

DIFFERENTIAL TOLERANCE TO INCREASING HETEROSEXUAL POLLEN DEPOSITION IN TWO SYMPATRIC SPECIES OF *BURMEISTERA* (CAMPANULACEAE: LOBELIOIDEAE)

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Premise of research. Heterospecific pollen deposition on stigmas can impact plant reproduction by decreasing seed set or inducing fruit abortion. Pollinating bats often carry pollen from many species on their fur, and thus bat-pollinated flowers may exhibit tolerance to heterospecific pollen deposition, but to our knowledge this has never been studied. We investigated the relative impact of increasing heterospecific pollen deposition on female reproduction of two sympatric species of *Burmeistera* that experience pollen transfer between them by their shared bat pollinators.

Methodology. We simulated heterospecific pollen deposition by applying pollen mixtures that differed in the ratio of heterospecific to conspecific flowers used to make them (3:1; 2:2, and 1:3) and measured female reproduction by quantifying fruit abortion, seed number, and seed mass.

Pivotal results. For *Burmeistera borjensis*, applying a higher relative amount of heterospecific pollen led to decreased seed production and lighter seed mass, whereas no significant effect of increasing heterospecific pollen was detected in *Burmeistera ceratocarpa*. *Burmeistera borjensis* aborted more fruits than *B. ceratocarpa* (44.1% vs. 18.8%); however, in both species the probability of fruit abortion was not affected by pollination treatment.

Conclusions. We detected asymmetric impacts on female reproduction between this pair of sympatric *Burmeistera*: while increasing heterospecific pollen deposition decreased seed production and seed mass for *B. borjensis*, it had no detectable effect on *B. ceratocarpa*. Prior work shows that in nature *B. borjensis* receives much lower amounts of heterospecific pollen than *B. ceratocarpa*, and thus results suggest that *B. ceratocarpa* may have stronger gametic or postzygotic isolating barriers to be able to tolerate heterospecific pollen deposition. Future work should explore how different isolation mechanisms contribute to the asymmetric effects on female reproduction that we report here and whether tolerance to heterospecific pollen deposition is a common response among bat-pollinated plants and other plant taxa relying on low-fidelity pollinators.

Keywords: bat pollination, interspecific pollen transfer, reproductive success, pollination competition, reproductive interference, reproductive isolation.

Online enhancement: appendix table.

Introduction

Interspecific pollen transfer is a form of reproductive interference among coflowering plants resulting from pollinators alternating foraging visits between coflowering species (Morales and Traveset 2008; Moreira-Hernández and Muchhala 2019). Such pollen transfer impacts male fitness via pollen misplacement, causing pollen losses during visits to competitors (Muchhala and Thomson 2012; Minnaar et al. 2019). Likewise, female fitness can be reduced when deposition of heterospecific pollen

grains in stigmas interferes with conspecific pollen arrival, germination, and performance, leading to fruit abortion or diminished seed set (Arceo-Gómez and Ashman 2011; Ashman and Arceo-Gómez 2013). Although several recent studies have shown that deposition of heterospecific pollen grains on stigmas is common in many plant communities (Fang and Huang 2013; Arceo-Gómez et al. 2016a; Tur et al. 2016), its ecological and evolutionary implications still remain poorly studied (Ashman and Arceo-Gómez 2013; Moreira-Hernández and Muchhala 2019).

Several factors may influence the likelihood of heterospecific pollen deposition and modify its fitness consequences, including the degree of overlap with competitors in geographic distribution and flowering time, the extent of pollinator sharing (in terms of attraction and pollen placement in their bodies), relative

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plant abundances, and pollinator behavior during visitation (Morales and Traveset 2008; Mitchell et al. 2009; Arceo-Gómez and Ashman 2014; Thomson et al. 2018). Once on the stigma, heterospecific pollen grains may interact with the stigma surface or directly with conspecific pollen grains, which can negatively affect the success of the latter (Caruso and Alfaro 2000; Brown and Mitchell 2001; Arceo-Gómez and Ashman 2011; Bruckman and Campbell 2016). When the species in question are closely related, interference may play out at later stages if heterospecific pollen grains are able to germinate, grow tubes down the style, or even reach the ovary and usurp ovules (Diaz and Macnair 1999; Fishman and Wyatt 1999; Ashman and Arceo-Gómez 2013). Through these varied processes, deposition of heterospecific pollen grains can negatively impact female reproduction by reducing conspecific seed set, inducing fruit abortion (if the number of conspecific seeds produced is low or if the number of nonviable hybrid seeds formed is high), or, in the case of viable hybrid seeds, by the decreased germination of these seeds or the diminished growth and survival of the seedlings they produce (Wang and Cruzan 1998; Wolf et al. 2001; Arceo-Gómez and Ashman 2011). Plants that rely on low-fidelity pollinators are thus expected to evolve mechanisms of avoidance or tolerance against the negative effects of heterospecific pollen (Huang et al. 2015; Arceo-Gómez et al. 2016b; Stewart and Dudash 2016).

Bat-pollinated plants are ideal candidates to study the effects of heterospecific pollen deposition on reproduction, yet to our knowledge no such study has been conducted to date. Flowers adapted to bat pollination have evolved in many tropical lineages, and along with the typical chiropterophilous traits such as strong musky smell, dull flower color, nocturnal anthesis, and abundant nectar, they are often characterized by relatively large stigmas and copious pollen production (Fleming et al. 2009; Muchhala and Thomson 2010). Nectar-feeding bats themselves are large-bodied pollinators that typically carry very large, multispecies pollen loads (Muchhala and Jarrín-V 2002; Muchhala 2008; Stewart and Dudash 2017), thereby potentially subjecting the plants they visit to substantial heterospecific pollen deposition. Given that the vast majority of studies on heterospecific pollen deposition and its consequences have been conducted on temperate plant taxa pollinated by insects and birds (e.g., Waser 1978; Wolf et al. 2001; Bell et al. 2005; Arceo-Gómez and Ashman 2011; Arceo-Gómez et al. 2016b; Bruckman and Campbell 2016), we aimed to expand current knowledge by testing whether simulated heterospecific pollen deposition decreases female reproduction in bat-pollinated plants.

Here we focus on the Neotropical bellflower genus *Burmeistera* (Campanulaceae: Lobelioideae) to explore the consequences of heterospecific pollen deposition on female reproduction. *Burmeistera* is characterized by high species richness in cloud forests of Central and South America, where multiple species co-occur, overlap in flowering time, and are mainly pollinated by nectar-feeding bats (Muchhala 2003, 2006; Lammers 2007; Moreno and Muchhala 2011). Bats commonly carry pollen from multiple *Burmeistera* species simultaneously (Muchhala and Jarrín-V 2002; Muchhala 2008) and cause high levels of heterospecific pollen deposition; on average, 20% of pollen grains deposited on *Burmeistera* stigmas are from other congeneric species (Muchhala 2006). The distance between the constriction of the corolla opening and the tip of the staminal column where the reproductive parts are located (hereafter, “exsertion length”; fig. 1A, 1B)

determines the precise pollen placement position on the head of the bats (Muchhala and Potts 2007; Muchhala 2008). Thus, differences in exsertion length affect the likelihood that bats will transfer pollen between any two *Burmeistera* species: species with similar exsertion lengths receive high deposition of each other’s pollen in their stigmas (Muchhala and Potts 2007). Moreover, exsertion length values in *Burmeistera* follow a bimodal distribution: out of 20 species, 11 could be categorized as long exserted (>20 mm; fig. 1A) and eight as short exserted (<18 mm; fig. 1B), with only one intermediate species (Muchhala 2008). In addition, local sites are overdispersed for this floral trait compared to null expectations (Muchhala and Potts 2007), suggesting that avoidance of interspecific pollen transfer influences *Burmeistera* assemblage composition (Muchhala and Potts 2007). However, differential pollen placement on the head of the bats does not completely eliminate competition. In fact, heterospecific pollen deposition occurs even among species with highly divergent exsertion lengths. Interestingly, field experiments have shown that more pollen from long-exserted species is deposited on stigmas of short-exserted species than vice versa (Muchhala and Potts 2007; Muchhala 2008). This asymmetric pollen flow should have different consequences for long- and short-exserted *Burmeistera*, leading to strong selection among short-exserted species to suppress heterospecific pollen performance and/or promote performance of conspecific pollen instead. Despite extensive research on the pollination ecology of *Burmeistera* (Muchhala 2003, 2006, 2008; Muchhala and Potts 2007), the consequences of heterospecific pollen deposition remain unexplored, and it is unknown whether they differ between long- and short-exserted species.

The goal of this study was to determine the relative impact of increasing heterospecific pollen deposition on female reproduction in two bat-pollinated *Burmeistera* species in a cloud forest site in Ecuador. We studied two focal species that represent extremes in terms of exsertion length and levels of heterospecific pollen deposition experienced in nature: the long-exserted *Burmeistera borjensis* Jeppensen (mean exsertion length = 23.8 mm; fig. 1A) and the short-exserted *Burmeistera ceratocarpa* Zahlbr (mean exsertion length = 15.2 mm; fig. 1B; Muchhala and Potts 2007). Given that previous work shows higher pollen transfer from *B. borjensis* to *B. ceratocarpa* than in the opposite direction (Muchhala 2006, 2008; Muchhala and Potts 2007), we hypothesized that impacts on female reproduction would be higher for *B. borjensis*, as this species has likely not undergone strong selection to overcome the negative effects of heterospecific pollen deposition. To test this hypothesis, we used a cross-pollination scheme using pollen mixtures as treatments simulating increasing amounts of heterospecific pollen deposition. Specifically, we addressed the following questions: (1) Does the probability of fruit abortion increase with heterospecific pollen deposition? (2) Does heterospecific pollen deposition negatively affect total seed production and seed mass per fruit? (3) Do these two *Burmeistera* species differ in their response to heterospecific pollen deposition?

Material and Methods

Study System and Site

Burmeistera H. Karst. & Triana (Campanulaceae: Lobelioideae) is a Neotropical genus of ~120 species of herbs, terrestrial and hemiepiphytic shrubs distributed from Guatemala to

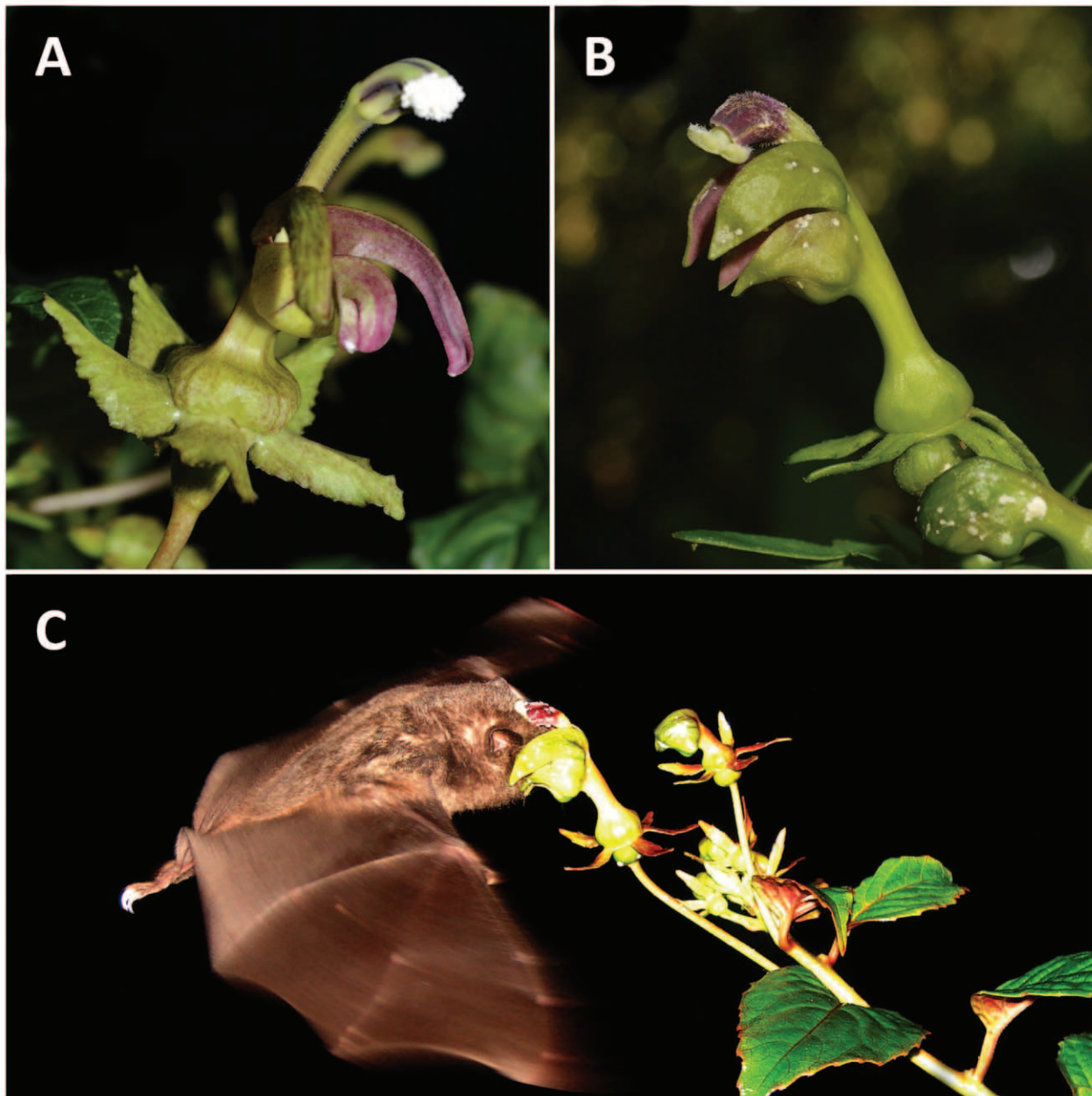


Fig. 1 Flowers of bat-pollinated *Burmeistera borjensis* and *Burmeistera ceratocarpa* (Campanulaceae: Lobelioideae). *A*, *Burmeistera borjensis* flower in male phase with copious pollen presented from the long-exserted anthers at the end of the staminal column. *B*, *Burmeistera ceratocarpa* flower in female phase showing its short-exserted bilobed stigma. *C*, Nectar-feeding bat (*Anoura caudifer*; Phyllostomidae) pollinating *B. ceratocarpa*. Photo credits: *A*, *B* = J. I. Moreira-Hernández; *C* = N. Muchhala.

northern Peru (Lammers 2007; Knox et al. 2008; Garzón-Venegas and González 2012; Lagomarsino et al. 2015). The genus attains its highest diversity in the cloud forests of Colombia and Ecuador, where up to six to eight species may co-occur at a given site (Moreno and Muchhala 2011; Vallejo et al. 2018). Overlap in flowering time between sympatric species is very high as individual plants remain in flower for several months and populations flower year-round (Muchhala 2006). Flowers are protandrous and zygomorphic (bilaterally symmetrical), with tubular corollas ending in a bell-shaped opening above which the re-

productive parts are exserted from the tip of a staminal column (Muchhala 2006; fig. 1*A*, 1*B*). Individual flowers last for 6–8 d, with an initial male phase occurring during the first couple of nights after anthesis. The female phase begins once the stigma protrudes from the staminal tube, pushing out any remaining pollen while expanding its lobes outward in a way that prevents self-pollination (Muchhala 2006). During the first couple of days of female phase, the wet stigmas are bright and smooth; afterward, the stigma withers, the flowers angle downward, and pollinator visitation stops (Muchhala 2006). Bats are the

primary pollinators of *Burmeistera*, with only a few species pollinated by hummingbirds (Muchhala 2003, 2006). *Burmeistera* fruits are berries that are either fleshy or inflated (hollow and with thin walls; Lammers 2002; Lagomarsino et al. 2015; Gamba et al. 2017) and contain hundreds to thousands of seeds <1 mm long (J. I. Moreira-Hernández and N. Muchhala, unpublished data).

Fieldwork was conducted in Yanayacu Biological Station (lat. 0°36'03"S, long. 77°53'22"W), Napo province, northeast Ecuador, during June and July 2014 and 2017. The station protects a combination of mature cloud forest, secondary growth, and abandoned pastures at an elevation of ~2200 m asl. Four *Burmeistera* species occur at the site: long-exserted *Burmeistera borjensis* and short-exserted *Burmeistera ceratocarpa*, *Burmeistera sodiroana*, and *Burmeistera succulenta*. We selected the former two as focal species because they are the most abundant at the study site; *B. sodiroana* and *B. succulenta* are far less common, reducing potential sample sizes. At the site, population-level flowering of both *Burmeistera* focal species occurs year-round, with individual plants continuously flowering at low intensity for several months (J. I. Moreira-Hernández and N. Muchhala, personal observation). *Burmeistera borjensis* is a terrestrial or hemiepiphytic shrub endemic to the Napo province in Ecuador from 1800 to 2400 m asl, while *B. ceratocarpa* is mostly terrestrial and has a wider distribution across the eastern slopes of the Colombian and Ecuadorian Andes between 1700 and 2500 m asl (Moreno and Muchhala 2011). Flowers of both species are of similar size for most floral measurements and differ mainly in exsertion length and the shape of the calyx lobes (fig. 1; Muchhala 2006). Previous work found that crosses between these two species using purely heterospecific pollen loads resulted in no seed set (J. I. Moreira-Hernández and N. Muchhala, unpublished data), suggesting that the formation of hybrid seeds in the wild is likely negligible.

Cross-Pollination Experiments

We used fully reciprocal cross-pollination experiments with pollen mixtures simulating increasing levels of heterospecific pollen deposition to estimate the effects of foreign pollen on female reproduction of *B. borjensis* and *B. ceratocarpa*. We selected experimental plants around the station and along forest trails, targeting plants with many flowers and flower buds ($N = 15$ and 17 for *B. borjensis* and *B. ceratocarpa*, respectively). Female-phase flowers were hand-pollinated using pollen mixtures containing different relative amounts of heterospecific and conspecific pollen. The mixtures were prepared in the late afternoon using pollen from four freshly opened male flowers in all cases. To simulate different levels of heterospecific pollen deposition, we created these mixtures while varying the ratio of heterospecific to conspecific flowers used in each case, resulting in three treatments: 1:3, 2:2, and 3:1. Throughout this article, we refer to our treatments as the ratio of heterospecific to conspecific flowers used in the mixtures and not the ratio of pollen because we lack data to ascertain whether pollen production per flower is comparable between our two study species. However, we compared their anther dimensions (anther length and anther width) with those of the only other *Burmeistera* species for which estimates of pollen production per flower are available (from Muchhala and Thomson 2010), and we present this data in table A1 (available online). The plants used in this study produced variable numbers of flowers. For those that produced only one, we

randomly chose a treatment, while for those that produced more, we continued to randomly apply one of each of the three treatments, rotating through all three treatments as more flowers opened through the course of the fieldwork.

Mixtures used on a given female flower never included self-pollen (e.g., pollen from another flower from the same plant). Female flowers were used for the experiments only if visual inspection with a hand lens showed the stigmatic surface to be free of any pollen. Although we cannot be 100% sure that there was no pollen present, we are confident that we reasonably minimized this possibility because pollinating bats bring in hundreds of grains of pollen per visit and change the appearance of stigmas from shiny to a dusty, matte look (J. I. Moreira-Hernández and N. Muchhala, personal observation). When the surface was not shiny, we did not use these flowers. In addition, repeated visits to the experimental plants allowed us to know in advance which flowers were going to transition from male to female phase each night so we could apply the pollen mixture treatments before any pollen deposition occurred.

To closely mimic natural pollen deposition, we applied the hand-pollination treatments using dry bat skins stuffed with cotton that were prepared for this study following standard protocols for mammal specimens destined for museum collections (Hall 1962). Pollen mixtures were placed on the bats' forehead and then applied to stigmas, mimicking the natural pollen-transfer process. Although we have not carried out pilot experiments to compare pollen loads from wild bats versus the bat specimens we used in the experiments, we suspect that these are similar to natural conditions based on previous work on how much pollen bats carry on their fur and transfer to *Burmeistera* stigmas in flight cage experiments (e.g., Muchhala and Thomson 2012) and how much they deposit in the wild (Muchhala 2003).

We used two different bat specimens to carry out the pollinations, and the pollen was mixed from newly opened flowers collected earlier that evening. On a given night, each bat was used for only one of the three mixture combinations, reloading with this same mixture before each pollination. Each night after all pollinations were carried out, we thoroughly removed all pollen from the specimens using clear tape.

Immediately after pollination, experimental flowers were covered with a small piece of a plastic straw closed at the tip for >24 h to prevent additional pollen deposition by floral visitors and were marked and labeled with tape. Five weeks after the experiments, plants were revisited to determine fruit fates (e.g., aborted or not). One fruit could not be reliably categorized because the whole mother plant died and was excluded from further analyses. Fruits were collected in 70% alcohol, and total seed production and total seed mass were estimated in the lab. The seeds were thoroughly washed out of each fruit using a squirt bottle and were collected on a paper filter. These filters were then weighed on an analytical scale with the seeds and then weighed again empty to calculate total seed mass of each fruit. For each fruit, approximately one-quarter of the seeds were counted and weighed to then extrapolate the total number of seeds produced by that fruit. When fruits contained relatively few seeds (<500), all seeds were counted.

Statistical Analyses

We used generalized linear mixed models to test whether the pollination treatments with different proportions of heterospecific

pollen affected the probability of fruit abortion, total seed production, and total seed mass of *B. borjensis* and *B. ceratocarpa*. The probability of fruit abortion was modeled with *Burmeistera* species and pollination treatment as fixed effects, specifying a logit link function and a binomial error distribution. Additional models for the total number of seeds produced and total seed mass per fruit (in milligrams) were built using negative binomial error distributions and logarithmic link functions. These last two models were run separately for each species given that they differ in fruit size (mean ± SE: for *B. borjensis*, 23.6 ± 1.2 mm; for *B. ceratocarpa*, 8.9 ± 0.2 mm; $U = 806, P < 0.001$) and seed number (mean ± SE: for *B. borjensis*, 1663 ± 131 seeds/fruit; for *B. ceratocarpa*, 1124 ± 116 seeds; $U = 570, P = 0.007$). In all models, focal plants were also included as random factors. When the effects of pollination treatment were significant, variation across levels was tested via Tukey’s post hoc test and P values were adjusted to avoid inflated type I error for these multiple comparisons. All analyses and graphs were made using the R statistical software, version 3.4.0 (R Development Core Team 2017).

Results

Across all treatments, we pollinated a total of 57 flowers of *Burmeistera borjensis* and 33 of *Burmeistera ceratocarpa* (table 1). Nearly half (45.6%) of *B. borjensis* fruits were aborted by the plants, while only 21.2% of *B. ceratocarpa* fruits were aborted (table 1), a significant difference ($\chi^2 = 3.88, P = 0.049$; fig. 2). However, and contrary to expectations, the relative proportion of heterospecific pollen in mixtures did not affect the probability of fruit abortion in either species ($\chi^2 = 0.00, P = 0.992$; fig. 2).

Total seed production per fruit decreased with increasing heterospecific pollen deposition in *B. borjensis* ($P = 0.031$; fig. 3A). Fruits from the treatment with the greatest relative amount of heterospecific pollen produced significantly fewer seeds than fruits from the treatment with the lowest relative amount of heterospecific pollen ($z = 2.541, P = 0.033$; fig. 3A). Pollination treatment also exhibited a negative trend on total seed mass per fruit for *B. borjensis*, but this effect was not significant ($P = 0.058$; fig. 3B). We did not find any effect of pollination treatment on female reproduction of *B. ceratocarpa* in terms of total seed production ($P = 0.876$; fig. 3A) or total seed mass per fruit ($P = 0.718$; fig. 3B).

Quantifying anther tube size demonstrates that *B. borjensis* anthers are somewhat larger than those of *B. ceratocarpa* (table A1). Anthers of *B. borjensis* (mean ± SD: length, 8.16 ±

1.12 mm; width, 3.69 ± 0.66 mm) are of similar size to those of *Burmeistera sodiroana* (mean ± SD: length, 8.83 ± 1.35 mm; width, 3.46 ± 1.24 mm), which produces 33,862 ± 2505 grains per flower (Muchhala and Thomson 2010). *Burmeistera ceratocarpa* anthers, on the other hand (mean ± SD: length, 5.44 ± 0.63 mm; width, 3.99 ± 0.56 mm), are roughly equal in size to those of *Burmeistera rubrosepala* (mean ± SD: length, 5.49 ± 0.00 mm; width, 3.14 ± 0.00 mm), which produces 23,001 ± 3956 grains per flower (Muchhala and Thomson 2010). Thus, our approach of combining pollen from four flowers to make our pollen mixtures may have led to an overrepresentation of *B. borjensis* pollen. To the degree that anther size is a good proxy for pollen production in *Burmeistera*, a possible overrepresentation of *B. borjensis* pollen in our mixtures would further support the significant effect of pollination treatment on female reproduction that we found for *B. borjensis* but not for *B. ceratocarpa*.

Discussion

We found that the consequences of simulated heterospecific pollen deposition on female reproduction differed for our two bat-pollinated *Burmeistera* species. *Burmeistera borjensis* showed significantly reduced seed production and lower seed mass when relatively higher proportions of heterospecific pollen were applied, while *Burmeistera ceratocarpa* was able to produce fruits with similar total number of seeds and total seed mass regardless of pollination treatment (fig. 3). Thus, female reproductive success in *B. ceratocarpa* was not affected by increasing heterospecific pollen deposition, while *B. borjensis* did not exhibit such tolerance. Moreover, assuming that anther size correlates with pollen production in *Burmeistera*, the mixtures we created most probably overrepresented *B. borjensis* pollen. The fact that *B. ceratocarpa* did not show any effect to our foreign pollen mixtures, despite being probably more numerically disadvantaged than we had assumed, if anything actually strengthens our conclusions. Conversely, *B. borjensis* showed reduced female reproductive success in response to foreign pollen, despite pollen mixtures being possibly biased in its favor.

Past assessments of natural rates of interspecific pollen flow show that the short-exserted *B. ceratocarpa* experiences much greater influx of foreign pollen than the long-exserted *B. borjensis* (Muchhala 2006, 2008; Muchhala and Potts 2007). However, the proportion of fruits that each species aborted before maturation did not change based on the relative amount of

Table 1

Fate of Fruits of *Burmeistera borjensis* and *Burmeistera ceratocarpa* after the Various Hand-Pollination Treatments

Species and ratio of heterospecific to conspecific flowers used to make pollen mixture	Total no. pollinations performed	Proportion of fruits (N)		
		Matured	Aborted	Lost
<i>B. borjensis</i> :				
1:3	24	.50 (12)	.50 (12)	0 (0)
2:2	18	.61 (11)	.39 (7)	0 (0)
3:1	15	.53 (8)	.47 (7)	0 (0)
<i>B. ceratocarpa</i> :				
1:3	12	.83 (10)	.17 (2)	0 (0)
2:2	10	.90 (9)	.10 (1)	0 (0)
3:1	11	.64 (7)	.36 (3)	.09 (1)

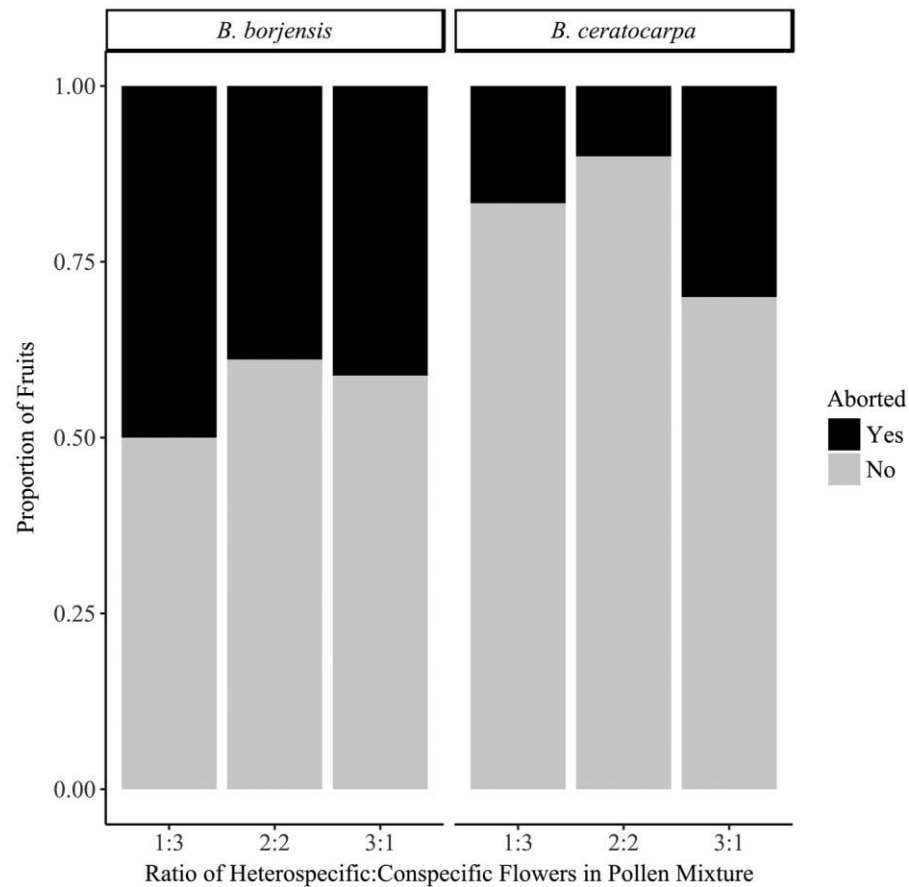


Fig. 2 Proportion of fruits aborted by *Burmeistera borjensis* ($N = 57$ flowers) and *Burmeistera ceratocarpa* ($N = 32$) for the three cross-pollination treatments defined by the ratio of heterospecific to conspecific flowers used to make the pollen mixtures.

heterospecific pollen that they received. *Burmeistera borjensis* consistently aborted around half (45.6%) of fruits across treatments, while *B. ceratocarpa* aborted around one-fifth (21.2%). If and how these rates might compare to patterns of abortion rates in natural conditions following mixed or pure conspecific pollen deposition warrants further investigation.

Although our study does not address what mechanisms are responsible for the differential effect of increasing heterospecific pollen deposition between our focal species, different gametic or postzygotic barriers act on heterospecific pollen receipt (Ashman and Arceo-Gómez 2013; Kostyun and Moyle 2017). One possibility is that after applying the pollen mixtures, pollen-stigma and pollen tube–pistil interactions could have resulted in faster rates of conspecific pollen germination and pollen tube growth in pistils of *B. ceratocarpa* but not those of *B. borjensis*, thus affecting seed production only for the latter species. This type of gametic barrier, termed “conspecific pollen precedence,” has been reported among several plant species pairs that commonly experience heterospecific pollen deposition from each other in sympatry (Figueroa-Castro and Holtsford 2009; Montgomery et al. 2010; Natalis and Wesselingh 2012). A second possibility would be that pollen tubes from *B. borjensis* fail to reach the ovary and fertilize ovules in *B. ceratocarpa* but not vice versa due to bio-

chemical or physical constraints (Carney et al. 1996; Lyu et al. 2016; Tong and Huang 2016); this would also affect *B. borjensis* more than *B. ceratocarpa*. Many studies find that gametic barriers often result in long-styled species outmatching pollen from short-styled relatives in either type of pistil (Carney et al. 1996; Diaz and Macnair 1999; Wolf et al. 2001; Rahmé et al. 2009), yet we found the opposite pattern, as seed production only decreased in long-exserted *B. borjensis* but not in short-exserted *B. ceratocarpa*. If, despite differences in exertion length, pollen transfer indeed occurs asymmetrically from *B. borjensis* to *B. ceratocarpa* (Muchhala 2006, 2008; Muchhala and Potts 2007), the latter might be undergoing selection for increased gametic isolation from the former (Kay and Schemske 2008; Arceo-Gómez et al. 2016b). Postzygotic barriers acting after ovule fertilization may also lead to differential abortion of hybrid seeds early in their development or may influence rates of fruit formation in ways that could explain our results (Wang and Cruzan 1998; Wolf et al. 2001; Arceo-Gómez and Ashman 2011). For example, if abortion of hybrid seeds occurs relatively early in development for *B. borjensis*, yet occurs later in *B. ceratocarpa*, this would falsely give the impression that the former aborts more seeds. However, we think that postzygotic barriers are unlikely to drive our results because pure heterospecific pollen

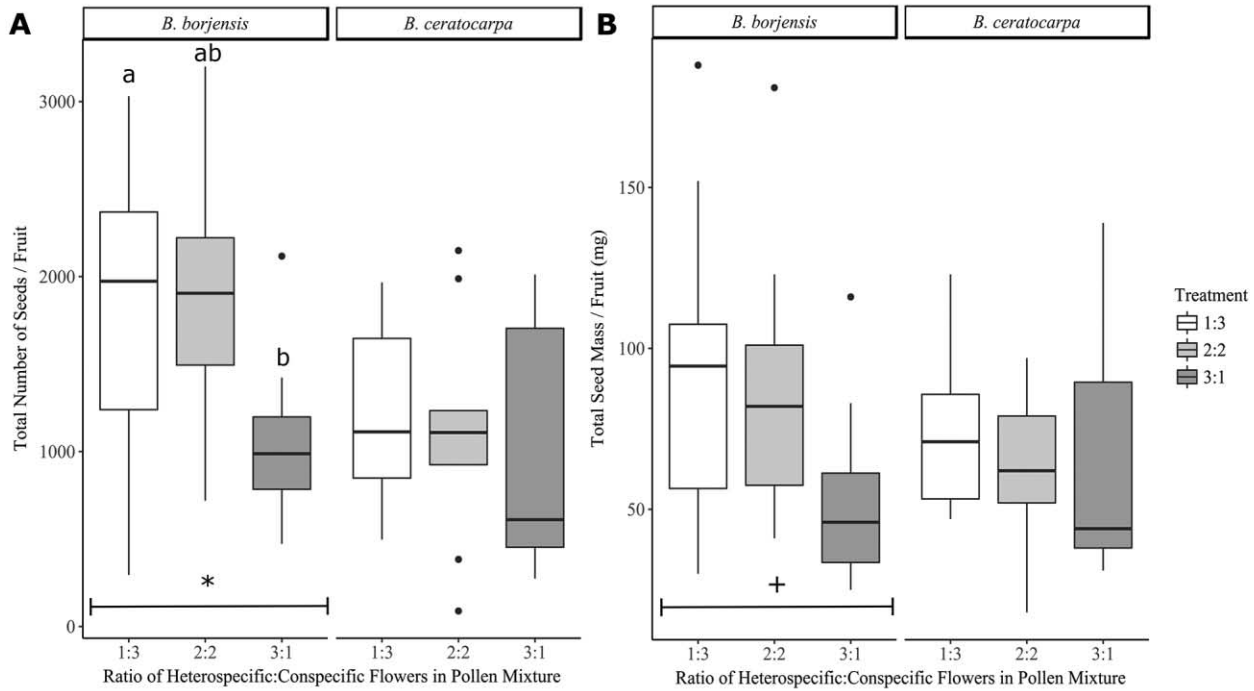


Fig. 3 Female reproductive success of *Burmeistera borjensis* ($N = 31$ flowers) and *Burmeistera ceratocarpa* ($N = 26$ flowers) for three cross-pollination treatments defined by the ratio of heterospecific to conspecific flowers used to make the pollen mixtures. **A**, Total number of seeds per fruit. **B**, Total seed mass per fruit. Significance levels: $0.10 > P > 0.05$ (plus sign); $P \leq 0.05$ (one asterisk). Different lowercase letters denote treatments that are significantly different from each other ($\alpha = 0.05$).

loads do not lead to fruit development in either species and aborted seeds were very rare in experimental fruits (J. I. Moreira-Hernández and N. Muchhala, data not shown). Future work should evaluate the strength of gametic and postzygotic barriers acting between these two species and help clarify whether the tolerance to increasing heterospecific pollen exhibited by *B. ceratocarpa* represents an evolutionary response to asymmetric rates of heterospecific pollen deposition in natural conditions.

Interspecific pollen transfer by shared pollinators can have profound implications for plant diversification because it influences reproduction and patterns of hybridization between closely related plant species (Morales and Traveset 2008; Moreira-Hernández and Muchhala 2019). Most of what we know about these consequences comes from studies on temperate species pollinated by insects and birds. Our study expands this understanding, with evidence suggesting that tolerance to heterospecific pollen deposition in bat-pollinated plants may facilitate coexistence in the face of low pollinator fidelity and high rates of interspecific pollen transfer (Muchhala 2006, 2008; Muchhala and Potts 2007). Differential pollen placement on bats' bodies is another mechanism that facilitates coexistence by minimizing interspecific pollen transfer (Tschapka et al. 2006; Muchhala and Potts 2007; Muchhala and Thomson 2012; Stewart and Dudash 2016). Differential pollen placement and tolerance to heterospecific pollen deposition represent complementary ways to ameliorate potential reproductive costs of relying on low-fidelity pollinators such as nectarivorous bats.

In conclusion, our data suggest that receiving heterospecific pollen has different consequences for female reproduction of these two *Burmeistera* species, with the species more exposed to heterospecific pollen deposition in nature being able to cope with high amounts of heterospecific pollen. Future work should test additional species pairs to determine the generality of these results across *Burmeistera* and explore in detail the specific isolating barriers that occur during pollen-pistil interactions and after ovule fertilization. Investigating the role of interspecific pollen transfer and its consequences during speciation is a necessary step to understanding the rapid diversification of many tropical plant clades.

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