January 2007

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## Recommended Citation

Muchhala, Nathan, "Adaptive Trade-off in Floral Morphology Mediates Specialization for Flowers Pollinated by Bats and Hummingbirds" (2007). *Biology Department Faculty Works*. 163.
DOI: https://doi.org/10.1086/512047
Available at: https://irl.umsl.edu/biology-faculty/163

vol. 169, no. 4 the american naturalist april 2007

# Adaptive Trade-Off in Floral Morphology Mediates Specialization for Flowers Pollinated by Bats and Hummingbirds

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*Submitted June 29, 2006; Accepted October 2, 2006;*
*Electronically published February 5, 2007*

abstract: Evolution toward increased specificity in pollination systems is thought to have played a central role in the diversification of angiosperms. Theory predicts that the presence of trade-offs in adapting to different pollinator types will favor specialization, yet few studies have attempted to characterize such interactions in nature. I conducted flight cage experiments with bats, hummingbirds, and artificial flowers to examine effects of corolla width on pollination. I videotaped visits to analyze pollinator behavior and counted pollen grains transferred to stigmas. Results demonstrated that flower-pollinator fit is critical to effective pollination; wide corollas guided bat snouts better, and narrow corollas guided hummingbird bills better. Poor fit resulted in variable entry angles and decreased pollen transfer. A model using these results predicts that wide corollas will be selected for when bats make more than 44% of the visits and narrow corollas when they make fewer. Intermediate corollas are never favored (i.e., generalization is always suboptimal). This is the first study to clearly document a pollinator-mediated fitness trade-off in floral morphology.

*Keywords: Burmeistera*, disruptive selection, chiropterophily, fitness trade-off, floral evolution, ornithophily.

When should pollination systems evolve toward increased specificity? Biologists have long recognized the potential importance of specialization in floral divergence and speciation (Darwin 1862). Extensive comparative studies across angiosperm families have revealed suites of traits, or pollination syndromes, that reflect adaptation of plants to pollination by particular animals (Pijl 1961; Stebbins 1970; Fenster et al. 2004), and there is some support for a link between specialized pollination and high species diversity (Eriksson and Bremer 1992; Dodd et al. 1999; Hodges et al. 2004). However, we still lack a clear understanding of the factors that promote specialization in pollination systems.

Stebbins (1970) suggested that the floral phenotype of a plant will evolve in response to the most frequent and effective pollinator in its habitat. Waser et al. (1996) formalized this idea with a simple mathematical model and suggested that specialization on the most effective pollinator will evolve when pollinator populations are relatively constant through time and a strong adaptive trade-off is involved, that is, when the adaptations that increase the effectiveness of one pollinator simultaneously decrease the effectiveness of the second by an equal amount. Aigner (2001, 2006) further explored this idea with optimality modeling and demonstrated that the nature of such pollinator-mediated adaptive trade-offs is critical to determining whether specialization will evolve. Sargent and Otto (2006) reached a similar conclusion with a population genetic model. They used a trade-off function to constrain the resources a hypothetical plant could invest in attracting different pollinators and found that the curvature of this function strongly affects whether specialization or generalization is favored.

Despite this theoretical framework stressing the importance of pollinator-mediated trade-offs to floral specialization, we have little empirical evidence as to the ubiquity and strength of such trade-offs in nature. This may reflect the inherent difficulty of examining the interaction between pollinator type and floral phenotype, which requires a factorial analysis with different species of pollinators and different floral phenotypes (Wilson and Thomson 1996). The few studies to do this have only rarely uncovered unambiguous trade-offs. Castellanos et al. (2004) experimentally altered four aspects of *Penstemon* floral morphology and found little evidence for a trade-off in selective pressures imposed by bees and hummingbirds; each manipulation affected pollen transfer only by one of the

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Am. Nat. 2007. Vol. 169, pp. 494–504. © 2007 by The University of Chicago. 0003-0147/2007/16904-41938$15.00. All rights reserved.

two pollinator types or affected both in the same direction. Galen et al. (1987; also see Galen 1996) found that bumblebees select for wide flowers in *Polemonium viscosum* but found only weak evidence for an opposing selective pressure exerted by flies. Although the purity of the pollen that flies deposited on narrow flowers was higher, there was no significant difference in the amount of conspecific pollen they deposited (Galen et al. 1987), and flower width did not significantly affect seed set for fly-pollinated flowers (Galen 1996). Similarly, interactions between various bee species and floral morphology did not take the form of a trade-off for *Impatiens pallidum*, *Erythronium grandiflorum*, or *Pontederia cordata* (Harder and Barrett 1993; Wilson and Thomson 1996). Aigner (2004, 2005, 2006) likewise found that hummingbirds and bees did not impose divergent selective pressures on *Dudleya* floral morphology in either natural or experimentally manipulated populations. Finally, Schemske and Bradshaw (1999) examined the effects of *Mimulus* floral traits on visitation by bees and hummingbirds and found that nectar volume and petal surface area had significant effects on only one of the two pollinator types. Flower color did impose a strong trade-off, affecting visitation rates of bees and hummingbirds in opposite directions (Schemske and Bradshaw 1999; Bradshaw and Schemske 2003).

The rarity of clear examples of trade-offs seems especially perplexing for floral morphology, since the fit between flower and pollinator should be critical to the successful transfer of pollen (Grant and Grant 1965). Numerous studies have demonstrated the strong selective pressures pollinators can exert on floral form (e.g., Nilsson 1988; Armbruster et al. 1994; Campbell et al. 1996; Smith et al. 1996; Cresswell 2000), and floral morphology figures prominently in classic descriptions of pollination syndromes (Baker 1961; Pijl 1961). Nevertheless, the existing data appear to support Waser et al.'s (1996) conclusion that only in extreme cases, such as the obligate mutualism between figs and fig wasps, are constraints of morphological fit strong enough to ensure specialization.

Here I examine the interaction between floral morphology and pollinator type for the Neotropical genus *Burmeistera* (Campanulaceae). Previous work suggests that most species of *Burmeistera* are highly specialized for bat pollination; although hummingbirds also visit their flowers, bats were responsible for 84%–100% of pollen flow in nine species (Muchhala 2006). A tenth species (*Burmeistera rubrosepala*) was pollinated exclusively by hummingbirds. Both bats and hummingbirds occur across the range of *Burmeistera*, and both visit their flowers. Why not generalize on both pollinator types? To look for fitness trade-offs that may favor specialization, I compared the floral phenotypes of the bat-pollinated species to those of the hummingbird-pollinated species. Differences closely matched those predicted by traditional chiropterophilous and ornithophilous pollination syndromes (e.g., Pijl 1961; von Helversen 1993) in terms of anthesis, color, exposure, odor, and corolla morphology (Muchhala 2006). Of these differences, width of the corolla aperture seemed to be the most critical for specialization; outer corolla width was much narrower for the hummingbird-pollinated species (1.9 mm) and varied little across the bat-pollinated species (16.4 mm ± 2.3 SD). I hypothesized that the fit between flower and pollinator imposes an adaptive trade-off in *Burmeistera*; wide flowers guide bat snouts better, and narrow flowers guide hummingbird bills better, resulting in less variation in the angle at which the pollinator enters the flowers and thus more efficient and consistent pollen transfer between flowers. In this study, I test whether such a trade-off exists, and if so, whether it is extreme enough to preclude generalization.

One difficulty in fully exploring selection gradients is that natural populations may not display sufficient phenotypic variation. For flowers, commonly employed methods to overcome this limitation include the use of hybrid swarms (e.g., Schemske and Bradshaw 1999) and experimental manipulation (e.g., Aigner 2004). An advantage of the latter is the ability to vary the desired trait while holding all other floral traits constant, thus eliminating the possibility of indirect selection via a correlated trait (Campbell et al. 1994). In this study, I experimentally manipulated floral width by using three types of artificial flowers: one with a wide corolla opening modeled after bat-pollinated *Burmeistera*, one with a narrow opening modeled after *B. rubrosepala*, and one with corollas of an intermediate width not known to occur in nature. I presented these flowers to bats and hummingbirds in flight cage experiments designed to test how corolla width affects (1) mean pollen transfer and (2) pollinator behavior in terms of entry angle and visit duration. If an adaptive trade-off existed, I expected to find a significant width × pollinator interaction for pollen transfer. With the second experiment, I hoped to elucidate the mechanism behind any differences in pollen transfer.

## Methods

### Study Site

This study was carried out in the Bellavista Cloud Forest Reserve, which is located in the Pichincha province of northwestern Ecuador (00°01′S, 78°43′W), from May to July of 2005. Bellavista is composed of 700 ha of primary and secondary premontane rainforest with an elevational range of 2,000–2,400 m. Experiments were conducted in flight cages set up near the research station.

### *Flowers*

Flowers of *Burmeistera* are zygomorphic (bilaterally symmetrical); corollas have a tubular base that expands distally into a bell shape. Long pedicels place the flowers at approximately 45° above horizontal, and a staminal column positions the reproductive parts above the corolla opening. Anthers are fused together to form a tube into which pollen is shed. During the male phase, pollen is released gradually through the open end of this tube via a "pump mechanism" as the style elongates within the tube (Erbar and Leins 1995). When the stigma emerges from the tube, its dorsal and ventral lobes open in a manner that prevents self-pollination, and the female phase begins (normally by the second day). With this floral morphology, flowers deposit and pick up pollen from the crown of the bat or hummingbird heads. The degree to which the staminal column is exserted beyond the corolla opening affects the exact position of the site of pollen deposition and varies between species. For *Burmeistera rubrosepala*, exsertion averaged 21.1 mm; for nine bat-pollinated species, exsertion ranged from 11.6 to 29.4 mm, with a mean of 18.8 ± 6.2 mm (Muchhala 2006). Full seed set probably requires thousands of pollen grains; hand-pollinated flowers of *Burmeistera sodiroana* produced on average 2,430 seeds ($\pm 388.9$, $N = 8$; N. Muchhala, unpublished data).

I made three types of artificial flowers that varied in the width of the corolla opening (or corolla flare; sensu Galen et al. 1987). The widest was designed after flowers of bat-pollinated species of *Burmeistera* and the narrowest after the hummingbird-pollinated *B. rubrosepala*. The intermediate flower had a width between these two extremes, a floral form not known to occur in nature. Specifically, the distal edge of the corolla opening was 12 mm wide × 20 mm high for wide flowers, 8 × 15 mm for intermediate flowers, and 4 × 10 mm for narrow flowers (fig. 1*A*). For each width, I made two identical flowers so that I could have one "male" and one "female" flower for experimental runs.

Corollas were created by stretching a layer of parafilm over wooden models of the three flower types. The parafilm was then covered with a layer of masking tape and removed from the mold. The parafilm served to waterproof the inside of the corollas, allowing the artificial flowers to be filled with a "nectar" solution of approximately one part honey and nine parts water. A small tube of masking tape (10 mm long × 3 mm in diameter) was affixed to the inner ventral surface of the corolla to facilitate removal and replacement of fresh floral reproductive parts. Finally, one end of a 20-cm-long wire was attached to the base of the artificial corolla and the other end to a glass jar. During experimental runs, jars supporting artificial flowers were placed on a bench so that flowers were positioned at 45° angles approximately 1 m above the ground (which mimics natural positioning of *Burmeistera* flowers; Muchhala 2006).

To make male and female artificial flowers, I collected fresh staminal columns in male or female phase from flowers of *B. sodiroana* and placed them in the tube described above. I trimmed staminal columns so that reproductive parts were exserted 20 mm outside of the corolla opening, which is similar to the exsertion of *B. rubrosepala* (21.1 mm) and the mean exsertion for bat-pollinated *Burmeistera* (18.8 mm; Muchhala 2006). Staminal columns were collected the night before or the day of experimental runs.

### *Pollinators*

The animals used as experimental subjects were captured with mist nets. For bats, I chose *Anoura geoffroyi* as the focal species for this study, since it is the most abundant of the two species of nectarivorous bats known to pollinate *Burmeistera* in Bellavista (N. Muchhala, unpublished data). I captured *A. geoffroyi* individuals at night with mist nets placed in front of flowering *B. sodiroana* and *Burmeistera succulenta* plants. *Adelomyia melanogenys* served as the focal species for hummingbirds. Videotaping demonstrated that this is the most common hummingbird visitor to bat-pollinated species of *Burmeistera* and is the exclusive visitor of the hummingbird-pollinated *B. rubrosepala* (Muchhala 2006). I captured *A. melanogenys* individuals during the day with mist nets placed in front of flowering *B. sodiroana* and an unidentified species of hummingbird-pollinated *Bomarea*.

Immediately following capture, animals were placed in one of two screen tents (3 m × 3 m). Only one animal was held in each tent at a time. On the first day (or night), I trained the animal to feed from the artificial flowers. I placed one artificial flower of each corolla width in the cage, filled each with honey water, and hand fed the animal with one of the flowers before releasing it inside the flight cage. For hummingbirds, when necessary, training was facilitated by affixing a bright red petal from a local ornithophilous flower to each artificial flower. For bats, training was facilitated by placing *Burmeistera* flowers near the artificial flowers in order to attract bats with odor. Animals that did not learn to feed within 3 h were released; those that did were left to habituate to the flight cage for the remainder of the day (or night). Experiments were run over the following 2 days (or nights). Between experimental sessions, animals were allowed to feed freely from test tubes filled with honey water. Animals were released after experiments were complete. I ran each experiment on five individuals each of *A. geoffroyi* and *A. melanogenys*.

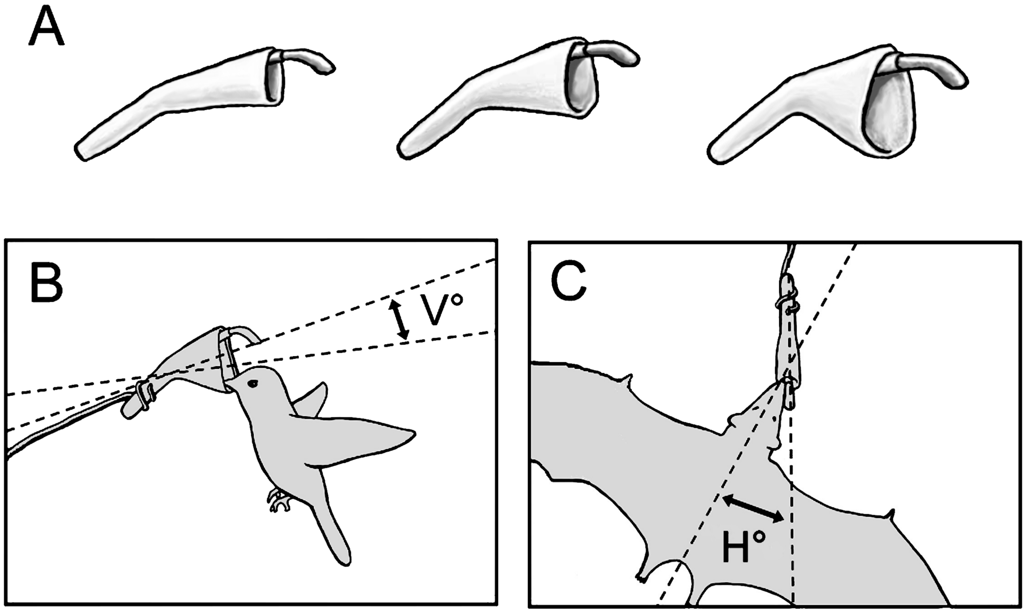

**Figure 1:** Illustrations of artificial flowers and methods. *A*, Narrow, intermediate, and wide corolla types with *Burmeistera sodiroana* staminal columns. *B*, Illustration of a screen-shot from the side-view camera (a hummingbird visit to a wide flower) showing the vertical angle ($V°$) formed by the main axes of the animal and flower (*dashed lines*). *C*, Illustration of a screen shot from the above-view camera (a bat visit to a narrow flower) showing the horizontal angle ($H°$) formed by the main axes of the animal and flower (*dashed lines*). Angles were measured with a protractor during replay of videotapes.

### *Experiment 1: Pollen Transfer*

To document single-visit effectiveness, I measured pollen transferred from one male to one female flower of each width. Male-phase staminal columns were replaced between trials when they no longer released pollen. In order to collect pollen transferred to female flowers, I wrapped the stigma in a layer of parafilm to which I affixed a small square of double-sided tape (see Muchhala 2006). After a visit, I removed this tape from the stigma, placed it on a slide, and covered it with single-sided tape. In the laboratory I examined the tape to quantify the number of grains of pollen transferred.

During experiments, I placed one male flower on the bench, filled it with approximately 0.5 mL of honey water, and waited for the animal to visit it. After a visit I replaced the male with a female flower of the same width (again filled with honey water). I repeated this procedure five times before switching to another flower type. For each pollinator individual (five hummingbirds and five bats), I performed a total of 25 repetitions for each of the three flower types (for a grand total of 750 experimental runs), haphazardly switching between types after each block of five to vary the order. I used the mean of these 25 repetitions for statistical analyses (i.e., I did not treat multiple observations of an individual as statistically independent).

To quantify the pollen grains transferred to female flowers, I counted grains along two transects for each tape sample. To define these transects, I cut a 5 × 10-mm hole in the middle of a 15 × 20-mm square of posterboard and affixed hairs in vertical and horizontal lines through the center of the hole. For each slide, I placed this square over the tape sample and used a light microscope to count all pollen grains along the two transects.

### *Experiment 2: Pollinator Behavior*

The morphology of a flower is critical to pollination because of its role in mediating the position and duration of contact between the pollinator and the plant's reproductive parts. Given that *Burmeistera* flowers deposit pollen on and receive pollen from the top of visitor's heads, the optimal corolla morphology should consistently orient the main axis of the pollinator's head to match the main axis of the flower. Poor orientation may preclude contact with the reproductive parts. Inconsistent orientation may cause reproductive parts of different flowers to contact the body in different areas, decreasing the efficiency of pollen transfer from males to females. Corolla morphology may also affect the handling time that a visitor requires to access the nectar and thus the duration of contact between pollinator and reproductive parts. The goal of this experiment was to analyze the affects of corolla width on pollinator behavior in order to elucidate the mechanism by which corolla width affects pollen transfer.

I videotaped flower visits with two cameras running simultaneously, one positioned above the flower and a second positioned at the side (fig. 1). I was interested in the entry angle, or the angle between the posterior-anterior axis of the animal and the posterior-anterior axis of the flower. Videotaping from both positions allowed me to analyze how the entry angle varies along both horizontal and vertical planes. Viewed from above the flower, the posterior-anterior axis corresponds to the staminal column for flowers, to the bill for hummingbirds, and to an imaginary line that passes from between the eyes through the center of the noseleaf for bats (see fig. 1*C*). The resulting angle between these axes (the horizontal angle, $H°$) will be 0° if the flower guides the pollinator accurately, negative if the pollinator enters from the left side of the corolla, and positive if it enters from the right side. Viewed from the side of the flower, the posterior-anterior axis again corresponds to the staminal column for flowers; for hummingbirds and bats, it corresponds to an imaginary line from the distalmost point of the bill or snout to the top of the head (see fig. 1*B*). The resulting angle (the vertical angle, $V°$) will be 0° if the flower guides the pollinator accurately, negative if the pollinator enters the corolla from below, and positive if it enters from above. When both the horizontal and vertical angles are 0°, the reproductive parts of the flower will successfully contact the center of the pollinator's head. Contact will not occur if the horizontal angle deviates more than approximately 10°; horizontal angles less than 10° will result in off-center contact. For the vertical angle, virtually any deviation from 0° will preclude contact.

For five bats (*A. geoffroyi*) and five hummingbirds (*A. melanogenys*), I videotaped 1 h of visits to each flower width (wide, intermediate, and narrow). Every 15 min I refilled the corolla with honey water. I replayed the tapes in slow motion to record data. For the above-view videotape, I recorded time of visit, duration of visit, and horizontal angle during the visit. I then used the time of visit while replaying the side-view videotape to match up the videos and recorded the vertical angle for each visit. I measured angles on a 20-in television screen with a protractor. If the position of a pollinator's head changed during a visit, I recorded the angle at which deviation from 0° was greatest. I recorded data on only the first 40 visits to each flower type. For each pollinator individual, I calculated the mean and the standard deviation of the horizontal and vertical entry angles. For mean values, I was interested in pollination accuracy, or how much the angle deviated from the "ideal" of 0°. In this sense, an angle of 15° is equally suboptimal whether from the left or right side of the flower; therefore, I used the absolute value of each measurement to calculate the means. For standard deviation, I was interested in pollination precision, or the extent to which the entry angle varied between visits. In this sense, an angle of 15° from the left is very different from an angle of 15° from the right; therefore, I did not change the sign of negative numbers when calculating standard deviations.

### *Statistical Analyses*

For both experiments, I analyzed differences for each pollinator type (hummingbird vs. bat) and corolla width (wide, intermediate, or narrow) with a two-way ANOVA. Pollinator type was treated as a between-subjects fixed factor and flower width as a within-subjects fixed factor. For experiment 1, mean pollen deposition served as the dependent variable. For experiment 2, separate ANOVAs were run for (1) mean visit duration, (2) mean horizontal entry angle, (3) standard deviation of the horizontal entry angle, (4) mean vertical entry angle, and (5) standard deviation of the vertical entry angle. For each ANOVA, within-cell replication was $N = 5$ because the experiments were repeated for five bat and five hummingbird subjects.

## Results

### *Experiment 1: Pollen Transfer*

The two-way ANOVA of the effects of pollinator type and corolla width on pollen transfer detected a highly significant pollinator × width interaction as well as significant main effects for pollinator and width (table 1). The interaction reflects a positive relationship between corolla width and pollen transfer for bats contrasted with negative relationship for hummingbirds (fig. 2). In other words, the wider the corolla, the more pollen transferred by bats and the less pollen transferred by hummingbirds. The main effect of pollinator reflects an overall pollen transfer by bats that was more than four times the pollen transfer by hummingbirds (means $= 136.8 \pm 12.14$ SE vs. $33.7 \pm 9.15$ SE, respectively).

Although I explicitly analyzed only pollen deposition on female flowers, I also noticed a difference in pollen removal from male flowers that deserves mention. It appears to be due to a behavioral difference between bats and hummingbirds; bats tend to treat flowers roughly, leaving the flower and often the whole plant swinging after a visit, while hummingbirds rarely physically displace flowers during their visits (see Muchhala 2006). Because of this, when bats visited male flowers at extreme angles (e.g., those with narrow corollas), they dislodged large amounts of pollen

**Table 1:** Two-way ANOVA table for pollen transfer (experiment 1) and pollinator behavior (experiment 2)

| Experiment, dependent variable, source of variation | df | MS | *F* | *P* |
|---|---|---|---|---|
| 1. Pollen transfer: | | | | |
| Mean pollen deposited: | | | | |
| Pollinator type | 1 | 79,732.40 | 448.86 | **<.001** |
| Corolla width | 2 | 1,355.30 | 9.23 | **.002** |
| Pollinator × width | 2 | 20,774.50 | 141.47 | **<.001** |
| Error (pollinator) | 8 | 485.20 | | |
| Error (width) | 16 | 146.90 | | |
| 2. Pollinator behavior: | | | | |
| Mean horizontal angle: | | | | |
| Pollinator type | 1 | 142.57 | 12.30 | **.008** |
| Corolla width | 2 | 1,355.30 | 9.23 | .135 |
| Pollinator × width | 2 | 160.91 | 19.17 | **<.001** |
| Error (pollinator) | 8 | 11.59 | | |
| Error (width) | 16 | 8.39 | | |
| SD of horizontal angle: | | | | |
| Pollinator type | 1 | 243.11 | 11.91 | **.009** |
| Corolla width | 2 | 25.90 | 2.34 | .129 |
| Pollinator × width | 2 | 190.59 | 17.20 | **<.001** |
| Error (pollinator) | 8 | 20.41 | | |
| Error (width) | 16 | 11.08 | | |
| Mean vertical angle: | | | | |
| Pollinator type | 1 | 51.22 | 26.29 | **.001** |
| Corolla width | 2 | 20.91 | 18.01 | **<.001** |
| Pollinator × width | 2 | 25.76 | 22.30 | **<.001** |
| Error (pollinator) | 8 | 1.95 | | |
| Error (width) | 16 | 1.16 | | |
| SD of vertical angle: | | | | |
| Pollinator type | 1 | 3.68 | 9.625 | **.015** |
| Corolla width | 2 | 1.26 | 7.233 | **.006** |
| Pollinator × width | 2 | 3.14 | 18.017 | **<.001** |
| Error (pollinator) | 8 | .38 | | |
| Error (width) | 16 | .17 | | |
| Visit duration: | | | | |
| Pollinator type | 1 | 4,018.26 | 5.60 | **.046** |
| Corolla width | 2 | 1,301.17 | 1.20 | .328 |
| Pollinator × width | 2 | 1,297.77 | 1.19 | .328 |
| Error (pollinator) | 8 | 717.63 | | |
| Error (width) | 16 | 543.26 | | |

Note: Fixed factors include pollinator type (bat or hummingbird) and corolla width (narrow, intermediate, or wide). Bold type indicates statistical significance ($P < .05$).

despite failing to contact the reproductive parts. Some of this was dispersed over the bat's head, but the majority fell to the ground. In contrast, when hummingbirds visited male flowers at extreme angles (e.g., those with wide corollas) no pollen was dislodged. These male flowers could be reused for multiple experiments since they still contained sufficient pollen, while reproductive parts for all bat-visited male flowers had to be replaced regularly. Such wasted pollen represents a significant fitness cost to the flower that was not formally analyzed in this study.

### *Experiment 2: Pollinator Behavior*

Data were recorded for a total of 1,000 visits, ranging from 89 to 120 visits per individual. The two-way ANOVAs of the effects of pollinator type and corolla width on pollinator behavior detected highly significant pollinator × width interactions for all four analyses of entry angle (table 1). Basically, bats contacted the reproductive parts of flowers with wide corollas more frequently, while hummingbirds contacted those of narrow corollas

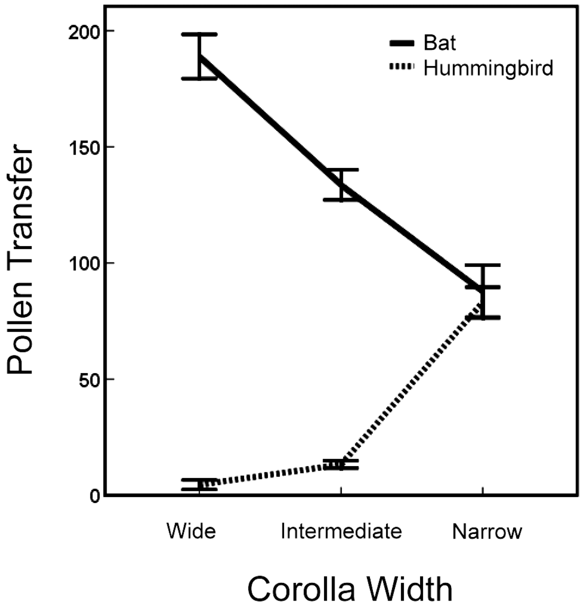

**Figure 2:** Mean single-visit pollen deposition (±1 SE) for bat and hummingbird visits to artificial flowers with three corolla widths. A two-way ANOVA demonstrates a significant pollinator × width interaction and significant main effects for pollinator type and corolla width.

more frequently. For mean horizontal angle and the standard deviation of the horizontal angle, the interactions reflect a positive relationship with corolla width for hummingbirds, contrasted with a negative relationship for bats (fig. 3). For mean vertical angle and the standard deviation of the vertical angle, the interactions reflect a positive relationship with corolla width for hummingbirds contrasted with no relationship for bats (fig. 4). In other words, wide corollas failed to effectively guide hummingbird bills; hummingbird entry angles varied more ( = low precision) and were farther from 0° ( = low accuracy) as floral width increased. In contrast, bat entry angles varied more and were farther from 0° as floral width decreased because bats could not fully insert their snouts into narrow corollas. While width affected both bats and hummingbirds in the horizontal plane, only hummingbird entry angles varied in the vertical plane. They tended to enter wide corollas from below, while bats entered at angles near 0° for all floral widths.

There were also significant main effects for pollinator for all four entry angle analyses. Bats showed overall greater mean entry angles and standard deviations in the horizontal plane, while hummingbirds showed greater mean entry angles and standard deviations in the vertical plane. In the vertical plane, there was a significant main effect for corolla width because visits to narrow corollas showed lower mean entry angles and standard deviations.

Corolla width did not affect visit duration, nor was there an interaction between pollinator and corolla width for visit duration. However, there was a significant main effect of pollinator type; hummingbird visits lasted longer than bat visits on average (1.39 vs. 0.62 s). This result corresponds with observations in nature; on average, hummingbird visits to flowers of *Burmeistera* last more than twice as long as bat visits (1.22 vs. 0.57 s; Muchhala 2006).

## Discussion

Results of this study strongly support the conclusion that the fit between flower and pollinator imposes an adaptive

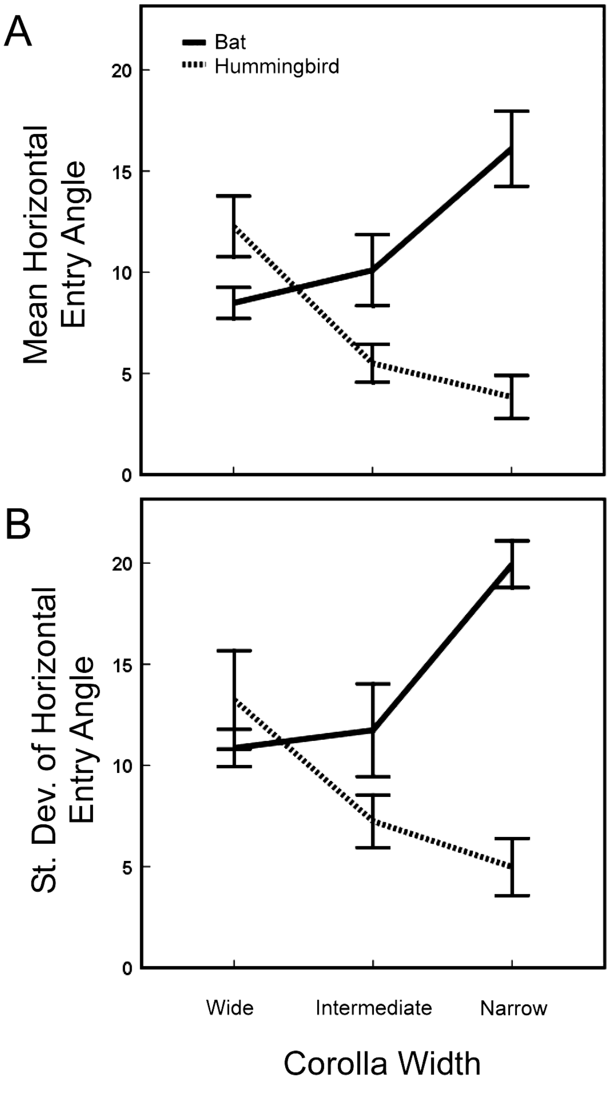

**Figure 3:** Mean horizontal entry angle (*A*) and standard deviation of the horizontal entry angle (*B*) for bat and hummingbird visits to artificial flowers with three corolla widths; entry angle in degrees (±1 SE). For each graph, two-way ANOVAs demonstrate significant pollinator × width interactions and significant main effects for pollinator type.

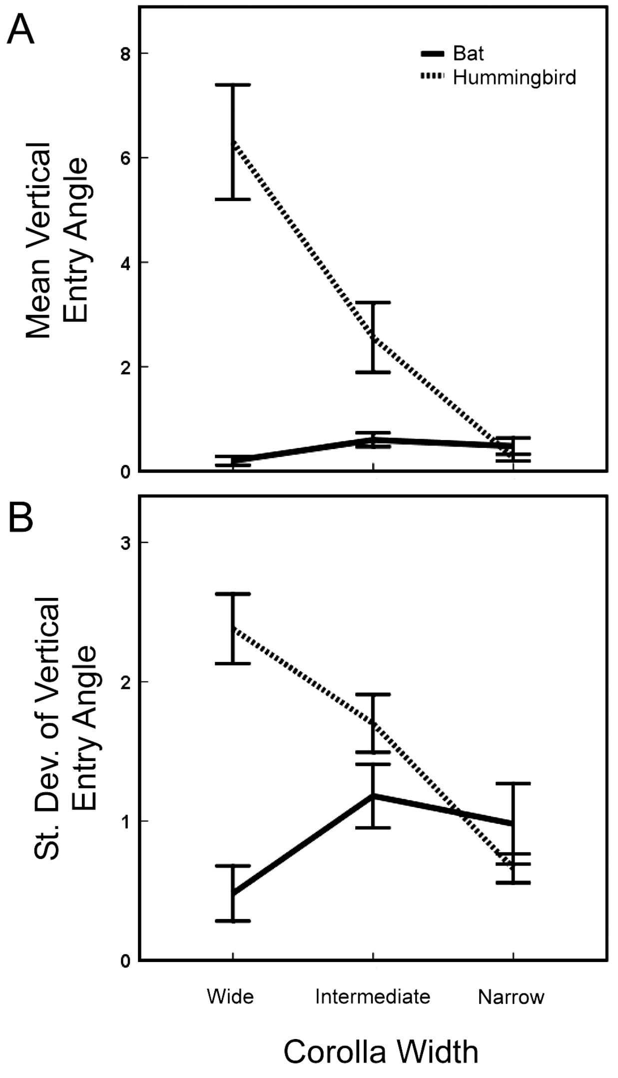

**Figure 4:** Mean vertical entry angle (*A*) and standard deviation of the vertical entry angle (*B*) for bat and hummingbird visits to artificial flowers with three corolla widths. Entry angle is in degrees (±1 SE). For each graph, two-way ANOVAs demonstrate significant pollinator × width interactions and significant main effects for pollinator type and corolla width.

trade-off for *Burmeistera* in specializing on bats or hummingbirds. The width of the corolla aperture affected pollination by each in different ways, as demonstrated by the highly significant pollinator type × corolla width interactions found in the two-way ANOVAs for pollen transfer and pollinator behavior (table 1). The first experiment showed that hummingbirds are significantly more effective at transferring pollen between narrow flowers, while bats are more effective at transferring pollen between wide flowers (fig. 2). The second experiment showed that this difference is not due to differences in visit duration. Rather, narrow flowers guide hummingbird bills better while wide flowers guide bat snouts better, resulting in less variable entry angles that are significantly closer to zero (figs. 3, 4). Only corollas that closely fit the mouthparts of visitors guarantee consistent and effective transfer of pollen. Poor fit decreases pollen transfer by decreasing the frequency of contact with floral reproductive parts and/or by increasing variation in the site of pollen transfer. Taken together, these experiments show that bats and hummingbirds impose disruptive selective pressures on floral form, with bats selecting for wide corollas and hummingbirds selecting for narrow corollas.

In order to adapt to one of the two pollinators, *Burmeistera* flowers must sacrifice the efficiency of the other pollinator. But is this trade-off strong enough to favor the specialized extremes, or would flowers of intermediate width (that utilize both pollinators) have the highest fitness? By assuming relative pollen transfer equates with relative fitness, we can use the results of single-visit pollen deposition (fig. 2) to predict the conditions under which each floral width would be favored. Since the fitness contribution that a pollinator makes to a plant's reproduction depends both on its quality (single-visit effectiveness) and its quantity (visitation rate; Herrera 1987, 1989) the fitness of a plant that utilizes bats and hummingbirds as pollinators can be mathematically modeled as

$$W_G = (V_{bat})g_{bat} + (1 - V_{bat})g_{hum},$$

where $V_{bat}$ represents, out of all visits a plant receives, the proportion that are performed by bats; $1 - V_{bat}$ represents the proportion of visits performed by hummingbirds; $g_{bat}$ represents the single-visit effectiveness of bats; and $g_{hum}$ represents the single-visit effectiveness of hummingbirds (adapted from Waser et al. 1996). Figure 5 uses single-visit effectiveness values from the pollen transfer experiment to estimate the fitness of wide, intermediate, and narrow flowers as the relative proportion of bat visits ($V_{bat}$) varies from 0 to 1. This analysis predicts that, of the three corolla types, wide corollas will have the highest fitness when bats make more than 44% of the visits ($V_{bat} > 0.44$), and narrow corollas will be favored at lower bat abundances ($V_{bat} < 0.44$). An important conclusion of this analysis is that intermediate corollas are never favored; in other words, the trade-off is strong enough to always select for morphological specialization. The absence of intermediate corollas across natural populations and species of *Burmeistera* supports this conclusion; known species have flowers that are either wide and bat-adapted or narrow and hummingbird-adapted (Muchhala 2006).

A limitation of this model is that it overlooks interactions between the pollinator types. That is, increased visits by one pollinator may decrease pollination effectiveness

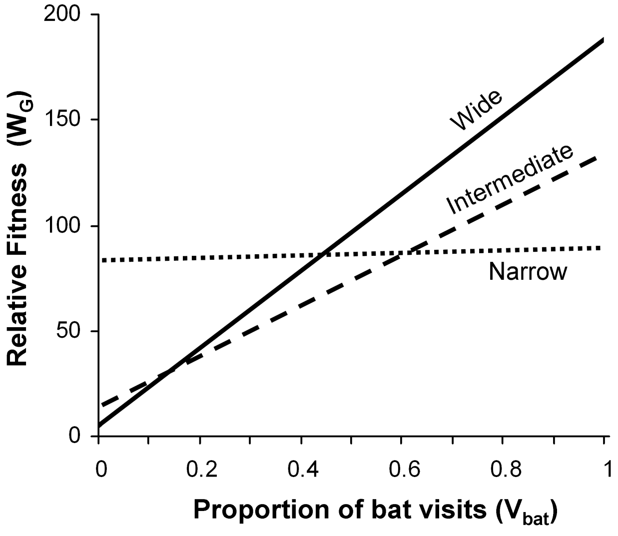

**Figure 5:** Graph of predicted relative fitness ($W_G$) of wide, intermediate, and narrow corollas as the proportion of bat visits ($V_{bat}$) varies from 0 to 1 (e.g., at $V_{bat} = 0$, hummingbirds perform all visits to the flower, while at $V_{bat} = 1$, bats perform all visits). Relative fitness is calculated as $W_G = (V_{bat})g_{bat} + (1 - V_{bat})g_{hum}$, using results from the pollen transfer experiment (fig. 3) for the single-visit effectiveness of bats ($g_{bat}$) and hummingbirds ($g_{hum}$).

of another (Aigner 2001; Thomson 2003). The potential for interaction becomes most apparent when considering that pollen is a finite resource; in other words, for every bat visit that removes pollen, there will be less pollen available for hummingbirds to transport. Although the presence of such an interaction will not change the conclusion that intermediate corollas are maladaptive, it may affect the evolution of narrow corollas. While bats and hummingbirds deposit similar amounts of pollen on narrow female flowers (fig. 2), bats appear to remove much larger amounts of pollen from narrow male flowers during their forceful visits. By wasting large amounts of pollen that would have been better dispersed by hummingbirds, bats may represent "ugly" pollinators for narrow flowers in the same way that pollen-collecting bees are ugly pollinators in the presence of hummingbirds (Thomson 2003). Thus, while the model predicts that a plant with narrow corollas should continue utilizing both bats and hummingbirds, it seems more likely that the plant would increase its fitness by shifting fully to bat pollination (via the evolution of wider corollas) or shifting fully to hummingbird pollination (by excluding bats via other means). It is interesting to note that the only known *Burmeistera* with narrow corollas, *Burmeistera rubrosepala*, is not pollinated by both bats and hummingbirds as the model would predict; it is exclusively pollinated by hummingbirds, despite the presence of nectar bats and bat-pollinated *Burmeistera* in its habitat (Muchhala 2006). Furthermore, it is the only species of *Burmeistera* that does not position its flowers beyond its foliage (Muchhala 2006). Well-exposed flowers are critical to bat pollination (von Helversen 1993; Muchhala 2003); the poorly exposed flowers of *B. rubrosepala* may represent an adaptation to decrease bat visitation and thus prevent bats from "wasting" pollen (and hence genome copies) that would be better dispersed by hummingbirds.

Some caution is warranted in extending the results of this study to other groups of plants, as the importance of flower-pollinator fit is probably highly system specific (Galen and Stanton 1989). The fact that *Burmeistera* flowers are zygomorphic, with joined and coordinated staminal and pistillate parts and precise pollen placement, probably accentuates the observed trade-off. In fact, there are various examples of actinomorphic flowers that are pollinator generalists on both bats and hummingbirds; these typically occur in areas where bat abundance is low or unpredictable (Buzato et al. 1994; Sahley 1996; Fleming et al. 2001; Dar et al. 2006). Flexible stigmas and anthers that transfer pollen indiscriminately over the visitor's body may make a close flower-pollinator fit less important for these plants. Even for zygomorphic flowers, special adaptations can allow generalization, as evidenced by the bat and hummingbird-pollinated *Siphocampylus sulfureus* (Sazima et al. 1994). The ventral corolla lobe of this species constricts the aperture of its corolla; it is firm enough to hold this shape when hummingbirds visit the flower (thus restricting the vertical entry angle and ensuring contact with its reproductive parts) yet flexible enough to bend downward during more forceful bat visits.

An interesting and somewhat unexpected result of this study was the significant main effect of pollinator type in the two-way ANOVA for pollen transfer (table 1; fig. 2). On a per-visit basis, bats transferred significantly more pollen than hummingbirds; averaged across the three flower widths, this corresponds to more than four times as many grains. Even at narrow flowers, hummingbirds did not transfer more pollen than bats. This was surprising because I modeled narrow flowers after those of the hummingbird-pollinated *B. rubrosepala.* These results suggest that there may be some inherent difference between bats and hummingbirds that makes bats more effective as pollinators in terms of the female component of pollination and fitness. Observed pollen transfer in natural populations of *B. rubrosepala* and bat-pollinated species of *Burmeistera* supports this idea. On average, bats transferred 57.4 pollen grains per night to flowers of bat-pollinated *Burmeistera*, while hummingbirds transferred only 15.6 pollen grains per day to *B. rubrosepala* (Muchhala 2006). This fourfold disparity was apparently not due to a difference in visitation rates, as hummingbirds averaged 0.24 visits per hour to *B. rubrosepala* and bats averaged 0.27 visits per hour to the bat-pollinated *Burmeistera* (Muchhala 2006). Why would bats be more effective at transferring pollen than hummingbirds? Two possible explanations are (1) they contact floral reproductive parts more forcefully, resulting in greater pick-up and deposition of pollen, or (2) fur holds pollen better than feathers, resulting in less pollen being lost during flights between flowers. Studies are currently under way to test these hypotheses. Regardless of the mechanism, this difference in effectiveness suggests that pollination by bats should be favored when bats are sufficiently abundant. I predict that phylogenetic studies of transition rates (e.g., Janson 1992, Armbruster 2002) between floral syndromes will reveal higher rates of switches from ornithophily to chiropterophily than vice versa.

This study further supports the importance of the mechanical fit between flower and pollinator (e.g., Armbruster et al. 1994, 2004, 2005; Nilsson 1988; Cresswell 2000). For many chiropterophilous flowers like those of bat-pollinated *Burmeistera*, corollas closely fit the heads of bats in what has been termed a "head-mask" morphology (von Helversen 1993). This study demonstrates the negative effects deviation from this morphology can have on fitness. The conclusion that bats may impose strong stabilizing selection on corolla width is further supported by the fact that out of 11 different measurements of floral dimensions across nine species of bat-pollinated *Burmeistera*, outer and inner corolla width showed the lowest coefficients of variation (Muchhala 2006). While other studies have quantified how floral morphology affects pollination by hummingbirds (Grant and Temeles 1992; Campbell et al. 1996; Smith et al. 1996; Temeles and Rankin 2000), to my knowledge, this is the first one to do so for bats. More importantly, this is the first study to show that flower-pollinator fit can be critical enough to impose an adaptive trade-off. The handful of other studies that have tested the interaction between pollinator type and floral morphology have found variation in the strength of the selective pressures exerted by different pollinators but no unambiguous example of pollinators selecting in different directions; instead, all pollinators select in the same direction, or only one type exerts significant selective pressures (Galen et al. 1987; Harder and Barrett 1993; Wilson and Thomson 1996; Aigner 2004).

This study is important not only in documenting a pollinator-mediated adaptive trade-off but in showing that the trade-off is extreme enough to select for floral specialization. Schemske and Bradshaw (1999) found that petal anthocyanin concentration correlated negatively with bee visitation and positively with hummingbird visitation, which is highly suggestive of a fitness trade-off, but they did not test how this affects fitness in terms of pollination success or seed set. Theoretical models demonstrate that the nature of such fitness trade-offs is critical to the evolution of floral specialization (Aigner 2001; Sargent and Otto 2006). Empirical data from other plant-pollinator systems are needed to fully understand the role trade-offs in floral phenotype may have played in the evolution of angiosperms.

## Acknowledgments

I am grateful to P. Aigner, S. Armbruster, T. Fleming, C. Horvitz, W. Searcy, and an anonymous reviewer for insightful comments on this manuscript, and to C. McCain, K. Murray, R. Muscarella, and S. Scobell for discussion of an earlier draft. I thank A. Caiza, A. Hubenthal, and J. C. Vizuete for invaluable help with fieldwork and R. Parsons and the staff of the Bellavista Cloud Forest Reserve for logistical support. Funding was provided by a Graduate Research Fellowship from the National Science Foundation and a Student Grant from Bat Conservation International.

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