

A study of correlational selection through floral manipulations of *Polemonium foliosissimum* (Polemoniaceae)

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Abstract: In this project I studied correlational selection upon *Polemonium foliosissimum* by observing insect response to floral manipulations. I independently manipulated color and shape to examine whether bee and fly response would comply with traditional pollination syndromes, which predict that bees will prefer tubular, purple flowers and that flies will prefer open, white flowers. Bees strongly preferred purple flowers to white flowers, whereas flies showed no detectable color preference. Both bees and flies preferred open and control flowers to tubular flowers. I then simultaneously manipulated color and shape to test for non-additive effects but was unable to detect correlational selection, as the effects of color and shape on insect visitation were not dependent upon each other.

Keywords: correlational selection, *Polemonium foliosissimum*, Polemoniaceae, floral manipulations, floral color, floral shape

Introduction:

Pollinators are incredibly diverse in morphology, sensory capacity and behavior and this diversity is thought to allow selection for a variety of floral traits. Flowers may attract animal pollinators through their shape, size, color, fragrance and edible rewards, and because pollinators can be limiting, flowers may target specific pollinators or groups of pollinators as a means of reducing competition for pollination (Mitchell et al. 2009; Zimmerman 1980). Often, specific visitors are attracted by multiple floral traits. For instance, rather than being simply red or sweet-smelling, a flower may be both red and sweet-smelling, which may increase floral attractiveness to a particular pollinator or set of pollinators. The theory of “pollination syndromes” proposes that certain linked suites of floral characteristics tend to attract a certain set of functional visitors (van der Pijl 1960-1961 reviewed by Baker & Hurd 1968). For instance, flowers that are yellow or purple, radially symmetrical, and tubular often attract bees. Flowers pollinated by flies are often white and open in shape, and may have a rancid odor reminiscent of decomposing meat (Baker & Hurd 1968).

Pollination syndromes are comprised of floral traits that correspond both with sensory abilities of pollinators and logistics of pollination mechanics. For example, flowers with deep tubes tend to be visited by pollinators with long tongues or beaks able to reach the nectar reward below (Benitez-Vieyra et al. 2006; Baker & Hurd 1968). However, complementary plant-pollinator fit is not enough to guarantee a successful pollination event. For instance, even if a bee is physically capable of pollinating a flower with a deep tube and a large nectar reward, if the flower is novel to that individual and is patterned in such a way that the individual is not able to distinguish it from background vegetation, the bee might not choose to visit (Martinez-Harms et al. 2010). However, through associative learning some pollinators are able to develop search images for flowers not typically part of their innate preference suite (Ings et al. 2009). Because floral communities may change greatly over the course of the flowering season, pollinator preferences may also change throughout a season (Internicola et al. 2009). Thus, pollinator response to floral availability dictates that the theory of pollination syndromes must be cautiously applied. Pollination syndromes are a widely accepted hypothesis---not a set of strict guidelines---and have been criticized because many pollinators are generalists and may visit flowers considered to be outside their pollination syndrome (Waser et al. 1996). Further research is required to examine how applicable pollination syndromes actually are to pollinator preferences.

Many studies have examined the importance of specific traits within a syndrome by examining how visitors respond to variation in plant traits. For instance, bumblebees are thought to prefer tubular flowers, and in a study of *Polemonium viscosum*, bumblebees preferred flowers with funnel-shaped corollas to flowers with short corollas

(Galen & Cuba 2001). Hummingbirds are also thought to preferentially select tubular flowers. In a manipulative study, corolla width influenced the ability of hummingbirds to insert their bills deeply into *Ipomopsis aggregata* flowers, which then influenced pollination success (Campbell et al. 1996).

While studies of individual floral traits are both interesting and informative, because pollinators are thought to select flowers based on groups of traits, it is highly useful to consider pollinator response to multiple traits. Responses to multiple floral characteristics may be interdependent, leading to correlational selection in which the attractiveness of one trait depends upon the expression of one or more other traits (Campbell 2009). Correlational selection occurs when traits interact in their effects on fitness, such that the effect of one trait is dependent upon at least one other trait. This joint selection shapes evolution of linked traits by favoring genetic correlation between traits (Sinervo & Svensson 2002).

Correlational selection has been studied in a variety of trait sets, including corolla shape and size (Gomez et al. 2009), inflorescence size and fragrance production (Valdivia & Niemeyer 2006), and flower color and fragrance (Odell et al. 1999) but is difficult to study experimentally in part due to the large number of replicates required to display all combinations of traits in factorial arrays. Observational studies of correlational selection are also challenging because interactions can be difficult to detect if one trait overpowers another. For example, Odell et al. (1999) found that although the combination of color and scent was likely important in pollinator selection, color was far more dominant than scent as a pollinator attractant, and statistical analyses were unable to show an interaction between the two characters.

In this study I examined the potential for correlational selection on floral traits in *Polemonium foliosissimum* through a set of manipulative experiments. I first examined pollinator response to single manipulations of floral color and shape and then combined the two types of manipulations to examine non-additive effects. In the floral color manipulations I examined whether changing *Polemonium* flowers from purple (typically thought of as a “bee” color) to white (typically thought of as a “fly” color) resulted in attraction of more fly visitors relative to bee visitors, as predicted by pollinator syndromes. For shape manipulations I flattened flowers into a more open shape to test whether the less tubular shape attracted fewer bees and more flies. Although I was unable to detect correlational selection through double-trait manipulations, bees and flies did differ in their responses to single-trait manipulations, and not always in line with the predictions of pollination syndromes.

Methods:

Study species

Polemonium foliosissimum (commonly known as “Towering Jacob’s Ladder”) is native to the Western and Southwestern United States and typically grows in montane or subalpine meadows (Darrow 2006). *Polemonium* flowers contain five bluish-purple petals, are generally self-incompatible, and plants may be female or hermaphroditic (Dorsett & Pike 1995, Zimmerman 1980). Near Gothic, CO, *Polemonium* blooms from late June to early August (Zimmerman 1980). Individual blossoms remain open for approximately five days. Although *P. foliosissimum* has been traditionally considered primarily bumblebee-pollinated, it is visited by a wide variety of flying insects including

butterflies, flies, and multiple bee families (Pleasants 1977 reviewed by Zimmerman 1980, M. Forster, pers. obs.).

Study site

I performed all surveys and experimental manipulations in a subalpine wet meadow at the Rocky Mountain Biological Laboratory in Gothic, Colorado with flowers collected from the main Gothic townsite. The observational plot was located near a natural patch of *P. foliosissimum* to best sample the pollinator community that would normally visit this species.

Assessment of natural population

I characterized the local population of *Polemonium foliosissimum* in order to develop a baseline from which to perform manipulations. This included measuring flower diameter for ninety-seven flowers from a natural patch in the Gothic townsite to determine the average display width as a proxy for “tubularness”. Diameter was measured along a bisecting axis from the tip of one petal and across the flower face to the tips of the two petals on the far side. Floral color was measured using a reflectance spectrometer that determined reflectance at wavelengths between 400-750 nm (Ocean Optics (Ocean Optics Inc., Dunedin, FL) Red Tide USB650 spectrometer, with a light source LS-1, and a fiber optic reflection probe R400-7-UV/VIS). These flowers do not reflect in the UV range of 250-400 nm (K. Whitney, pers. comm.). I also created and observed two arrays of thirty-six flowers each (6 x 6 in a 0.7 m² plot) at the beginning and the middle of the experimental period to characterize the natural pollinator community of

unmanipulated *P. foliosissimum*.

Single-trait manipulations (Pollinator Syndrome study)

Both floral color and floral “tubularness” were manipulated, with treatments first performed singularly to establish the impact that each individual manipulation had on visitation. For each set of manipulations I treated flowers the morning of the experiment to preserve floral integrity. I established experimental arrays in a 0.7 m x 0.7 m plot with thirty-six flowers randomly distributed and evenly spaced within the plot. To preserve freshness and provide support, individual flowers were placed with approximately 1.0 mL of plant food (Miracle-Gro® Quick Start Planting & Transplant Starting Solution) in microcentrifuge tubes attached to wooden skewers staked into the ground. Flowers were numbered 1-36 for ease in observation (Figure 1).

Color manipulations

For each color manipulation trial I painted eighteen of the thirty-six flowers white with water-based acrylic paint. The other eighteen flowers were painted purple as a control to ensure that color, not the presence of paint, was the main factor in impacting pollinator preference. Using a spectrometer to analyze floral and paint visible light reflectance, I matched the paint for the purple control flowers as closely as possible to the color of natural *Polemonium foliosissimum* blossoms (Figure 2). Acrylic paints have been used successfully to test pollinator preferences in other flower manipulation studies (Melendez-Ackerman and Campbell 1998). Nevertheless, to test for the effect of paint on pollinator visitation I performed eight hours of observation with control arrays of

eighteen natural flowers and eighteen flowers painted purple on the calyx so that pollinators could theoretically smell, but not see the purple paint.

Shape manipulations

Each shape manipulation array contained three treatments (“open”, “tubular”, and control) with twelve flowers of each treatment. I created open flowers by smoothing the petals onto five-pointed plastic stars covered in double-sided tape. Open flowers had the largest and flattest display area. Tubular flowers had the smallest display area and were created by inserting flowers into tubes of plastic sheeting, which greatly constricted the corolla. Control flowers with approximately the same average display and “tubularness” of natural flowers were created by forming shallow tubes from the five-pointed stars and resting the flowers inside. I measured the diameter of treated flowers as a proxy by which to compare “tubularness” among treatments and between manipulated and natural flowers (Figure 2).

Multi-trait manipulations

To examine all possible combinations of floral characteristics, I performed double manipulations in arrays with factorial sets of flower characteristics. Each array contained six types of flowers: control/purple, control/white, open/purple, open/white, tubular/purple, and tubular/white. These arrays were observed in precisely the same manner as the arrays receiving single manipulation treatments. Arrays contained thirty-six flowers with six individuals of each treatment. Two “blocks” of arrays with unique

spatial arrangements of the six floral treatments were performed with one block replicated for 13.5 hours of observation and the other for 7 hours.

Data collection

I began performing single manipulation arrays on July 6, 2010 and finished observations on July 27, 2010 and performed double manipulation arrays from July 30 to August 6. Each array was placed in the field from approximately 10:00 AM-3:00 PM to sample during the period when bees and flies are expected to be most active. I performed twenty hours of observations for each single manipulation, twenty hours of observation for the double manipulations, and eight hours of observation of painted/unpainted control flowers for a total of 68 hours of observation.

Each continuous sequence of probes by an insect from the time it entered the array until it exited the array was considered a foraging bout. For each bout, I recorded the identity of the visiting insect, the sequence of flowers probed, the size of the insect, and the approximate total time spent in the array. I considered any landing on a flower or close approach with hesitation over a flower to be a probe. Each fly and butterfly visitor was recorded to family level, and bees were identified to family or genus when possible. Because bumblebees are larger and more conspicuously marked than most other visitors I received, bumblebee visitors were typically identified to species level.

Data analyses

I divided all visits into category by insect type (bees, flies, and other) and calculated the proportion of probes to each floral treatment within each insect foraging

bout. For the single-trait arrays, I used a one sample t-test to compare the proportion of visits by each insect type to a particular treatment to the null hypothesis, which stated that in each type of array the visitors would probe each floral treatment equally. For example, in an array with purple and white flowers, I compared the proportion of visits to purple flowers to the null hypothesis of 0.5. In addition, I used a one-way ANOVA test to compare insect types with respect to the mean proportion of visits to a particular floral treatment. I also examined the type of flower first probed within a foraging bout. First probe choices were compared to the null hypothesis of equal frequencies using a chi-square test, and contingency analysis was used to compare the type of flower first probed across insect types.

For the double-trait arrays, a three-way contingency analysis with factors of color, shape, and type of insect (bee versus fly) was used to determine if the frequency of first probes to purple versus white depended on the shape of the flower. This analysis employed procedure CATMOD in SAS. Probes to double-trait flowers were also recorded and analyzed from the “floral perspective”. For this analysis, I grouped the data into two blocks of observation, each of which had different spatial arrangements of the six flower treatments in the array. For each of the 36 flower positions in a block, I determined the number of visits per hour to that flower. This visitation rate was analyzed with procedure GLM in SAS with the crossed class factors of flower color and flower shape, along with the blocking factor. A significant interaction between color and shape in this analysis would indicate the potential for correlational selection. All statistical analyses were performed using either SAS 9.1 or JMP 8.0.

Results

In twenty hours each of observation the color array received a total of 64 foraging bouts and 138 probes, and the shape array received a total of 80 foraging bouts and 333 probes. The paint control array received 33 foraging bouts for a total of 58 probes. The double manipulation array received 137 foraging bouts encompassing 288 probes.

Visitors included bombyliid, calliphorid, sarcophagid, syrphid and anthomyiid flies, andrenid, colletid, halictid, megachilid and bumblebees (Apidae), beetles, moths, and nymphalid and lycaenid butterflies (Table 2). All visiting individuals were grouped as bees, flies, or other insects. The “other” group (moths, butterflies, beetles) represented a very small minority of visits to each array, so only bees and flies were included in analyses. In most instances bees and flies were successfully identified to family level, except where insects were too small or were in the array for too brief of a time to be identified on the wing. The majority of visiting bumblebees were *Bombus bifarius*, but the arrays also received visits from *Bombus appositus* and one *Bombus californicus* individual. Bumblebees typically probed a greater number of flowers within a foraging bout than did other types of insects, with the most extreme case being one *Bombus bifarius* individual that probed thirty flowers within one bout, compared to an average of three or fewer flowers for other insect types (Table 1).

Paint control

I did not detect any effect of the paint on insect visitation during the paint control array observations. Bees made an average proportion of 0.70 of probes to painted flowers. However, only eleven bee foraging bouts were recorded and that value was not

significantly different from the null hypothesis that each type would receive a proportion 0.50 of probes ($t_{10}=1.52$, $P=0.16$). Bees first probed painted flowers during 73% of bouts, which was not significantly different from the null hypothesis that painted and unpainted flowers would receive equal percentages of first probes ($X^2=2.27$, $P=0.13$). Flies made an average proportion of 0.43 of probes to painted flowers ($t_{19}=-0.68$, $P=0.50$), and first probed painted flowers on 40% of visits ($n=20$, $X^2=0.81$, $P=0.37$).

Color

Bees greatly preferred purple to white flowers with an average proportion of 0.94 probes to purple flowers within a visit ($t_{25}=13.84$, $P<0.001$) (Figure 3). Because bumblebees performed the greatest number of bee-probes within color arrays, I also analyzed the data with bumblebees removed to examine whether bumblebees heavily weighted the results, but found that the preference for purple flowers did not change (0.94 purple probes/visit, $P<0.001$). First probes by bees to purple flowers were significantly more frequent than first probes to white flowers (96% purple, $X^2=22.15$, $P<0.0001$) (Figure 4). Only one of twenty-six bees (a halictid) visited a white flower on its first probe. Flies also preferred purple flowers, but not significantly so with a mean proportion of 0.62 probes per visit to purple flowers ($t_{36}=1.81$, $P=0.079$). Flies performed 39% of first probes to purple flowers ($X^2=2.19$, $P=0.14$). The bee preference for purple was stronger than the fly preference for purple (ANOVA on proportion of probes to purple, $F_{1,61}=15.44$, $P=0.0002$).

Shape

Measurements of floral diameter (as a proxy for “tubularness”) showed that tubular, open, and control flowers all had significantly different mean diameters and that control and natural flowers were not significantly different in diameter (one-way ANOVA/Tukey’s HSD post-hoc test, $P=0.64$: Figure 5). Thus, control flowers served to mimic natural flowers, and open, tubular, and control manipulations were each distinct enough to be considered separate treatments.

Bees did not make a significantly higher proportion of visits to open flowers than expected at random (null hypothesis=0.33, one-sample t test, $t_{57}=0.60$, $P=0.55$) but did significantly avoid tubular flowers ($t_{57}=-6.73$, $P<0.0001$: Figure 6). They also discriminated significantly against tubular flowers for first flower probed ($X^2=11.83$, $P=0.0027$: Figure 7). Flies did not make a higher proportion of visits to open flowers than expected at random ($t_{19}=1.32$, $P=0.20$) but did probe tubular flowers significantly less often per bout than expected at random ($t_{19}=-2.18$, $P=0.042$). Flies did not display a significant preference among shape treatments for first flower probed within a bout ($X^2=3.10$, $P=0.21$), but show similar trends to bees, with tubular flowers probed first less frequently than other types (16% vs. 37% and 47%). A contingency analysis did not detect difference between bees and flies in how often they first probed open vs. tubular vs. control flowers ($X^2=0.19$, $P=0.91$).

Double manipulations

For first probes made during a foraging bout, contingency analysis was unable to detect an interaction between shape and color ($X^2=1.91$, $P=0.38$). The three-way

interaction of shape x color x insect type was also not significant. ($X^2=2.36$, $P=0.31$). When the two types of insects were analyzed separately, neither bee ($n=33$, likelihood ratio $X^2 = 2.48$, $P=0.29$) nor fly ($X^2=0.7916$, $P=0.67$) first probe choice showed an interaction between shape and color. However, insect groups did show preferences for single traits in the multi-trait array. The collective group of insects significantly discriminated against tubular flowers in first probe choice (contingency analysis, $X^2=6.83$ $P=0.033$).

The most direct test of the potential for correlational selection comes from examining whether color and shape interacted in their effects of number of visits received by a flower (Figure 8). Using such a floral perspective analysis, flower color and shape both significantly influenced overall insect visits per hour ($F_{1,65}=4.28$, $P=0.043$; $F_{2,65}=4.88$, $P=0.011$). However, color and shape did not interact in their effects on visitation, so no correlational selection occurred ($F_{2,65}=0.96$, $P=0.39$). Color significantly affected bee visits per hour ($F_{1,65}=11.93$, $P=0.001$), but flower shape had no significant effect ($F_{2,65}=0.12$, $P=0.89$) and neither was there an interaction between color and shape ($F_{2,65}=0.90$, $P=0.41$). Flower color did not significantly influence fly visitation ($F_{1,65}=0.02$, $P=0.88$), but shape did ($F_{2,65}=7.47$, $P=0.0012$), and there was no interaction between color and shape ($F_{2,65}=0.33$, $P=0.72$). Purple/control flowers received the highest visitation rate from bees, while white/open flowers received the highest visitation rate from flies.

Discussion

Single-trait manipulations

Although it is not surprising that bees preferred purple flowers to white flowers in the color array, it is interesting that they preferred purple flowers so heavily. While the theory of pollinator syndromes predicts that bees will forage primarily for blue/purple and yellow flowers (Baker & Hurd 1968), bees can learn search images for other colors of flowers (Raine & Chittka 2007, Gumbert 2000). The facts that a) bees devoted such a small proportion of probes to white flowers and b) only one bee probed a white flower first on a foraging bout within the color array may indicate that visiting bees had not developed a search image for white flowers. This is unsurprising given that the experimental array was located in a meadow filled almost entirely with blue and yellow flowers. Solitary bees like andrenids and halictids typically forage between 300 and 1500 m from the nest and bumblebees forage 200 to 10,000 m from the nest depending on the species (Zurbuchen et al. 2010). Therefore, some of the visitors I received may have never foraged outside the experimental meadow. If so, they would have experienced few encounters with white flowers and may not have mentally linked a novel trait with rewards (Dyer & Murphy 2009).

It is also interesting that flies did not prefer white flowers to purple given that pollination syndromes predict flies to visit white flowers preferentially. Local variation in floral communities can lead to biases in floral preference in bumblebees and may also influence preferences in other types of insects---like flies---which could explain the unexpected result (Raine & Chittka 2007). Perhaps visiting flies also associate *P. foliosissimum* with the color purple and, like bees, discriminated against novel traits in

the painted flowers. Bees and flies may have preferentially visited the manipulated flower that appeared most like a natural flower (unmanipulated *Polemonium*) that they had encountered before.

Although the eight hours of observation with the paint control did not reveal any impact of paint on insect visitation, painted flowers received less than half the total number of insect probes (148 to 333) that flowers in the shape array received. The paint control study only examined the effect of applying paint to the calyx, and therefore only examined response to the paint odor. Insect preference may also have been affected by the appearance and the feel of the paint, so to improve the control study of paint impact on insect visitation, another array could be performed in which half of the flowers are left unmanipulated and the other half are painted purple.

The most interesting result of the shape manipulation array is that bees visited tubular flowers so infrequently. Bees often have much longer proboscises than flies and often visit closed or tubular flowers (Baker and Hurd 1968), so I expected they would visit tubular flowers at least as frequently as open and control flowers. However, the tubular flowers had much smaller corolla display sizes than open and control flowers, which may have made tubular flowers more difficult for visitors to perceive. Also, the anthers of tubular flowers protruded less above the floral plane than those of open and control flowers. If anthers are important visual attractants for insects, the lack of anther protrusion may help account for low rates of visitation and first probes to tubular flowers. Anecdotally, bumblebees showed the longest handling times for tubular flowers, which could mean that tubular flowers were difficult to extract rewards from, and thus less appealing to visitors. Finally, although tubular flowers were noticeably narrower than

flowers with the other treatments, open flowers were difficult for the human eye to discriminate from control flowers at a distance. Visiting insects may also have had trouble distinguishing between open and control flowers, which could explain why visitors did not preferentially choose between open and control flowers.

Double-trait manipulation

Neither bees nor flies exhibited correlational selection on doubly manipulated flowers, as the effects of color and shape on preference were not dependent on each other. However, visitors displayed preferences for single traits. Bees still preferred purple flowers to white flowers, as they had in the single-trait arrays, but they no longer discriminated against tubular flowers during visits to the double-trait array. Flies still discriminated against tubular flowers, and also did not display a color preference. Thus, responses to the double-trait array were more in line with pollination syndromes than responses to the single-trait arrays because bees were less hesitant to visit tubular flowers. A possible explanation for the reversal of the bee aversion to tubular flowers could be that the combinations of traits presented by the six different floral treatments were so novel to the bees that they honed in on the sensory cue that they had the strongest preference for--- purple color--- instead of shape, which they were not as particular about.

Furthermore, I performed the double-trait arrays at the end of the *P. foliossimum* flowering season, at a point where most patches were blooming only in small quantities. Thus, I received fewer bee visits than I had to the single-trait arrays because most bees had changed their preference to other types of flowers, such as the yellow *Helioeris multiflora*. Those bees that remained interested in *Polemonium* may have had a very

particular search image for it as a purple flower, but may have been less discriminatory against different shapes in the final stages of the *P. foliosissimum* blooming season.

The fly reversal to preferring white flowers over purple flowers may also be a reflection of seasonal changes. Many of the fly visitors to single-trait arrays were bombyliids, whereas many of the fly visitors to the double-trait arrays were sarcophagids. Perhaps sarcophagid flies have a stronger preference for white than do bombyliids. The majority of fly visitors to flowers in the double-trait array appeared to spend a lot of time resting on flowers. A flat, sturdy, open flower would provide more support and an easier place to rest than a tubular flower with a smaller display area.

If this study were continued in the future I would recommend performing double-trait arrays earlier in the summer when *P. foliosissimum* is in peak blooming season (early-mid July). Peak bloom during this study coincided with peak visitor diversity and peak bombyliid and bumblebee visitation, and it would be most meaningful to search for correlational selection when the primary pollinators (bumblebees) are most likely to visit. It would also be informative to perform a double-trait array with only two treatments: tubular purple and open white flowers. Both bees and flies preferred purple and open flowers during the first half of the experiment, so it would be interesting to examine their response to an array in which visitors would be choosing one “disliked” trait during a foraging bout. This might elucidate which trait (shape or color) is more important to each insect type, although the double-trait arrays performed in this experiment did tentatively suggest that color is more important to bees and shape more important to flies in choosing a first flower to probe within an array.

To help understand shape preferences, it would also be useful to examine the life history of *P. foliosissimum* flowers. For instance, does nectar production change over the course of the floral life cycle? Perhaps very young *Polemonium* flowers---which are more tubular than more mature flowers---provide little nectar reward to visitors, and tubular shape serves as a signal that the flowers are not yet ready to be visited. Very young and tubular flowers also may require time for the anthers to dehiscence, so tubular flowers may discourage pollen thieves, as well as pollinators, from visiting.

A final and critical component to advancing this study would be to perform single visit observations of emasculated flowers to test visitor efficiency in delivering pollen to virgin stigmas. While documenting visit frequency to manipulated flowers helps inform our knowledge of pollinator preference, it does not directly translate into an understanding of floral fitness as manipulated flowers will not set seed. Thus, pollinator efficiency must be examined to more comprehensively understand selection upon manipulated flowers. Although bumblebees are thought to be the major pollinators of *Polemonium foliosissimum* (Pleasants 1977), many other types of insects visit the flowers, and examining the efficiency and preference of the dominant pollinators may help explain why *P. foliosissimum* has evolved to look the way it does.

Figures and Tables

Insect type	B/F/O	Size	Total bouts	Total probes
Andrenidae	B	S, M, L	27	48
Bombus appositus	B	L	9	90
Bombus bifarius	B	L	28	213
Bombus californicus	B	L	2	6
Bombus sp.	B	L	23	45
Colletidae	B	S	5	8
Halictidae	B	S, M, L	26	47
Megachilidae	B	L	9	15
Unidentified bees	B	S, M	6	8
Anthomyiidae	F	M	3	3
Bombyliidae	F	M, L	37	100
Calliphoridae	F	M	2	2
Sarcophagidae	F	S, M, L	64	84
Syrphidae	F	S, M	31	88
Unidentified flies	F	V.S, S, M	47	52
Butterfly	O	L	2	2
Coleoptera	O	V.S	1	1
Moth	O	M	1	1

Table 1: Insect visitors to all three types of arrays. The “B/F/O” column shows insect group with “B” coding for bees, “F” for flies, and “O” for other insects. Insects approximately 0.25 cm in length were considered “very small” (v. s), approximately 0.5 cm in length were considered “small”, approximately 1.0 cm in length were considered “medium” (m), and approximately 1.5-2.0 cm were considered “large” (l). In this table the “bout” columns show the number of times an insect group type performed a foraging bout to a particular array and the “probe” columns display the number of times an insect type probed a flower within a particular array.

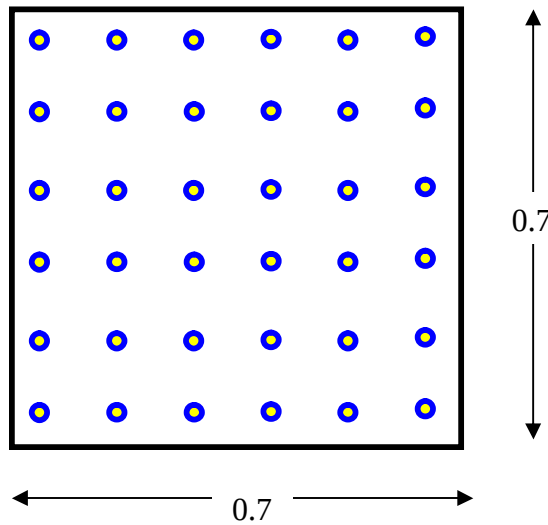


Figure 1: Sample experimental array: The sample plot was 0.7 m² with a total of thirty-six flowers in each trial. Flowers were spaced evenly apart (10 cm between individuals) and randomly distributed during each observing bout.

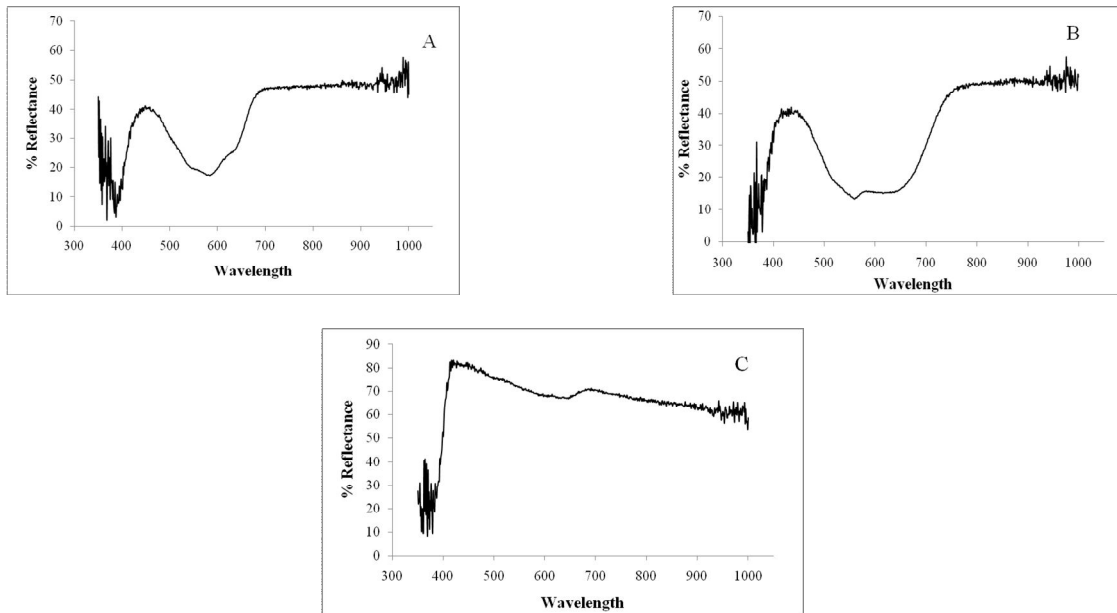


Figure 2: Light reflectance spectra for (a) unmanipulated *Polemonium foliosissimum* petals, (b) petals with purple paint, and (c) petals painted white. The spectrometer was used to match the purple color of the paint with the purple color of *Polemonium foliosissimum* as closely as possible to provide a control for the color manipulation arrays.

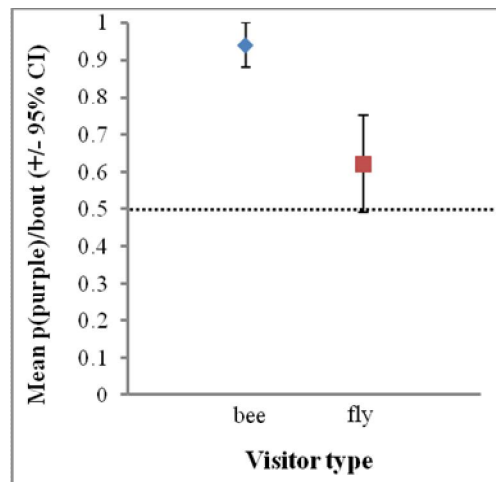


Figure 3: Average proportion of probes to purple flowers per bout in the color manipulation array. The horizontal line at 0.5 represents the null hypothesis that visitors would probe the same proportion of white and purple flowers within each foraging bout.

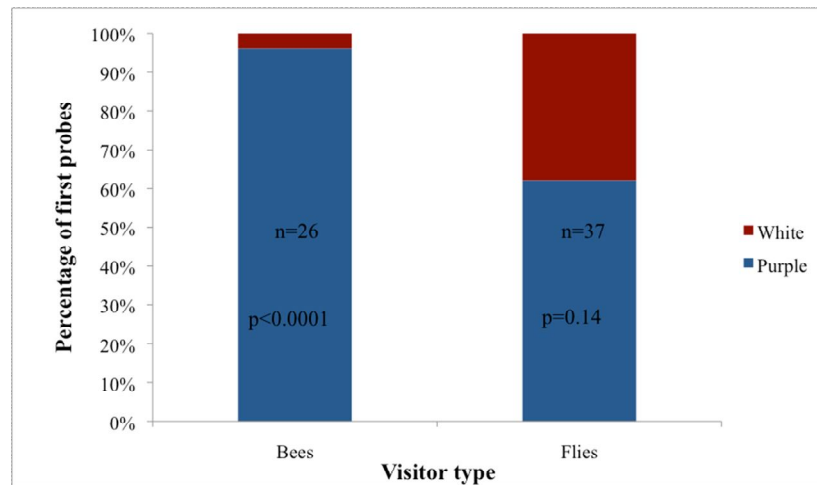


Figure 4: Percentage of first probes by color within a foraging bout on painted array. Bees significantly probed purple flowers first during visits, while flies did not.

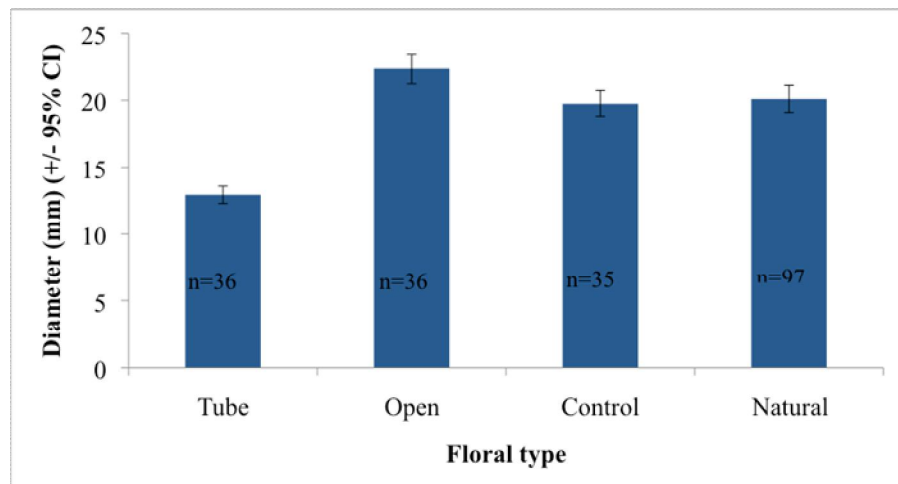


Figure 5: Average diameter of tubular, open, control, and natural flowers. The only floral treatments not significantly different from each other in diameter were control and natural.

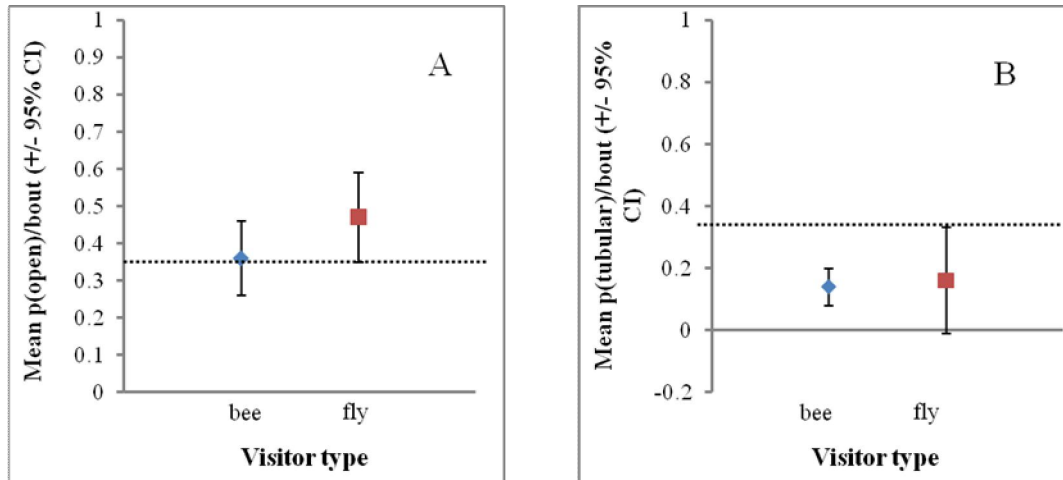


Figure 6: a) Average proportion of probes to open flowers per bout in the shape manipulation array. The horizontal line at 0.33 represents the null hypothesis that visitors would probe the same proportion of open, tubular, and control flowers within each foraging bout. b) Average proportion of probes to tubular flowers per bout in the shape manipulation array. The horizontal line at 0.33 represents the null hypothesis that visitors would probe the same proportion of open, tubular, and control flowers within each foraging bout.

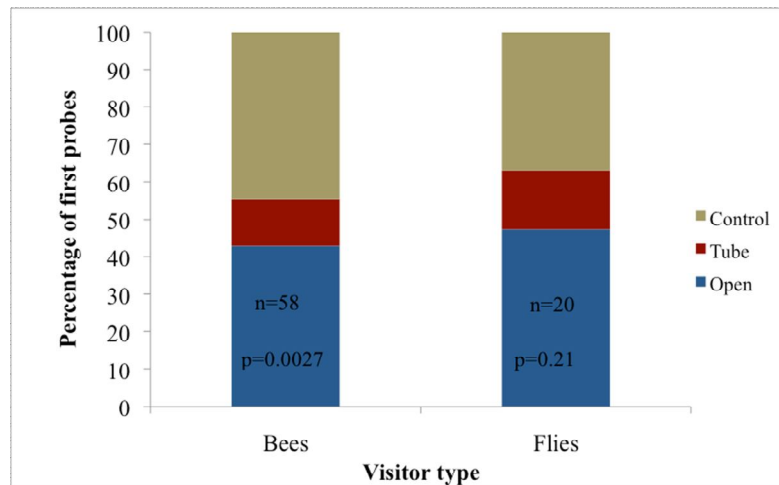


Figure 7: Percentage of first probes by shape type in the shape manipulation arrays. Bees displayed significant preferences in first probe choice among shape treatments ($p=0.004$). Flies did not show a significant preference in first probe choice among treatments ($p=0.229$) but did initially probe tubular flowers (15.79%) less frequently than open (47.37%) and control (36.84%) flowers.

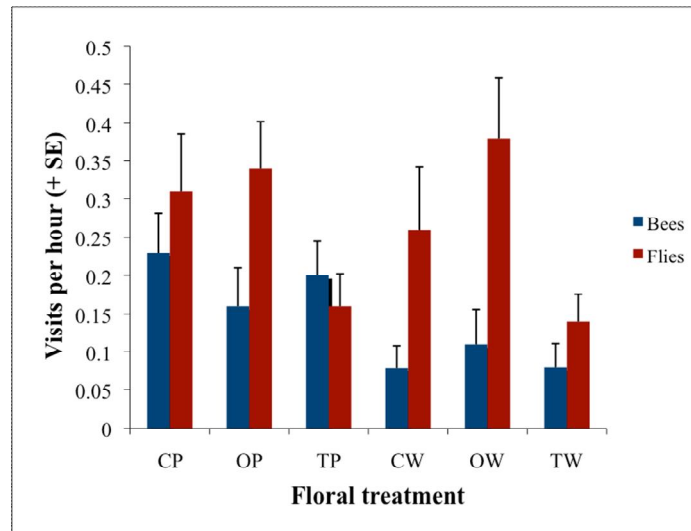


Figure 8: Average number of visits per hour by each insect type to each floral treatment. Although bee preference was dependent upon color and fly preference was dependent upon shape, neither bees nor flies showed an interaction between the effects of shape and color on visit rate. Therefore, correlational selection was not shown through this survey.

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