Gender inequality in predispersal seed predation contributes to female seed set advantage in a gynodioecious species

GRETEL L. CLARKE[1] AND ALISON K. BRODY

Department of Biology, University of Vermont, Burlington, Vermont 05405 USA and
The Rocky Mountain Biological Laboratory, Crested Butte, Colorado 81224 USA

Abstract. Most flowering plants are hermaphrodites. However, in gynodioecious species, some members of the population are male-sterile and reproduce only by setting seed, while others gain fitness through both male and female function. How females compensate for the loss of male function remains unresolved for most gynodioecious species. Here, as with many plants, fitness differences may be influenced by interactions with multiple species. However, whether multiple species interactions result in gender-specific fitness differences remains unknown. Using observational data from 2009–2010, we quantified seed set of the two sex morphs of *Polemonium foliosissimum* and asked how it is affected by pollination, and seed predation from a dipteran predispersal seed predator (Anthomyiidae: *Hylemya* sp.). We assessed seed production and losses to predation in 27 populations for one year and in six populations for a second year. Females set significantly more seed than did hermaphrodites in both years. Of the fitness components we assessed, including the number of flowers per plant, fruit set, seeds/fruit, and proportion of fruits destroyed by *Hylemya*, only fruit destruction differed significantly between the sexes. In one year, seeds/fruit and predation had a stronger effect on seed set for hermaphrodites than for females. Because predispersal seed predators do not pollinate flowers, their effects may depend on successful pollination of flowers on which they oviposit. To examine if genders differed in pollen limitation and seed predation and/or their interactive effects, in 2011 we hand-pollinated flowers and removed seed predator eggs in a fully factorial design. Both sexes were pollen limited, but their degree of pollen limitation did not differ. However, predation reduced seed set more for hermaphrodites than for females. We found no significant interaction between hand pollen and seed predation, and no interaction between hand pollination and gender. Our results suggest that while interactions with both pollinators and seed predators affect reproductive success, floral enemies can cause inequality in seed set between genders. The next step is to understand how the seed set advantage affects long-term fitness and persistence of females in gynodioecious populations.

Key words: gynodioecy; Hylemya *sp.; obligate outcrosser; plant–insect interactions;* Polemonium foliosissimum; *pollination; predispersal seed predation.*

INTRODUCTION

Most angiosperms are hermaphrodites, with plants accruing female and male fitness through the damming and siring of seed. Gynodioecy, however, is a relatively common alternative mating system that occurs in ~7% of all species of flowering plants (Richards 1986). In gynodioecious populations, some individuals are typical hermaphrodites, while others have lost the ability to produce pollen and function as females. The interest in gynodioecy is long standing (Darwin 1877), and continues, in large part, because it presents an intriguing evolutionary conundrum: how do females compensate for the loss of male fitness, and thereby persist alongside hermaphrodite counterparts?

Theory tells us that the loss of male function is evolutionarily stable only if females compensate via increased quantity or quality of seed (Lloyd 1975, Charlesworth and Charlesworth 1978). Females could produce more or better seeds by conserving resources that would otherwise go to the production of pollen and larger flowers typical of hermaphrodites (Vaughton and Ramsey 2002, Ashman 2006). Females could also reap an advantage by avoiding inbreeding depression (Charlesworth and Charlesworth 1978). Although these mechanisms provide a female seed set advantage in some cases (Sakai et al. 1997, Vaughton and Ramsey 2004, Ramsey et al. 2006, Collin et al. 2009), in others they don't (Shykoff 1992, Asikainen and Mutikainen 2005*a*, Miyake and Olson 2009).

Virtually all plants interact with a multitude of other species and do so simultaneously. These interactions can affect hermaphrodite and female success differently (e.g., Puterbaugh 1998, Marshall and Ganders 2001, Ashman 2002, 2006, Ashman et al. 2004, Asikainen and

Mutikainen 2005*b*). For example, pollinators often prefer hermaphrodites, which have larger flowers and provision visitors with pollen in addition to nectar (Delph and Lively 1992, Ashman 2000, Case and Ashman 2009). In addition, most gynodioecious species are self-compatible, and therefore hermaphrodites garner some reproductive assurance through the use of self-pollen (Lloyd 1975). One might thus expect females to be more strongly pollen limited than hermaphrodites, and experimental evidence supports this in some cases (e.g., Ramsey and Vaughton 2002, Dufay and Billard 2012), but not in others (reviewed by Shykoff et al. 2003, Knight et al. 2005, and see Asikainen and Mutikainen 2005*a*). An unexpected lack of difference in pollen limitation between sex morphs may occur for several reasons. For example, flower size, important to pollinator attraction, may enhance male function more than female function (Bell 1985, Stanton et al. 1986). Thus, although females produce smaller flowers than hermaphrodites and may experience reduced visitation as a result, visitation may be sufficient to insure fertilization of most ovules. In addition, hermaphrodites may suffer from geitonogamy (the transfer of self-pollen among flowers on the same inflorescence leading to stigmas clogged with self-pollen [Klinkhamer et al. 1989, de Jong et al. 1993, Alonso and Herrera 2001]), and/or lower seed quality through inbreeding depression (Sakai et al. 1997, Ramsey et al. 2006, Collin et al. 2009). Overall, we lack sufficient studies in gynodioecious species to understand how pollination success affects the relative seed set advantage of sex morphs (Dufay and Billard 2012).

There is also growing evidence that hermaphrodites suffer greater damage by floral and foliar herbivores and predispersal seed predators (Agren et al. 1999, Ashman 2002, Asikainen and Mutikainen 2005*b*, Cornelissen and Stiling 2005, Vega-Frutis et al. 2013; but see Alonso 2003, Collin and Shykoff 2010). For example, hermaphrodite-biased herbivory has been reported for bud-clipping weevils (Ashman et al. 2004, Ashman and Penet 2007), seed-feeding weevils (Marshall and Ganders 2001, Asikainen and Mutikainen 2005*b*), and lepidopteran frugivores and florivores (Collin and Shykoff 2010, McCall and Barr 2012). In each case, greater damage to hermaphrodites helped to explain the fitness advantage garnered by females. Yet the contributions to fitness differences often vary and can depend on the broader context in which interactions occur (Wootton 1994, Brody 1997, Strauss and Irwin 2004, Morris et al. 2007, Alarcon et al. 2008). Indeed, for many flowering plants, the effects of pollinators and herbivores are intertwined and the effects of one cannot be fully ascertained without the context of the other (Strauss and Irwin 2004, Irwin 2010). This may be especially true for predispersal seed predators that are not, themselves, pollinators. In this case, the effects of predispersal seed predation will depend on successful pollination of the fruits on which they oviposit, as well as on other sources of fruit failure

such as resource limitation or selective abortion of damaged fruits by plants. Moreover, opposing selection by multiple species can constrain plants' response to each (Strauss and Irwin 2004, Morris et al. 2007).

Here we examined how pollination success and predispersal seed predation affected seed set for hermaphrodites and females in gynodioecious populations of sticky polemonium, *Polemonium foliosissimum*. In addition, we examined if their effects were additive or not. If well-pollinated plants are disproportionately attractive to seed predators, one would expect to see nonadditive effects of pollination success and seed predation. These patterns will ultimately affect plant response to both parties and could lead to different types of selection operating on each gender (Ashman 2000).

First we asked: Do the genders differ in total seed production or any of its components including proportion of fruit set, seeds/fruit, or proportion of fruits destroyed? Second, we asked: Does the importance of individual fitness components to total seed production differ between the genders? Third, we experimentally evaluated the strength of pollen limitation and seed predation to ask: Do the effects of pollen limitation and seed predation differ for females and hermaphrodites and are they additive? By exploring these questions, we shed new light on the potential for biotic interactions to promote the maintenance of females in gynodioecious plants.

METHODS

Study system

Polemonium foliosissimum (Polemoniaceae) is a long-lived, herbaceous perennial that grows in subalpine meadows of the western United States. *Polemonium foliosissimum* is an obligate outcrosser, pollinated primarily by generalist bumble bees (Zimmerman 1980*a*). Seeds are consumed by a dipteran predispersal seed predator (Anthomyiidae: *Hylemya* sp.). *Hylemya* females oviposit eggs singly under sepals of buds. Larvae hatch, burrow into the developing fruit, and consume all developing seeds before burrowing out of the carpel wall and falling to the ground to pupate in the soil. Larvae do not move among fruits, completing development in the fruit on which they were laid. *Hylemya* does not pollinate flowers and, therefore, larval success depends on successful pollination of the flower on which eggs were laid (Zimmerman 1980*b*).

Near the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado, where we worked, many populations of *P. foliosissimum* are gynodioecious; sex ratios range from 100% hermaphrodite to ~40% female. We studied 27 populations in the Paonia Ranger District of the Gunnison National Forest. All populations occurred within 2 km of Kebler Pass Road, but were separated by 2–5 km (and, at minimum, 100 m) from each other. Population sizes ranged from 66 to 622 plants ($\bar{X} \pm$ SE = 242.93 $\pm$ 21.84). In 2009, we haphazardly tagged 10 female and 10 hermaphrodite

plants in each of 27 populations varying in sex ratio from 6% to 33% female. In 2010, in six of these populations we permanently tagged all plants within belt transects, 20–50 m long and 1.5 m wide. If necessary, we marked additional females outside the transect to reach at least 50 females/population, or all females in populations with <50 females.

Because *P. foliosissimum* is self-incompatible and requires animals to vector pollen, we began with an "all else equal" assumption that pollination success is a reasonable proxy for pollinator service. Indeed, low fruit set and low numbers of seeds/fruit can result from insufficient pollination (Zimmerman and Pyke 1988). Predispersal seed predation can account for significant, post-pollination losses, which could ameliorate or enhance sex morph-specific differences in pollination success. To quantify differences in pollination success and seed predation between the sex morphs, we measured the proportion of flowers that set fruit (hereafter fruit set), seeds/fruit from undamaged fruits, and the proportion of fruits destroyed by *Hylemya* from permanently marked plants in 2009 and 2010. We counted all reproductive stalks, and collected fruits from a subset of stalks, scoring each fruit as aborted (not expanded and lacking seeds) or filled, and examined each expanded fruit for damage by *Hylemya*. Fruit set was calculated as the proportion of flowers that set seed, regardless of whether seeds were destroyed or not, and fruit destruction was calculated as the proportion of set fruits destroyed. *Hylemya* destruction is easily identified by seed remnants and frass. The remaining undamaged fruits were used to estimate seeds/fruit. Last, we calculated seed set by multiplying the average numbers of flowers produced per stalk × proportion of flowers to set fruit × average seeds/fruit × proportion of fruits not destroyed × total number of reproductive stalks.

Do genders differ in total seed production or any of its components including proportion fruit set, seeds/fruit, or proportion of fruits destroyed?—We compared the fitness components, fruit set, seeds/fruit, and proportion of fruits destroyed, as well as total plant seed set, between the genders. We first ran a MANCOVA for 2009 and 2010, separately, and included all of the fitness components as dependent variables with gender as the single main effect, blocked by site, and total flowers per plant as the covariate. Site was treated as a random, blocking factor here and throughout. Finding gender significant in the MANCOVA, we ran two-way AN-OVAs (Scheiner 1993) using each fitness component as the dependent variable in separate tests, with gender as a main effect, blocked by site. The gender × site interaction was not significant, and was thus dropped from all subsequent models. In all analyses, we included only those plants with ≥20 fruits, resulting in a final sample size of 102 females and 113 hermaphrodites in 2009 and 59 females and 185 hermaphrodites in 2010. We used a cutoff of at least 20 fruits to ensure an accurate estimate of fitness components. On average,

plants produced 143.43 ± 9.16 fruits; those with <20 were heavily browsed or very small. Few plants (12 females and 14 hermaphrodites) were shared between 2009 and 2010 data sets; thus the data sets were largely independent. We log transformed total flowers per plant and seed set, and arcsine square-root transformed proportion fruit set and fruits destroyed. All analyses were performed in IBM SPSS v. 21 (IBM SPSS 2012).

Does the importance of individual fitness components to total seed production differ between the genders?—To determine if genders differ in the importance of individual fitness components to seed set we ran ANCOVAs using seed set as the dependent variable, fitness measures as covariates (each in a different model) with gender as a main effect, blocked by site. In each case, we started with a fully saturated model but dropped all nonsignificant interactions. We were most interested in whether genders differed in fitness measures. Therefore, we retained the gender × fitness measure interaction in all models.

Does pollen limitation and seed predation differ for females and hermaphrodites and are their effects additive?—To experimentally quantify the effects of pollen limitation and seed predation on seed set of female and hermaphrodite plants, we supplemented pollen and removed seed predator eggs in a fully factorial design in two populations in 2011. We used plants at two sites separated by ~300 m, in the vicinity of, but separate from, the populations used in previous years. The size and sex ratio of these populations were comparable to others (225 and 75 plants, with 14% and 20% female). Early in the season, we identified sets of eight plants (4 females and 4 hermaphrodites) that were similar in size and flowering phenology. We randomly assigned plants within each set (by gender) to one of four treatments: hand pollinated (yes/no) crossed with eggs removed (yes/no). Because plants can produce hundreds of flowers ($\bar{X}$ = 302.16 ± 25.2 flowers in this study), applying treatments to all flowers on plants was impossible. Instead, we applied treatments to all flowers on three stalks per plant and, on rare occasions, added additional stalks when those initially chosen ceased flowering. To examine if plants reallocated resources from untreated to treated stalks (Zimmerman and Pyke 1988), we included equal numbers of control stalks on which flowers were handled but not treated. On control plants, we also labeled and handled six stalks comparably but without treatment application. The experiment included a total of 88 plants across two sites: two genders × four treatments × 11 replicates.

For the hand pollination treatment, we applied pollen (using anther bouquets from multiple plants within 20 m of, but outside, the study population) to all receptive stigmas every three or four days. At the same time, for plants assigned to the egg removal treatment, we removed eggs from all flowers and buds on three stalks per plant. Stigmas are receptive for ~3 days, and *Hylemya* larvae emerge from eggs within 5–7 days after

oviposition. By applying treatments at 3–4 day intervals, we ensured that most flowers were treated. Treatments were applied from mid-June until plants finished flowering in early August. We collected fruits as they ripened and calculated components of female fitness as previously described. However, we calculated seed set over the stalks within a given plant and treatment and refer to this as "net seed set" to distinguish it from total plant seed set measured in the observational study.

Plants might allocate resources to hand-pollinated flowers, or flowers from which eggs were removed, at the expense of those on control stalks. If so, one would find a significant difference in fitness measures between control stalk flowers on treatment plants vs. those on control plants. To test for this, and to test for non-treatment differences among plants, we first ran a MANOVA using only control stalks, with fruit set, seeds/fruit, proportion of fruits destroyed, and net seed set, as response variables, and gender, hand pollination, and egg removal treatments as main effects, blocked by site. Finding gender significant, we ran ANOVAs for each response variable. We included flower number as a covariate, but subsequently deleted it from all analyses except net seed set due to its insignificance.

To examine the response of genders to treatments, we calculated response ratios for each component of female fecundity (fruit set, seeds/fruit, proportion of fruits destroyed, and total seed set). Response ratios measure proportional differences in treatments while correcting for natural differences among plants in these measures (Morris et al. 2007), and were calculated by taking the average of each variable across all flowers on treated stalks and dividing by the average of that same response variable on control stalks on the same plant. Thus values above 1.0 represent an increase in the fitness measure due the treatment, and those below 1.0 indicate a decrease due to the treatment (e.g., response ratios of 1.2 and 0.8 indicate a 20% increase and decrease).

We first ran a MANCOVA with gender, hand pollination, and egg removal treatments as main effects, blocked by site, and the ratio of flowers on treatment : control stalks as the covariate. Dependent variables included the response ratio (treatment : control stalk values) for each female fitness measure. We log transformed response ratio values for seeds/fruit and total seed set, and square-root transformed proportion of fruits destroyed, to meet assumptions for parametric analyses. Finding significant main effects of hand pollination and egg removal, we then ran individual ANCOVAs for fruit set, seeds/fruit, and proportion of fruits destroyed. In all cases, we first ran a full model with all interactions and then removed insignificant interactions. The covariate was significant only for total seed set and was dropped from other analyses. Because there were no treatment stalks on control plants, we randomly sampled without replacement from control fruits to derive separate control and "treatment" samples for control

plants and used these values to calculate fruit set, seeds/fruit, and net seed set for "treatment" stalks on control plants.

RESULTS

Females produced ~40% more seeds per plant than hermaphrodites in 2009 ($F_{1, 187} = 5.682$, $P = 0.018$) and ~11% more seeds per plant than hermaphrodites in 2010 ($F_{1, 236} = 4.492$, $P = 0.035$; Fig. 1; Appendices A and B). There was no significant gender × site interaction in either year, suggesting the female seed set advantage was similar across sites.

Of the components that comprise total seed set, only the proportion of fruits destroyed differed significantly between genders, and did so in both years. In 2009, females lost ~49% fewer fruits to seed predators than hermaphrodites ($F_{1, 188} = 33.529$, $P < 0.001$) and females lost ~36% fewer fruits to seed predators in 2010 ($F_{1, 236} = 21.337$, $P < 0.001$, Fig. 1; Appendices A and B). In 2010, both predation and seeds per fruit had a stronger effect on seed set of hermaphrodites than females (gender × predation interaction, $F_{1, 235} = 4.473$, $P = 0.035$; gender × seeds per fruit interaction, $F_{1, 235} = 5.277$, $P = 0.022$).

Results of our hand pollination and egg removal experiment agreed with those of the observational studies. As before, genders differed in overall seed production and its components ($\lambda = 0.521$, $F_{4,48} = 11.018$, $P < 0.001$). Female controls produced 29% more total seeds ($F_{1,63} = 21.892$, $P < 0.001$), 14% more seeds/fruit ($F_{1,64} = 6.406$, $P = 0.014$, Fig. 2; Appendices C and D), and lost 25% fewer fruits to seed predation, than hermaphrodites ($F_{1,64} = 30.559$, $P < 0.001$). Plants did not appear to reallocate resources from control to treatment stalks; we found no significant differences among control stalks of plants assigned to hand pollination or egg removal treatments vs. those on control plants (Appendix D).

Both hand pollination and egg removal significantly affected fitness components ($\lambda = 0.743$, $F_{2,57} = 6.570$, $P = 0.001$; $\lambda = 0.505$, $F_{2,57} = 18.642$, $P < 0.001$, respectively); there was a significant effect of gender ($\lambda = 0.873$, $F_{2,57} = 2.771$, $P = 0.05$) and a nearly significant gender × egg removal interaction ($\lambda = 0.889$, $F_{2,57} = 2.380$, $P = 0.079$). Egg removal reduced the proportion of fruits destroyed by ~61%, and hand pollination increased the number of seeds/fruit by ~20% ($F_{1,64} = 111.196$; $P < 0.001$ and $F_{1,64} = 15.855$; $P < 0.001$, respectively, compared to control stalks; Fig. 2; Appendices C and E).

On average, net seed set of hermaphrodites responded more strongly to hand pollination and egg removal than it did for females (a significant effect of gender overall; $F_{1,60} = 10.489$, $P = 0.002$, Fig. 2; Appendix E). There were also significant effects of hand pollination ($F_{1,60} = 6.658$, $P = 0.012$, Fig. 2) and egg removal ($F_{1,60} = 58.661$, $P < 0.001$; Fig. 2). Hand pollination alone resulted in an 11% increase (response ratio = 1.11) in net seed set, while egg removal alone increased it by 91% (response ratio =
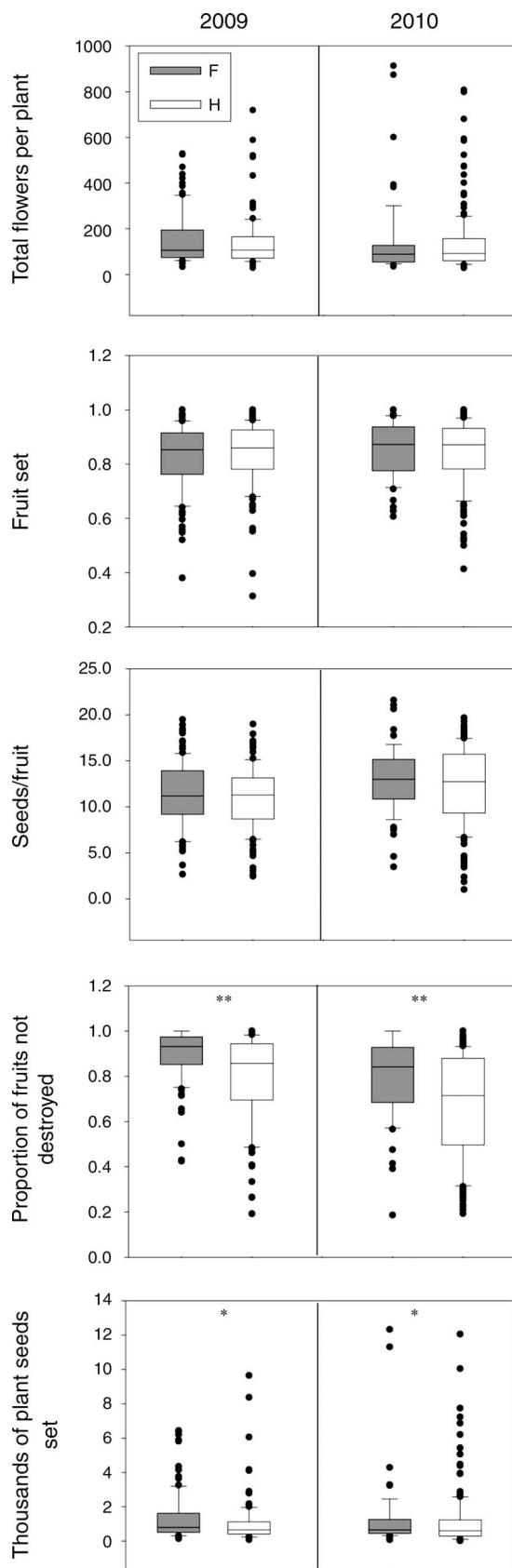
1.91). Net seed set increased by 144% in the hand pollination and egg removal treatment (response ratio = 2.44). Hand pollination and egg removal were additive: there was no significant interaction between the treatments, and no gender × hand pollination × egg removal interaction. However, we did find a significant interaction between gender and egg removal. Net seed set for hermaphrodites increased 135% in response to egg removal treatments (response ratio = 2.35), while the gain for females was 100% that of controls (response ratio 2.0; $F_{1,60} = 4.158$, $P = 0.046$, Appendix E).

DISCUSSION

Complex ecological interactions contribute to relative fitness differences among individuals and, in sexually dimorphic species, may act as important drivers in mating system evolution (Charlesworth 2006). Although sex morphs of gynodioecious *P. foliosissimum* did not differ in pollen limitation, females experienced significantly lower rates of predispersal seed predation and set significantly more seed than hermaphrodites. Predation had a stronger effect on seed set in hermaphrodites than in females in one year and, in a third year when eggs were removed, hermaphrodites experienced a greater net gain in seed production than did females.

A stronger effect of predispersal seed predation, rather than pollen limitation, on reproductive differences between the sex morphs was somewhat surprising. Pollen limitation might be expected in gynodioecious species because a significant fraction of the population does not produce pollen. Indeed, pollen limitation can become more severe as the sex ratio of females/hermaphrodites increases (e.g., Widen and Widen 1990, but see Asikainen and Mutikainen 2005*a*, reviewed in Ashman 2006). Our study plants were significantly pollen limited; however, the magnitude of pollen limitation did not differ between the genders. The similar degree of pollen limitation between the sex morphs of *P. foliosissimum* may be attributed to opposing mechanisms operating simultaneously. Hermaphrodites produce larger flowers, provide both nectar and pollen, and thus may be more attractive to pollinators than females (Ashman 2000). However, self-incompatibility prevents *P. foliosissimum* from gaining reproductive assurance through the use of its own pollen, and hermaphrodites may suffer from self-

←

FIG. 1. Fitness components of female and hermaphrodite plants from observational studies in 2009 and 2010. H = hermaphrodite and F = female. Note: the proportion of fruits not destroyed is shown so that in all panels an increase in value on the y-axis represents increased plant fitness. The line in each box represents the median. The upper and lower edges of the box are the 75th and 25th percentile, respectively. Upper and lower whiskers show the 90th and 10th percentile, respectively. All outliers higher than the 90th and lower than the 10th percentile are shown as dots. Asterisks denote level of significance between genders within years from ANOVAs (* $P < 0.05$; ** $P < 0.01$). (Appendix A).
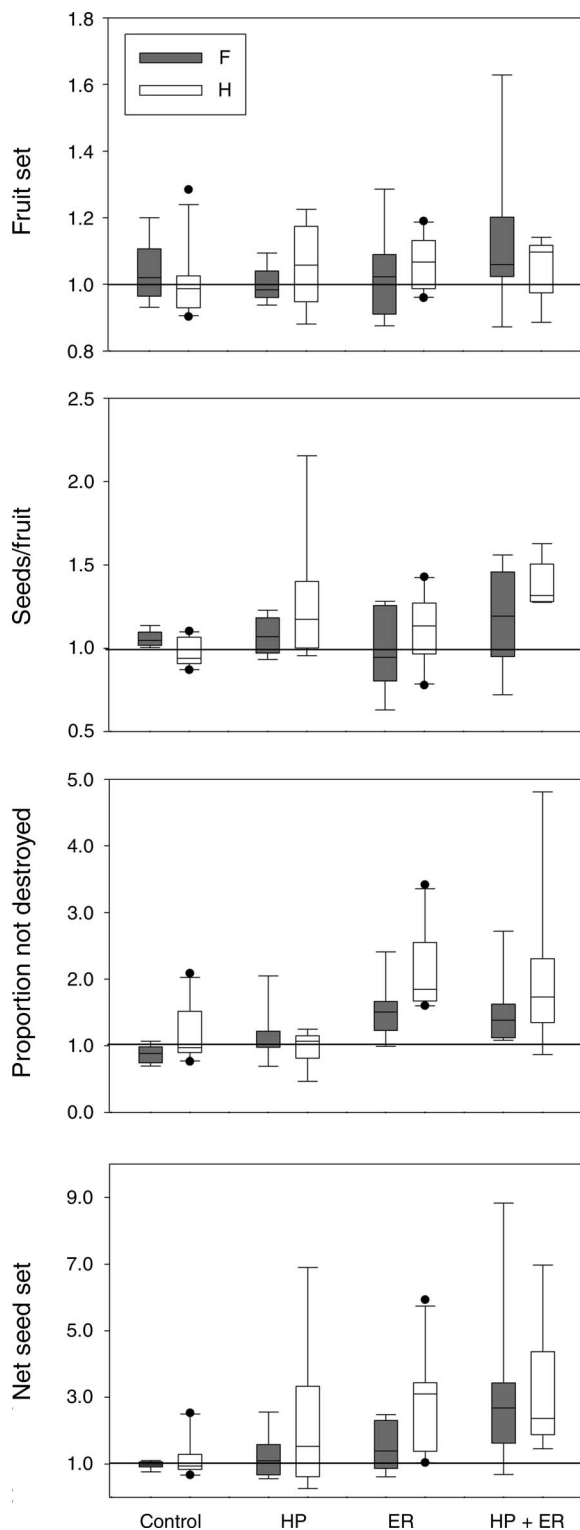
FIG. 2. Response ratios (treatment stalk values:control stalk values) for fruit and seed parameters. Note: proportion of fruits *not* destroyed is shown rather than proportion destroyed, so that all fitness measures are enhanced when the response ratio is >1.0. Treatments included controls, hand pollination (HP), egg removal (ER) and hand pollination and egg removal (HP and ER) for female (F) and hermaphrodite (H) plants.

pollen deposition (e.g., de Jong et al. 1993, Alonso and Herrera 2001, Sage et al. 2006). The relative importance of visitation rates and transfer of self pollen to inequality in seed set between sex morphs of self-incompatible species remains a question ripe for investigation. Interestingly, from our observational data, the difference in seed set between genders was uninfluenced by sex ratio. Although one might expect pollen limitation to increase with the proportion of females, a lack of frequency dependence in female fitness advantage is not unprecedented (e.g., Molina-Freaner and Jain 1992, Olson 2001).

Differences in the effects of seed predation could come about via several, nonexclusive mechanisms. First, ovipositing *Hylemya* could prefer one sex morph over the other. Our data support this: a greater percentage of hermaphrodite flowers were oviposited on by *Hylemya* than those of females in 2011 (66.37% ± 15.71% vs. 48.54% ± 16.93%, respectively). Second, egg or larval survivorship could be lower on females. Eggs may be more likely to desiccate on the smaller flowers characteristic of females. Zimmerman and Brody (1998) found significantly higher mortality due to egg desiccation under the narrower sepals of *Hylemya*'s alternative host, *I. aggregata*. Third, females may be more able to tolerate destruction of some fruits by increasing the seed set in undamaged fruits. Although we did not test tolerance per se, the slope of the relationship between predation and seed set in 2010 differed between the sexes, suggesting there may be differences in tolerance at least in some years.

Regardless of the underlying mechanisms, sex-differential predation resulted in greater seed set for females (here, and Uno 1982, Marshall and Ganders 2001). Yet the bias in sex-differential predation is not always consistent, even within the same system. For example, flower- and bud-clipping weevils preferentially damage hermaphrodite *F. virginiana* but, in some years, hermaphrodites are better able to tolerate damage than are females (Ashman et al. 2004, Ashman and Penet 2007). In *Dianthus sylvestris*, fruits on female plants may be more likely to escape predation by moth larvae, but these pollinating parasites also vector fungal pathogens to which female flowers are more susceptible than are hermaphrodites (Collin et al. 2002, Collin and Shykoff 2010). These examples underscore the need to assess multiple interactions and to do so over multiple years. The complex ways in which species interactions play out ultimately affect plants' ecological and evolutionary responses to their individual and combined effects.

Most studies testing the combined effects of herbivores and pollinators have found additive effects (Morris et al. 2007). Nonadditive, or interactive, effects are more likely where there are resource-based trade-offs, or where one species changes the strength or consequence of an interaction with another (Karban and Strauss 1993). Of the four studies we know of in which pollinators and seed predators were simultaneously

manipulated, two found interactive effects. In both, the seed predators were frugivores. Frugivores targeted successfully pollinated fruits, and thus the effects of an increase in pollination could only be realized when these animals were absent (Herrera 2000, Herrera et al. 2002). In the other two studies we know of, the pollinators were also seed predators and the fitness gains and losses had additive effects on seed set (Humphries and Addicott 2000, Holland 2002). Here, we might have expected interactive effects because seed predators rely on pollinators to successfully provision their offspring with seeds. Thus, a clever fly should target flowers that will ultimately be visited by pollinators. But, they don't (Brody and Waser 1995, Brody and Morita 2000). We also expected flies to target females if females have greater fruit set or produce more seeds per fruit. However, the opposite was true; flies preferentially oviposit on hermaphrodites. Therefore, the choices made by ovipositing females may be based more on the protective effects of larger flowers than the promise of successful pollination. Nonadditive or interactive effects would also occur if plants face resource-based trade-offs; for example if plants can re-allocate resources from fruits with larvae to those without. If plants can re-allocate in this way, we might expect seed set to have plateaued in the hand pollination and egg removal treatment where plants experienced low predation and high pollination success, yet we did not. Additive effects tend to make the strength of selection by animals on plants more predictable, and thus present stronger and more consistent opportunities for selection (Gomez 2005).

Although hermaphrodites consistently experienced greater levels of seed predation over three years and among plants in 29 different sites, we expect the relative importance of seed predation and pollination to vary over the lifetime of a long-lived, perennial plant. *P. foliosissimum* is pollen limited in some years but not in others (Zimmerman 1980a, Zimmerman and Pyke 1988), and fruit destruction varies among years as well (see Appendix A and Brody 1997). Predation may be more or less important in years of limited resources. The summer of 2013, for example, was extraordinarily dry. Many species around the RMBL, including *P. foliosissimum*, did not flower at all or produced few flowers and fruits despite an abundance of pollinators. In dry years, low resources and reduced flowering could ameliorate or exacerbate the differences in fruit destruction between sex morphs.

In the year we manipulated pollination and predation rates, egg loads were high and pollinator numbers low. We thus expected, and found, a strong response to both egg removal and hand pollination. Although one might expect the importance of seed predators to be enhanced on plants that were hand pollinated, we found no evidence for an interaction that could dilute the effects of seed predators (Strauss et al. 2005). Predation is variable, but can account for as much as 90% fruit loss

to an individual (Brody 1997). Over the three years of this study, fruit loss to *Hylmeya* ranged from 10.41% to 39.42% ($\bar{X} = 23\%$) for females and from 20.55% to 55.29% ($\bar{X} = 36\%$) for hermaphrodites. Adding to the handful of studies of how mutualists and antagonists simultaneously affect female reproductive advantage in a gynodioecious plant (e.g., Collin et al. 2002, Asikainen and Mutikainen 2005b, Cole and Ashman 2005, Ashman and Penet 2007, Zhang et al. 2007), our results provide strong evidence that the seed fitness advantage of females in *P. foliosissimum* is generated by hermaphrodite-biased predispersal seed predation. But in our study and in general, the effects of seed predation over multiple generations are largely unknown (but see Louda 1982, Leimu and Lehtila 2006, Maron and Crone 2006). The next challenge is to understand if the seed set advantage garnered by females in one or a few years translates into a lifetime fitness advantage in the context of the entire life cycle of plants (Silvertown et al. 1993) and thus helps explain the persistence of females in gynodioecious plants.

LITERATURE CITED

Agren, J. A., K. T. Danell, L. Elmquist, L. Ericson, and J. Hjalten. 1999. Sexual dimorphism and biotic interactions. Pages 217–246 *in* M. A. Geber, T. E. Dawson, and L. F. Delph, editors. Gender and sexual dimorphism in flowering plants. Springer-Verlag, Berlin, Germany.

Alarcón, R., N. M. Waser, and J. Ollerton. 2008. Year-to-year variation in the topology of a plant-pollinator interaction network. Oikos 117:1796–1807.

Alonso, C. 2003. Herbivores do not discriminate between leaves of female and hermaphrodite individuals of gynodioecious *Daphne laureola* (Thymelaeaceae). Oikos 101:505–510.

Alonso, C., and C. M. Herrera. 2001. Neither vegetative nor reproductive advantages account for high frequency of male-steriles in southern Spanish gynodioecious *Daphne laureola* (Thymelaeaceae). American Journal of Botany 88:1016–1024.

Ashman, T. L. 2000. Pollinator selectivity and its implications for the evolution of dioecy and sexual dimorphism. Ecology 81:2577–2591.

Ashman, T. L. 2002. The role of herbivores in the evolution of separate sexes from hermaphroditism. Ecology 83:1175–1184.

Ashman, T. L. 2006. The evolution of separate sexes: a focus on the ecological context. Pages 419–465 *in* S. C. H. Barrett and L. D. Harder, editors. The ecology and evolution of flowers. Oxford University Press, New York, New York, USA.

Ashman, T. L., D. H. Cole, and M. Bradburn. 2004. Sex-differential resistance and tolerance to herbivory in a gynodioecious wild strawberry. Ecology 85:2550–2559.

Ashman, T. L., and L. Penet. 2007. Direct and indirect effects of a sex-biased antagonist on male and female fertility: consequences for reproductive trait evolution in a gender-dimorphic plant. American Naturalist 169:595–608.

Asikainen, E., and P. Mutikainen. 2005a. Pollen and resource limitation in a gynodioecious species. American Journal of Botany 92:487–494.

Asikainen, E., and P. Mutikainen. 2005b. Preferences of pollinators and herbivores in gynodioecious Geranium sylvaticum. Annals of Botany 95:879–886.

Bell, G. 1985. On the function of flowers. Proceedings of the Royal Society B 224:223–265.

Brody, A. K. 1997. Effects of pollinators, herbivores, and seed predators on flowering phenology. Ecology 78:1624–1631.

Brody, A. K., and S. I. Morita. 2000. A positive association between oviposition and fruit set: female choice or manipulation? Oecologia 124:418–425.

Brody, A. K., and N. M. Waser. 1995. Oviposition patterns and larval success of a pre-dispersal seed predator attacking two confamilial host plants. Oikos 74:447–452.

Case, A. L., and T. L. Ashman. 2009. Resources and pollinators contribute to population sex-ratio bias and pollen limitation in Fragaria virginiana (Rosaceae). Oikos 118:1250–1260.

Charlesworth, B. 2006. The evolutionary biology of sex. Current Biology 16:R693–R695.

Charlesworth, B., and D. Charlesworth. 1978. A model for the evolution of dioecy and gynodioecy. American Naturalist 112:975–997.

Cole, D. H., and T. L. Ashman. 2005. Sexes show differential tolerance to spittlebug damage and consequences of damage for multi-species interactions. American Journal of Botany 92:1708–1713.

Collin, C. L., L. Penet, and J. A. Shykoff. 2009. Early inbreeding depression in the sexually polymorphic plant Dianthus sylvestris (Caryophyllaceae): effects of selfing and biparental inbreeding among sex morphs. American Journal of Botany 96:2279–2287.

Collin, C. L., P. S. Pennings, C. Rueffler, A. Widmer, and J. A. Shykoff. 2002. Natural enemies and sex: how seed predators and pathogens contribute to sex-differential reproductive success in a gynodioecious plant. Oecologia 131:94–102.

Collin, C. L., and J. A. Shykoff. 2010. Flowering phenology and female fitness: impact of a pre-dispersal seed predator on a sexually polymorphic species. Plant Ecology 206:1–13.

Cornelissen, T., and P. Stiling. 2005. Sex-biased herbivory: a meta-analysis of the effects of gender on plant-herbivore interactions. Oikos 111:488–500.

Darwin, C. R. 1877. The different forms of flowers on plants of the same species. Murray, London, UK.

de Jong, T. J., N. M. Waser, and P. G. L. Klinkhamer. 1993. Geitonogamy–the neglected side of selfing. Trends in Ecology and Evolution 8:321–325.

Delph, L. F., and C. M. Lively. 1992. Pollinator visitation, floral display, and nectar production of the sexual morphs of a gynodioecious shrub. Oikos 63:161–170.

Dufay, M., and E. Billard. 2012. How much better are females? The occurrence of female advantage, its proximal causes and its variation within and among gynodioecious species. Annals of Botany 109:505–519.

Gomez, J. M. 2005. Non-additive effects of herbivores and pollinators on Erysimum mediohispanicum (Cruciferae) fitness. Oecologia 143:412–418.

Herrera, C. M. 2000. Measuring the effects of pollinators and herbivores: evidence for non-additivity in a perennial herb. Ecology 81:2170–2176.

Herrera, C. M., M. Medrano, P. J. Rey, A. M. Sanchez-Lafuente, M. B. Garcia, J. Guitian, and A. J. Manzaneda. 2002. Interaction of pollinators and herbivores on plant fitness suggests a pathway for correlated evolution of mutualism- and antagonism-related traits. Proceedings of the National Academy of Sciences USA 99:16823–16828.

Holland, J. N. 2002. Benefits and costs of mutualism: demographic consequences in a pollinating seed-consumer interaction. Proceedings of the Royal Society B 269:1405–1412.

Humphries, S. A., and J. F. Addicott. 2000. Regulation of the mutualism between yuccas and yucca moths: intrinsic and extrinsic factors affecting flower retention. Oikos 89:329–339.

IBM SPSS. 2012. Version 21. IBM, Armonk, New York, USA.

Irwin, R. E. 2010. Evolutionary ecology: when pollinators are also herbivores. Current Biology 20:R100–R101.

Karban, R., and S. Y. Strauss. 1993. Effects of herbivores on growth and reproduction of their perennial host, Erigeron glaucus. Ecology 74:39–46.

Klinkhamer, P. G. L., T. J. de Jong, and G.-J. de Bruyn. 1989. Plant size and pollinator visitation in Cynoglossum officinale. Oikos 54:201–204.

Knight, T. M., J. A. Steets, J. C. Vamosi, S. J. Mazer, M. Burd, D. R. Campbell, M. R. Dudash, M. O. Johnston, R. J. Mitchell, and T. L. Ashman. 2005. Pollen limitation of plant reproduction: pattern and process. Annual Review of Ecology, Evolution, and Systematics 36:467–497.

Leimu, R., and K. Lehtila. 2006. Effects of two types of herbivores on the population dynamics of a perennial herb. Basic and Applied Ecology 7:224–235.

Lloyd, D. G. 1975. The maintenance of gynodioecy and androdioecy in angiosperms. Genetica 45:325–339.

Louda, S. M. 1982. Limitation of the recruitment of the shrub Haplopappus squarrosus (Asteraceae) by flower-feeding and seed-feeding insects. Journal of Ecology 70:43–53.

Maron, J. L., and E. Crone. 2006. Herbivory: effects on plant abundance, distribution and population growth. Proceedings of the Royal Society B 273:2575–2584.

Marshall, M., and F. R. Ganders. 2001. Sex-biased seed predation and the maintenance of females in a gynodioecious plant. American Journal of Botany 88:1437–1443.

McCall, A. C., and C. M. Barr. 2012. Why do florivores prefer hermaphrodites over females in Nemophila menziesii (Boraginaceae)? Oecologia 170:147–157.

Miyake, K., and M. S. Olson. 2009. Experimental evidence for frequency dependent self-fertilization in the gynodioecious plant, Silene vulgaris. Evolution 63:1644–1652.

Molina-Freaner, F., and S. K. Jain. 1992. Female frequencies and fitness components between sex phenotypes among gynodioecious populations of the colonizing species Trifolium hirtum in California. Oecologia 92:279–286.

Morris, W. F., et al. 2007. Direct and interactive effects of enemies and mutualists on plant performance: a meta-analysis. Ecology 88:1021–1029.

Olson, M. S. 2001. Patterns of fruit production in the subdioecious plant Astilbe biternata (Saxifragaceae). Journal of Ecology 89:600–607.

Puterbaugh, M. N. 1998. The roles of ants as flower visitors: experimental analysis in three alpine plant species. Oikos 83:36–46.

Ramsey, M., and G. Vaughton. 2002. Maintenance of gynodioecy in Wurmbea biglandulosa (Colchicaceae): gender differences in seed production and progeny success. Plant Systematics and Evolution 232:189–200.

Ramsey, M., G. Vaughton, and R. Peakall. 2006. Inbreeding avoidance and the evolution of gender dimorphism in Wurmbea biglandulosa (Colchicaceae). Evolution 60:529–537.

Richards, A. J. 1986. Plant breeding systems. George Allen and Unwin, London, UK.

Sage, T. L., M. V. Price, and N. M. Waser. 2006. Self-sterility in Ipomopsis aggregata (Polemoniaceae) is due to prezygotic ovule degeneration. American Journal of Botany 93:254–262.

Sakai, A. K., S. G. Weller, M. L. Chen, S. Y. Chou, and C. Tasanont. 1997. Evolution of gynodioecy and maintenance of females: the role of inbreeding depression, outcrossing rates, and resource allocation in *Schiedea adamantis* (Caryophyllaceae). Evolution 51:724–736.

Scheiner, S. M. 1993. MANOVA: multiple response variables and multispecies interactions. Pages 94–112 *in* S. M. Scheiner and J. Gurevitch, editors. Design and analysis of ecological experiments. Chapman and Hall, New York, NewYork, USA.

Shykoff, J. A. 1992. Sex polymorphism in *Silene acaulis* (Caryophyllaceae) and the possible role of sexual selection in maintaining females. American Journal of Botany 79:138–143.

Shykoff, J. A., S. O. Kolokotronis, C. L. Collin, and M. Lopez-Villavicencio. 2003. Effects of male sterility on reproductive traits in gynodioecious plants: a meta-analysis. Oecologia 135:1–9.

Silvertown, J., M. Franco, I. Pisanty, and A. Mendoza. 1993. Comparative plant demography–relative importance of life-cycle components to the finite rate of increase in woody and herbaceous perennials. Journal of Ecology 81:465–476.

Stanton, M., A. A. Snow, and S. N. Handel. 1986. Floral evolution: Attractiveness to pollinators increases male fitness. Science 232:1625–1627.

Strauss, S. Y., and R. E. Irwin. 2004. Ecological and evolutionary consequences of multispecies plant-animal interactions. Annual Review of Ecology, Evolution, and Systematics 35:435–466.

Strauss, S. Y., H. Sahli, and J. K. Conner. 2005. Toward a more trait-centered approach to diffuse (co)evolution. New Phytologist 165:81–89.

Uno, G. E. 1982. Comparative reproductive-biology of hermaphroditic and male-sterile *Iris douglasiana* herb (Iridaceae). American Journal of Botany 69:818–823.

Vaughton, G., and M. Ramsey. 2002. Evidence of gynodioecy and sex ratio variation in *Wurmbea biglandulosa* (Colchicaceae). Plant Systematics and Evolution 232:167–179.

Vaughton, G., and M. Ramsey. 2004. Dry environments promote the establishment of females in monomorphic populations of *Wurmbea biglandulosa* (Colchicaceae). Evolutionary Ecology 18:323–341.

Vega-Frutis, R., M. A. Munguia-Rosas, S. Varga, and M. M. Kytoviita. 2013. Sex-specific patterns of antagonistic and mutualistic biotic interactions in dioecious and gynodioecious plants. Perspectives in Plant Ecology, Evolution and Systematics 15:45–55.

Widen, B., and M. Widen. 1990. Pollen limitation and distance-dependent fecundity in females of the clonal gynodioecious herb *Glechoma hederacea* (Lamiaceae). Oecologia 83:191–196.

Wootton, J. T. 1994. The nature and consequences of indirect effects in ecological communities. Annual Review of Ecology and Systematics 25:443–466.

Zhang, Y. W., G. W. Robert, Y. Wang, and Y. H. Guo. 2007. Nectar robbing of a carpenter bee and its effects on the reproductive fitness of *Glechoma longituba* (Lamiaceae). Plant Ecology 193:1–13.

Zimmerman, M. 1980*a*. Reproduction in *Polemonium*: competition for pollinators. Ecology 61:497–501.

Zimmerman, M. 1980*b*. Reproduction in *Polemonium*: pre-dispersal seed predation. Ecology 61:502–506.

Zimmerman, M., and A. K. Brody. 1998. Choices and consequences of oviposition by *Hylemya* (Delia) sp. (Diptera: Anthomyiidae). Journal of Insect Behavior 11:371–381.

Zimmerman, M., and G. H. Pyke. 1988. Reproduction in *Polemonium*–assessing the factors limiting seed set. American Naturalist 131:723–738.

Supplemental Material

Ecological Archives

Appendices A–E are available online: http://dx.doi.org/10.1890/14-1513.1.sm