

## Pollination Ecology and Breeding Systems of Five *Gesneria* Species from Puerto Rico

SILVANA MARTÉN-RODRÍGUEZ<sup>1,3,\*</sup> and CHARLES B. FENSTER<sup>1,2</sup>

<sup>1</sup>Behavior, Ecology, Evolution and Systematics Program, <sup>2</sup>Biology Department, University of Maryland, College Park, MD 20742, USA and <sup>3</sup>Herbario de la Universidad de Costa Rica, Escuela de Biología, Ciudad Universitaria Rodrigo Facio Brenes, San José, Costa Rica

Received: 3 January 2008 Returned for revision: 28 February 2008 Accepted: 19 March 2008 Published electronically: 18 April 2008

- **Background and Aims** The genus *Gesneria* diversified in the Greater Antilles giving rise to various floral designs corresponding to different pollination syndromes. The goal of this study was to characterize the pollination and breeding systems of five Puerto Rican *Gesneria* species.
- **Methods** The study was conducted in Arecibo and El Yunke National Forest, Puerto Rico, between 2003 and 2007. Floral visitors were documented by human observers and video cameras. Floral longevity and nectar production were recorded for the five study species. Tests for self-compatibility and autonomous selfing were conducted through hand-pollination and bagging experiments.
- **Key Results** Floral phenology and nectar production schedules agree with nocturnal (in bell-shaped flowered *G. pedunculosa* and *G. viridiflora* subsp. *sintensisii*) or diurnal pollination syndromes (in tubular-flowered *G. citrina*, *G. cuneifolia* and *G. reticulata*). Nectar concentration is consistently low (8–13 %) across species. *Gesneria citrina* and *G. cuneifolia* are exclusively pollinated by hummingbirds, while *Gesneria reticulata* relies mostly on autonomous self-pollination, despite having classic ornithophilous flowers. A variety of floral visitors was recorded for the two species with bell-shaped flowers; however, not all visitors have the ability to transfer pollen. Bats are the primary pollinators of *G. pedunculosa*, with bananaquits probably acting as secondary pollinators. For *G. viridiflora* subsp. *sintensisii*, both bats and hummingbirds contact the flower's reproductive organs, thus, this species is considered to be a generalist despite its nocturnal floral syndrome. All species are self-compatible but only tubular-flowered *Gesneria* are capable of autonomous self-pollination.
- **Conclusions** The visitation patterns described in this study fit the predicted hummingbird and bat pollination syndromes and support both specialization and generalization of pollination systems in Puerto Rican *Gesneria*. Specialization is associated with low pollinator visitation, particularly by hummingbirds, which may explain the occurrence of autonomous selfing mechanisms in tubular-flowered species.

**Key words:** Autonomous selfing, bat pollination, breeding systems, *Gesneria*, hummingbird pollination, Puerto Rico.

### INTRODUCTION

Pollination specialization has long been considered an important process underlying the evolution of floral diversity. Consequently floral traits have been viewed as adaptations to attract specific pollinators and to enhance the efficiency of pollen transfer and outcrossing (Darwin, 1862; Stebbins, 1970). In recent years, this classic view of specialization has been a subject of contention (Waser *et al.*, 1996; Johnson and Steiner, 2000; Fenster *et al.*, 2004). Waser *et al.* (1996) argue that most plant species have generalized visitation patterns and that floral visitors rarely specialize on particular food sources. While it is true that generalization at the community level is common in temperate regions (e.g. Herrera, 1988; Gomez, 2002), specialization in functional groups of pollinators is also widespread (Armbruster *et al.*, 2000; Fenster *et al.*, 2004). More knowledge of the floral biology of plants in tropical regions is necessary to achieve a better understanding of the overall ecological and evolutionary patterns of specialization (Johnson and Steiner, 2000).

Island plants may provide further insights into the evolution of pollination systems. Islands tend to have

lower abundance and diversity of many important pollinator groups (Carlquist, 1974; Barrett, 1996), thus, the particular patterns of abundance, composition and behaviour of floral visitors on islands may create selective environments different from those that flowers are exposed to in mainland regions (Barrett, 1996; Armbruster and Baldwin, 1998). Two literature reviews suggest that islands generally have a greater representation of species pollinated by generalist insects compared with mainland regions (Carlquist, 1974; Barrett, 1996). However, the floral diversity of plant radiations from some tropical islands suggests that pollinator specificity may be a common feature of some insular plant taxa: e.g. Hawaiian mints (Lindqvist and Albert, 2002) and Hawaiian lobeliads (Lammers and Freeman, 1986). Here, the pollination and breeding systems are documented for five *Gesneria* species belonging to one such plant group, the tribe Gesnerieae from the Antillean islands (Skog, 1976).

Given that floral structures have functional significance for both pollination and breeding systems, simultaneous study of both will lead to a better understanding of the processes that drive floral trait diversification (Barrett *et al.*, 1996; Holsinger, 1996; Barrett, 2003). Plants on islands may benefit from having selfing breeding systems,

\* For correspondence. E-mail smartenr@gmail.com

particularly during colonization and establishment or when pollinator abundances are low (Baker, 1955; Gonzales-Diaz and Ackerman, 1988). However, reduced genetic diversity and higher levels of inbreeding depression have been found associated with selfing in some insular plant species (e.g. Naito *et al.*, 2005). Consequently, traits that promote outcrossing may be selected for after island colonization (Carlquist, 1974; Barrett, 1996).

This study provides the first step in the characterization of the floral biology of the tribe Gesnerieae (family Gesneriaceae). The tribe is a monophyletic clade of the family Gesneriaceae distributed primarily across the Antilles (Zimmer *et al.*, 2002). Field studies were conducted on five *Gesneria* species from the island of Puerto Rico in order to: (a) characterize their floral morphology, floral phenology and nectar production; (b) document floral visitor assemblages and behaviour of pollinating and non-pollinating flower visitors; and (c) characterize breeding systems (dichogamy, self-compatibility, autonomous selfing) and assess them in the context of the pollination system.

## MATERIALS AND METHODS

### *Study sites and species*

According to Zimmer *et al.* (2002) and preliminary phylogenies, the genus *Gesneria* is paraphyletic and the study species belong in at least two different clades. *Gesneria reticulata* and *G. cuneifolia* are sister species within a clade that comprises most species in the genus; *Gesneria citrina* groups within the genus *Rhytidophyllum*, while *G. pedunculosa* and *G. viridiflora* belong in two clades which have ambiguous placement in a preliminary phylogeny. The pollination biology of *Gesneria pedunculosa*, *G. citrina* and *G. cuneifolia* was studied in the forests surrounding the Arecibo Observatory (18°20'36.6"N, 66°45'11.1"W, approx. 300 m a.s.l.) in Puerto Rico. These three species are endemic to the island. The Arecibo Observatory is located in the northern karst region of the island, and is characterized by a topography of limestone hills and valleys with an underground drainage structure that includes extensive cave systems. Some of the caves in the region are known to host large bat populations, including the nectarivorous *Monophyllus redmanii*.

*Gesneria viridiflora* subsp. *sintenisii* and *G. reticulata* were studied at El Yunque National Forest, which rests within the boundaries of the Caribbean National Forest (18°19'N, 65°47'W). Plants of *G. viridiflora* subsp. *sintenisii* occur along rainforest streams, between 700 m and 1000 m. This species is also a Puerto Rican endemic. *Gesneria reticulata* has a narrow distribution in Puerto Rico, where it has only been collected along route 191, between kilometres 9 and 11; however, this species occurs in Cuba and Hispaniola.

### *Pollinator observations*

Characterization of the pollinator fauna of five Gesnerieae species was accomplished in January and June of 2003 and December to March 2005–2007. Floral visitors

were recorded throughout the day for 0.5-h periods by human observers and 1.5-h periods by video cameras. Observations were performed on 22–40 individuals per species, at different times of the day and on scattered days throughout the flowering period; total observation time per species is listed in Table 1. Nocturnal observations were conducted regardless of the floral syndrome to avoid biasing the sampling towards the expected pollinators; however, these observations were limited to 8 h in species with diurnal nectar production. For all nocturnal observations video cameras with infrared night vision were used (Sony Handycam DCR-HC42 and DCR-TRV350). The identity of visitors was recorded to the lowest possible taxonomic level as determined by examination of the video recordings. When possible, insect visitors were also collected, however, collections were not made during observation time to prevent altering natural visitation rates. Time and duration of the visit, number of flowers, type of reward (nectar or pollen), and contact with anthers or stigmas were recorded. Visitation rates per flower per hour were calculated for each species as the total number of visits divided by the observation time, divided by the number of flowers observed. The estimate was multiplied by 12 to obtain diurnal or nocturnal pollination rates per 12 h day or night, according to the visitation schedule of the pollinator.

### *Nectar production and floral phenology*

To determine the schedule of nectar production one to three flower buds from 12–32 plants were bagged and nectar was measured every 6 h for 2 d. Nectar amounts were measured using a 50- $\mu$ L syringe (Hamilton, NV, USA), and 5- $\mu$ L capillary tubes for species with low production. Nectar concentration was measured once or twice per flower using a hand-held refractometer (Sugar/Brix Refractometer, 0–32 % w/ATC, Sper Scientific, Scottsdale, AZ, USA). All nectar data were collected in December 2006 and January 2007. Differences in nectar volumes among time periods and sugar concentration among four species were tested with ANOVA using the GLIMMIX procedure in SAS version 9.1.3 (SAS Institute, 2004). A repeated measures model was specified for the analysis of time periods because the same flowers were measured at different times. *Gesneria reticulata* was excluded from the analysis because only three out of 32 flowers tested produced any measurable amount of nectar.

The floral phenology of all species was studied by following 15–20 flowers from bud until senescence in January 2005. Flowers were checked every 3 h to determine the timing of anther dehiscence. For the two species with bell-shaped flowers, once a range of times was established, flowers were observed every hour to document the specific time of pollen release. This was not done for tubular-flowered species because anthers are often positioned within the floral tube when they dehisce and it was difficult to assess this trait without damaging the flower. Stigma receptivity was determined four times a day (0600 h, 1200 h, 1800 h and 0000 h) by adding a drop of hydrogen

TABLE 1. Distribution, growth form and floral characteristics of five *Gesneria* species from Puerto Rico

Species	Distribution	Growth form	Floral design	Floral longevity (d)	Breeding system	Anthesis time (dehiscence)	Nectar production, mean per day [range] ( $\mu\text{L}$ )	% Sugar concentration, mean [range]
<i>G. citrina</i>	Central western karst	Scandent shrub	Tubular	4–5 <i>n</i> = 20	Protogynous	Before dawn (second day)	12.8 [0–28] <sup>a</sup> <i>n</i> = 18	13.3 [11–15] <sup>a</sup> <i>n</i> = 17
<i>G. cuneifolia</i>	Central western karst	Roseate	Tubular	3–4 <i>n</i> = 18	Protogynous	Before dawn (second day)	5.2 [1–20] <sup>a</sup> <i>n</i> = 16	13.4 [11–15] <sup>a</sup> <i>n</i> = 12
<i>G. reticulata</i>	North east Rainforest	Roseate	Tubular	3–4 <i>n</i> = 15	Adichogamous	Before dawn (first day)	0 [0–2] <i>n</i> = 32	8.3 [8–9] <sup>b</sup> <i>n</i> = 3
<i>G. pedunculosa</i>	Central western karst	Shrub	Campanulate	2–3 <i>n</i> = 20	Protandrous	1500–1700 h (after 1800 h)	60.3 [27–108] <sup>b</sup> <i>n</i> = 38	12.1 [11–14] <sup>ac</sup> <i>n</i> = 21
<i>G. viridiflora</i> subsp. <i>sintensis</i>	North east Rainforest	Shrub	Subcampanulate	2–3 <i>n</i> = 20	Protogynous	1500–1700 h (1700–1800 h)	62.0 [15–128] <sup>b</sup> <i>n</i> = 17	10.5 [7–15] <sup>bc</sup> <i>n</i> = 16

Sample size is the number of plants examined for each species. In the nectar volume and concentration columns identical letters indicate the means are not significantly different at alpha 0.05 (Tukey adjustment).

\* *Gesneria reticulata* also occurs in Cuba and Dominican Republic.

peroxide and checking for bubble formation (Kearns and Inouye, 1993) on different flowers of known age.

### Breeding systems

Hand-pollination experiments were used to test for self-incompatibility systems. Twelve plants per species were used for each of two treatments: hand self-pollination and hand outcross-pollination. Flower buds were covered with wedding-veil bags and stigmas were pollinated by rubbing anthers of donor flowers onto the stigmas of recipient flowers. Outcross pollen was collected from two or three donors growing at least 7 m away to reduce the chances of crossing with pollen from genetically related individuals. Self-pollinations were performed using pollen donor flowers from the same plant.

To evaluate potential for autonomous selfing, the ability to set seed in the absence of pollinators, all flower buds developing for a period of 1–2 weeks in 15–28 plants were bagged. Fruit set was determined 2 months later. An autofertility index was calculated by dividing the fruit set of bagged flowers by the fruit set of hand-outcrossed flowers (Lloyd and Schoen, 1992). Tests for apomixis were conducted by emasculating and bagging two to four flower buds from seven to ten individuals per species.

Differences in fruit set (fruits/flowers) and seed mass were tested for among hand-self, hand outcross and bagging with ANOVA using the GLIMMIX procedure in SAS (SAS Institute, 2004). Pairwise differences were tested specifying the Tukey adjustment for multiple comparisons. Seed mass of bagged flowers was not compared with hand-pollination treatments in *G. pedunculosa* and *G. viridiflora*, because most plants did not set fruit. Gesneriaceae fruit capsules contain many small seeds (300 to >3000), making counting of all seeds impractical. However, significant correlations between seed number and seed mass ( $n = 30$  capsules for each of five species,  $r = 0.87–0.93$ ,  $P < 0.0001$ ) were found for all species. Therefore, seed mass was used as a surrogate of seed set.

## RESULTS

### Plant habit and floral biology

*Gesneria* species included in this study span three distinct floral designs: tubular and two types of bell-shaped flowers (campanulate and subcampanulate). *Gesneria citrina* has tubular, yellow flowers that are protogynous (Fig. 1). Floral characteristics are listed in Table 1. Delayed self-pollination may or may not occur in *G. citrina* depending on the degree of herkogamy, which varies among plants (S. Martín-Rodríguez, pers. obs.). *Gesneria cuneifolia* and *G. reticulata*, sister species according to preliminary phylogenies, have tubular, red, flowers, that are also protogynous (Fig. 1). Floral phenology, pistil length and nectar production differ between the two species (Table 1). In *G. cuneifolia*, delayed self-pollination can be achieved by the third day of flowering when stamens have elongated fully to the length of the stigma. In *G. reticulata*, self-pollination occurs

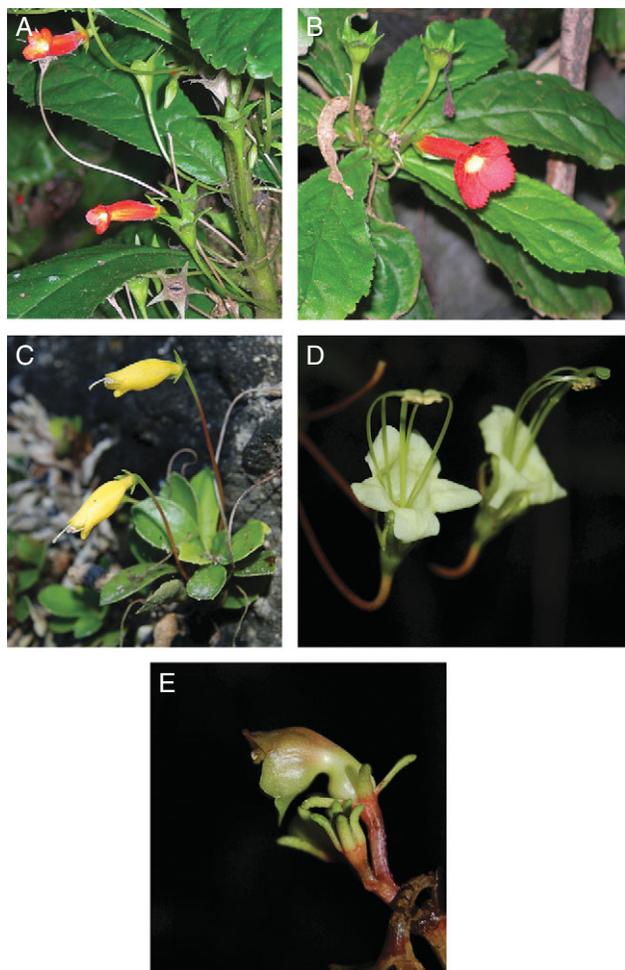


FIG. 1. Five species of *Gesneria* from Puerto Rico: (A) *Gesneria cuneifolia*, (B) *Gesneria reticulata*, (C) *Gesneria citrina*, (D) *Gesneria pedunculosa*, (E) *Gesneria viridiflora* subsp. *sintensisii*.

during stamen elongation on the first day, or when stamen filaments bend to contact stigmas by the second or third day.

*Gesneria pedunculosa* has inflorescences that bear three or four white campanulate flowers with exerted reproductive organs (Fig. 1). The flowers are protandrous and schedules of anther dehiscence and nectar production are mostly nocturnal (Table 1). In second-day flowers mature stigmas grow to reach anther level and stigma receptivity may last through the third night. However, self-pollination does not occur because stamens curl down below the stigmas after the first night of anthesis (also see bagging experiments below).

*Gesneria viridiflora* subsp. *sintensisii* has subcampanulate flowers that have a constriction above the nectar chamber; corollas are green, and sometimes tinted with brown or violet markings (Fig. 1). Anthesis and nectar production start in the afternoon but anther dehiscence is nocturnal (Table 1). In this protogynous species, stigma receptivity starts with anthesis and may last through the second night, but self-pollination is rare because the stigma contacts only the back wall of the anthers (also see bagging experiments below).

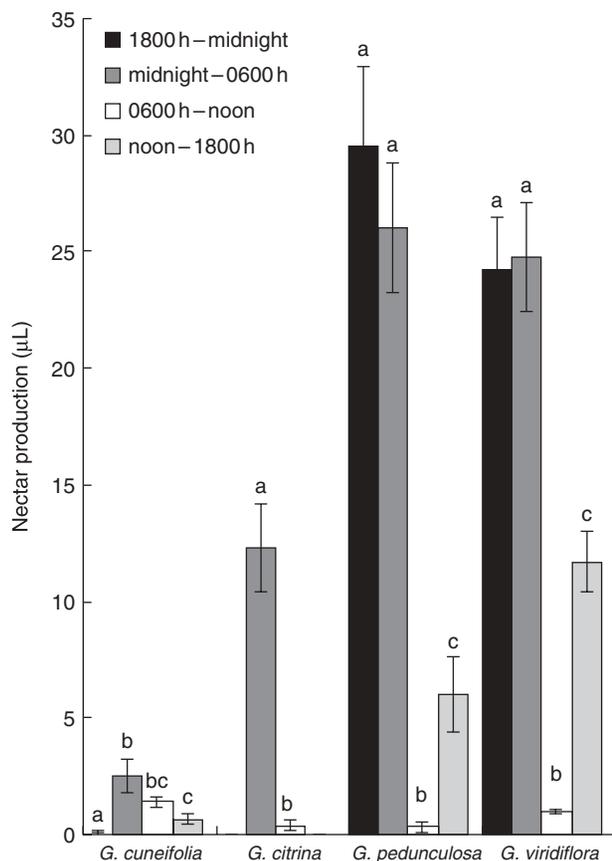


FIG. 2. Average nectar production per 6-h period recorded in January 2007 for four species of *Gesneria* endemic to Puerto Rico. Error bars represent s.e.m. Identical letters indicate the means among time periods within species are not significantly different. In *G. cuneifolia* and *G. citrina*, nectar production starts after 0300 h.

Like all members of the tribe Gesnerieae, the Puerto Rican species have dehiscent capsules that contain hundreds to thousands of small seeds. The seeds are most likely wind dispersed, although in the case of *G. viridiflora* subsp. *sintensisii*, and perhaps other species, water dispersal is also possible. Fruit development takes 2–3 months.

#### Nectar measurements

Nectar volumes and production schedules differed among species (Table 1 and Fig. 2). Species with bell-shaped flowers secreted significantly more nectar than tubular-flowered species ( $F_{3,96} = 76.1$ ,  $P < 0.0001$ ; Table 1). For tubular-flowered species, significant variation in nectar volume among time periods was detected ( $F_{3,17} = 37.9$ ,  $P < 0.0001$  for *G. citrina*, and  $F_{3,30} = 24.9$ ,  $P = 0.0004$  for *G. cuneifolia*). Nectar production in these species occurred mostly between 0300 and 0600 h, although in *G. cuneifolia* nectar accumulation continued throughout the morning (Fig. 2). *Gesneria reticulata* typically does not produce nectar, although very small amounts of nectar ( $1-2 \mu\text{L d}^{-1}$ ) were detected in three out of 32 plants tested (Table 1).

Nectar production in campanulate- and subcampanulate-flowered *Gesneria* averaged between 60 and 62  $\mu\text{L}$

(Table 1). Nectar volumes among time periods differed for both *G. pedunculosa* and *G. viridiflora* subsp. *sintensisii* ( $F_{3,15} = 35.9$ ,  $P < 0.0001$  and  $F_{3,31} = 83.1$ ,  $P < 0.0001$ , respectively); the largest volume of nectar was secreted during the night time periods in both species (Tukey adjusted  $P < 0.05$ ) followed by afternoon accumulation (between 1500 and 1800 h) and very little production during the morning hours (Fig. 2).

Sugar concentration values ranged from 8% to 13% with significant differences among species ( $F_{3,62} = 11.8$ ,  $P < 0.0001$ ). Sugar concentration was slightly but significantly lower for *G. viridiflora* subsp. *sintensisii* compared with *G. citrina* and *G. cuneifolia*, two tubular-flowered species (Tukey adjusted  $P < 0.05$ ). *Gesneria reticulata* had a significantly lower sugar concentration than other species (Table 1).

#### Pollination systems

Hummingbirds were the exclusive pollinators of tubular-flowered *Gesneria* (Table 2). However, *G. reticulata* received only a single visit by *Chlorostilbon maugeaus*, the Puerto Rican emerald, in 43 h of observation. None of the hummingbird visitors observed exhibited territorial behaviour. Visitation frequencies to tubular flowers were in the range of one visit per flower every 2 or 3 d. All visits resulted in contact between anthers or stigma and the hummingbird's beak or forehead (Table 3). *Chlorostilbon maugeaus* also visit campanulate- and subcampanulate-flowered *Gesneria*. While these hummingbirds did not contact the flower's reproductive organs in *G. pedunculosa*, they often did in flowers of *G. viridiflora* subsp. *sintensisii* (Table 3).

Bananaquits (*Coereba flaveola*) are occasional visitors to flowers of tubular-flowered *G. citrina* and *G. cuneifolia*; they pierce a hole at the base of the corolla and extract nectar without removing or depositing pollen. Bananaquits also visit bell-shaped flowers, feeding on nectar as nectar robbers (in *G. viridiflora* subsp. *sintensisii*), or by inserting their heads into the corolla and occasionally contacting the flower's reproductive organs (in *G. pedunculosa*).

Bats of the species *Monophyllus redmanii* are major pollinators of both *Gesneria pedunculosa* and *G. viridiflora* subsp. *sintensisii* (Table 2). Bat visits occur between 1900 h and 0600 h lasting <1 s with peak visitation

between 1900 h and 1100 h and then again from 0400 h to 0600 h. Visitation rates for years with bats present ranged between two and four visits per flower per night (Table 3); no bats were observed in 2003.

Moths also visit flowers of bell-shaped flowered *Gesneria*; however, moths rarely have the potential to be effective pollinators (Table 3). In *G. viridiflora* subsp. *sintensisii*, a number of noctuid moth species and perhaps other moth families visited some plants at high frequencies, particularly in 2003 and 2005. These moths crawl into the corolla moving in and out (by backing up) several times and visits may last several minutes.

Other visitors to *G. pedunculosa* included honey bees and flies (Muscidae and Syrphidae). Honeybees (*Apis mellifera*) are the most common visitors but they are not considered legitimate pollinators. Honeybees remove pollen from anthers right before dehiscence breaking down the connections among them and displacing anther filaments; this may affect future contact with legitimate pollinators. Honeybees do not contact stigmas while foraging for nectar. Flies forage for leftover pollen; occasionally they land on stigmas before moving to the anthers. *Gesneria pedunculosa* and *G. viridiflora* subsp. *sintensisii* are thus visited by varied assemblages of animal taxa, but the only visitors with potential to be important pollinators are bats in the former and bats and hummingbirds in the latter.

#### Self-compatibility and autonomous selfing

All five species of *Gesneria* are self-compatible; no significant differences in fruit set were found between hand-self pollinated and outcrossed flowers for any species (Table 4). There was no evidence of apomixis, none of the bagged emasculated flowers set seed. The levels of autonomous self-pollination varied among species (Table 4). The two species with campanulate and subcampanulate corollas had lower than 10% potential autonomous selfing rates. The three species with tubular flowers have relatively high potential autonomous selfing levels (25–90% fruit set); however, only for *G. cuneifolia* and *G. reticulata* was fruit set of bagged flowers the same as that of out-crossed flowers (Table 4). Seed mass did not differ among treatments for these two species either (*G. cuneifolia*,  $F_{1,42} = 0.97$ ,  $P = 0.37$ ; *G. reticulata*  $F_{2,48} = 1.7$ ,  $P = 1.9$ ). Seed mass of

TABLE 2. Floral visitors recorded for five *Gesneria* species from Puerto Rico between 2003 and 2007

Species	Floral design	Pollinators (common name)	Non-pollinating floral visitors (resource used)
<i>G. citrina</i>	Tubular yellow	<i>Anthracocharax viridis</i> (green mango) <i>Chlorostilbon maugeaus</i> (Puerto Rican emerald)	Pterophoridae (plume moth larva feeds on immature stamens) <i>Coereba flaveola</i> (nectar robber)
<i>G. cuneifolia</i>	Tubular red	<i>Chlorostilbon maugeaus</i>	<i>Coereba flaveola</i> (nectar robber)
<i>G. reticulata</i>	Tubular red	<i>Chlorostilbon maugeaus</i>	Not observed
<i>G. pedunculosa</i>	Campanulate	<i>Monophyllus redmanii</i> (Greater Antillean long-tongued bat) <i>Coereba flaveola</i> (bananaquit)	<i>Chlorostilbon maugeaus</i> (nectar) Money bee (nectar, pollen) Sphingid moth (nectar)
<i>G. viridiflora</i> subsp. <i>sintensisii</i>	Subcampanulate	<i>Monophyllus redmanii</i> <i>Chlorostilbon maugeaus</i> Noctuid moths (various morphospecies)	<i>Coereba flaveola</i> (nectar robber) Small moths (Nectar)

TABLE 3. Frequency of visitation per flower per day and contact with reproductive organs by potential pollinators for five species of *Gesneria* from Puerto Rico studied during 2003 and 2005–2007

Species	Pollinator	Mean no. of visits/flower per day [range across years]	Mean percentage contact with reproductive organs [range]	No. of visitors	[No. of years] no. of hours
<i>G. citrina</i>	Hummingbird	0.4 [0.3–0.5]	100	13	[3] 67
<i>G. cuneifolia</i>	Hummingbird	0.5 [0.4–0.6]	100	6	[3] 41
<i>G. reticulata</i>	Hummingbird	0.1 [0.0–0.2]	100	1	[3] 43
<i>G. pedunculosa</i>	Bat	3.9 [3.7–4.2]	100	39	[3] 91
	Bananaquit	1.0 [0.7–1.6]	41 [29–56]	7	
	Pollen collectors	0.6 [0.0–0.9]	68 [60–75]	6	
<i>G. viridiflora</i> subsp. <i>sintensisii</i>	Bat	2.5 [1.3–3.6]	100	6	[3] 84
	Hummingbird	3.0 [2.5–3.7]	61 [57–65]	12	
	Moth	3.3 [3.0–3.6]	7 [0–14]	16	

Number of visitors observed, number of study years and observation hours are indicated for each species.

TABLE 4. Tests for self-compatibility and autonomous selfing for five *Gesneria* species from Puerto Rico performed in January 2006

Species	Hand-outcross	Hand-self	Bagged	F value	P	AI*
<i>G. citrina</i>	64 ± 5.8 <sup>a</sup>	56 ± 5.0 <sup>a</sup>	24 ± 6.4 <sup>b</sup>	$F_{2,47} = 8.7$	<0.001	0.38
<i>G. reticulata</i>	89 ± 5.2 <sup>a</sup>	92 ± 5.4 <sup>a</sup>	90 ± 5.3 <sup>a</sup>	$F_{2,48} = 0.08$	0.92	0.98
<i>G. cuneifolia</i>	77 ± 7.1 <sup>a</sup>	82 ± 6.7 <sup>a</sup>	68 ± 9.3 <sup>a</sup>	$F_{2,42} = 0.75$	0.48	0.88
<i>G. pedunculosa</i>	60 ± 4.0 <sup>a</sup>	55 ± 3.3 <sup>a</sup>	1.2 ± 4.5 <sup>†</sup>	$F_{1,46} = 1.04$	0.32	0.02
<i>G. viridiflora</i> subsp. <i>sintensisii</i>	64 ± 5.1 <sup>a</sup>	59 ± 5.0 <sup>a</sup>	7.6 ± 2.8 <sup>b</sup>	$F_{2,51} = 24.5$	<0.001	0.09

Least square means (± SEM) for fruit set are reported for each pollination treatment. Identical letters indicate no significant differences among means at the 0.05 alpha (Tukey adjustment).

\* Autonomous selfing index = fruit set of bagged plants/fruit set of hand-outcrossed.

† Bagged treatment not statistically compared because most values were zeros.

bagged flowers of *G. citrina* was significantly lower than seed mass of hand pollination treatments ( $F_{2,46} = 7.61$ ,  $P = 0.001$ ) indicating a lower potential for autonomous seed set in this species. *Gesneria reticulata* has the highest potential autonomous selfing rate as can be noted by the autonomous selfing index (AI = 1). Considering the low hummingbird visitation, lack of nectar production, and high potential autogamy rate, this species can be considered predominantly selfing.

## DISCUSSION

### Pollination systems

Plant–pollinator interactions in islands tend to be more generalized than their mainland counterparts as a consequence of the reduced diversity and abundance of animal pollinators that characterize insular ecosystems (Carlquist, 1974; Barrett, 1996; Olesen and Jordano, 2002). However, highly specialized interactions have been documented in various islands, often associated with vertebrate pollination (e.g. Kodric-Brown *et al.*, 1984; Temeles and Kress, 2003; Micheneau *et al.*, 2006). Highly specialized hummingbird pollination in two species of *Gesneria* from Puerto Rico is reported here. Specialized pollination mutualisms are expected in the Caribbean islands where distance to the mainland is relatively short at both ends of the archipelagos, and animal

dispersal by island hopping is possible. Hummingbirds, in particular, have colonized remote islands such as Juan Fernandez, 667 km off the coast of Chile, where various species of plants with distinct hummingbird pollination syndromes are entirely dependant on these birds for outcross pollination (Bernardello *et al.*, 2006).

Bats also have the ability of long-distance dispersal and are known pollinators of various island plants (e.g. Elmquist *et al.*, 1992; Zusi and Hamas, 2001). In Puerto Rico, bats were the most frequent and potentially most effective pollinators of *Gesneria pedunculosa*, although this species had a fairly diverse visitor assemblage (Table 3). Some floral visitors, such as bananaquits and flies, had low potential to transfer pollen (Table 3), while others did not contact stigmas during their visits (e.g. hummingbirds, hawkmoths, honeybees). These animals are most likely to be antagonists of the system that reduce the amounts of floral rewards available for legitimate pollinators (Thomson, 2003). Nevertheless, the presence of secondary floral visitors may be advantageous when legitimate pollinators become absent or scarce (Schemske and Horowitz, 1989; Thomson, 2003). For instance, in *Pilocereus royenii* – a Puerto Rican cactus species with flowers characteristic of bat pollination – only carpenter bees affected fruit set during a year of low densities of nectar-feeding bats (Rivera-Marchand and Ackerman, 2006). Thus, temporal variation in pollinator assemblage

might favour the maintenance of pollination generalization in species with distinct adaptations to particular pollinator guilds (Waser *et al.*, 1996).

Temporal variation in bat visitation patterns was detected for subcampanulate-flowered *G. viridiflora* subsp. *sintenisii*; visitation rates ranged from one to four bat visits per flower per night (Table 3). *Gesneria viridiflora* subsp. *sintenisii* is a functionally generalized species pollinated primarily by bats and hummingbirds. Hummingbirds probably transfer little pollen during afternoon hours – before anther dehiscence time; however, early morning visits by hummingbirds may ensure pollination to flowers not visited during the night. Pollination by bats and hummingbirds has been reported for various groups of plants including *Abutilon* species from Brazil (Buzato *et al.*, 1994), *Marcgravia* from the island of Dominica (Zusi and Hamas, 2001), *Burmeistera* from South America (Muchhala, 2006). However, in *Burmeistera* most species specialize on bat pollination (Muchhala, 2006), and trade-offs for corolla shape appear to favour specialization to only one functional group, i.e. either bats or hummingbirds (Muchhala, 2007). The flowers of *G. viridiflora* subsp. *sintenisii* are intermediate in shape, but it is unclear whether or not subcampanulate flowers reflect selection imposed by two different pollinator guilds.

#### Floral rewards

Variation in nectar sugar concentration and amount of energy reward are known to influence attraction, foraging behaviour and energetics of different animal pollinators (Feinsinger, 1987; Baker and Baker, 1990). It was found that the amount and schedule of nectar production was consistent with the schedules and energetic needs of the primary pollinators of *Gesneria*; however, there was little variation in sugar concentration (10–13%; Table 1), suggesting phylogenetic conservatism. High nectar volumes and low sugar concentration are characteristic of chiropterophilous flowers (Baker *et al.*, 1998; Sanmartín-Gajardo and Sazima, 2005; Tschapka and von Helversen, 2007) and these nectar traits have also been recently associated with pollination by generalist birds (Johnson and Nicholson, 2008). Overall nectar concentration for tubular-flowered Puerto Rican *Gesneria* is in the low range reported for hummingbird-pollinated plants (Pyke and Waser, 1981; Stiles and Freeman, 1993; Perrett *et al.*, 2001; Nicolson and Fleming, 2003; Johnson and Nicholson, 2008); however, tubular-flowered *Gesneria* do not compensate by producing greater volumes of nectar. Low sugar concentration has been proposed as a strategy to deter bee visitation in ornithophilous flowers (Bolten and Feinsinger, 1978). However, this hypothesis is unlikely to explain the low nectar concentration in *Gesneria* because native bees are uncommon floral visitors of Antillean Gesneriaceae (S. Martén-Rodríguez and C. B. Fenster, unpubl. data).

#### Breeding systems

An alternative for plants that occur in environments where pollinators are scarce is to have breeding

systems that provide reproductive assurance when natural pollination fails (Eckert *et al.*, 2006; Fenster and Martén-Rodríguez, 2007). All Puerto Rican Gesneriaceae are self-compatible but most are also dichogamous and only the three tubular-flowered species had relatively high selfing potentials (Table 3). One species, *G. reticulata*, is predominantly selfing and has evolved features associated with this mode of reproduction (i.e. lack of dichogamy, shortened pistils and little or no nectar production). Only one hummingbird visit was observed to *G. reticulata*, suggesting hummingbirds have learned to avoid the nectarless flowers despite their attractive appearance. Inference of the pollination system based exclusively on external morphology for *G. reticulata* would have incorrectly led to hummingbird pollination. This highlights the importance of conducting both pollination and breeding system studies simultaneously.

#### Conclusions

Puerto Rican *Gesneria* species display ornithophilous and chiropterophilous flowers and primary visitors generally correspond to the expected by floral syndromes. The present results provide evidence for highly specialized pollination systems in insular plant species; in one case specialization to bat pollination occurs despite the presence of a diverse visitor assemblage (in *G. pedunculosa*). Nevertheless, evidence is also found for pollination generalization; *G. viridiflora* subsp. *sintenisii* shows a mixed pollination system with hummingbirds and bats as potential major partners of the mutualism. Generalized pollination might buffer against demographic fluctuations of pollinators and/or low floral visitation in tropical islands like Puerto Rico. Similarly, the presence of an autonomous selfing in morphologically specialized *G. reticulata* suggests self-pollination is used as reproductive assurance in an environment where hummingbird visitation is low. This work highlights the usefulness of studying sets of closely related species to improve our understanding of the evolutionary and ecological aspects involved in the diversification of island plants.

#### ACKNOWLEDGEMENTS

We thank Julie Cridland, Xin-Sheng Chen, Ana Chuquin and Carlo Moreno for invaluable help conducting field work, Richard Reynolds and five anonymous reviewers for insightful criticisms and suggestions to earlier versions of this manuscript. We thank the Arecibo Observatory for logistical support provided during field work. Funding for field work was provided by Sigma-Xi, Graduate Woman in Science, Explorer's Club of Washington Group, American Gloxinia and Gesneria Society and the University of Maryland to Silvana Martén-Rodríguez and NSF DDIG 0710196 to Silvana Martén-Rodríguez and Charles B. Fenster.

#### LITERATURE CITED

Armbruster WS, Baldwin BG. 1998. Switch from specialized to generalized pollination. *Nature* 394: 632.

- Armbruster WS, Fenster CB, Dudash MR. 2000. Pollination “principles” revisited: specialization, pollination syndromes and the evolution of flowers. *Det Norske Videnskaps-akademi. I. Matematisk Naturvidenskapelige Klasse, Skrifter, Ny Serie* 39: 179–200.
- Baker HG. 1955. Self compatibility and establishment after ‘long distance’ dispersal. *Evolution* 9: 347–349.
- Baker HG, Baker I. 1990. The predictive value of nectar chemistry to the recognition of pollinator types. *Israeli Journal of Botany* 39: 157–166.
- Baker HG, Baker I, Hodges SA. 1998. Sugar composition of nectars and fruits consumed by birds and bats in the tropics and subtropics. *Biotropica* 30: 559–586.
- Barrett SCH. 1996. The reproductive biology and genetics of island plants. *Philosophical Transactions of the Royal Society. B, Biological Sciences* 351: 725–733.
- Barrett SCH. 2003. Mating strategies in flowering plants: the outcrossing–selfing paradigm and beyond. *Philosophical Transactions of the Royal Society. B, Biological Sciences* 358, 991–1004.
- Barrett SCH, Harder LD, Worley AC. 1996. The comparative biology of pollination and mating in flowering plants. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences* 351: 1271–1280.
- Bernardello G, Anderson GJ, Stuessy TF, Crawford DJ. 2006. A survey of floral traits, breeding systems, floral visitors, and pollination systems of the angiosperms of the Juan Fernandez Islands (Chile). *Botanical Review* 67: 255–308.
- Bolten AB, Feinsinger P. 1978. Why do hummingbird flowers secrete dilute nectar? *Biotropica* 10: 307–309.
- Buzato S, Sazima M, Sazima I. 1994. Pollination of three species of *Abutilon* (Malvaceae) intermediate between bat and hummingbird flower syndromes. *Flora* 189: 327–334.
- Carlquist S. 1974. *Island biology*. New York, NY: Columbia University Press.
- Darwin C. 1862. *On the various contrivances by which British orchids and foreign orchids are fertilized by insects, and on the good effect of intercrossing*. London: Murray.
- Eckert CG, Samis KE, Dart S. 2006. Reproductive assurance and the evolution of uniparental reproduction in flowering plants. In: Harder LD, Barrett SCH, eds. *The ecology and evolution of flowers*. Oxford: Oxford University Press, 183–203.
- Elmqvist T, Cox PA, Rainey WE. 1992. Restricted pollination on oceanic islands – pollination of *Ceiba pentandra* by flying foxes in Samoa. *Biotropica* 24: 15–23.
- Feinsinger P. 1987. Approaches to nectarivore–plant interactions in the New World. *Revista Chilena de Historia Natural* 60: 285–319.
- Fenster CB, Martín-Rodríguez S. 2007. Reproductive assurance and the evolution of pollination specialization. *International Journal of Plant Sciences* 168: 215–228.
- Fenster CB, Armbruster WS, Thomson JD, Wilson P, Dudash MR. 2004. Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution and Systematics* 35: 375–403.
- Gómez JM. 2002. Generalización en las interacciones entre plantas y polinizadores. *Revista Chilena de Historia Natural* 75: 105–116.
- González-Díaz N, Ackerman JD. 1988. Pollination, fruit set and seed production in the orchid *Oeceoclades maculata*. *Lindleyana* 3: 150–155.
- Herrera CM. 1988. Variation in mutualisms: the spatio-temporal mosaic of an insect pollinator assemblage. *Biological Journal of the Linnean Society* 35: 95–125.
- Holsinger KE. 1996. Pollination biology and the evolution of mating systems in flowering plants. *Evolutionary Biology* 29: 107–149.
- Johnson SD, Nicolson SW. 2008. Evolutionary associations between nectar properties and specificity in bird pollination systems. *Biology Letters* 4: 49–52.
- Johnson SD, Steiner KE. 2000. Generalization versus specialization in plant pollination systems. *Trends in Ecology and Evolution* 15: 140–143.
- Kearns CA, Inouye DW. 1993. *Techniques for pollination biologists*. Boulder, CO: University Press of Colorado.
- Kodrick Brown NA, Brown JH, Byersa GS, Gori DF. 1984. Organization of a tropical island community of hummingbirds and flowers. *Ecology* 65: 1358–1368.
- Lammers TG, Freeman CE. 1986. Ornithophily among the Hawaiian Lobelioideae (Campanulaceae) – evidence from floral nectar sugar compositions. *American Journal of Botany* 73: 1613–1619.
- Lindqvist C, Albert VA. 2002. Origin of the Hawaiian endemic mints within North American *Stachys* (Lamiaceae). *American Journal of Botany* 89: 1709–1724.
- Lloyd DG, Schoen DJ. 1992. Self- and cross-fertilization in plants. I. Functional dimensions. *International Journal of Plant Sciences* 153: 358–369.
- Micheneau C, Fournel J, Paillet T. 2006. Bird pollination in an angraecoid orchid on Reunion Island (Mascarene Archipelago, Indian Ocean). *Annals of Botany* 97: 965–974.
- Muchhala N. 2006. The pollination biology of *Burmeistera* (Campanulaceae): specialization and syndromes. *American Journal of Botany* 93: 1081–1089.
- Muchhala N. 2007. Adaptive trade-off in floral morphology mediates specialization for flowers pollinated by bats and hummingbirds. *The American Naturalist* 169: 494–504.
- Naito Y, Konuma A, Iwata H, Suyama Y, Seiwa K, Okuda T, et al. 2005. Selfing and inbreeding depression in seeds and seedlings of *Neobalanocarpus heimii* (Dipterocarpaceae). *Journal of Plant Research* 118: 423–430.
- Nicolson SW, Fleming PA. 2003. Nectar as food for birds: the physiological consequences of drinking dilute sugar solutions. *Plant Systematics and Evolution* 238: 139–153.
- Olesen JM, Jordano P. 2002. Geographic patterns in plant–pollinator mutualistic networks. *Ecology* 83: 2426–2424.
- Perret M, Chautaems A, Spichiger R, Peixoto M, Savolainen V. 2001. Nectar and sugar composition in relation to pollination syndromes in Sinningieae. *Annals of Botany* 87: 267–273.
- Pyke GH, Waser NM. 1981. The production of dilute nectars by hummingbird and honeyeater flowers. *Biotropica* 13: 260–270.
- Rivera-Marchand B, Ackerman JD. 2006. Bat pollination breakdown in the Caribbean columnar cactus *Pilosocereus royenii*. *Biotropica* 38: 635–642.
- Sanmartín-Gajardo I, Sazima M. 2005. Chiropterophily in Sinningieae (Gesneriaceae): *Sinningia brasiliensis* and *Paliavana prasinata* are bat-pollinated, but *P. sericiflora* is not. *Annals of Botany* 95: 1097–1103.
- SAS Institute. 2004. *SAS for Windows*, version 9.1. Cary, NC: SAS Institute.
- Schemske DW, Horovitz CC. 1989. Temporal variation in selection in a floral character. *Evolution* 43: 461–465.
- Skog LE. 1976. A study of the tribe Gesnerieae with a revision of *Gesneria* (Gesneriaceae: Gesnerioideae). *Smithsonian Contributions to Botany* 29: 1–182.
- Stebbins GL. 1970. Adaptive radiation of reproductive characteristics in angiosperms: pollination mechanisms. *Annual Review of Ecology and Systematics* 1: 307–326.
- Stiles FG, Freeman CE. 1993. Patterns in floral nectar characteristics of some bird-visited plant species from Costa Rica. *Biotropica* 25: 191–205.
- Temeles EJ, Kress WJ. 2003. Adaptation in a plant–hummingbird association. *Science* 300: 630–633.
- Tschapka M, von Helversen O. 2007. Phenology, nectar production and visitation behaviour of bats on the flowers of the bromeliad *Werauhia gladioliflora* in a Costa Rican lowland rain forest. *Journal of Tropical Ecology* 23: 385–395.
- Thomson J. 2003. When is it mutualism? *The American Naturalist* 162: S1–S9.
- Waser NM, Chittka L, Price MV, Williams NM, Ollerton J. 1996. Generalization in pollination systems, and why it matters. *Ecology* 77: 1043–1060.
- Zimmer EA, Roalson EH, Skog LE, Boggan JK, Idnurm A. 2002. Phylogenetic relationships in the Gesnerioideae (Gesneriaceae) based on nr DNA ITS and cp DNA trn L-F and trn E-T spacer region sequences. *American Journal of Botany* 89: 296–311.
- Zusi RL, Hamas MJ. 2001. Bats and birds as potential pollinators of three species of *Marcgravia* lianas on Dominica. *Caribbean Journal of Science* 37: 274–278.