Pollinator recognition by a keystone tropical plant

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Understanding the mechanisms enabling coevolution in complex mutualistic networks remains a central challenge in evolutionary biology. We show for the first time, to our knowledge, that a tropical plant species has the capacity to discriminate among floral visitors, investing in reproduction differentially across the pollinator community. After we standardized pollen quality in 223 aviary experiments, successful pollination of *Heliconia tortuosa* (measured as pollen tube abundance) occurred frequently when plants were visited by long-distance traplining hummingbird species with specialized bills ($\bar{x}$ pollen tubes = 1.21 ± 0.12 SE) but was reduced 5.7 times when visited by straight-billed territorial birds ($\bar{x}$ pollen tubes = 0.20 ± 0.074 SE) or insects. Our subsequent experiments revealed that plants use the nectar extraction capacity of tropical hummingbirds, a positive function of bill length, as a cue to turn on reproductively. Furthermore, we show that hummingbirds with long bills and high nectar extraction efficiency engaged in daily movements at broad spatial scales (~1 km), but that territorial species moved only short distances (<100 m). Such pollinator recognition may therefore affect mate selection and maximize receipt of high-quality pollen from multiple parents. Although a diffuse pollinator network is implied, because all six species of hummingbirds carry pollen of *H. tortuosa*, only two species with specialized bills contribute meaningfully to its reproduction. We hypothesize that this pollinator filtering behavior constitutes a crucial mechanism facilitating coevolution in multispecies plant–pollinator networks. However, pollinator recognition also greatly reduces the number of realized pollinators, thereby rendering mutualistic networks more vulnerable to environmental change.

coevolution | hummingbirds | pollinator networks | mutualism | specialization

The remarkable diversity of angiosperms has been, in part, attributed to the evolution of complex floral structures and the variety of strategies for sexual reproduction (1). The resulting increase in genetic mixing facilitates adaptation to changing environmental conditions, enhances morphological diversification, and leads eventually to speciation (2).

Much research has focused on the genetic benefits afforded by long-distance pollen transfer among individuals, particularly in animal-pollinated plants (3, 4). However, pollen dispersal kernels are often short, resulting in self-pollination or gene exchange among closely related individuals (5, 6). In response, plants have evolved floral traits and attractants that influence the degree of pollinator specialization (7) and act as filters against inefficient pollinator services (8) or enhance pollen transport (9). Coevolution occurs when evolution of pollinator morphology tracks these floral changes, which in turn, drives reciprocal changes in floral traits (10).

Although the potential for coevolution in the most specialized obligate mutualisms is clear (11), the processes by which coevolution occurs in more complex interaction networks remains a central question in evolutionary biology (10, 12). The striking fit (trait matching) between morphologies of some plants and their pollinators in many systems (7, 13) suggests a high degree of plant–pollinator specialization. Such matches between flowers and groups of pollinators are the basis of the identification of floral syndromes (or pollination web modules), which link floral traits with particular types of pollinators (e.g., bird-pollinated and bee-pollinated) (14, 15). However, observational data of pollination mutualisms indicate a high degree of generality, even within floral syndromes (16, 17); plant species may exhibit highly specialized morphological traits (e.g., long, curved corollas) but are visited by many pollinators, most of which lack specialized foraging morphologies (18, 19). The importance of coevolutionary processes in the presence of many pollinator species, each of which may impose conflicting selection pressures, remains much debated (20). This situation is exemplified by the species-rich guild of tropical hummingbirds that shows strikingly high among-species diversity in bill length, bill curvature, and spatial behavior (21); however, both specialized and generalized hummingbird species visit plants with apparently high morphological specialization (22). How can strong morphological coevolution occur if plants are visited by many pollinator species with high trait variation?

In this paper, we report a previously unidentified mechanism that increases the realized specialization between a plant and its suite of floral visitors. Through a series of experiments in a tropical plant–hummingbird system, we show that a keystone understory herb (23), *Heliconia tortuosa* Griggs, has the capacity to discriminate among floral visitors representing a wide range of morphologies within a functional group. Flowers of *H. tortuosa* recognize specialized pollinators that potentially carry high-quality pollen and are most likely to facilitate mate selection.

Results

Testing the Pollinator Recognition Hypothesis. Our discovery of pollinator recognition was the direct result of original hand-pollination experiments designed to test the degree of pollen limitation in *H. tortuosa* in relation to landscape fragmentation (23). Pollen limitation was measured as the difference between hand pollination

Significance

By using structural characteristics, such as long tubular flowers, plants are known to achieve selective visitation by certain pollinator species. These morphological traits typically arise over evolutionary timescales. We show for the first time, to our knowledge, that at least one plant has also evolved the capacity to recognize pollinator species immediately after visitation, thereby increasing the likelihood that a flower visitor has delivered high-quality pollen. This novel responsiveness by the plant leads to functional specialization in an apparently generalized tropical plant–pollinator network. Such specialized linkages likely facilitate coevolution but also, render pollination mutualisms more vulnerable to environmental change.

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of covered flowers and those left unmanipulated with full exposure to pollinators (24). We measured pollination using the abundance of pollen tubes following methods by Kress (25). Surprisingly, hand-pollinated flowers averaged 5.04 times [95% confidence interval (95% CI) = 3.65–7.24] fewer pollen tubes per style than open-pollinated flowers [generalized linear model (GLM): $t = 8.36$, $P < 0.0001$; hand: $\bar{x} = 0.21$ (95% CI = 0.15–0.30) tubes per style; open: $\bar{x} = 1.08$ (95% CI = 1.01–1.16) tubes per style].

We hypothesized that such decreased plant reproduction after hand pollination could have two potential causes. The quality of pollen brought by hummingbirds could be higher (5), reflecting either longer distance or more genetically mixed pollen transfer by natural pollinators than in our hand-pollination experiments (the pollen-quality hypothesis). Alternatively, the plant might distinguish pollen quality indirectly based on a physical or chemical cue transmitted by the pollinator (the pollinator-recognition hypothesis). To distinguish these hypotheses, we designed an experiment in which we hand-pollinated flowers as before but introduced pollen-free clean hummingbird pollinators under controlled aviary conditions. Under the pollen-quality hypothesis, visitation by a pollen-free pollinator should not increase pollen tube abundance in flower styles. Under the pollinator-recognition hypothesis, visitation alone by hummingbirds—even in the absence of pollen on the bird—should increase the abundance of pollen tubes over pollinator-excluded controls.

To test these hypotheses, we captured 148 individual floral visitors representing six hummingbird species and one common species of butterfly—*Anartia fatima*. All species have been observed visiting flowers of *H. tortuosa*. After cleaning hummingbirds of all pollen (*Materials and Methods*), they were released individually into aviaries containing a single flower that we had hand pollinated with pollen from 30 to 100 m away. We released butterflies into mesh bags covering a hand-pollinated flower. Flower styles were collected the next day and inspected for pollen tubes using epifluorescence microscopy (25) (*SI Materials and Methods*).

Hummingbird flower visitation strongly influenced pollen tube abundance [generalized linear mixed model (GLMM): $F = 4.57$, $P < 0.0001$], supporting the pollinator-recognition hypothesis. However, this effect was mediated by specific species of hummingbirds (Table 1). For instance, flowers visited by violet sabrewings (*Campylopterus hemileucurus*) contained 3.10 times (95% CI = 2.33–6.17, $\bar{x}$ pollen tubes = 1.39 ± 0.22 SE) more pollen tubes than green-crowned brilliants (*Heliodoxa jacula*; $\bar{x}$ pollen tubes = 0.38 ± 0.18 SE) and 28.6 times (95% CI = 21.39–56.94) more pollen tubes than rufous-tailed hummingbirds (*Amazilia tzacatl*; $\bar{x}$ pollen tubes = 0.04 ± 0.04 SE) (Fig. 1). Visitation by *A. fatima* resulted in no pollen tubes (0 of 21). To our knowledge, this behavior in *H. tortuosa* is the first evidence that a plant has the capacity to distinguish among species of floral visitors and respond by preventing (or facilitating) pollen tube growth. We term this plant behavior pollinator recognition, because unlike previous forms of pollinator filtering, such as morphological differences in plant corollas (26–28), the mechanism that we report operates in physiological–ecological time rather than on an evolutionary timescale.

Table 1. Results of GLMM predicting differences in pollen tube abundance as a function of different hummingbird pollinator species released into aviaries with hand-pollinated *H. tortuosa*

Parameter	β	LCI	UCI	*Z*	*P*	*P-FDR*
Intercept: green-crowned brilliant	−1.10	−2.00	−0.20	−2.41	0.016	—
Self-pollen	−0.78	−1.31	−0.25	−2.92	0.004	—
Green hermit*	1.06	0.13	1.99	2.22	0.027	0.049
Rufous-tailed hummingbird	−2.23	−4.40	−0.06	−2.01	0.045	0.054
Scaly-breasted hummingbird	−0.16	−1.90	1.58	−0.18	0.854	0.561
Stripe-throated hermit	−0.35	−1.86	1.16	−0.46	0.646	0.483
Violet sabrewing*	1.33	0.37	2.29	2.71	0.007	0.015
Intercept: rufous-tailed hummingbird	−3.33	−4.34	−2.32	−3.28	0.001	—
Self-pollen	−0.78	−1.31	−0.25	−2.92	0.004	—
Green hermit*	3.28	2.26	4.30	3.21	0.001	0.004
Green-crowned brilliant	2.23	1.12	3.34	2.01	0.045	0.054
Scaly-breasted hummingbird	2.06	0.79	3.33	1.63	0.104	0.098
Stripe-throated hermit	1.87	0.68	3.06	1.58	0.114	0.098
Violet sabrewing*	3.56	2.53	4.59	3.46	0.001	0.003

We controlled for our two pollen quality treatments statistically by including self-pollen vs. outcrossed pollen as an indicator variable. *P-FDR*, *P* values that have been corrected for false discovery rates associated with multiple comparisons among species (*SI Materials and Methods*). LCI and UCI are lower and upper 95% confidence intervals, respectively.

*Species that differed significantly from the two reference species of green-crowned brilliant and rufous-tailed hummingbirds.

Testing the Mechanism Used in Pollinator Recognition. Next, we tested for the cue used by plants to distinguish among pollinators. Species of tropical hummingbirds differ strongly in their capacity to extract nectar from ornithophilous plants (29), and these differences correspond to the degree of specialization in hummingbird bill morphology (7, 29). The capacity to distinguish visitors that carry high-quality pollen loads from those that do not should be adaptive (5, 30). Morphological specialists are more likely to be faithful to a particular flower species (27), reducing the risk of mixed-species pollen loads. Specialization should also require greater movement distances among plants to acquire necessary resources (30), thereby increasing the potential for gene flow. We, therefore, hypothesized that capacity for nectar extraction is the mechanism used by *H. tortuosa* to recognize the identity of morphologically specialized floral visitors.

Three lines of evidence support this hypothesis. First, similar to the work by Wolf et al. (29), we found strong differences among pollinator species in their capacity to extract nectar (GLM: $F = 81.30$, $R^2 = 0.75$, $P < 0.0001$) (Fig. 2*A* and Table S1). Bill length directly reflected nectar extraction capacity of pollinators ($r = 0.85$, $P = 0.008$). Second, the nectar extraction capacity of each species was correlated with mean pollen tube abundance in experimentally pollinated flowers (Fig. 2*B*) ($r = 0.73$, $P = 0.039$). The outlier in this analysis was the scaly-breasted hummingbird (*Phaeochroa cuvierii*) (Fig. 2 *A* and *B*), which in our aviary experiments, we have only observed robbing nectar by piercing flower bases. Despite the fact that this species removed high nectar amounts, the damage that it inflicts on the flower apparently reduces pollen tube growth. Removing this species from analysis resulted in a stronger correlation between pollinator nectar extraction capacity and mean pollen tube abundance ($r = 0.83$, $P = 0.020$). Third, experimental extraction of nectar significantly increased the abundance of pollen tubes in hand-pollinated flowers (GLMM: $Z = 2.53$, $P = 0.011$) (*SI Materials and Methods*). Pollen tubes were 3.67 times (95% CI = 1.38–9.74) more common when we extracted nectar than when flowers were hand-pollinated without nectar extraction (Fig. 2*C*) (GLMM: $Z = 2.67$, $P = 0.008$).

Discussion

To our knowledge, these findings provide the first evidence of pollinator recognition in plants. *H. tortuosa* is capable of discerning morphologically specialized hummingbird species from those with generalized traits. Elegantly, the traits themselves (i.e., long, curved bills) enable some hummingbird species to extract more nectar, which in turn, is the very cue used by the plant to become receptive. This plant behavior raises the question of whether pollinator recognition confers any contemporary adaptive advantages to the plant. The fact that it is the specialized, traplining species that tend to induce pollen tube

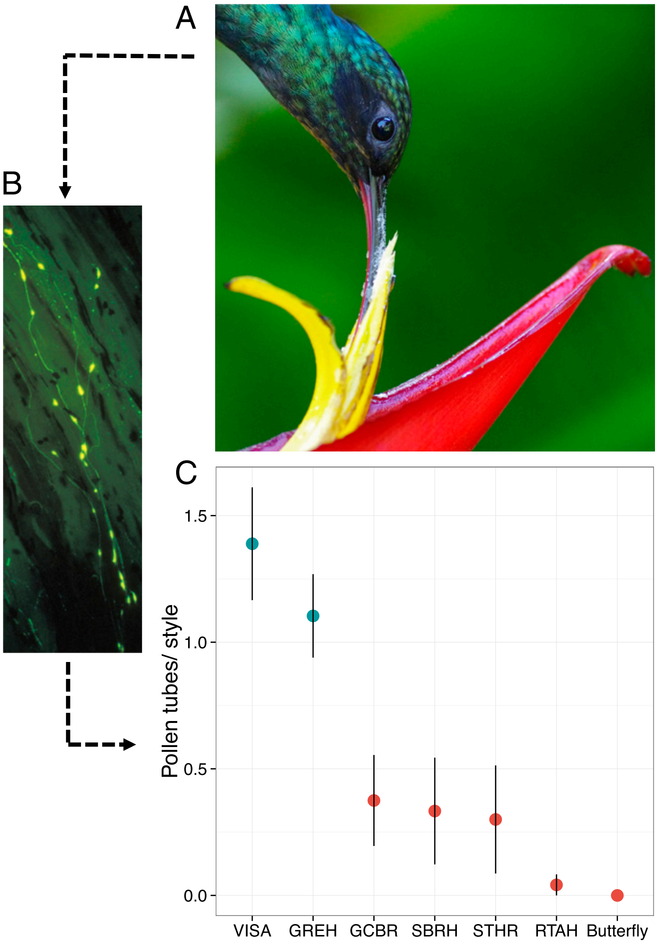

Fig. 1. Results of aviary experiments, where flowers were visited by pollen-free pollinators after being hand-pollinated under controlled conditions. (*A*) A bract of *H. tortuosa* with a male green hermit hummingbird. (*B*) Pollen tubes, the first step in reproduction, viewed in the style of *H. tortuosa* using epifluorescence microscopy. (*C*) Experimental addition of hummingbirds strongly influenced the abundance of pollen tubes, but this effect was species-dependent. Means and SEs are shown. *P* values for contrasts are reported in Table 1. Green represents those species stimulating pollen tube growth, and red indicates less-effective species. Butterfly, *A. fatima*; GCBR, green-crowned brilliant; GREH, green hermit (*Phaethornis guy*); RTAH, rufous-tailed hummingbird; SBRH, scaly-breasted hummingbird; STHR, stripe-throated hermit (*Phaethornis striigularis*); VISA, violet sabrewing.

growth suggests that pollinator recognition may increase the quality of pollen receipt, thereby enhancing fitness. This advantage could occur through three possible mechanisms. First, this plant behavior could facilitate long-distance gene transfer or at least, reduce the frequency of self-pollination. *H. tortuosa* is a clonal herb, with plants often occurring in clumps of >10 individuals (25, 31). This high concentration of nectar resources often results in defense of a single clonal clump by territorial hummingbirds, which strongly constrains pollen flow (28). However, the large body size and specialized morphology of traplining hummingbirds require them to move further to acquire necessary resources (21), thus potentially facilitating gene flow and enhancing plant fitness (32). To test this hypothesis, we assembled movement data on all seven pollinator species (*SI Materials and Methods*). Pollen tube abundance was positively correlated with the median movement distance that each species moved within 1 d (Fig. 3*A*) ($F = 48.98$, $R^2 = 0.87$, $P = 0.0004$). We also found a strong effect of movement behavioral strategy on the number of pollen tubes; traplining species, which regularly move long distances across landscapes to acquire nectar resources (32, 33), elicited greater pollen tube abundance than territorial species (6) (Fig. 3*B*) (GLMM: $Z = 4.52$, $P < 0.0001$).

Second, the capacity to recognize specialized pollinators could also function to maximize the diversity of conspecific pollen received. Because of the placement of the anthers in *H. tortuosa* and the foraging position of green hermit and violet sabrewing (Movie S1), these species are more likely, on average, to carry high pollen loads than nonspecialized species. Our data on hummingbird pollen loads support this hypothesis; traplining species carried significantly higher individual loads of *H. tortuosa* pollen ($\hat{x} = 154.47$; 95% CI = 129.02–183.09) than territorial species ($\hat{x} = 28.78$; 95% CI = 14.15–58.55; GLM: $t = 4.63$, $P < 0.0001$). Although *H. tortuosa* ultimately requires only three grains to fertilize all seeds in the ovary, evidence from other plant species suggests that high pollen abundance on the stigma increases pollen tube competition (or potentially, allows greater opportunity for female choice), thereby increasing the quality of pollen reaching the ovary (34). Indeed, an increase in the number of pollen donors has been shown to be positively correlated with seed weight (34).

Third, recognition of morphologically specialized pollinators might reduce the risk of pollen allelopathy or other types of pollen interference, whereby nonspecific pollen is deposited on the stigma by generalist pollinators and interferes with pollen receipt (35). In our study, both trapliners and territorial hummingbird species carried substantial non-*Heliconia* pollen, and we found no statistical differences between these groups (GLM: $t = 1.55$, $P = 0.121$). However, on average, trapliners carried lower loads of non-*Heliconia* pollen grains ($\hat{x} = 12.55$; 95% CI = 9.58–16.44) vs. territorial hummingbird species ($\hat{x} = 29.96$; 95% CI = 9.87–89.12).

Together, our results suggest that pollinator recognition by *H. tortuosa* facilitates mate selection and is most likely to do so by (*i*) increased outcrossing caused by receipt of longer-distance nonself-pollen or (*ii*) enhanced conspecific pollen diversity.

Species in the genus *Heliconia* lack two mechanisms that promote outcrossing in many flowering plants, namely physiological self-incompatibility and spatial/temporal separation of sexual function (25). However, the often long and highly curved flowers of *Heliconia* species, including *H. tortuosa*, are a striking floral mechanism to screen out certain visitors. In many bird-pollinated plant species, flower length has been proposed as an adaptation to allow plants to exclude pollinators that are unlikely to be carrying high-quality pollen (29). In *H. tortuosa*, the long, curved perianth reduces but does not preclude visitation by territorial hummingbirds, which still receive some nectar rewards (29). Repeated deposition of pollen by such locally foraging species of hummingbirds would clearly increase the levels of inbreeding in the plants as well as cause a reduction in the diversity and abundance of conspecific pollen. Because of the energetic costs of fruit and seed development (36), a mechanism that enables a plant to distinguish low- from high-quality pollinators before investing in seed production would confer a considerable adaptive advantage.

Plant recognition of pollinators may occur in other plant taxa, particularly in relatively stable tropical systems with high pollinator diversity. One hypothesis is that the fitness gains afforded by pollinator recognition in terms of increasing mutualist specialization and possibly, outcrossing rates may have provided an initial mechanism for corolla lengthening in other plant families. Minor microevolutionary increases in corolla length should carry no fitness benefits until they accumulate sufficiently to induce switches by poor-quality pollinators to alternative flower species (30). However, if plants use minor differences in nectar extraction as a cue to indicate visitation by a high-quality pollinator, corolla lengthening in tandem with pollinator recognition would have immediate benefits. An alternative hypothesis is that pollinator recognition may have evolved to more effectively prevent

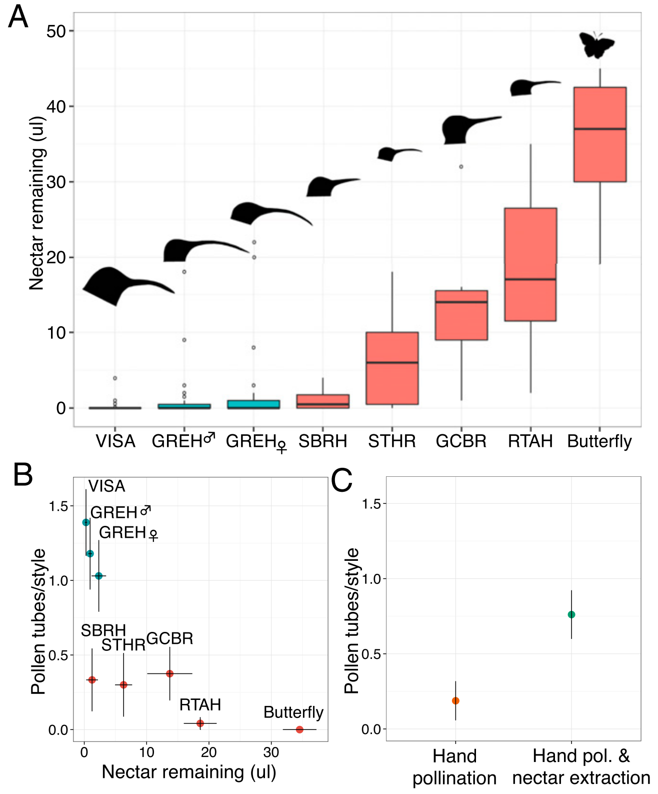

Fig. 2. (*A*) Boxplot showing how nectar extraction efficiency in *H. tortuosa* varies across pollinator species. Boxes show first and third quartiles of data. The bill shapes/sizes of the species are to scale in relative terms. Green represents successful pollen tube growth, and red represents limited growth. (*B*) Relationship between nectar remaining after pollinator visitation and the number of pollen tubes per style for each species (species abbreviations are the same as in Fig. 1). Nectar extraction capacity of hummingbirds strongly influenced the abundance of pollen tubes ($r = -0.73$). (*C*) Effect of hand pollination only vs. hand pollination combined with nectar extraction on pollen tube abundance. Pollen tubes were 3.5 times more common when nectar was experimentally extracted than when flowers were hand-pollinated only. Error bars in *B* and *C* are ± SE.

pollination by poor pollinators in plants that have already evolved long corollas. Regardless of the initial evolutionary mechanism, candidates to examine for such pollinator recognition are those with long corollas combined with high nectar rewards (e.g., Acanthaceae, Rubiaceae, and Malvaceae) that would result in strong pollinator motivation and a subsequent reduction in morphological filters.

Research over the past decade has emphasized the importance of understanding plant–pollinator interactions as networks of species rather than tight, parallel specializations (16). Such networks are highly nested, which means that the most specialized plant species are visited by generalist pollinators and vice versa. This structure has been hypothesized to buffer networks against loss of individual species (37). Our results indicate that, in this tropical system, plant–pollinator relationships are more specialized than assumed given the observational data used to quantify most pollination networks (16). Although we have frequently observed all six species of hummingbirds carrying pollen and visiting the flowers of *H. tortuosa* (Fig. 4*A*), pollination and successful fertilization are dominated locally by only two traplining species (i.e., violet sabrewing and green hermit). Indeed, when we compensated for this functional pollination effect, 80.1% of the reproductive contribution to *Heliconia* was provided by only two species (Fig. 4*B*); the contribution of all other species combined dropped from 53.7% to 19.9%. This hidden specialization has two important implications. First, pollination and subsequent plant demography could be more vulnerable to population declines or disruptions to movement of specialized pollinator species than would be expected given observations of floral visitors. Indeed, our previous work indicates that interpatch movement of traplining species is disrupted by tropical forest fragmentation (33, 38), with consequent negative impacts on seed set (23). Second, plant–pollinator mutualisms in this system may not be as generalized as superficially apparent, which may result in greater potential for rapid reciprocal selection and therefore, coevolutionary change in *H. tortuosa* and a few key specialized pollinators.

It is now well-known that the high cognitive capacity of many vertebrate pollinators allows them to recognize and specialize on particular flower species (30). However, a growing body of research indicates that plants may also exhibit complex decision-making behavior (39, 40). Here, we show for the first time, to our knowledge, that a plant has evolved an effective behavior that allows the recognition of specific hummingbird pollinators and thereby, regulates reproduction accordingly.

Materials and Methods

We conducted the study in the landscape surrounding the Organization for Tropical Studies Las Cruces Biological Station in southern Costa Rica (8° 47′ 7′′ N, 82° 57′ 32′′ W).

Hand-Pollination Experiments. To conduct our initial test of pollen limitation in *H. tortuosa*, we randomly attributed hand-pollination treatments to 159 flowers during the dry season (January to March) from 2011 to 2014. *H. tortuosa* flowers open for only a single day before abscising. Treatments constituted covering all inflorescences with fine-mesh bags the night before pollination. We then collected pollen from conspecific flowers 30–100 m away (to ensure no self-fertilization) and applied it immediately (41) to flower stigmas using a toothpick. Stigmas were cleaned of self-pollen using a cotton swab before pollination. All flowers were then rebagged, and styles were collected the next day. We compared treated flowers with 746 flowers that were allowed full pollinator access and collected from the same study area and time period. We applied a similar or greater amount to what we typically observe on captured green hermits and violet sabrewings. To test whether under- or overabundance of pollen during hand supplementations negatively affected pollen tube growth, we conducted an additional experiment, in which we hand-pollinated flowers using the methods above but left them open to pollinators. Our pollinator-accessible hand

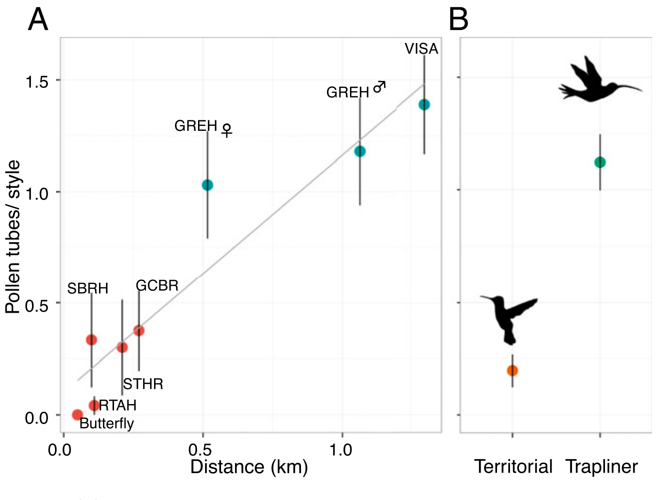

Fig. 3. (*A*) Relationship between median daily distance moved by pollinators and pollen tube abundance (± SE; $R^2 = 0.87$). Species moving longer distances stimulated significantly more pollen tubes than those tending to make short-distance movements. (*B*) Individual flowers experimentally exposed to traplining species (GREH, VISA, and STHR) grew significantly more pollen tubes than ones visited by territorial species (GCBR, RTAH, and SBRH). Species abbreviations are the same as in Fig. 1.

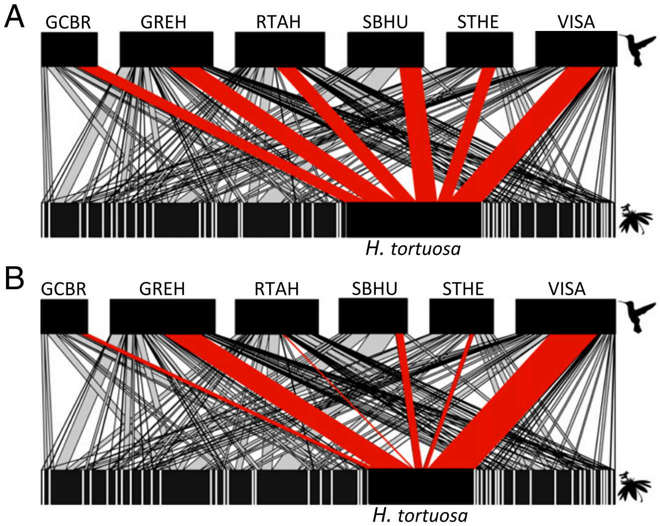

Fig. 4. Pollination network showing interactions between the six hummingbird pollinators that we examined (top row of black squares) and plants (bottom row of black squares). Width of connections represents strength of interactions. (*A*) Pollen-load data collected from the bills, nares, and heads of individual hummingbirds show that *H. tortuosa* seems to be a pollinator generalist, with contributions to its reproduction spread across all six hummingbird species. However, after taking the differential rates of plant investment after pollinator recognition into account (*B*), violet sabrewings and green hermits are likely responsible for 80% of successful reproduction. Pollinator recognition, therefore, promotes high levels of functional specialization within this complex network. GCBR, green-crowned brilliant (*H. jacula*); GREH, green hermit (*Phaethornis guy*); RTAH, rufous-tailed hummingbird; SBHU, scaly-breasted hummingbird; STHE stripe-throated hummingbird; VISA, violet sabrewing.

pollinations ($n = 24$, $\bar{x} = 0.85 \pm 0.29$ SE) did not alter pollination success in relation to unmanipulated open controls ($n = 21$, $\bar{x} = 0.95 \pm 0.30$ SE; GLM: $t = 0.36$, $P = 0.72$).

Aviary Experiment. We captured 128 individual hummingbirds representing six species using a combination of 12-m nets and hall traps (23, 33). Individuals were fitted with unique aluminum bands to allow identification of recaptures and hence, nonindependent samples. We randomly assigned birds to individual flowers within aviaries and randomly assigned treatment to flowers. In this experiment, treatments comprised (*i*) hand-pollinated flowers with pollen outcrossed from a conspecific flower collected from within 30–100 m and with pollinators excluded ($n = 92$) and (*ii*) hand-pollinated flowers using the same methods but with bird visitation ($n = 202$). To minimize the risk of pollen degradation (41), pollen was applied immediately after removal from pollen donors. Again, we cleaned stigmas with a cotton swab before pollination; although this process could have negatively affected reproduction, it does not constitute a bias, because it was conducted in all treatments. Floral emasculation is conducted in many hand-pollination studies to remove a potential confounding effect of self-pollen (42). However, in *H. tortuosa*, emasculation alters the positioning of the style and damages the flower, and both of which could result in estimates of reproductive success that are biased. Finally, floral emasculation risks altering pollinator behavior (42, 43).

In bird visitation treatments, we took extreme care to ensure that birds were completely clean of pollen (*SI Materials and Methods*). We removed pollen using fuchsin jelly (for later use in pollen samples), a photographer's brush (Fig. S1), and disposable lens-cleaning cloths. Bills, nares, heads, and throats were inspected with a hand lens after cleaning to ensure that no pollen grains remained. Birds were then released into the aviary and permitted to visit experimental flowers (Movie S1). Only one specific treatment flower was available to each bird within an experiment. We used three portable aviaries, each of which was assembled to cover individual plants. Aviaries were 2 × 2 × 3 m and covered in shade cloth mesh. Birds typically visited flowers in <30 min, and if they had not visited within 1 h, we fed and released them. Birds were permitted to visit individual flowers more than one time (number of visits: $\bar{x} = 1.78 \pm 1.17$ SD), but number of visits was not a significant predictor of the number of pollen tubes (GLMM: $Z = -0.23$, $P = 0.81$); therefore, we did not include this as a covariate in our final model.

In total, we conducted 223 aviary experiments comprising the following treatments/species: green hermits ($n = 105$), violet sabrewing ($n = 39$), rufous-tailed hummingbird ($n = 24$), green-crowned brilliant ($n = 16$), stripe-throated hermit ($n = 12$), scaly-breasted hummingbird ($n = 6$), and *A. fatima* ($n = 21$) (*SI Materials and Methods* and Fig. S2). All aviary experiments were approved under Oregon State University Animal Care and Use Protocol 4266.

Pollinator Nectar Consumption Observations. We measured nectar consumption by hummingbirds and butterflies by observing pollinator visitation to previously excluded flowers and then, measuring the nectar remaining after a single visit. We measured the volume of nectar using 70-μL capillary tubes and tested sugar content using a refractometer.

Pollination Network Data. We collected and identified 417 individual pollen samples for six species of hummingbirds from across 14 study sites surrounding Las Cruces Biological Station and preserved them in semipermanent slides of glycerin jelly (43). We then calculated a quantitative web using the number of times that each plant–pollinator interaction was detected normalized by the number of captures of each species (44) (*SI Materials and Methods*). To quantify the influence of pollinator recognition on web structure, we constructed a second web that accounted for the fitness contribution of each pollinator species by multiplying the proportion of pollen tubes per style for each species by the initial quantitative pollen web (Fig. 4*B*). Finally, to test the hypotheses that trapliners carry more *Heliconia* pollen and less heterospecific pollen than territorial species, we summed the number of *Heliconia* and non-*Heliconia* pollen grains for each individual bird and tested for statistical differences between these functional groups.

Statistical Methods. Pollen tube abundance data were Poisson-distributed, so we used generalized nonlinear mixed models [in the lme4 R package (45)] to test for differences in tube abundance as a function of pollinator species. We tested for the effects of pollen source (self-pollen vs. outcrossed) by including this term as a covariate in the model (Table 1). To account for lack of statistical independence caused by individual birds and plants being used for multiple trials, we specified individual bird and individual plant as partially crossed random effects. To limit the number of tests, we conducted pairwise comparisons with only two species: green-crowned brilliant and rufous-tailed hummingbird. We expected the green-crowned brilliant to be intermediate in pollination effectiveness because of its body size and bill length. Alternatively, we expected the rufous-tailed hummingbird to be the poorest of the hummingbird species because of its short bill length. This approach still resulted in a total of nine comparisons, which increases the risk of type I error. Sequential Bonferroni-type multiple comparisons are often used to account for such error inflation, but they are highly conservative (46). Therefore, we used a false discovery rate procedure (the graphically sharpened method) (46), which does not suffer from the same loss of power but corrects for multiple comparisons.

Models assuming a Poisson distribution sometimes suffer from lack of fit because of inflated zeros, sparse very high counts, or lack of independence among study subjects (47). We tested for model overdispersion ($\hat{c} > 1$) in all models that assumed a Poisson distribution by calculating the sum of squared Pearson residuals, the ratio of residuals to rdf (residual degrees of freedom), and the P value based on the appropriate χ^2 distribution (48) (Table S2). We detected significant but minor overdispersion in one of three models (Table S2), and therefore, we modeled pollen tube count data in this case using the quasi-Poisson family (49). As another test of whether our results were driven by poor match to the data distribution, we also modeled the presence/absence of pollen tubes in addition to pollen tube abundance. Results did not differ substantially from those assuming a Poisson distribution (*SI Materials and Methods* and Tables S3–S6).

We tested for differences in nectar consumption capacity across species using GLMs with a Gaussian distribution. Green hermit males and females were included separately in analysis of nectar extraction, because they show strong sexual dimorphism in bill length, which is likely to influence nectar extraction efficiency (50). Sexes of this species have different movement behavior, with male home ranges larger than female home ranges (33). We tested assumptions of regressions by inspecting regression residuals for normality against a q-q plot.

We tested the effect of experimental nectar extraction on pollen tube abundance using GLMMs with a Poisson distribution. The individual plant was specified as a random effect to account for occasional repeat sampling of the same plant. These data were analyzed two ways. First, we predicted pollen tube abundance as a function of the amount of nectar removed. Second, we tested the effect of nectar extraction treatments vs. hand pollination-only treatments.

Finally, we tested whether territorial species carried more non-*Heliconia* pollen than traplining species using GLMs with a Gaussian distribution. Data were log($x + 1$)-transformed to meet model assumptions. We used the same modeling approach to test whether traplining species carried more *Heliconia* pollen than trapliners.

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1. Kondrashov AS (1988) Deleterious mutations and the evolution of sexual reproduction. *Nature* 336(6198):435–440.
2. Fischer RA (1930) *The Genetical Theory of Natural Selection* (Clarendon, Oxford).
3. Darwin C (1877) *The Various Contrivances by Which Orchids Are Fertilised by Insects* (John Murray, London).
4. Dodd ME, Silvertown J, Chase MW (1999) Phylogenetic analysis of trait evolution and species diversity variation among angiosperm families. *Evolution* 53(3):732–744.
5. Aizen MA, Harder LD (2007) Expanding the limits of the pollen-limitation concept: Effects of pollen quantity and quality. *Ecology* 88(2):271–281.
6. Stiles FG, Wolf LL (1970) Hummingbird territoriality at a tropical flowering tree. *Auk* 87(3):467–491.
7. Temeles EJ, Koulouris CR, Sander SE, Kress WJ (2009) Effect of flower shape and size on foraging performance and trade-offs in a tropical hummingbird. *Ecology* 90(5):1147–1161.
8. Baker HG, Hurd PD (1968) Intrafloral ecology. *Annu Rev Entomol* 13(1):385–414.
9. Stanton ML, Snow AA, Handel SN (1986) Floral evolution: Attractiveness to pollinators increases male fitness. *Science* 232(4758):1625–1627.
10. Thompson JN (2005) *The Geographic Mosaic of Coevolution* (University of Chicago Press, Chicago).
11. Thompson JN (1999) The evolution of species interactions. *Science* 284(5423):2116–2118.
12. Guimarães PR, Jr, Jordano P, Thompson JN (2011) Evolution and coevolution in mutualistic networks. *Ecol Lett* 14(9):877–885.
13. Newman E, Manning J, Anderson B (2014) Matching floral and pollinator traits through guild convergence and pollinator ecotype formation. *Ann Bot (Lond)* 113(2):373–384.
14. Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD (2004) Pollination syndromes and floral specialization. *Annu Rev Ecol Evol Syst* 35(1):375–403.
15. Thomson JD, Wilson P (2008) Explaining evolutionary shifts between bee and hummingbird pollination: Convergence, divergence, and directionality. *Int J Plant Sci* 169(1):23–38.
16. Bascompte J, Jordano P, Olesen JM (2006) Asymmetric coevolutionary networks facilitate biodiversity maintenance. *Science* 312(5772):431–433.
17. Waser NM, Chittka L, Price MV, Williams NM, Ollerton J (1996) Generalization in pollination systems, and why it matters. *Ecology* 77(4):1043–1060.
18. Fenster CB (1991) Selection on floral morphology by hummingbirds. *Biotropica* 23(1): 98–101.
19. Nilsson LA, Johnsson L, Ralison L, Randrianjohany E (1987) Angraecoid orchids and hawkmoths in central Madagascar: Specialized pollination systems and generalist foragers. *Biotropica* 19(4):310–318.
20. Nuismer SL, Jordano P, Bascompte J (2013) Coevolution and the architecture of mutualistic networks. *Evolution* 67(2):338–354.
21. Stiles FG (1975) Ecology, flowering phenology, and hummingbird pollination of some Costa Rican Heliconia species. *Ecology* 56(2):285–301.
22. Feinsinger P (1976) Organization of a tropical guild of nectarivorous birds. *Ecol Monogr* 46(3):257–291.
23. Hadley AS, Frey SJK, Robinson WD, Kress WJ, Betts MG (2014) Tropical forest fragmentation limits pollination of a keystone understory herb. *Ecology* 95(8):2202–2212.
24. Knight TM, et al. (2005) Pollen limitation of plant reproduction: Pattern and Process. *Annu Rev Ecol Evol Syst* 36(1):467–497.
25. Kress WJ (1983) Self-incompatibility in Central American Heliconia. *Evolution* 37(4): 735–744.
26. Abrahamczyk S, Souto-Vilarós D, Renner SS (2014) Escape from extreme specialization: Passionflowers, bats and the sword-billed hummingbird. *Proc Biol Sci* 281(1795): 20140888.
27. Maglianesi MA, Blüthgen N, Böhning-Gaese K, Schleuning M (2014) Morphological traits determine specialization and resource use in plant-hummingbird networks in the Neotropics. *Ecology* 95(12):3325–3334.
28. Linhart YB, et al. (1987) Disturbance and predictability of flowering pattens in bird-pollinated cloud forest plants. *Ecology* 68(6):1696–1710.
29. Wolf LL, Hainsworth FR, Stiles FG (1972) Energetics of foraging: Rate and efficiency of nectar extraction by hummingbirds. *Science* 176(4041):1351–1352.
30. Heinrich B, Raven PH (1972) Energetics and pollination ecology. *Science* 176(4035): 597–602.
31. Linhart YB (1973) Ecological and behavioral determinants of pollen dispersal in hummingbird-pollinated Heliconia. *Am Nat* 107(956):511–523.
32. Stiles FG (1978) Ecological and evolutionary implications of bird pollination. *Am Zool* 18(4):715–727.
33. Volpe NL, Hadley AS, Robinson WD, Betts MG (2014) Functional connectivity experiments reflect routine movement behavior of a tropical hummingbird species. *Ecol Appl* 24(8):2122–2131.
34. Murdy WH, Carter MEB (1987) Regulation of the timing of pollen germination by the pistil in Talinum-mengesii (Portulacaceae). *Am J Bot* 74(12):1888–1892.
35. Loughnan D, Thomson JD, Ogilvie JE, Gilbert B (2014) Taraxacum officinale pollen depresses seed set of montane wildflowers through pollen allelopathy. *J Poll Ecol* 13(15):146–150.
36. Haig D, Westoby M (1988) On limits to seed production. *Am Nat* 131(5):757–759.
37. Memmott J, Waser NM, Price MV (2004) Tolerance of pollination networks to species extinctions. *Proc Biol Sci* 271(1557):2605–2611.
38. Hadley AS, Betts MG (2009) Tropical deforestation alters hummingbird movement patterns. *Biol Lett* 5(2):207–210.
39. Allmann S, Baldwin IT (2010) Insects betray themselves in nature to predators by rapid isomerization of green leaf volatiles. *Science* 329(5995):1075–1078.
40. Trewavas A (2002) Mindless mastery. *Nature* 415(6874):841.
41. Stone JL, Thomson JD, Dent-Acosta SJ (1995) Assessment of pollen viability in hand-pollination experiments: A review. *Am J Bot* 82(9):1186–1197.
42. Eckert CG, et al. (2010) Plant mating systems in a changing world. *Trends Ecol Evol* 25(1):35–43.
43. Kearns CA, Inouye DW (1993) *Techniques for Pollination Biologists* (University of Colorado Press, Niwot, CO).
44. Dormann CF, Gruber B, Frund J (2008) Introducing the Bipartite Package: Analysing ecological networks. *R-News* 8(2):8–11.
45. Bates D, et al. (2014) *lme4: Linear Mixed-Effects Models Using Eigen and S4* (R Core Team).
46. Pike N (2011) Using false discovery rates for multiple comparisons in ecology and evolution. *Methods Ecol Evol* 2(3):278–282.
47. Richards SA (2008) Dealing with overdispersed count data in applied ecology. *J Appl Ecol* 45(1):218–227.
48. Venables WN, Ripley BD (2002) *Modern Applied Statistics with S* (Springer, Berlin), 4th Ed.
49. Zeileis A, Kleiber C, Jackman S (2008) Regression models for count data in R. *J Stat Softw* 27(8):1–25.
50. Temeles EJ, Miller JS, Rifkin JL (2010) Evolution of sexual dimorphism in bill size and shape of hermit hummingbirds (Phaethornithinae): A role for ecological causation. *Philos Trans R Soc Lond B Biol Sci* 365(1543):1053–1063.

Supporting Information

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SI Materials and Methods

Aviary Experiment Details. In 37 of 202 bird visitation treatments, we hand-pollinated flowers with self-pollen rather than outcross pollen. In this treatment, we hand-pollinated using pollen from anthers adjacent to the stigma. *Heliconia tortuosa* are partially self-compatible (1), and therefore, this treatment should be expected to result in pollen tubes, albeit with lower frequency than outcrossed pollen. As in all of our hand-pollination experiments, we initially cleaned all flowers of self-pollen and covered flowers after each treatment. The purpose of the self-pollen treatment was to test whether residual pollen remained undetected on the cleaned hummingbirds. If birds carried extraneous, nonexperimentally controlled pollen, these experiments should be equivalent to our treatments with outcrossed pollen; however, we found substantially lower abundance of pollen tubes in self-pollen treatments than outcrossed treatments (Table 1), indicating that the pollen that we manually applied, and not extraneous pollen carried by hummingbirds, was driving pollen tube effects.

In butterfly experiments, we captured butterflies and contained them in a mesh bag on a live plant with an open, hand-pollinated flower for >10 h (Fig. S2). Butterflies were given longer access to flowers than birds for two reasons. First, we were concerned about the welfare of birds kept in an aviary longer than 1 h. Second, initial observations indicated that butterflies took longer to make contact with flower reproductive parts than hummingbirds. Although in this case, treatment (butterfly vs. bird) was confounded with exposure time, this procedure should bias our results in favor of butterflies, which had access to flowers for longer periods.

Pollination Success. Pollen tubes are the first critical step in plant reproduction and have been used extensively as an indicator of pollination success. Although fruit and seed sets are the most commonly reported evidence of reproductive success (2), we focused on pollen tubes, because (*i*) examination of fruit and seed set would not have enabled us to determine the timing of pollinator recognition (i.e., whether it occurred within the style or at some later point in reproductive development); (*ii*) pollen tubes can be examined rapidly (<6 d) after experiments, which allowed us to increase our sample size and hence, statistical power; and (*iii*) pollen tubes are highly correlated with fruit set across *Heliconia* species ($r = 0.91$, $n = 9$, $P = 0.0003$) (data are from ref. 1). Furthermore, in a separate effort, we collected data on both *H. tortuosa* fruit size (i.e., fruit diameter at the widest point 3 wk after flowering) and pollen tubes from the same open-pollinated flowers ($n = 50$). Number of pollen tubes strongly predicted fruit size ($Z = 5.9$, $P < 0.0001$). Although the presence of pollen tubes does not ensure reproduction, without them, no seeds or fruit can be produced. We applied standard epifluorescence microscopy techniques (3) to quantify pollen tube abundance. Pollen tubes are relatively easy to count in the *Heliconia* genus owing to low abundances typically present at flower bases ($\bar{x} = 2.56 \pm 0.87$ tubes per style) (1).

Pollen Tube Laboratory Methods. To assess pollen tubes, we collected styles of abscised *Heliconia tortuosa* flowers 1 d after each experimental treatment and fixed them in Formalin Aceto-Alcohol Solution for >24 h. We then stained the styles with aniline blue dye following the methods by Kearns and Inouye (3). Styles were rinsed in distilled water for 24 h and then soaked in an 8 M solution of sodium hydroxide for 24 h. Next, styles were rinsed two times in distilled water or a total of 48 h. We then soaked the styles in a 0.05% solution of aniline blue for at least 6 h. We mounted the styles on slides using a drop of aniline blue dye and flattened them under coverslips. Finally, we examined styles for the presence of pollen tubes using epifluorescence microscopy. Only two observers (M.G.B. and A.S.H.) examined styles, and in all cases, the observers were naïve to treatment.

Nectar Extraction Experiment. We randomly selected flowers to receive either hand pollination only or hand pollination combined with nectar extraction. We extracted nectar using flexible tip pipettes inserted into the flower through the corolla opening to access the nectar chamber, and measured the volume and concentration of nectar extracted using the methods above. To test the possibility that the apparent positive effect of nectar extraction on the success of hand pollinations was caused by pipette insertion rather than nectar extraction per se, we conducted an experiment where we inserted pipette tips into hand-pollinated flowers ($n = 13$) as in our nectar extraction treatment. We then tested for differences between this sham pipette treatment vs. outcrossed, hand-pollinated flowers ($n = 40$) and hand-pollinated flowers with nectar extracted ($n = 31$). Under the expectation that insertion of a pipette itself has a positive effect, we should have seen no difference between pollen tubes in nectar-extracted flowers vs. pipette-inserted flowers. We detected a difference between the pipette insertion treatment ($\bar{x} = 0.10 \pm 0.10$ SE tubes per style) and flowers with nectar extracted ($\bar{x} = 0.76 \pm 0.16$ SE tubes per style, $t = 2.0$, $P = 0.04$) but no significant difference between pipette insertions and hand pollinations alone ($\bar{x} = 0.19 \pm 0.13$ SE tubes per style, $t = 0.582$, $P = 0.560$). This evidence contradicts the hypothesis that the positive effect of nectar extraction on pollen tube growth is simply the physical effect of pipette insertion.

Hummingbird Movement Distances. From 2012 to 2014, we collected data on movement distances of pollinators using a combination of methods. We fitted radio-transmitter units (<0.25 g; Blackburn Transmitters) to green hermit ($n = 20$), violet sabrewing ($n = 2$), rufous-tailed ($n = 2$), and green-crowned brilliant ($n = 1$) hummingbirds. The relatively large size of these species (>5.0 g) made this a logistically feasible and efficient means to monitor movement (4, 5). Birds were caught using the methods described above. We attached radio transmitters to bare skin underneath feathers on the lower backs of hummingbirds using eyelash glue. We tracked birds from 2 to 7 d for a minimum of 4 h/d (detailed methods are in ref. 5) and followed birds as closely as possible with handheld radio receivers and Yagi antennas. We recorded locations whenever the bird was seen or located to within 30 m. Because of their small size (~2.7 g), we monitored stripe-throated hermits ($n = 3$) using radiofrequency identification devices subcutaneously-implanted into the upper back of hummingbirds (6). Movements were detected at radiofrequency identification device readers mounted on 17 hummingbird feeders positioned in a trapping grid. Each grid had a central feeder and four cardinal transects with feeders at 25, 75, 175, and 275 m (for a maximum reader array diameter of 550 m). Maximum recorded movements of stripe-throated hermits were <350 m, and therefore, we have some confidence that we captured typical daily movement distances for this species. We were not able to capture and radiotrack scaly-breasted and *Anartia fatima* because of their relative scarcity and small size, respectively. We, therefore, conducted detailed observational bouts of both species lasting 4–6 h on at least 4 separate d. Both species were highly visible and could be followed nearly continuously, and therefore, we can be reasonably certain that regular, diurnal, longer-distance movements did not go unobserved. We used median daily movement distance—the greatest width across the daily foraging area for each species—as a proxy for typical foraging movement distances of pollinators. Using median vs. maximum movement distance did not qualitatively influence our results. We classified traplining and territorial species according to the foraging behavior described in ref. 7, which also matches criteria established by Stiles (8), and the curvature of hummingbird bills (trapliners with curved bills: green hermit, violet sabrewing, and stripe-throated hermit; territorial with straight bills: rufous-tailed hummingbird, scaly-breasted hummingbird, and green-crowned brilliant).

Pollination Web. We identified pollen grains present in each sample (n = 417) to morphospecies under a microscope by comparing with a library of known pollen grains and calculated a quantitative pollen web using the number of times that each plant–pollinator interaction was detected (9) normalized by the total number of captures of each species (Fig. 4*A*); the presence of more than five grains of pollen on an individual constituted a single observation to avoid bias in estimates of pollinators caused by sample contamination. To construct webs, we used the number of individual birds observed to be carrying pollen rather than pollen quantity (i.e., number of pollen grains), because *H. tortuosa* only requires three grains of pollen for successful pollination; therefore, we considered this response variable to be more biologically meaningful.

1. Kress WJ (1983) Self-incompatibility in Central American Heliconia. *Evolution* 37(4):735–744.
2. Knight TM, et al. (2005) Pollen limitation of plant reproduction: Pattern and process. *Annu Rev Ecol Evol S* 36(1):467–497.
3. Kearns CA, Inouye DW (1993) *Techniques for Pollination Biologists* (University of Colorado Press, Niwot, CO).
4. Hadley AS, Betts MG (2009) Tropical deforestation alters hummingbird movement patterns. *Biol Lett* 5(2):207–210.
5. Volpe N, Hadley AS, Robinson WD, Betts MG (2014) Functional connectivity experiments reflect routine movement behavior of a tropical hummingbird species. *Ecol Appl* 24(8):2122–2131.
6. Brewer LW, Redmond CA, Stafford JM, Hatch GE (2011) Marking Ruby-throated hummingbirds with radio frequency identification tags. *J Wildl Manage* 75(7):1664–1667.
7. Fogden M, Fogden P (2006) *Hummingbirds of Costa Rica* (Firefly Books Ltd., Buffalo, NY).
8. Stiles FG (1975) Ecology, flowering phenology, and hummingbird pollination of some Costa Rican Heliconia species. *Ecology* 56(2):285–301.
9. Dormann CF, Gruber B, Frund J (2008) Introducing the Bipartite Package: Analysing ecological networks. *R-News* 8(2):8–11.

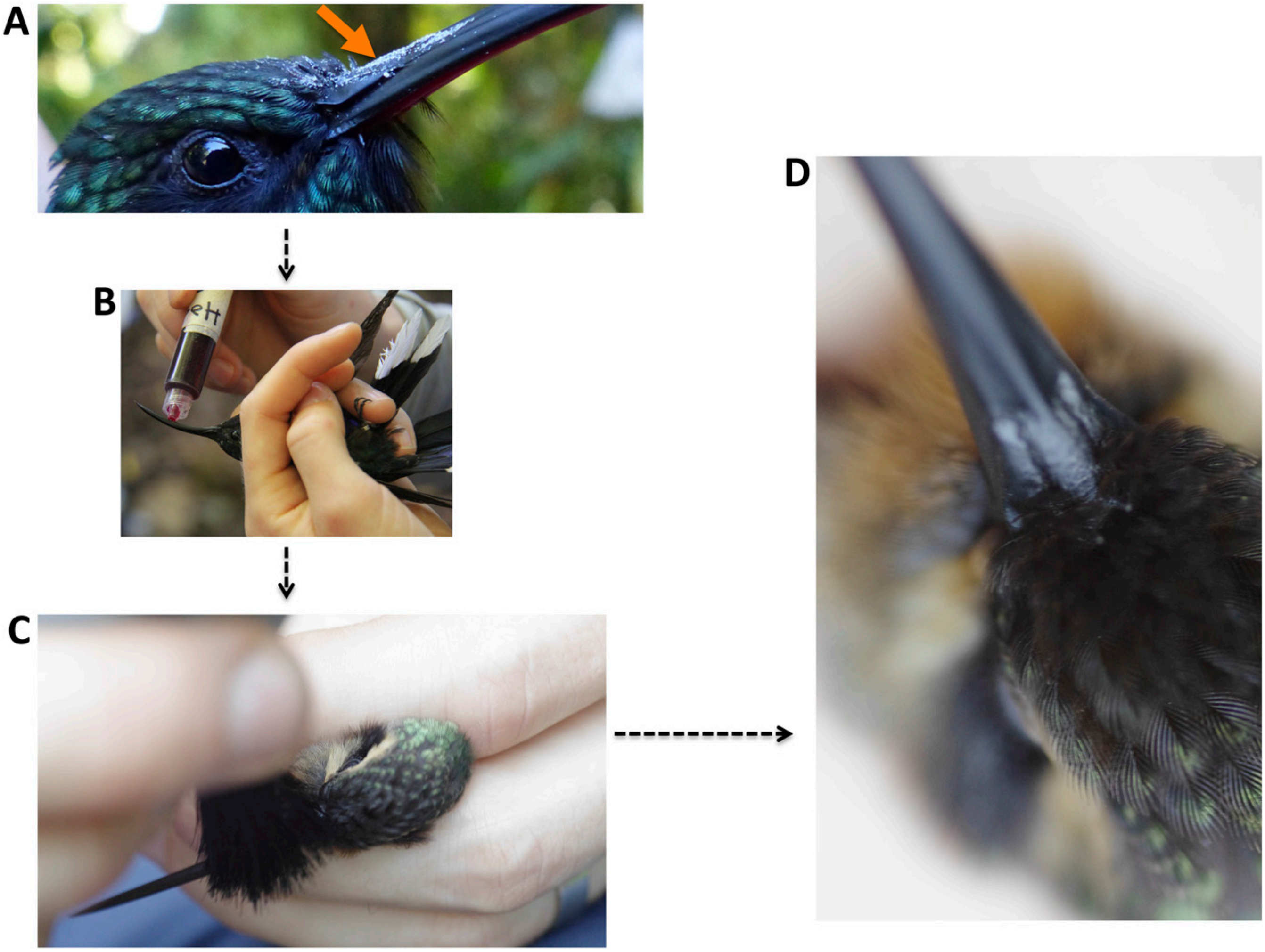


Fig. S1. We cleaned hummingbirds of all external pollen (indicated by the orange arrow) using three steps (*A*). First, we used glycerin jelly to remove the majority of the pollen (*B*). Second, we used an artist brush to remove remaining pollen from head, throat, bill, or nares. If any residue still remained, we removed it using lens-cleaning tissues and rebrushed the bird (*C*). Third, we verified that no pollen remained using a 30× hand lens. *D* shows a cleaned bird after pollen has been removed.

Fig. S2. For butterfly treatments, we placed *A. fatima* in meshed bags containing a treated flower for 10 h.

Table S1. Results of a GLM predicting nectar extraction efficiency across pollinator species

Species	β	SE	*Z*	*P*	*P-FDR*
Intercept: green-crowned brilliant	13.71	1.95	7.04	<0.0001	—
Butterfly*	20.83	2.49	8.36	<0.0001	<0.0001
Rufous-tailed hummingbird*	4.89	2.36	2.07	0.0398	0.0400
Stripe-throated hermit*	−7.41	2.30	−3.23	0.0015	0.0017
Scaly-breasted hummingbird*	−12.46	3.23	−3.86	0.0002	0.0002
Green hermit female*	−11.39	2.20	−5.17	<0.0001	<0.0001
Green hermit male*	−12.78	2.03	−6.31	<0.0001	<0.0001
Violet sabrewing*	−13.44	2.21	−6.07	<0.0001	<0.0001
Intercept: rufous-tailed hummingbird	18.60	1.33	13.97	< 0.0001	—
Butterfly*	15.95	2.05	7.79	<0.0001	<0.0001
Green-crowned*	−12.29	1.80	−6.82	0.0398	0.0400
Stripe-throated hermit*	−16.27	1.68	−9.66	<0.0001	<0.0001
Scaly-breasted hummingbird*	−17.66	1.44	−12.26	<0.0001	<0.0001
Green hermit female*	−17.35	2.90	−5.98	<0.0001	<0.0001
Green hermit male*	−4.89	2.36	−2.07	<0.0001	<0.0001
Violet sabrewing*	−18.32	1.70	−10.80	<0.0001	<0.0001

P-FDR, *P* values that have been corrected for false discovery rates associated with multiple comparisons among species (*Materials and Methods*).

*Species that differed significantly from the reference species of green-crowned brilliant and rufous-tailed hummingbirds (GLM: $F = 81.30$, $R^2 = 0.75$, $P < 0.0001$).

Table S2. Results of tests for overdispersed count data for three Poisson models of pollen tube abundance

Model	Model type	$\hat{c}$	*P*
Pollen tubes ~ hand-pollinated vs. open	GLM	1.22	0.002
Pollen tubes ~ pollinator species	GLMM	0.93	0.740
Pollen tubes ~ experimental nectar extraction	GLMM	0.79	0.964

Table S3. Results of the GLMM with a binomial distribution predicting differences in pollen tube presence as a function of different hummingbird pollinator species released into aviaries with hand-pollinated *H. tortuosa*

Parameter	β	SE	*Z*	*P*	*P-FDR*
Intercept: green-crowned brilliant	−3.19	1.04	−3.06	0.002	—
Self-pollen	−1.24	0.45	−2.79	0.005	—
Green hermit	1.35	0.65	2.07	0.039	0.068
Rufous-tailed hummingbird	2.07	1.19	1.74	0.083	0.097
Scaly-breasted hummingbird	0.40	1.07	0.37	0.709	0.551
Stripe-throated hermit	−0.38	1.00	−0.38	0.703	0.551
Violet sabrewing*	2.08	0.73	2.85	0.004	0.010
Intercept: rufous-tailed hummingbird	−3.19	1.04	−3.06	0.002	—
Self-pollen	−1.24	0.45	−2.79	0.005	—
Green hermit*	3.42	1.08	3.18	0.001	0.005
Green-crowned brilliant	2.07	1.19	1.74	0.083	0.097
Scaly-breasted hummingbird	2.47	1.36	1.81	0.070	0.097
Stripe-throated hermit	1.69	1.30	1.30	0.195	0.195
Violet sabrewing*	4.15	1.13	3.67	0.000	0.002

We controlled for our two pollen quality treatments statistically by including self-pollen vs. outcrossed pollen as an indicator variable. Results for alternative statistical tests, where rather than modeling the response (pollen tube abundance) as a Poisson-distributed response variable, all pollen tube data were reduced to zeros and ones and modeled using logistic regression (or mixed effects logistic regression models in the case of repeated sampling of individual flowers or pollinators). Reducing all nonzero integers to ones results in a loss of information on pollen tube abundance, and therefore, these results should be considered a highly conservative version of those reported in the text. *P-FDR* (false discovery rates) are *P* values that have been corrected for false discovery rates associated with multiple comparisons among species (*Materials and Methods*).

*Species that differed significantly from the two reference species of green-crowned brilliant and rufous-tailed hummingbirds.

Table S4. Results of the GLM predicting the probability of pollen tubes as a function of whether flowers were hand-pollinated and bagged ($\hat{p}$ = 0.14; 95% CI = 0.094–0.209) or had full access to pollinators ($\hat{p}$ = 0.60; 95% CI = 0.567–0.636)

	β	SE	*Z*	*P*
Intercept: open	0.41	0.07	5.53	<0.0001
Hand-pollinated	−2.19	0.24	−9.22	<0.0001

Results for alternative statistical tests, where rather than modeling the response (pollen tube abundance) as a Poisson-distributed response variable, all pollen tube data were reduced to zeros and ones and modeled using logistic regression (or mixed effects logistic regression models in the case of repeated sampling of individual flowers or pollinators). Reducing all nonzero integers to ones results in a loss of information on pollen tube abundance, and therefore, these results should be considered a highly conservative version of those reported in the text.

Table S5. Result of the GLMM with binomial distribution predicting the probability of pollen tubes as a function of how much nectar we experimentally extracted from *H. tortuosa*

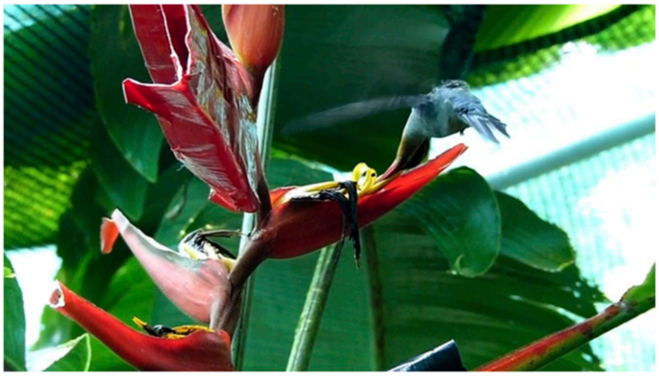
	β	SE	Z	P
Intercept	−2.30	0.68	−3.39	0.001
Amount of nectar	1.85	0.71	2.59	0.010

Results for alternative statistical tests, where rather than modeling the response (pollen tube abundance) as a Poisson-distributed response variable, all pollen tube data were reduced to zeros and ones and modeled using logistic regression (or mixed effects logistic regression models in the case of repeated sampling of individual flowers or pollinators). Reducing all nonzero integers to ones results in a loss of information on pollen tube abundance, and therefore, these results should be considered a highly conservative version of those reported in the text.

Table S6. Result of the GLM with binomial distribution predicting the probability of pollen tubes as a function of whether we attempted to extract nectar from *H. tortuosa* during hand pollinations ($\hat{p} = 0.39$; 95% CI = 0.16–0.747) or whether we hand-pollinated only ($\hat{p} = 0.094$; 95% CI = 0.024–0.22)

	β	SE	Z	P
Intercept: hand-pollinated	−2.27	0.61	−3.74	<0.0001
Nectar-extracted	1.83	0.68	2.70	0.007

Results for alternative statistical tests, where rather than modeling the response (pollen tube abundance) as a Poisson-distributed response variable, all pollen tube data were reduced to zeros and ones and modeled using logistic regression (or mixed effects logistic regression models in the case of repeated sampling of individual flowers or pollinators). Reducing all nonzero integers to ones results in a loss of information on pollen tube abundance, and therefore, these results should be considered a highly conservative version of those reported in the text.



Movie S1. Female green hermit hummingbird visiting *H. tortuosa* in an experimental aviary. Note the inverted bill insertion by the hermit, which is required by most curve-billed visitors to *H. tortuosa* because of the upturned curve of the flower. This approach to bill insertion shows a striking match between bird bill and flower corolla shape, which results in high nectar extraction efficiency (Fig. 2*A*).

Movie S1