

Evaluation of pollination syndromes in Antillean Gesneriaceae: evidence for bat, hummingbird and generalized flowers

Silvana Martén-Rodríguez^{1,2*}, Abel Almarales-Castro³ and Charles B. Fenster^{1,4}

¹Behavior, Ecology, Evolution and Systematics Program, University of Maryland, College Park, MD 20742, USA;

²Herbario de la Universidad de Costa Rica, Escuela de Biología, Ciudad Universitaria Rodrigo Facio Brenes, San José, Costa Rica; ³Centro Oriental de Ecosistemas y Biodiversidad (BIOECO), Museo de Historia Natural Tomás Romay, Enramadas No. 601, Esquina Barnada, Santiago, Cuba; and ⁴Biology Department, University of Maryland, College Park, MD 20742, USA

Summary

1. Current views about the predominance of generalization of pollination systems have stimulated controversy concerning the validity of pollination syndromes. In order to assess the extent to which floral characters reflect selection by the most important pollinators we evaluated pollination syndromes in a florally diverse plant group, the tribe Gesnerieae, a monophyletic plant radiation from the Antillean islands.
2. The study species include representatives of three groups of floral phenotypes, two of which chiefly correspond to ornithophilous and chiropterophilous syndromes. The third group includes subcampanulate flowers (characterized by a corolla constriction above the nectar chamber) with combinations of traits not fitting classic pollination syndromes.
3. Pollination systems were characterized for 19 Gesnerieae species in five Antillean islands between 2003 and 2007 and supplemented with observations of four Gesneriaceae species from Costa Rica. Pollinator visitation and frequency of contact with anthers or stigmas were used to calculate an index of pollinator importance. Eleven floral traits including morphology, phenology and rewards were used to assess clustering patterns in phenotype space.
4. Multidimensional scaling analysis of floral traits resulted in two clusters comprising: (i) tubular, red to yellow-flowered species with diurnal anthesis, (ii) bell-shaped-flowered species; two groups of floral phenotypes were evident within the latter cluster, campanulate nocturnal and subcampanulate flowers. Correlations between pollinator importance values and floral axes revealed strong associations with the expected pollinators, hummingbirds for tubular flowers, and bats for campanulate flowers; subcampanulate-flowered species had generalized pollination systems including bats, hummingbirds and insects. Discriminant analysis of the multivariate set of floral traits correctly classified 19 out of 23 species into the predicted pollination categories.
5. *Synthesis.* This study provides support for classic hummingbird and bat pollination syndromes, demonstrating the importance of pollinator-mediated selection in the floral diversification of Antillean Gesnerieae. However, there was evidence for generalized pollination systems in species characterized by a unique morphological trait (corolla constriction), but with variable combinations of other floral traits. These findings suggests that floral phenotypes might also evolve under selection by various functional groups of pollinators, and underscores the importance of considering the presence and effectiveness of all floral visitors in pollination studies.

Key-words: Antilles, bat pollination, Costa Rica, generalization, Gesneriaceae, hummingbird pollination, islands, pollination syndromes, specialization

*Correspondence author. E-mail: smartenr@gmail.com

Introduction

Closely related plant species often display great variation in flower form and function. Darwin (1862) proposed that the evolution of this floral diversity reflected pollinator-mediated selection. Patterns of convergence of floral phenotypes across the angiosperms provide strong support for Darwin's paradigm and suggest that suites of floral characteristics have evolved in association with particular groups of pollinators. These floral phenotypes are known as 'pollination syndromes' (Faegri & van der Pijl 1978), and they comprise morphological as well as biochemical (e.g. composition of attractants and rewards, Baker & Baker 1990) and phenological traits (e.g. patterns of anther dehiscence, Castellanos *et al.* 2006). For example, large bell-shaped flowers that produce large quantities of dilute nectar and shed pollen at night tend to be associated with bat pollination, whereas tubular red flowers with diurnal schedules are commonly associated with pollination by birds. Syndromes therefore imply that flowers have become specialized for pollination by specific groups of floral visitors, that is, floral traits have evolved to increase pollen transfer by the most effective visitors and to deter antagonistic visitors (Stebbins 1970; Faegri & van der Pijl 1978; Fenster *et al.* 2004).

During the past decade, however, the notion that pollination specialization underlies the observed patterns of floral convergence has been debated (Waser *et al.* 1996; Fenster *et al.* 2004). Community and taxon surveys predominantly from temperate regions reveal that many flowers have generalized visitation patterns (e.g. Robertson 1928; Lindsey 1984; Herrera 1996; Olesen *et al.* 2007), pollinator communities vary in time and space (e.g. Herrera 1995; Fenster & Dudash 2001; Horovitz & Schemske 2002), and animals often use floral resources from different plant species (e.g. Herrera 1996). Furthermore, syndromes do not predict all floral visitors, and flowers that conform to particular syndromes are sometimes pollinated by animals that do not fit the expectations (Ollerton *et al.* 2007). These observations have led some authors to question the validity of the pollination syndrome concept (Waser *et al.* 1996; Ollerton *et al.* 2007). While syndromes were not originally meant to be used as substitutes for field observations, there is a valid concern regarding the use of floral traits as predictors of the pollinators, particularly when biased or no field data have been collected (Feinsinger 1987; Waser *et al.* 1996). Obtaining impartial characterizations of pollinators at the level of communities or higher order plant taxa, particularly from understudied tropical regions, is critical to solving the apparent disagreement between observed evolutionary patterns of floral specialization and the patterns suggested by field ecology (Johnson & Steiner 2000). Furthermore, despite the clear difficulties involved in obtaining direct measures of pollinator efficiency, an attempt should be made at distinguishing between potential pollinators and non-pollinating floral visitors. This approach should lead us to a more comprehensive understanding of the different selective agents that have influenced the great floral diversification observed in some plant taxonomic groups.

Pollination studies of closely related insular species could provide important insights into the selective pressures that

underlie patterns of floral convergence. We studied the Antillean tribe Gesnerieae to determine to what extent floral phenotypes defined by morphology, phenology and rewards are explained by the pollinators. This group of plants provides an excellent study system for various reasons. First, the tribe Gesnerieae is a monophyletic radiation from the Antilles that consists of 73 species encompassing considerable floral diversity (Skog 1976; Smith 1996; Zimmer *et al.* 2002). Second, the tribe comprises floral phenotypes that have been traditionally associated with hummingbird and bat pollination, but previous to our studies, no field data were available to support these predictions. Furthermore, despite the great floral diversity displayed by Neotropical Gesneriaceae, only a limited number of studies have documented pollinators in the field (e.g. Podolsky 1992; Sazima *et al.* 1996; Lara & Ornelas 2002; Carlson 2008), and only for the tribe Sinningieae in Brazil has there been a systematic assessment of pollinators in a group of closely related species (Sanmartin-Gajardo & Sazima 2004, 2005a,b). Third, oceanic archipelagos provide unique conditions of natural selection and opportunities for evolutionary change that may differ from mainland regions. And last, our preliminary phylogenies indicate at least five independent origins of bell-shaped corollas that differ from the tubular corollas of the ancestral phenotype; this suggests pollinators played a significant role in the floral diversification of the clade. We included four species from three additional tribes of the family Gesneriaceae from Costa Rica to obtain phylogenetically independent evidence from mainland taxa. The selected species fall into three general classes of floral phenotypes corresponding to ornithophilous and chiropterophilous syndromes, and a class of more variable phenotypes that do not clearly match classic syndrome predictions (Fig. 1).

For this study, we specifically address the following questions: (i) what are the pollination systems of Gesneriaceae species representative of the different floral phenotypes?; (ii) when floral traits are used to search for patterns in multivariate space, is there evidence for discontinuous associations of species corresponding to traditional pollination syndromes?; (iii) which floral traits contribute most to distinguishing the floral associations defined in multivariate space?; and (iv) are pollination syndromes good predictors of the floral visitors for Antillean Gesneriaceae? We evaluate the predictions that tubular flowers in the Gesnerieae are primarily pollinated by hummingbirds, and bell-shaped (campanulate and subcampanulate) nocturnal flowers are primarily pollinated by bats. We also provide the first descriptions of pollinators for *Rhytidophyllum* species with mixed floral traits not fitting classic pollination syndrome categories.

Methods

STUDY SITES

Pollinator observations and floral biology studies were conducted in Costa Rica (February–March 2007), Cuba (September 2007–February 2008), the Dominican Republic (June–August 2004–2007), Jamaica



Fig. 1. Three predominant floral phenotypes in Antillean Gesneriaceae. Photos A–C correspond to *Gesneria fruticosa*, *G. viridiflora* subsp. *sintensisii* and *G. pedunculosa*, representing the bat-pollination syndrome (green or white bell-shaped flowers with nocturnal anthesis and high nocturnal nectar production). Photos D–G correspond to *Columnnea quercetii*, *Gesneria citrina*, *G. decapleura* and *G. pulverulenta* representing the hummingbird-pollination syndrome (tubular red or yellow corollas with diurnal anthesis and nectar production). Photos H–I correspond to *Rhytidophyllum leucomallon*, *R. vernicosum*, with mixed traits of diurnal and nocturnal-pollination syndromes (yellow to spotted red bell-shaped flowers with nocturnal and/or diurnal anthesis and nectar production). Corolla constriction indicated by white arrow.

(January 2004) and St. Lucia (June 2003). *Rhytidophyllum minus* was observed at Castillo San Pedro de la Roca located on coastal limestone cliffs, south of the city of Santiago, in western Cuba. In the Dominican Republic, plants were observed at various sites of Cordillera Central, Cordillera Septentrional, Parque Nacional Sierra de Bahoruco and Sierra Neiba. All of these sites are located in mountain regions between 300 and 2000 m. Most species occur in limestone soils but they occupy a diversity of habitats including pine forests, moist and cloud forests, and roadsides. In Jamaica, *Gesneria calycosa* plants were observed in the forest surrounding Windsor Biological station in the NW side of the island (a.k.a. cockpit country) and *Pheidonocarpa corymbosa* at Cane River Falls in the foothills of the Blue Mountains. In St. Lucia, observations of *G. ventricosa* were conducted in Edmund Forest along the road to En Vasseux Waterfall, 500 m. Specific localities and geographic coordinates for the study sites for each species are listed in Appendix S1 (Supporting Information).

To obtain phylogenetically independent samples, we also observed four species of Gesneriaceae from three different tribes (Beslerieae, Gloxinieae and Episcieae, according to Zimmer *et al.* 2002) that occur in Costa Rica. Observations for these species were conducted in the forest of the Biological Station in Monteverde (for *Besleria solanoides*) and in the rainforest of San Gerardo Biological Station (for *Capanea grandiflora*, *Columnnea consanguinea* and *C. quercetii*); these sites are located on the western and eastern slopes of the Tilarán Mountain range, respectively. We also used data from a detailed study of the pollination biology of five Puerto Rican *Gesneria* performed in January and March 2003–2007 in two regions of the island (Martén-Rodríguez & Fenster 2008).

POLLINATOR VISITATION AND IMPORTANCE

To document pollinator visitation we conducted field observations on 23 species of Gesneriaceae for a total of 602 h. Detailed descriptions of the methodology and floral biology of five Puerto Rican species of *Gesneria* are reported elsewhere (Martén-Rodríguez & Fenster 2008); thus, we only briefly describe the methods for pollinator observations here. The total number of individuals observed per species ranged from 80 to 60, depending on the population size and density of each species. The number of study years varied from one to three, but for each floral phenotype at least three species were observed for more than 1 year. For most species we made both direct observations and observations with video cameras (SONY Handycam DCR-HC42 and DCR-TRV350); the observer or the camera stood 2–5 m from the focal plant and recorded the time of visitation, type of visitor (e.g. bird species, bat, moth, and diurnal insect order or family), contact with the flower's reproductive organs, and the number of flowers visited. Both diurnal and nocturnal observations were conducted for most species. For species with nocturnal and diurnal visitors, approximately half to three quarters of the time reported was dedicated to nocturnal observation. The larger time effort put into night observations was necessary to compensate for the limited number of flowers that video cameras could be focused on at night (one to four), as opposed to the ability to conduct direct observation on patches of flowers during the day.

We classified pollinators into 'functional groups', defined on the basis of taxonomic affinity and similarity in feeding behaviour. Functional groups are expected to represent sets of animal taxa that exert similar selective pressures on floral traits, because they share

similar feeding behaviours, physiology and morphology (Fenster *et al.* 2004). For this study, the taxonomic classes of floral visitors differed primarily in feeding schedule (active at flowers during day or night), reward sought (pollen or nectar) and behaviour while feeding (e.g. hovering vs. perching). Thus, the functional groups of pollinators that visited Gesneriaceae species include hummingbirds, bats, moths and diurnal insects (small bees and flies that visited flowers primarily for pollen). For each year, pollinator visitation rates by each functional group were calculated as the number of visits per flower per hour; this rate was multiplied by 12 to obtain visitation frequencies per day or night, according to the schedule of the pollinator. At the latitudes where the study was conducted, daylight hours range between 12 and 13; thus, for the sake of consistency, we calculated visitation for 12-h days.

To distinguish non-pollinating floral visitors from animals that have the ability to transfer pollen we carefully observed visitor behaviour and frequency of contact with the reproductive organs. Whenever possible we observed virgin flowers and checked them after a visit to determine whether pollen had been removed from anthers or deposited onto stigmas. However, since these data were collected only for a subset of the flowers, we quantified efficiency as the number of times the visitor contacted stigmas or anthers divided by the total number of visits (Armbruster & Herzig 1984). We recognize that contact is an approximate measure of efficiency but due to the logistical difficulties of obtaining pollen removal and deposition data for a large group of species, we consider this approach provides a better characterization of the pollination system than a simple list of floral visitors. Pollinator importance values for each group of visitors were calculated as the product of visitation and efficiency. To obtain a comparable index of pollinator importance we standardized each value, dividing it by the sum of importance values across all functional groups of pollinators. Therefore, pollinator importance indices range from 0 to 1. We report mean pollinator visitation and range across years for species observed for more than 1 year. Importance values obtained from 1 year of sampling, particularly those of bats and infrequent insect visitors, may not be accurate estimators. However, we have a representative sample of species (including the principal floral phenotypes) that were observed for many hours in multiple years. Since these results are mostly consistent across the data set, we considered it appropriate to include the understudied species. We excluded visitors that were never observed contacting the reproductive organs or carrying pollen (e.g. grasshoppers, beetles) and the introduced honeybee *Apis mellifera*, since it is unlikely this species has been long enough in the New World (a few hundred years) to be responsible for evolutionary changes underlying the floral diversification of the tribe Gesneriaceae.

MEASUREMENTS OF FLORAL TRAITS

To characterize floral phenotypes we measured 11 floral characters from two to three flowers of 7–23 individuals per species. Flowers were collected from all plants available when population densities were low (< 20 individuals); otherwise, flowers were collected from a sample of the population. Flower measurements of fresh flowers included: (i) corolla length, the shortest length of the corolla tube; (ii) pistil exertion, measured in pistillate-phased flowers as the difference between pistil length and corolla length; (iii) diameter of the corolla opening; (iv) corolla constriction, coded as present or absent; (v) corolla curvature, taken with a protractor for curvature of the dorsal side of the corolla tube; (vi) nectar concentration (see below); (vii) symmetry, coded as (0) subactinomorphic (reproductive organs not symmetrically positioned, otherwise actinomorphic) or (1) zygomorphic; (viii) timing of anther dehiscence, coded as (0) nocturnal (18.00–06.00), (2) diurnal

(06.00–18.00), or (1) both; (ix) timing of nectar production, same coding as trait eight; (x) colour, coded as (1) green, (2) yellow, (3) orange or (4) red; (xi) spots: coded for (0) presence or (1) absence of dark red or brown markings on the inside of the corolla. Measurements of length, width and pistil exertion were taken with calipers and rounded to the nearest 0.1 mm. Floral measurements for most species were taken by one person (S. Martén-Rodríguez), except for measurements for *R. minus*, which were taken by A. Almarales-Castro; all measurements are listed in Appendix S2.

To document the timing of anthesis and nectar production, two species of each floral phenotype were selected (nocturnal campanulate and subcampanulate: *G. fruticosa*, *G. quisqueyana*; tubular: *G. pedicellaris*, *R. asperum*; subcampanulate mixed traits: *R. leucomallon*, *R. vernicosum*). We also used data for five Puerto Rican *Gesneria* previously studied (Martén-Rodríguez & Fenster 2008). Flower buds of one to two flowers per plant, in five to 14 plants per species were bagged and checked every 3 h for a continuous 24-h period starting at 15.00; the earliest time at which anther slits were noticed open was recorded. Nectar was extracted from bagged first-day flowers using capillary tubes or with a 50- μ L Hamilton Syringe (Hamilton, NV, USA), and sugar concentration was measured with a hand-held refractometer (Sugar/Brix Refractometer, 0–32% w/ATC, Sper Scientific, Scottsdale, AZ). For the remaining species, plants were checked at least four times over the course of 24 h, such that we could tell whether pollen shedding occurred at night (18.00–06.00) or day (06.00–18.00). Nectar concentration on these plants was measured in flowers that were not previously bagged. Nectar production coded as nocturnal started as early as 15.00 and generally stopped by 07.00; nectar production coded as diurnal started as early as 04.00 and stopped at different times of the day, depending on the species. For anther dehiscence, nocturnal schedules were generally from 18.00 to 20.00 while diurnal anther dehiscence started as early as 05.00. Nectar volume was not included because this measurement required bagging flowers and sample sizes were insufficient to obtain reliable measurements for almost half of the species. In order to identify scent production, first-day flowers of at least two species of each floral phenotype (nine species total) were left in glass containers for 2–3 h and then checked by smelling them. No perceivable scent was detected for any of these species; thus, this trait was not included in the analysis.

STATISTICAL ANALYSES

All analyses were performed in SAS version 9.1.3 (SAS Institute 2004). We used multidimensional scaling to examine patterns of association among species with floral characters traditionally linked with pollination syndromes. Eleven floral characters described above were used to calculate dissimilarity matrices using the DISTANCE procedure with method = dgorwer specified to calculate distances based on Gower's coefficient; this coefficient allows the use of nominal and different kinds of quantitative variables (Gower 1971). The MDS procedure using the ordinal level option was used to indicate non-metric ordinations. Two ordinations using floral characters were performed: one that included all species and one that excluded species with tubular flowers. The latter analysis was conducted to evaluate floral characters that might help discriminate between bat-pollinated and generalist species. Multidimensional scaling by pollinator visitation and importance values was also performed to compare the grouping patterns produced by floral characters with the groupings suggested by the pollinators. The patterns using visitation data were the same as those using importance values; therefore, we report only the latter below.

The sample of species used in the floral ordination was not phylogenetically corrected; therefore, some similarities among species may reflect common ancestry. Being aware of this problem, we attempted to ensure a higher level of independence by including, for both chiropterophilous and ornithophilous flowers, species from at least two clades within the Antillean tribe Gesnerieae (Martén-Rodríguez *et al.* unpublished data) and four Costa Rican species from three additional tribes (Zimmer *et al.* 2002). Therefore, we can be confident that the results reported below reflect more than taxonomic affinities.

To evaluate the association between floral characters or pollinator importance with the first two dimensions of the ordination, we conducted Spearman rank correlations. Correlation coefficients are reported significant at the $P = 0.05$ level after sequential Bonferroni correction to adjust for multiple comparisons. Likewise, Spearman rank correlations were used to assess the degree of association among floral characters.

To evaluate the predictability of pollination syndromes in Antillean Gesneriaceae, we conducted non-parametric discriminant analysis using the first two dimensions of the floral ordination (all species included) to represent the suite of floral traits. We used the DISCRIM procedure in SAS, specifying the 'kernel normal' option to allow for a nonlinear discriminant search. We made no assumptions about the underlying multivariate distribution and used a non-pooled covariance matrix. *A priori* groupings were based on the observed pollination systems (i.e. hummingbird specialists, bat specialists and generalists). To classify species as generalist or specialist we used our pollinator importance index, which combines visitation frequency and frequency of contact with the reproductive organs. Fenster *et al.* (2004) used a cut-off of a 75% visitation frequency for the most abundant pollinator to determine their categories of specialization and generalization. Here, we utilized a natural break in the data near that cut-off point and classified species as generalists if the importance index of the most important pollinator was 76% or below, and as specialists if the index was 77% or higher. The lowest most important pollinator index for species classified as specialists was 84% (for *G. pedunculosa*).

Results

POLLINATOR VISITATION AND IMPORTANCE

A list of the floral visitors observed in all Costa Rican and Antillean Gesneriaceae is provided in Appendix S3. Pollinators of tubular-flowered species were almost exclusively hummingbirds, usually one or two hummingbird species. Visitation rates by hummingbirds to Gesneriaceae from the Antillean islands ranged from one visit per flower every three days to two visits per flower per day; visitation rates to Costa Rican Gesneriaceae ranged between three and six visits per flower per day (Table 1). Hummingbird pollinator importance values between 0.96 and 1.00; other visitors included Halictid bees and butterflies, with low importance values (0.03–0.04). We found little temporal variation in visitation rates and pollinator importance values for species that were observed in multiple years (Table 1).

Gesneriaceae species with campanulate green or white flowers were primarily pollinated by bats; bird and insect visitors, when present, had low importance values (Table 1). Visitation rates by bats ranged between one and four visits per flower per night and importance values between 0.80 and 1.00 (Table 1). As reported in a previous study, subcampanulate *G. viridiflora* subsp. *sintensisii* from Puerto Rico was pollinated

both by bats and hummingbirds. Pollen is available for pollen transfer by hummingbirds in late afternoon (second-day flowers), and at dawn (unvisited third-day flowers); therefore we consider this species an ecological generalist despite its mostly nocturnal pollination syndrome (Martén-Rodríguez & Fenster 2008). In contrast, *G. quisqueyana* from the Dominican Republic and sister to *G. viridiflora* subsp. *sintensisii*, restricts access to diurnal visitors by an active exclusion mechanism. The flowers of *G. quisqueyana* are protogynous; however, unlike its bat-pollinated relative, which has mid-afternoon anthesis, flowers of *G. quisqueyana* open between 19.00 and 20.00 and the pistillate phase lasts only one night. Corollas close up completely the next morning between 06.00 and 07.00 h and open the second and last night in male phase; receptive stigmas are not exposed during the day.

Generalized pollination systems were characteristic of *Rhytidophyllum* species with subcampanulate corollas and mixed combinations of other floral traits. The two-day protogynous flowers were visited by different sets of animal taxa, including bats, hummingbirds, moths and small diurnal insects (Halictid bees and flies). All these animals contacted stigmas and anthers at least occasionally, but differences in efficiency among visitors may be considerable. Bat and hummingbird visits often result in pollen removal and deposition (checked on virgin flowers after one visit), and large pollen loads deposited on foreheads or bills. In contrast, most insect visitors carry little pollen. Overall visitation to generalist flowers ranged from 3 to 26 visits per flower per day (Table 1).

ORDINATIONS BY FLORAL TRAITS AND POLLINATOR IMPORTANCE

Two distinct clusters separate along dimension 1 of the floral ordination, corresponding to tubular and bell-shaped flowers (both campanulate and subcampanulate) (Fig. 2). The cluster of tubular flowers includes species from various clades (Zimmer *et al.* 2002, Martén-Rodríguez *et al.* unpublished data), and are all strictly hummingbird-pollinated. Within the cluster of species with bell-shaped flowers, two subgroups can be distinguished, one associated with bat pollination (above the zero value of dimension 2), and the other associated with generalized pollination (mostly below the zero value).

Most floral characters were highly correlated with the first dimension of the ordination plot. The correlations indicate that, moving towards the left side of the plot along dimension 1, flowers have wider corollas, some constriction above the nectar chamber, lower nectar concentration, nocturnal schedules of nectar production and anther dehiscence, colours towards the yellow/green part of the spectrum, and the presence of dark red spots (Fig. 2). In contrast, moving to the right side along dimension 1, the trend is for tubular corollas with solid bright colours, greater nectar concentration, and diurnal nectar production and anther dehiscence (i.e. hummingbird pollination syndrome). For the colour trait, which was coded as a multi-state character, the coding was set to reflect the colour spectrum; therefore, moving to the right along the dimension 1 indicates more orange and red corollas.

Table 1. Pollination system, visitation frequencies and pollinator importance values recorded for 23 species of Gesneriaceae from the Antillean islands (19 species) and Costa Rica (4 species). Pollinator visitation was calculated as the number of visits per flower per day. Mean values over the number of study years are presented followed by the range across years in brackets. Pollinator importance was calculated as the product of proportional visitation rates and a proxy for efficiency (proportion of contacts with anthers and stigmas). Importance values were scaled to proportions. Number of years and hours of observation are provided in the last column

Species	Locality	Floral phenotype	Pollinator	Pollinator visitation mean [range]	Scaled Importance mean [range]	Number of years [Number of hours]
<i>Besleria solanoides</i>	Costa Rica	Tubular	Hummingbird	3.0	0.97	1 [19]
			Butterfly	0.1	0.03	
<i>Capanea grandiflora</i>	Costa Rica	Campanulate	Bat	1.7	1.00	1 [43]
<i>Columnnea consanguinea</i>	Costa Rica	Tubular	Hummingbird	4.2	1.00	1 [13]
<i>Columnnea quercetii</i>	Costa Rica	Tubular	Hummingbird	6.4	1.00	1 [14]
<i>Gesneria acaulis</i>	Jamaica	Tubular	Hummingbird	0.3	1.00	1 [6]
<i>Gesneria calycosa</i>	Jamaica	Campanulate	Bat	0.9	1.00	1 [7]
<i>Gesneria citrina</i>	Puerto Rico	Tubular	Hummingbird	0.4 [0.3–0.5]	1.00	3 [67]
<i>Gesneria cuneifolia</i>	Puerto Rico	Tubular	Hummingbird	0.5 [0.4–0.6]	1.00	3 [51]
<i>Gesneria fruticosa</i>	Hispaniola	Campanulate	Bat	1.2 [0.9–1.6]	1.00	2 [21]
<i>Gesneria pedicellaris</i>	Hispaniola	Tubular	Hummingbird	0.4 [0.3–0.5]	1.00	2 [14]
<i>Gesneria pedunculosa</i>	Puerto Rico	Campanulate	Bat	3.9 [3.7–4.2]	0.84 [0.80–0.91]	3 [90]
			Bananaquit	1.0 [0.7–1.6]	0.09 [0.08–0.09]	
			Diurnal insects	0.6 [0.0–0.9]	0.07 [0.00–0.14]	
			Hummingbird	2.2 [1.7–2.7]	1.00	
<i>Gesneria pulverulenta</i>	Hispaniola	Tubular	Hummingbird	2.2 [1.7–2.7]	1.00	2 [12]
<i>Gesneria quisqueyana</i>	Hispaniola	Subcampanulate	Bat	1.5 [0.9–2.1]	0.95 [0.90–1.00]	2 [24]
			Hummingbird	0.05 [0.0–0.1]	0.05 [0.00–0.10]	
			Hummingbird	0.1 [0.0–0.2]	1.00	
<i>Gesneria reticulata</i>	Puerto Rico	Tubular	Hummingbird	0.1 [0.0–0.2]	1.00	3 [41]
<i>Gesneria ventricosa</i>	St. Lucia	Tubular	Hummingbird	0.5	1.00	1 [7]
<i>Gesneria viridiflora subsp. sintensisii</i>	Puerto Rico	Subcampanulate	Bat	2.5 [1.3–3.6]	0.52 [0.32–0.72]	2 [48]
			Hummingbird	3.0 [2.5–3.5]	0.42 [0.28–0.57]	
			Moth	3.3 [3.0–3.6]	0.06 [0.00–0.11]	
			Hummingbird	2.0	1.00	
<i>Pheidonocarpa corymbosa</i>	Jamaica	Tubular	Hummingbird	2.0	1.00	1 [6]
<i>Rhytidophyllum asperum</i>	Hispaniola	Tubular	Hummingbird	1.9 [1.5–2.1]	0.96 [0.93–1.00]	3 [26]
			Diurnal insects	0.1 [0.0–0.2]	0.04 [0.00–0.07]	
			Bat	0.0 [0.0–1.2]	0.20 [0.00–0.44]	
<i>Rhytidophyllum auriculatum</i>	Puerto Rico	Subcampanulate yellow/red	Bananaquit	[0.0–0.5]	0.10 [0.00–0.20]	2 [20]
			Hummingbird	2.5 [1.7–3.4]	0.70 [0.56–0.80]	
			Hummingbird	3.0	0.27	
<i>Rhytidophyllum grandiflorum</i>	Hispaniola	Subcampanulate yellow/red	Moth	13.7	0.23	1 [13]
			Diurnal insects	9.7	0.50	
			Bat	2.4 [0.0–4.7]	0.24 [0.00–0.48]	
			Hummingbird	8.1 [6.0–11.9]	0.65 [0.44–0.86]	
<i>Rhytidophyllum leucomallon</i>	Hispaniola	Subcampanulate yellow	Moth	4.1 [1.3–6.9]	0.07 [0.03–0.12]	2 [25]
			Diurnal insects	0.3 [0.3–0.4]	0.04 [0.03–0.04]	
			Hummingbird	4.7	1.00	
			Hummingbird	8.7 [7.0–10.4]	0.76 [0.75–0.77]	
<i>Rhytidophyllum minus</i>	Eastern Cuba	Subcampanulate yellow	Hummingbird	4.7	1.00	1 [39]
<i>Rhytidophyllum vernicosum</i>	Hispaniola	Subcampanulate yellow/red	Hummingbird	8.7 [7.0–10.4]	0.76 [0.75–0.77]	2 [28]
			Moth	3.3 [1.5–5.0]	0.14 [0.09–0.19]	
			Diurnal insects	1.1 [0.5–1.7]	0.10 [0.04–0.16]	

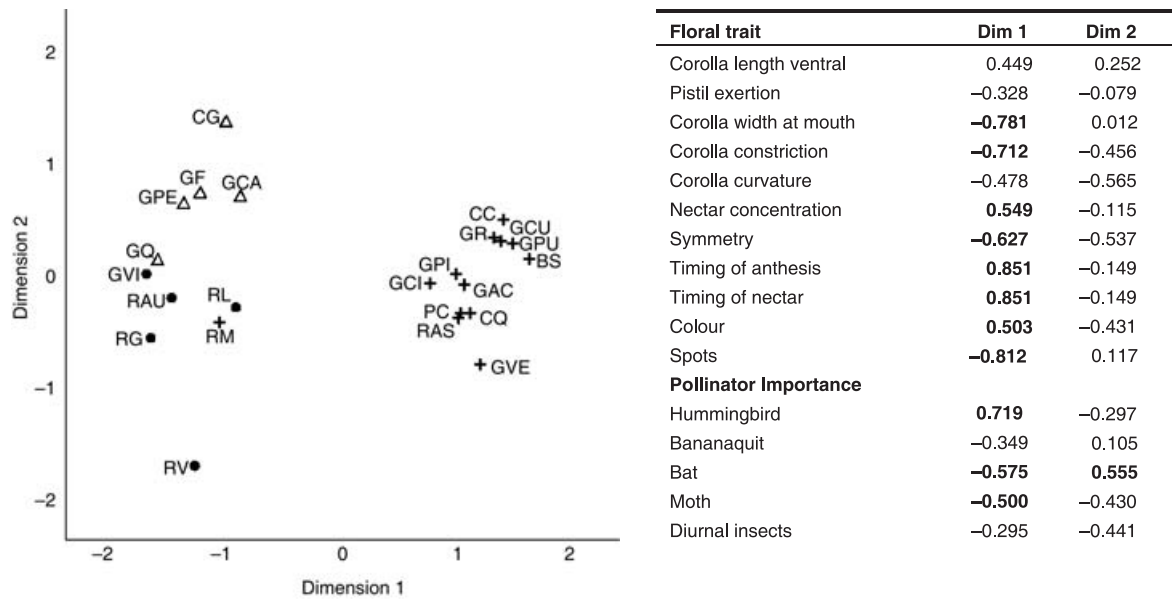


Fig. 2. Multidimensional scaling analysis of 23 Gesneriaceae species based on 11 floral characters. Triangles represent species that specialize on bat pollination, plus signs represent species that were exclusively hummingbird-pollinated and dots represent species with mixed hummingbird and nocturnal pollination (bats and/or moths). Spearman correlation coefficients are listed for associations of dimensions 1 and 2, with floral traits and with pollinator importance values. Coefficients in bold indicate significant correlations following sequential Bonferroni adjustment ($P < 0.05$).

The ordination conducted excluding tubular-flowered species shows a stronger separation of the two subgroups of bell-shaped flowers; however, two oddities are evident: *R. minus* (RM) appears clustered within the generalists but only hummingbirds were observed as native pollinators. Given its nocturnal schedule of nectar production and anther dehiscence, we cannot rule out the possibility of bat pollination until observations in multiple seasons are conducted. The second inconsistent case is *G. quisqueyana* (GQ), a strict bat specialist that was placed within the generalists cluster. This species restricts diurnal visitors by closing flowers during the day (see above description). Thus, although the floral morphology would allow a wider range of visitors, the floral phenology filters out diurnal visitors.

With tubular-flowered species excluded from the ordination, the corolla constriction became the single most important trait separating the two subgroups of bell-shaped flowers (associated with generalized and bat pollination) (Fig. 3).

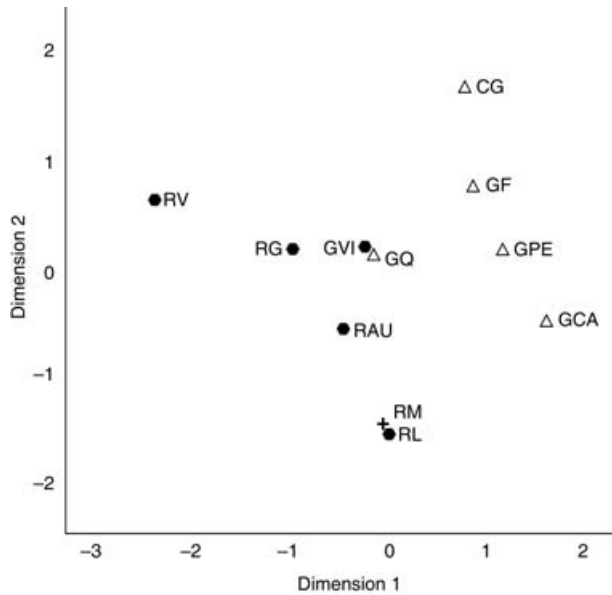
Other high correlations (significant before Bonferroni correction) included: pistil exertion, corolla curvature, and colour. Thus, moving to the right along dimension 1 (associated with specialized bat pollination), pistils tend to be more exerted, corollas less curved, light green or white, and not constricted above the nectar chamber.

Pollinators also separated Gesneriaceae species into clusters corresponding to ornithophilous and chiropterophilous flowers in the ordination using pollinator importance values (Fig. 4). However, in contrast with the clustering defined by floral traits (Fig. 1), species with subcampanulate flowers appeared scattered throughout the plot, reflecting the variability in pollinator importance values and pollinator assemblages (Fig. 4). The only trait that correlated with dimension 1 was colour, indicating red colours present in most species visited by hummingbirds, both specialists and generalists.

Correlations among floral traits revealed 11 significant associations (Table 2). These indicate flowers with wide

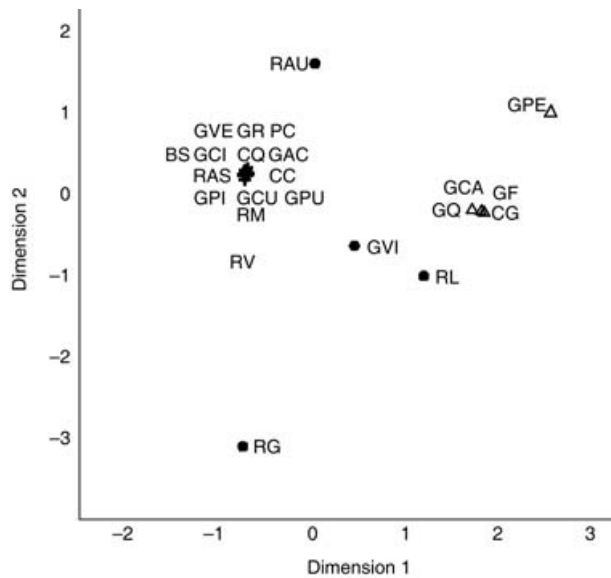
Table 2. Spearman correlation coefficients among all floral traits of 23 Gesneriaceae species used for floral ordinations. Numbers in bold indicate significant correlations after sequential Bonferroni adjustment

	PE	CWM	CC	CUR	NC	SYM	TAD	TNP	Colour	Spots
Corolla length (CL)	-0.02	-0.21	-0.53	-0.23	0.15	-0.36	0.38	0.38	0.26	-0.32
Pistil exertion (PE)		0.30	-0.07	0.26	-0.16	0.69	-0.25	-0.25	-0.37	-0.01
Corolla width at mouth (CWM)			0.50	0.28	-0.36	0.44	-0.84	-0.85	-0.54	0.70
Corolla constriction (CC)				0.28	-0.35	0.39	-0.65	-0.65	-0.15	0.51
Corolla curvature (CUR)					-0.27	0.56	-0.11	-0.11	0.08	0.42
Nectar concentration (NC)						-0.24	0.48	0.48	0.00	-0.55
Symmetry (SYM)							-0.36	-0.36	-0.22	0.23
Timing anther dehiscence (TAD)								1.00	0.66	-0.73
Timing nectar production (TNP)									0.66	-0.78
Colour										-0.42



Floral trait	Dim 1	Dim 2
Corolla length ventral	0.345	0.228
Pistil exertion	0.596	-0.182
Corolla width at mouth	0.068	0.687
Corolla constriction	-0.837	-0.418
Corolla curvature	-0.670	0.373
Nectar concentration	0.176	-0.200
Symmetry	0.200	-0.500
Timing of anthesis	-0.500	0.300
Timing of nectar	-0.500	0.300
Colour	-0.724	-0.261
Spots	0.387	0.710
Pollinator importance		
Hummingbird	-0.719	-0.330
Bananaquit	0.027	-0.242
Bat	0.796	0.278
Moth	-0.642	0.084
Diurnal insects	-0.357	0.094

Fig. 3. Multidimensional scaling analysis of 11 Gesneriaceae species based on 11 floral characters (excluding 12 species with tubular flowers). *Rhytidophyllum minus* (RM, plus sign next to RL) was included because with only one year of observation, the occurrence of bat pollination cannot be discarded. As above, triangles represent bat-pollinated species and dots represent species with mixed hummingbird and nocturnal pollination (bats and/or moths). Spearman correlation coefficients are listed for associations of dimensions 1 and 2, with floral traits and with pollinator importance values. Coefficients in bold indicate significant correlations following sequential Bonferroni adjustment ($P < 0.05$).



Floral trait	Dim 1	Dim 2
Corolla length ventral	-0.026	0.128
Pistil exertion	0.350	0.073
Corolla width at mouth	0.360	-0.595
Corolla constriction	-0.059	-0.476
Corolla curvature	0.160	-0.045
Nectar concentration	0.195	0.197
Symmetry	0.093	-0.156
Timing of anthesis	-0.549	0.504
Timing of nectar	-0.608	0.514
Colour	-0.724	0.192
Spots	0.381	-0.331
Pollinator Importance		
Hummingbird	-0.555	0.573
Bananaquit	0.357	0.511
Bat	0.875	-0.333
Moth	-0.177	-0.689
Diurnal insects	-0.397	-0.414

Fig. 4. Multidimensional scaling of 23 species of Gesneriaceae based on pollinator importance values. Importance was calculated as the product of visitation rates and effectiveness (contact with reproductive organs) and standardized as a proportional value. Note the hummingbird-pollinated species (plus sign) are mostly clustered in one point. Triangles indicate species primarily bat-pollinated and circles indicate generalist species. Spearman correlation coefficients are listed for associations of dimensions 1 and 2, with floral traits and with pollinator importance values. Coefficients in bold indicate significant correlations following sequential Bonferroni adjustment ($P < 0.05$).

corollas tend to have nocturnal schedules, green to white colours and dark red or brown spots, while flowers with narrow corollas tend to have diurnal schedules and solid bright red or orange colours. These associations reflect the suites of floral characters associated with classic bat and hummingbird pollination syndromes, respectively. The presence of a corolla constriction that makes subcampanulate corollas, was associated with nocturnal schedules of nectar

production and anther dehiscence. As a general rule, this subcampanulate floral phenotype indicates generalized pollination systems in the tribe Gesneriaceae.

EVALUATION OF POLLINATION SYNDROMES

Discriminant analysis was used to evaluate the ability of suites of floral traits to predict the pollination system; the

Table 3. Number of observations classified into expected pollination system and posterior probabilities (in parentheses) under cross-validation of discriminant analysis of multivariate set of floral traits of 23 Gesneriaceae species

<i>A priori</i>	Classified as			Total	Posterior probability Error rate
	Bat	Generalist	Hummingbird		
Bat	4 (0.820)	1 (0.530)	0	5	0.210
Generalist	1 (0.670)	3 (0.714)	1 (0.731)	5	0.274
Hummingbird	0	1 (0.958)	12 (1.000)	13	0.021

three *a priori* designated pollination system categories were based on our field observations: hummingbird, bat and generalist. When cross-validation was used to evaluate the ability of the model to classify species into expected pollination systems, floral traits were able to predict hummingbird pollination 12 out of 13 times; *R. minus* was classified as a generalist (Table 3). For the bat pollination category, one species out of five was misclassified (*G. quisqueyana* was classified as a generalist), and for the generalist, two out of five species were misclassified, one into the hummingbird (*R. leucomallon*) and one into the bat (*G. viridiflora* subsp. *Sintenisi*) pollination categories (Table 3).

Discussion

The validity of the pollination syndrome concept has been recently called into question based on an argument derived primarily from the observed widespread generalization of pollination systems in temperate regions (Waser *et al.* 1996; Ollerton *et al.* 2007), although some recent community-level studies suggest generalized pollination systems may be equally common in the tropics (Ollerton & Cranmer 2002). This study evaluated the correspondence between pollination ecology and patterns of floral diversity in the Antillean monophyletic tribe Gesnerieae. To obtain a better idea of the animals that could act as agents of selection on floral characters, we made an effort to distinguish floral visitors that have the ability to transfer pollen, from non-pollinating visitors. We also attempted to reduce underestimating the number of potential pollinators by surveying a subset of species for various years and at various sites. Our study provides evidence for both extreme ecological specialization and generalization within a group of Neotropical Gesneriaceae, and demonstrates that the occurrence of ecological generalization (visits by many species) has not precluded the evolution or maintenance of suites of floral traits that coincide with established pollination syndromes (Faegri & van der Pijl 1978).

The patterns for bat and hummingbird pollination syndromes were the same for Antillean and more distantly related mainland Gesneriaceae; no generalists from the mainland were identified in this study. Gesnerieae flowers show adaptations to bat pollination that correspond to traits described in over 700 bat-pollinated species of tropical and

subtropical plants (Tschapka & Dressler 2002), including other members of the family Gesneriaceae (e.g. floral morphologies that facilitate access to nectar, open corollas to facilitate detection by echolocation, exposed floral displays to enhance accessibility; Sanmartín-Gajardo & Sazima 2005b). Our results also support the existence of a distinct hummingbird pollination syndrome in the Gesneriaceae (tubular, red or yellow diurnal flowers with dilute nectar), one of the most widespread and accepted patterns of floral convergence (e.g. Sakai *et al.* 1999; Kay & Schemske 2003; Hargreaves *et al.* 2004; Sanmartín-Gajardo & Sazima 2005a; Wilson *et al.* 2006; Whittall & Hodges 2007). However, an intriguing finding was the occurrence of high hummingbird visitation to flowers that obviously do not correspond to the ornithophilous syndrome (i.e. visitation to bell-shaped, green/light yellow flowers).

Although hummingbird visitation to 'non-ornithophilous' flowers had been previously observed (e.g. Feinsinger 1976; Stiles 1976), the significantly greater visitation to the green flowers of *Gesneria* was unexpected. In a sample of Gesnerieae from Puerto Rico and the Dominican Republic we found that the average nectar volume was greater for bell-shaped-flowered species (range 60–82 µL, $n = 4$ species), than in tubular-flowered ones (range 5–16 µL, $n = 5$ species; Martén-Rodríguez & Fenster 2008 and unpublished data). Nectar volume, a trait that clearly separates bat-pollinated from hummingbird-pollinated species was not included in this study due to the small number of species for which accurate estimates were obtained. However, it appears that hummingbirds are attracted to green bell-shaped-flowered *Gesneria* species due to their higher nectar content. Other floral traits associated with the ornithophilous syndrome may serve to enhance efficiency of pollen transfer (tubular corollas; e.g. Castellanos *et al.* 2004), or signal the presence of a common food source (corolla colour), but are not the primary attractants for the birds (Stiles 1976).

The lack of fidelity by hummingbirds to species with tubular corollas explains the existence of generalized pollination systems. Gesnerieae species with subcampanulate corollas and mixed floral traits had nocturnal (bats and moths) and diurnal (hummingbirds and flies) visitors potentially contributing to fruit set. Floral traits that coincide with bat pollination are: nocturnal schedules of nectar production and anther dehiscence, abundant dilute nectar, and light

yellow-green corollas in most species. Although some of these traits also correspond with known adaptations to moth pollination, moths do not always contact stigmas or anthers. In contrast, some traits appear to be driven by selection to enhance hummingbird pollination. These traits vary among generalist Gesneriaceae, but they include narrower bell-shaped corollas, yellow colours with variable amounts of bright red markings, and diurnal as well as nocturnal nectar production and anther dehiscence (in *R. vernicosum*). No floral adaptations for the rare and inconsistent diurnal insect visitors were detected, as indicated by the lack of correlation between these visitors and floral traits (Figs 2–4). Species with intermediate phenotypes between ornithophily and chiropterophily have been described in at least two other plant families: the Lobeliaceae (e.g. *Syphocampylus sulfureus*, Sazima *et al.* 1994) and the Malvaceae (e.g. *Abutilon*, Buzato *et al.* 1994). In both cases, floral traits have been interpreted as transitional phenotypes along an evolutionary pathway to bat pollination. There are other instances where bat-pollinated and hummingbird-pollinated species occur within the same genus but no intermediate phenotypes are found in nature, reflecting trade-offs in corolla shape imposed by bats and hummingbirds (Muchhala 2007).

In Gesneriaceae, some traits display character states that appear to reflect selection by two different functional groups of pollinators, such as nocturnal and diurnal schedules of nectar production and anther dehiscence, and colour variation in some species. Whether these characters represent a transitional stage or an equilibrium point maintained by divergent selective pressures exerted by nocturnal and diurnal pollinators is not clear. However, these traits do not consistently explain the phenotypic clustering of species with generalized pollination systems. The single trait that distinguished generalists from their bat-pollinated relatives was the presence of a corolla constriction located right above the nectar chamber (see Fig. 1). Wolfe & Stiles (1989) proposed that corolla constrictions in hummingbird-pollinated flowers were part of an adaptive 'fail-safe' mechanism that enticed visitation by secondary pollinators, when the primary hummingbird specialists were absent. We hypothesize the corolla constriction in *Gesneria* and *Rhytidophyllum* facilitates nectar access to bats, while increasing the effectiveness of hummingbird pollination, thus promoting a dual pollination strategy in flowers predominantly adapted for bat pollination.

In the generalist species of *Gesneria* and *Rhytidophyllum*, hummingbird visits occur mostly in the late afternoon and early morning, which could be interpreted as thieving of early or leftover nectar. However, the stigmas of Gesneriaceae species remain receptive for nearly 30 straight hours, and pollen deposition was observed during hummingbird visits occurring at dawn and dusk. The corolla constriction appears to direct the hummingbird's bill to contact stamens and pistils in flowers that would otherwise be too wide for effective pollination to occur (video 1, Supporting Information). The constriction also makes nectar overflow accumulate as a nectar drop in the lower limb of the corolla, which is visible during the late night hours. This nectar drop may enhance the

chances of pollination to unvisited flowers, by making nectar more accessible or attractive to bats. Future work should address the functional significance of corolla constriction in relation to all observed functional groups of pollinators.

PREDICTABILITY OF POLLINATION SYNDROMES

The current debate on pollination syndromes has focused on two major issues: the role of pollination specialization and the predictive power of syndromes (Fenster *et al.* 2004; Ollerton *et al.* 2007). The notion that syndromes reflect natural selection to enhance pollen transfer by principal pollinators assumes that specialization into functional groups of pollinators (*sensu* Armbruster *et al.* 2000) has been important in shaping floral evolution. We have demonstrated that floral characteristics in Antillean Gesneriaceae assemble species into hummingbird and bat pollination syndromes as well as into an intermediate floral phenotype that is closer to chiropterophily. Phylogenetic relatedness cannot account for all of the similarity among species that fell into particular syndrome categories (Zimmer *et al.* 2002, Martén-Rodríguez *et al.* unpublished data phylogeny). For instance, the cluster that contains bat-pollinated specialists in the ordination includes at least three independent origins of this pollination system, while the cluster comprised by species with generalized pollination systems includes two independent origins. Hummingbird-pollinated species are distributed across at least four different clades, although in the tribe Gesneriaceae hummingbird pollination is most likely ancestral (Martén-Rodríguez *et al.* unpublished phylogeny). Significant correlations among traits and pollinators (e.g. timing of anthesis, timing of nectar production, corolla shape and colour) suggest that sets of floral characters have responded to selection to enhance pollination by the observed visitor guilds. Support for syndromes has been found in various other plant taxa using multivariate approaches (Sakai *et al.* 1999; Wilson *et al.* 2004; Wolfe & Sowell 2006). For example, in Bornean gingers, three clusters of floral phenotypes defined in multivariate space corresponded with pollination by spiderhunters (Nectarinidae) and two different groups of bees (Anthophoridae and Halictidae; Sakai *et al.* 1999). Likewise, in *Penstemon* clustering of ornithophilous and melittophilous species strongly corresponded with the predicted pollinators (Wilson *et al.* 2004).

Predictability is the second major issue concerning the debate on pollination syndromes (Ollerton *et al.* 2007). Our statistical evaluation indicates that floral traits are good predictors of specialized hummingbird and bat pollination in Antillean Gesneriaceae. However, the classificatory scheme was not perfect: *R. minus* (hummingbird-pollinated) and *G. quisqueyana* (bat-pollinated) were both misclassified as generalists. The first species has been observed for only 1 year and although nocturnal observation time (18 h) was within the range of other bat-pollinated species in our sample, it is possible that further observations will reveal the expected nocturnal pollinators. It is also possible that given the restricted present distribution of *R. minus* (one population in eastern Cuba isolated from undisturbed habitats), bat visits are

rare and thus, the species relies largely on hummingbirds for reproduction. The second misclassified species, *G. quisqueyana*, prevents hummingbird visitation by an active exclusion mechanism: the two-night flowers of *G. quisqueyana* close up during daytime. Therefore bat specialization is achieved by a unique phenological trait not included in the statistical analysis. This finding is evidence that, even within groups of related species, the pathways to specialization vary, resulting in different phenotypes associated with the same pollination syndrome.

In contrast to specialized Gesneriidae species, suites of floral traits did not consistently predict generalized pollination systems (Table 3). However, the presence of a constricted bell-shaped corolla was, in most cases, a good indicator of generalization. The variability of other floral traits in the generalists may reflect the more variable selective regimes to which species with nocturnal and diurnal pollinators are exposed. Alternatively, certain associations may reflect phylogenetic affinities rather than pollinator-mediated selection. For example, in a multivariate analysis of South African asclepiads, Ollerton *et al.* (2003) found distinct separation of wasp and beetle pollination syndromes, but the generalist *Xysmalobium gerrardi* was grouped near its beetle-pollinated sister species.

In general, we found little specialization of floral visitors to particular floral phenotypes. From an ecological standpoint, the asymmetric specialization observed in the interaction between flowers and hummingbirds in the Gesneriidae supports recent findings of mutualistic networks of species. Asymmetric interactions commonly characterize plant–pollinator food webs and networks (Bascompte *et al.* 2005, 2006) and have also been described for clades of species with highly specialized pollination systems (e.g. the South African orchid clade Coryciinae, where a single species of oil-collecting bee pollinates 15 species of orchids; Pauw 2006). In the Antilles, most Gesneriidae species have relatively short flowering periods and restricted distributions, while the animal pollinators have broader geographic ranges and need to feed year round. These results indicate that the evolution of pollination specialization does not have to be reciprocal; extreme specialization on the plant side has evolved without a corresponding specialization of the pollinators.

Because pollination syndromes are the result of convergent evolution across many different angiosperm flowers, they are not expected to describe the uniqueness of floral phenotypes evolved in different environments and phylogenetic backgrounds, nor can they be expected to predict unfailingly all floral visitors. This principle was stressed by the proponents of pollination syndromes (Faegri & van der Pijl 1978; Vogel 2006) and has been widely recognized by other researchers in the field (e.g. Stebbins 1970; Fenster *et al.* 2004; Ollerton *et al.* 2007). As exemplified in this study, pollination syndromes describe suites of traits that might evolve in a correlated way under selection by principal pollinators (Stebbins 1970). However, secondary floral visitors acting primarily as nectar and pollen robbers may also play important roles in floral evolution (Thomson 2003). Last, selection by different functional groups of pollinators may be responsible for the

existence of floral phenotypes that appear intermediate or that do not fit classic syndromes. The study of ecological interactions between plants and different kinds of floral visitors, including mutualists and parasites, remains a major task to complete in order to elucidate the evolutionary processes responsible for the floral diversification of Antillean Gesneriidae and other groups of tropical plants.

Acknowledgements

Authors are grateful to T. Clase, J. Cridland, X.S. Chen, A. Chuquin, M. Faife, D. Fernandez, D. Growald, J. Hereford, C. Moreno, D. Stanton, for help conducting field work and the Arecibo Observatory, the Institute of Jamaica, and Jardín Botánico de Santo Domingo for logistical support. Authors thank J. Ågren, A. Baier, M. Dudash, D. Inouye, J. Ollerton, L. Zimmer and one anonymous referee for insightful revisions to earlier versions of this article. Authors thank R. Reynolds for statistical advice and L. Skog for information and support provided during various stages of the project. Funding for field work was provided by American Gloxinia and Gesneria Society, The Explorers Club-Washington Group, Graduate Woman in Science, Sigma Xi, University of Maryland (Bamford Fund and BEES Program) to SMR, and NSF DDIG 0710196 to SMR and CBF.

References

- Armbruster, W.S. & Herzig, A.L. (1984) Partitioning and sharing pollinators by four sympatric species of *Dalechampia* (Euphorbiaceae) in Panama. *Annals of the Missouri Botanical Garden*, **71**, 1–16.
- Armbruster, W.S., Fenster, C.B. & Dudash, M.R. (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, H.G. & Baker, I. (1990) The predictive value of nectar chemistry to the recognition of pollinator types. *Israeli Journal of Botany*, **39**, 157–166.
- Bascompte, J., Jordano, P., Mejía, C. & Olesen, J.M. (2005) The nested assembly of plant–animal mutualistic networks. *Proceedings of the National Academy of Sciences*, **100**, 9383–9387.
- Bascompte, J., Jordano, P. & Olesen, J.M. (2006) Asymmetric coevolutionary networks facilitate biodiversity maintenance. *Science*, **312**, 431–433.
- 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.
- Carlson, J.E. (2008) Hummingbird responses to gender-biased nectar production: are nectar biases maintained by natural or sexual selection? *Proceedings of the Royal Society B-Biological Sciences*, **275**, 1717–1726.
- Castellanos, M.C., Wilson, P. & Thomson, J.D. (2004) 'Anti-bee' and 'pro-bird' changes during the evolution of hummingbird pollination in *Penstemon* flowers. *Journal of Evolutionary Biology*, **17**, 876–885.
- Castellanos, M.C., Wilson, P., Keller, S.J., Wolfe, A.D. & Thomson, J.D. (2006) Anther evolution: pollen presentation strategies when pollinators differ. *The American Naturalist*, **167**, 288–296.
- Darwin, C. (1862) *The Various Contrivances by Which Orchids are Fertilized by Insects*. Appleton and Company, New York.
- Faegri, K. & van der Pijl, L. (1978) *The Principles of Pollination Ecology*, 3rd edn Pergamon Press, Oxford, U.K.
- Feinsinger, P. (1976) Organization of a tropical guild of nectarivorous birds. *Ecological Monographs*, **46**, 257–291.
- Feinsinger, P. (1987) Approaches to nectarivore–plant interactions in the New World. *Revista Chilena de Historia Natural*, **60**, 285–319.
- Fenster, C.B. & Dudash, M.R. (2001) Spatiotemporal variation in the role of hummingbirds as pollinators of *Silene virginica*. *Ecology*, **82**, 844–851.
- Fenster, C.B., Armbruster, W.S., Thomson, J.D., Wilson, P. & Dudash, M.R. (2004) Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution and Systematics*, **35**, 375–403.
- Gower, J.C. (1971) A general coefficient of similarity and some of its properties. *Biometrics*, **27**, 857–874.
- Hargreaves, A.L., Johnson, S.D. & Nol, E. (2004) Do floral syndromes predict pollination specialization in plant pollination systems? An experimental test in an 'ornithophilous' African *Protea*. *Oecologia*, **140**, 295–301.
- Herrera, C.M. (1995) Microclimate variation and individual variation in pollinators: flowering plants are more than their flowers. *Ecology*, **76**, 1516–1524.

- Herrera, C.M. (1996) Floral traits and plant adaptation to insect pollinators: a devil's advocate approach. *Floral Biology* (eds S.C.H. Barrett & D.G. Lloyd), pp. 65–87. Chapman and Hall, New York, USA.
- Horowitz, C.C. & Schemske, D.W. (2002) Spatiotemporal variation in insect mutualists of a Neotropical herb. *Ecology*, **71**, 1085–1097.
- Johnson, S.D. & Steiner, K.E. (2000) Generalization versus specialization in plant pollination systems. *Trends in Ecology and Evolution*, **15**, 140–143.
- Kay, M.K. & Schemske, D.W. (2003) Pollinator assemblages and visitation rates for 11 species of Neotropical *Costus*. *Biotropica*, **35**, 198–207.
- Lara, C. & Ornelas, J.F. (2002) Effects of nectar theft by flower mites on hummingbird behavior and the reproductive success of their host plant, *Moussonia deppeana* (Gesneriaceae). *Oikos*, **96**, 470–480.
- Lindsey, A.H. (1984) Reproductive biology of Apiaceae. 1. Floral visitors to *Thaspium* and *Zizia* and their importance in pollination. *American Journal of Botany*, **71**, 375–387.
- Martín-Rodríguez, S. & Fenster, C.B. (2008) Pollination ecology and breeding systems of five *Gesneria* species from Puerto Rico. *Annals of Botany*, **102**, 23–30.
- Muchhala, N. (2007) Adaptive trade-off in floral morphology mediates specialization for flowers pollinated by bats and hummingbirds. *The American Naturalist*, **169**, 494–504.
- Olesen, J.M., Dupont, Y.L., Ehlers, B.K. & Hansen, D.M. (2007) The openness of a flower and its number of flower-visitor species. *Taxon*, **56**, 729–736.
- Ollerton, J. & Cranmer, L. (2002) Latitudinal trends in plant-pollinator interactions: are tropical plants more specialized? *Oikos*, **98**, 340–350.
- Ollerton, J., Johnson, S.D., Cranmer, L. & Kellie, S. (2003) The pollination ecology of an assemblage of grassland Asclepiads in South Africa. *Annals of Botany*, **92**, 807–834.
- Ollerton, J., Killick, A., Lamborn, E., Watts, S. & Whiston, M. (2007) Multiple meanings and modes: on the many ways to be a generalist flower. *Taxon*, **56**, 717–728.
- Pauw, A. (2006) Floral syndromes accurately predict pollination by a specialized oil-collecting bee (*Rediviva peringueyi*, Melittidae) in a guild of South African orchids (Coryciinae). *American Journal of Botany*, **93**, 917–926.
- Podolsky, R.D. (1992) Strange floral attractors - Pollinator attraction and the evolution of plant sexual systems. *Science*, **258**, 791–793.
- Robertson, C. (1928) Flowers and insects. *Lists of Visitors of Four Hundred and Fifty-Three Flowers*. C. Robertson. Carlinville, IL.
- Sakai, S., Kato, M. & Inoue, T. (1999) Three pollination guilds and variation in floral characteristics of Bornean gingers (Zingiberaceae and Costaceae). *American Journal of Botany*, **86**, 646–658.
- Sanmartín-Gajardo, I. & Sazima, M. (2004) Non-Euglossine bees also function as pollinators of *Sinningia* species (Gesneriaceae) in southeastern Brazil. *Plant Biology*, **6**, 506–512.
- Sanmartín-Gajardo, I. & Sazima, M. (2005a) Especies en *Vanhouttea* Lem. E *Sinningia* Nees (Gesneriaceae) polinizadas por beija-flores: interacciones relacionadas ao habitat da planta e ao nectar. *Revista Brasileira de Botanica*, **28**, 441–450.
- Sanmartín-Gajardo, I. & Sazima, M. (2005b) 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. SAS Institute, Cary, NC.
- Sazima, I., Buzato, S. & Sazima, M. (1996) An assemblage of hummingbird-pollinated flowers in a montane forest in southeastern Brazil. *Botanica Acta*, **109**, 149–160.
- Sazima, M., Sazima, I. & Buzato, S. (1994) Nectar by day and night – *Syphocampylus sulfurus* (Lobeliaceae) pollinated by hummingbirds and bats. *Plant Systematics and Evolution*, **191**, 237–246.
- Skog, L.E. (1976) A study of the tribe Gesnerieae with a revision of *Gesneria* (Gesneriaceae: Gesnerioideae). *Smithsonian Contributions to Botany*, **29**, 1–182.
- Smith, J.F. (1996) Tribal relationships within the Gesneriaceae: a cladistic analysis of morphological data. *Systematic Botany*, **21**, 497–513.
- Stebbins, G.L. (1970) Adaptive radiation of reproductive characteristics in angiosperms. Pollination mechanisms. *Annual Review of Ecology and Systematics*, **1**, 307–326.
- Stiles, G.F. (1976) Taste preferences, color preferences, and flower choice in hummingbirds. *The Condor*, **78**, 10–26.
- Thomson, J. (2003) When is it mutualism?. *The American Naturalist*, **162**, S1–S9.
- Tschapka, M. & Dressler, S. (2002) Chiropterophily: on bat-flowers and flower-bats. *Curtis's Botanical Magazine*, (ser. 6) **19**, 114–125.
- Vogel, S. (2006) Floral syndromes: empiricism versus typology. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie*, **127**, 5–11.
- Waser, N.M., Chitka, L., Price, M.V., Williams, N.M. & Ollerton, J. (1996) Generalization in pollination systems and why it matters. *Ecology*, **77**, 1043–1060.
- Whittall, J.B. & Hodges, S.A. (2007) Pollinator shifts drive increasingly long nectar spurs in columbine flowers. *Nature*, **447**, 706–709.
- Wilson, P., Castellanos, M.C., Hogue, J.N., Thomson, J.D. & Armbruster, W.S. (2004) A multivariate search for pollination syndromes among penstemons. *Oikos*, **104**, 345–361.
- Wilson, P., Castellanos, M.C., Wolfe, A.D. & Thomson, J.D. (2006) Shifts between bee and bird pollination in penstemons. *Plant-Pollinator Interactions: From Specialization to Generalization* (eds N.M. Waser & J. Ollerton), pp. 47–68. The University of Chicago Press, Chicago, IL.
- Wolf, L.L., & Stiles F.G. (1989) Adaptations for the 'fail-safe' pollination of specialized ornithophilous flowers. *American Midland Naturalist*, **121**, 1–10.
- Wolfe, L.M. & Sowell, D.R. (2006) Do pollination syndromes partition the pollinator community? A test using four sympatric morning glory species. *International Journal of Plant Sciences*, **167**, 1169–1175.
- Zimmer, E.A., Roalson, E.H., Skog, L.E., Boggan, J.K. & 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.

Received 11 April 2008; accepted 14 November 2008

Handling Editor: Jon Agren

Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Study locations for 23 species of Gesneriaceae studied in Costa Rica and five Antillean islands.

Appendix S2. Mean values for 11 morphological and phenological floral traits of 23 species of Gesneriaceae from Costa Rica and five Antillean islands.

Appendix S3. List of native pollinators, non-pollinating visitors and introduced pollinators observed on 23 species of Gesneriaceae from the Antilles and Costa Rica.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.