THE BREEDING SYSTEM AND EFFECTIVENESS OF INTRODUCED AND NATIVE POLLINATORS OF THE ENDANGERED TROPICAL TREE *GOETZEA ELEGANS* (SOLANACEAE)

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Abstract—The impact of introduced species on native organisms is one of the main conservation concerns around the world. To fully understand the effect of introduced pollinators on native plants, it is important to know the reproductive biology of the focal species, especially its pollination biology. In this study we examined the breeding system of the endangered tree *Goetzea elegans* (Solanaceae), and compared pollination effectiveness of the two main floral visitors, *Coereba flaveola* (an avian nectarivore), and *Apis mellifera* (the introduced European Honeybee). We assessed the breeding system of *G. elegans* by applying several pollination treatments to flowers of cultivated trees to test fruit set, seed set, and seed viability. We also examined the pollination efficiency of *A. mellifera* and *C. flaveola*, and compared all the treatments with positive and negative controls. Our results indicate that the introduced honeybee *A. mellifera* is as efficient as the native bird *C. flaveola* in pollinating the flowers of *G. elegans*. This study also showed that *G. elegans* requires cross–pollination for fruit and seed set, and to obtain high seed viability rates. Despite the fact that many studies report exotic species as detrimental for native organisms, we document a case where an introduced insect has a beneficial impact on the reproductive biology of an endangered tropical tree.

Key words: Apis mellifera, Coereba flaveola, *geitonogamy, outcrossing, Puerto Rico, selfing.*

INTRODUCTION

The negative impacts of introduced species on ecosystems are a major issue in conservation biology (e.g. Callaway et al. 2004; Lugo 2005; Nogueira-Filho et al. 2009; Davis et al. 2010; Nunez et al. 2010). Introduced species can disrupt native plant-animal interactions, such as plant-pollinator mutualisms, which can have negative effects on reproductive success (Traveset & Richardson 2006; Aizen et al. 2008; Bartomeus et al. 2008; Padron et al. 2009). One of the most common introduced pollinators around the world is the honeybee, *Apis mellifera* L. (Apidae). Honeybees are native to Europe, Africa and the Middle East (Winston 1987), and they have been intentionally introduced in many countries to improve crop pollination and to produce honey (Hansen et al. 2002). The impacts of the introduction of *A. mellifera* on plant communities are a focal point of debate (Goulson 2003; Stanley et al. 2004; Moritz et al. 2005; Traveset & Richardson 2006; Kaiser-Bunbury & Müller 2009), with studies showing honeybees to be neutral or beneficial for the pollination of native plants (Gross 2001; Dupont et al. 2004;

Fumero-Cabán & Meléndez-Ackerman 2007), especially for dispersing pollen in fragmented habitats (Dick 2001; Dick et al. 2003). Conversely, other studies on the pollination of tropical plants show that *A. mellifera* has negative impacts on the fitness of native plants (Gross & Mackay 1998; do Carmo et al. 2004), on interactions with native pollinators by displacing them (Kato et al. 1999; Hansen et al. 2002; Goulson 2003), and they tend to be less efficient pollinators than native species, including other bees and birds (Westerkamp 1991; Freitas & Paxton 1998; Gross & Mackay 1998; Hansen et al. 2002).

The ecological disruption of introduced species on plant-pollinator mutualisms is expected to be more severe for organisms restricted to islands because island populations are usually smaller, have limited dispersal, and are believed to be inferior competitors than continental populations (Cronk & Fuller 1995; Whittaker 1998). In addition, islands often have depauperate pollinator communities which provide services to a wide array of plants (Olesen et al. 2002). However, rare plant species are often less likely to receive pollinator visits due to competition with more abundant floral resources of surrounding common plant species (Rymer et al. 2005). Pollen limitation and the resultant low seed set are particularly detrimental for rare tropical trees, as most of the species in the tropics are dioecious or self-incompatible (Bawa et al. 1985; Ward et al. 2005). The combination of pollen

limitation and habitat destruction of tropical forests is believed to be especially harmful for rare self-incompatible trees in biodiversity hotspots. This is because in biodiversity hotspots, intense competition for pollinator services and high deforestation rates are prevalent (Vamosi et al. 2006).

The objectives of this study were to examine the impact of the introduced *A. mellifera* on the pollination system of the tropical tree *Goetzea elegans*, an endemic and rare tree found in the Caribbean Biodiversity Hotspot (Fig. 1a; Myers et al. 2000), to compare the pollinator effectiveness of *G. elegans* floral visitors, and to examine the *G. elegans* breeding system. Specifically, we hypothesized that (1) *A. mellifera* pollinates *G. elegans* less efficiently than its native pollinator, as found in previous studies on the impact of *A. mellifera* on pollination of native plants (Westerkamp 1991; Freitas & Paxton 1998; Hansen et al. 2002). We also hypothesized that (2) *G. elegans* is self-incompatible and requires outcrossing to set seeds, as this is the predominant reproductive strategy in tropical trees (Bawa et al. 1985, Nason & Hamrick 1997, Ward et al. 2005).

MATERIALS AND METHODS

Study species

Goetzea elegans Wydler is an endangered tree of the subfamily Goetzeoideae in the Solanaceae (sensu Olmstead et al. 2008) and only found in populations on the northwestern and the eastern region of the island of Puerto Rico (including Vieques Island; USFWS 1987; IUCN 2010 [C2a version 2.3]). Although *G. elegans* was once considered critically endangered, totalling around 50 individuals in three populations (USFWS 1987), new populations have been recently discovered. The total population is now estimated at around 820 individuals in 12 populations, most of them fragmented and restricted to ravines. As for most of the species designated as endangered, very little is known of its life history, such as breeding system, pollinators, dispersal agents and demographic characteristics. Preliminary studies by Santiago-Valentín (1995) suggest that *G. elegans* requires cross pollen to set fruits. However, additional experiments are needed to confirm these preliminary data. Although the populations of *G. elegans* have declined mainly due to deforestation and land-use changes (USFWS 1987), they may also be suffering the impact of introduced species.

In the wild, *G. elegans* trees can reach up to 18 m in height, and produces flowers and fruits throughout the year, with peak flowering occurring between February and July (Little et al. 1974; Santiago-Valentín 1995). The flowers are perfect with no fragrance perceptible to humans. The pale yellow corolla is funnel shaped, up to 2 cm long and 1.3 to 2 cm across (Fig. 1a). Six slender stamens are borne near the base of the corolla and are exserted. The pistil has a slender style with a bilobed stigma, and a hairy 2-celled ovary bearing few ovules. The fruits are orange drupes about 4 cm long covered by velvety hairs with a persistent calyx (Little et al. 1974). Fruit shapes are variable among trees (from round to pear–shaped), with shape being consistent within one tree. Fruits are commonly one-seeded, but fruits with two and up to nine seeds (more rarely) have been observed in the wild

(Santiago-Valentín 1995). The seeds are elliptic and about 0.7 cm in size (Little et al. 1974).
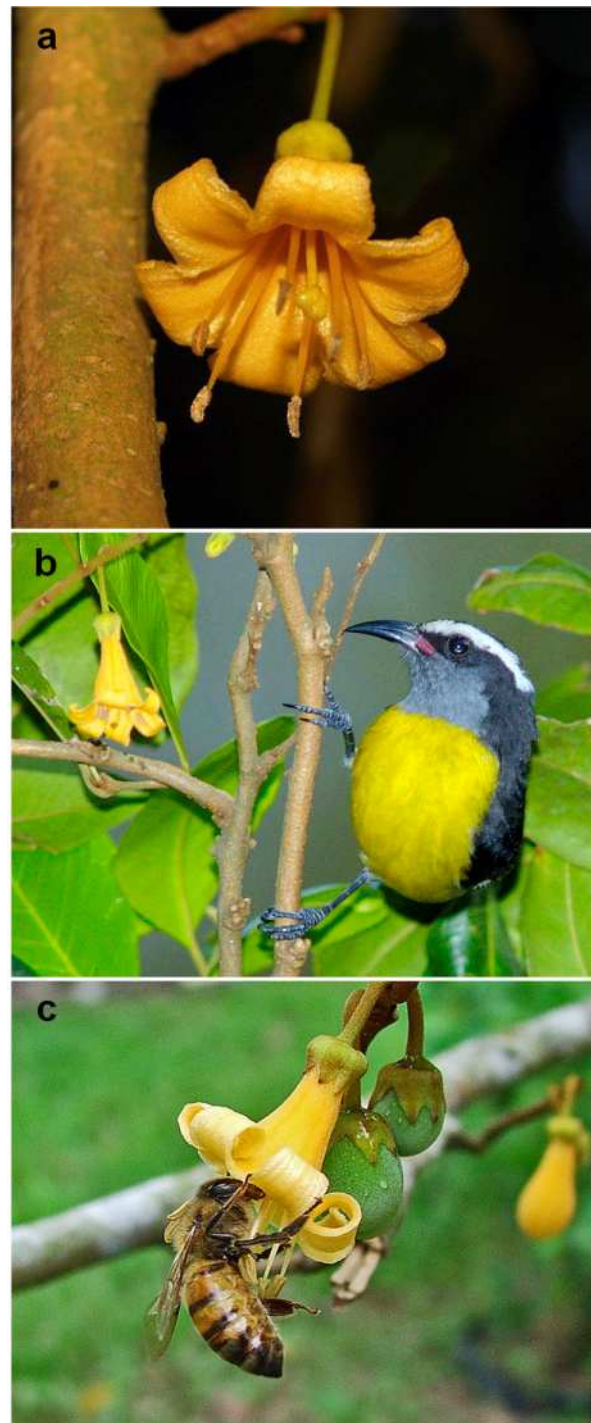

FIG. 1. Flower of *Goetzea elegans* at anthesis (a), receiving a visit from *Coereba flaveola* (b), and receiving a visit from *Apis mellifera* (c). Photo credits: W. Hernández Aguiar (a), T. A. Carlo Joglar (b), and M. A. Caraballo Ortiz (c).

The flowers of *G. elegans* are visited by both birds and insects. The most frequent flower visitor is the native nectarivorous bananaquit *Coereba flaveola* Bryant (Coerebidae; Fig. 1b; USFWS 1987; Santiago-Valentín 1995), which is considered the most common bird in Puerto Rico (Raffaele et al. 1998). *Coereba flaveola* has a reputation

for robbing the nectar of many plant species (Kodric-Brown et al. 1984, Ricart 1992; Fumero-Cabán & Meléndez-Ackerman 2007), and although the culmen of *C. flaveola* appears to contact the reproductive structures of *G. elegans*, we lack experimental data confirming its role as a pollinator of this tree species. Another common visitor of *G. elegans* is the common honeybee *Apis mellifera* L. (Apidae; Fig. 1c; Santiago-Valentín 1995). This introduced bee has been observed foraging on flowers of many native species throughout the island in both managed and wild colonies. Both *C. flaveola* and *A. mellifera* are common visitors of *G. elegans* flowers in all of the wild populations of the species (M. A. Caraballo-Ortiz, pers. obs.). Aside from *C. flaveola* and *A. mellifera*, *G. elegans* flowers are infrequently visited by two native hummingbird species (*Anthracothorax dominicus aurulentus* Audebert & Vieillot and *Eulampis holosericeus holosericeus* L.; Santiago-Valentín 1995; M. A. Caraballo-Ortiz, pers. obs.). Although there are other common pollinator species in Puerto Rico (e.g. *Xylocopa* carpenter bees), they have not been observed interacting with *G. elegans* flowers.

Study site

Our study was conducted at the Botanical Garden of the University of Puerto Rico in San Juan, Puerto Rico (18°23'38.17" N, 66°03'46.35" W; 19 m asl), located within the subtropical moist forest zone (Holdridge system: Ewel & Whitmore 1973). All *G. elegans* plants used in this study were grown from seeds collected randomly from a wild population of about 50 trees at the municipality of Isabela, in northwestern Puerto Rico (Rafael Rivera-Martínez [Conservation Trust of Puerto Rico], pers. comm.). Experiments were performed from November 2003 to January 2005 with cultivated trees of *G. elegans* that had reached maturity. Under cultivation conditions, *G. elegans* begin first flowering during the first two or three years of age. Accessibility to an adequate number of trees and flower samples, as well as the fact that the two putative pollinators are common residents of the Botanical Garden, made the cultivated setting an ideal set-up for the pollination experiments.

Pollination treatments

We selected 20 cultivated adult trees of *G. elegans* to examine its breeding system. The trees were planted in 30 L pots, reaching a mean height of 2.5 m (S.E. ± 0.035 m) and all of them were positioned within an area of about 50 m². Each of the 20 experimental trees was subjected to 13 pollination treatments. Each pollination treatment was replicated three times (i.e. on three different flowers) on each tree (i.e. 39 flowers on each of the 20 trees: 780 flowers in total). The 13 pollination treatments were: (1) visits limited to *A. mellifera*, (2) visits limited to *C. flaveola*, (3) exposed flowers, (4) bagged flowers (see details of bagging technique below), (5) outcrossed hand pollination, (6) geitonogamous hand pollination, and (7) selfed (same flower) hand pollination. To exclude the possibility that experimental flowers were contaminated with their own pollen, treatments 1-6 were repeated with emasculated flowers (representing treatments 8-13, respectively). The following describes details of each treatment.

For the exposed pollination treatment, flowers were left open continuously to all visitors until abscission. In the bagged treatments, flowers were kept covered to exclude all pollinators. Flowers used to test pollination by animal vectors (*A. mellifera* and *C. flaveola*) were kept bagged and only exposed to allow a single visit per flower. Immediately after the vector visit, we re-bagged the flower and waited for fruit production. In the hand-pollinated treatments, flowers were bagged at all times except while performing manual pollinations and emasculations. For the outcrossing treatment, pollen was manually transferred between trees, and for the geitonogamy treatment, pollen was manually applied among flowers of the same tree. To examine within-flower selfing, pollen was manually transferred from anthers to the stigma of the same flower.

Hand-pollinations were performed using a fine brush with a long, pointed tip. The brush was rinsed thoroughly with water and dried between each pollen transfer to avoid contamination. Emasculations were performed one day prior to flower anthesis (and dehiscence of anthers) by removing all anthers in the flower bud with forceps. For all treatments involving bagged flowers, flower buds were covered one day prior to anthesis using a pellon cloth bag. Pellon is a lightweight, non-woven fabric used in the clothing industry that allows light and air to pass through. It has previously been used to cover flowers (Wyatt *et al.* 1992) without altering their development or longevity (Santiago-Valentín 1995). For each of the pollination treatments, fecundity was measured in three different ways: as fruit set, as seed set, and seed viability. Seedless fruits were classified as aborted and considered as zero in the fruit set analysis and not included in the seed set analysis. Seed viability tests were carried out at the Conservation Trust of Puerto Rico nurseries (located within the grounds of the Botanical Garden of the University of Puerto Rico in Río Piedras, Puerto Rico) by germinating the seeds in a humid peat moss bed with low sunlight conditions (to simulate natural germinating conditions for this species) and verifying normal shoot and root growth from January 2004 to April 2005. All seeds collected from an individual tree were planted in a block, and all the blocks were positioned at random along the peat moss bed.

Statistical analyses

We tested for differences in fruit set, seed set and seed viability among the 13 pollination treatments first by using the fraction of fruit set as the response variable. Fractions were calculated on a per-flower basis ($N = 3$ flowers per treatment per tree, thus, possible outcomes were 0, 0.33, 0.66 and 1). Seed set data were normalized using a logarithmic transformation, while seed viability data were normalized using an arcsine transformation. In statistical analyses, trees ($N = 20$ individuals) were treated as blocks where all treatments were represented and replicated. First we analyzed fecundity measures altogether using a multivariate analysis of variance (MANOVA; SAS Institute 2000) given that we measured multiple and correlated response variables on the same treatments. In the MANOVA, the responses were the percentage of fruit set per flower, seed number per flower and, percentage of seed viability per flower, while the factors were pollination treatments, individual, and the interaction between

pollination treatments and individual. Following significance of this analysis (and as recommended by Scheiner 2001), we conducted post hoc univariate tests using two-way ANOVA to test the effects of pollination treatments (at 13 levels), individual, and the interaction terms using JMPIN (SAS Institute 2000). In the case of fruit set, we used a Kruskal-Wallis ANOVA (and post hoc pairwise Wilcoxon Signed Rank tests with Bonferroni correction) given that data structure violated ANOVA assumptions. Results are presented as mean ± standard deviation.

RESULTS

Pollinator visits

Visits by *A. mellifera* produced fruit on 28% of the unemasculated flowers, and 35% of the emasculated flowers (Fig. 2a). The average number of seeds recorded for *A. mellifera* visits was 0.7 ± 0.2 seeds in the unemasculated flowers, and 0.9 ± 0.2 seeds in the emasculated flowers (Fig. 2b). Lastly, seed viability in the unemasculated and in the emasculated flowers was 12% and 22%, respectively (Fig. 2c).

Following a similar pattern to *A. mellifera*, visits by *C. flaveola* produced fruit on 22% of the unemasculated flowers and 18% of the emasculated flowers (Fig. 2a). Seed set and seed viability for the unemasculated flowers visited by *C. flaveola* were 0.5 ± 0.2 seeds and 10% respectively, whereas the values for the emasculated flowers were 0.6 ± 0.2 seeds and 15%, respectively (Fig. 2b, c).

Although *A. mellifera* and *C. flaveola* were effective pollinators of *G. elegans*, in contrast to our expectations, both animals were statistically similar for all of the fecundity measures (Fig. 2). Also, we did not detect statistical differences between any of the unemasculated and emasculated pollination treatments for either *A. mellifera* and *C. flaveola* (Fig. 2).

Breeding system

The unemasculated exposed flowers showed 50% fruit set, while the emasculated exposed flowers yield a fruit set of 47% (Fig. 2a). For seed set and seed viability, the values for the exposed unemasculated flowers were 1.7 ± 0.3 seeds and 32% respectively, and for the emasculated flowers were 1.8 ± 0.3 seeds and 26%, respectively (Fig. 2b, c). On the other hand, the bagged treatment produced the lowest values for fruit set, with 3% for unemasculated flowers and 0% for emasculated flowers (Fig. 2a). Additionally, the unemasculated bagged flowers had a seed set of 0.2 ± 0.1 seeds, and a seed viability of 3% (Fig. 2b, c).

The highest fruit yield (70%) was recorded in the unemasculated outcrossed flowers, followed by its emasculated counterpart (57%; Fig. 2a). Similarly, the outcrossed pollination treatment obtained the highest seed set and seed viability for the unemasculated (2.7 ± 0.3 seeds and 52% viability) and for the emasculated (2.2 ± 0.4 seeds and 34% viability) flowers when compared to the other pollination treatments (Fig. 2b, c). In sharp contrast to the outcrossed treatment, the selfed treatment produced a fruit set of 7% and the lowest values for seed set and for seed viability,

with 0.1 ± 0.7 seeds and 2% viability (Fig. 2). The geitonogamy pollination treatment followed the same pattern as the selfed treatment, with a fruit set of 8%, a seed set of 0.2 ± 0.1 seeds, and a seed viability of 5% in the unemasculated flowers (Fig. 2). Likewise, emasculated flowers in the geitonogamy treatment produced a fruit set of 17%, a seed set of 0.4 ± 0.1 seeds, and a seed viability of 4% (Fig. 2).
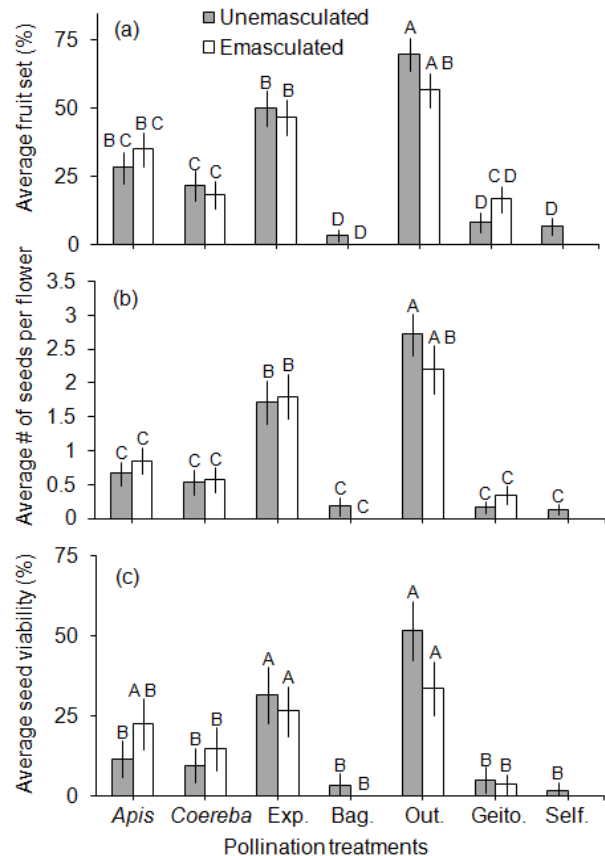

FIG. 2. Pollination treatments among unemasculated and emasculated flowers of *Goetzea elegans*. Columns show standard error bars, and different letters indicate statistical differences among treatments (α = 0.05). Pollination treatments include visits of *Apis mellifera* (*Apis*), visits of *Coereba flaveola* (*Coereba*), exposed flowers (Exp.), bagged flowers (Bag.), outcrossed pollination (Out.), geitonogamous pollination (Geito.), and selfed pollination (Self.). (a) Average percentage of fruit set (%). (b) Average number of seeds per flower. (c) Average percentage of seed viability (%).

While the bagged, selfed and geitonogamy treatments were not significantly different from each other, they were different from the exposed and outcrossed pollination treatments for all fecundity measures. The fruit set of the pollinator visitations was significantly different from the bagged, selfed and geitonogamy treatments (except for the emasculated geitonogamy treatment; χ^2 = 178, df = 12, P = < 0.0001); however, seed set and seed viability values were not significantly different among pollination treatments (Fig. 2). Moreover, we did not detect significant differences among any of the unemasculated and emasculated pollination treatments for the breeding system (Fig. 2). The MANOVA test detected significant differences among the pollination treatments, the individual *G. elegans* tree, and the interaction

between pollination treatments and individual (Tab. I). In the model, the pollination treatments were the most important factor explaining the variance (46%), followed by the individual (8%) and the interaction between pollination treatments and the individual (3%). After performing the two-way ANOVA tests, we found significant effects of pollination treatments ($F_{12,520} = 22.5$, P < 0.0001), individual ($F_{19,520} = 3.9$, P < 0.0001), and the interaction between pollination treatments and individual ($F_{228,520} = 1.4$, P = 0.002) on the seed set. Similarly, for the seed viability tests, we found significant effects of pollination treatments ($F_{12,520} = 17.1$, P < 0.0001), individual ($F_{19,520} = 3.1$, P < 0.0001), and the interaction between pollination treatments and individual ($F_{228,520} = 1.4$, P = 0.001).

Table 1. Results for the multivariate analysis of variance (MANOVA) for the whole model, for pollination treatments, for individual trees of *Goetzea elegans*, and for the interaction between pollination treatments and individual trees.

Source	Pillai's trace	Approx. *F*	df	P
Whole model ($R^2 = 0.57$)	0.93	1.75	518, 1040	<0.0001
Pollination Treatments	0.31	7.88	24, 1040	<0.0001
Individual Tree	0.19	2.81	38, 1040	<0.0001
Pollination Treatments x Individual Tree	0.75	1.36	456, 1040	<0.0001

DISCUSSION

We found that both *A. mellifera* and *C. flaveola* were legitimate pollinators of *G. elegans*, with similar pollination effectiveness per visit. In addition, *G. elegans* is partially self-incompatible, requiring pollen outcrossing to produce a high fruit set. Outcrossing is the most common breeding system in tropical trees, although there are varying degrees of self-compatibility (Bawa et al. 1985; Doligez & Joly 1997; Nason & Hamrick 1997; Dick et al. 2003; Ward et al. 2005).

Pollinator visits

The legitimate pollination of *G. elegans* by *A. mellifera* corroborates results from some studies showing the beneficial role of the introduced honeybee as a pollinator of native plant species (Vaughton 1992; Dick 2001; Gross 2001; Dick et al. 2003; Fumero-Cabán & Meléndez-Ackerman 2007), and differs from those where *A. mellifera* has been found to be less effective than native nectarivorous birds (Vaughton 1996; Hansen et al. 2002). However, our results support the idea that the effectiveness of *A. mellifera* as pollinator is contingent on the structural attributes (size and shape) of flowers, as well as on the foraging behaviour of *A. mellifera* (Vaughton 1996). For example, when *A. mellifera* visited *G. elegans* flowers to collect pollen, it landed and crawled throughout the anthers and usually made contact with the nearby stigma, thus promoting pollination (Fig. 1b).

Conversely, when *A. mellifera* foraged for nectar, it landed on the inner surface of the corolla lobes from which it inserted its proboscis into the nectaries (located at the base of the ovary), which did not usually result in pollination (M. A. Caraballo-Ortiz, unpubl. data).

The pollination role of *C. flaveola* in *G. elegans* contrasts with their robbing behaviour reported in several studies on flower visitation (Kodric-Brown et al. 1984; Askins et al. 1987; Fumero-Cabán & Meléndez-Ackerman 2007). Ricart (1992) determined that robbing or legitimate pollination behaviour of *C. flaveola* was dependent on corolla length. The short size of the corolla tube in *G. elegans* (11.1 ± 1.6 mm in length and 10.2 ± 1.6 mm in width) provides easy access for *C. flaveola* to nectar (culmen length = 13.2 ± 0.7 mm), and the exserted stamens promote pollen deposition on the bird's forehead and consequently, pollen transport. Robbing by *C. flaveola* has been reported only in other plant species bearing longer corolla tubes (> 19 mm in length, unpublished data), where legitimate access to the flower nectaries by those birds with short bills is prevented by the disparity between bill and corolla length.

Breeding system

We detected a strong effect of inbreeding depression in the seed viability of the selfed treatments, suggesting that a single generation of selfing is sufficient to lower the germination vigour of a mainly outcrossing species such as *G. elegans*. By being partially self-incompatible, *G. elegans* decreases the deleterious effects of inbreeding and promotes genetic diversity in its populations (Charlesworth & Charlesworth 1979). Outcrossing plants generally have higher recombination rates, and thus, are more genetically variable and have higher levels of heterozygosity within populations than selfing plants (Hamrick et al. 1979; Loveless & Hamrick 1984; Hamrick & Godt 1989). Recombination is particularly important for plants that exist in small populations, as they often suffer from lower genetic diversity due to founder effects (Pfosser et al. 2005). Although outcrossing assures genetic variability of populations, a degree of self-compatibility enables reproduction in isolation when cross pollen limits seed set. In the Solanaceae family, there are several mechanisms through which the self-incompatibility system of a species can allow self-fertilization. These include changes in environmental conditions, the age of the flowers, and mutations in the *S*-alleles, among others (e.g., Levin 1996; Tsukamoto et al. 2003; Travers et al. 2004). It is possible that the partial self-compatibility in *G. elegans* evolved as a mutation that became fixed in the population, due to the fact that it increases the probability of reproduction in isolation or in fragmented habitats. As with *G. elegans*, other plants in Solanaceae have been found with partial self-incompatibility, such as the weed *Solanum carolinense* L., which has a plastic self-incompatible system (Travers et al. 2004).

The low fecundity exhibited by single visits of both *C. flaveola* and *A. mellifera* when compared to the exposed pollination treatment suggests that repeated visits to the flowers of *G. elegans*, which remained open an average of 3.4 days (S.D. ± 0.82), are necessary to increase chances of pollen deposition. However, the outcrossed pollination treatment

achieved higher fecundity rates than the exposed treatment, indicating that a significant proportion of pollen deposition in exposed flowers might be the result of either heterospecific pollen or geitonogamy. Cultivated *G. elegans* trees used in this study usually displayed several dozens of flowers at once, which increases the probability of geitonogamous pollination. We predict that geitonogamy is frequent in larger and isolated wild trees, where thousands of flowers are presented to visitors at once.

The statistical similarity between the emasculated and unemasculated pollination treatments suggests that the pollen present in mature anthers does not interfere (i.e. blocking or clogging) with pollen deposition on the stigma, or that outcross pollen is more vigorous and fertilizes the ovaries faster than selfed pollen (Cruzan 1989; Aizen et al. 1990; Snow & Spira 1993). The fecundity of unemasculated bagged flowers was similar to the selfed treatment, demonstrating the ability of *G. elegans* flowers to set fruit in the absence of pollinators (i.e., autogamy). Autogamy was facilitated by the proximity of the anthers to the stigma due to the similar length of the reproductive whorls in most of the examined flowers. We observed, however, that a few trees presented a degree of herkogamy. Herkogamy - a disparity in the length of stamens and style of flowers - prevented those *G. elegans* trees from performing autogamy, and could be interpreted as an additional mechanism to promote outcrossing (Motten & Antonovics 1992). Finally, the lack of fruits in the emasculated bagged treatment suggests that *G. elegans* does not produce seeds by agamospermy, thus it requires pollen for sexual reproduction.

Conclusions

Unlike many introduced species, *A. mellifera* has a positive effect on the fecundity of the tropical endangered tree *G. elegans*. Besides being an additional pollinator, the ability of *A. mellifera* to connect isolated populations through pollen outcrossing in fragmented landscapes (Dick 2001; Dick et al. 2003) is of particular importance for rare and partially self-incompatible species such as *G. elegans*, since cross-pollination is critical for their reproduction. At present, the scattered distribution of *G. elegans* trees in the wild and the considerable geographic distance among populations (mean: 36.1 Km ± 55.3) could prevent optimal outcrossing, thus jeopardizing the continuous genetic diversity of the species. Currently, the main threats to *G. elegans* survival are habitat destruction and modification, such as deforestation for agriculture, selective logging for fence posts, and limestone quarrying, as these activities promote fragmentation of populations, decline of reproductive trees, and reproductive isolation of remnant trees (USFWS 1987). Although nearly all *G. elegans* populations are restricted to small canyons or ravines, which also shelter important populations of other rare and endangered plants and animals, none of these areas have legal designation for protection. However, propagation efforts and establishment of *ex situ* populations in protected areas has already been initiated (Rafael Rivera-Martínez [Conservation Trust of Puerto Rico], pers. comm.). The establishment of these *ex situ* populations, in conjunction with the conservation of extant wild populations and the effective pollination of wild trees by *C. flaveola* and *A.*

mellifera, are essential to maintain viable populations and to promote the conservation of *G. elegans*.

REFERENCES

Aizen MA, Searcy KB, Mulcahy DL (1990) Among- and within-flower comparisons of pollen tube growth following self– and cross–pollinations in *Dianthus chinensis* (Caryophyllaceae). American Journal of Botany 77:671–676

Aizen MA, Morales CL, Morales JM (2008) Invasive mutualists erode native pollination webs. PLoS Biology 6:e31

Askins RA, Ercolino KM, Waller JD (1987) Flower destruction and nectar depletion by avian nectar robbers on a tropical tree, *Cordia sebestena*. Journal of Field Ornithology 58:345–349

Bartomeus I, Vila M, Santamaria L (2008) Contrasting effects of invasive plants in plant-pollinator networks. Oecologia 155:761–770

Bawa KS, Perry DR, Beach JH (1985) Reproductive biology of tropical lowland rainforest trees. I. Sexual systems and incompatibility mechanisms. American Journal of Botany 72:331–345

Callaway RM, Thelen GC, Rodriguez A, Holben WE (2004) Soil biota and exotic plant invasion. Nature 427:731–733

Charlesworth D, Charlesworth B (1979) The evolutionary genetics of sexual systems in flowering plants. Proceedings of the Royal Society of London, Series B, Biological Sciences 205:513–530

Cronk QCB, Fuller JL (1995) Plant invaders. Chapman and Hall, London, UK, 256 pp

Cruzan MB (1989) Pollen tube attrition in *Erythronium grandiflorum*. American Journal of Botany 76:562–570

Davis NE, O'Dowd DJ, Mac Nally R, Green PT (2010) Invasive ants disrupt frugivory by endemic island birds. Biology Letters 6:85–88

Dick CW (2001) Genetic rescue of remnant tropical trees by an alien pollinator. Proceedings of the Royal Society of London, B, Biological Sciences 268:2391–2396

Dick CW, Etchelecu G, Austerlitz F (2003) Pollen dispersal of tropical trees (*Dizinia excelsa*: Fabaceae) by native insects and African honeybees in pristine and fragmented Amazonian rainforest. Molecular Ecology 12:753–764

do Carmo RM, Franceschinelli EV, da Silveira FA (2004) Introduced honeybees (*Apis mellifera*) reduce pollination success without affecting the floral resource taken by native pollinators. Biotropica 36:371–376

Doligez A, Joly HI (1997) Mating system of *Carapa procera* (Meliaceae) in the French Guiana tropical forest. American Journal of Botany 84:461–470

Dupont YL, Hansen DM, Valido A, Olesen 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

Ewel JJ, Whitmore JL (1973) The ecological life zones of Puerto Rico and U.S. Virgin Islands. U.S. Department of Agriculture, Forest Service Research Report ITF–18, Río Piedras, Puerto Rico, USA, 72 pp

Freitas BM, Paxton RJ (1998) A comparison of two pollinators: the introduced honeybee *Apis mellifera* and an indigenous bee *Centris tarsata* on cashew *Anacardium occidentale* in its native range of NE Brazil. Journal of Applied Ecology 35:109–121

Fumero-Cabán JJ, Meléndez-Ackerman EJ (2007) Relative pollination effectiveness of floral visitors of *Pitcairnia angustifolia* (Bromeliaceae). American Journal of Botany 94:419–424

Goulson D (2003) Effects of introduced bees on native ecosystems. Annual Review of Ecology, Evolution, and Systematics 34:1–26

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

Gross CL, Mackay D (1998) Honeybees reduce fitness in the pioneer shrub *Melastoma affine* (Melastomataceae). Biological Conservation 86:169–178

Hamrick JL, Linhart YB, Milton JB (1979) Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. Annual Review of Ecology and Systematics 10:173–200

Hamrick JL, Godt MJ (1989) Allozyme diversity in plant species. In: Brown AHD, Clegg MT, Kahler AL, Weir BS (eds). Plant Population Genetics, Breeding and Germplasm Resources. Sinauer, Sunderland, Massachusetts, USA, pp. 43–63

Hansen DM, Olesen JM, Jones CG (2002) Trees, birds, and bees in Mauritius: exploitative competition between introduced honey bees and endemic nectarivorous birds? Journal of Biogeography 29:721–734

IUCN [International Union for Conservation of Nature] (2010) IUCN Red List of Threatened Species. Version 2010.4. [online] URL: http://www.iucnredlist.org, accessed on 03 December 2010

Kaiser-Bunbury CN, Müller CB (2009) Indirect interactions between invasive and native plants via pollinators. Naturwissenschaften 96:339–346

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

Kodric-Brown A, Brown JH, Byers GS, Gori DF (1984) Organization of a tropical island community of hummingbirds and flowers. Ecology 65:1358–1368

Levin DA (1996) The evolutionary significance of pseudo self-fertility. The American Naturalist 148:321–332

Little EL, Woodbury RO, Wadsworth FH (1974) Trees of Puerto Rico and the Virgin Islands. USDA Agriculture Handbook No. 449, US Forest Service, Washington, D.C., USA, 1024 pp

Loveless MD, Hamrick JL (1984) Ecological determinations of genetic structure in plant populations. Annual Review of Ecology and Systematics 15:65–95

Lugo AE (2005) The outcome of alien tree invasions in Puerto Rico. Frontiers in Ecology and the Environment 2:265–273

Moritz RFA, Hartel S, Neumann P (2005) Global invasions of the western honeybee (*Apis mellifera*) and the consequences for biodiversity. Ecoscience 12:289–301

Motten AF, Antonovics J (1992) Determinants of outcrossing rate in a predominantly self–fertilizing weed, *Datura stramonium* (Solanaceae). American Journal of Botany 79:419–427

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403: 853–858

Nason JD, Hamrick JL (1997) Reproductive and genetic consequences of forest fragmentation: two case studies of Neotropical canopy trees. Journal of Heredity 88:264–276

Nogueira-Filho SLG, Nogueira SSC, Fragoso JMV (2009) Ecological impacts of feral pigs in the Hawaiian Islands. Biodiversity and Conservation 18:3677–3683

Nunez MA, Bailey JK, Schweitzer JA (2010) Population, community, and ecosystem effects of exotic herbivores: a growing global concern. Biological Invasions 12:297–301

Olesen JM, Eskildsen LI, Venkatasamy S (2002) Invasion of pollination networks on oceanic islands: importance of invader complexes and endemic super generalists. Diversity and Distributions 8: 181–192

Olmstead RG, Bohs L, Migid HA, Santiago-Valentín E, García VF, Collier SM (2008) A Molecular Phylogeny of the Solanaceae. Taxon 57:1159–1181

Padron B, Traveset A, Biedenweg T, Diaz D, Nogales M, Jens M (2009) Impact of alien plant invaders on pollination networks in two archipelagos. PLoS One 4:e6275

Pfosser M, Jakubowsky G, Schlüter PM, Fer T, Kato H, Stuessy TF, Sun B-Y (2005) Evolution of *Dystaenia takesimana* (Apiaceae), endemic to Ullung Island, Korea. Plant Systematics and Evolution 256:159–170

Raffaele H, Wiley J, Garrido O, Keith A, Raffaele J (1998) A guide to the birds of the West Indies. Princeton University Press, Princeton, New Jersey, USA. 512 pp

Ricart CM (1992) Feeding ecology of nectar–feeding birds in the lower montane wet forest life zone, Maricao, Puerto Rico. Acta Científica 6:41–48

Rymer PD, Whelan RJ, Ayre DJ, Wetson PH, Russell KG (2005) Reproductive success and pollinator effectiveness differ in common and rare *Persoonia* species (Proteaceae). Biological Conservation 123:521–532

Santiago-Valentín E (1995) Reproductive and population ecology of *Goetzea elegans* Wydler (Solanaceae or Goetzeaceae). M.S. thesis, University of Puerto Rico, Mayagüez, Puerto Rico

SAS Institute (2000) JMPIN. Version 4.0.2, SAS Institute, Cary, North Carolina, USA

Scheiner SM (2001) MANOVA: multiple response variables and multispecies interactions. In Scheiner SM, Gurevitch J (eds). Design and analysis of ecological experiments (2nd edition). Oxford University Press, New York, USA, pp. 99–115

Snow AA, Spira TP (1993) Individual variation in the vigor of self pollen and selfed progeny in *Hibiscus moscheutos* (Malvaceae). American Journal of Botany 80:160–164

Stanley SS, DeGrandi-Hoffman G, Smith DR (2004) The African Honeybee: Factors contributing to a successful biological invasion. Annual Review of Entomology 49: 351–376

Travers SE, Mena-Alí JI, Stephenson AG (2004) Plasticity in the self-incompatibility of *Solanum carolinense*. Plant Species Biology 19:127–135

Traveset A, Richardson DM (2006) Biological invasions as disruptors of plant reproductive mutualisms. Trends in Ecology and Evolution 21:208–216

Tsukamoto T, Ando T, Takahashi K, Omori T, Watanabe H, Kokubun H, Marchesi E, Kao T-H (2003) Breakdown of self-incompatibility in a natural population of *Petunia axillaris* caused by loss of pollen function. Plant Physiology 131:1903–1912

USFWS [U.S. Fish & Wildlife Service] (1987) Beautiful *Goetzea* Recovery Plan. U.S. Fish and Wildlife Service, Atlanta, Georgia, USA, 35 pp

Vamosi JC, Knight TM, Steets JA, Mazer SJ, Burd M, Ashman T-L (2006) Pollination decays in biodiversity hotspots. Proceedings of the National Academy of Sciences of the United States of America 103: 956–961

Vaughton G (1992) Effectiveness of nectarivorous birds and honeybees as pollinators of *Banksia spinulosa* (Proteaceae). Australian Journal of Ecology 17:43–50

Vaughton G (1996) Pollination disruption by European honeybees in the Australian bird-pollinated shrub *Grevillea barklyana* (Proteaceae). Plant Systematics and Evolution 200:89–100

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

Westerkamp C (1991) Honeybees are poor pollinators– why? Plant Systematics and Evolution 177:71–75

Whittaker RJ (1998) Island biogeography: ecology, evolution, and conservation. Oxford University Press, Oxford, New York, USA, 304 pp

Winston ML (1987) The biology of the honey bee. Harvard University Press, Cambridge, UK, 294 pp

Wyatt R, Broyles SB, Derda GS (1992) Environmental influences on nectar production in milkweeds (*Asclepias syriaca* and *A. exaltata*). American Journal of Botany 79:636–642