

## RESEARCH PAPER

# Florivory increases selfing: an experimental study in the wild strawberry, *Fragaria virginiana*

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**ABSTRACT**

Florivores are antagonists that damage flowers, and have direct negative effects on flowering and pollination of the attacked plants. While florivory has mainly been studied for its consequences on seed production or siring success, little is known about its impact on mating systems. Damage to flowers can alter pollinator attraction to the plant and may therefore modify patterns of pollen transfer. However, the consequences of damage for mating systems can take two forms: a decrease in flower number reduces opportunities for intra-inflorescence pollen deposition (geitonogamy), which, in turn, may lead to a decrease in selfing; whereas a decrease in floral display may also reduce overall visitation and thus increase the chances of self-pollination *via* facilitated or autonomous autogamy. We investigated the effects of damage by a bud-clipping weevil (*Anthonomus signatus*) in *Fragaria virginiana* in an experimental setting mimicking natural conditions. We found that increased damage led to an increase in selfing, a result consistent with the increased autogamy pathway. We discuss the implications of this finding and evaluate the generality of florivore-mediated mating system expression.

**INTRODUCTION**

Ever since Darwin (1876) noted that the quality of offspring resulting from selfing was reduced relative to that from outcrossing, the persistence of self-fertilisation in plants has been an important evolutionary question (Barrett & Harder 1996; Holsinger 2000). The broad range of species with intermediate selfing rates (*i.e.* mixed mating) is still puzzling, given that several influential theoretical models predict a preponderance of either outcrossing or selfing as stable outcomes of the evolutionary process (*e.g.* Lande & Schemske 1985). In fact, some authors have gone so far as to label mixed mating as a ‘paradox’ (Vogler & Kalisz 2001; Goodwillie *et al.* 2005). Theoretical studies emphasise the role of genetic factors in the evolution of plant sexual and mating systems (Charlesworth & Charlesworth 1987; Lloyd & Schoen 1992; Schoen & Lloyd 1992). But, although many factors thought to influence the different modes of selfing derive from plant or flower characteristics (*e.g.* cleistogamous selfing, prior, competing or delayed selfing; see Schoen &

Lloyd 1992), others are considered side effects of adaptation to cross fertilisation by pollinators, such as geitonogamous (intra-inflorescence pollen transfer) or facilitated selfing (intra-flower pollen deposition attributable to pollinator activity) and are largely dependent on the ecological context in which selfing occurs (Lloyd 1992; Barrett & Harder 1996; Barrett *et al.* 1996). Ecological factors that may influence selfing range from plant–plant competitive interactions to plant–insect interactions, but these have received less attention than plant characteristics (but see Holsinger 1991; Ashman 2006; Steets *et al.* 2007).

Pollinators are indeed expected to play a primary role in pollen movement and thus not only influence reproductive success but also mating system of the plants they visit. For instance, because different pollinator species differ in their ability to carry pollen and in the way they visit the flowers, their abundance can influence plant outcrossing rate (Brunet & Sweet 2006). In particular, an increase in pollinator visitation can increase the likelihood of geitonogamous pollination and thus can have an influence on selfing rate (Galloway *et al.* 2002; Karron *et al.*

2004; Brunet & Sweet 2006), but the possibility of geitonogamy also increases with the number of flowers open simultaneously (*i.e.* floral display size; *e.g.* Klinkhamer *et al.* 1989; de Jong *et al.* 1993).

While studies of how pollinators impact pollen limitation, pollen transfer among plants and selfing rates are numerous, biologists have tended to overlook the influence of other ecological factors (but see Strauss & Whittall 2006), notably with regard to the evolution of plant mating systems (Steets *et al.* 2007). Such other factors include nectar robbing (Irwin 2006), sap-sucking herbivory (Ivey & Carr 2005), folivory (Ashman 2002; Steets & Ashman 2004) and florivory (McCall & Irwin 2006), and studies reveal that these factors can also dramatically alter self-fertilisation in plants. This oversight is especially surprising given that many pollinators are also often antagonists (*e.g.* florivores, folivores or seed predators; Dufaÿ & Anstett 2003; Wright & Meagher 2003; Adler & Bronstein 2004, and references therein).

Herbivores are well known for their negative effects on plant reproductive success. Folivores damage vegetative tissues and thus reduce resource acquisition (Strauss 1997). They most often have indirect effects on flower production and flower size (*e.g.* Strauss *et al.* 1996; Zamora *et al.* 1999; Mothershead & Marquis 2000; Gomez 2003), and this can translate into increased selfing (Ivey & Carr 2005) *via* reduced herkogamy (Elle & Hare 2002) and pollinator visitation (Lehtilä & Strauss 1997). The net effect of herbivory on plant reproduction is, however, not always negative. Herbivory may increase investment in male function and male fitness (Strauss *et al.* 2001; Avila-Sakar *et al.* 2003), so even if selfing increases in damaged plants it may be compensated by reproductive gains *via* male function (but see Quesada *et al.* 1995). On the other hand, florivores directly damage flowers and buds and thus can directly affect reproductive success and plant mating. In fact, they have been seen to mediate selection, which not only opposes that by pollinators but is often higher in magnitude (*e.g.* Ashman *et al.* 2004; Cariveau *et al.* 2004; Ashman & Penet 2007).

While much recent work has investigated how the mating system affects the level of herbivory (*e.g.* Carr & Eubanks 2002; Hayes *et al.* 2004; Ivey *et al.* 2004; Hull-Sanders & Eubanks 2005), far fewer studies have addressed the causal effect of herbivory on mating systems (see Table 1), and those have found mixed results. This variation suggests that there is more than one way that herbivores can affect plant mating system. Herbivores primarily modify resource allocation to reproduction, thus influencing selfing *via* a decrease in flower size or herkogamy, whereas florivores directly damage reproductive units/structures. There are two non-exclusive processes by which florivores may alter the mating system in self-compatible plants. In the first case, florivores reduce plant attractiveness, either by reducing/damaging the floral display, reducing rewards or deterring pollinators directly. As a consequence of reduced visitation, pollen loads in dehiscing anthers remain high, which increases the opportunity for self-pollen to fall passively onto stigmas, or to be transferred to stigmas when the rare visitor arrives. Here, damage would lead to increased selfing because of the increased chance of autonomous or facilitated autogamy. Moreover, flowers with high per-flower pollen production may be particularly susceptible. In the second case, damage reduces floral display size, which directly reduces the opportunity for pollinator movement between flowers within a plant. Hence, damage would lead to decreased selfing as a consequence of decreased opportunity for geitonogamous pollen movement. This latter hypothesis has received more attention, although both processes can occur in most plant species. Thus, it is important to measure both the damage levels and the selfing rates, as well as components or indicators of these pathways, to determine the likely contribution of these florivore-mediated effects on selfing rate.

Most estimates of mating system parameters are calculated at the population level. This situation is connected to the lower levels of polymorphism in historically used markers (isozymes) and other pitfalls (*e.g.* linkage disequilibrium and underestimation of outcrossing rate due to

**Table 1.** Effects of herbivory on mating system. An increase (+) or a decrease (–) in overall selfing, geitonogamous and autonomous selfing is indicated whenever it has been documented.

plant species	type of damage	effect on			references
		selfing	geitonogamy	autogamy	
<i>Datura wrightii</i>	folivory	–	–		Elle & Hare (2002)
<i>Fragaria virginiana</i>	florivory (bud clipping)	+		+	this study
<i>Impatiens capensis</i> <sup>a</sup>	folivory	+		+	Steets & Ashman (2004)
		–	–		Steets <i>et al.</i> (2006)
<i>Isomeris arborea</i>	folivory	no effect			Krupnick & Weis (1999)
<i>Lespedeza cuneata</i> <sup>a</sup>	folivory	+		+	Schutzenhofer (2007)
<i>Lespedeza virginica</i> <sup>a</sup>	folivory	+		+	Schutzenhofer (2007)
<i>Mimulus guttatus</i>	folivory (spittlebugs)	+			Ivey & Carr (2005)

<sup>a</sup> Species with flower mating dimorphism (cleistogamy and chasmogamy); for these species the increase in autogamy occurs *via* cleistogamous flowers only, *i.e.* by autonomous selfing.

the lack of diagnostic markers for different individuals in natural populations; Hill 1974; Ritland 1989; Morgan & Barrett 1990). Thus, many early attempts to detect associations between selfing and morphological or ecological factors met with mixed success (Crawford 1984; Glover & Barrett 1986; Ritland 1989; but see van Kleunen & Ritland 2005 in wild and Karron *et al.* 2004 in experimental populations). In this study, we take advantage of the high polymorphism level of microsatellite markers and of completely experimental populations of unrelated genotypes, to study selfing rates at the individual level.

This approach allowed us to investigate the impact of the flower clipping weevil *Anthonomus signatus* (Curculionidae) Say, on the selfing rate of its host *Fragaria virginiana* Mill., the Virginian wild strawberry. Specifically, we posed the following questions: (i) How does weevil damage impact selfing rates in hermaphrodite plants of *F. virginiana*? (ii) What is the magnitude of the weevil effect on selfing rate compared to that of a floral trait, *i.e.* pollen production per flower, or display size? Our study addressed the unexplored impact of florivores on selfing rate and found that weevil damage increased self-fertilisation, which is consistent with an increase in autogamous selfing relative to geitonogamy.

## MATERIALS AND METHODS

### Study system

The Virginian wild strawberry, *Fragaria virginiana* (Rosaceae), is a perennial herb that grows in North America and has a gynodioecious sexual system, where females coexist with hermaphrodites (Staudt 1989), and both sexes reproduce asexually *via* runners and sexually *via* seeds. Fruit production varies widely in hermaphrodite plants, with an average 20% fruit set, and a large portion of hermaphrodite plants do not set any fruit (Ashman 1999, 2003). Inflorescences contain five to ten flowers in the wild and usually have two to four flowers open simultaneously (T-L Ashman, unpublished data, with flowers having a typical lifespan of 1–2 days (Ashman & Hitchens 2000)). Wild strawberry flowers are visited by a variety of species belonging to three main taxonomic groups (Ashman 2000): small bees (Halictidae, Apidae, Andrenidae, Megachilidae), flies (Syrphidae, Bombyliidae, Conopidae) and ants (Formicinae, Dolichoderinae). These floral visitors often visit multiple flowers per plant within a foraging bout (CL Collin, L Penet, and T-L Ashman, unpublished data), and thus potentially transfer self-pollen both geitonogamously and autogamously.

The strawberry clipper weevil, *Anthonomus signatus*, is a common florivore of *F. virginiana* (Kovach *et al.* 1999). Weevil females emerge in early spring and lay eggs inside unopened buds before clipping bud pedicels (Kovach *et al.* 1999). Larvae develop within these buds and pollen may be required for successful development (Jones & Peruyero 2002). Weevils show dramatic sex morph-biased attack, damaging hermaphrodites seven-times as often as

females (Ashman *et al.* 2004; Ashman & Penet 2007). Moreover, weevil damage increases significantly with increasing production of flower buds and pollen per flower by hermaphrodites (Ashman *et al.* 2004; Ashman & Penet 2007).

### Experimental material

Plants used in the current study were part of a larger experiment addressing the effect of florivory on natural selection in this gynodioecious species, thus here we briefly describe the experimental set up (for greater detail see Ashman & Penet 2007). We set up experimental gardens of potted plants (45 females and 45 hermaphrodites) in each of 2 years (2003, 2004) at the Pymatuning Laboratory of Ecology (PLE), Crawford County, PA, USA. Each garden had a plant density similar to that observed in wild populations. Gardens contained numerous unique genotypes, and most genotypes were used in both years. Flowering in the gardens was synchronized with the native wild strawberries. We collected weevils from a wild population and released them in the gardens. Daily surveys confirmed weevil residence and florivory. Plants received pollination by native pollinators, and water as needed. At the end of flowering, we moved plants to a greenhouse at the University of Pittsburgh to protect maturing fruits and to facilitate seed collection.

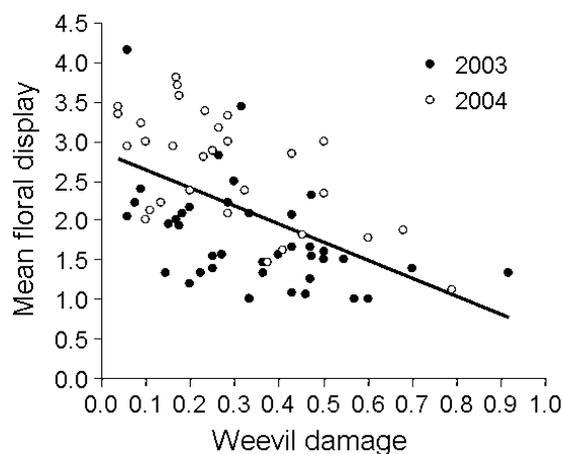
In the context of this study we measured several traits on hermaphrodite plants that had been attacked by weevils (mean  $\pm$  SE and range are presented;  $n = 40$  in 2003 and 30 in 2004). We counted the number of flower buds produced ( $14.3 \pm 0.8$ , range 4–30) and the number of these that were clipped by weevils ( $4.3 \pm 0.4$ , range 1–17) to calculate the intensity of florivore damage as the proportion of buds that were clipped per plant over the season. We estimated floral display as the average number of open flowers per plant, from surveys taken every 2 to 3 days over the flowering season ( $2.1 \pm 0.1$ , range 1.0–4.3). Finally, pollen per flower ( $1.65 \pm 0.11 \times 10^5$ , range  $1.03\text{--}2.59 \times 10^5$ ;  $n = 18$ ) was estimated as the product of pollen per undehisced anther and the number of anthers per flower for one representative flower per inflorescence (*i.e.* secondary position). We determined pollen per anther with the aid of an Elzone particle counter, as described in Ashman & Hitchens (2000).

### Selfing rates and statistical analyses

We estimated selfing rate at the individual level for hermaphrodites that were damaged by weevils and that produced at least 10 seeds. Eighteen plants met these criteria. We planted seeds from single fruits in Sunshine germination mix, and maintained them in growth chambers under standard conditions (20 °C, 14-h day length). Under these conditions, germination rate of selfed and outcrossed progenies do not differ significantly (CL Collin, AS Krieger, L Hefferman, T-L Ashman, unpublished data). When seedlings had two to three true leaves we

harvested aboveground tissue and froze it at  $-80^{\circ}\text{C}$ . Leaf tissue was collected from maternal plants and treated similarly. DNA was extracted from young leaves after being frozen with liquid nitrogen and ground, following a procedure adapted from Keim *et al.* (1989), where thawing in extraction buffer was reduced to two successive periods of 10 and 20 min, respectively, while  $100\ \mu\text{l}$  of a 5 M potassium acetate solution were added before cooling and centrifugation. We genotyped seedlings and maternal tissue at four microsatellite loci (Fv9, Fv11, FAC\_002 and ARSFL\_99, with 10, 9, 9 and 13 alleles, respectively) following published protocols for wild strawberries (Ashley *et al.* 2003; Lewers *et al.* 2005). We used the Expectation-Maximisation method of the MLTR program (Ritland 2002) to estimate individual selfing rates. This method provides estimates that converge between 0 (complete outcrossing) to 1 (complete selfing). In total, we analysed 126 progeny on 11 plants in the 2003 garden, and 62 progeny on seven plants in the 2004 garden (mean  $\pm$  SD =  $10.4 \pm 4.1$  progeny per maternal plant; range 6–26). Genetic variance of individual selfing rate estimates by MLTR were very low owing to the experimental setting, where alleles were diagnostic of individual plants and progenies could not possibly be inferred as selfed when resulting from an outcross fertilisation event (average SD of 0.068, range 0–0.187).

In the experimental gardens, damage by weevils was diverse (percentage of buds clipped ranged from none to above 60%) and negatively affected floral display ( $r = -0.53$ ,  $P < 0.0001$ ,  $n = 70$ ; Fig. 1), which, in turn, negatively affects total pollinator visitation rate (Ashman & Penet 2007) and incidence of geitonogamous visits (T-L Ashman, unpublished data). Variation in floral display is affected by both genotypic (number of buds



**Fig. 1.** Impact of weevil damage on floral display in wild strawberry, *Fragaria virginiana*. The intensity of weevil damage was calculated as the proportion of buds that were clipped. Floral display corresponds to the mean number of flowers open simultaneously. Data points are showed separately for 2003 and 2004, although the trend line represents the overall effect. Regression is as follows: number of open flowers =  $-2.28 \times$  proportion of buds clipped + 2.87 ( $R^2 = 0.28$ ).

produced) and abiotic (weather) components in addition to the weevil-mediated effect. By including both the effects of floral display and weevil damage in the analysis of covariance of selfing rates, we investigated whether weevils have an effect that is not mediated through floral display. The analysis also contained year as a fixed main effect and pollen production per flower. Although there was no year effect in damage levels (Ashman & Penet 2007), we included this factor in the model to take into account other environmental and genetic differences that occurred between years, *i.e.* not all replicates from each genotype were attacked by weevils or produced seeds.

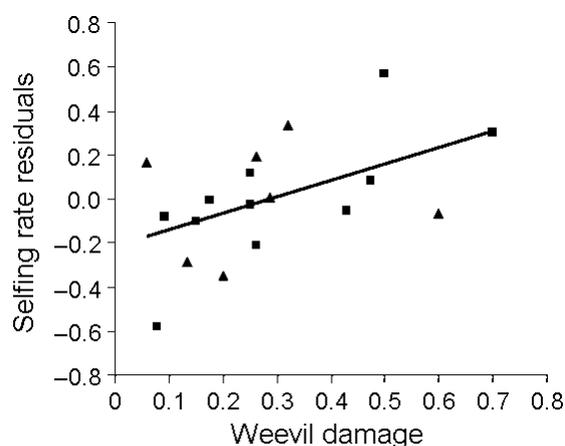
## RESULTS

Individual selfing rates varied widely, ranging from 0 to 1 (mean  $\pm$  SE:  $0.59 \pm 0.07$ ;  $n = 18$ ), but there was no difference between years (Table 2). Selfing rates increased significantly with increasing intensity of damage by weevils and with increasing pollen per flower (Table 2, Fig. 2). The effect of weevils on selfing rates was independent of the effect of floral display, which was not statistically significant in our analysis (Table 2). The effects of

**Table 2.** Analysis of covariance of selfing rate in wild strawberry, *Fragaria virginiana*.

source	DF	MS	F	P	$\beta'$
Year	1	0.01	0.18	0.676	–
Weevil damage	1	0.36	5.47	0.036	0.50
Pollen production	1	0.42	6.43	0.025	0.57
Floral display	1	0.07	1.11	0.311	0.32
Error	13	0.07			

The standardised regression coefficients ( $\beta'$ ) are given for the continuous effects.



**Fig. 2.** Impact of weevil damage on selfing rates in wild strawberry *Fragaria virginiana*. Regression of selfing rate residuals (after controlling for year, pollen production and floral display effects; see Table 2) on weevil damage (calculated as the proportion of buds clipped). Squares represent data from 2003, triangles are data from 2004.

weevil damage and pollen production on selfing rate were similar in strength, as evidenced by their standardized partial regression coefficients ( $\beta' = 0.50$  versus  $0.57$ , respectively).

## DISCUSSION

Flower-clipping weevils reduce floral display of hermaphrodites and consequently reduce their outcross siring success (Ashman & Penet 2007). Here, our results suggest that weevil attack also impacts mating system of fruiting hermaphrodites: selfing rates increased with the intensity of clipping. We attribute the increase in selfing rate to an increase in autogamy (*i.e.* either autonomous or facilitated selfing). This was mostly an indirect consequence of reduced floral display and plant attractiveness, although we acknowledge that there may have been transient deterrent effects of weevils on pollinators at the time of clipping. Our conclusion is supported by the fact that moderately high florivore damage (40–50% buds clipped) reduces floral display to less than two simultaneously open flowers a day (see Fig. 1; Ashman & Penet 2007), which, in turn, dramatically decreases pollinator visitation and the opportunities for geitonogamous selfing (Ashman & Penet 2007; T-L Ashman, unpublished data). The impact of weevils occurred in addition to the effect of floral display on selfing rate, which was not significant, although positive and relatively strong ( $\beta' = 0.32$ , Table 2); a result that suggests that geitonogamy contributes less to overall selfing rate. Moreover, given our experimental set up, where gardens were composed of unique genotypes (no genotypic replication within gardens), selfing could not occur *via* pollen transfer among clonal ramets. Our interpretation is strengthened by the fact that selfing is also associated with high pollen production, because greater amounts of pollen within flowers provide greater opportunity for autogamy.

Although an intuitive consequence of flower damage is a decrease in selfing due to reduced geitonogamy, the possibility of increased autonomous or facilitated selfing resulting from lower pollinator visitation has previously been overlooked. This is probably because damage to flowers often translates into changes in visitation (*e.g.* Krupnick *et al.* 1999; Leavitt & Robertson 2006) and thus alters geitonogamous pollen transfer, leading to the intuitive association of florivory with a reduction in selfing opportunity. Among the numerous negative consequences of florivory that have been emphasised (*e.g.* loss of gametes at individual and population levels, decrease in attractiveness to pollinators; for a review see McCall & Irwin 2006), increased autogamy had not previously been suggested as a possible outcome of florivore attack. Our results indicate that increased autogamy may be an important consequence of florivory.

It is interesting to compare effects of florivores on selfing to those of folivores; however, too few studies are available to make generalisations about the impact of florivory on mating systems. Indeed, florivores affect floral

display after buds are produced, so their net impact on selfing is thus mostly pollinator-mediated, as compared to folivores. Folivores, on the other hand, can affect the plant before the inflorescences are actually produced, and can thus alter flowering in a more diverse fashion (decrease in floral display or morphological allometry altering herkogamy or both). At first sight, the impact of herbivory or florivory on selfing offers a mixed picture – selfing can either decrease or increase as a result of damage (Table 1). On closer examination, however, the pattern fits expectations of the two mutually non-exclusive processes hypothesised. A decrease in selfing seems to be associated with a decrease in geitonogamy; while, on the other hand, an increase in selfing seems associated with increased autogamy. Overall, the impact of herbivores on mating systems in plants may have a lot to do with species characteristics: less selfing is expected in species with features preventing autogamy (*e.g.* dichogamy in *Ipomopsis aggregata*; Juenger & Bergelson 2000), especially if herbivore damage further affects those features when altering resource allocation (*e.g.* herkogamy in *Datura wrightii*, Elle & Hare 2002). More selfing is expected in species with adaptations for selfing (*i.e.* with specialised cleistogamous flowers; Table 1) or species such as *Fragaria virginiana* where selfing proclivity may be an indirect consequence of selection for increased male reproductive success (*i.e.* producing more pollen per flower).

Our understanding of the influence of herbivores and florivores on mating systems would benefit from integrating details of ecological interactions and of plant life history. As an example, the dichotomy of selfing responses to herbivory is particularly evident in plants with both cleistogamous and chasmogamous flowers. In *Impatiens capensis*, herbivory has been shown to increase plant reliance on selfing fitness, *i.e.* investment in cleistogamous flower production increases with herbivore attack (Steets & Ashman 2004), whereas it also decreases geitonogamous selfing in chasmogamous flowers (Steets *et al.* 2006). Identifying the flower and inflorescence characteristics that lend themselves to one pathway over the other (*i.e.* decreased geitonogamy or increased autogamy) under herbivore/florivore damage represents an important prospect for research. For instance, it would be interesting to investigate whether herbivore-mediated increases in selfing result from either lowered attraction (*e.g.* decreased flower size such as documented by Mothershead & Marquis 2000) or reduced herkogamy, or both. This would be particularly important when the pathway to increased selfing depends on alteration in resource allocation to reproduction following damage (*e.g.* attack by spittlebugs resulting in doubled selfing in *Mimulus guttatus*; Ivey & Carr 2005).

Species characteristics may indeed interact with damage by antagonists and constrain the response to one direction or another, even though decreased geitonogamy and increased autogamy are not mutually exclusive processes. For instance, in the work presented here, pollen production is an important factor influencing selfing.

Standardised partial regression coefficients suggest that both pollen production and damage by weevils affect selfing with similar strength. Because stamens are close to stigmas, the damage-driven decrease in pollinator visitation results in higher selfing rates *via* autogamy. This may also have another consequence on plant reproductive success: pollen falling from stamens onto the stigmas might be eliminated from the pool available for export by pollinators, therefore potentially leading to pollen discounting in addition to the direct loss due to bud clipping. High levels of selfing, however, are not always associated with pollen discounting, as long as highly selfing genotypes are also the most successful sires (Rausher *et al.* 1993). The interactive effect of florivore damage, selfing and outcross siring success is an area that is wide open for study.

The present results suggest a role for weevils in increasing autogamy of hermaphrodites, and thus incur a reproductive cost *via* selfing and inbreeding depression; this is predicted to contribute to a female advantage (obligate outcrossers) and to favourably select for gynodioecy (Ashman 2002) if everything else is equal. Moreover, our work joins a growing number of studies demonstrating that herbivory can have important influences on the expression of mating system and potentially impact the evolution of mating systems. The effect of florivory (as opposed to folivory), nevertheless, is not well described with regard to its implication on mating system evolution. More data are needed before we can conclude that general effects of herbivory do not differ between folivory and florivory. We have clarified two ways that direct flower antagonists can bring about opposite changes in plant mating systems, and stress that the outcome of this plant–antagonist interaction will depend on the importance of the various modes of selfing available to a plant.

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## REFERENCES

- Adler L.S., Bronstein J.L. (2004) Attracting antagonists: does floral nectar increase leaf herbivory? *Ecology*, **85**, 1519–1526.
- Ashley M.V., Wilk J.A., Styan S.M.N., Craft K.J., Jones K.L., Feldheim K.A., Lewers K.S., Ashman T.-L. (2003) High variability and disomic segregation of microsatellites in the octoploid *Fragaria virginiana* Mill. (Rosaceae). *Theoretical and Applied Genetics*, **107**, 1201–1207.
- Ashman T.-L. (1999) Determinants of sex allocation in a gynodioecious wild strawberry: implications for the evolution of dioecy and sexual dimorphism. *Journal of Evolutionary Biology*, **12**, 648–661.
- Ashman T.-L. (2000) Pollinator selectivity and its implications for the evolution of dioecy and sexual dimorphism. *Ecology*, **81**, 2577–2591.
- Ashman T.-L. (2002) The role of herbivores in the evolution of separate sexes from hermaphroditism. *Ecology*, **83**, 1175–1184.
- Ashman T.-L. (2003) Constraints on the evolution of males and sexual dimorphism: field estimates of genetic architecture of reproductive traits in three populations of gynodioecious *Fragaria virginiana*. *Evolution*, **57**, 2012–2025.
- Ashman T.-L. (2006) The evolution of separate sexes: a focus on the ecological context. In: Barrett S.C.H., Harder L.D. (Eds), *The Ecology and Evolution of Flowers*. Oxford University Press, Oxford, UK: 419–465.
- Ashman T.-L., Hitchens M.S. (2000) Dissecting the causes of variation in intra-inflorescence allocation in a sexually polymorphic species, *Fragaria virginiana* (Rosaceae). *American Journal of Botany*, **87**, 197–204.
- Ashman T.-L., Penet L. (2007) Direct and indirect effects of a sex-biased antagonist on male and female fertility: consequences for reproductive trait evolution in a gender-dimorphic plant. *The American Naturalist*, **169**, 595–608.
- Ashman T.-L., Cole D.H., Bradburn M. (2004) Sex-differential resistance and tolerance to herbivory in a gynodioecious wild strawberry. *Ecology*, **85**, 2550–2559.
- Avila-Sakar G., Leist L.L., Stephenson A.G. (2003) Effects of the spatial pattern of leaf damage on growth and reproduction: nodes and branches. *Journal of Ecology*, **91**, 867–879.
- Barrett S.C.H., Harder L.D. (1996) Ecology and evolution of plant mating. *Trends in Ecology and Evolution*, **11**, 73–79.
- Barrett S.C.H., Harder L.D., Worley A.C. (1996) The comparative biology of pollination and mating in flowering plants. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **351**, 1271–1280.
- Brunet J., Sweet H.R. (2006) Impact of insect pollinator group and floral display size on outcrossing rate. *Evolution*, **60**, 234–246.
- Cariveau D., Irwin R.E., Brody A.K., Garcia-Mayeya L.S., von der Ohe A. (2004) Direct and indirect effects of pollinators and seed predators to selection on plant and floral traits. *Oikos*, **104**, 15–26.
- Carr D.E., Eubanks M.D. (2002) Inbreeding alters resistance to insect herbivory and host plant quality in *Mimulus guttatus* (Scrophulariaceae). *Evolution*, **56**, 22–30.
- Charlesworth D., Charlesworth B. (1987) Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics*, **18**, 237–268.
- Crawford T.J. (1984) What is a population? In: Shorrocks B. (Ed), *Evolutionary Ecology*. Blackwell Scientific Publications, Oxford: 135–173.
- Darwin C.R. (1876) *The Effects of Cross- and Self-Fertilisation in the Vegetable Kingdom*. Murray, London.

- Dufay M., Anstett M.-C. (2003) Conflicts between plants and pollinators that reproduce within inflorescences: evolutionary variations on a theme. *Oikos*, **100**, 3–14.
- Elle E., Hare J.D. (2002) Environmentally induced variation in floral traits affects the mating system in *Datura wrightii*. *Functional Ecology*, **16**, 79–88.
- Galloway L.F., Cirigliano T., Gremski K. (2002) The contribution of display size and dichogamy to potential geitonogamy in *Campanula americana*. *International Journal of Plant Sciences*, **163**, 133–139.
- Glover D.E., Barrett S.C.H. (1986) Variation in the mating system of *Eichhornia paniculata* (Spreng.) Solms. (Pontederiaceae). *Evolution*, **40**, 1122–1131.
- Gomez J.M. (2003) Herbivory reduces the strength of pollinator-mediated selection in the Mediterranean herb *Erysimum mediohispanicum*: consequences for plant specialization. *The American Naturalist*, **162**, 242–256.
- Goodwillie C., Kalisz S., Eckert C.G. (2005) The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. *Annual Review of Ecology, Evolution and Systematics*, **36**, 47–79.
- Hayes C.N., Winsor J.A., Stephenson A.G. (2004) Inbreeding influences herbivory in *Cucurbita pepo* ssp. *texana* (Cucurbitaceae). *Oecologia*, **140**, 601–608.
- Hill W.G. (1974) Estimation of linkage disequilibrium in randomly mating populations. *Heredity*, **33**, 229–239.
- Holsinger K.E. (1991) Mass-action models of plant mating systems: the evolutionary stability of mixed mating systems. *The American Naturalist*, **138**, 606–622.
- Holsinger K.E. (2000) Reproductive systems and evolution in vascular plants. *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 7037–7042.
- Hull-Sanders H.M., Eubanks M.D. (2005) Plant defense theory provides insight into interactions involving inbred plants and insect herbivores. *Ecology*, **86**, 897–904.
- Irwin R.E. (2006) The consequences of direct versus indirect species interactions to selection on traits: pollination and nectar robbing in *Ipomopsis aggregata*. *The American Naturalist*, **167**, 315–328.
- Ivey C.T., Carr D.E. (2005) Effects of herbivory and inbreeding on the pollinators and mating system of *Mimulus guttatus* (Phrymaceae). *American Journal of Botany*, **92**, 1641–1649.
- Ivey C.T., Carr D.E., Eubanks M.D. (2004) Effects of inbreeding in *Mimulus guttatus* on tolerance to herbivory in natural environments. *Ecology*, **85**, 567–574.
- Jones R.W., Peruyero D.B. (2002) Reproductive ecology of two species of the *Anthonomus grandis* species group (Coleoptera: Curculionidae) on *Hampea* (Malvaceae: Gossypieae) host plants in southern Mexico. *Environmental Entomology*, **31**, 693–701.
- de Jong T.J., Waser N.M., Klinkhamer P.G.L. (1993) Geitonogamy: the neglected side of selfing. *Trends in Ecology and Evolution*, **8**, 321–325.
- Juenger T., Bergelson J. (2000) Does early season browsing influence the effect of self-pollination in scarlet gilia? *Ecology*, **81**, 41–48.
- Karron J.D., Mitchell R.J., Holmquist K.G., Bell J.M., Funk B. (2004) The influence of floral display size on selfing rates in *Mimulus ringens*. *Heredity*, **92**, 242–248.
- Keim P., Paige K.N., Whitham T.G., Lark K.G. (1989) Genetic analysis of an interspecific hybrid swarm of *Populus*: occurrence of unidirectional introgression. *Genetics*, **123**, 557–565.
- van Kleunen M., Ritland K. (2005) Estimating heritabilities and genetic correlations with marker-based methods: an experimental test in *Mimulus guttatus*. *Journal of Heredity*, **96**, 368–375.
- Klinkhamer P.G.L., de Jong T.J., de Bruyn G.-J. (1989) Plant size and pollinator visitation in *Cynoglossum officinale*. *Oikos*, **54**, 201–204.
- Kovach J., Rieckenberg R., English-Loeb G., Pritts M. (1999) Oviposition patterns of the strawberry bud weevil (Coleoptera: Curculionidae) at two spatial scales and implications for management. *Journal of Economic Entomology*, **92**, 1358–1363.
- Krupnick G.A., Weis A.E. (1999) The effect of floral herbivory on male and female reproductive success in *Isomeris arborea*. *Ecology*, **80**, 135–149.
- Krupnick G.A., Weis A.E., Campbell D.R. (1999) The consequences of floral herbivory for pollinator service to *Isomeris arborea*. *Ecology*, **80**, 125–134.
- Lande R., Schemske D.W. (1985) The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution*, **39**, 24–40.
- Leavitt H., Robertson I.C. (2006) Petal herbivory by chrysomelid beetles (*Phyllotreta* sp.) is detrimental to pollination and seed production in *Lepidium papilliferum* (Brassicaceae). *Ecological Entomology*, **31**, 657–660.
- Lehtilä K.P., Strauss S.Y. (1997) Leaf damage by herbivores affects attractiveness to pollinators in wild radish, *Raphanus raphanistrum*. *Oecologia*, **111**, 396–403.
- Lewers K.S., Styan S.M.N., Hokanson S.C., Bassil N.V. (2005) Strawberry GenBank-derived and genomic simple sequence repeat (SSR) markers and their utility with strawberry, blackberry, and red and black raspberry. *Journal of the American Society for Horticultural Science*, **130**, 102–115.
- Lloyd D.G. (1992) Self- and cross-fertilization in plants. II. The selection of self-fertilization. *International Journal of Plant Sciences*, **153**, 370–380.
- Lloyd D.G., Schoen D.J. (1992) Self- and cross-fertilization in plants. I. Functional dimensions. *International Journal of Plant Sciences*, **153**, 358–369.
- McCall A.C., Irwin R.E. (2006) Florivory: the intersection of pollination and herbivory. *Ecology Letters*, **9**, 1351–1365.
- Morgan M.T., Barrett S.C.H. (1990) Outcrossing rates and correlated matings in *Eichhornia paniculata* (Pontederiaceae). *Heredity*, **64**, 271–280.
- Mothershead K., Marquis R.J. (2000) Fitness impacts of herbivory through indirect effects on plant–pollinator interactions in *Oenothera macrocarpa*. *Ecology*, **81**, 30–40.

- Quesada M., Bollman K., Stephenson A.G. (1995) Leaf damage decreases pollen production and hinders pollen performance in *Cucurbita texana*. *Ecology*, **76**, 437–443.
- Rausher M.D., Augustine D., Vanderkooi A. (1993) Absence of pollen discounting in a genotype of *Ipomoea purpurea* exhibiting increased selfing. *Evolution*, **47**, 1688–1695.
- Ritland K. (1989) Correlated matings in the partial selfer *Mimulus guttatus*. *Evolution*, **43**, 848–859.
- Ritland K. (2002) Extensions of models for the estimation of mating systems using  $n$  independent loci. *Heredity*, **88**, 221–228.
- Schoen D.J., Lloyd D.G. (1992) Self- and cross-fertilization in plants. III. Methods for studying modes and functional aspects of self-fertilization. *International Journal of Plant Sciences*, **153**, 381–393.
- Schutzenhofer M.R. (2007) The effect of herbivory on the mating system of congeneric native and exotic *Lespedeza* species. *International Journal of Plant Sciences*, **168**, 1021–1026.
- Staudt G. (1989) The species of *Fragaria*, their taxonomic and geographical distribution. *Acta Horticulturae*, **265**, 23–33.
- Steets J.A., Ashman T.-L. (2004) Herbivory alters the expression of a mixed-mating system. *American Journal of Botany*, **91**, 1046–1051.
- Steets J.A., Hamrick J.L., Ashman T.-L. (2006) Consequences of vegetative herbivory for maintenance of intermediate outcrossing in an annual plant. *Ecology*, **87**, 2717–2727.
- Steets J.A., Wolf D.E., Auld J.R., Ashman T.-L. (2007) The role of natural enemies in the expression and evolution of mixed mating in hermaphroditic plants and animals. *Evolution*, **61**, 2043–2055.
- Strauss S.Y. (1997) Floral characters link herbivores, pollinators, and plant fitness. *Ecology*, **78**, 1640–1645.
- Strauss S.Y., Whittall J.B. (2006) Non-pollinator agents of selection on floral traits. In: Barrett S.C.H., Harder L.D. (Eds), *The Ecology and Evolution of Flowers*. Oxford University Press, Oxford: 120–138.
- Strauss S.Y., Conner J.K., Rush S.L. (1996) Foliar herbivory affects floral characters and plant attractiveness to pollinators: implications for male and female plant fitness. *The American Naturalist*, **147**, 1098–1107.
- Strauss S.Y., Conner J.K., Lehtilä K.P. (2001) Effects of foliar herbivory by insects on the fitness of *Raphanus raphanistrum*: damage can increase male fitness. *The American Naturalist*, **158**, 496–504.
- Vogler D.W., Kalisz S. (2001) Sex among the flowers: the distribution of plant mating systems. *Evolution*, **55**, 202–204.
- Wright J.W., Meagher T.R. (2003) Pollination and seed predation drive flowering phenology in *Silene latifolia* (Caryophyllaceae). *Ecology*, **84**, 2062–2073.
- Zamora R., Hódar J.A., Gómez J.M. (1999) Plant–herbivore interaction: beyond a binary vision. In: Pugnaire F., Valladares F. (Eds), *Handbook of Functional Plant Ecology*. Dekker, New York: 677–718.