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# A generalized pollination system in the tropics: bats, birds and *Aphelandra acanthus*

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• *Background and Aims* A number of different types of flower-visiting animals coexist in any given habitat. What evolutionary and ecological factors influence the subset of these that a given plant relies on for its pollination? Addressing this question requires a mechanistic understanding of the importance of different potential pollinators in terms of visitation rate (pollinator 'quantity') and effectiveness at transferring pollen (pollinator 'quality') is required. While bat-pollinated plants typically are highly specialized to bats, there are some instances of bat-pollinated plants that use other pollinators as well. These generalized exceptions tend to occur in habitats where bat 'quantity' is poor due to low or fluctuating bat densities.

• *Methods Aphelandra acanthus* occurs in tropical cloud forests with relatively high densities of bat visitors, yet displays a mix of floral syndrome characteristics, suggesting adaptation to multiple types of pollinators. To understand its pollination system better, aspects of its floral phenology and the 'quantity' and 'quality' components of pollination by its floral visitors are studied here.

• *Key Results* Flowers were found to open and senesce throughout the day and night, although anther dehiscence was restricted to the late afternoon and night. Videotaping reveals that flowers are visited nocturnally by bats and moths, and diurnally by hummingbirds. Analysis of pollen deposition shows that bats regularly transfer large amounts of conspecific pollen, while hummingbirds occasionally transfer some pollen, and moths rarely do so.

• *Conclusions* Hummingbirds and bats were comparable in terms of pollination 'quantity', while bats were the most effective in terms of 'quality'. Considering these components together, bats are responsible for approx. 70 % of *A. acanthus* pollination. However, bats also transferred remarkably large amounts of foreign pollen along with the conspecific grains (three of four grains were foreign). It is suggested that the negative effects of interspecific pollen transfer may decrease bat 'quality' for *A. acanthus*, and thus select for generalization on multiple pollinators instead of specialization on bats.

**Key words:** Specialization, generalization, pollinator effectiveness, hummingbirds, floral syndrome, bat pollination, chiropterophily, ornithophily, cloud forest, heterospecific pollen transfer.

## INTRODUCTION

It is widely accepted that evolutionary specialization in pollination systems has been a central process in the divergence and diversification of the angiosperms (Grant, 1949; Stebbins, 1970; Johnson and Steiner, 2000; Fenster *et al.*, 2004). However, we still lack a solid understanding of the factors that favour generalization or specialization. Ecological studies demonstrate that plants range from generalists pollinated by many animals (e.g. Gomez and Zamora, 1999) to extreme specialists which depend exclusively on one pollinator species (e.g. Muchhala, 2006*a*; also see reviews in Waser and Ollerton, 2006). Typically a diverse array of potential pollinators occurs in any given habitat; what determines the identity and number of pollinators a particular plant depends on?

The effectiveness of a pollinator can be divided into a 'quantity' and 'quality' component; respectively, its visitation rate and the amount of pollen it transfers per visit. Different pollinator types often differ in the 'quantity' and 'quality' of the pollination services they provide (e.g. Schemske and Horvitz, 1984; Fishbein and Venable, 1996). Given such

variation, Stebbins (1970) suggested that a plant can be expected to specialize on its most effective pollinator. However, Aigner (2004, 2006) used optimality modelling to show that generalization can also evolve (via floral adaptations to less effective pollinators), irrespective of the magnitude of the differences in pollinator effectiveness, as long as it provides the plant with a marginal fitness gain. Two circumstances can increase the likelihood of evolutionary specialization on the most effective pollinator. First, pollinators often differ in morphology, behaviour, and floral preferences, and thus can select in different directions on floral phenotype. If such pollinator-mediated adaptive trade-offs are strong enough, generalization will become suboptimal (Wilson and Thomson, 1996; Muchhala, 2007). Secondly, multiple pollinator types may negatively impact each other's effectiveness. Considering that a flower produces a finite amount of pollen, a less effective pollinator may waste pollen which would have been better dispersed by the primary pollinator (Lau and Galloway, 2004). In this sense, a pollinator can become a 'conditional parasite' (rather than a mutualist) in the presence of a more effective pollinator, since its visits will actually reduce plant fitness (Thomson, 2003). In the absence of either strong pollinator-mediated

trade-offs or negative pollinator interactions, theory predicts that generalization should be favoured. Advantages of generalization include a simple increase in the number of pollen vectors per unit of time, and the flexibility to be able to withstand spatio-temporal fluctuations in pollinator abundance (Waser *et al.*, 1996).

Although bat-pollinated plants in the New World are believed to have evolved from hummingbird-pollinated ancestors in the majority of cases (Baker, 1961; Helversen, 1993; Helversen and Winter, 2003), there are relatively few examples of plants known to be pollinated by both types of animals (but see Buzato *et al.*, 1994; Sazima *et al.*, 1994; Sahley, 1996; Fleming *et al.*, 2001; Dar *et al.*, 2006). These generalized exceptions deserve further study as they can shed light on the selective pressures that drive switches between pollination systems. Hummingbirds and bats differ in morphology (bills vs. jaws), activity patterns (diurnal vs. nocturnal), and means of locating flowers (visual vs. echolocation and smell), and previous work demonstrates that these differences can impose adaptive trade-offs in floral phenotype (Muchhala, 2007). Additionally, bats transferred nearly four times as much pollen as hummingbirds in flight cage experiments (Muchhala, 2007) and in the wild (Muchhala, 2006*b*). This suggests that hummingbirds may become conditional parasites in the presence of bats, leading to selective pressures that favour specialization on bats. This hypothesis accords with the distribution of species pollinated by both bats and hummingbirds; these tend to occur in areas where bat abundances are either low or highly variable from year to year (Sahley, 1996; Fleming *et al.*, 2001). Decreased bat 'quantity' may make generalization optimal despite high bat 'quality'.

This study documents the pollination system of *Aphelandra acanthus*, a shrub from the cloud forests of Ecuador (Wasshausen, 1975). Preliminary observations suggest that this species is adapted to pollination by both bats and hummingbirds. Its flowers have narrow, tubular corolla bases similar to those of other hummingbird-pollinated plants, yet the distal portion flares into a wide opening like those of some bat-pollinated flowers. The flowers vary in colour from bright yellow to a pale cream. Many other members of the genus *Aphelandra* are hummingbird-pollinated (McDade, 1985, 1992; Rengifo *et al.*, 2006), and hummingbirds have been observed visiting *A. acanthus*, but its pollen has also been found on the fur of mist-netted bats (N. Muchhala and A. Caiza, unpubl. res.). Here the 'quantity' and 'quality' components of the effectiveness of bats and hummingbirds as pollinators of *A. acanthus* in the wild is documented. Visitation rates were calculated by videotaping flowers nocturnally and diurnally, and single-visit effectiveness estimated by counting pollen transferred to flowers. Floral phenology was also studied to determine whether flowers and anthers open during the day or night, and how long they remain open.

## METHODS

### Study site and study organism

Research was carried out in 2005 (7–26 February), 2007 (6–12 December) and 2008 (12–17 January) in the Bellavista Cloud Forest Reserve (00°01's, 78°41'W), which is located on the western slopes of the Andes of Ecuador. Bellavista ranges from 2000 to 2400 m in elevation, with temperatures from 12 to 17° C. It is characterized by a rainy season from December to May and a dry season from June to November.

*Aphelandra acanthus* Nees (Acanthaceae) is a spiny shrub (up to 3 m tall) distributed from Colombia to Peru in Andean cloud forests at elevations from 2000–3400 m (Wasshausen, 1975). The Bellavista population of *A. acanthus* flowers from November to early March. Inflorescences are terminal spikes, up to 30 cm long. These bear from several to tens of buds, which open sequentially (basically to apically) over 1–2 months, with one to three flowers open at any given time. Anthesis lasts for several days, after which the corolla abscises. Stigmas are receptive during the day and night, as evidenced by the fact that both diurnal and nocturnal hand-pollinations set fruit (N. Muchhala, unpubl. res.). Corollas are curved tubes (4–6 cm long, 5 mm wide at the base, and 8–10 mm wide at the throat) that range in colour from bright yellow to pale cream, occasionally tinged with red. The distal opening of the corolla is 8–10 mm wide and 7–9 mm high, with two upper and three lower lobes. Flowers are presented horizontally, and anthers and stigmas are positioned just beneath the dorsal corolla lobes, so that pollen is deposited and picked-up from the dorsal surface of bat or hummingbird heads (Fig. 1). Stigmas are exserted 4–6 mm beyond the anthers. Such herkogamy is frequent among *Aphelandra*, and probably minimizes autogamy (i.e. pollination from own anthers; McDade, 1985). In fact, hand-crosses with geitonogamous pollen demonstrate that *A. acanthus* flowers are self-compatible (7 of 15 crosses set fruit; N. Muchhala, unpubl. res.). Fruits of *Aphelandra* typically dehisce explosively in the dry season, and produce one to four seeds each (McDade, 1984).

### Floral phenology

In order to characterize the floral phenology of *Aphelandra acanthus*, the inflorescences of 14 plants were tracked from 6 to 12 December 2007. Every 2 h from 0800 to 2400 h the status of 29 flowers was recorded throughout anthesis. Timing of bud opening, dehiscence of the four anthers, and senescence (abscission of corollas) were noted.

### Pollinator 'quantity'

To identify floral visitors, flowers were videotaped with Sony (Tokyo, Japan) Nightshot Digital Camcorders from 11 to 26 February 2005 and 12 to 17 January 2008. Three cameras were used, simultaneously videotaping three different *Aphelandra acanthus* plants. Each camera was placed on a tripod approx. 2 m away from the inflorescence and sheltered from the rain. Each videotape records for 4 h. Flowers from a total of 23 plants were filmed during the day and night. During the day, flowers were videotaped twice; once from approx. 0630 to 1030 h and again from 1430 to 1830 h (dusk and dawn in Bellavista are approx. 0600 and 1800 h year-round). At night, typically videotaping was from 1900 to 2300 h, and occasionally from 2330 to 0330 h. In total, 32 videotapes were recorded during the day and 36 at night

FIG. 1. An inflorescence of *Aphelandra acanthus*, with a visit by a bat (*Anoura fistulata*) on the left and a hummingbird (*Heliodaxa rubinoides*) on the right. Note the loop of tape marked by an arrowhead, which was used to quantify pollen transfer.

(using the Nightshot mode), for a grand total of 253·7 h of footage. Because inflorescences contained from one to three open flowers at any given time, it was often possible to video-tape more than one flower with each camera (on average, 2·1 flowers diurnally and 1·8 nocturnally). Visitation rates were defined as visits per flower per hour; for this calculation, the total number of flower-hours was 243·2 diurnally and 235·5 nocturnally.

### Pollinator 'quality'

In order to evaluate pollen deposition by visitors on the flowers videotaped, a loop of clear tape was placed around the flower's reproductive parts (anthers and stigma) and the two dorsal corolla lobes, with the adhesive facing out (see Fig. 1). This tape was collected and replaced after 2 h of exposure (thus twice for each videotape) by placing a microscope slide underneath and unrolling the loop so that it adhered to the slide. Immediately after collecting it, the tape was marked with a felt-tip marker to delineate the portion that was under-neath the flower's reproductive parts. In total, 95 tape samples were collected and analysed. Pieces of tape were occasionally lost, especially in heavy rains or when the corolla senesced and fell during videotaping; thus the tape sample size is some-what lower than the total number of flowers filmed.

A light microscope was used to count and identify pollen present along two transects across each tape sample. To define these transects, a 15 × 20 mm square of posterboard with a 10 × 15 mm hole cut out of the middle and two hairs affixed in vertical and horizontal lines through the centre of the hole was used. For each slide, this square was placed over the tape

sample and all pollen grains counted along the two transects. While these grains may not all have been deposited on the flower's stigma, this analysis serves as a standardized estimate for pollen deposition, and allowed the relative amounts of pollen deposited by different types of pollinators to be compared. Pollen was identified through comparison with a reference col-lection of pollen taken directly from different species of flowers in Bellavista. All 95 tape samples were analysed; later, the videotaping results were used to identify which of the corre-sponding flowers had actually been visited. The majority of these tape samples corresponded to a single visit. Those with multiple visits by a single pollinator type were categorized with the appro-priate type. Four samples included visits from different pollinator types (robbing and legitimate hummingbird visits); these were categorized with the legitimate hummingbird samples because robbing hummingbirds were found not to touch the plant's repro-ductive parts or transfer pollen.

## RESULTS

### Floral phenology

Flower buds typically opened in stages, with first the lower central, then the two lower lateral and finally the two upper petal lobes unfolding. For the 29 flowers tracked, this process took from 2 to 10 h. At anthesis, anthers initially remained closed, and eventually dehisced along a lateral line, presenting large amounts of pollen. Flowers remained viable for 2 ($n = 12$) to 3 d ($n = 17$), at which point the corolla abscised from the hypanthium. No clear pattern can be seen in terms of timing of either flower anthesis or senescence (Fig. 2); i.e.
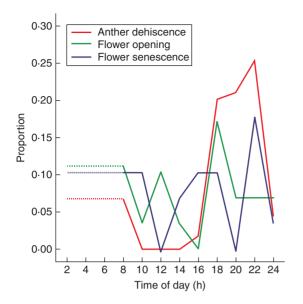
FIG. 2. Daily flower phenology of *Aphelandra acanthus*: for 29 flowers from 12 plants, shows the proportion per 2-h period of anthers that dehisce (red), flowers that open (green), and flowers that senesce (blue) (i.e. the number per 2-h period over the total number). Flowers were not tracked from 0200 to 0800 h; the dashed lines for these four time periods show the proportion of dehiscences/openings/senescences recorded at 0800 h divided by four (i.e. the mean over the time periods).

flowers opened and senesced throughout the day and night. However, anther dehiscence was found to be restricted to the late afternoon or night, occurring most frequently from 1800 to 2200 h (Fig. 2). Dehisced anthers often still held small amounts of pollen on the following morning, but were 'empty' by the afternoon. For each flower, timing of the dehiscence of its four anthers was staggered over one or two nights. For half of the flowers (n = 15 of 29), all four anthers opened in the same night. Of the rest, some had one anther open one night and three another night (n = 9), and the remaining had two open on each of two nights (n = 5). About half of the time (n = 16), anthers did not begin to dehisce until the second night following anthesis. Because anthers always remained closed for at least several hours when flowers first open, *Aphelandra acanthus* are likely protogynous (i.e. first female, then male), although more detailed analyses of stigma receptivity would be necessary to confirm this.

*Pollinator 'quantity'*

Hummingbirds visited the flowers during the day, while bats and moths visited at night (Table 1). *Trigona* bees (Hymenoptera: Apidae) were also frequently encountered in flowers during the day, most often consuming nectar through holes they chewed at the base of the corolla. They also occasionally collected pollen from the anthers. Because of their small size and the spatial separation between stigma and anthers, they did not contact stigmas during visits, and therefore these visits were ignored in analyses of pollen transfer. Hummingbird visits were made by collared incas (*Inca torquata*; n = 57), fawn-breasted brilliants (*Heliodoxa rubinoides*; n = 5) and speckled hummingbirds (*Adelomyia melanogenys*; n = 20). Of these, the former two species always made legitimate visits, inserting their bills in the corolla opening. Fawn-breasted brilliants appeared to 'fit' the flowers better, and always touched the reproductive parts during the visits. Collared incas have longer bills, resulting in less predictable contact; often the head remained visibly outside of the corolla throughout visits (thus failing to contact reproductive parts). Speckled hummingbirds always nectar-robbed, perching on top of the flower and inserting their bills into the holes chewed by *Trigona* bees in the base of the corolla. These were clearly non-pollinating visits. All five moth visits in 2008 were made by hawkmoths (Sphingidae), while the seven visits in 2005 were by smaller, unidentified lepidopterans. Although the latter did not contact the reproductive parts of the flowers, hawkmoths occasionally did. Bats always contacted reproductive parts during the visits (n = 45). Although the bats could not be reliably identified in the videos, analysis of pollen loads on bats captured with mist nets shows that *Anoura geoffroyi*, *A. fistulata* and *A. caudifer* all visit *Aphelandra acanthus* (N. Muchhala and A. Caiza, unpubl. res.). Of these, *A. geoffroyi* are the most abundant and rely heavily on *A. acanthus*, while *A. fistulata* are less common and visit the flowers less frequently, and *A. caudifer* are very rare in Bellavista.

*Pollinator 'quality'*

Based on videotaping results, each tape sample was categorized according to the visits received. Table 2 summarizes the pollen deposited on these samples. Whether pollen is deposited on a particular visit depends on whether the visitor makes

TABLE 1. *Visitation rates for visitors to* Aphelandra acanthus *flowers*

| | | Visitation rate | | | | |
|---|---|---|---|---|---|---|
| | | Hummingbirds | | | | |
| Time | n (fl × h) | Legitimate | Robbing | Bats | Hawkmoths | Other moths |
| Day | 243·2 | 0·26 (62) | 0·08 (20) | −(0) | −(0) | −(0) |
| Night | 235·5 | −(0) | −(0) | 0·19 (45) | 0·02 (5) | 0·03 (7) |

Sample size is given as the total number of flower-hours, i.e. the number of hours for each videotape multiplied by the number of flowers (1–3) in that videotape.

The raw number of visits is given in parenthesis.

Hummingbird visits are categorized as legitimate or robbing depending on whether the hummingbird entered from the corolla opening or a hole in the base of the corolla.

TABLE 2. *Median, range, mean and standard deviation of conspecific and heterospecific pollen grains deposited by different floral visitors on tape samples from* A. acanthus *flowers*

| Visitor | $n$ | Conspecific | | Heterospecific | |
|---|---|---|---|---|---|
| | | Median (range) | Mean ($\pm$ s.d.) | Median (range) | Mean ($\pm$ s.d.) |
| Bats | 31 | 20·0 (0–551) | 57·2 ($\pm$118·3) | 30·0 (0–1455) | 156·3 ($\pm$316·5) |
| Hummingbirds | 30 | 3·5 (0–299) | 17·1 ($\pm$54·7) | 0·0 (0–10) | 1·2 ($\pm$2·2) |
| Hawkmoths | 5 | 2·0 (0–54) | 11·8 ($\pm$23·6) | 0·0 (0–3) | 1·0 ($\pm$1·4) |
| Other moths | 3 | 0·0 (0–0) | 0·0 (–) | 0·0 (0–0) | 0·0 (–) |

contact with the flower's reproductive part, and on whether it was carrying pollen from a previous visit. This leads to extensive variation in the amount of pollen transferred, and many visits which fail to transfer any pollen, as can be seen in the measurements of mean, median and variance shown in Table 2. Bats were the most consistent in transferring pollen, and transferred the greatest amounts (mean $= 57·2$, $n = 31$). Robbing hummingbird visits did not transfer pollen, as would be expected based on the fact that they fail to contact the plant's reproductive parts. Legitimate hummingbird visits were quite variable (range 0–299 grains), with many failing to transfer any pollen, and a mean transfer of 17·1 grains ($n = 30$). Of the five hawkmoth samples, four transferred little or no pollen, and one transferred 54 grains. The visits by other moths did not transfer pollen, again consistent with the videotaping results suggesting they do not contact floral reproductive parts.

Bat visits transferred significantly more *A. acanthus* pollen grains (mean $= 57·2$, $n = 31$) than legitimate hummingbird visits (mean $= 17·1$, $n = 30$, Mann–Whitney $U$: 242·0, $P <$ 0·001). Closer examination of these pollen loads, however, reveals an interesting difference in their composition. Hummingbirds deposited a mean of only 1·2 heterospecific pollen grains, while bats deposited 156·3 (Table 2). Thus, although bats deposited significantly more conspecific pollen, 73 % of the pollen they deposited was heterospecific (vs. 6 % for hummingbirds). The majority (68 %) of this heterospecific pollen belonged to bat-pollinated Campanulaceae flowers (*Burmeistera* spp. or *Centropogon nigricans*); the rest consisted of *Meriania* (Melastomataceae), *Marcgravia* (Marcgraviaceae) and unidentified grains.

## DISCUSSION

Flowers of *Aphelandra acanthus* present a mix of characteristics representative of different pollination syndromes. Flower anthesis cannot be classified as either nocturnal or diurnal. Rather, flowers open throughout the day and night (Fig. 2), and last for several days. Similarly, stigmas are receptive diurnally and nocturnally, as shown by hand-crossing experiments (N. Muchhala, unpubl. res.). Although nectar production was not explicitly measured, the fact that hummingbirds visit flowers throughout the day and bats throughout the night suggests constant production. In contrast, for bat-pollinated *Burmeistera* flowers in this forest, the same species of hummingbirds opportunistically visit only in the early morning and late afternoon (Muchhala, 2006*b*). One aspect of floral phenology of *A. acanthus*, the fact that anthers dehisce in the early evening, does suggest adaptation

to nocturnal pollinators. Meanwhile, the often bright yellow colour of the flowers suggests adaptation to hummingbirds. And finally, the flowers produced a faint sweet odour typical of hawkmoth pollination.

Analysis of visitation rates and pollen transfer demonstrate that both bats and hummingbirds effectively pollinate *Aphelandra acanthus*. In terms of pollinator 'quantity', both vertebrates visited the flowers at similar rates: 0·26 visits flower$^{-1}$ h$^{-1}$ for hummingbirds and 0·19 visits flower$^{-1}$ h$^{-1}$ for bats. In terms of pollinator 'quality', bats deposited significantly more conspecific pollen per tape sample than hummingbirds (57·2 vs. 17·1 grains, respectively). Considering these two components of pollination together (quantity $\times$ quality), bats performed 72·1 % of pollen transfer to flowers of *A. acanthus* while hummingbirds performed 27·9 %. Fenster *et al.* (2004) consider a plant to be specialized if one type of floral visitor is responsible for $>75$ % of its pollination; following this criterion, *A. acanthus* can be considered a pollinator generalist.

According to the above analysis, bats are the most effective pollinators of *Aphelandra acanthus* because of their higher 'quality'. However, this study also revealed a potential cost to bat pollination for this plant. On average, bats transferred 156·3 grains of heterospecific pollen to *A. acanthus* flowers per tape sample. Thus only about one-quarter of the total pollen load was conspecific. In contrast, hummingbirds only transferred 1·2 heterospecific pollen grains per visit. The majority of foreign pollen came from species of *Burmeistera* (67 %), bat-pollinated plants which also deposit and pick-up their pollen using the crown's of bats' heads (Muchhala, 2006*a*, 2008). Four species of *Burmeistera* are relatively abundant in Bellavista.

Interspecific pollen transfer can decrease reproductive success through the loss of pollen to foreign flowers and the blocking or clogging of stigmas with foreign pollen (Armbruster and Herzig, 1984; Fishman and Wyatt, 1999; Caruso and Alfaro, 2000; Bell *et al.*, 2005). In the face of high levels of interspecific pollen transfer, selection is predicted to favour specialization on a more constant pollinator (Sargent and Otto, 2006). We suggest that, in the case of *Aphelandra acanthus*, interspecific pollen transfer actually favours increased generalization by decreasing the 'quality' component of the primary pollinator. That is, while specialization on bats (and the exclusion of hummingbirds) might be selected for in the absence of other bat-pollinated plants, the large levels of interspecific pollen that bats transfer decreases their 'quality' and favours the inclusion of secondary pollinators in the pollination system of *A. acanthus*.

Another factor which may favour generalization is spatio-temporal variation in pollinator assemblages. *Aphelandra acanthus* has a relatively large range, occurring at 2000–3400 m elevation from Colombia to Peru (Wasshausen, 1975), and subpopulations are likely to face different pollination regimes. For example, in this study no hawkmoth visits were recorded in 2005 and only five in 2008, but the fact that one such visit transferred 54 grains of pollen suggests that hawkmoths may serve as important pollinators in regions/years that they are more abundant. Similarly, only five visits by fawn-breasted brilliants were recorded in 2005, and none in 2008. This hummingbird species is rare where the present research was conducted, but becomes abundant at lower elevations in the reserve.

In conclusion, the present results show that the mix of floral syndrome characteristics found in *Aphelandra acanthus* corresponds to a relatively generalized pollination system. Bats serve as the primary pollinators and hummingbirds and possibly hawkmoths serve as secondary pollinators. Bats and hummingbirds are similar in the 'quantity' component of pollination effectiveness, while bats are more effective in terms of the 'quality' component, as they consistently transfer greater amounts of conspecific pollen. However this 'quality' component may be reduced by the remarkably high levels of interspecific pollen transfer that bats incur. Further studies would be useful to better understand the possible negative effects. We argue that pollination biologists should pay careful attention to interspecific pollen transfer when evaluating pollinator 'quality', especially in species-rich tropical communities where competition for pollination may be particularly important given the large number of potential competitors (Vamosi *et al.*, 2006).

## ACKNOWLEDGEMENTS

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