



Floral isolation and pollination in two hummingbird-pollinated plants: the roles of exploitation barriers and pollinator competition

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Abstract

Differences in feeding performance and aggressive abilities between species and sexes of hummingbirds are often associated with the partitioning of their food sources, but whether such partitioning results in floral isolation (reproductive isolation at the stage of pollination) has received little attention. We examined components of floral isolation and pollinator effectiveness of *Heliconia caribaea* and *H. bihai* on the island of Dominica, West Indies. The short flowers of *H. caribaea* match the short bills of male *Anthracothorax jugularis*, its primary pollinator, whereas the long flowers of *H. bihai* match the long bills of female *A. jugularis*, its primary pollinator. In pollination experiments, both sexes of *A. jugularis* were equally effective at pollinating the short flowers of *H. caribaea*, which they preferred to *H. bihai*, whereas females were more effective at pollinating the long flowers of *H. bihai*. Moreover, an average difference in length of 12 mm between *H. caribaea* and *H. bihai* flowers did not prevent heterospecific pollen transfer, and both sexes transported pollen between the two plant species. In field studies using powdered dyes as pollen analogs, however, heterospecific pollen transfer was minimal, with only 2 of 168 flowers receiving dye from the other species. The length of *H. bihai* flowers acted as an exploitation barrier to male *A. jugularis*, which were unable to completely remove nectar from 88% of the flowers they visited. In contrast, interference competition combined with high floral fidelity through traplining prevented female *A. jugularis* from transferring pollen between the two *Heliconia* species. A combination of exploitation barriers, interference and exploitative competition, and pollinator preferences maintains floral isolation between these heliconias, and may have contributed to the evolution of this hummingbird-plant system.

Keywords Pollination · Floral isolation · Hummingbird · *Heliconia* · Exploitation barrier · Competition

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Introduction

Plants and their pollinators have fascinated biologists for centuries owing to the variety of flower shapes and sizes and the tendency for the flowers of some plants to match the trophic morphology of their pollinators. Darwin (1862) was among the first to put these apparent matches in an evolutionary context by suggesting that the great diversity of floral structures of plants evolved in response to interactions with their different animal pollinators. Studies since Darwin's time have demonstrated that pollinators may not only contribute to floral diversification (Fenster et al. 2004), but also may contribute to reproductive isolation of co-flowering plant species through differences in pollinator morphology and behavior (Scheistl and Schlüter (2009).

Such reproductive isolation at the stage of pollination has been termed floral isolation (and occasionally, pollinator isolation; Scheistl and Schlüter 2009), and may be achieved through mechanical and ethological isolation (Grant 1949, 1992, 1994). In mechanical isolation, heterospecific pollen transfer is prevented by differences between two plant species in floral structure. The flowers of two plant species may place pollen on different parts of a pollinator's body or alternatively, may be adapted for pollination by animal pollinators with differing morphologies. In ethological isolation, heterospecific transfer is prevented by differences between plant species in floral structures that influence pollinator behavior: pollinators may preferentially visit flower species that they recognize by various cues such as shape, color, or scent (Grant 1994). In addition to fitting or attracting certain pollinators, traits involved in floral isolation may exclude or discourage non-preferred visitors by acting as exploitation barriers that make access to floral resources such as nectar more difficult for non-preferred visitors (Grant and Grant 1968; Grant and Temeles 1992; Temeles et al. 2002; Rodríguez-Gironés and Santamaría 2005, 2006, 2010). Floral isolation may evolve as a secondary result of allopatric speciation and adaptation to different pollinators, as a direct result of selection for reproductive isolation to avoid hybridization, or in response to competition for pollinators or reproductive interference via heterospecific pollen transfer, i.e., character displacement (Grant 1994; Scheistl and Schlüter 2009).

In his seminal review of floral isolation, Verne Grant (1949) noted the tremendous variation in length and shape of the flowers of tropical hummingbird-pollinated plants, and hypothesized that such variation might be associated with mechanical and ethological reproductive isolation. Subsequent studies of floral isolation between hummingbird and non-hummingbird pollinated plants have, in fact, provided evidence that differences between hummingbird and non-hummingbird-pollinated plants in the morphology and orientation of their flowers contribute to mechanical and ethological isolation (e.g., Fulton and Hodges 1999; Queiroz et al. 2015; Shaw et al. 2017). Less attention has been given to whether differences in floral length and shape between closely-related species of hummingbird-pollinated plants result in floral isolation, and the physical and behavioral mechanisms by which such isolation happens (but see Kay 2006; Cuevas et al. 2018).

Recent studies of hummingbird foraging behavior suggest that differences in the size and shape of the flowers of hummingbird-pollinated plants might contribute to floral isolation as a result of asymmetries between hummingbirds in exploitative and interference competition and subsequent resource partitioning. First, differences in feeding performance between hummingbirds of different bill morphologies at natural and artificial flowers are realized only at the extremes of floral shape and size, with short-billed hummingbirds outperforming long-billed hummingbirds at short, straight flowers and long-billed hummingbirds outperforming short-billed hummingbirds at

long flowers (Temeles et al. 2009). Second, short-billed hummingbirds visited plants with short flowers whereas long-billed hummingbirds visited plants with long flowers under natural conditions, but both short and long-billed hummingbirds preferred artificial feeders with short flowers when given a choice among artificial flowers of varying size and shape (Maglianesi et al. 2015). Both of these studies suggest that the size and shape of the flowers of some hummingbird-pollinated plants may act as exploitation barriers that either prevent access to nectar by some hummingbirds or increase foraging costs so that it is more profitable to feed at other food sources, and that competitive interactions may mediate choices of food plants and resource partitioning. Lastly, both theoretical and empirical studies indicate that resource partitioning of floral phenotypes by pollinators may result in assortative mating among phenotypes and floral isolation (Rodríguez-Gironés and Santamaría 2004, 2005; Temeles et al. 2016).

Here we present a study of factors contributing to floral isolation, especially exploitation barriers and pollinator competition, between *Heliconia caribaea* and *Heliconia bihai* on the island of Dominica, West Indies. *Heliconia caribaea* and *H. bihai* are the only heliconias native to the Eastern and Northern Caribbean (Berry and Kress 1991; Kress et al. 1999). The two species evolved in separate clades in Section *Heliconia* and came into contact through range expansion into the Eastern Caribbean (Iles et al. 2017). Our work over the past 20 years has determined that these heliconias are pollinated primarily by the purple-throated carib hummingbird, *Anthracothorax jugularis*, and more specifically, that the two *Heliconia* species are partitioned by sexes of purple-throated caribs (Temeles et al. 2000, 2005, 2006, 2009; Temeles and Kress 2003, 2010). *Heliconia caribaea* is pollinated primarily by male caribs, and has many bracts, and hence flowers, per inflorescence and short flowers corresponding to the short bills, larger size, and higher energy requirements of its male hummingbird pollinator (Fig. 1). *Heliconia bihai* is pollinated primarily by female caribs, and has fewer bracts per inflorescence with long flowers corresponding to the long bills, smaller size, and lower energy requirements of its female hummingbird pollinator (Fig. 1).

Although we have demonstrated that male and female purple-throated caribs differentially visit the two *Heliconia* species, we do not know whether such differential visitation results in floral isolation. Accordingly, we conducted experiments with captive birds on the pollination abilities and preferences of each sex of purple-throated carib for the two *Heliconia* species, as well as on the opportunity for heterospecific pollen transfer. We also conducted field experiments and observations on the potential for heterospecific pollen transfer among sympatric populations of the two *Heliconia* species. We show that (1) in experiments with captive birds, differences in flower length do not prevent heterospecific pollen transfer between the two *Heliconia* species; (2) both sexes of caribs are equally effective at removing and depositing pollen onto flowers of *H. caribaea*, whereas female caribs are more effective at pollinating *H. bihai*; (3) both sexes of caribs, but especially females, are capable of carrying pollen between the two *Heliconia* species; (4) the long corolla of *H. bihai* acts as an exploitation barrier to male caribs by hindering access to nectar; and (5) where the two *Heliconia* species are sympatric, heterospecific pollen transfer between the two heliconias is prevented by a combination of exploitation barriers, interference and exploitative competition, and pollinator preferences. We conclude with a discussion of the implications of exploitation barriers and pollinator competition for floral isolation and divergence between plant species.



Fig. 1 Bills of female (a) and male (b) *Anthacothorax jugularis*, flowers of *H. bihai* (c) and *H. caribaea* (d), and feeding positions of female *A. jugularis* at *H. bihai* (e) and male *A. jugularis* at *H. caribaea* (f). a–d Modified from Temeles and Kress (2003)

Materials and methods

We conducted field observations and experiments on the pollination and floral isolation of *H. caribaea* and *H. bihai* on the island of Dominica, West Indies, from March to July 2014, and from mid-May to July, 2016. Pollination and pollinator preference experiments were conducted in secondary rainforest on the property and with the permission of Mr. Mervin Thomas, Warner Rd., Dominica, West Indies (15°23'54"N, 61°23'21"W).

The plants

Heliconias are perennial, tree-like (1–6 m height) herbs that occur primarily along roads, trails, rivers, and in forest light gaps. They have rhizomatous growth, a musoid growth habit, and produce multiple inflorescences (1–35) that generate flowers over a period of 1–3 months (Berry and Kress 1991). Inflorescences are formed by large showy bracts (modified leaves) and in *H. bihai* and *H. caribaea*, bracts may vary in color both between species on the same island and within species from island to island (see Temeles and Kress 2003). On Dominica, *H. bihai* has a single color morph whereas *H. caribaea* has two color morphs, one with red bracts and the other with yellow bracts (Temeles and Kress 2003). In addition to having more bracts per inflorescence and shorter flowers than *H. bihai* (Temeles and Kress 2003), *H. caribaea* also produces more nectar per flower per 24 h (Mean \pm SE: 158.8 ± 2.8 μ l, $N=42$ flowers and plants) than *H. bihai* (123.2 ± 13.7 μ l, $N=11$ flowers

and plants; unpublished data from Temeles and Kress 2003, 2010). Nectar concentrations of the two species, however, are similar (*H. caribaea*: $21.8 \pm 0.4\%$ sucrose, $N=42$ flowers; *H. bihai*: $22.4 \pm 1.0\%$ sucrose, $N=11$; unpublished data from Temeles and Kress 2003, 2010).

In *H. bihai* and *H. caribaea*, each bract holds a range of 1–30 flowers over the season, but no more than one flower is produced daily within a bract; anthesis lasts 1 day (Temeles et al. 2005). The flowers of both *H. bihai* and *H. caribaea* are bisexual, zygomorphic, tubular, and greenish-white in color (Temeles and Kress 2003). Both species produce a single fruit per flower containing 0–3 seeds. On Dominica, *H. bihai* is visited primarily by trampling female purple-throated caribs (Temeles and Kress 2003; Gowda and Kress 2013). *Heliconia caribaea* is visited by female and especially male purple-throated caribs, with the latter defending clumps of the plants as territories (Temeles and Kress 2003, 2010; Gowda and Kress 2013). Green-throated caribs (*A. holosericeus*) and Antillean crested hummingbirds (*Orthorhyncus cristatus*) are minor visitors to *H. caribaea*, accounting for less than 5% of visits in our 18 years of work on Dominica (Temeles and Kress 2003, 2010; Gowda and Kress 2013). Our past work indicates that sexes of purple-throated caribs differentially visit color morphs of *H. caribaea* in some but not all populations (Temeles and Kress 2003; Temeles et al. 2013).

Pollination and preference experiments

Purple-throated caribs were captured by using mist-nets strategically placed within clumps of *H. caribaea*. Following capture, individual birds were housed in 3 m × 3 m screenhouses and provided with natural perches from cut foliage. Each bird was provided with two hummingbird feeders as well as natural *Heliconia* inflorescences filled with 20% sucrose solution by volume; the screenhouses also contained small insects that the birds gleaned from foliage and the tent surfaces. Each bird was given 24 h to acclimate to captivity prior to the start of experiments. Five males and seven females were used in the experiments.

Our previous field studies indicate that male purple-throated caribs visit *H. bihai* at much lower frequency than females (Temeles and Kress 2003; Temeles et al. 2013). Because some or all males may have lacked familiarity with *H. bihai* as a food source, we began all experiments by offering each bird *H. caribaea* first, followed by *H. bihai*, so that birds would learn to associate cut inflorescences with food. We also checked *H. bihai* flowers for residual nectar following male visits using a pipetter with a gel-loading pipette tip. To motivate birds to feed, we fasted them for 1 h prior to the start of a day's experiments by removing their food (Temeles et al. 2009).

Pollen removal experiments

To compare pollen removal between male and female purple-throated caribs, we cut inflorescences containing newly-dehisced flowers. Inflorescences of *H. caribaea* were collected from the surrounding rainforest, whereas inflorescences of *H. bihai* were collected from 20 plants maintained in an enclosed garden at the study site (see Temeles et al. 2016 for description). Because nectar volume and concentration influence the amount of time hummingbirds spend feeding at flowers (Temeles et al. 2009) and hence pollen removal (Temeles and Rankin 2000), we removed a flower's natural nectar using a pipetter with a gel-loading pipette tip and replaced it with 20 μ l of 20% sucrose solution by volume. Our choice of volume is similar to standing crops of heliconias (Temeles and Kress 2010) and

also avoided satiating the birds during trials. Our choice of concentration approximated those found in earlier studies (see above). Inflorescences were secured to straight wooden branches using cable ties, and the branches with inflorescences were then placed inside the screenhouse. Birds were presented with five flowers in total on two inflorescences. Once the bird had visited all five flowers, we captured it and removed pollen from the bird using clear scotch tape, marking where the tip of the bill occurred on the tape with a permanent black marker. The tape was then affixed to a microscope slide. A metric ruler was used to divide the tape into 5 mm segments starting from the bill tip. The slides were then later examined under a microscope for pollen counts. Prior to the start of the next trial, the bird's bill and crown were rinsed with water and patted dry with paper towels. Each bird visited each of the five flowers once, and trials usually lasted less than 20 min.

Pollen deposition experiments

To compare pollen deposition within and between the two *Heliconia* species, inflorescences of *H. caribaea* and *H. bihai* were collected as described above and secured to straight branches using cable ties. Natural nectar was removed and replaced with 20 μ l of 20% sucrose solution by weight. Five newly-dehisced flowers of either *H. caribaea* or *H. bihai* were used as pollen donors for the experiment, and five newly-dehisced flowers of either *H. caribaea* or *H. bihai* were used as recipient flowers for the experiment (two inflorescences for each). We removed all the anthers from recipient flowers, and used brushes and scotch tape to remove all pollen from recipient stigmas, verifying that pollen had been removed using a 10 \times hand-lens. The five donor flowers were presented to a bird first, and once the bird had visited all five flowers, the donor flowers were immediately removed and replaced with the inflorescences containing the recipient flowers. Once the hummingbird had visited all five recipient flowers once, we removed them. We cleaned the bills and crowns of birds with water between trials.

Fuschin jelly is the standard technique for removing pollen grains from stigmas for pollen counts (Kearns and Inouye 1983; Temeles and Pan 2002), but we found that the jelly liquefied in the rainforest heat and humidity. As a result, we used scotch tape to remove pollen deposited on stigmas by dabbing the tape onto the stigma. To reduce the possibility of counting pollen grains deposited onto the tape by accident, we circled the location of where the tape was applied to the stigma. The tape and the stigma were then affixed to a microscope slide. Pollen grains on the microscope slides were later counted under a light microscope. Six of the 240 slides had pollen counts that were significant outliers (beyond 95% of the distribution); because these slides had pollen on other parts of the tape, we regarded them as contaminated by hand contact and excluded them from the analyses. We measured the corolla lengths of all flowers used in all treatments. Corolla lengths of *H. caribaea* averaged 35.50 ± 0.09 mm ($N = 120$ flowers), whereas corolla lengths of *H. bihai* averaged 47.32 ± 0.15 mm ($N = 120$ flowers).

Preference experiment

Following the pollen removal and deposition experiments, we conducted choice tests in which the experimental subject was offered a choice between the two *Heliconia* species. We matched inflorescences in this experiment for both the number of bracts and the number of flowers. A positive choice was scored when the bird landed and began to feed from a flower (Temeles et al. 2009).

Field studies pollen transfer

On Dominica, *H. caribaea* and *H. bihai* have a predominantly parapatric distribution, with *H. caribaea* occurring on rain forest edges and clearings from approximately 70 to 600 m elevation and *H. bihai* occurring on elfin forest edges at elevations above 600 m (Temeles and Kress 2003). We studied the opportunity for pollen transfer between the two *Heliconia* species at three of their contact zones.

Our studies of banded purple-throated caribs indicate that they are site-faithful with both males and females remaining on the same patches of heliconias for up to 6 years (Gowda et al. 2012). We thus considered distances of > 3 km to be sufficient barriers to gene flow and selected study populations based on this criterion. To examine conspecific and heterospecific pollen transfer where *H. bihai* and *H. caribaea* co-occurred, we studied three mixed populations of *H. bihai* and *H. caribaea* (Canicules, 15°27'42"N, 61°23'59"W; Haut Jean, 15°28'9"N, 61°23'57"W; Imrays View, 15°30'22"N, 61°25'29"W). The yellow morph of *H. caribaea* was rare at both the Canicules and Haut Jean sites (≤ 6 plants), so we only analyzed data from the red morph at these sites. In contrast, the red morph was rare at the Imrays View site (3 plants), so we only analyzed data for the yellow morph.

Because pollen of the two *Heliconia* species is indistinguishable under microscopic examination, we used powdered dyes to study conspecific and heterospecific pollen transfer (Risk Reactor® short-wave ultra-violet dyes in blue, green, and yellow; see Temeles et al. 2016). At each of the three sympatric sites (Canicules, Imrays View, and Haut Jean), we applied dyes to the anthers of flowers of *H. bihai* (Canicules: $N=28$; Imrays View: $N=21$; Haut Jean: $N=31$) and *H. caribaea* (Canicules: $N=40$; Imrays View: $N=52$; Haut Jean: $N=62$) on two different days, alternating colors between species on the two different days (applications at each site were separated by a minimum of 6 days to avoid cross-contamination and only one flower was dyed per plant). Dye was applied to flowers within the 2 h after sunrise, and bracts containing dyed flowers were marked for identification. We also marked the bracts of an approximately equal sample of undyed flowers (one per plant) of *H. bihai* (Canicules: $N=31$; Imrays View: $N=21$; Haut Jean: $N=36$) and *H. caribaea* (Canicules: $N=38$; Imrays View: $N=47$; Haut Jean: $N=65$). All flowers were then collected in the early afternoon and inspected under a microscope with UV lighting for the presence or absence of dye particles on stigmas. To further assess the potential for pollen movement between *Heliconia* species, for each pollinator foraging bout recorded (see below), we examined pollinator transitions within and between flowers of the two *Heliconia* species at these three sites. At each of the three sites, we estimated plant species constancy by using counts of pollinator transitions within and between species. For both sexes, the number of transitions between flowers was small (Females, Imrays View: 2.4 ± 0.3 , $N=11$ individuals; Haut Jean: 3.0 ± 1.3 , $N=5$ females; Canicules: 3.7 ± 0.9 , $N=6$ females; Males, Imrays View: 2.7 ± 0.4 , $N=4$ males; Haut Jean: 2.0 ± 0.2 , $N=11$ males; Canicules: 1.9 ± 0.3 , $N=4$). Consequently, we pooled transitions across all observations for each sex at each of the three sites. These pooled data were then used to calculate Bateman's Constancy Index (Bateman 1951). Bateman's index (BCI) is $BCI = ((AD)^{1/2} - (BC)^{1/2}) / ((AD)^{1/2} + (BC)^{1/2})$, where A is the number of transitions from *H. bihai* to *H. bihai*, B is the number of transitions from *H. bihai* to *H. caribaea*, C is the number of transitions from *H. caribaea* to *H. bihai*, and D is the number of transitions from *H. caribaea* to *H. caribaea*. Values of this index range from -1 to 1 , with a value of -1 indicating movements alternating

between *Heliconia* species and a value of 1 indicating complete fidelity to a single species (Bateman 1951). One difficulty with the application of this index is when a pollinator visits only one species while foraging. Bateman (1951) noted that in this situation, constancy is at a maximum; thus, we assigned a *BCI* of 1 (complete constancy) in these situations.

Statistical analyses

We used *t* tests to compare pollen removal and deposition between sexes of purple-throated caribs. We used the averages for each of the five males and seven females in these analyses to avoid pseudo replication, and sample sizes in all *t* tests involved five males and seven females. Means are presented along with standard errors (SE). We used binomial probability tests to compare choices by male and female purple-throated caribs for either *H. caribaea* or *H. bihai*.

Studies of natural selection on corolla lengths by hummingbirds indicate that when bill lengths and corolla lengths are well-matched, selection is either weakly directional or stabilizing, whereas when bill lengths and corolla lengths are poorly-matched, selection is strongly directional (Nattero et al. 2010). Accordingly, we estimated directional and stabilizing selection on corolla length using standard selection analysis (Lande and Arnold 1983) as a means to assess the degree of matching between male and female caribs and the two heliconias. Prior to analysis we standardized each corolla length trait to a mean equal to zero and to a variance equal to one. Our fitness measure, dye grains deposited per stigma per flower, was converted to relative fitness by dividing individual fitness by mean fitness (Lande and Arnold 1983). Fitness measures were relativized and floral traits were standardized separately for each *Heliconia* donor–*Heliconia* recipient trial for each sex (N =eight trials). We estimated net selection (direct and indirect selection resulting from phenotypically correlated traits) on a given floral trait using univariate regression models. Directional selection is estimated by the linear model ($w_i' = c + \beta_{uni} z_i$) whereas stabilizing or disruptive selection is estimated by the quadratic model ($w_i' = c + \beta_1' z_i + \beta_2 z_i^2$), where ($\gamma_i = 2 \beta_2$) is the univariate nonlinear selection gradient. In these analyses, the univariate linear selection gradient is equivalent to the selection intensity (standardized selection differential; Fairbairn and Reeve 2001; Foellmer and Fairbairn 2005). Note that in the heterospecific pollen deposition trials, coefficients indicate selection against values of corolla length. All regressions were performed using PROC GLM in SAS Version 9.3 (SAS Institute, Cary, North Carolina, U. S. A.).

We used Monte Carlo estimates of exact tests to compare dye transfer within and between *Heliconia* species in the three sympatric populations in two different ways. First, for each of the three populations, we compared the observed frequency of heterospecific dye transfer to the observed frequency of conspecific dye transfer. Second, we compared the observed frequencies of conspecific and heterospecific dye transfers to expected frequencies calculated from the relative frequencies of donor and recipient flowers of the two *Heliconia* species. We also used Monte Carlo estimates of exact tests to compare the observed numbers of transitions within and between *Heliconia* species to the expected number of transitions based upon observed visits to the two *Heliconia* species by each sex at each site. In cases where observed cell frequencies were zero, we added one for statistical tests. Monte Carlo exact tests were performed with 10^6 draws to compute *P* values using PROC FREQ in SAS Version 9.3 (SAS Institute, Cary, North Carolina, U. S. A.).

The datasets analyzed for the current study are available in the Dryad Digital Repository (<https://doi.org/10.5061/dryad.5732bd7>).

Results

Pollination experiments with captive birds

Both male and female purple-throated caribs were efficient at removing pollen from *H. caribaea* flowers and the total number of pollen grains removed did not differ between the sexes (Mean \pm SE, five males: 880 ± 77 pollen grains; seven females: 768 ± 115 pollen grains; $t = 0.81$, $P = 0.44$). Sexes differed in where pollen was placed on their bills and heads, however, and males received significantly more pollen than females 16–20 mm along the bill whereas pollen tended to be placed in the 26–35 mm range on females (bill and crown; see Fig. 2a). In contrast, females were significantly more efficient at removing pollen from *H. bihai* than males (Males: 123 ± 72 pollen grains; Females: 598 ± 157 pollen grains; $t = 2.75$, $P = 0.025$; Fig. 2b). For both sexes, pollen of *H. bihai* tended to be placed further back from the bill than pollen of *H. caribaea*, but locations of pollen on birds' bills overlapped considerably between the two plant species (Fig. 2).

Our experiments on pollen deposition on stigmas yielded similar results. Both male and female purple-throated caribs were efficient at depositing pollen onto stigmas of *H. caribaea* flowers and the total number of pollen grains deposited on stigmas did not differ between the sexes (24.2 ± 6.6 pollen grains per flower; 22.5 ± 5.1 pollen grains; $t = 0.25$, $P = 0.81$). In contrast, females were significantly more efficient at depositing pollen onto stigmas of *H. bihai* than males (Males: 4.7 ± 1.6 pollen grains per flower; Females: 18.4 ± 3.1 pollen grains; $t = 3.96$, $P = 0.004$).

Sexes did not differ significantly in their efficiency at depositing pollen from *H. bihai* onto the stigmas of *H. caribaea* (Males: 5.5 ± 2.5 pollen grains per flower; Females: 5.9 ± 3.3 pollen grains; $t = 0.09$, $P = 0.90$). Females purple-throated caribs, however, were marginally better at carrying pollen from *H. caribaea* to the stigmas of *H. bihai* than males (Males: 6.4 ± 3.1 pollen grains per flower; Females: 14.7 ± 3.1 pollen grains; $t = 1.88$; $P = 0.09$).

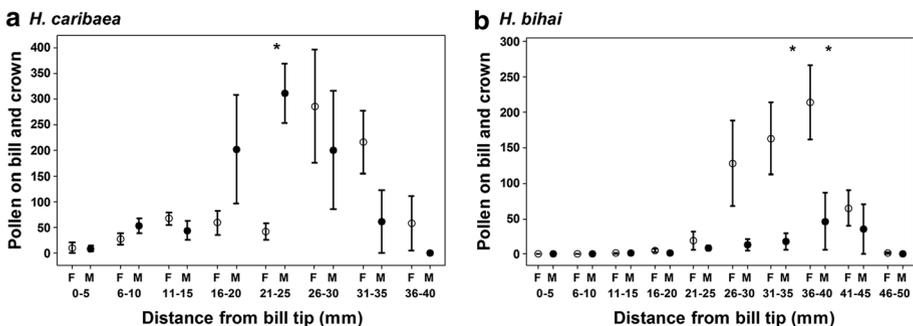


Fig. 2 Placement of pollen on bills and crowns of male (solid circles) and female (open circles) purple-throated caribs (number of pollen grains, mean \pm SE) after visits to flowers of *H. caribaea* (a) and *H. bihai* (b). Ranges on X-axis refer to distances (in mm) from bill tip. Significant differences between the sexes are indicated by asterisks (* $P < 0.05$; t tests)

When offered a choice between *H. caribaea* and *H. bihai*, five of five males and six of seven females preferred *H. caribaea* (P 's ≤ 0.05 , binomial tests). Lastly, although males visited *H. bihai* in our pollination experiments, they left residual nectar in 88% of the flowers offered to them, and flowers from which nectar was completely removed were significantly shorter (46.02 ± 0.47 mm, $N=15$) than flowers containing residual nectar (47.60 ± 0.16 mm, $N=110$). Females did not leave residual nectar in the flowers of *H. bihai*.

Males exerted weak stabilizing selection on corolla lengths of *H. caribaea*, using the number of pollen grains deposited on stigmas as a proxy for fitness (Table 1). Males did not exert any form of selection in the other three treatments. In contrast, females exerted weak stabilizing selection on corolla lengths of *H. bihai* (Table 1) whereas directional and stabilizing selection on corolla lengths of *H. caribaea* were not significant (Table 1). In terms of heterospecific pollen transfer, assuming greater heterospecific pollen deposition reduces fitness, females exerted significant selection against long flowers of *H. caribaea* (Table 1) and significant selection against short flowers of *H. bihai* (Table 1).

Dye transfer and pollinator constancy

At the Canicules site, 17 of 38 (45%) recipient *H. caribaea* flowers received dye from donor *H. caribaea* flowers and 8 of 31 (26%) recipient *H. bihai* flowers received dye from donor *H. bihai* flowers. The pattern was similar at the Haut Jean sympatric site, with 35 of 65 (54%) recipient *H. caribaea* flowers receiving dye from donor *H. caribaea* flowers and 10 of 36 (28%) recipient *H. bihai* flowers receiving dye from donor *H. bihai* flowers. At the Imrrays View site, 26 of 47 (55%) recipient *H. caribaea* flowers received dye from donor *H. caribaea* flowers and 4 of 21 (19%) recipient *H. bihai* flowers received dye from donor *H. bihai* flowers. In contrast, dye transfer between species was extremely rare, with 1 of 59 donor and recipient *H. bihai* flowers receiving dye from a donor *H. caribaea* flower at Canicules and 1 of 42 donor and recipient *H. bihai* flowers receiving dye from a donor *H. caribaea* flower at Imrrays View. No dye transfer between *Heliconia* species was recorded

Table 1 Selection gradients ($\pm SE$, N) for corolla length (standardized) versus relative fitness (pollen grains deposited on stigma) for male and female purple-throated caribs in four pollen donor—pollen recipient treatments (DBRB donor *H. bihai*, recipient *H. bihai*; DCRC donor *H. caribaea*, recipient *H. caribaea*; DBRC donor *H. bihai*, recipient *H. caribaea*; DCRB donor *H. caribaea*, recipient *H. bihai*). β_{uni} = univariate linear gradients; γ_{uni} = univariate nonlinear gradients. Note that for treatments involving pollen transfer between species (DBRC, DCRB), significant gradients imply selection against specific corolla lengths, assuming that heterospecific pollen transfer reduces fitness

Sex	Male		Female	
	β_{uni}	γ_{uni}	β_{uni}	γ_{uni}
DBRB	0.24 ± 0.20 (25)	0.70 ± 0.45 (25)	0.16 ± 0.11 (34)	$-0.26 \pm 0.14^{\ddagger}$ (34)
DCRC	-0.02 ± 0.17 (25)	$-0.64 \pm 0.38^{\dagger}$ (25)	-0.04 ± 0.15 (33)	0.01 ± 0.18 (33)
DBRC	0.15 ± 0.23 (25)	0.15 ± 0.30 (25)	$0.43 \pm 0.20^*$ (34)	0.13 ± 0.26 (34)
DCRB	0.12 ± 0.26 (25)	-0.82 ± 0.64 (25)	$-0.47 \pm 0.19^*$ (34)	0.44 ± 0.32 (34)

* $P < 0.05$; $^{\ddagger}P < 0.075$; $^{\dagger}P < 0.09$

Table 2 Observed (Obs.) and expected (Exp.) frequencies of dye transfer between flowers of *H. bihai* (HB) and *H. caribaea* (HC) by female and male purple-throated at three sites on Dominica. Expected frequencies were determined from the relative frequencies of donor and recipient flowers of *H. bihai* and *H. caribaea* at each of the three sites. Because we used different colors of dyes for each of the two species, we pooled donor and recipient flowers within each species for analyses of heterospecific dye transfer. Where observed cell frequencies were zero, one was added for statistical tests (denoted by asterisks). Statistical significance was determined from Monte Carlo Exact Tests with 10^6 random draws

Dye transfer	Canicules				Haut Jean				Imrays view			
	Obs.	Exp.	<i>P</i>	<i>N</i>	Obs.	Exp.	<i>P</i>	<i>N</i>	Obs.	Exp.	<i>P</i>	<i>N</i>
HB to HB	0.26	0.05	<0.001	31	0.28	0.03	<0.001	36	0.19	0.02	<0.001	21
HB to HC	0.0*	0.12	<0.01	78	0.0*	0.10	<0.001	127	0.0*	0.10	<0.01	99
HC to HB	0.01	0.13	<0.01	59	0.0*	0.11	<0.02	67	0.02	0.10	0.12	42
HC to HC	0.45	0.08	<0.001	38	0.54	0.11	<0.001	65	0.55	0.12	<0.001	47

at the Haut Jean site. For all three populations, differences in the frequencies of conspecific versus heterospecific dye transfer were highly significant (P 's < 0.001, Monte Carlo Exact tests). Similarly, in all populations, conspecific dye transfer was more frequent than expected, whereas heterospecific dye transfer was less frequent than expected (Table 2).

At all three sites in this study, male *A. jugularis* were observed aggressively chasing female *A. jugularis* and other avian species from their food plants, which they defended as territories. Sexual differences in visit frequencies to *Heliconia* species were significant at all three sites, with males accounting for a higher proportion of visits to *H. caribaea* and females accounting for a higher proportion of visits to *H. bihai* (Table 3).

Observations of within-bout pollinator constancy were consistent with our studies of dye transfer. For female purple-throated caribs, transitions between flowers of the same *Heliconia* species were significantly more frequent than expected at all three sites (Canicules: 23 of 26 transitions, $BCI=0.77$, $P<0.001$; Haut Jean: 15 of 15 transitions, $BCI=1$, $P<0.001$; Imrays View: 25 of 26 transitions, $BCI=1$, $P<0.001$). A similar result was observed for male purple-throated caribs (Canicules: 23 of 23 transitions, $BCI=1$, $P<0.001$; Haut Jean: 75 of 75 transitions, $BCI=1$, $P<0.001$; Imrays View: 42 of 43 transitions, $BCI=1$, $P<0.001$). Based on expected transitions calculated from observed visits to the two *Heliconia* species (Table 3), female purple-throated caribs made more within-species transitions and fewer between-species transitions at all three sites (Table 4). Differences between observed and expected transition frequencies for

Table 3 Visits per plant by female and male purple-throated caribs to *H. bihai* and *H. caribaea* for three sympatric of *H. bihai* and *H. caribaea*. Statistical significance of differences between sexes was determined by Monte Carlo Exact tests with 10^6 random draws

	Males		Females		<i>P</i>
	<i>H. bihai</i>	<i>H. caribaea</i>	<i>H. bihai</i>	<i>H. caribaea</i>	
Canicules	0	16	12	11	<0.001
Haut Jean	2	49	9	3	<0.001
Imrays View	1	28	10	14	<0.001

Table 4 Observed (Obs.) and expected (Exp.) frequencies of transitions between flowers of *H. bihai* (HB) and *H. caribaea* (HC) by female and male purple-throated at three sites on Dominica. Expected frequencies were determined from the relative frequencies of visits by each sex to *H. bihai* and *H. caribaea* at each of the three sites (see Table 3). Where observed cell frequencies were zero, one was added for statistical tests (denoted by asterisks). At all three sites, observed transitions for females differed significantly from expected transitions (P 's < 0.01, Monte Carlo Exact Tests), whereas observed transitions for males did not differ significantly from expected transitions

Transition	Canicules			Haut Jean			Imrays View		
	Obs.	Exp.	<i>N</i>	Obs.	Exp.	<i>N</i>	Obs.	Exp.	<i>N</i>
<i>Females</i>									
HB to HB	0.27	0.27	7	0.65	0.56	11	0.33	0.18	9
HB to HC	0.08	0.25	2	0.06	0.19	0*	0.04	0.24	1
HC to HB	0.04	0.25	1	0.06	0.19	0*	0.04	0.24	0*
HC to HC	0.61	0.23	16	0.23	0.06	4	0.59	0.34	16
<i>Males</i>									
HB to HB	0.04	0.01	0*	0.03	0.01	2	0.02	0.01	0*
HB to HC	0.04	0.06	0*	0.01	0.04	0*	0.02	0.03	0*
HC to HB	0.04	0.06	0*	0.01	0.04	0*	0.02	0.03	1
HC to HC	0.87	0.87	23	0.95	0.91	73	0.93	0.93	42

males were not significant, but this was a result of males visiting *H. caribaea* to the near exclusion of *H. bihai* (Table 3).

Discussion

Exploitation barriers and pollinator competition

Despite the close correspondence between the bill morphology of male and female purple-throated caribs and the morphology of the flowers they visit (Fig. 1), both sexes of caribs were capable of removing and depositing pollen onto flowers of *H. caribaea* and *H. bihai*. Male and female purple-throated caribs were equally efficient at pollinating flowers of *H. caribaea*, whereas females were significantly better at pollinating flowers of *H. bihai*. Moreover, even though corollas of *H. caribaea* and *H. bihai* differ in length by approximately 12 mm, such differences in length did not prevent heterospecific pollen transfer in our experiments with captive hummingbirds. Both sexes of purple-throated caribs, especially females, were capable of transferring pollen between the two species. Yet heterospecific pollen transfer was virtually absent in our experiments on natural populations where the two *Heliconia* species co-occur on Dominica. The absence of heterospecific pollen transfer between the two *Heliconia* species resulted from a combination of preferences, exploitation barriers, interference competition, and floral fidelity.

Both sexes of purple-throated caribs preferred *H. caribaea* to *H. bihai* in choice tests, consistent with our earlier experiments on St. Lucia (Temeles et al. 2009). Such preferences are likely a result of the greater nectar volume per flower, greater numbers of flowers per plant, and shorter flowers of *H. caribaea*, which make nectar more accessible and easier to extract (Temeles et al. 2009). In this regard, male preference for *H. caribaea* also was influenced by the inability to remove some or all nectar from

most (88%) flowers of *H. bihai*. While the length of *H. bihai* flowers acted as an exploitation barrier to males, our observations suggest that the orientation of flowers within bracts also may influence nectar accessibility. Flowers of *H. caribaea* are oriented with their long axis at a vertical angle within bracts. To feed at these flowers, both male and female purple-throated caribs insert the bill into the tip of the flower and then down the length of the floral tube, accessing the flower's nectar supply (Fig. 1f). In contrast, flowers of *H. bihai* are oriented with their long axis parallel to the bract. The curved bill of female purple-throated caribs allows them to access the nectar of *H. bihai* because the curved bill tip runs parallel to the floral tube while the curved base of the bill contacts the flowers' reproductive parts. The straight bills of males, however, are poorly matched for feeding from flowers of *H. bihai*. When males insert their straight bills into *H. bihai* flowers, the bill is at a right angle to the long axis of the flower. Any further "pushing" of their bills by males only pressed into the middle of *H. bihai* flowers and not towards the base of the flower, and males could only access nectar from the shortest flowers of *H. bihai*. Thus, the length and orientation of *H. bihai* flowers act as exploitation barriers to male purple-throated caribs. Differences in flower orientation resulting from twisting of the pedicel (resupination) and from position of the inflorescence (erect or pendant) are widespread with the Heliconiaceae, and have been used in the taxonomic classification of subgroups (Iles et al. 2017). Such differences in flower orientation are often associated with visits by hummingbird pollinators differing in bill length and shape (Stiles 1975; Taylor and White 2007). Thus, exploitation barriers may have had a major role in the evolution of bird-flower relationships within this group of plants.

In contrast to the exploitation barriers, female purple-throated caribs were deterred from feeding at the flowers of *H. caribaea* by interference competition with larger, dominant males. Rodríguez-Gironés and Santamaría (2010) did not specifically address interference competition in their models, but noted that it could result in floral isolation, assortative mating, and floral divergence in a manner similar to exploitation barriers and exploitative competition. We have demonstrated elsewhere through experiments that interference competition between sexes of purple-throated caribs can result in resource partitioning of heliconias and cause assortative mating within floral phenotypes and divergent selection on corolla length (Temeles et al. 2016). Females' exclusion from *H. caribaea* was not absolute, however, and at all three sites, we observed some females visit flowers of *H. caribaea*, either by visiting undefended plants or by intruding onto male territories. Within a foraging bout, however, females exhibited strong constancy to one or the other *Heliconia* species, minimizing heterospecific pollen transfer. Both Grant (1994) and Rodríguez-Gironés and Santamaría (2007) suggested that if floral constancy improves the foraging efficiency of a pollinator, it can result in floral isolation. In previous work (Temeles et al. 2006), we have shown that female purple-throated caribs primarily forage by traplining at undefended plants on a regular route and schedule and that they will alter that schedule in the presence or absence of competitors. Their ability to alter their traplining schedule in the presence of competitors requires fidelity to specific plants, which could reduce heterospecific pollen transfer. Thus, as demonstrated here, traplining may result in floral constancy and floral isolation. Heterospecific pollen transfer also may have been prevented by grooming of the crown between foraging bouts. A final factor that could have contributed to ethological floral isolation in our study is that females intrude onto male *H. caribaea* territories to steal nectar more frequently in the late morning or early afternoon, after nectar has been depleted in undefended plants (Temeles and Kress 2010;

also see Gill and Wolf 1975). The temporal shift from traplining at *H. bihai* to nectar thievery from *H. caribaea* territories defended by males also may have reduced pollen transfer between the two plant species if females divided their foraging on the two species by time of day.

Selection on floral traits

As a result of exploitation barriers and interference competition, male purple-throated caribs were the most frequent pollinator of *H. caribaea* whereas female purple-throated caribs were the most frequent pollinator of *H. bihai* (Table 1). Male purple-throated caribs exerted weak stabilizing selection on corolla lengths of *H. caribaea* whereas female purple-throated caribs exerted weak stabilizing selection on corolla lengths of *H. bihai*. Although our sample size for quadratic regression was small and the resulting power was low, these results suggest that corolla lengths of *H. caribaea* and *H. bihai* may be well-matched to the bills of male and female purple-throated caribs in terms of pollen deposition and receipt, leading to stabilizing selection (Nattero et al. 2010; Temeles et al. 2016). In contrast, work by Nattero et al. (2010) suggests that selection should be strong and directional when bills and flowers are poorly-matched. Bills of males are not well matched to corollas of *H. bihai*, yet we did not observe selection for shorter corollas as inferred from dye deposition. The lack of selection for shorter corollas of *H. bihai* by males may result from their inability to feed successfully from flowers of this plant due to the length and orientation of flowers (see above). *Heliconia bihai* is more abundant on islands in the southern Lesser Antilles, such as St. Lucia, however, and is visited by male purple-throated caribs on these islands (Temeles et al. 2005; Gowda and Kress 2013). Consistent with visitation by males, flowers of *H. bihai* are shorter on these islands (Temeles et al. 2000). We did not detect selection by female purple-throated caribs for longer corollas of *H. caribaea* in the *H. caribaea* donor-recipient trials, but we did detect greater pollen deposition on long corollas of *H. caribaea* in the *H. bihai* donor–*H. caribaea* trials, suggesting that females would select for longer corollas of *H. caribaea* as in our earlier experimental and field studies (Temeles et al. 2013, 2016). The absence of selection for long corollas of *H. caribaea* in this one trial may have been an artifact of an insufficient number of long flowers in our test sample.

The models of Rodríguez-Gironés and Santamaría (2005, 2006, 2007) indicate that partitioning of floral resources by competing pollinators can lead to divergence in floral traits such as corolla length and color. As shown here, the long corollas of *H. bihai* do not prevent pollen transfer from the short corollas of *H. caribaea*, but they do exclude shorter-billed male purple-throated caribs from accessing nectar. Thus, the divergence in corolla lengths between the two *Heliconia* species on islands where they co-occur is consistent with mechanisms of floral divergence based on exploitation barriers and resource competition between pollinators, and not plants (Rodríguez-Gironés and Santamaría (2005, 2006, 2007).

The absence of significant heterospecific pollen transfer observed here does not mean that it might not have occurred in the past and influenced floral divergence between *H. bihai* and *H. caribaea* through reproductive character displacement (e.g., selection for mechanical isolation). Although rare, we identified 3 hybrids at our Haut Jean study site and found a hybrid zone consisting of approximately 20 plants at our Imrays View site. Such hybrids indicate the breakdown of resource partitioning between sexes of purple-throated caribs. Manipulative experiments of both plants and pollinators are necessary to understand the dynamics of the interaction between pollinator competition and competition

for pollination, but a breakdown of resource partitioning of plants by pollinators is most likely to occur when plants are super-abundant relative to pollinators or when a dominant competing pollinator is rare or absent. Under such conditions, the subordinate pollinator would not be excluded from the preferred plant species, which could result in heterospecific pollen transfer. Consistent with this hypothesis, the Imrays View hybrid zone was the only site where we observed more visits by female purple-throated caribs to *H. caribaea* than to *H. bihai*, and also was the site where we observed the fewest male purple-throated caribs.

Rodríguez-Gironés et al. (2015) suggested that the optimal foraging strategy of two types of pollinators that differ in the relative efficiency with which they exploit two types of flowers for which they compete is resource partitioning, with one type of pollinator behaving as a specialist and the other type of pollinator behaving as a specialist or generalist depending on ecological conditions. Our results support findings from their models: male purple-throated caribs are specialists on *H. caribaea*, whereas female purple-throated caribs are generalists capable of feeding from flowers of both *H. bihai* and *H. caribaea* owing to their longer bills (Temeles et al. 2009). In the presence of larger, dominant males, female purple-throated caribs specialize on *H. bihai*, but when males are absent or *H. caribaea* is abundant, females may also visit *H. caribaea* and in the absence of males can drive selection on its floral traits (Temeles et al. 2016). In a review of the ecological roles of different species in hummingbird communities, Feinsinger and Colwell (1978) noted that sexes of purple-throated caribs were the island analog of mainland species, with short-billed males having the role of territorial species and long-billed females having the role of traplining species. Studies of these mainland hummingbird communities indicate that short-billed species visit plant species with short flowers whereas long-billed species often visit plant species with short as well as long flowers and that differences in plant use between hummingbird species are often driven by both exploitative and interference competition (e.g., Feinsinger 1976; Snow and Snow 1980; Arizmendi and Ornelas 1990; Cotton 1998; Temeles et al. 2002; Maglianesi et al. 2015; Cuevas et al. 2018). We suggest that the evolution of long bills and long flowers in hummingbird communities may be driven by exploitation barriers and pollinator competition, although this hypothesis clearly warrants further study.

Implications

Rodríguez-Gironés and Santamaría have emphasized that to understand the evolution of floral traits, we must consider the foraging behavior of pollinators, but unfortunately, pollinator behavior is often overlooked (Rodríguez-Gironés and Santamaría 2010; Santamaría and Rodríguez-Gironés 2015). As shown here, both male and female purple-throated caribs are equally effective pollinators of *H. caribaea*, and both are capable of transferring pollen between *H. caribaea* and *H. bihai*. Yet where the two plant species co-occur, they are effectively isolated as a result of exploitative and interference competition between sexes of their pollinators. Thus, studies of floral isolation and divergence should consider the role of pollinator behavior and community context, especially when observations and experiments on plant-related mechanisms (e.g. mechanical isolation) yield negative results. For pollinator taxa in which interference and exploitative competition are strong components of community assembly, such as hummingbirds, the competitive behavior of pollinators may play a major role in floral isolation and divergence.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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