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Direct and Indirect Effects of a Sex-Biased Antagonist on Male and Female Fertility: Consequences for Reproductive Trait Evolution in a Gender-Dimorphic Plant

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ABSTRACT: Gender-dimorphic plants are often subject to sex-differential enemy attack, but whether and how this contributes to trait evolution is unknown. To address this gap, we documented the spatiotemporal prevalence of sex-biased weevil damage in a gynodioecious strawberry. We then conducted path analysis to evaluate the direct and indirect pathways for weevils to affect female and male fertility and to mediate selection in two experimental gardens. Direct effects of weevils significantly reduced fertility and mediated selection on reproductive traits, even in the nonpreferred sex (females). Weevils significantly reduced floral display size in hermaphrodites in both gardens, and this translated into a substantial negative indirect effect on male fertility in the garden where the pathway to fertility via display was stronger. Thus, indirect effects of weevils can contribute to selection in hermaphrodites, which gain the majority of their fitness via male function. Our results also indicate that weevils often play a larger role than pollinators in shaping reproductive phenotype and thus raise the intriguing possibility that antagonists may be drivers of sexual dimorphism. Finally, our results support the view that mutualists, antagonists, and the abiotic environment should be considered when attempting to understand reproductive trait evolution in gender-dimorphic species.

Keywords: dioecy, florivory, natural selection, plant-animal interactions, pollination, sexual dimorphism.

It is generally acknowledged that antagonists, such as herbivores, can have direct as well as indirect negative effects

on fitness in hermaphrodite plants (Gronemeyer et al. 1997; Galen and Cuba 2001; Strauss et al. 2001; Herrera et al. 2002; Gomez 2003). In addition, several authors have suggested that enemies may be important in sexual system evolution (e.g., Bawa and Opler 1978; Cox 1982; Muenchow 1998; Ashman 2002); however, no study has characterized the direct and indirect effects of an antagonist in a gender-dimorphic plant (e.g., hermaphrodites and females). In sex-dimorphic plants, not only does damage differ between the morphs (Muenchow 1998; Ågren et al. 1999; Ashman 2002; Cornelissen and Stiling 2005), but also it varies within morphs (Ashman et al. 2004; Verdu et al. 2004; Hemborg and Bond 2005). So, herbivore-mediated selection is possible both across and within sex morphs. In fact, because antagonist-mediated selection can oppose pollinator- or resource-mediated selection (Armbruster 1997; Adler et al. 2001; Cariveau et al. 2004; Strauss and Irwin 2004; Irwin 2006), sex-differential selection via herbivores could also be an important driver of sexual dimorphism (Hemborg and Bond 2005). Currently, we have an incomplete understanding of whether sex-biased enemy attack differentially impacts realized male and female fertility, whether attack has direct and/or indirect effects on fertility, and whether it contributes to differential trait evolution in the sex morphs.

Sex-differential effects of antagonists could come about via direct or indirect pathways or both. Direct effects of antagonists include reduced quantity or quality of gametes (e.g., Mothershead and Marquis 2000). However, the consequence of direct effects for male and female fertility will depend on the degree to which fertility via a given sex is gamete limited. For instance, if females are not mate limited but are gamete production limited, then the loss of ovules would directly result in reduced female fertility. On the other hand, if pollen is produced in huge excess, the loss of some pollen to florivores may have little or no impact on realized male fertility. Indirect or trait-mediated effects of antagonists can manifest via the action of pollinators (Strauss and Irwin 2004). For instance, damage

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to both leaves and flowers can reduce pollinator visitation by reducing attraction or reward traits, for example, open flowers, petal size, and nectar production (Krupnick et al. 1999; Irwin 2006; Steets et al. 2006). The consequence of this type of indirect effect for female and male fertility will depend not only on how damage intensity affects attraction and pollinator visitation but also on how important pollinator visitation is to fertility. Male fertility is sometimes more dependent on pollinator visitation than is female fertility (e.g., Stanton et al. 1991) but not always (e.g., Ashman and Morgan 2004), and being highly attractive can have negative consequences for outcross siring success (Klinkhamer and de Jong 1993; Klinkhamer et al. 1994). Thus, it may be difficult to predict the total effect of an antagonist on fertility in gender-dimorphic plants based solely on damage differentials. Moreover, only by separating direct and indirect effects of an antagonist can we shed light on the mechanism behind sex-differential fertility effects.

We studied the interactions between a flower-clipping weevil (*Anthonomus signatus*) and a gender-dimorphic wild strawberry (*Fragaria virginiana*) to address these issues. We asked the following questions. First, is weevil antagonism a persistent feature of the wild strawberry system? Second, do weevils have direct and/or indirect effects on realized male and/or female fertility? Third, which reproductive traits are subject to weevil-mediated selection? Finally, how do weevils contribute as agents of selection? To answer the first question, we surveyed weevil damage in wild populations several times over a decade. To answer the remaining questions, we used experimental gardens, paternity analysis, and path analysis combined with structural equation modeling.

Methods

Study System

Fragaria virginiana (Rosaceae), the Virginian wild strawberry, is a spring-flowering perennial herb native to eastern North America (Staudt 1989). Gynodioecy (the occurrence of females and hermaphrodites) in *F. virginiana* is under nuclear control (Ahmadi and Bringham 1989). Females have consistently high fruit set (~90%), whereas hermaphrodites are variable in functional gender (sensu Lloyd 1980) and on average have low fruit set (<20%; Ashman 2003). Flowers are visited by a variety of insects, including small solitary bees (Halictidae, Apidae, Andrenidae, Megachilidae), flies (Syrphidae, Bombyliidae, Conopidae), and ants (Formicinae, Dolichoderinae; Ashman 2000; Ashman and King 2005). Many of these are attracted to plants

with more open flowers, larger petals, and greater nectar and pollen production (Ashman 2000; Ashman et al. 2000).

Anthonomus signatus (Coleoptera, Curculionidae), the strawberry clipper weevil, is a univoltine florivore of strawberry plants in eastern North America (Kovach et al. 1999). Weevil females emerge in early spring, oviposit within unopened flower buds, and then girdle ("clip") the pedicels, leaving the buds to fall to the ground (Kovach et al. 1999). Weevil larvae develop within these unopened buds, and pollen may be a requirement for their successful development (Jones and Peruyero 2002). Adult weevils feed on pollen but cause only minor damage to petals (T.-L. Ashman, personal observation). Weevils attack hermaphrodites more than they do females, and in northwestern Pennsylvania, weevil damage can be extensive (up to 70% of hermaphrodite plants per population; Ashman et al. 2004; this study) and intensive at both patch (mean 30%, range 0%–100% of hermaphrodites damaged per patch; C. L. Collin, L. Penet, and T.-L. Ashman, unpublished data) and plant levels (mean of 37% of buds damaged per hermaphrodite; Ashman et al. 2004). In the wild, weevil damage increases significantly with increasing pollen and flower bud production in hermaphrodites and with increasing flower bud production in females (Ashman et al. 2004).

Wild Population Surveys

To determine whether weevil damage is a consistent feature of wild strawberry populations, we resurveyed five of the northwestern Pennsylvania populations (Housing Railroad, Hartstown, Old Erie Canal, Pymatuning Railroad, and Warren) that were surveyed in 1995 (Ashman et al. 2004). From 2003 to 2006, we ran transects through each population at the end of flowering and recorded plant sex, number of flowers, and clipped buds for 50–100 plants per population. Weevil damage is very distinctive because buds are often left dangling or are found directly below the plant. We estimated damage incidence as the proportion of plants damaged and damage intensity as the proportion of flower buds clipped. We analyzed spatial and temporal variation in damage for the sex morphs separately. Within a sex morph, we conducted ANOVAs on damage incidence or intensity, with population as a fixed factor and year as a random factor (SAS 1996). To compare damage levels between sex morphs, we pooled data across all wild populations and years. All proportions were arcsine-square root transformed before analysis to improve normality.

Experimental Gardens

Garden Setup. We created two experimental gardens, one in each of 2 years. Each garden was 1.3 m² and contained 90 potted plants spaced ~12 cm apart to create a density similar to that observed in the wild. An equal number of females and hermaphrodites were arranged in a checkerboard pattern. We propagated plants for these gardens by clonal replication in the greenhouse and used a single ramet of each genet within each garden. We overwintered potted plants in the field at the Pymatuning Laboratory of Ecology, Crawford County, Pennsylvania (41°34'N, 80°27'W) so that they flowered synchronously with the native strawberries in the area.

Because we were interested in allowing weevils not only to damage plants but also to select their hosts, we collected actively clipping female *A. signatus* from a wild population of strawberry (Hartstown; see Ashman et al. 2004) and released them individually from random points in the gardens a few days after the first plant flowered (May 6 in both years). This approach realistically captures both host location (apparency) and selection (suitability) components of the weevil-plant interaction. Frequent monitoring revealed that weevils were resident and active (i.e., clipping buds and moving among plants) within the gardens throughout the entire 3–5-week flowering period. Plants were visited by native pollinators, were fertilized once, and received supplemental water when needed. When flowering ceased, all plants were moved to a greenhouse at the University of Pittsburgh to facilitate fruit and seed collection.

Plant Measurements. To characterize plant phenotypes, we measured several reproductive traits. Petal size was measured as the product of petal length and width of one petal per flower at the secondary position on each inflorescence (flowers at this position are representative of the average produced per plant; T.-L. Ashman, unpublished data). We enumerated ovules and anthers in each of these flowers, and if the plant was hermaphroditic, we also collected one undehisced anther. Pollen per anther was determined with the aid of an Elzone particle counter, as in work by Ashman and Hitchens (2000). We estimated total pollen production per flower as the product of pollen per anther and the number of anthers per flower. Every 2–3 days, we recorded the number of open flowers per plant and calculated the average for each plant. At the end of flowering, we recorded the total number of flower buds, fruits, and seeds produced. We calculated the proportion of fruit set as the number of fruits divided by the number of flowers that opened. For each flower-level trait, we averaged across all sampled flowers per plant in analyses described in “Path Analysis.”

Weevil Damage and Pollinators. We enumerated and collected clipped buds on each plant. We recorded pollinator visitation rate per plant during peak visitation hours (10:00 a.m.–4:00 p.m.) on 12–15 days in May and June of each year by observing one-quarter of each garden for 20 min at a time and rotating among quarters. In total, we conducted 25 and 27.3 h of observation and recorded 1,050 and 2,275 visits per garden in 2003 and 2004, respectively.

Male and Female Fertility. Here, we consider relative female fertility of females only because less than one-third of the hermaphrodites set fruit, and these contributed <7% to the total seeds produced in each garden. Thus, the vast majority of hermaphrodites' fertility is gained through their male function. Relative female fertility was determined as the number of seeds produced per plant relative to the mean per female within each garden. Male fertility was determined with the aid of paternity analyses. In each garden, 750 progeny (selected in proportion to each plant's seed production) and all parents were assayed at four microsatellite loci (Fv 9, Fv 11, FAC 002, ARSFL 99), following published protocols (Ashley et al. 2003; Lewers et al. 2005). We used the Gradient Estimate program described by Morgan and Conner (2001) to determine paternity of each progeny. Exclusion probabilities were greater than 95% in both years, and genetic data excluded all but one paternal parent for 407 and 366 of the progeny (45% of the 910 and 24% of the 1,505 possible parent-offspring triplets) in the 2003 and 2004 gardens, respectively. In addition, 76% and 96% of the progeny had genotypes consistent with sires within the gardens. The remaining progeny represent mistyping or gene flow from outside the experimental gardens and were not included in the analyses. Gene flow was not accounted for in the fractional paternity assignments, but its presence will most likely serve to undermine the precision with which we assign paternity rather than to bias our analysis; thus, our conclusions are likely (statistically) conservative. To estimate male fertility, we summed fractional paternities derived from the Gradient Estimate program (Morgan and Conner 2001) for each hermaphrodite, and these were relativized by the mean per garden.

Path Analysis. We used path analysis combined with structural equation modeling (reviewed in Mitchell 1992; Mitchell 2001) to test the fit of competing a priori hypotheses (i.e., models) concerning the role of direct and indirect effects of weevils on fertility and to identify the traits subject to selection via weevils (Conner 1996; Scheiner et al. 2000). We devised two path models, one for male fertility (fig. 1A) and one for female fertility (fig. 1B). In the full model for male fertility, we hypothesized that two

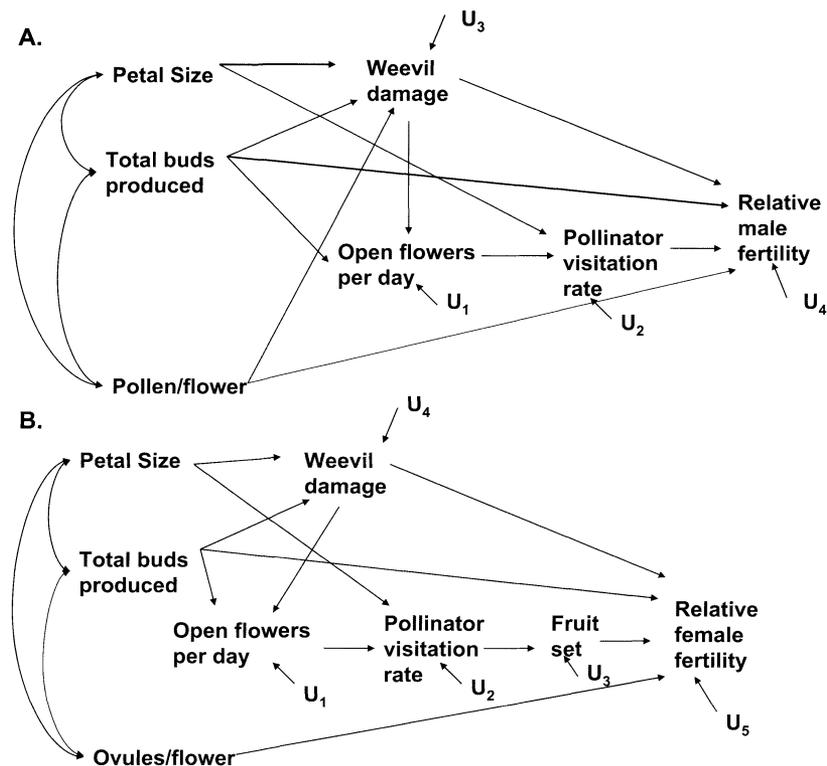


Figure 1: Hypothesized path models for the effects of flower-clipping weevils on male fertility (A) and female fertility (B) in *Fragaria virginiana*. Straight arrows reflect causal paths; curved arrows designate correlations. Residual-variation-associated unmeasured variables are denoted by arrows labeled with U_1 – U_5 .

flower-level traits (petal size and pollen production per flower) and one plant-level trait (number of flower buds) attract weevils and thus will affect weevil damage levels. Further, we hypothesized that weevils could affect male fertility directly by clipping buds or consuming or damaging pollen or indirectly via the effects of damage on display size (open flowers per day), which in turn affects pollinator visitation and seed siring. In addition, some phenotypic traits, such as petal size, affect fertility indirectly via attraction of pollinators (Ashman et al. 2000), whereas others (e.g., pollen per flower, flower buds per plant) directly affect fertility by putting an upper limit on the number of gametes. The full model for female fertility is similar, but because females do not produce pollen, only petal size and flower buds are linked to weevil damage. In addition, pollinator visitation affects the probability that a flower becomes a fruit, which in turn, along with ovules per flower (A. L. Case and T.-L. Ashman, unpublished manuscript) and weevil damage, has direct effects on seed production. All models included correlations among plant phenotypic traits.

For each fertility mode, we statistically tested three nested models relative to the full models (table 1, models

F_A and M_A). The first model(s) assumes that weevils have no direct effect on fertility (F_B and M_B); that is, the path from weevil damage to relative fertility was constrained to 0. Our second model(s) assumes that weevils have no indirect effects (F_C and M_C); that is, the path from weevil damage to open flowers per plant (display size) was constrained to 0. This model retains the pathway from display size to fertility via pollinator visitation because we wanted to continue to account for the effects of pollinators on fertility (i.e., in the absence of a weevil effect) in the model and thereby isolate the effect of weevils on this pathway via display size. The third model(s) assumes that weevils have neither direct nor indirect effects (F_D and M_D); that is, both the paths described above were constrained to 0.

Using the model(s) with the strongest support (see statistical analyses below), we assessed which traits were possible targets of weevil-mediated selection using two nested models. First, we tested a model where the path from flower buds to weevil damage was constrained to 0. Then, we tested a model where the paths from flower-level traits to weevil damage were constrained to 0. For male fertility, we simultaneously constrained to 0 paths from petal size and pollen to weevil damage because these traits are tightly

Table 1: Summary of hypothesized path models and fit statistics used to test for the effects of weevil antagonists on male and female fertility of *Fragaria virginiana* in two experimental gardens

Model and hypothesis	Paths set to 0	2003 garden					2004 garden				
		χ^2	df	<i>P</i>	AIC	w_i	χ^2	df	<i>P</i>	AIC	w_i
A. Male fertility:											
M_A : both IE and DE	None	11.89	7	.10	-2.11	.91	9.47	7	.22	-4.52	.44
M_B : no DE	Weevil damage to relative fertility	19.89*	8	.01	3.89	.04	11.02	8	.20	-4.98	.56
M_C : no IE	Weevil damage to open flowers	19.81*	8	.01	3.81	.05	30.16*	8	.0002	14.17	.000
M_D : neither IE nor DE	Weevil damage to open flowers and to relative fertility	27.81*	9	.001	9.81	.002	31.71*	9	.0002	13.70	.000
B. Female fertility:											
F_A : both IE and DE	None	12.26	14	.58	-15.73	.13	16.89	14	.26	-11.11	.29
F_B : no DE	Weevil damage to relative fertility	13.64	15	.55	-16.35	.18	20.26	15	.16	-9.73	.14
F_C : no IE	Weevil damage to open flowers	12.68	15	.62	-17.32	.29	18.38	15	.24	-11.62	.38
F_D : neither IE nor DE	Weevil damage to open flowers and to relative fertility	14.07	16	.59	-17.93	.40	21.76	16	.15	-10.23	.19

Note: Fit statistics: χ^2 goodness of fit, Akaike Information Criterion (AIC), and AIC weights (w_i). For each fertility mode, the full model (F_A , M_A) is followed by three nested alternative models: those including only indirect effects (IE) of weevils (F_B , M_B), only direct effects (DE; F_C , M_C), or no weevil effects (F_D , M_D). Values of w_i in bold are included in the 95% confidence set (for explanation, see "Methods").

* Significantly different ($P \leq .05$) from the full model based on χ^2 .

positively correlated (Ashman 2003). If constraining any of these paths to 0 reduces the fit of the model to the data, we can conclude that these traits are likely targets of weevil-mediated selection.

Statistical Analyses. We used PROC CALIS in SAS (METHOD=ML) to calculate both the goodness of fit (χ^2) and the Akaike Information Criterion (AIC) for each model. A nonsignificant χ^2 value indicates that the model-derived correlation matrix does not deviate significantly from the observed one (Shipley 2000) and the model can be considered an adequate description of the data (Mitchell 1992). We used differences in χ^2 to assess the fit of each nested model relative to the full model (Mitchell 2001). We also compared models using the AIC. The AIC for a given model is a function of its maximized log likelihood (l) and the number of estimable parameters (K ; $AIC = -2l + 2K$). The model with the lowest AIC is preferred (Akaike 1983; Burnham and Anderson 2003). AIC is designed to estimate the predictive accuracy of competing hypotheses, and thus the differences in AIC between models was assessed. Given a set of candidate models, a model that differs from the model with the smallest AIC value by <2 units receives a similar level of support, whereas one that differs by >10 units has very little support (Burnham and Anderson 2003, p. 70). We ranked competing models by their AIC weights (w_i), which can be interpreted

as the approximate probability of a model being the actual best model, given the data (Burnham and Anderson 2003, pp. 71, 171). We further used w_i to evaluate the relative likelihood of two competing models (i.e., the evidence ratio; Burnham and Anderson 2003, p. 78) and to produce a 95% confidence set of models when a single best model (e.g., $w_i \geq 0.90$) was not identified (Burnham and Anderson 2003, pp. 169–171). We produced an average model based on this set (weighted by w_i) to provide robust multimodel inference from the data (Burnham and Anderson 2003, pp. 150–153).

For the single or model-averaged path diagram for each fertility avenue in each year, we decomposed the total effects of weevils and plant traits on fertility into their direct and indirect effects and calculated significance values for individual paths in PROC CALIS. Model-averaged path coefficients and standard errors were estimated following Burnham and Anderson (2003). We used PROC CORR to generate the observed correlation matrix (tables A1, A2 in the online edition of the *American Naturalist*).

The recommended sample size for path analysis is at least five times the number of paths (Petraitis et al. 1996). With 45 plants of each sex and 10–11 paths per full model, we are slightly below this threshold value. Because the goodness-of-fit χ^2 test depends on large sample sizes to use the asymptotic χ^2 distribution, small sample sizes may result in conservative probability estimates and thus a re-

duced ability to reject an ill-fitting model (Shipley 2000; Mitchell 2001). To address this issue, we calculated Shipley's empirical probability estimates using the MCX2 program (Shipley 2000) and compared these with the probability values for χ^2 goodness-of-fit tests from PROC CALIS. In all cases, the use of the asymptotic probability was justified. Because collinearity can also be a problem in path analysis (Petraitis et al. 1996), we tested for multicollinearity by calculating variance inflation factors (VIF in PROC REG), and all were <3 , suggesting that multicollinearity was not a significant issue in our analyses (Meiers 1990).

Results

Is Weevil Antagonism a Persistent Feature of the Wild Strawberry System?

In wild populations of *Fragaria virginiana*, weevil damage is significantly sex biased (fig. 2; $F = 36.25$, $df = 1, 49$, $P < .0001$): an average of 27% (range 0%–70%) of hermaphrodites and 4% (range 0%–24%) of females suffer weevil damage. For hermaphrodites, damage incidence varied significantly among populations ($F = 5.88$, $df = 3, 12$, $P < .005$) but not years ($F = 2.34$, $df = 3, 12$, $P > .05$). For females, neither source of variation was significant (both $P > .20$). Damage intensity was higher for hermaphrodites ($32\% \pm 1\%$ of buds clipped) than for females ($23\% \pm 3\%$; $F = 6.06$, $df = 1, 493$, $P < .02$) and showed significant spatial and temporal variation for hermaphrodites (mean range 11%–44%; population: $F = 4.07$, $df = 4, 14$, $P < .001$; year: $F = 7.92$, $df = 4, 450$, $P < .0001$). Data on damage intensity for females was not sufficient for analysis.

Patterns of damage in the experimental gardens mirrored those seen in the wild populations in most respects. As in wild populations, hermaphrodites had a higher probability of damage than did females in both gardens (H vs. F, plants damaged, 2003: 91% vs. 40%; 2004: 66% vs. 16%; both $P < .001$). While these incidences correspond to the high end of population averages (fig. 2), they are not uncommon within populations; that is, 25% of local patches experience damage at these levels or higher (C. L. Collin, L. Penet, and T.-L. Ashman, unpublished data). Damage intensity in experimental gardens also differed between morphs in the direction observed in wild populations (mean \pm SE, H vs. F, buds clipped per plant: $33\% \pm 3\%$ vs. $11\% \pm 2\%$; $P < .0001$). There was no difference between gardens or any sex-garden interaction (both $P > .15$). This intensity of damage is similar to, or below, the average observed in wild populations.

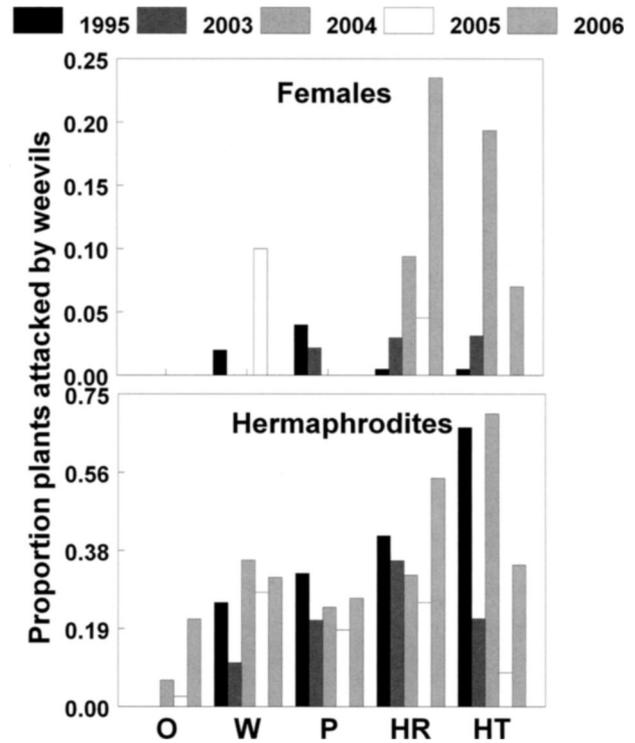


Figure 2: Incidence of clipper weevil damage in five wild populations (Old Erie Canal [O], Warren [W], Pymatuning Railroad [P], Housing Railroad [HR], and Hartstown [HT]) of *Fragaria virginiana* in northwestern Pennsylvania over 5 years (1995, 2003–2006). Note that the scale of the Y-axis is different for females (top) and hermaphrodites (bottom).

Do Weevils Have Direct and/or Indirect Effects on Male and/or Female Fertility?

Male Fertility. For male fertility in the 2003 garden, the full model did not deviate significantly from the observed data ($P < .10$; table 1, pt. A), whereas all three of the nested models (M_B , M_C , M_D) differed significantly from the observed data and from the full model (table 1, pt. A). The full model also had the largest AIC weight (0.91) and an evidence ratio of nearly 20 more than the next-closest model (M_C), making it the best supported of all models. Taken together, this suggests that both direct and indirect weevil effects are important to an acceptable description of the relationship among variables in this year (fig. 3A).

In the 2004 garden, the full model (M_A) and the model with no direct effects (M_B) did not deviate significantly from the observed data and or from each other (table 1, pt. A). Similarly, the difference in their AIC was small (<1 unit), and their AIC weights were nearly identical (0.44 and 0.56), indicating that they receive nearly equivalent support. In contrast, both M_C and M_D were poor fits to the data based on all criteria (table 1, pt. A). Thus, both

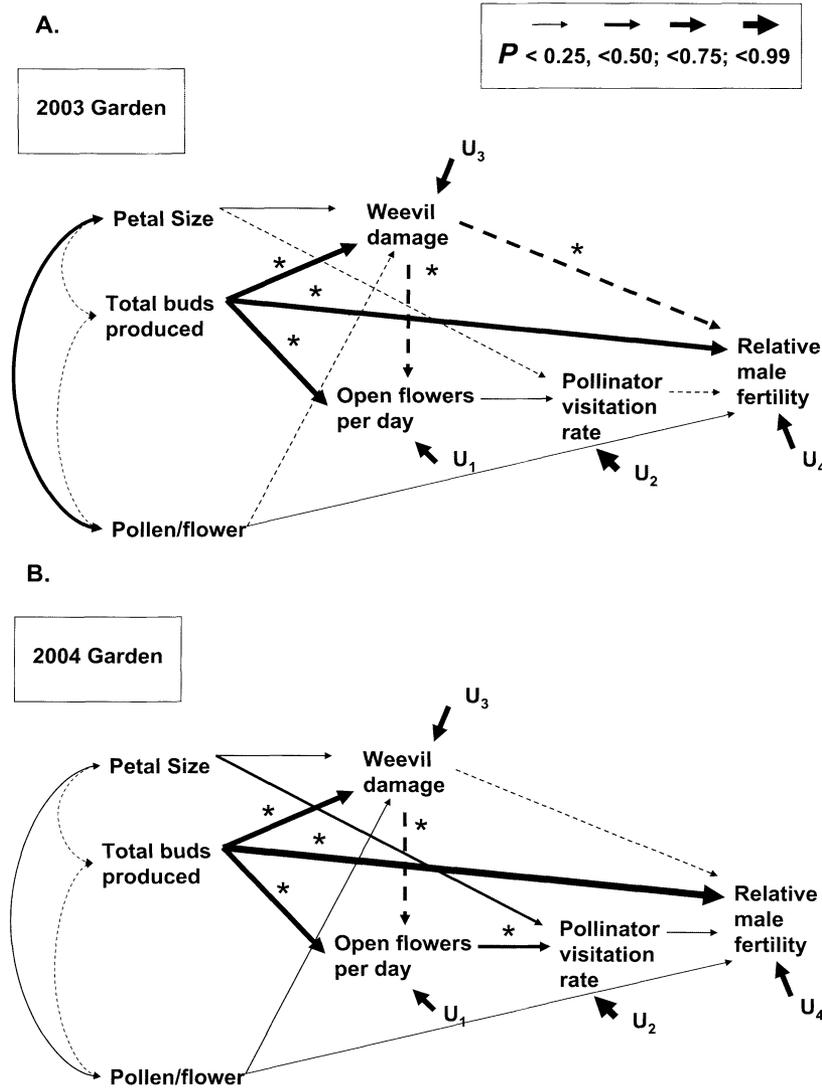


Figure 3: Empirically derived path models for the effects of flower-clipping weevils on male fertility in *Fragaria virginiana* in 2003 (A) and 2004 (B) gardens. Straight arrows reflect causal paths; curved arrows designate correlations. Positive effects are shown by solid arrows, whereas negative effects are shown by dashed arrows. Strength of the relationship is designated by arrow thickness. Asterisks denote paths significant ($P < .05$) as determined by *t*-tests. Residual-variation-associated unmeasured variables are denoted by arrows labeled with U_1 – U_4 .

models M_A and M_B are retained, and together they constitute the 95% confidence set for this year’s male fertility data. The model-averaged path diagram includes both direct and indirect effects, but the direct effects are reduced in magnitude relative to model M_A (fig. 3B).

Female Fertility. In the 2003 garden, none of the models deviated significantly from the observed data, and none of the nested models differed significantly from the full model (table 1, pt. B). All models were within 2.2 units of the model with the minimum AIC (F_D : -17.93), and

model weights ranged from 0.40 to 0.13, indicating that although model F_D has approximately three times the support of F_A , no model received greater support than any other (table 1, pt. B). Given that no model could be excluded, the 95% confidence set includes all four, and a model-averaged path diagram was constructed based on their relative probabilities (i.e., w_i ; fig. 4A).

Similarly, in the 2004 garden, all models fit the observed data, and no nested model deviated significantly from the full model (table 1, pt. B). The model with only direct effects (F_C) had the lowest AIC value (-11.62) and the

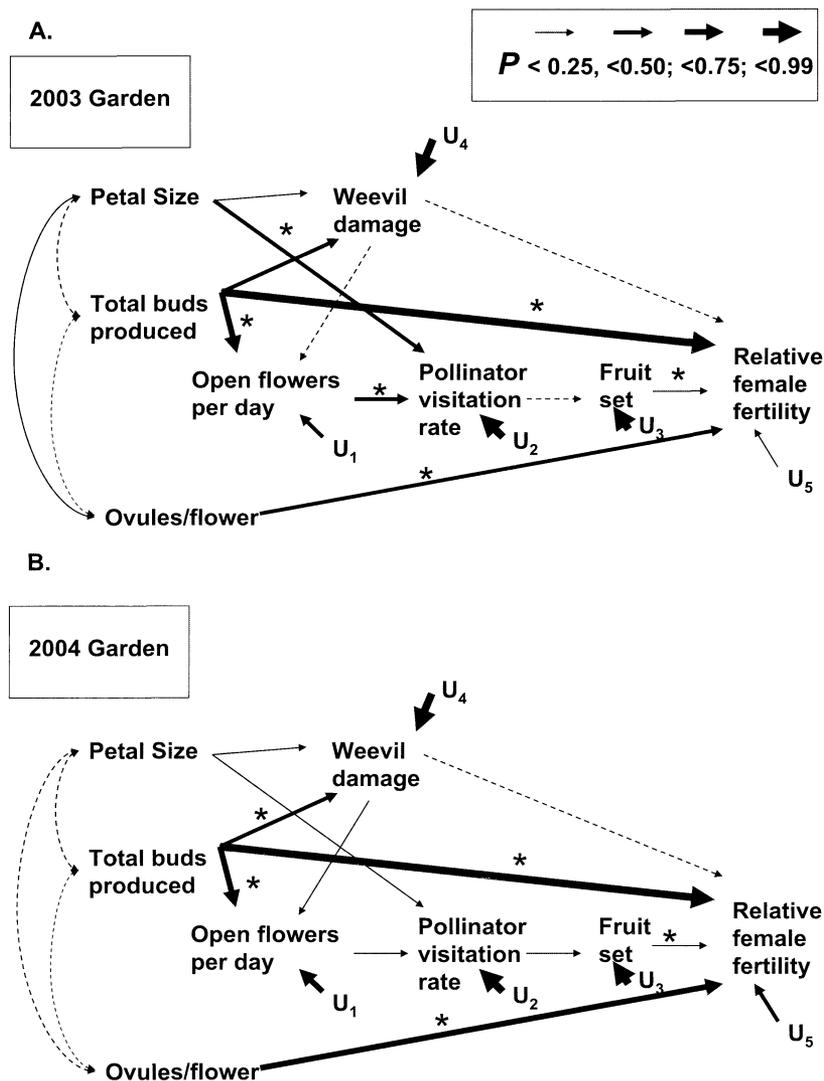


Figure 4: Empirically derived path models for the effects of flower-clipping weevils on female fertility in *Fragaria virginiana* in 2003 (A) and 2004 (B) gardens. Straight arrows reflect causal paths; curved arrows designate correlations. Positive effects are shown by solid arrows, whereas negative effects are shown by dashed arrows. Strength of the relationship is designated by arrow thickness. Asterisks denote paths significant ($P < .05$) as determined by t -tests. Residual-variation-associated unmeasured variables are denoted by arrows labeled with U_1 – U_5 .

greatest AIC weight (0.38), but its evidence ratio ranged from 1.3 (over the next most likely model: F_A) to 2.7 (over the least likely model: F_B ; table 1, pt. B). Thus, the 95% confidence set includes all four models, and the model-averaged path diagram was based on these (fig. 4B).

Total Effect of Weevils on Male and Female Fertility. To evaluate the sex-specific effect of weevils on fertility, we calculated the total, direct, and indirect effects of weevil damage in each garden based on the models presented in

figures 3 and 4. In the 2003 garden, the total effect of weevils on male fertility was large (-0.462) and mostly due to direct negative effects (fig. 3A; table 2). Similarly, weevils had mainly negative direct effects on female fertility in this garden (fig. 4A; table 2), although they were less than one-tenth the magnitude of those on male fertility. In the 2004 garden, however, the total effects of weevils on male and female fertility were similar in magnitude and, if anything, were slightly stronger on female fertility (-0.088 vs. -0.108). In addition, here, negative

Table 2: Decomposition of the total effect of weevils on male and female fertility of *Fragaria virginiana* into their direct and indirect components

Garden and fertility mode	Weevil effects on relative fertility		
	Direct	Indirect	Total
A. 2003:			
Male	-.464	.002	-.462
Female	-.037	.0001	-.037
B. 2004:			
Male	-.072	-.016	-.088
Female	-.106	.002	-.104

Note: Two experimental gardens are presented, one in each of two years 2003 (A) and 2004 (B). Total effect is the model-defined correlation between weevil damage and fertility, direct effects are the path coefficients between weevil damage and fertility, and indirect effects are the sum of all indirect paths (see Mitchell 2001).

indirect effects on male fertility were strong and accounted for 20% of the total effect of weevils.

Which Reproductive Traits Are Subject to Weevil-Mediated Selection?

For each path diagram, we compared two reduced models nested within the full model to test hypotheses concerning the targets of selection (table A3 in the online edition of the *American Naturalist*). For male fertility in the 2003 garden, the model without the plant trait was not a good fit to the data and had a negligible w_i . In contrast, the full model and the model without floral traits did not deviate from the observed data and had much higher values of w_i (0.33 and 0.66, respectively). Taken together, these data provide the strongest support for flower buds as a target of weevil-mediated selection. Again, in the 2004 garden, the model without the plant trait was the only one to deviate significantly from the observed data and had a minute w_i (table A3). Neither the full model nor the one without floral traits deviated significantly from the observed data, but the full model had an evidence ratio of ~ 3 over the latter model. Thus, the 2004 data provide the strongest support for both flower number and floral traits as targets of weevil-mediated selection via male fertility.

For female fertility in 2003 and 2004, none of the models deviated significantly from the observed data (table A3). In the 2003 garden, the model with only flower number had twice the support of the full model ($w_i = 0.55$ vs. $w_i = 0.28$), whereas in the 2004 garden, the reverse was true ($w_i = 0.38$ vs. $w_i = 0.52$), and here the model without flower number deviated significantly from the full model based on the χ^2 test (table A3). Taken together, we

can conclude that flower number is highly likely but petal size is less likely to be under weevil-mediated selection via female fertility. In sum, these results suggest that while the number of flower buds is consistently a target of weevil-mediated selection via both male and female fertility, flower-level traits may be targets only in some years (2004) and more likely through male than female fertility.

How Do Weevils Contribute as Agents of Selection?

To evaluate the effect of weevils relative to other potential selective agents, we partitioned the total effect of each plant trait on relative fertility into its direct effects and indirect effects via weevils (either directly or via pollination) or pollinators alone (table 3). Total flowers produced per plant stands out as being a trait consistently subject to significant negative selection via weevils, and this opposes the mostly positive selection via pollinators and the positive direct effects of this trait on fertility (table 3). For example, in the 2003 garden, weevil-mediated effects (-0.273) were nearly half the magnitude of the positive direct effects of flower buds on male fertility (0.669) and greatly outweighed the contribution made by pollinators alone on the relation of this trait to fertility (-0.003). Even though the animal-mediated effects of flower number on male and female fertility in 2004 were an order of magnitude lower (weevils vs. pollinators, male: -0.046 vs. 0.014; female: -0.036 vs. 0.003) than the direct effects of flower bud number on fertility (0.672 and 0.750), weevil-mediated indirect effects were still three to 10 times greater than pollinator-mediated ones.

The contribution of weevil damage to selection on flower-level traits was more variable and not as often significant (table A3). In the garden where there was good support for flower-level traits being subject to weevil-mediated selection, weevil effects opposed the direct positive effect of pollen per flower on male fertility (-0.020 vs. 0.077). Likewise, in this garden, weevil-mediated selection on petal size opposed and was equivalent to or stronger than that via pollinators alone (male fertility: -0.017 vs. 0.014; female fertility: -0.025 vs. 0.002).

Discussion

The work presented here suggests that flower-clipping weevils that exhibit sex-dependent attack can be consistent features of wild strawberry populations and can significantly reduce both male and female fertility as well as mediate selection on reproductive traits. However, the complexity of interacting biotic effects leads to variable total effects and strength of selection across years and fertility modes. These results are discussed in greater detail, as are the potential consequences for sexual dimorphism.

Table 3: Decomposition of total effect of traits on male and female fertility of *Fragaria virginiana* into direct and indirect effects

Plant trait	Direct	Indirect via		Total
		Weevils	Pollinators	
A. Male:				
2003:				
Petal size	...	-.086	.004	-.082
Total flower buds	.669	-.273	-.003	.393
Pollen per flower	.146	.082228
Open flowers per day	-.005	-.005
2004:				
Petal size	...	-.017	.014	-.003
Total flower buds	.672	-.046	.014	.640
Pollen per flower	.077	-.020057
Open flowers per day025	.025
B. Female:				
2003:				
Petal size	...	-.005	-.003	-.008
Total flower buds	.863	-.010	-.003	.850
Ovules per flower	.374374
Open flowers per day	-.004	-.004
Fruit set	.152152
2004:				
Petal size	...	-.025	.002	-.023
Total flower buds	.750	-.036	.003	.729
Ovules per flower	.480480
Open flowers per day004	.004
Fruit set	.206206

Note: Effects are based on the models depicted in figs. 3 and 4. Indirect effects are further separated into those mediated by weevils (via pollination or directly) or by pollinators alone (see path diagrams). Two experimental gardens (2003, 2004) are presented. Total effect is the model-defined correlation between a trait and fertility, direct effects are the path coefficients between the trait and fertility, and indirect effects are the sum of all indirect paths connecting the trait and fertility via either weevil damage or pollinators alone. Ellipsis indicates that path was not part of path model (see figs. 3, 4).

Total, Direct, and Indirect Effects of Weevils on Fertility

While sex-differential damage is common in gender-dimorphic plants, the consequences of this damage for fertility and phenotypic selection, especially through realized male fertility, have largely been undocumented (but see Muenchow 1998; Ashman et al. 2004; Verdu et al. 2004; Hemborg and Bond 2005). The results presented here suggest that simple extrapolation of damage differentials to fertility effects could be erroneous at least some of the time. Specifically, even though hermaphrodite wild strawberries are always damaged more than females, the differential effect on fertility was mirrored only by the 2003 findings, where weevils had strong negative direct (and total) effects on male fertility but only weak negative direct

effects on female fertility. In contrast, in the 2004 garden, damage had slightly greater negative effects on the fertility of females than on the male fertility of hermaphrodites. This was the case even in spite of the fact that weevils had both negative direct and indirect effects on male fertility this year.

These results underscore the importance of studying both direct and indirect effects to understand the impact of sex-biased antagonists on plant fertility. We found that the pathway from weevil damage to floral display was an important contributor to the fit of the model for male fertility in both years but less so for female fertility (table 1; figs. 3, 4). This suggests that only when damage intensity is high, as with hermaphrodites, does weevil damage have the potential to exert an indirect effect on relative fertility via display size. The consequence of this indirect weevil effect for male fertility, however, can be diminished by the weak links between floral display, pollinators, and fertility. This was especially apparent in the 2003 garden, where despite significant weevil effects on display (fig. 3A), the net indirect effect was very small (table 2). Weak indirect effects via female fertility reflect the combination of limited effects on floral display and a weak link between pollinators and seed fertility (fig. 4).

Although there are no data in gender-dimorphic species to draw on for comparison, consideration of the few studies in hermaphroditic systems suggests that the patterns of weak indirect effects relative to direct effects or larger indirect effects through male fertility may be common. In the only other study to estimate both male and female components of fertility, Krupnick and Weis (1999) found direct effects of a beetle florivore on *Isomeris arborea* via both male success (pollen analog export) and female success (pollen receipt and seed production) but indirect effects only via male success. The indirect effects, however, were estimated to be only one-tenth the magnitude of the direct effects. Studying female fertility only, both Adler and colleagues (2001) and Irwin and colleagues (2004, 2006) found that the indirect effects of floral antagonists were weaker than their direct effects, but Mothershead and Marquis (2000) found the opposite pattern. A common theme that emerges from these studies is that indirect effects were observed on female fertility only when seed production was pollen limited. Too few studies exist for any strong consensus regarding male fertility, but the two cases studied thus far (*I. arborea* [Krupnick and Weis 1999] and *Fragaria virginiana* [this study]) demonstrate that indirect effects can occur via male fertility, but these can be relatively weak in contrast to the direct damage to pollen and flowers by florivores.

Minor and variable indirect effects of weevils are partly a function of a weak and sometimes negative link between pollinator visitation and fertility (figs. 3, 4). Perhaps sur-

prisingly, the link between pollinator visitation and annual or lifetime fertility is often one of the weakest in path analyses of selection on reproductive traits (e.g., Conner et al. 1996; Gomez 2000; Sanchez-Lafuente 2002; Cariveau et al. 2004). This link may be weak because it embodies a great deal of biological complexity (i.e., pollinator diversity, pollen transfer dynamics, and postpollination processes). Stronger causal associations may be found only when we tease apart some of this complexity. For example, pooling across a diverse pollinator fauna could obscure the importance of individual taxa. Using path analysis, Stanton and colleagues (1991) revealed that visitation by some members of the pollinator fauna had negative while others had positive or neutral contributions to paternity in *Raphanus sativus*. It is also possible that biological properties not included in the path model are important. For instance, increased pollinator visitation can have diminishing returns for male (e.g., pollen discounting; Klinkhamer et al. 1994) as well as female fertility (e.g., dislodging of pollen already deposited on stigmas; Engel and Irwin 2003), and the possibility for nonlinearity has not been accounted for in the present model. One must also acknowledge that pollinator visitation rates are based on a necessarily limited sampling of the total flowering time of a plant and thus may be inherently imprecise. All of these issues will require greater attention before we can make generalizations concerning the relative weight of indirect effects mediated via pollination in this or any plant-herbivore system.

Targets of Weevil-Mediated Selection

Our analysis identified several traits as likely targets of selection via weevils. The number of flower buds per plant, in particular, was often a target of weevil-mediated selection, a finding that corroborates patterns found in both wild and cultivated populations of *F. virginiana* (Ashman et al. 2004). In addition, similar to several other studies (e.g., Brody and Mitchell 1997; Ehrlen 1997; Fenner et al. 2002; Leimu et al. 2002; Cariveau et al. 2004), antagonist-mediated selection on this trait opposed that via pollinators or resources (i.e., the direct effects of flower number on fertility). In fact, weevil-mediated selection on flower number was always stronger than pollinator-mediated selection in *F. virginiana* (table 3), suggesting a greater role for antagonists in the evolutionary trajectory of this trait than for pollinators. As a whole, these studies suggest that antagonists reduce the marginal gains of producing more flowers and could cause balancing selection on flower bud number. When this occurs, antagonists could be an important force maintaining genetic variation in this trait. The number of flowers per plant has high heritability relative to other reproductive traits in *F. virginiana* (Ashman

2003) and relative to the average for this trait across species (Ashman and Majetic 2006). A more general test of this idea, however, will require knowledge of genetic variation for flower number in other species subject to this sort of balancing selection.

Flower-level traits (petal size and/or pollen per flower), on the other hand, received strong support as targets of selection in only one year (2004) and mostly via male fertility. One possibility for the greater importance in 2004 than in 2003 is that the temperature was warmer this year (mean maximum temperature 2003 vs. 2004: 65° vs. 70°F), and this may have led to tighter linkages between plant traits and insect behaviors. Weevils and pollinators are known to respond to both visual and olfactory floral cues in this system (Ashman et al. 2004; T.-L. Ashman, unpublished data), and flowers emit greater amounts of volatiles under higher temperatures (R. Raguso and T.-L. Ashman, unpublished data), so both insect activity level and floral signals may have been higher in this year. The strong link between pollinator visitation and male fertility also contributed to the significance of floral traits as targets of selection via male fertility in this year. In contrast, there was relatively weak support for petal size as a target of selection via female fertility. This may not be surprising, given that seed production was not pollen limited in either garden (T.-L. Ashman, unpublished data). In fact, other studies of *F. virginiana* have shown that petal size is under selection via female fertility only when seed production is limited by the availability of pollen donors or pollinators (Ashman and Diefenderfer 2001; A. L. Case and T.-L. Ashman, unpublished manuscript). Our results add breadth to this pattern by suggesting that in the presence of weevils and when pollen is not limiting, petal size may be under negative directional selection via female fertility. A rigorous assessment of the causes of the variation in floral traits as targets of selection, however, will require greater replication and explicit manipulation and measurement of these putative agents.

Consequences for Sexual Dimorphism

Sexual selection and sex-differential selection are two forces that can promote sexual dimorphism (Geber 1999), and the conflict between sexual and natural selection is thought to govern the evolution of secondary sexual traits (Darwin 1871). Results from the 2004 garden raise the intriguing possibility that flower-level traits of hermaphrodites may be subject to this type of conflicting selection. In particular, hermaphrodite plants that produce copious pollen per flower and have large petals have increased siring success but also receive more damage by weevils. The plant-level trait (flower buds per plant), on the other hand, was targeted by weevils similarly in the sex morphs

but more strongly in hermaphrodites than in females. Such sex-differential selection could still lead to sexual dimorphism because all the traits studied here are heritable and have between-sex genetic correlations of less than 1 (Ashman 2003, 2005). The outcome of selection, however, is not likely to be straightforward because within-sex between-trait genetic correlations can constrain potential response to selection. For instance, two traits targeted by weevils in hermaphrodites, flower bud number and pollen per flower, are negatively genetically correlated (Ashman 2003). So to predict the outcome of selection, we will need to combine traditional estimates of selection gradients with estimates of the genetic variance-covariance matrix (Scheiner et al. 2000).

Conclusions

The work presented here indicates that weevils can exert both direct and indirect effects on fertility. Further, they can play a large role, often larger than pollinators, in shaping reproductive phenotype in *F. virginiana*. The direct effects of weevils can significantly reduce fertility and mediate selection on reproductive traits, even in the non-preferred sex. In contrast, indirect effects of weevils are important contributors to selection on reproductive phenotype only in hermaphrodites, which gain the majority of their fitness via male function. The results presented here also raise the intriguing possibility that antagonists play a role in the evolution of sexual dimorphism.

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