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UNIVERSITY OF CALIFORNIA, SAN DIEGO

Pollinator specificity and pollen limitation in the San Diego mesa mint, *Pogogyne abramsii,* a vernal pool endemic

A Thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Biology

by

Justin Anthony Scioli

Committee in charge:

Professor David Holway, Chair
Professor Joshua Kohn
Professor James Nieh

2014

The Thesis of Justin Anthony Scioli is approved and it is acceptable in quality and form for publication on microfilm and electronically:

Chair

University of California, San Diego

2014

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ABSTRACT OF THE THESIS

Pollinator specificity and pollen limitation in the San Diego mesa mint, *Pogogyne abramsii*, a vernal pool endemic

by

Justin Anthony Scioli

Master of Science in Biology

University of California, San Diego, 2014

Professor David Holway, Chair

Vernal pool ecosystems in California support a variety of narrowly distributed annual plants. As a result of the destruction, fragmentation, and degradation of vernal pool habitat, some vernal pool endemic plants are now considered threatened or endangered. The federally endangered San Diego mesa mint (*Pogogyne abramsii*) is a vernal pool endemic now found only in a few locations in coastal San Diego County, California. To learn more about the pollination biology of this species, we conducted a survey of its floral visitors and measured the extent to which natural populations experience pollen limitation. The assemblage of floral visitors present at sites supporting mesa mint did not differ from that present in nearby chaparral sites. The most frequent visitors to *P. abramsii* flowers were two bee species in the genus *Anthophorula*. By performing hand cross pollination, we found evidence for pollen limitation in our study population. The degree of pollen limitation was independent of floral density. We found a negative correlation between visitation rate per unit area and pollen limitation, however we found

no correlation between visitation rate and floral density. Our results indicate that conservation of this rare plant species likely requires the maintenance of pollination services through healthy populations of generalist pollinators and that individuals will produce more seeds in areas with higher visitation rates regardless of floral density.

The introduction, materials and methods, results and discussion sections are currently being prepared for submission for publication of the material. The thesis author was the primary investigator and author of this material.

Introduction

Vernal pool habitats are seasonally flooded wetlands that usually form above an impermeable soil layer, typically hardpan or claypan soil (Keeley & Zedler 1998). Winter and early spring rains fill these depressions, which gradually dry during the late spring to early summer. Vernal pools in California support numerous plant species that are restricted to this type of ecosystem. Vernal pool annuals germinate only in the wetting or inundation period and begin flowering once the pools dry out (Keeley & Zedler 1998). Vernal pool plants often rely on insects for pollination; some of these insect pollinators may specialize on vernal pool plants (Thorp & Leong 1998).

In the Central Valley region of California, for example, specialist bee pollinators associate with four different genera of vernal pool plants (Thorp & Leong 1998). Clarifying whether or not specialist pollinators visit vernal pool plant species serves as essential baseline information for strategies aimed at increasing plant abundance or at reintroducing plants into sites where they have been extirpated. Even in the absence of specialists, a unique assemblage of pollinators visiting vernal pool plants (i.e. compared to those present in the surrounding habitat) might still be essential to maintaining stable populations of particular vernal pool plant species.

Studies investigating the reproductive biology of *P. abramsii* have demonstrated that it produces higher seed set when outcrossed (Zedler 1987). This finding suggests that *P. abramsii* could experience pollen limitation when insufficient outcrossed pollen is received. Although knowledge of the pollinators that visit *P. abramsii* is incomplete, Schiller et al. (2000) found that per-flower visitation by insects to *P. abramsii* decreased with plant density but that seed production was independent of plant density. This result suggests that higher frequency of floral visits may not correlate with higher seed set, despite evidence that outcrossing leads to higher seed set in this species. The role for pollination services as a limiting factor in the reproduction of *P. abramsii* is still unclear.

Here we address three questions centered on the pollination biology of *P. abramsii*. First, do specialist pollinators visit *P. abramsii* flowers? Second, are pollinator assemblages around vernal pools different from those found in the surrounding habitat? Third, do *P. abramsii* populations experience pollen limitation and, if so, is pollen limitation density-dependent? To answer these questions we first conducted surveys of floral visitors (primarily bees) at vernal pool sites supporting *P. abramsii* and adjacent chaparral sites that do not support *P. abramsii*. We then cross pollinated individual *P. abramsii* plants to determine if plants at our sites are pollen limited.

The introduction section is currently being prepared for submission for publication of the material. The thesis author was the primary investigator and author of this material.

Materials and Methods

Study Area

We surveyed bees (Hymenoptera: Anthophila) on 22 days between 12 March and 20 June, 2012 at three different sites in San Diego Co., CA: Marine Corps Air Station Miramar, Del Mar Mesa, and Otay Mesa. All three sites contained vernal pool endemic flora, and pool depressions were embedded within chaparral habitats. Dominant flora in these sites included *Eriogonum fasciculatum*, *Acmispon glaber*, *Adenostoma fasciculatum,* non-native *Erodium* sp., and *Salvia mellifera*. We sampled most extensively at Marine Corps Air Station Miramar because this site contained the largest spatial extent of vernal pool habitat and the largest populations of *P. abramsii*. Although *P. abramsii* does not occur at Otay Mesa, we used this study site because of the presence of vernal pool endemic species to see if unique pollinators visit the flowers of other vernal pool plant species. Sampling took place at Miramar 17 times, at Otay Mesa 4 times, and at Del Mar Mesa once.

Bee Sampling

To sample bees, we used fluorescently painted bowl traps and aerial nets (Roulston et al. 2007). Bowl traps were deployed in 4-8 study plots per site (each arranged according the size of the *P. abramsii* patch it surrounded, roughly 15-20 m^2, hereafter referred to as transects). Each bowl transect was made up of three white bowls, three bowls painted fluorescent blue, and three bowls painted fluorescent yellow, to simulate the coloration of native flora. Each bowl trap was filled with soapy water. The bowl traps were set out in the field before 09h00 and retrieved after 15h30 to encompass the peak pollinator activity period.

Plots were divided into two groups. Pool plots were centered upon distinct vernal pools, as indicated by characteristic ground depressions and the remains of vernal pool indicator flora (e.g. *Pogogyne abramsii, Downingia cuspidata, Eringium aristulatum, and Psilocarphus tenellus*). Non-pool plots were placed in areas at least 50 m from the nearest vernal pool depression or remains of indicator flora. Bowl traps were set in groups of nine in a roughly elliptical formation either surrounding the perimeter of a pool depression (in the case of pool transects) or a similarly shaped area (in the case of non-pool transects).

Net sampling was conducted in the surrounding area of each bowl trap transect on each day of bowl trap sampling. All bees collected from bowl traps and net collection were curated and identified to at least the genus level, and in many cases to the species level.

We compared the assemblages represented in our specimens through a permutational multivariate analysis of variance (PERMANOVA). This analysis was conducted using the Adonis function in the VEGAN package (Okansen et al. 2013) in R 2.15.2 (R Development Core Team 2008) by inputting the abundances of different taxa. Because a large portion of the collected bees were not identified below the level of genus, analysis was conducted at the genus level.

Pollen limitation of Pogogyne abramsii

We conducted data collection at 29 different quadrats (each 3 X 3 m) within the Marine Corps Air Station Miramar. Quadrat locations were selected once *P. abramsii* began flowering. Each quadrat was placed at least 50 m away from all other quadrats; quadrats were placed across a gradient of estimated *P. abramsii* density based on germination at the time of selection. Once weekly between 15 April and 26 May, 2013, the density of open flowers (not individual plants) was recorded in each quadrat, either in the field or by visual analysis of high definition photographs of the quadrats.

In each quadrat, one to two individual plants were hand pollinated with conspecific pollen from five haphazardly selected individuals from the same quadrat as the focal plant. Because the individual flowers of *P. abramsii* typically bloom throughout the course of the plant's lifetime, hand pollination was conducted once weekly during the six-week sampling season. When some hand-pollinated plants were lost to wind or herbivory during the flowering season, nearby plants that had not yet begun flowering were selected and hand pollinated in their place.

In each quadrat, we also measured seed set in three to four *P. abramsii* individuals that were not manipulated. These measurements allowed us to estimate pollen limitation experienced by plants in each plot. Unmanipulated plants were nearest neighbors of hand pollinated plants and were comparable in size and number of flowers.

After flowers had completed blooming, mesh bags were placed over entire plants to prevent seed predation and to contain any fruits or seeds that may have fallen off the plant. After plants senesced, we collected all seeds present. Collected seeds were removed from the dried calyx of the senesced plant and placed into 1.5mL microfuge tubes, then set in a drying oven at 40°C for 24 hours. All seeds from a given plant were weighed together and the average mass per seed was calculated for each plant. Seed set per flower was calculated by dividing the total number of seeds on a given individual plant by the number of flowers on the plant.

Pollinator Monitoring & Sampling

To quantify patterns of pollinator visitation to *P. abramsii*, we observed plants between 29 April and 21 May, 2013. Each 20-min monitoring period was conducted between 10h00 and 15h00. Monitoring was conducted at 22 of the 29 quadrats used in the pollen limitation experiment, and some quadrats were monitored multiple times (7 quadrats had completed flowering before we were able to sample them, and therefore we allocated the time to resample other quadrats at different points in the season). During the 20 min observation period, we recorded the identity of each floral visitor visiting *P. abramsii* flowers within the quadrat. In instances where the visitor was difficult to identify to species, the visitor was collected to be identified in the laboratory.

Statistical Analysis

We calculated a pollen limitation index (PLI) for each quadrat by using a modified formula by Larson & Barrett (2000):

$$PLI = 1 - \frac{Average\ Seed\ Set\ per\ Unmanipulated\ Flower}{Average\ Seed\ Set\ per\ Hand\ Pollinated\ Flower}$$

In instances where the control plants had a higher mean seed set per flower than did the hand-pollinated plants (7 of 29 quadrats), we used a negative PLI value as calculated by this formula. In two instances , hand-pollinated plants produced no seeds, and we therefore removed these quadrats from this analysis. In comparing the fecundity of hand-pollinated and control plants across the entire study site, we generated a mean seed set value for hand-pollinated plants and a mean seed set value for control plants, and compared the two treatments using a paired *t*-test. We used the same method to compare average mass per seed. When testing for relationships between floral density and reproductive variables such as seed set, seed mass or PLI, we used a linear model to determine if our results suggest a correlation between the variables. In instances of non-normally distributed data, we instead used a generalized linear model.

The materials and methods section is currently being prepared for submission for publication of the material. The thesis author was the primary investigator and author of this material.

Results

Floral Visitor Assemblages

In total we collected 2,064 bee specimens. Across all sites, 26 genera of bees were represented in our samples. Of these, 25 genera were represented in our samples from Miramar and 13 were represented in our samples from Otay Mesa. Nearly 50% (984 of 2,064) of the collected specimens were members of the genus *Lasioglossum* (family Halictidae).

Due to the difference in number of sampling days between sites, we generated values of mean frequency per sampling day for each of the taxa that were collected. When corrected for sampling effort, we found no significant difference in the genus composition of floral visitor assemblages in pool transects compared to non-pool transects. (PERMANOVA, $F_{1,21} = 1.99$, $R^2 = 0.08$, $p = 0.07$, Fig. 1). There was also no significant difference in comparing pool and non-pool samples from only Miramar (PERMANOVA, $F_{1,10}=1.64$, $R^2 = 0.15$, $p = 0.14$) or from only Otay Mesa (PERMANOVA, $F_{1,10} = 1.76$, $R^2= 0.16$, $p = 0.20$).

Pollen Limitation

We found a significant difference between mean seed set per flower of hand pollinated flowers and unmanipulated flowers (paired *t*-test, $t_{29} = 2.17$, $p = 0.04$, Fig. 2) However, we did not find a significant difference between average seed mass of hand pollinated flowers and unmanipulated flowers (paired *t*-test, $t_{29} = -1.21$, $p= 0.24$).

Floral Density

Mean floral density of all quadrats decreased over the sampling season (Fig. 3). Only four quadrats had floral density peaks after the first week of the sampling season. Initial (week 1) floral densities varied from 42 flowers per m^2 to 994 flowers per m^2. However, the single highest density recording of 2,807 flowers per m^2 occurred during the second week (4/22/13-4/28/13)

Effects of Floral Density on Fecundity

We found no relationship between floral density and PLI of *P. abramsii* patches (linear regression, $F_{(1, 25)} = 0.1994$, $p = 0.66$). Similarly, we found no significant relationship between flower density and seed set per flower for unmanipulated plants (GLM, $z = 0.438$, $p = 0.66$) or for hand pollinated plants (GLM, $z = 0.050$, $p = 0.96$). We also found no significant correlation between average seed mass and floral density for unmanipulated plants (linear regression, $F_{(1,27)} = 0.802$, $p = 0.38$) or hand pollinated plants (linear regression, $F_{(1, 27)} = 1.941$, $p = 0.17$) (Fig. 4).

Floral Visitation

A total of thirty 20-min observational periods were conducted over the course of the season, amounting to 10 total hours of observational data. Five major floral visitor taxa were recorded during floral monitoring. A majority (24 of 40 recordings, 60%) of visitors were bees in the genus *Anthophorula* (Apidae): *A. torticornis* (Cockerell, 1927) and *A. nitens* (Cockerell, 1915). Other floral visitors included non-native honey bees (*Apis mellifera*), *Bombyliid* flies, *Lasioglossum* bees and one Hesperiid butterfly (*Hylephila phyleus*) (Table 1).

We found no relationship between floral density and number of floral visits per quadrat per unit time (linear regression, $F_{(1, 28)} = 0.91$, $p = 0.35$). We also found no relationship when comparing floral density with the number of visits by only the most frequent visitors, bees in the genus *Anthophorula* (linear regression, $F_{(1, 28)} = 0.15$, $p = 0.70$) or when considering floral density and the number of visits by non-*Anthophorula* visitors (Linear regression, $F_{(1, 28)} = 0.97$, $p = 0.33$).

We found a significant negative correlation between visitation rate per quadrat and PLI (linear regression, $F_{(1, 16)} = 6.421$, $p=0.02$, Fig. 5). However, we found no correlation between visitation rate per flower and PLI (linear regression, $F_{(1, 16)} = 0.0021$, p=0.96). We found no relationship between seed set per flower of unmanipulated plants and visitation rate per quadrat (linear regression, $F_{(1, 16)}= 3.169$, p=0.09) or visitation rate per flower (linear regression $F_{(1, 16)}= 0.19$, p=0.67). We also found no relationship between average seed mass of unmanipulated plants and visitation rate per quadrat (linear regression, $F_{(1, 16)} = 0.134$, p=0.10) or visitation rate per flower (linear regression, $F_{(1, 16)} = 0.225$, p=0.64).

We found no relationship when comparing only the visitation rate per quadrat of *Anthophorula* and PLI (linear regression, $F_{(1, 16)} =0.81$, p=0.37). Similarly, we found no relationship between the visitation rate per quadrat of non-*Anthophorula* visitors and PLI (linear regression, $F_{(1,16)}$ = F=0.24, p=0.63).

The results section is currently being prepared for submission for publication of the material. The thesis author was the primary investigator and author of this material.

Discussion

Our results suggest that *P. abramsii* is a species whose seed production is limited by the receipt of pollination services. This is not an unusual result, as reviews have reported that as much as 73% of angiosperm species experience pollen limitation (Ashman et al. 2004). Our visitation data suggest that *P. abramsii* populations receive pollination services from generalist insect pollinators. The assemblage of bee species surveyed around plots that supported *P. abramsii* was not significantly different from the assemblage surveyed in plots that did not support *P. abramsii*. This result suggests that the visitors of nearby chaparral plants are also present in areas supporting *P. abramsii*.

Floral Visitor Assemblages in Vernal Pools

Unlike the results of Thorp & Leong (1998) in the Central Valley region, we found no evidence of specialist pollinators in San Diego's vernal pools. In 2012, all visitors sampled near vernal pools were either also found elsewhere or were known to visit plants that are not pool-endemic (such as *Eriastrum* sp., *Malacothamnus fasciulata*, *Chorizanthe* sp., *Calochortus splendens*, *Cryptantha intermedia*, *Acmispon glaber, Deinandra fasciculata*). Also, our assemblage comparison between pool and non-pool transects found no significant differences with respect to bee genera assemblage composition. These results suggest that the vernal pool endemic plants present in our study sites are largely, if not entirely, visited by generalist bee floral visitors. There are several evolutionary reasons why a flowering plant may not develop specialist pollinators. Models suggest that this would occur in cases where the fitness cost of losing generalists is greater than the fitness cost of developing specialists (Aigner 2001). An analysis comparing the plant communities of Central Valley vernal pools to those of San Diego County might shed light on the evolutionary context that led one group of flowering plants to develop specialist pollinators, while another did not. Another possibility is that the destruction of the vast majority of vernal pool habitat in San Diego Co. and the reduction of *P. abramsii* populations may have led to the extinction or extirpation of any specialist pollinators that were previously present.

Floral Visitation of P. abramsii

The floral visitor assemblage observed through floral monitoring of *P. abramsii* was in similar to that recorded by Schiller et al. (2000). Unlike their results, however, we found no evidence of density-dependent visitation rate by visitors in general, or by any specific visitor group. The lack of positive correlation between floral density and observed floral visitation may be the result of too small of a sample size to demonstrate density-dependent visitation, Schiller et al. (2000) included 46 total cumulative hours of observation while ours was limited to 10. Also, it may be the result of differences in flowering phenology. Because January, February, and April 2013 received lower than modal rainfall, we suggest that our visits per m^2 are likely unusual with regard to the density dependence of floral visits in more typical rainfall years when mean floral densities are higher.

The most frequent floral visitors to *P. abramsii* were two bee species in the genus *Anthophorula*. A previous study suggested that these species could be effective pollinators for *P. abramsii* because they carry large quantities of *P. abramsii* pollen and have high visitation frequencies (Zedler 1987). However, although these species were the most frequent floral visitors, their rate of visitation showed no correlation with the seed set of unmanipulated flowers or their degree of pollen limitation. This suggests that although *Anthophorula* may be the most frequent floral visitor group, it may not be the most effective pollinator. Studies comparing the relative effectiveness of pollinators of a single plant have shown that only a minority are effective pollinators (Castro et al. 2013).

The significant negative correlation between floral visitation rate per quadrat and PLI demonstrates that hand pollinated plants produced higher seed set relative to the seed set of unmanipulated plants in areas where floral visitation was higher. This suggests that higher floral visitation reduces the degree of pollen limitation in this species. However, when comparing per-flower visitation rates to PLI values, we found no relationship. The non-significant result when standardizing visitation rates by density is likely the result of the observed lack of correlation between floral density and visitation rates.

Pollen Limitation

When examining all of our quadrats, we found significant pollen limitation in our populations of *P. abramsii*. The significantly higher mean seed set in hand-pollinated plants versus unmanipulated plants occurred despite unmanipulated plants outperforming hand pollinated plants in 7 of 27 quadrats (resulting in negative PLI values for those quadrats) and two quadrats where hand pollinated individuals failed to produce any seeds.

Because low rainfall years result in poorer performance and greater competition with non-native invasive weeds for vernal pool plants in San Diego (Bauder 2000), it is possible that low winter rainfall could lead to water limitation that may potentially supersede pollen limitation as a limiting factor for *P. abramsii* populations. Although we found a pollen limitation effect in a poor rainfall year, we suggest that pollen limitation as a limiting factor may be more pronounced in years when water and potential competition with non-natives are less limiting.

Contrary to our expectations, floral density had no correlation with seed set of unmanipulated plants or on the extent of pollen limitation. These data suggest that plants in denser patches do not receive significantly different pollination services from those in less dense patches, and also do not perform significantly differently in seed set. We found significantly reduced pollen limitation in quadrats with higher per-quadrat visitation rates, but found no relationship when we standardized those visitation rates by density. Thus, it appears that the studied *P. abramsii* population is limited by pollinator visitation without any effect of floral density. Schiller et al. (2000) also found no significant difference in mean seed set between denser and less dense patches, suggesting this is likely the case for *P. abramsii* populations in general. This information is potentially useful for restoration efforts that develop artificial pools, as their lower-than-natural floral density should not impact their fecundity with regard to pollen services received. Based on our results, increasing the visitation rate per unit area will reduce pollen limitation for individuals in a given patch regardless of the floral density of that patch.

The discussion section is currently being prepared for submission for publication of the material. The thesis author was the primary investigator and author of this material.

Figures and Tables

Table 1: Sum of number of observed floral visits across 2013 sampling season on *P. abramsii* flowers by visitor group.

Visitor group	Floral density in flowers/m^2 1-100	101-500	501-1000	>1000	**Total**
Genus *Anthophorula*	2	10	2	10	24
Genus *Lasioglossum*	0	1	0	4	5
Honeybees (*Apis mellifera)*	0	1	0	4	5
Bombyliid Flies	0	3	1	1	5
Hesperiid Butterflies	0	0	0	1	1
Total	2	15	3	20	40

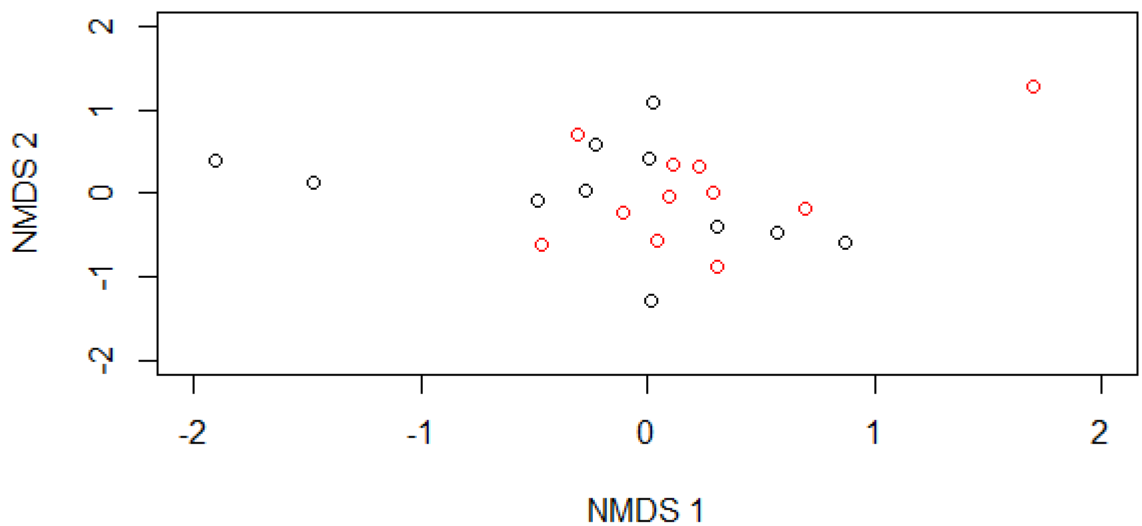

Figure 1: NMDS of community data of bee genera. Non pool plots are indicated in black, pool plots are indicated in red.

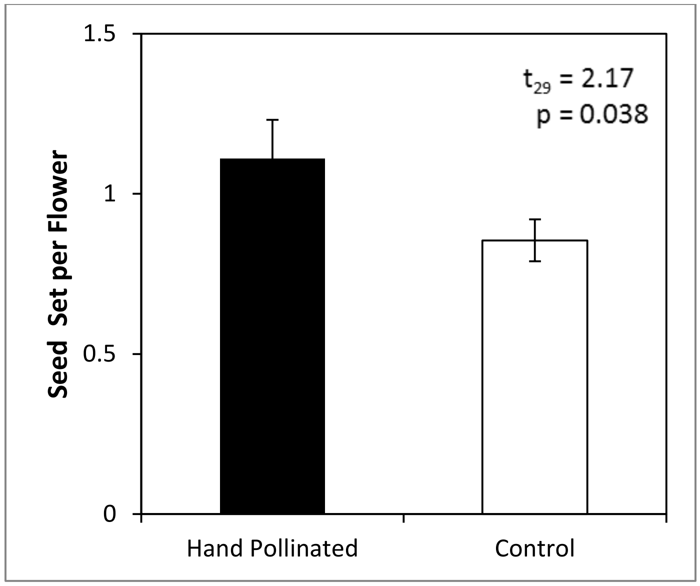

Figure 2: Seed set per flower for hand pollinated and control plants. Hand pollinated plants produced significantly higher seed set per flower.

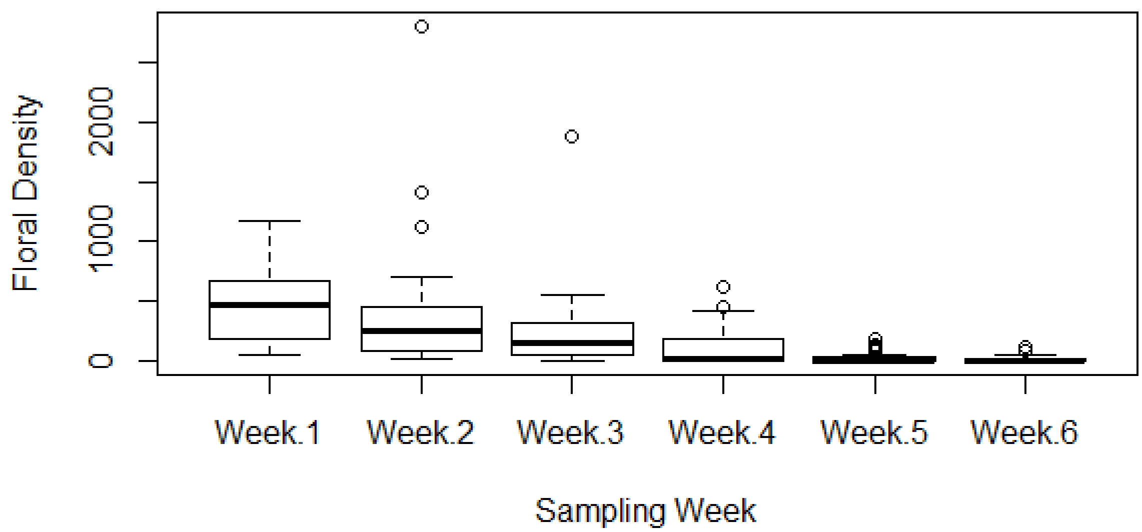

Figure 3: Mean density of quadrats over the six weeks of the sampling period (4/15-5/26)

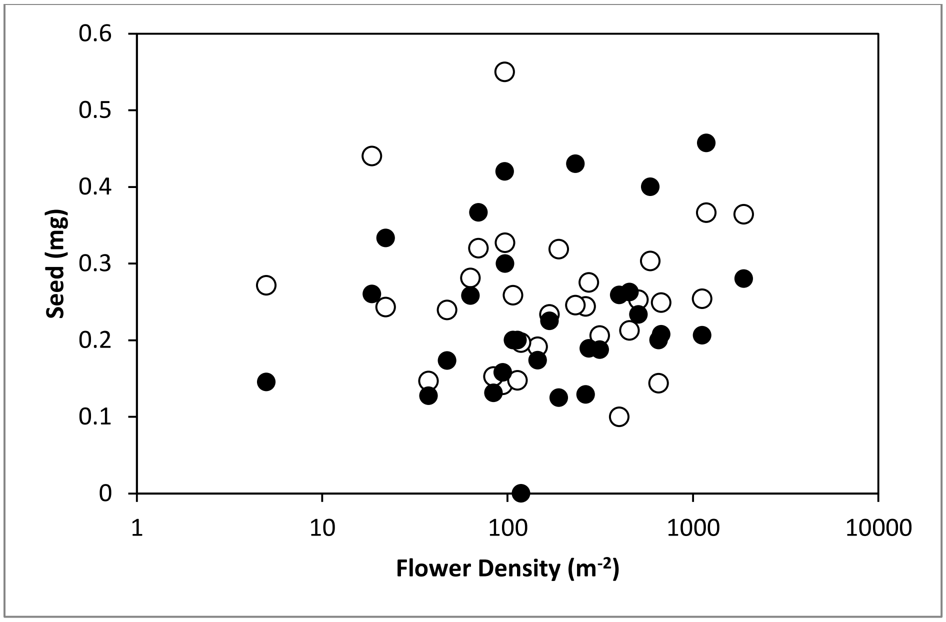

Figure 4: Average seed mass by flower density. Each quadrat's hand pollinated average is shown in black, and the control average is shown in white.

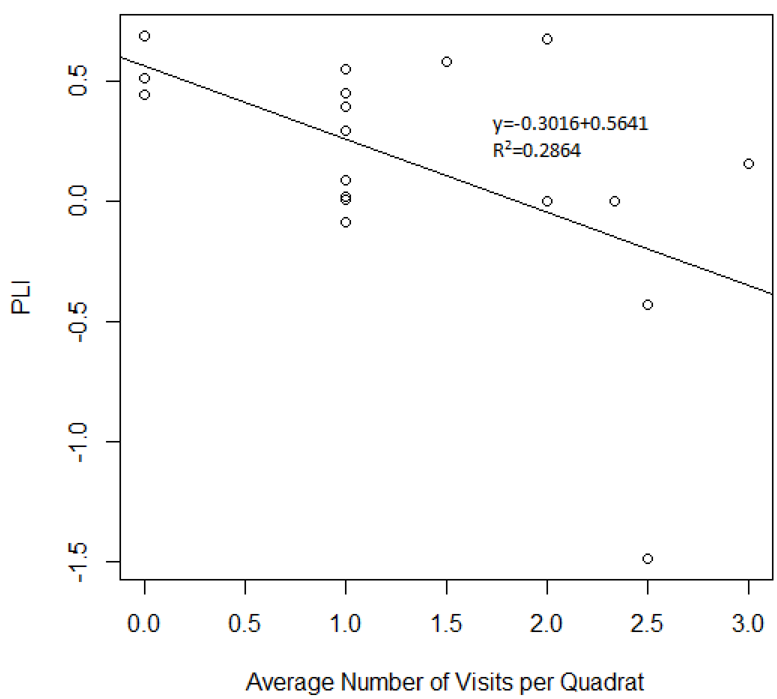

Figure 5: PLI by average number of visits per quadrat. The trend line is the linear regression.

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