Different foraging preferences of hummingbirds on artificial and natural flowers reveal mechanisms structuring plant–pollinator interactions

María A. Maglianesi[1,2]*, Katrin Böhning-Gaese[1,3] and Matthias Schleuning[1]

[1]*Biodiversity and Climate Research Centre (BiK-F) and Senckenberg Gesellschaft für Naturforschung, 60325 Frankfurt am Main, Germany;* [2]*Vicerrectoría de Investigación, Universidad Estatal a Distancia (UNED), De la Rotonda La Betania 500m al este, Carretera a Sabanilla Mercedes de Montes de Oca, San José, Costa Rica; and* [3]*Department of Biological Sciences, Johann Wolfgang Goethe University Frankfurt, 60438 Frankfurt am Main, Germany*

Summary

1. In plant–pollinator networks, the floral morphology of food plants is an important determinant of the interaction niche of pollinators. Studies on foraging preferences of pollinators combining experimental and observational approaches may help to understand the mechanisms behind patterns of interactions and niche partitioning within pollinator communities.

2. In this study, we tested whether morphological floral traits were associated with foraging preferences of hummingbirds for artificial and natural flower types in Costa Rica. We performed field experiments with artificial feeders, differing in length and curvature of flower types, to quantify the hummingbirds' interaction niche under unlimited nectar resources. To quantify the interaction niche under real-world conditions of limited nectar resources, we measured foraging preferences of hummingbirds for a total of 34 plant species.

3. Artificial feeders were visited by *Eupherusa nigriventris* and *Phaethornis guy* in the premontane forest, and *Lampornis calolaemus* in the lower montane forest. Under experimental conditions, all three hummingbird species overlapped their interaction niches and showed a preference for the short artificial flower type over the long-straight and the long-curved flower types. Under natural conditions, the two co-occurring hummingbird species preferred to feed on plant species with floral traits corresponding to their bill morphology. The short-billed hummingbird *E. nigriventris* preferred to feed on short and straight flowers, whereas the long- and curved-billed *P. guy* preferred long and curved natural flowers. The medium-size billed species *L. calolaemus* preferred to feed on flowers of medium length and did not show preferences for plant species with specific corolla curvature.

4. Our results show that floral morphological traits constrain access by short-billed hummingbird species to nectar resources. Morphological constraints, therefore, represent one important mechanism structuring trophic networks. In addition, other factors, such as competition and differences in resource quantity or quality, define the interaction niches of consumer species in real-world communities, enforcing patterns of niche segregation between co-occurring consumer species. This suggests that experimental studies are needed to disentangle effects of morphological constraints from those of competition for resources in plant–pollinator interactions and other types of trophic interactions.

Key-words: artificial feeders, biotic interactions, Costa Rica, floral morphology, floral traits, foraging preferences, hummingbirds, interaction niche, morphological constraints, resource partitioning

*Correspondence author. E-mail: mmaglianesi@uned.ac.cr

Introduction

Plant and animal species are embedded in complex ecological networks and are linked by trophic interactions (Bascompte & Jordano 2007). In such trophic networks, plant species provide food resources for the dependent consumer guild, whereby plants represent an important niche dimension for the consumer species (Blüthgen 2010). This idea is consistent with the traditional niche concept proposed by Hutchinson (1957), who described the fundamental niche of a species as a multidimensional hypervolume, in which the dimensions include the resources that define the requirements of a species. In the presence of other species, however, this fundamental niche may be reduced to a smaller realized niche because of ecological constraints such as competition for resources (see Colwell & Fuentes 1975).

In plant–pollinator networks, the interaction niche of the pollinators is commonly defined by the range of plant species that are visited (i.e. the interaction or trophic niche; cf. Devictor et al. 2010). Previous research has demonstrated that floral morphology largely influences patterns of interactions of pollinators by constraining the number and strength of interactions (Stiles 1975; Stang, Klinkhamer & van der Meijden 2006; Maglianesi et al. 2014; Vizentin-Bugoni, Maruyama & Sazima 2014). For instance, specific floral morphologies may facilitate or hinder access to nectar rewards (Ornelas et al. 2007; Maglianesi et al. 2014), leading to preferences of pollinators for particular plant species. Thus, floral traits may be an important determinant of the interaction niche of pollinators. The full range of plants that pollinators are potentially able to use (i.e. potential interactions) may be restricted by additional factors (e.g. interspecific competition with other pollinators) to the subset of plant species they actually use (i.e. realized interactions) (Pauw 2013).

Hummingbirds (Trochilidae) are important pollinators in the Neotropics (Stiles 1981). Hermit hummingbirds have mostly long and curved bills and are specialized on specific plant species (Linhart 1973; Stiles 1978), whereas non-hermit hummingbirds exhibit a wider range of bill morphologies and degrees of specialization (Cotton 1998; Stiles 2004). Morphological fitting between pairs of corresponding traits in plant and hummingbird species influences foraging preferences by hummingbirds (Colwell 1973; Temeles, Miller & Rifkin 2010). Particularly close morphological matches, such as those observed between *Heliconia* flowers and hermit hummingbirds, may impose strong constraints in these systems (Stiles 1975; Feinsinger 1976). These morphological constraints may be an important factor defining the potential interactions of hummingbirds with plant species. Furthermore, direct competition for nectar resources has been found to be intense in hummingbird assemblages, especially at low resource availability (Colwell 1973; Brown & Bowers 1985). It is likely that competition plays an important role

in determining foraging preferences of hummingbirds and therefore in defining their realized interactions.

A successful approach to quantify potential and realized interactions between plants and pollinators is to combine experimental and observational data. Potential interactions can be derived from controlled experiments in which pollinators' foraging preferences are measured across a range of resource types (e.g. different flower types) (Devictor et al. 2010). Experiments designed to detect foraging preferences of consumers require offering a selection of food types to individual consumers. In order to give consumers the opportunity to express a foraging choice, multiple resource types should be offered simultaneously (Peterson & Renaud 1989). In contrast, the realized interactions of pollinators can be estimated from observations of plant–pollinator interactions in the real world. From these interactions, the range of plant species visited by pollinators in natural conditions can be determined (Benadi et al. 2014). Previous work combining experimental with observational data has demonstrated that interspecific exploitation competition appears to drive resource partitioning between two species of bumblebess (*Bombus* spp.; Inouye 1978). Here, we measured foraging preferences of hummingbird species by quantitative comparisons of their flower choices under experimental and natural conditions. We performed field experiments with artificial feeders (differing in length and curvature of artificial flower types) in order to quantify the interaction niche of hummingbird species with unlimited nectar resources. We also measured foraging preferences of hummingbirds for plant species in natural conditions, in order to quantify their interaction niche with limited nectar resources. We address the following questions: (i) Do hummingbird species prefer to feed on specific flowers types under experimental conditions? (ii) Do hummingbird species prefer to feed on flowers with specific morphological traits under natural conditions? and (iii) Do hummingbirds' foraging preferences under unlimited nectar resources differ from those in the real world?

Materials and methods

STUDY AREA AND DATA COLLECTION

The study was conducted in north-eastern Costa Rica within the Braulio Carrillo National Park on the Caribbean slope of the Cordillera Central. The park encompasses an area of *c.* 45.000 ha, of which about 67% is old-growth forest. Our study included two sites, placed in two different tropical forest types of different elevations: pre-montane forest (1.000 m; 10°16′N, 84°05′W) and lower montane wet forest (2.000 m; 10°11′N, 84°07′W) (Holdridge 1967). Canopy heights were *c.* 30 m at 1.000 m and 20 m at 2.000 m (Hartshorn & Peralta 1988). Rainfall reported for elevations similar to our study sites in the national park is 3.200 and 2.200 mm for the pre-montane and lower montane forest, respectively (Hartshorn & Peralta 1988). Mean annual temperatures recorded during the last 5 years are 20 and 14 °C, respectively (Blake & Loiselle 2000; TEAM 2013).

The dry season lasts from late December to April, and the wet season reaches a peak during July and October–November in both study sites.

The study was conducted from May 2011 to April 2012, covering an entire study year. During the year of sampling, we collected data on hummingbird abundances and bill traits, morphological floral traits and plant–hummingbird interactions across seven sampling periods per site, each lasting for about 10 days. We conducted field experiments with artificial feeders for a subset of two sampling periods per forest type, covering both seasons. Experiments were carried out during 5 days in August (wet season) and 4 days in February–March (dry season).

FLOWER CHOICE ON EXPERIMENTAL FEEDERS

To assess hummingbird preferences for different floral morphologies under experimental conditions, we set up self-built feeders with different artificial flower types differing in length and curvature (Fig. 1a). Each feeder consisted of a 350-ml water bottle attached to a small container with three tubes (i.e. the number of entrances per feeder was the same across feeders) representing one of the following flower types: short (10 mm), long-straight (40 mm) and long-curved (40 mm). These three flower types represented floral trait values from the natural communities. For the long-curved type (30° of deflection), the location of the bend was one-third the distance from the base of the flower, approximating the shape of natural flowers frequently visited by hummingbirds (e.g. *Heliconia* flowers) (Temeles *et al.* 2009). The tubes of all flower types had the same internal diameter (4 mm). In order to make feeders more attractive to hummingbirds, we painted them bright red because previous studies showed that hummingbirds prefer red over other colours (Grant & Grant 1968). Feeders were filled with the same amount of 25% sucrose solution (mass:mass) that represents the common concentration and dominant sugar found in hummingbird-visited flowers (McDade 2004; Rodríguez-Flores & Stiles 2005; Chalcoff, Aizen & Galetto 2006). We established four feeding stations at each study site separated by at least 200 m. We installed feeders 1 month before the experiment started to allow hummingbirds to get used to them. At each of these stations, an array of three artificial feeders, each representing one flower type, was placed about 1·8 m above the ground. The feeders were spaced *ca.* 25 cm from one to another, that is they were close enough to offer hummingbirds a choice between the different artificial flower types.

We refilled the feeders with new sucrose solution each day of the experiment to avoid fungal infection that could affect hummingbirds' health. We were careful to ensure that the feeders were permanently filled with artificial nectar, so that hummingbirds had a much larger amount of nectar resources available than they utilized at a given site. To discard the possibility that hummingbirds choose feeders for their particular spatial position, feeders were rotated within the array on different days of the experiment. Thus, the hummingbird choice for different flower types was enforced on every day of the experiment.

To record visitation frequency by hummingbirds to artificial feeders, we fixed unattended cameras about 10 m from the feeders for a 6-h period between 06:00 and 14:00. From the videotapes, we identified the hummingbird species and recorded the flower type used by each individual at each single visit. A visit was scored whenever a hummingbird was observed to probe an artificial flower of the feeders. We defined the total number of visits per hummingbird species on each flower type as interaction frequency. We constructed one interaction matrix for each study site, feeding station and season (pooled across the observation days within a season). Overall, we recorded 6.974 visits of four hummingbird species over 1.800 hours of videotapes. We excluded visits from *Heliodoxa jacula* from further analyses because we had too few observations of interactions between this species and plant species under natural conditions (four interactions in the two forest types).

FLOWER CHOICE IN NATURAL PLANT COMMUNITIES

To record the interactions between hummingbird and plant species, we carried out observations of flowering plants in the understorey (up to 10 m above the ground). We chose plant species fitting the traditional ornithophilous pollination syndrome (Faegri & van der Pijl 1979). As hummingbird-pollinated flowers do
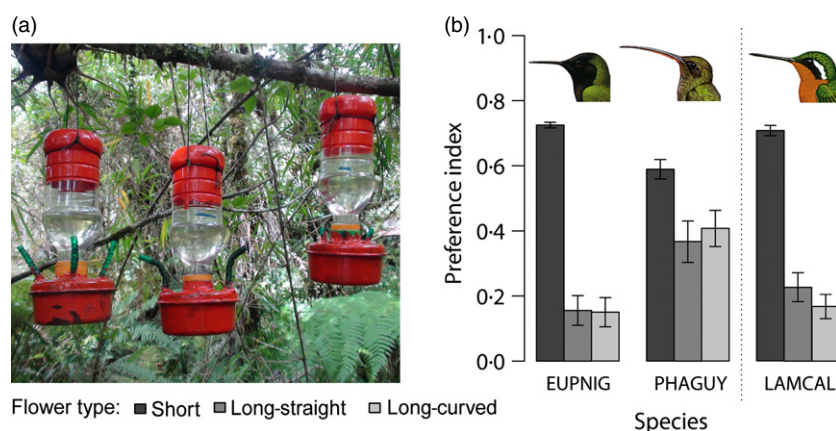
Fig. 1. (a) Artificial feeders used to assess hummingbird preferences for a flower types under experimental conditions in two tropical forests in Costa Rica. Each feeder included three tubes representing one of the following flower types: short, long-straight and long-curved. The number of entrances per feeder was the same across feeders. (b) Means and standard errors of preference index for three hummingbird species: *Eupherusa nigriventris* (EUPNIG) and *Phaethornis guy* (PHAGUY) in the pre-montane forest and *Lampornis calolaemus* (LAMCAL) in the lower montane forest. Statistics are provided in Table 1. Hummingbird illustrations were taken from del Hoyo, Elliott & Christie (2005).

not always fit into this syndrome (Ollerton *et al.* 2009), we also considered plant species fitting other pollination syndromes (e.g. bat- or insect-pollinated flowers) that were likely to be visited by hummingbirds. We randomly chose 4–12 plant individuals per species at each study site and sampling period. We videotaped 8–12 individuals of the more abundant plant species and 4–6 individuals of the less abundant species. For recording visits of hummingbirds to plant individuals, we fixed unattended cameras (Sony DCR-HC51) about 10 m from open flowers for periods of 120 min between 06:00 and 14:00 (see Robertson *et al.* 1999; Maglianesi *et al.* 2014). We recorded 1.073 flowering plant individuals of 65 species and over 2.000 hours of videotapes. We recorded the hummingbird species identity of each visit to natural flowers. A visit was scored whenever a hummingbird was observed to probe at least one flower of the observed plant individual.

Because we were interested in investigating patterns of hummingbird preferences for plant species with specific morphological floral traits, we excluded all illegitimate visits in which the hummingbird did not access the flower through the corolla entrance. For further analysis, we only considered visitation data of the hummingbird species that were observed to visit artificial feeders. Interaction frequency was defined as the total number of legitimate visits of each hummingbird species on each plant species. We constructed one interaction matrix for each study site and season.

FLORAL TRAITS

To be able to quantify hummingbird preferences, we measured for all videotaped plant species two floral traits: length and curvature of the floral corolla. We do not exclude the possibility that other, unmeasured traits may contribute to hummingbird preferences (see Temeles *et al.* 2002), but the selected traits have been reported as the primary floral constraints determining nectar accessibility for hummingbird species (see Temeles *et al.* 2009; Maglianesi *et al.* 2014; Vizentin-Bugoni, Maruyama & Sazima 2014). Length of the corolla (from the base to the tip) was measured to the nearest 0·10 mm with a dial calliper. To measure corolla curvature, the flower was placed on 1 mm graph paper so that the straight part of the corolla near the base was in line with the first 5 mm and digital photographs were taken (Kershaw 2006). From these photographs, we measured the deflection of the tip of the corolla from the line through the base of the corolla to the nearest 0·10 mm. To determine the angle of deflection, we divided the deflection by the corolla length and calculated the angle of deflection by using the sine rule. Corolla curvature was arcsin-sqrt-transformed for statistical analysis (i.e. angular transformation). For both morphological traits, we calculated mean values from 2–4 individual flowers for 88 plant species.

HUMMINGBIRD ABUNDANCE AND TRAITS

To evaluate the abundance of the studied hummingbird species in the natural communities, we placed 12–14 standard mist nets (12 × 3 m) at each study site for *c.* 6 h after dawn (Ralph *et al.* 1993). Mist nets were operated 4 days in each of the seven sampling periods. Overall, our sampling effort was about 33.160 mist-net hours (one standard mist net operated for 1 h is a net hour); sampling effort was very similar for the two study sites. We used the number of hummingbird individuals captured per species, summed across sampling periods, as an estimate of hummingbird abundance per species at each site. All hummingbirds captured were identified to species level (according to Stiles & Skutch 1989) and banded with aluminium-numbered bands. To avoid overestimation of hummingbird abundance, we excluded recaptured hummingbird individuals from abundance estimates. We did not employ standard capture–recapture approaches for estimating population sizes because only 16% of all captured hummingbirds were recaptures and sample sizes were too small for the studied species.

We measured two morphological traits that have been reported to influence plant–hummingbird interactions: bill length and curvature (Hainsworth & Wolf 1972; Temeles *et al.* 2009). Bill length (exposed culmen) was measured to the nearest 0·10 mm on captured hummingbird individuals using dial callipers. To measure bill curvature, we placed the bill on graph paper following the same procedure as for corolla curvature (Kershaw 2006). Bill curvature was arcsin-sqrt-transformed for statistical analyses (i.e. angular transformation).

STATISTICAL ANALYSIS

To investigate patterns of flower choice by pollinator species, we used a preference index (PI) to measure the preference of a hummingbird species for a specific plant species (Williams 2005; Fründ, Linsenmair & Blüthgen 2010). The same PI was used for the experimental data and the natural visits. We applied the formula $PI_k = p_{obs,k}/(p_{obs,k} + p_{null,k})$, where $p_{obs,k}$ is the proportion of visits to plant species *k* among all visits by a hummingbird species, and p_{null} is the expected proportion of visits to a particular plant species. We defined p_{null} as the proportion of observation time of plant species *k* among the total observation time for all plant species in a given study site and season. We thus assumed that under a random interaction scenario, the visitation frequency of a hummingbird species to a particular plant species is proportional to the observation time dedicated to this plant species (Fründ, Linsenmair & Blüthgen 2010). For completely opportunistic interactions (null hypothesis), the focal hummingbird species would visit the focal flower species with a probability of p_{null}. PI ranges between 0 and 1, being 0 for unvisited plant species and 0·5 for plant species visited as frequently as expected under the null hypothesis, and approaches 1 for strong preferences for a particular plant species (Fründ, Linsenmair & Blüthgen 2010).

We calculated PI for each hummingbird species and each artificial flower type as well as for each plant species observed in the natural communities. Since PI ranges between 0 and 1, it was logit-transformed for statistical analysis. To test whether hummingbird species preferred a particular flower type of the artificial feeders, we fitted a linear mixed effects model with PI as the response variable and hummingbird species identity and flower type as fixed effects (main and interaction effects). To account for random variation in foraging preferences, we included season and feeding station nested within study site as random effects. Similarly, we performed a linear mixed effects model to test for preferences of hummingbird species for plant species with specific floral morphology in natural communities. In this model, we included main and interaction effects of hummingbird species identity and floral traits (corolla length and corolla curvature) as fixed effects. Site and season were included as random effects. We fitted separate models for each floral trait.

To explore whether there is a linear or a hump-shaped relationship between the foraging preference of hummingbird species and floral morphological traits, we added an additional quadratic term of corolla length and corolla curvature to the linear models (i.e. fitted polynomial models). We compared the fits between linear and polynomial models using Akaike information criterion (AIC) and χ^2 distributed likelihood ratio tests. We used maximum likelihood (ML) approximation for model comparisons and for estimating model parameters. All statistical analyses were performed with R statistical software ver. 3.0.0 (R Development Core Team 2013).

Results

FLOWER CHOICE ON EXPERIMENTAL FEEDERS

We recorded a total of 4.503 interactions among the three artificial flower types and the three selected hummingbird species (visits by *H. jacula* were excluded). Artificial feeders were used by *Eupherusa nigriventris* and *Phaethornis guy* in the pre-montane forest and *Lampornis calolaemus* in the lower montane forest. All three hummingbird species had a preference for the short flower type (Table 1, Figs 1b and 3). PI values for the long-curved and the long-straight flower types were lower than 0·5 in all cases (Fig. 1b). Foraging preferences of *P. guy* for the short flower type was significantly lower than for the two other hummingbird species (see the respective interaction term in Table 1).

FLOWER CHOICE IN NATURAL PLANT COMMUNITIES

At the two study sites, we observed 324 interactions among 34 flowering plant species and the three selected hummingbird species (see Table S1, Supporting information for a

Table 1. Linear mixed effects model of the relationships between foraging preference indices and predictor variables (species identity and artificial flower types) in two forest types in Costa Rica. *Eupherusa nigriventris* (EUPNIG) and *Phaethornis guy* (PHAGUY) visited the artificial feeders in the submontane forest and *Lampornis calolaemus* (LAMCAL) in the lower montane forest. Artificial flower types were short, long-straight and long-curved. Season and feeding station nested within forest type were included as random effects in the model. Parameters were estimated with maximum likelihood approximation. The reference level (intercept) is given by EUPNIG and the long-curved flower type. Significant effects are shown in bold

Predictor variable	β	*t* value	*P*
Intercept	−1·66	−5·03	**< 0·001**
LAMCAL	0·07	0·18	0·857
PHAGUY	1·27	3·27	**0·002**
Long-straight	0·04	0·09	0·932
Short	2·64	5·98	**< 0·001**
LAMCAL × long-straight	0·31	0·56	0·574
LAMCAL × short	−0·22	−0·40	0·690
PHAGUY × long-straight	−0·38	−0·70	0·486
PHAGUY × short	−1·91	−3·52	**< 0·001**

complete plant species list, Supporting Information). The two measured morphological traits of plants (length and curvature of corolla) had significant effects on foraging preferences of all three hummingbird species. In the pre-montane forest, *E. nigriventris* preferred to feed on flowers with short and straight corollas, whereas *P. guy* preferred to feed on flowers with long and curved corollas (Table 2, Fig. 2a,b,d,e). In the lower montane forest, *L. calolaemus* preferred to feed on flowers of medium length, as indicated by a hump-shaped relationship between the preference index and corolla length (Fig. 2c). The species showed no preference for plant species with specific corolla curvature (Fig. 2f). The best model of the relationship between the preference index and corolla length included a linear and a quadratic term of corolla length (difference to the linear model: ΔAIC = −2·1; $\chi^2 = 8·1$, $P = 0.04$). In the case of corolla curvature, the best model included only the linear term of corolla curvature (difference to the polynomial model: ΔAIC = −2·6; $\chi^2 = 3·4$, $P = 0.33$). Niche plots indicate that preferences of hummingbird species for specific floral types under natural conditions strongly differed from those under experimental conditions with unlimited nectar resources (Fig. 3).

HUMMINGBIRD ABUNDANCE AND TRAITS

The three studied hummingbird species contributed considerably to total hummingbird abundance at each study site. *P. guy* was the most abundant species at the pre-montane forest site followed by *E. nigriventris*, representing 42% and 15% of the total number of hummingbird individuals captured in that forest type. *L. calolaemus* was the dominant species in the lower montane forest, representing 56% of all captured hummingbird individuals. Mean values of bill length (±1 SE) were 15·6 ± 0·3, 40·5 ± 0·4 and 21·5 ± 0·2 mm for *E. nigriventris*, *P. guy* and *L. calolaemus*, respectively. Mean values of bill curvature were 3·80 ± 0·12, 15·71 ± 0·40 and 3·34 ± 0·06° for the same species, respectively.

Discussion

Under experimental conditions, the three selected hummingbird species overlapped in their interaction niches showing preferences for the short artificial flower type over the long-straight and the long-curved flower types. Under natural conditions, the short-billed species *E. nigriventris* preferred to feed on short and straight flowers. In contrast, the long-curved bill species *P. guy* preferred plant species with long and curved flowers, whereas the medium-size billed species *L. calolaemus* showed weaker preferences for plant species with specific floral traits. Our findings show that preferences of hummingbird species for specific floral morphologies strongly differed between experimental and natural conditions (i.e. under unlimited and limited nectar resources).

Table 2. Linear mixed effects models of the relationships between foraging preference indices and predictor variables (species identity and floral traits) in two forest types in Costa Rica. *Eupherusa nigriventris* (EUPNIG) and *Phaethornis guy* (PHAGUY) visited plant species in the submontane forest and *Lampornis calolaemus* (LAMCAL) in the lower montane forest. Floral traits were corolla length (a) and corolla curvature (b). Season and forest type were included as random effects in the models. Parameters were estimated with maximum likelihood approximation. For corolla length, the best model includes a linear and a quadratic term, and for corolla curvature, the best model only includes a linear term. The reference level (intercept) is given by EUPNIG; parameter estimates are based on scaled values (mean = 0, standard deviation = 1) for corolla length and curvature. Significant effects are shown in bold

Predictor variable	Model with linear and quadratic term		
	β	*t* value	*P*
(a) Corolla length			
Intercept	−2·14	−6·70	**< 0·001**
LAMCAL	0·18	0·41	0·680
PHAGUY	−0·24	−0·59	0·559
Corolla length	−4·07	−2·57	**0·012**
(Corolla length)^2	2·72	1·73	0·088
LAMCAL × corolla length	7·11	3·43	**< 0·001**
PHAGUY × corolla length	5·99	2·69	**0·009**
(LAMCAL × corolla length)^2	−5·79	−2·80	**0·006**
(PHAGUY × corolla length)^2	−3·59	−1·62	0·110

Predictor variable	Model with linear term		
	β	*t* value	*P*
(b) Corolla curvature			
Intercept	−2·24	−6·27	**< 0·001**
LAMCAL	−0·16	0·34	0·732
PHAGUY	−0·04	−0·08	0·935
Corolla curvature	−1·03	−3·02	**0·003**
LAMCAL × corolla curvature	1·21	2·55	**0·013**
PHAGUY × corolla curvature	1·71	3·56	**< 0·001**

INTERACTION NICHE UNDER EXPERIMENTAL CONDITIONS

Foraging preferences for the short flower type under experimental conditions were more pronounced in the short-billed and the medium-size billed hummingbird species than in the long- and curved-billed species. The importance of morphological matching in determining patterns of interactions between plant and consumer species has been demonstrated for different types of mutualistic interaction networks such as plant–pollinator (Stang, Klinkhamer & van der Meijden 2006; Ibanez 2012; Maglianesi *et al.* 2014) and plant–frugivore networks (Moran & Catterall 2010; Dehling *et al.* 2014). In plant–hummingbird interactions, morphological floral traits of plants may act as barriers allowing only certain hummingbirds access to nectar rewards (Temeles *et al.* 2009; Vizentin-Bugoni, Maruyama & Sazima 2014). The strong preference of the short and medium-size billed hummingbirds

for the short flower type in the experiment is very likely to result from morphological constraints of accessibility to nectar imposed by long-straight and long-curved flower types. Our results indicate that length and curvature of corolla are important floral traits that limit the access to nectar resources and therefore determine the interaction niche of short-billed hummingbird species within the trait space that was experimentally explored.

Phaethornis guy with its long and curved bill was able to utilize all three artificial flower types in our field experiment. Wiklund, Eriksson & Lundberg (1979) proposed that elongated mouthparts in pollinators offer them the opportunity to exploit a greater diversity of floral morphologies, which has been confirmed by several studies (Feinsinger 1976; Corbet 2000; Goldblatt & Manning 2000). The long bill of the hermit species enables it to access nectar resources from the long and curved flower types unlike the shorter billed species. Even though *P. guy* used all three artificial flower types, it preferred the short flower type. This may be the result of easier nectar intake from short flowers (e.g. by faster bill insertion into the short tube). However, the preference for the short artificial flowers also resulted in an overlapping interaction niche with those of the two shorter billed species. The unlimited nectar resources provided to hummingbirds in the experiment are likely to have contributed to niche overlap among the hummingbird species in the experiment.

Some hummingbirds visiting the artificial feeders were observed to chase other individuals, which may be an indicator of direct competition for the offered nectar resources. This aggressive behaviour may have influenced how many birds had the opportunity to feed at the feeders, but it is unlikely to influence the foraging preferences of hummingbirds because all three flower types were blocked together within the same feeding station. An individual that had conquered a feeding station had the opportunity to choose among the three different flower types. Thus, competition between hummingbird individuals at the artificial feeders with unlimited nectar resources is very unlikely to have influenced foraging decisions of hummingbirds. We propose that experiments with artificial flowers and unlimited nectar resources can be utilized to quantify better the interaction niche of hummingbirds in the absence or, at least, with strongly reduced intensity of competition.

INTERACTION NICHE UNDER NATURAL CONDITIONS

Under natural conditions, the two co-occurring hummingbird species in the pre-montane forest showed strong foraging preferences for plant species with floral traits matching their bill morphology. The short-billed species *E. nigriventris* preferred to feed on short and straight flowers, whereas the long-billed species *P. guy* preferred to feed on long and curved flowers. This is consistent with other studies showing that nectar-feeding birds were specialized on plant species with flowers traits that matched
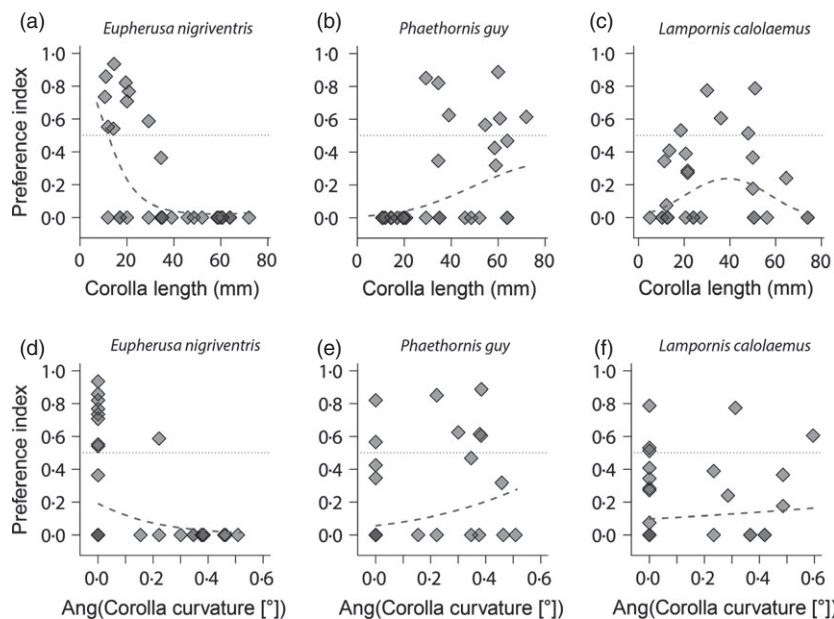
Fig. 2. Associations between the foraging preference index (PI) of hummingbird species and floral traits in two tropical forests in Costa Rica. Each data point represents one of the following hummingbird species: *Eupherusa nigriventris* (a, d), *Phaethornis guy* (b, e) and *Lampornis calolaemus* (c, f). For each hummingbird species, we calculated the corresponding PI for each videotaped plant species; plant species are represented by their mean floral trait values of length and curvature of corolla, respectively. The horizontal dashed line indicates the interactions exactly matching the null hypothesis where the visitation frequency of hummingbird species is proportional to the observation time. We used the predicted values from the linear mixed effects models in Table 2 to fit a trend line in each plot. Due to numerous plant species unvisited by the respective hummingbird species (PI = 0 in these cases), the models predicted comparatively weak preferences. Corolla curvature was angular-transformed (ang) for statistical analyses.
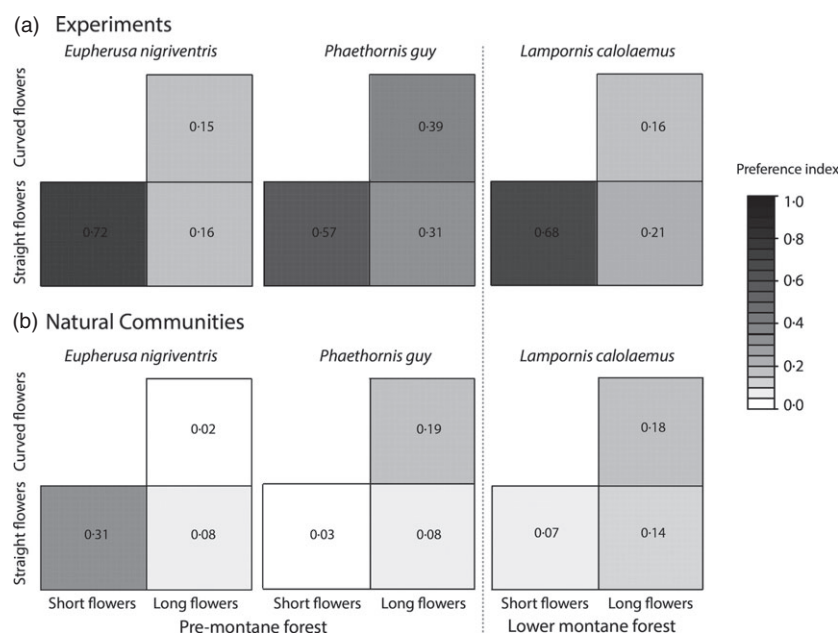
Fig. 3. Flower choice of hummingbird species in two tropical forests in Costa Rica on artificial (a) and natural flowers (b). Flower types were short, long-straight and long-curved. *Eupherusa nigriventris* and *Phaethornis guy* visited feeders and plant species in the submontane forest and *Lampornis calolaemus* in the lower montane forest. Different shades of grey indicate preference indices (PI; range: 0–1) of hummingbird species for the three types of artificial flowers and their corresponding morphologies under natural conditions, as derived by predictions from linear mixed effects models (see Tables 1 and 2). In the case of natural flowers, PI values were derived separately for the specific length (10 and 40 mm) and curvature values (0 and 30°) and then the geometric mean of the two predicted PI values was calculated. Predictions of PI values under natural conditions were always lower than those under experimental conditions because several of the observed plant species were not visited by the respective hummingbird species (PI = 0 in these cases; see Fig. 2). There were no short-curved flowers in the experimental set-up.

their bill morphology (Wolf, Stiles & Hainsworth 1976; Dalsgaard *et al.* 2009; Geerts & Pauw 2009; Maglianesi *et al.* 2014). The interaction niche of *E. nigriventris* under natural conditions corresponded to the interaction niche under unlimited nectar resources at the feeders within the range of trait values that was experimentally investigated (Fig. 3). These results suggest that foraging preferences of short-billed hummingbird species are likely driven by morphological constraints as it has been shown in other studies including hummingbird (Snow & Snow 1972) and insect pollinators (Inouye 1977, 1978). For instance, Inouye (1980) found that bumblebee species with long proboscis foraged significantly faster than those with short proboscis on flowers with long corolla tubes. Likewise, Stang, Klinkhamer & van der Meijden (2006) found that the number of insect species decreased with an increasing nectar holder depth in a Mediterranean plant–flower visitor community.

Foraging preferences by *P. guy* for long and curved flowers may result from factors other than morphological constraints because interaction niches differed considerably between experimental and natural conditions (Fig. 3). First, the long and curved flowers may be more attractive for the long- and curved-billed hummingbird species because these flowers may *per se* offer higher nectar rewards (Ornelas *et al.* 2007; Geerts & Pauw 2009). Secondly, the long and curved flowers may be more attractive for the hermits because the short- and straight-billed hummingbird species are unable to access most of these flowers, resulting in higher standing crops of nectar rewards in these flowers. The long and curved bills of the hermit species may hence contribute to reduce competition with the other species through resource exploitation. This explanation is consistent with the idea that competition for resources contributes to floral resource partitioning in hummingbird communities (Stiles 1981). Thirdly, short flowers could be defended by short-billed hummingbird species, and thus, competitive interference may limit access to these flowers (Case & Gilpin 1974; Feinsinger 1976). Our data do not enable us to disentangle these three potential causes for niche segregation of hermit species relative to other hummingbird species under natural conditions. Nevertheless, the comparison of experimental and real-world data shows that resource use in natural pollinator communities is strikingly different from that under controlled experimental conditions with unlimited and equally rewarding resources.

Lampornis calolaemus preferred to feed on flowers of medium length corresponding to its bill morphology, and it did not show a preference for plant species with specific corolla curvature. Although *L. calolaemus* showed a preference for flowers of medium size, preferences for specific flower types were overall weaker than for the two other species. These results suggest a rather high flexibility in foraging behaviour of this species. The interaction niche of this species under natural conditions strongly differed from that under experimental conditions where the species

showed a strong preference for short artificial flowers. Our results are consistent with previous studies in which *L. calolaemus* was classified as a generalist and described as a species with an opportunistic ecological role in natural plant communities (Feinsinger & Colwell 1978). In the lower montane forest, *L. calolaemus* was the dominant species (>50% of the captured individuals belonged to the species), suggesting that *intraspecific* competition for floral resources might be intense among hummingbird individuals and potentially was more important than *interspecific* competition. Species abundance affects individual foraging decisions because foraging choices of individuals influence those of other individuals depleting similar or the same floral resources (Tur *et al.* 2014). Consequently, high levels of intraspecific overlap in plant resource use may result in individuals expanding their interaction niches to a wide range of potential resources (Bolnick *et al.* 2003; Maruyama *et al.* 2013). However, *L. calolaemus* avoided very short flowers, potentially due to intense competition with short-billed hummingbird species for these flowers. *L. calolaemus* was able to use some flowers longer than their bills. This may be possible because this species, like other hummingbird species, is able to extend its tongue to harvest nectar from long flowers (Paton & Collins 1989). Furthermore, in unvisited flowers, the nectar volume may exceed the nectar chamber and therefore may be accessible for shorter billed hummingbirds (Wolf & Stiles 1989). Nevertheless, *L. calolaemus* did not visit very long flowers, which confirms that morphological constraints may be an important factor defining the realized niche also for this medium-size billed species.

Conclusions

Comparisons between experimental and observational data of foraging preferences of pollinators aid our understanding of the factors defining species' interaction niches and the mechanisms driving floral resource partitioning within communities. Our experimental results reveal that floral traits limit the access to nectar resources for short-billed hummingbird species and therefore constrain certain interactions in plant–hummingbird networks ('forbidden link hypothesis'; Jordano, Bascompte & Olesen 2003). However, pollinators may change foraging preferences in response to additional factors, such as reward quantity and quality and competition for floral resources, resulting in partitioning or expansion of their interaction niche. This is supported by our results with two co-occurring hummingbird species segregating their overlapping interaction niches under experimental conditions to non-overlapping realized interaction niches in the real world. Correspondingly, a species with strong foraging preferences for short flowers in the absence of competition expanded its interaction niche to a wider range of flower resources, most likely driven by high intraspecific competition. We conclude that morphological constraints are one important mechanism structuring trophic networks,

albeit other factors, such as as inter- and intraspecific competition, additionally define interaction niches of consumer species in real-world communities.

Acknowledgements

Data accessibility

The data associated with this article are publicly accessible in the BiK-F Data & Metadata Repository, http://dx.doi.org/10.12761/SGN.2014.4 (Maglianesi, Böhning-Gaese & Schleuning 2015).

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Supporting Information

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

Table S1. List of plant families, species and the number of visits they received from the three selected hummingbird species in Costa Rica.