

The Influence of Aboveground and Belowground Resources on the Persistence of Females for a Gynodioecious Plant

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Abstract:

Gynodioecy is prolific within some angiosperm populations. These coexisting individuals within the same population will either be females that can't produce pollen, or hermaphrodites which can. Our study attempted to peer into the reasoning behind female persistence in *Polemonium foliosissimum*, a perennial wildflower native to the Rocky Mountains USA. Through examining resource limitation effects on hermaphrodites and pollinator visitation rates to manipulated hermaphrodites, no significant interactions between sexes and treatments were found. What was found, unexpectedly, was a correlation between floral display number per stalk and pollinator visitation suggesting that perhaps pollinators are indifferent between females and hermaphrodites.

Introduction:

Most flowering plants are hermaphroditic. They produce female gametes (ovules that, when fertilized, become seeds) and male gametes (pollen that sire seeds). However, approximately 7% of all angiosperms are gynodioecious, whereby some members of the population have lost male function and function only as females (Renner & Ricklefs 1995). Yet, these females coexist with hermaphrodites (Darwin 1877; Toivonen & Mutikainen 2012). It is suggested that in female plants there is selective pressure to remain in a population of hermaphroditic plants. This is likely due to their need to only allocate resources to survival and seed production/fitness, a female advantage as suggested by Darwin (1877). To persist, females must produce both more numerous, and more fit seeds than their hermaphrodite neighbors (Darwin 1877; Lloyd 1975; Charlesworth & Charlesworth 1978; Mutikainen & Delph 1998; Asikainen & Mutikainen 2003).

Interactions with pollinators may also affect the success of female and hermaphrodite flowering. Hermaphrodites occasionally show higher visitation rates than females when looking at gynodioecious species (Eckhart 1991; Asikainen and Mutikainen 2003). Hermaphrodites generally have larger flowers and it may be that size of the floral display plays a large role in visitation frequency (Conner and Rush 1996). Hermaphrodites also exhibit pollen which many pollinators can use as a source of protein, which leads to higher rates of visitation (Harder 1990). Pollinators may be biased based on these floral traits which could lead to pollen limitation in females.

Resource limitation, pollinator preference, and persistence of females were elucidated by using *Polemonium foliosissimum*, a gynodioecious perennial wildflower native to the Rocky Mountain range in the USA. Abiotic factors such as nutrients were manipulated to try and solicit a response to resource limitation exhibited by hermaphroditic *P. foliosissimum* individuals within a gynodioecious population. It was expected that hermaphrodites will be more resource limited (Darwin 1877; Primack & Hall 1990; Asikainen & Mutikainen 2003; Toivonen & Mutikainen 2012), therefore by providing additional nutrients to these plants, they should exhibit larger seed and fruit sets. It can be inferred that the smaller flowers displayed by female *P. foliosissimum* individuals may experience less visitation from pollinators such as flies and some bees that mainly consume pollen or consume pollen as a protein source along with nectar (Asikainen and Mutikainen 2003).

The following questions were addressed within this study: (1) are female *P. foliosissimum* individuals resource limited in below ground resources, and (2) do pollinators discriminate against female flowers over those of hermaphrodites when given a choice. These answers may provide a firm basis for why females persist within gynodioecious populations as a whole, and especially those of *P. foliosissimum*.

Methods:

Field Site and Natural History

Polemonium foliosissimum (Polemoniaceae) is a long-living perennial native to the Rocky Mountains, USA. Blue, five-petal flowers crown the stalks of *P. foliosissimum* from late June until early August. Some populations of *P. foliosissimum* are gynodioecious consisting of typical, hermaphroditic individuals and females that have lost male function and produce small anthers that lack pollen (Renner & Ricklefs 1995). On average, Hermaphroditic plants produce larger flowers than female flowers, and display pronounced anthers. (Brody personal communication)

During the summer of 2013, Experiments were conducted on two populations at the Rocky Mountain Biological Laboratory (RMBL) in Gothic, CO USA. One population was located near the Enders Dining Hall which will hereafter be referred to as the Dining Hall (DH) population. The DH population is purely hermaphroditic. The second

population is gynodioecious, consisting of ca. 50% females and located in a small meadow along the Gothic Main Road near the Willey Stand Pipe (WSP) (Figure 1).

These populations will be used to reinforce the understanding of resource limitation in hermaphrodites within gynodioecious populations of *P. foliosissimum*. In another meadow located at RMBL, only hermaphroditic plants are found (Martin & Brody, 2013 personal observation). These plants will be used to quantify the selection and preference of pollinators based on floral traits such as petal size and anther presence/ absence. *Bombus bifarius* and *Bombus flavifrons* are the two primary pollinators of *P. foliosissimum* (Zimmerman 1980).

Pollinator visitation and visitor preference

To examine if pollinators discriminate against females on the basis of floral size, floral traits of hermaphrodite flowers were manipulated to mimic those characteristics displayed by females. To do so, the size of petals were reduced, and anthers were removed in a factorial design. Plants were selected with at least four flowering stalks and each of the four stalks were assigned randomly to one of four treatments: (i) no manipulations, (ii) anther removal and petal size reduction, (iii) petal size reduction only, and (iv) a sham control for damage where small cuts were made into the petals, but no tissue was removed. A total of 48 stalks were subject to treatment and marked according to treatment with stringettes. Newly-formed flowers were treated appropriately prior to each daily observation period. Pollinator visitation was observed beginning July 19th until August 10th every day alternating morning (~9:30-12:00) and afternoon (~13:00-15:00) observation times. Times varied due to intense downpours experienced in the monsoon season which began on July 8th.

Nutrient limitation and floral trait response

To examine if plant resource status affected investment in floral traits differently between hermaphrodites and females, a resource limitation experiment was conducted where floral and flowering traits were measured over the course of the season as response variables. Prior to flowering, 30 triplets of plants were matched on the basis of size and stalk number (total of 90 individual plants, ~45 females and ~45

hermaphrodites). Each plant in the triplet was assigned randomly one of three treatments; (i) water, (ii) water plus 30mL/gallon of fertilizer, and (iii) an unmanipulated control.

A cup with a single drip emitter was placed near the base of each plant and the cup was filled with 12oz(30mL) of the appropriate solution every other day. Total flower count as well as petal length, petal width, corolla width, sepal length, sepal width, and carpel length were taken on July 5th, July 19th and August 2nd and used as response variables. Fruit set were also collected after the flowering season ended August 4th by counting the number of fruits and the number of aborted fruits on each of three stalks per plant.

Data Analysis

To examine if pollinators discriminated against small “female” flowers, an ANCOVA was run with treatment (control, petal reduction, petal reduction and anthers removed, and sham control), to treated stalks, and first visits to treated stalks as response variables. We did this for all visitors combined, and then ran separate analyses for bumble bees, solitary bees, and for flies.

To examine if plant genders responded differently to treatments, an analysis of variance was run with treatment (control, water, and water +fertilizer), gender, and measurement period (set) as main effects and flower size measurements and flower number as response variables. Also all two-way interactions were included.

Results:

Pollinator visitation and visitor preference

When all visitors were combined, there was no significant effect of flower size manipulation on either visitation rate or first flowers visited by organism (Fig.2) however, pollinator visitation responded significantly to flower number. (Fig. 3)

Nutrient limitation and floral trait response

When female floral trait measurements were compared to those of hermaphrodites, female plants produced significantly smaller flowers overall (all

measurements: petal length, petal width, corolla width, carpel length, sepal length and sepal width) (Fig. 4). There were no significant interactions between Treatment and Sex, and no Treatment Effects (Fig. 5).

When examining total flower number across measuring periods (set), there was an interaction where female flower numbers decreased over time while hermaphrodite flowers peaked during the second set of measurements before declining (Figure 6).

-----Still collecting/analyzing fruit set-----

Discussion:

In contrast to other studies (eg cite sources ashman 2000, Brody and Mitchell 1997), there was no significant effect of floral traits on visitation by bumble bees or solitary bees. Unexpectedly, a significant effect on visitation rates was due to flower number on stalks. When looking at flower number produced based on sex and available resources, a correlation was anticipated, however there was no significant effect of sex on flower number per stalk found.

Pollinators did not respond differently to treatments which may suggest that pollinator attraction may not be an important factor in differential fitness between females and hermaphrodites. Previous studies have shown that some pollinators are able to associate floral rewards with specific locations (Dukas & Real 1991). Hermaphrodites are known to produce higher quality nectar in addition to pollen and thus provide greater rewards (Kytöviita et al. 2013). Further consideration into future experimental design, reproducing a study such as Makino and Sakai (2007) could potentially elucidate a response between hermaphrodite and female flowers, suggesting reward-biased foraging.

Though no relevant significance was uncovered, females still persist within gynodioecious populations of *P. foliosissimum* suggesting that there are still unknown elements to their persistence. However it can be concluded that, for one reason or another that are as of now unrelated, pollinators prefer plants with greater numbers of floral displays per stalk, and that females are significantly smaller than their hermaphroditic counterparts.

Acknowledgements:

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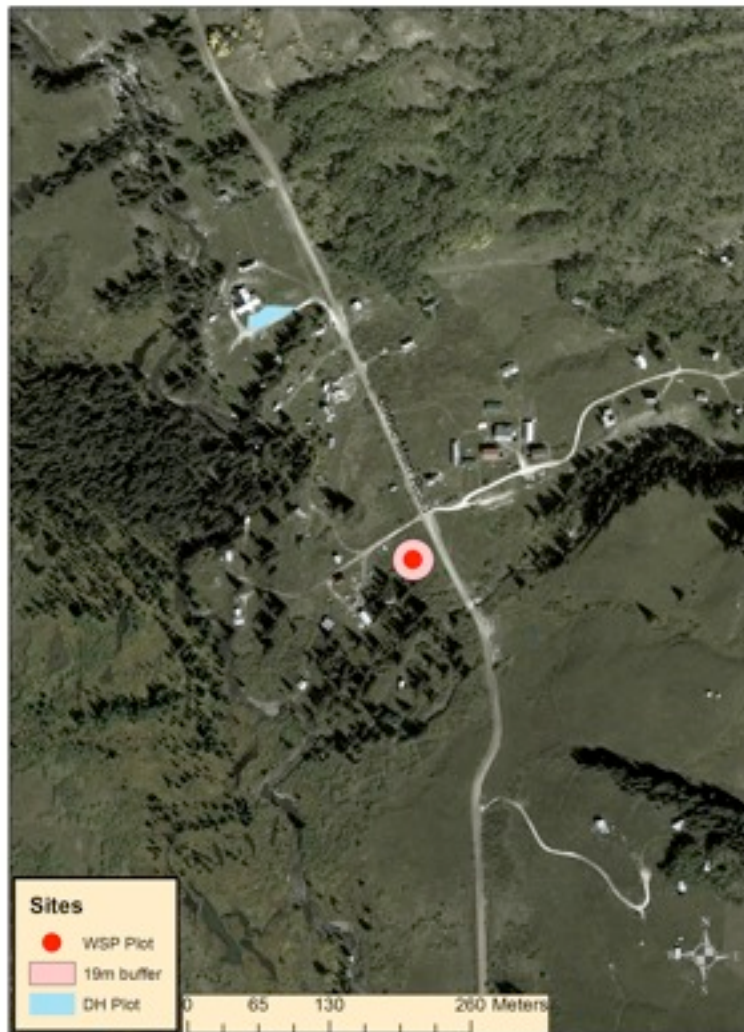


Figure 1. Aerial photograph of the Gothic Townsite and research sites.

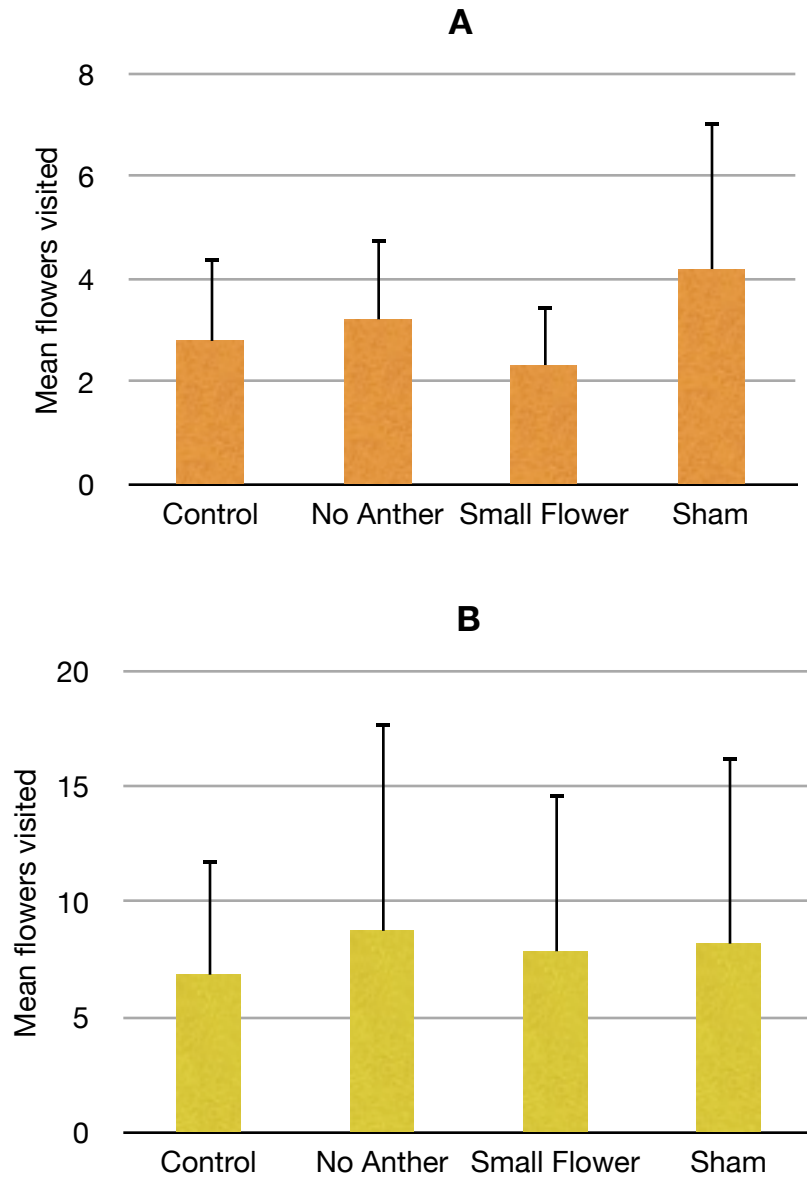


Figure 2 Mean number of visits for each group A, Solitary Bees. ($F_{25}=0.9489$, $P=0.4391$) B, Bumble Bees. ($F_{157}= 0.5753$, $P=0.6321$)

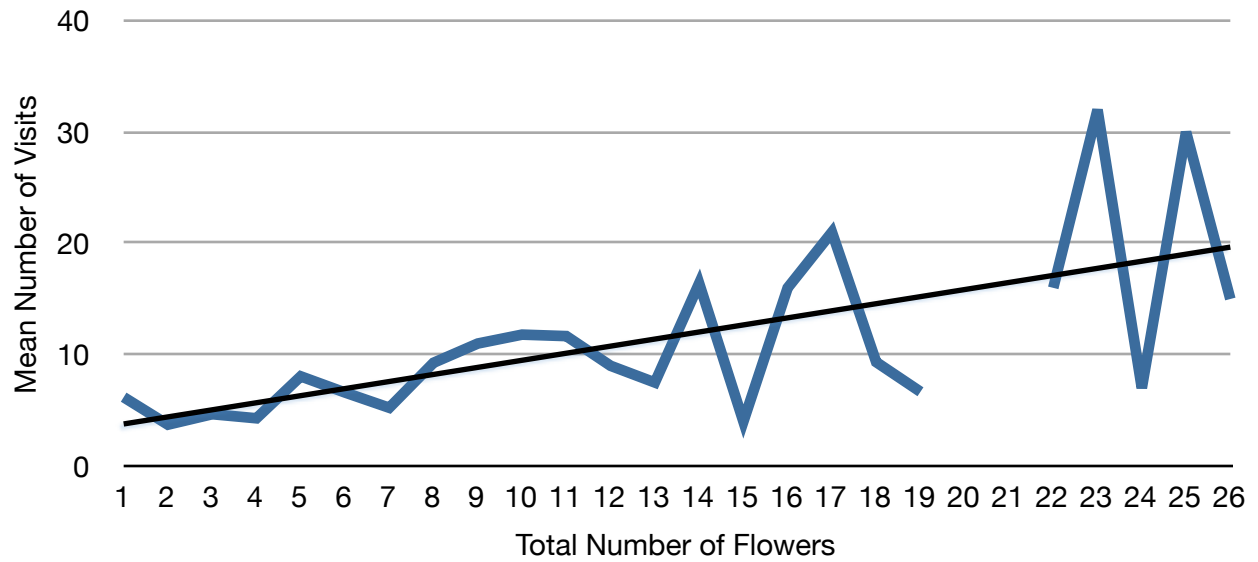


Figure 3 Visitation rate based on the number of flowers. Gap represents the absence of flowers with 20 and 21 flowers. ($P=0.0013$)

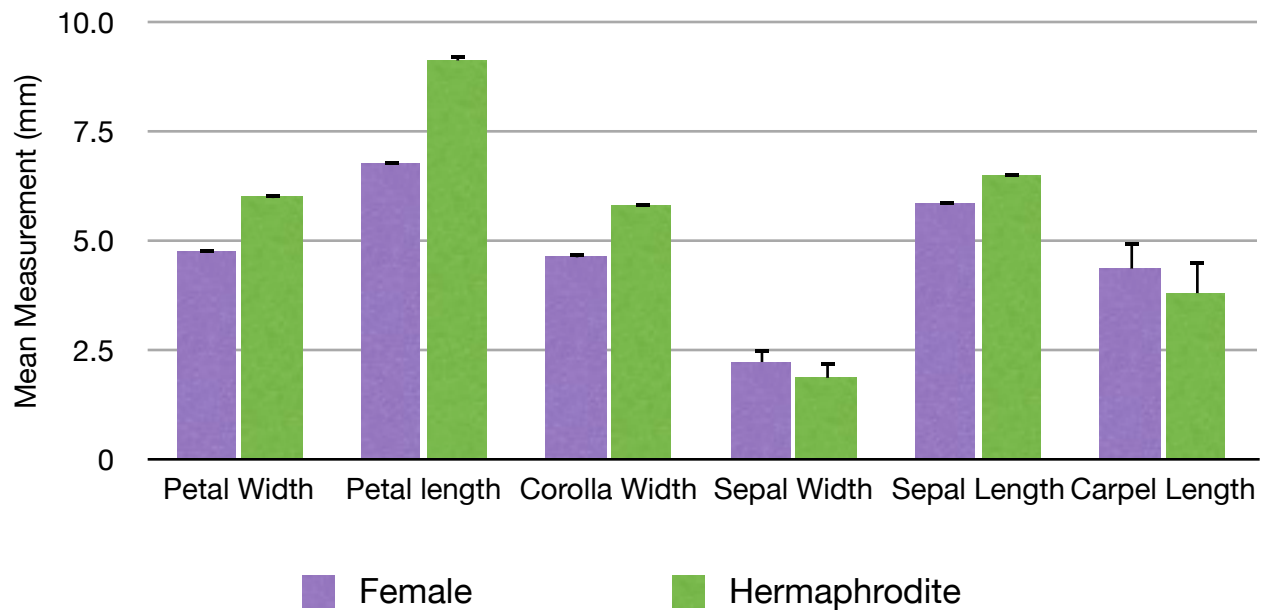


Figure 4 Mean measurements of floral traits comparing females and hermaphrodites. For all floral traits, the difference was highly significant.

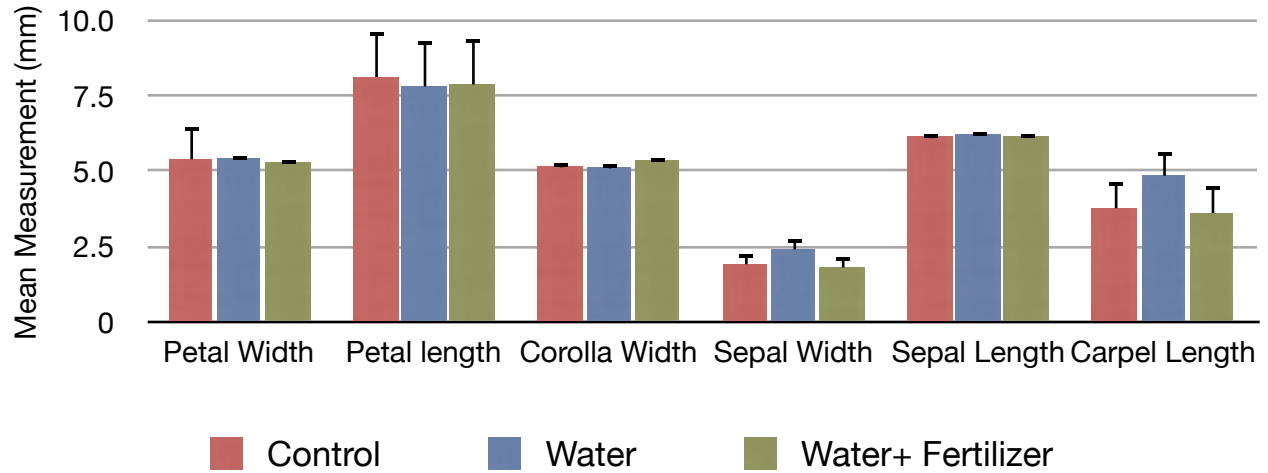


Figure 5 Mean measurements of floral traits comparing treatments. ($F_{224}=0.61$, $P=0.49$; $F_{224}=1.13$, $P=0.33$; $F_{224}=1.17$, $P=0.31$; $F_{224}=0.6$, $P=0.56$; $F_{224}=0.2$, $P=0.85$; $F_{224}=0.58$, $P=0.56$ respectively)

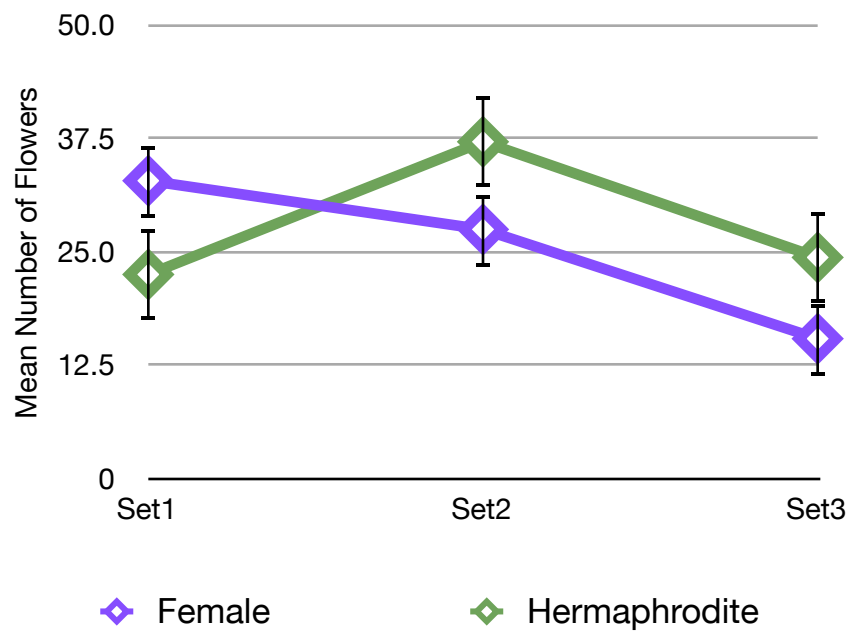


Figure 6 Mean number of flowers observed during each set of measuring. ($F_{224}=4.5197$, $P=0.0120$)