

Effects of multi-species interactions in gynodioecious populations of *Polemonium foliosissimum*.

Abstract

Gynodioecy is a dimorphic breeding system in which male sterile individuals coexist with hermaphroditic individuals in the same populations. In order for female plants to be maintained within these populations, considering that they do not achieve fitness through male function, they must have a fitness advantage through female function. We looked at mutualists such as pollinators and antagonists such as pollen thieves that steal pollen without performing pollination service and in gynodioecious species could leave hermaphrodites functionally female. Although pollen theft is highly understudied, it could be enormously important in influencing pollen limitation. At this time all results are very preliminary and need further analysis, but we hope to gain understanding into how interactions with other species might provide trade-offs for females and hermaphrodites in terms of attracting both mutualists and enemies. To do this we asked if floral visitors showed a preference for hermaphrodites or females, and if *P. foliosissimum* is pollen limited all within the context of different sex ratios. Preliminary results suggest significant effects of site on pollen production as well as abortion rates on fruit set.

Introduction

Gynodioecy is a dimorphic breeding system in which male sterile individuals coexist with hermaphroditic individuals in the same populations. In order for female plants to be maintained within these populations, considering that they do not achieve fitness through male function, there must be some benefit that allows for their selection. For example, Asikainen and Mutikainen (2005) found that female plants produce a significantly higher seed set than hermaphroditic counterparts (also see Shykoff et al. 2003). In some species females also suffer less seed predation (e.g., Marshall and Ganders 2001). Such differences between sex morphs could explain the perpetuation of females and highlights the fact that interactions with mutualists or antagonists may affect the sexes differently.

Because females do not produce pollen it might be expected that female plants would put more energy into floral display; however, this is not always the case. Galen and Stanton (1989) have shown that floral display is more important to male than female function which may lead to larger more showy flowers in hermaphroditic plants. In some species pollinators preferentially visit larger flowers of hermaphrodites (Asikainen and Mutikainen 2005) perhaps due to the larger flowers or pollen rewards they offer. However, it is also possible that herbivores may also be choosing plants with showier flowers and higher rewards (Knight et al. 2008). In addition hermaphroditic plants, by virtue of producing pollen, incur risk that male function is reduced by pollen thieves.

Pollen thieves steal pollen without performing pollination service, and in gynodioecious species could leave hermaphrodites functionally female. Although pollen theft is highly understudied, it could be enormously important in influencing pollen limitation. Furthermore, pollen theft has been shown to reduce overall fitness at not only the plant level but at the population level as well. Plants have many ways of responding to pollen theft. Flower bearing plants reproduce sexually and therefore undergo genetic diversification generationally through out-crossing of gametes. Pollen theft could select for

greater pollen production but some think it could also promote gynodioecy. (Hargreaves et al. 2009).

Here, I examined if floral visitors showed a preference for hermaphrodites or females through frequency of visits per plant, time spent visiting each plant, and time spent manipulating each flower. I also considered pollinators and pollen thieves within populations of *P foliosissimum* that are of high sex ratio and low sex ratio respectively to determine if my study plants were pollen limited and whether this is exacerbated by theft and or a low fraction of hermaphrodites in populations. Without the effect of pollen thieves, pollen limitation would be higher in populations with more female plants. However, if pollen thieves target populations with more hermaphrodites, plants could conceivably be more pollen limited in populations with more hermaphrodites.

Ashman et al (2004) showed that clipper weevil damage was significantly reduced in female plants of the gynodioecious strawberry that had smaller floral displays and less pollen production. Ashman also suggests in her 2002 paper that these interactions may have multiple effects within the system of interaction and could have a great deal of interdependency with one another. In order for us to be able to fully understand how any particular plant is affected by an antagonist or a mutualist we must not only understand how these organisms affect the plant, but we must also understand how they affect each other when present or absent.

The goal of this study was to determine if floral visitors showed a preference for female or hermaphroditic plants, and if *P foliosissimum* is pollen limited in the context of populations with different sex ratios.

Methods

Study Species

Polemonium foliosissimum is an herbaceous, iteroparous perennial that produces blue/purple, campanulate flowers on numerous flowering stalks, and they exist within gynodioecious populations (D Henrie, personal observations). It is primarily pollinated by two species of *Bombus* (*B. flavifrons*, and *B. bifarius*; Zimmerman et al. 1980) with a variety of other small pollinators such as flies and solitary bees. Hermaphroditic variants of *P. foliosissimum* also generally have larger flowers than their female counterparts (Brody unpublished).

Experimental Design

To examine the extent of pollen limitation in females and hermaphrodites and evaluate how both sex ratio and rate of pollen thieving influence it, we chose four *P foliosissimum* populations, 2 in Gothic (Willey stand pipe (WSP), and dining hall (DH)) and 2 at Kebler pass (road 1 and road 3). At each site we had one low (Road 1 had 14% female, and DH had 0% female) and one high sex ratio population (Road 3 had 30% and WSP had 64%). We randomly chose 20 females and 20 hermaphrodites at Willie stand pipe, and 30 hermaphrodites at our dining hall populations in Gothic. We then chose 14 hermaphrodites and 12 females at road 3 as well as 16 hermaphrodites and 10 females at road 1 in Kebler pass. At each site, plants were randomly assigned to either a pollen addition or control treatment.

On pollen addition plants, I applied pollen to all open flowers on 1-3 stalks depending on the size of the plant. In addition, 1-3 stalks were also marked on both pollen addition and control plants to which no pollen was added. Untreated stalks on pollen addition plants will allow us to examine whether there is within plant redistribution of resources in response to our pollen addition treatments. Control plants received mock treatments to mimic the handling done to hand pollinated plants.

To examine if populations experience different visitation rates by both pollinators and pollen thieves, and to assess if these correspond to levels of pollen limitation, I conducted observations of

floral visitors. We then coupled our observational and experimental data in order to discover whether or not pollen theft and pollen limitation share a common link. At each population, I observed plants for a total of 1 hour per day periodically throughout the week and at varying times of day in order to gain an understanding of visitation rates. To do this, I walked throughout the population recording all floral visitors, including potential pollinators and pollen thieves. For bumblebees, Once a visitor was observed I followed it, as long as possible, and recorded the sequence of plants visited, their gender, and the approximate size of their floral display. I also noted the number of flowers visited per plant and the duration of the visit to each plant. I also conducted observations of individual focal plants at the Kebler sites to gain data concerning floral damage as well as beetle visitation. To do this, I spent 2 minutes at each plant and recorded all open flowers, and the number of flowers on each plant with beetle damage.

In addition to conducting observations, we used pollen removal rates as indices of visitation by pollinators and removal by pollen thieves. To do so, we collected anthers from 10 additional hermaphrodites per population, separate from those used for the pollen limitation experiment. During three consecutive weeks, we collected anthers from 3 flowers per plant prior to anthesis (“pre-opening” flowers). On the same day that we removed anthers from these flowers, We marked three flowers at a similar stage. To assess pollen loss, we collected anthers from these marked flowers 48 hours later. We dried anthers in open microcentrifuge tubes prior to counting pollen. To count pollen, we added 5 ml of 95% ethanol to which a small amount of fuschin dye had been added to stain the pollen grains. We then sonicated the anthers to remove pollen grains as well homogenize the sample. I estimated the amount of pollen in the sample using a hemocytometer (Kearns and Inouye 1993).

Results

There were no significant differences in any measures of visitation rate or efficiency to plants of different genders (Table 2). There was no difference in duration of visits to plants ($F=0.668$, $p = 0.554$), average duration of visits to flowers (handling time; $F= 0.023$, $df = 2$, $p =0.997$), or percent of open flowers visited on a plant between females and hermaphrodites ($F= 0.689$, $df=2$, $p = 0.504$). Sample sizes were not large enough to permit comparisons among treatments or sites. I also tested whether pollinators showed a preference either gender, once sex ratios are accounted for (Table 3; sample sizes permitted comparison only at two sites). Visitors do not visit the genders at a different frequency than would be expected from their frequencies in the populations at either Road 3 ($\chi^2= 0.036$, $df = 1$, $p = 0.85$) or Willey Stand Pipe ($\chi^2= 1.731$, $df = 1$, $p = 0.188$), however there is a trend toward females being visited more frequently than expected at the latter.

There was a significant effect of site on pollen loss from stamens as well as on pre-opening stamen pollen loads (Table 4). Pollen loss was significantly greater at both sites at Kebler Pass (Road 1 and Road 3 populations) than sites at the RMBL (DH and WSP; $F= 4.89$, $df = 3$, $p =0.006$). However, initial pollen loads on stigmas was also significantly higher at the Kebler Pass populations than the RMBL populations ($F= 5.946$, $df = 3$, $p =0.001$).

Discussion

Our visitation data showed no significant difference between frequency of visits per plant, time spent per plant, or time spent manipulating flowers. There was also no difference between site or gender which is counter intuitive to what (Shykoff et al. 2003) suggested. Shykoff's study showed that female plants had larger floral displays and thus experienced higher fecundity. Our sample sizes were too small to tease apart pollinator and antagonists and may also be responsible for some of these results. We did, however, show that there was a significant effect of site, not gender, when anther removal data was analyzed. Our Kebler sites produced far more pollen initially than did those of our Gothic sites. Yu Ming-Wu (2010) showed that nitrogen and phosphorous treatments had no effect on pollen production, but did increase plant size and floral traits. It is not clear yet whether or not nutrients played a significant role in this study.

Seed set data is still being collected and will be analyzed as soon as possible. From this we hope to ascertain whether plants at different sites are pollen limited as well as if they are re-directing resources within the plant by looking at control stalk on control plants and comparing them to control stalks on hand pollinated plants. We are also still processing anther collections which we hope will continue a trend of difference as sample sizes increase.

This study was integral to understanding multi-species interactions of gynodioecious species in that we considered all potential interactions and their effects at the plant level. To further this study I would like to consider soil nutrient levels at each population to see if that could be a potential factor in pollen production. I would also like to identify and study all species involved to better understand how they affect not only the plant, but each other as well.

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Tables and Figures

Table 1. ANOVA results from tests of three metrics of visitation, testing for the differences between genders. Data were pooled across treatments and sites due to small sample sizes.

variable	source of variation	df	MS	F	p
Duration of visits to plants (sec)	gender	2	1276.345468	0.668	0.514
Duration of visits to flowers (visits/ second)	gender	2	0.003	0.023	0.977
Percent of open flowers visited	gender	2	270.007	0.689	0.504

Table 2. Visitation frequency to hermaphrodite and female plants at two sites. Visitation rates are compared to expected frequencies (sex ratios in populations) with a Chi-square test. Sites with sufficient sample sizes were included in analysis (without expected frequencies of <5%).

Population	H	F	expected ratio (H:F)	χ^2	df	p
Rd3	22	6	22:6	0.036	1	0.85
Willey Stand Pipe	31	73	37:67	1.731	1	0.188

Table 3. ANOVA results from two tests of pollen loads on stamens, comparing across sites. The first compares pollen loss from stamens (loads from flowers prior to opening minus that found two days later). The second compares pre-opening loads (those found in anthers collected prior to flowers opening).

variable	source of variation	df	MS	F	p
pre-post pollen loads	site	3	1553.419	4.89	0.006
pre opening pollen loads	site	3	2808.661	5.946	0.001

Figure 1: Total number of flowers visited by gender among all observations. Data are shown for each site.

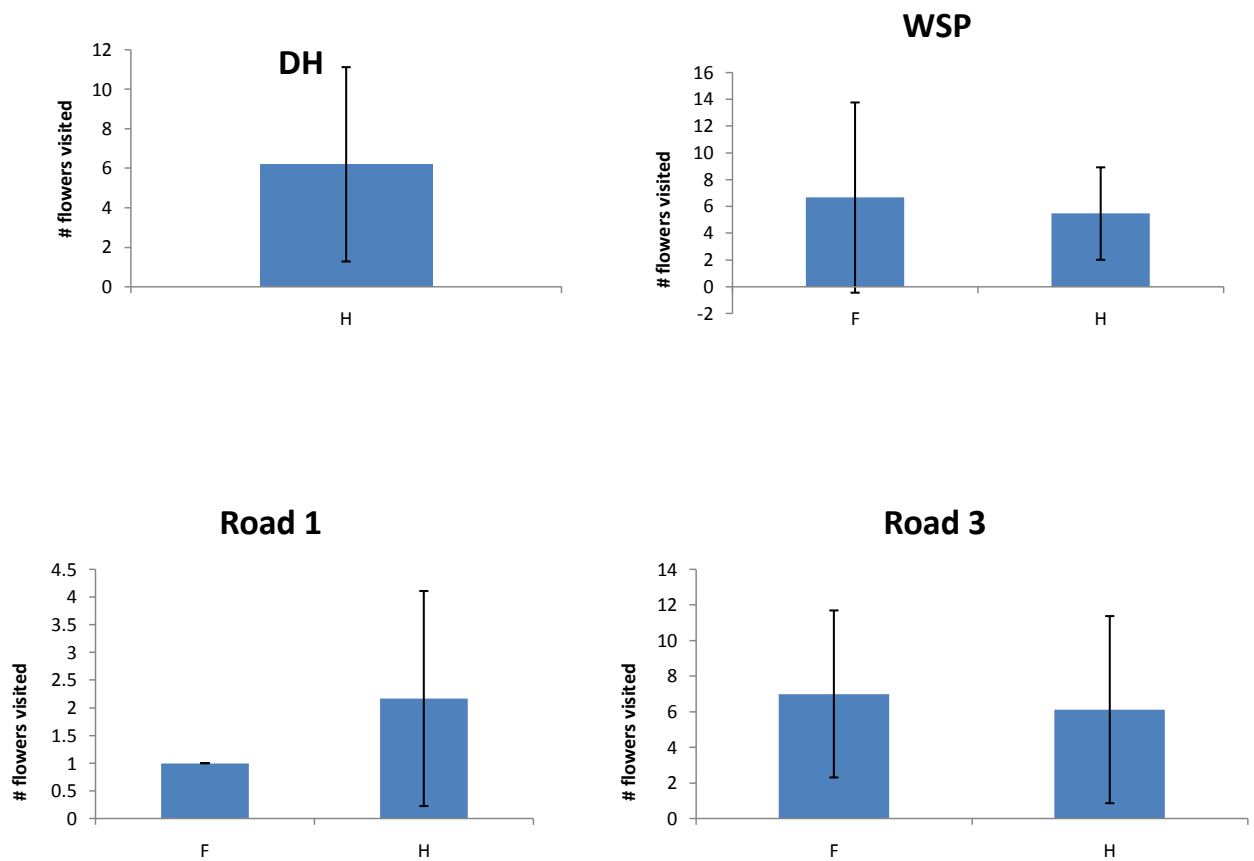


Figure 2: Describes total time spent on each flower by visitors in seconds. These data were computed by dividing the number of flowers visited on a plant by the time spent on a plant.

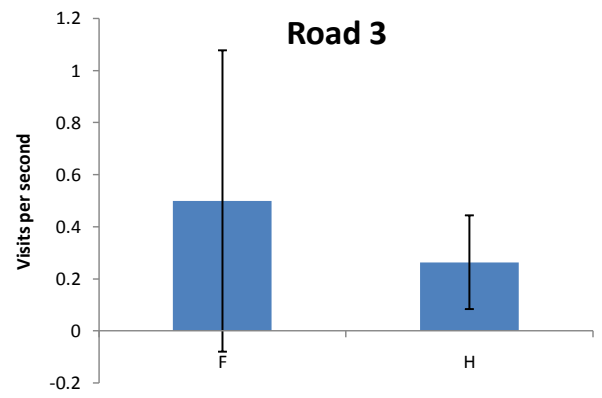
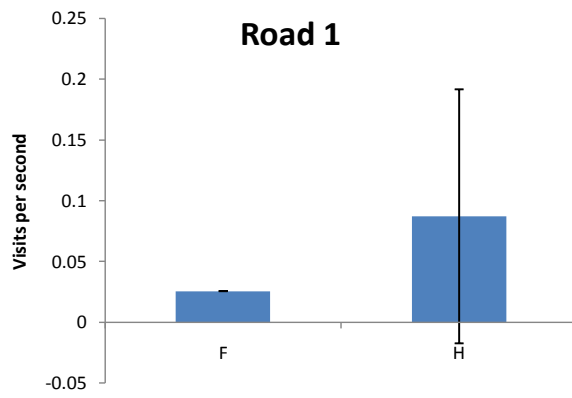
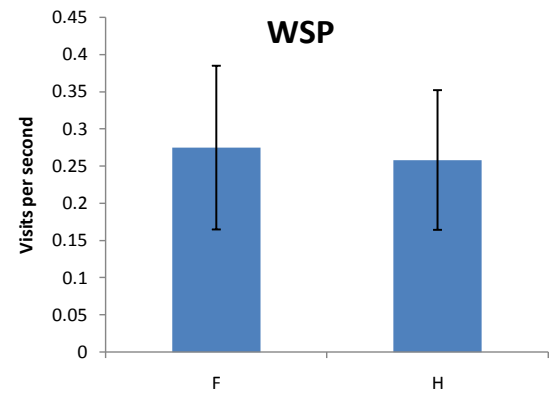
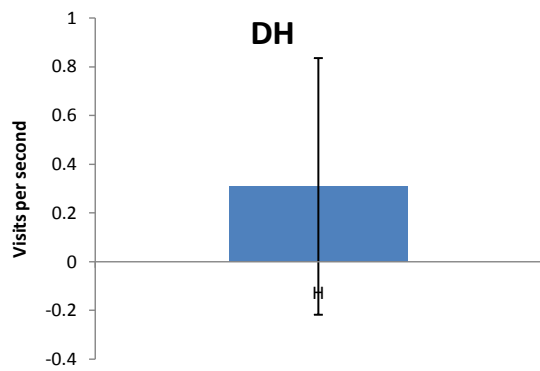


Figure 3: Describes the time spent on each individual plant by pollinators and thieves in seconds.

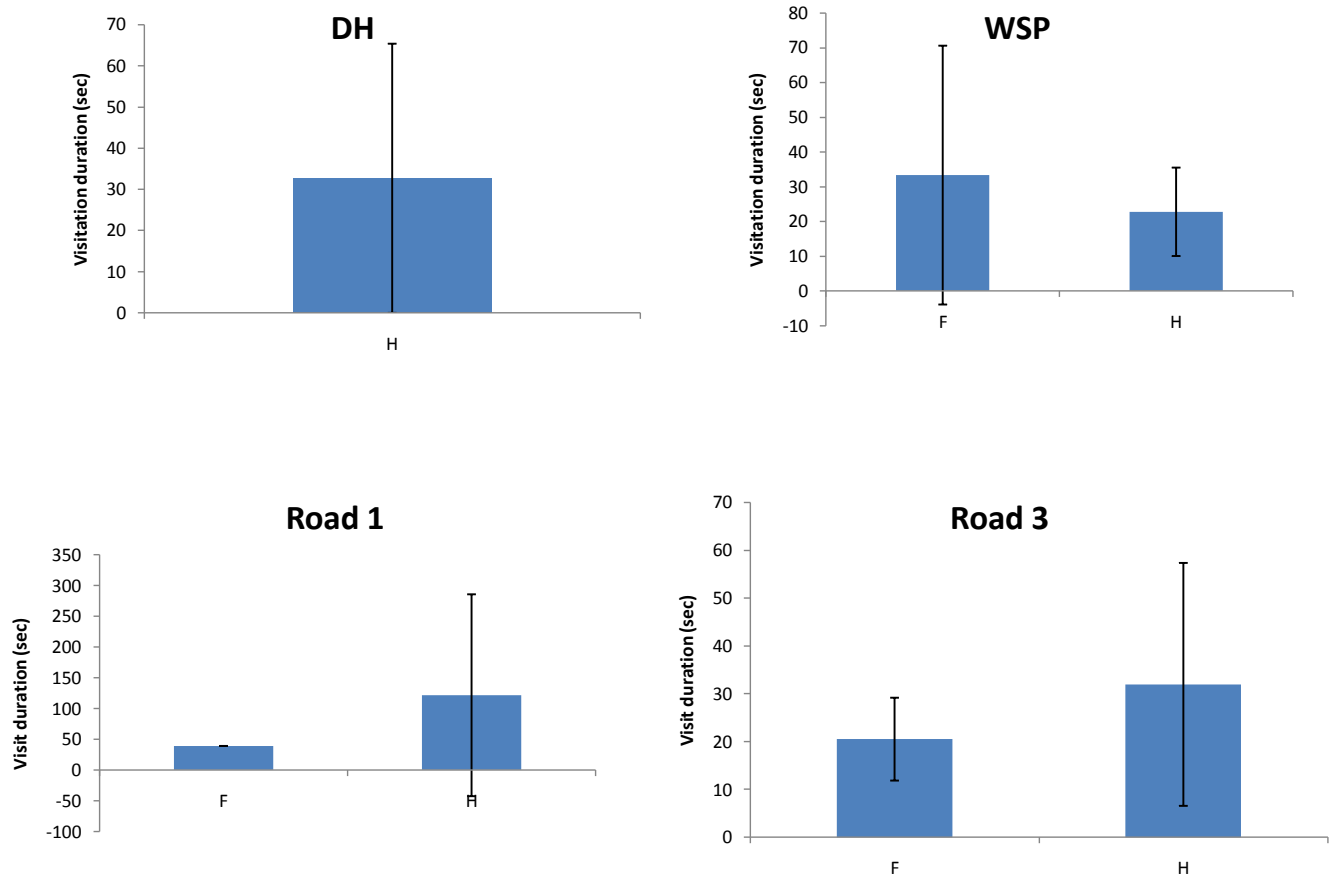


Figure 4: Shows initial pollen loads from pre-opening flowers at all four sites.

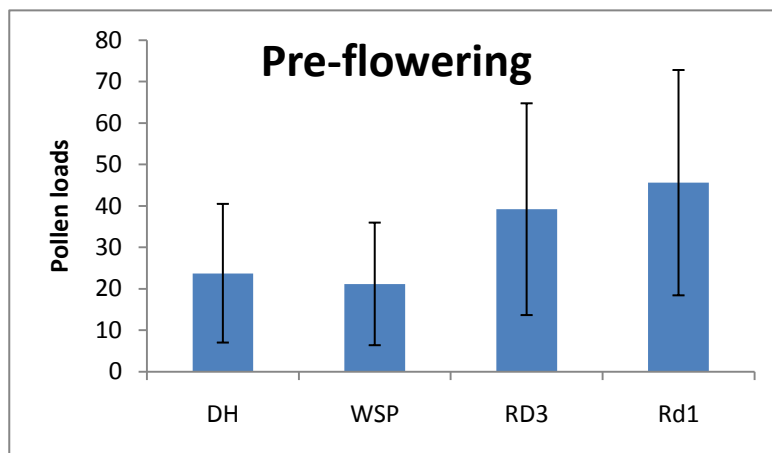


Figure 5: Shows the amount of pollen that was lost in a two day period by subtracting the post pollen counts from the pre pollen counts.

