# ROLES OF POLLINATORS, SEED PREDATORS, AND VERTEBRATE HERBIVORES IN MAINTAINING FEMALES IN THE GYNODIOECIOUS POLEMONIUM FOLIOSISSIMUM

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## ABSTRACT

Most flowering plants are hermaphroditic, reproducing through seed set and seed siring. In gynodioecious species, some individuals are female and only reproduce through seed set. Because females do not gain fitness through pollen, they must have a seed fitness advantage over hermaphrodites to be maintained. Mutualists and antagonists interacting with plants may generate this advantage. We evaluated whether pollinators (*Bombus* sp.), pre-dispersal seed predator flies (*Hylemya* sp.), and vertebrate herbivores help explain the persistence of females in the gynodioecious *Polemonium foliosissimum*.

We found no evidence that pollinator service differed between the sex morphs; neither fruit set nor seeds per fruit differed between them. However, fruits on female plants were attacked less often by *Hylemya*, and females set more seed. Physical removal of seed predator eggs increased seed set more than did supplemental pollen addition, and hand pollination and reduction in seed predation had additive effects on seed set. In addition, destruction of fruits by seed predators reduced seed set of hermaphrodites more than females. Thus, we concluded that seed predators, not pollinators, most strongly affect the female seed set advantage in *P. foliosissimum*.

We tested the mechanisms underlying sex-biased predation and found that flies preferentially oviposit on hermaphrodites and may be partly explained by earlier flowering phenology and larger flowers of hermaphrodites; oviposition rates decline over the season and with flower size. Larval survival was also higher on hermaphrodites, and may be partly explained by the earlier phenology and larger flowers of hermaphrodites; larval survival also declined over the season and with flower size. Thus flies appear to be 'wise' in preferentially ovipositing on hermaphrodites. From the plant point of view, females experience a lower rate of predation both because they receive fewer eggs and because larval mortality is higher on females than hermaphrodites.

All of the studies we know of to evaluate whether herbivores generate fitness differences between the sex morphs used seed set in one or a few years. However, one should ideally use demographic modeling to compare lifetime seed production, incorporating differences in survivorship or growth, likely important to lifetime seed production. Females had a higher germination rate of seeds, and greater survival and growth of medium sized reproductive plants. Females also produced more seed in high sex ratio populations (high proportion of female plants), where seed predation was hermaphrodite-biased. However, even in these populations, females did not have a lifetime advantage (female : hermaphrodite $\lambda$ was $< 1$) in preliminary models where we assumed females and hermaphrodites produced 30% and 5% female progeny, respectively. Females only had a lifetime advantage if they produced more than ~ 50% female offspring. Thus, female persistence in *P. foliosissimum* may be context-dependent, perhaps occurring where cytoplasmic male sterility alleles are at a high frequency due to stochastic processes such as drift and founder effects.

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# CHAPTER 1: GENERAL INTRODUCTION

## 1.1 Introduction

Most flowering plants are hermaphroditic—garnering offspring through both female function (seeds) and male function (the siring of seeds; Richards 1986). However, mixed sexual strategies, whereby individuals differ in gender, occur in ~10% of angiosperm species (Renner and Ricklefs 1995). Among plants employing a mixed strategy, gynodioecy is one of the most common types whereby some members of the population are female while others are typical hermaphrodites. Given that they have lost male function and sire no seeds, females must have a seed fitness advantage over hermaphrodites to be maintained (Lewis 1941, Lloyd 1975, Charlesworth and Charlesworth 1978).

In the past decade, the role that mutualists and antagonists may play in generating fitness differences and maintaining females within populations has come into the limelight (see reviews by Ashman 2002, 2006). However, few studies have quantified the effects of multi-species interactions (but see Collin et al. 2002, Asikainen and Mutikainen 2005, Cole and Ashman 2005, Ashman and Penet 2007, Zhang et al. 2008) and none that I know of have employed experimental manipulations of mutualists and antagonists to rigorously quantify the outcome of multi-species interactions. Beyond this, I know of no studies which have assessed the effects of mutualists and antagonists on the fertility of

the two sex morphs of a gynodioecious species within the context of the entire life cycle using demographic modeling. Using demographic modeling, it is possible to quantify the effect of multi-species interactions on the lifetime fertility advantage females attain through seed production. Moreover, within a demographic modeling framework, it is possible to evaluate the importance of any numerical seed set advantage females have over hermaphrodites in the context of other lifestage transitions, namely growth and survivorship transitions, which may be more important to population growth (Silvertown et al. 1993). Finally, if sex morphs differ in survivorship or growth transitions (which has rarely been investigated but see Morris and Doak 1998, Ramula et al. 2007) it may help explain how females persist. Here I examine what if any lifetime fitness advantage females attain through seed production and the extent to which mutualists and antagonists generate fitness differences that may help females persist.

## 1.2 Gynodioecy

The vast majority of angiosperms can be classified as hermaphroditic, monoecious (with separate female and male flowers on the same plants) or into two main types of dimorphic systems- dioecy, and gynodioecy (Renner and Ricklefs 1999). Of the dimorphic sexual systems, gynodioecy is second most common following dioecy – or complete separation of the sexes, where individuals are separate male and female plants. Gynodioecy is thought to be the most likely evolutionary intermediate between hermaphroditism and dioecy (Delannay 1979, Charlesworth and Charlesworth 1999,

Weiblen 2000), and this transition has occurred in approximately 38% of angiosperm families (Renner and Ricklefs 1995). Thus, elucidating the role ecological forces play in maintaining this sexual system also sheds light on its contribution to the evolution of a much wider array of plant mating systems.

The debate over how females can be maintained alongside hermaphrodites, considering that females are at a disadvantage on account of the fact that they forgo one of two main avenues for gaining fitness, is not a new one (Darwin 1877). Early theoretical studies showed that females, in lacking any fitness gain through male function, should have twice the female (seed) fitness advantage of hermaphrodites to be maintained. However, it is also known that some species display cytoplasmic or maternal inheritance of "male sterility", in which case females only require a slightly greater seed fitness advantage than hermaphrodites, since feminizing genes do not segregate and are passed to all offspring (Lewis, 1941; Lloyd, 1975). Female advantage could be expressed as an increase in offspring quality (Shykoff 1988, Ashman 1992, Eckhart 1992, Puterbaugh et al. 1997, Chang 2006) and/or quantity (Shykoff et al 2003, Ashman 2006). Offspring quality may be enhanced in females due to avoidance of inbreeding depression (Darwin 1877, Charlesworth 1999, Ashman 2006) or a greater maternal provisioning of seeds. However, these mechanisms do not consistently explain female advantage (e.g., Thompson and Tarayre 2000, Marshall and Ganders 2001, reviewed in Ashman 2006 and Dufay and Billard 2012).

### 1.3 Mutualist and antagonist effects on seed set advantage

The outcome of interactions with mutualists and antagonists often differs between sex morphs in sexually dimorphic species (reviewed in Ashman 2002, Cornelissen and Stiling 2005, Ashman 2006, Vega-Frutis et al. 2013). For example, pollinators often preferentially visit the larger flowers of hermaphrodites over females (e.g. Delph and Lively 1992, Ashman 2000, Case and Ashman 2009), which could make females more pollen-limited, likely reducing the relative seed set advantage of females over hermaphrodites. In addition, pollinator movements control how much self pollen is deposited on hermaphrodite stigmas. In self-incompatible species, the transfer of self pollen can reduce seed quality in hermaphrodites through increased rates of inbreeding depression (Sakai et al. 1997, Dufay and Billard 2012, Thompson and Tarayre 2000), or in self-incompatible species through pollen clogging (De Jong et al. 1993). Sex differential herbivory is also common in sexually dimorphic species. In dioecious species, male biased herbivory is well documented and likewise in gynodioecious species it is generally hermaphrodite biased (e.g. Uno 1982, Case 2000, Marshall and Ganders 2001, Ashman and King 2005, Ashman and Penet 2007, Collin and Shykoff 2010). Sex morphs can differ in floral display and scent (Shykoff 2003, Asikainen and Mutikainen 2005, Ashman et al. 2005), growth rate (Agren et al. 1999), allocation to reproduction (Lloyd and Webb 1977), allocation to defense (Jing and Coley 1990, Cornelissen and Stiling 2005, and Tsuji and Sota 2010, Tsuji and Sota 2013), and phenology (Uno 1982, Asikainen and Mutikainen 2005, Ashman and Stanton 1991) which could explain the differences in apparency or suitability to mutualists and antagonists. Even where

herbivory is not biased toward one sex morph or another – as is likely the case with vertebrate herbivores – sex morphs may differ in their tolerance to predation due to differences in phenology or allocation to reproduction (e.g., Ashman et al. 2004).

Evidence that sex-differential interactions do result in seed set differences between sex morphs has emerged in several systems (Ashman 2002, 2006). For example, Puterbaugh (1998) found that ants, which act as pollinators in their alpine Rocky Mountain system, increased female seed set advantage in two gynodioecious plants. In *Eritrichum aretioides*, this was the result of ants inflicting greater damage to hermaphrodites than females, and in *Paronychia pulvinata*, ants effected greater pollination service to females than hermaphrodites, possibly as a result of high rate of self pollen transfer among hermaphrodite flowers. For *Sidalcea hendersonii*, damage by pre-dispersal seed eating weevils is hermaphrodite-biased. In the absence of weevil damage, females and hermaphrodites had similar seed set, but as a result of hermaphrodite-biased attack, females had 1.6 times greater seed set (Marshall and Gander 2001). Moreover, models that included this sex differential predation more accurately predicted female frequencies in natural populations. Similarly, model predictions of female frequency in *F. virginiana* were closer to those seen in the wild when hermaphrodite-biased weevil florivory (Ashman 2006) was accounted for, and, the extent to which models under-predicted female frequency was correlated with predation intensity in populations. These

studies suggest that understanding plant-animal interactions may be crucial to understanding how gynodioecy is maintained.

Most studies have addressed the effects of mutualists and antagonists on different sex morphs individually. Yet, most flowering plants interact simultaneously with both mutualists and antagonists, and, to understand the consequences of these interactions they must be studied simultaneously (Strauss and Irwin 2004, Vega-Frutis et al. 2013). For example, herbivory can change interactions with pollinators and seed predators through altering flower shape, floral displays, or flowering phenology (e.g., Mothershead and Marquis 2000, Stowe et al. 2000, Strauss et al. 2002). Moths which pollinate, and are seed predators of, *Dianthus sylvestris* preferentially oviposit on hermaphrodites, which would benefit females, yet fungal infections vectored by moths are more likely to destroy female flowers (Collin et al. 2002). The identity of pollinators visiting plants altered the female seed set advantage in *Fragaria virginiana* (Ashman and King, 2005). Ants in this system were nearly as effective as pollinators as were bees and flies, but ants damaged the pistils of female plants while foraging. Ants also preferentially visited hermaphrodites when bees and flies were excluded. The net effect of ants was to reduce the female seed set advantage when only ants were allowed access to plants, in comparison to when bees were allowed access. Cole and Ashman (2005) also found that spittlebug feeding reduced bud clipping weevil damage in hermaphrodites, but not females, of *Fragaria virginiana*. Thus hermaphrodites were more tolerant of spittlebug damage in terms of growth and

floral display in one season. However, spittlebug damage increased the level of fungal damage to females but not hermaphrodites, suggesting it may be females which suffer greater long-term fitness reductions following spittlebug damage.

### 1.4 Mutualist and antagonist effects on lifetime fitness advantage

Demographic models use survivorship, growth, and fertility "vital rates" - probabilities of survival, growth, and reproduction over set intervals of time generally obtained by marking and tracking individuals in the wild - to project population growth (Caswell 2001). Demographic models incorporate the full lifecycle of organisms -for example, rates of germination, survivorship, growth, and seed set- and thus realistically project population growth in light of those vital rates which are most limiting. Most studies exploring the effect of mutualists and antagonists on plant fitness use seed set as their fitness measure (Strauss and Irwin 2004). However, since germination rates are often very low in plants, population growth may be relatively insensitive to seed set (Silvertown et al. 1993, Ehrlen 1995, Heppell et al. 2000, Crone 2001, Knight 2004; but see Maclean et al. 2011). Thus, demographic models make it possible to assess the importance of mutualists and antagonists on lifetime fitness by providing a context with which to assess those effects. Ultimately, the extent to which a mutualist or antagonist can affect lifetime fitness of a plant will depend on the vital rates they affect and the sensitivity of population growth to those vital rates (Caswell 2001).

Demographic studies have rarely been done with gynodioecious species (but see Morris and Doak 1998, Ramula et al. 2007), and we do not know whether vital rates apart from fertility (i.e. survivorship and growth) generally differ between sex morphs. If growth and survivorship vital rates differ between the sex morphs- as has been shown in one study (Morris and Doak 1998)- they may help explain female advantage. In addition, interactions between mutualists and antagonists could "interact" with these vital rates and alter sex morph fitness. For example, vertebrate herbivores could reduce survival or slow growth to larger size classes and therefore change the importance of all vital rates to population growth. Vertebrate herbivory can affect a host of vital rates including growth, survival, and fertility (Stowe et al. 2000), though surprisingly very few studies have experimentally tested the effects of vertebrate herbivores on non-fertility vital rates (but see Maclean et al 2011, Knight 2004). Particularly in long-lived species, the effects of vertebrate herbivory may have a much more profound effect on population growth, than effects of pollinators, for example, which are generally thought to only influence fertility vital rates (Knight 2004).

### 1.5 Rationale and objectives

My research tests what if any lifetime fitness advantage females attain through seed production in *Polemonium foliosissimum* as well as what role mutualists and antagonists play in generating differences in vital rates that contribute to a fitness advantage for females. Although there has been growing interest in characterizing the

role of biotic interactions in maintaining gynodioecy (Ashman 2002, Ashman 2006), few studies have used demographic modeling to project the lifetime fitness consequences of these interactions. This is despite the fact that there has been increased use of demographic modeling to understand lifetime fitness consequences of biotic interactions in other systems (Calvo and Horwitz 1990, Parker 1997, Garcia and Ehrlen 2002, Rooney and Gross 2003, Knight 2004, Halpern and Underwood 2006, Maron and Crone 2006, Knight et al. 2009).

*Polemonium foliosissimum* (Polemoniaceae) is a long-lived herb of Rocky Mountain subalpine meadows. At study sites near the Rocky Mountain Biological Lab in Gothic, Colorado, it is pollinated by generalist bumblebees and, because it is self-incompatible (Zimmerman 1980a), both females and hermaphrodites require visitation by pollinators to set seed. It incurs fruit destruction by a pre-dispersal fly predator (Diptera: *Hylemya* sp.; Brody and Waser 1995, Zimmerman 1980b) which does not pollinate and, therefore, relies on pollinators for provisioning offspring. Thus, I tested whether sex morphs differ in pollen limitation as well as in susceptibility to seed predation. Finally plants are often browsed by deer. Thus, it is important to assess the importance of any differences in seed set which result from the activities of pollinators and seed predators, in light of potentially more profound effects of vertebrate herbivores.

Here I collected demographic data for female and hermaphrodite plants to determine whether sex morphs differ in survivorship, growth, or fertility vital rates. I

employed realistic manipulative experiments in the field to quantify the effects of pollinators and seed predators on seed set. Next, I quantified the effects of vertebrate herbivores on vital rates of plants. Using these data, I then determined what role pollinators and seed predators have in influencing lifetime female fitness advantage in light of vertebrate herbivore effects on a wider range of vital rates. My research has allowed me to determine 1) which life stages limit population growth, 2) what life stages are altered by antagonists and mutualists, and 3) what effects these mutualists and antagonists have in light of the entire life cycle of plants.

The strength of my work is that it combines empirical data collected under experimental field conditions with that of demographic modeling to understand the long-term lifetime fitness consequences of pollinators and seed predators and of vertebrate herbivores for female and hermaphrodite sex morphs of a gynodioecious species. In this way my research is one of the first to have evaluated the role biotic interactions play in shaping the lifetime fitness advantage of female versus hermaphrodite sex morphs, and thus to realistically evaluate the role biotic interactions play in the maintenance of females, and of gynodioecy.

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# CHAPTER 2: GENDER INEQUITY IN PRE-DISPERSAL SEED PREDATION CONTRIBUTES TO FEMALE SEED SET ADVANTAGE IN A GYNODIOECIOUS SPECIES

## 2.1 Abstract

Most flowering plants are hermaphroditic, reproducing through seed and seed-siring. In gynodioecious species, females do not produce pollen and reproduce only through seed. Because of the loss of male fitness, females must have a seed fitness advantage to be maintained alongside hermaphrodites. Although the importance of interactions with mutualist pollinators and antagonist seed predators has been examined before, rarely are they studied together. Here, we assessed how the relative fitness of the two sex morphs of *Polemonium foliosissimum* is affected by interactions with both mutualist pollinators (primarily bumble bees) and a dipteran pre-dispersal seed predator (Anthomyiidae: *Hylemya* sp.). *Polemonium foliosissimum* is an obligate outcrosser that relies on pollinators to effect both male and female function. Over two years, we marked approximately 150 plants of each sex morph. These plants occurred in 27 populations. We followed fruit fate in all marked plants in both years. In a third year, we experimentally quantified the individual and combined effects of pollinators and antagonist seed predators by measuring the degree to which seed set is pollen limited, limited by seed predators, or both. We found no clear evidence that pollinator service differed between the sex morphs; neither fruit set nor the average number of seeds per fruit differed between females and hermaphrodites. However, fruits on female plants

were attacked significantly less often by *Hylemya* sp., and females set significantly more seed. In 2010, a year of high rates of predation, hermaphrodites with a higher rate of predation also had lower rate of fruit set and had fewer seeds per fruit. Removing seed predator eggs increased seed set more than did pollen addition on both morphs, and hand pollination and reduction in seed predation had additive effects on seed set. In addition, destruction of fruits by seed predators reduced seed set of hermaphrodites more than females, corroborating two years of observational results. Thus, we conclude that seed predators, not pollinators, most strongly affect the female seed set advantage in *Polemonium foliosissimum*.

## 2.2 Introduction

Most angiosperms are hermaphrodites; flowers accrue female fitness through the production of seed and male fitness through the production of pollen and the siring of seed. Yet, gynodioecy—whereby some plants produce hermaphroditic flowers while others are female—is a relatively common, dimorphic breeding system, that occurs in ca. 7% of flowering plant species (Richards 1986). In gynodioecious populations, individuals are either typical hermaphrodites or they have lost the ability to produce pollen and reproduce only as females. Gynodioecy is thought to be the most likely evolutionary intermediate from the ancestral hermaphroditism to dioecy (Weiblen et al. 2000), but how

females persist and gain a reproductive advantage over their hermaphrodite counterparts remains a topic of considerable debate (Dornier and Dufay 2013).

Interest in gynodioecy dates back over a century (Darwin 1877) in large part because it presents an evolutionary conundrum: how do females compensate for loss of male function? Theory tells us that the loss of male function is expected to be evolutionarily stable only if females are able to compensate via increased quantity of seed or enhanced seed quality, for example through the avoidance of inbreeding depression (Lewis 1941, Lloyd 1975, Charlesworth and Charlesworth 1978, 1999, but see Dufay and Billard 2012). In addition, the conservation of resources associated with the typically smaller flowers of females and their lack of pollen may enhance female seed production over hermaphrodites (Delph 1996, Shykoff et al. 2003, Vaughton and Ramsey 2002). Empirical studies have supported these predictions in some, but not all, cases. Females of gynodioecious populations often produce only slightly more seeds than hermaphrodites (e.g., Alonso and Hererra 2001, Asikainen and Mutikainen 2005b, reviewed in Shykoff et al. 2003, Dufay and Billard 2012) and evidence that outcrossing rescues plants from inbreeding depression is rare (but see Sakai et al. 1997, Ramsey et al. 2006, Collin et al. 2009).

The role of plant enemies in generating fitness differences sufficient to support a female advantage may be critically important to the persistence of females in gynodioecious populations. Although we might expect females to incur higher rates of

pre-dispersal seed predation due to their enhanced investment in seeds, in several cases the opposite has been found For example, damage by seed-feeding weevils was higher on hermaphrodites in two species of *Sidalcea* (Marshall and Ganders 2001), and female *Hadena* moths preferentially oviposit on hermaphrodites on *Dianthus sylvestris* as well (Collin and Shykoff 2010). However, in the latter system, although more fruits of females escaped predation, this was insufficient to provide a reproductive advantage to females because they produced fewer flowers than their hermaphrodite counterparts. In *Fragaria virginiana*, bud clipping weevils preferentially attack hermaphrodites; however, hermaphrodites tolerate attack better than do females, and ultimately suffer a lesser reduction in seed set than females (Ashman et al. 2004). Thus, the jury remains out on how females persist for many gynodioecious species, but direct and indirect effects of floral enemies may be key and results from a broader array of study systems are needed (Ashman 2002, Ashman and Penet 2007, Penet et al. 2009).

Here, we examined how interactions with mutualist pollinators and pre-dispersal seed predators affected the relative success of females and hermaphrodites in gynodioecious populations of sticky polemonium, *Polemonium foliosissimum* (Polemoniaceae). Our work differs from most previous systems studied because *P. foliosissimum* is an obligate outcrosser (Zimmerman 1980a). Therefore, the effects of inbreeding depression are irrelevant for this plant, and not only the quantity but also the quality of pollination is critical. If ignored by pollinators, flowers of *P. foliosissimum* will not set fruit. However, the movement of pollen among flowers within a plant may also

reduce fertilization if stigmas get clogged with self-pollen (De Jong et al. 1993). Given this, hermaphrodites should be equally, or possibly more, pollen limited than females. Although female-biased predation is predicted in other systems because females should allocate more resources to seed production, here there may be an additional reason for predation to be female-biased if, indeed, hermaphrodites are more pollen limited than females.

We first gathered two years of observational data and asked: Do the direct and indirect effects of species interactions differentially affect seed set in female and hermaphrodite plants? Specifically, we asked whether there are differences in fitness measures or predation rates to the sex morphs. In addition, we asked whether the cost of predation differs between the sex morphs, as evidenced by a reduction (or increase) in the quantity or quality of undamaged fruits. If seed predators affect reproductive success in ways that would suggest indirect effects through, e.g., resource-based tradeoffs and reallocation of resources away from damaged fruits, then seed predation may be less limiting to plant reproduction as long as flowers are amply pollinated. In a third year, we experimentally manipulated levels of seed predation and pollination to ask: Do the effects of pollen limitation and seed predation differ for females and hermaphrodites and are they additive? We evaluated 1) the relative importance of pollen limitation and pre-dispersal seed predation and 2) whether the combined effects of pollen limitation and seed predators were additive or interactive. Whether the effects of pollinators and seed

predators are additive or interactive can have profound effects on the fitness outcome and consequences of their interactions (Strauss and Irwin 2004).

## 2.3 Methods

### 2.3.1 Study system

*Polemonium foliosissimum* (Polemoniaceae) is a long-lived, herbaceous perennial that grows in subalpine meadows of the western United States. Near the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado, many populations are gynodioecious; sex ratios range from 100% hermaphrodite to nearly 40% female. Our work was conducted in 27 populations in the Paonia Ranger District of the Gunnison National Forest, Colorado. All populations occur within 2 km of Kebler Pass Road, but are separated by 2-5 km from each other. The population sizes ranged from 66 to 622 plants ($\overline{X}$= 242.93 ± 21.84). In 2009, we haphazardly tagged ten female and ten hermaphrodite plants in each of 27 populations that varied in sex-ratio from 6 to 33% female. Populations were separated by at least 100 m, a distance exceeding that travelled by most bumblebees (Elliott 2009). In 2010, in six of these populations we identified and permanently tagged all plants within belt transects, 20-50m long and 1.5 m wide. Due to the relative rarity of females, we marked additional females outside of transects, to sample a minimum of 50 females/population, or all females in populations with fewer than 50 females.

*Polemonium foliosissimum* is an obligate outcrosser, pollinated primarily by generalist bumblebees (Zimmerman 1980a). Seeds are frequently consumed by a dipteran pre-dispersal seed predator (Anthomyiidae: *Hylemya* sp.). *Hyleyma* females oviposit eggs singly under sepals while a flower is still in bud stage. 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 and complete development in the fruit on which they were laid. Because *Hylemya* does not pollinate flowers, female flies rely on pollinators to visit flowers they choose as oviposition sites to insure larval provisioning (Zimmerman 1980b, Brody 1997).

### 2.3.2 Do species interactions differentially affect seed set in female and hermaphrodite plants?

To quantify differences in the relative effects of pollinators and seed predators on sex morph seed set we quantified flower fate from permanently marked plants in 2009 and 2010. We counted all reproductive stalks, and collected fruits from a subset of these stalks, scoring each as aborted (not set and lacking filled seeds), destroyed by *Hylemya*, or filled. Fruit set was calculated as the proportion of flowers that set fruit, regardless of whether fruits were destroyed or not, and fruit destruction was calculated as the proportion of set fruits that were destroyed. *Hylemya* destruction is easily identified by seed remnants and frass. Remaining undamaged fruits were used to estimate seeds per fruit. The number of flowers on, and seed set for, entire plants were computed by

multiplying the respective values for sampled stalks by the total number of stalks and dividing by the number of stalks sampled. Because *P. foliosissimum* is an obligate outcrosser, we assumed fruit set and seeds per fruit were a proxy for pollinator service. Both low fruit set and low seed numbers per fruit can result from insufficient pollination (Zimmerman 1980a, Zimmerman and Pyke 1988).

To compare fitness measures between the genders, and explore the potential role of pollinators and seed predators to differences in seed set, we compared total flowers per plant, fruit set, seeds per fruit, proportion of fruits destroyed, and total seed set between the genders. First, we ran a MANOVA, separately in 2009 and 2010, including each of the fitness measures and proportion destroyed as dependent variables, with gender as the single main effect, blocked by site. Finding gender significant in the MANOVA, we then ran two-way ANOVAs (Scheiner 1993) for each fitness measure using gender as a main effect, and blocking by site. In all analyses, we included only those plants from which we collected $\geq$ 20 fruits, resulting in a final sample size of 102 females and 113 hermaphrodites in 2009 and 59 females and 185 hermaphrodites in 2010. Few plants (12 females and 14 hermaphrodites) were shared between the 2009 and 2010 datasets; thus, the datasets were largely independent. We log transformed total flowers per plant and seed set, and arcsine square root transformed proportion fruit set and fruit destruction data to meet ANOVA assumptions. All analyses were performed in SPSS v 21.

We used structural equation modeling (SEM; Mitchell 1992, 1993) to quantify sex-specific differences in the strength of pollinators and direct and indirect effects of seed predators on female reproductive success. We first tested whether our data supported a simpler model in which seed predators do not have indirect effects on seed set, against a more complex model in which they do. Our first model (Figure 2A) proposed that total flowers per plant, fruit set, seeds per fruit, and proportion of fruits destroyed directly affected seed set. Because plant size influences predation rates (Zimmerman 1980b, Brody and Mitchell 1997) we also included an indirect effect of total flowers on seed set via an influence on predation rates. To test whether seed predators have an indirect effect on seed set, we compared the fit of the first model to that of a second model in which we included indirect effects of seed predators on seed set via effects on fruit set and seeds per fruit (Figure 2b). An indirect effect of predation rate on seed set could occur if, for example, plants re-allocate resources away from fruits harboring larvae (in which case predation rates might be positively correlated to seeds in undamaged fruits). We used Akaike Information Criterion (AIC) to determine which of the models represented a better fit to the observed covariance matrix (Burnham and Anderson 2002).

First, we determined if the simpler model — including only direct effects of seed predators— or the more complex model — including both direct and indirect effects of seed predators— was supported in each of the years 2009 and 2010. Then, separately for each year, we used a multi-group analysis to determine the degree to which the female

and hermaphrodite subsets fit the overall model, and to identify significant differences in parameters between the datasets. We used multi-group analysis to simultaneously fit the data from both female and hermaphrodite plants to the same model, first constraining all parameters (path coefficients, variances, and covariances) to be equal between groups and (Grace and Pugseek 1998, Grace and Jutila 1999). Next, we progressively relaxed the constraints on the parameters, until an adequate fit to actual covariance structure was achieved, as assessed by AIC (Bollen 1989). Significant differences between groups were indicated by parameters which needed to be relaxed to obtain a reasonable fit. Final model estimation was based on relaxed parameter values. Structural equation models were fit with the lavaan package in R.

### 2.3.3 Do the individual and combined effects of pollinators and seed predators on seed set differ for females and hermaphrodites?

To experimentally quantify the individual and combined effects of pollinators and seed predators 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 meters, 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). Treatments included pollen addition (yes/no) and egg removals (yes/no). Early in the season, we identified sets of eight plants (4 females and 4 hermaphrodites) that were similar in size and flowering phenology. We then randomly assigned plants within each

set (by gender) to one of four treatments: hand pollinated (yes/no) crossed by eggs removed (yes/no), matching sets of eight plants within a site. Because plants are large and produce hundreds of flowers ($\overline{X}$=302.16 ± 25.2 in this study), applying treatments to all flowers on plants was not feasible. Instead, we applied treatments to all flowers on three stalks per plant and, on rare occasion, added additional stalks when those initially chosen ceased flowering before mid-July. 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—those assigned to no pollen addition and no egg removal—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.

To test if genders differed in pollen limitation and response to seed predation, 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, we removed eggs from all flowers and buds on stalks assigned to the egg-removal treatment. Stigmas are receptive for ca. 3 days, and *Hylemya* larvae emerge from eggs within five to seven 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 from experimental and control stalks just prior to dehiscence. Fruits were categorized as filled, aborted, or destroyed, and all seeds were counted.

We calculated fruit set, seeds per fruit, and proportion destroyed, as described previously, and net seed set (total seeds produced by all stalks of a given treatment), and summed each of these over the stalks of a given treatment per plant. If plants reallocate resources away from control stalks to treatment stalks in response to pollen addition or egg removal treatments, one would expect to find a significant difference in one or more fitness measures of unmanipulated, control stalks among treatments. To test this, and to test for baseline, non-treatment differences among plants we first ran a MANOVA, and finding gender significant, followed it with ANOVA with proportion of fruits set, fruits destroyed, seeds per fruit, and net seed set as the response variables, using only control stalk data and including gender, hand pollination, and egg removal treatments as main effects, blocked by site. We included flower number as a covariate in ANOVAs, but finding it insignificant, we deleted it from all final analysis except net seed set.

To examine the response of genders to hand pollination and egg removal treatments, we first ran a MANOVA with the response ratio (treatment/control stalk values) for each female fitness measure, along with gender, hand pollination, and egg removal treatments as main effects, blocked by site. Control stalk values were used as the denominator of the response ratio, thus values above 1 represent an increase in fitness measure above the control stalk and those below 1 represent a decrease in fitness measure relative to the control stalk (e.g., response ratios of 1.2 and 0.8 indicate a 20% increase and decrease respectively). We used response ratios because they measure proportional

changes, and correct for background differences among plants in fitness measures (e.g., Hawkes and Sullivan 2001). The difference between treatment and control stalks for net seed set quantifies treatment effects on overall reproductive success, incorporating both pollination and seed predation effects.

Finding both hand pollination and egg removal significant in the MANOVA, we ran individual ANOVAs for fruit set, seeds per fruit, and proportion of fruits destroyed. In all cases, we first ran a full model with all interactions and then removed interactions which were not significant. For net seed set, we used an ANCOVA, and included the ratio of flowers on treatment:control stalks as a covariate, which was not significant for proportion fruit set, seeds per fruit, and proportion destroyed. We log transformed (treatment/ control stalk values for) seeds per fruit and seed set data, and square root transformed proportion of fruits destroyed data, to meet assumptions for parametric 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 per fruit and net seed set for "treatment" stalks on control plants.

## 2.4 Results

### 2.4.1 Do species interactions differentially affect seed set in female and hermaphrodite plants?

In 2009 and 2010, fitness measures were significantly different between genders (2009: $\lambda = 0.819$, $F_{5,163} = 7.222$, $p < 0.001$; 2010: $\lambda = 0.946$, $F_{5,244} = 2.780$, $p = 0.018$). Females suffered significantly lower rates of seed predation than hermaphrodites in both years (2009: $\overline{X} = 0.10 \pm 0.01$ proportion of fruits destroyed versus $\overline{X} = 0.21 \pm 0.02$; $F_{1,167} = 30.127$, $p < 0.001$; in 2010: $\overline{X} = 0.21 \pm 0.02$ versus $\overline{X} = 0.33 \pm 0.02$, $F_{1,233} = 14.798$, $p < 0.001$; Figure 1, Appendix 1), and did not differ in total flowers, fruit set, or seeds per fruit in either year. Females also had higher total seed set per plant in both years (in 2009: 1482.10 ± 188.78 versus 1060.85 ± 129.60, $F_{1,167} = 4.182$, $p = 0.042$; in 2010: 1291.36 ± 279.19 versus 1159.80 ± 123.92, $F_{1,233} = 5.813$, $p = 0.022$).

In 2009, results of structural equation modeling showed no support for seed predators having an indirect effect on fitness measures. The simplest model including only direct effects provided a good fit ($\chi_{2152} = 13.865$, $p = 0.460$, Figure 2a) and explained 65% of the variation in seed set for females and hermaphrodites. Due to the difference in predation rates between the sex morphs (proportional fruit loss of 0.10 versus 0.21, for females and hermaphrodites respectively) the importance of predation to seed set was significantly less for females than for hermaphrodites (-0.17 versus -0.49 respectively, $p < 0.05$). In 2010, we found significant indirect effects of seed predators on

seed set (Figure 2b) and the more complex model was a better fit ($\chi_{2632}$ = 7.403, p = 0.687), explaining 94 and 97% of the variation in female and hermaphrodite seed set respectively. As in 2009, given the difference in predation rates between the sex morphs (0.21 versus 0.33 for females and hermaphrodites respectively), there was a stronger direct effect of predation on seed set for hermaphrodites than females (-0.300 vs -0.366 respectively, $p < 0.05$). Fruit set and seeds per fruit were also significantly lower in hermaphrodites that experienced high levels of predation, but those correlations were not significant for females (Figure 2b). The difference between the sex morphs in the effects of seed predation was even more dramatic when the total effects (including direct and indirect effects) of seed predators on seed set were considered (-0.26 versus -0.61 for females and hermaphrodites respectively).

### 2.4.2 Do the individual and combined effects of pollinators and seed predators on seed set differ for females and hermaphrodites?

Gender significantly affected control stalk fitness measures ($\lambda$= 0.521, $F_{4,48}$ = 11.018 , $p < 0.001$). Females experienced higher rates of fruit set than hermaphrodites, although the difference was not statistically significant ($F_{1,64}$ = 2.550, p = 0.115; Appendix 2). However, females produced significantly more seeds per undamaged fruit ($F_{1,64}$ = 6.406, p=0.014) and experienced over 25% lower rates of seed predation than did hermaphrodites. ($F_{1,64}$ = 30.559; $p <0.001$). Control females set ca. 1.3 times more seeds than did hermaphrodites ($F_{1,63}$ = 21.892, $p <0.001$). We also found no significant effects of hand pollination or egg removal treatments on any fitness measures among control

stalks (suggesting plants do not re-allocate from control to treatment stalks in response to any of the treatments).

Both hand pollination ($\lambda = 0.743$, $F_{2,57} = 6.570$, $p = 0.001$) and egg removal treatment ($\lambda = 0.505$, $F_{2,57} = 18.642$, $p < 0.001$) significantly affected response ratios for female fitness measures in the MANOVA. Furthermore, genders responded differently to treatments ($\lambda = 0.873$, $F_{2,57} = 2.771$, $p = 0.05$) and there was a nearly significant gender × egg removal interaction $\lambda = 0.889$, $F_{2,57} = 2.380$, $p = 0.079$).

Egg removal significantly reduced the proportion of fruits destroyed by *Hylemya* by 0.37 times (a reduction from 49% to 18%; $F_{1,64} = 111.196$; $p < 0.001$; Figure 3; Appendix 2 and 4). Neither hand pollination nor egg removal increased the proportion of fruits to set seed ($F_{1,64} = 0.725$; $p = 0.398$, and $F_{1,64} = 1.913$, $p = 0.171$, respectively). However, seeds per fruit increased in response to hand pollination by 1.2 times (an increase from 9.95 to 11.89 seeds per fruit on average; $F_{1,64} = 15.855$; $p < 0.001$).

On average, net seed set increased more in hermaphrodites in response to treatments; the increase in seed set was by 1.6 and by 2.0 times respectively for females and hermaphrodites ($F_{1,60} = 10.489$; $p = 0.002$; Figure 2, Appendix 4). Hand pollination increased net seed set significantly by 1.27 times (from 514.16 to 650.84 for control and hand pollination treatments respectively; $F_{1,60} = 6.658$; $p = 0.012$). The egg removal treatment increased net seed set significantly by 1.48 times (from 514.16 to 761.52 for

controls and egg removal treatments respectively; $F_{1,60}$ =58.661; p <0.001). Hand pollination and egg removal were additive: there was no significant interaction between the two treatments. However, we did find a significant interaction between gender and egg removal. Hermaphrodites in the egg removal treatments had 2.2 times the net seed set of controls, while the gain for females was 1.8 times that of controls ($F_{1,60}$ =4.158; p =0.046).

## 2.5 Discussion

The complex interplay of ecological interactions contribute to relative fitness differences among individuals and may act as important drivers to mating system evolution and differentiation of the sexes (Charlesworth 2006). Here, we found that although sex morphs of gynodioecious *P. foliosissimum* did not differ in pollen limitation, females experienced significantly lower rates of pre-dispersal seed predation and set significantly more seed than their hermaphrodite neighbors. Hermaphrodites experienced a greater net gain in seed set by the removal of seed predator eggs than did females because hermaphrodites suffered a greater rate of predation.

Pre-dispersal seed predation is often hermaphrodite-biased in gynodioecious species (Uno 1982, Marshall and Ganders 2001, Collin and Shykoff 2010, but see Asikainen and Mutikainen 2005b). However, to the extent that the quality of a fruit for

larval development can be measured by the probability of fruit set and seed numbers within fruits, females, not hermaphrodites, should make better hosts (Shykoff et al. 2003). In the three years of this study, we found evidence for a difference in quality of fruits between the sex morphs only in 2011, when females produced more seeds per fruit. The probability of fruit set, in particular, should be important to *Hylemya* because females do not pollinate the fruits on which they lay eggs, and lack of fruit set will spell certain mortality for their offspring. But our results combined with others suggest that either 1) fruit set and seeds per fruit are not good indicators of resource quality for larval development, or 2) that resource quality is not driving sex-biased predation. Other factors could contribute to sex-differential predation such as gender differences in phenology or defensive chemistry (Cornelissen and Stiling 2005). For *P. foliosissimum*, *Hylemya* may preferentially oviposit on hermaphrodites because of their larger flowers whish reduce the likelihood that eggs will desiccate under wider sepals (Brody 1992, Zimmerman and Brody 1998).

Pollen limitation is perhaps expected in gynodioecious species, since a significant fraction of the population does not produce pollen (14% and 19% of plants in the sites where our experiment was conducted). Indeed, the sex ratio in gynodioecious populations is sometimes correlated with pollen limitation (e.g., Widen and Widen 1990, Ashman and Diefenderfer 2001; but see Asikainen and Mutikainen 2005a, Cuevas et al. 2008, Case and Ashman 2009). Here, both female and hermaphrodite sex morphs were significantly pollen limited but not differentially so.

The similar extent of pollen limitation between the sex morphs of *P. foliosissimum* may be attributed to different mechanisms operating simultaneously between the genders. Hermaphrodites produce larger flowers that reward visitors with both nectar and pollen. Thus they may be more attractive to pollinators than females (e.g., Ashman 2000) and we might expect them to be less pollen (or pollinator) limited. However, self-incompatibility significantly alters the equation. *Polemonium foliosissimum* cannot gain reproductive assurance through the use of its own pollen and, may suffer from self-pollen deposition (e.g., De Jong et al. 1993, Sage et al. 2006) while females benefit from receiving only outcross pollen. Thus, we might expect the sexes to be equally pollen limited but for different reasons. Although we did not examine the underlying mechanisms, we indeed found that the two sexes were equally pollen limited.

Whether selfing is possible or not, resource limitation may differ between sex morphs of gynodioecious species because hermaphrodites incur the cost of producing larger flowers and pollen (Eckhart and Chapin 1997, Dawson and Geber 1999, Case and Ashman 2005). Although we did not test for resource limitation directly, our results suggest it may be important for *P. foliosissimum*. In 2010, the year with the higher rates of predation, we found significant, negative indirect effects of fruit destruction on fruit set and seeds per fruit in hermaphrodites. These results suggest that hermaphrodites cannot reallocate limited to resources to undamaged fruits and suffer an overall reduction in reproductive success as a result. We did not find the same pattern in females. Whether

this means that females reallocate resources to undamaged fruits, or simply have ample resources to maximize fruit and seed set regardless, we don't know.

In any case, our results suggest that the effects of pollinators and seed predators are additive for *P. foliosissimum*, such that seed predators reduce total seed set of those flowers that get pollinated (Irwin and Brody 2011). Non-additive effects of pollinators and seed predators are more likely where there are resource-based tradeoffs, where one species changes the strength or consequence of an interaction with another species (e.g., Karban and Strauss 1993, Ashman and King 2005, Cole and Ashman 2005). Most studies testing the combined effects of herbivores and pollinators have found additive effects (Morris et al. 2007). For example, the effects of nectar robbing bumblebees and pre-dispersal seed predators had additive on the fitness of *Ipomopsis aggregata* (Brody et al. 2008, Irwin and Brody 2011). Of the four studies we know of in which pollinators and seed predators were simultaneously manipulated, two found interactive effects. However, in both cases frugivory increased in response to successful pollination of fruits such that pollinators only increased seed set when herbivores were absent (Herrera 2000, and Herrera et al. 2002). For *P. foliosissimum*, it is unlikely that seed predators could target well-pollinated fruits; seed predators oviposit when flowers are still in bud. It is also unlikely that the presence of seed predator eggs alters pollinator behavior for *P. foliosissimum* as egg removal did change fruit set or seeds per fruit (here and Brody and Waser 1995).

Our study is among few to have tested how mutualists and antagonists simultaneously affect female reproductive advantage in a gynodioecious plant (e.g., Collin et al. 2002, Asikainen and Mutikainen 2005b, Ashman and Penet 2007, Zhang et al. 2007, Cole and Ashman 2005), and is the only study we know of to have addressed multi-species interactions for an obligately outcrossing gynodioecious species. Our results provide strong evidence that the seed fitness advantage of females in *P. foliosissimum* is generated by hermaphrodite biased pre-dispersal seed predation. In the year we manipulated pollination and predation rates, egg loads were high and pollinator numbers were low. We would have thus expected a strong response to both egg removal and hand pollination. Indeed, seed production was limited by pollen and seed predation, but the genders differed only for the latter and we found no evidence for interactive effects between pollinators and seed predators which could dilute the strength of seed predator effects (Strauss et al. 2005).

The cumulative effect of seed predators on seed fitness over the long lifespan of plants may be important to explaining why females persist. Predation rates are variable, but can account for as much as 90% fruit loss on an individual plant (Brody 1997). Over the three years of this study, fruit loss to *Hylmeya* averaged over 23% to females and 36% to hermaphrodites. Yet, seed set differences between may be less important than growth or survivorship vital rates of plants (Silvertown et al. 1993). How seed predation ultimately affects long-term, multi-generational fitness is largely unknown (but see Louda 1982, Leimu and Lehtila 2006, Maron and Crone 2006). We are currently using

demographic modeling to understand whether the seed set advantage females reap ultimately translates into a lifetime fitness advantage for females and thus helps explain the persistence of females in gynodioecious plants.

## 2.6 Literature cited


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## 2.7 Figure Legends

Figure 1. Structural equation models showing the relative importance of components of reproductive success and predation rates to total seed set for two sex morphs in two years, from observational data of ca. 150 plants in 27 populations. In both years, we evaluated first whether a more complex model - including indirect effects of seed predators on seed set, via effects on fruit set and seeds per fruit - was warranted. The width of each line is proportional to strength of the relationship and dashed lines denote negative relationships. Path coefficients are standardized and all are significant at $p < 0.05$ (non-significant paths are denoted by "ns"). For each regression, the $r^2$ value is noted. Numbers associated with curved lines are Pearson correlation coefficients. Asterisks indicate statistical differences between genders (* < 0.05).

Figure 2. Box plots showing proportion of destroyed fruits and seed set for female and hermaphrodite plants from observational studies in 2009 and 2010.

Figure 3. Response to treatments (treatment/control) for A) fruit set (proportion of flowers that set fruit); B) seeds / fruit; C) proportion of fruits not destroyed; D) net seed set. Note: proportion of fruits *not* destroyed is shown rather than proportion destroyed, so that all fitness measures are enhanced (from the plant perspective) when the response ratio is > 1. Plant-level treatments include controls, hand pollination (HP), egg removal (ER) and hand pollination and egg removal (HP&ER) for female (F) and hermaphrodite (H) plants. A response of one represents no difference between treatment and control

stalks on a given plant. For control plants, a random sampling of control stalk fruit data were used to generate “treatment” stalk values to have a balanced design.

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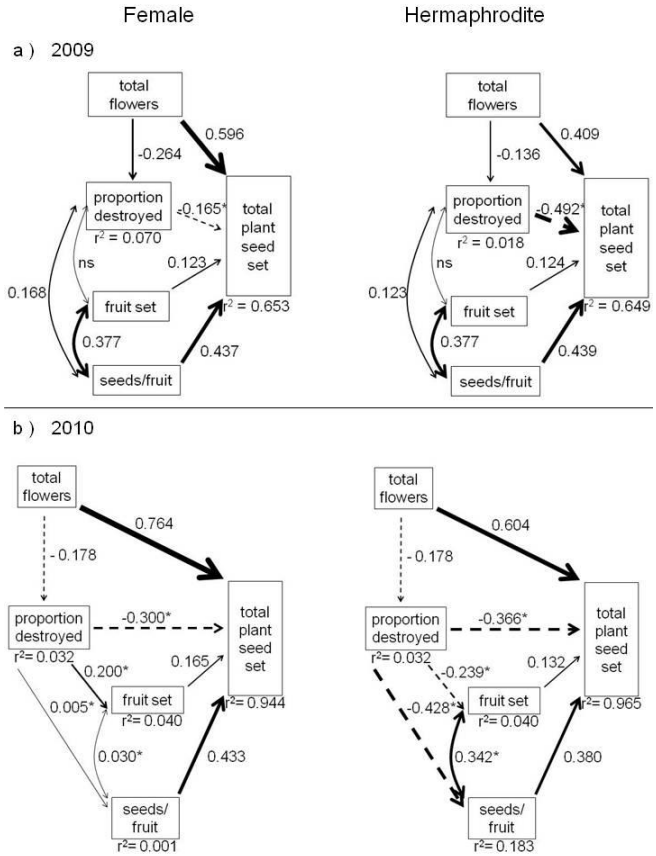

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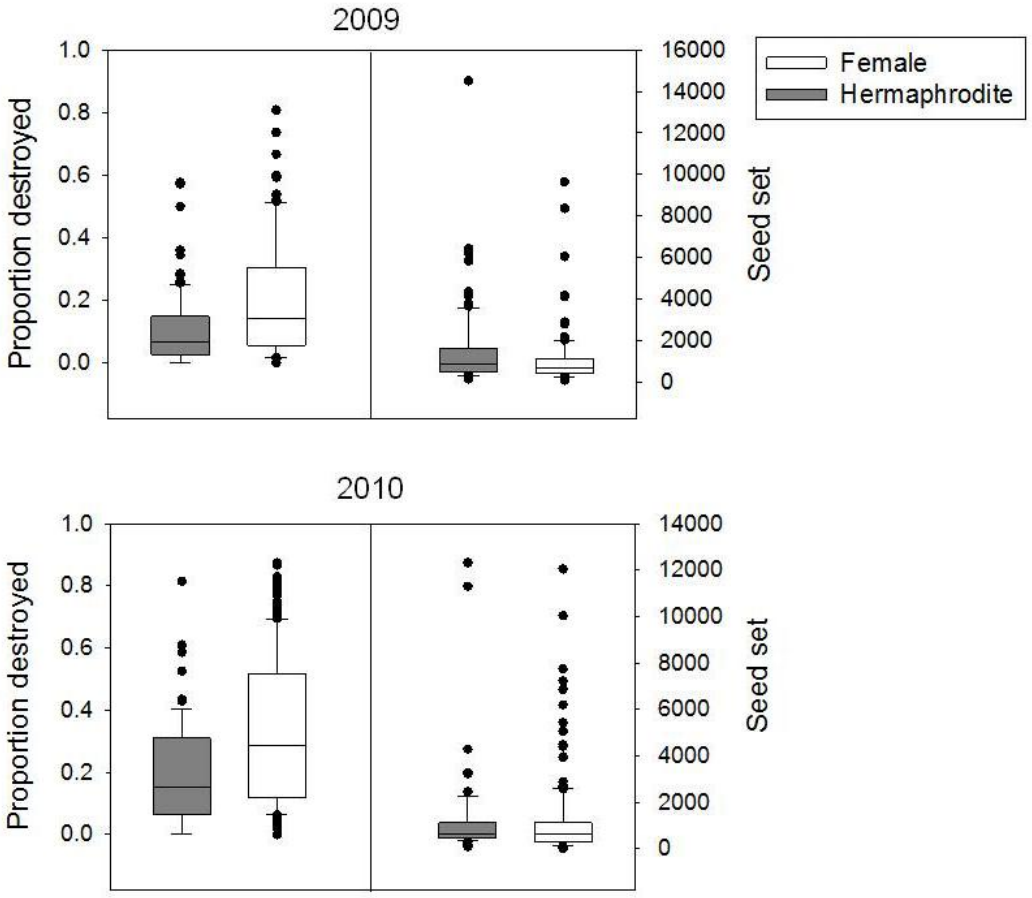

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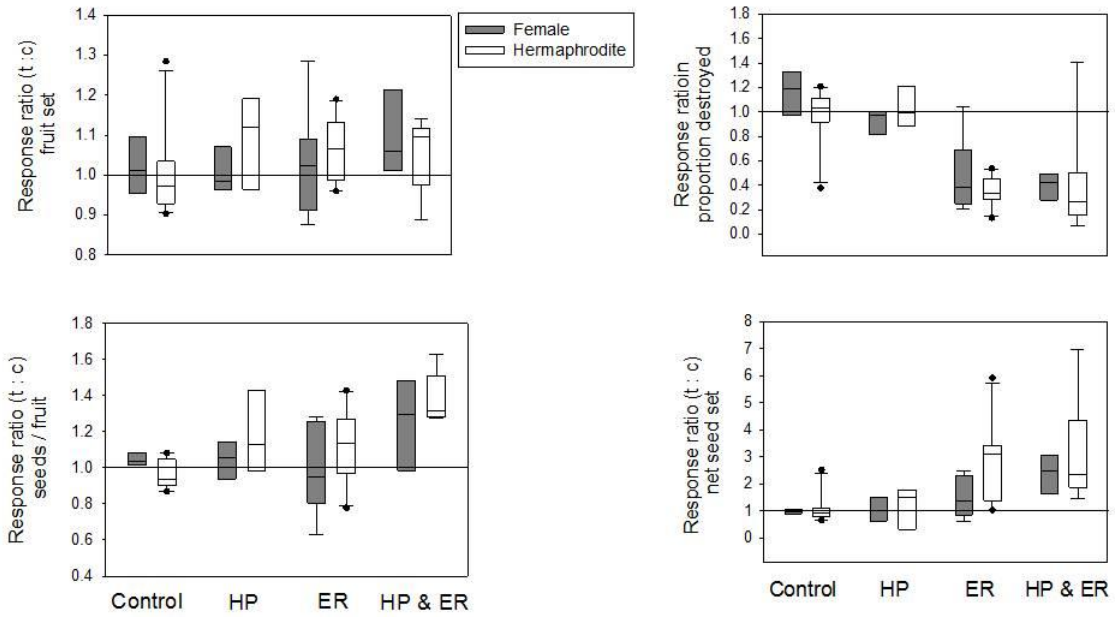

# CHAPTER 3: FLY PREFERENCES FOR HOSTS AGREE WITH LARVAL SURVIVAL OUTCOMES IN THE GYNODIOECIOUS POLEMONIUM FOLIOSISSIMUM

## 3.1 Abstract

Insects should select oviposition sites 'wisely' to insure the success of their offspring. How those choices are made remain a mystery in many systems. We investigated whether the pre-dispersal seed predator, *Hylemya* sp. (Diptera: Anthomyiidae), preferentially oviposits on female or hermaphrodite sex morphs of its gynodioecious host plant, *Polemonium foliosissimum* and tested whether preferences were beneficial to larval survival. We evaluated whether differences in oviposition were explained by differences in phenology or flower size between the sex morphs and whether gender and plant traits important to *Hylemya* were good indicators of resources in fruits (fruit set and seeds per fruit), which may be important to *Hylemya*. Last, we determined whether larval survival differed between the sex morphs, and whether these differences were explained by differences in flowering phenology or flower size between sex morphs.

Ovipositing *Hylemya* significantly preferred to oviposit on hermaphrodites over females. Flowers produced early in the season were more likely to be oviposited on than later ones. Some of the difference in oviposition between the sex morphs may be

explained by differences in phenology; female flowers opened ca. 3 days later than hermaphrodites. Larger flowers also tended to have more eggs than smaller ones. Hermaphrodites had larger flowers and this may also partly explain hermaphrodite biased oviposition. Finally, the preference of *Hylemya* for hermaphrodites was despite female flowers providing potentially more larval resources as demonstrated by greater numbers of seeds per fruit ($F_{1,48} = 5.675$, $p = 0.021$). Larval survivorship was higher on hermaphrodites. Survivorship was also higher earlier in the season and on larger flowers. Thus, females appear to making wise choices that insure survivorship of their offspring. Gynodioecious species may provide ideal systems in which to test links between preference and performance since sex morphs differ in floral traits which 1) may make it easy to distinguish them, and, 2) may matter to offspring performance.

## 3.2 Introduction

The green world faced by herbivorous insects is not a uniform one; individual plants and plant parts differ dramatically in their quality as hosts for herbivores (Price et al. 1980, Bernays and Graham 1988, Bernays and Chapman 1994, Merritt 1996, Stowe 1998, Zalucki et al. 2002, Nyman et al. 2011, Liu et al. 2012, Potter et al. 2012). Thus, selection of optimal oviposition sites can have important consequences for growth and survival of an insect's offspring (Price et al.1980, Atsatt 1981, Rausher and Papaj 1983, Awmack and Leather 2002). If females make poor choices, their larvae may perish. Therefore, natural selection should favor females that oviposit 'wisely' (Rausher 1979). If females are indeed adapted to oviposit where their offspring fare better, then we should find a correspondence between oviposition choices and larval success. Yet, studies linking oviposition preference and larval performance have yielded mixed results (see reviews by Thompson 1988, Mayhew 1997, Gripenberg et al. 2010, Refsnider and Janzen 2010). A number of explanations help to explain the lack of agreement between preference and performance (e.g., Rausher 1985, Cronin et al. 2001, Singer et al. 2002, Scheirs and De Bruyn 2002). One theme which regularly emerges is the potentially limiting role of insect neural capacity (Bernays 1998; Bernays 2001, Janz 2003), which could limit the ability of insects to distinguish among hosts of different quality.

If insect neural capacity limits preference, agreement may be more likely when host choice is among individuals of a sexually dimorphic species, since sex morphs often show extreme differences in phenotype (e.g., Shykoff et al. 2003) which may facilitate discrimination by insects. Moreover, sex morphs often differ in morphology, physiology (Obeso 2002, Agren et al. 1999, Lloyd and Webb 1977) and defense (Jing and Coley 1990) which could be important to larval performance. Thus, sex morphs may differ in traits which enhance the ability of insects to distinguish them, and may differ in their quality as food and host resources for insects, giving insects a reason to discriminate between them.

Indeed, insects discriminate between sex morphs of sexually dimorphic plants—sex -biased herbivory and predation is common (reviewed in Agren et al. 1999, Ashman 2002, Cornelissen and Stiling 2005, Vega-Frutis et al. 2013). Herbivory is most often heaviest on  males in dioecious species and hermaphrodites in gynodioecious species (Boecklen and Hoffman 1993, Agren et al 1999, Ashman 2002) though not always (e.g., Ashman 2002, Cornelissen and Stiling 2005, Asikainen and Mutikainen 2005). Sex-biased herbivory appears to be strongest where herbivory is to sinks (i.e. flowers and fruits) rather than sources (e.g. leaves; Ashman 2002, Cornelissen and Stiling 2005). Although a number of studies have documented sex-biased preference in gynodioecious species, few have investigated if offspring performance differs as well (Ashman 2002, Cornelissen and Stiling 2005), thus, it is difficult to draw generalizations about whether

'wise' choice is a common feature of these systems helping to explain sex-biased herbivory.

Sex morphs differ in a variety of ways that could affect insect performance, including phenology (Asikainen and Mutikainen 2005), allocation of resources to growth (e.g., Eckhart 1992a, Agren et al. 1999, Cornelissen and Stiling 2005) and defense (Jing and Coley 1990, Cornelissen and Stiling 2005, Tsuji and Sota 2010) and floral morphology (Shykoff et al. 2003). Of the seven studies that we know of to test insect growth or survivorship on sexually dimorphic species, three found differences in larval performance (Fritz et al. 2003, Tsuji and Sota 2010, 2013, Tsuji and Sota 2013). Two of these showed a corresponding bias in insect preference (Tsuji and Sota 2010, 2013, Tsuji and Sota 2013). In both cases, the insects were florivores, suggesting that differences in the host quality of sex morphs may be most pronounced in flowers and fruits. If, indeed, there is a greater incidence of sex-biased performance for florivores than other herbivores, this may help explain why there is a greater incidence of sex-biased damage for these herbivores and predators.

In addition to differences in tissue quality, sex morphs sometimes differ in flowering phenology (e.g. Asikainen and Mutikainen 2005) which may be important to floral herbivores and seed predators. In this way, plants can escape herbivory through mismatches in phenology with herbivores (e.g., English-Loeb and Karban 1992, Pilson 2000). Thus, even where insect performance differs among plants and plant parts, insects

may not choose favorable oviposition sites if the highest quality plants or plant parts are unavailable when insects are ovipositing. Whether differences in phenology contribute to differences in rates of herbivory in sexually dimorphic species is virtually unexamined (but see Collin and Shykoff 2010).

Here we compared oviposition choices and larval performance of a pre-dispersal seed predator fly, *Hylemya* sp., on hermaphrodite and female plants in two gynodioecious populations of *Polemonium foliosissimum*. Predation rates are higher on hermaphrodites than females in *P. foliosissimum* (Clarke and Brody in prep.). However, rates of oviposition decline over the flowering season at our study sites (Zimmerman 1980b, Brody 1997). Thus, we examined if differences in predation rates between sex morphs might be due to differences in flowering phenology rather than preference and performance- of sex morphs. In addition, as in many gynodioecious plants (Delph 1996, Shykoff et al. 2003), hermaphrodites produce larger flowers than females. Because eggs are prone to desiccation (Zimmerman and Brody 1998), we hypothesized that females would choose plants with larger flowers and thus prefer hermaphrodites, and we hypothesized that larval survivorship would be positively correlated with flower size. Finally, females produce more seeds than their hermaphrodite counterparts. Therefore, we also examined if fly preference correlated with fruit set or seeds per fruit. To examine these questions, we compared oviposition rates on hermaphrodites and females and quantified the importance of flowering phenology and flower size to egg loads and larval success on the sex morphs.

## 3.3 Methods

### 3.3.1 Study system

*Polemonium foliosissimum* (Polemoniaceae) is a long-lived, herbaceous perennial that grows in subalpine meadows of the western United States. At our study sites near the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado, many populations are gynodioecious, occasionally reaching sex ratios of ca. 50% female. *Polemonium foliosissimum* seeds are frequently consumed by a dipteran, pre-dispersal seed predator (Anthomyiidae: *Hylemya* sp.). *Hyleyma* females oviposit eggs singly under sepals of *Polemonium* buds (Zimmerman 1980b). After larvae hatch, they burrow into fruits and consume all developing seeds before tunneling out of carpel wall to fall to the ground where they pupate. Larvae do not move among fruits and consume only resources within the fruit chosen by their mother. Although flies oviposit on buds, they may use flowers already open on plants as cues in choosing among plants as oviposition sites. *Polemonium foliosissimum* is pollinated primarily by generalist bumblebees (Zimmerman 1980a). *Hylemya* does not pollinate, thus, female flies rely on pollinators to visit flowers with their larvae, to insure these flowers set fruit, and thus produce seeds which larvae consume (Zimmerman 1980b, Brody 1997).

To test whether gender, flowering phenology, or flower size are important to oviposition choices, resources in fruits (fruit set and seeds per fruit), and larval survival,

we censused and marked flowers with eggs throughout the 2011 flowering season and followed the fates of *Hylemya* eggs and fruits. We tagged ca. 20 plants of each gender at each of two populations at the onset of flowering. The two populations were ~ 300 m apart, and had a similar sex ratio of plants (14 versus 20% female), although the populations differ in size (~200 versus 70 plants). At five day intervals throughout the flowering season, from mid-July to mid- August, we censused flowers and late-stage buds for seed predator eggs on three stalks per plant. Flowers last ca. 5 days, thus we censused most flowers on stalks. We marked flowers with a small dot of non-toxic, acrylic paint, using a different color each week, and marked the calyx of buds and flowers on which we found an egg using an indelible black pen.

### 3.3.2. Are gender, flowering phenology, and flower size important to flies in making oviposition decisions?

All flowers were marked with a dot, color coded for the week they were open. Thus, at the end of the season, we knew how many flowers were open each week for each plant (for three stalks per plant). We calculated phenology as the average week of opening of a given plant's flowers. Thus, the average week of opening of the flowers on a given plant is an accurate measure of average phenology of plants. In addition, we measured flower size by measuring its individual components of sepal length, sepal width, petal length, and petal width on 3 flowers per plant. Petal size is a measure of floral display, which may influence attractiveness to *Hyleyma*, while sepal size may be

important to *Hylemya* larval survival, if larger sepals afford greater protection to larvae (Zimmerman and Brody 1998).

We performed a series of analyses to quantify the importance of gender, phenology, sepal, and flower size measures to 1) oviposition, 2) fruit set and seeds per fruit, and 3) larval survival (described below). Thus, we also wanted, first, to determine whether there were significant differences between genders in phenology, sepal area, and petal area which may help explain differences between genders in oviposition, resources in fruits (fruit set or seeds per fruit), or larval survival. To quantify the extent to which phenology, petal area, and sepal area were confounded with gender, we compared each of the traits between genders using T-tests.

To test whether sex morphs differ in oviposition rates, and to determine whether differences in phenology and flower size between the sex morphs contribute to differences in oviposition rates, we used an ANCOVA with gender as a main effect, population as a blocking effect, and including phenology, sepal area (length * width), and petal area (length * width), as covariates, with the proportion of flowers with eggs (arcsine square root transformed) over the entire season as the dependent variable. Because phenology and sepal area were strongly correlated (see results), we evaluated the extent to which co-linearity influenced these ANCOVA, and all subsequent ANCOVA, results by running models with each covariate sequentially removed. Here, and all

subsequent ANCOVAs, we first ran a complete model that included all possible interactions and removed interactions which were not significant.

### 3.3.3. Are plant traits associated with oviposition indicators of high quality resources, and do choices by *Hylemya* agree with outcomes for larval survival?

We used individually censused plants from 2011, described above, to 1) assess whether flies chose wisely with respect to putative larval resources in fruits (fruit set and seeds per fruit), 2) quantify the importance of phenology and flower size to putative larval resources, and 3) to determine whether fly choices resulted in increased larval survival. We visited plants every ca.5 days at the end of the season, collected fruits just prior to dehiscence, and scored fruit set (proportion of flowers that set fruit) and counted the number of seeds per fruit for those not destroyed by *Hylemya* larvae. We calculated fruit set as the percentage of expanded fruits/the total number of fruits, averaged over three stalks. Fruit set included all expanded fruits, whether destroyed or not. We calculated larval survival as the percentage of all buds previously marked as having an egg that were destroyed. Fruit destruction is easily identified by ruined seeds and frass in fruits. Fly mortality either occurred when 1) a bud with an egg did not develop into a fruit or 2) the egg or larva died as evidenced by lack of fruit destruction. Because larvae complete development (through last larval instar) in fruits (Zimmerman 1980b), our approach accurately measured larval success through the start of pupation.

To test whether gender, phenology, or flower size were important to potential measures of resource quality of fruits, we ran a MANCOVA including gender as a main effect, blocking by population, with phenology, petal area, and sepal area as covariates, and fruit set (arcsine square root transformed) and seeds per fruit as dependent variables. Finding the MANCOVA significant, we ran two separate ANCOVAs for the two dependent variables.

To test whether larval survival differed between the genders, and to determine whether differences in phenology, and flower measures between the genders contribute to differences in larval survival, we used an ANCOVA including gender as a main effect, blocking by population, with phenology, petal area, and sepal area as covariates, and larval survival (arcsine square root transformed) as a dependent variable.

## 3.4. Results

### 3.4.1 Are gender, flowering phenology, or flower size important to flies in making oviposition decisions?

Female plants flowered significantly later than hermaphrodites; on female plants, flowers had an average week of flowering of 2.68 ± 0.09 versus 2.29 ± 0.07 weeks for hermaphrodites ($T_{76} = 3.602$, $p = 0.001$). Hermaphrodites also produced larger

flowers than females. Sepal size (area) of hermaphrodites was 29.76 % larger than females, and petal size was 54.20% larger than that of female flowers (sepal area: 12.34 ± 1.37 versus 9.51 ± 0.74 $mm^2$; petal area: 70.70 ± 3.56 versus 45.85 ± 2.35 $mm^2$; sepals: $T_{65} = 2.219$, $p = 0.031$; petals: $T_{65} = 5.897$, $p < 0.001$; Figure 4).

*Hylemya* females preferentially oviposited on hermaphrodites; 67.27% ± 2.58 of flowers on hermaphrodite plants were oviposited on versus 45.99 % ± 3.42 of flowers on female plants ($F_{1, 61}= 4.815$, $p = 0.032$; Figure 5, Table 1). Oviposition rates were higher at population 1 (97.79% ± 3.08) than at population 2 (77.33% ± 3.44; $F_{1, 61}= 5.421$, $p = 0.024$). Plants which flowered later also had fewer eggs ($F_{1, 61}= 10.987$, $p = 0.002$). Phenology and sepal area were strongly correlated ($r^2= 0.220$, df = 61, $p <0.001$), but there were no other significant correlations among covariates. The ANCOVA results were stable; phenology was significant and sepal and petal area were not, no matter which covariates were included in the model. However, since gender is confounded with phenology, if phenology is dropped from the model, gender explains more of the model variation ($F_{1, 61}= 11.476$, $p = 0.001$) than it does with phenology also in the model. Also, a significant effect of sepal area on oviposition appears to be lost in the model because sepal area is confounded with gender. Sepal area (unlike petal area) was significantly correlated with oviposition rate ($r^2= 0.515$, df = 61, $p <.001$), however, with gender in the model the effect of sepal area was not itself significant.

### 3.4.2 Are plant traits associated with oviposition indicators of high quality resources, and do choices by *Hylemya* agree with outcomes for larval survival?

Gender influenced measures of fruit level resources (fruit set and seeds per fruit) in the MANCOVA ($\lambda = 0.878$, $F_{2,48} = 3.337$, $p = 0.044$). Flower measures, however, did not influence fruit set or seeds per fruit of plants (sepal area: $\lambda = 0.956$, $F_{2,48} = 1.116$, $p = 0.336$; petal area: $\lambda = 0.988$, $F_{2,48} = 0.303$, $p = 0.740$). Female plants produced more seeds per fruit than hermaphrodites: 10.41 ± 0.415 versus 9.47± 0.383 seeds per fruit for females and hermaphrodites respectively ($F_{1,53} = 5.811$, $p = 0.020$; Table 2).

Survival was higher overall on hermaphrodite plants than on females; the percent of larvae to survive on hermaphrodites was 67.58% ±2.40 versus 56.19% ± 4.67 on females (Figure 5), however, with phenology and flower measures in the model, gender itself was not significant ($F_{1,47} = 3.108$, $p = 0.085$; Table 3). Larval survival was greater on plants with flowers open earlier in the season ($F_{1,47} = 5.375$, $p = 0.025$) and those with larger sepals ($F_{1,42} = 5.734$, $p = 0.021$). When either phenology or flower measures (or both) were dropped from the model, gender was significant. Despite the significant correlation between phenology and sepal area (see above), the significance of the three covariates were not altered by sequential removal of other covariates.

## 3.5 Discussion

The link between oviposition preference and larval performance is fundamental to the ecology and evolution of herbivore-host plant interactions. If poor choices are made, offspring may perish. If good choices are made, host fitness may suffer as a result. Female herbivores should choose oviposition sites 'wisely' to increase the performance of their offspring, unless doing so reduces their overall fitness or unless there are constraints preventing such optimal behavior (Gould and Lewontin 1979, Krebs and Davies 1997). Here, we found significant positive effects of choices made by female, pre-dispersal seed predators and larval success. Oviposition rates by *Hylemya* sp. were higher on hermaphrodites than on females. In addition, larval survival was greater on hermaphrodites, likely in part because their larger sepals afford greater protection to fly eggs and larvae.

Sex morphs of sexually dimorphic species often differ in floral display (e.g., Shykoff et al. 2003, Asikainen and Mutikainen 2005), the presence or quantity of pollen or nectar, and in scent, which can all serve as cues for insects (e.g., Ashman et al. 2005). In addition, sex morphs can differ in the number and size of leaves and stems (Cornelissen and Stiling 2005), also used as oviposition cues (Renwick and Chew 1994). Differences in appearance and scent between sex morphs presumably make it relatively easy for insects to distinguish them. This is likely one of the reasons sex-biased feeding

and damage is the norm in these species (reviewed in Agren et al. 1999, Ashman 2002, Cornelissen and Stiling 2005). In *P. foliosissimum*, hermaphrodites have significantly larger flowers, particularly larger petals, but also larger sepals than females, and have pollen which females lack. In addition, hermaphrodites have longer stems of greater diameter than females (Gonzalez, unpublished). *Hylemya* preferentially oviposited on hermaphrodites, and may use any of these cues to do so. The magnitude of the preference was similar at the two populations, though the overall rate of oviposition differed (population 1: 76.85% ± 1.73 versus 56.03 % ± 3.16, and at population 2: 57.72 ± 3.23 versus 36.78 ± 3.52 for hermaphrodites and females respectively). *Hylemya* may also use sepal size in selecting host plants. However, because sepal size was confounded with gender, determining the importance of sepal size to differences in oviposition rates between the genders would require experimental manipulations to isolate the two. However, hermaphrodites had a higher rate of oviposition at all sepal sizes, suggesting that flies are likely to use other cues in addition to sepal size. In any case, whichever cues *Hylemya* uses, they lead to a preferential use of hermaphrodite hosts.

Females may receive fewer eggs than hermaphrodites in part due to a mismatch in phenology with *Hylemya*. It has been well documented that *Hylemya* oviposition and destruction decline over the season at our field sites (our results and Zimmerman 1980a, Zimmerman 1980b, Brody 1997). Moreover, females flowered later than hermaphrodites (average week of opening of flowers differed by 0.39, or ca. 3 days), which may partly explain why they receive fewer eggs. However, phenology was confounded with gender,

which limits our capacity to disentangle the effects of gender and phenology on oviposition. However, in our analysis both gender and phenology were significant which suggests that, at most, phenology can be only part of the explanation behind why oviposition rates are lower to females. Among gynodioecious species, differences in phenology have been observed (Uno 1982, Ashman and Stanton 1991, Eckhart 1992b, Ehlers and Thompson 2004, Asikainen and Mutikainen 2005). To our knowledge, only one study has explicitly quantified differences in phenology between sex morphs of a gynodioecious species to evaluate whether differences in phenology were important to herbivory or predation. In that study Collin and Shykoff (2010) found no differences in phenology between sex morphs. However, results from both Uno (1982) and Asikainen and Mutikainen (2005) are suggestive that phenology could contribute to differences in rates of herbivory between sex morphs.

*Hylemya* larval survival was significantly greater earlier in the season and under larger sepals, and both may help explain lower rates of larval survival on females. Declining survival of larvae could be the result of increasing rates of predation or egg desiccation later in the season, however they may also be the result of reduced provisioning of eggs by female flies later in the season (Jaenike 1990). Whatever the cause, larval survival rates may be lower on females in part because they are available slightly later in the season, when larvae have a lower probability of survival. Larval survival also increased with sepal area of plants. Previous work in this system has shown that sepal area influences larval success. Exposed *Hylemya* eggs have 1.7 times higher

mortality than those enclosed by sepals (Zimmerman and Brody 1998). Likely in part due to the differences in their flower sizes, survivorship was 20% greater on hermaphrodite as opposed to female plants (67.58 % ± 2.40 versus 56.19% ± 2.42). Higher rates of mortality of exposed *Hylemya* eggs may be due to desiccation of eggs, or perhaps to natural enemies (e.g., Bernays and Graham 1988). However, given that the egg stage is short for *Hyleyma* (only lasting about nine days) and wasp predation rates to larvae within fruits appears low (Brody per comm., pers. obs.), desiccation may be the most likely cause of egg mortality on small flowers. Unlike for oviposition rate, gender itself was not a significant effect in the larval survivorship model (unless phenology and sepal area were dropped) suggesting differences in phenology and sepal area between genders are sufficient to explain differences in larval survivorship between genders. In all gynodioecious species in which sex-biased predation has been recorded (Uno 1982, Marshall and Ganders 2001, Collin and Shykoff 2010), hermaphrodite flowers are larger than female flowers (although this is also true for gynodioecious species in general, Delph 1996), thus, it is possible that protection afforded by larger hermaphrodite flowers is important in other systems as well.

The protective role of larger sepals on hermaphrodite plants appears to be more important to larval survival than the fewer seeds – and potentially fewer resources – in fruits on hermaphrodite plants. *Hylemya* survivorship was greater on hermaphrodites than on females in spite of hermaphrodites having fewer seeds per fruit. Moreover, this is in spite of evidence which suggests that *Hylemya* is not able to select fruits, or manipulate

them, to enhance the probability of fruit set, or the number of seeds in fruits they choose as oviposition sites (Brody and Waser 1995, and Clarke unpublished), as it does on *I. aggregata* (Brody and Morita 2000). However, the differences in seeds per fruit were small between the sex morphs (10.41 ± 0.415 versus 9.47± 0.383) and are not seen in all years (Clarke and Brody, in prep.). Moreover, while it is assumed the number of seeds in a fruit is a measure of its food quality for larvae (Zimmerman 1979a, Zimmerman 1980 selective deposition), this is difficult to test since *Hylemya* consumes all seeds in fruits during development.

A common question which emerges in the preference-performance literature when performance preference agreement is uncovered, is why the suboptimal host is still in the insect's diet at all. In our system, why are females used as hosts given that egg survivorship is significantly lower on females than on hermaphrodites of *Polemonium foliosissimum*? One possibility is that *Hylemya* uses sepals as an honest cue in selecting oviposition sites. Considering the overlap in sepal size between females and hermaphrodites, complete discrimination against females might not be desirable. In addition, enhanced discrimination between the sex morphs might result in avoidance of other similar hosts (Fox and Lalonde 1993, Larsson and Ekbom 1995), in this case, *Ipomopsis*. Although it may be possible to distinguish between lower and higher quality hosts and to avoid the latter, plasticity in host choice could be important, particularly where 'wise' choices can be made. Indeed 'wise' choice by *Hylemya* may extend beyond sex morphs of *P. foliosissimum*. *Hylemya* may preferentially oviposit, and exhibit greater

survival, on *P. foliosissimum* over *I. aggregata*. Moreover, *Hylemya* may 'wisely' exhibit a greater degree of discrimination between *P. foliosissimum* and *I. aggregata* than between sex morphs of *P. foliosissimum.* There is perhaps a 7 fold difference in egg loads between the species (Brody 1997, but see Zimmerman 1980, Zimmerman 1982) in comparison to 1.5 fold difference here between sex morphs. In addition, there is perhaps a 3.7 or 5.7 fold difference in larval survival between the species (Zimmerman and Brody 1998, and Zimmerman 1980b) compared to a 1.2 fold difference between the sex morphs here. Moreover, *Hylemya* may use other behavioral adaptations such as the selective use of an oviposition deterring pheromone, applied to it's preferred host *P. foliosissimum* but not *I. aggregata* (Zimmerman 1979a, 1979b, 1980b), to maximize the benefit of its use of two hosts of different quality.

The broader explanation for why *Hylemya* continues to use inferior hosts may relate to variability in host availability and competition intensity, in space and time (Ward 1987). Plastic defensive strategies- such as choice- are particularly advantageous in variable environments (Agrawal 2001). When preferred resources are infrequent, suboptimal hosts should be used (Fretwell 1972). Indeed, the rate of *Hylemya* attack is highly variable among sites (e.g. between populations 1 and 2 in this study) and from year to year (e.g., Clarke and Brody in prep.), and thus competition intensity is likely highly variable as well. Moreover, resource limitation may be exasperated by low mobility of *Hylemya* females, which may not disperse far from where they emerge and thus may rely on resources within the populations where they pupate. That flies generally

make choices on a small spatial scale seems to be supported by the finding that *Ipomopsis* experiences higher rates of attack when *Polemonium* does not occur at a site (Brody 1997).

Some studies which have uncovered differences in growth or survivorship of insects on sexually dimorphic plants have found dramatic effects of plant gender. For example, for the galling sawfly, *Phyllocolpa leavitti*, survivorship was more than twice as high on females than on males of its dioecious host, *Salix discolor*. Despite this difference, there was no sign that insects preferred one sex morph over another in the field (Fritz et al. 2003). *Chloroclystis excise* larvae, which consume flower buds on its' host plants, polygamodioecious *Eurya japonica,* did not survive on female flowers due to high concentrations of defensive compounds in their flowers. *Chloroclystis excise* larvae avoid females in favor of males and hermaphrodites in the field (Tsuji and Sota 2010). On the other hand, *Alcis angulifera*, which consume leaves and flowers of *Eurya japonica* larvae had significantly but not dramatically slower development (1.04 x slower) on female versus male plants, yet larvae preferentially feed on males in the lab (Tsuji and Sota 2013). Interestingly, these latter two studies are the only ones we know of to have tested for preference-performance agreement for florivores on a sexually dimorphic plant. While it is tempting to take these results along with ours as evidence that preference-performance agreement on sexually dimorphic hosts may be more common among florivores than other herbivores, more work is needed before we can begin to describe such trends.

As often as not, herbivore preference has been found to not agree with herbivore performance. Among the explanations invoked to explain the mismatches are neglected aspects of larval fitness such as density dependence in egg survival (e.g. Siemens and Johnson 1992), or female fitness such as adult survivorship or fecundity (Rausher and Papaj 1983, Wan et al.1996, Weisser et al. 1994, Scheirs and De Bruyn 2002), and evolutionary constraints to adaptations to enhance discrimination (Hopper 1999, Grippenberg et al. 2007, Cunningham 2012). What aspects of *Hylemya* biology or of this system may predispose *Hylemya* to choose optimal oviposition sites? First, *Hylemya* larvae are immobile and complete development within the fruit chosen for oviposition, which increases the stakes for oviposition choices (Thompson 1988, Craig and Itami 2008). Second, *Hylemya* is oligophagous, using a small range of hosts, and specialization on a small number of hosts is thought to permit greater "fine tuning" to them (Levins and MacArthur 1969, Janz 2003, Roslin and Salminen 2008). In agreement with this, there is greater agreement in preference and performance among insects with narrow versus wide host ranges (Gripenberg et al. 2010). Third, in sexually dimorphic species, within species differences in traits important to host discrimination and larval performance may be especially large. Last, adaptations which permitted *Hylemya* to distinguish *P. foliosissimum* and *I. aggregata* may have served as pre-adaptations to discriminate between sex morphs of *P. foliosissimum*, assuming gynodioecy evolved relatively recently in this system.

Given that phytophagous insects are highly diverse, and that preference and performance traits may be particularly important mechanism driving divergent selection and speciation (Matsubayashi et al. 2011), there is understandable interest in searching for wider explanations to explain variable results from preference –performance investigations. Yet traits presumed to be related to insect offense are generally not tested as rigorously as have been traits related to plant defense, often not measuring, or demonstrating, measurable fitness advantages for alleged offensive traits (Karban and Agrawal 2002). Here we found *Hylemya* employed an effective offensive strategy in choosing among potential host plants as oviposition sites which resulted in increased survival of their larvae. This study is among the few to have tested for differences in insect performance among sex morphs of sexually dimorphic plant species, despite the fact that differences in preference (or damage) among sex morphs is widespread (e.g., Cornelissen and Stiling 2005, Vega-Frutis et al. 2013). More work will be needed if we are to determine whether, perhaps, aspects of these systems—such as the consistent differences and simple cues which exist to distinguish among sex morphs—foster a greater incidence of what has proven to be an elusive behavioral trait: wise adult preference.

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Table 1. Summary of ANCOVA results testing the effect of gender, population, phenology (average week of flower opening), and flower measures on the rate of oviposition (proportion of flowers with eggs). Significance is denoted as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

| Source | df | F | P | $\eta_p^2$ |
|---|---|---|---|---|
| Gender | 1 | 4.815* | 0.032 | 0.079 |
| Population | 1 | 5.421* | 0.024 | 0.088 |
| Phenology | 1 | 10.987** | 0.002 | 0.164 |
| Petal area | 1 | 1.959 | 0.167 | 0.034 |
| Sepal area | 1 | 0.932 | 0.338 | 0.016 |
| Residual | 56 | | | |

Table 2. Summary of ANCOVAs testing the effect of gender, population, phenology (average week of flower opening) and flower measures on A) fruit set, and, B) seeds per fruit of plants. Significance is denoted as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

| | Fruit Set | | | | Seeds per fruit | | | |
|---|---|---|---|---|---|---|---|---|
| Source | df | F | p | $\eta^2$ | df | F | P | $\eta^2$ |
| Gender | 1 | 1.167 | 0.285 | 0.020 | 1 | 5.811* | 0.020 | 0.108 |
| Population | 1 | 0.040 | 0.949 | 0.000 | 1 | 0.038 | 0.846 | 0.001 |
| Phenology | 1 | 0.032 | 0.859 | 0.001 | 1 | 1.563 | 0.217 | 0.032 |
| Petal area | 1 | 0.600 | 0.442 | 0.011 | 1 | 0.013 | 0.908 | 0.000 |
| Sepal area | 1 | 0.103 | 0.749 | 0.002 | 1 | 0.567 | 0.455 | 0.012 |
| Residual | 56 | | | | 48 | | | |

Table 3. Summary of ANCOVA testing the effect of gender, population, phenology (average week of flower opening) and flower measures on larval survival (proportion of flowers with eggs from which larvae successfully emerged) on plants. Significance is denoted as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

| Source | df | F | p | $\eta^2$ |
|---|---|---|---|---|
| Gender | 1 | 3.108 | 0.085 | 0.069 |
| Population | 1 | 0.169 | 0.683 | 0.004 |
| Phenology | 1 | 5.735* | 0.025 | 0.113 |
| Petal area | 1 | 0.826 | 0.369 | 0.019 |
| Sepal area | 1 | 5.734* | 0.021 | 0.12 |
| Residual | 42 | | | |

## 3.7 Figure legends

Figure 4. Histograms showing the distribution of petal areas and sepal areas (both computed as length x width) for female and hermaphrodite plants (female n = 27, hermaphrodite n = 37).

Figure 5. Scatterplots showing the relationship between A) phenology (average week of flower opening) and B) sepal area (length x width) and oviposition rate (proportion of flowers with *Hylemya* eggs), for female and hermaphrodite plants. The bottom graphs show the relationship between C) phenology and D) sepal area and proportion larval survival (the proportion of flowers with eggs to have successful larval development).

Figure 4. Histograms showing the distribution of petal areas and sepal areas (both computed as length x width) for female and hermaphrodite plants (female n = 27, hermaphrodite n = 37).

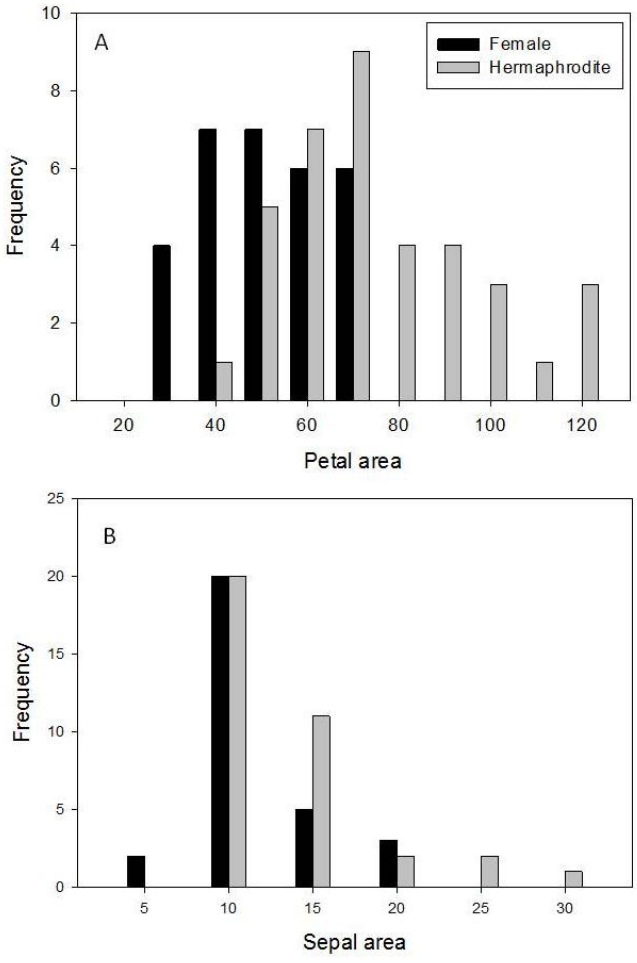
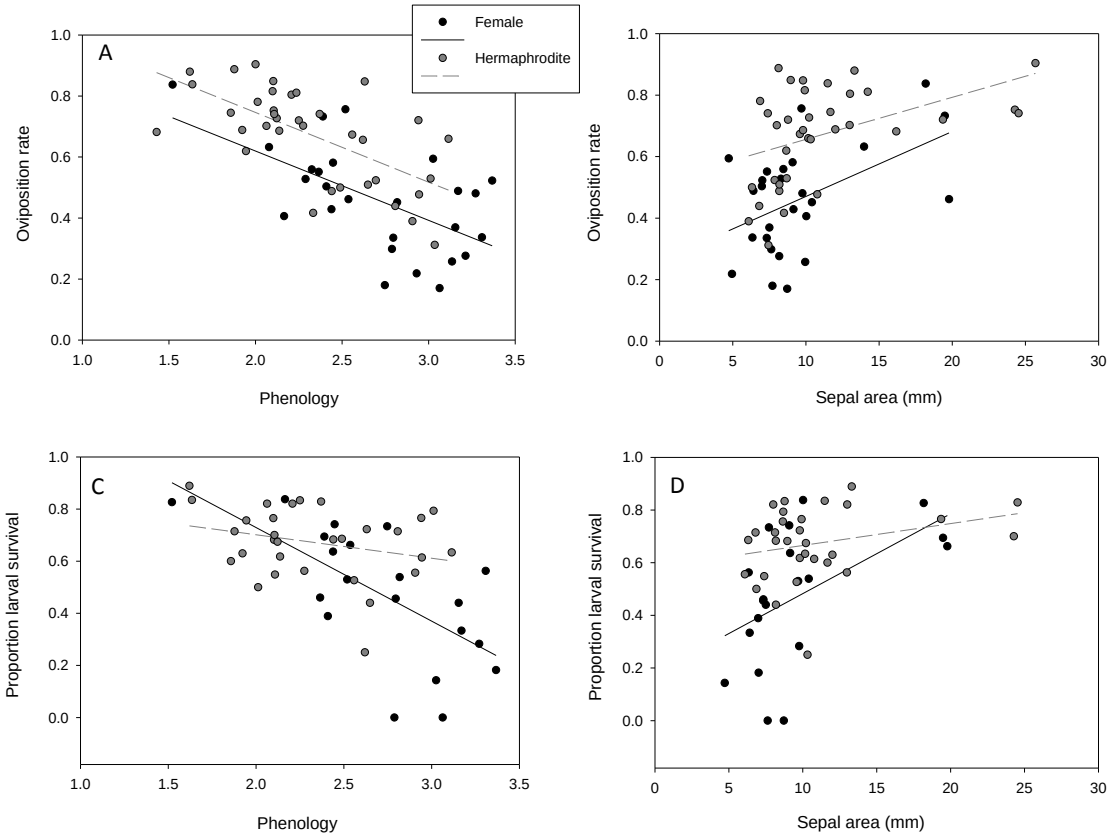


Figure 5. Scatterplots showing the relationship between A) phenology (average week of flower opening) and B) sepal area (length x width) and oviposition rate (proportion of flowers with *Hylemya* eggs), for female and hermaphrodite plants. The bottom graphs show the relationship between C) phenology and D) sepal area and proportion larval survival (the proportion of flowers with eggs to have successful larval development).



# CHAPTER 4: DEMOGRAPHIC EFFECTS OF HERBIVORES AND SEED PREDATORS ON THE SEX MORPHS OF A GYNODIOECIOUS SPECIES, POLEMONIUM FOLIOSISSIMUM

## 4.1 Abstract

In gynodioecious species —in which there are separate hermaphrodite and female sex morphs — females should produce more seed than hermaphrodites to compensate for their lack of pollen production. In some cases, herbivores may generate fitness differences which allow females to persist, as herbivory is often hermaphrodite-biased in these systems. However no studies I know of have evaluated the effects of herbivores on fitness beyond seed set in one or a few years. Here I evaluated the effects of vertebrate herbivores and pre-dispersal seed predators on the lifetime seed production (i.e., fitness attained through seed set, or $\lambda$) of sex morphs, separately, in populations of high (high female frequency) and low (low female frequency) sex ratio. I characterized the life history of plants by quantifying survival and growth from 2010 to 2011, reproduction in 2010, and germination rates of seeds from both sex morphs in the greenhouse. I also quantified the effects of vertebrate herbivores on growth transitions (using fenced exclosures), and the effect of vertebrate herbivores and seed predators on seed set of plants. I used these data to construct "ambient", "no herbivory", "no predation", and "no herbivory & no predation" matrices, and used these to evaluate the lifetime fitness advantage of females in each condition, in low and high sex ratio populations, and evaluated the sensitivity of the results to seed sex ratio.

Female medium sized reproductive plants survived more often, and advanced to the large size class more often than did hermaphrodites (16% versus 10% of plants advanced respectively). Seeds from female plants had a higher rate of germination than seeds from hermaphrodites (17% versus 14% respectively). In high sex ratio populations, pre-dispersal seed predation was hermaphrodite-biased, and as a result, females set 1.4 x as many seed as hermaphrodites. In low sex ratio populations, there was no difference in predation rates, or in seed set, between the sex morphs. Herbivores reduce the rate at which small reproductive plants advance to a medium size (35% versus 14% for fenced and unfenced plants respectively). Females did not have a seed set advantage in any other scenario except in the high sex ratio ambient condition.

In high sex ratio populations, where females had an advantage in germination rates, growth of medium sized plants, and seed set, females did not have a lifetime fitness advantage over hermaphrodites (female : hermaphrodite $\lambda = 0.994$). However, this result was sensitive to the sex ratio of seeds to female and hermaphrodite plants. In my preliminary model, females and hermaphrodites produced 30% and 5% female seeds respectively. If, instead, females produce >50% female seed, females would have a lifetime fitness advantage over hermaphrodites. This result suggests that the persistence of females may be context-dependent, perhaps only occurring where cytoplasmic sterility alleles are maintained at a high frequency by stochastic population genetic processes, such as drift and founder effects.

## 4.2 Introduction

In gynodioecious species — in which there are separate hermaphrodite and female sex morphs — females are at a potential disadvantage because they have no male function. Therefore, females must produce more seeds than hermaphrodites to be maintained (Lewis 1941, Lloyd 1974, Charlesworth and Charlesworth 1978). Such an advantage could be expressed as an increase in offspring quality (Shykoff 1988, Ashman 1992, Eckhart 1992, Puterbaugh et al. 1997, Chang 2006) and/or quantity (Shykoff et al. 2003, Ashman 2006). Offspring quality may be enhanced in females due to avoidance of inbreeding depression (Darwin 1877, Charlesworth 1999, Ashman 2006) or a greater maternal provisioning of seeds. In some cases, female advantage may be due to their suffering lower rates of consumption by herbivores (Ashman 2006) or seed predators (Marshall and Ganders 2001), or lower reductions in seed set in response to damage, than hermaphrodites (e.g. Cole and Ashman 2005). Although the role of consumers in fitness differences for sexually dimorphic plants has been addressed for a variety of species (Agren et al. 1999, Marshall and Ganders 2001, Ashman 2002, Collin et al. 2002, Ashman et al. 2004), the long-term lifetime fitness consequences are virtually unknown.

In sexually dimorphic plant species, herbivory is often biased toward one sex morph over another (Agren et al. 1999, Marshall and Ganders 2001, Ashman 2002, Collin et al. 2002, Ashman et al. 2004). Male-biased herbivory is well-documented in

dioecious systems (reviewed in Agren et al. 1999 and Ashman 2002). In gynodioecious systems, florivory and seed predation are often hermaphrodite-biased (e.g. Uno 1982, Marshall and Ganders 2001, Ashman and King 2005, Ashman and Penet 2007, Collin and Shykoff 2010, but see Asikainen and Mutikainen 2005b). In addition, in several systems, there is compelling evidence that sex-biased predation contributes to the maintenance of females. For example, for *Sidalcea hendersonii*, models including mode of inheritance, selfing rate, and inbreeding depression vastly under-predicted female frequencies in natural populations (Marshall and Ganders 2001). However, models that included sex differential predation (which differs by more than 30%, and increased the relative seed fitness advantage of females from 1.0 to 1.6) more accurately predicted female frequencies. Similarly, model predictions of female frequency in *F. virginiana* were closer to those seen in the wild when sex differential herbivory was accounted for, and, the extent to which models under-predicted female frequency was correlated with predation intensity in populations (Ashman 2006).

Virtually all studies investigating how females are maintained alongside hermaphrodites in gynodioecious species use seed produced within a single year as a measure of fitness (but see Morris and Doak 1998, Ramula et al. 2007); however, seed production in a single, or even few years, can be an inadequate measure of lifetime fitness. Ideally, one should compare sex morph differences in lifetime seed production. A lifetime measure of seed production incorporates measures of survival and growth which, particularly for perennials, are critical components of lifetime fitness (Silvertown et al.

1993, Ehrlen 1995, Morris and Doak 1998, Crone 2001). Because few demographic studies have used gynodioecious species, we do not know if sex morphs often differ in vital rates, apart from fertility. The two demographic investigations of gynodioecious species which have been done suggest important roles for fertility differences between the sex morphs in one instance, and in growth and survivorship differences in the other. In *Silene acaulis*, females had an ca. 4-fold advantage in net reproductive rates over their hermaphrodite neighbors, largely due to their higher seed set in all size classes (Morris and Doak 1998). On the other hand, Ramula et al. (2007) found no fertility advantage for females in *Geranium sylvaticum*. However, in this self-compatible species, they found that selfed hermaphrodite offspring suffered lower growth and survivorship than outcrossed female offspring, which may help explain why females persist (Ramula et al. 2007).

Although there is growing interest in understanding the role that herbivores play in the maintenance and evolution of sexual systems, no studies I know of have explored the effect of herbivores or other consumers on the lifetime reproductive advantage of females in a gynodioecious species. Herbivores can significantly reduce seed set (reviewed in Crawley 1989, Stowe et al. 2000), but their effects on survivorship and growth of plants of different life stages is less well known (but see Ehrlen 1995, Ehrlen and Eriksson 1995, Parker 1997, Garcia and Ehrlen 2002, Knight 2004, Maclean et al. 2011, reviewed in Crawley 1989, Strauss and Zangrel 2002 ). For example, florivores and seed predators likely only influence fertility vital rates, whereas herbivores can effect

survivorship and growth transitions (e.g., Garcia and Ehrlen 2002). Thus the capacity of these different consumers to effect lifetime reproduction may be very different, depending on how sensitive population growth is to these transitions (e.g., Garcia and Ehrlen 2002, Knight 2004, Leimu and Lehtila 2006). Demographic modeling makes it possible to quantify the effects of herbivores on population growth (or lifetime fitness advantage of sex morphs) by 1) quantifying the effects of herbivores on different vital rates, 2) determining how sensitive population growth is to those vital rates, and 3) determining how herbivore effects scale up to influence λ (Caswell 2001). These same techniques make it possible to evaluate the roles consumers have on the lifetime reproductive advantage females have over hermaphrodites in gynodioecious species.

One important theme that has emerged from the growing body of research on gynodioecious species is the important role of population sex ratio to female fitness advantage (e.g., Widen and Widen 1990, McCauley and Brock 1998, Ashman and Diefenderfer 2001). For one, females across a number of gynodioecious species are associated with lower resource sites (e.g. Darwin 1877, Alonso and Herrera 2001, Delph and Carroll 2001, Vaughton and Ramsey 2002), and although the ultimate causes behind this association are debated, it is generally assumed that the proximate cause is likely related to females maintaining a higher quality of seed in these sites (reviewed in Ashman 2006). On the other hand, females may become more pollen limited, and seed set may decline, with the decreasing frequency of the pollen bearing morph, hermaphrodites (e.g., Molina-Freaner and Jain 1992, Widen and Widen 1990), though not always (Asikainen

and Mutikainen 2005a). In addition, sex-biased visitation by pollinators may change across populations of different sex ratio (e.g., Ashman and Diefenderfer 2001, Case and Ashman 2009). Thus, I ask here whether females have a greater lifetime fitness advantage in high sex ratio sites with a higher frequency of females – in spite of the high frequency of females – which would support the idea that whatever factors enhance fitness at those sites, may also drive the greater frequency of females.

Here, I used a demographic modeling approach to quantify how vertebrate herbivores and pre-dispersal seed predators affect the lifetime reproductive advantage of females in *Polemonium foliosissimum,* a long-lived, gynodioecious plant. Seed predation rates are lower on females than on hermaphrodites (Clarke and Brody, in review) and, as a result, females have a numerical seed set advantage over hermaphrodites. Rates of vertebrate herbivory also may differ between sex morphs, or herbivory may have different effects on the survivorship or growth of the sex morphs. I quantified the effect of vertebrate herbivores and seed predators on the lifetime fitness ($\lambda$) advantage of females. Specifically, I asked: 1) whether sex morphs differ in survivorship or growth vital rates and 2) how herbivores affect survivorship and growth using experimental herbivore exclosures, and 3) how herbivores and seed predators affect fertility of plants and 4) whether females have a lifetime fitness ($\lambda$) advantage over hermaphrodites, and whether this advantage differs between high and low sex ratio populations and finally 5) I quantified the extent to which herbivores and/or invertebrate seed predators contribute to the lifetime fitness ($\lambda$) advantage of females.

## 4.3 Methods

### 4.3.1 Study system

*Polemonium foliosissimum* (Polemoniaceae) is a long-lived, herbaceous perennial that grows in subalpine meadows of the western United States. At my study sites along Kebler Pass Road, Gunnison County, Colorado (Lat: 38.864ºN, Long: -107.121 ºW), many populations are gynodioecious and vary in sex ratio from 40% female to 100% hermaphrodites. Six populations were included in the present study varying in size from ca. 250-650 plants. Of these populations, three had a low sex ratio of plants (ca. 6-8% female), and three had a high sex ratio (20-31% female). All six populations occurred within 2 km of Kebler Pass Road in Gunnison CO, and within 2-5 km of each other. Because sample sizes were insufficient to treat each population as a separate replicate, I combined data across the three sites for low and high sex ratio populations.

*Polemonium foliosissimum* is a long-lived perennial that does not reproduce clonally (Zimmerman 1980a). Adults produce vegetative and flowering stalks after snowmelt in May or June and flowering begins in early July. Most plants flower for 4-6 weeks, begin setting seed by early- to mid-August, and have finished setting seed by mid-September. Seedlings can emerge throughout the summer, but the majority emerge early in the season (Clarke pers. obs.).

*P. foliosissimum* plants are often browsed by mule deer. Deer most often browse stems at the ground and stalks are not regrown within the same season. *Polemonium foliosissimum* seeds are frequently consumed by a dipteran pre-dispersal seed predator (Anthomyiidae: *Hylemya* sp.). *Hyleyma* females oviposit single eggs under sepals while the flower is still in bud stage. The larvae then hatch, burrow into the developing fruit and consume all developing seeds before burrowing out of the carpal wall and falling to the ground to pupate in the soil. Because *Hylemya* does not pollinate flowers, female flies rely on pollinators to visit flowers they chose as oviposition sites, to insure they set fruit and provide provisioning for fly larvae (Zimmerman 1980b, Brody 1997). *Polemonium foliosissimum* is self-incompatible and requires animals - primarily generalist bumblebees - to vector out-cross pollen (Zimmerman 1980a) - in order to set seed. In addition, because *P. foliosissimum* is self-incompatible, and hermaphrodite offspring are never the result of selfed crosses, inbreeding avoidance can not be invoked as a mechanism by which females gain an advantage in this system.

### 4.3.2 Survivorship, growth, seed set, and germination rates

To estimate stage-specific vital rates and to compare vital rates between sex morphs in low and high sex-ratio populations, I permanently marked plants in six populations and recorded their yearly survivorship and growth from 2010-2012, recording whether plants were alive, dead or missing, the and the number of reproductive

and vegetative stalks, and number of those which were browsed and unbrowsed, each year. Vegetative stalks have a different appearance (namely smaller stem diameter) than reproductive stalks, thus it was also possible to categorize browsed stalks as vegetative or reproductive. In 2010, I tagged ca. 150 plants within 20-50m long x 1.5m wide belt transects in each population. Because females are infrequent in low sex-ratio populations, I marked additional females outside of transects, to sample a minimum of 50 females/population, or all females in populations with fewer than 50 females. To estimate the effect of vertebrate herbivory on survival and growth vital rates for female and hermaphrodite plants, I enclosed a subset of each of four populations (2 low and 2 high sex ratio populations) in 2012 in deer-proof fences. Fences were erected in early June, prior to deer browsing, and were removed at the end of the season after the plants had senesced. Early in 2013, I censused all plants along transects which had been either fenced or unfenced the previous summer, as described above.

To quantify seed set, and seed loss to herbivores and seed predators, I counted reproductive stalks at the end of the season in 2010 and collected fruits from a subset of stalks. Fruits were scored as aborted, set, or destroyed by *Hylemya*. *Hylemya* destruction was easily identified by seed remnants and frass. The remaining set, but undamaged, fruits were used to estimate seed per fruit, which was used to compute seed set of plants. Seed set was calculated as the number of set, non-destroyed fruits * average seed per fruit. However, since fruits were collected from only a subset of reproductive stalks, this number was divided by the ratio of reproductive stalks from which fruits were collected

on the plant, to estimate seed set for the entire plant. This actual entire plant seed set is referred to as "ambient" seed set throughout. I also used the proportion of stalks browsed and proportion of fruits destroyed to estimate what seed set would have been in the absence of herbivores and pre-dispersal seed predators. For example, seed set in the absence of herbivory, which is referred to as "seed set without herbivory" was computed as "ambient seed set" divided by one minus the proportion of browsed stalks. Similarly, seed set in the absence of predators, or "seed set without predation" was computed as ambient seed set divided by one minus the proportion of destroyed fruits. A similar approach was used in computing "seed set without herbivores or seed predators".

To estimate seed germination probabilities for the two sex morphs, I did the following. First, I censused transects for seedlings in 2011 and estimated the overall rate of germination in the field as # seedlings in 2011/ number of seeds set in plots in 2010. Next, I conducted a greenhouse experiment to estimate the difference in germination rate of seeds from female and hermaphrodite plants. I bulked seeds collected in 2010 from each gender, cold stratified them for three months at 4º C and planted 25 seeds/pot for a total of 44 and 96 pots (or 1100 and 2400 seeds) for females and hermaphrodites respectively. Pots were watered and fertilized weekly and under simulated spring conditions of 13 hours of sunlight, and temperatures ranging daily from ~ 19º C to ~ 5ºC. After ~6 weeks, germination rates declined rapidly, and I ended the experiment after 8 weeks, after having no germinants emerge for two weeks.

### 4.3.3 Demographic modeling

I constructed a ten-stage demographic matrix model encompassing five stages for both sexes, to describe the life history stages of female and hermaphrodite sex morphs (Figure 6, Table 4). Stage classes were defined as seedlings (1st year), juveniles (2nd year until reproductive) and three reproductive size classes: small (SR), medium (MR), and large (LR) reproductive plants, characterized as <= 5, 6-10, and 11+ reproductive stalks (browsed or not) respectively (Figure 6, Table 4). First year seedlings had a higher mortality rate than juveniles ($\chi_{21}$= 4.364 p = 0.026; Table 5), justifying the distinction between seedlings and juveniles, and this categorization of reproductive plants afforded sufficient sample sizes in each size class (Caswell 2001). Reproductive classes also differed in mortality rates from 2011 to 2012, justifying the use of this stage based model.

In my demographic model, seedlings either die or advance to juvenile stage. All other life-stages can remain in their stage, advance, or regress to a previous stage. Survivorship and transition probabilities (vital rates) were garnered from transitions of marked plants from 2010 to 2011 (Table 5). For each size class, I used chi square tests to compare the number of plants to survive versus die (or grow or regress versus remain in the same class) between 1) low and high sex ratio populations, and separately between the sex morphs. Significant differences (in seedling survival between low and high sex ratio populations, and between sex morphs in the MR-LR transition; see results) were incorporated into models. Although all transitions were derived from the 2010 to 2011 transition, I confirmed all plants documented as dead in 2011 were again absent in 2012.

In this model seeds must germinate in the year after they were released (i.e. there is no seed bank). Although I have assumed here that there is no seed bank, this is a simplification; two year old seed has a germination rate of ~5%, and three year old seed perhaps ~3% germination rate in the greenhouse (Clarke, unpublished). Future models will include seed bank dynamics. Fertility transitions (plant to seed) included seed set, germination probabilities, and sex ratio of seeds. I used two-way ANOVAs to compare seed set among size classes of plants, and sex morphs, separately for populations of different sex ratio (see "effects of herbivory and seed predation on the relative lifetime female fitness advantage" for more detail). I used a chi-square test to compare the number of seeds that germinated (versus those that did not) from females versus hermaphrodite plants in the greenhouse experiment. Because the germination rates differed between the sex morphs (see results), the overall germination rate from the field was used, however, the magnitude of the difference in rates between the genders from the experiment was maintained. I do not know the sex ratio of seeds to plants of different maternal genders. However, I assume the mode of inheritance of gender in *P. foliosissimum* is nuclear-cytoplasmic (Clarke and Brody, in prep; Chase 2007) thus I assumed conservatively (e.g., McCauley et al. 2000, Bailey and McCauley 2005, Byers et al. 2005) that hermaphrodites produce seeds that are 5% female, and females produce seeds that are 30% female. In refer to all matrices with these seed sex ratios as preliminary to distinguish them from matrices in which I vary seed sex ratio to conduct a sensitivity analysis of my results to this assumption (see below).

Each Lefkovitch matrix (A) can be used to project population growth with the formula $N_{t+1} = A N_t$, where $N_t$, and $N_{t+1}$ are vectors containing the number of individuals in each size class in time t or t+1 respectively. The survival, growth, and reproduction rates from one year to the next (2010 to 2011, Table 5) are the vital rates which make up the 28 elements ($a_{ij}$) in the matrix (Table 4). All possible transitions in the life stage model (Figure 6), are represented by an element in the transition matrix A. I projected population-level parameters including the dominant eigenvector (population growth rate, $\lambda$), the right eigenvector (stable stage distribution, w), and the left eigenvector (reproductive value of each stage class, v; Caswell, 2001). I also calculated sensitivity ($S_{ij}$) of $\lambda$ to changes in matrix elements ($a_{ij}$) from the dominant left and right eigenvectors ($v_i$ and $w_j$, respectively; Caswell 1978 and 2001, Morris and Doak, 2002). I also calculated elasticises as the proportional change in $\lambda$ which results from a proportional change in vital rate (Caswell et al. 1984), which is a standard way to rescale sensitivities, to account for the different magnitude of different vital rates.

To model the differences in relative lifetime fitness advantage of female and hermaphrodite plants, I ran deterministic matrix projections for separate female and hermaphrodite matrices. In the female matrix, all hermaphrodite fertility transitions were set to zero, and vice versa for the hermaphrodite matrix. Thus each matrix summarizes (along with survivorship and growth transitions for both sex morphs) seed production for one sex morph, permitting progeny to be either gender. This approach accurately

measures the lifetime fitness of a given sex morph by accounting for the fitness of all seeds produced regardless of their gender (Morris and Doak 1998). Relative lifetime fitness of female sex morphs was calculated as λ of the female matrix / λ of the hermaphrodite matrix, such that values >1 indicate a greater lifetime fitness advantage of females.

### 4.3.4 Effects of herbivory and seed predation on lifetime female fitness advantage

I quantified the effect of herbivores and seed predators on the relative female lifetime fitness advantage (λ) incorporating both 1) herbivore effects on growth transitions from the fencing experiment, and, 2) estimates of the effects of herbivory and seed predation on seed set. I used previous female and hermaphrodite matrices developed using the broader 2010- 2011 transitions described above, adjusting one growth vital rate influenced by herbivores (described below), and adjusting fertility vital rates influenced by herbivores and pre-dispersal seed predators. In this way, I created separate matrices for each of the: "ambient", "no herbivory", "no predation", and "no herbivory & no predation" conditions, starting from the ambient matrices, and adding significant differences (Caswell, 2001) from analyses described below.

First, I evaluated whether herbivory altered growth transitions of plants. Using chi-square tests, I compared the number of plants to grow (or regress) versus remain in the same stage from 2011 to 2012 between plants fenced during the 2011 season versus those not fenced using chi-square tests for each size class and gender of plants separately

(mortality was too infrequent to test for differences in survivorship). Having found a significant effect of herbivory on the rate of transition of SR to MR plants (see results), this difference was incorporated into "no herbivory" matrices.

To evaluate the influence of herbivores and seed predators on fertility, I ran a series of ANOVAs, one for each dependent variable: "ambient seed set", "seed set without herbivory", "seed set without predation", and "seed set without herbivory & predation". Separately for low and high sex ratio populations, I performed four two-way ANOVAs with gender and size class as main effects with the following dependent variables 1) "ambient seed set", 2) "seed set without herbivory", 3) "seed set without predation", and 3) "seed set without herbivory and predation". Differences in vital rates between genders and size classes identified from these analyses (see results) were used to construct sixteen separate matrices (Caswell 2001), for each population sex ratio, both genders, and for "ambient", "no herbivory", "no predation", and "no herbivory & no predation" conditions. Relative female advantage was quantified in each case as female $\lambda$ : hermaphrodite $\lambda$. Because females only had a seed set advantage in high sex ratio populations under ambient conditions (see results), I performed a sensitivity analysis to seed sex ratio by varying the sex ratio of seeds from female plants from 10% to 60% female using the high sex ratio ambient matrix. These seed sex ratios are not uncommon for females of nuclear-cytoplasmic species (e.g., McCauley et al. 2000, Bailey and McCauley 2005, Byers et al. 2005).

## 4.4 Results

### 4.4.1 Survivorship, growth, seed set, and germination rates

Mortality rates were highest in the smallest reproductive size class (Table 5) and very low for larger reproductive plants (2% for MR and 1% for LR plants). The probability of plants advancing from one life stage to the next was low (ca. 10% for each life-stage) except seedlings which, if they survive, automatically advance to the juvenile stage. Only one vital rate differed between populations of different sex ratio; seedling survivorship was significantly lower in high sex ratio populations (86.32% of 95 seedlings) than low sex ratio populations (62.5% of 48 seedlings; $\chi_{21,143} = 10.652$, $p = 0.002$). Survival of MR plants was greater for females than hermaphrodites (for females 2 of 63 died versus 19 of 190 for hermaphrodites; $\chi_{21,252} = 2.896$, $p = 0.067$). Female MR plants advanced to LR plants (rather than remaining MR or regressing to SR) more often than did hermaphrodites. For females, 15.8% of MR plants advanced to LR plants in the next year, whereas only 9.5% of hermaphrodites did so ($\chi_{21,273} = 9.964$, $p = 0.007$).

In high sex ratio populations, where predation rates were higher to hermaphrodites than to females (24.34% ± 2.57 vs. 38.35% ± 2.14 of fruits destroyed on females and hermaphrodites respectively; $t_{1,126} = 4.097$, $p < 0.001$, Appendix 5), "ambient" seed set was 1.4 times greater for females than for hermaphrodites (Figure 7). Females set on average 684.68 ± 71.5 seeds in comparison to 488.56 ± 45.0 seeds for hermaphrodites ($F_{1,24} = 8.703$, $p = 0.0004$, Table 6). However, size classes did not differ

in seed set ($F_{2,124} = 1.214$, $p = 0.300$). In low sex ratio populations where predation rates did not differ between the sex morphs (21.93% ± 4.80 vs. 29.09% ± 2.01 of fruits destroyed on females and hermaphrodites respectively; $t_{1,152} = 1.449$, $p = 0.163$, Appendix 5), sex morphs did not differ in "ambient" seed set (Figure 7). Females set on average 1835.68 ± 665.04 seeds in comparison to hermaphrodites which set 1276.82 ± 156.99 seeds ($F_{1,150} = 0.998$, $p = 0.319$, Table 6). As in high sex ratio populations, size classes did not differ in seed set ($F_{2,150} = 1.921$, $p = .150$).

On average, 31,054.46 ± 2009.58 seeds were shed by all plants within transects in six populations in 2010 (for females 7,435.5 ± 5,040.4 and 9,870.5 ± 9,720.6 in low and high sex ratio populations respectively, and for hermaphrodites 14,832.9 ± 939.6 and 50,934.3 ± 46, 084.3 in low and high sex ratio populations respectively). On average there were 15.17 ± 0.95 seedlings in the same transects in 2011, yielding an average germination rate across all transects of 0.0015 ± 0.0005%. In the greenhouse 195 out of 1100 seeds from females germinated, whereas 337 of 2400 seeds from hermaphrodites germinated (17.7% versus14.0%; $\chi_{21,3500} = 7.949$, $p = 0.005$).

### 4.4.2 Effects of herbivory and seed predation on lifetime female fitness advantage

Exclosures significantly increased the probability that plants transition from the SR to MR reproductive class (35.1% versus 13.8% for SR plants that were not fenced; $\chi_{21,131} = 7.576$, $p = 0.013$). There were no significant differences between genders, or between populations of different sex ratio, in the effect of fencing on growth transitions

for any size classes of plants. Although "ambient seed set" differed between the sex morphs in high sex ratio populations (see above), seed set in the absence of herbivory, predation, or both did not differ between the sex morphs ($F_{1, 108} = 2.366$, $p = 0.127$, $F_{1,124} = 3.323$, $p = 0.071$, $F = 0.348$, $p = 0.557$ for "no herbivory", "no predation", and "no herbivory & no predation" respectively, Table 6). In low sex ratio populations, where sex morphs did not differ in "ambient seed set" (see above), sex morphs also did not differ in seed set in the absence of herbivory, predation, or in the absence of both herbivory and predation (Figure 7). However, whereas "ambient seed set" did not differ among size classes in either low or high sex ratio populations, size classes differed significantly in "seed set without herbivory", "seed set without predation", and "seed set without herbivory & predation" in both high and low sex ratio populations ( Table 6).

Despite females' advantage in seed set, germination rates, and more rapid transitioning from MR to LR plants, population growth was lower for females than for hermaphrodites under ambient conditions, in my preliminary "ambient" model in which hermaphrodites produce 5% female progeny and females produce 30% female progeny (F:H $\lambda$ was 0.994, Table 7). In the remainder of my preliminary models (assuming this same sex ratio of seeds to females and hermaphrodite plants) including "no herbivory", "no predation", or "no herbivory & no predation" models for high and low sex ratio populations, females did not have a numerical seed set advantage. However, simulating the removal of herbivores and predators changed the importance (sensitivities and elasticizes) of vital rates to $\lambda$ (Tables 10-11, Appendices 6-7). Removing herbivory

decreased the elasticity, or importance, of SR – SR transition, and increased the importance of the SR-MR transition (Table 11). Removing predation, but especially removing herbivory, reduced the importance of the LR-LR transition. Removing either herbivory or predation increased the importance of all fertility vital rates (SR-S, MR-S, and LR-S) and this effect was stronger in the hermaphrodite than the female matrix since, by virtue of the seed sex ratios in these models, a greater percentage of the seeds set in one generation, enter the system in the next generation in the hermaphrodite matrix. As in the preliminary ambient high sex ratio matrix, female λ did not exceed hermaphrodite λ in the preliminary "no herbivory", "no predation", or "no herbivory & no predation" models in low sex ratio populations, which also used 5% and 30% female progeny to hermaphrodite and female plants respectively (Table 7).

The sensitivity analysis (Figure 8) shows how the female lifetime fitness advantage (female λ : hermaphrodite λ) would be altered if females have a greater numerical seed set advantage, or if females produce a greater proportion of female progeny than in the preliminary "ambient seed set" matrix (where the female advantage was 1.4 x that of hermaphrodites and where females produced 30% female progeny). In order for the lifetime fitness advantage of females to exceed that of hermaphrodites (or for female λ : hermaphrodite λ >1), the numerical seed set advantage of females would have to be at least twice that of hermaphrodites or females would have to produce at least 50% female progeny.

## 4.5 Discussion

Despite the growing interest in the role ecological factors play in sexual system evolution (e.g., Ashman 2002 and 2006, Cornelissen and Stiling 2005, Vega-Frutis et al. 2013) this is the first study I know of to have quantified the effects of consumers such as herbivores and seed predators on the lifetime fitness of sex morphs of a gynodioecious species. I found no evidence that rates of vertebrate herbivory, or effects of vertebrate herbivory on growth transitions, differed between the sex morphs of *P. foliosissimum*. However, my results suggest that the seed set advantage females have over hermaphrodites, because they suffer lower rates of pre-dispersal seed predation at least in some populations, may contribute to the maintenance of females under some circumstances. I found that in addition to females setting 1.4 times as many seed as hermaphrodites, females also have an advantage in that their seeds have a higher probability of germinating, and female plants also transition more rapidly from MR to LR size class. In spite of these differences, in my preliminary model females did not have a lifetime fitness advantage over hermaphrodites. However, in these preliminary models, I conservatively assumed that the progeny from hermaphrodites are only 5% female and progeny from females are 30% female. If either sex morph produces a greater percentage of female progeny (e.g., if females produce >50% female progeny), the female lifetime fitness advantage would exceed that of hermaphrodites. These results highlight the fact that the germination rate, growth transition, and fertility advantage females have would

lead to a lifetime fitness advantage for females in some cases, depending on the sex ratio of seeds.

*Polemonium foliosissimum* vital rates were typical of those seen in long lived species. Mortality rates declined sharply across size classes, with the largest size classes experiencing very low rates of mortality, 2% and 1% of MR and LR plants respectively (Silvertown et al. 1993). Females had a greater survivorship (3.2% versus 10.0%) and growth of MR plants (15.8% versus 9.5% advanced to LR), than hermaphrodites. Considering the sensitivity of population growth to the MR-MR and, to a lesser extent the MR-LR, vital rates, these differences between the sex morphs undoubtedly contribute to the (seed sex ratio dependent) lifetime female fitness advantage I measured. The difference in seedling survivorship between the sex morphs likely contributed less to any lifetime fitness advantage; the sensitivity of population growth to the S-J transition was relatively low.

I found no evidence that vertebrate herbivores either preferentially browse one sex morph over another or that herbivory differently affected the growth rates of sex morphs. Herbivory slowed the transition of SR-MR plants, significantly reduced seed set, and, in this year of relatively high rate of browse (on average plants lost 57.14% ± 1.25 of stems to herbivory), removed the differences in seed set among size classes of plants. Pre-dispersal seed predation, which was also relatively high in 2010 (accounting for an average of19.48% ± 2.09 versus 33.26% ± 1.71 of fruits destroyed for females and

hermaphrodites respectively), also removed differences in seed set among size classes. In addition, seed predators preferentially attacked hermaphrodites in high sex ratio populations. I found no difference in any other fitness component (e.g. total fruits per plant, fruit set or seeds per fruit) between sex morphs in either low or high sex ratio populations (see Appendix 5). Thus the significant difference in seed set in high sex ratio populations seems to be driven by sex-biased predation. This is also supported by the lack of difference in seed set between the sex morphs in the low sex ratio populations, where predation did not differ between the sex morphs.

Collectively, my results suggest that it was only in high sex ratio populations, and only in the presence of herbivores and seed predators that females had a fertility, or numerical seed set, advantage. Because predation is hermaphrodite-biased, I expected the removal of predation to remove the female seed set advantage. However, I expected the opposite for herbivores. I expected my simulation of the removal of herbivory to magnify the differences in seed set between the sex morphs, since I simply assumed seed set in the absence of herbivory would increase in proportion to the proportion of stems that were browsed. Removing herbivory did not magnify the female seed set advantage, however, because hermaphrodites were slightly larger than females in high sex ratio populations (females had 11.12 stems in comparison to 12.49 for hermaphrodites; $t_{1, 126} = -1.107$, $p = 0.271$). If this is an artifact, the removal of herbivory might actually tend to increase the seed set advantage (and relative lifetime fitness advantage) of females. Moreover, if the female seed set advantage is actually magnified when herbivores are removed, my results

also suggest that the greater sensitivity of population growth to fertility vital rates when herbivores are absent would tend to magnify the effect of any difference in seed set on the difference in lifetime fitness advantage.

Two prior demographic studies with gynodioecious species found opposing results with respect to the importance of fertility differences to female lifetime fitness advantage. Ramula et al. (2007) found that fertility differences were unlikely to be important to the relative lifetime fitness of sex morphs of *Geranium sylvaticum*. They found only small differences in seed set between sex morphs and no differences in growth or survivorship transitions in the field. However, they found that seeds from selfed hermaphrodite crosses had a lower rate of germination, survivorship, and growth than outcrossed seeds in the greenhouse, and found the lifetime female fitness advantage was sensitive to these effects of inbreeding depression. Conversely, Morris and Doak (1998) found that the lifetime female fitness advantage in *Silene acaulis* was likely driven by a 2-10 times greater seed advantage across all reproductive classes of plants. Although they found females also reached maturity more quickly than hermaphrodites, the female lifetime advantage was relatively insensitive to this difference in growth rate because of the relatively low seed set of plants in the smallest size classes. Collectively the numerical seed advantage females had resulted in females having 4.4 times the net reproductive rate of hermaphrodites.

I measured a seed set advantage of females that was 1.4 times that of hermaphrodites (in a subset of populations). This result may lend further support to the idea that gender inheritance is cyto-nuclear in *P. foliosissimum*; twice the seed fertility advantage is required for females to be maintained under strict nuclear inheritance (Lewis 1941, Lloyd 1975, Charlesworth and Charlesworth 1978), whereas only a slight advantage is needed where inheritance is cyto-nuclear (Gouyon et al., 1991, Charlesworth 1999, Bailey et al. 2003). Most gynodioecious species, regardless of mode of inheritance, have less than a two-fold seed fertility advantage, although they generally do have some advantage (Dufay and Billard 2012). My results also show how, even a fairly substantial seed set advantage, however, will not lead to a lifetime fitness advantage for females unless the sex ratio of progeny is relatively high (to one or both genders). Under cyto-nuclear inheritance, hermaphrodites are expected to produce a greater proportion of hermaphrodite than female progeny (Charlesworth and Laporte 1998, Bailey et al. 2003, Ramula et al. 2007). The actual sex ratio of seeds could be quite high, though, even for hermaphrodites and would be dependent in the frequencies of cytoplasmic and restorer types in populations (McCauley and Olson 2003). Although the seed sex ratios required for females to have a lifetime fitness advantage are probably not uncommon, they will be highly dependent on population structure and genetics (e.g. Gouyon et al. 1991, Bailey et al. 2003, Dufay et al. 2007, McCauley and Bailey 2009). My results, therefore, contrast with those of Morris and Doak (1998) where females had >4 times greater advantage of females, and where female occurrence is likely less sensitive to vagaries of population dynamics that *P. foliosissimum* may be subject to.

The differences I found in vital rates between low and high sex ratio populations are intriguing and may have implications for sexual system evolution in gynodioecious species. First, it is interesting that high sex ratio populations had overall higher rates of seed predation, but also significant sex-differential rates of attack (Appendix 5). This suggests the possibility that sex-biased attack drives a female seed set advantage which drives the high (female-biased) sex ratios found at those sites. I also found a significantly lower probability of seedling survival in high sex ratio, in comparison to low sex ratio populations (86.32% versus 62.5% respectively). Considering the large body of literature which suggests that lower resource conditions favor the occurrence of females (Darwin 1877, Alonso and Herrera 2001, Delph and Carroll 2001, Vaughton and Ramsey 2002), one wonders whether my high sex ratio sites may be harsher habitats, and that this in some way encourages the occurrence of females. Different explanations have been put forward to explain a greater advantage for females on poorer quality sites (see Ashman 2006). For example, females would be associated with lower quality sites if hermaphrodites are more plastic in their seed production, or are more-resource limited than females, because either would lead to a greater discrepancy in hermaphrodite seed quality or quantity in low quality sites. However, my data do not support this idea. Although seedling survival was lower in high sex ratio (perhaps lower quality) sites, I found no evidence that vital rates differed between the sex morphs differently between populations of different sex ratio. On the other hand, I did not track seeds according to their maternal gender, had I done so I might have found that there were differences in

vital rates between low and high sex ratio populations depending on the maternal gender of plants.

The female lifetime fitness advantage I measured may under-estimate the true female lifetime fitness advantage. The high rates of herbivory in 2010, combined with the fact that larger plants are more likely to get browsed and to lose a larger proportion of stalks than smaller plants, resulted in (ambient) seed set not differing among size classes. In other years, seed set increases across size classes of plants (Clarke unpublished, Brody pers.com). Had seed set increased with size class, the greater MR-LR transition rates of females would have had a greater effect on the female lifetime fitness advantage because advancing to the largest size class would have entailed — not only the slight reduction in mortality — but also an increase in seed set. On the other hand, the lifetime female fitness advantage I measured could also be an over-estimate of the female fitness advantage if there are density dependent effects on growth, survivorship, or fertility in this system, which I did not measure. Density dependence is often overlooked and assumed unimportant (Menges 2000, but see Augustine et al. 1998, Kauffman and Maron 2006). There are several reasons to think density dependence may not be strong in this system. First, sites where *Polemonium foliosissimum* occurs are open grasslands with abundant bare ground. It is likely that in these sites germination is more seed than safe site limited, which could mean a stronger correlation between seed set and seed germination rates (Crawley 1989). Several studies which have explored the effect of seed predators across more open as opposed to more closed habitats have found a greater

effect of seed predators in more open habitats where safe sites are less limiting (Louda and Potvin 1995, Maron et al. 2002). I also found a positive, though not statistically significant, trend between seed set in transects in 2010 and seedling establishment in 2011 ($\rho = .639$, $p = 0.172$) which may suggest density-dependence is not strong in habitats where *P. foliosissimum* occurs.

Here, I documented that several vital rates differ between female and hermaphrodite sex morphs of *Polemonium foliosissimum*. Females have several vital rate advantages over hermaphrodites in that they set more seed than hermaphrodites in the presence of herbivores and seed predators (at least in some populations), their seeds have a greater probability of germination, and female medium reproductive plants advance more often to a large reproductive size class than do hermaphrodite plants. These differences would translate to a lifetime fitness advantage if at least 50% of female offspring are female, which is not uncommon in gynodioecious species (e.g. Byers et al. 2005). In addition, this requirement would also be relaxed if hermaphrodites produce more than 5% female offspring, which was assumed conservatively in my models (e.g. Bailey and McCauley 2005). However, my results highlight that the lifetime fitness advantage that females have is not sufficient to suggest that female persistence is secure. Rather, females will only have a lifetime fitness advantage under specific population genetic situations, namely where the frequency of cytoplasmic male sterility alleles are common, and their nuclear restorers are rare (Chase 2007). It is also worth noting that this assumes that the mode of inheritance of gender in this system is nuclear-cytoplasmic.

If instead, gender inheritance is strictly nuclear, then females should have a two-fold lifetime fitness advantage over hermaphrodites (Lewis 1941, Lloyd 1974, Charlesworth and Charlesworth 1978). Thus, my results do not suggest that females have an adequate advantage to be supported under nuclear inheritance, in which case the existence of females might simply be the result of mutations maintained by stochastic population genetic processes, such as drift and founder effects (Nilsson and Agren 2006).

On the other hand, a two-fold seed set advantage is rarely seen in gynodioecious species (although mode of inheritance is generally not known; reviewed in Dufay and Billard 2012). Thus, perhaps other conditions such as costs of restorer alleles (e.g., Dufay et al. 2007), or frequent founder effects and meta-population dynamics (e.g., Couvet et al. 1998, McCauley and Bailey 2009) are important to maintaining females across a host of gynodioecious species. In concert with these other forces, under a cyto-nuclear mode of inheritance, any lifetime advantage could contribute to the maintenance of females (Gouyon et al. 1991, Bailey et al. 2003). In my research I have found evidence that females have inherent advantages in some vital rates, and that seed predators generate additional seed set advantages which may help contribute to the maintenance of females in *Polemonium foliosissimum*.

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Table 4. Matrix model showing all possible transitions from any given stage in time t to any other stage in time t+1. For each matrix element, the table shows how the element is calculated from individual survivorship (S), growth (T), and fertility (F) vital rates. Numbered subscripts indentify specific transitions in the model/ transition diagram (see Figure 6; for example, $S_1$ refers to survivorship of a female seedling, and, $T_{34}$ indicates the probability that a stage 4, or female MR plant, will regress to stage 3, or SR plant). For simplicity, seed sex ratio is not shown but all fertility transitions also incorporate sex ratio of seeds.

| | | Stage in time t | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 ($S_F$) | 2 ($J_F$) | 3 ($SR_F$) | 4 ($MR_F$) | 5 ($LR_F$) | 6 ($S_H$) | 7 ($J_H$) | 8 ($SR_H$) | 9 ($MR_H$) | 10 ($LR_H$) |
| Stage in time t+1 | 1 ($S_F$) | | | $F_3$ *$G_F$ | $F_4$*$G_F$ | $F_5$*$G_F$ | | | $F_8$*$G_H$ | $F_9$*$G_H$ | $F_{10}$*$G_H$ |
| | 2 ($J_F$) | $S_1$ * $T_{21}$ | $S_2$* $T_{22}$ | | | | | | | | |
| | 3 ($SR_F$) | | $S_2$*$T_{32}$ | $S_3$*$T_{33}$ | $S_4$*$T_{34}$ | | | | | | |
| | 4 ($MR_F$) | | | $S_3$*$T_{43}$ | $S_4$*$T_{44}$ | $S_5$*$T_{45}$ | | | | | |
| | 5 ($LR_F$) | | | | $S_4$*$T_{54}$ | $S_5$*$T_{55}$ | | | | | |
| | 6 ($S_H$) | | | $F_3$ *$G_F$ | $F_4$*$G_F$ | $F_5$*$G_F$ | | | $F_8$*$G_H$ | $F_9$*$G_H$ | $F_{10}$*$G_H$ |
| | 7 ($J_H$) | | | | | | $S_6$*$T_{76}$ | $S_7$*$T_{77}$ | | | |
| | 8 ($SR_H$) | | | | | | | $S_7$*$T_{87}$ | $S_8$*$T_{88}$ | $S_9$*$T_{89}$ | |
| | 9 ($MR_H$) | | | | | | | | $S_8$*$T_{98}$ | $S_9$*$T_{99}$ | $S_{10}$*$T_{910}$ |
| | 10 ($LR_H$) | | | | | | | | | $S_9$*$T_{109}$ | $S_{10}$*$T_{1010}$ |

Table 5. Transition rates between different stages of plants (see Figure 6) in female and hermaphrodite matrices, based on field transitions between 2010 to 2011, used to construct "ambient" matrices.

| | High sex ratio | | Low sex ratio | |
|---|---|---|---|---|
| | Female | Hermaphrodite | Female | Hermaphrodite |
| $S_F$ - $J_F$ | 0.625 | 0.625 | 0.86 | 0.86 |
| $J_F$-$J_F$ | 0.77 | 0.77 | 0.77 | 0.77 |
| $J_F$-$SR_F$ | 0.11 | 0.11 | 0.11 | 0.11 |
| $SR_F$-$S_F$ | 0.0411 | | 0.0833 | |
| $SR_F$-$SR_F$ | 0.79 | 0.79 | 0.79 | 0.79 |
| $SR_F$-$MR_F$ | 0.12 | 0.12 | 0.12 | 0.12 |
| $SR_F$-$S_H$ | 0.0959 | | 0.1945 | |
| $MR_F$-$S_F$ | 0.0411 | | 0.0833 | |
| $MR_F$-$SR_F$ | 0.15 | 0.15 | 0.15 | 0.15 |
| $MR_F$-$MR_F$ | 0.67 | 0.67 | 0.67 | 0.67 |
| $MR_F$-$LR_F$ | 0.16 | 0.16 | 0.16 | 0.16 |
| $MR_F$-$S_H$ | 0.0959 | | 0.1945 | |
| $LR_F$-$S_F$ | 0.0411 | | 0.0833 | |
| $LR_F$-$MR_F$ | 0.23 | 0.23 | 0.23 | 0.23 |
| $LR_F$-$LR_F$ | 0.76 | 0.76 | 0.76 | 0.76 |
| $LR_F$-$S_H$ | 0.0959 | | 0.1945 | |
| $S_H$-$J_H$ | 0.625 | 0.625 | 0.86 | 0.86 |
| $J_H$-$J_H$ | 0.77 | 0.77 | 0.77 | 0.77 |
| $J_H$-$SR_H$ | 0.11 | 0.11 | 0.11 | 0.11 |
| $SR_H$-$S_F$ | | 0.0041 | | 0.0116 |
| $SR_H$-$S_H$ | | 0.0776 | | 0.2204 |
| $SR_H$-$SR_H$ | 0.79 | 0.79 | 0.79 | 0.79 |
| $SR_H$-$MR_H$ | 0.12 | 0.12 | 0.12 | 0.12 |
| $MR_H$-$S_F$ | | 0.0041 | | 0.0116 |
| $MR_H$-$S_H$ | | 0.0776 | | 0.2204 |
| $MR_H$-$SR_H$ | 0.15 | 0.15 | 0.15 | 0.15 |
| $MR_H$-$MR_H$ | 0.73 | 0.73 | 0.73 | 0.73 |
| $MR_H$-$LR_H$ | 0.1 | 0.1 | 0.1 | 0.1 |
| $LR_H$-$S_F$ | | 0.0041 | | 0.0116 |
| $LR_H$-$S_H$ | | 0.0776 | | 0.2204 |
| $LR_H$-$MR_H$ | 0.23 | 0.23 | 0.23 | 0.23 |
| $LR_H$-$LR_H$ | 0.76 | 0.76 | 0.76 | 0.76 |

Table 6. Results from ANOVAs evaluating whether sex morphs and size classes of plants differ in seed set. For both high and low sex ratio populations, separate ANOVAs were run for "ambient", "no herbivory", "no predation", and "no herbivory & no predation matrices".

High sex ratio populations

| | Ambient | | | No herbivory | | | No predation | | | No herbivory & No predation | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SS | df | F (p) | SS | df | F (p) | SS | df | F (p) | SS | df | F (p) |
| Gender | 1.116 | 1 | 8.703 (0.004) | 0.516 | 1 | 2.366 (0.127) | 0.317 | 1 | 3.323 (0.071) | 0.069 | 1 | 0.348 (0.557) |
| Size class | 0.156 | 2 | 1.214 (0.300) | 4.951 | 2 | 11.360 (<0.001) | 0.731 | 2 | 3.829 (0.024) | 6.88 | 2 | 17.427 (<0.001) |
| Error | 15.9 | 124 | | 23.534 | 108 | | 11.834 | 124 | | 21.318 | 108 | |

Low sex ratio populations

| | Ambient | | | No herbivory | | | No predation | | | No herbivory & No predation | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SS | df | F (p) | SS | df | F (p) | SS | df | F (p) | SS | df | F (p) |
| Gender | 0.314 | 1 | 0.998 (0.319) | 0.745 | 1 | 1.864 (0.174) | 0.109 | 1 | 0.500 (0.480) | 0.371 | 1 | 1.270 (0.262) |
| Size class | 1.207 | 2 | 1.921 (0.150) | 3.733 | 2 | 4.67 (0.011) | 1.568 | 2 | 3.604 (0.030) | 4.562 | 2 | 7.811 (0.001) |
| Error | 47.115 | 150 | | 53.557 | 134 | | 32.631 | 150 | | 39.127 | 134 | |

Table 7. Population growth rates ($\lambda$) from female ($\lambda_F$) and hermaphrodite ($\lambda_H$) matrix projections in "ambient", "no herbivory", "no predation", and "no herbivory & no predation" conditions from high and low sex ratio populations, and relative lifetime fitness advantage in each condition, expressed as F$\lambda$ : H $\lambda$. In each of these matrices, hermaphrodites produce 5% female offspring and females produce 30% female offspring.

| High sex ratio populations | | | |
|---|---|---|---|
| | $\lambda_F$ | $\lambda_H$ | F:H $\lambda$ |
| Ambient seed set | 0.970945 | 0.976944 | 0.99386 |
| Seed set without herbivory | 1.017434 | 1.049865 | 0.96911 |
| Seed set without predation | 0.972188 | 0.98632 | 0.985672 |
| Seed set without herbivory & predation | 1.030805 | 1.071175 | 0.962312 |

| Low sex ratio populations | | | |
|---|---|---|---|
| | $\lambda_F$ | $\lambda_H$ | F:H $\lambda$ |
| Ambient seed set | 0.988374 | 1.023015 | 0.966139 |
| Seed set without herbivory | 1.054494 | 1.111684 | 0.948556 |
| Seed set without predation | 0.991753 | 1.025209 | 0.967367 |
| Seed set without herbivory or predation | 1.110412 | 1.216094 | 0.913098 |

Table 8. Proportion of plants in each stage (see Figure 6) at the stable stage distribution in "ambient", "no herbivory", "no predation", and "no herbivory & no predation" matrices for female (F) and hermaphrodite (H) matrices in high sex ratio and low sex ratio populations.

| | High sex ratio populations | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Ambient | | No browse | | No predation | | No browse & No predation | |
| | F | H | F | H | F | H | F | H |
| $S_F$ | 0.013 | 0.003 | 0.026 | 0.007 | 0.014 | 0.004 | 0.034 | 0.009 |
| $J_F$ | 0.039 | 0.009 | 0.066 | 0.016 | 0.042 | 0.012 | 0.082 | 0.018 |
| $SR_F$ | 0.112 | 0.018 | 0.056 | 0.008 | 0.109 | 0.016 | 0.054 | 0.008 |
| $MR_F$ | 0.106 | 0.016 | 0.096 | 0.012 | 0.102 | 0.013 | 0.083 | 0.010 |
| $LR_F$ | 0.080 | 0.011 | 0.059 | 0.006 | 0.077 | 0.009 | 0.049 | 0.005 |
| $S_H$ | 0.029 | 0.057 | 0.061 | 0.139 | 0.032 | 0.077 | 0.079 | 0.169 |
| $J_H$ | 0.091 | 0.171 | 0.155 | 0.310 | 0.098 | 0.222 | 0.190 | 0.351 |
| $SR_H$ | 0.226 | 0.319 | 0.139 | 0.169 | 0.227 | 0.306 | 0.133 | 0.158 |
| $MR_H$ | 0.206 | 0.271 | 0.246 | 0.246 | 0.204 | 0.237 | 0.215 | 0.206 |
| $LR_H$ | 0.098 | 0.125 | 0.095 | 0.085 | 0.096 | 0.105 | 0.080 | 0.066 |

| | Low sex ratio populations | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Ambient | | No browse | | No predation | | No browse & No predation | |
| | F | H | F | H | F | H | F | H |
| $S_F$ | 0.019 | 0.006 | 1.000 | 0.009 | 0.020 | 0.006 | 0.053 | 0.013 |
| $J_F$ | 0.074 | 0.019 | 1.226 | 0.022 | 0.079 | 0.019 | 0.133 | 0.025 |
| $SR_F$ | 0.097 | 0.014 | 3.171 | 0.008 | 0.095 | 0.014 | 0.045 | 0.006 |
| $MR_F$ | 0.074 | 0.008 | 3.386 | 0.008 | 0.070 | 0.008 | 0.047 | 0.004 |
| $LR_F$ | 0.052 | 0.005 | 4.158 | 0.004 | 0.048 | 0.005 | 0.022 | 0.002 |
| $S_H$ | 0.044 | 0.105 | 0.000 | 0.168 | 0.047 | 0.107 | 0.123 | 0.248 |
| $J_H$ | 0.172 | 0.357 | 0.000 | 0.424 | 0.184 | 0.362 | 0.311 | 0.478 |
| $SR_H$ | 0.225 | 0.270 | 0.000 | 0.148 | 0.222 | 0.268 | 0.109 | 0.113 |
| $MR_H$ | 0.171 | 0.158 | 0.000 | 0.163 | 0.164 | 0.154 | 0.121 | 0.091 |
| $LR_H$ | 0.075 | 0.060 | 0.000 | 0.046 | 0.071 | 0.058 | 0.035 | 0.020 |

Table 9. Reproductive values for each stage class (see Figure 6) in "ambient", "no herbivory", "no predation", and "no herbivory & no predation" conditions for female (F) and hermaphrodite (H) matrices in high sex ratio and low sex ratio populations.

| | High sex ratio populations | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Ambient | | No browse | | No predation | | No browse & No predation | |
| | F | H | F | H | F | H | F | H |
| $S_F$ | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 |
| $J_F$ | 1.554 | 0.000 | 1.628 | 0.000 | 1.556 | 0.000 | 1.649 | 0.000 |
| $SR_F$ | 2.838 | 0.000 | 3.662 | 0.000 | 2.859 | 0.000 | 3.910 | 0.000 |
| $MR_F$ | 3.937 | 0.000 | 3.811 | 0.000 | 3.994 | 0.000 | 4.140 | 0.000 |
| $LR_F$ | 4.487 | 0.000 | 4.310 | 0.000 | 4.603 | 0.000 | 4.930 | 0.000 |
| $S_H$ | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 |
| $J_H$ | 0.000 | 1.563 | 0.000 | 1.680 | 0.000 | 1.578 | 0.000 | 1.714 |
| $SR_H$ | 0.000 | 2.941 | 0.000 | 4.274 | 0.000 | 3.103 | 0.000 | 4.693 |
| $MR_H$ | 0.000 | 3.935 | 0.000 | 4.485 | 0.000 | 4.160 | 0.000 | 5.022 |
| $LR_H$ | 0.000 | 4.529 | 0.000 | 5.686 | 0.000 | 4.906 | 0.000 | 6.966 |

| | Low sex ratio populations | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Ambient | | No browse | | No predation | | No browse & No predation | |
| | F | H | F | H | F | H | F | H |
| $S_F$ | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 |
| $J_F$ | 1.149 | 0.000 | 1.153 | 0.000 | 1.226 | 0.000 | 1.291 | 0.000 |
| $SR_F$ | 2.282 | 0.000 | 2.325 | 0.000 | 3.171 | 0.000 | 3.996 | 0.000 |
| $MR_F$ | 3.078 | 0.000 | 3.259 | 0.000 | 3.386 | 0.000 | 4.035 | 0.000 |
| $LR_F$ | 3.464 | 0.000 | 3.886 | 0.000 | 4.158 | 0.000 | 4.190 | 0.000 |
| $S_H$ | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 |
| $J_H$ | 0.000 | 1.190 | 0.000 | 1.192 | 0.000 | 1.293 | 0.000 | 1.414 |
| $SR_H$ | 0.000 | 2.736 | 0.000 | 2.766 | 0.000 | 4.015 | 0.000 | 5.735 |
| $MR_H$ | 0.000 | 3.476 | 0.000 | 3.702 | 0.000 | 4.310 | 0.000 | 5.771 |
| $LR_H$ | 0.000 | 3.878 | 0.000 | 4.717 | 0.000 | 6.170 | 0.000 | 6.043 |

Table 10. Sensitivity of population growth to each matrix element or stage class transition (see Figure 6) in "ambient", "no herbivory", "no predation", and "no herbivory & no predation" conditions for female (F) and hermaphrodite (H) matrices, from high sex ratio populations.

| | Ambient | | No browse | | No predation | | No browse & No predation | |
|---|---|---|---|---|---|---|---|---|
| | F | H | F | H | F | H | F | H |
| $S_F$ - $J_F$ | 0.017 | 0.000 | 0.045 | 0.000 | 0.018 | 0.000 | 0.058 | 0.000 |
| $J_F$-$J_F$ | 0.052 | 0.000 | 0.113 | 0.000 | 0.057 | 0.000 | 0.139 | 0.000 |
| $J_F$-$SR_F$ | 0.095 | 0.000 | 0.253 | 0.000 | 0.105 | 0.000 | 0.330 | 0.000 |
| $SR_F$-$S_F$ | 0.096 | | 0.058 | | 0.095 | | 0.055 | |
| $SR_F$-$SR_F$ | 0.271 | 0.000 | 0.213 | 0.000 | 0.271 | 0.000 | 0.217 | 0.000 |
| $SR_F$-$MR_F$ | 0.376 | 0.000 | 0.222 | 0.000 | 0.379 | 0.000 | 0.230 | 0.000 |
| $SR_F$-$S_H$ | 0.000 | | 0.000 | | 0.000 | | 0.000 | |
| $MR_F$-$S_F$ | 0.091 | | 0.100 | | 0.088 | | 0.086 | |
| $MR_F$-$SR_F$ | 0.257 | 0.000 | 0.365 | 0.000 | 0.253 | 0.000 | 0.337 | 0.000 |
| $MR_F$-$MR_F$ | 0.357 | 0.000 | 0.380 | 0.000 | 0.353 | 0.000 | 0.357 | 0.000 |
| $MR_F$-$LR_F$ | 0.407 | 0.000 | 0.430 | 0.000 | 0.407 | 0.000 | 0.426 | 0.000 |
| $MR_F$-$S_H$ | 0.000 | | 0.000 | | 0.000 | | 0.000 | |
| $LR_F$-$S_F$ | 0.069 | | 0.062 | | 0.067 | | 0.051 | |
| $LR_F$-$MR_F$ | 0.271 | 0.000 | 0.236 | 0.000 | 0.266 | 0.000 | 0.211 | 0.000 |
| $LR_F$-$LR_F$ | 0.309 | 0.000 | 0.267 | 0.000 | 0.307 | 0.000 | 0.251 | 0.000 |
| $LR_F$-$S_H$ | 0.000 | | 0.000 | | 0.000 | | 0.000 | |
| $S_H$-$J_H$ | 0.000 | 0.031 | 0.000 | 0.079 | 0.000 | 0.042 | 0.000 | 0.096 |
| $J_H$-$J_H$ | 0.000 | 0.093 | 0.000 | 0.175 | 0.000 | 0.122 | 0.000 | 0.200 |
| $J_H$-$SR_H$ | 0.000 | 0.174 | 0.000 | 0.446 | 0.000 | 0.240 | 0.000 | 0.547 |
| $SR_H$-$S_F$ | | 0.000 | | 0.000 | | 0.000 | | 0.000 |
| $SR_H$-$S_H$ | | 0.110 | | 0.057 | | 0.106 | | 0.052 |
| $SR_H$-$SR_H$ | 0.000 | 0.324 | 0.000 | 0.243 | 0.000 | 0.330 | 0.000 | 0.246 |
| $SR_H$-$MR_H$ | 0.000 | 0.433 | 0.000 | 0.255 | 0.000 | 0.442 | 0.000 | 0.263 |
| $MR_H$-$S_F$ | | 0.000 | | 0.000 | | 0.000 | | 0.000 |
| $MR_H$-$S_H$ | | 0.094 | | 0.083 | | 0.082 | | 0.069 |
| $MR_H$-$SR_H$ | 0.000 | 0.276 | 0.000 | 0.354 | 0.000 | 0.256 | 0.000 | 0.322 |
| $MR_H$-$MR_H$ | 0.000 | 0.369 | 0.000 | 0.372 | 0.000 | 0.343 | 0.000 | 0.345 |
| $MR_H$-$LR_H$ | 0.000 | 0.424 | 0.000 | 0.471 | 0.000 | 0.404 | 0.000 | 0.478 |
| $LR_H$-$S_F$ | | 0.000 | | 0.000 | | 0.000 | | 0.000 |
| $LR_H$-$S_H$ | | 0.043 | | 0.029 | | 0.036 | | 0.022 |
| $LR_H$-$MR_H$ | 0.000 | 0.170 | 0.000 | 0.128 | 0.000 | 0.152 | 0.000 | 0.111 |
| $LR_H$-$LR_H$ | 0.000 | 0.196 | 0.000 | 0.163 | 0.000 | 0.179 | 0.000 | 0.154 |

Table 11. Elasticity of population growth to each matrix element or stage class transition (see Figure 6) in "ambient", "no herbivory", "no predation", and "no herbivory & no predation" conditions in female (F) and hermaphrodite (H) matrices in high sex ratio populations. Elasticities measure proportional effects on λ of small changes in matrix elements.

| | Ambient | | No browse | | No predation | | No browse & No predation | |
|---|---|---|---|---|---|---|---|---|
| | F | H | F | H | F | H | F | H |
| $S_F$ - $J_F$ | 0.011 | 0.000 | 0.027 | 0.000 | 0.012 | 0.000 | 0.035 | 0.000 |
| $J_F$-$J_F$ | 0.041 | 0.000 | 0.085 | 0.000 | 0.045 | 0.000 | 0.104 | 0.000 |
| $J_F$-$SR_F$ | 0.011 | 0.000 | 0.027 | 0.000 | 0.012 | 0.000 | 0.035 | 0.000 |
| $SR_F$-$S_F$ | 0.004 | | 0.005 | | 0.004 | | 0.006 | |
| $SR_F$-$SR_F$ | 0.221 | 0.000 | 0.132 | 0.000 | 0.220 | 0.000 | 0.133 | 0.000 |
| $SR_F$-$MR_F$ | 0.047 | 0.000 | 0.076 | 0.000 | 0.047 | 0.000 | 0.078 | 0.000 |
| $SR_F$-$S_H$ | 0.000 | | 0.000 | | 0.000 | | 0.000 | |
| $MR_F$-$S_F$ | 0.004 | | 0.008 | | 0.004 | | 0.010 | |
| $MR_F$-$SR_F$ | 0.040 | 0.000 | 0.054 | 0.000 | 0.039 | 0.000 | 0.049 | 0.000 |
| $MR_F$-$MR_F$ | 0.246 | 0.000 | 0.250 | 0.000 | 0.243 | 0.000 | 0.232 | 0.000 |
| $MR_F$-$LR_F$ | 0.067 | 0.000 | 0.068 | 0.000 | 0.067 | 0.000 | 0.066 | 0.000 |
| $MR_F$-$S_H$ | 0.000 | | 0.000 | | 0.000 | | 0.000 | |
| $LR_F$-$S_F$ | 0.003 | | 0.014 | | 0.004 | | 0.019 | |
| $LR_F$-$MR_F$ | 0.064 | 0.000 | 0.053 | 0.000 | 0.063 | 0.000 | 0.047 | 0.000 |
| $LR_F$-$LR_F$ | 0.242 | 0.000 | 0.199 | 0.000 | 0.240 | 0.000 | 0.185 | 0.000 |
| $LR_F$-$S_H$ | 0.000 | | 0.000 | | 0.000 | | 0.000 | |
| $S_H$-$J_H$ | 0.000 | 0.020 | 0.000 | 0.047 | 0.000 | 0.027 | 0.000 | 0.056 |
| $J_H$-$J_H$ | 0.000 | 0.073 | 0.000 | 0.129 | 0.000 | 0.095 | 0.000 | 0.144 |
| $J_H$-$SR_H$ | 0.000 | 0.020 | 0.000 | 0.047 | 0.000 | 0.027 | 0.000 | 0.056 |
| $SR_H$-$S_F$ | | 0.000 | | 0.000 | | 0.000 | | 0.000 |
| $SR_H$-$S_H$ | | 0.009 | | 0.012 | | 0.012 | | 0.015 |
| $SR_H$-$SR_H$ | 0.000 | 0.262 | 0.000 | 0.146 | 0.000 | 0.264 | 0.000 | 0.145 |
| $SR_H$-$MR_H$ | 0.000 | 0.053 | 0.000 | 0.085 | 0.000 | 0.054 | 0.000 | 0.086 |
| $MR_H$-$S_F$ | | 0.000 | | 0.000 | | 0.000 | | 0.000 |
| $MR_H$-$S_H$ | | 0.007 | | 0.018 | | 0.009 | | 0.020 |
| $MR_H$-$SR_H$ | 0.000 | 0.042 | 0.000 | 0.051 | 0.000 | 0.039 | 0.000 | 0.045 |
| $MR_H$-$MR_H$ | 0.000 | 0.275 | 0.000 | 0.259 | 0.000 | 0.254 | 0.000 | 0.235 |
| $MR_H$-$LR_H$ | 0.000 | 0.043 | 0.000 | 0.045 | 0.000 | 0.041 | 0.000 | 0.045 |
| $LR_H$-$S_F$ | | 0.000 | | 0.000 | | 0.000 | | 0.000 |
| $LR_H$-$S_H$ | | 0.003 | | 0.017 | | 0.006 | | 0.021 |
| $LR_H$-$MR_H$ | 0.000 | 0.040 | 0.000 | 0.028 | 0.000 | 0.035 | 0.000 | 0.024 |
| $LR_H$-$LR_H$ | 0.000 | 0.152 | 0.000 | 0.118 | 0.000 | 0.138 | 0.000 | 0.109 |

## 4.7 Figure legends

Figure 6. Life-cycle graph depicting ten life stages and twenty eight vital rates forming the basis of the two-sex stage-based population projection matrix for *Polemonium foliosissimum*. Abbreviations are as follows: seedling (S), juvenile (J), and small (SR), medium (MR), and large (LR) reproductive. Genders are noted by the subscripts F (for female) and H (for hermaphrodite). This lifecycle depicts the female matrix, in which hermaphrodite fertility transitions were set to zero. Thus this matrix summarizes survivorship and growth transitions for both sex morphs, along with seed production for females, permitting their progeny to be either gender. A separate hermaphrodite matrix (not shown) is identical except that instead of including female fertility, it includes only hermaphrodite fertility.

Figure 7. Seed set of female and hermaphrodite plants in high and low sex ratio populations under "ambient", "no herbivory", "no predation", and "no herbivory & no predation" conditions.

Figure 8. Relative female lifetime fitness advantage (female $\lambda$ : hermaphrodite $\lambda$) under varying magnitudes of seed set advantage and varying progeny sex ratio to female plants. In all models hermaphrodite progeny sex ratio was 5% female. However, the sex ratio of female progeny was varied from 10% to 60% female.

Figure 6. Life-cycle graph depicting ten life stages and twenty eight vital rates forming the basis of the two-sex stage-based population projection matrix for *Polemonium foliosissimum*. Abbreviations are as follows: seedling (S), juvenile (J), and small (SR), medium (MR), and large (LR) reproductive. Genders are noted by the subscripts F (for female) and H (for hermaphrodite). This lifecycle depicts the female matrix, in which hermaphrodite fertility transitions were set to zero. Thus this matrix summarizes survivorship and growth transitions for both sex morphs, along with seed production for females, permitting their progeny to be either gender. A separate hermaphrodite matrix (not shown) is identical except that instead of including female fertility, it includes only hermaphrodite fertility.

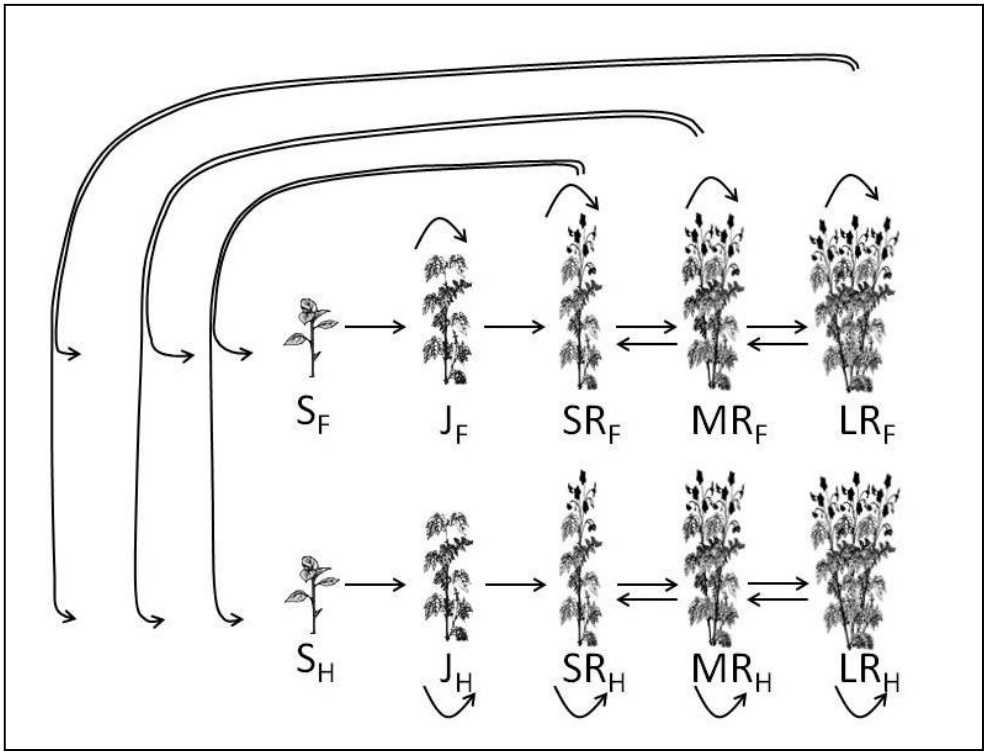


Figure 7. Seed set of female and hermaphrodite plants in high and low sex ratio populations under “ambient”, “no herbivory”, “no predation”, and “no herbivory & no predation” conditions.

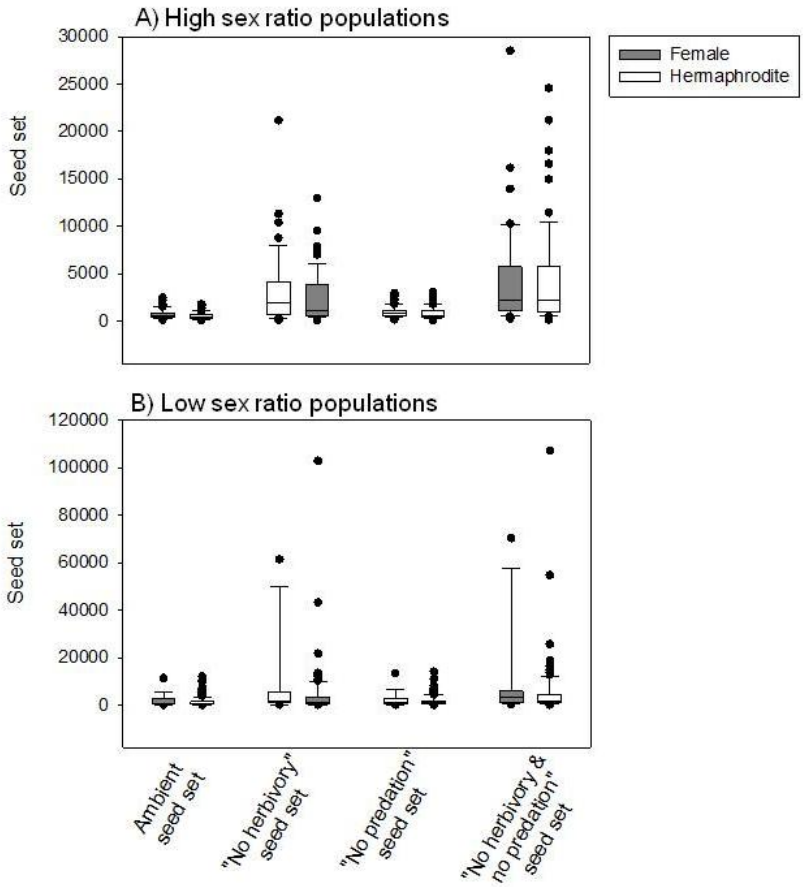
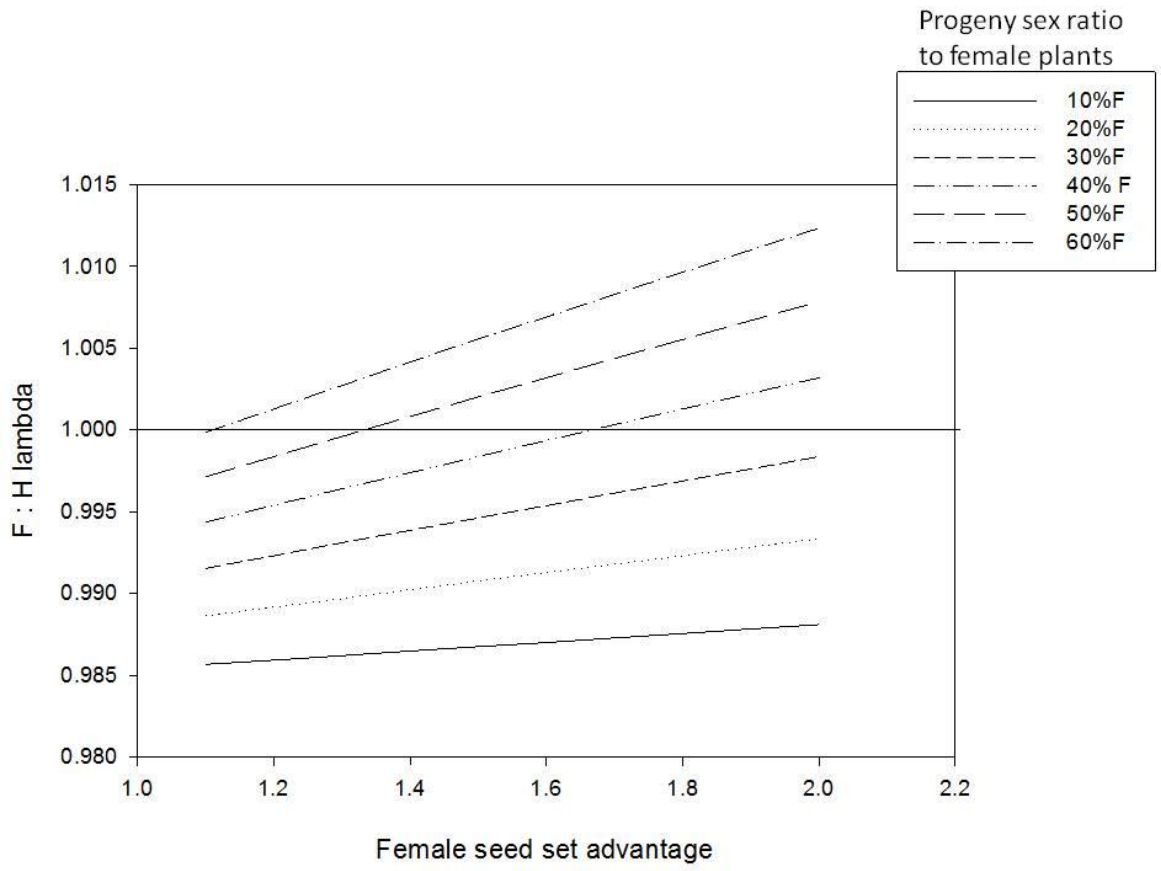


Figure 8. Relative female lifetime fitness advantage (female λ : hermaphrodite λ) under varying magnitudes of seed set advantage and varying progeny sex ratio to female plants. In all models hermaphrodite progeny sex ratio was 5% female. However, the sex ratio of female progeny was varied from 10% to 60% female.



## APPENDIX 1

Summary of ANOVAs (F statistics, followed by p values in parentheses) testing the effects of gender and site and their interaction on measures of reproductive success and predation rates from observational studies in 2009 and 2010. Significant factors and interactions ($p < 0.05$) are bolded.

| | | 2009 | | | | |
|---|---|---|---|---|---|---|
| Source | df | Total flowers | Fruit set | Seeds/fruit | Proportion destroyed | Total plant seed set |
| Gender | 1 | 0.475 (0.492) | 0.530 (0.468) | 0.001 (0.986) | **30.127 (<0.001)** | **4.182 (0.042)** |
| Site | 25 | **3.445 (< 0.001)** | **3.340 (<0.001)** | **2.581 (<0.001)** | **7.645 (<0.001)** | **5.158 (<0.001)** |
| Gender x site | 21 | 0.566 (0.936) | 0.705 (0.825) | 0.843 (0.664) | 1.147 (0.306) | 0.773 (0.750) |
| Residual | 167 | | | | | |

| | | 2010 | | | | |
|---|---|---|---|---|---|---|
| Source | df | Total flowers | Fruit set | Seeds/fruit | Proportion destroyed | Total plant seed set |
| Gender | 1 | 0.027 (0.872) | 3.379 (0.101) | 4.519 (0.043) | **14.798 (<0.001)** | **5.813 (0.022)** |
| Site | 5 | 4.202 (0.111) | 1.310 (0.413) | **7.855 (0.049)** | **185.289 (0.008)** | **18.486 (0.015)** |
| Gender x site | 4 | 0.932 (0.446) | 2.321 (0.058) | 0.681 (0.606) | 0.179 (0.949) | 0.556 (0.695) |
| Residual | 233 | | | | | |

## APPENDIX 2

Mean values (± 1 SE) of reproductive success and predation rates for females (F) and hermaphrodites (H) in the 2011 hand pollination and egg removal experiment. Treatmeants at the plant level include controls (C), hand pollination (HP), egg removal (ER), and hand pollination and egg removal (HP & ER). Controls stalks are denoted "c" and treatment stalks "t".

| | | Fruit set | | Seeds/fruit | | Proportion destroyed | | Net seed set | |
|---|---|---|---|---|---|---|---|---|---|
| Trt | gdr | c stalks | t stalks | c stalks | t stalks | c stalks | t stalks | c stalks | t stalks |
| C | F | 0.85 ± 0.04 | 0.88 ± 0.03 | 10.36 ± 0.85 | 10.87 ± 0.79 | 0.38 ± 0.05 | 0.45 ± 0.06 | 633.50 ± 166.25 | 627.75 ± 177.78 |
| | H | 0.82 ± 0.03 | 0.82 ± 0.02 | 9.88 ± 0.68 | 9.59 ± 0.72 | 0.59 ± 0.05 | 0.55 ± 0.06 | 380.62 ± 91.59 | 364.03 ± 76.26 |
| HP | F | 0.90 ± 0.03 | 0.90 ± 0.02 | 10.79 ± 0.82 | 11.71 ± 1.01 | 0.47 ± 0.07 | 0.44 ± 0.06 | 699.15 ± 188.83 | 726.46 ± 203.50 |
| | H | 0.81 ± 0.04 | 0.84 ± 0.02 | 9.90 ± 0.93 | 11.90 ± 0.45 | 0.54 ± 0.04 | 0.56 ± 0.05 | 479.53 ± 122.01 | 565.76 ± 147.37 |
| ER | F | 0.81 ± 0.05 | 0.82 ± 0.04 | 10.21 ± 1.05 | 9.68 ± 0.67 | 0.43 ± 0.06 | 0.19 ± 0.04 | 490.38 ± 103.66 | 660.82 ± 94.18 |
| | H | 0.81 ± 0.02 | 0.86 ± 0.02 | 9.54 ± 0.54 | 10.69 ± 0.49 | 0.60 ± 0.03 | 0.21 ± 0.03 | 365.62 ± 92.99 | 843.90 ± 150.70 |
| HP & ER | F | 0.83 ± 0.04 | 0.91 ± 0.02 | 11.08 ± 0.80 | 12.34 ± 1.08 | 0.38 ± 0.07 | 0.16 ± 0.03 | 526.47 ± 186.66 | 1401.08 ± 534.88 |
| | H | 0.81 ± 0.03 | 0.81 ± 0.04 | 7.92 ± 0.47 | 11.19 ± 0.61 | 0.50 ± 0.06 | 0.17 ± 0.03 | 593.58 ± 182.28 | 1149.24 ± 244.93 |

## APPENDIX 3

Summary of ANOVAs (F statistics, followed by p values in parentheses) for different measures of reproductive success and predation rates, and ANCOVA for seed set, testing the effects of gender, hand pollination (HP), egg removal (ER), site, and their interactions, using only control stalk data (except for total flowers per plant) from the 2011 hand pollination and egg removal experiment. Significant factors and interactions ($p < 0.05$) are bolded.

| Source | df | Total flowers | Fruit set | Seeds / fruit | Proportion destroyed | df | Net seed set |
|---|---|---|---|---|---|---|---|
| Gender | 1 | 1.381 (0.244) | 2.550 (0.115) | **6.406 (0.014)** | **30.559 (<0.001)** | 1 | **21.892 (< 0.001)** |
| HP | 1 | 0.864 (0.356) | 0.503 (0.481) | 0.019 (0.892) | 0.761 (0.386) | 1 | 0.035 (0.852) |
| ER | 1 | 0.139 (0.710) | 2.014 (0.161) | 1.081 (0.302) | 0.160 (0.690) | 1 | 2.211 (0.142) |
| Site | 1 | 0.113 (0.738) | 0.848 (0.361) | 1.144 (0.289) | **34.127 (<0.001)** | 1 | **15.885 (<0.001)** |
| Gender x HP | 1 | 0.249 (0.620) | 0.845 (0.361) | 1.806 (0.184) | 1.968 (0.165) | 1 | 0.007 (0.934) |
| Gender x ER | 1 | 3.207 (0.078) | 1.757 (0.190) | 1.150 (0.288) | 0.079 (0.780) | 1 | 0.001 (0.981) |
| HP x ER | 1 | 0.001 (0.993) | 0.106 (0.746) | 0.274 (0.602) | 2.687 (0.106) | 1 | 0.796 (0.376) |
| Gender x HP x ER | 1 | 2.410 (0.126) | 0.525 (0.471) | 0.810 (0.371) | 0.183 (0.670) | 1 | 1.592 (0.212) |
| # flowers on control stalks | | | | | | 1 | **203.645 (<0.001)** |
| Residuals | 64 | | | | | 63 | |

## APPENDIX 4

Summary of ANOVAs (F statistics, followed by p values in parentheses) using the response to treatments (treatment/control) for different measures of reproductive success and predation rates, and ANCOVA for seed set, testing the effects of gender, hand pollination (HP), egg removal (ER), site, and their interactions, from the hand pollination and egg removal experiment. The ANCOVA for seed set included the treatment : control stalk ratio of flowers as a covariate. Significant factors and interactions ($p < 0.05$) are bolded.

| Source | df | Fruit set | Seeds/ fruit | Proportion destroyed | Source | df | Net seed set |
|---|---|---|---|---|---|---|---|
| Gender | 1 | 0.011 (0.917) | 3.390 (0.052) | 2.237 (0.140) | Gender | 1 | **10.489 (0.002)** |
| HP | 1 | 0.725 (0.398) | **15.855 (<0.001)** | 0.339 (0.562) | HP | 1 | **6.658 (0.012)** |
| ER | 1 | 1.913 (0.171) | 1.672 (0.201) | **111.196 (<0.001)** | ER | 1 | **58.661 (<0.001)** |
| Site | 1 | 0.028 (0.868) | 1.066 (0.306) | 0.148 (0.701) | Site | 1 | 2.465 (.122) |
| Gender x HP | 1 | 0.051 (0.822) | 2.625 (0.110) | 1.332 (0.253) | Gender x HP | 1 | 0.730 (0.396) |
| Gender x ER | 1 | 0.288 (0.594) | 2.575 (0.113) | 0.116 (0.735) | Gender x ER | 1 | **4.158 (0.046)** |
| HP x ER | 1 | 0.489 (0.487) | 0.708 (0.403) | 0.004 (0.947) | HP x ER | 1 | 0.673 (0.415) |
| Gender x HP x ER | 1 | 3.649 (0.061) | 1.124 (0.293) | 0.709 (0.403) | Gender x HP x ER | 1 | 0.088 (0.767) |
| Residuals | 64 | | | | t:c total flowers | 1 | **9.617 (0.003)** |
| | | | | | HP x t:c total flowers | 1 | **6.078 (0.017)** |
| | | | | | ER x t:c total flowers | 1 | **6.632 (0.012)** |
| | | | | | HP x ER x t:c total flowers | 1 | **6.439 (0.014)** |
| | | | | | Residuals | 60 | |

## APPENDIX 5

Means and standard error values for components of fitness for female and hermaphrodite plants in 2010 in high and low sex ratio populations (high and low frequency of females respectively). For each fitness measure, t test results are included comparing each fitness measure between female and hermaphrodite sex morphs.

| | High sex ratio | | |
|---|---|---|---|
| | Females | Hermaphrodites | t-test results |
| Total stalks | 11.12 ± 1.12 | 12.49 ± 0.91 | $t_{126}$ = -1.107; p = 0.271 |
| Percent of stalks browsed | 62.38 ± 4.63 | 64.91 ± 3.53 | $t_{126}$ = -0.373; p = 0.710 |
| Total flowers per plant | 93.58 ± 10.66 | 82.08 ± 5.84 | $t_{126}$ = -0.908; p = 0.366 |
| Percent of fruits destroyed | 24.34 ± 2.57 | 38.35 ± 2.14 | $t_{126}$ = -4.097; p <0.001 |
| Percent of fruits set | 84.24 ± 1.63 | 84.31 ± 1.10 | $t_{126}$ = 0.248; p = 0.805 |
| Seeds per fruit | 14.01 ± 0.42 | 13.16 ± 0.38 | $t_{126}$ = 1.557; p = 0.131 |
| Total seed set | 684.68 ± 71.50 | 488.56 ± 45.00 | $t_{126}$ = 2.972; p = 0.004 |
| Total stalks | 11.12 ± 1.12 | 12.49 ± 0.91 | $t_{126}$ = -1.107; p = 0.271 |

| | Low sex ratio | | |
|---|---|---|---|
| | Females | Hermaphrodites | |
| Total stalks | 8.88 ± 1.33 | 8.23 ± 0.44 | $t_{126}$ = 0.524; p = 0.606 |
| Percent of stalks browsed | 55.21 ± 8.87 | 43.57 ± 2.92 | $t_{126}$ = 1.271; p = 0.219 |
| Total flowers per plant | 176.82 ± 55.41 | 139.34 ± 11.66 | $t_{126}$ = 0.375; p = 0.712 |
| Percent of fruits destroyed | 21.93 ± 4.80 | 29.09 ± 2.01 | $t_{126}$ = -1.449; p =0.163 |
| Percent of fruits set | 83.26 ± 4.07 | 84.41 ± 1.14 | $t_{126}$ = -0.106; p = 0.917 |
| Seeds per fruit | 14.62 ± 0.93 | 13.26 ± 0.35 | $t_{126}$ = 0.676; p = 0.521 |
| Total seed set | 1835.68 ± 665.04 | 1276.82 ± 156.98 | $t_{126}$ = 0.812; p = 0.427 |

## APPENDIX 6

Sensitivity of population growth to each matrix element or stage class transition (see Figure 6) in "ambient", "no herbivory", "no predation", and "no herbivory & no predation" conditions for female (F) and hermaphrodite (H) matrices, from low sex ratio populations.

| | Ambient | | No browse | | No predation | | No browse & No predation | |
|---|---|---|---|---|---|---|---|---|
| | F | H | F | H | F | H | F | H |
| $S_F$ - $J_F$ | 0.029 | 0.000 | 0.059 | 0.000 | 0.011 | 0.000 | 0.099 | 0.000 |
| $J_F$-$J_F$ | 0.116 | 0.000 | 0.178 | 0.000 | 0.041 | 0.000 | 0.251 | 0.000 |
| $J_F$-$SR_F$ | 0.230 | 0.000 | 0.460 | 0.000 | 0.011 | 0.000 | 0.776 | 0.000 |
| $SR_F$-$S_F$ | 0.132 | | 0.072 | | 0.004 | | 0.066 | |
| $SR_F$-$SR_F$ | 0.302 | 0.000 | 0.228 | 0.000 | 0.221 | 0.000 | 0.263 | 0.000 |
| $SR_F$-$MR_F$ | 0.407 | 0.000 | 0.243 | 0.000 | 0.047 | 0.000 | 0.266 | 0.000 |
| $SR_F$-$S_H$ | 0.000 | | 0.000 | | 0.000 | | 0.000 | |
| $MR_F$-$S_F$ | 0.101 | | 0.097 | | 0.004 | | 0.069 | |
| $MR_F$-$SR_F$ | 0.231 | 0.000 | 0.307 | 0.000 | 0.040 | 0.000 | 0.275 | 0.000 |
| $MR_F$-$MR_F$ | 0.311 | 0.000 | 0.328 | 0.000 | 0.246 | 0.000 | 0.278 | 0.000 |
| $MR_F$-$LR_F$ | 0.350 | 0.000 | 0.403 | 0.000 | 0.067 | 0.000 | 0.288 | 0.000 |
| $MR_F$-$S_H$ | 0.000 | | 0.000 | | 0.000 | | 0.000 | |
| $LR_F$-$S_F$ | 0.071 | | 0.053 | | 0.003 | | 0.031 | |
| $LR_F$-$MR_F$ | 0.218 | 0.000 | 0.178 | 0.000 | 0.064 | 0.000 | 0.127 | 0.000 |
| $LR_F$-$LR_F$ | 0.245 | 0.000 | 0.219 | 0.000 | 0.242 | 0.000 | 0.132 | 0.000 |
| $LR_F$-$S_H$ | 0.000 | | 0.000 | | 0.000 | | 0.000 | |
| $S_H$-$J_H$ | 0.000 | 0.061 | 0.000 | 0.095 | 0.000 | 0.060 | 0.000 | 0.158 |
| $J_H$-$J_H$ | 0.000 | 0.207 | 0.000 | 0.238 | 0.000 | 0.203 | 0.000 | 0.305 |
| $J_H$-$SR_H$ | 0.000 | 0.477 | 0.000 | 0.740 | 0.000 | 0.472 | 0.000 | 1.237 |
| $SR_H$-$S_F$ | | 0.000 | | 0.000 | | 0.000 | | 0.000 |
| $SR_H$-$S_H$ | | 0.132 | | 0.064 | | 0.126 | | 0.051 |
| $SR_H$-$SR_H$ | 0.000 | 0.361 | 0.000 | 0.258 | 0.000 | 0.349 | 0.000 | 0.292 |
| $SR_H$-$MR_H$ | 0.000 | 0.458 | 0.000 | 0.277 | 0.000 | 0.467 | 0.000 | 0.294 |
| $MR_H$-$S_F$ | | 0.000 | | 0.000 | | 0.000 | | 0.000 |
| $MR_H$-$S_H$ | | 0.077 | | 0.071 | | 0.073 | | 0.041 |
| $MR_H$-$SR_H$ | 0.000 | 0.210 | 0.000 | 0.285 | 0.000 | 0.201 | 0.000 | 0.235 |
| $MR_H$-$MR_H$ | 0.000 | 0.267 | 0.000 | 0.306 | 0.000 | 0.269 | 0.000 | 0.236 |
| $MR_H$-$LR_H$ | 0.000 | 0.298 | 0.000 | 0.438 | 0.000 | 0.342 | 0.000 | 0.247 |
| $LR_H$-$S_F$ | | 0.000 | | 0.000 | | 0.000 | | 0.000 |
| $LR_H$-$S_H$ | | 0.029 | | 0.020 | | 0.027 | | 0.009 |
| $LR_H$-$MR_H$ | 0.000 | 0.102 | 0.000 | 0.087 | 0.000 | 0.101 | 0.000 | 0.052 |
| $LR_H$-$LR_H$ | 0.000 | 0.113 | 0.000 | 0.125 | 0.000 | 0.129 | 0.000 | 0.054 |

## APPENDIX 7

Elasticity of population growth to each matrix element or stage class transition (see Figure 6) in "ambient", "no herbivory", "no predation", and "no herbivory & no predation" conditions in female (F) and hermaphrodite (H) matrices in low sex ratio populations. Elasticities measure proportional effects on λ of small changes in matrix elements.

| | Ambient | | No browse | | No predation | | No browse & No predation | |
|---|---|---|---|---|---|---|---|---|
| | F | H | F | H | F | H | F | H |
| $S_F$ - $J_F$ | 0.011 | 0.000 | 0.027 | 0.000 | 0.012 | 0.000 | 0.035 | 0.000 |
| $J_F$-$J_F$ | 0.041 | 0.000 | 0.085 | 0.000 | 0.045 | 0.000 | 0.104 | 0.000 |
| $J_F$-$SR_F$ | 0.011 | 0.000 | 0.027 | 0.000 | 0.012 | 0.000 | 0.035 | 0.000 |
| $SR_F$-$S_F$ | 0.004 | | 0.005 | | 0.004 | | 0.006 | |
| $SR_F$-$SR_F$ | 0.221 | 0.000 | 0.132 | 0.000 | 0.220 | 0.000 | 0.133 | 0.000 |
| $SR_F$-$MR_F$ | 0.047 | 0.000 | 0.076 | 0.000 | 0.047 | 0.000 | 0.078 | 0.000 |
| $SR_F$-$S_H$ | 0.000 | | 0.000 | | 0.000 | | 0.000 | |
| $MR_F$-$S_F$ | 0.004 | | 0.008 | | 0.004 | | 0.010 | |
| $MR_F$-$SR_F$ | 0.040 | 0.000 | 0.054 | 0.000 | 0.039 | 0.000 | 0.049 | 0.000 |
| $MR_F$-$MR_F$ | 0.246 | 0.000 | 0.250 | 0.000 | 0.243 | 0.000 | 0.232 | 0.000 |
| $MR_F$-$LR_F$ | 0.067 | 0.000 | 0.068 | 0.000 | 0.067 | 0.000 | 0.066 | 0.000 |
| $MR_F$-$S_H$ | 0.000 | | 0.000 | | 0.000 | | 0.000 | |
| $LR_F$-$S_F$ | 0.003 | | 0.014 | | 0.004 | | 0.019 | |
| $LR_F$-$MR_F$ | 0.064 | 0.000 | 0.053 | 0.000 | 0.063 | 0.000 | 0.047 | 0.000 |
| $LR_F$-$LR_F$ | 0.242 | 0.000 | 0.199 | 0.000 | 0.240 | 0.000 | 0.185 | 0.000 |
| $LR_F$-$S_H$ | 0.000 | | 0.000 | | 0.000 | | 0.000 | |
| $S_H$-$J_H$ | 0.000 | 0.020 | 0.000 | 0.047 | 0.000 | 0.027 | 0.000 | 0.056 |
| $J_H$-$J_H$ | 0.000 | 0.073 | 0.000 | 0.129 | 0.000 | 0.095 | 0.000 | 0.144 |
| $J_H$-$SR_H$ | 0.000 | 0.020 | 0.000 | 0.047 | 0.000 | 0.027 | 0.000 | 0.056 |
| $SR_H$-$S_F$ | | 0.000 | | 0.000 | | 0.000 | | 0.000 |
| $SR_H$-$S_H$ | | 0.009 | | 0.012 | | 0.012 | | 0.015 |
| $SR_H$-$SR_H$ | 0.000 | 0.262 | 0.000 | 0.146 | 0.000 | 0.264 | 0.000 | 0.145 |
| $SR_H$-$MR_H$ | 0.000 | 0.053 | 0.000 | 0.085 | 0.000 | 0.054 | 0.000 | 0.086 |
| $MR_H$-$S_F$ | | 0.000 | | 0.000 | | 0.000 | | 0.000 |
| $MR_H$-$S_H$ | | 0.007 | | 0.018 | | 0.009 | | 0.020 |
| $MR_H$-$SR_H$ | 0.000 | 0.042 | 0.000 | 0.051 | 0.000 | 0.039 | 0.000 | 0.045 |
| $MR_H$-$MR_H$ | 0.000 | 0.275 | 0.000 | 0.259 | 0.000 | 0.254 | 0.000 | 0.235 |
| $MR_H$-$LR_H$ | 0.000 | 0.043 | 0.000 | 0.045 | 0.000 | 0.041 | 0.000 | 0.045 |
| $LR_H$-$S_F$ | | 0.000 | | 0.000 | | 0.000 | | 0.000 |
| $LR_H$-$S_H$ | | 0.003 | | 0.017 | | 0.006 | | 0.021 |
| $LR_H$-$MR_H$ | 0.000 | 0.040 | 0.000 | 0.028 | 0.000 | 0.035 | 0.000 | 0.024 |
| $LR_H$-$LR_H$ | 0.000 | 0.152 | 0.000 | 0.118 | 0.000 | 0.138 | 0.000 | 0.109 |