Variable and sexually conflicting selection on *Silene stellata* floral traits by a putative moth pollinator selective agent

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Conflicting selection is an important evolutionary mechanism because it impedes directional evolution and helps to maintain phenotypic variation. It can arise when mutualistic and antagonistic selective agents exert opposing selection on the same trait and when distinct phenotypic optima are favored by different fitness components. In this study, we test for conflicting selection through different sexual functions of the hermaphroditic plant, *Silene stellata* during its early and late flowering season. We find selection is consistently stronger during the early flowering season, which aligns with the activity peak of the pollinating seed predator *Hadena ectypa*. Importantly, we observe sex-specific selection on petal dimensions to have opposite signs. We propose that the observed sexually conflicting selection on petal design results from the negative selection through female function for the avoidance of oviposition and the subsequent fruit predation by *H. ectypa* larvae and the positive selection through male function for pollen export by *H. ectypa* adults. The *Silene–Hadena* interaction has previously been considered to be largely parasitic. Our findings suggest a trade-off mechanism that could thwart the evolution of an "escape route" from the nocturnal pollination syndrome by *Silene spp*. and contribute to the long-term maintenance of the *Silene–Hadena system*.

KEY WORDS: Natural selection, nursery pollination, paternity analysis, plant-animal interaction, pollinating seed predator, sexual conflict.

Natural selection is prevalent in wild populations (Endler 1986). An important form of natural selection is conflicting selection, which occurs when opposing selective pressures are exerted on the same trait. Conflicting selection is an important evolutionary mechanism because the associated evolutionary trade-offs (Schluter et al. 1991) limits directional evolution and helps to maintain phenotypic variation within the population (Siepielski and Benkman 2010).

Flowering plants provide excellent systems for studying conflicting selection since they often interact with multiple species that could affect various fitness components and life history traits, therefore enriching the opportunity for selective pressures exerted by different agents to be of opposite signs. For instance, conflicting selection can arise when a plant species interacts with multiple pollinator species that show opposite preferences for the same trait (Sahli and Conner 2011; Kulbaba and Worley 2012).

Furthermore, antagonistic actors such as herbivores, nectar robbers, predispersal seed predators, and pathogens (Strauss and Whittall 2006; Irwin and Brody 2011; Maruyama et al. 2015; Caruso et al. 2019) have also been observed to exert selection on floral traits. Since mutualistic and antagonistic interactions often co-occur (Strauss and Armbruster 1997), their positive and negative ecological effects can lead to conflicting selection if they are associated with the same plant traits (Galen and Cuba 2001; Adler and Bronstein 2004; Sletvold et al. 2015) or indirectly through phenotypic correlation between traits (Gómez 2003).

In addition to the multitude of selective agents, conflicting selection may also arise when a trait exhibits distinct fitness optima for different life history stages or fitness components (Schluter et al. 1991). Because approximately 90% of flowering plants exhibit hermaphroditic sex expression (Barrett and Hough 2013), where total individual reproductive fitness involves both male and female functions, it is important to examine how phenotypic selection is exerted differentially through the two sexes, as evolutionary theory suggests the possibility of prevalent conflicting selection for hermaphroditic plants (Morgan 1992). However, most natural selection studies of hermaphroditic plants that have quantified selection using various female fitness components as the proxy for total fitness while rarely quantifying male reproductive success through paternity analyses. To date, the majority of the small number of studies examining selection on floral design for both sexual functions do not reveal conflict (Conner et al. 1996; Morgan and Schoen 1997; Delph and Ashman 2006; Hodgins and Barrett 2008; Sahli and Conner 2011; Rosa et al. 2017).

One possible reason for the lack of evidence of sexually conflicting selection in plants is that these studies have mostly been restricted to various stages of pollination, where pollen receipt and export are used as the proxies of female and male fitness, respectively (Delph and Ashman 2006), while overlooking other potentially equally important interactions listed above. In contrast, there is more potential for sexually conflicting selection to arise if one considers both mutualistic and antagonistic interactions and the possible differential responses of the two sexual functions to them. For example, predispersal seed predation may lower female reproduction of the source plant, but may have little effect on pollen export. Therefore, a trait profile that attracts both predispersal seed predators and pollinators may be under sexually conflicting selection, which may remain unknown if one only focuses on pollination. To date, studies that integrate various fitness components and ecological interactions remain scarce.

Furthermore, few studies have quantified male reproductive success by directly examining siring ability using genetic markers (e.g., Conner et al. 1996, Hodgins and Barrett 2008, Sahli and Conner 2011). Instead, most studies have used pollen export as a surrogate measure for male reproductive success (but see Inouye et al. 1994). While the correlation between siring success

and pollen export has been confirmed in a few systems (Galen 1992; Nilsson et al. 1992; Ashman 1998), it has been rejected in a number of other studies (e.g., Kobayashi et al. 1999, Conner et al. 2003). Consequently, we have a limited understanding of the prevalence of sexually conflicting selection for phenotypic traits in wild plant populations.

Here, we examine and compare selection on floral traits across the flowering phenology, and through both male and female functions, using the North American species *Silene stellata*. We think this system is excellent for studying conflicting selection for two main reasons. First, a well-documented interaction occurs between the genus *Silene* and the nocturnal moth genus *Hadena*, the latter serving as both an important pollinator and a strong seed predator at its larval stages (Kephart et al. 2006). We have confirmed this interaction between North American *S. stellata* and *H. ectypa* (Reynolds et al. 2012; Kula et al. 2013). The positive and negative interactions therefore provide ample opportunities for conflicting selection to arise. Second, in addition to *H. ectypa*, a number of generalist nocturnal moths also provide important mutualistic pollination service to *S. stellata*. Importantly, during over 15 years of field work (Dudash and Fenster pers. obs.) we have observed consistent phenological separation between *H. ectypa* and the copollinating moths (Reynolds et al. 2012). Therefore, we can also ask whether conflicting selection arises at different stages of the flowering phenology that aligns with the shift of pollination community composition. We measure selection through male fitness by directly assessing siring success using highly polymorphic microsatellite markers. Additionally, we complement this field experiment with a previous multi-year field dataset that quantified total selection through female reproductive success. We focus on three main questions: (1) Is there sexually conflicting selection on the floral design of *S. stellata* through male and female reproductive success? (2) Does conflicting selection occur across the flowering phenology of *S. stellata*? (3) Can we interpret the findings in light of the interaction with *H. ectypa* and the copollinating moths? We then examine the consequences of our findings on the evolution of the *Silene–Hadena* interaction.

Methods
STUDY SYSTEM

The study system includes the North American hermaphroditic plant *Silene stellata*, its obligate pollinating seed predator, the noctuid moth, *Hadena ectypa*, and a group of co-pollinating nocturnal moths, including the noctuid moths *Amphipoea americana*, *Feltia herelis*, *Autographa precationis*, and *Cucullia asteroids*, the arctiid *Halysidota tessellaris*, and the notodontid, *Lochmaeus manteo* (Reynolds et al. 2009). *Silene stellata* L. is an infrequent, iteroparous, long-lived perennial herb that is

distributed throughout the eastern half of the United States. In our Virginia study sites, *S. stellata* flowering occurs from early July through early September and is characterized by panicle inflorescences with white, hermaphroditic, protandrous flowers (average of 25 ± 2.5 ovules/pistil, Reynolds et al. 2009). An individual plant at our study site usually produces multiple stems and on average produces approximately 40 flowers each flowering season (Reynolds et al. 2012). Outcrossing rate is relatively high based on estimates from both pollinator exclusion experiments (>73%, Reynolds 2008) and mating system estimation using microsatellite markers (∼83%, Zhou et al. 2018b).

Hadena ectypa is an obligate pollinating seed predator of *S. stellata*, although occasionally observed on co-occurring *S. virginica* (M. Dudash and C. Fenster pers. obs) and on introduced *S. vulgaris* (Nelson 2012). *Hadena ectypa* is distributed from Massachusetts west to Minnesota and Kansas, and south to northern Georgia, concordant with the distribution of *S. stellata* (Schweitzer et al. 2011; Nelson 2012). Adult male and female *H. ectypa* extract nectar in the flowers of *S. stellata* with pollination taking place simultaneously. Oviposition behavior follows nectaring, as female moths oviposit singly or in mass of two to three eggs at the base of the ovary or on the ovary wall (Zhou et al. 2016a). A single flower may receive up to 20 eggs from multiple female *H*. ectypa moths over the course of 3 days (J. Zhou personal observation). Forty percent of all visits are followed by oviposition (Kula et al. 2013). Newly hatched larvae bore into the ovary and develop through the third instar therein, before starting to forage between flowers and plants. A larva can consume up to 40 flowers and/or unhardened fruits under lab conditions (Reynolds et al. 2012). The larvae pupate underground, and *H. ectypa* exhibits a univoltine life history at our study sites. The co-pollinating generalist moths are equally effective in pollen transfer (Reynolds et al. 2012), and do not exhibit seed predation, thus are strict mutualists. There is no significant difference in outcrossing rates between early and late flowering seasons and across years (∼83%, Zhou et al. 2018b).

FIELD EXPERIMENT 2012–2013 TO EXAMINE SELECTION THROUGH BOTH MALE AND FEMALE REPRODUCTIVE SUCCESS

Experimental setup

To quantify selection on floral traits of *S. stellata* through both male and female reproductive success, we conducted field experiments in 2012 and 2013 in an open meadow under a power line cut near the University of Virginia's Mountain Lake Biological Station in Giles County, Virginia, U.S.A. (37.3471, –80.5426, elevation ≈ 1100–1300 meters). The power line cut traverses the southwest side of Salt Pond Mountain and our study population occurs in the upper elevation of the power line cut. The experimental plot was near the top of the naturally occurring

population denoted by a 20 m × 20 m fenced exclosure (2 m high) built to prevent deer herbivory.

At this study site, adult *H. ectypa* abundance peaks early in the flowering season, when co-pollinator species are rare and drops quickly as co-pollinating moths become the primary pollinators (Kula 2012; Reynolds et al. 2012; Dudash and Fenster pers. obs.). Therefore, in each year, two experiments were conducted during the *Hadena*-dominant and co-pollinator-dominant periods to quantify selection exerted presumably by *H. ectypa* and the co-pollinating moths, respectively. We confirmed the dominant status of *H. ectypa* and the co-pollinating moths during this study by conducting pollinator surveys throughout the flowering season following the method by Reynolds et al. (2012) (Fig. 1). For each day following night-time walks, we dissected approximately 20 randomly chosen flowers collected from the same site and counted *H. ectypa* eggs. The average number of eggs per flower was used to represent *H. ectypa* oviposition activity of the night before the collection date (Fig. 1).

Each experiment was carried out for a week with an "in situ" experimental population consisting of approximately 60 equally spaced, haphazardly chosen adult plants that would flower during each experimental period (details below). To minimize the probability of pollen flow from outside the experimental population, an exclusion zone was created by removing all above ground *S. stellata* flowering stems within 10 m of the fenced area. We considered this an adequate safeguard because earlier work based on a fluorescent dye survey (Reynolds et al. 2009) and an isolation experiment (Kula et al., 2014) suggested that pollen dispersal distances in *S. stellata* are 1–2 m. Subsequent work with the microsatellite markers used here confirmed limited pollen dispersal distances (Zhou et al. 2018b). Thus, the vast majority of potential pollen donors were restricted to the experimental plants.

We divided the study plot into eight transects along the elevation gradient, with each transect further divided into eight evenly spaced quadrants (each quadrant had a radius of 1 m) containing multiple *S. stellata* plants. For the "early" experiment, we randomly picked one *S. stellata* plant from each quadrant and removed all non-experimental plants that were flowering or had flower buds expected to open within 1 week. We also removed all plants outside the quadrants to produce a thinned population of equally spaced experimental plants, each separated by approximately 1.5 m. We thinned the study population primarily because our sampling regime resulted in a constant trampling of plants. The remaining experimental plants could then be sampled without the worry of broken stems. Our design resulted in a uniform plant density in the plot, likely artificially increasing pollen dispersal distances (e.g., Fenster 1991). The design also reduced the number of potential sires, thus increasing the resolution of the paternity assignment tests.

Table 1. Summary statistics of experimental *Silene stellata* populations near Mountain Lake Biological Station studied during the early (*Hadena ectypa* dominant) and late (co-pollinators dominant) flowering season for 2012 and 2013. Fruit initiation proportion is calculated as the proportion of flower that initially set fruit.

Experiment	Adult plants	Fruit initiation proportion (Mean ± SE)	Successful fruits (Mean ± SE)	Predation rate (Mean ± SE)	Seedlings genotyped
2012 Early	59	0.91 ± 0.19	2.66 ± 2.95	0.59 ± 0.36	583
2012 Late	58	0.92 ± 0.19	3.91 ± 4.13	0.41 ± 0.35	673
2013 Early	55	0.93 ± 0.10	9.77 ± 6.91	0.20 ± 0.30	545
2013 Late	55	0.93 ± 0.10	8.60 ± 7.01	0.19 ± 0.24	570
Overall	227	0.92 ± 0.15	6.36 ± 6.34	0.34 ± 0.35	2371

We kept a number of non-flowering experimental plants in each quadrant to be used as backups or to be used in the "late" experiment. One week prior to the onset of the "late" experiment, a second experimental population of the same size was set up by randomly selecting from each quadrant a new flowering plant or one bearing large flower buds expected to open within 1 week. To avoid repeated measurements, plants used in a previous experiment or in the previous year were excluded from future experiments. On average approximately 57 plants were used per experiment (2012 Early, $n = 59$; 2012 Late, $n = 58$; 2013 Early, $n = 55$; 2014 Late, $n = 55$; see Table 1). Plant density of this experimental design was well within the range of naturally occurring *S. stellata* populations.

Oviposition and flower fate

All flowers initiated during the 1-week experimental period were tagged in their male phase during the first day of opening. We followed each labeled flower for three consecutive days (1 day in male phase and 2 days in female phase). For each day, we counted the number of eggs in the flower using a jeweler's eyepiece. We only carried out egg counting during the early experiment for each year since oviposition becomes rare in the late experiment. We measured a total of 1094 flowers across 2 years (mean flower number measured per plant: 2012 early: $N = 280$, 4.8; 2012 late: $N = 239$, 4.1; 2013 early: $N = 294$, 5.4; 2013 late: $N = 281$, 5.1).

FIELD SURVEY 2003–2011 TO QUANTIFY SELECTION THROUGH FEMALE REPRODUCTIVE SUCCESS

Seventy-one plants were randomly selected across the whole study site along three transects in each of the years 2003 and 2005 for a total of 142 plants for use in the multiyear study of phenotypic selection on *S. stellata* floral traits. Three linear and parallel transects marked every 10 m for 250 m were used to select the study plants. Each transect was spaced by 10–15 m. At each 10-m interval the nearest flowering plant was located and marked for future study. The group from 2003 was studied each year from 2003 to 2011. The group from 2005 was monitored

from 2005 to 2011. As these were mature plants with many flowers, a subsample of five flowers was selected from each of the plants to estimate average floral trait expression per plant. Since stigmas continue to grow and the delicate petals become easily damaged, all traits were measured on flowers in female phase within 24 hours of receptivity. If fewer than five flowers were available to measure at any given time, the plant was visited 2–3 days later to measure the remaining flowers at the appropriate stage. After flowering ended, the number of stems and the heights of each stem were measured on all transect plants as a vegetative vigor covariate. About 2–3 weeks after each plant flowered one to two mature fruits were opened and the seed inside were inspected for the onset of sclerification. If browning was noted, then all fruits that appeared to be at a similar developmental stage were removed and stored. At this time, flowers failing to set seed were collected. The monitoring, inspection, and removal of fruits from each transect plant was repeated —three to four times until all reproductive units were removed from the transect plants.

TRAITS STUDIED

For both studies described above, phenotypic measurements were conducted on all experimental plants with a caliper with a precision of 0.1 mm for seven floral traits (Fig. 2; Table S1): (1) length of the corolla tube; (2) width of the corolla tube; (3) length of the largest petal; (4) width of the largest petal (petal width); (5) number of fringes (indentations $\geq$ 1 mm) on the distal margin of a randomly chosen petal; (6) distance from the nectary at the base of the flower to the tip of the anther (nectar–anther distance); (7) nectar–stigma distance. Anther exertion or stigma exertion was calculated as the difference between nectar–anther or nectar–stigma distance and corolla tube length. We quantified selection on these floral characters because shifts in their character states are often identified with pollinator-mediated selection (Fenster et al. 2004; de Boer et al. 2012) and pollinator-mediated selection has been detected on these traits for many different flowering species (Fenster et al. 2004; Parachnowitsch and
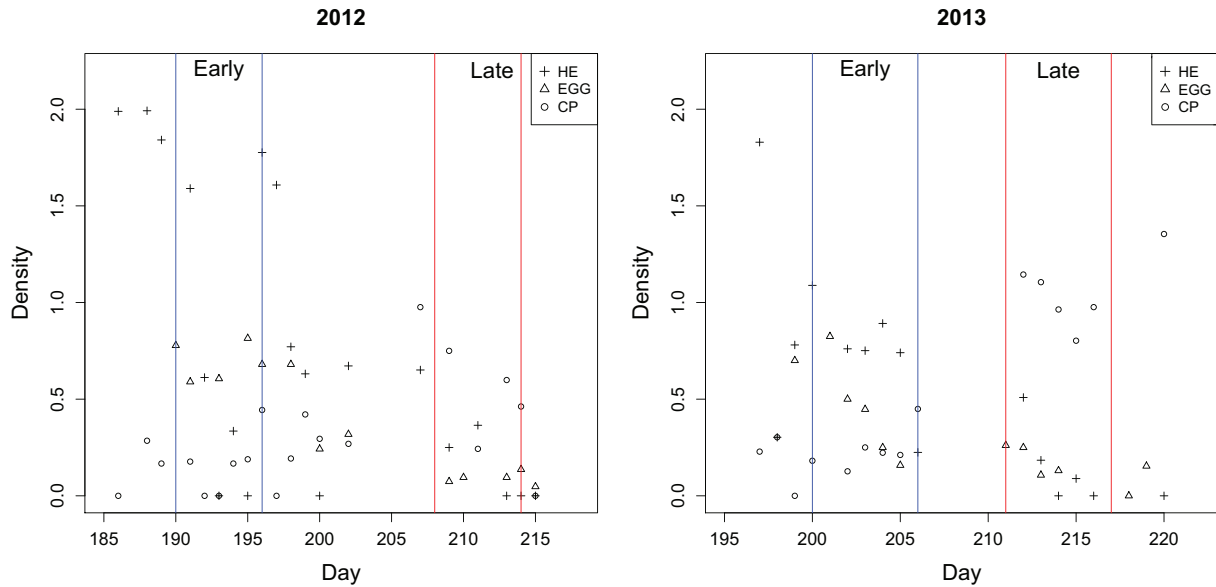
Figure 1. Phenological data of moth and egg densities of 2012 and 2013 for one population of *Silene stellata* near Mountain Lake Biological Station. Moth density was calculated as number of *Hadena ectypa* or co-pollinating moths per flower × 100. Egg density was calculated as mean number of eggs per flower. CP: co-pollinators; HE: *Hadena ectypa*; EGG, egg density.
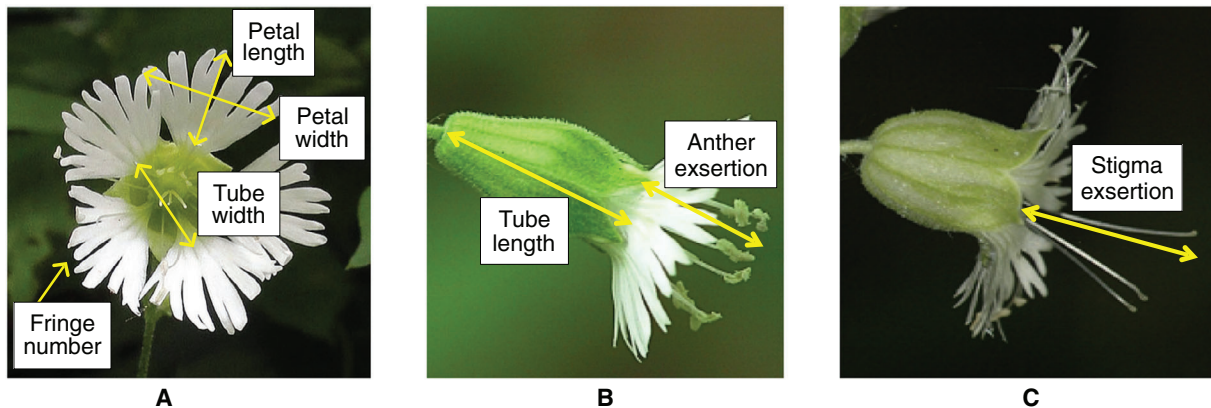
Figure 2. Diagram of *Silene stellata* floral traits measured. All floral traits except stigma exertion were measured on flowers during the first day of opening (the male phase, panel A and B). Stigma exsertion was measured on the first day of the female phase (C).

Kessler 2010; Sletvold 2019), including one related species, *Silene virginica* (Reynolds et al. 2010).

All floral traits except stigma exertion were measured on flowers during the first day of opening (the male phase). Stigma measurements were taken on the first day of the female phase. On average, five of the labeled flowers were measured on each plant. Repeatability (Falconer and Mackay 1996) of the seven floral traits are all significantly different from zero and range between 0.39 (stigma exertion) to 0.89 (petal length). All floral traits except stigma exertion and anther exertion manifest significant heritability ranging between 0.36 (petal width) and 0.44 (petal length) (Zhou 2017). Additionally, we recorded the total number of flowers open throughout the experimental period (number of

flowers) and height of the longest stem (display height) to the nearest cm, for both of which we found no significant heritability (Zhou 2017).

Means of floral-trait measurements from all measured flowers on a plant were calculated to obtain one representative measure for each trait per plant per year. The mean trait measures were z-transformed within each experiment to mean = 0 and variance = 1.

FEMALE FITNESS COMPONENTS

We harvested all labeled reproductive units (RUs) from the study plants after fruit maturation and before fruit dehiscence. In the laboratory, all pistils were scored for whether they initiated a

fruit, had been predated by *H. ectypa* larvae, or matured a fruit without experiencing predation. The proportion of flowers to initially form fruits (hereafter referred to as "fruit initiation proportion") is indicative of the portion of flowers that were pollinated because there is little fruit abortion in *S. stellata* (Kula et al. 2013) and was calculated as the total number of initiated fruits divided by the total number of labeled flowers. For RUs not entirely consumed by *H. ectypa* larvae, we determined fruit initiation occurrence if the ovary was enlarged while a shriveled ovary was indicative of its absence. Larvae sometimes consume the entire pistil of flowers and leave only the petals and calyx (flower predation). In such cases, it was impossible to determine if the RU was enlarged or not. Therefore, we excluded such RUs from the calculation of fruit initiation proportion. If an initiated fruit was consumed late in its development by *H. ectypa* larvae, the hardened ovary wall was left in place with a feeding hole that was 1–2 mm in diameter. We used the number of successful fruits (fruit set) as the measure of female reproductive success, where a successful fruit must have been initiated and free from *H. ectypa* predation. Fruit set is highly correlated with seed set in *S. stellata* (Pearson correlation coefficient $r = 0.88$, $P < 0.001$; Reynolds 2008) thus is a good proxy measure of female reproductive success.

MALE FITNESS COMPONENTS

We used paternity assignment tests to directly estimate male reproductive success with eight highly polymorphic microsatellite markers (mean number of alleles per marker $= 27$, Zhou et al. 2016b, Zhou et al. 2018a; see Supporting Information Methods). We assigned paternity to individual seedlings categorically to the candidate sires using the paternity exclusion program PolyPatEx (Zwart et al., 2016) in the R environment (R Core Team, 2018). PolyPatEx is designed for polyploids and matches the presence/absence pattern of alleles at each locus to determine whether the allele set observed at a given locus of a candidate sire is compatible with the corresponding allele sets in an offspring–mother pair. In our analysis, the set of candidate sires consisted of all study plants of a given experimental period. Because *S. stellata* is a tetraploid, the underlying genotypes of partial heterozygotes cannot be readily resolved (e.g., an individual showing two alleles A and B on a given locus can have AAAB, AABB, or ABBB as the underlying genotype). PolyPatEx addresses this issue by assessing the genotypic compatibility between the progeny, the mother, and the candidate sires through the enumeration of all possible allelic configurations given the presence or absence of alleles. Although the PolyPatEx algorithm is based on Mendelian inheritance, to accommodate the possibility of double reduction as well as genotyping error, we set the maximum number of mismatch loci to three and assigned paternity to the candidate sire with the least number of mismatches with the parent–offspring pair. We included the mother in the set of candidate sires to allow for self-pollination.

We genotyped a total of 2371 seedlings with a mean of 593 seedlings per experiment (2012 early $N = 583$; 2012 late $N = 673$; 2013 early $N = 545$; 2013 late $N = 570$. Table 1), yielding an average of 10.4 seedlings genotyped per plant. The mean percentage of seedlings with unambiguous paternity was 92%, resulting in 2181 seedlings assigned categorically to unique fathers.

TESTING FOR SELECTION DIFFERENCES BETWEEN EARLY AND LATE FLOWERING SEASON AND FEMALE AND MALE FUNCTIONS

We used analysis of covariance (ANCOVA) to test for differences in selection between period (early vs. late) and sex (male vs. female). To avoid over-parameterization and to test if certain interactions were important predictors, we followed a model selection procedure using the Akaike Information Criteria (AIC). Specifically, in addition to the full model, we also fit and compared the performance of four models where the nonzero parameters are (1) traits + sex + year + period; (2) traits + sex + year + period + trait × pollinator, (3) traits + sex + year + period+ trait × sex, (4) traits + sex + year + period + trait × pollinator + trait × sex; and (5) the full model: traits + sex + year + period + trait × pollinator + trait × sex + trait × pollinator × sex. Male and female fitness scores were scaled by the mean fitness of a particular pollinator type-by-sex combination such that the main effects of sex and period and sex × period interaction were eliminated from the model and only the standardized selection gradients were compared across treatment levels.

We interpret significant trait × period interactions as an indication that significantly different patterns of selection are present between the early and late flowering seasons. We interpret significant trait × sex interactions as evidence that selection differs between the two sexual functions. All ANCOVA analyses were performed in R (R Core Team 2018) using the function lmer of the package lme4 1.1-12 (Bates et al. 2015) with "plant" included as a random effect.

ESTIMATING SELECTION GRADIENTS

To quantify phenotypic selection through the two sexual functions and during the early and late flowering seasons, we first pooled the early or late experiments across the 2 years of study and estimated selection coefficients exerted on the floral design of *S. stellata* during a given period of the flowering season that corresponds to the *H. ectypa* dominant or the co-pollinator dominant phase. Year was included as a random effect. The random effect approach estimates overall selection gradients and a random year-specific deviation from the overall mean selection gradients. The variance component for the deviations provides an indicator of the variation in selection across

years, so that substantial variance among years would suggest against pooling data across years and restrict our analyses within years.

Next, we pooled the data across all four experiments for a given sexual function. In addition to the linear terms, we also included quadratic terms in order to quantify nonlinear selection. Nonlinear selection coefficients were doubled according to Stinchcombe et al. (2008).

Multiple regression analyses were carried out in R using the function lmer. To obtain standardized regression coefficients (Lande and Arnold 1983), we normalized the phenotypic measurements by subtracting off the population mean and dividing by the population standard deviation, and rescaled the male or female fitness scores by the mean of all plants of a given experiment. To account for any potential violation of non-normality of the residuals, we generated permutation P-values for testing statistical significance of the selection coefficients. Specifically, for regression analysis, we generated 1000 permutations of the vector of fitness scores and recorded the F-statistics of the regression coefficients for each trait. Permutation P-values were calculated as the frequency of permutations with F-statistics larger than the observed F-statistic. We permuted the fitness vector, instead of individual vectors of trait measurements to preserve the observed phenotypic covariance. In our selection analyses we ascertain significance using type I error rate < 0.05 and False Discovery Rate < 0.10 (Benjamini and Hochberg 1995).

Because *S. stellata* is a predominantly outcrossing species and inbreeding depression is inferred to be high based on mating system estimates (Zhou et al. 2018b) and has been observed in the closely related *S. virginica* (Dudash and Fenster, 2001) and *S. caroliniana* (M. R. Dudash and C. B. Fenster, unpubl. data), we quantified selection only through outcrossing male fertility to test for conflicting selection on traits between sexual functions. We also conducted multiple regressions using data pooled across the early and late experiments for a given year, in order to test for conflicting selection between sexual functions averaged over the two pollinator types.

For the two early experiments during peak adult *H. ectypa* activity with egg count data, we also modeled mean number of eggs per flower as a linear function of the seven floral traits using multiple Gaussian regression to quantify oviposition preference of female *H. ectypa*. Specifically, we fit a linear mixed model on the flower level with egg count as the response variable and floral traits as the predictor variables while having plant as a random effect. We performed the analysis in R using the function lmer of the package lme4 and generated permutation P-values using the same procedure mentioned above.

Results
PHENOTYPIC CORRELATIONS
Phenotypic correlations range between 0.00 and 0.70. The strongest correlation is between petal width and petal length ($r = 0.59$–0.7, $P < 0.001$; Table 2). The two vegetative traits, number of flowers and display height, are significantly positively correlated in all experiments ($r = 0.36$–0.47; Table 2). Correlation between floral and the two vegetative traits is generally low (Table 2) and insignificant, except for anther exertion versus number of flowers ($r = 0.26$, $P < 0.05$; Table 2) in early 2012 and petal length versus display height ($r = 0.27$, $P < 0.05$; Table 2) in late 2012. Mean phenotypic values of the measured traits are summarized in Table S1.

FRUIT-SET
Fruit initiation proportion is high (mean $\pm$ SE $= 0.92 \pm 0.15$, Table 1) and does not significantly differ among the four experiments (F $= 0.47$, DF $= 3,224$, $P > 0.10$) nor between years when data are pooled across pollinator types (F $= 0.22$, DF $= 1,226$, $P > 0.10$). There are significant differences in fruit predation rates among experiments (F $= 48.1$, DF $= 3, 224$, $P < 0.001$). Fruit predation rate is highest in early 2012 (mean $\pm$ SE $= 0.59 \pm 0.36$, Table 1) and lowest in late 2013 (0.19 ± 0.24). When averaging across the early and late experiments for each year, predation rate is significantly higher in 2012 than 2013 (F $= 45.35$, DF $= 1, 226$, $P < 0.001$). Across all of our surveys, fruit predation rates range between 10% (2005) and 50% (2013) for the whole duration of the flowering season.

SELECTION DURING THE EARLY AND LATE FLOWERING SEASON
For selection on both male and female reproductive success, we found the variance explained by year to be close to zero, indicating the lack of significant variation in selection gradients across the 2 years of study. Therefore, we present the results acquired by pooling across years for the early and late flowering season and the two sexual functions as the main findings of this paper.

For the early, adult *Hadena*-dominant experiments, we detected strong patterns of contrasting selection gradients through the two sexual functions on petal length (Female: $\beta = -0.267$, $P = 0.006$; Male: $\beta = 0.645$, $P < 0.001$; Table 3; Figure 3) and petal width (Female: $\beta = 0.269$, $P = 0.004$; Male: $\beta = -0.479$, $P = 0.012$). Additionally, we found significant positive selection for number of flowers in both sexes (Female: $\beta = 0.691$, $P < 0.001$; Male: $\beta = 0.376$, $P = 0.027$; Table 3; Figure 3) and significant positive selection for petal fringes through male function ($\beta = 0.289$, $P = 0.04$; Table 3; Figure 3).

Table 2. Phenotypic Pearson-correlation matrices of the nine traits measured in the early (upper triangular) and late flowering season (lower triangular) of *Silene stellata* near Mountain Lake Biological Station in 2012 and 2013.

2012	TL	TW	PL	PW	FR	ST	AN	HT	NF
TL		0	0.3*	0.22	0.16	0.46***	0.64***	−0.06	0.21
TW	−0.19		0.42***	0.54***	0.29*	0.51***	0.13	0.05	0
PL	0.27*	0.44***		0.59***	0.45***	0.37***	0.16	0	0.09
PW	0.28*	0.51***	0.61***		0.08	0.38***	0.27*	−0.07	−0.01
FR	0.02	0.02	0.26*	−0.03		0.38***	0.23	0.03	−0.03
ST	0.4***	0.41***	0.46***	0.59***	0.11		0.6***	0.17	0.11
AN	0.74***	0.09	0.38***	0.37***	0.25	0.56***		0.07	0.26*
HT	0.07	0.1	0.27*	0.11	0.17	0.22	0.12		0.36**
NF	0.01	0.02	0.12	0.2	0	0.21	0	0.47***	
2013	TL	TW	PL	PW	FR	ST	AN	HT	NF
TL		0.08	0.33**	0.4*	0.04	0.41***	0.69***	0.07	−0.13
TW	−0.09		0.46***	0.42***	0.09	0.33*	0.21	−0.18	−0.07
PL	0.09	0.31*		0.7***	0.16	0.21	0.24	−0.08	−0.09
PW	0.16	0.38***	0.69***		0.07	0.16	0.3*	−0.03	−0.11
FR	0.03	0.31*	0.4***	0.18		0.06	0.09	−0.05	−0.07
ST	0.45***	0.24	0.33**	0.44***	0.19		0.47***	0.13	−0.02
AN	0.46***	0.18	0.38***	0.55***	0.19	0.71***		0.03	−0.05
HT	0.04	−0.04	0.07	0	0.06	0.24	0.1		0.42***
NF	−0.25	0	0.16	−0.02	0.02	0.11	0.04	0.37**	

TL, corolla tube length; TW, corolla tube width; PL, petal length; PW, petal width; FR, number of petal fringes; ST, stigma exertion; AN, anther exertion; HT, display height; NF, number of flowers.
***$P < 0.001$;
**$P < 0.01$;
*$P < 0.05$

Table 3. Results of multiple linear regressions through male and female functions using data pooled across years for the early and late experiments for *Silene stellata* near Mountain Lake Biological Station.

	Early				Late			
	Male		Female		Male		Female	
	β	P	β	P	β	P	β	P
Tube length	0.101	0.477	−0.108	0.137	0.027	0.84	−0.024	0.712
Tube width	−0.095	0.524	−0.029	0.718	−0.078	0.603	0.118	0.141
Petal length	0.645	<0.001*	−0.267	0.006*	0.102	0.47	−0.034	0.704
Petal width	−0.479	0.012*	0.269	0.004*	0.114	0.462	−0.03	0.734
Fringe number	0.289	0.04*	−0.061	0.345	−0.14	0.25	−0.033	0.632
Stigma exertion	−0.165	0.321	0.057	0.502	−0.011	0.934	0.025	0.756
Anther exertion	−0.008	0.947	0.04	0.566	0.192	0.081	0.076	0.289
Flower number	0.376	0.027*	0.691	<0.001*	0.074	0.562	0.798	<0.001*
Display height	0.257	0.065	−0.002	0.976	−0.049	0.708	0.091	0.239

Nonparametric *P*-values based on 1000 permutations are shown next to the standardized linear regression coefficients (β). Asterisks indicate *P*-values significant at FDR <0.10.
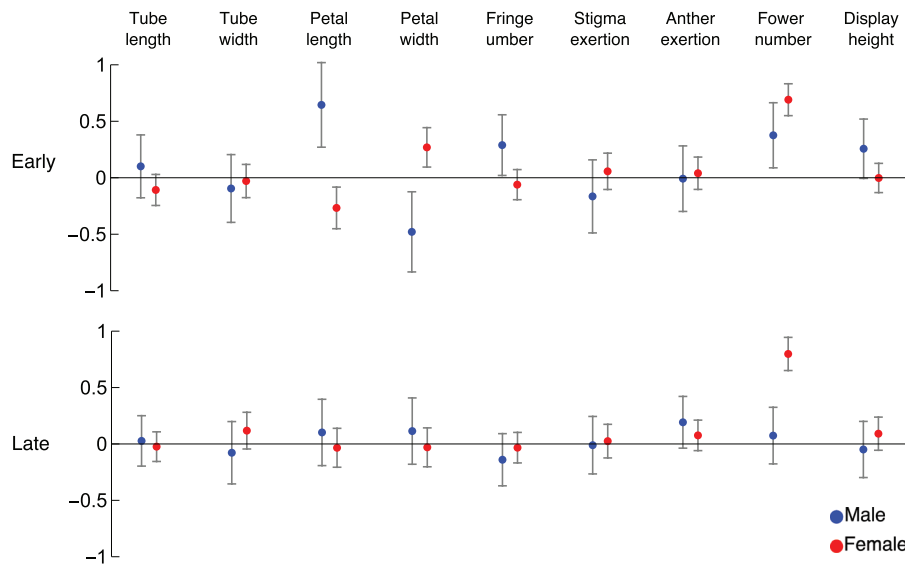
Figure 3. Selection gradients for the phenotypic traits of *Silene stellata* through male and female functions during early and late flowering season of 2012 and 2013 near Mountain Lake Biological Station. Data were pooled across years for a given period (early vs. late). Error bars indicate 95 percent confidence interval.

We detected no significant selection for the late experiment except the positive selection for number of flowers through female function ($\beta = 0.798$, $P < 0.001$).

OVERALL SELECTION THROUGH MALE AND FEMALE REPRODUCTIVE SUCCESS

When data for a given sex were pooled across years and flowering seasons, we found the selection gradients to have consistent signs with those of the early experiments for both sexes but smaller in magnitude for traits that were found to be significant in the previous section. We found the only significant selection coefficients to be petal length through male function ($\beta = 0.254$, $P = 0.039$; Table S2) and number of flowers through female function ($\beta = 0.783$, $P < 0.001$; Table S2). We found no significant nonlinear selection through both sexual functions using data pooled across all four experiments.

SELECTION THROUGH FEMALE REPRODUCTIVE SUCCESS 2003–2011

Phenotypic selection through only female function throughout the whole flowering seasons of 2003–2011 detected significant positive selection for flower number in all years (Table S3), consistent with the results for 2012–2013. Additionally, we found significant negative selection for tube width in 2003 ($\beta = -0.27$, $P < 0.01$) and 2006 ($\beta = -0.13$, $P = 0.05$) (Table S3). We also found significant negative selection for tube length ($\beta = -0.22$, $P = 0.02$) in 2004.

SEXUALLY CONFLICTING SELECTION

The AIC model comparison supported model 3 (traits + sex + period + year + trait × sex), for which ANCOVA detected significant differences in selection between sexual functions (significant trait × sex interactions) for three traits: petal length (F = 15.39, DF = 1, $P < 0.001$; Table 4), petal width (F = 3.97, DF = 1, $P = 0.048$; Table 4), and number of flowers (F = 62.42, DF = 1, $P < 0.001$; Table 4).

COMPARISON OF MAGNITUDE OF SELECTION BETWEEN SEXES AND POLLINATOR TYPES

When pooled across years and sexes, absolute values of selection gradient β's are higher in the early experiments than the late experiments (early: median ($|\beta|$) = 0.14, late: median ($|\beta|$) = 0.07, Wilcoxon rank sum W = 216, $P = 0.09$). When selection gradients are pooled across years and pollinator types, selection through male function is stronger than through female function (Male: median ($|\beta|$) = 0.11, Female: median ($|\beta|$) = 0.06, W = 203, $P = 0.20$).

OVIPOSITION PREFERENCE OF *Hadena ectypa*

The mean number of eggs per flower was significantly higher in 2012 than 2013 (2012: Mean ± SE = 1.04 ± 0.82; 2013: Mean ± SE = 0.55 ± 0.32; $t = 4.27$, $P < 0.001$, Welch two sample *t*-test). There is a significant negative correlation between oviposition (mean egg number per flower) and female fitness across years (Spearman rank correlation, 2012: $r = -0.26$, $P = 0.049$; 2013: $r = -0.40$, $P = 0.003$). Correlations between the mean number of eggs per flower and male fitness are close to

Table 4. ANCOVA results testing for differential selection on floral traits of *Silene stellata* between sexual functions in 2012 and 2013 near Mountain Lake Biological Station. Trait main effects represent total selection averaged over male and female reproductive success, and trait × sex interactions test for differences in selection through male vs. female functions. Plant is included as a random effect. Main effects of sex, experiment (early vs. late), and year are also included in the model but are not shown for simplicity. Effects in bold are significant at *P* < 0.05.

Effect	SS	df	F ratio	P
Tube length	0.031	1	0.035	0.853
Tube width	0.007	1	0.007	0.932
Petal length	2.373	1	2.648	0.105
Petal width	0.447	1	0.499	0.481
Fringe number	0.038	1	0.042	0.837
Stigma exsertion	0.304	1	0.339	0.561
Anther exsertion	1.815	1	2.025	0.156
Flower number	**62.419**	**1**	**69.66**	**<0.001**
Display height	2.188	1	2.442	0.12
Sex × Tube length	0.77	1	0.859	0.355
Sex × Tube width	2.332	1	2.603	0.108
Sex × Petal length	**13.786**	**1**	**15.385**	**<0.001**
Sex × Petal width	**3.556**	**1**	**3.968**	**0.048**
Sex × Fringe number	1.876	1	2.094	0.149
Sex × Stigma exsertion	0.597	1	0.666	0.415
Sex × Anther exsertion	0.045	1	0.05	0.823
Sex × Flower number	**13.695**	**1**	**15.284**	**<0.001**
Sex × Display height	0.028	1	0.031	0.861

Table 5. Results of linear mixed model testing *Hadena ectypa* oviposition preference for floral traits of *Silene stellata* on the flower level using egg count data collected during the early flowering seasons of 2012 and 2013. Trait values were normalized by subtracting off the population mean and dividing by the population standard deviation. Number of eggs per flower was used as the response variable with plant included as a random effect. Nonparametric p-values based on 1000 permutations are shown next to the standardized linear regression coefficients.

Trait	Coefficient	P
Tube length	−0.157	0.485
Tube width	−0.067	0.145
Petal length	0.291	0.001
Petal width	0.019	0.87
Fringe number	0.025	0.39
Stigma exsertion	−0.056	0.882
Anther exsertion	−0.108	0.181

itation in *S. stellata* (J. Zhou, unpublished data). In contrast, we found high mean and high variance of fruit predation rate among plants during the two early experiments. Therefore, we consider fruit predation to be the most likely pathway for selection through female function. This is consistent with the findings by Burkhardt et al. (2012) where for the dioecious *Silene latifolia*, its pollinating seed predator *Hadena bicuris* exerts stronger selective pressure on female plants through predation than pollination.

Selection for avoidance of predation by *H. ectypa* larvae can occur mainly during two stages of interaction with *H. ectypa*, namely oviposition by female moths and fruit predation by larvae. Using egg count data, we found a strong oviposition preference for longer petals on the flower level. Newly hatched larvae (first and second instars) feed on *S. stellata* ovules within the flower and have limited ability to move between flowers (J. Zhou personal observation) and are possibly incapable of moving between plants. Therefore, large number of *H. ectypa* eggs can be directly linked to increase seed predation by young larvae. In contrast, in the late flowering season, young *H. ectypa* larvae (first and second instars) became rare and seed predation was primarily carried out by large larvae that can move between plants (J. Zhou personal observation), which is concordant with the observation that selection gradients on traits other than flower number vanished in the late season. Therefore, we consider female oviposition preference to be the primary mechanism for selection through female function of *S. stellata*.

zero and insignificant in either year. Linear mixed model found petal length to have a strong positive correlation with higher oviposition by female *H. ectypa* (*P* = 0.001; Table 5).

Discussion

Using multi-year field experiments combined with paternity analysis, we found strong evidence of sexually conflicting selection acting through male and female functions on the floral design of the hermaphroditic *Silene stellata*. The conflicting selection pressures acted on petal length and petal width, for which we found significant trait by sex interaction as well as consistent contrasting selection gradients during the early flowering season, when *Hadena ectypa* was the dominant pollinator for *S. stellata*. Below we synthesize the selection patterns on floral traits and discuss these findings within the context of pollinator-mediated selection.

POSSIBLE MECHANISMS FOR THE OBSERVED CONFLICTING SELECTION PATTERNS
Selection through female function
We observed high fruit initiation rate with low variance in all four experiments, consistent with the lack of evidence for pollen lim-

Selection through male function
The pathway to paternity for hermaphroditic plants is complex and involves multiple stages (Inouye et al. 1994). Natural selection may occur during any stage where nonpollinator and/or

abiotic agents may be the primary sources of selective pressures. However, the observed pattern of selection is consistent with *H. ectypa* as the selective agent since the trait preference we observed is largely congruent with moth pollination behavior. Longer petal or larger flower size has been previously documented to be associated with higher visitation. For example, petal length is positively correlated with visitation rate by syrphid flies in *Raphanus raphanistrum* (Conner et al. 1996). In *S. virginica*, a related species to *S. stellata*, large flower size is found to increase hummingbird visitation (Fenster et al. 2006; Reynolds et al. 2009). More relevant, large flowers are correlated with higher pollinator visitation in F2 hybrids between *Silene diclinis* and *Silene latifolia*, for which *Hadena bicuris* serve as an important pollinator (Brothers and Atwell 2014). At the same time, we emphasize that our result is distinct from previous studies, in that long and narrow petal size is selected for through male function, in contrast to the commonly measured petal or flower size. We speculate that the phenotypic selection through male function for longer and narrower petals and possibly for more fringes may indicate a trait profile that helps to increase the visibility of the flower under low light conditions and thus increase pollinator visitation and possibly pollen export (Delpino 1870; Vogel 1954; de Boer et al. 2012). Furthermore, flower fringes may increase the surface area of petals, increasing the dispersal of attractive fragrances (Mitchell et al. 2015).

We propose that the observed sexually conflicting selection in *S. stellata* can be explained by a simple mechanism that the floral design with long and narrow petals increases visitation rate by both the male and female *H. ectypa* moths due to its high visibility. Higher *H. ectypa* visitation can be directly linked to increased pollen export and male fertility. On the other hand, higher visitation by female *H. ectypa* adults can also lead to higher oviposition frequency, which has strong negative consequence on female fitness. While there is indirect evidence for the pathway through female function provided by our egg count data, behavioral observation of pollination visitation and oviposition will help to elucidate the mechanism for the observed sexually conflicting selection.

Selection during early versus late flowering phenology

Although the ANCOVA analysis does not support a model with strong trait × period interaction, we did observe stronger selection on more traits during the two early experiments versus the later periods. Specifically, we found no significant selection through male or female function in the late experiment on traits except flower number. As discussed above, we consider selection through female function to be largely through the avoidance of oviposition by the *H. ectypa* larvae. Therefore, we interpret the lack of significant selection through female function in the late

season to be related to the drop in oviposition activity during the two years of study.

Given that the flowering phenology was largely coincidental with the decline of *H. ectypa* density and the rise of copollinators density, we propose that the variation of selection magnitude on *S. stellata* floral traits through male function is manifested within the whole functional group of nocturnal moth pollinators. More specifically, *H. ectypa*, as an obligate pollinating seed predator possesses specific preferences for the floral design of its host, whereas the group of purely generalist moths likely has less distinct trait preferences. Therefore, the collective selective pressure they exert is likely to be diffuse (Strauss et al. 2005; Sahli and Conner 2011) and difficult to detect. While differential selection by various pollinator groups might implicate conflicting selection and trade-offs (Kulbaba and Worley 2013), our result of a lack of divergent selection between different pollinator cohorts suggests that a trade-off between pollinator groups is unlikely (Aigner 2004, 2006). Last, in addition to the variational selective pressure across the flowering season, we also observed variation in selection through female reproductive success across years based on the 2003–2011 data. This could be the result of variation in *H. ectypa* activity across years and/or the general pollinator community dynamics (Reynolds et al. 2012).

About a third of the 600+ *Silene spp.* manifest traits consistent with nocturnal moth pollination (Kephart et al. 2006). Here, we demonstrate that even within a "generalized" nocturnal moth pollination system, *H. ectypa* is potentially imposing the strongest selection and may be the most important selective agent on floral design in moth pollinated *Silene* species. There are few studies of pollinator mediated selection that distinguish the amount and type of selection exerted within or between different functional groups on a single plant. Given the frequent evolution of floral form corresponding to generalized and specialized pollination systems and the consequences for clade diversification associated with these transitions (O'Meara et al. 2016), we need more investigations of the selective pressures on the maintenance and origin of pollination generalization.

Conclusion

Here, we show that floral traits, most notably petal shape, of *S. stellata* are under opposing selection through the two sexual functions of the hermaphroditic plant. We propose that this observed pattern can be understood largely in light of the interaction with the obligate pollinating seed predator *H. ectypa*, which serves as both a pollinator and a seed predator. More specifically, male and female fitness components of *S. stellata* potentially have differential sensitivity to these two types of interactions, giving rise to the observed conflict between male

and female function. If the source of the sexually conflicting selection is indeed *H. ectypa*, our results may have implications for the evolutionary dynamics of the *Silene–Hadena* systems. The role of *H. ectypa* was previously classified as parasitic due to the large quantities of fruits the *H. ectypa* larvae consume as well as to the presence of mutualistic co-pollinators that are equally effective at pollen transfer (Reynolds et al. 2012). Consequently, an "escape route" from *H. ectypa* or nocturnal pollination syndrome in general should be evolutionarily favored (Kephart et al. 2006). Here, we show that the same floral traits (petal shape) potentially under selection to avoid fruit predation could be under opposing selective pressure to increase male fertility, possibly providing a strong counterbalancing force to stabilize the *S. stellata* population at an evolutionary equilibrium and contribute to the long-term maintenance of this interaction.

More generally, our study indicates that conflicting selection may result from differential responses of different fitness components to mutualistic and antagonistic interactions. This form of conflicting selection may remain undetected if the experiment is restricted to only one aspect of interaction or one fitness component. Therefore, future research conducted using a more integrated procedure may help to elucidate the prevalence and importance of conflicting selection in plants.

AUTHOR CONTRIBUTIONS
J.Z., M.R.D, and C.B.F designed and performed field experiments for 2012 and 2013; J.Z and E.A.Z designed and performed molecular experiments; R.J.R, M.R.D, and C.B.F designed and performed field experiment for 2003–2009; J.Z., R.J.R, E.A.Z, M.R.D, and C.B.F wrote the paper.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Supplemental Table 1. Mean (SE) of floral and plant traits of *Silene stellata* near Mountain Lake Biological Station assessed during the early (*Hadena ectypa* dominant) and late (co-pollinators dominant) flowering season for 2012 and 2013. Floral traits are in unit of mm.
Supplemental Table 2. Results of multiple linear regressions with linear and quadratic terms through male and female reproductive success using data pooled across all four experiments conducted for *Silene stellata* in 2012 and 2013 near Mountain Lake Biological Station.
Supplemental Table 3. Results of generalized linear multiple regressions through female function throughout the flowering season of 2003-2011 for *Silene stellata* near Mountain Lake Biological Station.