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2010

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Submitted July 2, 2009; Accepted February 12, 2010; Electronically published April 21, 2010

ABSTRACT: One floral characteristic associated with bat pollination (chiropterophily) is copious pollen production, a pattern we confirmed in a local comparison of hummingbird- and bat-adapted flowers from a cloud forest site in Ecuador. Previous authors have suggested that wasteful pollen transfer by bats accounted for the pattern. Here we propose and test a new hypothesis: bats select for increased pollen production because they can efficiently transfer larger amounts of pollen, which leads to a more linear male fitness gain curve for bat-pollinated plants. Flight cage experiments with artificial flowers and flowers of *Aphelandra acanthus* provide support for this hypothesis; in both instances, the amount of pollen delivered to stigmas by birds is not related to the amount of pollen removed from anthers on the previous visit, while the same function for bats increases linearly. Thus, increased pollen production will be linearly related to increased male reproductive success for bat flowers, while for bird flowers, increased pollen production leads to rapidly diminishing fitness returns. We speculate that fur takes up and holds more pollen than feathers, which seem to readily shed excess grains. Our gain-curve hypothesis may also explain why evolutionary shifts from bird to bat pollination seem more common than shifts in the opposite direction.

Keywords: sex allocation theory, male fitness gain curves, chiropterophily, ornithophily, *Aphelandra acanthus*, *Burmeistera*.

Introduction

Although flower-visiting bats have evolved only recently relative to other flower-visiting animals (Fleming and Muchhala 2008), many angiosperm species from a wide range of families have adapted to bat pollination (Dobson and Peikert-Holle 1985; Fleming et al. 2009). Chiropterophilous flowers typically display a predictable suite of traits, including nocturnal anthesis, dull coloration, musty odor, wide flower openings, large amounts of pollen, and large amounts of hexose-rich nectar (Faegri and van der

Pijl 1979; von Helversen 1993). Most bat-pollinated flowers in the New World are thought to have evolved from hummingbird-pollinated ancestors (Skog 1976; Gottsberger 1986; Sazima and Sazima 1988; Buzato et al. 1994; Sazima et al. 2003; Martén-Rodríguez et al. 2009), and several recent phylogenetic studies support this hypothesis (Kay 2003; Perret et al. 2007; Knox et al. 2008; Martén-Rodríguez 2008; E. Tripp, unpublished data), although a shift from euglossine bee pollination has also been documented (Mori and Boeke 1987; Mori et al. 2007).

Although many chiropterophilous traits can readily be explained as adaptations to optimize pollination by large, nocturnal, nonvisual, olfactory-oriented animals, the adaptive significance of increased pollen production is not obvious. This increase is achieved through greater pollen quantity per anther (Sazima et al. 2003), greater number of anthers per flower (von Helversen 1993), or a greater proportion of male flowers relative to hermaphrodite flowers (i.e., andromonoecy; Heithaus et al. 1974; Ramirez et al. 1984). Earlier authors simply noted the correlation (Pijl 1961; Skog 1976), whereas later authors offered brief adaptive hypotheses. These suggest that increased pollen production is selected for because bats are inefficient pollinators, either because they groom their fur and consume pollen (Faegri and van der Pijl 1979; von Helversen 1993) or because their body surface area is much larger than the stigma surface area of the flowers they pollinate (Heithaus et al. 1974; Proctor et al. 1996; also see Cruden and Miller-Ward 1981). According to the latter idea, a plant that switches to a large-bodied pollinator spreads its pollen over a larger area; thus, given the same size of stigma, more pollen is needed to ensure that a similar number of grains reach the stigma.

As presented, both of these efficiency hypotheses fall short by failing to separate the male and female functions of a flower, as is done more explicitly in sex allocation theory. Inefficient pollinators may bring less pollen to stigmas, but this can only affect selection on female function

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(to increase pollen receipt). Changes in pollen production undergo selection through male function; that is, selection will favor those individuals in the population that successfully father the most offspring. Therefore, optimal pollen production depends on intraspecific competition with other males, and the fitness of a given male individual relative to that of other males is not affected by the general level of wastefulness of the pollinator that the species uses. This idea has been formalized through evolutionarily stable strategy (ESS) models of sex allocation; changes in the efficiency of the pollination mode lead to changes in the intensity of male-male competition but do not affect the ESS allocation to male function (Charnov 1982; Lloyd 1984).

Furthermore, recent work calls into question the assumption that bats are inefficient pollinators. Despite similar visitation rates, primarily bat-pollinated species of *Burmeistera* receive more than four times as much pollen as the hummingbird-pollinated *Burmeistera rubrosepala* (Muchhala 2006). On average, bats transferred more than four times as much pollen as hummingbirds per visit to artificial flowers of various widths in flight cage experiments; they even transferred more than hummingbirds to artificial flowers with narrow corollas, which were modeled after hummingbird-adapted flowers (Muchhala 2007).

While conducting these flight cage experiments, N. Muchhala noticed that hummingbirds that flew away from a donor flower with a bright white patch of pollen on their feathers would typically have only a light gray dusting on their subsequent visit to a recipient flower. In contrast, bats that left with large amounts of pollen returned with those loads apparently undiminished. This observation prompted an alternative hypothesis for the adaptive significance of increased pollen production in bat-pollinated flowers. We suggest that the feathered surfaces of hummingbirds can become "saturated" with a modest load of pollen, beyond which excess grains are simply shed or blown off in flight. In contrast, the furred surfaces of bats can take up and hold larger amounts before any excess is shed. As a result, male fitness returns for hummingbird-pollinated flowers would level off at lower amounts of pollen removal from anthers per visit and, all else being equal, selection should favor higher pollen production in bat-pollinated flowers. This argument thus depends on the shapes of male gain curves or the relationship between the resources invested in male function (e.g., pollen production) and the corresponding fitness gain accrued (Charnov 1982; Brunet 1992; de Jong and Klinkhamer 2005). Specifically, we predict that the male gain curve for pollen production decelerates less rapidly for bat-pollinated plants than for hummingbird-pollinated ones.

Our study had three phases. First, we determined the amount of pollen produced by flowers from a number of

different bat- and hummingbird-pollinated species from one Neotropical habitat. Second, we ran flight cage experiments with bats, hummingbirds, and artificial flowers with three differing amounts of pollen and examined how this affected subsequent pollen delivery. Third, we ran similar flight cage experiments with *Aphelandra acanthus*, a species known to be pollinated by both animals (Muchhala et al. 2009), and measured both pollen removal and delivery. We compared the relation between pollen removal and pollen delivery for bat and hummingbird pollination.

Methods

Part 1: Pollen Counts

While many descriptions of pollination syndromes mention that bat-pollinated flowers produce copious amounts of pollen, evidence is typically anecdotal, and the magnitude of the difference is not known. We sampled a set of local species during our study of pollen transfer. We collected anthers from all known bat-pollinated species in the Bellavista Cloud Forest Reserve (Pichincha Province, Ecuador: 00°01'S, 78°41'W) and a number of hummingbird-pollinated species with flowers of a similar size. Hummingbird flowers can be roughly divided into two groups: those that place pollen on the bill and those that use the feathers (typically of the head). The former have smaller flowers with correspondingly small anthers and little pollen production. Therefore, to be conservative, we decided to focus on the latter group, which have flowers comparable to bat flowers in overall size and function. Before anthesis, we bagged flowers to prevent visitation. After anthesis, we measured greatest flower length (from base of corolla to distal end of the corolla lobes or petals) and greatest flower width (greatest width of corolla tube or petals), removed the anthers, and placed them in microcentrifuge tubes with 0.2 mL of 70% ethanol. We later estimated pollen production and pollen grain size using an Elzone 282PC electronic particle counter (Particle Data, currently Micromeritics, Norcross, GA; see Harder 1990b). Data were collected for three flowers for each species (listed in table 1), from different individuals in all cases except *Passiflora* sp., for which we found only one individual in flower. We analyzed differences in pollen production, pollen size, flower length, and flower width between bat and hummingbird flowers with a *t*-test or a Mann-Whitney *U* when assumptions of normality or homogeneity of variance were not met.

Part 2: Pollen Delivery with Artificial Flowers

This experiment tested how pollen delivery for bats and hummingbirds differed given low, medium, or high pollen

Table 1: Flower characteristics, including pollen production per flower (\pm SE), for eight hummingbird-pollinated and five bat-pollinated species from a cloud forest site in Ecuador

Family and species	Flower color	Anthers per flower	Corolla length (mm)	Corolla width (mm)	Pollen grains per flower	Pollen diameter (μ m)
Hummingbird pollinated:						
Campanulaceae:						
<i>Burmeistera rubrosepala</i>	Red/yellow	5	18.6 \pm .20	10.2 \pm .24	23,001 \pm 3,956.2	29.7
<i>Centropogon aequatorialis</i>	Purple	5	46.4 \pm 2.48	10.0 \pm .46	20,546 \pm 3,330.2	29.3
<i>Centropogon solanifolius</i>	Red	5	53.1 \pm 1.89	9.7 \pm .07	25,425 \pm 1,939.5	29.4
Gesneriaceae:						
<i>Kohleria affinis</i>	Pink	4	55.7 \pm 1.96	21.3 \pm .6	66,777 \pm 4,187.4	20.8
<i>Glossoloma oblongicalyx</i>	Red	4	58.9 \pm 1.91	16.0 \pm .9	26,591 \pm 1,979.6	30.6
<i>Columnnea strigosa</i>	Orange	4	69.6 \pm .98	17.0 \pm .96	40,775 \pm 3,100.8	28.6
Onagraceae:						
<i>Fuchsia macrostigma</i>	Red	8	97.1 \pm 3.78	31.2 \pm 1.8	3,194 \pm 649.1	...
Solanaceae:						
<i>Ichroma calycinum</i>	Purple	5	73.0 \pm 2.0	16.1 \pm .2	38,807 \pm 2,921	27.2
Mean			59.1 \pm 8.01	16.4 \pm 2.56	30,639 \pm 7,505.1	27.9
Bat pollinated:						
Campanulaceae:						
<i>Burmeistera sodiroana</i>	Green/purple	5	27.7 \pm .91	17.8 \pm .18	33,862 \pm 2,505.2	36.7
<i>Centropogon nigricans</i>	Green	5	87.1 \pm 6.68	19.4 \pm 1.46	142,776 \pm 10,462.3	31.6
Melastomataceae:						
<i>Meriania pichinchensis</i>	Orange	12	34.3 \pm 1	36.0 \pm 1.82	519,503 \pm 32,425.2	16.4
Passifloraceae:						
<i>Passiflora</i> sp.	Green	5	73.8 \pm 1.6	44.4 \pm 2.02	47,110 \pm 7,622.1	51.7
Solanaceae:						
<i>Trianaea</i> sp.	Green	4	105.0 \pm 1.97	47.5 \pm 1.64	349,173 \pm 40,194.4	31.4
Mean			65.6 \pm 15.00	33.0 \pm 6.19	218,485 \pm 94,042.2	33.6

Note: $N = 3$ for each species.

availability from male flowers. We captured animals with mist nets and placed them in 3 \times 3-m flight cages set up in the field. Experiments were conducted over the following 2–3 d, after which animals were released. To test pollen delivery, we used semiartificial flowers modeled after hummingbird- and bat-adapted species of *Burmeistera*. These incorporated real sex organs in artificial flowers, which were constructed by stretching a layer of Parafilm over a wood mold and covering this with a layer of masking tape (see Muchhala 2007). Corollas were bell-shaped, with a flaring mouth 4 mm wide for hummingbirds and 12 mm wide for bats, and oriented at approximately 45° relative to the horizon to mimic *Burmeistera* flower orientation. A small tube of masking tape (10 mm long \times 3 mm in diameter) was affixed to the inner ventral surface of the corolla to facilitate removal and replacement of fresh floral reproductive parts. We installed male-phase anther tubes from *Burmeistera sodiroana* flowers for “male” artificial flowers. For “female” flowers, we installed a female-phase anther tube (with stigma emerged) in the flower, covered the distal portion in a layer of Parafilm, and affixed a small square of double-sided tape to the end. After a pollinator

visit, we placed this tape on a microscope slide and covered it with a layer of single-sided tape. We later estimated pollen transfer by counting all pollen grains along two transects (vertical and horizontal) through the geometrical center of the tape sample.

Because we wanted to vary the amount of pollen available to visitors, we used the natural pollen presentation mechanism to produce three treatment levels. *Burmeistera* flowers exhibit secondary pollen presentation; pollen is shed into the anther tube and made available to pollinators through an opening at the distal end of the tube. Throughout the male phase (1–2 days), the stigma elongates within this tube and, like a piston, pushes more pollen toward the opening. If undisturbed, pollen remains clumped together as it pushes out of the opening, potentially presenting a large dose to the next visitor. For the “low” treatment, we brushed off any excess pollen from the end of the anther tube before presenting the flower to the pollinator, minimizing the pollen dose size. For the “medium” treatment, we gently squeezed the anther tube until a clump of pollen emerged from the end of the tube, thus making a large dose available. For “high” treatments, we

allowed the animal to visit two male-phase flowers with large doses (as in the medium treatment) in rapid succession before visiting the female flower.

After an animal picked up pollen in one of these three treatments, we allowed it to visit a single female recipient flower. We performed 27 replicates of each level for one hummingbird individual (*Adelomyia melanogenys*) and 21 replicates of each treatment level for one bat individual (*Anoura geoffroyi*), with the treatment order randomized in blocks of three. Hummingbird experiments were performed on July 1–2, 2005, and bat experiments were performed on August 9–10, 2005. Unequal variances precluded analyzing the hummingbird and bat data together in a two-way ANOVA (Levene's test: $F_{3,135} = 8.61$, $P < .0005$); therefore, we analyzed results with a separate one-way ANOVA for each pollinator type (these met assumptions of normality and homogeneity of variance). Treatment level (low, medium, or high pollen) served as a fixed factor and pollen delivery to recipient flowers as the dependent variable.

Part 3: Pollen Removal and Delivery with Aphelandra acanthus Flowers

With this second set of experiments, we wanted to measure pollen removal more precisely and test more individuals and species of bats and hummingbirds. Rather than artificial flowers, we used flowers of *Aphelandra acanthus*, which are visited and pollinated by both bats and hummingbirds at Bellavista (Muchhala et al. 2009). And rather than using three treatment levels of pollen available for removal, in this case we estimated actual pollen removal. We ran experiments with two species of bats (*A. geoffroyi* and *Anoura fistulata*) and two species of hummingbirds (*A. melanogenys* and *Heliodoxa rubinoides*) from January 11 to March 9, 2008. As in the previous experiment, animals were captured with mist nets and held for 3–4 d in 3×3 -m flight cages set up in the field.

Aphelandra acanthus flowers have four anthers that are positioned under the upper petals, such that pollen transfer occurs on the dorsal surfaces of the heads of bats and hummingbirds. For experimental runs, we either left the anthers of the pollen donor flowers untouched (whole treatments) or gently scraped the distal half of each anther head to remove a portion of the pollen (half treatments). Anthers were scraped with scissors, and we were careful to remove the same portion from each of the four anthers. Before we began experiments, one anther was selected at random, removed from the flower with scissors, and placed in a microcentrifuge tube with 0.2 mL of 70% alcohol. By counting these grains and assuming uniformity among the anthers, we could estimate the amount of pollen present before the visit. The animal was then allowed to visit the

donor flower, followed by a single recipient flower. A loop of single-sided tape was placed around the reproductive parts and the two dorsal petals of each recipient flower, such that the adhesive surface was facing out just under the stigma (i.e., covering the stigma) and could collect any pollen deposited (see Muchhala et al. 2009). Tape samples were placed on a microscope slide. Following this run, a second anther was collected from the donor flower and placed in a microcentrifuge tube so that per-visit removal could be estimated. The donor flower, with its two remaining anthers now somewhat depleted of pollen, was then used again for a second run; the same animal was allowed to visit it, followed by a pollen recipient. After this visit, one of the two remaining anthers was collected and the donor flower was discarded. Thus, for these experiments, there were four treatment levels of pollen available in donor flowers: (1) full anthers, first visit, (2) full anthers, second visit, (3) half anthers, first visit, and (4) half anthers, second visit. By varying pollen availability in this way, we hoped to obtain experimental runs that displayed a wide range of amounts of pollen removal. Pollen counts for the tape samples from recipient flowers were estimated with a light microscope. We counted all pollen grains along two transects (vertical and horizontal) through the sample, aligning the vertical transect with the greatest visual density of the pollen deposit before viewing through the microscope. Pollen counts for the anthers collected in microcentrifuge tubes were estimated in the lab with the Elzone particle counter. Because some of these tubes opened in transit, eight *H. rubinoides*, one *A. melanogenys*, and four *A. fistulata* samples were lost.

For statistical analyses, we produced plots of "stigma" counts of pollen delivery to recipients versus estimates of pollen removed from the preceding donor flower. We then compared the slopes of the least squares regression lines for bats and hummingbirds with a Mann-Whitney *U*-test. The stochasticity inherent in the mechanical processes of pollen pickup and delivery, in combination with the errors arising from imprecise estimation of removal rates, contributed great variability to these plots. For example, some pollen removal estimates were negative, in part because of the assumption that all four anthers were equal in terms of their pollen production and subsequent depletion during visits. Given that such negative estimates were likely counterbalanced by similar variance in the opposite direction, we opted to include these points in the regression analyses.

The corolla apertures of *A. acanthus* flowers are wide enough that hummingbirds occasionally visit without contacting the reproductive parts (Muchhala et al. 2009; see also Muchhala 2007). In preliminary trials, this was especially common for *A. melanogenys*, as it is relatively small. Therefore, for experiments with this species, we

narrowed the corollas (also see Castellanos et al. 2004) by cutting two longitudinal slits along the right and left sides of the corolla tube and wrapping a “belt” of masking tape around the corolla; this approximately halved the diameter of the corolla opening (from 8 to 4 cm). Additionally, since we were interested in the dynamics of pollen removal and delivery rather than the frequency of effective visits, for both species of hummingbirds we allowed individuals to perform a second visit in cases where they clearly did not contact anthers or stigmas on the first visit.

Before presenting each donor flower, we checked to verify that there were no visible amounts of pollen on the head of the animal. For bats, there were often large amounts of pollen left over from the previous experiment. In these instances, we allowed the bat to feed from a test tube with bridal veil wrapped around a wire ring (slightly wider than a bat's head) affixed immediately in front of the opening. As the bat pushed its head through the ring to reach sugar-water at the base of the tube, excess pollen was brushed off on the bridal veil.

Results

Part 1: Pollen Counts

The bat-pollinated species produced 218,485 ($\pm 94,042.2$) pollen grains per flower on average (table 1), significantly more than hummingbird-pollinated species ($30,639 \pm 7,505.1$; Mann-Whitney $U = 4$, $P = .019$) by a factor of 7.1. Bat flowers were also significantly wider by a factor of 2.0 (Mann-Whitney $U = 4$, $P = .019$), and the diameter of bat flower pollen grains was larger by a factor of 1.2, although this trend was not quite significant (Mann-Whitney $U = 7$, $P = .088$). There was no difference in flower length ($t = -0.42$, $df = 11$, $P = .68$); thus, differences in flower size do not appear to be driving differences in pollen production. One species that is pollinated by both bats and hummingbirds, *Aphelandra acanthus*, produced 6,892 (± 188.3 , $N = 134$) pollen grains per flower. This is lower than the bat-pollinated species yet significantly higher than primarily hummingbird-adapted species of Acanthaceae; Linhart et al. (1987) reported that *Razisea spicata* produce a mean of 3,094.4 grains per flower (one-sample t -test: $t = 20.17$, $df = 133$, $P < .0005$) and *Hansteinia blepharorachis* produce 5,696.3 grains per flower (one-sample t -test: $t = 6.35$, $df = 133$, $P < .0005$).

Part 2: Pollen Delivery with Artificial Flowers

A one-way ANOVA testing the effect of pollen availability on pollen delivery for the bat was highly significant (between-groups mean square = 2.02×10^5 , $F_{2,60} =$

21.59, $P < .0005$). Pollen delivery increased as pollen availability increased over the three levels (fig. 1). For the hummingbird, a similar one-way ANOVA found no significant differences in pollen delivery (between-groups mean square = 2,155, $F_{2,78} = 1.57$, $P = .21$).

Part 3: Pollen Removal and Delivery with *Aphelandra acanthus* Flowers

For all six individuals from both species of bats, there were significant linear correlations between pollen removal and subsequent delivery (pooling all individuals: $r^2 = 0.17$, $P < .0005$). For all six individuals from both species of hummingbirds, there were no correlations between the variables (pooling all individuals: $r^2 = 0.002$, $P = .49$; fig. 2). Comparison of the slopes of regression lines shows significantly greater slopes for bats (Mann-Whitney $U = 36$, $P < .0005$).

Discussion

Flowers adapted for pollination by bats have long been thought to produce more pollen than flowers of other animal-pollinated plants (Pijl 1961; Skog 1976; von Helversen 1993). Quantification of pollen production in our study site reveals that the bat-pollinated flowers produced more pollen than hummingbird-pollinated flowers by a

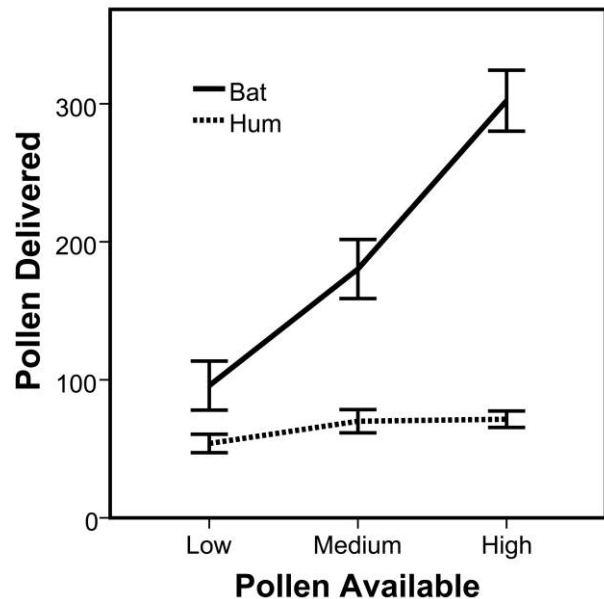


Figure 1: Results for part 2. Mean pollen delivery (\pm SE) to artificial female flowers by a bat and a hummingbird after visiting male flowers with three different levels of pollen availability (low, medium, and high).

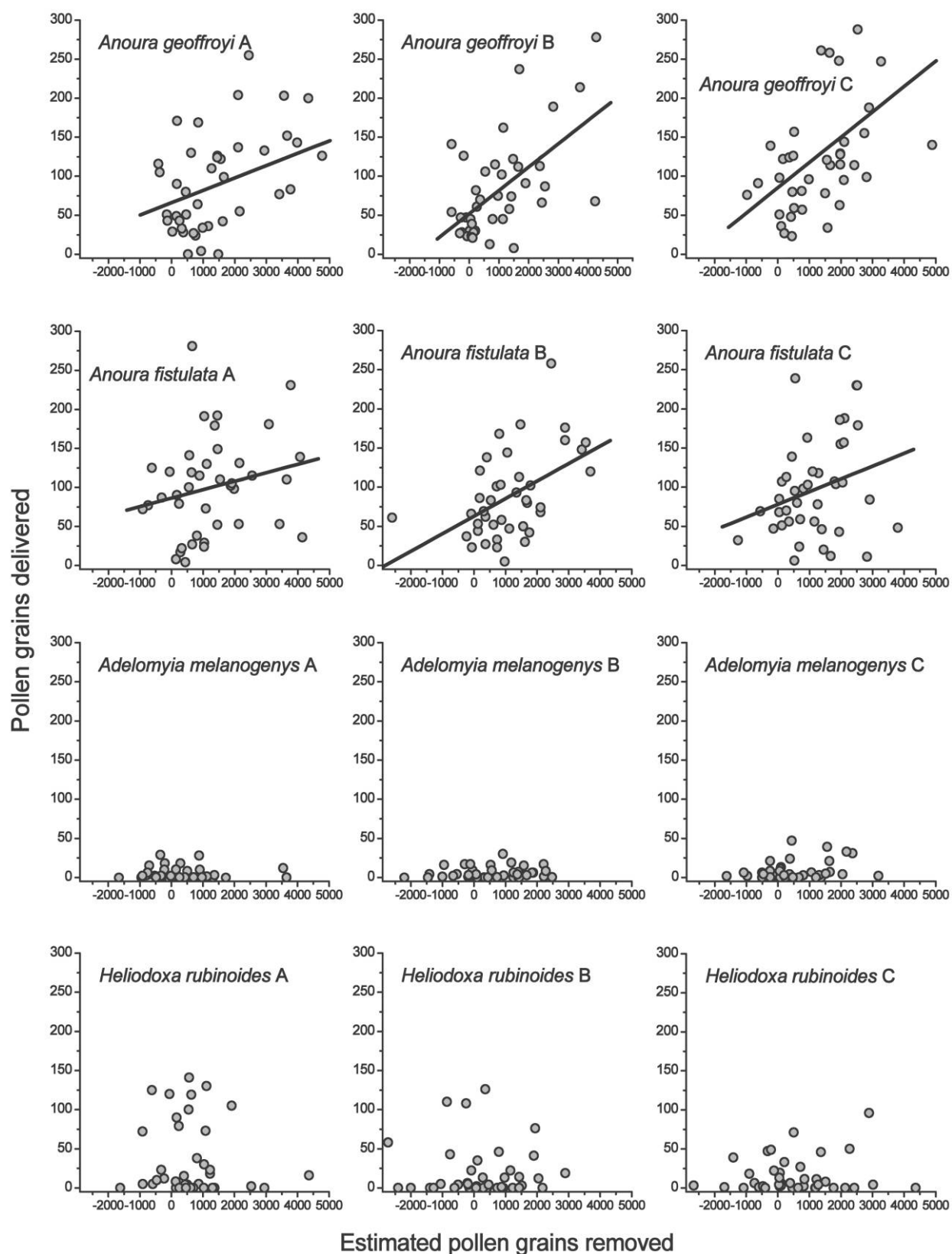


Figure 2: Results for part 3. Pollen delivery to *Aphelandra acanthus* flowers as a function of estimated pollen removal for bats (*Anoura geoffroyi* and *Anoura fistulata*) and hummingbirds (*Adelomyia melanogenys* and *Heliodoxa rubinoides*). Letters correspond to different individuals.

factor of seven on average (218,485 vs. 30,639 pollen grains; table 1). Flower size alone cannot account for this magnitude of difference; although bat flowers were twice as wide on average (also see Muchhala 2007), there was no significant difference in flower length (table 1). Our analysis should be considered preliminary; comparisons of sister taxa would be more informative than our opportunistic sampling from a local flora, but it is interesting to note that the bat-pollinated *Centropogon* species produced about six times more pollen than the two hummingbird-pollinated *Centropogon* species (table 1). Additionally, *Burmeistera rubrosepala*, a hummingbird-pollinated species (Muchhala 2006) that evolved very recently from bat-pollinated ancestors (Knox et al. 2008), produced about two-thirds as much pollen as *Burmeistera sodiroana*.

We hypothesized that increased pollen production in chiropterophilous flowers is favored because the corresponding male gain curve is nearly linear, whereas the male gain curve for hummingbird-pollinated flowers is more sharply saturating because of differences in pollen-transfer mechanics. Our observations suggest that limited amounts of pollen can be profitably placed on the heads of a hummingbird; excess is quickly shed. Our results support this hypothesis. In our experiments with artificial flowers, pollen transfer by the bat *Anoura geoffroyi* increased over the three levels of pollen availability, whereas pollen transfer by the hummingbird *Adelomyia melanogenys* was not significantly affected by pollen level (fig. 1). In other words, presenting a hummingbird with a high pollen load did not provide any increase in pollen transfer over presenting a medium-sized pollen load. Our experiments with flowers of *Aphelandra acanthus* further supported these results and demonstrated that the difference in pollen deposition on recipient flowers was not simply due to differences in pollen removal from donor flowers. For the nectar bats *A. geoffroyi* and *Anoura fistulata*, there were significant positive correlations between pollen removal and subsequent pollen delivery, whereas no correlations were detected for the hummingbirds *A. melanogenys* or *Heliodoxa rubinoides* (fig. 2).

Although it is encouraging to find similar results for both our artificial flowers and those of *A. acanthus*, two caveats suggest caution in evaluating the gain-curve hypothesis. First, the relationship between pollen removal and subsequent delivery in nature may be further influenced by behavioral factors not measured in our flight cage experiments, such as floral fidelity (Flanagan et al. 2009), distances flown between plants (Ghazoul 2005), pollen carryover (Thomson and Thomson 1989; Fenster et al. 1996), or geitonogamous pollen transfer (Galloway et al. 2002; Karron et al. 2009). Any differences between bats and hummingbirds in these factors could further influence differences in the corresponding male gain curves.

Second, the *Burmeistera* flowers used in the first experiment are primarily bat adapted (Muchhala 2006), and bats provide more pollen transfer than hummingbirds for flowers of *A. acanthus* (Muchhala et al. 2009); thus, it is possible that the pollen of these species has evolved special adaptations to maximize adhesion to bat fur (e.g., differences in pollenkitt or electrostatic charges; Vuknin et al. 2001; Pacini and Hesse 2005). It would be useful to perform similar pollen-transfer experiments with other angiosperm species. However, with these caveats in mind, our results provide initial support for the idea that bats select for increases in pollen production by providing less saturating male gain curves.

Phylogenetic evidence demonstrates that most bat-pollinated flowers in the Neotropics evolved from hummingbird-pollinated ancestors (Kay 2003; Perret et al. 2007; Knox et al. 2008; Martén-Rodríguez 2008; E. Tripp, unpublished data). The gain-curve hypothesis suggests that, following this pollinator shift, male-male competition will rapidly select for increased pollen production because those flowers that produce more pollen will sire more offspring. In plants that retain hummingbird pollination, increases in pollen production will not be selected for because they will not increase male fitness. This is analogous to the way in which sperm production (testes size) is affected by mating systems in primates; monogamous or polygamous mating systems limit the amount of sperm that can be profitably produced, while sperm competition in promiscuous mating systems selects for increased testis size (see, e.g., Harcourt et al. 1981). Shifts in the opposite direction (back to hummingbird from bat pollination) will select for decreased pollen production because excess pollen represents wasted resources that could be better allocated to female function (or plant survival). In fact, a shift back to hummingbird pollination in *Burmeistera* (*B. rubrosepala*) shows a concomitant reduction in pollen production (table 1), despite apparently having evolved quite recently (Knox et al. 2008).

What mechanism may account for the observed differences in pollen transfer? We suggest that the physical differences between fur and feathers are key; feathers can hold only limited amounts of pollen, whereas pollen can continue to be packed into the interstitial spaces between mammal hair (also see Law and Lean 1999). Alternatively, behavioral differences between bats and hummingbirds may be responsible. For example, we noted that hummingbirds in the experiments often ruffled their head feathers, which may dislodge excess pollen. Differences in wing motion or flight speed may also shake excess pollen off of hummingbirds. Further experimental work would be useful in clarifying the influences of these physical and behavioral differences on pollen transfer.

In addition to supporting the gain-curve hypothesis, our

results also provide evidence against previous hypotheses. These propose that increased pollen production is selected for in bat flowers because bats are particularly wasteful of pollen, either through grooming (Faegri and van der Pijl 1979; von Helversen 1993) or because of their large surface area relative to stigma size (Heithaus et al. 1974; Proctor et al. 1996). Although bats did occasionally groom during our experiments, clearly neither this grooming nor their body size diminished their efficiency in transferring pollen; in fact, on average they transferred 97.8 (± 4.19) grains per visit to *A. acanthus* recipient flowers, whereas hummingbirds transferred only 10.1 (± 1.20) grains. This accords with previous comparisons of bat and hummingbird pollen transfer in nature (Muchhala 2006) and flight cage experiments (Muchhala 2007).

Similar studies in the bumblebee-pollinated lily genus *Erythronium* found that pollen delivery was a decelerating function of pollen removal (Harder and Thomson 1989; Thomson and Thomson 1989). In this case, the deceleration appears to arise from bumblebee grooming, since bees groom more intensively after receiving large doses of pollen (Harder 1990a, 1990b). Note that, although bats also groom, the fact that our data do not show a similar deceleration (fig. 2) implies that grooming is not similarly dependent on pollen load size for bats, at least not over the range of pollen amounts we studied.

Harder and Thomson (1989) suggested that bee-pollinated plants can evade diminishing returns on male investment (i.e., “linearize” the male gain curve) by presenting pollen in small doses to a large number of bees (also see Thomson and Thomson 1992), an idea that was subsequently supported by comparisons of pollen presentation schedules of bee- versus bird-pollinated *Penstemon* (Thomson et al. 2000; Castellanos et al. 2006). In a similar way, hummingbird flowers might be expected to be able to successfully disperse the same amount of pollen as bat flowers if they increased visitor attraction and presented the pollen in small doses. However, this does not seem to happen in nature, given that hummingbird flowers produce less pollen than bat flowers. Perhaps hummingbirds, unlike bees, are simply not abundant enough to sufficiently increase visitation rates. Alternatively, any increases in nectar production to encourage increased visitation may elicit a strong opposing selective pressure by encouraging territorial behavior in the hummingbirds, leading to defense of the plant by a single hummingbird individual and a concordant decrease in pollen dispersal to other plants (see Linhart 1973; Linhart et al. 1987).

Tripp and Manos (2008) proposed that bat pollination may represent an evolutionary “dead end,” given that they found several shifts to bat pollination in the genus *Ruellia* and no reversions back to other pollinators. Our results may explain this pattern; we predict that once a flower

becomes bat adapted, switches to other pollinators become unlikely because they would transport such a small proportion of the total pollen produced. In fact, hummingbirds may even become “conditional parasites” of these flowers (sensu Thomson 2003) by wasting pollen that would have been better dispersed by bats. At the same time, hummingbirds may provide a backup mechanism (sensu Wolf and Stiles 1989) for bat flowers that do not receive bat visits. We suggest that this is the case for *A. acanthus*. Flowers of this species present a mix of chiropterophilous and ornithophilous traits, in that they open throughout the day and night and are brightly colored, yet anthers only dehisce at night (Muchhala et al. 2009). This means that the large amounts of pollen produced are initially available only to bats, but undispersed grains are available for hummingbird dispersal the following day. In fact, in all instances of angiosperms pollinated by both bats and hummingbirds that we are aware of, flowers initially present their pollen nocturnally (Buzato et al. 1994; Sahley 1996; Fleming et al. 2001; Dar et al. 2006) or in the late afternoon (Sazima et al. 1994). This pattern makes sense in light of our pollen-transfer results, as it should maximize male fitness.

A final point regarding selection on pollen production in bat flowers concerns the fact that bats, like bees, eat pollen. It has been argued that pollen may serve as an attractant for bats (Faegri and van der Pijl 1979), such that plants with higher pollen production are selected for because they receive more visits. True, nectar bats are known to consume pollen groomed from their fur, and their digestive tracts are adapted to efficiently extract the contents of pollen grains (Herrera and Martínez del Río 1998). Additionally, captive bats allowed to feed for many days on a nectar-only diet will consume pollen directly from anthers when presented with a flower (Tschapka and Dressler 2002), and rare instances of pollinivory have been documented for visits to *Ceiba pentandra* in Mexico (Quesada et al. 2003). However, we have never observed this behavior in approximately 650 h of videotaped footage of bat flowers (corresponding to 179 bat visits; Muchhala 2006; Muchhala et al. 2009; N. Muchhala, unpublished data). Bats appear to visit flowers primarily for nectar, and only later, in intermittent pauses during foraging bouts, do they stop to groom their fur and consume pollen. Thus, it does not seem likely that they exert a direct pressure on pollen production by opting to visit flowers based on pollen availability (unlike bumblebees; see, e.g., Harder 1990a).

Acknowledgments

We are grateful to K. O'Neill, D. Proaño, and J. C. Vizúete for assistance in the field, to B. Thomson for help with

statistical analyses, and to M. Burd, B. Cole, J. Forrest, J. Ogilvie, and anonymous reviewers for comments on the manuscript. We thank S. Barrett for use of the particle counter, M. Vallejo-Marin for explaining how to use it, and B. Cole for keeping it usable. F. Werner kindly provided translations of German literature. Fieldwork was funded in part by Bat Conservation International.

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Associate Editor: Christopher G. Eckert
Editor: Mark A. McPeck



A nectar bat (*Anoura geoffroyi*) visiting a flower (*Aphelandra acanthus*) in a flight cage experiment designed to quantify pollen transfer. Photograph by Nathan Muchhala.