

# POLLINATION ECOLOGY AND BREEDING SYSTEM OF THE TROPICAL TREE *GUAIAECUM SANCTUM* ON TWO CARIBBEAN ISLANDS WITH CONTRASTING POLLINATOR ASSEMBLAGES

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**Abstract**—Insular pollination systems are more extinction-prone and vulnerable to invasive species than mainland ones. They often have plants with reproductive mechanisms allowing for self-compatibility and low species-rich communities of pollinators. Here, we document different reproductive traits of the tropical tree *Guaiaecum sanctum* on two insular populations with contrasting pollinator assemblages: Guánica in Puerto Rico with alien honeybees and Mona Island where honeybees do not occur. Using field observations and pollination experiments, we evaluated pollinator species richness, visitation rates, breeding system, and the fitness of selfed- vs. crossed-progenies. We found that flowers are pollinated by insects on both islands, but while the species richness of pollinators was higher on Mona, the visitation rates were considerably higher in Guánica where trees are almost exclusively visited by the introduced *Apis mellifera*. Flowers are not apomictic, and autogamy is negligible indicating that pollinators are required to set fruits. Outcrossing yielded nearly twice the number of fruits and seeds than selfing and these differences were consistent between populations, which might reflect early acting inbreeding depression, partial self-incompatibility, or differences in resource allocation between selfed and outcrossed fruits. Our combined results suggest that the substantial reduction in pollinator visitors in areas dominated by *A. mellifera* may add an additional level of vulnerability to these threatened populations. Although reproductive fitness is higher in Guánica, mostly due to the pollination services provided by *A. mellifera*, this population may be more susceptible to environmental changes and large-scale disturbances affecting pollinator abundance given the reduced diversity of flower visitors.

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## INTRODUCTION

Pollination is a mutualistic interaction in which plants offer a reward to flower visitors that transport pollen among conspecific plants resulting in plant reproduction (Waser 1983; Fenster et al. 2004). Approximately 82% of all

known angiosperms depend on animal-mediated pollination (Ollerton et al. 2011). In plants, breeding systems include a variety of morphological and/or physiological reproductive mechanisms that can influence the quality and quantity of seeds (Richards 1997). While some systems may promote outcrossing to avoid the

effects of inbreeding depression (Charlesworth & Charlesworth 1987; Keller & Waller 2002), others may allow for complete self-fertilization to ensure reproduction (Barrett 2003; Busch & Delph 2012). Several studies have shown that many rare and endangered plant species have breeding systems requiring pollinator services to achieve sexual reproduction (Kwak & Bekker 2006). Therefore, knowledge of plants' breeding systems as well as assessments on the health of pollinator communities, are essential to developing effective management and recovery programs (Buchmann & Nabhan 1996; Allen-Wardell et al. 1998; Barrett 2003; Liu & Koptur 2003; Kwak & Bekker 2006).

On islands, pollination systems have distinctive ecological traits compared to mainland areas mostly due to the differential pool of species inhabiting insular areas. For example, island pollinators are often depauperate due to the poor dispersal abilities of insects (Bernardello et al. 2001; Gillespie & Roderick 2002; Whittaker & Fernández-Palacios 2007). Lower diversity and abundance of pollinators on islands may result in lower visitation rates and pollination limitation for insular plants (Anderson et al. 2001). A long-standing hypothesis about insular pollination systems suggests that plant species on islands are more likely to exhibit reproductive mechanisms that allow for self-compatibility or self-fertilization and mixed mating systems (Baker 1955; Anderson et al. 2001; Grossenbacher et al. 2017). The rationale of this hypothesis is that these reproductive conditions can confer colonization advantages to plants during the first stages of colonization. Moreover, autogamous self-fertilization and mixed mating systems may provide reproductive assurance when pollinators are absent, unreliable, or not abundant (Anderson et al. 2001; Martén-Rodríguez & Fenster 2008; Rojas-Sandoval & Meléndez-Ackerman 2011). On the other hand, empirical data for tropical regions suggests that trees are predominantly outcrossing (Bawa et al. 1985; O'Malley & Bawa 1987; Murawski & Hamrick 1991; Ward et al. 2005). However, most studies evaluating the breeding systems of tropical trees have been focused on continental regions, while assessments on islands are still scarce (but see Humeau et al. 1999; Lhuillier et al. 2006; Caraballo-Ortiz & Santiago-Valentin 2011). Therefore, data on tree breeding systems and pollination ecology from islands are needed to

evaluate to which extent the breakdown of self-incompatibility mechanisms on insular trees is a common occurrence.

Given its unique evolutionary history, insular biotas are more prone to extinction and particularly vulnerable to human-related disturbances and invasive species than mainland ones. Currently, biological invasions are one of the major threats to the conservation of biodiversity and have particularly impacted island communities (Traveset & Richardson 2006; Simberloff et al. 2013). With at least 80 species regarded as invaders, bees are among the most successful invasive species worldwide (Russo 2016). Invasive *Apis* and *Bombus* species are appreciated for their pollinator services in both natural and managed ecosystems, but there is also a growing concern about their impact on native species. Invasive bees have the potential to compete with native species for floral resources and nest sites, disrupt plant-pollinator networks, reduce reproductive success for native plants, and displace native pollinators (Goulson 2003; Paini 2004; Aizen et al. 2009; Stout & Morales 2009; Russo 2016; Ackerman 2021, Garibaldi et al. 2021). In Puerto Rico, the Africanized honeybee *Apis mellifera* was first reported in 1994, and now all feral colonies on the island are classified as Africanized honeybees (Cox 1994; Rivera-Marchand et al. 2012; Galindo-Cardona et al. 2013). On this island, native bees are primarily solitary and considerably less common than the alien *A. mellifera* (Torres & Snelling 1992; Rivera-Marchand et al. 2012) that seems to be playing a key role as a novel pollinator of many native plants (Fumero-Cabán & Meléndez-Ackerman 2007; Caraballo-Ortiz & Santiago-Valentin 2011). In contrast, on the nearby small island of Mona, *A. mellifera* is still absent, presenting a unique opportunity to evaluate the potential effects of an alien pollinator on native plants and pollinators.

*Guaiacum sanctum* is a tropical tree of widespread distribution that inhabits dry habitats across the Neotropics (López-Toledo et al. 2011), where it has been heavily exploited as a timber species (Grow and Schwartzman 2001). High deforestation of its prime habitat and over-exploitation have led to considerable reductions and isolation of *G. sanctum*' wild populations throughout its native distribution range

(McCauley et al., 2008; López-Toledo et al., 2011). Currently, *G. sanctum* is considered a rare tree with wild populations decreasing through its native range and thus it is listed in the IUCN Red List of Threatened Species with the status of “near threatened” (Rivers, 2017). It is also listed under CITES Appendix II, so that it may be protected from international trade (Gonzalez-Espinosa, 2009). A study performed in Costa Rica showed that flowers of *G. sanctum* are mostly visited by bee species in the genera *Apis* and *Melipona*, and that local populations of *G. sanctum* have mixed mating systems that are predominantly outcrossing (Fuchs & Hamrick 2011). However, for the Caribbean region, despite the long history of human use and its critical conservation status, basic data on the pollination ecology and the breeding systems of *G. sanctum* is lacking. This knowledge gap limits our ability to monitor the stability and vigour of natural populations and their vulnerability related to reproduction, fitness, and genetic diversity across the region (McCauley et al. 2008).

In this study, we documented several aspects of the reproductive biology of *G. sanctum* on two insular populations: Guánica, in the main island of Puerto Rico, with alien *A. mellifera* and Mona Island where alien honeybees do not occur. We used *G. sanctum* as a study case to address the following questions: (1) What is the breeding system of *G. sanctum* on these islands? (2) What are the pollinators of *G. sanctum* on each island? (3) Are there differences in pollinator species richness and/or visitation rates between islands? Based on the literature available we expected that *G. sanctum* would be visited and pollinated by insects, and it would show a mixed breeding system. We also expected populations in Guánica to receive a higher diversity of pollinators and to have lower self-compatibility values and a more depressed seed production under self-pollination based on the assumption that on larger islands (e.g., Puerto Rico) a higher diversity of pollinators would provide more opportunities for the maintenance of outcrossing breeding systems compared to smaller islands (e.g., Mona Island). In terms of the potential effects of *A. mellifera*, because evidence shows that introduced honeybees can have negative, neutral, or even positive effects on insular floras (Ackerman 2021), we could not anticipate the direction of potential effects on

pollinator assemblages and visitation frequency. The results of this study provide relevant information on the breeding system of an insular tree as well as the role of alien pollinators on islands, which may well serve to inform the conservation and management of insular floras.

## MATERIALS AND METHODS

### STUDY SITE

This study was conducted on two islands within the archipelago of Puerto Rico: the main island of Puerto Rico and the nearby Mona Island (Fig. 1). On the main island of Puerto Rico, our study site was located within the Guánica Forest Biosphere Reserve (hereafter Guánica). Guánica is the largest and best-preserved remnant of Caribbean dry forest on the main island of Puerto Rico, and it harbors the last wild population of *G. sanctum* on this island (Fumero-Cabán 2019). The northern part of the reserve is dominated by a mixture of deciduous and semi-evergreen forest, whereas the southern portion presents a combination of cacti and scrubby vegetation on a limestone plateau along the coastline forest (Colón & Lugo 2006). The mean annual rainfall and the mean annual temperatures are 860 mm and 24°C respectively (Murphy & Lugo 1986; Colón & Lugo 2006) with a seasonality pattern of wet and dry periods (Ewel & Whitmore 1973). Guánica has a long history of forest research and is one of the best understood Caribbean dry forests, designated as a UNESCO Biosphere Reserve in 1981 (Colón & Lugo 2006). Our second study site was located on Mona Island. Mona is the second largest (total area = 55 km<sup>2</sup>) of Puerto Rico's offshore islands located between Puerto Rico and Hispaniola (Fig. 1). Since 1973, the entire island is a natural reserve protecting one of the most important remnants of Caribbean dry forests (Cintrón & Rogers 1991). The mean annual precipitation and mean annual temperatures are 1,114 mm and 25°C respectively with two seasonal wet and dry periods (Ewel & Whitmore 1973). Populations of *G. sanctum* on these two islands are in the eastern limit of its distribution range (Liogier 1988; Fumero-Cabán 2019).

### STUDY SYSTEM

*Guaiacum sanctum* L. (Zygophyllaceae) is a slow-growing tree emblematic of Neotropical dry ecosystems (Wendelken & Martin, 1987). This



**Figure 1.** Map of the Caribbean region showing the location of the Archipelago of Puerto Rico and the location of the two islands used as study sites (red circles).

species is threatened by habitat loss, deforestation, and fragmentation across its native range, and it is now listed as “near threatened by the IUCN Red List of Threatened Species (Rivers, 2017). Its native distribution range includes the Bahamas, Florida, the Greater Antilles, Mexico, Central America (from Yucatán to southern Costa Rica), and northern South America (Wendelken & Martin 1987; Liogier 1988). In Puerto Rico, *G. sanctum* can reach heights of up to 10 m, with a dense, spreading crown and a thick trunk up to 30 cm in diameter. Trees grow naturally in dry forest remnants and coastal thickets, often on limestone soils (Liogier 1988). Flowers are bisexual, actinomorphic, with five blue to purple petals 7 to 12 mm long, ten anthers, and 8-10 ovules. Generally, flowers are solitary, but they can also form umbelliferous clusters (Fig. 2). Flower anthesis begins at dawn, stigma appears to mature in the afternoon following anthesis and flowers

remain viable for up to two days after which petals change colour from blue to white (Fumero-Cabán 2019). The fruit is a 2–5-winged capsule, yellow or orange, with several 1 cm ellipsoid seeds covered with a bright red aril. In Guánica and Mona Island, flowers are produced from mid-January to early May and the fruits appear from late August to December (Fumero-Cabán 2019). Studies from Central America have indicated that *G. sanctum* trees are primarily bee-pollinated (Fuchs & Hamrick 2011), but no quantitative assessment of the number and abundance of visiting species has been made until this study.

#### POLLINATOR SPECIES RICHNESS AND VISITATION RATES

We selected 4 to 8 focal individuals of *G. sanctum* (depending on date and availability of individuals flowering) to perform observations of flower visitors on the populations of Guánica and Mona. Observations were carried out over two time periods within the day: (1) Morning



**Figure 2.** Flowers, details of reproductive structures, and visitors of *Guaiacum sanctum*.

observations (7:00 to 11:00 h) and (2) Afternoon observations (14:00 to 18:00 h). These observations were used to: (1) identify the species of insects visiting the flowers and (2) estimate the flowers' visitation rates. Each observation period was subdivided into "observation segments" where a 0.5 m<sup>2</sup> section of the crown of each focal tree was observed continuously for 15 minutes either using a video camera or a human observer. Camera recordings were performed using camcorders (SONY HANDYCAM DCR-DVD-408, Tokyo, Japan) fixed in a tripod or attached to branches for bigger trees (Gardener & Daehler 2006). For each observation segment, we recorded the following variables: (1) number of open flowers, (2) identity of flower visitors to species level, when possible, (3) time of the day at which the observation period was performed, and (4) total number of pollinator visits per observation period. All observation periods were performed on either sunny or partly cloudy days during the flowering season over three years (2008, 2009, 2010). For pollinators that could not be identified in the field, voucher specimens were collected and identified in the laboratory. All the specimens collected were

deposited in the Museum of Zoology at the University of Puerto Rico in Rio Piedras (MZUPRRP). A total of 180 hours of observations were performed, distributed in 732 segments of 15 minutes. We used these data to calculate flower visitation rates (number of visits/flower/hr) for each focal tree. Tree sample sizes were unequal across sites and across years due to logistic difficulties and ranged from 4-8 at each site. To test for differences in visitation rates between islands we fitted Generalized Linear Models (GLMs) with Gaussian error structure and the identity link function using the year as a fixed factor.

#### POLLINATION EXPERIMENTS

We combined data on open natural pollination and hand-pollination experiments to determine the breeding system of *G. sanctum* and to determine whether this species needs a pollen vector to set fruit. To perform hand-pollination experiments, we selected 10 trees of *G. sanctum* separated by at least 10 m on each island with the maximum distance being 3 km. On each tree, five different pollination treatments with five replicate flowers per treatment were performed. The

pollination treatments performed included: (1) Selfing: hand-pollinations using pollen from the same flower, (2) Outcrossing: hand-pollinations using a mixture of pollen from two or three individuals from at least 10 m away, (3) Autogamy: mature flower buds were bagged and isolated from visitors to test for spontaneous self-pollination, (4) Agamospermy: mature buds were bagged, and when flowers opened they were emasculated (anthers were removed) and bagged again immediately after emasculation, and (5) Open pollination: flowers were not manipulated. Mature flower buds were bagged with bridal veil the day before the experimental use. For all treatments involving manipulation, we performed emasculations before anthers dehisced (early in the morning, often before 8:30 hr) to avoid contamination of the stigmas with self-pollen. On both islands, pollination experiments were performed during the flowering seasons 2009-2010.

For each pollination treatment, we calculated the mean fruit-set as the number of flowers that successfully produced fruits. We also reported seed-set as the number of seeds per fruit per tree and seed size using an area index that was obtained by multiplying length  $\times$  width. In this study, the pollination experiment was conducted as a block design, with all the treatments nested within each tree. Therefore, every tree had one fruit-set value for each treatment expressed as the proportion of successful pollinations. To test for differences in fruit-set among pollination treatments, we used Generalized Linear Mixed Models (GLMMs) with a binomial error distribution and logit link function. Treatment was set as a fixed factor while the ID of individual trees was included as a random effect. Differences in seed-set (number of seeds per fruit) and seed size among treatments were analysed fitting GLMs with Poisson and Gaussian error distributions respectively and with pollination treatment as a fixed factor.

To characterize the breeding system of *G. sanctum*, we used the ISI incompatibility index (Ruiz-Zapata & Katling-Arroyo 1978). This index is calculated by dividing the mean seed-set produced via selfing by the mean seed-set produced via outcrossing. Values of  $ISI > 1$  indicate self-compatibility; between 0.2 and 1 indicate

partial self-incompatibility and values  $< 0.2$  indicate self-incompatibility (Ruiz-Zapata & Katling-Arroyo 1978). A t-test was performed to test for differences in the mean ISI values between the two islands.

#### GERMINATION SUCCESS

Following pollination experiments, flowers remained bagged and were monitored until fruit abortion or fruit maturation (ca. 6 months). Fruits were also kept bagged to avoid seed predators and seed dispersers. Once fully developed, fruits were collected and taken to the laboratory where seeds were extracted and washed in a 5% sodium hypochlorite solution to prevent fungal infections and they were scarified using single-edge razor blades to remove a small portion of the seed coat. Seed germination experiments were performed in a greenhouse by planting individual seeds in 6 cm<sup>3</sup> plastic pots. Seed germination was monitored weekly for 6 months. Differences in the percentage of germinated seeds derived from each pollination treatment were analysed by fitting GLMs adjusted to a binomial error distribution and with a logit link function.

#### MULTIPLICATIVE FITNESS

For each tree in each pollination treatment, we calculated a multiplicative fitness ( $w$ ) using the following formula:  $w = \text{fruit-set} \times \text{seed-set} \times \text{seed germination}$ . The multiplicative fitness is the product of the probability of setting fruits, the number of seeds produced per fruit, and the proportion of seeds germinated (Rojas-Sandoval & Melendez-Ackerman 2009). The differences in multiplicative fitness among pollination treatments were analyzed using GLMM adjusted to Gaussian distribution error with an identity link function and tree ID was included as a random effect. For all models (GLMM and GLM), when significant differences were found, differences between levels of each effect were tested using multiple pairwise comparisons with Tukey contrasts. All statistical analyses were performed in R version 4.2.

## RESULTS

#### POLLINATOR SPECIES RICHNESS AND VISITATION RATES

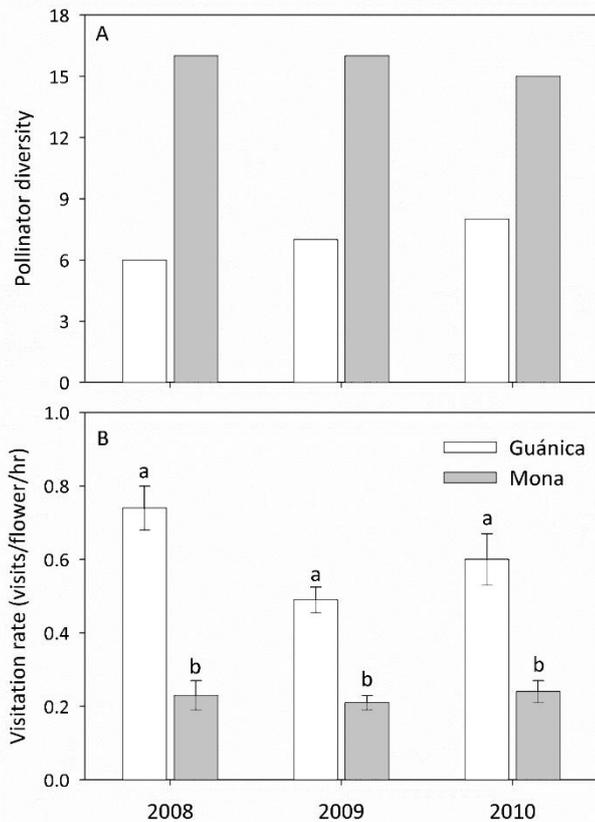
The pollinator species richness was very different between the two islands, with only eight species of flower visitors observed in Guánica

compared to a total of 17 species observed on Mona Island (Tab. 1). On both islands, all visits to *G. sanctum* flowers were performed by insects. In Guánica, flowers were visited almost exclusively by the alien honeybees *Apis mellifera*. In contrast, flowers on Mona Island had a higher pollinator species richness and flower visitors included different species of native bees, flies, and

butterflies with visits being dominated by native bees (Tab. 1, Fig. 3A). However, visitation rates were significantly higher in Guánica compared to Mona Island ( $\chi^2_{1,34} = 27.22$ ,  $P < 0.001$ , Fig. 3B). Mean visitation rates for Guánica were twice as high as those observed on Mona Island, but there were no differences in the visitation rates among years ( $\chi^2_{2,34} = 5.42$ ,  $P = 0.06$ , Fig. 3B).

**Table 1. Flower visitor species and total number of visits to *Guaiacum sanctum***

	Total number of visits per year		
	2008	2009	2010
Guánica			
Hours of observations	16	60	16
Total number of flowers observed	602	2,397	661
Total number of trees observed	4	8	4
Species			
<i>Apis mellifera</i>	1,706	6,191	1,385
<i>Centris lanipes</i>	7	33	5
<i>Centris decolorata</i>	0	6	4
<i>Centris haemoroidalis</i>	11	9	1
<i>Xylocopa mordax</i>	1	1	3
<i>Lassioglossum</i> sp.	0	4	3
<i>Ascia monuste</i>	9	46	8
<i>Pirgus</i> sp.	7	0	3
Mona Island			
Hours of observations	16	60	16
Total number of flowers observed	653	2,504	682
Total number of trees observed	8	8	4
Species			
<i>Centris lanipes</i>	196	817	154
<i>Centris decolorata</i>	78	345	82
<i>Centris smithi</i>	16	79	16
<i>Xylocopa mordax</i>	8	22	5
<i>Megachile holosericea</i>	46	258	85
<i>Agapostemon viequesensis</i>	46	92	70
<i>Anthophora tricolor</i>	60	207	28
<i>Lasioglossum</i> sp.	7	21	11
Tachinidae (Diptera)	10	70	21
Syrphidae (Diptera)	10	11	11
<i>Bombylius</i> sp.	1	0	0
<i>Ascia monuste eubolea</i>	78	239	107
<i>Antheos maerulea</i>	2	9	4
<i>Urbanus darantes camptoni</i>	1	9	8
<i>Perichares philites philites</i>	1	4	1
<i>Composia credula</i>	0	1	0
<i>Epryriades arcas philemon</i>	2	6	3



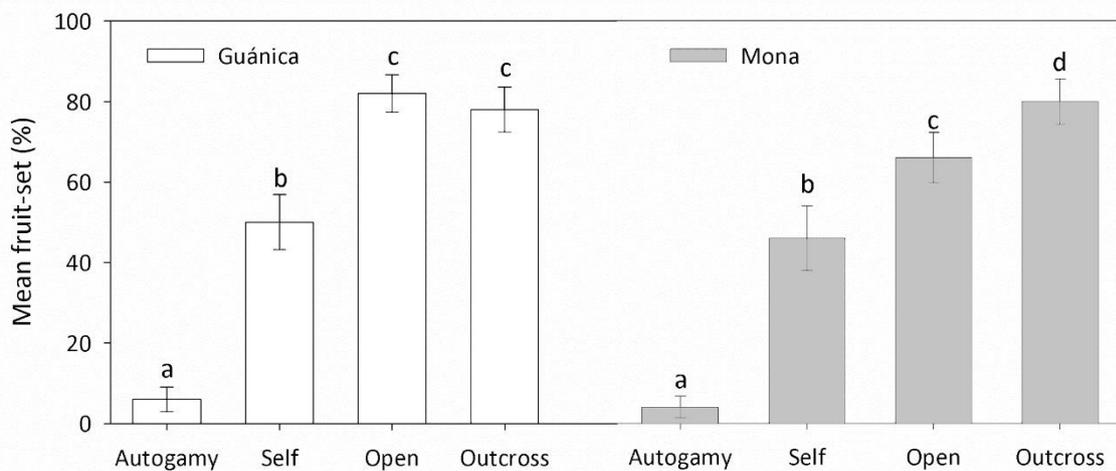
**Figure 3.** Pollinator diversity (A) and flower visitation rates (B) across years for Guánica and Mona Island. Error bars are standard error of the mean. Different letters indicate significant differences (Tukey's post hoc tests,  $P < 0.05$ ) between islands.

#### POLLINATION EXPERIMENTS

The agamospermy treatment produced no mature fruits indicating that no seeds are

produced from unfertilized ovules in *G. sanctum*, and thus, this treatment was excluded from further analyses. Our GLMMs show that mean fruit-set differed significantly among the remaining four pollination treatments for both islands (Guánica:  $\chi^2_{3,9} = 9.02$ ,  $P = 0.01$ ; Mona:  $\chi^2_{3,9} = 12.41$ ,  $P < 0.01$ ; Fig. 4). On both islands, autogamy treatment produced almost no fruits (only 3 fruits in Guánica and 2 fruits on Mona Island) while the self-pollination treatment was significantly lower compared to the open and outcross-pollination treatments ( $P < 0.001$ , Fig. 4). On Mona Island, the highest fruit set was achieved by outcross-pollination followed by open pollination, but in Guánica this trend was the opposite, and the highest fruit set was achieved by open pollination followed by outcrossing (Fig. 4). In Mona, the outcross-pollination treatment significantly increased the fruit-set compared to the open pollination treatment ( $P < 0.001$ ; Fig. 4). Finally, because the number of fruits produced by autogamy was negligible, this treatment was also excluded from further analyses.

Significant differences were also detected for the number of seeds produced per fruit (seed-set) across pollination treatments ( $\chi^2_{2,59} = 77.14$ ,  $P < 0.0001$ , Fig. 5). We found that seed production was significantly lower for selfing compared to outcross- and open-pollination treatments ( $P < 0.05$ , Fig. 5), but we did not detect differences between islands ( $\chi^2_{1,59} = 4.74$ ,  $P = 0.09$ ). The mean value for the ISI index for *G. sanctum* was 0.60 and 0.63 in Guánica and Mona respectively. These ISI values were not significantly different between the



**Figure 4.** Mean fruit-set for different pollination treatments for each island. Error bars are standard error of the mean. Different letters above the columns indicate significant differences (Tukey's post hoc tests,  $P < 0.05$ ) among pollination treatments.

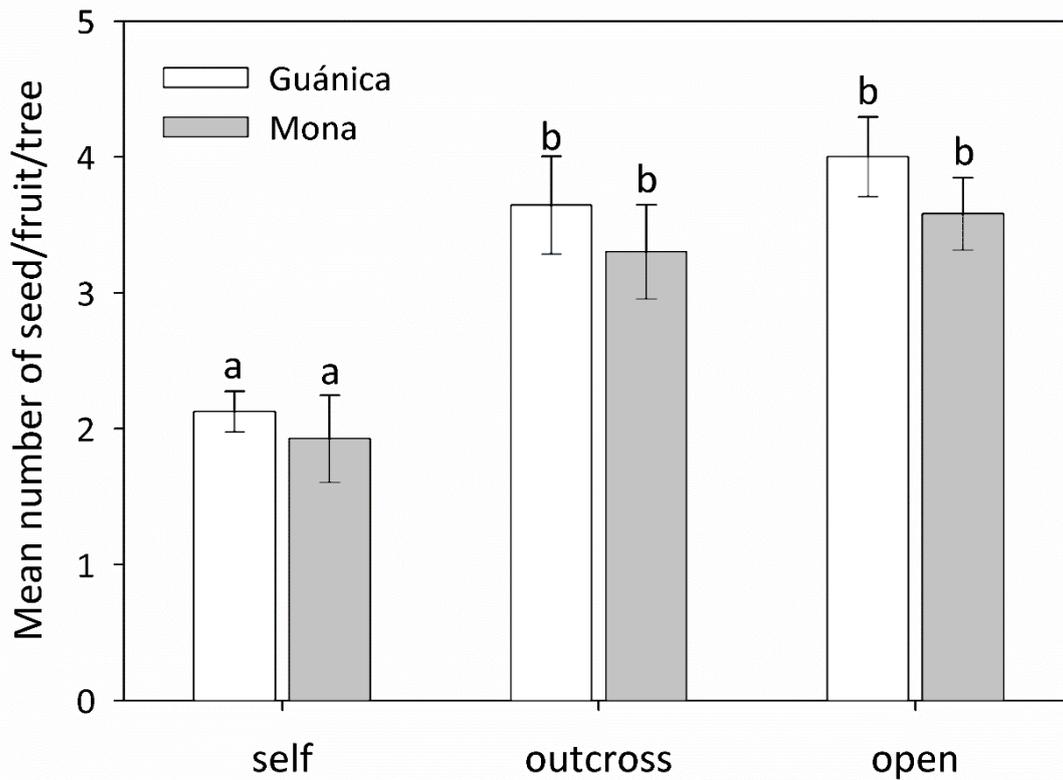


Figure 5. Mean seed-set for different pollination treatments performed in Guánica and Mona Island. Error bars are standard error of the mean. Different letters indicate significant differences (Tukey's post hoc tests,  $P < 0.05$ ) among treatments.

two islands ( $t = -0.29$ ,  $df = 18$ ,  $P = 0.77$ ). Finally, significant differences were detected for seed size among treatments ( $\chi^2_{2,89} = 13.22$ ,  $P < 0.001$ , Fig. 6A). Seeds produced via selfing were significantly smaller than seeds from outcross- and open-pollination treatments ( $P < 0.05$  in all cases), but this pattern was the same on both islands ( $\chi^2_{1,89} = 1.12$ ,  $P = 0.29$ , Fig. 6A).

#### GERMINATION SUCCESS AND MULTIPLICATIVE FITNESS

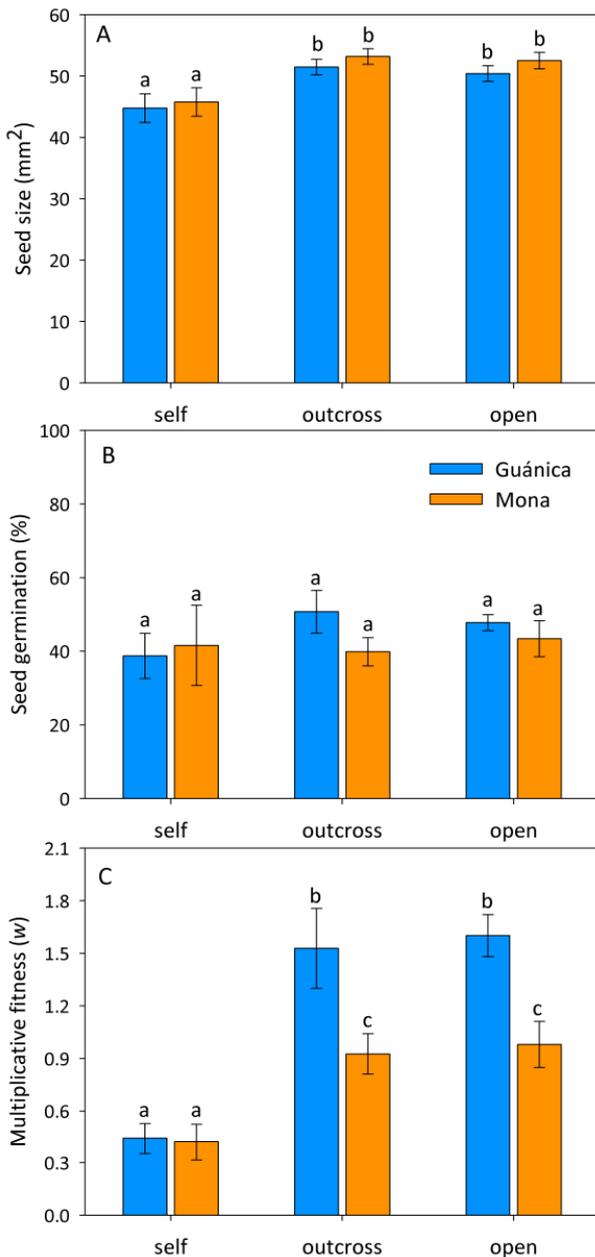
Overall, germination rates were low ( $< 50\%$ ) regardless of pollination treatment and island. Moreover, no significant differences were detected for the percentage of seed germination when comparing the different pollination treatments ( $\chi^2_{2,59} = 0.76$ ,  $P = 0.68$ ) and islands ( $\chi^2_{1,59} = 0.87$ ,  $P = 0.39$ , Fig. 6B). Significant differences were observed when comparing the multiplicative fitness among pollination treatments ( $\chi^2_{2,59} = 14.76$ ,  $P < 0.001$ , Fig. 6C). In Guánica, multiplicative fitness was highest for the outcrossed ( $w_x = 1.5$ ) and open progenies ( $w_n = 1.6$ ) compared to the selfed progeny ( $w_s = 0.43$ ). A similar pattern was found on Mona, where the multiplicative fitness was highest for the outcrossed ( $w_x = 0.9$ ) and open progenies ( $w_n = 0.97$ ) relative to the selfed progeny ( $w_s = 0.40$ ; Fig.

6C). These results also showed a significant island effect ( $\chi^2_{1,59} = 6.33$ ,  $P = 0.01$ , Fig. 5C), indicating that Guánica has significantly higher multiplicative fitness than Mona for the outcrossed and open progenies ( $P < 0.05$  in all cases).

#### DISCUSSION

Our results for flower visitors showed that, as expected, *G. sanctum* is visited and pollinated by insects (mainly bees) on both islands. This species did not exhibit agamospermy and the fruit production in the autogamy treatment was negligible indicating that in *G. sanctum* a pollen vector is needed for effective pollination and reproduction. We also detected that outcrossing yielded nearly twice the number of fruits and seeds than selfing and these differences were consistent between populations, which might reflect early acting inbreeding depression, partial self-incompatibility, or differences in resource allocation between selfed and outcrossed fruits.

Results for fruit and seed production suggest that under natural conditions, these reproductive structures are likely generated primarily by outcrossing given that fruit-set and seed-set values



**Figure 6.** Means for (A) seed size (area in mm<sup>2</sup>), (B) percentage of seed germination, and (C) multiplicative fitness estimates ( $w$ ) for the different pollination treatment performed in Guánica and Mona Island. Error bars are standard error of the mean. Different letters indicate significant differences (Tukey's post hoc tests,  $P < 0.05$ ) among treatments.

resulting from our pollination experiments were similar between the open and outcross treatments for both islands. Thus, outcrossed mating's are likely to predominate at these sites. In general, these results are consistent with previous evidence from continental areas showing that *G. sanctum* has a mixed mating system that is predominantly outcrossing (Fuchs & Hamrick 2011).

Nevertheless, further research is needed to improve our understanding of the breeding system of *G. sanctum* on insular ecosystems.

As expected, we found differences in the diversity of flower visitors between Guánica and Mona Island, but contrary to our original expectations, the pollinator species richness in Guánica (bigger island) was lower than on Mona (smaller island). These differences may be explained, at least in part, by the presence of the alien pollinator *A. mellifera* in Guánica. *Apis mellifera* is a super-generalist that often competes for floral resources with native pollinators (Roubik 1983; Kato et al. 1999; Paini 2004; Dohzono & Yokoyama 2010). There is also evidence showing that alien honeybees can disrupt plant-pollination interactions as they have the capacity to displace other flower visitors (Roubik 1983, Gross 2001; Hansen et al. 2002, Traveset & Richardson 2006; Valido et al. 2019). Indeed, we found that the community of pollinators observed on Mona Island, where *A. mellifera* does not occur, is considerably more diverse than the community observed in Guánica. However, despite the lower pollinator species richness observed in Guánica, flower visitation rates in this location were considerably higher than those recorded on Mona Island. In this regard, our results clearly showed that in Guánica visitation rates are twice as high as those observed on Mona, but they also showed that Guánica trees are almost exclusively visited by *A. mellifera* with very few visits by native pollinators. In agreement with previous studies, our data show that *A. mellifera* may be playing a role as a novel pollinator of native insular plants (Fumero-Cabán & Meléndez-Ackerman 2007; Caraballo & Santiago-Valentín 2011). However, in terms of fruit and seed production, it seems that the higher visitation rates by *A. mellifera* could compensate for the lower pollinator diversity observed in Guánica. Several studies have shown that even when *A. mellifera* may not be as effective as native pollinators, their social structure and high numerical abundances may level plant reproductive outputs (Vaughton 1992, Gross 2001, Fumero-Cabán & Meléndez-Ackerman 2007, 2012). However, the effectiveness of *A. mellifera* as a pollinator of native plants varies enormously among plant species and systems (Aslan et al. 2016; Ackerman 2021).

On the other hand, the apparent reproductive compensation by *A. mellifera* in Guánica appears not to be proportional to the overabundance of their visits. The fruit-set and seed-set resulting from open pollination treatments were similar in Guánica and Mona, suggesting that even when flowers in Guánica are more visited than on Mona Island, more visits did not translate into higher fruit and/or seed production. One potential explanation may lie on the foraging behaviour of *A. mellifera*. This species typically forages in large numbers, visiting many flowers within a single individual and restricting flights between plants before moving to a different source (Michaelson-Yeates et al. 1997; Gross 2001, Dupont et al. 2004). This type of foraging behaviour by *A. mellifera* is likely to impact the pollination mating patterns by increasing both selfing and geitonogamy (intraspecific pollen deposition, England et al. 2001; Dick 2003; Celebreze & Paton 2004; Dupont et al. 2004; Saez et al. 2014). In our study, selfed progenies have significantly lower reproductive success and lower multiplicative fitness relative to outcrossed and open progenies. In this regard, our results indicate that reductions in reproductive success either via self-incompatibility mechanisms or by early acting inbreeding depression are likely to counteract any potential increase in the pollination services provided by *A. mellifera* in Guánica and could also reduce inbreeding offspring in this location.

In terms of genetic diversity, seeds produced via outcrossing promote variability, enhance vigour, and may provide natural populations with the capacity to effectively respond to environmental changes (Barrett 1995, 2003; Apsit et al. 2001, Eckert et al. 2009). Most tropical trees, including *G. sanctum* in continental areas, have mating systems that are predominantly outcrossing (Murawski & Hamrick 1991; Apsit et al. 2001 Fuchs & Hamrick 2011). In this study, we have shown that selfed progenies of *G. sanctum* have lower reproductive success and lower fitness than outcrossed progenies. Under this scenario, it is expected for populations to experience strong selection favouring outcrossing mechanisms. Our findings for *G. sanctum* suggest that some level of selfing may be occurring in populations on both islands. One plausible explanation could be related to the irregular and infrequent availability of pollinators on these islands. Caribbean islands are

characterized by highly variable and unpredictable climates, and they are periodically impacted by large-scale natural disturbances such as hurricanes and tropical storms. These natural events may lower dramatically the abundance of local pollinators and create periods of low pollinator availability and thus lead to pollen-limitation conditions (Wunderle 1995; Rathcke 2000, 2001; Rivera Marchand & Ackerman 2006; Rojas Sandoval & Meléndez-Ackerman 2011). Conditions of low pollinator abundance may favour and select for self-compatibility as a mechanism of reproductive assurance which may be advantageous in these unstable environments and depauperate pollinators communities that are Caribbean islands (Martén-Rodríguez & Fenster 2008; Martén-Rodríguez et al. 2010; Pérez et al. 2018).

#### IMPLICATIONS FOR CONSERVATION

*Guaicum sanctum* exhibits a partial self-compatible breeding system but under natural conditions this species is producing more fruits and seeds via outcrossing. Because this species needs insect pollinators to successfully reproduce, we recommend that assessment on the population dynamics and health of pollinators should be included in its conservation and management plans. Even when our results show evidence of inbreeding depression, the similarity observed in the fitness of progenies produced by natural open pollination and those produced by artificial outcrossing suggest that managers wanting to create new *in situ* populations of *G. sanctum* could effectively use seeds collected directly from natural populations. In terms of pollinators, our result suggests that *A. mellifera* is a viable novel pollinator of *G. sanctum*. Nevertheless, this result needs to be interpreted carefully because even when *A. mellifera* is pollinating and producing viable seeds on *G. sanctum* trees, this species is an introduced pollinator and the long-term effects of its invasion/presence on this island are still uncertain. It is also important to consider that even when the pollination services provided by *A. mellifera* are positive and similar to those provided by native pollinators, our data also suggest that the substantial reduction in pollinator diversity in areas dominated by *A. mellifera* may be adding an additional level of vulnerability to these endangered populations. These may be

particularly relevant for Caribbean islands that are inherently exposed to hurricanes and tropical storms and where the reduction of pollinator populations has been documented to occur following these natural catastrophic events (Wunderle 1995; Rathcke 2000; 2001, Martinez-Llaurador 2021). Under this scenario, a higher diversity of pollinators may offer more redundancy in pollination services and a higher adaptive capacity in response to environmental change and large-scale natural disturbances. Long-term monitoring of *G. sanctum* populations and other native plants in areas with and without *A. mellifera* could be useful to evaluate whether differences in pollinator diversity may affect reproductive success over time and to assess how vulnerable are native populations to the presence of alien pollinators and the fluctuations in pollinator diversity and abundances over time.

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#### AUTHOR CONTRIBUTION

JFC and EMA conceived the study and designed the experiments. JFC set up the experiments and collected the data. JRS analyzed the data. All authors contributed significantly to the drafts and gave final approval for publication.

#### REFERENCES

Ackerman JD (2021) Island invasions by introduced Honey Bees: what can be expected for Puerto Rico and the Caribbean? *Frontiers in Ecology and Evolution* 8:519.

Aizen MA, Harder LD (2009) The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. *Current Biology* 19:915-918.

Allen-Wardell G, Bernhardt P, Bitner R, Burquez A, Buchmann S (1998) The potential consequences of pollinator decline on the conservation of biodiversity and stability of food crop yields. *Conservation Biology* 12:8-17.

Anderson GJ, Bernadello G, Stuessy TF, Crawford DJ (2001) Breeding system and pollination of selected plants endemics to Juan Fernández Islands. *American Journal of Botany* 88: 220-233.

Aslan CE, Liang CT, Galindo B, Kimberly H, Topete W (2016) The role of honey bees as pollinators in natural areas. *Natural Areas Journal* 36:478-488.

Apsit VJ, Hamrick JL, Nason JD (2001) Breeding population size of a fragmented population of a Costa Rican dry forest tree species. *The Journal of Heredity* 92: 415-420.

Baker HG (1955). Self-compatibility and establishment after long-distance dispersal. *Evolution* 9:347-349.

Barrett SCH (1995) Mating-system evolution in flowering plants: micro- and macroevolutionary approaches. *Acta Botanica Neerlandica* 44:385-402.

Barrett SCH (2003) Mating strategies in flowering plants: the outcrossing-selfing paradigm and beyond. *Philosophical Transactions of the Royal Society of London B* 358:991-1004.

Bawa KS, Perry DR, Beach JH (1985) Reproductive biology of tropical lowland rain forest trees. I. Sexual system and incompatibility Mechanisms. *American Journal of Botany* 72:331-345.

Bernadello G, Anderson GJ, Stuessy TF, Crawford DJ (2001) A survey of floral traits, breeding systems, floral visitors, and pollination systems of the angiosperms of the Juan Fernández Islands (Chile). *The Botanical Review* 67:255-308.

Buchmann SL, Nabha GP (1996) *The forgotten pollinators*. Island Press, Washington DC. USA.

Busch JW, Delph LF (2012) The relative importance of reproductive assurance and automatic selection as hypotheses for the evolution of self-fertilization. *Annals of Botany* 109:553-562.

Caraballo M, Santiago-Valentín E (2011) The breeding system and effectiveness of introduced and native pollinators of the endangered tropical tree *Goetzea elegans*. *Journal of Pollination Ecology* 4:26-33.

Celebrezze T, Paton C (2004) Do introduced honeybees (*Apis mellifera*, Hymenoptera) provide full pollination service to bird-adapted Australian plants with small flowers? An experimental study of *Brachyloma ericoides* (Epacridaceae). *Austral Ecology* 29:129-136.

Charlesworth D, Charlesworth B (1987) Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology, Evolution and Systematics* 18:237-268.

- Cintrón B, Rogers L (1991) Plant communities of Mona Island. *Acta Científica* 5:10-64.
- Colón SM, Lugo AE (2006) Recovery of a Subtropical Dry Forest After Abandonment of Different Land Uses. *Biotropica* 38:354-364.
- Cox B (1994) AHB in Puerto Rico. *American Bee Journal* 134:668-669.
- Dick CW (2003) Pollen dispersal of tropical trees (*Dinizia excelsa*: Fabaceae) by native insects and African honeybees in pristine and fragmented Amazonian rainforest. *Molecular Ecology* 12:753-764.
- Dohzono I, Yokoyama J (2010) Impacts of alien bees on native plant-pollinator relationships: A review with special emphasis on plant reproduction. *Applied Entomology and Zoology* 45:37-47.
- Dupont YL, Hansen DM, Alfredo V, Oleson JM (2004) Impact of introduced honeybees on native pollination interactions of the endemic *Echium wildpretii* (Boraginaceae) on Tenerife, Canary Islands. *Biological Conservation* 118:301-311.
- Eckert CG, Kalisz S, Geber MA, Elle E, Cheptou PO, Goodwile C, Johnston MO, Kelly JK, Moeller DA, Porcher E, Ree RH, Vallejo-Marín M, Winn AA (2009) Plant mating systems in a changing world. *Trends in Ecology and Evolution* 25:35-42.
- England PR, Beynon F, Ayre DJ, Whelan RJ (2001) A molecular genetic assessment of mating system variation in a naturally bird-pollinated shrub: contributions from birds and introduced honeybees. *Conservation Biology* 15:1645-1655.
- Ewel JJ, Whitmore JL (1973) The ecological life zones of Puerto Rico and the US Virgin Islands. USDA Forest Service, Institute of Tropical Forestry, Research Paper ITF-018, 18.
- Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD (2004) Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics* 35:375-403.
- Fuchs EJ, Hamrick JL (2011) Mating system and pollen flow between remnant populations of the endangered tropical tree, *Guaiaicum sanctum* (Zygophyllaceae). *Conservation Genetics* 12:175-185.
- Fumero-Cabán JJ, Meléndez-Ackerman EJ (2007) Relative pollination effectiveness of floral visitors of *Pitcairnia angustifolia* (Bromeliaceae). *American Journal of Botany* 94:419-424.
- Fumero-Cabán JJ (2019) Reproductive Ecology and Population Structure of *Guaiaicum sanctum* (Zygophyllaceae) in Two Caribbean Islands: Implication for Conservation. University of Puerto Rico, Rio Piedras (Puerto Rico).
- Galindo-Cardona A, Acevedo-Gonzalez JP, Rivera-Marchand B, Giray T (2013) Genetic structure of the gentle Africanized honey bee population (gAHB) in Puerto Rico. *BMC Genetics* 14:1-12.
- Gardener MC, Daehler CC (2006) Documenting floral visitors to rare Hawaiian plants using automated video recordings. *Pacific Conservation Biology* 12:189-194.
- Garibaldi LA, Pérez-Méndez N, Cordeiro GD, Hughes A, Orr M, Alves-dos-Santos I, Freitas BM, Freitas de Oliveira F, LeBuhn G, Bartomeus I, Aizen MA (2021) Negative impacts of dominance on bee communities: Does the influence of invasive honey bees differ from native bees? *Ecology* doi: 10.1002/ECY.3526
- Gillespie RG, Roderick GK (2002) Arthropods on islands: colonization, speciation, and conservation. *Annual Review of Entomology* 47:595-632.
- Gross CL (2001) The effect of introduced honeybees on native bee visitation and fruit-set in *Dillwynia juniperina* (Fabaceae) in a fragmented ecosystem. *Biological Conservation* 102:89-95.
- Grossenbacher DL, Brandvain Y, Auld JR, Burd M, Cheptou PO, Conner JK, Grant AG, Hovick SM, Pannell JR, Pauw A, Petanidou T (2017) Self-compatibility is over-represented on islands. *New Phytologist* 215:469-478.
- González-Espinosa M (2009) *Guaiaicum sanctum*. IUCN Red List of Threatened Species.
- Goulson D (2003) Effects of introduced bees on native ecosystems. *Annual Review Ecology Evolution and Systematics* 34:1-25.
- Grow S, Schwartzman E (2001) The status of *Guaiaicum* species in trade. *Medicinal Plant Conservation* 7:19-21.
- Hansen DM, Olesen MJ, Jones CG (2002) Trees, birds and bees in Mauritius: exploitative competition between introduced honeybees and endemic nectarivorous birds. *Journal of Biogeography* 29:721-734.
- Humeau L, Pailler T, Thompson JD (1999) Variation in the breeding system of two sympatric *Dombeya* species on La Réunion island. *Plant Systematics and Evolution* 218:77-87.
- Kato M, Shibata A, Yasui T, Nagamasu H (1999) Impact of introduced honeybees, *Apis mellifera*, upon native bee communities in the Bonin (Ogasawara) Islands. *Researches on Population Ecology* 41:217-228.
- Kearns CA, Inouye DW (1993) Techniques for pollination biologist. University Press of Colorado, Niwot, Colorado, USA.
- Keller LF, Waller DM (2002) Inbreeding effects in wild populations. *Trends in Ecology and Evolution* 17:230-241.
- Kwak MM, Bekker RM (2006). Ecology of plant reproduction: Extinction risk and restoration perspectives of rare plant species. In: Waser NM, Ollerton J (eds) *Plant-Pollinator interactions from*

- specialization to generalization. University of Chicago Press, Chicago, USA.
- Lhuillier E, Butaud JF, Bouvet JM (2006) Extensive clonality and strong differentiation in the insular Pacific tree *Santalum insulare*: implications for its conservation. *Annals of Botany* 98:1061-1072.
- Liogier HA (1988) Descriptive flora of Puerto Rico and adjacent islands, Spermatophyta. Vol. 2. Puerto Rico.
- Liu H, Koptur S (2003) Breeding systems and pollination of a narrowly endemic herb of the Lower Florida Keys: Impacts of the urban-wild land interface. *American Journal of Botany* 90:1180-1187.
- López-Toledo L, Gonzalez-Salazar C, Burslem DF, Martínez-Ramos M (2011) Conservation assessment of *Guaiacum sanctum* and *Guaiacum coulteri*: historic distribution and future trends in Mexico. *Biotropica* 43:246-255.
- Martínez-Llaurador NA (2021). Resistance and resilience of a flower-visitor network to a severe hurricane in an insular tropical beach dune system (Doctoral dissertation, University of Puerto Rico, Rio Piedras, Puerto Rico.
- McCauley RA, Cortés-Palomec AC, Oyama K (2008) Isolation, characterization, and cross-amplification of polymorphic microsatellite loci in *Guaiacum coulteri* (Zygophyllaceae). *Molecular Ecology Resources* 8:671-674.
- Martín-Rodríguez S, Fenster CB (2008) Pollination ecology and breeding systems of five *Gesneria* species from Puerto Rico. *Annals of Botany* 102:23-30.
- Marten-Rodriguez S, Fenster CB, Agnarsson I, Skog LE, Zimmer EA (2010) Evolutionary breakdown of pollination specialization in a Caribbean plant radiation. *New Phytologist* 188: 403-417.
- Michaelson-Yeates TPT, Marshall AH, Williams IH, Carreck NL, Simpkins JR (1997) The use of isoenzyme markers to determine pollen flow and seed paternity mediated by *Apis mellifera* and *Bombus* spp. in *Trifolium repens*, a self-incompatible plant species. *Journal of Apicultural Research* 36:57-62.
- Murawski DA, Hamrick JL (1991) The effect of the density of flowering individuals on the mating systems of nine tropical tree species. *Heredity* 67:167-174.
- Murphy PG, Lugo AE (1986) Structure and biomass of a subtropical dry forest in Puerto Rico. *Biotropica* 1:89-96.
- Ollerton J, Winfree R, Tarrant S (2011) How many flowering plants are pollinated by animals? *Oikos* 120:321-326.
- O'Malley DM, Bawa KS (1987) Mating system of a tropical rain forest tree species. *American Journal of Botany* 74:1143-1149.
- Paini DR (2004) Impact of the introduced honey bee (*Apis mellifera*) (Hymenoptera: Apidae) on native bees: a review. *Austral Ecology* 29:399-407.
- Pérez ME, Meléndez-Ackerman EJ, Monsegur-Rivera OA (2018) Breeding system and pollination of *Gesneria pauciflora* (Gesneriaceae), a threatened Caribbean species. *Flora* 242:8-15.
- Rathcke BJ (2000) Birds, pollination reliability, and green flowers in an endemic island shrub, *Pavonia bahamensis* (Malvaceae). *Rhodora* 1:92-414.
- Rathcke BJ (2001) Pollination and predation limit fruit set in a shrub, *Bourreria succulents* (Boraginaceae), after Hurricanes on San Salvador Island, Bahamas. *Biotropica* 33:330-338.
- Richards AJ (1997) Plant breeding systems. Second Edition. Chapman & Hall. USA.
- Rivera-Marchand B, Ackerman JD (2006) Bat Pollination Breakdown in the Caribbean Columnar Cactus *Pilosocereus royenii*. *Biotropica* 38:635-642.
- Rivera-Marchand B, Oskay D, Giray T (2012) Gentle Africanized bees on an oceanic island. *Evolutionary Applications* 5:746-756.
- Rivers MC (2017) *Guaiacum sanctum*. The IUCN Red List of Threatened Species 2017: e.T32955A68085952. <https://dx.doi.org/10.2305/IUCN.UK.2017-3.RLTS.T32955A68085952.en>. Accessed on 13 February 2022.
- Rojas-Sandoval J, Meléndez-Ackerman E (2009) Pollination biology of *Harrisia portoricensis* (Cactaceae), an endangered Caribbean species. *American Journal of Botany* 96:2270-2278.
- Rojas-Sandoval J, Melendez-Ackerman E (2011) Wind facilitated self-pollination in *Harrisia portoricensis* (Cactaceae): A mechanism for reproductive assurance. *International Journal of Plant Reproductive Biology* 3:1-5.
- Roubik DW (1983) Experimental community studies: time-series tests of competition between African and Neotropical bees. *Ecology* 64:971-978.
- Russo L (2016) Positive and negative impacts of non-native bee species around the world. *Insects* 7:69.
- Ruiz-Zapata T, Arroyo MTK (1978) Plant reproductive ecology of a secondary deciduous tropical forest in Venezuela. *Biotropica* 1:221-230.
- Sáez A, Morales CL, Ramos LY, Aizen MA (2014) Extremely frequent bee visits increase pollen deposition but reduce drupelet set in raspberry. *Journal of Applied Ecology* 51:1603-1612.
- Stout JC, Morales CL (2009) Ecological impacts of invasive alien species on bees. *Apidologie* 40:388-409.
- Simberloff D, Martin JL, Genovesi P, Maris V, Wardle DA, Aronson J, Courchamp F, Galil B, García-Berthou E, Pascal M, Pyšek P (2013) Impacts of biological

- invasions: what's what and the way forward. *Trends in Ecology & Evolution* 28:58-66.
- Torres JA, Snelling RR (1992) Los himenópteros de Isla de Mona. *Acta Científica* 6:87.
- Traveset A, Richardson DM (2006) Biological invasions as disruptors of plant reproductive mutualisms. *Trends in Ecology & Evolution* 21:208-216.
- Valido A, Rodríguez-Rodríguez MC, Jordano P (2019) Honeybees disrupt the structure and functionality of plant-pollinator networks. *Scientific Reports* 9:1-11.
- Vaughton G (1992) Effectiveness of nectarivorous birds and honeybees as pollinators of *Banksia spinulosa* (Proteaceae). *Australian Journal of Ecology* 17:43-50.
- Ward M, Dick CW, Gribel R, Lowe AJ (2005) To self, or not to self... A review of outcrossing and pollen-mediated gene flow in neotropical trees. *Heredity* 95:246-254.
- Waser NM (1983) The adaptive nature of floral traits: ideas and evidence. *Pollination Biology* 1:241-285.
- Wendelken PW, Martin RF (1987) Avian consumption of *Guaiacum sanctum* fruit in the arid interior of Guatemala. *Biotropica* 1:16-121.
- Whittaker RJ, Fernández-Palacios JM (2007) *Island biogeography: ecology, evolution, and conservation*. Oxford University Press.
- Wunderle JM (1995) Responses of bird populations in a Puerto Rican Forest to Hurricane Hugo: the first 18 months. *The Condor* 97 879-896.