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Morphological traits determine specialization and resource use in plant–hummingbird networks in the neotropics

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Abstract. Ecological communities are organized in complex ecological networks. Trait-based analyses of the structure of these networks in highly diversified species assemblages are crucial for improving our understanding of the ecological and evolutionary processes causing specialization in mutualistic networks. In this study, we assessed the importance of morphological traits for structuring plant–hummingbird networks in Neotropical forests by using a novel combination of quantitative analytical approaches. We recorded the visitation of hummingbirds to plant species over an entire year at three different elevations in Costa Rica and constructed quantitative networks based on interaction frequencies. Three morphological traits were measured in hummingbirds (bill length, bill curvature, and body mass) and plants (corolla length, curvature, and volume). We tested the effects of avian morphological traits and abundance on ecological specialization of hummingbird species. All three morphological traits of hummingbirds were positively associated with ecological specialization, especially bill curvature. We tested whether interaction strength in the networks was associated with the degree of trait matching between corresponding pairs of morphological traits in plant and hummingbird species and explore whether this was related to resource handling times by hummingbird species. We found strong and significant associations between interaction strength and the degree of trait matching. Moreover, the degree of trait matching, particularly between bill and corolla length, was associated with the handling time of nectar resources by hummingbirds. Our findings show that bill morphology structures tropical plant–hummingbird networks and patterns of interactions are closely associated with morphological matches between plant and bird species and the efficiency of hummingbirds' resource use. These results are consistent with the findings of seminal studies in plant–hummingbird systems from the neotropics. We conclude that trait-based analyses of quantitative networks contribute to a better mechanistic understanding of the causes of specialization in ecological networks and could be valuable for studying processes of complementary trait evolution in highly diversified species assemblages.

Key words: biotic interactions; Costa Rica; fourth-corner analysis; hummingbirds; mutualistic networks; Neotropical forest; optimal foraging; pollination; specialization; trait complementarity; Trochilidae.

INTRODUCTION

Specialization is a central concept in community ecology because it influences species coexistence and the structure and stability of ecological communities (Thompson 1994, Waser et al. 1996). In recent years, interactions between plants and pollinators have often been interpreted in the context of plant–pollinator networks (e.g., Blüthgen et al. 2007, Olesen et al. 2007). This approach is a powerful tool to analyze the complexity of ecological communities (Ings et al. 2009).

Plant–pollinator networks systematically vary in their degree of specialization (Dalsgaard et al. 2011, Schleuning et al. 2012), and this variability may be associated with the morphological traits of interacting plants and pollinators (Ings et al. 2009). Although a number of earlier studies have tested the importance of species traits for plant–pollinator interactions (Linhart 1973, Stiles 1975, Temeles and Kress 2003), studies using trait-based analyses in the community context of ecological networks are still rare (but see Stang et al. 2009, Junker et al. 2013).

Specialization occurs when plants are visited by a relatively small proportion of the available pollinators in a community (Armbruster et al. 2000, Johnson and Steiner 2000) and, vice versa, when pollinators restrict the use of flower resources to a subset of plant species in

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relation to overall resource availability. Specialization can be viewed from an ecological perspective in which ecological specialization refers to the state of being specialized under current ecological conditions or an evolutionary perspective, in which evolutionary specialization describes the process of evolving toward greater specialization (Armbruster 2006). Several non-mutually exclusive mechanisms have been proposed to explain causes of ecological specialization of species in plant–animal interaction networks. The species trait hypothesis has received particular attention (Santamaría and Rodríguez-Gironés 2007, Stang et al. 2009). This hypothesis states that morphological, behavioral, and life history traits constrain the type, number, and strength of interactions exerted by a species (e.g., Linhart 1973, Stiles 1975, Stang et al. 2009, Junker et al. 2013). The neutral hypothesis, in contrast, assumes that network structure largely results from random interactions between interacting species of different abundances (Dupont et al. 2003, Vázquez 2005). Here, we employed mutualistic plant–hummingbird networks to test the trait hypothesis and to identify which avian morphological traits are most important in determining ecological specialization (i.e., niche partitioning of plant resources) of hummingbird species.

Trait matching or complementarity between pairs of interacting species can be considered as a consequence of specialization, either through reciprocal co-evolutionary processes or through ecological fitting between pairs of species with independent trait evolution (Janzen 1980, 1985, Guimarães et al. 2011). According to Blüthgen et al. (2008), trait matching refers to the partitioning of interaction partners between species, resulting from the correspondence of phenotypic traits of the interacting species (i.e., phenotypic specialization). Several studies have shown that trait matching influences patterns of interactions between plant and pollinator species (Stiles 1975, Wolf et al. 1976, Dalsgaard et al. 2009, Stang et al. 2009). However, most research has been focused on specific pollinator species and their food plants (e.g., Temeles et al. 2009, Dohzono et al. 2011), calling for more integrated studies at the community level. A high degree of matching in morphological traits between flowers and their pollinators may contribute to a high quality of pollination services and a high efficiency in resource use by faster nectar intakes, leading to fitness benefits for both plants and pollinators (Temeles 1996, Dohzono et al. 2011). In this study, we assess the relationship between interaction strength in plant–hummingbird networks and the degree of trait matching in corresponding pairs of morphological traits of plant and hummingbird species. In addition, we examined whether increased trait matching between plants and hummingbirds corresponds to decreased hummingbird handling times on flowers.

In the neotropics, hummingbirds (Trochilidae) are considered to be the most specialized nectar-feeding birds (Linhart 1973, Stiles 1981). Hermit hummingbirds

have evolved exclusive morphological adaptations in bill traits toward corresponding flower morphologies, as well as a high degree of ecological specialization (Stiles 1978). By contrast, non-hermit hummingbirds exhibit a wider range of bill morphology and degrees of ecological specialization (Cotton 1998). The variety of morphological types and the degree of specialization on their nectar plants make hummingbirds an ideally suited study system to assess the influence of morphological traits on interaction patterns in pollination networks (see Plate 1). The goal of this study is to evaluate the role of morphological traits (i.e., phenotypic specialization) for ecological specialization in plant–hummingbird networks in three types of Neotropical forests at different elevations. We addressed the following questions: (1) Are morphological traits of hummingbird species associated with the degree of ecological specialization in plant–hummingbird networks? (2) Is the degree of trait matching between plant and hummingbird species associated with their pairwise interaction strength in the network? (3) Does increased trait matching between plants and birds correspond to a decreased handling time of nectar resources by hummingbirds?

METHODS

Study area and sampling design

The study was conducted in northeastern Costa Rica within the forest of the La Selva–Braulio Carrillo corridor on the Caribbean slope of the Cordillera Central. This area extends from La Selva Biological Station (LS; ~1500 ha) to the Braulio Carrillo National Park (~45 000 ha). Our study sites included three tropical forest types located at different elevations: wet forest (50 m; 10°26' N, 84°01' W) in LS, pre-montane forest (1000 m; 10°16' N, 84°05' W), and lower montane wet forest (2000 m; 10°11' N, 84°07' W) in the park (Holdridge 1967). All sites were located in old-growth forest. Canopy heights were ~30–40 m at LS, 30–35 m at 1000 m, and 20 m at 2000 m (Hartshorn and Peralta 1988). Mean annual temperature ranges from 25°C in the lowlands to 14°C in the highlands, while mean annual precipitation ranges from 4300 mm in the lowlands to 2200 mm in the highlands (data provided by the Tropical Ecology Assessment and Monitoring network [TEAM], Volcán Barba, Costa Rica; Blake and Loiselle 2000). The dry season lasts from January to April, and the wettest months are July and October–November.

The study was conducted from May to September 2011 and from December 2011 to April 2012. During the period of sampling, we collected data on abundances and morphological traits of flower resources and hummingbirds and on plant–hummingbird interactions across seven sampling periods per site, each lasting for ~10 days.

Flower abundance and traits

To estimate the abundance of floral resources in the forest understory, we established five transects of 100 ×

5 m at each study site, with transects being separated from one another by at least 50 m. Along these transects, open flowers of all plant species fitting the traditional ornithophilous syndrome (Faegri and van der Pijl 1979) were counted up to 10 m above the ground. Because hummingbird-pollinated flowers do 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 as well. Transect counts were done once during each sampling period, that is, we collected abundances of plant species blooming at different times of the year. We used the total number of flowers per plant species summed over all transects and sampling periods as an estimate of plant species-specific resource abundance for hummingbirds at each of the three elevations.

We measured the following plant morphological traits that have been reported to affect plant–hummingbird interactions: external diameter, length, and curvature of the corolla (Costigan 2008, Temeles et al. 2009). External diameter (maximum width at the opening of the corolla tube) and total corolla length (from the base to the corolla opening) were measured to the nearest 0.10 mm with a dial caliper. To measure corolla curvature, the flower was placed on graph paper so that the angle of deflection could be calculated using simple trigonometry (see Kershaw 2006). Corolla curvature was arcsine square-root transformed for statistical analysis (i.e., angular transformation). Using corolla length and external diameter, we additionally calculated corolla volume as $C_{VOL} = \text{corolla length } \pi (\text{external diameter}/2)^2$ for all plant species. Corolla volume can be considered as an integrated measure of flower size, and flower size has been found to be correlated with nectar production rates per flower (Ornelas et al. 2007). Corolla volume was log-transformed prior to statistical analyses. For all three morphological traits, we calculated mean values from 3–4 individual flowers for 133 plant species (mean values are given in the Appendix: Table A1). In addition to morphological traits, we measured nectar volume for a subset of the plant species ($n = 41$ species). We sampled nectar from unvisited flowers on inflorescences that had been bagged with nylon netting prior to anthesis. Nectar from 3–5 flowers of each plant individual was extracted with capillary tubes until no further nectar could be extracted. We sampled nectar from 3–15 individuals per plant species between 10:00 and 13:00 (approximately 24 h after flowers had been bagged), which approximates the daily nectar production of each flower.

Hummingbird abundance and traits

We placed 12–14 standard mist nets (12 × 3 m) at each study site for ~6 h after dawn (Ralph et al. 1993). Mist nets were operated four days in each sampling period. To calculate the sampling effort, one standard mist net operated for one hour was considered as a net hour.

Overall, our sampling effort was about 52 300 mist-net hours and was similar across the three study sites. We used the number of hummingbird individuals captured per species, summed across sampling periods, as an estimate of hummingbird abundance at each site. All hummingbirds captured were identified to species level (according to Stiles and Skutch 1989) and banded with aluminum numbered bands. To avoid overestimation of hummingbird abundance, we excluded recaptured hummingbird individuals from abundance estimates. We measured avian morphological traits that have been found to affect plant–hummingbird interactions, including bill length and curvature (Hainsworth and Wolf 1972, Temeles et al. 2009), as well as body mass (Temeles and Kress 2003, Dalsgaard et al. 2009); mean values are given in the Appendix: Table A2. For each captured individual, we measured bill length (exposed culmen) to the nearest 0.10 mm using a dial caliper. To measure bill curvature, we placed the bill on graph paper following the same procedure as for corolla curvature (Kershaw 2006). Bill curvature was arcsine square-root transformed for statistical analyses (i.e., angular transformation). We used a digital scale to the nearest 0.10 g to record body mass.

Network observations

To record the interactions between plant and hummingbird species in the understory, we carried out observations of flowering plants up to 10 m above the ground. Plant species were chosen following the same criteria as for transect counts of floral resources. 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 within 10 m from open flowers for periods of 120 min between 06:00 and 14:00. In most cases, all open flowers on a plant individual were videotaped together.

We recorded 1073 plant individuals and over 2000 hours of videotapes. Of the videotaped plants, 35% were visited by hummingbirds during the recording period. We could not identify 45 of the total hummingbird visitors (5%), which were excluded from further analysis. The parameters recorded from the videotapes were as follows: the number of flowers probed at each visit, the feeding time at each visit, and the contact with reproductive structures of the flower. A visit to a plant individual was recorded whenever an individual hummingbird was observed to probe at least one flower of the observed plant individual. We excluded all illegitimate visits in which the hummingbird did not access the flower through the corolla entrance. Since these illegitimate visits represented only 2.9% of the total visits, including these visits in the analyses did not modify the results. We used the interaction frequency as the currency in the networks, that is, the total number of

legitimate visits of each hummingbird species on each plant species.

We compiled one interaction matrix for each study site lumped across all sampling periods in order to assess hummingbird specialization across the entire study year. We were not interested in phenological differences in hummingbird specialization. In addition to the plant–hummingbird networks, we calculated the mean foraging time per flower as a measure of individual resource handling time (i.e., the cumulative foraging time on all individual flowers during a visit divided by the number of flowers probed at this visit). Foraging time on each flower was considered as the time it took a bird to insert its bill, lick up nectar, and withdraw its bill from a flower (Montgomerie 1984), excluding the transit time between flowers and the time that hummingbirds spent hovering around the flowers.

Statistical analyses

To investigate patterns of specialization in plant–hummingbird interactions, we determined specialization at the community level with the standardized two-dimensional Shannon entropy (specialization index H'_2) and at the species level with the conceptually related index d' (Blüthgen et al. 2006). The specialization index H'_2 quantifies the degree of niche divergence (i.e., niche complementarity) between flowering plant species and between hummingbird species in the interaction networks and thus estimates the degree of complementary specialization in a network. We used null models based on the Patefield algorithm (Patefield 1981), assuming random interactions between species constraining species' total frequencies, to assess whether network specialization H'_2 at each study site was higher than expected at random. To test for differences from randomness, we compared observed H'_2 values with those obtained from 10 000 permutations of randomized networks. The species-level network index d' is derived from the Kullback-Leibler distance and measures how strongly a pollinator species deviates from an expected random choice of available interaction partners (Blüthgen et al. 2006). To calculate d' , we derived expected interaction frequencies between hummingbirds and flowers according to the independent estimates of flower abundance at each study site. According to this concept of ecological specialization, a generalized species uses floral resources proportional to floral abundances, whereas a specialized species strongly deviates in its interaction pattern from the distribution of floral abundances (Blüthgen et al. 2006). Both indices, H'_2 and d' , range from 0 to 1 and have the advantage of being largely unaffected by the number of interacting species and by differences in sampling intensity (Blüthgen et al. 2006).

To assess whether avian morphological traits and abundance were associated with hummingbird specialization at the species level, we used (1) univariate linear models for bill length, curvature, body mass, and

abundance, and (2) a multivariate model including all traits and abundance as predictors of hummingbird specialization. We fitted linear mixed-effects models with traits and abundance as fixed effects and accounted for random variation among the three different networks from different elevations by including elevation as a random effect. In all models, d' values were weighted by total interaction frequencies of hummingbirds (square-root transformed) because the observed interaction links may be incomplete for species with very few observations. To explore whether the association between morphological traits of hummingbird species and specialization was context dependent (i.e., varied between elevations), we additionally fitted random-slope models that allowed trait-specialization relationships to vary between elevations (Zuur et al. 2009). We compared the fits between random-intercept and random-slope models for testing whether trait-specialization relationships varied among elevations; this would be the case if random-slope models were more supported than random-intercept models. For the model comparison, we used χ^2 distributed likelihood-ratio tests. In addition, we accounted for potential differences between hermit and non-hermit species and between genera by adding hermit/non-hermit and genus as nested random-intercept factors to the models. To compare models with and without these additional random effects, we used χ^2 distributed likelihood-ratio tests. We also tested whether morphological traits differed between hermit and non-hermit hummingbirds. To identify minimal adequate models from the full multivariate model, we fitted models with all possible combinations of the four predictor variables and chose the most parsimonious model according to the corrected Akaike information criterion (AIC_c).

To assess whether the degree of trait-matching affects interaction strength in the networks, we performed fourth-corner analyses on the interaction frequencies of bird and plant species in the three study sites (Fig. 1). We used the following combinations of corresponding hummingbird and plant traits that may influence the interaction patterns between species (Dalsgaard et al. 2009, Temeles et al. 2009): (1) bill–corolla length, (2) bill–corolla curvature, and (3) body mass–corolla volume. Prior to analysis, traits were standardized to zero mean and unit variance. The fourth-corner method was proposed to measure and test the relationships between species traits and environmental variables (Legendre et al. 1997, Dray and Legendre 2008). We adapted the method to detect significant associations in interaction strength between plant and hummingbird species with specific morphologies at each of the three elevations. Specifically, we tested whether interaction strength between pairs of species with high degrees of matching in the corresponding traits was higher than expected from a null model (for details see Dehling et al. 2014). Interaction strength was the relative frequency of a hummingbird species on a particular plant species

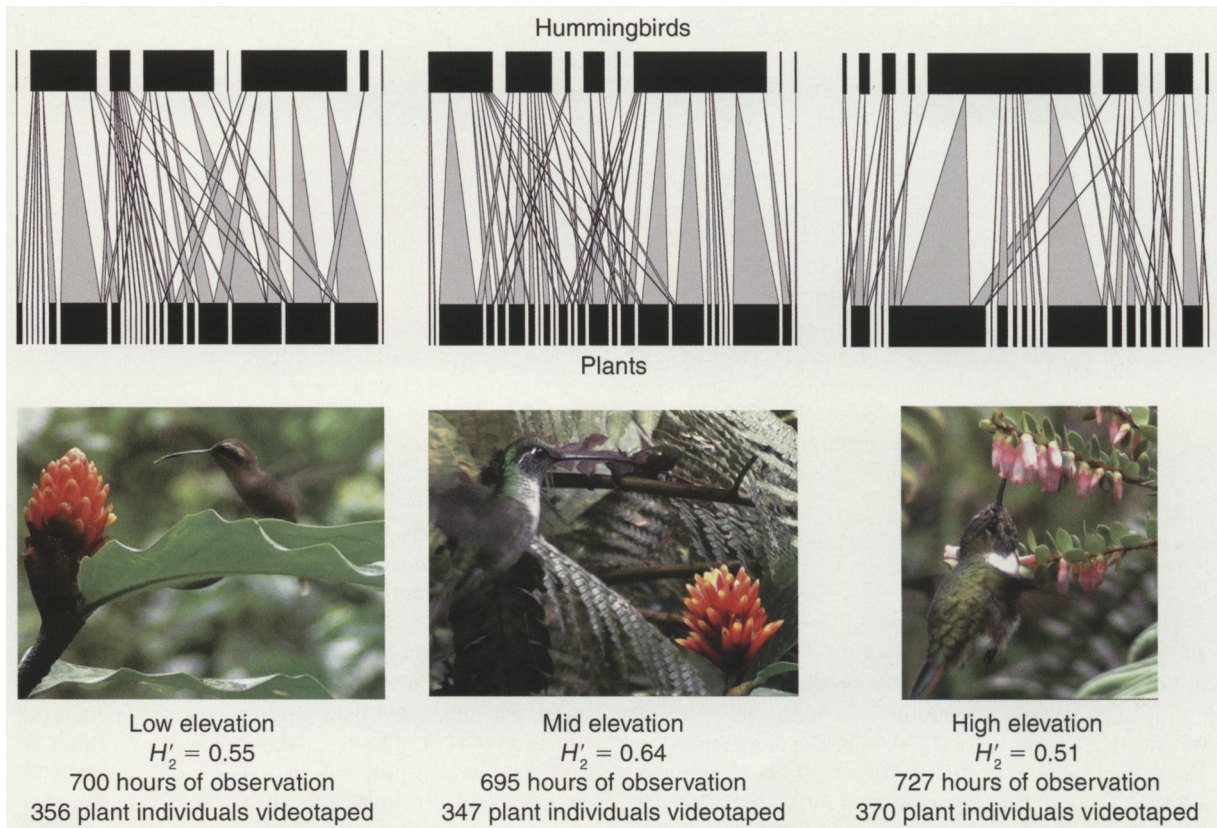


FIG. 1. Plant-hummingbird interaction networks in tropical forests at three elevations in Costa Rica. Hummingbird and plant species are indicated by black boxes at top and bottom, respectively. Box width corresponds to the proportion of interactions contributed by each species to the network. Links between species are indicated by lines that are proportional to interaction strength. Complementary specialization H'_2 (standardized two-dimensional Shannon entropy) is reported for each elevation. Examples of interactions between hummingbird and plant species are shown for each elevation: *Phaethornis striigularis* and *Lampornis hemileucus* interacting with *Renalmia cernua* (Zingiberaceae) at low and mid elevation, respectively; and *Selasphorus flammula* feeding on *Disterigma humboldtii* (Ericaceae).

(number of interactions between hummingbird species j and plant species i divided by the total number of visits of hummingbird species j to all plant species). We considered three tables: a matrix \mathbf{L} ($n \times p$) describing the interaction strength of p species of hummingbirds with n plant species, a second matrix \mathbf{R} ($n \times m$) with m trait values for the n plant species, and a third matrix \mathbf{Q} ($p \times s$) containing s trait values for the p species of hummingbirds. For testing the significance of the correlations between corresponding pairs of traits, we used a combination of permutation models 2 (i.e., permutation of entire rows in matrix \mathbf{L}) and 4 (i.e., permutation of entire columns in matrix \mathbf{L}) (Dray and Legendre 2008). From these models, we chose the larger of the two P values as suggested by Ter Braak et al. (2012), as the most conservative approach.

To analyze the influence of trait matching on the efficiency in hummingbirds' resource use, we used the trait distances of bill-corolla length and bill-corolla curvature as a measure of the degree of mismatching between pairs of traits. These distances were calculated

as absolute differences between mean values of corresponding pairs of morphological traits in plant and hummingbird species. We fitted linear mixed-effects models with handling time as the response variable and the degree of trait mismatching as the fixed effect, analyzing each trait combination separately. To account for differences in nectar production per flower among plant species, we included the mean nectar volume per flower as an additional fixed effect. We could not account for the actual standing crop (i.e., the amount of nectar present at the time of a visit), which was impossible to measure for all observed plant individuals. To account for additional random variation among sites, species, and plant individuals, we included the following random effects in these models: site, hummingbird species identity, and plant individual identity nested within plant species identity. In addition to the two univariate models, we fitted a multivariate model to simultaneously test the effect of mismatches in length and curvature on handling time. We selected a minimal

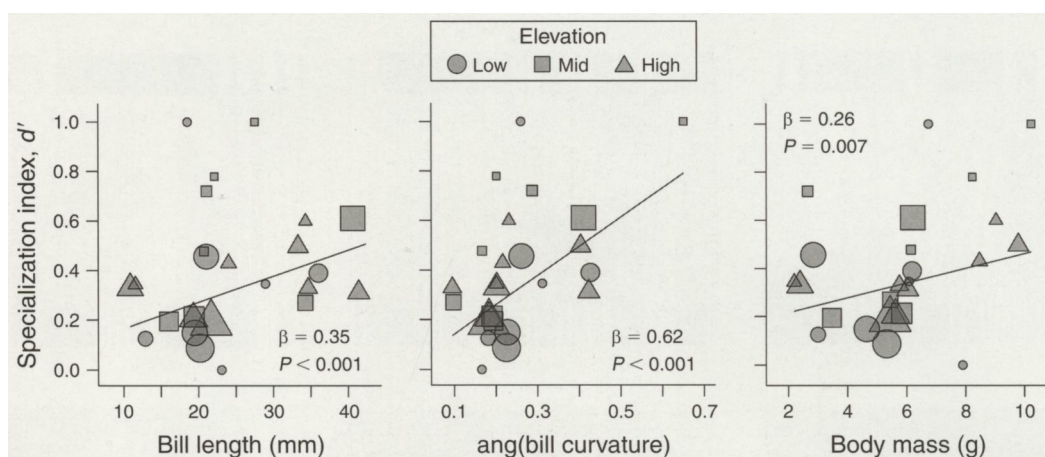


FIG. 2. Relationships between species-level network specialization, d' , and morphological traits for 20 hummingbird species from three hummingbird communities in Costa Rica at different elevations (five species occurred at two elevations). Coefficients of determination (R^2) were 0.22, 0.47, and 0.12 for single-predictor models including bill length, bill curvature, and body mass, respectively. Bill curvature was angular-transformed (ang; originally measured in degrees). Symbol size corresponds to weights by the number of interactions observed for each hummingbird species at the respective elevation.

adequate model among the three possible model combinations as described in the previous paragraph.

All statistical analyses were conducted with R statistical software version 3.0.0 (R Development Core Team 2013) and dedicated packages. We used marginal and conditional R^2 as goodness of fit statistics for all mixed-effects models (Nakagawa and Schielzeth 2013).

RESULTS

We captured a total of 762 individuals belonging to 28 hummingbird species at the three study sites (Appendix: Table A3). Across all study sites, we observed a total of 823 visits of hummingbirds to plant individuals between 65 flowering plant species and 20 hummingbird species. Networks at each elevation included 23×8 , 25×8 , and 20×9 (plant \times hummingbird species) at low, mid, and high elevation, respectively (see the Appendix: Tables A3 and A4 for complete species lists). Among the 20 hummingbird species, 5 were hermits and 15 were non-hermits. Non-hermit hummingbirds had shorter ($F_{1,23} = 6.5$, $\beta = -1.02$, $P = 0.018$) and less curved bills ($F_{1,23} = 24.9$, $\beta = -0.19$, $P < 0.001$) compared to hermits, whereas body mass did not differ ($F_{1,23} = 0.006$, $\beta = 0.04$, $P = 0.94$). Plant–hummingbird networks at all elevations were highly specialized (Fig. 1). In all networks, specialization H'_2 was higher than expected at random ($P < 0.001$ in all cases).

Species-level specialization (d') increased with bill length, bill curvature and body mass (Fig. 2). Hummingbird abundance did not affect specialization of hummingbird species ($t = -0.58$, $P = 0.57$). When we accounted for differences between hermits and non-hermits and the taxonomic relatedness among species, this improved the fit only for the body mass model (model with vs. model without taxonomy: $\chi^2 = 7.55$, $P = 0.023$), while the models with bill length ($\chi^2 = 1.16$, $P = 0.56$) and bill curvature ($\chi^2 < 0.1$, $P > 0.9$) were less

supported. Models that allowed for random slopes of trait-specialization relationships at each elevation were always less supported than random-intercept models ($\chi^2 < 0.1$, $P > 0.9$). The best fitting model, according to AIC_c , contained only bill curvature as predictor of specialization ($R^2 = 0.47$, $P < 0.001$; ΔAIC_c to all other models > 2), in multivariate models both with and without taxonomic effects.

Fourth-corner analyses revealed that interaction strength in the networks was associated with the degree of trait matching in corresponding morphological traits of hummingbirds and plants, especially at mid and high elevations (Fig. 3, Table 1). Interaction strength was stronger between plant and hummingbird species with a high degree of matching in bill–corolla length in all three communities. Interaction strength was associated with matches in body mass and corolla volume at mid and high elevation, and in bill and corolla curvature at mid elevation only.

The degree of mismatching between pairs of corresponding morphological traits increased resource handling time by hummingbirds, accounting for the differences in nectar volume among plant species. Together with nectar volume, mismatches between bill length and corolla length explained about 20% of the variation in bird's handling time, while mismatches between bill curvature and corolla curvature explained much less variation (Table 2). The best fitting model included nectar and mismatches between bill length and corolla length as predictors, while the second best model additionally included mismatches between bill curvature and corolla curvature (ΔAIC_c to the best model 1.87).

DISCUSSION

Our results show high levels of ecological specialization in plant–hummingbird networks at all three elevations. Morphological traits of hummingbird species

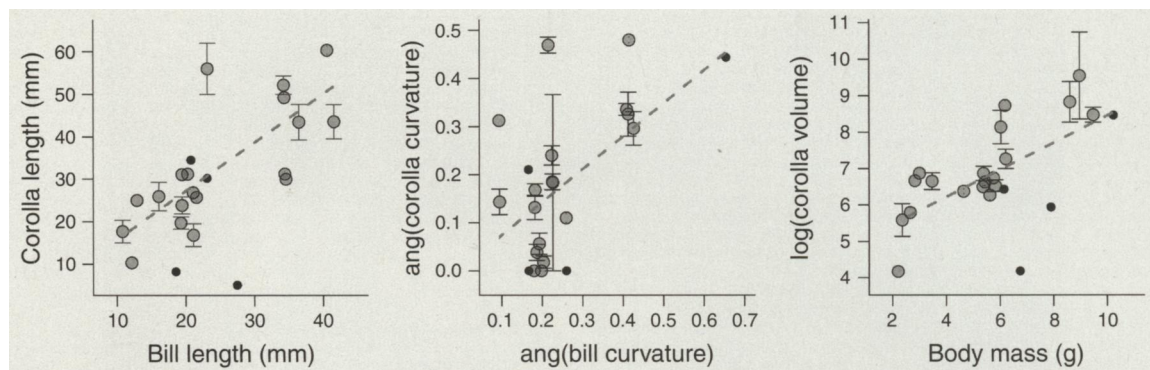


FIG. 3. Associations between morphologies of plant and hummingbird species in mutualistic networks at three study sites in Costa Rica. Each data point represents one hummingbird species at a given elevation ($n = 23$); five species occurred at two study sites. For each hummingbird species, we computed the corresponding mean floral trait value across all observed interactions at a given elevation. Shown are mean (\pm SE) floral trait values (length, curvature, and volume of corolla [originally measured in mm^3]) against the respective hummingbird trait value (bill length, bill curvature, and body mass). Bill curvature was angular-transformed (ang; originally measured in degrees). Black dots represent hummingbird species that were only observed once. We fitted a simple linear model with the respective mean values to indicate a trend line in each plot.

influenced patterns of ecological specialization and bill traits were more relevant than body mass in determining niche partitioning within the community. Interaction strength in the networks was stronger between plant and hummingbird species with close matches in their corresponding morphological traits. Trait matching was associated with a decreased handling time of nectar resources by hummingbirds. These findings indicate the high sensitivity of quantitative network analyses for detecting trait associations in mutualistic plant–animal systems.

Network specialization

Recent network analyses showed moderate specialization in most plant–pollinator associations (Blüthgen et al. 2007, Schleuning et al. 2012). We found that complementary specialization (H'_2) of plant–hummingbird networks was high compared to the specialization reported for 25 tropical pollination networks in a previous meta-analysis ($H'_2 = 0.43 \pm 0.03$ [mean \pm

SE]; Schleuning et al. 2012). The high degree of specialization in the plant–hummingbird networks was consistent across elevations ($H'_2 > 0.5$ in all three elevations). All networks were more specialized than one would expect in a randomly interacting community. These findings suggest a pronounced flower partitioning among hummingbird species, which is consistent with previous research (e.g., Dalsgaard et al. 2011). In specialized systems, consumer species tend to be adapted to use their resources effectively (Blüthgen et al. 2007). The use of a subset of available floral resources by a given species may increase the resource use efficiency and/or reduce interspecific competition (Linhart 1973, Stiles 1981). Since hovering flight implies a high metabolic cost for hummingbird species (Suarez 1998), ecological specialization could reduce the foraging costs of non-perching hummingbird species because of less

TABLE 1. Statistics of the fourth-corner analyses for corresponding pairs of traits in plant–hummingbird networks at three elevations in Costa Rica.

Trait combination	Elevation					
	Low		Mid		High	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Bill–corolla length	0.491	0.040	0.573	0.045	0.599	0.002
Bill–corolla curvature	0.295	0.141	0.661	0.025	0.346	0.130
Body mass–corolla volume	0.007	0.808	0.542	0.040	0.642	0.015

Notes: Given are correlation coefficients and their respective *P* values from permutation tests. To test whether the degree of trait-matching was significantly associated with the interaction strength between plant and hummingbird species, we used two different permutation tests and report the larger of the two *P* values (see *Methods* for details). Significant associations are shown in boldface type.

TABLE 2. Linear mixed-effects models testing the relationship between resource handling time by hummingbirds and the degree of mismatch between morphological bird and plant traits, accounting for differences in nectar volume in both models.

Predictor	β	<i>t</i>	<i>P</i>	R^2	
				marginal	conditional
Δ bill–corolla length	0.25	3.35	<0.001	0.20	0.60
Nectar volume	0.32	2.55	0.011
Δ bill–corolla curvature	0.14	1.23	0.221	0.17	0.63
Nectar volume	0.39	2.58	0.010

Notes: Pairs of corresponding traits were bill–corolla length and bill–corolla curvature, and mismatches were defined as absolute differences between the mean values of the corresponding pairs of morphological traits. Observations included 557 individual plant–hummingbird interactions. Marginal and conditional R^2 are shown as goodness of fit statistic for each of the two models (not for the respective predictor variable, as indicated with ellipses).



PLATE 1. Hummingbirds (Trochilidae) are important pollinators in Neotropical forests and differ widely in bill morphology: (A) *Threnetes ruckeri*, (B) *Phaethornis longirostris*, (C) *Eutoxeres aquila*, (D) *Selasphorus scintilla*, (E) *Campylopterus hemileucurus* and (F) *Doryfera ludovicae*; images (A–C) correspond to hermit and (D–F) to non-hermit hummingbirds. Photo credit: M. A. Maglianesi.

interference competition with other species (see Feinsinger 1976).

Species-level specialization

Our findings support the idea that certain morphological traits of hummingbirds contribute to their specialization on specific plant resources. Bill traits had a stronger effect on specialization than body mass. Long-billed and curved-billed hummingbird species were particularly specialized, i.e., deviated strongly from a random interaction pattern that would be driven by the abundances of floral resources at a given elevation. Hence, species with long and curved bills were more specific in their resource choice and foraged preferably on relatively rare, but rewarding resources, whereas species with short and uncurved bills foraged mostly on the most abundant plant resources, and thus, followed a more random interaction pattern. Bill morphology in hummingbirds has long been known to be associated with the efficiency of resource use (Wolf et al. 1972, Temeles et al. 2009) and has been proposed to determine interaction patterns in plant–hummingbird assemblages (Feinsinger 1976, Brown and Bowers 1985). Hummingbird species with strongly curved bills reach nectar from curved flowers that straight-billed species are not able to access or only access with greater difficulty. Hence, interspecific competition for curved-billed hummingbird species is likely to be reduced (Linhart 1973, Stiles 1981). For instance, the long, curved bills of most hermit species enable them to reach nectar from flowers that short and uncurved billed species are not as easily able to access. Correspondingly, our results indicate that bill morphology, in particular bill curvature, influence resource use and niche partitioning in hummingbird assemblages, which is likely to reduce competition for floral resources.

Body mass has also been associated with the foraging efficiency for nectar in hummingbirds (Hainsworth and Wolf 1972). However, this morphological trait is rather associated with the dominance relationships among co-occurring hummingbirds than to foraging efficiency and flower choice (Feinsinger 1976, Altshuler 2006). This may explain why body mass had a weaker influence on ecological specialization than bill morphology and suggests that niche partitioning of floral resources within hummingbird assemblages is mostly determined by variability in bill morphology.

Trait matching and foraging efficiency

We found that interaction strength in the networks was associated with the degree of trait matching in corresponding morphological traits of hummingbird and plant species. First, large hummingbird species preferred to feed on large flowers at mid and high elevations, which may be related to a high nectar production of these flowers (Rodríguez and Stiles 2005, Ornelas et al. 2007). This finding suggests that high energy requirements in the harsh environmental conditions at higher elevations may require large-bodied hummingbird species to specialize on floral resources with large nectar crops. Second, long-billed and curve-billed hummingbird species preferred plant species with long and curved flowers, respectively, indicating high degrees of trait complementarity between bill and corolla shape. The findings revealed by the novel and fully quantitative approach of fourth-corner analysis of interaction matrices (Dehling et al. 2014) is thus consistent with earlier studies showing high degrees of trait matching between hummingbirds and their foraging plants (Snow and Snow 1980, Dalsgaard et al. 2009, Temeles et al. 2009).

Our results reveal that hummingbirds spent more foraging time not only on flowers with high nectar

volume, but also on flowers that did not match well with their bill morphology. This is consistent with optimal foraging theory (Krebs and Davies 1993), which predicts that high trait matching should lead to an increased efficiency of resource use, reflected in shorter handling times (Temeles 1996). Hence, a reduced cost in resource handling makes long-billed hummingbirds more efficient feeders on flowers with extended corollas. Consequently, a plant species that offers a greater profitability for pollinators, either through a high reward or a high efficiency in resource use, will be visited more frequently. The relationship between resource handling efficiency and trait matching is a plausible explanation for the close association between interaction strength and trait matching found in all networks. Network analyses combining trait and interaction data with a novel combination of analytical approaches may also be valuable for studying linkages between phenotypic and ecological specialization in other types of ecological networks.

Conclusions

Consistent with previous studies, we show that morphological traits, particularly avian bill morphology, shape the specialization of hummingbird species in plant–hummingbird networks. We present evidence that the close morphological matches between interacting plant and hummingbird species contribute to a high efficiency in hummingbirds' resource use. Similar mechanisms of trait complementarity between interacting species, associated with a high efficiency in resource use, may also structure many other types of ecological networks. We conclude that network analysis of specialization and trait complementarity represents a powerful methodological approach that is likely to contribute to a better mechanistic understanding of the evolutionary and ecological causes of specialization in highly diversified species assemblages.

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SUPPLEMENTAL MATERIAL

Ecological Archives

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