Pollen limitation and reproductive assurance in Antillean Gesnerieae: a specialists vs. generalist comparison

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Abstract. Pollen limitation of female fecundity is widespread among angiosperms, a signal that pollinators frequently fail to transfer pollen to fertilize all ovules. Recent surveys have suggested that pollen limitation is associated with floral specialization. This study uses a group of Antillean Gesneriaceae with contrasting pollination systems (bat, hummingbird, and generalist) to assess the premise that plants with specialized pollination systems and infrequent pollinator visitation experience greater pollen limitation of fruit and seed set than their generalist congeners. Alternatively, specialists may possess mechanisms that reduce pollen limitation, such as autonomous self-pollination. A survey of autonomous self-pollination conducted on 13 *Gesneria* and *Rhytidophyllum* species during 2006–2008 revealed no significant association between reproductive assurance mechanisms and pollination system specialization. However, high levels of potential autonomous self-pollination were only found among specialized hummingbird-pollinated species. A comparison of fruit and seed set between emasculated and unmanipulated flowers provided evidence for autonomous selfing acting as a reproductive assurance mechanism in three out of four ornithophilous species. Furthermore, the Puerto Rican population of *G. reticulata* relies almost exclusively on self-pollination for reproduction. Two-year pollen supplementation experiments conducted on nine Gesnerieae species from the Dominican Republic and Puerto Rico provided evidence for significant pollen limitation associated with pollination specialization including both bat- and hummingbird-pollinated Gesnerieae; no pollen limitation was detected in any of the four generalist species. No pollen limitation was detected either in two ornithophilous *Gesneria* species with low hummingbird visitation and high levels of autonomous self-pollination. This study provides support for the idea that generalized pollination systems may, in some cases, buffer against fluctuations in the pollinator environment. However, the use of reproductive assurance mechanisms allows the maintenance of highly specialized pollination systems in pollinator depauperate environments.

Key words: autonomous self-pollination; Dominican Republic; generalized pollination; Gesneriaceae; pollen limitation; Puerto Rico; reproductive assurance; specialized pollination.

Introduction

Most flowering plants rely on external agents to produce outcrossed seed. Consequently, failures in the process of pollen transfer among conspecifics can severely impact plant female reproductive success (Wilcock and Neiland 2002). Evidence for widespread pollen limitation has been found in recent surveys reflecting inadequacies in pollen supply that prevent full seed set, even when resources are plentiful (Burd 1994, Larson and Barrett 2000, Knight et al. 2005). The prevalence of pollen limitation across unrelated angiosperm lineages suggests that pollinators frequently do not effect successful cross-fertilization (Ashman et al. 2004, Aizen

and Harder 2007). A potentially important determinant of pollination success is the degree of floral specialization, i.e., the degree of dependence of a plant species on particular species or functional groups of pollinators. However, the association between floral specialization and pollen limitation has only been assessed in a few literature and community-level surveys (Larson and Barrett 2000, Knight et al. 2005, Merrett et al. 2007) and not through direct comparisons of related species.

Floral specialization has traditionally been considered an evolutionary pathway to promote effective outcross pollen transfer (Stebbins 1970, Faegri and van der Pijl 1978, Fenster et al. 2004). However, specialization can make plants vulnerable to temporal fluctuations in pollinator visitation or permanent loss of important pollinators (Wilcock and Neiland 2002). In contrast, plants with generalized flowers are buffered against fluctuations of individual pollinator species and are thus assumed to experience less variation of reproductive success than specialists (Waser et al. 1996). These views

underlie the prediction that female fertility in specialized species should be more pollen limited than in generalists (Larson and Barrett 2000), and would suggest that the evolution of pollination specialization reflects selection acting to increase male rather than female reproductive success. Support for the prediction of increased pollen limitation in species with specialized pollination systems was found in two recent studies, a large survey of pollen limitation across flowering plants (Knight et al. 2005), and a field study of a shrub assemblage in New Zealand (Merrett et al. 2007). However, deviations from the association between pollen limitation and floral specialization are expected as a result of variation in pollinator efficiency. Pollen limitation may result not just from insufficient pollen transfer, but from deposition of low quality pollen, i.e., genetically related, incompatible, or heterospecific pollen (Aizen and Harder 2007). Therefore, if floral visitors do not transfer adequate amounts of compatible pollen, then generalized species could also experience significant pollen limitation (Wilcock and Neiland 2002). Comparisons of closely related species in similar ecological settings but with varying degrees of floral specialization are necessary to evaluate these two contrasting hypotheses.

Assessments of pollen limitation within groups of related species also allow us to examine the frequently overlooked association between pollination and breeding system evolution. For instance, when plants experience intense pollen limitation of female fertility, natural selection should favor traits or trait combinations that increase attraction of existing pollinators (e.g., Hansen and Totland 2006), attract more pollinator species, and/or decrease pollinator dependence (e.g., Fishman and Willis 2008). Thus, autonomous breeding systems may evolve to reduce reliance on unpredictable or ineffective floral visitors (Darwin 1877, Lloyd 1979). Available evidence shows that autonomous selfing mechanisms are widespread across different angiosperm lineages encompassing a wide variety of pollination systems (Lloyd and Schoen 1992, Fenster and Martén-Rodríguez 2007). However, the pattern of association between levels of floral specialization and the evolution of autonomous self-pollination remains unclear (Fenster and Martén-Rodríguez 2007). This study evaluates the hypothesis that specialization increases vulnerability to pollination failure and makes plants more likely to exhibit reproductive assurance mechanisms in a group of closely related Antillean Gesneriaceae.

The tribe Gesnerieae is a clade of ~75 species that radiated in the Antilles from a single common ancestor (Zimmer et al. 2002). Approximately 60% of species in the tribe are pollinated by hummingbirds; the remaining species are pollinated by bats or have generalized pollination systems (including different combinations of bats, birds, and insects); a few species are pollinated by bees (Martén-Rodríguez et al. 2009). Molecular and morphological phylogenies including half of the extant members of the tribe indicate that hummingbird-pollinated, tubular flowers are most likely ancestral, with at least two independent origins of bat pollination and three origins of generalized pollination systems (Martén-Rodríguez 2008). Transitions from hummingbird to bat and generalized pollination systems are associated with changes in corolla shape, color and schedules of anther dehiscence and nectar production. In one unusual case, bat pollination evolved from generalized ancestors by restricting anthesis time to strictly night hours (in *Gesneria quisqueyana*). Autonomous self-pollination mechanisms evolved at least twice independently in ornithophilous lineages from self-compatible but non-autogamous ancestors (Martén-Rodríguez 2008).

Pollinator visitation frequencies vary among pollination systems. For instance, in terms of the number of visits per flower per day, generalists have the highest visitation rates (mean $\pm$ SE $= 13 \pm 1.8$, $n = 5$), followed by bat-pollinated species (2 ± 1.8, $n = 5$), and hummingbird-pollinated species (1 ± 1.5, $n = 9$) (Martén-Rodríguez et al. 2009; Appendix A). Thus, visitation rates are, on average, 10 times lower for specialists than for generalists. We used a group of Gesnerieae species with the three contrasting pollination systems to address the following questions: (1) Are autonomous selfing mechanisms more common in species with specialized pollination systems? (2) Do autonomous selfing mechanisms provide reproductive assurance? (3) Is female fertility limited by pollen availability? (4) If so, is the occurrence and magnitude of pollen limitation associated with pollination specialization? Pollinator observations underlie the prediction that species with specialized pollination systems and infrequent pollinator service will be more likely to use autonomous self-pollination as a reproductive assurance mechanism. We also predict that specialized Gesnerieae species will experience greater pollen limitation of fruit and seed set than their generalist relatives.

METHODS

Study sites and species descriptions

All members of the tribe Gesnerieae are perennial plants that range from rosettes to small trees. All species have woody stems, at least at the base of the plant (Skog 1976). The flowers are gamopetalous and they fall primarily into three main floral phenotypes: tubular, campanulate (bell-shaped), and subcampanulate (bell-shaped with a corolla constriction above the nectar chamber; see Plate 1). Tubular-flowered species are bright red, orange, or yellow, and have diurnal anther dehiscence and nectar production; these species are pollinated exclusively by one to two species of hummingbirds in each island. Campanulate (bell-shaped) flowered species are pollinated primarily by a few species of bats (Martén-Rodríguez and Fenster 2008, Martén-Rodríguez et al. 2009). We designate levels of specialization following Fenster et al. (2004), where pollinators fall into functional groups, generally defined by taxonomic relatedness and the potential to exert similar

TABLE 1. Potential autonomous fruit set for 13 Gesnerieae species from Puerto Rico and the Dominican Republic studied in 2005–2007.

Species	Pollination system	Floral morphology	Autonomous fruit set (%)	n	AFI
Gesneria citrina†	hummingbird	tubular	24	30	0.38
G. cuneifolia†	hummingbird	tubular	68	15	0.88
G. pulverulenta	hummingbird	tubular	13	8	0.19
G. reticulata†	self-pollination	tubular	90	28	0.98
G. tuberosa Peguero & Marten (inedit)	hummingbird	tubular	4	21	0.07
Rhytidophyllum asperum	hummingbird	tubular	12	23	0.17
G. fruticosa	bat	bell-shaped	0	10	0.00
G. pedunculosa†	bat	bell-shaped	1	22	0.02
G. quisqueyana	bat	bell-shaped	1	21	0.02
G. viridiflora subsp. *sintenisii*†	generalist	bell-shaped	8	15	0.09
R. auriculatum	generalist	bell-shaped	4	19	0.06
R. leucomallon	generalist	bell-shaped	3	19	0.03
R. vernicosum	generalist	bell-shaped	2	30	0.03

Notes: The autofertility index (AFI) was calculated as the average fruit set of bagged flowers (autonomous fruit set) divided by the average fruit set of hand-pollinated outcrossed flowers. Sample sizes (*n*) are the numbers of plants used for bagging and hand pollination treatments.

† Autonomous fruit set from Martén-Rodríguez and Fenster (2008).

selective pressures on flower structure (e.g., hummingbirds or nectar-feeding bats). Consequently, we consider Gesnerieae species pollinated primarily by hummingbirds or bats as specialists. We define generalization as effective pollination by two or more functional groups. Generalized Gesnerieae have subcampanulate flowers and they receive visits from different pollinator functional groups, including bats, birds, and insects: all of these have the potential to transfer pollen. Pollination systems for all study species are listed in Appendix A. Study sites and plant characteristics are listed in Appendix B.

All Gesnerieae species studied to date are self-compatible and do not produce seed by apomixis (Martén-Rodríguez and Fenster 2008; S. Martén-Rodríguez and C. B. Fenster, *unpublished data*). Breeding systems vary in the temporal separation and spatial placement of the reproductive organs. Most study species are protogynous (except for *G. pedunculosa*, which is protandrous). The female phase in protogynous Gesnerieae lasts two to three days and anther dehiscence generally occurs by the second day. The spatial separation of anthers and stigmas observed the first day usually decreases by the time of anther dehiscence; therefore both temporal and spatial overlap between male and female functions provide the potential for delayed self-pollination. However, for most species with bell-shaped flowers, contact between the stigmatic surface and pollen is prevented by the specific positioning of the reproductive organs, with anthers contacting only the back side of the stigma. This type of placement of anthers and stigma is less common in tubular flowers.

Autonomous self-pollination survey

We compiled data for 13 Gesnerieae species to determine whether the degree of autonomous self-pollination is associated with particular pollination systems. The data set includes seven species from the Dominican Republic and one from Puerto Rico surveyed in 2006–2007, and five species of Puerto Rican *Gesneria* reported in an earlier study (Martén-Rodríguez and Fenster 2008). To determine the potential for autonomous selfing, 2–10 flowers per plant were bagged in 6–30 individuals per species, depending on the population density. A comparable set of hand-pollinated outcrossed flowers was included to account for the variability in fruit production due to environmental or physiological conditions. Bridal veil fabric bags were used to cover flower buds until fruit development. Mature fruits were counted six to nine weeks later. We report two measures of potential autonomous self-pollination: the fruit set of bagged flowers, and the autofertility index proposed by Lloyd and Schoen (1992), hereafter designated as AFI. This index is calculated by dividing the fruit set of bagged flowers by the fruit set of hand-outcrossed flowers.

To test for differences in the levels of autonomous self-pollination among pollination systems we conducted non-parametric Kruskal-Wallis tests (proc NPAR1-WAY) in SAS 9.1.3 (SAS Institute 2005). We used the same test to evaluate differences in levels of autonomous selfing between species with tubular flowers and species with bell-shaped flowers (campanulate and subcampanulate); the EXACT option was specified to obtain exact *P* values because the normal approximation may not be accurate with small sample sizes.

Reproductive assurance field experiments

In our sample, only hummingbird-pollinated Gesnerieae had significant potential to set seed in the absence of pollinators (see results below). Thus, to determine whether these plants actually use autonomous selfing as a reproductive assurance mechanism, we selected four species with autofertility indices greater than 10% (fruit set from bagged flowers/hand-pollinated flowers; Table 1), and large population sizes to allow for adequate sampling.

The species were *G. citrina*, *G. cuneifolia*, *G. reticulata*, and *R. asperum*. Prior to the start of these experiments, we tested for the effects of emasculation on two flowers of 10–15 plants per species by comparing two treatments: (T1) hand-pollination and no emasculation (T2) hand-pollination and emasculation. No significant differences in mean fruit set among treatments were found, indicating emasculation has no negative effects on fruit production for these species (*G. citrina* T1 = 62 ± 8.6 [mean ± SE], N = 9; T2 = 61 ± 7.9, N = 9, P = 0.535; *G. cuneifolia* T1 = 84 ± 7.1, N = 8; T2 = 86 ± 7.0, N = 8, P = 0.772; *G. reticulata* T1 = 94 ± 5.6, N = 9, T2 = 88 ± 6.1, N = 10, P = 0.399; *R. asperum* T1 = 57 ± 8.2, N = 12, T2 = 65 ± 8.0, N = 11, P = 0.558).

Reproductive assurance experiments were conducted in 2006 and 2007 on 21–33 individuals per species. We followed plants for two to three weeks, tagged all flower buds, and assigned pairs at random to the following treatments: (1) emasculation—flowers had their anthers removed in bud and were left uncovered to allow for pollinator visitation, a measure of geitonogamy and outcrossing; (2) control—flower buds were tagged and left intact to serve as a measure of natural pollination (geitonogamy, outcrossing, and autonomous self-pollination). The contrast between the two treatments (control − emasculation) reflects the contribution of self-pollination. Fruit set was determined six to nine weeks after flowering and seeds were extracted and weighed to the nearest 0.001 g. Tests of the reproductive assurance hypothesis were conducted by comparing control and emasculated treatments for each hummingbird-pollinated species. We used the GLIMMIX procedure in SAS, with treatment, year and their interaction specified as fixed effects in the model, and fruit set (fruits/flowers) and seed mass set as the response variables. For fruit set, the binomial distribution and logit link function were specified; to obtain back-transformed least-square means we specified the *ilink* option under the LSMEANS statement. For seed mass the residual variance was normally distributed; thus, no transformation was necessary. The covariance between emasculated and control treatments applied to the same individuals was accounted for by a random residual statement where plant was stated as the subject of repeated measures. Degrees of freedom were estimated using the Satterthwaite option. Differences between emasculated and open pollination treatments within each year were tested using orthogonal contrast statements.

Pollen limitation field experiments

To determine whether pollination system is associated with the degree of pollen limitation, we selected nine Gesnerieae species that encompass the three main pollination modes described above. Five species were studied in Puerto Rico (January–March, 2006 and 2007) and four in the Dominican Republic (May–August, 2006 and 2007). Given that sister species pairs were not available at particular sites or in large enough numbers for experimentation, we ensured some level of phylogenetic independence by selecting, at each of three sites, at least a pair of related species with contrasting pollination systems (Martén-Rodríguez 2008). The groups include: (1) *Rhytidophyllum vernicosum* (generalist moth and hummingbird pollination), *R. leucomallon* (generalist bat, hummingbird, and moth pollination), and *Rhytidophyllum asperum* (hummingbird specialist) (these species occur in Sierra de Bahoruco National Park, the Dominican Republic); (2) *G. pedunculosa* (bat pollination), *G. citrina* (hummingbird pollination), and *G. cuneifolia* (hummingbird pollination) (these species occur in the karst hills surrounding the Arecibo Observatory, Puerto Rico); (3) *G. viridiflora sintenisii* (generalist bat, hummingbird, moth pollination) and *G. reticulata* (self/hummingbird pollination) (from El Yunque National Forest, Puerto Rico). We also included *G. quisqueyana* (bat pollination) from Cordillera Septentrional in the Dominican Republic, because it is sister to *G. viridiflora* (Martén-Rodríguez 2008) and it allows a direct specialist vs. generalist sister-species comparison. These two species are morphologically similar, and they occur at similar elevations with comparable weather conditions; however, the flowers of *G. quisqueyana* open only during the night (making this species a strictly bat specialist), whereas in *G. viridiflora* flowers are open both day and night (Martén-Rodríguez et al. 2009).

We tagged 21–33 pairs of plants per species; these pairs encompassed a wide representation of the different sizes and ages of plants found in each population. Each pair consisted of individuals of similar size growing within 5 m of each other. Within each pair, plants were assigned at random to a pollen supplementation treatment or an open pollination control. All flowers produced by an individual were subject to the same treatment. Pollen-supplemented flowers received a mixture of pollen from at least two different donors. Donor plants were located at least seven m away from the recipient plant to prevent matings between close relatives. Hand pollinations were conducted by rubbing dehisced anthers onto receptive stigmas. Timing of stigma receptivity and anther dehiscence were previously determined using the peroxidase test (Kearns and Inouye 1993). Open-pollinated flowers were unmanipulated to serve as controls for natural pollination. Because the study species are perennials and flowering generally lasts several weeks or months, field work was conducted for 14–27 days, depending on the per-day flower output of the species, i.e., species with longer flowering seasons and lower daily flower production (one to two flowers) were studied for longer periods to ensure adequate sample sizes. Ideally, measurements should be taken over the entire life span of the plant (Ashman et al. 2004), but there are obvious logistical difficulties for implementing this approach on long-lived perennials. Thus, to reduce variation in potential conflicts of resource allocation with fruits developed

before and after the study period, we removed mature fruits at the time of tagging and large flower buds present at the end of the experiment. By taking a sample representative of the population size and age classes, applying treatments to entire plants, and measuring fruit and seed set on the same individuals for two years, we expect to provide representative estimates of pollen limitation during the study period, as well as a uniform setting to compare species with different degrees of pollination specialization.

Fruit set was determined seven to nine weeks after flowering and seeds were extracted and weighed to the nearest 0.001 g. Seed mass was used as a surrogate for seed set due to the large number of seeds contained in each capsule. We previously determined seed mass to be significantly correlated with seed number in all nine study species ($0.87 < r > 0.94$; $P < 0.0005$). A measure of whole-plant seed production was calculated by multiplying mean fruit set by mean seed mass per plant. We calculated the pollen limitation index as ($1 -$ [open/pollen-supplemented whole-plant seed mass]) after Larson and Barrett (2000).

We tested for pollen limitation of fruit set and seed mass with repeated-measures ANOVA, using proc GLIMMIX in SAS (SAS Institute 2005). Model terms included treatment (open vs. pollen supplemented), specified as a fixed effect; year, specified as a random effect; and plant, as the subject of repeated measures. This model accounts for the potential covariation that may arise from applying particular treatments to the same plants for two years. Furthermore, this approach provides a single random estimate of pollen limitation for each plant, which represents a sample taken across a wide range of age classes within the population. As above, the binomial distribution was specified for the variable fruit set, and back-transformed means were obtained stating the *ilink* option. For seed mass, the default options for a normally distributed variable were used. Degrees of freedom were estimated stating the Satterthwaite option.

Differences in the levels of pollen limitation among pollination systems were evaluated with a Kruskal-Wallis test (proc NPAR1WAY); the EXACT option was used to obtain *P* values. We excluded the two species with high levels of autonomous self-pollination to avoid a comparison of pollen limitation that was confounded by variation in the breeding system.

Results

Autonomous self-pollination survey

The ability to set seed autonomously was predominantly associated with hummingbird pollination, but there was great variation among species (Table 1). Fruit set upon bagging for hummingbird specialists ranged between 4% and 90%, and the autofertility index (AFI) was equally dispersed (0.07–0.98). Autonomous fruit set was 0–1% for bat-pollinated species (AFI values $\leq$ 0.02), and 3–8% for generalist species (AFI values $\leq$ 0.09), indicating low or no potential for autonomous self-pollination in the latter two pollination categories. No differences in the autofertility index between specialized and generalized species were detected (Wilcoxon two-sample test, $P = 0.51$), however the three pollination systems differed significantly (Kruskal-Wallis test, $\chi^2 = 9.8$, df = 2, $P = 0.0002$). Floral morphology also influenced autofertility levels, with tubular-flowered species showing significantly higher AFI values than bell-shaped-flowered ones (Wilcoxon two-sample test, $P = 0.017$).

Reproductive assurance field experiments

Autonomous self-pollination significantly increased fruit set in three ornithophilous Gesnerieae species (Fig. 1, Table 2), and marginally so in a fourth species, *G. citrina* ($P = 0.07$). There were no significant effects of treatment for mean seed mass per fruit for any of the four species, suggesting that when flowers are visited, hummingbirds effect full seed set (Fig. 1, Table 2). The highest contribution of autonomous selfing to total female fertility or $1 -$ ([mean fruit set $\times$ mean seed mass/fruit] of emasculated/control flowers) was for *G. reticulata* (100% in 2006 and 96% in 2007), followed by *G. cuneifolia* (56% in 2006 and 45% in 2007). The contribution of self-pollination was more variable across time in *G. citrina* (7% in 2006 and 19% in 2007) and *R. asperum* (0% and 18% in 2006 and 2007, respectively), and significant only for the latter species in 2007 (Fig. 1).

Pollen limitation field experiments

We detected pollen limitation in four species with specialized pollination systems and no autonomous self-pollination, while we found no evidence for pollen limitation in five species that had either generalized pollination systems or high autonomous selfing levels (Fig. 2, Table 3). Specifically, we documented significant differences in fruit set and seed mass between pollen-supplemented and open-pollination treatments for bat specialist *G. pedunculosa* and hummingbird specialists *G. citrina* and *R. asperum*. Pollen limitation of fruit set, but not seed set was detected for the bat specialist, *G. quisqueyana*. There were no significant differences between pollen supplementation and open pollination treatments for any of the three species with mixed pollination systems, i.e., *G. viridiflora*, *R. vernicosum*, and *R. leucomallon*. No pollen limitation was detected for *G. cuneifolia* and *G. reticulata*, the two species with ornithophilous flowers but high levels of autonomous self-pollination. Pollen limitation was not associated with habitat as both pollen-limited and non-pollen-limited species were present at all sites (Fig. 2).

The degree of pollen limitation, as determined by the pollen limitation index (PLI), was highest (PLI > 25%) in bat-pollinated *Gesneria* and the two species with hummingbird-pollinated flowers and low reproductive assurance (Table 3). Intermediate values of pollen
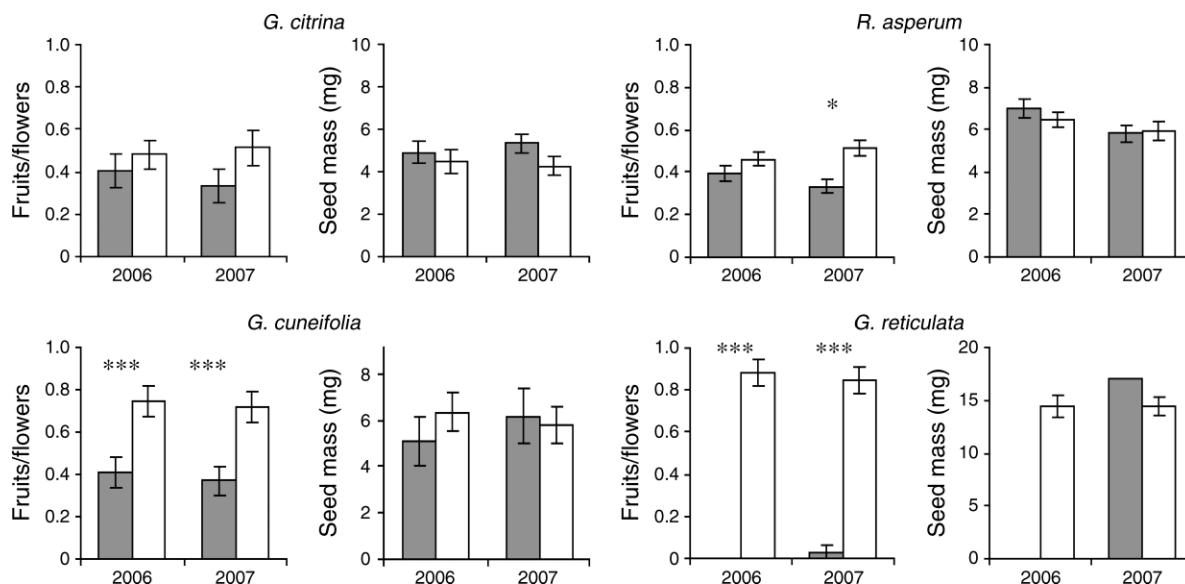
Fig. 1. Least-square mean proportion of fruit set and seed mass of emasculated (gray bars) and open-pollination (open bars) treatments for four ornithophilous species of Antillean *Gesneria* and *Rhytidophyllum* studied in 2006 and 2007. Means for fruit set were back-transformed using the *ilink* option in SAS (SAS Institute 2005). Error bars indicate ± SE. Asterisks indicate a significant contribution of autonomous self-pollination to fruit set.
* $P < 0.05$; ** $P < 0.005$; *** $P < 0.0005$.

limitation (8–26%) were characteristic of most other species, while no pollen limitation was detected for autogamous *G. reticulata*. However, outcrossed seed set was reduced by 97% in *G. reticulata* when the opportunity for self-pollination was eliminated (Table 3). Despite the small sample size (seven species with low autonomous selfing), we detected a trend for higher

pollen limitation in the specialized species (Wilcoxon two-sample test, $P = 0.0571$).

Discussion

Pollen limitation and pollination specialization

Specialized pollination is thought to have evolved in response to selection for higher precision and effective-

TABLE 2. ANOVA quantifying effects of reproductive assurance treatment (emasculation vs. control) and year (2006 vs. 2007) on fruit set and seed mass of four tubular-flowered species of *Gesneria* and *Rhytidophyllum* from the Dominican Republic and Puerto Rico.

	Fruit set			Seed mass		
Source of variation	df	F	P	df	F	P
G. citrina						
Treatment	1	3.27	0.074	1	2.07	0.161
Year	1	0.07	0.795	1	0.03	0.853
Treatment × year	1	0.59	0.445	1	0.42	0.520
Error	64			53		
R. asperum						
Treatment	1	4.29	0.043	1	0.13	0.715
Year	1	0.01	0.913	1	4.80	0.034
Treatment × year	1	1.28	0.262	1	0.37	0.546
Error	57			82		
G. cuneifolia						
Treatment	1	24.30	<0.0001	1	0.25	0.618
Year	1	0.34	0.564	1	0.10	0.752
Treatment × year	1	0.02	0.902	1	0.92	0.342
Error	66			78		
G. reticulata						
Treatment (year 2007)†	1	21.89	<0.0001			
Error	32					

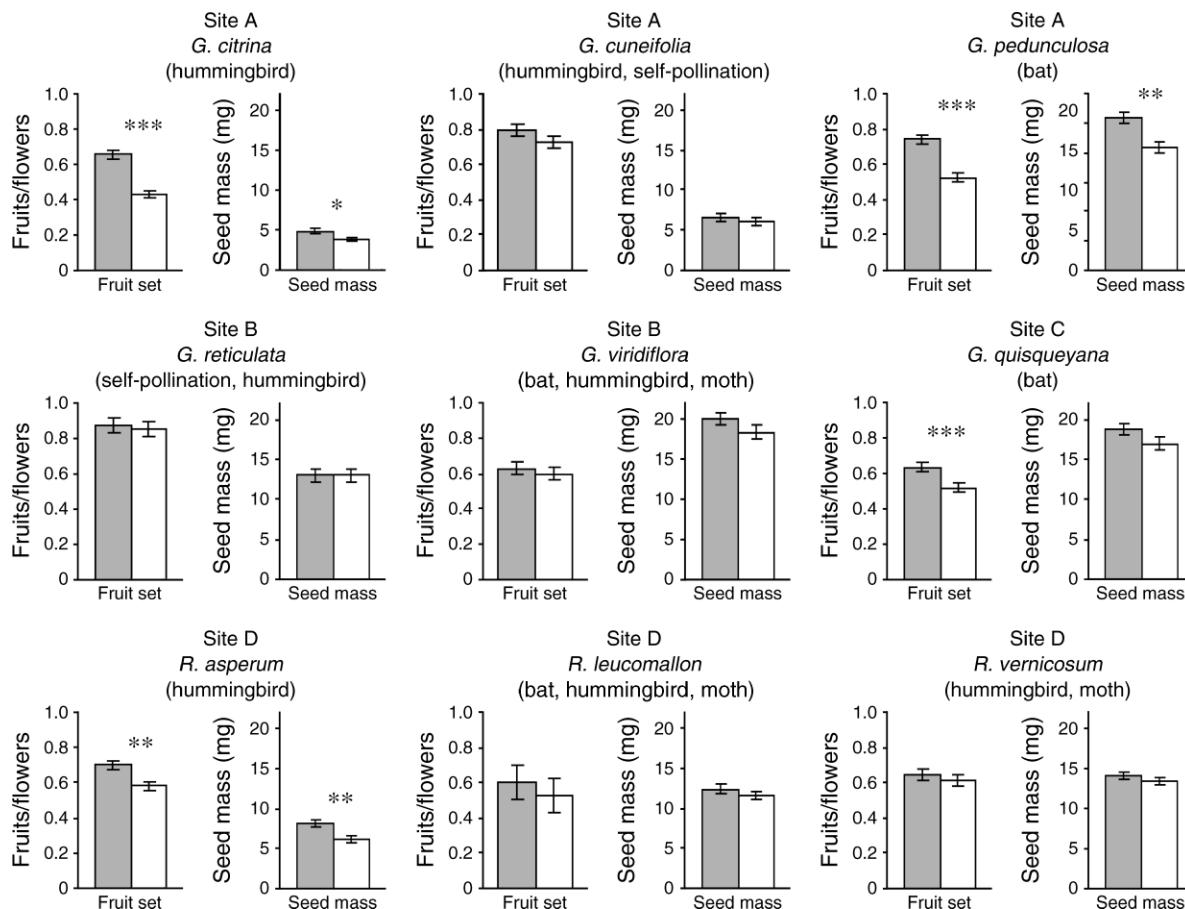† Means were compared for fruit set in 2007 only; emasculated flowers set no fruit in 2006.

FIG. 2. Least-square mean proportion of fruit set and seed mass of pollen-supplemented treatment (gray bars) and open-pollinated control (open bars) of nine species of *Gesneria* and *Rhytidophyllum* from Puerto Rico (sites A and B) and the Dominican Republic (sites C and D) studied in 2006 and 2007. Means for fruit set were back-transformed using the *ilink* option in SAS (SAS Institute 2005). Error bars indicate ±SE. Asterisks indicate a significant contribution of autonomous self-pollination to fruit set. * *P* < 0.05; *** *P* < 0.0005.

TABLE 3. Pollen limitation index (PLI) and repeated-measures ANOVA effects of pollination treatment (hand-pollination vs. open pollination) on fruit set and seed mass of nine species of *Gesneria* and *Rhytidophyllum* from the Dominican Republic and Puerto Rico studied in 2006 and 2007.

Species	Pollination system	PLI (%)	Fruit set			Seed mass		
			F	df	*P*	*F*	df	*P*
G. citrina	hummingbird	48	41.63	1, 69	<0.0001	5.76	1, 41	0.020
R. asperum	hummingbird	36	9.07	1, 63	0.004	8.80	1, 44	0.005
G. cuneifolia	hummingbird/self	17 [51]	2.02	1, 67	0.160	0.67	1, 64	0.415
G. reticulata	self-pollination	2 [97]	0.10	1, 51	0.758	0.00	1, 52	0.996
G. pedunculosa	bat	44	36.84	1, 53	<0.0001	12.07	1, 47	0.001
G. quisqueyana	bat	26	13.46	1, 43	0.0007	2.88	1, 38	0.098
G. viridiflora	generalist	12	0.28	1, 75	0.596	2.06	1, 67	0.156
R. leucomallon	generalist	8	0.35	1, 58	0.558	0.96	1, 56	0.331
R. vernicosum	generalist	18	0.41	1, 68	0.524	0.67	1, 55	0.416

Notes: The index is calculated as 1 − (open/pollen-supplemented total seed) (Larson and Barrett 2000); where total seed = fruit set × mean seed mass per fruit. For two *Gesneria* species with significant self-pollination, an approximate measure of pollen limitation for emasculated flowers is provided as 1 − (emasculated/open), in brackets.

PLATE 1. Five Gesnerieae species representing different pollination and breeding systems. Clockwise from upper left: *Rhytidophyllum asperum*, hummingbird pollination; *Rhytidophyllum auriculatum*, generalized pollination; *Gesneria pedunculosa*, bat pollination; *Gesneria reticulata*, primarily self-pollination; *Gesneria tuberosa* (ined.), hummingbird pollination. Photo credits: S. Martén-Rodríguez.

ness of outcross pollen transfer (Darwin 1877). However, a recent literature survey documented a negative association between the number of pollinator species and the level of pollen limitation (Knight et al. 2005), probably reflecting lower pollinator service to specialized species. This study provides the first empirical evidence for an association between pollen limitation and pollination specialization within a group of closely related species. Our results support the notion that, in insular environments, inadequate visitation to specialists increases their risk of reproductive failure in contrast with their more frequently visited generalist congeners.

Pollen limitation has been related to low pollinator visitation in plant species with small population sizes (e.g., Baker et al. 2000), while other studies have not found a relationship between floral visitation and pollen limitation (e.g., Duan et al. 2007). These contrasting findings may reflect the importance of pollination effectiveness in addition to visitation frequency as determinants of the degree of pollen limitation experienced by plants (Aizen and Harder 2007). There are various reasons why visitation frequency appears to have a greater impact in the studied Gesnerieae species. First, differences in visitation among the different pollination systems are large,

ranging from an average of one visit per flower per day for hummingbird-pollinated species to 13 visits per flower per day for generalist species (Appendix A). Second, considering that average visitation can be as low as one visit per flower every three days, it is reasonable to conclude that some flowers do not receive a single visit during their receptive period (stigmas are generally receptive for two to three days in Gesnerieae). Last, generalized Gesnerieae species are visited by the same pollinators that visit specialists, in addition to other pollinator functional groups. For example, specialized *Rhytidophyllum asperum* is pollinated exclusively by hummingbirds, while generalized *R. leucomallon* is pollinated by hummingbirds, bats and moths. The same species of hummingbird, *Chlorostilbon swainsonii*, visits both *Rhytidophyllum* species in the same region of the Dominican Republic (Martén-Rodríguez et al. 2009). Since having specialized flowers is generally associated with greater precision and effectiveness of pollen transfer on a per visit basis (Wilcock and Neiland 2002), it is difficult to envision that the reduced fruit and seed set of tubular-flowered *R. asperum* would be due to lower quality pollen transfer by the Hispaniolan Emerald. However, having additional pollinators such as bats may increase both the quantity and quality of pollen received by generalists, because bats fly long distances and are likely to transfer outcross pollen. Studies that involve measures of pollinator effectiveness will be extremely useful to assess the role of pollen quality as a determinant of the frequency and magnitude of pollen limitation.

Autonomous self-pollination and pollination specialization

In a survey of the literature Fenster and Martén-Rodríguez (2007) found that mechanisms that facilitate autonomous self-pollination were found both in species with generalized (e.g., *Sanguinaria canadensis*; Schemske et al. 1978), and specialized pollination systems (e.g., *Ophrys apifera*; Darwin 1877). The prevalence of autonomous selfing in specialized species was an unexpected finding, given the long-standing notion that pollination specialization reflects selection for outcrossing (Darwin 1877, Zhang et al. 2005). However, theory predicts that autonomous self-pollination should be favored if it ensures seed set when pollinators are scarce (Lloyd 1992); thus, we expected that autonomous breeding systems would be more prevalent in specialized Gesnerieae species, which have low pollinator service. This prediction was only partially supported by the data. Significant levels of potential autonomous self-pollination were detected only in five species with highly specialized hummingbird pollination, while no autonomous selfing characterized the three species with specialized bat pollination systems (Table 1). Overall, the results demonstrate that the relationship between specialization and reproductive assurance is more complex than we predicted.

The occurrence of pollen limitation in both bat and hummingbird specialists suggests that they should be under similar selective pressures to evolve reproductive assurance mechanisms. However, a scarcity of autonomous selfing mechanisms in bat-pollinated species is also evident from literature surveys (Lloyd and Schoen 1992, Fenster and Martén-Rodríguez 2007). There are at least two reasons that might explain why only hummingbird-pollinated Gesnerieae exhibit the capacity for autonomous self-pollination. First, flower morphology facilitates contact between stigmas and anthers due to the proximity of these organs in narrow tubular corollas. Thus, accidental autonomous self-pollination could be frequent in tubular flowers, making this trait an easy target of selection. Second, the incidence of self-fertilization may be related to life history traits and growth habit (Wiens 1984, Barrett et al. 1996). For instance, high levels of inbreeding depression in long-lived woody perennials (like most bat-pollinated species) may hinder the evolution of selfing (Barrett et al. 1996). Herbaceous species generally exhibit higher frequencies of self-fertilization than woody species, which has been attributed to the greater ability of small plants with shorter life cycles to purge deleterious mutations and reduce genetic loads (Barrett et al. 1996). The few cases of autonomous selfing in bat-pollinated species documented in the literature are understory or epiphytic herbaceous plants (e.g., *Irlbachia alata* [Machado et al. 1998], *Werahuia gladioliflora* [Tschapka and von Helversen 2007]). Likewise, in our study, only the two small-sized hummingbird pollinated *Gesneria*, *G. cuneifolia* and *G. reticulata*, exhibited a high contribution of self-pollination to fruit set. Studies that compare levels of inbreeding depression between closely related species that differ in size and habit are necessary to achieve a more comprehensive understanding of the factors that underlie variation in breeding systems in Gesnerieae.

Reproductive assurance and hummingbird pollination

Our study provides evidence for the role of autonomous self-pollination as a reproductive assurance mechanism in hummingbird-pollinated Gesnerieae. However, the lack of differences in seed mass between emasculated and unmanipulated flowers reveals that, while hummingbirds are infrequent visitors, they are effective pollinators. Furthermore, the temporal variation in the contribution of autonomous selfing indicates that hummingbirds can be fully effective under certain conditions, perhaps when other floral resources are scarce or when the plant has more limited resources to develop fruit.

A number of studies conducted primarily in temperate regions have empirically demonstrated that autonomous self-pollination confers reproductive assurance when opportunities for outcrossing are low (e.g., Eckert and Schaefer 1998, Elle and Carney 2003, Kalisz 2004, Jacquemyn and Brys 2008). However, not in all cases

does self-pollination increase plant fitness; for instance, high inbreeding depression and seed discounting reduce the net advantage of reproductive assurance in *Aquilegia Canadensis* (Herlihy and Eckert 2004).

It is still unknown whether hummingbird pollinated *Gesneria* experience inbreeding depression. However, contrary to *Aquilegia*, which lacks a mechanism to temporally separate pistillate and staminate functions, most hummingbird-pollinated Gesnerieae are protogynous, and self pollination occurs mostly at the end of the flower's life. Since delayed selfing mechanisms provide opportunities for outcrossing when pollinators are present (Lloyd and Schoen 1992), seed discounting is probably less relevant in our study species.

Autonomous selfing and generalized pollination systems: An escape from pollen limitation?

An obvious disadvantage of specialization in pollination mutualisms is that the scarcity or absence of a pollinator jeopardizes the reproductive success of the plant. Thus, selection to reduce pollen limitation is likely an underlying cause for the overwhelming presence of generalized pollination systems in some pollinator depauperate environments such as islands (Carlquist 1974, Barrett 1996). Alternatively, insular plants might exhibit autonomous breeding systems that provide reproductive assurance (Baker 1955, Barrett 1996). While selection to reduce inbreeding depression favors floral phenotypes that promote outcrossing, selection for reproductive assurance might be strong in specialized island plants, particularly if pollinators are rare or inefficient. In spite of the relative geographic proximity of the Greater Antilles to the American continent, the pollinator fauna in these islands is reduced when compared to mainland regions at similar latitudes. For example, Costa Rica has 52 species of hummingbirds (Garrigues and Dean 2007) and 10 species of nectar-feeding bats (Laval and Rodríguez Herrera 2002), while Cuba—twice the size of Costa Rica—has three species of hummingbirds (Raffaele et al. 1998) and four species of nectar-feeding bats (Koopman 1981). The less diverse pollinator fauna, combined with high levels of habitat degradation in the Antilles, probably increase the risk of reproductive failure in narrow endemic plant species. In the tribe Gesnerieae approximately 60 out of 75 species are restricted to one island, and many species have restricted distribution within islands. Overall the results indicate that autonomous breeding systems in Gesnerieae safeguard against fluctuations in the composition and abundance of floral visitors and help reduce pollen limitation; thus, the presence of autonomous breeding systems is likely to contribute to the maintenance of specialized hummingbird-pollinated systems in Antillean Gesnerieaceae. Likewise, having additional species of effective pollinators appears to reduce susceptibility to pollination failure. Our study suggests that pollen limitation may be a strong selective force promoting the evolution of generalization and the evolution of

breeding mechanisms that ensure reproduction under variable pollination environments.

LITERATURE CITED

Aizen, M. A., and L. D. Harder. 2007. Expanding the limits of the pollen limitation concept: effects of pollen quantity and quality. Ecology 88:271–281.

Ashman, T. L., T. M. Knight, J. A. Steets, P. Amarasekare, M. Burd, D. R. Campbell, M. R. Dudash, M. O. Johnston, S. J. Mazer, R. J. Mitchell, M. T. Morgan, and W. G. Wilson. 2004. Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. Ecology 85:2408–2421.

Baker, A. M., S. C. H. Barrett, and J. D. Thompson. 2000. Variation in pollen limitation in the early flowering *Narcissus assoanus* (Amarylidaceae). Oecologia 124:529–535.

Baker, H. G. 1955. Self compatibility and establishment after "long distance" dispersal. Evolution 9:347–349.

Barrett, S. C. H. 1996. The reproductive biology and genetics of island plants. Philosophical Transactions Royal Society B 351:725–733.

Barrett, S. C. H., L. D. Harder, and A. C. Worley. 1996. The comparative biology of pollination and mating in flowering plants. Philosophical Transactions of the Royal Society B 351:1271–1280.

Burd, M. 1994. Bateman's principal and plant reproduction: the role of pollen limitation in fruit and seed set. Botanical Review 60:83–139.

Carlquist, S. 1974. Island biology. Columbia University Press, New York, New York, USA.

Darwin, C. 1877. The various contrivances by which orchids are fertilized by insects. Second edition. D. Appleton and Company, New York, New York, USA.

Duan, Y.-W., T.-F. Zhang, and J.-Q. Liu. 2007. Interannual fluctuations in floral longevity, pollinator visitation and pollination limitation of an alpine plant (*Gentiana straminea* Maxim., Gentianaceae) at two altitudes in the Qinghai-Tibetan Plateau. Plant Systematics and Evolution 267:255–265.

Eckert, C. G., and A. Schaefer. 1998. Does self-pollination provide reproductive assurance in *Aquilegia canadensis* (Ranunculaceae)? American Journal of Botany 85:919–924.

Elle, E., and R. Carney. 2003. Reproductive assurance varies with flower size in *Collinsia parviflora* (Scrophulariaceae). American Journal of Botany 90:888–896.

Faegri, K., and L. van der Pijl. 1978. The principles of pollination ecology. Pergamon, Oxford, UK.

Fenster, C. B., W. S. Armbruster, J. D. Thomson, P. Wilson, and M. R. Dudash. 2004. Pollination syndromes and floral specialization. Annual Review of Ecology, Evolution and Systematics 35:375–403.

Fenster, C. B., and S. Martén-Rodríguez. 2007. Reproductive assurance and the evolution of pollination specialization. International Journal of Plant Sciences 168:215–228.

Fishman, L., and J. H. Willis. 2008. Pollen limitation and natural selection on floral characters in the yellow monkey-flower, *Mimulus guttatus*. New Phytologist 177:802–810.

Garrigues, R., and R. Dean. 2007. Birds of Costa Rica. Cornell University Press, Ithaca, New York, USA.

Hansen, V. I., and O. Totland. 2006. Pollinator visitation, pollen limitation and selection on flower size through female function in contrasting habitats within a population of *Campanula persicifolia*. Canadian Journal of Botany 84:412–420.

Herlihy, C. R., and C. G. Eckert. 2004. Experimental dissection of inbreeding and its adaptive significance in a flowering plant, *Aquilegia canadensis* (Ranunculaceae). Evolution 58:2693–2703.

Jacquemyn, H., and R. Brys. 2008. Density-dependent mating and reproductive assurance in the temperate forest herb *Paris quadrifolia* (Trilliaceae). American Journal of Botany 95:294–298.

Kalisz, S. 2004. Context-dependent autonomous self-fertilization yields reproductive assurance and mixed mating. Nature 430:884–887.

Kearns, C., and D. W. Inouye. 1993. Techniques for pollination biologists. University Press of Colorado, Niwot, Colorado, USA.

Knight, T. M., J. A. Steets, J. C. Vamosi, S. J. Mazer, M. Burd, D. R. Campbell, M. R. Dudash, M. O. Johnston, R. J. Mitchell, and T. Ashman. 2005. Pollen limitation of plant reproduction: pattern and process. Annual Review of Ecology and Systematics 36:467–497.

Koopman, K. F. 1981. The distributional patterns of New World nectar-feeding bats. Annals of the Missouri Botanical Garden 68:352–369.

Larson, B. M. H., and S. C. H. Barrett. 2000. A comparative analysis of pollen limitation in flowering plants. Biological Journal of the Linnean Society 69:503–520.

Laval, R., and B. Rodríguez Herrera. 2002. Costa Rican bats. INBIO, Santa Domingo de Heredia, Costa Rica.

Lloyd, D. G. 1979. Some reproductive factors affecting the selection of self-fertilization in plants. American Naturalist 113:67–79.

Lloyd, D. G. 1992. Self- and cross-fertilization in plants. II. The selection of self-fertilization. International Journal of Plant Sciences 153:370–380.

Lloyd, D. G., and D. J. Schoen. 1992. Self- and cross-fertilization in plants. I. Functional dimensions. International Journal of Plant Sciences 153:358–369.

Machado, I. C. S., I. Sazima, and M. Sazima. 1998. Bat pollination of the terrestrial herb *Irlbachia alata* (Gentianaceae) in northeastern Brazil. Plant Systematics and Evolution 209:231–237.

Martén-Rodríguez, S. 2008. Evolution of pollination and breeding systems of Antillean Gesneriaceae. Dissertation. University of Maryland, College Park, Maryland, USA.

Martén-Rodríguez, S., A. Almarales-Castro, and C. B. Fenster. 2009. An evaluation of pollination syndromes in Caribbean Gesneriaceae: evidence for hummingbird, bat and generalized flowers. Journal of Ecology 97:348–359.

Martén-Rodríguez, S., and C. B. Fenster. 2008. Pollination ecology and breeding systems of five *Gesneria* species from Puerto Rico. Annals of Botany 102:23–30.

Merrett, M. F., A. W. Robertson, and P. G. Peterson. 2007. Pollination performance and vulnerability to pollination breakdown of sixteen native shrub species from New Zealand. New Zealand Journal of Botany 45:579–591.

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

SAS Institute. 2005. SAS for Windows, version 9.1.3. SAS Institute, Cary, North Carolina, USA.

Schemske, D. W., M. F. Willson, M. N. Melampy, L. J. Miller, L. Verner, K. Schemske, and L. B. Best. 1978. Flowering ecology of some spring woodland herbs. Ecology 59:351–366.

Skog, L. 1976. A study of the tribe Gesnerieae with a revision of *Gesneria* (Gesneriaceae: Gesnerioideae). Smithsonian Contributions to Botany 29:1–182.

Stebbins, G. L. 1970. Adaptive radiation of reproductive characteristics in angiosperms. Pollination mechanisms. Annual Review of Ecology and Systematics 1:307–326.

Tschapka, M., and O. von Helversen. 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.

Waser, N. M., L. Chittka, M. V. Price, N. M. Williams, and J. Ollerton. 1996. Generalization in pollination systems, and why it matters. Ecology 77:1043–60.

Wiens, D. 1984. Ovule survivorship, brood size, life history, breeding systems, and reproductive success in plants. Oecologia 64:47–53.

Wilcock, C., and R. Neiland. 2002. Pollination failure in plants: why it happens and when it matters. Trends in Plant Science 7:270–277.

Zhang, L., S. C. H. Barrett, J.-Y. Gao, J. Chen, W. W. Cole, Y. Liu, Z. L. Bai, and Q.-J. Li. 2005. Predicting mating patterns from pollination syndromes: The case of "sapromyophily" in *Tacca chantrieri* (Taccaceae). American Journal of Botany 92:517–524.

Zimmer, E. A., E. H. Roalson, L. E. Skog, J. K. Boggan, and A. Idnurm. 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.

APPENDIX A

Growth habit and floral characteristics and pollinator visitation frequency for 13 species of *Gesneria* and *Rhytidophyllum* from Puerto Rico and the Dominican Republic (*Ecological Archives* E091-014-A1).

APPENDIX B

Sites and geographic coordinates of studied populations of 13 *Gesneria* and *Rhytidophyllum* surveyed in Puerto Rico and the Dominican Republic in 2006 and 2007 (*Ecological Archives* E091-014-A2).