

RMBL

Testing the Oviposition Cues That Drive Preference of Hylemya for Different Sexes of Polemonium foliosissimum

Independent Research

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Abstract

This investigation focused on how mutualists and antagonists affect the flowers of different sexes within the same species. My study examined plant and insect interactions in *Polemonium foliosissimum* to better understand the selective pressures that influenced the evolution of gynodioecy in this plant species. My manipulative experiment and observations were designed to determine (1) if the pre-dispersal seed predator *Hylemya* chooses floral cues such as flower size and presence of pollen in selecting a plant to lay eggs on and (2) if *Hylemya* chooses the same floral cues as pollinators in plant selection. I found that plant sex and floral traits seemed to not be a factor in plant selection by these insects, but a trend in un-manipulated plant selection indicated that they preferred visiting undamaged plants. The results of this study imply that other mechanisms such as plant defense or perhaps plant appearance may influence *Hylemya* and pollinator preference.

Key Words: *Polemonium foliosissimum*; Gynodioecy; pre-dispersal seed predator; pollinator; *Hylemya*.

Introduction

It has been argued that not only abiotic factors, but plant and animal interactions have had an impact on the evolution of Hermaphroditism to Dioecy through Gynodioecy in plants (Ashman 2002). Gynodioecy describes a mating system of some plant species in which some individuals are hermaphrodites and others are male-sterile and function only as females (Ashman 2006). Considering that most plants are hermaphrodites and females give up male function, the environmental influences that maintain females are not well understood.

Although many plant and insect interactions can be mutualistic, as apparent in pollinators, they can also be hostile as apparent in pre-dispersal seed predators. These interactions may have resulted in selective pressures that influenced plant traits (Brody 1997). Hermaphrodites in many gynodioecious species have larger flowers than the female plants (Ashman 2006). A large floral display enables the plant to take full advantage of the male function. It is positively correlated with pollen output, which in turn increases the likelihood of pollinators receiving pollen (Galen and Stanton 1989), but can be a hindrance to the plant if it attracts herbivores and pre-dispersal seed predators (Marshall and Ganders 2001).

In addition, for self-pollinating plants, hermaphrodites can suffer from inbreeding depression while females avoid this problem (Ashman 2006). Since female plants do not achieve any fitness through male function, it seems that most resources are allocated to female parts to produce more or higher quality seeds than hermaphrodites (Shykoff et al. 2003). However, seed predators may benefit from the high yield of quality seeds in females that feed their larvae. If seed predators could be choosy, we might expect them to oviposit on female plants.

The plant being studied that displays gynodioecy is *Polemonium foliosissimum* (Jacob's Ladder). This plant is a perennial, non-woody herb present in montane and subalpine regions (Brody and Waser 1995). *P. foliosissimum* is pollinated by generalist bumblebees while its fruits suffer from attacks by the pre-dispersal fly seed predator *Hylemya* sp. (Diptera). *Hylemya* preferentially oviposits on hermaphrodites and this might be because *Hylemya* larvae survival is higher on plants with larger flowers (Zimmerman and Brody 1998).

The goal of my work was to determine if pre-dispersal seed predators use cues that are the same or correlated with those used by mutualist pollinators in selecting a site to lay their eggs. More specifically, I addressed the following questions 1) Does *Hylemya* prefer to oviposit in hermaphrodites or females? Does this put either the hermaphrodites or females at greater risk to seed predation? 2) Does *Hylemya* use the same floral cues used by pollinators in determining where to lay its eggs?

Methods

Study Species and Study Site

Polemonium foliosissimum is a perennial, non-woody herb from the family Polemoniaceae. It is commonly found in montane and sub-alpine regions. The study took place at Rock Mountain Biological Laboratory in Gothic, Colorado from July 4 to July 28, 2010. I selected a plot of a gynodioecious population of *Polemonium foliosissimum*.

Experimental Design and Set-up

To test for preference of *Hylemya* between hermaphrodite and female plants, I conducted two separate 2-way factorial experiments (Table 1 & 2). I haphazardly selected ten plants of each sex (hermaphrodite and female) to be the control group, in which they were not manipulated in any way, and ten other plants for each gender were manipulated. For the manipulated hermaphrodite plants, I chose three stalks to be experimental and three other stalks to be the sham or in-plant control for any response *Hylemya* might have towards flower damage. I trimmed the flower petals and removed anthers from the flowers on the experimental stalks to make them appear as female flowers and nipped the flower petals on the in-plant control stalks. For female plants, I removed the rudimentary anthers and nipped the flower petals on the

experimental stalks to simulate hermaphrodite manipulation and just nipped the petals on the in-plant control stalks for damage control. Since the *Hylemya* oviposits on buds and not open flowers, this experiment tested for indirect cues that *Hylemya* chooses to oviposit. However, flower size and the presence of functional stamens are obvious differences between hermaphrodites and females and could be cues used by pollinators. Once or twice a week, I took a pre-manipulation egg census for all plants then manipulated the experimental plants and took another egg census for all plants the next day in the afternoon or early evening along with pollinator observations. I recorded oviposition rate as number of eggs per bud. To relate oviposition preference of *Hylemya* to pollinator preference, I monitored each plant for twenty minutes during observations and recorded the number of visits on flowers per twenty minutes.

I conducted a two-way analysis of variance with oviposition rate and pollinator visitation with plant sex (hermaphrodite vs. female) and treatment (experimental vs. sham control) as factors. In addition, I conducted a two-way analysis of variance for oviposition rate and pollinator visitation with plant sex and treatment (sham control vs. no manipulation) as factors. The software used for the 2-way ANOVA was SAS JMP 8 (SAS Institute, Cary, NC). The alpha level was 0.05 for all tests.

Results

Plant Gender and Experimental vs. Sham Control Treatments: Figures 1 and 2.

The effect of plant sexes:

Oviposition rate by *Hylemya* was not significantly different between hermaphrodite and female plants ($F=0.065$, $P=0.79$). Pollinator visitation also did not differ between hermaphrodite and female plants ($F=0.21$, $P=0.64$).

The effect of plant treatment:

Oviposition rate did not differ between experimental and sham control plants ($F=0.002$, $P=0.95$). Pollinator visitation also did not differ between experimental and sham control plants ($F=0.0003$, $P=0.98$).

The effect of treatment between plant sexes:

The effect of treatment on oviposition rate did not differ between hermaphrodite and female plants ($F=0.085$, $P=0.77$). The effect of treatment on pollinator visitation did not differ between hermaphrodite and female plants ($F=1.05$, $P=0.31$).

Plant Gender and Control vs. Sham Control Treatments: Figures 3 and 4, Table 3.

The effect of plant sexes:

Oviposition rate did not differ between hermaphrodite and female plants ($F=0.47$, $P=0.49$). Pollinator visitation also did not differ between hermaphrodite and female plants ($F=0.002$, $P=0.96$).

The effect of plant treatment:

There was a significant difference between oviposition rate and treatment of plants ($F=5.12$, $P=0.03$). However, there was no significant difference between pollinator visitation and treatments ($F=1.27$, $P=0.26$).

The effect of treatment between plant sexes:

The effect of treatment on oviposition rate did not differ between hermaphrodite and female plants ($F=0.41$, $P=0.52$). Treatment effect on pollinator visitation also did not differ between hermaphrodites and females ($F=0.21$, $P=0.64$).

Discussion

The purpose of this experiment was to examine which floral traits, such as petal size and presence of pollen, the pre-dispersal seed predator *Hylemya* sp. used in selecting a plant to oviposit on and if it chooses the same cues of floral traits as pollinators in plant selection. Although there seemed to be no preference in plant gender or floral traits, *Hylemya* preferred to oviposit on undamaged plants and although not statistically significant, pollinators showed the same preference as well. Previous studies have shown that *Hylemya* often oviposit on plants with high pollinator activity, but that study dealt only with hermaphrodite plants in *Polemonium foliosissimum* (Zimmerman 1980).

In the case of the experimental vs. sham control experiment, there was no significant difference in oviposition rate and pollinator visits on manipulated hermaphrodite and female plants. This indicated that the insects were not selecting plants that were damaged or handled most of the time. For the control vs. sham control experiment, I compared plants that were unmanipulated to the damage control stalks of the plants of both genders. There appeared to be a trend in higher oviposition rate and pollinator visits for control plants. Although there was no significant difference in pollinator visitation between control and sham control plants, perhaps due to a low sample size, figure 3 shows a trend of higher visitation on control plants for both genders.

This experiment did not support whether *Hylemya* preferred hermaphrodite or female plants to oviposit on or whether it chooses the same cues of floral traits as pollinators in my attempt to better understand the selective pressures that influenced gynodioecy in *Polemonium foliosissimum*. However, the trend shows that the insects were selecting un-manipulated plants regardless of gender. This could possibly be that plant damage induces a chemical defense mechanism that repels the insects or that the insects prefer the appearance of unhandled plants. In a study done on *Ipomopsis Aggregata*, a plant from the family *Polemoniaceae*, browsed plants by vertebrates seemed to decrease oviposition rate and plant damage increased plant fitness. There was also some evidence that plants that produced high amounts of Cucurbitacin, a defense chemical, had less seed predator damage (Juenger et al. 2005). These hypotheses must be addressed in order to support the results of this experiment and help determine how insect and plant interactions have shaped plant phenology of *Polemonium foliosissimum*.

Two-way factorial-Plant sex and treatments:

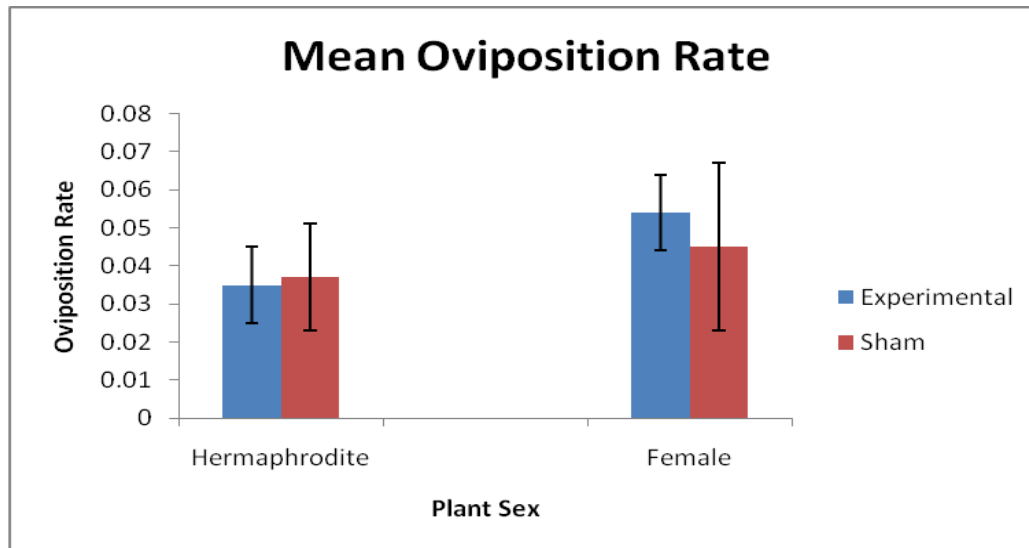
Table 1

	Hermaphrodite		Female	
Sham	Experimental	N=10		N=10
		N=10		N=10

Table 2

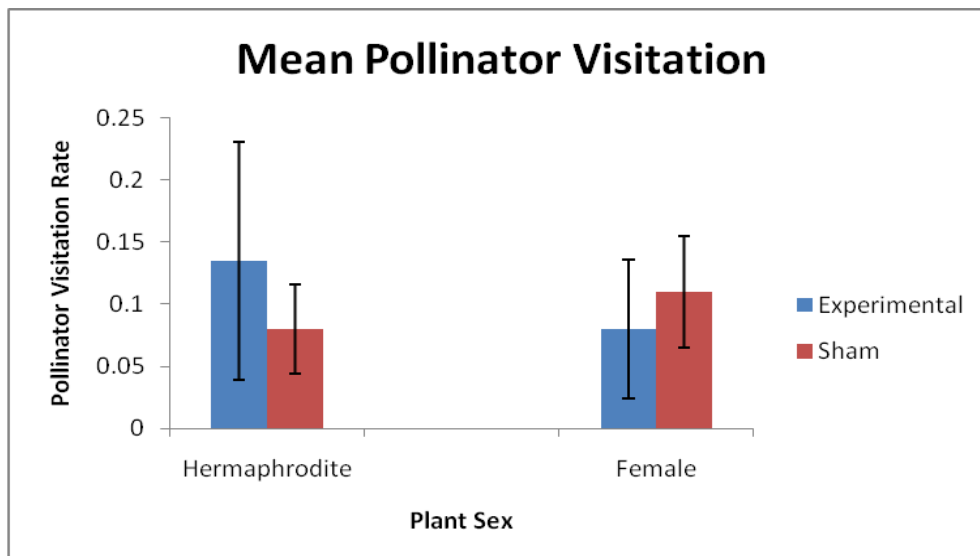
	Hermaphrodite		Female	
Sham	Control	N=10		N=10
		N=10		N=10

Figure 1



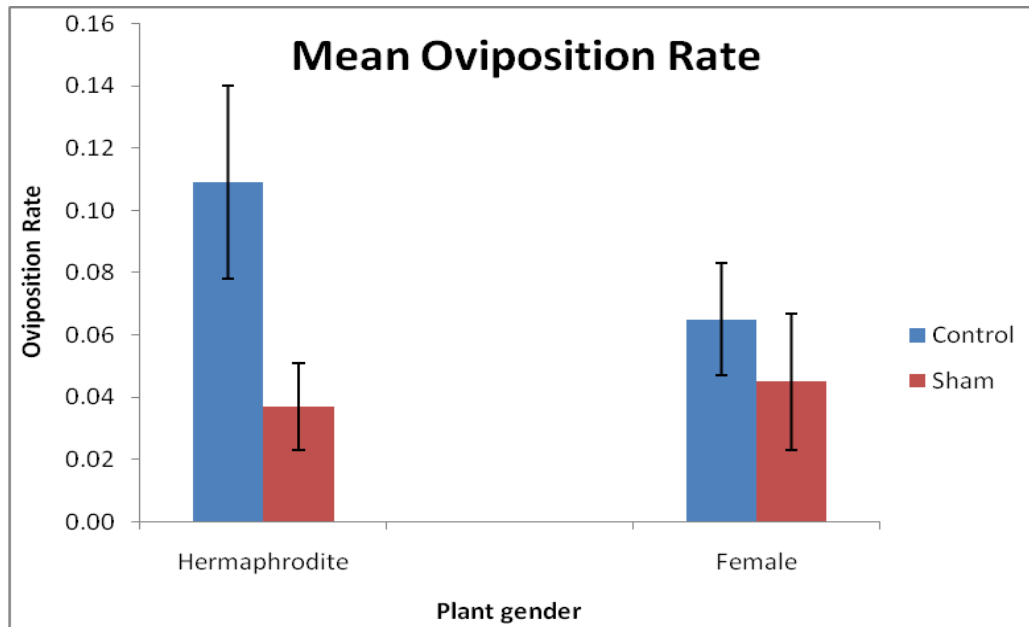
- Mean oviposition rate between hermaphrodite and female plants at two different treatments (experimental vs. sham)

Figure 2



- Mean pollinator visitation between hermaphrodite and female plants at two different treatments (experimental vs. sham).

Figure 3



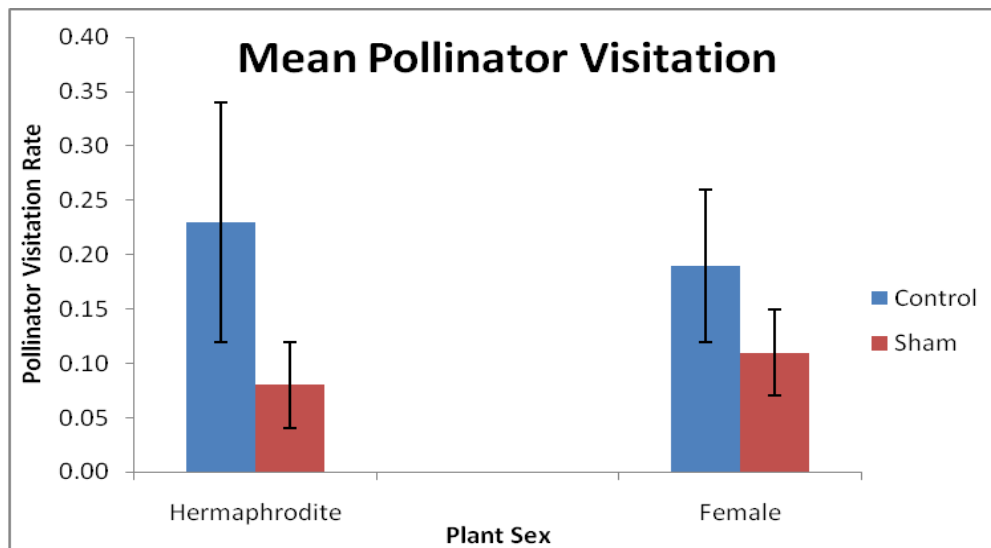
- Mean oviposition rate between hermaphrodite and female plants at two different treatments (control vs. sham).

Table 3- Mean oviposition rate ($\pm$ standard error)

Treatment	Plant Sex	
	Females	Hermaphrodites
Control	0.065($\pm$ 0.018)	0.109($\pm$ 0.031)
Sham	0.045($\pm$ 0.022)	0.037($\pm$ 0.014)

- The mean oviposition rate was higher in control plants than the sham control plants of both plant sexes.

Figure 4



- Mean pollinator visitation between hermaphrodite and female plants at two different treatments (control vs. sham).

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Literature Cited

- Ashman, T.L. 2002. The Role of Herbivores In The Evolution of Separate Sexes From Hermaphroditism. *Ecology* 83: 1175-1184.
- Ashman, T. L. 2006. The Evolution of Separate Sexes: A Focus On The Ecological Context. The Ecology and Evolution of Flowers. S. C. H. Barrett, Harder, L.D., Oxford University Press: 419-465.
- Brody Alison K., Nickolas M. Waser. 1995. Oviposition Patterns and Larval Success of A Pre-dispersal Seed Predator Attacking Two Confamilial Host Plants. *OIKOS* 74: 447-452.
- Brody Alison K. 1997. Effects of Pollinators, Herbivores, and Seed Predators On Flowering Phenology. *Ecology* 78(6): 1624-1631.
- Galen Candace, Maureen L. Stanton. 1989. Bumble Bee Pollination and Floral Morphology: Factors Influencing Pollen Dispersal In The Alpine Sky Pilot, *Polemonium Viscosum* (*Polemoniaceae*). *American Journal of Botany* 76: 419-426.
- Juenger Thomas, Timothy C. Morton, Rick E. Miller and Joy Bergelson. 2005. Scarlet Gilia Resistance To Insect Herbivory: The Effects of Early Season Browsing, Plant Apparency, and Phytochemistry On Patterns of Seed Fly Attack. *Evolutionary Ecology* 19: 79-101.
- Marshall Melanie, Fred R. Ganders. 2001. Sex-Biased Seed Predation and The Maintenance of Females In A Gynodioecious Plant. *American Journal of Botany* 88: 1437-1443.

Shykoff, J. A., S. O. Kolokotronis, Carine L. Collin and Manuela Lopez-Villavicencio. 2003.

Effects of Male Sterility On Reproductive Traits In Gynodioecious Plants: A Meta-analysis. Oecologia 135(1): 1-9.

Zimmerman, M. 1980. Reproduction in *Polemonium*: Pre-dispersal Seed Predation. *Ecology* 61(3): 502-506.

Zimmerman, M. and A. K. Brody. 1998. Choices and Consequences of Oviposition By *Hylemya* (Delia) sp. (Diptera : Anthomyiidae)." *Journal of Insect Behavior* 11(3): 371-381.