Breeding system and pollination of Gesneria pauciflora (Gesneriaceae), a threatened Caribbean species

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Mervin Perez
University of Puerto Rico at Rio Piedras
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Elvia Melendez-Ackerman
University of Puerto Rico at Rio Piedras
83 PUBLICATIONS **1,154** CITATIONS

Omar A. Monsegur-Rivera
U.S. Fish and Wildlife Service
4 PUBLICATIONS **2** CITATIONS

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Breeding system and pollination of *Gesneria pauciflora* (Gesneriaceae), a threatened Caribbean species

Mervin E. Pérez[a,*], Elvia J. Meléndez-Ackerman[a,b], Omar A. Monsegur-Rivera[c]

[a] Environmental Sciences Department, College of Natural Science, University of Puerto, Rico-Río-Piedras, Puerto Rico
[b] Center for Applied Tropical Ecology and Conservation, College of Natural Science, University of Puerto, Rico-Río Piedras Campus, Puerto Rico
[c] Caribbean Ecological Services Field Office, Fish and Wildlife Service, Boquerón, Puerto Rico

ABSTRACT

Knowledge of plant breeding systems may be critical to facilitate recovery of species considered at risk. *Gesneria pauciflora* is a threatened, rupiculous, and riverine species with floral-traits that suggest a hummingbird-pollination system. Because these habitat-pollinator conditions have been linked to pollinator-independent reproductive systems, we expected a breeding system that provides reproductive assurance. We assessed the breeding system and potential vulnerabilities during pollination phase using field observations and pollination experiments, to determine pollinator dependency and the existence of strategies for reproductive assurance. *Gesneria pauciflora* was pollinated by two hummingbird species but visitation rates were low, probably due to low nectar rewards. Fruit set and seed production were significantly higher in flowers when pollen was artificially delivered (self- and cross-pollination) compared to when it was not (control and autogamy), suggesting that reproduction is pollen-limited. *Gesneria pauciflora* is self-compatible, with some seeds produced via autonomous selfing without any effects of pollination mechanism on seed germination rates. Florivory by the native plume-moth *Postplatyptilia caribica* was observed, but its incidence was low (12%). Our findings are consistent with theory on the evolution of plant breeding systems on islands, and contribute to the understanding of the evolution of breeding systems in Caribbean gesneriads. Overall, data do not show immediate vulnerabilities to this listed species at the pollination phase, and autogamy may represent reproductive assurance counteracting unpredictable environmental factors that influence its reproduction. However, its capacity for selfing is small relative to other tubular Puerto Rican *Gesneria* which may explain its rare status.

1. Introduction

Information on breeding systems may be critical for the development of successful strategies to facilitate recovery in rare, endemic, threatened, or endangered plant species (Cao et al., 2006; Pino-Torres and Koptur, 2009; Robertson et al., 2004; Rodríguez-Pérez, 2005). Breeding systems may influence processes at the reproductive stage that in turn influence the type of mating (selfing vs. outcrossing), the likelihood of fruit and seed production, and plant fitness. For example, *Astrophytum asterias* (Zucc.) Lem., a self-incompatible endangered cactus with a restricted distribution range, has a reproductive system that is highly dependent on the availability and effectiveness of its pollinators (Strong and Williamson, 2007). The same phenomenon has been shown for the threatened shrub *Tetratheca juncea* Sm. (Gross et al., 2003) and the endemic perennial shrub *Polygala vayredae* Costa (Castro, 2009; Castro et al., 2008). All of which faced reproductive constraints due to low biotic visitation rates and high pollinator dependence, which

led to recommendations for management of their habitat as well as their plant-pollinator interactions to improve fruit and seed production. However, not all endangered or threatened species have an obligate pollination interaction but instead exhibit mixed pollination systems that may reduce their vulnerability (Yates and Ladd, 2004). The endangered herbs *Helianthemum marifolium* Mill. and *H. caput-felis* Boiss. are both self-compatible species that produce fruit and seeds in self-pollinated and outcrossed flowers (Rodríguez-Pérez, 2005); the endemic herb *Petrocoptis viscosa* Rothm, has an autonomous facultative self-pollination system that has the potential for outcrossing (Navarro and Guitián, 2002); and the endangered climbing vine species *Jacquemontia reclinata* House ex Small, is predominantly outcrossing but is also capable of producing fruits and seeds via late autogamy (Pino-Torres and Koptur, 2009). Thus, the observed variability in breeding system mechanisms across rare and endemic species may also be accompanied by variation in their reproductive capacity and in their demographic vulnerability.

Plants may exhibit a reduction in fitness during the pollination phase due to variation in biotic and abiotic factors, or by human-disturbance impacts through a variety of mechanisms. Large-scale disturbance events such as selective logging, metal soil contamination, wetland creation, may reduce the availability of pollinators and increase pollen limitation, but also diminish pollen loads on stigmas and in the process reduce outcrossing (Eckert et al., 2009). Likewise, forest patch isolation (habitat fragmentation) can reduce pollinator network connectivity and increase vulnerability in outcrossing species by reducing plant fitness (Vanbergen et al., 2014). Changes in climate (i.e. temperature and precipitation) by inducing early or late flowering events (Hamann, 2004; Iler and Inouye, 2013; Menzel et al., 2006) can lead to mismatches in plant-pollinator interaction that in turn may decrease fruit production (Hegland et al., 2009). Other vulnerabilities during the pollination phase may result from antagonist processes such as flower herbivory, in which pollinator' visitation rates might decrease due to a reduction in flower attractiveness, nectar production and delayed flowering (McArt et al., 2014; Schiestl et al., 2014). Given the tight link between pollination and plant fitness, it is important to evaluate the factors that influence pollination success directly or indirectly, especially for rare and endangered species as a way to gather information that may help improve natural seed production, seedling recruitment, and long-term population persistence.

For plants endemic to insular systems, the evolution of breeding systems may be influenced by ecological conditions that may characterize island ecosystems (Barrett, 1996; Charlesworth, 2006). This hypothesis, also known as Baker's rule (Baker, 1955; Barrett, 1996), states that species with self-compatible and autonomous pollination systems should be more common in island ecosystems (relative to continental ones); where pollinator faunas are less diverse or where pollinators of species dispersing into islands are absent (Anderson et al., 2001; Ayre et al., 1994; Barrett et al., 2008; Jacquemyn et al., 2005; Wheelwright et al., 2006). Alternatively, novel pollinator interactions and generalized pollination systems are also expected in these environments (Martén-Rodríguez and Fenster, 2010; Martén-Rodríguez et al., 2009). Recent analyses comparing Gesneriaceae in mainland and island ecosystems suggested no differences in autofertiliy index between geographic areas, yet generalized systems were more evident in insular species (Martén-Rodríguez et al., 2015). While studies by Marten-Rodríguez and collaborators support some hypotheses on the evolution of plant breeding systems on islands, they also suggest that local habitats may also influence plant breeding systems. For example, rupiculous (i.e. growing on rocks) and epiphytic (i.e. growing on trees) gesneriad species exhibit autonomous breeding systems more often that terrestrial ones. One hypothesis is that on rupiculous and epiphytic habitats, autonomous breeding systems may be favored to maximize the use of available resources to reproduction under unpredictable pollinator environments (Martén-Rodríguez et al., 2015). Our target species, *Gesneria pauciflora* Urb., is a rupiculous species that grows along riverbanks that may experience periodic flash floods (or extended dry periods). Assuming that these habitats may offer uncertain pollinator environments as in other gesneriads, *G. pauciflora* may also exhibit an autonomous breeding system for reproductive assurance.

We evaluated the breeding system and pollinators of the endangered species *Gesneria pauciflora* Urb. (Gesneriaceae) on the island of Puerto Rico. There are five additional species of *Gesneria* [*G. citrina* Urb., *G. cuneifolia* (DC.) Fritsch, *G. pedunculosa* (DC.) Fritsch, *G. reticulata* (Griseb.) Urb., and *G. viridiflora* subsp. *sintenisii* (Urb) L.E. Skog], all of which are self-compatible species as one would expect on insular ecosystems (Martén-Rodríguez and Fenster, 2008). With the exception of *G. reticulata*, all *Gesneria* species reported for Puerto Rico are endemic and only *G. pauciflora* is classified as a threatened species under the US Endangered Species Act. This species was listed due to its narrow distribution (endemic to serpentine soils in south-western Puerto Rico) and the potential for population extinction due to large-scale natural (i.e. hurricanes, landslides or severe flooding) and human disturbances

(i.e. changes in the hydrology due to water extraction) (U.S. Fish and Wildlife Services, 1995). The study of this species' reproductive biology and ecology are identified as recovery actions in the "Recovery Plan for *G. pauciflora*" (U.S. Fish and Wildlife Services, 1998). However, we lack information on its reproductive biology, potential pollinators, and on vulnerabilities experienced during the pollination phase. *Gesneria pauciflora* is a rupiculous and riverine species that may experience periodic disturbances. Assuming that *G. pauciflora*'s breeding system behaves in similar ways to other rupiculous gesneriads, we hypothesized that the species may exhibit an autonomous breeding system for reproductive assurance. Herein we address the information gaps in its reproductive biology by asking the following questions: What are the main pollinator (s) of *G. pauciflora*? Is fruit or seed production influenced by the type of pollination (self- vs. cross)? Is the breeding system of *G. pauciflora* different to those reported for other *Gesneria* species on Puerto Rico? Our goal was to carry out a comprehensive study to evaluate the reproductive biology of *G. pauciflora* focusing on events occurring at the pollination stage. We also compared the breeding system characteristics of *G. pauciflora* to those reported for other *Gesneria* species in the Caribbean to explore the ecological and evolutionary context of *G. pauciflora*'s breeding system.

2. Materials and methods

2.1. Study site

The Maricao State Forest has an area of 4150 ha and lies at the western end of the Cordillera Central mountain system of Puerto Rico within the municipalities of Maricao, Sabana Grande, and San Germán (Anadón-Irizarry, 2006). The protected area's topography is dominated by rugged mountains and hills with steep slopes (between 40 and 60%), the majority of which (90%) is characterized by serpentine soils (Ricart Pujals and Padrón Vélez, 2010). This particular geological substrate distinguishes the Maricao Commonwealth Forest as an important ecological area due to a high number of associated endemic species (Ricart Pujals and Padrón Vélez, 2010), and its contribution to the higher plant diversity in Puerto Rico (Cedeño-Maldonado, 1997; Cedeño-Maldonado and Breckon, 1996). The Reserve's mean temperature and precipitation are 21.7 °C and 2326 mm, respectively (Tossas, 2006). The forest reserve area has an elevation range from 150 to 875 m that includes subtropical wet, moist forest, and lower montane wet life zones (Tossas, 2006). Our study site was located within the Maricao River watershed where one of the larger populations of *G. pauciflora* is reported. We selected three patches for breeding system experiments that ranged from 500 to 700 m asl.

2.2. Study system

Gesneria pauciflora (Gesneriaceae) is an acaulescent or suffruticose plant with suffrutescent basal stem; branches are erect or decumbent (Liogier, 1995) and large plants form creeping mats with fragile branches. Inflorescences produce one to three nectar-producing tubular flowers that are about 2 cm long and have a yellowish-orange color (Fig. 1). Flowering appears to occur throughout the year with peaks happening between August and October (Liogier, 1995; U.S. Fish and Wildlife Services, 1998). The species was listed in 1995 as threatened due to its narrow distribution and the potential for population extinction thru large-scale natural and human disturbances (U.S. Fish and Wildlife Services, 1995). The 12 known subpopulations have an estimated 780–1425 individuals (U.S. Fish and Wildlife Services, 2013). Inspection of these patches suggests that the number of individuals among sub-populations is highly variable with some containing less than five individuals and others more than 600. The species grows along streambeds on exposed rock in the lower parts of the river, and on step slopes in the upper watershed.
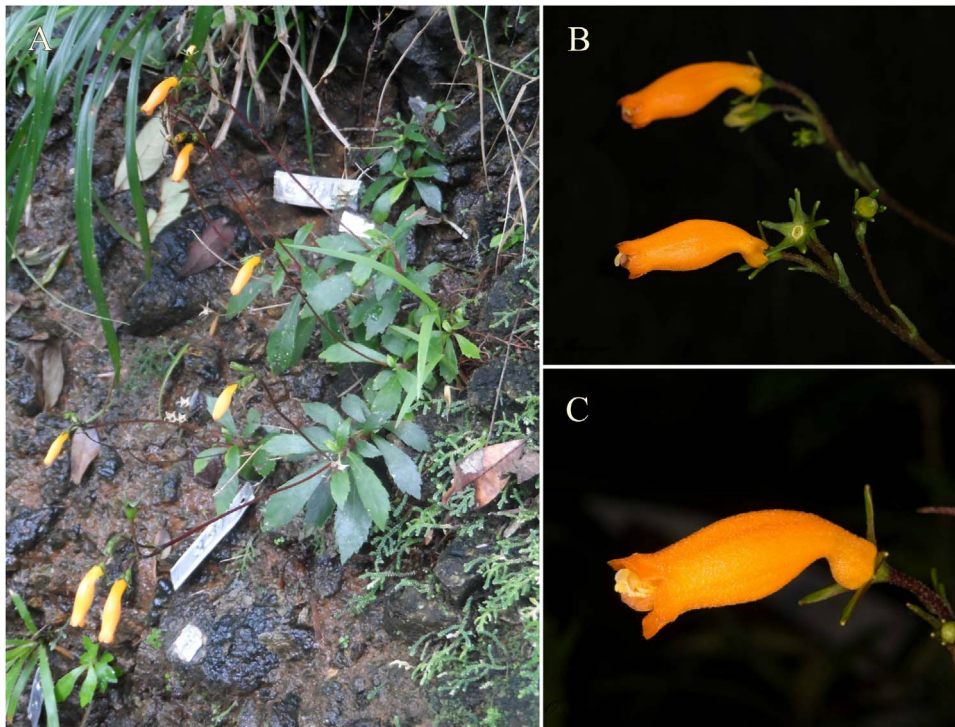
Fig. 1. *Gesneria pauciflora* inhabiting rocky wet habitats (A). Flowers when stigma was first exposed (B) and when stamens reached the stigma level (C). Images by Mervin E. Pérez (A) and Omar A. Monsegur-Rivera (B and C).

2.3. Pollination activity

Pollinator observations were initially conducted during September and October of 2012 (29.5 h) and 2013 (24 h) for a total of 53.5 h that included more than 100 plants in both years. As the reproductive phenology of the species extended to other months of the year, we expanded observations to include the following periods: December 2015, January–February 2016, July–December 2016, and January–March 2017. Biweekly observations were performed during these periods amounting to 65.5 h in more than 150 plants. In addition to human observations, from September 14th to October 10th we placed a LTLACON scouting camera (LTL-6310, 12 mega pixels) facing five reproductive plants to record diurnal and nocturnal (infrared vision) pollination activity. We set the camera's motion sensor to high sensitivity mode and when activated it recorded three pictures and a 30-s video with a sensor time lag of 10 min between activations. The LTLACON camera recorded 763 videos equivalent to 6.36 h of shooting. Additionally, 2310 pictures were recorded during the same period. The LTLACON camera recordings, however, did not yield pollinator visits. Overall, observation activities covered 10 plant colonies varying in the number of reproductive individuals (from one to more than 50) distributed throughout the forest.

Direct human flower observations covering diurnal activity of pollinators ranged from early in the morning (6 a.m.) to late in the afternoon (6 p.m.) during which we recorded for each plant the number of open flowers, the number of flowers visited, and the identity of pollinator species. Visitation rate was calculated as the number of flowers visited per plant per hour. Regardless of the year, hummingbirds visiting flowers of *G. pauciflora* were observed only from September to October, and thus data from these two months were used to assess annual differences in visitation rate (2012, 2013, and 2016). The data did not meet normality or homoscedasticity assumptions, thus we used a non-parametric Kruskal-Wallis analysis and Nemenyi post-hoc comparison with Chi-square correction (Hicks et al., 2016) to test for differences in annual visitation rates. To run these tests, we used the PMCMR package (Pohlert, 2014) in R (R Core Team, 2013).

2.4. Nectar volume and concentration

We marked 24 flowers for the measurement of nectar volume and concentration; however, flowers were extremely fragile so that measurements were only possible in 12 flowers from different plants. Nectar volume was measured with 5 μL capillary tubes and nectar concentration was determined using a hand-held refractometer (Brix scale range 0–32%, Atago, U.S.A., Inc.). Each flower was bagged and measured four times during a 24-h period as follows: 12:00 p.m. and 6:00 p.m. (first day), 6:00 a.m. and 12:00 p.m. (second day). After four nectar measurements, flowers become more delicate and obtaining readings was less successful. A repeated measure ANOVA was used (using the "Holm-Bonferroni" method for multiple paired comparisons to reduce type I error) to compare nectar volume and concentration at four different times within a 24-h period across two days in the lifetime of the flowers.

2.5. Breeding system experiment

From September 2016 to January 2017, we tagged 131 plants and used four different hand-pollination treatments to assess the need for pollinators to produce fruit: 48 plants for the control (110 flowers), 31 for autogamy (66 flowers), 27 in the self- (59 flowers) and 25 cross-pollination (41 flowers) treatments. Pilot experiments assessing asexual reproduction at flower level (apomixes) failed to produce fruits. Therefore, this treatment was excluded from the analyses. With the exception of those flowers selected for the control treatment, all flower buds were covered using organza mesh bags before opening to exclude flower visitors. All hand pollinations (self- and cross-pollinations) were executed when pistils and stamens were exerted. For self-pollinations, we used pollen from the same flower or plant, and for cross-pollinations, we used pollen from donors located in different colonies or plants located at least nine meters away from focal plants to avoid crossing genetically identical individuals (Martén-Rodríguez and Fenster, 2010). Autonomous autogamy, the capacity to produce fruits and seeds when isolated from pollination (Eckert et al., 2009), was determined by bagging flower buds which were followed for flower development and

fruit maturation. All treatments were monitored weekly for the first month (September 2016) when the experiment was set and biweekly thereafter to follow flower fate and record events of fruit initiation, production, abortion, or any other factor influencing fruit development and maturation. Following fruit progress, ripe fruits were collected and transported to the laboratory where we counted the number of healthy seeds per fruit. We defined healthy seeds as those that were oblong in shape with light/dark brown color discarding those flat and translucent seeds or twisted linear seeds with dark color. A generalized linear model was used to test for differences in fruit set among pollination treatments with fruit set expressed as a binomial response (pollinations that produced fruits vs. those that did not), and pollination treatment as a categorical explanatory factor with the control treatment as the reference category. This analysis was run in R using the nlme package (Pinheiro et al., 2017) assuming a binomial distribution and a logit link function (Dart and Eckert, 2015). To test for differences in the number of healthy seeds among reproductive treatments, we ran an ANOVA and a post hoc Tukey test evaluating all possible pollination treatment contrasts. For plants that had replicated pollination treatments, we used the average number of seeds to generate a representative value per plant. For these analyses, we did not use information from fruits that were damaged by herbivory (two plants excluded), or seeds that were dispersed before fruits were collected (seven plants excluded), and not all the plants developed fruits (54 plants in total). Therefore, the number of plants evaluated for the analyses of seed production was lower than the ones used for fruit set (19 in the control, eight in the autogamy, 20 for the self- and 21 for the cross-pollination treatments).

Using the fruit set per treatment, we calculated a series of indexes to evaluate the potential influence of pollen limitation, autofertility, and self-compatibility on reproduction of *G. pauciflora*. First, we calculated an indicator of total pollen limitation using the pollen limitation index (PL): 1–(control/cross-pollinated); this index compares the relative success of control vs. artificial cross-pollinations assuming no manipulation effect (Vaughton and Ramsey, 2010). Values near zero indicate no pollen limitation. We also calculated an autofertility index (AF), which represents the relative fruit production success of the autonomous autogamy treatment to those produced by cross-pollinations; an AF index near zero denotes low auto-fertility in the species (Eckert et al., 2009). Last, we calculated an index of self-compatibility (SC) by dividing the relative fruit set of self-pollinations by the fruit set of cross-pollination treatments. An index from zero to 0.75 indicates a self-incompatible breeding system (Lloyd and Schoen, 1992).

2.6. Seed germination rates

A germination experiment was run from January to May of 2017 using fruits from the pollination experiments generated above. Fruits were collected from November 2016 to March 2017 and stored at 5 °C before starting the experiment to prevent degradation. The seeds from the four reproductive treatments were placed in a growth chamber programmed to have light for a 12 h-period with a diurnal and nocturnal temperature of 24 and 18 °C, respectively, resembling temperature conditions of the Maricao Forest Reserve. We randomly choose fifty healthy seeds per fruit (or less if the fruit had a lower number of seeds or if fruits were open just before collection) and placed them in petri dishes with one layer of filter papers wet with demineralized water. The number of seeds selected per plant varied from 17 to 300 depending on the number of fruit per plant. Seeds were weekly humidified and monitored to count the numbers of germinated seeds until no additional seeds germination was observed. Some petri-dishes showed signs of contamination with biological agents (i.e. fungi) and they were discarded resulting in a lower number of plants per treatment (control = 11, autogamy = 7, self-pollination = 14, and cross-pollination = 13). Data on germination rate was arcsine transformed to meet normality and homoscedasticity assumptions of ANOVA, which was used to test differences among treatments in the proportion of germinated seeds.

3. Results

3.1. Plant-animal interaction

During the 26 days in which the LTLACON camera was placed in the field, nine flowers were open at different times but no pollinator visits were recorded. Other animals (mouse, coqui frog, crabs, dragonflies, moths, and birds) were captured by the camera confirming that the equipment was working well. The overall visitation rate was low in the species averaging 0.07 visits/plant/hour. The years 2012 and 2016 scored the lowest and highest hummingbird visitation rate with 0.05 (± 0.02) and 0.1 (± 0.03) visits/plant/hour, respectively, while the year 2017 got 0.07 (± 0.02) visits/plant/hour. Nevertheless, there were no significant differences in visitation rates among years (Kruskal-Wallis Chi-square = 4.61; $P = 0.099$). Human observations outside the month of September and October did not capture any flower visits by pollinators.

The hummingbird species, *Anthracocorax viridis* Audebert & Vieillot (Green Mango) and *Chlorostilbon maugaeus* Audebert & Vieillot (Puerto Rican Emerald) were the only pollinators recorded visiting flowers of *G. pauciflora* during field observations. Hummingbirds visited flowers only in the morning from 7:00 to 11:00 AM with a maximum visit time length of approximately three seconds. In addition to being seen visiting *G. pauciflora*, these hummingbird species were flying around all sites most of the time (determined by sight or sound) and performing activities related to resting, bathing, or foraging on other species during scheduled and non-scheduled observations periods.

3.2. Nectar volume and concentration

Flowers of *G. pauciflora* produced up to 13 μL nectar standing crops with sugar concentrations of up to 13%. Their nectar volume did not change significantly through time (F = 1.17; $P = 0.337$; Fig. 2A) but their concentration did (F = 33.2; $P < 0.001$; Fig. 2B). Average nectar concentration tends to decrease significantly over time from 10.3% to 4.2% from the first to the last measurement. The overall mean volume and concentration was 6.6 μL (± 0.78 μL) and 7.11% (± 0.94%), respectively.

3.3. Breeding system and germination rate experiments

Approximately twelve percent from the 131 plants used in the breeding system experiment exhibited some degree of floral herbivory by the plume-moth *Postplatyptilia caribica* or by other unidentified insects. In addition, a small fraction of ripe fruits in the control treatment (8%) was also lost to herbivory also by *P. caribica* and other unidentified species.

The highest average fruit set proportion was reported for the supplemented pollination treatment [fruit set: 84.5% (± 6.0 Standard Error) and 74.1% (± 6.9) for cross- and self-pollination, respectively]. The control and autonomous treatment had lower fruit sets with values of 31.5% (± 5.7) and 18.7% (± 6.2), respectively. There were significant differences in fruit set among treatments ($X^2 = 26.74$; $P < 0.0001$; Fig. 3). The cross-pollination and self-pollination treatments had a significantly higher fruit set when compared with the control group (Z's > 3.0; all $P < 0.01$), but the control did not differ significantly from the autogamy treatment (Z = −1.67; $P = 0.09$; Fig. 3). We also observed significant differences in the mean number of healthy seeds among treatments (F = 9.25; $P < 0.001$). Averages were statistically similar between the control (140.3 ± 26.2) and autogamy (73.3 ± 20.0), and between self-(231.7 ± 25.9) and cross- (267.3 ± 22.0) pollination treatments (all $P > 0.05$; Fig. 4). Yet, the self- and cross-pollinations had a significantly higher average numbers of healthy seeds when compared with control and autogamy treatments (all $P < 0.05$; Fig. 4). No significant differences were found in the average number of germinated seeds among pollination treatments (F = 1.18; $P = 0.33$). Seed germination rates for *G. pauciflora*
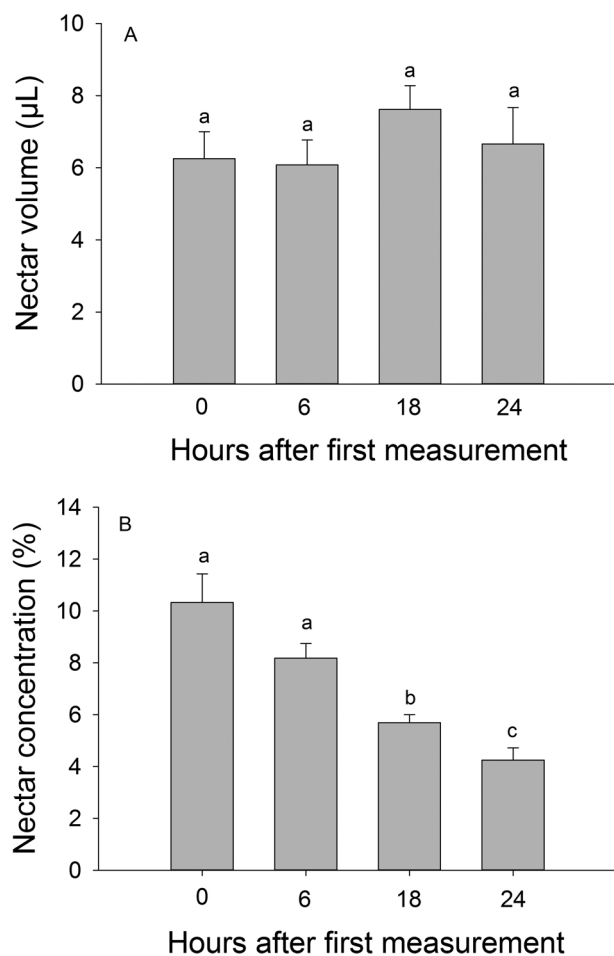
Fig. 2. Repeated measured ANOVA using Holm-Bonferroni method to test for differences in nectar production (A) and nectar concentration (B) in *G. pauciflora* over a 24 h period. Different lowercase letters indicate significant differences at *P* = 0.05.
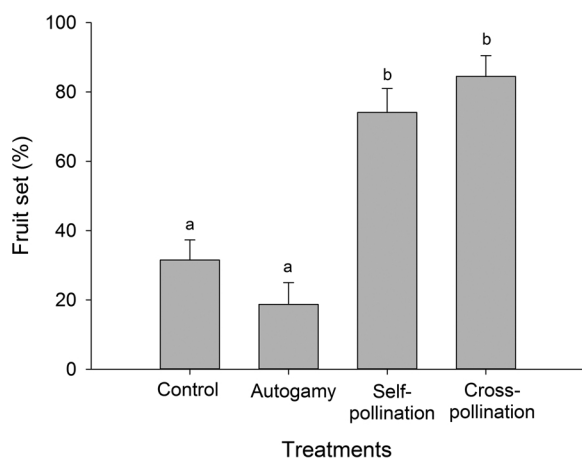

Fig. 3. Generalized linear model testing for differences in fruit set among pollination treatments in *Geseneria pauciflora*. Different lowercase letters means significant differences among pollination treatments at *P* = 0.05.

averaged 72.4% with all treatments being equal or higher than 59.5% [Control = 67.5% (± 8.1), Autogamy = 59.5% (± 12.8), Self-pollination = 76.6% (± 4.1), Cross-pollination = 78.9% (± 7.4)]. Using fruit set information; the reproductive indexes for *G. pauciflora* indicated that this species is self-compatible (SC = 0.88) with the capability of some autonomous pollination (AI = 0.22) but with a high pollen limitation index (PL = 0.63).
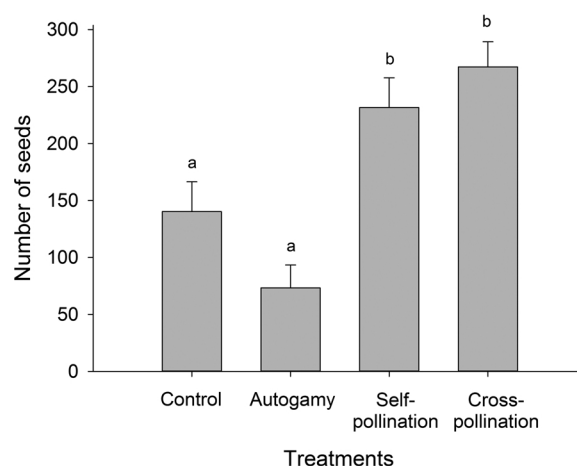

Fig. 4. Post hoc Tukey analyses (following ANOVA) performing pairwise comparisons in the average number of seeds per plant produced among pollination treatments in *Geseneria pauciflora*. Different letters means significant differences among pollination treatments at *P* = 0.05.

4. Discussion

Based on floral morphology and the ancestral pollination system in the insular clade of the Gesneriaceae (Martén-Rodríguez et al., 2010), hummingbirds are likely the sole pollinator of *G. pauciflora*. Although we had observed visits by two hummingbird species, visitation rates were very low yet consistent with other island species of Gesneriaceae, ranging from 0.01 to 0.21 visits/flower/hour (Martén-Rodríguez et al., 2015). In *G. pauciflora*, nectar production was constant in time, yet replenished sugar was more diluted. Temporal differences in flower nectar concentration in *G. pauciflora* may partially explain why hummingbirds only visited flowers in the morning. It has been suggested that the production of diluted sugar after an initial nectar removal may be associated with the translocation of energy resources to support fruit development but that this strategy may also carry trade-offs by reducing subsequent flower visitation (Ordano and Ornelas, 2004). Despite the morphology-pollinator syndrome fit, average nectar concentration values were somewhat atypical for hummingbird-pollinated flowers. Nectar concentration range values in *G. pauciflora* (0–13%) were in the lower end of those reported for hummingbird-pollinated species in continental areas (12–25%; Baker, 1975; Roberts, 1996) as well as in the Caribbean (11–30%; Bolten and Feinsinger, 1978; Dalsgaard et al., 2009; Percival, 1974; Rodríguez-Robles et al., 1992), or even within the *Gesneria* species reported for Puerto Rico (Table 1). Indeed, *G. pauciflora* has the lowest nectar concentration among all Puerto Rican *Gesneria* (Table 1). Hummingbird species visiting *G. pauciflora* are known pollinators of other *Gesneria* species (Martén-Rodríguez and Fenster, 2008) and of a variety of plant taxa in Puerto Rico including *Comparettia falcata* Poeppig & Endlicher (Ackerman et al., 1994; Rodríguez-Robles et al., 1992), *Heliconia caribaea* Lamarck (Martén-Rodríguez et al., 2011), *Pitcairnia angustifolia* Aiton (Fumero-Cabán and Meléndez-Ackerman, 2012), and *Melocactus intortus* (Mill.) Urb. (Fagua and Ackerman, 2011), including other 16 species listed for the island (see Dalsgaard et al., 2009). Some of the plant species listed above also co-exist with *G. pauciflora* in the Maricao Forest Reserve and offer higher nectar rewards in term of volume and concentration (i.e. *G. pedunculosa:* 12.1% and 60.3 μL, Martén-Rodríguez and Fenster, 2008; *P. angustifolia:* 26.6% and 70.5 μL, Fumero-Cabán and Meléndez-Ackerman 2012), which could make them more attractive to their shared pollinators. Indeed, we observed frequent hummingbird visits to *G. pedunculosa*, *P. angustifolia* and other co-existing species in our study site [*Odontonema cuspidatum* (Nees) Kuntze, *Passiflora tulae* Urb., *Poitea punicea* Urb. Lavin, *Renealmia jamaicensis* (Gaertn.) Horan, *Hirtella rugosa* Pers., and *Neorodolphia volubilis* (Willd.) Britton. M. Pérez, personal

Table 1
Variation in floral traits, visitation rate, and reproductive indices associated to *Gesneria* species reported for Puerto Rico. Recorded variables were labeled as follows: tubular (T) and non-tubular (NT) flower type (FT), nectar concentration (NC) and production (NP), corolla length (CL), visitation rate (VR-visit/flower/plant/day), number of pollinators (PR), and reproductive indexes (pollen limitation-PL, autofertility-AI, and self-compatibility-SC). Species with tubular flower correspond to the hummingbird's syndrome. Data is sort by flower type.

Species[a,b]	FT	NC (%)	NP (μL)	CL (mm)	VR	PR	PL	AI	SC
G. pedunculosa (DC.) Fritsch	NT	12.1	60.3	18.1	5.5[c]	4	0.44	0.02	0.92
G. viridiflora subsp. *sintenesii* (Urb.) L.E. Skog	NT	10.5	62.0	18.1	8.8[c]	2	0.12	0.09	0.92
G. citrina Urb.	T	13.3	12.8	21.0	0.4	2	0.48	0.38	0.88
G. cuneifolia (DC.) Fritsch	T	13.4	5.2	26.2	0.5	1	0.17	0.88	1.06
G. pauciflora Urb.	T	7.11	6.6	20.2	0.8	2	0.63	0.22	0.88
G. reticulata (Griseb.) Urb.	T	8.30	0.0	19.1	0.1	1	0.02	0.98	1.03

[a] Data of each species (with exception of *G. pauciflora*) were extracted from Martén-Rodríguez and Fenster (2008,2010, Martén-Rodríguez et al. (2015), and in Chen et al. (2009).

[b] Visitation rate mean value for *G. pauciflora* was multiplied by 12 to obtain comparable hummingbirds' diurnal visitation rates with other species reported by Martén-Rodríguez' studies.

[c] If the species reported more than one pollinator taxa, the value represent the sum of the entire visitation rate average.

observation]. By producing more rewards, these species' flowers can be more attractive to pollinators shared with *G. pauciflora*. While hummingbirds may prefer certain types of flowers, they often are generalist pollinators (Las-Casas et al., 2012) and that respond to nectar resource availability (Garrison and Gass, 1999). Therefore, the availability of a variety floral resources coupled with low nectar rewards for this species may explain observed low hummingbird visitation rates for *G. pauciflora*, and its higher pollen limitation index relative to other *Gesneria* in Puerto Rico (Table 1).

Pollen limitation via low pollinator activity or by lower quality or quantities of pollen delivered may result in low fruit and seed production rates (Ashman et al., 2004; Knight et al., 2005). The fact that *G. pauciflora* flowers had low visitation rates and scored higher probabilities to produce fruits and seeds in pollen supplemented treatments (self- and cross-pollination), suggests that pollen limitation may relate at least in part to poor hummingbird visitation. Even though visits were scarce, it has been shown that hummingbirds can be very effective at transferring pollen in tubular flowers after single visits (Fumero-Cabán and Meléndez-Ackerman, 2007; Martén-Rodríguez and Fenster, 2010), which could offset the effects of low visitation if pollen transfers lead to increases in fruit and seed production and seed quality when outcrossing occurs. Lower pollen quality may result from self-fertilization and mating between related when recessive deleterious alleles are expressed in selfed embryos that fail to develop seeds (Aizen and Harder, 2007). Nevertheless, given our data, we can discard lower seed quality (via inbreeding depression) as a factor leading to pollen limitation in *G. pauciflora* because cross-pollinations and self-pollinations were equally successful at producing and germinating seeds.

Plants that experience pollen limitation may evolve floral traits or reproductive assurance mechanism to lessen pollination constraints (Barrett, 1996; Fenster and Martén-Rodríguez, 2007; Martén-Rodríguez et al., 2010). This hypothesis has been tested in *Clarkia xantiana* ssp. *parviflora* (Eastw.) Harlan Lewis where populations show variable breeding systems and evidence links the origin of autogamous populations in sites where pollinators (and consequently visitation rates) and mates where infrequent (Fausto et al., 2001). Similar results have been shown for *Eichhornia paniculata* (Spreng.) Solms where the dominance of tristylous flowers, predominantly outcrossing, diminished during invasion to the Central America and the Caribbean where autonomous selfing flowers were favored (Barrett et al., 2008). In the case of the gesneriad species of Caribbean islands, narrow tubular flowers (that allow anther and stigma contact) and presence of herkogamy may provide reproductive assurance (through autonomous selfing mechanisms) in periods when pollinators are limited (Chen et al., 2009; Martén-Rodríguez and Fenster, 2010). Indeed, *G. pauciflora* has narrow tubular flowers and presented herkogamy. Similar to *G. citrina* (Chen et al., 2009), stigma-anther separation in *G. pauciflora* decreased over the life span of flowers in *G. pauciflora* (M.E. Pérez, personal observation) and self-pollination was thus possible. Autonomous delayed

selfing in this species might have an advantage under conditions of low hummingbird visitation rates and an apparent lack of inbreeding depression.

In the Caribbean, hurricanes may reduce vertebrate pollinators and be a selective force favoring breeding mechanisms that provide reproductive assurance (Rivera-Marchand and Ackerman, 2006; Rojas-Sandoval and Meléndez-Ackerman, 2011). While hummingbird populations were shown to dramatically decrease following Hurricane Hugo at the Luquillo Forest in northeastern of Puerto Rico (Wunderle, 1995), these reductions need not be uniform across events. Indeed, and contrary to expectations, a separate study conducted at the Maricao Forest Reserve following Hurricane Georges (a category 3 hurricane) showed an increase in hummingbird capture rates two years after the hurricane event even when other bird populations decreased or disappeared from the study site (Tossas, 2006). While the combined results may weaken the hypothesis that hummingbird pollinator services are always reduced following hurricane events, one cannot rule out the possibility that these or other large-scale events may lower hummingbird populations and create even worse conditions of pollinator service. This may be even more critical for *G. pauciflora* than for other *Gesneria* in Puerto Rico since this species exhibits higher pollination limitation index and lower autofertility of all tubular species combined (Table 1) and may explain its rarity.

The potential for autofertility in Caribbean gesneriads pollinated solely by hummingbirds is diverse ranging from 0.00 to 1.10, with *G. decapleura* and *G. pedicellaris* representing the lower and upper boundaries of this reproductive index, respectively (Martén-Rodríguez et al., 2015). Interspecific variability in autofertility rates seems to be negatively associated with interspecific variability in visitation rates in Puerto Rico and values for *G. pauciflora* seem to fit this pattern (Table 1). Considering that pollen limitation is an issue under the current pollination environment in *G. pauciflora*, the presence of autogamy in this system and associated morphological features (narrow tubular flowers and presence of herkogamy) may have evolved to buffer the lower hummingbird visitation rate, even when autogamy does not allow individual plants to achieve their full reproductive potential.

Despite the fact that pollen limitation is a potential cause of infrequent fruit and seed production in the species, we cannot discard other potential constraints to fruit and seed production. In our experiments, not all supplemented pollinations lead to fruit production and not all ovules produced seeds in the pollen supplementation treatments. That would suggest reproductive constraints related to resource availability. Resource limitation to fruit production has been demonstrated in many plant systems, and in many circumstances, can either override or alter the severity of limited pollination services (i.e. Ackerman and Montalvo, 1990; Calvo, 1993; Campbell and Halama, 1993; Kudo et al., 2001; Meléndez-Ackerman et al., 2000; Rodríguez-Robles et al., 1992). *Gesneria pauciflora* inhabits rocky wet habitats at or near the stream flow and may be influenced by natural disturbances such as flash floods

or drought events. To the extent that stream bank disturbances might lead to reduced nutrients or induce water stress temporally, inundate areas, uproot or damage individuals causing a reduction in growth (Richardson et al., 2007), resources to produce fruits and seeds could also become limited in *G. pauciflora*; even though our design did not allow us to test for that. Flower herbivory of *G. pauciflora* by *Postplatyptilia caribica* Gielis (Pterophoridae), a microlepidopteran native to the Caribbean (Gielis, 2006; Matthews and Pérez, 2014) may also limit reproduction in this system, but it is a factor that we consider to be less important relative to pollination constraints given that only a small fraction of plants (12%) failed to produce fruits.

4.1. Management implications and recommendations

Current habitat protection of the Maricao Reserve (the only place where this species has been reported to occur) likely benefits both *G. pauciflora* and its two-pollinator species. However, the specificity of *G. pauciflora* on hummingbird pollination highlights the importance of avoiding further habitat fragmentation, and maintaining forested buffer areas on the periphery of the Maricao Forest to support healthy populations of hummingbirds. On the other hand, even when its pollinator habitat is protected, pollination service is scarce which may limit population growth rates and result in naturally rare populations. Temporal dynamics of herbivores and pollinators are likely to be influenced by temporal changes in climate (Dukes et al., 2009). Thus, short- and long-term monitoring on different phenophases (from flower bud to seeds production) would help us to understand the role of current and future variability in pollination service and antagonist interactions (i.e. florivory, frugivory), and how vulnerable *G. pauciflora* population might be following natural disturbances. A concern is that reproductive success in *G. pauciflora* may suffer declines under current climate change scenarios anticipated for the Caribbean, which predict an increase in hurricane disturbances (Lugo, 2000; Pulwarty et al., 2010). Long-term monitoring of natural fruit set levels may should reveal the variability in reproductive output. On the other hand, this study shows that *G. pauciflora* has high germination rates, which facilitates the development of propagation protocols for *G. pauciflora* to establish recovery strategies when needed.

4.2. Conclusions

We were unable to detect immediate vulnerabilities for *G. pauciflora* at the pollination stage. Fruit production is naturally low and likely the result of low frequency of pollinator visits. Yet, the species is self-compatible and autonomous, and also presents morphological features that may help ensure fruit production. The occurrence of an autonomous breeding system in *G. pauciflora,* as a reproductive assurance mechanism, could also be associated to a habitat type (rocky habitat) in which resources are limited and that may inherently offer uncertain pollinator environments as well. Such changes or transitions more often related to the environmental/habitat contexts than to phylogenetic constraints within the Gesneriaceae clade in the Caribbean (Martén-Rodríguez et al., 2015). Finally, the high germination rates observed for *G. pauciflora* may facilitate its propagation and allow for the development of re-introduction and planting programs towards recovery efforts.

Acknowledgements

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