

Interspecific competition in pollination systems: costs to male fitness via pollen misplacement

Nathan Muchhala^{*1} and James D. Thomson²

¹*School of Biological Sciences, University of Nebraska, Lincoln, NE 68588, USA; and* ²*Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, ON M5S 3G5 Canada*

Summary

1. Although competition for pollination is often invoked as a driver of broad-scale evolutionary and ecological patterns, we still lack a clear understanding of the mechanics of such competition. When flower visitors alternate between two species of flower, heterospecific pollen transfer takes place. The impact of these mixed loads on the female reproductive success of a recipient has received considerable attention, but the concomitant loss of male reproductive success – because of pollen grains being lost to foreign stigmas – has received less. Furthermore, pollen losses are not limited to grains that land on stigmas, but can also include deposition on non-stigmatic surfaces of the intervening flowers, or loss from the animal's body through passive detachment or active grooming. We collectively term these losses because of competition 'pollen misplacement'.

2. Here, we quantify pollen transferred by nectar bats between focal flowers (*Aphelandra acanthus*) with and without intervening visits to one of two competitor species. One competitor (*Centropogon nigricans*) places its pollen in the same region of bats' heads as the focal species, while the other (*Burmeistera sodiroana*) places its pollen farther forward.

3. We found that (i) any intervening visit caused some reduction in the number of pollen grains transferred, (ii) competitor flowers with similar pollen-placement locations caused greater reductions in pollen transfer and (iii) of these competitors, those in male phase (dispensing pollen) caused greater pollen loss than those in female phase (without pollen).

4. This study provides rare empirical support for the detrimental effects of competition for pollination on male fitness via pollen misplacement and is the first to show an added cost imposed by male-phase competitors. Although this competition is especially strong when competitors overlap in pollen placement, diverging in pollen placement will not completely eliminate pollen loss during visits to foreign flowers, simply because pollen sheds or is groomed from pollinator's bodies at some background rate over time. This suggests that any angiosperms that share pollinators face pervasive selection through male fitness to diverge in floral traits, alleviating competition by attracting different pollinators, altering floral phenology or encouraging floral constancy.

Key-words: conspecific pollen loss, competition for pollination, interspecific pollen transfer, mechanism, pollen wastage, pollen placement, pollinator sharing, *Aphelandra acanthus*, *Anoura*

Introduction

There has been a recent resurgence of interest in how competition for pollination drives broad-scale ecological and evolutionary patterns. Pollinator-mediated competition is considered an important component of the negative effects invasive plants can have on local communities (Traveset & Richardson 2006) and has been invoked to explain angiosperm community structure (Aizen & Vázquez 2006; McEwen & Vamosi 2010; Waterman *et al.* 2011), floral

diversification (Whitney 2009; Alcantara & Lohmann 2011; Waterman *et al.* 2011) and global patterns of pollen limitation (Vamosi *et al.* 2006). However, it is difficult to ascribe causation without a clear understanding of the assumed competitive interactions. Although studies of broad-scale patterns frequently conclude with calls for closer investigation of underlying processes, less research has been devoted to understanding the mechanistic details of competition for pollination.

Such competition can take two non-exclusive forms: competition through pollinator preference or competition through interspecific pollen transfer (Waser 1978a,b). The

*Correspondence author. E-mail: n_muchhala@yahoo.com

former involves competitors drawing away pollinators, thus decreasing visit rates. The latter is a form of reproductive interference and can lead to pollen loss during visits to foreign flowers, or heterospecific pollen deposition on stigmas (potentially blocking the stigmatic surface, clogging the stigma with foreign pollen tubes, usurping ovules and/or resulting in hybrid offspring; reviewed in Morales & Traveset 2008; Mitchell *et al.* 2009). Pollen loss to foreign flowers has recently been termed ‘conspecific pollen loss’ (Morales & Traveset 2008); however, we note that it is awkward to refer to pollen deposited on foreign flowers as ‘conspecific’. In fact this pollen is heterospecific relative to these flowers, and relative to the source flower, it is not merely conspecific but from the same individual. Here, we introduce a new term that avoids these referential difficulties: pollen misplacement, defined as all pollen losses incurred during competitive interactions with other flowers.

Of the three possible competitive mechanisms outlined previously (pollinator preference and the two forms of competition through interspecific pollen transfer), pollen misplacement has received by far the least attention (Morales & Traveset 2008). Many studies have examined pollinator preference in terms of how the presence of competitors affects visit rates (especially invasive competitors, e.g. Brown, Mitchell & Graham 2002; Kandori, Hirao & Matsunaga 2009; Yang, Ferrari & Shea 2011), and many others have used hand-pollinations to test effects of heterospecific pollen deposition on seed set (e.g. Thomson, Andrews & Plowright 1982; Waser & Fugate 1986; Caruso & Alfaro 2000; Brown & Mitchell 2001), but only a handful have looked at how visits to competitors reduce pollen export (Campbell 1985; Campbell & Motten 1985; Feinsinger, Busby & Tiebout 1988; Feinsinger & Tiebout 1991; Murcia & Feinsinger 1996; Flanagan *et al.* 2009). Nevertheless, mounting evidence suggests that this may be the most powerful and prevalent of the three forms of competition (Morales & Traveset 2008). For example, the elegant study by Flanagan *et al.* (2009) found that experimental augmentation of a competitor’s population did not affect visit rates to focal flowers and led to only small amounts of heterospecific pollen deposition on focal stigmas (which had no detectable negative effect on fitness), yet caused an overall 34% reduction in seed set because of pollen misplacement (also see Flanagan, Mitchell & Karron 2010, 2011).

It is important to note that, although pollen misplacement may or may not decrease a population’s seed set (and thus female fitness), it will always decrease male fitness because of reduced pollen export. For example, even if all focal individuals in the study by Flanagan *et al.* (2009) had produced full seed set over all treatments, selection would still favour reducing competition because individuals that lose less pollen to interspecific visits will father more seeds. This fact is frequently overlooked, and indicative of a frequent lack of attention to male function in empirical studies of plant reproduction. Even though it is widely acknowledged that both male and female function

contribute to floral evolution (e.g. Willson 1979; Ashman & Morgan 2004), mathematical models of pollination similarly tend to focus exclusively on seed set, often because they are concerned with population dynamics rather than selection (e.g. Levin & Anderson 1970; Devaux & Lande 2009; Montgomery 2009; but see Rodríguez-Gironés & Santamaría 2007). However, simulation models that explicitly tracked pollen fates demonstrated that competitive costs to male fitness alone can select for traits that reduce competition under a wide range of conditions (Muchhala *et al.* 2010).

Because of the scarcity of empirical work on the topic, we still do not understand the magnitude, generality or specific mechanisms involved in pollen misplacement. For example – does the sex of competing flowers affect pollen loss? Although female-phase competitors may dislodge pollen from pollinators, male-phase competitors could also bury or displace grains with their own pollen (Mitchell *et al.* 2009). And where exactly is the pollen lost during visits to foreign flowers? Although early reviews emphasized pollen deposited on foreign stigmas and anthers (Rathcke 1983; Waser 1983), Murcia & Feinsinger (1996) found that pollen on these parts accounted for only a fraction of the total pollen loss. They suggested that foreign petals and other flower parts must be removing the majority of pollen. However, Flanagan *et al.* (2009) found that all deposition on foreign flowers (including stigmas, petals and other parts) still only accounted for a small fraction of total pollen loss. Thus, the majority of losses must occur between flowers, either through grooming or through grains simply falling off pollinator’s bodies (Flanagan *et al.* 2009; Mitchell *et al.* 2009). The previously mentioned simulation models similarly stress the importance of non-stigmatic losses (Muchhala *et al.* 2010), as do experiments that track pollen fates during visits to a series of flowers of a single species. For example, bumblebees were found to deposit about 1/10th as many grains on stigmas than on non-stigmatic flower parts during visits to *Erythronium grandiflorum* (Thomson & Eisenhart 2003), and stigma deposition only accounts for about 1/40 of total pollen loss between visits to *Echium vulgare* (Rademaker, De Jong & Klinkhamer 1997).

The costs of pollen misplacement could be reduced by partitioning the pollination ‘resource’ (via character displacement) along several axes (Armbruster & Muchhala 2009). Competing plants could flower at different times of the year or day (Stone, Willmer & Rowe 1998; Aizen & Vázquez 2006), specialize on different taxa of pollinators (Armbruster & Herzig 1984) or diverge in the location of pollen placement on the bodies of pollinators. This latter idea makes intuitive sense, but support has been mixed (Murray *et al.* 1987; Armbruster, Edwards & Debevec 1994; Murcia & Feinsinger 1996; Muchhala & Potts 2007). One reason to doubt the importance of pollen placement is the fact that diverging in pollen placement will only reduce the pollen lost to foreign stigmas/anthers; it will not affect the other pollen losses discussed in the previous paragraph. On the other hand,

diverging in pollen placement will prevent pollen from being displaced or buried by foreign grains from male-phase competitors.

Here, we attempt to quantify the negative effects of pollen misplacement through a controlled set of pollen-transfer experiments. We focus on *Aphelandra acanthus* (Acanthaceae), a neotropical shrub previously found to be pollinated by bats and hummingbirds (Muchhala *et al.* 2009). Although bats are responsible for the majority of pollen transfer, they also deposit large amounts of foreign pollen on *A. acanthus* flowers. This indicates a lack of constancy and suggests that the bats must be simultaneously losing *A. acanthus* pollen during visits to foreign flowers. To test this hypothesis and the magnitude of any such competitive effects, we measure how much pollen bats transfer between pairs of *A. acanthus* flowers with and without intervening visits to competitor flowers. We use one competitor species with similar pollen placement and another that deposits pollen on a different region of bats' heads to test whether diverging in pollen placement reduces pollen loss. We also use both male- and female-phase flowers for each competitor species, predicting that competitive effects will be greater when intervening flowers are male phase.

Materials and methods

STUDY SPECIES

Our focal species, *A. acanthus* Nees (Acanthaceae), is a spiny shrub distributed in Andean cloud forests from Colombia to Peru (Wasshausen 1975). This study was carried out in the Bellavista Cloud Forest Reserve (Pichincha Province, Ecuador: 00° 01' S, 78° 41' W), where the local population of *A. acanthus* flowers from November to early March (Muchhala *et al.* 2009). 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. Previous work demonstrated that bats are responsible for c. 70% of pollen transfer and hummingbirds are responsible for the rest (Muchhala *et al.* 2009). Bat visitors include *Anoura geoffroyi*, *Anoura fistulata* and *Anoura caudifer*. Along with conspecific pollen, these bats also transferred large amounts of foreign pollen to stigmas of *A. acanthus* flowers. The majority of this pollen was from bat-pollinated Campanulaceae, including *Centropogon nigricans* and several species of *Burmeistera*, and a separate study of *Burmeistera* pollination shows similarly that bats often transfer *A. acanthus* pollen to *Burmeistera* stigmas (Muchhala 2006). Additional evidence of the inconstant foraging patterns of *Anoura* can be seen in the pollen loads found on their bodies; one study found pollen from two or more plant species in 74% of samples taken from bats' fur and 96% of samples from faeces (Muchhala & Jarrin-V 2002).

What are the fitness consequences for *A. acanthus* of such high levels of interspecific pollen transfer? To address this question, we tracked how much pollen bats transfer between *A. acanthus* flowers with and without intervening visits to flowers from competitor species. We used *Burmeistera sodiroana* and *C. nigricans* as competitor species. Although all three of our study species transfer pollen on the dorsal surfaces of bats' heads, there is some spatial segregation: *A. acanthus* places the majority of its pollen on the bat's crown, *B. sodiroana* places pollen further anteriorly on the forehead and

C. nigricans places pollen in a swath from between the eyes and across the forehead to the back of the crown (Fig. 1). Thus, *C. nigricans* pollen placement overlaps extensively with that of *A. acanthus*, while *B. sodiroana* overlaps relatively little.

Nectar bats from the genus *Anoura* were captured with mist nets set in front of bat-pollinated flowers and across trails. Bats were placed in separate flight cages (3 × 3 m screen tents) and maintained on a diet of sugar-water presented in test tubes. Those that failed to feed within 3 h were released; others were held for up to five nights for the experimental trials. We used *A. geoffroyi* for experiments with *B. sodiroana* competitors and *A. fistulata* for experiments with *C. nigricans* competitors; we could not use the same bat for both because *A. fistulata* is the only known pollinator of *C. nigricans* and does not visit flowers of *Burmeistera* (Muchhala 2006).

EXPERIMENTS

We conducted experiments from January 16th to March 8th 2008. Each experimental run consisted of one visit to a male-phase *A. acanthus* flower followed by one visit to a female-phase *A. acanthus* flower, with or without an intervening visit to a competitor flower. All flowers were picked in the afternoon on the day before or the day of the trials. For male-phase *A. acanthus* flowers, we used only those with all four of their anthers dehisced. For female-phase *A. acanthus* flowers, we placed a loop of clear tape around the two dorsal petals and the flower's stigma, with the adhesive side facing out (see Muchhala *et al.* 2009). Thus, the tape 'intercepted' any pollen that would have been transferred to the stigma. After the bat visited the female flower, we placed this tape on a microscope slide to be able to quantify pollen transfer.

We ran four treatments for each of six bat individuals, with 10 repetitions per treatment. These included (i) the 'control' treatment, with no intervening flowers between the male and female *A. acanthus*, (ii) the 'straw' treatment, with an intervening visit to a drinking straw (closed off at the base and filled with sugar-water), (iii) the 'female' treatment, with an intervening visit to a female-phase competitor and (iv) the 'male' treatment, with an intervening visit to a male-phase

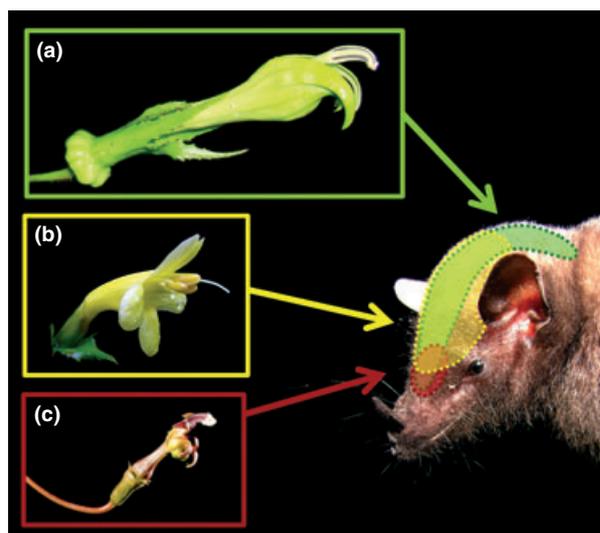


Fig. 1. A nectar bat (*Anoura geoffroyi*) and the three species of flowers used in the experiments. Dashed lines indicate location of pollen placement: green for *Centropogon nigricans* (a), yellow for the focal species *Aphelandra acanthus* (b) and red for *Burmeistera sodiroana* (c).

competitor. There was an average of 2 min between visits; new flowers or straws were made available 1 min after the previous visit, and at 2 min, resting bats were encouraged to visit by gently tapping the tent next to the bat. Thus, on average, 2 min passed for pollen transfer between *A. acanthus* flowers for the 'control', and 4 min for all other treatments. A straw was used for the second treatment because bats cannot insert their heads inside, so pollen on their heads will not be disturbed. Intervening visits to female competitors may displace pollen, and those to male competitors may displace or block pollen as competitor pollen is deposited. The 'straw' treatment eliminates these effects, such that comparison of the 'control' and 'straw' treatments will isolate the effects of time on pollen transfer. We ran treatments in blocks of four, with a random treatment order within blocks, and were typically able to run five blocks per night. In total, we conducted 240 experimental runs: ten replicates of each treatment level for three *A. fistulata* individuals (with *C. nigricans* as the competitor), and for three *A. geoffroyi* individuals (with *B. sodiroana* as the competitor).

STATISTICAL ANALYSES

We estimated pollen transfer to tape samples by counting all *A. acanthus* pollen grains along two transects (vertical and horizontal) through the sample. Before viewing through the microscope, we aligned the vertical transect with the greatest visual density of the pollen deposit; this increases repeatability of counts by minimizing spurious variation when pollen loads are off-centre. We conducted a repeated-measures ANOVA with competitor type (control, straw, female or male) as a within-subjects fixed factor and competing flower type (*B. sodiroana* or *C. nigricans*) as a between-subjects fixed factor. Individual bats served as the unit of replication, and the dependent variable was the mean number of *A. acanthus* pollen grains transferred (averaged over the ten replicates for each treatment level).

Results

The two-way repeated-measures ANOVA found no main effect of competitor flower species (*C. nigricans* or *B. sodiroana*) on pollen transfer ($F_{1,12} = 2.352$, $P = 0.199$) and a significant main effect for treatment type (control, straw, female competitor or male competitor; $F_{3,12} = 32.833$, $P < 0.001$). There was also a significant interaction between these factors ($F_{3,12} = 3.699$, $P = 0.043$; Fig. 2). For the group of experiments involving *B. sodiroana* flowers and *A. geoffroyi* bats, the control had the highest levels of pollen transfer, and pollen transfer was similar for the other three treatments. For the experiments with *C. nigricans* flowers and *A. fistulata* bats, pollen transfer decreased across treatments in this order: control, straw, female competitor and male competitor (Fig. 2).

For experiments with intervening male-phase flowers, tape samples often had large amounts of foreign pollen: 95.4 grains (± 16.84 SE) on average from *B. sodiroana* and 115.7 (± 17.1 SE) on average from *C. nigricans*. However, as discussed previously, *B. sodiroana* places its pollen farther forward on the foreheads of bats (Fig. 1). Consistent with this observation, *B. sodiroana* pollen typically occurred in the lower quarter of tape samples, while *A. acanthus* and *C. nigricans* typically occurred throughout.

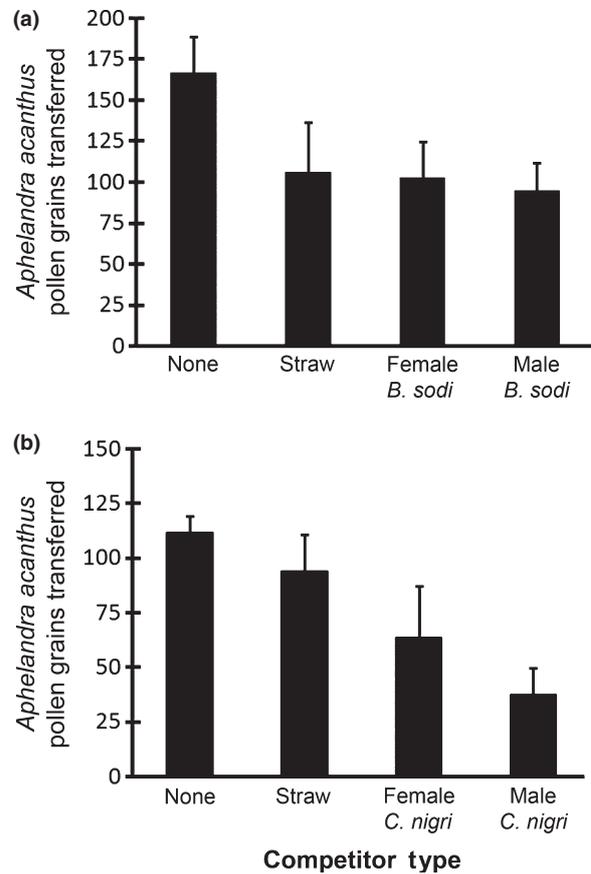


Fig. 2. Number of pollen grains (\pm SE) that bats transferred between *Aphelandra acanthus* flowers for four treatment levels: without intervening visits, with a visit to a straw or with an intervening visit to a competitor flower in female or male phase. (a) Experiments with *Burmeistera sodiroana* competitor flowers and *Anoura geoffroyi* bats. (b) Experiments with *Centropogon nigricans* competitor flowers and *Anoura fistulata* bats.

Discussion

These pollen-transfer experiments highlight a little-studied aspect of interspecific competition for pollination: pollen loss during visits to foreign flowers, or pollen misplacement. There are three important results. First, any visit that interrupted a sequence of visits between *A. acanthus* flowers, whether to a straw, female or male competitor, led to some reduction in the number of pollen grains transferred (Fig. 2). Second, competitor flowers that overlapped with *A. acanthus* in terms of pollen placement on bats' bodies caused greater reductions in pollen transfer. And third, male-phase competitors caused greater pollen loss than female-phase competitors. We will discuss each of these conclusions in turn.

COST OF INTERVENING VISITS

The experimental treatment involving an intervening visit to a sugar-water-filled straw was designed to isolate any pollen losses incurred during visits to foreign flowers, yet not directly attributable to pollen deposition on these flowers. Bats

cannot insert their heads into the straw (see Fig. S1, online appendix), so the straw never directly contacts the pollen load. Nonetheless, pooling the two sets of experiments, there was a 29.2% decrease in pollen transfer for the straw treatment relative to the control. What causes this decrease? Because every subsequent straw or flower was presented 2 min after the previous one, there were 2 min from *A. acanthus* pollen pickup to transfer in the control treatment, and 4 min from pickup to transfer in the straw treatment (and all other treatments). We suspect that pollen loads on bat bodies steadily decrease over time, through the grains either being groomed off, settling deeper into the fur, or simply falling off in flight. Flanagan *et al.* (2009) similarly found that deposition on foreign flowers only accounted for a small fraction (*c.* 1/7) of pollen loss during visits to foreign flowers. Simulation models of competition for pollination further stress the importance of such losses (Muchhala *et al.* 2010). These results imply that intervening visits to any foreign flowers, regardless of their morphology, will decrease male fitness simply by increasing the delay until the pollinator reaches conspecific flowers. More generally, they suggest that angiosperms that share pollinators face pervasive selection (through male fitness) for divergence in terms of flowering time and pollinator attraction, or for floral traits that encourage pollinator constancy.

POLLEN PLACEMENT

Using two different types of competitor flowers reveals that pollen placement can affect interspecific competition. The reproductive parts of *A. acanthus* contact the crowns of bats' heads, while those of *B. sodiroana* contact slightly farther forward on the forehead, and those of *C. nigricans* move in a swath through both of these regions (Fig. 1). Indeed, *B. sodiroana* pollen was typically found in the bottom portions of the tape samples, rarely overlapping *A. acanthus* pollen. If neither stigmas nor anthers of *B. sodiroana* disturb *A. acanthus* pollen loads, an intervening visit to a male- or female-phase *B. sodiroana* flower might be functionally equivalent to a straw visit; and in fact, no difference can be seen between these three treatment levels (Fig. 2a). However, *C. nigricans* flowers cause further decreases in pollen transfer beyond those in the straw treatment (Fig. 2b). It should be noted that both the competitor species (*B. sodiroana* or *C. nigricans*) and bat species (*A. geoffroyi* or *A. fistulata*) vary between these sets of experiments, so these further decreases could be due to either. However, it is not readily clear how bat differences might explain the results, and these bats are actually morphologically very similar except for an overall 5% difference in size (Muchhala, Mena & Albuja 2005), thus different floral morphologies seem to provide the most plausible explanation. These results provide rare empirical support for the commonly cited idea that competing flowers with similar pollen placement face stronger fitness costs (also see Armbruster, Edwards & Debevec 1994; Muchhala & Potts 2007). Murcia & Feinsinger (1996) did not find support for this idea in their experiments with a hummingbird-pollinated guild of

flowers, perhaps because their focal flower placed pollen on hummingbird bills, and any foreign flower parts (not just stigmas and anthers) appeared to readily scrape off this pollen.

SEX OF COMPETITOR FLOWERS

Our results further suggest that when flowers overlap in pollen placement, the sex of the competitor flower impacts the amount of pollen loss. Female-phase *C. nigricans* flowers reduced pollen transfer by 43.1%, while those in male phase reduced pollen transfer by 66.1%. A greater cost to male-phase competitors makes sense because, although both stigmas and anthers are likely to dislodge previous pollen loads, foreign pollen from anthers can also displace or bury this pollen (Lertzman 1981; Mitchell *et al.* 2009). However, to our knowledge, ours is the first study to find empirical support for this idea. An alternate explanation is that the additional pollen stimulates more grooming by the bats, as is known for bumble bees (Harder & Thomson 1989).

CAVEATS

These experiments provide important estimates for the sources and amounts of pollen loss induced by interspecific competition, estimates which can be useful to parameterize models of pollination (e.g. Waser 1978b; Harder & Thomson 1989; Sargent & Otto 2006; Montgomery 2009; Muchhala *et al.* 2010). However, more pollen misplacement studies are needed, as results could be highly system specific. For example, bat-adapted flowers are known to produce very large amounts of pollen (Muchhala & Thomson 2010), which may make male-phase bat-flowers especially potent competitors. Additionally, female-phase *C. nigricans* flowers have an unusually large stigmatic surface (*c.* 5 mm²), which likely increases competitive effects. We also note that our experiments were conducted with a single competitor flower and a single *A. acanthus* recipient. It would be useful to extend experiments to longer series of flowers. For example, one intervening visit to a female-phase *C. nigricans* flower reduces pollen transfer by 48 grains on average, or 43.1% – would each additional visit reduce transfer by a similar number of grains, a similar percentage of remaining grains, or would the function between competitor number and pollen loss take some other form? Finally, it is unclear whether pollen buried by foreign pollen would 'resurface' in subsequent visits; tracking pollen transfer to a series of recipients would help to address this question.

Conclusions

Our study provides rare empirical support for the negative effects of competition for pollination on male fitness (also see Campbell 1985; Campbell & Motten 1985; Feinsinger, Busby & Tiebout 1988; Feinsinger & Tiebout 1991; Murcia & Feinsinger 1996; Flanagan *et al.* 2009). Additionally, it is the first to show an added cost imposed by male-phase

competitors, presumably because of pollen burial. Although pollen loss is especially severe when competitors overlap in pollen placement, our results suggest that diverging in pollen placement will not completely eliminate pollen loss during visits to foreign flowers simply because pollen sheds or is groomed from pollinator's bodies at some background rate over time. Given that all pollinators likely lose granular pollen at some rate over time, and that switching between flower species will increase time between conspecific visits, it follows that any angiosperms that share pollinators likely face pervasive selection through male fitness to diverge in floral traits. Such divergence could help to alleviate pollen misplacement by attracting different pollinators, altering floral phenology or encouraging floral constancy. This selection may have been an important driver of the incredible floral diversity observed among angiosperms (Grant 1949; Whitney 2009).

Acknowledgements

We are grateful to Juan Carlos Vizuete, Daniela Proaño and Kylie O' Neill for assistance in the field, and to Bat Conservation International for financial support. This research was approved by the Ministry of the Environment of Ecuador (permit no. 007-2007-IC-FLO/FAU-DRFP/MA).

References

- Aizen, M.A. & Vázquez, D.P. (2006) Flowering phenologies of hummingbird plants from the temperate forest of southern South America: is there evidence of competitive displacement? *Ecography*, **29**, 357–366.
- Alcantara, S. & Lohmann, L.G. (2011) Contrasting phylogenetic signals and evolutionary rates in floral traits of Neotropical lianas. *Biological Journal of the Linnean Society*, **102**, 378–390.
- Armbruster, W.S., Edwards, M.E. & Debevec, E.M. (1994) Floral character displacement generates assemblage structure of Western Australian trigger-plants (*Stylidium*). *Ecology*, **75**, 315–329.
- Armbruster, W.S. & Herzig, A.L. (1984) Partitioning and sharing of pollinators by four sympatric species of *Dalechampia* (Euphorbiaceae) in Panama. *Annals of the Missouri Botanical Garden*, **71**, 1–16.
- Armbruster, W.S. & Muchhala, N. (2009) Associations between floral specialization and species diversity: cause, effect, or correlation? *Evolutionary Ecology*, **23**, 159–179.
- Ashman, T.L. & Morgan, M.T. (2004) Explaining phenotypic selection on plant attractive characters: male function, gender balance or ecological context? *Proceedings of the Royal Society B*, **271**, 553–559.
- Brown, B.J. & Mitchell, R.J. (2001) Competition for pollination: effects of pollen of an invasive plant on seed set of a native congener. *Oecologia*, **129**, 43–49.
- Brown, B.J., Mitchell, R.J. & Graham, S.A. (2002) Competition for pollination between an invasive species (purple loosestrife) and a native congener. *Ecology*, **83**, 2328–2336.
- Campbell, D.R. (1985) Pollen and gene dispersal: the influences of competition for pollination. *Evolution*, **39**, 418–431.
- Campbell, D.R. & Motten, A.F. (1985) The mechanism of competition for pollination between two forest herbs. *Ecology*, **66**, 554–563.
- Caruso, C.M. & Alfaro, M. (2000) Interspecific pollen transfer as a mechanism of competition: effect of *Castilleja linariaefolia* pollen on seed set of *Ipomopsis aggregata*. *Canadian Journal of Botany*, **78**, 600–606.
- Devaux, C. & Lande, R. (2009) Displacement of flowering phenologies among plant species by competition for generalist pollinators. *Journal of Evolutionary Biology*, **22**, 1460–1470.
- Feinsinger, P., Busby, W.H. & Tiebout III, H.M. (1988) Effects of indiscriminate foraging by tropical hummingbirds on pollination and plant reproductive success: experiments with two tropical treelets (Rubiaceae). *Oecologia*, **76**, 471–474.
- Feinsinger, P. & Tiebout III, H.M. (1991) Competition among plants sharing hummingbird pollinators: laboratory experiments on a mechanism. *Ecology*, **72**, 1946–1952.
- Flanagan, R.J., Mitchell, R.J. & Karron, J.D. (2010) Increased relative abundance of an invasive competitor for pollination, *Lythrum salicaria*, reduces seed number in *Mimulus ringens*. *Oecologia*, **164**, 445–454.
- Flanagan, R.J., Mitchell, R.J. & Karron, J.D. (2011) Effects of multiple competitors for pollination on bumblebee foraging patterns and *Mimulus ringens* reproductive success. *Oikos*, **120**, 200–207.
- Flanagan, R.J., Mitchell, R.J., Knutowski, D. & Karron, J.D. (2009) Interspecific pollinator movements reduce pollen deposition and seed production in *Mimulus ringens* (Phrymaceae). *American Journal of Botany*, **96**, 809–815.
- Grant, V. (1949) Pollination systems as isolating mechanisms in angiosperms. *Evolution*, **3**, 82–97.
- Harder, L.D. & Thomson, J.D. (1989) Evolutionary options for maximizing pollen dispersal of animal-pollinated plants. *The American Naturalist*, **133**, 323–344.
- Kandori, I., Hirao, T. & Matsunaga, S. (2009) An invasive dandelion unilaterally reduces the reproduction of a native congener through competition for pollination. *Oecologia*, **159**, 559–569.
- Lertzman, K.P. (1981) *Pollen transfer: processes and consequences*. M.Sc. Thesis, University of British Columbia, Vancouver, Canada.
- Levin, D.A. & Anderson, W.W. (1970) Competition for pollinators between simultaneously flowering species. *The American Naturalist*, **104**, 455–467.
- McEwen, J.R. & Vamosi, J.C. (2010) Floral colour versus phylogeny in structuring subalpine flowering communities. *Proceedings of the Royal Society B*, **277**, 2957–2965.
- Mitchell, R.J., Flanagan, R.J., Brown, B.J., Waser, N.M. & Karron, J.D. (2009) New frontiers in competition for pollination. *Annals of Botany*, **103**, 1403–1413.
- Montgomery, B.R. (2009) Do pollen carryover and pollinator constancy mitigate effects of competition for pollination? *Oikos*, **118**, 1084–1092.
- Morales, C.L. & Traveset, A. (2008) Interspecific pollen transfer: magnitude, prevalence and consequences for plant fitness. *Critical Reviews in Plant Sciences*, **27**, 221–238.
- Muchhala, N. (2006) Nectar bat stows huge tongue in its rib cage. *Nature*, **444**, 701–702.
- Muchhala, N. & Jarrin-V., P. (2002) Flower visitation by bats in cloud forests of western Ecuador. *Biotropica*, **34**, 387–395.
- Muchhala, N., Mena, P.V. & Albuja, L.V. (2005) A new species of *Anoura* (Chiroptera: Phyllostomidae) from the Ecuadorian Andes. *Journal of Mammalogy*, **86**, 457–461.
- Muchhala, N. & Potts, M.D. (2007) Character displacement among bat-pollinated flowers of the genus *Burmeistera*: analysis of mechanism, process and pattern. *Proceedings of the Royal Society B*, **274**, 2731–2737.
- Muchhala, N. & Thomson, J.D. (2010) Fur versus feathers: pollen delivery by bats and hummingbirds, and consequences for pollen production. *American Naturalist*, **175**, 717–726.
- Muchhala, N., Caiza, A., Vizuete, J.C. & Thomson, J.D. (2009) A generalized pollination system in the tropics: bats, birds, and *Aphelandra acanthus*. *Annals of Botany*, **103**, 1481–1487.
- Muchhala, N., Brown, Z., Armbruster, W.S. & Potts, M.D. (2010) Competition drives specialization in pollination systems through costs to male fitness. *The American Naturalist*, **176**, 732–743.
- Murcia, C. & Feinsinger, P. (1996) Interspecific pollen loss by hummingbirds visiting flower mixtures: effects of floral architecture. *Ecology*, **77**, 550–560.
- Murray, K.G., Feinsinger, P., Busby, W.H., Linhart, Y.B., Beach, J.H. & Kinsman, S. (1987) Evaluation of character displacement among plants in two tropical pollination guilds. *Ecology*, **68**, 1283–1293.
- Rademaker, M.C.J., De Jong, T.J. & Klinkhamer, P.G.L. (1997) Pollen dynamics of bumble-bee visitation on *Echium vulgare*. *Functional Ecology*, **11**, 554–563.
- Rathcke, B.J. (1983) Competition and facilitation among plants for pollination. *Pollination Biology* (ed. L. Real), pp. 305–329. Academic Press, New York.
- Rodríguez-Gironés, M.A. & Santamaría, L. (2007) Resource competition, character displacement, and the evolution of deep corolla tubes. *American Naturalist*, **170**, 455–464.
- Sargent, R.D. & Otto, S.P. (2006) The role of local species abundance in the evolution of pollinator attraction in flowering plants. *The American Naturalist*, **167**, 67–80.
- Stone, G.N., Willmer, P. & Rowe, J.A. (1998) Partitioning of pollinators during flowering in an African *Acacia* community. *Ecology*, **79**, 2808–2827.
- Thomson, J.D., Andrews, B.J. & Plowright, R.C. (1982) The effect of foreign pollen on ovule development in *Diervilla lonicera* (Caprifoliaceae). *New Phytologist*, **90**, 777–783.
- Thomson, J.D. & Eisenhart, K.S. (2003) Rescue of stranded pollen grains by secondary transfer. *Plant Species Biology*, **18**, 67–74.

- Traveset, A. & Richardson, D.M. (2006) Biological invasions as disruptors of plant reproductive mutualisms. *Trends in Ecology & Evolution*, **21**, 208–216.
- Vamosi, J.C., Knight, T.M., Steets, J.A., Mazer, S.J., Burd, M. & Ashman, T.L. (2006) Pollination decays in biodiversity hotspots. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 956–961.
- Waser, N.M. (1978a) Competition for hummingbird pollination and sequential flowering in two Colorado wildflowers. *Ecology*, **59**, 934–944.
- Waser, N.M. (1978b) Interspecific pollen transfer and competition between co-occurring plant species. *Oecologia*, **36**, 223–236.
- Waser, N.M. (1983) Competition for pollination and floral character differences among sympatric plant species: a review of the evidence. *Handbook of Experimental Pollination Biology* (eds C.E. Jones & R.J. Little). pp. 277–293. Van Nostrand Reinhold, New York.
- Waser, N.M. & Fugate, M.L. (1986) Pollen precedence and stigma closure: a mechanism of competition for pollination between *Delphinium nelsonii* and *Ipomopsis aggregata*. *Oecologia*, **70**, 573–577.
- Wasshausen, D.C. (1975) The genus *Aphelandra* (Acanthaceae). *Smithsonian Contributions to Botany*, **18**, 1–157.
- Waterman, R.J., Bidartondo, M.I., Stofberg, J., Combs, J.K., Gebauer, G., Savolainen, V., Barraclough, T.G. & Pauw, A. (2011) The effects of above- and belowground mutualisms on orchid speciation and coexistence. *The American Naturalist*, **177**, E54–E68.
- Whitney, K.D. (2009) Comparative evolution of flower and fruit morphology. *Proceedings of the Royal Society B*, **276**, 2941–2947.
- Willson, M.F. (1979) Sexual selection in plants. *American Naturalist*, **113**, 777–790.
- Yang, S., Ferrari, M.J. & Shea, K. (2011) Pollinator behavior mediates negative interactions between two congeneric invasive plant species. *American Naturalist*, **177**, 110–118.

Received 27 July 2011; accepted 18 November 2011

Handling Editor: Gaku Kudo

Supporting Information

Additional Supporting information may be found in the online version of this article.

Figure S1. *Anoura fistulata* visiting a straw (closed off at one end and filled with sugar-water) in the ‘straw’ treatment. Note that the straw does not touch the top of the bat’s head, leaving the pollen load undisturbed.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.