

Herbivory as an agent of natural selection for floral-sex ratio in horsenettle (*Solanum carolinense*)

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ABSTRACT

Hypothesis: Floral herbivory can affect the strength or pattern of natural selection for floral-sex ratio in a host plant with sexually dimorphic flowers.

Organisms: The andromonoecious herb horsenettle (*Solanum carolinense*) and its main floral herbivore, the potato bud weevil (*Anthonomus nigrinus*).

Methods: In a controlled experiment with potted plants, we exposed a group of 27 plants to simulated-weevil herbivory on 50% of their flower buds, the florivory rate found in a field study. A control group of 27 plants received no florivory. We used phenotypic-selection analyses to look for directional, disruptive, and stabilizing selection on floral-sex ratio in the two groups and to determine whether florivory changed the optimum ratio.

Results: In the absence of florivory, directional selection acted to increase the percentage of male flowers, at least to a ratio of 67% males and 33% perfect flowers. Florivory changed the pattern to stabilizing selection, with an optimum of 9% male and 91% perfect flowers.

Keywords: andromonoecy, floral-sex ratio, florivory, phenotypic-selection analysis, plant-breeding system.

INTRODUCTION

Evolutionary ecologists have long been interested in the impact of herbivores on the evolution of defence traits in their host plants (Ehrlich and Raven, 1964; Rausher, 1996). The set of plant traits hypothesized to be influenced by herbivores is steadily growing, with a recent emphasis on the subtle but significant roles herbivores may play in the reproductive strategies of plants. For instance, herbivory can have different effects on plant fitness through paternal and maternal reproductive routes (Strauss *et al.*, 1996; Agrawal *et al.*, 1999). Herbivores with dioecious hosts often preferentially attack one sex over the other (Bawa and Opler, 1978; Boecklen and Hoffman, 1993), and herbivores with monoecious hosts may prefer parts bearing female (or male) structures (Muenchow and Delesalle, 1992; Cobb *et al.*, 2002). In addition, some

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monoecious and andromonoecious plants have been shown to alter their floral-sex ratios in response to herbivore damage (Hendrix and Trapp, 1981; Hendrix, 1984; Krupnick and Weis, 1998; Biernacki and Lovett Doust, 2002). Similarly, Steets and Ashman (2004) showed that herbivory can alter the ratio of cleistogamous to chasmogamous flowers in a plant with a mixed-mating system. Clearly, herbivores can affect the ecology of plant reproduction in several interesting ways.

Several authors have recently suggested that herbivores may play a role not only in the ecology of host-plant reproduction, but also in the evolution of plant-breeding systems (Marshall and Ganders, 2001; Collin *et al.*, 2002). For instance, Muenchow (1998) and Ashman (2002) hypothesized that male-biased herbivory may facilitate the evolution of gynodioecy and dioecy from hermaphroditic ancestors. What is currently most needed to support any hypothesized evolutionary role for herbivores is empirical evidence that herbivores actually act as agents of natural selection on the sexual strategies of their hosts. Evidence of sex-biased herbivore attack and phenotypic plasticity in host-plant sex expression are suggestive of a potential evolutionary role. However, to conclude that a herbivore is an agent of selection requires a demonstration that the relationship between plant fitness and a component of a plant's sexual strategy depends on whether the herbivore is present or absent. Here, we report on such an experiment in which simulated floral herbivory had a major effect on the pattern of selection for floral-sex ratio in the andromonoecious plant *Solanum carolinense* L. (Solanaceae).

MATERIALS AND METHODS

Natural history

Solanum carolinense, or horsenettle, is a perennial herbaceous weed native to the southeastern United States (Bassett and Munro, 1986). Its flowers are borne on racemes that average 7–8 flowers each, and an individual ramet (stem) may possess from none to over a dozen racemes (Wise and Cummins, 2006). Within a ramet, the maturation of racemes is staggered over several weeks. Flower buds mature sequentially from the base to the tip of racemes, and each flower is open for 3–4 days. Horsenettle is often characterized as weakly andromonoecious, with mostly perfect (hermaphroditic) flowers and a small, variable proportion of male (staminate) flowers, often concentrated near the end of racemes (Elle, 1998; Steven *et al.*, 1999). A similar pattern of andromonoecy is found in a large number of species in the genus *Solanum* (Whalen and Costich, 1986; Diggle, 1991). The floral-sex ratio of horsenettle is a genetically controlled trait, with heritabilities in previous studies ranging from 0.25 to 0.50 (Elle, 1998; Wise *et al.*, in press). Thus, although environmental conditions can influence the ratio (Solomon, 1985), the floral-sex ratio is a trait that can evolve in response to natural selection.

Although potentially fertile, often a relatively large fraction of a ramet's perfect flowers do not set fruit, suggesting a strategy of 'reserve ovaries' to compensate for damaged or unpollinated flowers (Solomon, 1985; Wise and Cummins, 2002, 2006). When mature, the fruits are roughly spherical, yellow berries with a diameter usually of 1–2 cm and an average of about 150 seeds.

A large proportion of horsenettle's flowers are regularly destroyed by herbivores, primarily by *Anthonomus nigrinus* Boheman (Curculionidae), the potato bud weevil (Wise, 2003, 2007). A female weevil lays her eggs in immature flower buds, then chews through the buds' pedicels, killing the flowers before they have a chance to open (Wise and Cummins, 2002). At this stage in bud development, sex differences are not generally apparent even upon

dissection, as the pistils of perfect flowers are not yet elongated. Therefore, it is unlikely that floral sex has much to do with weevil oviposition decisions (i.e. there is no sex-biased herbivory). Horsenettle's floral-sex ratio can show a small plastic response to florivory, however. A florivory rate of 50% has been shown to decrease the proportion of male flowers by about 6% (Wise and Cummins, 2006).

Experimental design

This study was performed in 1997–1998 at the University of Virginia's Blandy Experimental Farm in Boyce, VA, USA. In May 1997, roots from 26 horsenettle genets (i.e. genetic individuals) were collected from an old-field population and were grown in pots in a commercial growing medium (WESCO growing media III®, Wetsel Seed Company, Harrisonburg, VA) through autumn. New roots from these plants were refrigerated over the winter and served as the source of plants in 1998. In the spring of 1998, individual ramets were propagated in a greenhouse in 3.8-litre pots from bare root cuttings of nine randomly selected genets. After one month in the greenhouse, six healthy ramets of each of the nine genets were transplanted into 7.6-litre plastic pots. Tomato cages were attached to the pots and covered with fine mesh bags, and the pots were placed in rows in randomized positions on wooden pallets outdoors in an unshaded area. Three ramets from each genet were randomly assigned to a 50% simulated-weevil florivory treatment, and three to a no-florivory control.

To simulate weevil florivory, we cut off the first half of the buds to mature on each raceme as each bud reached the size at which weevils would find it acceptable for oviposition. While the pattern of bud clipping did not necessarily mimic weevil behaviour, it allowed the flower-bud loss to be spread out spatially and temporally, which does occur in the field. Moreover, it allowed the pattern to be consistent among all the treatment plants. It is important to note that plant reactions to simulated herbivory are not always identical to actual herbivory, particularly if real herbivore damage accrues gradually (Baldwin, 1990; Hjäältén, 2004; Lehtilä and Boalt, 2004). However, potato bud weevils clip buds rapidly after oviposition, and all of the larval feeding occurs after the buds have been severed. Therefore, horsenettle's reaction to actual weevil florivory and our method of flower-bud clipping are likely to be very similar.

Because the mesh bags precluded access by pollinators, the flowers had to be hand pollinated to set fruit. Each ramet was checked every 2–3 days, and all open flowers were hand pollinated with pollen collected daily from a mixture of horsenettle genets in field populations at Blandy Farm. [Details on the pollination technique can be found in Wise and Cummins (2002, 2006).] A separate experiment conducted concurrently at Blandy Farm revealed that fruit set and seed production were not pollen limited in the field, regardless of the level of florivory (Wise and Cummins, 2002). Each flower was recorded as either male or perfect and, following convention, we defined a ramet's floral-sex ratio as the percentage of its flowers that were male.

The number of seeds a ramet produced served as its fitness measure. To estimate the number of seeds, we measured the diameter of each fruit in late October, after all fruits had ripened. To determine the relationship between fruit diameter and seed number, we dissected 114 fruits with diameters between 6 and 24 mm and counted their seeds. A regression of seed number on fruit diameter (d) yielded the following prediction equation: $\text{Seeds} = 70.1 - 23.0d + 2.18d^2 - 0.0415d^3$ ($P < 0.0001$; $r^2 = 0.90$). Because horsenettle is

obligately outcrossing, the number of seeds a ramet produces represents the maternal component of fitness only. Male and perfect flowers of horsenettle have been found to contain equivalent amounts of pollen and to be equally attractive to pollinators (Solomon, 1986); therefore, floral-sex ratio itself is not likely to affect paternal fitness (Vallejo-Marín and Rausher, 2007; but see Elle and Meagher, 2000). Consequently, any impact of herbivory on selection for floral-sex ratio is not likely to occur through the paternal route.

Selection analysis

To assess the influence of florivory on selection for floral-sex ratio, we employed standard phenotypic-selection analyses, which involve regressions of fitness on floral-sex ratio to determine linear-selection differentials (Lande and Arnold, 1983; Brodie *et al.*, 1995). We also performed selection analyses including both floral-sex ratio and the square of floral-sex ratio to determine quadratic-selection differentials. A negative quadratic coefficient would be consistent with stabilizing selection and an intermediate optimum ratio, while a positive quadratic coefficient would be consistent with disruptive selection. Separate regressions were run for the simulated-florivory and control groups. The floral-sex ratios were arcsine-square-root transformed prior to the analyses to better meet distributional assumptions of the regressions. (Analyses with untransformed ratios provided nearly identical results.) The ratios were standardized to a mean of zero within each treatment and expressed in units of standard deviation from the treatment mean. This standardization provided selection differentials whose magnitudes can readily be compared between the two treatment groups.

Traditional phenotypic-selection analyses are at risk of bias due to potential covariances of both the trait being analysed and fitness with an unmeasured variable in the environment (Rausher, 1992; Mauricio and Mojonner, 1997). A solution is to use a genotypic analysis, which employs breeding-value, family, or genet means in the regressions. However, genotypic analyses were not practical in this study because it only involved nine genets. Nevertheless, the design of the experiment minimized differences among plants, thus making environmental bias in the phenotypic analyses unlikely (Stinchcombe *et al.*, 2002). In addition, we were able to incorporate some genetic information into the phenotypic analyses by including genet as a categorical variable in the regressions (cf. Pilson, 1996).

Finally, we performed a selection analysis with combined data for both treatments and included treatment as a categorical variable. A significant treatment $\times$ floral-sex ratio interaction term in this model would indicate that florivory significantly altered the pattern or strength of selection on floral-sex ratio.

RESULTS

In the control group, the floral-sex ratio of the 27 ramets ranged from 0 to 69% male flowers, with a mean and standard deviation of $13 \pm 16\%$ male. The floral-sex ratio in the simulated florivory group was slightly lower, with a range of 0–39% male flowers and a mean ratio of $8 \pm 11\%$ male. This result indicates an average plastic shift in floral-sex ratio of 5% in response to the loss of 50% of the flower buds.

The 54 ramets opened between 3 and 130 flowers, and the ramets that opened the most flowers tended to have the greatest proportions of male flowers, as the correlation coefficient, r , between flowers opened and floral-sex ratio was 0.66 ($P < 0.0001$) (Fig. 1). While the ramet with 130 flowers was an outlier in terms of flowers opened, it was not

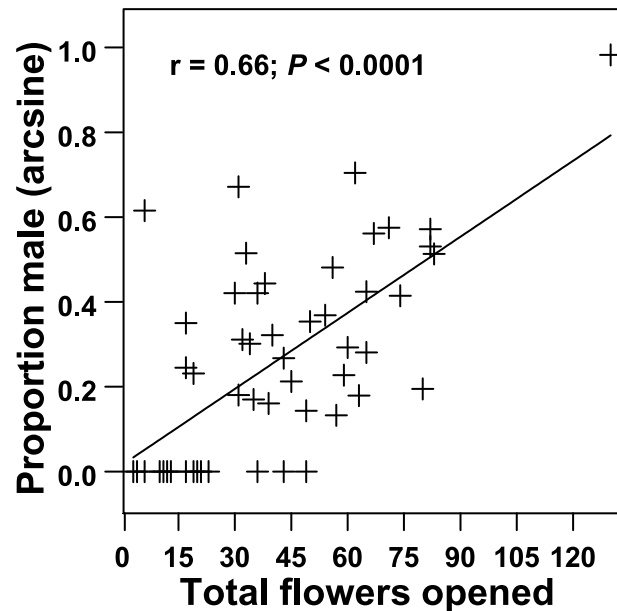


Fig. 1. The ramets' floral-sex ratios (arcsine square root-transformed proportion of male flowers) and the total number of flowers they opened were strongly positively correlated. $N = 54$ ramets; simulated-weevil florivory and no-florivory control groups combined.

particularly influential on r . With this ramet removed, the correlation was still strongly positive ($r = 0.57$, $P < 0.0001$).

In the simulated-florivory group, plants experienced significant stabilizing selection, but not directional selection, on floral-sex ratio (Table 1; Fig. 2A). The ratio at which seed production was maximized was 9% male flowers. The pattern of selection in the no-florivory control group was quite different: plants experienced significant directional selection for an increased floral-sex ratio (Fig. 2B). Stabilizing selection was only marginally significant in the no-florivory group ($F_{1,24} = 2.9245$, $P = 0.10$). Still, the apparent curvature in the relationship suggests that seed production would be maximized at a ratio of 67% male flowers in the absence of florivory. Finally, the combined regression analysis showed that selection acting on floral-sex ratio was significantly different between the two treatments (treatment $\times$ floral-sex ratio interaction: $F_{1,42} = 5.5671$, $P = 0.023$).

DISCUSSION

Horsenettle ramets with simulated florivory experienced stabilizing selection for floral-sex ratio towards an optimum of 9% male flowers. Without florivory, the plants instead experienced strong directional selection to increase the proportion of male flowers, at least up to 67% male flowers. Because the only difference between the two groups was the simulated florivory, we conclude that florivory can substantially alter the pattern of selection on floral-sex ratio in horsenettle. This result offers some of the clearest evidence to date that herbivory can impose natural selection for a key breeding-system attribute in a host plant.

Table 1. Standardized linear (β) and quadratic (γ) selection differentials for floral-sex ratio

Treatment	Selection differentials $\pm$ 1 standard error		Optimal ratio
	β	γ	
Florivory	0.039 ± 0.066 ($P = 0.57$)	-0.188 ± 0.059 ($P = 0.0058$)	9% male: 91% perfect
No florivory	0.288 ± 0.076 ($P = 0.0009$)	-0.099 ± 0.058 ($P = 0.10$)	67% male: 33% perfect

Notes: Plant genet ($n = 9$) was used as a categorical variable in the regressions. The model and error sums of squares (and degrees of freedom) for the linear selection models were 5.553 (9) and 1.061 (17) for the florivory treatment, and 3.789 (9) and 2.151 (17) for the no-florivory control, respectively. The model and error sums of squares (and degrees of freedom) for the quadratic selection models were 5.956 (10) and 0.650 (16) for the florivory treatment, and 3.889 (10) and 2.051 (16) for the no-florivory control, respectively.

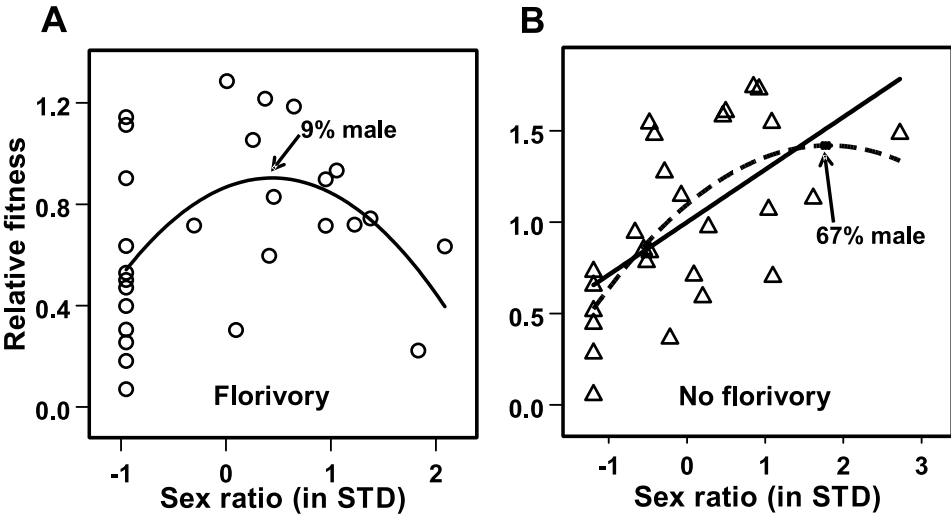


Fig. 2. Selection on floral-sex ratio. (A) Ramets exposed to simulated weevil florivory on 50% of their flower buds. (B) Ramets were not exposed to any florivory. The x-axes show the floral-sex ratios (i.e. the proportion of a ramet’s flowers that were male) in units of standard deviations (STD) from the mean for each group. Each point represents an individual ramet, and the solid lines represent statistically significant patterns of natural selection. Arrows mark the ratios with the maximum fitness as predicted from the quadratic selection models.

Regardless of whether the plants experienced simulated florivory, selection acted against plants with low ratios (i.e. high proportions of perfect flowers). This result may seem counterintuitive because, all else being equal, ramets with a greater proportion of perfect flowers should be able to produce more seeds. However, not all else was equal: the number of flowers a ramet produced was positively correlated with its floral-sex ratio. In fact, plants with the fewest flowers in total tended to produce only perfect flowers. With few ovaries available to set fruit, these low sex-ratio plants were incapable of producing many seeds, even when none of the flower buds were lost to florivory. Therefore, selection against low

floral-sex ratios in both treatments was likely an indirect effect of correlated selection against low flower production in general.

The critical difference in the patterns of selection between the two treatments occurred at the high floral-sex ratios. Plants with a high proportion of male flowers were only at a disadvantage if the plants experienced florivory. This disadvantage can best be understood in terms of ovary limitations and opportunity costs. If a plant loses a substantial proportion of its flowers to herbivory, then its reproduction is more likely to be ovary limited, and the production of a male flower instead of a perfect flower costs the plant an opportunity for setting a fruit. Therefore, selection would be expected to act against ovary-limited plants with higher floral-sex ratios, as was seen in the florivory treatment in this study. Without florivory, reproduction is less likely to be ovary limited, and a plant will incur no reproductive opportunity cost as a result of producing additional male flowers instead of surplus perfect flowers (i.e. flowers that will not set fruit). Thus, we did not observe selection against high floral-sex ratios in the control group.

It is worth emphasizing that the selection coefficients presented in this study are selection differentials rather than gradients, and as such they estimate the total, net selection on floral-sex ratio rather than just direct selection on floral-sex ratio (Brodie *et al.*, 1995). As described above, part of the pattern we observed was likely an indirect result of selection on flower number. A future study is planned to distinguish direct and indirect selection on floral-sex ratio in the field. In the current study, our interest was in isolating the potential effects of florivory on the total strength and pattern of selection for floral-sex ratio.

It is also important to note that fitness (seeds produced) in the selection analysis was a measurement of reproduction through the maternal route only. Because self-fertilization does not occur in horsenettle, and because the mesh bags in this experiment prevented pollen export, the plants did not sire any seeds. Nevertheless, it is of interest to speculate how paternal fitness might be affected by florivory. In an andromonoecious plant, all flowers produce pollen, and previous studies have found that male and perfect flowers of horsenettle produce equivalent quantities and quality of pollen (Solomon, 1985, 1986; Vallejo-Marín and Rausher, 2007). Therefore, florivory in an andromonoecious plant may be expected to decrease a plant's paternal fitness to the same extent regardless of what proportion of the flowers are male versus perfect. Moreover, the opportunity cost observed for the florivory-treatment plants is exacted in the currency of maternal fitness only. Thus, an inclusion of paternal fitness would not be expected to affect the patterns of selection for floral-sex ratio.

Some data on florivory of horsenettle and floral-sex ratios in field populations are available for comparison with the results of this experiment. In a field study of the horsenettle population from which the plants used in the current experiment originated, florivores destroyed 50–52% of horsenettle's flowers (Wise, 2003). The results of the current study suggest that the optimal floral-sex ratio for these plants would be 9% male. Indeed, at 8% male, the mean floral-sex ratio of the ramets in the florivory treatment was nearly identical to this optimum.

In most published surveys of horsenettle populations, less than a quarter of the flowers are male (Elle, 1998; Steven *et al.*, 1999). However, Solomon (1985) reported a mean ratio of 71% male flowers in a horsenettle population in Indiana. A major difference between this population and the field populations in Virginia is an apparent lack of florivory in the Indiana population. It is interesting to note that the floral-sex ratio of 71% males in Indiana is very close to the optimum ratio of 67% males suggested by the no-florivory control group of our experiment. While such comparisons are necessarily speculative, the similarities between the

optimal floral-sex ratios observed in the present study and the ratios found in field populations with similar florivory levels suggest that floral-sex ratios may have evolved at least partly in response to selection from floral herbivores.

Similar to some previous studies on other andromonoecious plants, the floral-sex ratio showed a plastic response to florivory (Hendrix and Trapp, 1981; Hendrix, 1984; Krupnick and Weis, 1998). Specifically, when buds were lost to florivory, the plants tended to make more perfect flowers instead of male flowers, which could help the plants compensate for the loss of ovaries and maintain seed production (Wise and Cummins, 2006). This decrease in floral-sex ratio in response to florivory was in the same direction as the change in the selective-optimum ratio when florivory is present. However, the change allowed by plasticity is very small compared with the differences in selective optima. Specifically, the optimum floral-sex ratio increased 58% (from 9% to 67% males) when plants went from 50% to zero florivory, while plasticity only allowed a 5% increase (from 8% to 13% males). In the field, if florivore populations crashed, or if horsenettle were to evolve resistance to florivory, plasticity alone could not get the plants to the optimum ratio. Nevertheless, with ample genetic variation for floral-sex ratio, horsenettle could evolve relatively rapidly in response to changes in selection for floral-sex ratio.

In conclusion, this study is a significant advance in elucidating the role of herbivores on the evolution of plant-breeding systems in at least three ways. First, phenotypic-selection analyses provided much needed empirical evidence that natural levels of herbivory can alter greatly the pattern of selection on a key attribute of a plant's breeding system. Second, unlike previous scenarios, the mechanism by which herbivores affected selection for floral-sex ratio does not require any sex-biased feeding preference on the part of the herbivores. Finally, this study expands the proposed realm of influence of herbivores to a wider variety of plant-breeding systems. While previous reports highlighted the potential role of herbivores in the pathway of the evolution of dioecy through gynodioecy, this study suggests that herbivores may also play an important role in the evolution of floral-sex ratios in the roughly 20% of angiosperm species (Bertin, 1982) that are andromonoecious, monoecious, or gynomonoecious.

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