

**The effects of floral traits on the behaviors of
pollinators and pre-dispersal seed predators
in a gynodioecious species, *Polemonium
foliosissimum*.**

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Abstract

In order to maintain in the population, female plants of gynodioecious species must compensate for their loss of male function. Understanding how mutualist pollinators and antagonists such as seed predators respond to floral cues presented by female and hermaphrodite flowers in ways that could contribute to differences fitness among the sexes. In this study, I investigated the effects of floral traits on visitation rates by pollinators and pre-dispersal seed predators in a gynodioecious species, *Polemonium foliosissimum*. I manipulated the petals and anthers of hermaphrodites to make them look like females and examined visitation rates by putative pollinators and rates of oviposition by a common pre-dispersal seed predator. Results showed that pollinator preference and oviposition rate did not differ among the treatment types, which indicated the pollinator and pre-dispersal seed predator did not use the size of petal and the presence of anther as cue to locate their host plants.

Introduction:

Gynodioecy is a relatively common breeding system of angiosperms in which females (male steriles) coexist with hermaphrodites in a population (Darwin 1877, Richards 1997). Flowers of hermaphrodites produce both seed and pollen while female flowers produce only seed. Gynodioecy is thought to be an intermediate sexual system in the evolution of dioecy from hermaphroditism (Charlesworth & Charlesworth 1978, Thomson & Brunet 1990).

Theory tells us that for females to be maintained, they must compensate for their loss of male function. Female fitness advantages can be realized through reallocation of resources from producing pollen to increased seed production, or through an increase in seed quality (Lloyd 1975, Charlesworth and Charlesworth 1978). Although avoidance of inbreeding depression, leading to higher seed quality, is often cited as a primary advantage garnered by females, a recent meta-analysis of 48 gynodioecious species showed that female advantage due to reduced selfing is relatively rare (Dufay and Billard 2012). In addition, the avoidance of inbreeding cannot explain how females persist in self-incompatible gynodioecious species where both females and hermaphrodites produce outcrossed seeds.

Interactions between plants and their mutualist pollinators and antagonists such as seed predators and herbivores have received notable recent attention as means by which females are favored. Hermaphrodites sometimes have a higher rate of visitation by pollinators than females in several gynodioecious species (eg. Eckhart 1991, Williams et al. 2000, Asikainen and Mutikainen 2003). In most gynodioecious species, female flowers are significantly smaller than those of hermaphrodites and this reduced display size may affect floral visitors. Large-flowered plants often have higher visitation rates than those with smaller corollas (eg. Conner and Rush 1996, Fenster et al. 2006, Galen 1989). Additionally, pollen rich flowers receive relatively higher visitation (eg. Harder 1990, Cresswell and Robertson 1994). The larger petals and the presence of pollen on hermaphrodites, may contribute to sex-biased foraging preference of pollinators.

Floral enemies, such as pre-dispersal seed predators and florivores, may contribute to

fitness differences among females and hermaphrodites (Parachnowitsch and Caruso 2008). Empirical studies have identified a positive relationship between pollinator visitation and pre dispersal seed predation (Cariveau et al. 2004) and revealed that ovipositing females tend to prefer larger flowers (Brody 1992a, Parachnowitsch and Caruso 2008). Hermaphrodites experienced higher seed predation than females in a number of species (e.g., Brody unpublished, Marshall and Ganders 2001, Collin et al. 2002). If seeds of females are less often consumed than those of hermaphrodites, females may acquire a fitness advantage over hermaphrodites which can help explain their persistence.

The purpose of my study was to investigate the effects of floral traits on visitation rates by pollinators and pre- dispersal seed predators. My goal was to better understand how these animals respond to floral cues presented by female and hermaphrodite flowers in ways that could contribute to differences in seed set among the sexes. To examine visitor responses to floral cues, I manipulated floral traits in a gynodioecious species, *Polemonium foliosissimum*. I manipulated the petals and anthers of hermaphrodites to make them look like females and examined visitation rates by putative pollinators and rates of oviposition by a common pre-dispersal seed predator. I hypothesized that if floral visitors use morphological cues and, in particular, floral size and the presence of pollen, in foraging decisions, then stalks in which the flowers were reduced would have lower visitation rates than unmanipulated stalks.

Methods:

Study system

Polemonium foliosissimum is a long lived perennial that is native to the Rocky Mountains. Plants bloom from late June through early August. Individuals produce several to many stalks with clusters of blue, bell-shaped flowers on the top of the stalk. Some populations of *P. foliosissimum* are gynodioecious. Female plants produce smaller flowers than hermaphrodites and contain non-functional stamens. *P. foliosissimum* is self incompatible and is mainly pollinated by two species of bumblebees, *Bombus bifarius* and *Bombus flavifrons* (Zimmerman 1980a). It also experiences high level of pre-dispersal seed predation by anthomyiid fly larvae, *Hylemya* sp (Zimmerman 1980b, Brody 1997). Females lay eggs singly underneath the sepal of flowers while flowers are still in bud stage. The larvae hatch, burrow into the ovary and eat the developing seeds (Zimmerman 1980b). Each individual can lose up to 80% of their seeds to *Hylemya* (Brody 1997). *Hylemya* does not pollinate *P. foliosissimum* (Brody and Waser 1995).

Experimental design

To examine if floral visitors use the size of petals and the presence of anthers as cues in choosing flowers, I conducted an experiment in which I manipulated floral traits of hermaphrodites to mimic females. A hermaphrodite plant would be included in the study when it had four stalks with at least one open flower on each stalk. I randomly assigned individual stalks of the plant to one of the four following treatments: (i) petal reduced and anther removed; (ii) petal reduced only; (iii) nicking the petal without change their size (sham control for damage); (iv) true control in which no manipulation is performed. The

treatment of anther removed alone was not included in the experiment because large female flowers do not exist in nature. All four treatments were applied to stalks within a plant. I monitored plants every day after treatments were initiated and I treated all new blooms on experimental stalks. I began treatments on June 25 and added additional plants when they began to bloom until ending the experiment on July 26, 2012. In total thirty plants were included in this study.

To estimate the rate of oviposition on buds, I censused all elongated buds for eggs every day after I applied the treatments. On each day, I recorded the number of buds and the number of buds with eggs on each experimental stalk. Buds with eggs were marked with indelible red Sharpie and would not be censused anymore because *Hylemya* only lay egg on flowers singly. If a bud already contained an egg before the treatment was applied, I marked that bud with a black Sharpie and excluded from further consideration. I censused eggs from June 25 to July 26 for all thirty plants.

To measure the visitation rates by pollinators, I observed pollinator visits to experimental plants on all non-rainy days. Prior to visitor observations, the total numbers of opened flowers were recorded. Each plant was observed for a ten-minute period every day in the afternoon. I recorded the identity of the visitor (bumblebees, solitary bee, flies, etc.), the activities of the visitors (collecting nectar, consuming pollen, etc.), the number of flowers visited on each stalk, and the sequence of plants visited. In total, 26 plants were observed.

Statistical analyses

Effects of manipulated floral traits on visitors

To quantify if visitation differed among treatments, I used a randomized block analysis of variance. Plants were treated as blocks, with treatment stalks nested within blocks. All visitors combined, visits by all bees, visits by flies, visits by bumblebees, and visits by solitary bees were used as response variables. For each group, I analyzed the total number of times stalks were visited and the number of times each particular treatment was visited first in a given foraging sequence. For each category, I first calculated the average number of visits per day and then these were averaged over the season because stalks differed in the number of days observed. In addition, I analyzed the number of flowers on each treatment.

Effects of manipulated floral traits on pre—dispersal seed predators

To determine whether ovipositing female flies response to treatments differentially, I used a randomized block ANOVA with the percentage of eggs (arcsine square root transformed) as the response variable. The four treatments were considered as main effects and individual plants were treated as the blocking factor. The percentage of buds with eggs was averaged over all the days of each plant, due to differences in the number of census dates available for different stalks and treatments.

Results

Effects of manipulated floral traits on visitors

The total number of visits by all visitors ($F=2.3342$, $df=3$, $p > 0.0807$), all bees ($F=2.3810$, $df=3$, $p > 0.0762$), bumblebees ($F=1.1654$, $df=3$, $p > 0.3377$) and solitary bees ($F=2.3106$, $df=3$, $p > 0.0831$) did not differ among the treatment types. The average number of first visits per day by all visitors ($F=1.3420$, $df=3$, $p > 0.2672$), all bees ($F=1.0188$, $df=3$, $p > 0.3892$), bumblebees ($F=1.0087$, $df=3$, $p > 0.4012$) and solitary bees ($F=1.0821$, $df=3$, $p > 0.3619$) also did not differ significantly among treatments. Specifically there was a trend for control stalks to have a greater rate of total visitation by all visitors and solitary bees than the other treatments.

The total number of visits by flies differed significantly among the treatments ($F = 4.2642$, $df = 3$, $p > 0.0095$). The average number of first visits per day also differed significantly among treatments ($F=4.0846$, $df=3$, $p > 0.0116$).

Effects of manipulated floral traits on pre—dispersal seed predators

Oviposition preference by *Hylemya* was not significantly different between each treatment stalks ($F=1.7827$, $df=3$, $p > 0.1564$).

Discussion

In contrast to other studies (eg. Ashman 2000, Ashman et al. 2000, Brody and Mitchell 1997), we found little effect of floral traits on visitation by either bumble bees or solitary bees. However, flies were more likely to visit larger flowers first, in any given foraging bout, and visited stalks with larger flowers more often than those with smaller flowers. Although we did not individually identify these flies, nor do we know to what degree they are legitimate pollinators, most of these flies appeared to be eating pollen. Perhaps flower size is more strongly correlated with pollen production, for which these flies were foraging, than for nectar production.

Unexpectedly, pre-dispersal seed predators also showed no preference for large- or small-flowered plants as oviposition sites. Again, our results contrast with others in which *Hylemya* showed a preference for flowers with wider sepals (Zimmerman and Brody 19XX). Two possible explanations for such unexpected results might be that pollinators and pre-dispersal seed predator either have no preference for hermaphrodites and females or use other cues rather than the size of petal and the presence of anther to discriminate against females.

Pollinators did not differentially respond to treatments which may suggest that pollinators may not be an important agent of selection on floral traits and the fitness differences observed between females and hermaphrodite plants. To further examine the role of pollinators in floral trait selection, it would be useful to know if plants within these populations suffer from pollen limitation. If the populations do not suffer from pollen limitation, pollinator selectivity will not affect the fitness of females and hermaphrodites (Parachnowitsch and Caruso 2008).

The flowering plants in and around the study site were fewer than previous years (Brody, personal observation), probably due to current extreme drought in Colorado (USA). Since some pollinators are able to adjust their foraging behaviors and preferences depending on the availability of resources (Fontaine and Collin 2008), the pollinators studied here might expand their diet as a result of scarce food supply this year and thus did not distinguish between hermaphrodites and manipulated hermaphrodites. Further studies will be necessary to determine if the non selective behaviors of pollinator and pre dispersal seed predator are consistent among years and among sites

Previous studies showed that some pollinators are able to associate floral rewards with locations (Dukas and Real 1991, Keasar et al. 1996). As hermaphrodite provides greater reward, including pollen and nectar, the pollinators in this study may have learnt the location of hermaphrodite flowers before the experiment and thus did not discriminate between hermaphrodites and manipulated hermaphrodites.

The manipulations of petal size to hermaphrodites were done by cutting the tip of the petal. The petal width of manipulated hermaphrodites was still larger than females. Pollinators may use the petal width alone or petal length and width in combination to discriminate against females.

Hermaphrodites and females may differ in flower height, floral scent, floral number, ultraviolet pattern and nectar production that might contribute to hermaphrodite-biased visitation. The sexual dimorphism of my study model- *P. foliosissimum* beyond the size of petals and the presence of pollen needed to be investigated in order to further explore their effects on the foraging behavior of pollinator and in turn, how pollinator selectivity contributes to the evolution of gynodioecy.

One possible cause for *Hylemya* not discriminating between hermaphrodites and manipulated hermaphrodites would be *Hylemya* employ the same cues as pollinators in their oviposition choice and the pollinators use indications other than the length of petal and the presence of anther to locate their host plants. On the other hand, *Hylemya* females have been found to oviposit on flowers of *Ipomopsis aggregata* that have a probability of setting seed (Brody 1992b, Brody and Waser 1995, Brody and Morita 2000). If *Hylemya* does not oviposit on flowers that are preferred by pollinators, then *Hylemya* might be able to manipulate flowers in some way to ensure seed production. Brody and Morita (2000) suggested that oviposition by *Hylemya* may delay flower abscission and increase resource allocation to developing fruits. Further experiment will be necessary to determine if *Hylemya* can manipulate *P. foliosissimum* flowers and how.

Without discriminating between the two sex morphs, pre-dispersal seed predators may still have potential to play a main role in the maintenance of gynodioecy. Zimmerman and Brody (1997) found that *Hylemya* eggs oviposited on plants with significantly thinner and shorter sepals had lower survival rate because the sepal failed to protect the eggs. Hermaphrodites of *P. foliosissimum* with larger corolla may have larger sepal than those of females. Thus, hermaphrodites studied here might suffer greater damage than females

even though they experienced a similar level of oviposition rate. Evaluating egg mortality, sepal morphology and net fruit set of sex morphs in *P. foliosissimum* can help to determine whether *Hylemya* have different effects on hermaphrodites and females fitness by reducing hermaphrodite's seed production.

Our results also showed that the difference between individual plants had significantly greater effects than treatments on the foraging behavior of visitor and oviposition preference of *Hylema*. These results indicated that performing different treatments to stalks within one plant is a better experimental design than assigning treatments to each plant to reduce differences that can be attributed to between-plant variation. To minimize the plant effects in the future experimental study, I suggest collecting seeds from five to six hermaphrodite plants from the same population this year and germinating them for the study next year so that the focal plants will be similar to each another as much as possible because they are siblings.

The purpose of this study is to investigate whether unmanipulated hermaphrodites and manipulated hermaphrodites (mimic female) differ in attractiveness to pollinator and pre-dispersal seed predators. Pollinators and pre-dispersal seed predators did not appear to use the size of petal and the presence of anther as cues to locate their host.

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Appendix

Table 1. Mean $\pm$ std error of first visits to flowers on stalks in each treatment.

Visitor	Petals reduced, anthers removed	Petals reduced	Petal nicked, sham control	Control	F; P
All	2.60 $\pm$ 0.29	2.57 $\pm$ 0.27	3.13 $\pm$ 0.33	3.88 $\pm$ 0.32	1.3420; 0.2672
Bees	2.52 $\pm$ 0.29	2.47 $\pm$ 0.26	2.90 $\pm$ 0.31	3.63 $\pm$ 0.31	1.0188; 0.3892
Bumblebees	1.23 $\pm$ 0.29	0.77 $\pm$ 0.17	0.90 $\pm$ 0.19	1.68 $\pm$ 0.28	1.0087; 0.4012
Solitary bees	2.04 $\pm$ 0.23	2.18 $\pm$ 0.27	2.53 $\pm$ 0.27	3.00 $\pm$ 0.31	1.0821; 0.3619
Flies	0.13 $\pm$ 0.08	0.36 $\pm$ 0.10	0.60 $\pm$ 0.12	0.80 $\pm$ 0.16	4.0846; 0.0116

Table 2. Mean $\pm$ std error of total visits to flowers on stalks in each treatment.

Visitor	Petals reduced, anthers removed	Petals reduced	Petal nicked, sham control	Control	F; P
All	3.94 $\pm$ 0.39	3.99 $\pm$ 0.38	5.05 $\pm$ 0.46	5.70 $\pm$ 0.41	2.3342; 0.0807
Bees	3.84 $\pm$ 0.38	3.92 $\pm$ 0.37	4.78 $\pm$ 0.45	5.46 $\pm$ 0.40	2.3810; 0.0762
Bumblebees	1.95 $\pm$ 0.36	2.05 $\pm$ 0.39	1.82 $\pm$ 0.32	2.65 $\pm$ 0.37	1.1654; 0.3377
Solitary bees	3.10 $\pm$ 0.32	3.10 $\pm$ 0.32	4.08 $\pm$ 0.44	4.46 $\pm$ 0.36	2.3106; 0.0831
Flies	0.13 $\pm$ 0.08	0.38 $\pm$ 0.10	0.62 $\pm$ 0.16	0.82 $\pm$ 0.16	4.2642; 0.0095

Table 3. Mean $\pm$ std error of ovipositon rate by Hylemyain each treatment

Petals reduced, anthers removed	Petals reduced	Petal nicked, sham control	Control	F; P
0.71 $\pm$ 0.03	0.69 $\pm$ 0.03	0.75 $\pm$ 0.03	0.78 $\pm$ 0.03	1.7827; 0.1564

Figure 1. The number of times each stalk visited first per day

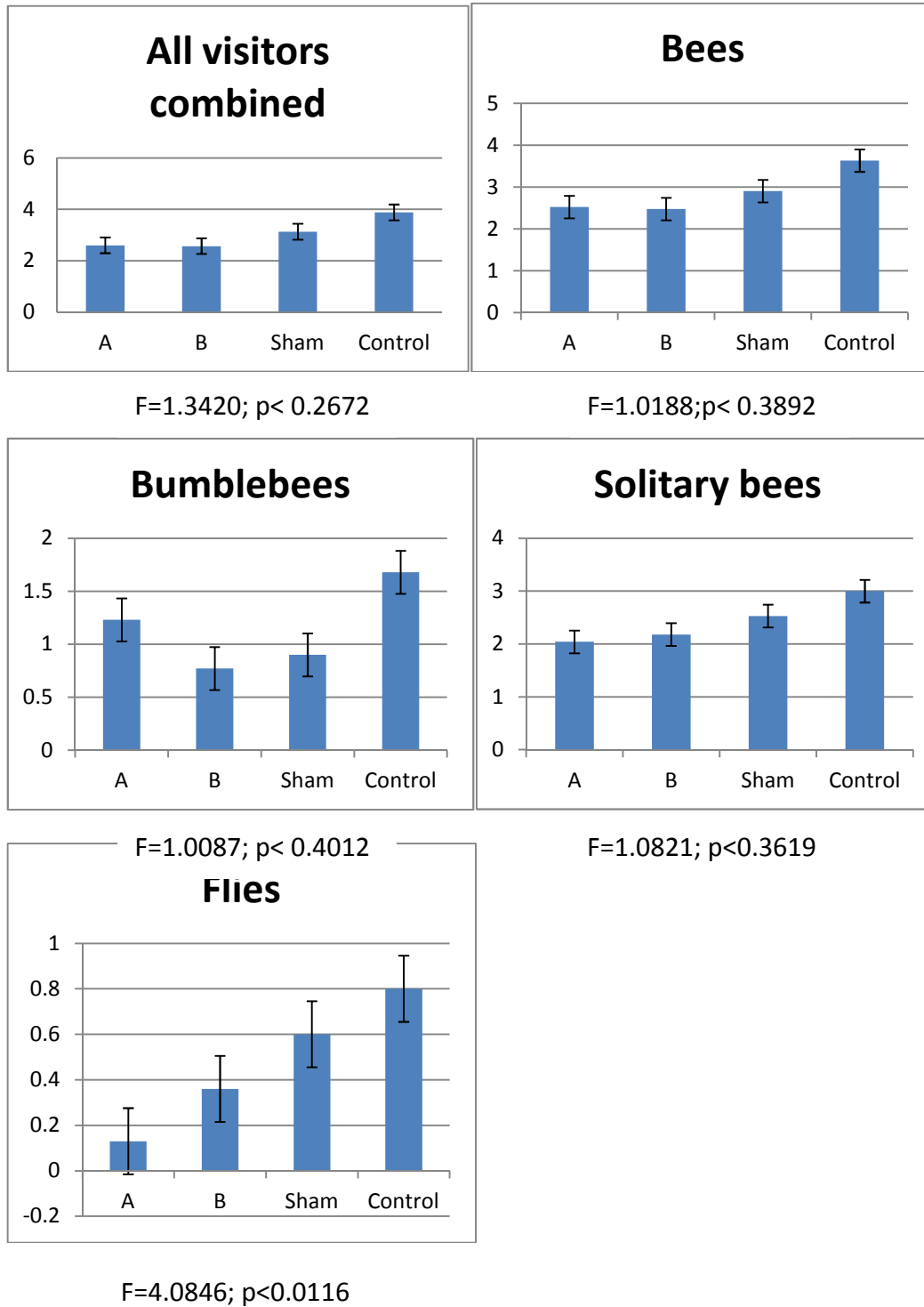


Figure 2. The total number of visits

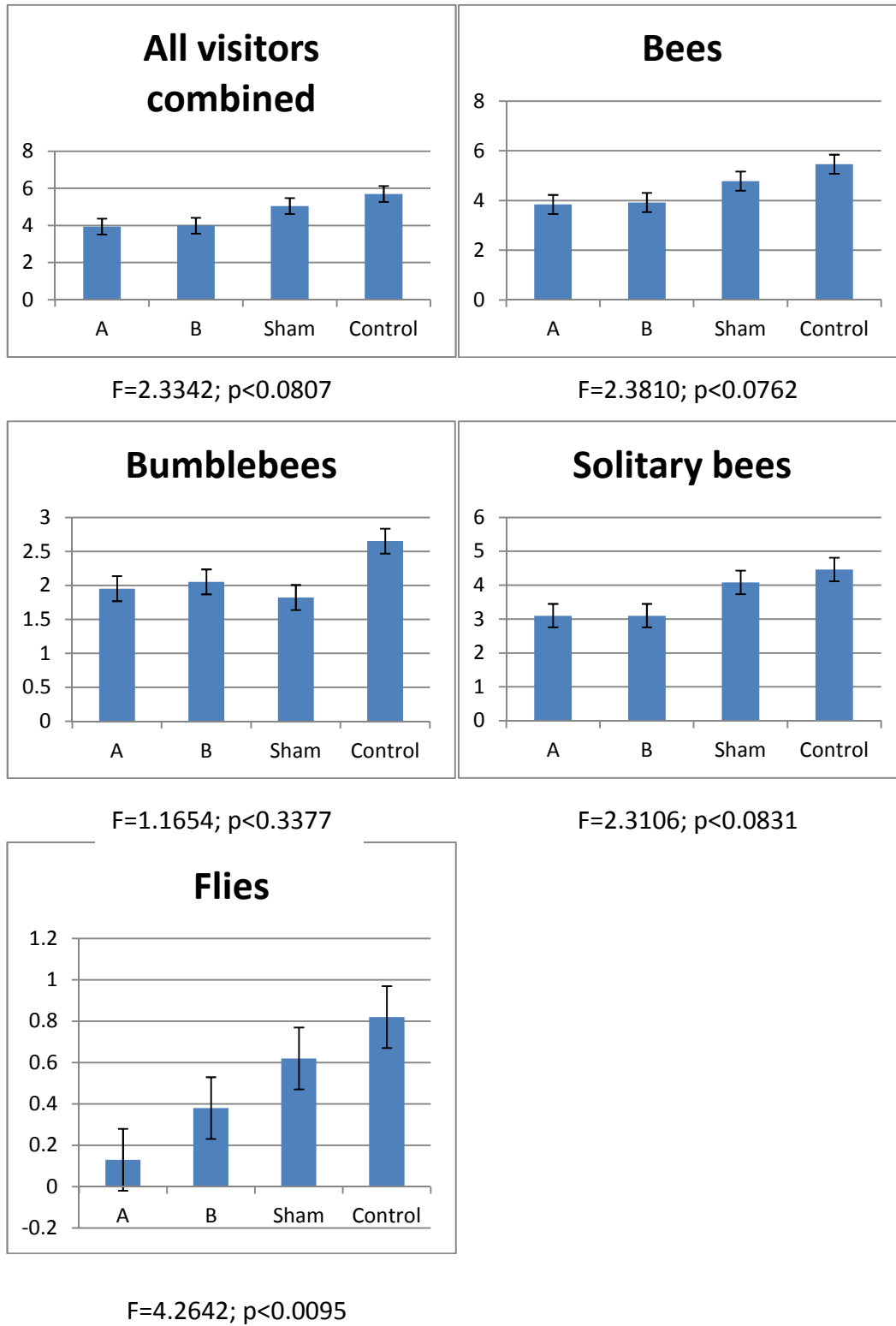
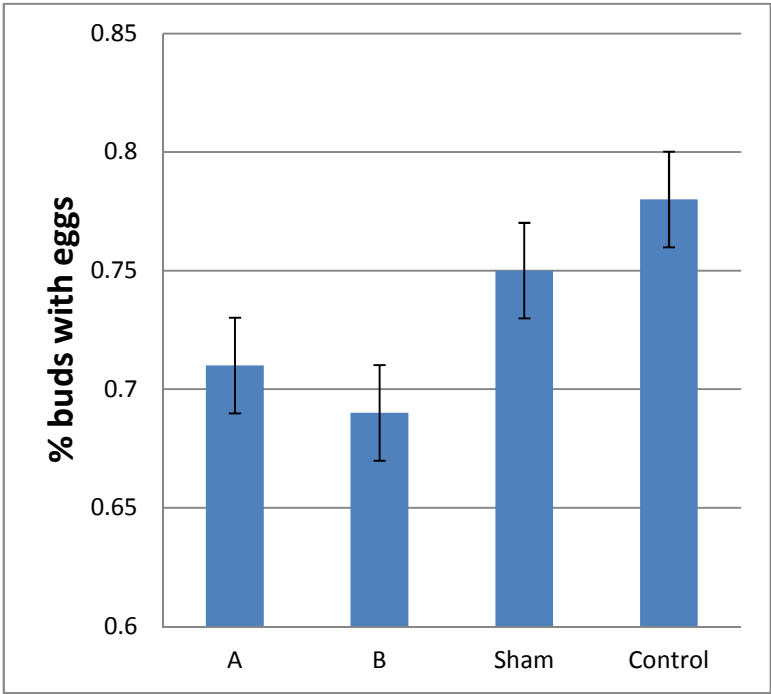


Figure 3. Oviposition preferences by *Hylemya*



F=1.7827; p<0.1564