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Effects of Florivory and Inbreeding on Reproduction in Hermaphrodites of the Wild  
Strawberry *Fragaria virginiana*

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## EFFECTS OF FLORIVORY AND INBREEDING ON REPRODUCTION IN HERMAPHRODITES OF THE WILD STRAWBERRY *FRAGARIA VIRGINIANA*

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Recently, the biotic context for sexual and mating system evolution in plants has received special attention; however, the significance of interactions with antagonists has only begun to be revealed. We investigated the effect of florivory on reproduction and inbreeding depression by simulating damage on selfed and outcrossed progeny of hermaphrodites of *Fragaria virginiana* and recording the response of reproduction, as well as measuring tolerance to florivory. While both florivory and inbreeding affected reproduction, their effects were independent with respect to sexual traits but not an asexual trait; inbreeding depression was florivory and family dependent, specifically, for plantlet production. Plants were intolerant of florivory in terms of flowers, moderately tolerant in terms of fruit, and most tolerant in terms of plantlets. However, only under severe damage was intolerance statistically significant. Inbreeding did little to change these patterns. Our findings indicate that florivory does not consistently influence inbreeding depression, suggesting that it is not likely to affect sex ratio through this mechanism. However, our results combined with knowledge of the sex determination system and evidence that florivory increases autogamous selfing suggest a new way florivores could affect sex ratio in this system: that is, by increasing the production of high-fruited hermaphrodites.

*Keywords:* *Anthonomus*, dioecy, *Fragaria*, mating system, tolerance, weevils.

### Introduction

The biotic context for sexual and mating system evolution in plants has received special attention in the last several years. While interactions with mutualists have long been recognized to be important in influencing key parameters of these evolutionary dynamics (Ashman 2000; Barrett 2003; Karron et al. 2004), the significance of interactions with antagonists has only just begun to be revealed (McCall and Irwin 2006; Ashman and Penet 2007; Steets et al. 2007; Wise and Cummins 2007). With respect to sexual system evolution, Ashman (2002, 2006) outlined the myriad ways that antagonists can affect the evolution of dioecy (separate males and females) from hermaphroditism (combined sexes). In particular, antagonists can affect the first step in this transition—that is, the evolution of gynodioecy (females and hermaphrodites) from hermaphroditism—via specific effects on relative seed production of the sex morphs, the selfing rates of hermaphrodites, and the expression of inbreeding depression in selfed offspring (Ashman 2002). While evidence for the effects of antagonists on seed production is substantial in hermaphroditic systems (e.g., Krupnick and Weis 1999; Mothershead and Marqui 2000; Sánchez-Lafuente 2007; Wise and Cummins 2007) and is steadily growing in gynodioecious ones (Puterbaugh 1998; Marshall and Ganders 2001; Collin et al. 2002; Ashman et al. 2004), only a few studies have addressed the effects of antagonists on the latter two compo-

nents, and these studies were mostly conducted in hermaphroditic systems (Krupnick and Weis 1999; Juenger and Bergelson 2000a; Irwin 2003; but see Penet et al. 2008). Thus, whether plant-antagonist interactions impact the first step in the evolution of dioecy via effects on mating system or inbreeding depression remains a largely open question.

Herbivores or florivores can affect the relative frequency of females and hermaphrodites in a gynodioecious population when their interaction with plants differs between sex morphs. In particular, if hermaphrodites are less resistant to or less tolerant of damage than females, then females may more easily achieve the seed advantage needed for their maintenance and spread when herbivores are present (Ashman 2002; Cole and Ashman 2005). This may indeed be the case, because herbivores preferentially attack hermaphrodite plants over female plants in many plant-herbivore systems (reviewed in Ashman 2002); this is especially prominent in the case of damage to flowers or seeds, as is seen in studies of flower-clipping weevils in *Fragaria virginiana* (Ashman et al. 2004), flower-visiting ants in *Eri-trichium aretoide* (Puterbaugh 1998), and seed-consuming weevils in *Sidalcea hendersonii* (Marshall and Ganders 2001). Whether sex morph-differential damage leads to differences in seed production is due in part to sex differences in tolerance of damage (Cole and Ashman 2005), but little is known about the extent or cause of variation in tolerance in sexually dimorphic species (but see Ashman et al. 2004; Cole and Ashman 2005). Regardless, in a few cases, damage has been demonstrated to increase the contribution of females to the seed pool relative to that of hermaphrodites (Puterbaugh 1998; Collin et al. 2002). In addition to the relative quantity of seeds produced by sex

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morphs, the relative quality of these seeds is also an important contributor to sexual system evolution (reviewed in Charlesworth 1999). Herbivores may primarily affect seed quality by influencing the selfing rate of self-compatible hermaphrodites and the expression or magnitude of inbreeding depression of selfed offspring.

Herbivore damage may modify hermaphrodite selfing rates by altering flowering characteristics such as display size or the proximity of anthers and stigmas within flowers and/or by influencing pollinator movements between or within flowers on a plant (Karron et al. 2004; Ivey and Carr 2005; Penet et al. 2008). Although not in all cases, selfing rate can increase with herbivore damage, for example, in several hermaphrodite species (Ivey and Carr 2005; Steets et al. 2006; Schutzenhofer 2007) and one gynodioecious species (Penet et al. 2008). In the latter case, such an effect could contribute to the maintenance of females. In fact, antagonists may have their greatest impact on the first step in the evolution of dioecy if the antagonist-mediated increase in selfing is combined with an antagonist-mediated increase in the expression of inbreeding depression (Ashman 2002).

It is well known that the expression of inbreeding depression can vary with environmental conditions (Hauser and Loeschcke 1996; Cheptou et al. 2000; Steets et al. 2006; Botham et al. 2009), and the herbivore environment is no exception (Carr and Eubanks 2002; Ivey et al. 2004; Koslow and Clay 2007; Leimu et al. 2008); however, the direction of the effect can be quite variable. For instance, in *Lychnis flos-cuculi*, inbreeding depression was reduced in the presence of snail herbivores because the snails depressed the fruit production of outcrossed plants more than that of selfed plants (Leimu et al. 2008). In contrast, inbreeding depression in *Mimulus guttatus* increased in the presence of spittlebug herbivores. Spittlebugs increased (or had no effect on) flower production and/or aboveground biomass of outcrossed plants but reduced these traits in selfed plants (Carr and Eubanks 2002; Ivey et al. 2004). The mechanism(s) underlying such variable responses is not often known, but they could be a function of variation in inbreeding history of the population, the individuals studied, or the type of herbivore involved (e.g., Ivey et al. 2004; Leimu et al. 2008). In addition, since mating system can affect both plant resistance to (Strauss and Karban 1994; Hayes et al. 2004; Du et al. 2008) and tolerance of (Carr and Eubanks 2002; Hull-Sanders and Eubanks 2005) herbivore damage, either could underlie an herbivore-context dependency of inbreeding depression. A recent review (Núñez-Farfán et al. 2007), however, provides some insight into these two mechanisms and suggests that, while inbreeding generally has mixed effects on resistance, it consistently has a negative effect on tolerance. However, because all the studies on the effects of herbivore context on inbreeding depression have been conducted in hermaphroditic species, we do not yet know whether such effects occur in gynodioecious species or if they occur in a direction that would compound the antagonist-mediated effects on selfing rate and, thus, could ultimately impact sexual system evolution.

In this study, we aim to address this gap in our understanding of the role of the antagonist context in the evolution of gynodioecy by examining the effect of simulated weevil florivory on reproduction and the expression of inbreeding de-

pression in *F. virginiana*. The specific questions we addressed were as follows: (1) Does florivory affect reproductive allocation of hermaphrodite plants and does the impact of florivory depend on inbreeding level—that is, the cross type that produced the plant—or the severity of damage? (2) Does the magnitude of inbreeding depression depend on florivore environment? (3) Does plant tolerance to florivory depend on the inbreeding level, the severity of damage, or both?

## Material and Methods

### Study System

*Fragaria virginiana* is an herbaceous perennial native to the eastern United States (Staudt 1989) that has a gynodioecious to subdioecious sexual system (Staudt 1989; Spigler et al. 2008). Thus, populations can contain females, hermaphrodites, males (pollen-bearing plants that produce no fruit), and, occasionally, neuters (plants that produce neither pollen nor fruit; Stahler et al. 1995; Ashman 1999; Spigler et al. 2008). Recent work suggests that sex is determined by two linked loci (or gene regions) with major effects (Spigler et al. 2008), with limited recombination between the loci that can explain the variation in sexual phenotypes observed in wild populations.

In northwestern Pennsylvania, plants flower between April and June and produce ~12–14 flowers per ramet (Ashman 2003). Hermaphrodites produce perfect flowers and are self-compatible. Both autogamous and geitonogamous self-pollination are possible (Penet et al. 2008), and the rate of self-fertilization for hermaphrodites in the source population of the plants studied here (“PR” in Ashman 1999) is  $0.722 \pm 0.004$  (family-level selfing ranges from 0.167 to 1; A. S. Rohde, C. L. Collin, L. Penet, A. Johnson, and T.-L. Ashman, unpublished data). Starting in the spring and continuing through the summer, *F. virginiana* also reproduces asexually via plantlets produced along stolons.

*Anthonomus signatus* (Coleoptera, Curculionidae), the strawberry bud-clipping weevil, is a significant florivore on *F. virginiana* (Kovach et al. 1999; Ashman and Penet 2007). *Anthonomus signatus* females oviposit in flower buds and then sever the pedicel, excising the bud. Larvae develop in the excised bud. Hermaphrodite plants can suffer consistent and high levels of damage by *A. signatus* (Ashman and Penet 2007). For instance, up to 70% of hermaphrodites in a population can be damaged, and on average these individuals experience a damage intensity of  $4.3 \pm 0.4$  buds clipped per plant (Penet et al. 2008). In extreme cases, all flowers per plant can be clipped (Ashman et al. 2004; Ashman and Penet 2007). Previous experiments have confirmed that plants respond to simulated weevil clipping (i.e., clipping buds off with forceps) in a manner that is similar to how they respond to natural clipping by *A. signatus* (Ashman et al. 2004).

### Experimental Design

*Production of experimental plants.* Hermaphrodite genotypes that are the subject of this experiment were the same as those used in Botham et al. (2009); thus, the methods for creating them are described only briefly here (refer to Botham et al.

2009 for additional details). We selected several hermaphrodite *F. virginiana* that were known to be capable of producing fruit. These plants originated from a wild population ("PR" in Ashman 1999) but have been under greenhouse cultivation for several years. In the greenhouse at the University of Pittsburgh, we emasculated flowers of each hermaphrodite before anthesis and conducted hand pollinations with either self-pollen or a mixture of pollen from other pollen-bearing plants (i.e., hermaphrodites and males) from the same population of origin to produce plants of two inbreeding levels (selfed and outcrossed). Hereafter, the outcrossed and selfed seeds of a given hermaphrodite genotype are referred to as a maternal family. Individual seeds within maternal families are referred to as outcrossed or selfed progeny. We chose eight maternal seed families from these crosses for this study on the basis of two conditions: (1) they had enough seeds to conduct the study and (2) they presented signs that were consistent with inbreeding depression at the seedling stage in a pilot study. While this may result in an overestimation of inbreeding depression for the population of origin, the main purpose of our study was not to gain an estimate of the magnitude of inbreeding depression per se but to determine whether florivory could alter its expression or whether there was an effect of inbreeding on tolerance to florivory.

In July 2007, we randomly selected five selfed and five outcrossed progeny from each maternal family and generated nine clonal replicates from each (hereafter, clones), for a total of 684 clones. Clones were initially grown in 4-cm-tall pots containing a 2 : 1 mix of Fafard #2 soil and sand. Approximately 2 mo later, these clones were transplanted into 10-cm-tall pots containing the same potting soil. After an additional 2 mo of growth in the greenhouse under natural day lengths (9.5–11 h) and temperatures of 7°–20°C, we transferred the plants to an outside plot for overwintering. In March 2008, we returned all clones to the greenhouse, where they were maintained under natural day lengths (12–14 h) and temperatures of 10°–20°C for the duration of the experiment. At this time, we randomly assigned each plant to one of three blocks and one of three florivory treatments (described below). Each block contained one clone of each progeny genotype  $\times$  florivory treatment. We watered plants daily and fertilized twice during the experiment, once in the fall with an application of 50 ppm Plantex (20-20-20 N-P-K) and once in the spring with 0.104 g of 100-d release Nutricote fertilizer beads (13-13-13 N-P-K). We hand-pollinated flowers three times a week with pollen collected from a pool of pollen donors, to ensure full fruit set.

**Florivory treatment.** To create the florivory treatments, we clipped buds from plants with forceps when buds were within 2 d of opening. To create the average level of damage, we clipped four buds per clone; to create the severe level of damage, we clipped eight buds per clone. The plants in the "none" group received no clipping (i.e., control). The average treatment is similar to the average damage observed in hermaphrodites in natural populations (Ashman et al. 2004), whereas the severe treatment reflects  $\sim 1$  SD greater than the mean, which is still a natural level of damage (T.-L. Ashman, personal observation).

**Reproductive and vegetative traits scored.** For each clone, we recorded sexual (number of inflorescences, flowers, and

fruits produced) and asexual (number of stolons and plantlets produced) traits and plant size. We used the product of the number of leaves and the diameter of the largest leaflet at the end of the experiment as an estimate of plant vegetative size. This value is a good estimate of aboveground biomass in *F. virginiana* (Ashman 1999). In addition, we produced an index of relative allocation to sexual versus asexual reproduction (hereafter, RSAR) that was based on meristem allocation. In strawberries, an axillary meristem can develop into an inflorescence or a stolon (Hancock 1999). For each plant, we calculated the proportion of axillary meristems allocated to sexual reproduction as the number of inflorescences divided by the sum of the number of inflorescences and stolons. Thus, a value of 0.5 for RSAR indicates that a plant allocated an equal proportion of its meristems to sexual and asexual reproduction, a value of  $>0.5$  indicates greater allocation of meristems to sexual reproduction, and a value of  $<0.5$  indicates greater allocation of meristems to asexual reproduction.

### Analysis

**Reproductive allocation.** To determine whether florivory affects reproductive investment (flowers, fruits, plantlets) and allocation (RSAR) of hermaphrodite plants, and whether this varies with inbreeding level, we conducted a fixed-effects ANCOVA, using PROC GLM in SAS (SAS 1996). Florivory treatment, inbreeding level, maternal family, and their interactions were fixed effects. We included maternal family in the design to account for the potential effects of inbreeding history, but maternal family was considered to be a fixed effect (because of the limited number of nonrandomly chosen families; Gotelli and Ellison 2004). In addition, the model included block and plant size to account for their effects on reproduction. All reproductive trait values except RSAR were transformed to conform to the assumptions of ANOVA (flower number and plant size were square-root transformed; numbers of inflorescences, fruits, and plantlets were natural-log transformed). We present least squares means (controlling for plant size) on untransformed data in figures and tables. Analyses were performed with a sample size of 593 clones because 92 clones were excluded because they never flowered, they died, or they did not fully receive their assigned florivory treatment (see below). These exclusions were evenly distributed across experimental factors.

In this analysis, we were particularly interested in the effects of florivory treatment and inbreeding level and their interaction. A significant effect of florivory treatment would indicate that plants allocate to reproduction differently in the face of florivore damage. A significant effect of inbreeding level would indicate that selfed and outcrossed plants differed in their reproductive investment or allocation, while a significant interaction between the two would indicate that the effects of florivory differ for selfed and outcrossed plants. An interaction of any of these with family would indicate that the maternal families in the experiment differed in their response to inbreeding or florivory or both. When a significant overall effect of a factor was found, we used Tukey's tests to identify the levels of the factor that were significantly different.

**Inbreeding depression.** A further examination of florivory and inbreeding level was conducted by comparing inbreeding

depression in fruit, flower, and plantlet production and RSAR expressed at the maternal family level within each of the three florivory treatments. To do this, we calculated means for selfed and outcrossed progeny for each family-florivory combination within each block. We then calculated inbreeding depression for each family and florivory level as  $\delta = 1 - \bar{X}_s/\bar{X}_o$ , where  $\bar{X}_s$  is the family mean of selfed individuals and  $\bar{X}_o$  is the family mean of outcrossed individuals (Johnston and Schoen 1994) under a given level of florivory within a block. This yielded 72 values of inbreeding depression for each trait. We determined whether maternal family or florivore treatment affected inbreeding depression expressed in each trait separately, using a fixed-effects ANOVA with family, florivory, their interaction, and block as class variables. Tukey's tests were used to identify the levels of a given factor that were significantly different. Before analysis, inbreeding depression for fruit, total flower, and plantlet production were square-root transformed to improve normality.

**Tolerance.** To determine whether inbreeding level or maternal family affects plant tolerance to florivory, we calculated tolerance for selfed and outcrossed progeny under conditions of average and severe damage separately. First, we calculated clone mean values for each fitness component (flowers, fruits, and plantlets) for each progeny-by-florivory treatment. Then we calculated tolerance as the difference in mean fitness of clones experiencing florivory and those not experiencing florivory, divided by the severity of florivory (Strauss and Agrawal 1999; Wise and Carr 2008). On the basis of this calculation, a value of 0 reflects full compensation (i.e., tolerance), whereas negative values reflect undercompensation and positive values reflect overcompensation. Because we were mainly interested in determining whether inbreeding level affected tolerance, we calculated mean tolerance for selfed and outcrossed progeny separately and tested each for a significant difference from 0, using *t*-tests (PROC TTEST; SAS 1996). We also determined whether tolerance of selfed progeny differed from that of outcrossed progeny, using paired *t*-tests. Finally, we determined whether severity of

florivory affected tolerance by testing whether the difference between tolerance under severe and average florivory was significantly different from 0, using *t*-tests. Bonferroni correction was applied to account for multiple tests.

## Results

### Reproductive Allocation

Simulated florivory reduced flower number (table 1). On average, plants exposed to severe florivory produced the fewest flowers (mean  $\pm$  SE:  $13.6 \pm 0.7$ ), and those exposed to the average florivory level in turn produced significantly fewer flowers ( $15.7 \pm 0.6$ ) than did undamaged plants ( $18.8 \pm 0.6$ ). In contrast, florivory did not significantly impact the average number of fruits, plantlets, or RSAR (table 1). However, there was a significant family-by-florivory interaction for RSAR (table 1); as florivory severity increased, variation in RSAR among families was reduced, and values converged on an RSAR that reflects equivalent allocation of meristems to sexual and asexual organs (fig. 1).

Inbreeding level significantly affected fruits, plantlets, and RSAR, but not flower number (table 1). Moreover, the effect of inbreeding on these traits varied greatly among families. On average, selfed plants produced significantly more fruits ( $5.52 \pm 0.26$ ) than did outcrossed plants ( $3.32 \pm 0.24$ ), but this effect was most pronounced in three families (17, 254, and 425; fig. 2B). Outcrossed plants produced significantly more plantlets ( $3.31 \pm 0.10$ ) than did selfed plants ( $3.01 \pm 0.11$ ), and this difference was quite extreme for one family (425; fig. 2C). Although the difference was small, selfed plants on average allocated a significantly greater fraction of meristems to sexual reproduction ( $0.52 \pm 0.01$ ) than did outcrossed plants ( $0.49 \pm 0.01$ ), but again, two families (254, 425) had the most pronounced pattern (fig. 2D).

The only reproductive trait to show an interaction between inbreeding and florivory was plantlet production (table 1). Here, outcrossed plants produced  $\sim 15\%$  more plantlets than did selfed ones when damaged; this was significant under av-

Table 1

ANCOVAs for Reproductive Traits in Hermaphrodite *Fragaria virginiana*

| Factor                          | Flowers |           | Fruits |           | Plantlets |           | RSAR |          |
|---------------------------------|---------|-----------|--------|-----------|-----------|-----------|------|----------|
|                                 | df      | F         | df     | F         | df        | F         | df   | F        |
| FLOR                            | 2       | 20.46**** | 2      | 2.06      | 2         | .03       | 2    | .51      |
| INB                             | 1       | .12       | 1      | 28.49**** | 1         | 4.51**    | 1    | 6.40**   |
| MFAM                            | 7       | 1.72      | 7      | 26.59**** | 7         | 11.28**** | 7    | 7.71**** |
| FLOR $\times$ INB               | 2       | .35       | 2      | .19       | 2         | 4.35***   | 2    | .69      |
| MFAM $\times$ INB               | 7       | 1.84      | 7      | 9.57****  | 7         | 4.50****  | 7    | 7.15**** |
| FLOR $\times$ MFAM              | 14      | .85       | 14     | .70       | 14        | .72       | 14   | 2.27***  |
| FLOR $\times$ MFAM $\times$ INB | 14      | .77       | 14     | .62       | 14        | 1.68*     | 14   | .85      |
| Plant size                      | 1       | 58.40**** | 1      | 12.94**** | 1         | 15.79**** | 1    | .97      |
| Block                           | 2       | .42       | 2      | .84       | 2         | .71       | 2    | .18      |

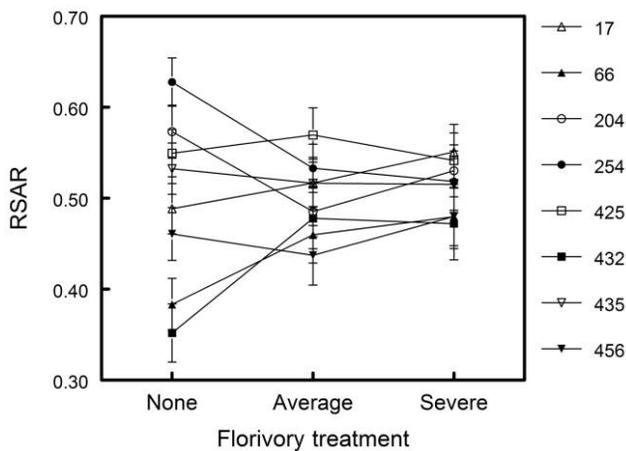
Note. Inbreeding level (INB), florivory treatment (FLOR), maternal family (MFAM), and block are fixed effects. RSAR = relative allocation to sexual versus asexual reproduction.

\*  $P < 0.06$ .

\*\*  $P < 0.05$ .

\*\*\*  $P < 0.01$ .

\*\*\*\*  $P < 0.001$ .



**Fig. 1** Least squares means ( $\pm$ SE) for relative meristem allocation to sexual and asexual reproduction (RSAR) under three florivory treatments (none, average, and severe) in eight maternal families of *Fragaria virginiana*.

erage damage, and the same pattern was present although nonsignificant under severe damage (fig. 3).

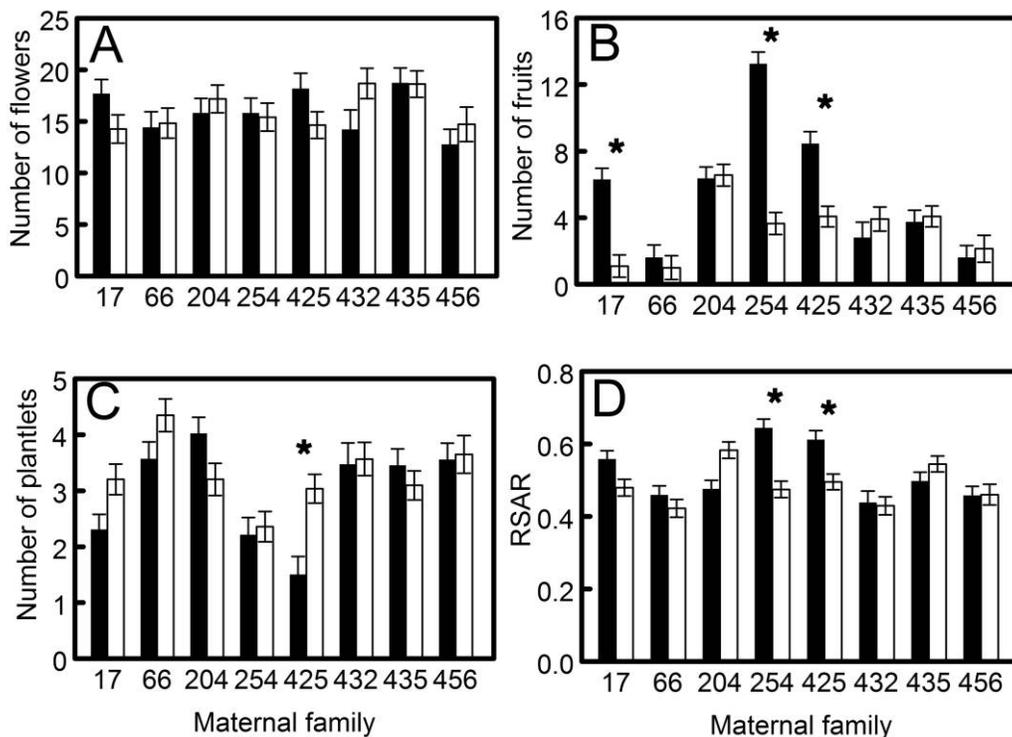
*Inbreeding Depression*

Interestingly, there was no significant overall effect of florivory treatment on the expression of inbreeding depression

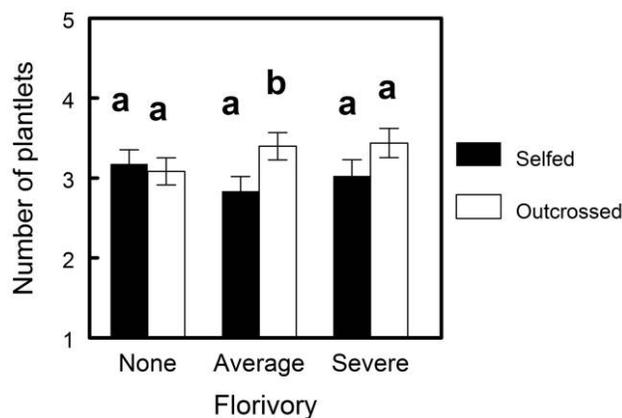
(table 2), but there was a maternal family-by-florivory interaction for inbreeding depression in plantlets (table 2; fig. 4). In response to increasing florivory, two families expressed significant or nearly significant increases in inbreeding depression (425, 435), while another showed a significant directional change, that is, from an expression of inbreeding depression under average florivory to outbreeding depression under severe florivory (254; fig. 4). In fact, maternal families varied significantly for inbreeding depression in all traits except flower number (table 2). Three families (17, 254, and 425) exhibited strong outbreeding depression in fruit production (fig. 5B); two of those families (17 and 425) also exhibited moderate inbreeding depression in the number of plantlets produced (fig. 5C). Both plantlet production and RSAR also showed maternal family variation ranging from inbreeding to outbreeding depression (fig. 5C, 5D).

*Tolerance*

Plants showed variable patterns of tolerance, ranging from an almost complete lack of compensation (flowers) to slight overcompensation (plantlets; fig. 6). Under average florivory, indices of tolerance were generally negative, reflecting undercompensation, but none were significantly different from 0. However, under conditions of severe florivory, indices of tolerance for flowers (fig. 6A) and fruits (fig. 6B) were significantly negative, reflecting a lack of tolerance. Tolerance indices did not, however, differ between severe and average damage (all  $|t| < 1.06$ ,  $df = 7$ ,  $P > 0.32$ ), suggesting that the



**Fig. 2** Least squares means ( $\pm$ SE) of flower production, fruit production, plantlet production, and relative meristem allocation to sexual and asexual reproduction (RSAR) for selfed (black bars) and outcrossed (white bars) progeny for eight maternal families of *Fragaria virginiana*. Significant differences ( $P < 0.05$ ) between selfed and outcrossed progeny within a given maternal family are indicated by an asterisk above the columns.



**Fig. 3** Plantlet production (least squares means  $\pm$ SE) of selfed and outcrossed *Fragaria virginiana* under three levels of florivory. Within a treatment level, selfed and outcrossed means that do not share a letter are significantly different at  $P < 0.05$ .

difference in statistical significance was largely due to a reduction in variance in tolerance under severe damage. Likewise, in no case was tolerance of selfed and outcrossed plants significantly different (all  $|t| < 1.96$ ,  $df = 7$ ,  $P > 0.09$ ), although one needs to keep in mind that these  $t$ -tests had limited power.

### Discussion

By exposing outcrossed and selfed clones of *Fragaria virginiana* hermaphrodites to simulated weevil florivory, we demonstrated that both damage and inbreeding affect reproduction, but their effects are largely independent with respect to the sexual traits examined. Interestingly, this independence was not seen for the asexual reproductive trait of plantlet production. Thus, it was only for this trait that inbreeding depression was florivory dependent, and even here the pattern varied among families. In fact, inbreeding depression was maternal family dependent for the majority of traits examined. Lastly, while plants largely did not compensate for florivory, this was only significant under severe damage, and inbreeding level did little to change this pattern. In the fol-

lowing paragraphs, we interpret these results in light of other studies of antagonist-dependent inbreeding depression and their potential role in sexual system evolution.

### Independent versus Interactive Effects of Inbreeding and Florivory

A particularly interesting outcome of the work presented here is that florivory and inbreeding level had largely independent effects because each affected different sexual traits. Florivory reduced flower number but had no effect on fruit production, whereas inbreeding increased fruit production but had no effect on flower number. The absence of an inbreeding effect on flower number could be due to an absence of genetic variation in this trait or to its tolerance to florivory. While flower number can be a highly heritable trait (Hof et al. 1999; Ashman 2003; Caruso 2004), the limited sampling of genotypes used in this study may have restricted genetic variation. However, Ashman et al. (2004), using a larger set of maternal genotypes, also did not detect genetic variation in tolerance to florivory with respect to flower number, which suggests that a paucity of variation for tolerance to florivory is not uncommon. This might not be surprising given that traits like tolerance, which may be subject to strong selection and are tightly linked to fitness, are expected to have low genetic variation (Geber and Griffen 2003; Blows and Hoffmann 2005) or because tolerance trades off with resistance such that highly resistant plants are not subject to selection for tolerance; if tolerance is costly, then variation for tolerance is not expected to be maintained (Fineblum and Rausher 1995). Other studies, however, have found significant genetic variation in tolerance (Shen and Bach 1997; Stinchcombe and Rausher 2002; Fornoni et al. 2003), including one that examined tolerance to florivory (Wise et al. 2008; but see Juenger and Bergelson 2000b; Ivey et al. 2009), indicating that the magnitude of variation may depend heavily on the system.

The absence of a florivory effect on fruit number is in line with the finding of Ashman et al. (2004) for hermaphrodite genotypes that indicated that hermaphrodites are generally tolerant to average levels of damage for this fitness component, although they may be intolerant at severe damage levels (fig. 6). The finding of an effect of inbreeding on fruit num-

**Table 2**

**ANOVAs Testing for the Effects of Florivory Treatment and Maternal Family on Inbreeding Depression in Sexual and Asexual Reproductive Traits of *Fragaria virginiana***

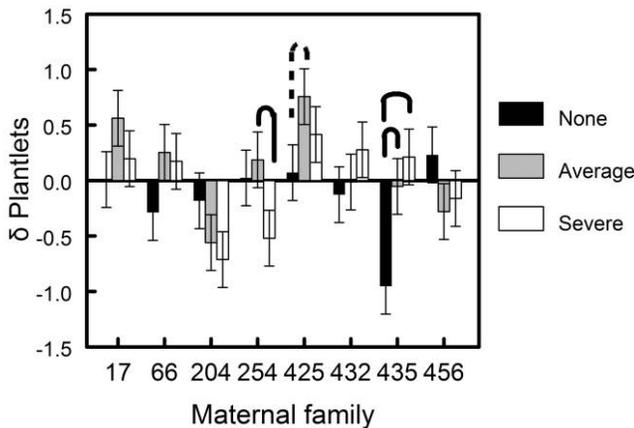
| Factor             | Flowers |     | Fruits |          | Plantlets |        | RSAR |        |
|--------------------|---------|-----|--------|----------|-----------|--------|------|--------|
|                    | df      | F   | df     | F        | df        | F      | df   | F      |
| FLOR               | 2       | .34 | 2      | .73      | 2         | 1.61   | 2    | 1.23   |
| MFAM               | 7       | 1.3 | 7      | 13.05*** | 7         | 3.88** | 7    | 5.5*** |
| FLOR $\times$ MFAM | 14      | .44 | 14     | .64      | 14        | 2.22*  | 14   | .95    |
| Block              | 2       | .21 | 2      | .55      | 2         | .34    | 2    | .12    |

Note. Florivory treatment (FLOR), maternal family (MFAM), and block are fixed effects. RSAR = relative allocation to sexual versus asexual reproduction.

\*  $P < 0.05$ .

\*\*  $P < 0.01$ .

\*\*\*  $P < 0.001$ .

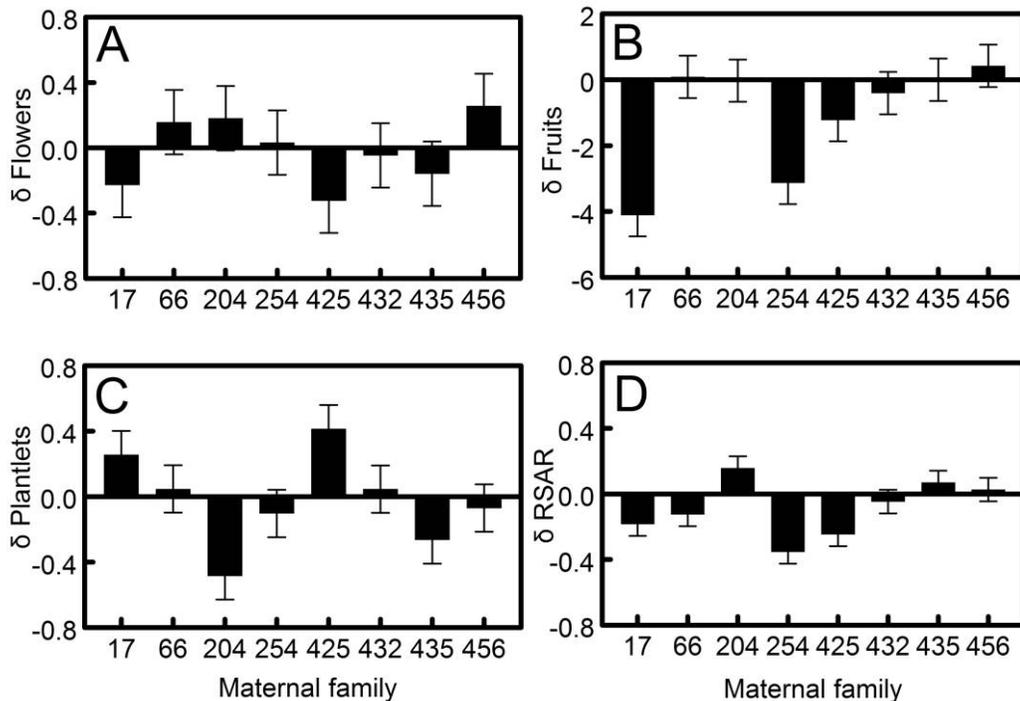


**Fig. 4** Mean ( $\pm$ SE) inbreeding depression ( $\delta$ ) in plantlet production for *Fragaria virginiana* hermaphrodites by maternal family under three levels (none, average, or severe) of simulated florivory. Treatments within a family that were significantly different are connected by curved solid lines ( $P < 0.05$ ) or curved dashed lines ( $P = 0.059$ ).

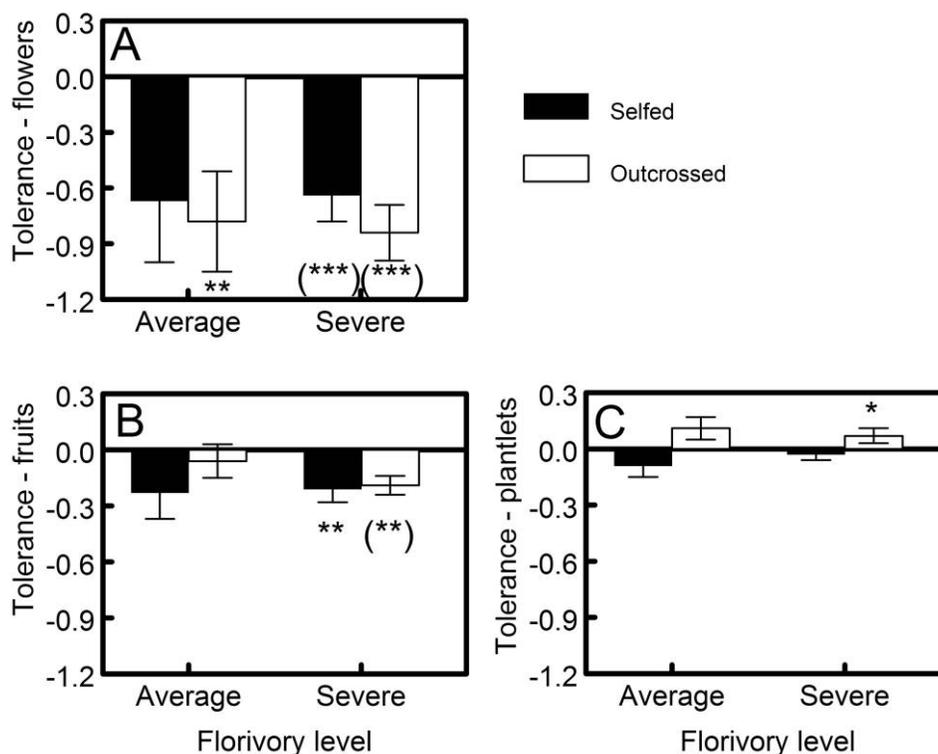
ber, however, is novel given the direction of this effect, which is pronounced in three families (fig. 2B). For these families, selfed progeny had higher fruit production than did outcrossed progeny, a finding similar to outbreeding depression (Lynch 1991) for fruit production, although here it occurs within a population. This result may be best explained by considering

the genetics underlying fruit production in this gyno(sub)dioecious species. Recently, Spigler et al. (2008) demonstrated that two linked gene regions are responsible for sexual phenotype. Their model postulates that at the female function (fruit production) locus there is an allele  $G$  that codes for fertility; this locus could be dominant or codominant to an allele  $g$  that codes for sterility, such that hermaphrodite individuals are  $GG$  or  $Gg$  at this locus and  $gg$  individuals produce no fruit (i.e., are males; Spigler et al. 2008). Since the pollen pool contains pollen carrying the sterility allele ( $g$ ), outcross progeny of  $GG$  hermaphrodites would have lower fruiting ability than would selfed progeny if  $G$  is codominant. If hermaphrodites were  $Gg$  and the outcrossed pollen pool was  $>50\%$   $g$  (i.e., because males were potential donors), then on average, outcrossed progeny would have lower fruit production than would selfed progeny, regardless of whether  $G$  is codominant or dominant. While we do not yet know which scenario was the case—in fact, family variation in the effects of selfing suggest that hermaphrodites included here could be  $GG$  or  $Gg$ —controlled crosses to evaluate the putative genotypes at the  $G$  locus for the maternal hermaphrodites used in this study and, thus, to test this hypothesis, are under way. Regardless, this finding reveals a particularly intriguing cost to outcrossing for hermaphrodite genotypes in a subdioecious species, and it suggests that there could be selection for autonomous selfing to maintain them.

Plantlet production was the only trait for which inbreeding and florivory had interactive effects; thus, inbreeding depression was florivory dependent, although the pattern varied



**Fig. 5** Mean ( $\pm$ SE) inbreeding depression ( $\delta$ ) in eight maternal families of *Fragaria virginiana*. Variation among families was significant for fruits and plantlets but not for flowers or relative allocation to sexual versus asexual reproduction (RSAR; table 2). Note that Y-axis scaling for fruits is different from the rest.



**Fig. 6** Mean ( $\pm$ SE) tolerance of selfed and outcrossed *Fragaria virginiana* exposed to average and severe simulated florivory. Tolerance indexes are in the units of fitness components per bud removed (see “Material and Methods” for details); those that are significantly different from 0 based on *t*-tests are noted as follows: one asterisk indicates  $P < 0.05$ , two asterisks indicate  $P < 0.01$ , and three asterisks indicate  $P < 0.001$ . Tests that retained significance after Bonferroni correction are indicated in parentheses.

among families (fig. 4). Selfed progeny produced fewer plantlets than did outcrossed progeny under florivory, and significantly so under average damage levels (fig. 3). The higher plantlet production under florivory may reflect resource redistribution from the combination of reduced fruit production of outcrossed plants (see above) and lower investment in flower expansion and maintenance when buds are lost to florivory. Such an interpretation is supported most clearly by patterns seen in two families (17 and 425), which both showed outbreeding depression in fruits and inbreeding depression in plantlets that increased with severity of florivory (although the latter pattern only reached marginal statistical significance for family 425). Such results lend credence to the idea that the presence of antagonists can lead to different fitness outcomes for a given reproductive strategy (i.e., that a shift from sexual to asexual reproduction may be favored when florivores are present), but to understand whether these responses are adaptive we will need a broader sampling and an assessment of fitness consequences under field conditions.

Maternal family variation in inbreeding depression was seen for plantlets (table 2) and was also evident from an interaction between maternal family and inbreeding level for fruits and RSAR in the ANCOVA (table 1). Variation among families in inbreeding depression is a common phenomenon that could suggest several causes. First, family variation in inbreeding depression has been linked to among-lineage variation in selfing rate and a subsequent purging of deleterious

recessive alleles (Lande and Schemske 1985). However, some systems have shown no consistent relationship between inbreeding history and inbreeding depression (Carr et al. 1997; Stone and Motten 2002). Second, Dudash et al. (1997) suggested that family differences may be due to the nature of deleterious mutations carried by individuals that undergo selfing, that is, if traits are affected by deleterious recessive alleles, overdominance, or epistatic interactions. Third, family variation in inbreeding depression in response to a stressor could result from family variation in the magnitude of phenotypic variation, where families with higher phenotypic variance for a given trait have a higher likelihood of displaying inbreeding depression (Waller et al. 2008). Both variation in inbreeding history and extent of phenotypic variation are likely causes for the patterns seen here because (1) although the selfing rate of maternal hermaphrodites used in this study are not known, selfing rates of hermaphrodites from the source population are known to vary widely (range, 0.167–1; A. S. Rohde, C. L. Collin, L. Penet, A. Johnson, and T.-L. Ashman, unpublished data), and (2) families varied twofold in coefficient of variation for plantlet, RSAR, and fruit production (A. S. Rohde and T.-L. Ashman, unpublished data).

#### *Tolerance and Inbreeding Depression*

Our study joins a small but growing number of studies that have examined the effects of inbreeding on tolerance (re-

viewed in Núñez-Farfán et al. 2007). We found that tolerance varied with trait and plants were significantly intolerant under severe damage levels but inbreeding had little effect on tolerance. This latter result contrasts with studies that show negative effects of inbreeding on tolerance (Carr and Eubanks 2002; Ivey et al. 2004; Hull-Sanders and Eubanks 2005), but this could be due to many factors, including the type of damage inflicted, whether that damage imposes a significant carbon loss to the plant, the fitness traits measured, the amount of genetic variation for tolerance, or experimental sample size. Given the few studies conducted, that no prior studies have examined inbreeding's effects on tolerance to florivory, and the variability of responses demonstrated thus far, it may be premature to draw conclusions about the prevalence of negative effects of inbreeding on tolerance; instead, it underscores the need for studies of inbreeding and tolerance to be conducted in more systems and with more types of antagonists and response variables.

#### *Implications for Sexual System Evolution*

Theory suggests that florivory may affect sexual system evolution if it affects the relative seed production of hermaphrodites and females, the selfing rate of hermaphrodites, and/or inbreeding depression (Ashman 2002, 2006). Ashman et al. (2004) addressed the first of these mechanisms for the strawberry bud-clipping weevil system and found that weevil damage did not significantly alter relative seed production of females and hermaphrodites. Our results confirm those of Botham et al. (2009) in that they reveal mild but highly variable inbreeding depression, and our study extends this earlier

work by demonstrating that florivory did not, in general, increase inbreeding depression. Thus, when taken at face value, these findings suggest that weevil damage will not have its greatest influence on population sex ratio in *F. virginiana* through its effects on inbreeding depression. Our results, however, suggest that weevil damage may instead affect sex ratio by affecting the production (or maintenance) of high-fruited (GG) hermaphrodites. Specifically, this is because weevil damage can increase autogamous selfing (Penet et al. 2008), and more GG hermaphrodites would be produced under selfing than under outcross or panmictic pollination in the wild, owing to preponderance of males (gg) and low-fruited (putative Gg) hermaphrodites contributing to the xenogamous pollen pool in wild populations (Ashman 1999; R. Spigler and T.-L. Ashman, unpublished data). A test of this possibility requires an understanding not just of variation in phenotypic gender and its association with sex ratio but also of the frequency of genotypes at the female function locus and its association with sex ratio and weevil damage and of the net effects of weevil damage on selfing rate, that is, whether geitonogamous selfing plays a role. Such work is under way.

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