



Temporal variation in pollination services to *Cucurbita moschata* is determined by bee gender and diversity

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Abstract. It has been proposed that species-rich insect communities and species turnover across landscapes enhance the pollination efficiency of crops through complementarity, where both the dominant and less abundant species contribute to reaching a yield threshold from pollination. Alternatively, fluctuations in the most abundant pollinator species, rather than changes in species richness, may drive temporal variation in pollination services. In this study, we used *Cucurbita moschata* as a model to investigate temporal variation in pollinator communities in a Mexican tropical dry forest region. We sampled floral visitors in the coastal region of Jalisco during the wet and dry seasons and determined the pollination efficiency of all floral visitors. Our results showed that there was temporal variation in the pollinator community and in the pollination efficiency of the main pollinators of *Cucurbita moschata* crops. In the wet season, native bees of the genus *Peponapis* were the most frequent and effective pollinators of *C. moschata*, whereas in the dry season, *Peponapis* bees were scarce and *Apis mellifera* became the most frequent floral visitor. *Apis mellifera* transfers smaller pollen loads than *Peponapis*, but it provides an effective pollination service in conjunction with other native bees during the dry season. There was also an interaction between flower gender and pollinator species, where *A. mellifera* had higher visitation rate to female *C. moschata* flowers, and *Peponapis* bees to staminate flowers. Mean visitation rate by *Peponapis* female bees was 17 times higher than visitation rate by male bees. This is the first report of a vis-à-vis relationship of pollinator gender with respect to plant gender in which plants of the genus *Cucurbita* that produce unisexual staminate and pistillate flowers are differentially visited by *Peponapis* male and female bees, where females are the main pollinators. Understanding the temporal variation in pollinator communities and the contribution of the different species of pollinators to the reproductive success of different crop species and varieties can be crucial to maintaining pollination services under the current global pollination crisis.

Key words: *Apis mellifera*; bees; crop pollination; fruit set; native pollinators; *Peponapis*; plant reproductive success; pollination service; pollinator gender; seed production; squash; tropical dry forest.

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INTRODUCTION

Recent evidence of the decline of introduced and wild pollinators in North America and Europe has caused great concern worldwide given its potentially catastrophic consequences on food security (Potts et al. 2010*a, b*). The global decline in pollinator abundance has caused a reduction in pollination services and crop yields (Millennium Ecosystem Assessment 2005). Under this scenario, the conservation of native pollinators may be crucial to ensure crop pollination success (Winfree et al. 2007, Garibaldi et al. 2013), since the contribution of native pollinators to fruit and seed production is often higher than the contribution of introduced pollinators (Canto-Aguilar and Parra-Tabla 2000, Gibbs et al. 2016). This is possibly the result of a shared evolutionary history between plants and native pollinators that has often resulted in specialized plant–pollinator interactions (Ashworth et al. 2009, Gibbs et al. 2016). Studies investigating pollination services of native pollinators to crop species are increasingly important to ensure global food security, crop productivity, and yield stability.

Despite the importance of native pollinators for crop productivity, only a few studies have documented the performance of native pollinator species and the impact of seasonal changes of the pollinator community on crop productivity. Diverse pollinator communities provide more stable and efficient pollination services (Hoehn et al. 2008, Klein et al. 2009, Rogers et al. 2014, Winfree et al. 2018), and temporal changes of pollinator assemblages through the year may contribute to the diversity of pollinator communities and to the stability of crop production. Alternatively, seasonal variation in primary pollinators or the lack of efficient pollinators in one season may result in a seasonal reduction in crop production. Temporal changes in pollinators have been documented for *Citrullus lanatus* and *Vaccinium angustifolium* in North America (Kremen et al. 2002, Bushmann and Drummond 2015); however, little is known about temporal variation in pollinator communities and pollinator efficiency in tropical regions. To propose viable strategies for the management and conservation of pollinator services for tropical crops, it is necessary to understand how changes in pollinator communities impact crop production across different seasons.

Temporal variation in pollinator services in tropical regions is relevant to annual and perennial crops with long or inducible flowering seasons. Most crop species are annual and have a single flowering season per year. However, irrigation allows cultivation at different times of the year, enabling farmers to harvest more than once a year, especially in areas near rivers and wetlands (Armillas 1949). This is the case of crops that are cultivated in Neotropical dry forest regions during the dry and wet seasons, such as the squash, *Cucurbita moschata*. Given that plant flowering time and pollinator activity should be evolutionarily tuned to maximize both plant and pollinator fitness, native pollinators should be present or active during the natural flowering season of a native crop species. Moreover, the native pollinators of a crop species should be more effective than introduced pollinators. However, if native pollinators are absent when native crops are cultivated out of their season, then introduced pollinators may provide alternative pollination services.

In agricultural fields of the tropical dry forest regions of Mesoamerica, farmers take advantage of natural rainwater in the wet season to grow crops like maize, backyard squash (*Cucurbita moschata*) and crops for livestock food. Crops of *C. moschata* are mainly cultivated for self-consumption. In the dry season, agriculture is more technified because farmers need to irrigate their crops from nearby water sources. Farmers generally use the alluvial soils of riverbanks that flood during the wet season but remain arable land during the dry season. This practice eliminates the risk of flooding and loss of crops that often occurs in the wet season. Furthermore, because the populations of many adult insect herbivores are reduced during the dry season, there should be lower herbivory (Dirzo and Domínguez 2002, Cuevas-Reyes et al. 2006), which is expected to increase crop yields. In Mexico, some crops are grown in both the dry and wet seasons, and include various species of squashes, such as *C. moschata* and *C. pepo*. These species are ideal to evaluate temporal and spatial variation in pollinator assemblages and pollination services for native crop species.

The genus *Cucurbita* is a group of monoecious, self-compatible plants, pollinated mainly by bees, and cultivated worldwide (Hurd et al. 1971, Lira-Saade 1995). There are about 27

species, of which five species have been domesticated: *C. pepo*, *C. ficifolia*, *C. moschata*, *C. maxima*, and *C. argyrosperma* (Whitaker 1974, Lira-Saade 1995). Mexico is considered an important center of genetic diversity of the genus *Cucurbita* (Lira-Saade and Montes 1992, Lira-Saade 1995); since pre-Hispanic times, four of the five species have been domesticated in the country, and 11 wild-type taxa have been recorded (Lira-Saade 1995). Bees of the genus *Peponapis* have been documented as the main pollinators of *Cucurbita* (Hurd et al. 1971, 1974, Tepedino 1981, Canto-Aguilar and Parra-Tabla 2000), although some studies report other bee genera as frequent floral visitors (Artz et al. 2011, Enríquez et al. 2015). *Peponapis* is exclusive to the American continent, and in particular, *Peponapis pruinosa* and *P. crassidentata* share a close evolutionary history with the distribution and domestication of *Cucurbita* (López-Urbe et al. 2016).

We evaluated the pollination services to *Cucurbita moschata* in the coastal region of Jalisco, Mexico, during the wet and dry seasons. *Cucurbita moschata* is an ideal study species because it is highly dependent on pollinators for seed production (Free 1993, Klein et al. 2007); in North America, native and introduced bees perform most of the pollination (Tepedino 1981, Canto-Aguilar and Parra-Tabla 2000, Artz et al. 2011), and in tropical dry forest habitats, crops are cultivated during both dry and rainy seasons. We studied the pollinator community composition, foraging behavior, and efficiency of pollinators for fruit and seed production of *C. moschata* across two seasons. *Peponapis* and *Xenoglossa* bee species are pollen specialists on *Cucurbita*, and have active nests during the wet season, coinciding with the natural flowering time of *Cucurbita* (Rozen and Ayala 1987, Ayala 2004, Delgado-Carrillo et al. 2017). Therefore, we predict that during the wet season, the pollinator community of *C. moschata* crops will be dominated mainly by native bees with high pollination efficiency. In the dry season, the pollinator community should change to more generalized and less efficient pollinators.

MATERIALS AND METHODS

Study plant

Cucurbita moschata is an annual herbaceous, climbing, and monoecious plant. Full development

of plants occurs within one to three months, and flowering lasts approximately two to three months (Lira-Saade and Montes 1992). *Cucurbita moschata* has staminate and pistillate yellow-orange flowers on the same individual plant. Floral anthesis lasts approximately six hours, with flowers opening just before dawn and closing at noon. Both types of flowers produce abundant nectar. Staminate flowers have a long pedicel, measuring between 16 and 18 cm. Pistillate flowers have shorter pedicels, 3–8 cm long, three-lobed stigmas, and an ovary that contains approximately 700 ovules (Canto-Aguilar and Parra-Tabla 2000). The pollen grains are large and sticky; thus, their transfer requires a biotic vector (Free 1993). Pollinators of *C. moschata* are essential for fruit and seed production (Klein et al. 2007).

Study site

We conducted the study in the municipalities of Cihuatlan and La Huerta, located in the southwest coast of Jalisco, Mexico. The altitude in this region ranges from 0 to 1300 m.a.s.l. The climate is predominantly warm subhumid with summer rains, with a mean annual temperature of 20–28°C, and a mean annual precipitation of 600–2000 mm. Agriculture occupies 21–25% of the area of the municipalities (INEGI 2009), while tropical dry forest occupies 56.1% of the coastal area of Jalisco (Sánchez-Azofeifa et al. 2009).

Crops of *Cucurbita moschata* are cultivated during the wet and the dry seasons. The dry season in this tropical dry forest begins in November and lasts to the end of May. This season coincides with the cultivation of other pollinator-dependent crops such as watermelon (*Citrullus lanatus*), tomatillo (*Physalis ixocarpa*), chili (*Capsicum annum*), cucumber (*Cucumis sativus*), chayote (*Sechium edule*), and tomato (*Solanum lycopersicum*). The wet season begins in June and lasts until October–November. Cultivation of other pollinator-dependent crops (e.g., tomatillo, *Physalis ixocarpa*; and watermelon, *Citrullus lanatus*) in this season is rare because farmers avoid cultivating these crops to prevent the risk of losing crop production to flooding or pests. We conducted our sampling at five *Cucurbita moschata* cultivated fields, two field plots during the wet season of 2015 (mid-September–October) and three field plots during the dry season of 2016

(January–March). Specific coordinate locations are provided in the supplementary material (Appendix S1: Fig. S1).

Pollination community and foraging behavior

We evaluated differences in the composition of the pollinator community and in visitation patterns between seasons, flower genders, and pollinator species. We conducted 190 h of video recording, focusing on staminate and pistillate flowers from 7:00 to 11:30 hour, in both the dry and the wet seasons. In the wet season, we filmed 10 staminate flowers and ten pistillate flowers on 20 individual plants (i.e., one flower observed per plant) in two different plots, plot one with 8 flowers and plot two with 12 flowers (Appendix S1: Fig. S1). In the dry season, we filmed 15 staminate flowers and 17 pistillate flowers on 32 individual plants (i.e., one flower observed per plant) in three different plots, plot three with 13 flowers, plot four with 9 flowers, and plot five with 10 flowers (Appendix S1: Fig. S1). For each floral visit, we recorded the pollinator species (to the lowest possible taxonomic level), time of arrival at the flower, and duration of the visit; we also quantified pollination events by registering visitors' contact with the reproductive organs of the flower. For identification, we collected individuals of each floral visitor species and used the bee species guide and world checklist (Asher and Pickering 2017). We collected two species of *Peponapis*, but due to the difficulty of identifying species in video recordings, we report visitation by bees in the genus *Peponapis*, rather than by species. We were able to separate *Peponapis* by sexual gender using the sexual characters antennae length (longer in male bees) and presence of scopae in female bees (Canto-Aguilar and Parra-Tabla 2000, Cane et al. 2011).

We calculated visitation rates (visits per flower per hour) for each pollinator species and observation period (i.e., for each flower filmed). We evaluated the foraging behavior of insect species that contacted the reproductive organs of the flower by assessing the duration in minutes of each pollination event.

Pollen loads on pollinators' bodies

To determine the capacity of different floral visitors to carry pollen of *C. moschata*, we captured visitors at staminate flowers (six species of

visitors, 3–15 individuals of each species) and examined pollen loads. Captures were performed between 8:00 and 10:00 hours. Each individual was captured on a separate killing vial. We removed pollen from each animal body dabbing one piece of fuchsin gel over four different parts of the pollinator body, maintaining the samples separated: back, head, ventral abdomen, and ventral torso. We did not remove pollen from specialized structures for pollen transport (i.e., corbiculae, scopae). We deposited each pollen sample on a slide, and counted the number of pollen grains of *C. moschata* with a stereoscopic microscope and the Zen program V 1.1.2 (Zen 2012). For statistical analyses of pollen samples, we considered *Peponapis crassidentata* and *P. utahensis* as a single taxon to be consistent with other analyses because we could not discriminate among these species on video recordings. We analyzed pollen loads separately for male and female *Peponapis* because they visit flowers at different rates and carry different amounts of pollen.

Pollinator efficiency experiments

To evaluate pollinator efficiency and the relative contribution of the different species to the pollination of *C. moschata*, we performed exclusion experiments at the five field plots during the flowering season of the crop. These experiments were conducted only for *Peponapis* sp., *Apis mellifera*, and *Trigona fulviventris*, because sample sizes were too low for the remaining species. In each plot, we examined all plants on the day of sampling and covered with a mesh bag five to ten large flower buds. The following day, upon opening, flowers were exposed to natural floral visitation and assigned to one of the following six treatments according to the number of visits received by particular insect species: (1) one visit by female *Peponapis* spp. ($n = 16$ plants), (2) one visit by *Trigona fulviventris* ($n = 13$ plants), (3) one visit by *Apis mellifera* ($n = 28$ plants), (4) three visits by *A. mellifera* ($n = 15$ plants), (5) all visitors excluded, a control for autonomous self-pollination ($n = 15$ plants), (6) open pollination control in wet season ($n = 28$ plants), and (7) open pollination in dry season ($n = 42$ plants). We only conducted treatment number 5 (pollinator exclusion) during the wet season. We did not repeat this treatment in the following dry season because the flowers that received the exclusion treatment in

the wet season did not produce fruit. After floral visitation, flowers were labeled and re-bagged for the rest of the day, removing the bag the following day upon flower senescence. In the case of open-pollinated flowers, the bag was removed before anthesis and the flower was marked. A week later, we counted the flowers that initiated fruit development, and a month later, we collected mature fruits and counted all viable seeds in each fruit. Variation in sample sizes among treatments was the result of different visitation frequencies of the different bee species.

Statistical analyses

To determine whether pollinator visitation rates and duration of pollinator visits differed between pollinator species, seasons, and flower gender, we used the GLIMMIX procedure in SAS version 9.4 (SAS Institute Inc 2014). We consider pollinator visitation rate as the number of visits by each pollinator to each flower per hour and the duration of pollinator visits as the time in minutes that each pollinator made to each flower. We conducted generalized linear mixed models to analyze: (1) the effect of season (dry or wet), pollinator species and their interaction (fixed effects) on pollinator visitation rates, and duration of individual pollinator visits (response variables); (2) the effect of flower gender, pollinator species, and their interaction on the same response variables. In both analyses, field plot was included as a random effect in the model. We specified a Poisson distribution and a log link function for both response variables. We considered male and female *Peponapis* as separate entities of the variable pollinator species to determine the contribution of each gender to pollination. In all analyses, we specified the ILINK option of the LS-MEANS statement to obtain back-transformed least square means and a Tukey adjustment for multiple comparisons.

To evaluate the capacity of different floral visitors to carry pollen of *C. moschata*, we performed a generalized linear mixed model with GLIMMIX procedure in SAS version 9.4 (SAS Institute 2014). The model included pollinator species as a fixed effect and pollen count as response variable. This analysis used a Poisson distribution and a log link function, the ILINK option of the LS-MEANS statement was used to obtain back-transformed least square means, and field plot

was included as a random effect in the model. All bees contacted stigmas of pistillate flowers of *C. moschata* with the lower part of their body; thus, for analyses, we used the sum of pollen loads from ventral abdomen and ventral torso. Because we only captured three species in both seasons (*Apis mellifera*, *Agapostemon* sp., *Augochloropsis metallica*), we considered bees collected in the dry and wet seasons as separate entities in this statistical analysis; for example, we considered *Apis mellifera* in wet season different from *A. mellifera* in dry season. To compare the relative contribution of the different pollinator species to fruit set and seed set, we performed generalized linear models with GENMOD procedure in SAS version 9.4 (SAS 2014). The model used pollination treatment as the independent variable with the proportion of flowers that developed into fruit and number of seeds produced per fruit as response variables. The analysis used a binomial distribution and a logit link function for fruit set and a normal distribution for seed set. We used the Tukey-adjusted *P*-values for multiple comparisons.

RESULTS

Pollinator community and foraging behavior

The most frequent floral visitors in the wet season were bees of the genus *Peponapis*, accounting for 92% and 95% of the total visits to staminate and pistillate flowers, respectively. Peak visitation occurred between 07:30 and 08:30 hours, with female *Peponapis* leading the visits to both staminate and pistillate flowers. In contrast, in the dry season, the most frequent visitor was *Apis mellifera*, accounting for 77% and 70% of the total visits to staminate and pistillate flowers, respectively; peak visitation in this season occurred between 09:00 and 10:00 hours (Fig. 1).

During the dry season, we observed 15 species of floral visitors; five of these were excluded from statistical analyses because they were all recorded a single time the same sampling day. In the wet season, we recorded a subgroup of six pollinator species. We did not find significant differences in pollinator visitation rates between seasons ($F_{(1,3)} = 2.33$, $P = 0.2$), but we found significant differences in pollinator visitation rates between pollinator species ($F_{(9,3)} = 13.08$, $P = 0.02$) and a significant interaction between season and

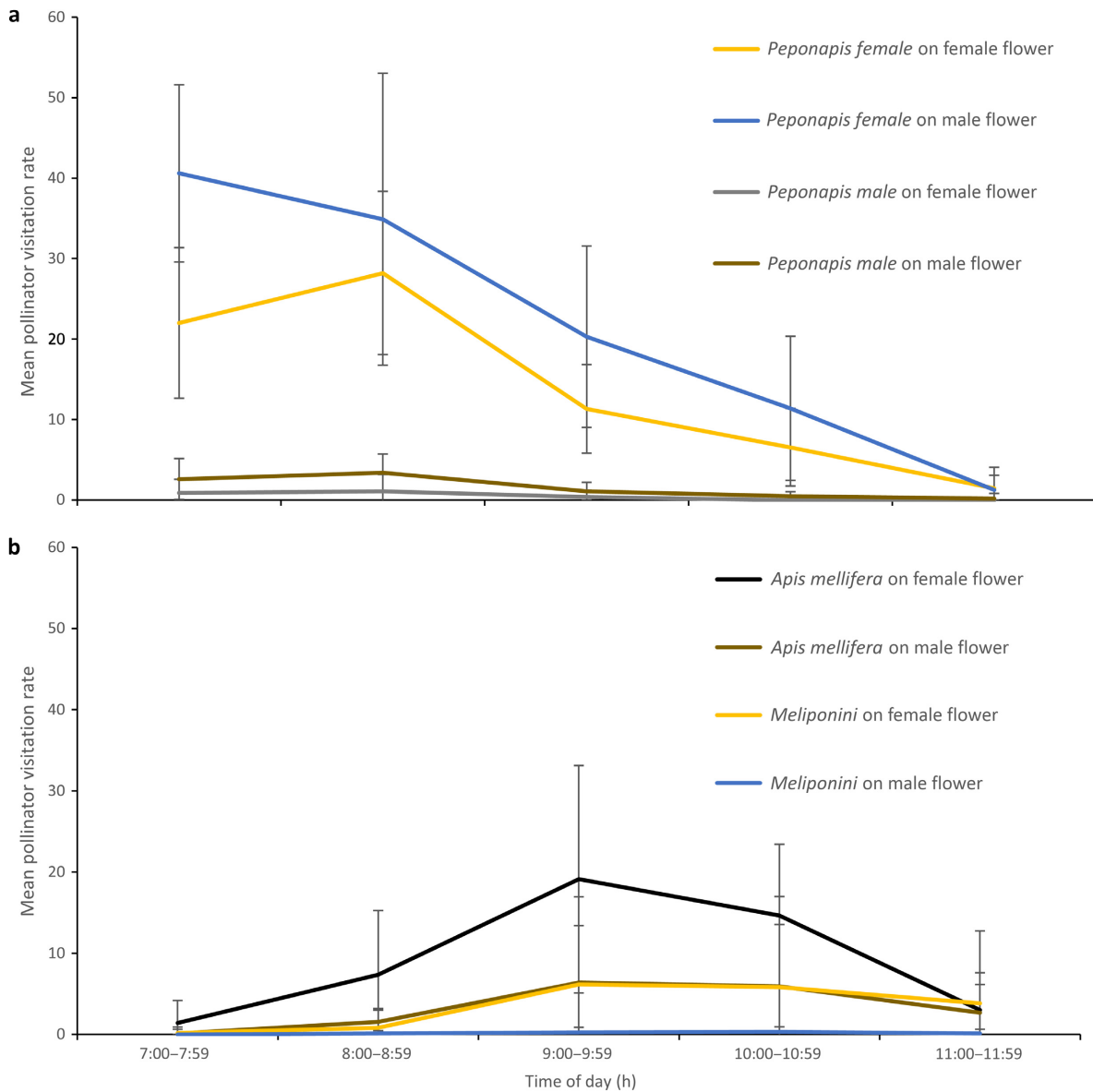


Fig. 1. Mean (\pm SE) number of pollinator visits-flower⁻¹·h⁻¹ to female and male flowers of *Cucurbita moschata* throughout floral anthesis in (a) wet season and (b) dry season. Bee species not included in (a) (*Apis mellifera*, meliponini and halictid bees) and (b) (*Peponapis* and halictid bees) had less than one visit-flower⁻¹·h⁻¹. Meliponini bees include *Scaptotrigona mexicana*, *Trigona fulviventris*, and *T. nigra*.

pollinator species ($F_{(5,3)} = 13.32$, $P = 0.02$), with *Peponapis* as the main floral visitor in the wet season, and *Apis mellifera* as the main visitor in the dry season; pollinators from nine other species were only present in the dry season (Fig. 2).

We did not find significant differences in pollinator visitation rates by flower gender ($F_{(1,3)} = 2.04$, $P = 0.2$), but we found significant

differences between pollinator species ($F_{(9,3)} = 29.39$, $P = 0.009$) and a significant interaction between flower gender and pollinator species ($F_{(9,3)} = 12.6$, $P = 0.03$), where we observed a strong preference for staminate flowers by *Peponapis* and by pistillate flowers in *Apis mellifera*, *Trigona fulviventris*, and *Scaptotrigona mexicana*, and for staminate flowers in *Peponapis* female bees (Fig. 3).

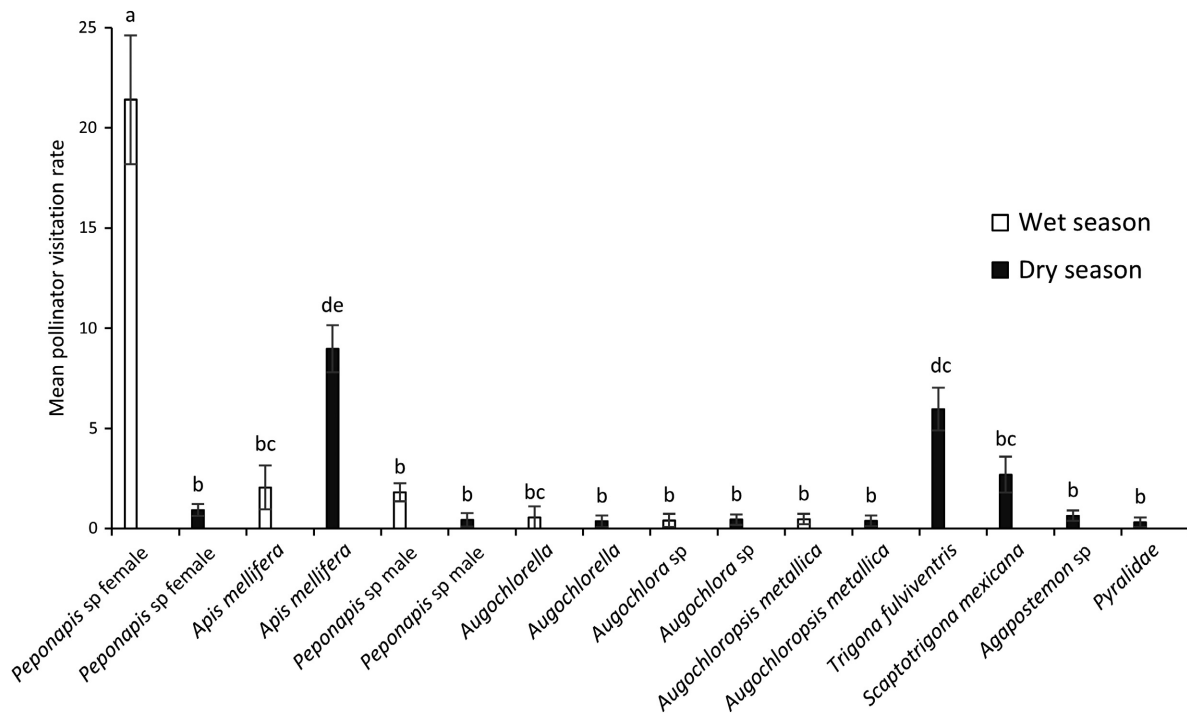


Fig. 2. Mean (\pm SE) number of pollinator visits-flower⁻¹·h⁻¹ by pollinator taxa in the wet season (white bars) and dry season (black bars). Different letters indicate significant differences between groups ($P < 0.05$) with Tukey's ad hoc test.

We did not find significant differences in the duration of visits to flowers by season ($F_{(1,3)} = 1.57$, $P = 0.29$), but we found significant differences between pollinator species ($F_{(9,3)} = 42.8$, $P = 0.005$) and in the interaction between season and pollinator species ($F_{(5,3)} = 10.45$, $P = 0.04$; Fig. 4). Both *Apis mellifera* and *Peponapis* made significantly longer visits to flowers in the wet season than in the dry season (Fig. 4). We did not find significant differences in the duration of flower visits by flower gender ($F_{(1,3)} = 6.42$, $P = 0.08$), nor in the interaction between flower gender and pollinator species ($F_{(8,3)} = 6.22$, $P = 0.08$), but we found significant differences in the duration of flower visits by pollinator species ($F_{(9,3)} = 73.34$, $P = 0.002$). The duration of flower visits by *A. mellifera* was at least three times higher than the duration of other pollinator species.

Pollen load on pollinators' body

We collected 93 individuals from six bee species. All the bees captured in staminate flowers had pollen in some part of their body. Since we found

flowering individuals of *C. argyrosperma* in the wet season and it was difficult to differentiate the pollen of each *Cucurbita* species, we report pollen counts for *Cucurbita* spp. We found significant differences among pollinator species ($F_{(9,80)} = 674.56$, $P < 0.0001$; Fig. 5). In the dry season, *A. mellifera* carried pollen loads that were four times greater than the loads carried by this species in the wet season; other bees did not change their efficiency or were simply present in one season. The pollen load of *Peponapis* female bees was similar to the pollen load of other pollinator species that carry large amounts of pollen in their bodies.

Pollinator efficiency experiments

Due to the changes in pollinator assemblages between seasons, we only performed efficiency experiments with *Peponapis* spp in the wet season and with *Apis mellifera* and *Trigona fulviventris* in the dry season. We found significant differences in fruit set between pollination treatments ($\chi^2 = 64.2$, $df = 6$, $P \leq 0.0001$). Flowers under complete pollinator exclusion and flowers

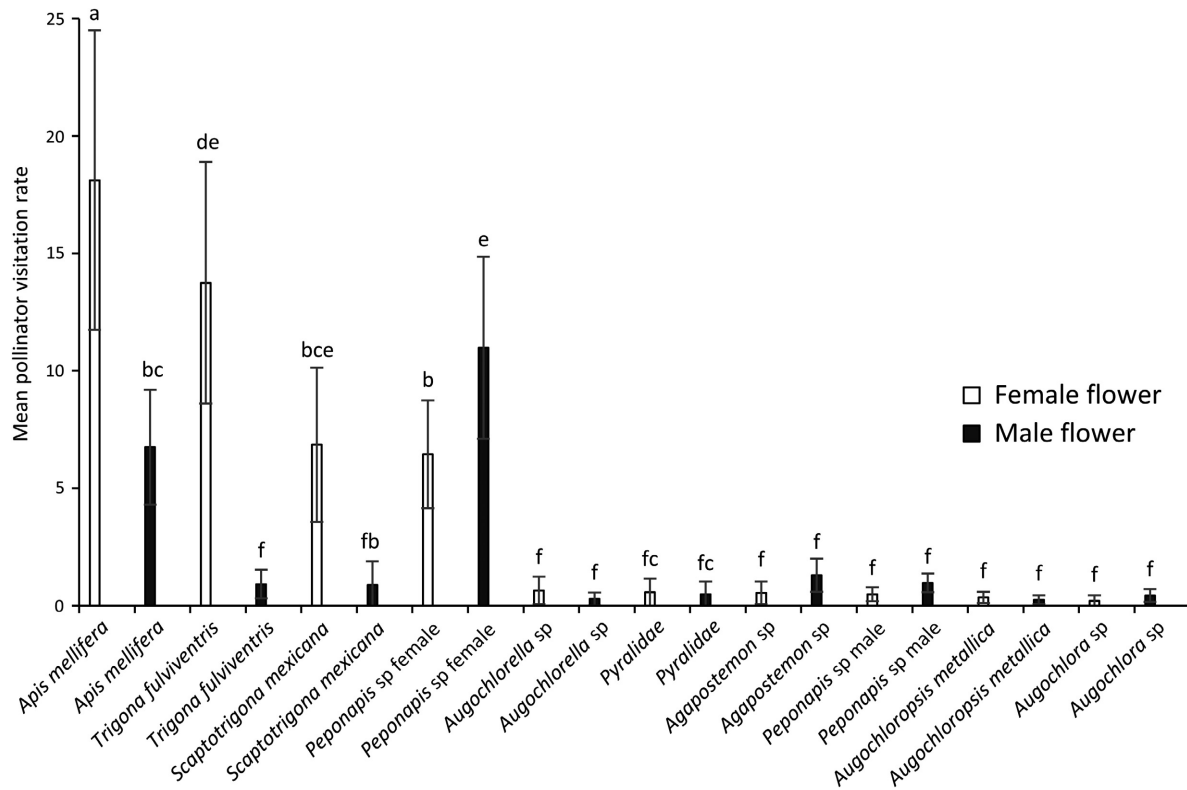


Fig. 3. Mean (\pm SE) number of pollinator visits-flower⁻¹·h⁻¹ by pollinator taxa in female (white bars) and male flowers (black bars). Different letters indicate significant differences between groups ($P < 0.05$) with Tukey's ad hoc test.

exposed to a single visit of *T. fulviventris* did not produce any fruit. We did not find significant differences in fruit set after one visit of *Peponapis* female bees and open pollination, and these two treatments produced the highest fruit set. Fruit set after one and three visits of *A. mellifera* was significantly lower than the fruit set of open-pollinated flowers and the fruit set of one *Peponapis* visit treatment (Fig. 6). For seed production, we collected a total of 56 fruits of *C. moschata*; on average, fruits from open-pollinated flowers produced 538 ± 32.4 seeds ($n = 22$) in the wet season and 510 ± 29.8 seeds ($n = 26$) in the dry season, fruits from the single female *Peponapis* visit produced 446 ± 61.9 ($n = 6$) seeds, and fruits developed from flowers that received three *A. mellifera* visits produced 384 (± 69.3 , $n = 2$) seeds. There were no differences in seed number per fruit between the single *Peponapis* visit treatment and the open pollination treatment in wet and dry seasons ($\chi^2 = 1.81$, $df = 2$, $P = 0.4$). The

three *A. mellifera* visits treatment was excluded from the statistical analysis due to the small sample size ($n = 2$ fruits).

DISCUSSION

Few studies have shown that species-rich insect communities enhance the pollination efficiency of native plants and crops through complementarity, where both the dominant and less abundant pollinator species contribute to reaching a yield threshold (Hoehn et al. 2008, Winfree et al. 2018). Moreover, species turnover across landscapes can also be important to attain this threshold (Winfree et al. 2018). However, in some agricultural systems, fluctuations in the frequency of the most abundant pollinator species, rather than changes in species richness, may drive temporal variation in pollination services (Genung et al. 2017). This study showed that temporal variation in the pollinator community

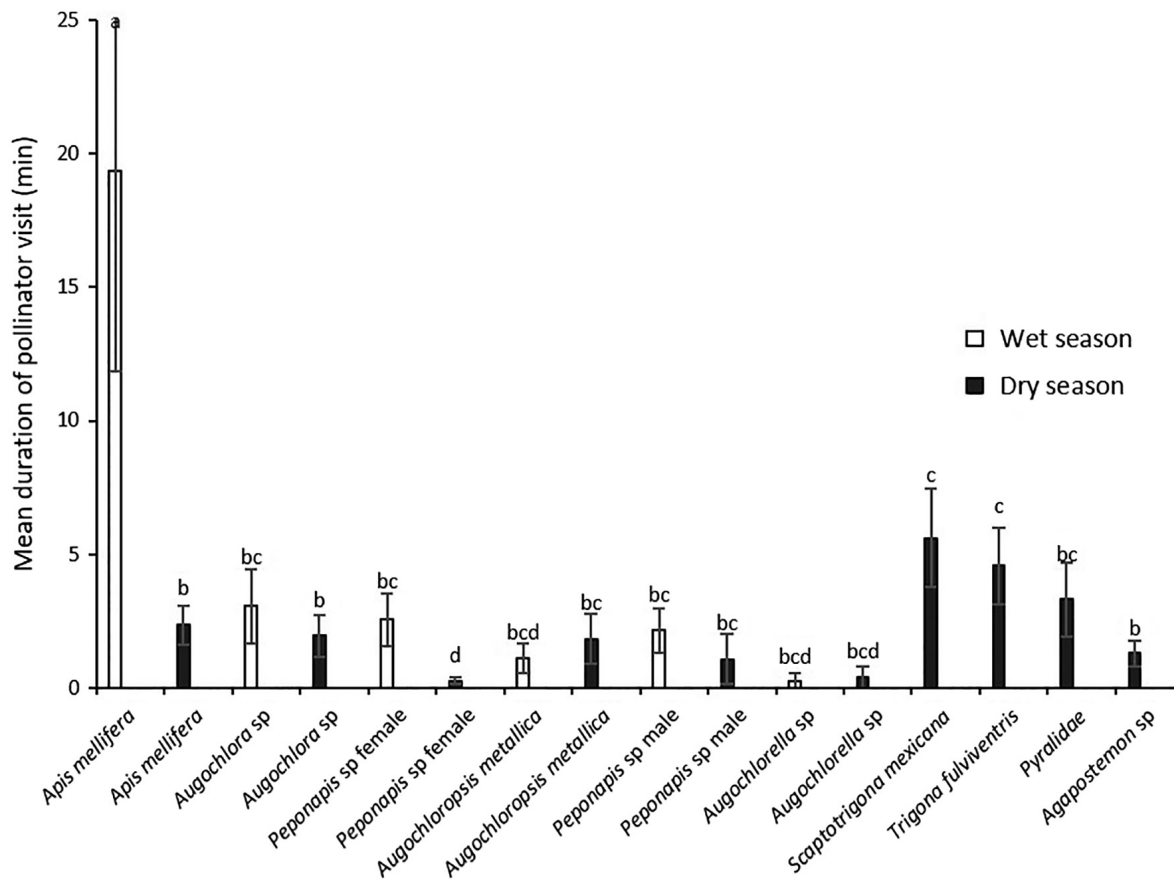


Fig. 4. Mean (\pm SE) duration (min) of pollinator visits-flower⁻¹·h⁻¹ by pollinator taxa in wet (white bars) and dry season (black bars). Different letters indicate significant differences between groups ($P < 0.05$) with Tukey's ad hoc test.

significantly affected the pollination efficiency of *Cucurbita moschata* crops between wet and dry seasons in cultivated areas surrounded by tropical dry forest. In the wet season, native bees of the genus *Peponapis* were the most frequent and effective pollinators of *C. moschata*, with little contribution from other bee species. In contrast, in the dry season, *Peponapis* bees were scarce and *Apis mellifera* became the main floral visitor of *C. moschata* crops. The pollination efficiency results of our study indicate that three visits of *A. mellifera* did not increase fruit set over one visit of *A. mellifera*, but the fruit set of open pollination was similar in both seasons; therefore, in the dry season, complementarity by other bee species contributes to fruit production in *C. moschata* crops. Similarly, complementarity by native bees in conjunction with *A. mellifera*

contributes to pollination services in other crops (Greenleaf and Kremen 2006, Brittain et al. 2013).

Temporal variation in pollinator visitation and efficiency determined the reproductive success of *C. moschata*. In the wet season, *Peponapis* bees visit *C. moschata* flowers early in the morning before other species arrive at flowers, removing and depositing more viable pollen than other pollinator species; furthermore, *Peponapis* bees had four times greater pollen loads and visitation rates to pistillate flowers than *A. mellifera*. These results suggest that when *Peponapis* bees are present, they are the most effective pollinators of *C. moschata*. However, the importance of *Peponapis* bees in the pollination of *Cucurbita* species is not consistent across the geographic distribution of these crops. In the Yucatan Peninsula in Mexico and in western United States, the genus

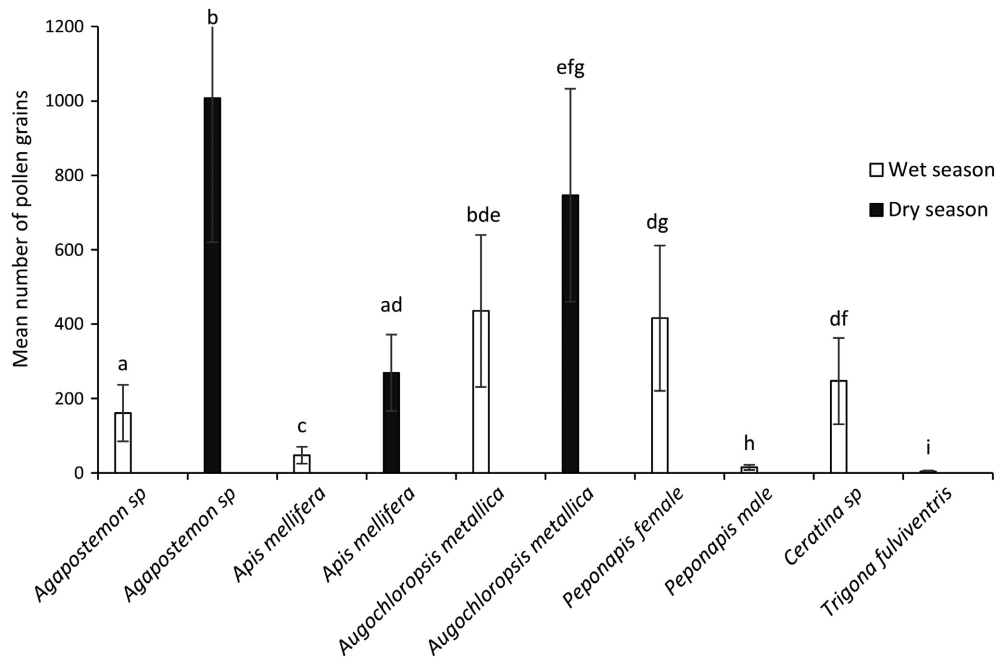


Fig. 5. Mean (\pm SE) number of *Cucurbita* pollen grains on the bodies of each pollinator taxon. White bars represent the pollinators in the wet season and black bars pollinators in the dry season. Different letters indicate significant differences between groups ($P < 0.05$) with Tukey ad hoc test.

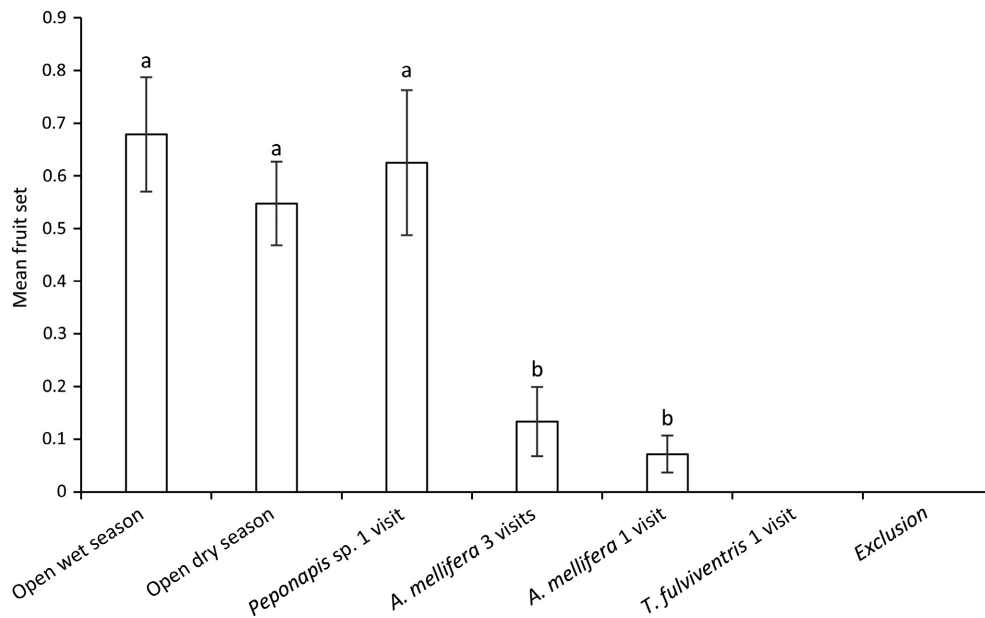


Fig. 6. Mean fruit set (\pm SE) of pollinator efficiency treatments. Different letters indicate significant differences between treatments ($P < 0.05$) with Tukey ad hoc test. The treatments pollinator exclusion and one visit of *Trigona fulviventris* did not produce fruits.

Peponapis is reported as one of the main pollinators of squash crops (Tepedino 1981, Canto-Aguilar and Parra-Tabla 2000); however, at other sites in North America, Guatemala, and Brazil, this group of bees is apparently poorly represented or not observed in squash crops (Serra and Campos 2010, Petersen et al. 2013, Enríquez et al. 2015, Phillips and Gardiner 2015). One possible explanation for the apparent incongruence in the importance of *Peponapis* bees for squash crops from different geographic regions is that most studies have not considered temporal variation in floral visitation to these crops.

The phenology of *Peponapis* bees in seasonal environments is probably an important determinant of the temporal variation in pollinator assemblages documented in this study. As observed by Delgado-Carrillo et al. (2017), the life cycle of *Peponapis* bees in the Central Pacific coast of Mexico is synchronized with the natural blooming period of squash crops in the wet season. Species of *Peponapis* are reported as annuals; for example, *Peponapis pruinosa* in North Eastern United States overwinters as a prepupa in the soil, pupation begins in mid-June and adult activity ceases in early September (Mathewson 1968, Julier and Roulston 2009). Active nests of *Peponapis crassidentata* and *P. utahensis* have been described in the late wet season in Mexican dry forests (Rozen and Ayala 1987, Delgado-Carrillo et al. 2017), and the few *Peponapis* individuals observed during the dry season in this study were probably the last adults of the previous year. Because both species of *Peponapis* at the study site spend the majority of the dry season as a prepupa, they are not important pollinators of *C. moschata* crops during this season. *Peponapis* bees are fully dependent on the resources provided by flowers of the genus *Cucurbita* for nutrition of both larvae and adults (Hurd et al. 1971, Delgado-Carrillo et al. 2017). Furthermore, the phenology of *Peponapis* is synchronized with the flowering phenology of the wild gourd *C. argyrosperma*—a species that occurs along riverbanks and seasonal wetlands along the Pacific coast of Mexico (Mariano and Dirzo 2002, Balvino-Olvera et al. 2017). Similar synchronicity has been found for bat pollinators and the flowering phenology of Bombacaceous trees in Neotropical dry forests (Lobo et al. 2003). Therefore, the association between *Peponapis* and *Cucurbita* reflects a long

shared evolutionary history between plants and bees that has probably led to the observed synchrony in their reproductive phenologies.

Bees of the genus *Peponapis* and squash crops of the genus *Cucurbita* have their center of origin in Mexico and naturally occur throughout Mesoamerica (Hurd et al. 1971, 1974, Lira-Saade 1995, Ayala and Griswold 2012). Cultivated species of *Cucurbita* have been used by humans for thousands of years, and the distributions of some *Peponapis* species have been associated with human dispersal of squash crops (Nee 1990, Lira-Saade 1995, López-Urbe et al. 2016, Balvino-Olvera et al. 2017). At present, these crops are dynamically cultivated in small orchards and fields, according to peasants' necessities and market prices in Mexico; thus, cultivated squashes are not always available to *Peponapis* bees. Nevertheless, the strong interaction between *Peponapis* and *Cucurbita* species (both wild and cultivated) has resulted in high pollination efficiency, as was demonstrated in this and other studies between native bees and native crops (Tepedino 1981, Canto-Aguilar and Parra-Tabla 2000, Isaacs and Kirk 2010, Frier et al. 2016, Gibbs et al. 2016). Therefore, native species are expected to be the most efficient pollinators of native crops.

As stated before, *Cucurbita moschata* crops are cultivated using artificial irrigation during the dry season, when annual species of *Cucurbita* and *Peponapis* bees do not naturally occur. The absence of native effective pollinators would suggest a reduction in the fruit set of *Cucurbita moschata* crops in the dry season. However, we did not find significant differences in natural fruit set or seed production between the two seasons. Our result suggests that introduced and native bee species maintain pollination services for this crop during the dry season. Specifically, *Apis mellifera* is a less efficient pollinator than *Peponapis* at single flower visits, but low pollination efficiency is compensated by its high visitation rate and by the pollination effected by native halictid and stingless bees during the dry season. Africanized *Apis mellifera* colonized Mexico in 1986 and gradually expanded its prolific populations to most agroecosystems, natural areas, and even urban regions of Mexico (Lobo et al. 1989, Lobo 1995, Quezada-Euán 2007). In our study region, we did not find managed hives near

Cucurbita crops; therefore, honey bees pollinating *Cucurbita* flowers most likely come from feral Africanized bee populations. A previous study in the same region indicates that generalist pollinators, such as *Apis mellifera*, are common in areas under ecological succession and these abundant bees are also capable of providing pollination services to the plants in the surrounding tropical dry forest (Lopezaraiza-Mikel et al. 2013). This study shows that naturalized Africanized bees that inhabit natural forests in conjunction with native bees allow offseason pollination of squash crops when *Peponapis* bees are absent or in low numbers.

Another group of potential pollinators are the native halictid bees, *Ceratina* bees, and stingless bees. *Augochloropsis metallica* and *Agapostemon* sp. visit squash flowers in both seasons, and they carry large loads of *Cucurbita* pollen on their bodies. Although the pollination efficiency of these bees was not measured in this study, halictid bees have been described in other studies as frequent visitors and efficient squash pollinators (Ali et al. 2015). However, visitation rates of halictid bees to *Cucurbita moschata* are low for both flower genders compared to *Peponapis*. *Ceratina* bees carried pollen loads comparable to *Peponapis*, but their visitation rates were low, only 10 individuals were captured in the wet season, and no *Ceratina* bees were observed in video recordings. One species of stingless bee is apparently an important pollinator of *Cucurbita pepo* in a cloud forest in Guatemala, but the pollination efficiency of *Peponapis* was not measured at that site (Enriquez et al. 2015). In Chamela during the dry season, we observed stingless bees taking nectar from pistillate flowers and a few individuals visiting staminate flowers with low pollen loads on their bodies; however, the *Trigona fulviventris* one-visit treatment did not produce any fruits, suggesting that more than one visit is required to develop a fruit, or that stingless bees act as nectar and pollen robbers in squash flowers. Halictid and *Ceratina* bees carry similar pollen loads as female *Peponapis* bees; however, they have little impact on the pollination service provided to *Cucurbita moschata* due to their low abundance.

Our study represents the first report of a vis-à-vis relationship of pollinator gender with respect to plant gender. Monoecious plants of the genus

Cucurbita produce unisexual staminate and pistillate flowers, which are differentially visited by *Peponapis* male and female bees; female bees visited staminate flowers nearly twice as often as pistillate flowers, while male bees visited both flower genders at a low frequency. This result is possibly explained by the high dependence of female *Peponapis* bees to the pollen provided by staminate flowers, which they use to feed their larvae (Hurd et al. 1971, Delgado-Carrillo et al. 2017). There was also a strong gender bias in *Peponapis* visitation rates to *Cucurbita* staminate flowers, where mean visitation rate by female bees was 17 times higher than the visitation rate by male bees (Fig. 3). This result is in apparent contradiction with a study that showed higher visitation rates of *P. pruinosa* males than females to flowers of *C. pepo* (Cane et al. 2011); however, the latter study did not differentiate visitation to pistillate and staminate flowers and did not analyze the contribution of male and female bees to the plant's reproductive success. Female *Peponapis* bees also visit pistillate *Cucurbita* flowers in search of high-energy sources, because pistillate flowers of some *Cucurbita* species like *C. pepo* tend to produce higher quantities of nectar with greater sucrose concentration (Nepi et al. 2001). Therefore, *Peponapis* female bees are the major contributors to the pollination service and fruit production of squash crops during the wet season. Differential use of floral resources by male and female pollinators of the same species has been associated with differences in energetic requirements or feeding behaviors related to the pollinator gender (e.g., Hymenoptera, Ne'eman et al. 2006, Lepidoptera, Alarcón et al. 2010, Diptera, de Jager and Ellis 2012, hummingbirds, Temeles et al. 2009). Likewise, in plant species with unisexual flowers, pollinators may prefer a particular flower gender due to differential production of floral rewards in pistillate and staminate flowers of the same plant species and this pollinator selectivity can influence the evolution of floral sexual dimorphism (e.g., Begonia, Le Corff et al. 1998, Ashman 2000). However, the reciprocal impact that male and female pollinators of the same species have on male and female function of a particular plant species has been little studied and deserves further attention.

Although fruit set and seed production are similar in open pollination treatments in both

seasons, other studies have shown that pollen competition affects seed quality and progeny viability due to differences in the quality and quantity of *Cucurbita* pollen (Quesada et al. 1991, 1993, Winsor et al. 2000). Flowers of *Cucurbita* species are ephemeral, and their pollen loses viability within a few hours (Nepi and Pacini 1993, Agbagwa et al. 2007, Franchi et al. 2014). *Peponapis* bees, which arrive in great numbers at flowers early in the morning when pollen is more abundant and viable, might carry higher loads of viable pollen, which may in turn result in better quality progeny. In contrast, *Apis mellifera* and the other native bees in dry season arrive at flowers later in the day, a behavior that might be associated with decreased progeny quality. This hypothesis, which deserves experimental testing, predicts changes in the seed quality and progeny viability of fruits produced at different time during the day, and at different seasons by different types of pollinators. Furthermore, knowledge of pollen viability and seed quality in association with progeny performance are key for the management of native and introduced pollinators to obtain better seeds and fruits of economically important crops.

Finally, natural protected areas provide an important pollination ecosystem service to crops because they provide nesting sites and floral resources for squash pollinators. Riparian habitats and forest reserves can maintain native and feral pollinators of crops (Brito et al. 2017), and a good example was shown for the provisioning of floral resources and nesting sites of *Peponapis crassidentata* in the study area (Delgado-Carrillo et al. 2017). Currently, only 4% of this study area is riparian habitat; thus it is crucial to protect this environment for providing resources for bees and other pollinator species (Sánchez-Azofeifa et al. 2009).

In Mexico, 85% of the crop species depend to some degree on pollinators (Ashworth et al. 2009); therefore, it is necessary to study spatial and temporal variation of pollinator communities and the contribution of the different species of pollinators to the reproductive success of crop species. A better understanding of these ecological factors will allow improved management and conservation actions in agroecosystem landscapes that ensure the presence of insect pollinators throughout the year.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/ecs2.2506/full>