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Pollinator Effectiveness of Peponapis pruinosa and Apis mellifera on Cucurbita foetidissima

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Pollinator Effectiveness of *Peponapis pruinosa* and *Apis mellifera* on *Cucurbita foetidissima*

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

in

Biology

by

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2017

The Thesis of Jeremy Raymond Warner is approved and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

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ABSTRACT OF THE THESIS

Pollinator Effectiveness of *Peponapis pruinosa* and *Apis mellifera* on *Cucurbita foetidissima*

by

Jeremy Raymond Warner

Master of Science in Biology

University of California, San Diego, 2017

Professor David Holway, Chair

Differences between specialist and generalist pollinators have long been a topic of interest for biologists. Plants of the genus *Cucurbita* (Cucurbitaceae) are visited by generalist pollinators, such as honey bees (*Apis mellifera*), and by specialist pollinators, known as squash bees (e.g., *Peponapis pruinosa*). Previous studies have examined pollinator effectiveness between *Apis* and *Peponapis* on agricultural *Cucurbita* species, but few have investigated the effectiveness of these pollinators in a non-agricultural context. In the summer of 2017, I conducted single visit pollination trials on 22 buffalo gourd (*Cucurbita foetidissima*) plants at Los Peñasquitos Canyon Preserve, San Diego

County, CA to measure pollinator effectiveness between honey bees and squash bees. The percentage of fruit set from single visits made by female *P. pruinosa* (57.9%) was higher than that of male *P. pruinosa* (23.5%). Single visits by *Apis* never resulted in successful fruit set. Control fruit, by comparison, set at a percentage of 85.7%. The average weight of seeds from fruit resulting from female *P. pruinosa* single visits was significantly higher (by 28.6%) than for single visits by males, but neither seed number nor fruit volume differed between male and female single visits. These results indicate that *Apis* and male *P. pruinosa* are less effective at pollinating buffalo gourd compared to female *P. pruinosa*. Differences in how these pollinators gather nectar from *Cucurbita* may be one explanation for differences in fruit set. These differences may be exaggerated in non-agricultural systems, where generalist pollinators like *Apis* have a variety of floral resources to choose from.

Introduction

Pollinator species differ in the degree to which they specialize on the plants that they visit (Waser et al. 1996). A pollinator may be a generalist, collecting nectar or pollen from many different plant species, or may instead be a specialist, collecting nectar or pollen from only a few select plant species. Bees more often specialize on pollen sources compared to sources of nectar (Wcislo and Cane 1996). In many cases, the floral preferences of specialists are not affected by other floral resources that are available (Wcislo and Cane 1996). It may seem intuitive that specialists would be the most effective pollinators for the plants they visit when compared to generalists, but this is not always the case (Motten et al. 1981, Neff and Simpson 1990, Olsen 1997). For example, a pollen specialist might sequester pollen from a focal plant species in order to feed its offspring. Pollen that could have gone towards pollinating another flower has thus been removed from the system. However, in some systems that have been studied, specialists are more effective pollinators compared to generalists (Larsson 2005, Lindsey 1984).

Squash bees (*Peponapis* and *Xenoglossa*) (Apidae) provide examples of highly specialized pollinators. Squash bees are solitary, ground-nesting bees found in the New World (Hurd and Linsley 1964). Squash bees gather pollen and nectar almost solely from squash and gourd plants in the genus *Cucurbita* (Hurd and Linsley 1964). Squash bees nest near these plants, mate inside their flowers, and use closed and wilted *Cucurbita* flowers as overnight shelters (Hurd and Linsley 1964).

Cucurbita plants are not only visited by specialists, however. *Cucurbita* flowers are visited by numerous pollinator species (McGregor 1976). Some of the pollinators that visit *Cucurbita* are generalists that gather nectar and pollen from a large number of plant species. One such generalist is the western honey bee (*Apis mellifera*) (vanEngelsdorp and Meixner 2010). Honey bees are important pollinators in agricultural systems because of their generalist habits (Artz et al. 2011, Garibaldi et al. 2013, Smith et al. 2013, vanEngelsdorp and Meixner 2010) and readily pollinate agricultural species of *Cucurbita.* Declines in managed honey bee populations (Smith et al. 2013) may enhance the importance of native bees as pollinators in agricultural systems (Winfree et al. 2007). Squash bees, which readily pollinate both agricultural and non-agricultural varieties of *Cucurbita*, provide pollination services for agricultural systems.

Studies on pollinator efficiency and effectiveness between squash bees and honey bees have been completed on several species of agricultural *Cucurbita* with mixed results. One study found that female squash bees were able to remove and deposit significantly more pollen compared to both male squash bees and honey bees (Canto-Aguilar and Parra-Tabla 2000), but other studies have found no difference in fruit set or seed set between squash bees and honey bees (Artz and Nault 2011, Tepedino 1981). However, the nature of agricultural systems may influence the ability of squash bees and honey bees to transfer pollen. For example, the diversity of floral resources present in non-agricultural systems may decrease the likelihood that a generalist will visit any one plant species, potentially altering the generalist's ability to successfully transfer pollen between plants of the same species. A specialist, by comparison, might be less influenced by the presence of other floral resources. If so, the effectiveness of specialists and generalists may differ in agricultural versus non-agricultural systems. For this reason, I examined the effectiveness of these pollinators in a non-agricultural setting.

I conducted my study on a species of wild *Cucurbita* known as buffalo gourd (*Cucurbita foetidissima*). Buffalo gourd has a distribution that extends from northern Mexico into the southwestern and midwestern U.S. (Bemis et al. 1978). Buffalo gourd is considered native to southern California, but it is possible that populations in southern California may have been introduced by humans (Bemis et al. 1978). Buffalo gourd is a gynodioecious, xerophytic gourd with an extensive perennial root system and produces large, yellow flowers (Dittmer and Talley 1964). In this region, buffalo gourd flowers between April and September. Individual flowers open early in the morning and close completely by midday; flowers last one day. Previous work on buffalo gourd has explored sex allocation (Kohn 1989), pollen competition and pollen dispersal (Ordway et al. 1987, Winsor et al. 2000), and its potential as an arid land crop (Bemis et al. 1978, DeVeaux and Shultz 1985).

For my study, I conducted single visitor pollination trails on buffalo gourd to independently assess the effectiveness of squash bees versus honey bees as pollinators of these plants. I tested if there were differences in the ability of these pollinators to set fruit, and also if differences exist in the quality of fruit resulting from single visits by *Apis*, female *Peponapis pruinosa*, and male *P. pruinosa*. Because previous research found a relationship between pollen load size and seed number (Winsor et al. 2000), I predicted that fruit set would be highest from single visits by female *P. pruinosa*, and that these fruit would have higher seed set and average seed weight compared to fruit resulting from single visits by either *Apis* or male *P. pruinosa.* These comparisons will provide insight into the differing ability of these pollinators to provide pollination services to *Cucurbita* in a non-agricultural setting.

Methods

Study System

This study took place at the Los Peñasquitos Canyon Preserve, in San Diego County, CA, which encompasses an area of over 1600 hectares of coastal sage scrub, chaparral, grassland, and riparian habitats. Buffalo gourd can be found in open areas along Los Peñasquitos Creek. Flowers of buffalo gourd at this location are visited by several species of native pollinators, including squash bees (*Peponapis pruinosa* and *Xenoglossa strenua*) and the non-native western honey bee (*Apis mellifera*). Honey bees are common throughout San Diego County (Kono and Kohn 2015).

Pollinator effectiveness

In April 2017, the locations of buffalo gourd plants (n = 45) were mapped in Los Peñasquitos Canyon Preserve (Appendix 1). I considered root crowns separated by at least 13 m to be separate individuals, because vines produced by buffalo gourd can grow up to 12 m long (Kohn 1989). I collected data on 20 hermaphrodite plants and 4 female plants. I was not able to collect single visit data on 21 plants because these plants were discovered later in the season and had already stopped flowering, or they emerged late in the season and did not produce female flowers.

I conducted single visit pollinator effectiveness trials from June to August 2017. Each day, plants were selected for single visit trials to be completed on the following day based on the availability of female flowers on each plant. I identified female flower buds that were going to open the next day based on the size and color of the bud. Flower buds turn from green to yellow on the day before they open. Prior to every trial, each bud was covered with an S27 Seedboro Pollinating Bag to ensure that pollinators could not visit the flowers prior to bag removal. No bees were ever observed in the bagged flowers (or in the bags themselves) upon removal of the bags the next day.

Single visit pollination trials allowed me to compare the effectiveness of *Apis mellifera* (♀) and *Peponapis pruinosa* (♂ and ♀) separately. To conduct each trial, I would arrive at a focal plant between 0600 and 0630 hours, unbag the focal flower, and observe it from a distance that allowed pollinators to visit the flower without apparent disturbance but that also allowed me to clearly see the inside of the flower. These distances varied slightly depending on the available space around that particular flower (approximately 0.5 m – 1.5 m). Once a pollinator entered the flower, I timed the duration of its visit. To prevent any additional pollinators from entering during trials, I held a small insect net (rim diameter = 30 cm) horizontally over the opening of the flower at a distance of approximately 10 – 20 cm. The net never contacted focal flowers and did not ever appear to alter the behavior of the pollinator inside the flower. Single visit trials interrupted by additional pollinators were discontinued. For each trial, I recorded the time the flower was opened, the time the visit began and ended, and the species and sex of the pollinator. Once the pollinator had left the flower, I covered the flower with a mesh bag with drawstrings to prevent any further pollinators from entering the flower. I tightened the drawstrings below the base of the ovary. Bees were never observed in these bagged flowers. If a particular flower had no visitors for an extended period of time (approximately 45 minutes), the single visit trial was discontinued. After each single visit trial, I gave each flower a unique tag (labelled flagging tape) for later identification.

Single visit trials were conducted until either all bagged flowers for the day had been used or until between 0930 and 1000 hours, when flowers began to wilt and close.

Starting in July 2017, I selected open female flowers to serve as controls to compare with single visit flowers. These flowers were tagged with labelled flagging tape for later identification and left open for pollinators. In September 2017, I harvested additional gourds to serve as control seed and fruit data for plants on which I was unable to tag open pollinated control flowers.

Fruit weight of buffalo gourd tends to reach its maximum 10 days after pollination, and seed development continues until seeds reach their maximum weight between 32 and 34 days after pollination (Awdh ba-amer and Bemis 1968). All fruits in this study were harvested a minimum of 35 days after pollination. Before harvesting a fruit, I measured the distance from that fruit along the vine to where it emerged from the ground. I also counted the number of leaves on the vine within this interval. I made these same measurements on fruits that aborted, and recorded which flowers failed to develop into fruit. I harvested developed fruits by cutting the stem approximately 1 – 2 cm above the fruit using scissors.

In the lab, I weighed fruit and measured their height and width on the date of harvesting. I then cut fruit open and counted all fully developed seeds. I also collected undeveloped seeds from control fruits harvested in September. Developed seeds, on average, weighed 327.3% more than undeveloped seeds (Fig. 1; two sample *t*-test: $t = 12.043$, df = 17.483, $P < 0.001$). Developed seeds were thick and tan in color, whereas undeveloped seeds were smaller, thin, and white or translucent. Once counted, I set seeds aside on a paper towel to allow them to dry at room temperature (approximately 23 °C). The seeds from each fruit were kept separately in their respective mesh bags and left on paper towels to dry for 3 or more days. Once dried, I measured the total weight of seeds from each fruit to obtain seed weights. Only developed seeds were used in my seed number and seed weight analyses. Fate of fruit, seed number, average seed weight, and fruit volume data for control and single visit fruit are provided in Appendix 2.

Data analysis

All statistical analyses were run in r (r Core Team 2016). To test for relationships between reproductive performance variables (seed and fruit traits) and plant traits (blooming time and position on vine), I used simple linear regressions. I used general linear mixed models to compare fruit set and fruit traits between single-visits by male and female *P. pruinosa.* For the analyses of the fruit set data, *Apis* was excluded from the model because single visits by this species resulted in no fruit being set and the general linear mixed models would not run with this species included. In this latter analysis, fruit set was treated as a binomial variable, buffalo gourds plants were treated as a random factor, and pollinator treated as a fixed factor. To compare seed number, seed weight, and fruit volume for fruit that resulted from single visits by male and female *Peponapis pruinosa*, we also used general linear mixed models. In these analyses, separate models were run for each of the three continuous response variables; buffalo gourds plants were treated as a random factor, and pollinator treated as a fixed factor. For the analyses of the fruit trait data, *Apis* could not be included because single visits by this species resulted in no fruit being set. These analyses were run using the ‘lme4’ package (Bates et al. 2015) in r. These analyses excluded female plants (n = 3), which differ from hermaphrodites; inclusion of these female individuals does not alter the results.

Results

Plant trait regressions

I used simple linear regressions to test for relationships between reproductive performance variables and different plant traits (Table 1). Linear regressions for number of leaves to root crown, distance to root crown, and pollination date against seed number and average seed weight of control fruit showed no significant relationships. Linear regressions for number of leaves to root crown, distance to root crown, pollination date, time of pollination visit end, and pollinator time spent against seed number and average seed weight of single visit fruit also showed no significant relationships.

Fruit set

The proportion of fruit produced from single visits varied greatly depending on pollinator identity (Table 2). *Apis* single visits never resulted in successful fruit set, but the small number of visits (n = 10) on relatively few plants (n = 5) precludes statistical comparisons between this species and squash bees. The percent fruit set on flowers visited by single female *P. pruinosa* (57.9%) was higher than that for flowers visited by single male *P. pruinosa* (23.5%)(Table 2; linear mixed model: pollinator $z = 2.03$, $P = 0.021$, n = 17 plants with 96 observations). Fruit set data for male and female *X. strenua* are also included in Table 2, but small sample sizes precluded their inclusion in the fruit set analyses.

Fruit volume, seed number, and seed weight

I used linear mixed models to compare fruit and seed variables for fruit that resulted from single visits by either male or female *P. pruinosa*. Fruit resulting from single visits by female *P. pruinosa* produced significantly heavier seeds (by 28.6%) compared to those produced by fruit that resulted from single visits by male *P. pruinosa* (Fig. 2; linear mixed model: pollinator t = 3.28, df = 22.9, P = 0.0033). The number of seeds produced per fruit resulting from single visits by male and female *P. pruinosa* did not differ (Fig. 2; linear mixed model: pollinator t = 0.38, df = 27.2, P = 0.70). The volume of single visit fruit resulting from single visits by male and female *P. pruinosa* also did not differ (Fig. 2c; linear mixed model: pollinator t = 0.26, df = 18, P = 0.80).

Discussion

This study system provides a unique view of the pollinator effectiveness of specialists and generalists between agricultural and non-agricultural systems because buffalo gourd is a wild, native plant with a floral morphology very similar to many agricultural species in the same genus (McGregor 1976, Bemis et al. 1978). Several studies have compared the pollinator effectiveness of honey bees and squash bees on agricultural *Cucurbita*, but the nature of agricultural systems may change how pollinators interact with these plants when compared to non-managed systems. The pollinators I examined in this study express differences in their morphology and how they behave in buffalo gourd flowers (Hurd and Linsley 1964, Tepedino 1981, Williams et al. 2009). These differences may help to explain the differing abilities of these pollinators to provide pollination services for *Cucurbita*.

While I was able to conclude that single visits by female *P. pruinosa* resulted in higher fruit set compared to single visits by male *P. pruinosa*, additional single visit data for *Apis* is needed to clarify the effectiveness of this species of pollinator on buffalo gourd. Although both *Apis* and male *P. pruinosa* have relatively low effectiveness, I recorded more than eight times as many male *P. pruinosa* single visits compared to *Apis* single visits. I also recorded more than four times as many male *P. pruinosa* single visits compared to female *P. pruinosa* single visits. Relatively high visitation by male *P. pruinosa* may compensate for their lower per visit effectiveness (Bruckman and Campbell 2014, Canto-Aguilar and Parra-Tabla 2000). Although I recorded only a small number of *X. strenua* single visits (Table 2), these data provide potential evidence that this less common species of squash bee may also be a highly effective pollinator for buffalo gourd.

Although visits to buffalo gourd by squash bees appeared to result in higher fruit set compared to honey bees, my results contrast with those of studies on *Cucurbita* from agricultural systems. A study on summer squash (*Cucurbita pepo*) found that there was no difference in fruit set between *P. pruinosa* and *Apis* single and double visits (Tepedino 1981). These opposing results may be explained by differences between agricultural and non-agricultural systems. In an agricultural system, plants of the same species are often grown together in large, homogenous fields. A lack of alternate floral resources may alter the effectiveness of pollinators visiting those plants, making it more likely for generalists (e.g., *Apis*) to collect and successfully transfer pollen. In the present study, the buffalo gourd plants were patchily distributed over a large area and interspersed with many other flowering plant species (e.g., *Brassica nigra* and *Eriogonum fasciculatum*) attractive to honey bees (*personal observation*). While I observed honey bees in buffalo gourd flowers fairly frequently early in the season, their visitation to buffalo gourd seemed to cease in the later summer months. The generalized behavior of honey bees may mean that they prefer to visit larger floral patches in the area if they are available (Sih and Baltus 1987), so the pollination efficiency of *Apis* may be influenced by the availability of other resources (Canto-Aguilar and Parra-Tabla 2000).

Pollinator effectiveness may also be altered by preferences for one floral sex over the other. When a specialist is a more effective pollinator than a generalist, a strong preference for one flower sex may hinder its ability to successfully transfer pollen

(Larsson 2005). In the *C. pepo* system, it was found that honey bees prefer female *Cucurbita* flowers, whereas squash bees have a preference for male *Cucurbita* flowers (Tepedino 1981). The preference for female flowers by honey bees likely results from female flowers producing more nectar compared to male flowers (Tepedino 1981). Honey bees also have difficulty collecting pollen grains from *Cucurbita* because of their large size (Michelbacher et al. 1964). Female squash bees likely prefer male flowers because of their need to collect pollen, and male squash bees likely prefer male flowers due to the higher probability of finding a mate (Tepedino 1981). Pollen deposition and removal data would be valuable to record for pollinators on buffalo gourd to examine the capability of these pollinators to transfer pollen.

Research on summer squash (Tepedino 1981), as well as a study conducted on pumpkins (*Cucurbita pepo* L.) (Artz and Nault 2011) found no differences in pollen deposition between squash bees and honey bees. However, a study on *Cucubrita moschata* found that female squash bees removed and deposited more pollen grains per visit compared to both honey bees and male squash bees (Canto-Aguilar and Parra-Tabla 2000). Behavioral differences between honey bees and squash bees may contribute to their differing ability to successfully transfer pollen onto stigmas of female flowers. Upon entering squash flowers, honey bees often make their way to the base of the corolla tube to drink nectar (Tepedino 1981, *personal observation*), often only briefly contacting the stigma if they touch it at all. Squash bees, on the other hand, often land on the stigma and remain there while drinking nectar (Michelbacher et al. 1968, Hurd et al. 1974). I also frequently observed squash bees grooming themselves while on stigmas; this behavior may cause more pollen grains to fall off of their body and elevate stigmatic pollen deposition.

A previous study examining pollen competition on buffalo gourd demonstrated a relationship between the size of a pollen load and the number of seeds produced by the resulting fruit (Winsor et al. 2000). If female *P. pruinosa* deposit more pollen compared to male *P. pruinosa* per visit, then I would have expected fruit resulting from female *P. pruinosa* single visits to produce a higher number of seeds. Despite this, I found no difference in the number of seeds produced by single visits made by male squash bees versus female squash bees (Figure 2a). One study showed no difference in the number of seeds produced by honey bee and squash bee single visits on pumpkin (Artz and Nault 2011), but I was not able to make this comparison on buffalo gourd because none of my honey bee single visits resulted in successful fruit set. On other *Cucurbita,* a relationship between pollen load size and seed quality has been described (Hayase 1953 in McGregor 1976, Quesada et al. 1993). I did find that the average seed weight from fruit resulting from female *P. pruinosa* single visits was significantly higher when compared to that of male *P. pruinosa*, but these results should be followed up on to see if they are repeatable. Observed differences in seed weight may be driven by particular plants on which I only had successful single visits for one type of pollinator. This pattern highlights one weakness of the single visit pollination method; it allows for no control over which pollinator species visits each flower on each plant.

The single visit pollination method has several limitations. One limitation is that it only considers female reproductive fitness. Pollen deposition and pollen removal data could be used to examine differences in pollination efficiency between pollinator taxa and to see how these differences compare with agricultural studies. Such information could clarify why differences exist in the ability of these pollinators to initiate fruit set. Another limitation of the single visit pollination method with respect to buffalo gourd is that fruit set from single visits was fairly low overall when compared to the fruit set of controls (Table 2), making it more difficult to compare reproductive variables of single visit fruit pollinated by different species. For example, single visits by *Apis* didn't set any fruit, so I was unable to compare reproductive variables for *Apis*-pollinated fruit. An alternate approach would be to examine how plant reproductive performance increases with increasing consecutive visits by pollinators (i.e., one visit, two visits, four visits), although this presents the challenge of obtaining consecutive visits by the same pollinator species.

In our system, specialist squash bees seem to be superior pollinators of *C. foetidissima* when compared to generalist honey bees. However, in other systems, specialist pollinators are not always the most effective pollinators (Motten et al. 1981, Neff and Simpson 1990). The effectiveness of generalists may change based on the availability of other floral resources, so it's possible that differences between the pollination abilities of specialists and generalists might vary across different plant and pollinator species, across different regions, or even across different years. Further work is needed within this system to test if my results are consistent on a larger spatial and temporal scale. Research should be conducted on visitation of these pollinators to determine if there are any effects of flower sex preference on their ability to transfer pollen. A close examination of the behavioral differences between honey bees and squash bees in agricultural and non-agricultural systems would be an important step towards discovering why there may be differences in the effectiveness of these pollinators on buffalo gourd, and why these relationships differ on agricultural *Cucurbita*.

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Tables

Table 1. Results of simple linear regressions testing for relationships between plant characteristics or pollination timing (independent variables) and reproductive performance (dependent variables) using (a) control and (b) single visit fruit.

(a)

Dependent Variable	**Independent Variable**	***F***	***P***	**Adjusted R^2**	**slope ± SE**
Seed number	Date pollinated	$F_{1,20} = 0.073$	0.789	-0.046	0.551 ± 2.033
Seed number	Distance from center	$F_{1,35} = 0.957$	0.335	-0.001	-0.125 ± 0.127
Seed number	Leaf number	$F_{1,35} = 0.569$	0.456	-0.012	-1.589 ± 2.107
Seed weight	Date pollinated	$F_{1,20} = 1.238$	0.279	0.011	$-9.204 \times 10^{-5} \pm 8.274 \times 10^{-5}$
Seed weight	Distance from center	$F_{1,35} = 1.958$	0.171	0.026	$-1.316 \times 10^{-5} \pm 9.409 \times 10^{-6}$
Seed weight	Leaf number	$F_{1,35} = 2.990$	0.093	0.052	$-2.643 \times 10^{-4} \pm 1.529 \times 10^{-4}$

(b)

Dependent Variable	**Independent Variable**	***F***	***P***	**Adjusted R^2**	**slope ± SE**
Seed number	Date pollinated	$F_{1,34} = 1.021$	0.320	0.001	-0.905 ± 0.896
Seed number	Distance from center	$F_{1,34} = 0.017$	0.897	-0.029	0.020 ± 0.156
Seed number	Leaf number	$F_{1,34} = 0.109$	0.743	-0.026	-0.673 ± 2.036
Seed number	Pollinator time spent	$F_{1,34} = 1.157$	0.290	0.004	0.216 ± 0.201
Seed number	Time visit end	$F_{1,34} = 0.015$	0.904	-0.029	-0.051 ± 0.417
Seed weight	Date pollinated	$F_{1,34} = 3.396$	0.074	0.064	$1.559 \times 10^{-4} \pm 8.461 \times 10^{-5}$
Seed weight	Distance from center	$F_{1,34} = 1.538$	0.223	0.015	$1.851 \times 10^{-5} \pm 1.492 \times 10^{-5}$
Seed weight	Leaf number	$F_{1,34} = 3.265$	0.080	0.061	$3.436 \times 10^{-4} \pm 1.902 \times 10^{-4}$
Seed weight	Pollinator time spent	$F_{1,34} = 0.051$	0.824	-0.028	$4.472 \times 10^{-6} \pm 1.989 \times 10^{-5}$
Seed weight	Time visit end	$F_{1,34} = 0.785$	0.382	-0.006	$3.562 \times 10^{-5} \pm 4.022 \times 10^{-5}$

Table 2. Fruit set for open pollinated control flowers and single visit flowers for different pollinator taxa. Percent fruit set is the percentage of fruit that successfully developed over the total for that treatment. SV = single visit.

	Fruited	**Aborted**	**Total**	**Percent Fruit Set**
Apis mellifera SV	0	10	10	0.0%
Female *Peponapis pruinosa* SV	11	8	19	57.9%
Male *Peponapis pruinosa* SV	20	65	85	23.5%
Female *Xenoglossa strenua* SV	5	3	8	62.5%
Male *Xenoglossa strenua* SV	1	1	2	50.0%
Control	24	4	28	85.7%

Figures

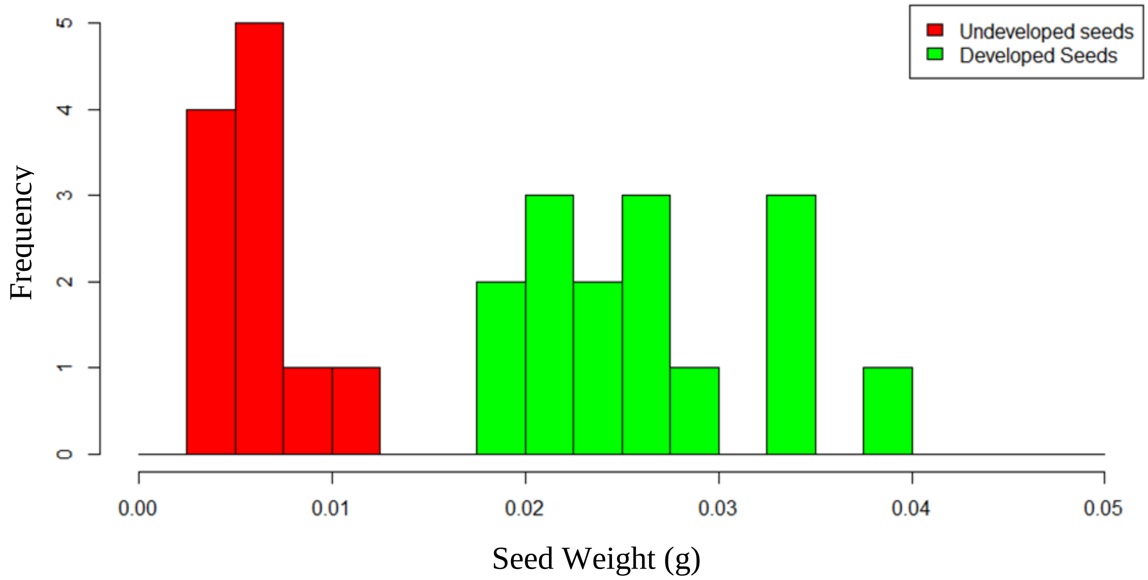
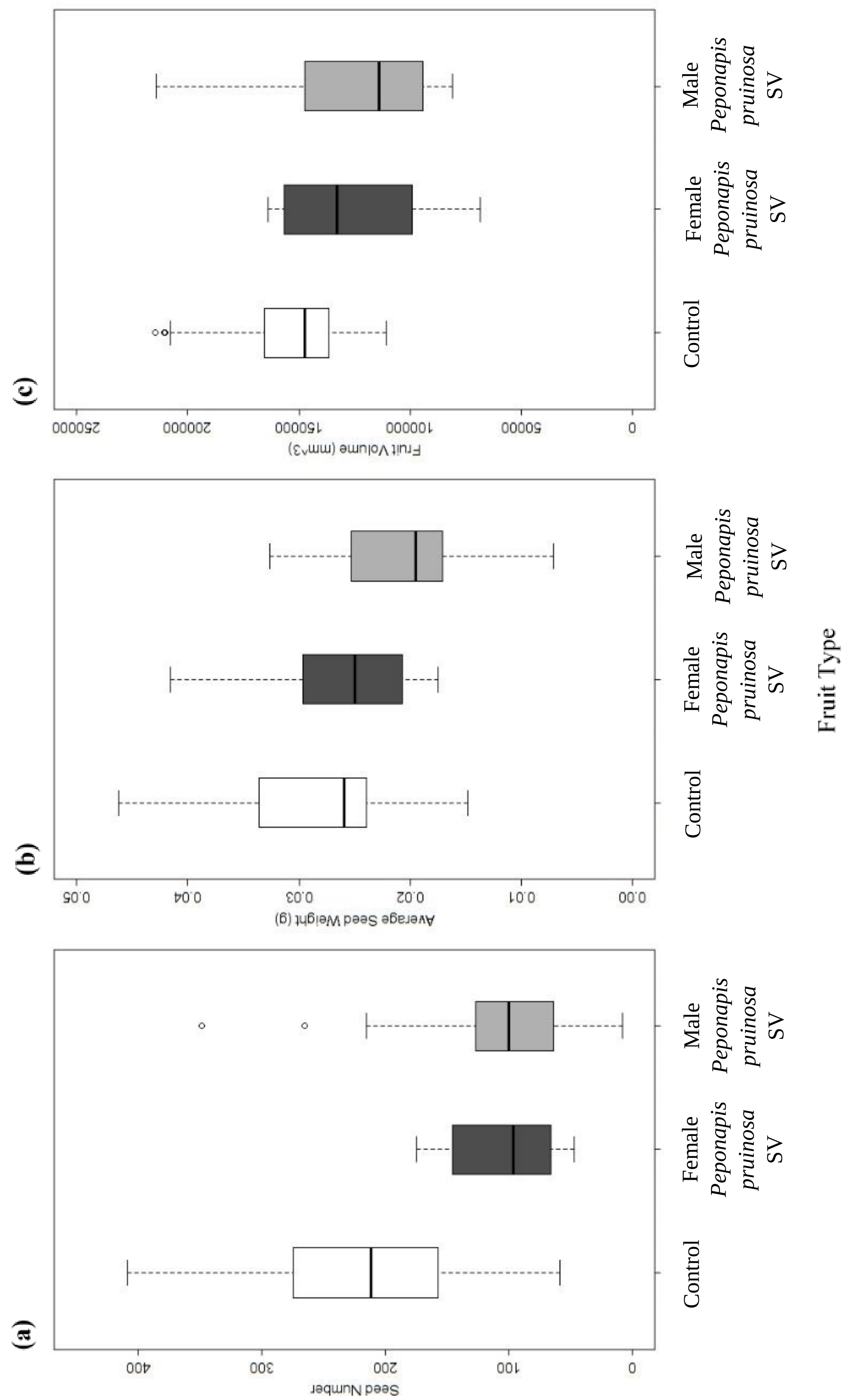


Figure 1. Mean seed weight distributions of developed and undeveloped seeds for control fruit from hermaphrodite buffalo gourds. Developed seeds were collected from n = 38 control fruit and undeveloped seeds were collected from n = 17 control fruit. Mean seed weights were obtained by weighing developed and undeveloped seeds for each fruit and dividing them by the total number of seeds. For plants with more than one fruit, seed weights were averaged across fruit from that plant.

Figure 2. Seed number (a), average seed weight (b), and fruit volume (c) of control fruit and fruit pollinated by single visits by male and female *Peponapis pruinosa* on female and hermaphrodite buffalo gourd. SV = single visit.


(a)
Seed Number
0
100
200
300
400
Control
Female Peponapis pruinosa SV
Male Peponapis pruinosa SV
(b)
Average Seed Weight (g)
0.00
0.01
0.02
0.03
0.04
0.05
Control
Female Peponapis pruinosa SV
Male Peponapis pruinosa SV
(c)
Fruit Volume (mm^3)
0
50000
100000
150000
200000
250000
Control
Female Peponapis pruinosa SV
Male Peponapis pruinosa SV
Fruit Type

Appendices

Appendix 1. GPS coordinates for all buffalo gourd plants (n = 45) mapped at Los Peñasquitos Canyon Preserve. "Plant" refers to separate individual buffalo gourd plants, each of which has been assigned a number (for plants I collected data on) or a letter (for plants I did not collect any data on).

Plant	**LAT**	**LONG**	**Plant**	**LAT**	**LONG**
1	32.90667	-117.20025	A	32.90695	-117.19795
4	32.90715	-117.19725	B	32.90704	-117.19768
5	32.90729	-117.19702	C	32.90728	-117.19695
7	32.91001	-117.20509	D	32.90680	-117.20500
8	32.91048	-117.20531	E	32.91176	-117.20532
10	32.91109	-117.20531	F	32.91305	-117.20440
11	32.91125	-117.20526	G	32.91454	-117.20329
12	32.91141	-117.20527	H	32.91457	-117.20292
13	32.91138	-117.20531	I	32.91534	-117.20402
15	32.91159	-117.20525	J	32.91535	-117.20419
17	32.91466	-117.20284	K	32.91536	-117.20423
19	32.91774	-117.19468	L	32.91568	-117.20197
20	32.91768	-117.19455	M	32.91600	-117.20175
22	32.91756	-117.19426	N	32.91933	-117.19961
24	32.91748	-117.19371	O	32.91961	-117.19921
27	32.91482	-117.20085	Q	32.91949	-117.19577
28	32.91124	-117.20528	R	32.91713	-117.19895
29	32.91969	-117.19860	S	32.91761	-117.19448
30	32.91971	-117.19844	T	32.91726	-117.19372
31	32.91963	-117.19544	U	32.91426	-117.20373
32	32.90700	-117.19816	V	32.90735	-117.21186
33	32.91950	-117.19549	W	32.91792	-117.19702
34	32.91074	-117.20529			

Appendix 2. Fruit set, seed number, seed weight, and fruit volume data for control fruit and fruit resulting from single visits. Pollinator identity is also included for single visit fruit. "Plant" refers to the number assigned to each individual plant. Plants 8, 15, 17, and 19 were female, all the rest were hermaphrodite. "Type" refers to the fruit type (C = control fruit, G = additional fruit harvested in September 2017, SV = single visit fruit).

Plant	Pollinator	Fate of Fruit	Seed Number	Average Seed Weight (g)	Fruit Volume (m^3)	Type
1	-	-	377	0.0262	204191.3117	G
1	-	-	281	0.0259	179363.4204	G
1	M Pepo	Fruited	95	0.0170	94638.0249	SV
4	-	-	258	0.0423	214505.5470	G
4	-	-	130	0.0339	165436.3335	G
4	M Pepo	Aborted	-	-	-	SV
4	M Pepo	Aborted	-	-	-	SV
4	M Pepo	Aborted	-	-	-	SV
4	F Pepo	Fruited	47	0.0416	99126.0544	SV
4	F Pepo	Fruited	105	0.0281	164055.6860	SV
4	F Pepo	Fruited	157	0.0297	156634.3643	SV
4	M Pepo	Fruited	128	0.0237	205704.2489	SV
4	M Pepo	Fruited	93	0.0290	163115.4720	SV
4	M Pepo	Fruited	112	0.0242	140173.8561	SV
4	M Pepo	Fruited	114	0.0296	180673.9503	SV
5	-	-	362	0.0257	207902.7686	G
5	-	-	259	0.0239	159675.0376	G
5	Honey bee	Aborted	-	-	-	SV
5	M Pepo	Aborted	-	-	-	SV
5	M Pepo	Aborted	-	-	-	SV
5	M Pepo	Aborted	-	-	-	SV
5	M Pepo	Aborted	-	-	-	SV
5	F Pepo	Fruited	90	0.0175	68520.3773	SV
5	M Pepo	Fruited	49	0.0146	81081.3764	SV
5	M Pepo	Fruited	91	0.0152	87542.0218	SV
5	M Pepo	Fruited	79	0.0171	110293.3055	SV
7	-	-	203	0.0223	140958.2874	G
7	M Pepo	Fruited	100	0.0195	111297.3180	SV
8	-	-	203	0.0462	144591.0565	G
8	-	-	285	0.0397	167705.5615	G
8	M Pepo	Aborted	-	-	-	SV
8	M Pepo	Aborted	-	-	-	SV
10	-	Aborted	-	-	-	C
10	-	Fruited	356	0.0241	132237.8034	C

10	-	Fruited	105	0.0166	138419.5375	C
10	F Xeno	Aborted	-	-	-	SV
10	M Pepo	Aborted	-	-	-	SV
10	M Pepo	Aborted	-	-	-	SV
10	M Pepo	Aborted	-	-	-	SV
10	M Pepo	Aborted	-	-	-	SV
10	M Pepo	Aborted	-	-	-	SV
10	M Pepo	Aborted	-	-	-	SV
10	F Xeno	Fruited	285	0.0208	153286.0008	SV
10	F Xeno	Fruited	223	0.0218	131236.1642	SV
10	F Xeno	Fruited	0	-	44298.7851	SV
10	M Pepo	Fruited	103	0.0193	122090.6560	SV
10	M Pepo	Fruited	144	0.0222	114118.1718	SV
11	-	Fruited	275	0.0192	171409.1739	C
11	-	-	315	0.0336	181370.3373	G
11	F Pepo	Aborted	-	-	-	SV
11	M Pepo	Aborted	-	-	-	SV
11	M Pepo	Aborted	-	-	-	SV
11	M Pepo	Aborted	-	-	-	SV
12	-	Aborted	-	-	-	C
12	-	Aborted	-	-	-	C
12	-	Fruited	62	0.0148	129371.7479	C
12	-	Fruited	59	0.0234	128384.6526	C
12	-	-	222	0.0157	210202.3688	G
12	F Pepo	Aborted	-	-	-	SV
12	M Pepo	Aborted	-	-	-	SV
12	M Pepo	Aborted	-	-	-	SV
12	M Pepo	Aborted	-	-	-	SV
12	F Pepo	Fruited	70	0.0391	136551.6398	SV
12	F Pepo	Fruited	103	0.0238	146128.5353	SV
12	F Xeno	Fruited	21	0.0174	114288.9222	SV
12	F Xeno	Fruited	310	0.0193	177829.7527	SV
12	M Pepo	Fruited	28	0.0163	94136.7652	SV
12	M Pepo	Fruited	38	0.0071	86119.6303	SV
12	M Pepo	Fruited	8	0.0185	95949.6208	SV
12	M Pepo	Fruited	20	0.0180	89462.7883	SV
12	M Xeno	Fruited	164	0.0253	167264.7469	SV
13	-	Fruited	253	0.0246	134700.6224	C
13	-	-	265	0.0388	140565.7070	G
13	-	-	290	0.0391	146464.2056	G
13	F Pepo	Aborted	-	-	-	SV
13	M Pepo	Aborted	-	-	-	SV

13	M Pepo	Aborted	-	-	-	SV
13	M Pepo	Aborted	-	-	-	SV
13	M Pepo	Aborted	-	-	-	SV
13	M Pepo	Aborted	-	-	-	SV
13	M Pepo	Fruited	265	0.0254	154605.6283	SV
13	M Pepo	Fruited	215	0.0251	121496.5419	SV
13	M Pepo	Fruited	126	0.0327	122686.7037	SV
15	-	-	264	0.0418	150806.2877	G
15	-	-	264	0.0453	176456.9486	G
15	F Xeno	Aborted	-	-	-	SV
15	Honey bee	Aborted	-	-	-	SV
15	M Pepo	Aborted	-	-	-	SV
15	M Pepo	Aborted	-	-	-	SV
15	M Pepo	Fruited	0	-	104610.3580	SV
17	-	Fruited	175	0.0327	110794.5535	C
17	-	Fruited	222	0.0330	136615.7686	C
17	M Pepo	Aborted	-	-	-	SV
17	M Pepo	Aborted	-	-	-	SV
17	M Pepo	Aborted	-	-	-	SV
19	-	-	212	0.0422	136679.9175	G
19	-	-	164	0.0357	124908.9453	G
19	Honey bee	Aborted	-	-	-	SV
19	Honey bee	Aborted	-	-	-	SV
20	-	Fruited	278	0.0217	151561.1183	C
20	-	Fruited	409	0.0246	210544.4857	C
20	F Pepo	Aborted	-	-	-	SV
20	Honey bee	Aborted	-	-	-	SV
20	Honey bee	Aborted	-	-	-	SV
20	Honey bee	Aborted	-	-	-	SV
20	M Pepo	Aborted	-	-	-	SV
20	M Pepo	Aborted	-	-	-	SV
20	M Pepo	Aborted	-	-	-	SV
20	M Pepo	Aborted	-	-	-	SV
20	M Pepo	Fruited	349	0.0258	214418.9131	SV
22	-	Fruited	185	0.0238	147474.3000	C
22	-	Fruited	88	0.0218	136872.4847	C
22	-	Fruited	131	0.0252	134954.9344	C
22	-	Fruited	205	0.0240	155442.9671	C
22	-	Fruited	189	0.0245	154188.0890	C
22	-	Fruited	136	0.0271	119843.2828	C
22	-	Fruited	123	0.0244	145191.3877	C
22	Honey bee	Aborted	-	-	-	SV

22	Honey bee	Aborted	-	-	-	SV
22	Honey bee	Aborted	-	-	-	SV
22	M Pepo	Aborted	-	-	-	SV
22	M Pepo	Aborted	-	-	-	SV
22	M Pepo	Aborted	-	-	-	SV
22	M Pepo	Aborted	-	-	-	SV
22	M Pepo	Aborted	-	-	-	SV
22	M Pepo	Aborted	-	-	-	SV
22	M Pepo	Aborted	-	-	-	SV
22	M Pepo	Aborted	-	-	-	SV
22	M Pepo	Aborted	-	-	-	SV
22	M Pepo	Aborted	-	-	-	SV
22	M Pepo	Aborted	-	-	-	SV
22	M Pepo	Aborted	-	-	-	SV
22	F Pepo	Fruited	146	0.0232	129062.7391	SV
24	-	Fruited	190	0.0263	149644.6676	C
24	-	Fruited	137	0.0274	151698.6303	C
24	-	Fruited	161	0.0249	147204.4889	C
24	F Pepo	Aborted	-	-	-	SV
24	M Pepo	Aborted	-	-	-	SV
24	M Pepo	Aborted	-	-	-	SV
24	M Pepo	Aborted	-	-	-	SV
24	M Pepo	Aborted	-	-	-	SV
24	M Pepo	Aborted	-	-	-	SV
24	M Pepo	Aborted	-	-	-	SV
24	M Xeno	Aborted	-	-	-	SV
27	-	Fruited	141	0.0241	124426.2059	C
27	-	-	205	0.0316	174637.5587	G
27	F Pepo	Aborted	-	-	-	SV
27	M Pepo	Aborted	-	-	-	SV
27	M Pepo	Aborted	-	-	-	SV
27	M Pepo	Aborted	-	-	-	SV
28	F Pepo	Fruited	0	-	104771.4898	SV
29	-	Aborted	-	-	-	C
30	-	Fruited	223	0.0198	164854.0680	C
30	-	Fruited	157	0.0170	135975.3833	C
30	M Pepo	Aborted	-	-	-	SV
30	F Pepo	Fruited	66	0.0207	94537.6310	SV
30	F Pepo	Fruited	57	0.0261	100061.1997	SV
30	F Pepo	Fruited	175	0.0197	159106.3780	SV
31	-	Fruited	227	0.0301	154954.1531	C
31	-	-	136	0.0386	160102.4204	G

31	F Pepo	Aborted	-	-	-	SV
31	F Pepo	Aborted	-	-	-	SV
31	M Pepo	Aborted	-	-	-	SV
31	M Pepo	Aborted	-	-	-	SV
31	M Pepo	Aborted	-	-	-	SV
31	M Pepo	Aborted	-	-	-	SV
31	M Pepo	Aborted	-	-	-	SV
31	M Pepo	Aborted	-	-	-	SV
32	M Pepo	Aborted	-	-	-	SV
33	-	-	298	0.0322	131924.2456	G
33	-	-	279	0.0333	138031.6872	G
33	M Pepo	Aborted	-	-	-	SV
34	F Xeno	Aborted	-	-	-	SV