of this work may be particularly important for conservation biology. As human induced changes in habitats increase, populations of native plants are often isolated and reduced in size. The effects of ecological interactions on inbreeding depression may be amplified in these situations and in order to conserve these populations the effects of ecological interactions may need to be included in management plans.

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CHAPTER I:

Herbivory by *Clastoptera xanthocephala* (Hemiptera: Cercopidae) can increase inbreeding depression in *Mimulus guttatus* (Phrymaceae)

One of the benefits of self pollination is that it provides plants with reproductive assurance when they are isolated or when pollinators are limited (Jain, 1976). In addition, when plants both self-pollinate and outcross, they contribute 100% of the genes to offspring from self-fertilization and 50% of the genes to offspring from the fertilization of other plants. This results in a 50% gene transmission advantage over outcrossing alone and strong selection for self pollination (Fisher 1941). Inbreeding, however, reduces plant fitness by reducing heterozygosity within individuals and increasing the expression of deleterious, recessive alleles. This reduction in fitness is called inbreeding depression and is thought to be the primary barrier to the evolution of self-pollination in plants (Lande and Schemske, 1985; Charlesworth and Charlesworth, 1987; Hamrick and Godt, 1989; Husband and Schemske, 1996; Lynch and Walsh, 1998). In order to maintain outcrossing as a mode of reproduction, however, inbreeding depression must be greater than 50% to overcome the gene transmission advantage of selfing (Charlesworth and Charlesworth, 1979, 1987, 1990; Charlesworth, 1980; Lande and Schemske, 1985; Charlesworth et al., 1990; Keller and Waller, 2002). Consequently, many studies have quantified inbreeding depression in a large number of plant species to help estimate the rate and magnitude of mating system evolution (e.g., Charlesworth and Charlesworth, 1987; Husband and Schemske, 1996). The vast majority of these studies have been conducted in greenhouses under a single set of environmental conditions. Inbreeding depression, however can vary with environmental conditions (Dudash, 1990; Carr and Dudash, 1995; Ramsey and Vaughton, 1998; Hayes et al., 2005). For example, in rose-pink (*Sabatia*) plants, inbreeding depression was higher in the field than in the greenhouse (Dudash, 1990) and other studies have shown that competition and drought can alter the expression of inbreeding depression (Carr and Dudash, 1995; Cheptou et al., 2000a,b). Thus, by altering the magnitude of inbreeding depression, environmental and ecological interactions could alter the rate and even the direction of mating system evolution.

An important ecological interaction that affects plant fitness is herbivory (Crawley, 1985; Maschinski and Whitham, 1989). Because self-pollination alters genetic variation, it is likely that inbred offspring will have pronounced physiological and morphological differences compared to outbred offspring and these differences may alter interactions with herbivores. For example, if self-pollination alters levels of defensive chemicals, morphological traits such as trichome density, or the concentration of nutrients in plants, then self-pollination will almost certainly alter plant – herbivore interactions. Plants defend themselves from herbivores in two main ways: tolerance and resistance (Strauss and Agrawal 1999). Tolerance is the ability of a plant to maintain relatively high fitness despite herbivory, and is often thought of as outgrowing herbivory (Stowe et al. 2000). Resistance is the ability of a plant to negatively affect the fitness of an herbivore through physical or chemical means, thereby decreasing herbivory.

Given that ecological interactions can increase or decrease inbreeding depression and that herbivory is an important ecological interaction for plants, herbivory could affect the expression of inbreeding depression and ultimately the evolution of plant mating systems (Ashman, 2002; Carr and Eubanks, 2002). Very few studies, however, have investigated the effects of herbivory on the intensity of inbreeding depression. The few studies that have addressed this issue suggest that these effects may be widespread. For example, herbivory can double inbreeding depression in *Cucurbita pepo* spp. *texana* plants (Hayes et al., 2004) and can double or even triple inbreeding depression in the yellow monkeyflower, *Mimulus guttatus* (Carr and Eubanks, 2002, Ivey et al., 2004).

The objective of this study was to document the effect of herbivory by a widespread spittlebug on the expression of inbreeding depression in a common wildflower. This study also explores the possibility that changes in a morphological trait in inbred plants (trichome density) are responsible for changes in the interaction between spittlebugs and inbred plants. Specifically, I quantified the effects of inbreeding on the tolerance and resistance of yellow monkeyflower, *M. guttatus*, to the sunflower spittlebug, *Clastoptera xanthocephala* (Cercopidae) by measuring the performance of both the plant and the herbivore. I then compared inbreeding depression in monkeyflowers with and without spittlebugs to quantify the effects of herbivory on inbreeding depression. It is essential that we understand factors that alter the expression of inbreeding depression because it is the major impediment to the evolution of self-pollination (Lande and Schemske, 1985). Understanding factors that exacerbate inbreeding depression is also important because inbreeding depression may significantly increase the risk of extinction for small and fragmented populations (Frankham, 1995a,b;

Heschel and Paige,1995; Newman and Pilson, 1997; Saccheri et al., 1998; Reed et al., 2003).

MATERIALS AND METHODS

Study organisms---The yellow monkeyflower is an herbaceous plant native to open, wet habitats throughout much of western North America. Though it is usually an annual, it can be a perennial in sites that are wet all year long (Dole, 1992). The yellow monkeyflower initially produces a rosette of leaves and then bolts to flower later in the season. Due to common and extreme fluctuations in population size, inbreeding and inbreeding depression are common in *M. guttatus* populations (Fenster and Ritland, 1992; Carr and Dudash, 1996; Dudash et al., 1997). Reproduction varies from 0% to 75% selfing among populations, but on average, *M. guttatus* plants self 40% of the time (Ritland and Ritland,1989; Ritland, 1990; Dudash and Ritland, 1991).

A xylem-feeding herbivore was used in this study, *Clastoptera xanthocephala* (Hemiptera: Cercopidae), the sunflower spittlebug. The sunflower spittlebug is a polyphagous, xylem feeder found throughout the United States. It feeds on shrubs, trees, grasses, and many herbaceous plants including monkeyflowers (Hamilton, 1982; Carr and Eubanks, unpublished data). The adults are small (2.4-4.7 mm long) and compact with a flattened body and shiny, dark pronotum (Hamilton, 1982). They are generally dark brown to black with a whitish spot at the middle of the outer edge of each forewing and the apex of the head is distinctly paler than the rest of the body. Because they feed on xylem, spittlebug performance is generally unaffected by the allelochemistry of plants, but is often affected by morphological traits of plants such as trichomes (Llewellyn, 1982, Raven, 1983, Hoffman and McEvoy, 1985). I chose to study the effects of this spittlebug on inbreeding depression in yellow monkeyflowers for two reasons. First, previous work with a different spittlebug (*Philaenus spumarius*) suggested that inbred monkeyflowers had reduced tolerance to spittlebug herbivory and, hence, spittlebug herbivory increased inbreeding depression (Carr and Eubanks, 2002, Ivey et al., 2004). By using a different spittlebug, I test the hypothesis that insects of the same feeding mode (xylem feeders) affect inbred plants in a similar manner (Hull-Sanders and Eubanks, 2005). The previous study by Carr and Eubanks, 2002 however, suggested that inbred monkeyflowers might be more resistant to spittlebugs than outbred monkeyflowers (i.e., spittlebugs developed more slowly when reared on inbred plants than outbred plants). Furthermore, a preliminary study of monkeyflowers suggested that trichome production may be altered in inbred plants and that differences in trichome production may explain differences in spittlebug performance (Eubanks and Carr, unpublished data). I tested this hypothesis by quantifying trichome density on inbred and outbred plants and correlating trichome density with spittlebug performance.

Experimental design---Single, randomly chosen plants from field-collected seed from multiple California *M. guttatus* populations were grown in a pollinator-free greenhouse in 2003 and hand-pollinated to produce self and full-sib outcross progeny following the methods of Carr and Eubanks (2002). Seeds were collected from ripe fruits and stored at room temperature. For this experiment, I sowed seeds in the spring of 2005 from 22 full-sib families representing seven California populations and individual plants were transplanted into 10-cm pots after two weeks. The experiment was originally designed as a completely randomized block design with 22 plant families, two pollination treatments (selfed and outcrossed), and two herbivory treatments [control (no spittlebug) or treatment (one spittlebug)]. One treatment and one control plant were assigned per pollination treatment per family per block and the experiment was replicated in nine blocks. Low germination in some families, however, resulted in fewer than nine replicates for some family/pollination/treatment combinations, so I analyzed the experiment as a partially balanced incomplete block design using Proc Mixed in SAS (Littell et al., 1996; SAS Institute, 2001).

Plants were "moated" by placing them in trays of water. Each pot was at least 3 cm from the edges of the trays and from other pots to prevent spittlebug escape. Second- and third-instar spittlebug nymphs were collected from goldenrod plants (*Solidago*) in Auburn, AL during late spring. One randomly selected spittlebug nymph was placed on each treatment plant. Plants were surveyed twice daily for spittlebug location. As spittlebug adults emerged, they were removed, dried to a constant weight at 50° C, and weighed. Development time and spittlebug mass were recorded.

After the last spittlebug was removed, flowers were counted, and plants were harvested at the soil level. Plant material was dried to a constant weight at 50° C and weighed to the nearest 1 mg. Biomass was used as a surrogate for fitness because in natural populations of *M. guttatus,* biomass and flower production are highly correlated ($r = 0.89$; Galloway 1995), as are biomass and total seed production ($r = 0.68$-0.80; Fenster and Ritland, 1994).

To measure trichome density, I took leaf samples from ten plants from each of the maternal families used in the experiment. I sampled two leaves from the second node of each plant by removing small leaf disks with a cork borer (12 mm^2 disks). Two samples were taken from each leaf, one from either side of the mid-vein while avoiding all other major veins. The disks were set at 45 degree angles to make the trichomes more visible and trichomes were counted using a dissecting microscope.

Analyses---Two variables were used to estimate resistance (spittlebug mass and development time) and two variables were used to estimate tolerance (flower count and aboveground biomass). I used mixed model analysis of variance (PROC MIXED; SAS Institute, 2001) to examine inbreeding effects on resistance and tolerance to herbivory. Four similar mixed-model analyses of variances were used to measure the effects of inbreeding on flower production and spittlebug mass. Spittlebug mass and flower production were square-root transformed to fit the assumptions of ANOVA. The models had five fixed effects: population, pollination treatment, herbivory, population x pollination, and pollination x herbivory. The models also had three random effects: block, family nested within population, and herbivory X pollination X family nested within population.

Inbreeding depression was calculated by comparing estimated fitness levels of selfed and outcrossed plants using the equation:

$$\delta = (W_O - W_S)/W_O$$

where δ is the inbreeding coefficient, W_O is the fitness of the outcrossed plants, and W_S is the fitness of the selfed plants (Darwin, 1876, Charlesworth and Charlesworth, 1987, Keller and Waller, 2002 for review). Fitness was estimated by both aboveground biomass and flower production.

I used mixed model analysis of variance to determine the effects of family, pollination, spittlebug herbivory, and their interactions on trichome production in *M. guttatus* plants. I used correlation analysis to measure the relationship between trichome density and spittlebug performance (biomass or development time).

RESULTS

Resistance---No effects of selfing on resistance were found. The effects of pollination, population, family, and their interactions on development time and adult mass were all statistically nonsignificant (Tables 1 and 2). Spittlebug nymphs began molting into adults on the 5th day of the experiment, with the last three adults emerging on the 20th day (average development time = 12.6 d ± 0.3 SE). Development time did not vary by pollination (Self 12.6 ± 0.4 mg vs. Outcross 12.6 ± 0.4 mg). Spittlebug biomass averaged 0.98 ± 0.04 mg, and did not significantly vary by pollination (Self 0.9 ± 0.05 mg vs. Outcross 1.0 ± 0.06 mg).

Tolerance---Inbreeding altered the ability of *M. guttatus* plants to tolerate spittlebug herbivory, but this effect varied among *M. guttatus* families (significant family x pollination x herbivory interactions for both aboveground biomass and flower production) (Tables 3, 4, 5 and 6). For example, in family AL 41, selfing decreased biomass by 31% in control plants while decreasing biomass 71% in plants with spittlebugs (Table 5). In family DP 8, the opposite effect was observed; biomass of control plants was decreased 70% by selfing on average, while the biomass of plants with spittlebugs was only decreased 32% on average (Table 5).

Inbreeding depression---Inbreeding depression as estimated by flower production was -14% in control plants, and 4% in plants with spittlebugs (Fig. 1). Likewise, inbreeding depression as estimated by aboveground biomass was increased by herbivory (16% in control plants vs. 29% in treatment plants). Inbreeding depression as estimated by biomass also varied widely among families, ranging from -6% in family M6 33 to 93% in family SB 5.

Trichomes---Self pollination significantly affected trichome density ($F_{1,21} = 7.79$, $p = 0.01$). Inbred plants produced approximately 15% more trichomes than outbred plants (3.9 ± 0.2 per mm^2 versus 3.4 ± 0.2 per mm^2, respectively). However, trichome density and spittlebug mass ($r = 0.01$, $p = 0.96$) and trichome density and spittlebug development time ($r = 0.03$, $p = 0.85$) were not significantly correlated.

DISCUSSION

This study strongly suggests that herbivory by the spittlebug *C. xanthocephala* can increase the magnitude of inbreeding depression in *M. guttatus*. Herbivory changed the inbreeding depression estimate based on flower production from -14% to 4% and nearly doubled the inbreeding depression estimate based on aboveground biomass. These changes appear to be due to changes in the ability of inbred plants to tolerate spittlebug herbivory. Inbreeding depression is the primary barrier to the evolution of self-pollination (Lande and Schemske, 1985). Given the effect of herbivory on inbreeding depression in this study, it appears that herbivory can play an important role in maintaining outcrossing in some populations. Because herbivory increased estimates of inbreeding depression based on both flower production and aboveground biomass, herbivory could slow the evolution of selfing in some *M. guttatus* populations (Charlesworth and Charlesworth, 1979, 1987, 1990; Lande and Schemske, 1985). The effect of herbivory on inbreeding depression in this study, however, was not strong enough to select for complete outcrossing (> 50%). It is possible that herbivory is only one of the environmental or ecological interactions that increases inbreeding depression. Herbivory may interact with other factors such as drought to additively or even synergistically increase inbreeding depression and provide strong selection for outcrossing.

This study also suggests that, by exacerbating inbreeding depression, herbivory could increase the probability of extinction of small, isolated plant populations. For example, inbreeding depression has been shown to increase extinction probability in small populations of *Ipomopsis aggregata* (Heschel and Paige, 1995) and *Clarkia pulchella* (Newman and Pilson, 1997). Furthermore, theoretical models suggest that even relatively small increases in inbreeding depression, even smaller than the increases observed in this study, can significantly increase the risk of extinction in small populations of outbreeding species (Dobson et al., 1992, Mills and Smouse, 1994, Reed et al., 2003). Conservation biologists should consider the potential effects of herbivory on inbreeding depression when making management decisions concerning plant populations. For instance, small, isolated plant populations may need to be protected from herbivores if herbivory significantly increases inbreeding depression.

Because inbreeding changes the expression of genetic variation, it likely changes many traits associated with host plant quality and resistance. In this study, I found that inbred plants produced significantly more trichomes than outbred plants, suggesting that resistance to herbivores such as spittlebugs should be increased. Indeed, Carr and Eubanks (2002) found an increase in resistance to spittlebugs in one of the two *M. guttatus* populations that they studied. Resistance, however, as estimated by both spittlebug development time and spittlebug biomass was unaffected by self pollination in this study. The different effects of selfing on resistance to spittlebugs could reflect differences in the response to trichomes of sunflower spittlebugs used in this study (*C. xanthocephala*) and meadow spittlebugs used in the Carr and Eubanks study (*P. spumarius*). The performance of meadow spittlebugs was negatively correlated with trichome density ($r = 0.48$, $p = 0.04$) in a preliminary study (Tindle et al., unpublished data), but the performance of sunflower spittlebugs was not correlated with trichome density in this study ($r = 0.00996$, $p = 0.9623$). Sunflower spittlebugs may be able to avoid damage from trichomes because of their relatively small size, different feeding behaviors, or tougher exoskeletons (e.g., Barker, 1990; Tuberville et al., 1996; Kellogg et al., 2002).

In conclusion, this study suggests that herbivory can increase the magnitude of inbreeding depression and potentially alter the evolution of plant mating systems. This study joins a growing body of work that suggests that environmental and ecological interactions can alter the rate and even the direction of mating system evolution (Ashman, 2002; Carr and Eubanks, 2002; Ivey et al., 2004; Hayes et al., 2004, 2005). Future work should focus on how widespread similar effects of environmental and ecological factors on inbreeding depression are in nature, what factors explain variation in these effects, and whether environmental and ecological factors can interact to significantly alter inbreeding depression.

Table 1. Variance components for the effects of population, pollination, family, and their interactions on square-root transformed adult spittlebug mass.

Source	Var comp	χ^2 or F
Block	0.000003	1.6
Family	0	0
Population	---	$0.47_{5,3}$
Pollination	---	$1.24_{1,3}$
Population*Pollination	---	$0.41_{4,3}$
Pollination*Family	0	0
Residual	0.000018	---

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

Notes: Random effects were tested as one degree of freedom Chi-square tests (Littell et al. 1996).

Table 2. Variance components for the effects of population, pollination, family, and their interactions on spittlebug development time.

Source	Var comp	χ^2 or F
Block	0.0304	0
Family	1.3138	1.6
Population	---	$0.5_{6,9}$
Pollination	---	$0.84_{1,9}$
Population*Pollination	---	$0.88_{6,9}$
Pollination*Family	0	0
Residual	8.4647	---

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

Notes: Random effects were tested as one degree of freedom Chi-square tests (Littell et al. 1996).

Table 3. Variance components for the effects of herbivory, population, pollination, family, and their interactions on aboveground biomass.

Source	Var comp	χ^2 or F
Block	685.08	30.9***
Family	2185.48	16.6***
Population	---	$0.52_{6,14}$
Pollination	---	$1.64_{1,14}$
Herbivory	---	$1.61_{1,15}$
Population*Pollination	---	$2.81_{6,14}$
Pollination*Herbivory	---	$0.00_{1,15}$
Pollination*Family	1581.29	9.5**
Family*Herbivory	110.26	0.1
Pollination*Family*Herbivory	1056.03	19.3***
Residual	5120.51	---

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

Notes: Random effects were tested as one degree of freedom Chi-square tests (Littell et al. 1996).

Table 4. Variance components for the effects of population, pollination, family, and their interactions on flower production.

Source	Var comp	χ^2 or F
Block	0.04823	9.8**
Family	0.1011	5.2*
Herbivory	---	$1.95_{1,13}$
Population	---	$4.89_{6,13}$**
Pollination	---	$0.08_{1,14}$
Population*Pollination	---	$1.88_{6,14}$
Pop*Herbivory	---	$1.28_{6,13}$
Poll*Herbivory	---	$0.23_{1,15}$
Pollination*Family	0.1594	4.3*
Family*Herbivory	0.03382	0.6
Pollination*Family*Herbivory	0.08939	6.6**
Residual	0.8218	---

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

Notes: Random effects were tested as one degree of freedom Chi-square tests (Littell et al. 1996).

Table 5. The effects of pollination and herbivory on aboveground biomass (mg) in different *Mimulus guttatus* families.

	Control		**Herbivory**	
Family	**Outcross**	**Self**	**Outcross**	**Self**
AL 1	349 ± 31.1	113 ± 22.4	281 ± 66.1	107 ± 22.5
AL 3	158 ± 27.0	65 ± 15.4	119 ± 12.9	---
AL 19	94 ± 24.9	98 ± 16.3	120 ± 52.9	117 ± 28.2
AL 41	154 ± 27.2	106 ± 19.6	217 ± 35.4	62 ± 7.0
AL 48	304 ± 39.5	148 ± 35.1	377 ± 59.8	116 ± 45.5
DP 5	335 ± 56.0	234 ± 49.0	193 ± 44.0	197 ± 23.7
DP 8	184 ± 15.6	55 ± 29.8	81 ± 4.0	55 ± 13.0
HC2 1	140 ± 25.0	111 ± 14.9	109 ± 16.6	111 ± 0
HC2 30	102 ± 8.8	125 ± 12.2	25 ± 0	116 ± 0
M13 1	111 ± 20.8	218 ± 29.7	---	174 ± 52.1
M13 3	85 ± 11.7	85 ± 21.8	70 ± 9.5	185 ± 0
M13 16	135 ± 33.8	39 ± 0	---	---
M6 16	114 ± 26.9	199 ± 33.0	91 ± 0	146 ± 29.2
M6 33	58 ± 11.3	155 ± 22.0	---	194 ± 34.4
M6 36	120 ± 24.9	142 ± 13.0	112 ± 19.0	91 ± 25.5
SB 2	173 ± 28.1	106 ± 8.9	155 ± 0.5	77 ± 25
SB 3	102 ± 14.3	125 ± 21.7	88 ± 20.3	76 ± 0
SB 5	174 ± 39.5	151 ± 63.3	182 ± 37.3	133 ± 48.5

SB 7	182 ± 23.0	182 ± 19.4	170 ± 15.6	168 ± 19.6
SB 17	139 ± 15.1	121 ± 29.7	---	57 ± 0
SC 9	106 ± 14.1	112 ± 13.6	152 ± 37.7	107 ± 0

Notes: Means ± standard error. Missing values are due to spittlebug rejection, or plant mortality.

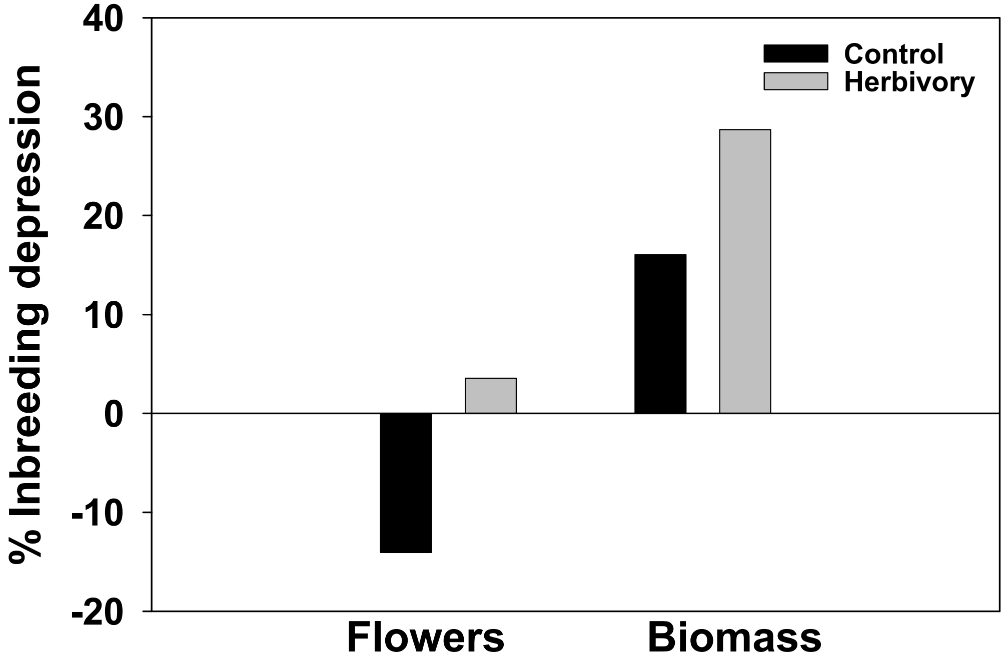
Table 6. The effects of pollination treatment and herbivory on flower production (flowers per plant) in different *Mimulus guttatus* families.

	Control		**Herbivory**	
Family	**Outcross**	**Self**	**Outcross**	**Self**
AL 1	2.8 ± 1.8	5.3 ± 1.4	0.7 ± 0.4	2.3 ±1.2
AL 3	7.1 ± 1.0	0.7 ± 0.7	1.7 ± 0.7	---
AL 19	1.2 ± 0.8	0.3 ± 0.3	0.7 ± 0.7	0.0 ± 0.0
AL 41	0.8 ± 0.6	2.1 ± 1.1	0.0 ± 0.0	0.5 ± 0.5
AL 48	2.4 ± 1.1	0.8 ± 0.8	2.6 ± 1.6	2.5 ± 2.5
DP 5	5.7 ± 5.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
DP 8	3.7 ± 3.7	3.5 ± 3.5	0.0 ± 0.0	1.0 ± 1.0
HC2 1	9.3 ± 3.2	8.0 ± 0.9	2.0 ± 2.0	2.0 ± 0.0
HC2 30	3.5 ± 2.1	10.3 ± 1.7	2.0 ± 0.0	11.0 ± 0
M13 1	0.0 ± 0.0	0.0 ± 0.0	---	0.0 ± 0.0
M13 2	---	---	0.0 ± 0.0	---
M13 3	3.1 ± 1.9	0.0 ± 0.0	4.5 ± 1.5	0.0 ± 0.0
M13 16	1.0 ± 1.0	0.0 ± 0.0	---	---
M6 16	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
M6 33	0.0 ± 0.0	1.1 ± 0.7	---	0.0 ± 0.0
M6 36	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
SB 2	1.4 ± 1.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
SB 3	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.0

SB 5	1.9 ± 1.1	4.3 ± 2.4	1.6 ± 0.7	6.5 ± 4.5
SB 7	0.2 ± 0.2	1.6 ± 1.1	0.0 ± 0.0	0.0 ± 0.0
SB 17	0.0 ± 0.0	0.4 ± 0.4	---	0.0 ± 0.0
SC 9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Notes: Means ± standard error. Missing values are due to spittlebug rejection, or plant mortality.

Figure 1.



40
30
20
10
0
-10
-20
% Inbreeding depression
Control
Herbivory
Flowers
Biomass

CHAPTER II:

The Effects of Inbreeding on Plant Defense of *Mimulus guttatus* (Phrymaceae) to *Junonia coenia* (Nymphalidae)

Eighty percent of all plant species exhibit self-fertilization, a form of inbreeding (Vogler and Kalisz 2001). One of the benefits of selfing is that it provides plants with reproductive assurance when plants are isolated or when pollinators are limited (Jain 1976). In addition, when plants both self-fertilize and outcross, they contribute 100% of their genes to offspring from self-fertilization, plus 50% of the genes to offspring from the fertilization of other plants (Fisher 1941), resulting in a 50% gene transmission advantage over outcrossing alone. Selfing, however, reduces genetic variation, increases the expression of deleterious alleles, and typically results in reduced fitness (Lande and Schemske 1985; Charlesworth and Charlesworth 1987; Hamrick and Godt 1989; Husband and Schemske 1996; Lynch and Walsh 1998). This reduction in fitness (inbreeding depression) is thought to be the primary barrier to the evolution of selfing. Due to the 50% gene transmission advantage, inbreeding depression must be greater than 50% to maintain outcrossing as a mode of reproduction (Charlesworth and Charlesworth 1979, 1987, 1990; Charlesworth 1980; Lande and Schemske 1985; Charlesworth et al. 1990; Keller and Waller 2002).

Many studies have quantified inbreeding depression (e.g., Charlesworth and Charlesworth 1987; Husband and Schemske 1996). Inbreeding depression, however, is not static and varies with environmental conditions (Dudash 1990; Carr and Dudash 1995; Ramsey and Vaughton 1998; Hayes et al. 2005). Dudash (1990) found inbreeding depression in *Sabatia angularis* was higher under field conditions than in the greenhouse or garden plots, suggesting that inbreeding depression can be expressed to different degrees depending on environmental factors and ecological interactions. Similarly, Hayes et al. (2005) found that inbreeding depression in *Cucurbita pepo* spp. *texana* plants was more severe for plants that did not receive a fertilizer treatment. Very few studies, however, have investigated the specific factors that increase or decrease the intensity of inbreeding depression. In order to further our understanding of the evolution of plant mating systems it is essential that we understand these factors because variation in inbreeding depression is likely to strongly affect the evolution of plant mating systems (Lande and Schemske 1985). Understanding interactions that exacerbate inbreeding depression is also important for conservation biology because as habitats become altered due to human activity, the effects of inbreeding become increasingly important for the management of small and fragmented populations (Heschel and Paige 1995).

Because self-fertilization decreases genetic variation, it is likely that inbred offspring will have pronounced physiological and morphological changes and this almost certainly will alter interactions with other organisms. If self-pollination alters concentrations of defensive chemicals, morphological traits such as trichome density or leaf toughness, and/or the concentration of nitrogen or carbohydrates in plants, then it is very likely that self-pollination will alter plant – insect herbivore interactions (Carr and

Eubanks 2002; Ashman 2002). Plants defend themselves from herbivores in two main ways: tolerance and resistance (Strauss and Agrawal 1999). Tolerance is the ability of a plant to maintain relatively high fitness despite herbivory, and is often thought of as outgrowing herbivory (Stowe et al. 2000). Resistance is the ability of a plant to negatively affect the fitness of an herbivore through physical or chemical means, thereby decreasing herbivory.

A few studies have examined the effects of inbreeding on plant-herbivore interactions. Núñez-Farfán et al. (1996) found no effect of inbreeding in Jimsonweed (*Datura stramonium*) on resistance to flea beetles and grasshoppers. This highly inbred plant species, however, displayed no inbreeding depression for any post germination trait so it is likely that inbreeding had no significant effects on this plant's physiology and/or morphology. In contrast, Hayes et al. (2004), reported that selfing reduced resistance to *Diabrotica* beetles in *Cucurbita pepo* spp. *texana* plants. In a field experiment, forty-two percent of outcrossed plants were damaged by beetles, whereas seventy-five percent of selfed plants were damaged. Hull-Sanders and Eubanks (2005) found that selfing in the entire-leaf morning glory, *Ipomoea hederacea* var. *integriuscula,* altered both resistance and tolerance. The effect of selfing, however, varied. In some cases, selfing increased resistance, and in other cases selfing reduced resistance. Likewise, selfing altered the tolerance and resistance of yellow monkeyflowers (*Mimulus guttatus*) to spittlebugs in both greenhouse and field experiments (Carr and Eubanks 2002; Ivey et al. 2004). The effects of selfing, however, varied among populations and full-sib families. In a greenhouse experiment, Carr and Eubanks (2002) found that spittlebugs had no effect on outbred plants in one population, but reduced the biomass of selfed plants by 30% and flower production by 13% in this population. A second population did not display any differences in tolerance, but selfing decreased resistance. Adult spittlebugs reared on selfed plants from this population were 16% larger than those reared on outbred plants. Likewise, Ivey et al. (2004) found that inbreeding reduced tolerance to spittlebug herbivory in *M. guttatus* in a field experiment. Overall, spittlebugs did not decrease the performance of outbred plants, but reduced the biomass of selfed plants by 25%. However, similar to the greenhouse experiment of Carr and Eubanks (2002), the effect of selfing varied among plant populations and full-sib families.

In this study, I examined the effects of inbreeding on the tolerance and resistance of yellow monkeyflower to a common leaf-chewing herbivore, caterpillars of the common buckeye, *Junonia coenia* (Nymphalidae). The primary goal of this study is to explain variation in the effects of selfing on plant-herbivore interactions within and among populations of yellow monkeyflowers. I hypothesize that variation in the effect of selfing on plants may be explained by the mating history of plant populations or lineages. Plants with a history of outcrossing are often dramatically affected by selfing and exhibit high levels of inbreeding depression (Lande and Schemske 1985; Husband and Schemske 1996; Willis 1999). Conversely, plants with a long history of selfing are typically not affected strongly by selfing and exhibit low levels of inbreeding depression, likely the result of the purging of deleterious alleles (Lande and Schemske 1985; Husband and Schemske 1996; Willis 1999). Husband and Schemske (1996) estimated inbreeding depression from 58 populations with varying inbreeding histories by surveying the literature. The average inbreeding depression for predominately selfing populations was 43% less than that for predominately outcrossing populations. I hypothesize, therefore, that selfing significantly alters plant defense in yellow monkeyflowers with a history of outbreeding, thus making them more susceptible to insect herbivores. In contrast, I hypothesize that selfing does not significantly alter plant defense in populations or lineages of yellow monkeyflowers with a long history of inbreeding. I tested this hypothesis by comparing the effect of selfing on plant tolerance and resistance in two populations of *M. guttatus* with different mating histories.

Methods

Study organisms: The yellow monkeyflower is an herbaceous plant native to open, wet habitats throughout much of western North America. Though it is usually an annual, it can be a perennial in sites that are wet all year long (Dole 1992). The yellow monkeyflower initially produces a rosette of leaves and then bolts to flower later in the season. Reproduction varies from 0% to 75% selfing among populations but, on average, *M. guttatus* plants self 40% of the time (Ritland and Ritland 1989; Ritland 1990; Dudash and Ritland 1991). Due to common and extreme fluctuations in population size, inbreeding and inbreeding depression are common in *M. guttatus* populations (Fenster and Ritland 1992; Carr and Dudash 1996; Dudash et al. 1997).

Plants in the family Phrymaceae produce defensive chemicals called iridoid glycosides (El-Naggar and Beal 1980). Thus, a leaf chewing herbivore that can tolerate consumption of iridoid glycoside containing plants was used in this study. *Junonia coenia* (Nymphalidae), the common buckeye butterfly, sequesters iridoid glycosides during larval and pupal stages for its own defenses (Nishida 2002). Buckeye caterpillars are commonly found on *M. guttatus* plants in the field (Eubanks & Carr, personal obs). Late-instar caterpillars can consume large amounts of plant material before they pupate, so the amount of time they were allowed to feed on individual plants during my experiments was limited.

Experimental design: Single, randomly chosen plants from each of 26 field-collected seed families were grown in a pollinator-free greenhouse and hand-pollinated to produce self and full-sib outcross seeds following the methods of Carr and Eubanks (2002). To produce outcross seeds, maternal plants were randomly paired with other plants in the same population, each family serving only once as pollen donor. These seeds were then planted in the fall of 2004, and transplanted (one plant per 10-cm pot) after two weeks. We selected 882 greenhouse-grown *M. guttatus* plants from two populations (AL and M6), 26 families within these populations (12 families in population AL and 14 families in M6) and two pollination treatments (selfed and outcrossed). Plants were randomly assigned to one of two herbivory treatments: control (no caterpillar) or treatment (one caterpillar). One treatment and one control plant were assigned per pollination treatment per family per block and the experiment was replicated in 10 blocks. The plants were measured nine days after transplanting (one day before caterpillars were placed on plants) to estimate their initial size. True leaves were counted and the length and width of the largest leaf was measured.

The two populations used were M6 from Santa Clara County, CA, (37° 16.29′ N, 121° 08.82′ W) and AL from Abotts Lagoon, Marin County, CA (38° 07.39′ N, 122° 56.33′ W). Population AL is from a marshy area that is wet throughout the year.

Population M6 is from an ephemeral wet area that is dry for a large part of the year. Plants from population AL are typically large and produce larger flowers than M6. The average corolla width for population AL was 26.9 ± 0.4 (SE) mm, while M6 averaged 24.1 ± 0.3 mm.

Plants were "moated" by placing their pots in trays of water so that there was at least 3 cm of open water between them and the edges of the trays and other pots in the same tray to prevent caterpillar escape. Caterpillars were weighed to the nearest 0.01 mg, and one caterpillar was added to each plant assigned to the herbivore treatment. Plants were surveyed twice daily for caterpillar location and to estimate the percent leaf area removed or damaged. When mean damage reached 50% (4 days after caterpillars were placed on the plants), the caterpillars were removed and reweighed.

Plants were allowed to continue growth for 3 weeks after caterpillars were removed, then flowers were counted, bolt height was measured, and plants were harvested at soil level. Each plant was dried to a constant weight at 50° C and weighed to the nearest 1 mg. Biomass was used as a surrogate for fitness because in natural populations of *M. guttatus,* biomass and flower production are highly correlated (r=0.89; Galloway 1995), as are biomass and total seed production (r= 0.68-0.80; Fenster and Ritland 1994).

Analysis: Two variables were used to estimate herbivore resistance (caterpillar weight gain and % plant damage) and four variables were used to estimate tolerance (flower count, aboveground biomass, bolt height, and probability of bolting).

Inbreeding depression was calculated by comparing estimated fitness levels of selfed and outcrossed plants using the equation:

$$\delta = (W_O - W_S)/W_O$$

where δ is the inbreeding coefficient, W_O is the measured fitness of the outcrossed plants, and W_S is the measured fitness of the selfed plants (Darwin 1876, Charlesworth and Charlesworth 1987, Keller and Waller 2002 for review). Fitness was estimated using both aboveground biomass and flower production.

I used mixed model analysis of covariance and logistic regression (PROC MIXED and PROC LOGISTIC; SAS Institute 2001) to examine inbreeding effects on resistance and tolerance to herbivory.

To measure the effect of inbreeding on aboveground biomass I used mixed model analysis of covariance. The model had five fixed effects: population, pollination treatment, herbivory, population X pollination, and pollination X herbivory. The model also had three random effects: block, family nested within population, and pollination X family nested within population. Initial leaf area and initial caterpillar mass were covariates. Four similar mixed-model analyses of covariance were used to measure the effects of inbreeding on bolt height, percent damage, flower production, and caterpillar growth.

To measure the effect of inbreeding on plant bolting I used logistic regression by categorizing plants that bolted as ‘1’ and plants that failed to bolt as ‘0’. The model had seven fixed effects: herbivory, population, pollination treatment, population X herbivory, pollination X herbivory, population X pollination and block. A similar logistic regression was used to measure the effects of inbreeding on caterpillar survival. Caterpillar mass and number of flowers were square-root transformed to fit the assumptions of analyses. Percent damage was converted to a proportion and arcsine square-root transformed.

To test the hypothesis that the mating history of a population explains variation in the effects of pollination treatment on plant defense, I correlated inbreeding depression due to herbivory with stigma-anther separation (SAS) and corolla width. I used SAS and corolla width as estimates of mating history because plants with larger corollas and greater stigma-anther separation generally have a long history of outcrossing and are less likely to self-pollinate (Ritland and Ritland 1989; Carr and Fenster 1994). Plants with smaller corollas and less stigma-anther separation typically come from lineages with a long history of inbreeding and deleterious alleles are more likely to have been purged, reducing inbreeding depression (Ritland and Ritland 1989; Carr and Fenster 1994). To quantify inbreeding depression due to herbivory, I used the difference between the inbreeding coefficients of plants subjected to herbivory and plants not subjected to herbivory. Ten plants from each of the maternal families were used to measure corolla width and stigma-anther separation. Floral measurements were taken within two days of first flowering on one fresh flower per plant taken from the first or second node. Using digital calipers (Mitutoyo Corporation Digimatic Plastic Caliper), I measured the width of the corolla at its widest point. I then measured the stigma and anther heights by peeling open the corolla and pressing it flat with a microscope slide. Stigma height was measured from the point of floral insertion to the hinge of the forked stigma, and anther height was measured from the floral insertion point to the top of the tallest stamen. This makes the difference between pistil height and stamen height equal to the narrowest distance between the stigma and anther.

Results

Main effect of pollination treatment: There was a significant two-way interaction between pollination and family, indicating that the effect of selfing on aboveground biomass varied among families (Table 1, Figure 1). For instance, in family M6 14, inbred plants weighed 83% more than outcrossed plants (self 146 ± 20 (SE) mg, outcross 80 ± 11 mg); family AL 21, on the other hand, had opposite results, with inbred plants weighing on average 77% less than outbred plants (self 52 ± 10 mg, outcross 225 ± 19 mg).

There was a significant two-way interaction between population and pollination treatment for the likelihood of plant bolting ($\chi^2 = 9.5$, $P = 0.002$). In population AL, 91% of outcrossed plants bolted, while 71% of the inbred plants bolted. In population M6, 39% of the outcrossed plants bolted while 35% of the inbred plants bolted. There was also a significant pollination by family interaction for both plant height and the likelihood of plant bolting (plant height: $\chi^2 = 44.1$, $P < 0.001$, Figure 2; bolting probability: $\chi^2 = 42.3$, $P < 0.001$, Figure 3).

Inbreeding depression was higher in population AL than in population M6 (Table 2). As estimated by flower count, inbreeding depression was 59% in population AL. As estimated by aboveground biomass, inbreeding depression was 44%. In contrast, for population M6, inbreeding depression was -19% (outcrossing depression) as estimated by flower count and 8% as estimated by biomass.

Main effect of herbivory: There were significant effects of herbivory on plant height, the probability of bolting, aboveground biomass, and flower production (plant height: $F_{1,23}$ = 77.1, $P < 0.001$; bolting: $\chi^2 = 20.8$, $P < 0.001$; aboveground biomass: $F_{1,366}$ = 42.03, $P < 0.001$; flowers: $F_{1,23}$ = 27.60, $P < 0.001$). Plants subjected to herbivory were, on average, 36.3 ± 2.7 mm tall, whereas control plants were, on average 57.8 ± 3.4 mm tall. Herbivory decreased aboveground biomass by 7.48%. Plants subjected to herbivory weighed, on average, 84 ± 3 mg, while control plants weighed, on average, 91 ± 3 mg. For bolting probability, there was a significant interaction between population and herbivory ($\chi^2 = 5.7$, $P = 0.017$).

Interaction of pollination and herbivory: Caterpillar survival was high throughout the experiment (88%) and pollination treatment did not affect caterpillar survival. However, inbreeding significantly decreased caterpillar weight gain ($F_{1,24}$ = 4.92, $P < 0.05$, Self 15.05 ± 1.25 mg vs. Outcross 16.91 ± 1.61 mg, Figure 4) and on the amount of plant damage ($F_{1,24}$ = 6.92, $P < 0.05$). On average, caterpillars gained 20% more weight when reared on outcrossed plants than inbred plants. The interactions between pollination and population, and pollination and family, were not significant. On average, outbred plants sustained less damage than inbred plants (28% vs. 34% respectively). The effects of selfing on resistance, however, varied among *M. guttatus* populations (Table 3, Figure 5). In population AL, for example, inbred plants sustained more damage than outbred plants (54% damage vs. 36% damage), but, there was no effect for population M6 (22% damage vs. 23% damage). The effect of pollination on the amount of damage from herbivory also varied among families ($\chi^2 = 8.4$, $P < 0.01$, Figure 6).

No effects of selfing on tolerance were found among the *M. guttatus* populations. The interaction between pollination and herbivory did not significantly effect aboveground plant biomass or the likelihood of bolting. There was also no effect of the interaction between pollination and damage on plant height, aboveground plant biomass, or flower production. This may be because flower production was very low. When flowering is so low it makes it very difficult to detect differences in flower production among treatments. The 3-way interactions between pollination, damage, and population; and pollination, damage, and family, were also not significant.

Inbreeding depression was exacerbated by herbivory in population AL. Herbivory increased inbreeding depression by 21.4% as estimated by flower production and 15.8% as estimated by aboveground biomass. In contrast, herbivory decreased inbreeding depression in population M6 by 57.5% as estimated by flower production, while increasing inbreeding depression by 1.9% as estimated by biomass.

Mating System History: Corolla width was larger in population AL (26.93 ± 0.37 mm) than in population M6 (24.08 ± 0.31 mm) ($F_{1, 24} = 17.48$, $P = 0.0003$). Stigma-anther separation did not vary significantly between populations ($F_{1, 24} = 0.04$, $P = 0.8$: AL, 2.334 ± 0.098 mm vs. M6, 2.277 ± 0.090 mm). As corolla width increased, inbreeding depression due to herbivory increased. Corolla width was significantly correlated with inbreeding depression due to herbivory when we used biomass as the fitness trait ($r_{1, 25} = 0.40$, $P = 0.04$, Figure 7) and was nearly significantly correlated when flower count was the fitness trait used ($r_{1, 25} = 0.34$, $P = 0.08$). Stigma-anther separation was not significantly correlated with inbreeding depression due to herbivory.

Discussion

This study tested the hypothesis that self-pollination affects the interactions between plants and herbivores by altering plant defenses, and that variation in the effect of selfing on plant-herbivore interactions may be explained by the mating history of plant populations or lineages. My results generally support this hypothesis: specifically I found that selfing decreased resistance in some families and that this effect was exacerbated in lineages with a history of outcrossing.

Similar to Carr and Eubanks (2002) and Ivey et al. (2004), the effects of selfing varied between different plant populations and families. Plant defense was generally decreased by inbreeding in population AL, but was not affected by inbreeding in population M6. This may be explained in part by the much higher levels of inbreeding depression in population AL compared to M6. Population AL also produced flowers with larger corollas and stigma anther separations compared to population M6, suggesting that population AL has a history of outbreeding, whereas population M6 has a history of selfing. Differences in the degree of inbreeding depression can often be attributed to differences in mating history (Husband and Schemske 1996). Plants with large corollas and stigma anther separation generally have a long history of outcrossing and are less likely to self-pollinate (Ritland and Ritland 1989; Carr and Fenster 1994). These plants are less likely to self-pollinate because the stigma-anther separation is too far apart for contact during or prior to corolla abscission (Dole 1992), and are more likely to be cross-pollinated because the larger corollas are more attractive to pollinators. Plants with large corollas and large stigma-anther separations are often dramatically affected by selfing and should theoretically have higher levels of inbreeding depression; therefore, inbreeding would strongly affect resistance and tolerance to herbivory. In plants with small corollas and small stigma-anther separation, selfing should have little affect on resistance and tolerance because purging of deleterious alleles in populations of selfing plants results in reduced inbreeding depression.

Self-pollination affects both plants and herbivores. Because inbreeding changes the expression of genetic variation, it likely changes many aspects of host plant quality and defense. In this study, damage data indicated a decrease in resistance in selfed plants similar to the findings of Carr and Eubanks (2002) and Ivey et al. (2004). However, caterpillar weight increased on outcrossed plants. This could have been because outcrossed plants were higher in nutritional value. Nutritional differences could also account for the fact that inbred plants sustained more damage than outcrossed plants (Figure 4). If inbred plants are lower in nutritional value, then herbivores must consume more to fulfill their nutritional needs (Slansky & Feeny 1977). Normally, being a poor host for herbivores is thought to be advantageous for a plant (Hunter and Price 1992; Karban 1992; Underwood and Rausher 2000), but in this case, the decrease in host plant quality resulted in increased damage.

Inbreeding depression is the major barrier preventing self-pollination from becoming the dominant form of reproduction in plants (Lande and Schemske 1985). Environmental stressors can increase inbreeding depression (Dudash 1990). Given the effect of herbivory on the populations in this study, it appears that herbivory helps maintain outcrossing because herbivory can increase inbreeding depression in populations with a history of outcrossing. Without herbivory, the primarily outcrossing population (AL) had 36.42% inbreeding depression (estimated by change in biomass). This relatively low level of inbreeding depression would result in selection for selfing (Charlesworth and Charlesworth 1979, 1987, 1990; Lande and Schemske 1985). With herbivory, however, AL had 52.2% inbreeding depression, which would result in selection for outcrossing or mixed mating (Charlesworth and Charlesworth 1979, 1987, 1990; Lande and Schemske 1985). Similarly, herbivory increased estimates of inbreeding depression when flower count was used. Population AL had 51.01% inbreeding depression when herbivores were not present. This level of inbreeding depression would result in selection mixed mating. With herbivory, however, AL had 72.41% inbreeding depression, which would result in selection for outcrossing. It is likely that inbreeding depression from many stressors is responsible for the maintenance of outcrossing. In this primarily outcrossing population, however, herbivory alone is a strong enough force to increase inbreeding depression above 50%. This is of particular importance when a primarily outcrossing population becomes fragmented and inbreeding increases. For these fragmented populations, conservation biologists must consider mating system history when making management decisions. For instance, populations with a history of outcrossing may need to be protected from herbivores because herbivory is likely to exacerbate inbreeding depression.

In population M6, a primarily selfing population, herbivory increased inbreeding depression as estimated by change in biomass from 7.39% to 9.29%. These low levels of inbreeding depression would both result in selection for selfing. Without herbivory, population M6 had an inbreeding coefficient of -1.41% (outcrossing depression) as estimated by flower count. In this highly selfing population, herbivory increased outcrossing depression to -58.93%. Outcrossing depression obviously results in strong selection for a selfing mating system.

This study demonstrates that inbreeding can affect interactions between plants and herbivores by altering plant defenses and reducing plant fitness. These effects vary between different plant populations and families depending on their mating history. In some conditions, interactions involving inbreeding, herbivory, and mating history could affect both host plant and herbivore populations.

Table 1. Variance components for the effects of herbivory, population, pollination, family, and their interactions on aboveground biomass.

Source	**Var comp**	**χ^2 or F**
Block	270.2	127.8***
Family	17.7	0
Leaf area	---	$878.2_{1, 808}$***
Initial Wt	---	$21.8_{1, 808}$***
Population	---	$0.1_{1, 24}$
Pollination	---	$0.0_{1, 24}$
Herbivory	---	$0.3_{1, 808}$
Population*Pollination	---	$1.2_{1, 24}$
Pollination*Herbivory	---	$0.0_{1, 808}$
Pollination*Family	453.6	100.8***
Residual	1187.1	---

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

Random effects were tested as one degree of freedom Chi-square tests (Littell et al. 1996).

Table 2. Estimates of inbreeding depression (ID) [(1-self mean/outcross mean) X 100] using aboveground biomass and flower production as estimates of fitness for plants with herbivory, and plants without herbivory.

A. Population AL

	Herbivory	Self	Outcross	ID
Biomass (mg)	w/o caterpillar	55.47	87.25	36.42%
	with caterpillar	40.76	85.29	52.20%
Flower production	w/o caterpillar	0.78	1.60	51.01%
	with caterpillar	0.28	1.01	72.41%

B. Population M6

	Herbivory	Self	Outcross	ID
Biomass (mg)	w/o caterpillar	99.54	107.48	7.39%
	with caterpillar	93.00	102.53	9.29%
Flower production	w/o caterpillar	0.95	0.93	-1.41%
	with caterpillar	0.67	0.42	-58.93%

Table 3. Variance components for the effects of population, pollination, family, and their interactions on arcsine-square-root transformed percent plant damage.

Source	**Var comp**	**χ^2 or F**
Block	0.004	3.3
Family	0.005	0.5
Initial Wt	---	$145.7_{1, 372}$***
Population	---	$28.6_{1, 24}$***
Pollination	---	$6.9_{1, 24}$*
Population*Pollination	---	$5.5_{1, 24}$*
Pollination*Family	0.01	8.4**
Residual	0.1	---

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

Random effects were tested as one degree of freedom Chi-square tests (Littell et al. 1996).

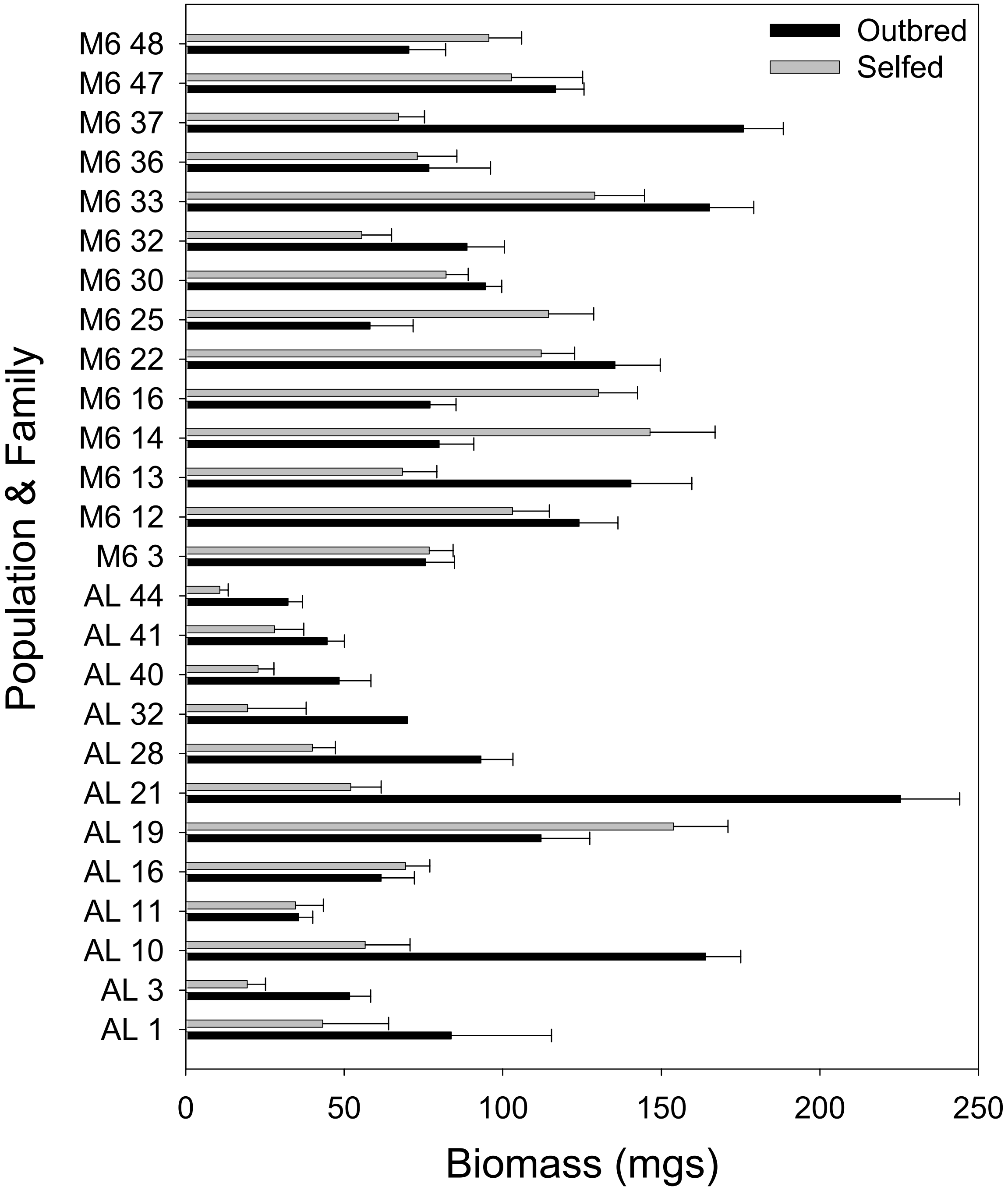

Outbred
Selfed
M6 48
M6 47
M6 37
M6 36
M6 33
M6 32
M6 30
M6 25
M6 22
M6 16
M6 14
M6 13
M6 12
M6 3
AL 44
AL 41
AL 40
AL 32
AL 28
AL 21
AL 19
AL 16
AL 11
AL 10
AL 3
AL 1
Population & Family
0
50
100
150
200
250
Biomass (mgs)

Figure 1

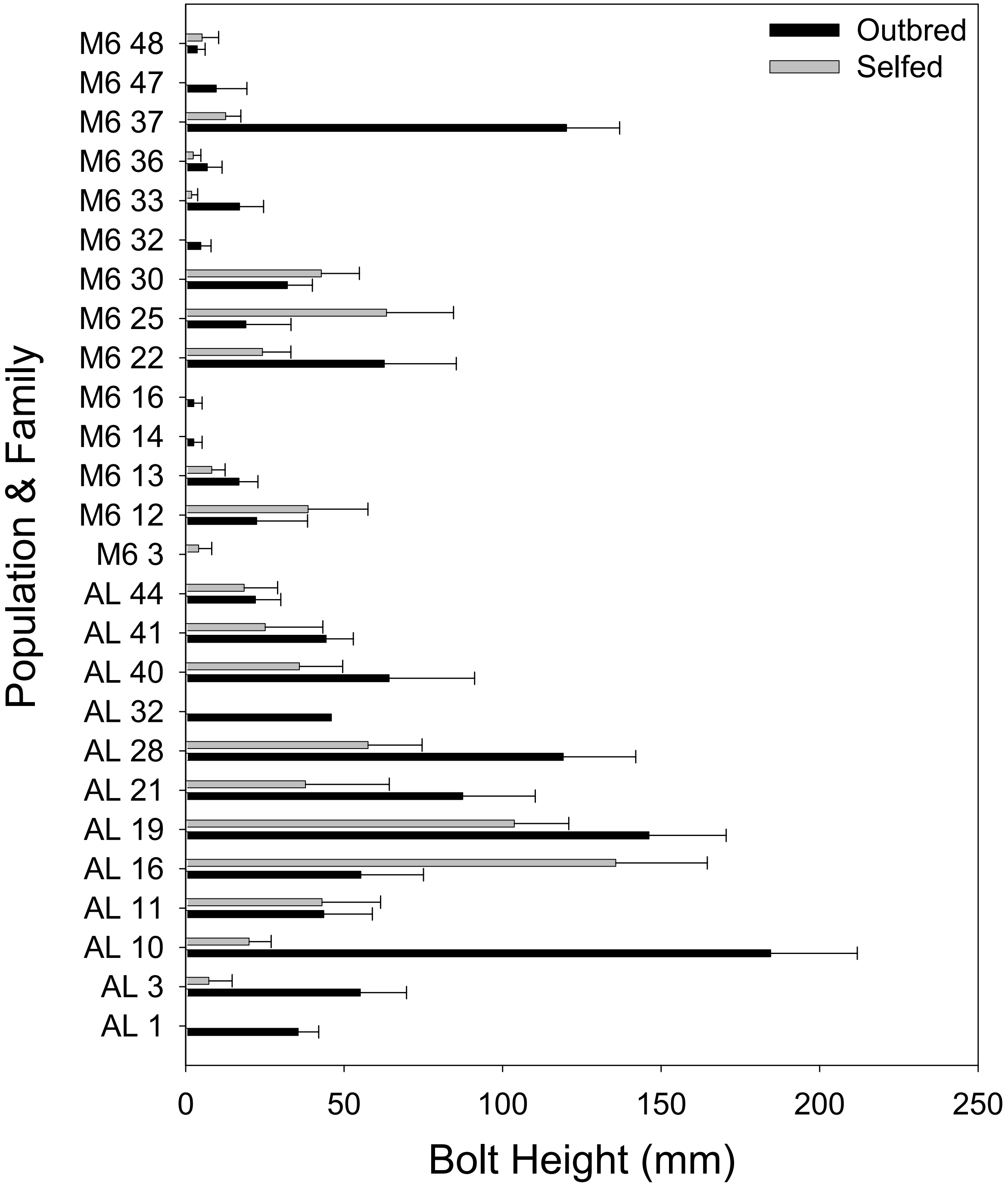
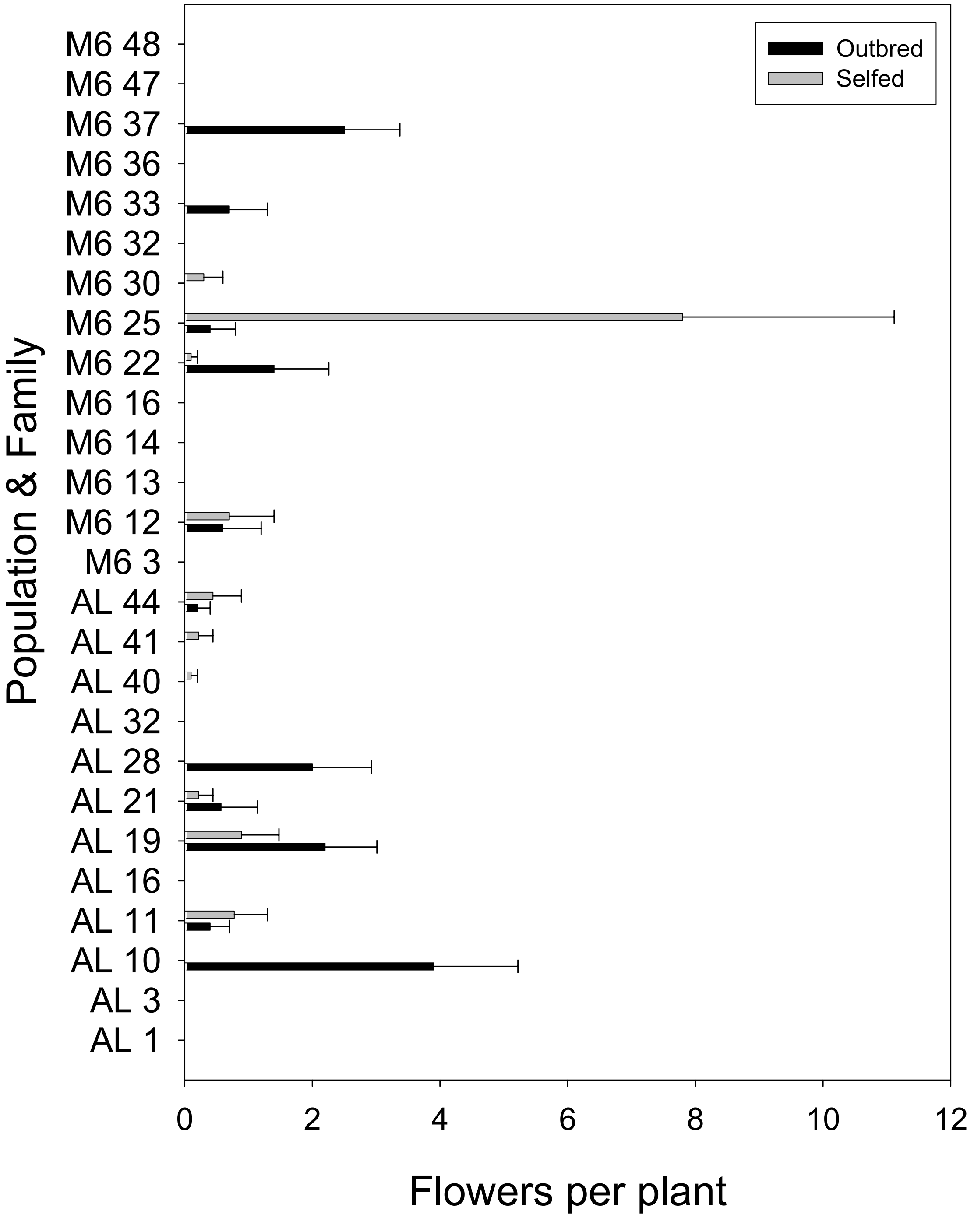

Outbred
Selfed
Population & Family
M6 48
M6 47
M6 37
M6 36
M6 33
M6 32
M6 30
M6 25
M6 22
M6 16
M6 14
M6 13
M6 12
M6 3
AL 44
AL 41
AL 40
AL 32
AL 28
AL 21
AL 19
AL 16
AL 11
AL 10
AL 3
AL 1
0
50
100
150
200
250
Bolt Height (mm)

Figure 2



Outbred
Selfed
Population & Family
M6 48
M6 47
M6 37
M6 36
M6 33
M6 32
M6 30
M6 25
M6 22
M6 16
M6 14
M6 13
M6 12
M6 3
AL 44
AL 41
AL 40
AL 32
AL 28
AL 21
AL 19
AL 16
AL 11
AL 10
AL 3
AL 1
0
2
4
6
8
10
12
Flowers per plant

Figure 3

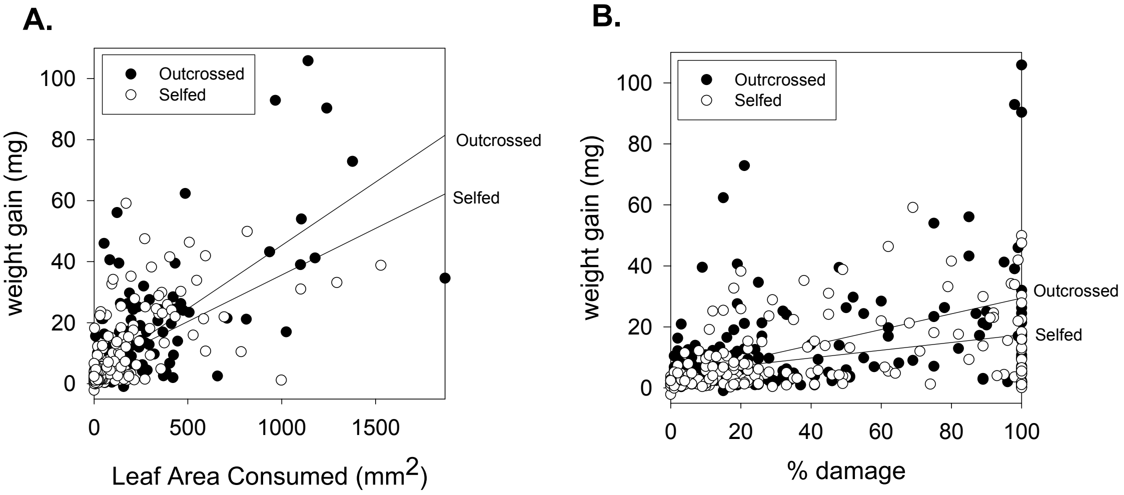

A.
● Outcrossed
○ Selfed
Outcrossed
Selfed
weight gain (mg)
0
20
40
60
80
100
0
500
1000
1500
Leaf Area Consumed (mm^2)
B.
● Outrcrossed
○ Selfed
Outcrossed
Selfed
weight gain (mg)
0
20
40
60
80
100
0
20
40
60
80
100
% damage

Figure 4

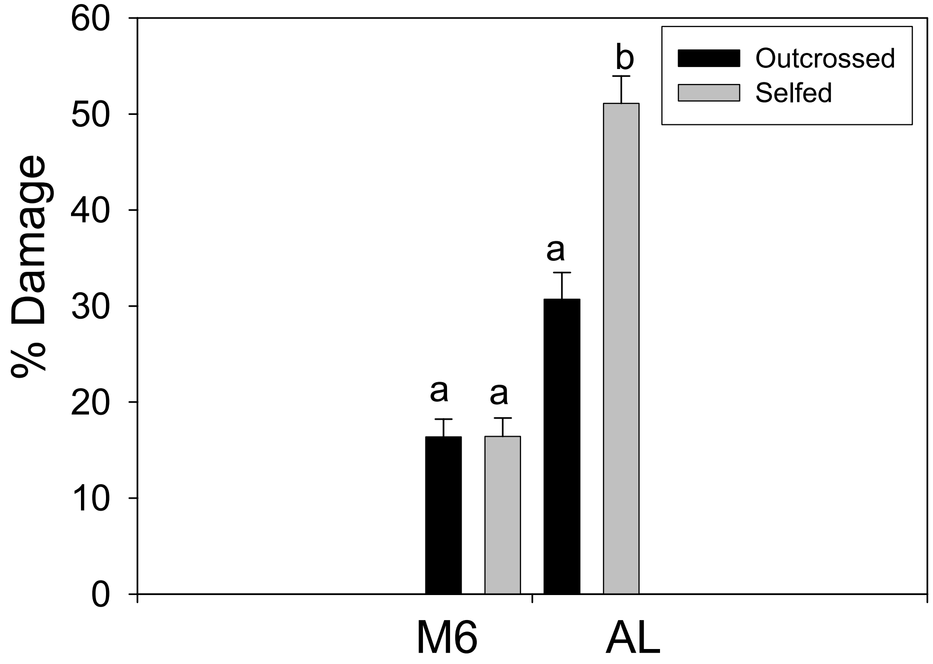
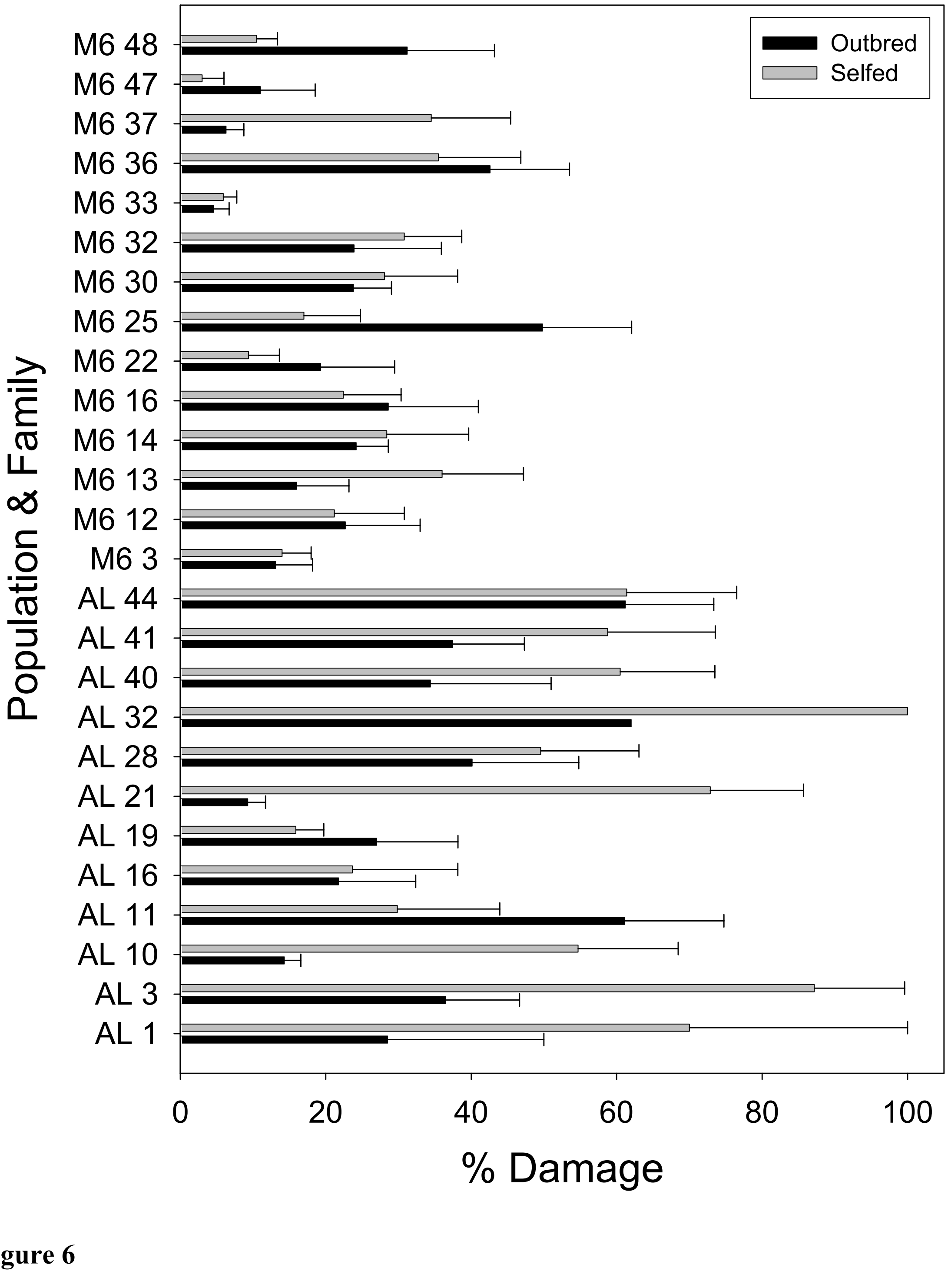


Figure 5



Outbred
Selfed
M6 48
M6 47
M6 37
M6 36
M6 33
M6 32
M6 30
M6 25
M6 22
M6 16
M6 14
M6 13
M6 12
M6 3
AL 44
AL 41
AL 40
AL 32
AL 28
AL 21
AL 19
AL 16
AL 11
AL 10
AL 3
AL 1
Population & Family
0
20
40
60
80
100
% Damage

Figure 6

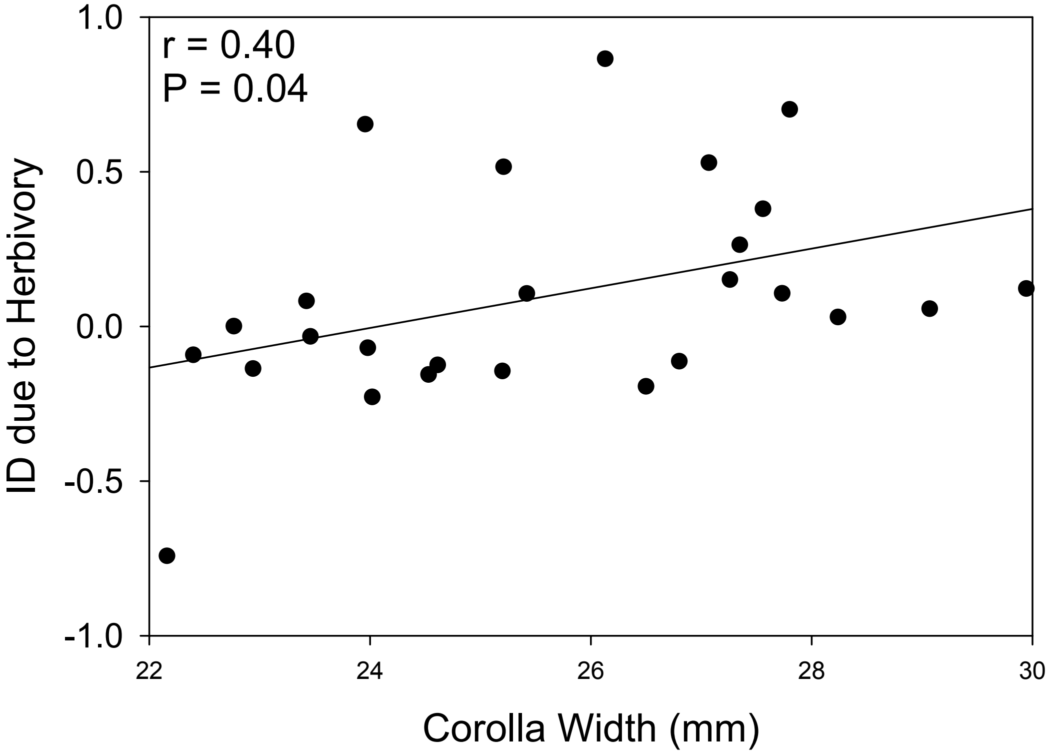

r = 0.40
P = 0.04
ID due to Herbivory
1.0
0.5
0.0
-0.5
-1.0
22
24
26
28
30
Corolla Width (mm)

Figure 7

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