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Exploring the boundary between pollination syndromes: bats and hummingbirds as pollinators of *Burmeistera cyclostigmata* and *B. tenuiflora* (Campanulaceae)

Abstract In this study I documented the degree of specialization in the pollination systems of *Burmeistera cyclostigmata* and *B. tenuiflora* (Campanulaceae) to explore the potential role of floral isolation in the diversification of the genus. I asked which floral characteristics are important in specializing on either bat or hummingbird pollination, and whether overlap between these floral syndromes can exist. I examined nocturnal and diurnal pollen deposition, pollinator visitation rates, and single visit effectiveness and related them to intra- and interspecific variation in *Burmeistera* floral characteristics at Monteverde, Costa Rica. Bats and hummingbirds visited both *Burmeistera* species, and bats pollinated both species. Owing to differences in floral morphology, however, hummingbirds effectively pollinated only *B. tenuiflora*. The generalized pollination system of *B. tenuiflora* demonstrates that there can be overlap in the boundary between ornithophily and chiropterophily, and that nectar production and timing of anthesis do not serve as barriers between these syndromes. The high intraspecific variation in floral color from green to red or purple did not correlate with either nocturnal or diurnal pollen deposition. Degree of flower accessibility did affect pollination; nocturnal pollen deposition significantly decreased as flowers become more obstructed. In *Burmeistera*, floral morphology and accessibility appear to be the most important floral characteristics for specialization at the boundary between ornithophily and chiropterophily.

Keywords Chiropterophily · Costa Rica · Floral isolation · Monteverde · Ornithophily · Pollinator specialization versus generalization

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Introduction

Pollination syndromes are text-book examples of convergent evolution (Baker 1961; van der Pijl 1961; Stebbins 1970). When utilizing the services of the same pollinator, unrelated taxa of angiosperms often converge independently on predictable sets of floral characteristics. For example, neotropical flowers pollinated by bats tend to have nocturnal anthesis, dull colors, hexose-dominated nectar, and a distinctive odor (Helversen 1993). However, the actual predictive value of pollination syndromes has never been rigorously statistically verified (Ollerton and Watts 2000). Some authors warn that the widespread use of syndromes has biased pollination biology (Ollerton 1996; Waser et al. 1996; Johnson and Steiner 2000). Once the 'correct' pollinator for a particular plant has been identified, researchers may consciously or unconsciously overlook other potential pollinators. Recent studies suggest that generalization on multiple taxa of pollinators may be more common than previously thought (Waser et al. 1996; Fleming et al. 2001; Mayfield et al. 2001).

The degree of specialization found in pollination systems has implications for angiosperm diversification. There is support for a link between animal pollination and high species diversity (Dodd 1999); however the actual role pollinators may play in increasing speciation rates or decreasing extinction rates is still poorly understood (Johnson and Steiner 2000). One idea is that specialization on different pollinators creates a reproductive barrier between populations (termed 'floral isolation'), thereby directly increasing angiosperm speciation rates (Grant 1949, 1994). Generalized pollination systems cannot reproductively isolate populations; therefore, to evaluate the possible role of floral isolation it is important to know the degree to which different floral traits restrict pollinator assemblages (Baker 1961; Waser 1998).

The goal of this study was to examine the nature of the boundary between chiropterophilous (bat-pollinated) and ornithophilous (bird-pollinated) floral syndromes in species of *Burmeistera* (Campanulaceae). Are these syndromes mutually exclusive, or can overlap exist? Which

specific traits contribute to pollinator specialization? And is the degree of specialization sufficient that switches between these floral syndromes will result in floral isolation?

Despite being a widespread and highly diverse genus (Lammers 1998), *Burmeistera* and its pollination biology are poorly known. Flowers of its species tend to be small, robust, and campanulate with greenish to maroon corollas (Stein 1987). Based on these characteristics, several authors have proposed that it is likely a bat-pollinated genus (Vogel 1969; Dobat and Peikert-Holle 1985). In support of this prediction, bats in cloud forests of Ecuador were found to be carrying pollen of four different species of *Burmeistera* on their fur (Muchhala and Jarrin-V 2002). However, pollination studies in Monteverde, Costa Rica, have placed the local *Burmeistera* (including *B. tenuiflora* and *B. cyclostigmata*) in the ornithophilous syndrome corresponding to the short-billed hummingbird guild (Feinsinger et al. 1987; Murray et al. 1987; Stratton 1989). While Feinsinger et al. (1987) suggested that *B. cyclostigmata* may also be bat-pollinated based on its strong odor, they considered hummingbirds to be legitimate pollinators of both *B. tenuiflora* and *B. cyclostigmata*.

A number of traits have traditionally been used to differentiate the ornithophilous and chiropterophilous floral syndromes. Timing of nectar production and anthesis is important. Color is also often mentioned. Bright red, yellow, or orange flowers are believed to attract hummingbirds, which are highly visually oriented (van der Pijl 1961). Chiropterophilous flowers tend to be more drab, typically green or white (Helverson 1993). Floral morphology differs in that hummingbird-pollinated flowers are often tubular while bat-pollinated flowers are more open, commonly bell- or bowl-shaped. Finally, accessibility seems to be more important to chiropterophilous flowers, which normally project into the open away from obstructions. It is believed that differences in flight patterns are responsible for this trait. While hovering, bats sweep their wings in a large arc around them (Helverson 1993). Hummingbirds, in contrast, are more maneuverable, and wing movement is restricted to an area directly behind the animal's back. Bat pollinated plants use various strategies to increase flower exposure, including hanging inflorescences below the canopy (flagelliflory), situating them on the trunk (cauliflory), or raising them on long stems above the foliage (Baker 1961; Helverson 1993).

In this study I documented the relative importance of bats and hummingbirds to the pollination biology of *B. tenuiflora* and *B. cyclostigmata*. I related various floral traits, including timing of anthesis and nectar production, morphology, color, and accessibility, to the relative effectiveness of each taxon as pollinators. The latter two traits varied within as well as between the species of *Burmeistera*, allowing me to look for an association between them and intraspecific variation in pollinator effectiveness. Specific hypotheses I addressed are: (1) *B. tenuiflora* is primarily hummingbird pollinated, while *B. cyclostigmata* is primarily bat pollinated, (2) bat pollination is positively correlated with flower accessibility, and (3) hummingbird pollination is positively correlated with red coloration.

To test these hypotheses, I devised and employed a novel technique for measuring relative pollination effectiveness. I placed small pieces of double-sided tape on parafilm-covered floral reproductive parts to collect pollen deposited by visitors. This allowed me to quantify nocturnal and diurnal pollen flow as well as pollination effectiveness of individual flower visits. Techniques traditionally used to obtain such information include 'stigma squashes' and pollinator exclusion experiments (Kearns and Inouye 1993). The new method involves fewer materials and is less time-consuming than either traditional method. Furthermore, it allows a single flower to provide multiple data points; i.e., one flower can provide paired data points for nocturnal and diurnal pollen deposition or can be re-used in analyses of single-visit effectiveness. This is especially important for studying the pollination biology of relatively rare species such as those of *Burmeistera*.

Materials and methods

Study site

Field work was conducted in the Monteverde Cloud Forest Reserve, Provincia de Puntarenas, Costa Rica (10°18'N, 84°49'W) from 11 January to 2 February 2002. Monteverde is classified as Lower Montane Wet Forest (Feinsinger et al. 1991; cf. Holdridge 1967), with an average yearly temperature of 18.5°C and average yearly rainfall of approximately 2,500 mm. The *Burmeistera* individuals I studied occurred near the continental divide, in the highest parts of the reserve (approx. 1,600 m), along the Pantanoso and Nuboso trails. See Lawton and Dryer (1980) for further details on the study site.

Twenty-one species of hummingbirds occur in Monteverde. Of these, Feinsinger et al. (1987) found that *Lampornis calolaema* made 96.9% of the visits they observed to 'short' flowers (the guild of flowers with short corollas, which includes *B. tenuiflora* and *B. cyclostigmata*), and *Eupherusa eximia* was responsible for most of the remaining visits. Six species of nectarivorous bats have been caught in Monteverde. However, only *Anoura geoffroyi*, *Hylonycteris underwoodi*, and *Glossophaga commissarisi* occur in the higher parts of the reserve where this study was conducted (Richard LaVal, personal communication).

Burmeistera

The 96 species of *Burmeistera* range from Costa Rica to Peru, reaching their highest species diversity in montane wet forests (Stein 1987). Flowers of the genus are protandrous. Anthers are fused together to form a tube into which the pollen is shed. Pollen is released gradually throughout the male phase via a 'pump mechanism' as the style elongates within this tube. When fully exserted (normally by the third day) the stigma becomes receptive and the female phase begins (Erbar and Leins 1995). The flower senesces 6–8 days after opening (Stratton 1989). Individual plants remain in flower for months, with one to four flowers open at a time (personal observation).

Ten species of *Burmeistera* occur in Costa Rica (Wilbur 1975), of which the following four are present in the Monteverde Cloud Forest Preserve: *B. cyclostigmata*, *B. tenuiflora*, *B. parviflora*, and

B. microphylla. This study focuses on the former two species, since only one individual of each of the latter was found to be in flower in January. Both *B. cyclostigmata* and *B. tenuiflora* are epiphytes found on the trunks of living or dead trees. Flowers of both species open nocturnally, between 1800 and 2000 hours (personal observation). They have high intraspecific variation in color; flowers of *B. cyclostigmata* range from light green to deep purple while flowers of *B. tenuiflora* range from light green to red.

Flowers of both species of *Burmeistera* are highly exposed, suggesting that pollinators may have exerted selective pressure on flower accessibility in the past. Unopened buds are often hidden beneath an individual's foliage, but before anthesis they are raised on long pedicels at a 45° angle above the foliage. Furthermore, flowers only occur near the ends of branches and only rarely beneath other branches.

Unfortunately *B. cyclostigmata* and *B. tenuiflora* pollen is indistinguishable via light microscopy; thus throughout this study, I used congeneric pollen deposition as a proxy for conspecific deposition in determining pollinator effectiveness.

Flower morphology and nectar production

I measured various aspects of floral morphology in the field with a plastic ruler (to the nearest mm), including length of the sepals, hypanthium, and pedicel, corolla width, and anther-nectar distance. Corolla width was calculated as the greatest width of the corolla opening, and anther-nectar distance was calculated as the length from the end of the anther tube to the base of the corolla. I recorded nocturnal and diurnal nectar production for 6 flowers (from different individuals) for both species of *Burmeistera*. Nectar was measured at dawn (0600 hours) and dusk (1800 hours) using a microsyringe with a blunt tip. To prevent visitor removal of nectar over the measurement periods, I covered flowers with mosquito netting affixed to the pedicel with twist-ties. I began nectar measurements in the male phase of the flowers and continued until nectar production had stopped. This occurred by the second day of the female phase for all flowers except for two (one *B. tenuiflora* and one *B. cyclostigmata*), which produced nectar on the third day of the female phase.

Nocturnal and diurnal pollen deposition

To determine the relative effectiveness of bats and hummingbirds as pollinators, I quantified nocturnal and diurnal pollen deposition on the reproductive parts of the flowers. To do this the anther tube of each flower was wrapped with a layer of parafilm, which was left on throughout the male and female phases of the flower. A small rectangle of Scotch double-sided tape (10x5 mm) was affixed to the parafilm. I collected the tape every dawn and dusk, placed it on a slide, and covered it with Scotch single-sided tape. Using a light microscope, I examined these slides for pollen. The presence of pollen indicates pollinator contact with the reproductive parts of the flower. During the male phase of the flower, this contact would correspond to the pick-up of pollen and potential transfer to conspecifics. Thus the presence of pollen on tape indicates potential pollen flow regardless of its identity. It should be noted that this method will underestimate pollen pick-up to some degree, since a visitor which was not carrying pollen will leave no record of its visit. For the female phase of the flower, the identity of the pollen deposited becomes important; only conspecific pollen can pollinate the flower. Therefore for each slide I recorded (1) total amount of pollen, (2) number of morphologically distinguishable types of pollen (morphotypes), (3) amount of *Burmeistera* pollen, and (4) percent of total pollen represented by *Burmeistera*. Morphotypes corresponded to different families (e.g., Ericaceae, Bromeliaceae) or genera (e.g., *Markea*, *Burmeistera*, *Passiflora*). The amount of pollen was approximated as one of four categories, with 1 representing less than 50 grains, 2 representing 50–1,000, 3 1,000–5,000, and 4 more than 5,000. A total of 437 slides was analyzed, corresponding to 29 *B. tenuiflora* flowers from 12 individuals and 12 *B. cyclostigmata* flowers from 8 individuals.

In the course of examining the slides, I noticed that on a number of the diurnal samples pollen was deposited only in a corner of the tape. Since the corners of the tape extend beyond the anther tube when affixed to the flower, these samples represent visits in which the reproductive parts of the flower were not contacted. Without contact, pollen will not be removed from anthers or deposited on stigmas. Therefore these 'corner' samples (*n*=15) were excluded from analyses of pollen deposition.

I statistically compared the relative importance of diurnal vs nocturnal pollen deposition for *B. cyclostigmata* and *B. tenuiflora* with paired *t*-tests. The dependent variable (pollen deposition) was calculated for each flower as the number of days (or nights) during which it received pollen over the total number of days (or nights) for which tape was collected. Only flowers for which I collected at least 3 days and 3 nights of data were included in this analysis (*n*=22 for *B. tenuiflora*; *n*=12 for *B. cyclostigmata*).

Visitation rates and pollinator effectiveness

I observed flowers of two focal individuals of *B. tenuiflora*. This allowed me to determine exactly which floral visitors contributed to nocturnal and diurnal pollen deposition. Furthermore, it allowed me to quantify pollination effectiveness of individual visits. Flowers were prepared with parafilm and double-sided tape as above. Immediately after observing a visit, I collected and replaced the tape. Using slides made with these pieces of tape, I counted the number of pollen grains of each morphotype deposited in a single visit. Observations were made in blocks of 0.5–2.5 h from 24 January to 2 February 2002, for a total of 17.6 h during the day and 17.6 h during the night. During observations I sat more than 5 m from the flowers to minimize disturbance, and at night illuminated the flowers using a flashlight covered with red cellophane.

Flower color and accessibility

I recorded the color and accessibility of each flower used in the analysis of nocturnal and diurnal pollen deposition in order to analyze their effects on pollen flow. For color, each flower was placed in one of five categories ranging from green to red (*B. tenuiflora*) or green to purple (*B. cyclostigmata*). Thus, for *B. tenuiflora* the categories were (1) solid green, (2) green suffused with some red, (3) approximately 50% green and 50% red, (4) red suffused with some green, and (5) solid red. Flower accessibility was ranked in three categories: 'open,' 'semi-obstructed,' and 'obstructed.' For the 'open' category, a flight path to the flower was unobstructed; that is, an imaginary cone originating at the flower and extending roughly 50 cm in front of it was completely free of obstructing undergrowth. 'Semi-obstructed' flowers had some leaves or other obstruction within this cone that might hamper access, while 'obstructed' flowers were heavily blocked by undergrowth. While such obstruction was often caused by foliage from other plants, and thus outside of the plant's 'control,' effects of any obstruction on visitation will offer insight into selective pressures on those aspects of flower exposure which are under the plant's control (e.g., length and angle of pedicel, location of flowering branches on plant, location of flowers on branches, etc.).

ANOVA was used to determine whether color or accessibility was significantly related to diurnal (hummingbird) or nocturnal (bat) visitation. Again, the dependent variable (pollen deposition) was calculated for each flower as the number of days (or nights) during which it received pollen over the total number of days (or nights) for which tape was collected, with only those flowers for which I collected at least 3 days and 3 nights of data included in the analysis. However in this case I included 'corner' samples since they indicated visitation. Since diurnal pollen deposition occurred only once on *B. cylostigmata*, only *B. tenuiflora* was used in diurnal analyses. For nocturnal deposition, I performed separate analyses for each *Burmeistera* species, as well an analysis pooling the two.

Table 1 Quantitative floral traits of *Burmeistera cyclostigmata* and *B. tenuiflora*. Values represent mean (SE) in mm

Species	n	Anther-nectar distance	Corolla width	Pedicel length	Hyphanthium length	Sepal length
B. cyclostigmata	7	41.0 (2.2)	23.3 (2.7)	43.0 (10.6)	8.3 (1.0)	7.0 (1.3)
B. tenuiflora	18	27.3 (1.4)	13.3 (2.3)	48.6 (10.8)	10.1 (1.2)	22.8 (7.1)

Table 2 Nocturnal and diurnal pollen flow to *B. cyclostigmata* and *B. tenuiflora* expressed as percent of flower-nights and flower-days during which pollen was deposited

Species	Nocturnal		Diurnal	
	Percent w/pollen	n = flower-nights	Percent w/pollen	n = flower-days
B. cyclostigmata	52.6	76	1.2	81
B. tenuiflora	54.3	140	20.7	140

Table 3 Type and amount of pollen deposited on *B. cyclostigmata* and *B. tenuiflora* nocturnally and diurnally. Amount of pollen represents the mode of the categories assigned to each sample, where 1 is <50 pollen grains, 2 is 50–1,000, 3 is 1,000–5,000, and 4 is >5,000

		n	Amount of pollen	Average no. of morphotypes per sample (SE)		No. of samples with *Burmeistera*	Amount of *Burmeistera* pollen
B. cyclostigmata	Nocturnal	39	3	2.4	(0.11)	36	2
	Diurnal	1	1	1.0	–	0	–
B. tenuiflora	Nocturnal	78	3	2.3	(0.09)	76	3
	Diurnal	29	1	2.0	(0.13)	23	1

Results

Flower morphology and nectar production

Burmeistera cyclostigmata flowers were larger than *B. tenuiflora* flowers; corollas were approximately 75% wider and anther-nectar distance was approximately 50% greater (Table 1). Pedicel length was highly variable, yet on average comparably long for both species (*B. cyclostigmata*: 43 mm, *B. tenuiflora*: 49 mm). Nectar was not produced diurnally by either *B. tenuiflora* or *B. cyclostigmata*. Mean nocturnal production was 0.57 ml (±1.1 SE) for *B. tenuiflora* and 0.56 ml (±1.0 SE) for *B. cyclostigmata*.

Nocturnal and diurnal pollen deposition

Diurnal and nocturnal pollen deposition on the reproductive parts of the flowers of *B. cyclostigmata* and *B. tenuiflora* are summarized in Table 2. Pollen was brought to *B. cyclostigmata* almost exclusively at night. Out of 81 flower-days, only one instance of diurnal pollen deposition occurred. In 40 (53%) of 76 flower-nights pollen was deposited. In *B. tenuiflora*, pollen deposition occurred both diurnally and nocturnally, although nocturnal deposition was more frequent. Out of 140 flower-nights, 76 (54%) received pollen while out of the same number of flower-days 29 (21%) received pollen.

Further analysis of the pollen deposited shows that in addition to providing more frequent pollen deposition, nocturnal pollinators were more effective in terms of the quantity of *Burmeistera* grains deposited per load (Table 3). Most nocturnal pollen loads of *B. cyclostigmata* and *B. tenuiflora* (93% and 97%, respectively) contained *Burmeistera* pollen. The average amount of *Burmeistera* pollen in these loads was approximately 50–1,000 grains for *B. cyclostigmata* (the modal category was 2) and 1,000–5,000 grains for *B. tenuiflora* (modal category 3). In contrast, only 76% of the diurnal *B. tenuiflora* pollen loads had *Burmeistera* pollen, and the average number of grains was less than 50 (modal category 1). On average nocturnal pollen loads contained an order of magnitude times more *Burmeistera* pollen than diurnal pollen loads.

Nocturnal pollen loads also tended to have a slightly higher number of morphotypes of pollen per load (2.4 and 2.3 vs 2.0; Table 3). However these other morphotypes typically were represented by only a few grains per load. After *Burmeistera*, Bromeliaceae pollen was the most common morphotype in nocturnal pollen loads; it was present in 85% of the 117 nocturnal loads. Pollen of Ericaceae was the most common morphotype in diurnal pollen loads; it was present in 80% of the 30 diurnal loads.

For both species of *Burmeistera*, nocturnal pollen deposition was significantly greater than diurnal pollen deposition (*B. cyclostigmata*: $t=5.23$, $df=11$, $P<0.001$; *B. tenuiflora*: $t=4.60$, $df=21$, $P<0.001$). Flowers of *B. cyclostigmata* received pollen on average 50% of the nights (±9.2 SE) and 1% of the days (±1.4 SE) they were open. In *B. tenuiflora*, pollen deposition occurred on 55% of nights (±6.6 SE) and 21% of days (±4.3 SE).

Table 4 One-way analyses of variance (ANOVAs) testing the effects of color and accessibility on nocturnal and diurnal pollen deposition. Pollen deposition, the dependent variable, was calculated for each flower as the number of days (or nights) with pollen divided by the total number of days (or nights). Note that diurnal pollen deposition on *B. cyclostigmata* was not analyzed since it only occurred once

	Color			Accessibility		
	df	F	P	df	F	P
Nocturnal						
B. tenuiflora	(3,18)	0.11	0.952	(2,19)	5.34	0.014
B. cyclostigmata	(2,9)	1.52	0.271	(2,9)	4.94	0.036
Both species pooled	(3,30)	0.39	0.763	(2,31)	8.78	0.001
Diurnal						
B. tenuiflora	(3,18)	1.24	0.325	(2,19)	1.42	0.265

Visitation rates and pollinator effectiveness

In 35.2 h of observation, only hummingbirds and bats visited flowers of *B. tenuiflora*. Visitation rates for both taxa were very low. In 17.6 h of diurnal observation, 17 visits by eight different hummingbird individuals were observed. When divided by the number of flowers I watched during each observation period, this gives a visitation rate of 0.28 visits flower^{-1} h^{-1}. In 17.6 h of nocturnal observation, four visits by four bats were recorded. This corresponds to a visitation rate of 0.07 visits flower^{-1} h^{-1}. In only seven (41%) of the 17 hummingbird visits was pollen deposited on the flower's reproductive parts. These pollen loads contained 1–2 pollen morphotypes and a mean of 233 (±73 SE) pollen grains. Four of the loads contained *Burmeistera* pollen, with a mean of 140 (±118 SE) *Burmeistera* grains per load. In three (75%) of the four bat visits pollen was deposited. *Burmeistera* pollen comprised the majority of all three of these loads; mean total number of grains was 2,639 (±1,050 SE) while mean number of *Burmeistera* grains was 2,573 (±1,024 SE). The composition of these single-visit pollen loads support the conclusion that, in the nocturnal versus diurnal pollen deposition analysis, pollen found on the nocturnal tape corresponded to bat visitation and that found on diurnal tape corresponded to hummingbird visitation. Ericaceae was only found on diurnal tape and after hummingbird visits, and the three bat visits brought an unidentified pollen morphotype which was only found on nocturnal tape.

Hummingbirds appear to only visit *Burmeistera* flowers in the early morning or late afternoon. Although I would end morning observation sessions around 0900 hours, the majority of visits (n=5) occurred before 0730 hours. The latest visit occurred at 0745 hours. Furthermore, pollen was never deposited on tape left on the flowers from 0900 to 1630 hours (n=20 flower-days). I observed two late afternoon hummingbird visits; one at 1730 hours and one at 1715 hours.

Flower color and accessibility

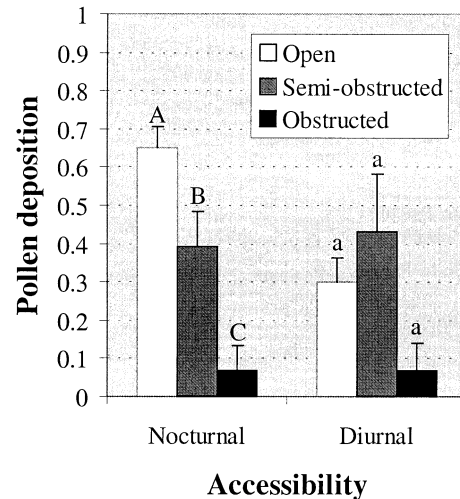Flower accessibility significantly affected nocturnal pollen deposition when the *Burmeistera* species were pooled

Fig. 1 Pollen deposition, as proportion of days or nights with pollen, by level of accessibility of flowers. *Error bars* represent 1 SE of the mean. Note that diurnal results represent only *Burmeistera tenuiflora* since there was only one instance of diurnal pollen deposition on *B. cyclostigmata*, while nocturnal results are pooled over both species. Means shown below the same letter are not significantly different at the *P*<0.05 level of significance

(*df*=2,31; *F*=8.78; *P*=0.001; Table 4). The proportion of flower-nights with pollen was highest for 'open' flowers (0.57±0.06), followed by 'semi-obstructed' (0.39±0.09) and 'obstructed' flowers (0.07±0.07; Fig. 1). This pattern also occurred when *B. cyclostigmata* and *B. tenuiflora* were analyzed separately (Table 4). No significant effect of accessibility was found for diurnal deposition (*df*=2,19; *F*=1.42; *P*=0.265; Table 4). Color did not significantly affect either nocturnal or diurnal deposition (Table 4).

Discussion

Results of this study show that *B. cyclostigmata* has a specialized pollination system which relies almost exclusively on bats. Out of 81 flower-days, only one instance of diurnal pollen deposition occurred, whereas pollen was deposited nocturnally in 53% of 76 flower-nights. *B. tenuiflora*, on the other hand, is regularly pollinated by both bats and hummingbirds. Pollen was deposited on 54% of 140 flower-nights and 21% of 140 flower-days.

Initially one might conclude that *B. cyclostigmata* flowers do not receive pollen diurnally because they do not attract hummingbirds. During this study, however, I incidentally observed several hummingbird visits to *B. cyclostigmata*. Furthermore, Feinsinger et al. (1987) reported an average of approximately 0.2 hummingbird visits per hour to *B. cyclostigmata* flowers. Thus hummingbirds do visit both species of *Burmeistera*, but due to differences in floral morphology only deposit pollen on flowers of *B. tenuiflora*. *B. cyclostigmata* flowers are nearly twice as wide as those of *B. tenuiflora* and their reproductive parts are exserted farther out (Table 1). This morphology allows hummingbirds to access the nectar in flowers of *B. cyclostigmata* without receiving or depositing any pollen.

Even with a more restricted opening and shorter anther tube, hummingbirds only made contact with the reproductive parts of *B. tenuiflora* flowers 41% of the time (7 of 17 visits). The 'poor fit' between hummingbirds and *B. tenuiflora* flowers means that they pick up and deposit less congeneric pollen than bats. *Burmeistera* pollen was deposited in only 4 of the 17 hummingbird visits, compared with three of the four bat visits. And the four hummingbird pollen loads with *Burmeistera* contained a mean of only 140±118 *Burmeistera* pollen grains, while the bat pollen loads contained a mean of 2,573±1,024 *Burmeistera* pollen grains per load. This 'poor fit' with hummingbirds, along with the fact that it only produces nectar nocturnally, implies that *B. tenuiflora* is primarily evolved to exploit bat pollination. It also implies that more extreme changes in morphology (e.g., an even smaller opening or shorter anther tube) would be necessary to take full advantage of hummingbird pollination.

Interestingly, neither *B. tenuiflora* nor *B. cyclostigmata* produce nectar during the day. Why, then, do hummingbirds even visit them? Some hummingbird visits occur just before dusk, probably as nocturnal nectar production begins. However the majority of visits occur before 0745 hours. Most likely these hummingbirds are opportunistically foraging for nectar left over from the previous night. Assuming bat visitation rates stay relatively constant throughout the night, the average *B. tenuiflora* flower receives 0.88 visits per night (12×0.07 visits flower^{-1} h^{-1}). *B. tenuiflora* flowers produce 0.56 ml (±1.0 SE) of nectar per night. With only 0.88 visits per flower per night, more than 10% of the flowers will receive no visits and the rest will still have a significant proportion of their nectar left in the morning for hummingbirds to exploit. Similar patterns of early morning and/or late afternoon hummingbird visitation have been documented for other plants with night-blooming flowers (Ramirez et al. 1984; Sazima and Sazima 1988; Sazima et al. 1989; Gribel and Hay 1993; Kay 2001).

Although flower color and accessibility figure prominently in vertebrate pollination syndromes, my results suggest that only accessibility is important in these species of *Burmeistera*. As predicted, bat visitation rates decreased sharply as the flight path towards the flower became more obstructed with undergrowth, while no significant difference was found between the three accessibility categories for hummingbirds. This supports the importance of accessibility in the traditional chiropterophilous floral syndrome (Dobat and Peikert-Holle 1985; Helversen 1993). In contrast, I found no support for a hummingbird preference for red coloration. No correlation was found between hummingbird visits and the degree of pigmentation of flowers of *B. tenuiflora*. Schemske and Bradshaw (1999) also failed to find a strong relationship between hummingbird visitation and flower color in *Mimulus*, but did find that red coloration decreased bee visitation. This is consistent with the hypothesis that the red coloration common in ornithophilous flowers may serve primarily to deter insect visitation rather than to attract hummingbirds.

This study provides further evidence that there can be an overlap between the chiropterophilous and ornithophilous pollination syndromes. *B. tenuiflora*, like *Siphocampylus sulfureus* (Sazima et al. 1994) and *Abutilon rufinerve* and *A. regnellii* (Buzato et al. 1994), is able to exploit the pollination services of both hummingbirds and bats. Both Sazima et al. (1994) and Buzato et al. (1994) proposed that these are examples of plants 'in transition' from the hummingbird to the bat pollination syndromes. I argue that generalization on bats and hummingbirds may actually be an evolutionary endpoint. In the case of *B. tenuiflora*, the costs of generalizing appear to be small; no nectar is produced during the day, and the negative impact of hummingbirds consuming 'leftover' nectar is probably minimal. The benefits include a greater total number of pollen vectors throughout the flower's lifetime, and the flexibility to still be able to set seed should either pollinator taxon become scarce.

This study also sheds light on the evolution of pollinator specialization. That is, what adaptations would a species of *Burmeistera* need to evolve to switch from a generalized pollination system to one specialized solely on bats or hummingbirds? Of the various syndrome traits explored, only flower accessibility and morphology appear capable of restricting the number of legitimate pollinators. Timing of nectar production fails to restrict visitation; although both *Burmeistera* only produced nectar nocturnally, hummingbirds still opportunistically visited the flowers. And given the fact that flowers of *Burmeistera* remain open for several days, timing of anthesis similarly fails to restrict visitation. Finally, no correlation was found between flower color and hummingbird visits. Although it is possible that small sample sizes may have masked a more subtle effect of color on visitation, the fact remains that hummingbirds will visit green *B. tenuiflora* flowers. Thus a shift in coloration would not be sufficient to exclude either bat or hummingbird visitation.

Accessibility of flowers does affect bat visitation. Thus it may play a role in specializing on hummingbirds (though not vice-versa). By decreasing the exposure of their flowers, for example by moving them from the ends of the branches or by decreasing pedicel length, bat

visitors could be excluded. Floral morphology also affects visitation, and may be the most important factor for pollinator specialization. With its large flowers, *B. cyclostigmata* effectively eliminates hummingbird pollen transfer despite visitation. Further support for the importance of morphology in *Burmeistera* pollination comes from several Ecuadorian species (Muchhala and Jarrin-V 2002). Of five sympatric *Burmeistera* species, bats (*Anoura geoffroyi* and *A. caudifera*) were carrying the pollen of four. Flowers of the fifth species, *B. crassifolia*, were comparatively small, thin, and tubular. Furthermore, the pollen of the smallest of the other four species (*B. succulenta*) was found only on the smaller bat (*A. caudifera*). Thus *B. succulenta* appears to restrict visitation by the larger bat, while *B. crassifolia* restricts visitation of any bats.

Waser et al. (1996) held that the classical view that morphological fit ensures pollinator specialization is rarely correct, and thus 'mechanical' isolation of sympatric congeners is not possible. I argue that my results for *Burmeistera* demonstrate that floral morphology can affect pollinator specialization, and that floral isolation is possible at the boundary between ornithophilous and chiropterophilous pollination syndromes. Furthermore, I propose that the high diversity found in *Burmeistera* is a direct result of an increased rate of speciation caused by floral isolation. Similar pollination studies of some of the other 96 *Burmeistera* species in combination with a phylogenetic analysis of the group would be useful in testing this hypothesis.

References

Baker HG (1961) The adaptation of flowering plants to nocturnal and crepuscular pollinators. Q Rev Biol 36:64–73

Buzato S, Sazima M, Sazima I (1994) Pollination of 3 species of Abutilon (Malvaceae) intermediate between bat and hummingbird flower syndromes. Flora 189:327–334

Dobat K, Peikert-Holle T (1985) Bluten und Fledermause. Waldemar Kramer, Frankfurt am Main

Dodd ME (1999) Phylogenetic analysis of trait evolution and species diversity variation among angiosperm families. Evolution 53:732–744

Erbar C, Leins P (1995) Portioned pollen release and the syndromes of secondary pollen presentation in the Campanulales-Asterales-complex. Flora 190:323–338

Feinsinger P, Beach JH, Linhart YB, Busby WH, Murray KG (1987) Disturbance, pollinator predictability, and pollination success among Costa Rican cloud forest plants. Ecology 68:1294–1305

Feinsinger P, Tiebout III HM, Young BE (1991) Do tropical bird-pollinated plants exhibit density-dependent interactions? Field experiments. Ecology 72:1953–1963

Fleming TH, Sahley CT, Holland JN, Nason JD, Hamrick JL (2001) Sonoran Desert columnar cacti and the evolution of generalized pollination systems. Ecol Monogr 71:511–530

Grant V (1949) Pollination systems as isolating mechanisms in angiosperms. Evolution 3:82–97

Grant V (1994) Modes and origins of mechanical and ethological isolation in angiosperms. Proc Natl Acad Sci USA 91:3–10

Gribel R, Hay JD (1993) Pollination ecology of Caryocar brasiliense (Caryocaraceae) in Central Brazil cerrado vegetation. J Trop Ecol 9:199–211

Helversen O von (1993) Adaptations of flowers to the pollination by glossophagine bats. In: Barthlott W, Naumann CM, K. S-L, Schuchmann KL (eds) Animal-plant interaction in tropical environments. Museum Koenig, Bonn, pp 41–59

Holdridge LR (1967) Life zone ecology. Tropical Science Center, San Jose, Costa Rica

Johnson SD, Steiner KE (2000) Generalization versus specialization in plant pollination systems. Trends Ecol Evol 15:140–143

Kay E (2001) Observations on the pollination of Passiflora penduliflora. Biotropica 33:709–713

Kearns CA, Inouye DW (1993) Techniques for pollination biologists. University Press of Colorado, Niwot, Colorado

Lammers TG (1998) Review of the neotropical endemics Burmeistera, Centropogon, and Siphocampylus (Campanulaceae: Lobelioideae), with description of 18 new species and a new section. Brittonia 50:233–262

Lawton R, Dryer V (1980) The vegetation of the Monteverde Cloud Forest Reserve. Brenesia 18:101–116

Mayfield MM, Waser NM, Price MV (2001) Exploring the 'most effective pollinator principle' with complex flowers: Bumblebees and Ipomopsis aggregata. Ann Bot 88:591–596

Muchhala NC, Jarrin-V P (2002) Flower visitation by bats in cloud forests of western Ecuador. Biotropica 34:387–395

Murray KG, Feinsinger P, Busby WH, Linhart YB, Beach JH, Kinsman S (1987) Evaluation of character displacement among plants in two tropical pollination guilds. Ecology 68:1283–1293

Ollerton J (1996) Reconciling ecological processes with phylogenetic patterns: the apparent paradox of plant-pollinator systems. J Ecol 84:767–769

Ollerton J, Watts S (2000) Phenotype space and floral typology–towards an objective assessment of pollination syndromes. In: Nor Vidensk-AkadMat Naturvidensk Klas Skr Ny Ser 39:149–159

Pijl L von de (1961) Ecological aspects of flower evolution. II. Zoophilous flower classes. Evolution 15:44–59

Ramirez N, Sobrevila C, Deenrech NX, Ruizzapata T (1984) Floral biology and breeding system of Bauhinia benthamiana (Leguminosae), a bat-pollinated tree in Venezuelan llanos. Am J Bot 71:273–280

Sazima M, Sazima I (1988) Helicteres ovata (Sterculiaceae), pollinated by bats in southeastern Brazil. Bot Acta 101:269–271

Sazima I, Vogel S, Sazima M (1989) Bat pollination of Encholirium glaziovii, a terrestrial bromeliad. Plant Syst Evol 168:167–179

Sazima M, Sazima I, Buzato S (1994) Nectar by day and night - Siphocampylus sulfureus (Lobeliaceae) pollinated by hummingbirds and bats. Plant Syst Evol 191:237–246

Schemske DW, Bradshaw HDJ (1999) Pollinator preference and the evolution of floral traits in monkeyflowers (Mimulus). Proc Natl Acad Sci 96:11910–11915

Stebbins GL (1970) Adaptive radiation of reproductive characteristics in angiosperms, I: Pollination mechanisms. Annu Rev Ecol Syst 1:307–326

Stein BA (1987) Synopsis of the genus Burmeistera (Campanulaceae: Lobelioideae) in Peru. Ann Mo Bot Gard 74:494–496

Stratton DA (1989) Longevity of individual flowers in a Costa Rican cloud forest: ecological correlates and phylogenetic constraints. Biotropica 21:308–318

Vogel S (1969) Chiropterophilie in der neotropischen Flora. II. Flora 158:185–350

Waser NM (1998) Pollination, angiosperm speciation, and the nature of species boundaries. Oikos 81:198–201

Waser NM, Chittka L, Price MV, Williams NM, Ollerton J (1996) Generalization in pollination systems, and why it matters. Ecology 77:1043–1060

Wilbur RL (1975) A synopsis of the Costa Rican species of Burmeistera (Campanulaceae: Lobelioideae). Bull Torrey Bot Club 102:225–231