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The influence of biogeographical and evolutionary histories on morphological traitmatching and resource specialization in mutualistic hummingbirdplant networks

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6 **Article title:** The influence of biogeographical and evolutionary histories on morphological trait-

7 matching and resource specialization in mutualistic hummingbird-plant networks

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128 AMMG collected the data; BD, PKM, KH, TBZ, JVB and AMMG compiled and checked the

129 database; JS (with help from BD, PKM, JDK, MSc) analysed the data; BD wrote the first draft of

130   the manuscript with inputs from PKM, JS, KH, BIS, MSc, AMMG. All authors contributed to the

131   manuscript and gave final approval for publication.

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133   **Data availability statement:** Upon the acceptance of the manuscript, all raw quantitative

134   hummingbird-plant networks and complementary morphological trait data supporting the results

135   will be archived in DRYAD and the data DOI will be included in the reference list.

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Abstract

1. Functional traits can determine pairwise species interactions, such as those between plants and pollinators. However, the effects of biogeography and evolutionary history on trait-matching and trait-mediated resource specialization remain poorly understood.

2. We compiled a database of 93 mutualistic hummingbird-plant networks (including 181 hummingbird and 1,256 plant species), complemented by morphological measures of hummingbird bill and floral corolla length. We divided the hummingbirds into their principal clades and used knowledge on hummingbird biogeography to divide the networks into four biogeographical regions: Lowland South America, Andes, North & Central America, and the Caribbean islands. We then tested: (i) whether hummingbird clades and biogeographical regions differ in hummingbird bill length, corolla length of visited flowers and resource specialization, and (ii) whether hummingbirds' bill length correlates with the corolla length of visited food plants and with their level of resource specialization.

3. Hummingbird clades dominated by long-billed species generally visited longer flowers and were the most exclusive in their resource use. Bill and corolla length and the degree of resource specialization were similar across mainland regions, but the Caribbean islands had shorter flowers and hummingbirds with more generalized interaction niches. Bill and corolla length correlated in all regions and most clades, i.e. trait-matching was a recurrent phenomenon in hummingbird-plant associations. In contrast, bill length did not generally mediate resource specialization, as bill length was only weakly correlated with resource specialization within one hummingbird clade (Brilliants) and in the regions of Lowland South America and the Andes in which plants and hummingbirds have a long co-evolutionary history.

4. These results demonstrate how biogeographical and evolutionary histories can modulate the effects of functional traits on species interactions, and that traits better predict functional groups

161   of interaction partners (i.e. trait-matching) than resource specialization. These findings reveal

162   that functional traits have great potential, but also key limitations, as a tool for developing more

163   mechanistic approaches in community ecology.

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168   **Keywords:** biogeography, island ecology, niche-partitioning, plant-animal interactions, resource

169   specialization, species traits, specificity, trait-matching

Introduction

Species do not live and evolve in isolation, but are entangled within networks of interactions with other species (Bascompte & Jordano, 2007). As species' interactions play a key role in species coexistence and speciation (Phillips et al., 2020), it is important to understand when and why co-occurring species interact and specialize on each other. Recently, there is growing interest in the role of functional traits in determining pairwise interactions between species (McGill, 2006; Maruyama et al., 2018; Pigot et al., 2020; Schleuning et al., 2020; Sonne et al., 2020). If two co-occurring species have matching traits, the probability and efficiency of their interaction should increase (Eklöf et al., 2013; Maglianesi et al., 2014). Otherwise, mismatches in traits could render interactions inefficient or even impose barriers to interactions, resulting in so-called 'forbidden links' (Jordano et al., 2003). In other words, species' traits determine whether and how frequently co-occurring species interact and so species' traits should match and influence resource specialization (Maglianesi et al., 2014; Klumpers et al., 2019).

Mutualistic systems of plant-pollinator interactions contain classic examples of trait-matching that have been highlighted as textbook examples of coevolution (e.g. Thompson, 2005). For instance, the length of a moth's proboscis, or the length of a hummingbird's bill, often match the corolla length of their food plants (Nilsson, 1988; Temeles & Kress, 2003). Trait-matching and floral specificity may be energetically advantageous for the pollinators and increase pollination efficiency (Montgomerie et al., 1984). Accordingly, both trait-matching and trait-mediated resource specialization have been reported for local plant-pollinator interaction networks (Stang et al., 2009; Maglianesi et al., 2014; Vizentin-Bugoni et al., 2014; Weinstein & Graham, 2017; Klumpers et al., 2019). However, drawing general conclusions in community ecology requires comparative studies of local communities across biogeographic regions (Lessard et al., 2012), and biogeographical history has been suggested to influence the role traits play in mediating plant-pollinator interactions

194 (Dalsgaard et al., 2018). Notably, theory predicts that biogeographical regions where species have

195 co-occurred for longer should contain species with more specialized associations and greater trait

196 matching (Dalsgaard et al., 2011; Sonne et al., 2016, 2020). At the other extreme, pollinators on

197 oceanic islands are predicted to have generalized feeding behaviours, probably because it is

198 advantageous to be a generalist to colonize and establish on islands (Olesen et al., 2002). Moreover,

199 as oceanic islands have an impoverished insect pollinator fauna (Olesen & Jordano, 2002),

200 evolutionary processes may drive island pollinators, especially vertebrates, to evolve novel and

201 generalized feeding niches (Olesen et al., 2002; Olesen & Valido, 2003; Traveset et al., 2015).

202 Taken together, theory suggests that biogeographical history may influence trait-matching and how

203 well traits predict resource specialization in plant-pollinator networks, but large-scale analyses

204 across biogeographical regions are rare (Dalsgaard et al., 2018; Sonne et al., 2020).

205   In addition to biogeographical history, if trait-matching and trait-mediated resource

206 specialization are invariant properties of pollination networks, they should manifest repeatedly

207 among distinctly related groups within a pollination system. For example, the mutualistic

208 association between hummingbirds and their nectar-food plants is the most specialized avian

209 pollination system (Fleming & Muchhala, 2008; Zanata et al., 2017), largely manifested in the

210 match between the length of hummingbird bills and the length of the flowers they feed on

211 (Feinsinger & Colwell, 1978; Stiles, 1981; Cotton, 1998; Dalsgaard et al., 2009; Maruyama et al.,

212 2014; Vizentin-Bugoni et al., 2014; Weinstein & Graham, 2017; Sonne et al., 2020). However,

213 hummingbirds consist of nine evolutionary distinct clades (McGuire et al., 2014), which differ

214 greatly in their bill morphology and floral preferences (Feinsinger & Colwell, 1978; Bleiweiss,

215 1988). Thus, if trait-matching is universal for hummingbird-plant associations, bill length and

216 corolla length of visited flowers should co-vary between hummingbird clades. In other words,

217 hummingbird clades consisting of long-billed species should prefer flowers with long corollas and

218 *vice versa* for clades with shorter bills. Moreover, bill length should correlate with corolla length

219 both across all hummingbird species and across the species within each hummingbird clade.

220 Likewise, if bill length predicts resource specialization (Maglianesi et al., 2014), then bill length

221 should co-vary with resource specialization both between and within hummingbird clades.

222 However, although evolutionary relatedness is known to structure plant-pollinator interaction

223 networks (Rezende et al., 2007; Martín González et al., 2015), the role of evolutionary history in

224 influencing trait-matching and trait-mediated resource specialization remains poorly understood.

225   To examine whether evolutionary and biogeographical histories influence the

226 generality of trait-matching and trait-mediated resource specialization in assemblages of plants and

227 pollinators, we compiled a database of 93 quantitative hummingbird-plant networks distributed

228 widely across continental America and the Caribbean islands. Each network represents the

229 mutualistic interactions occurring within local assemblages of hummingbirds and their food plants

230 (Dalsgaard et al., 2011), for which we gathered data on hummingbird bill length and the effective

231 floral corolla length (*sensu* Wolf et al., 1976). To test the generality of trait-matching and trait-

232 mediated resource specialization across evolutionary and biogeographical histories, we divided the

233 hummingbirds into their nine principal clades and used knowledge on hummingbird biogeography

234 to divide the networks into four biogeographical regions: Lowland South America, Andes, North &

235 Central America, and the Caribbean islands (McGuire et al., 2014). We used this unique set of

236 hummingbird-plant networks and trait data to test: (*i*) whether hummingbird clades and

237 biogeographical regions differ in hummingbird bill length, corolla length of visited flowers and

238 resource specialization, and (*ii*) whether hummingbirds' bill length correlates with the corolla

239 length of their food plants and with their level of resource specialization, which we examined both

240 for the entire dataset and within each hummingbird clade and biogeographical region.

241

Materials and methods

Datasets: hummingbird-plant networks and traits

We compiled a dataset consisting of 93 quantitative hummingbird-plant interaction networks distributed widely across the Americas. The networks were sampled to represent all hummingbird clades and hummingbird-visited plant families without any taxonomic bias. Each of the 93 networks describes interaction frequencies within assemblages of hummingbirds and their food plants in a specific location. We only included mutualistic interactions in which a given hummingbird was observed drinking nectar and touching the stigma / anthers of the given flower, thereby potentially acting as a pollinator (see Appendix 1). Species names of the hummingbirds follow the International Ornithological Committee World List (IOC version 9.2; www.worldbirdnames.org; Appendix 2). Hummingbirds were divided into nine clades following McGuire et al. (2014). Species names and families of the plants follow 'The Plant List' (TPL version 1.1; www.theplantlist.org), with a few exceptions where species names of recorded plants were not found in TPL (specified in Appendix 3). The networks can be downloaded from DRYAD [Link to be inserted upon acceptance of the manuscript].

We compiled two trait datasets: one for all hummingbird species and one for all plant species observed in the 93 networks (Appendix 2 and 3). In total, these contained 181 hummingbird species and 1,256 plant species for which we gathered data on hummingbird bill length and the effective floral corolla length (*sensu* Wolf et al., 1976). Data on hummingbird bill length was based on museum specimens, most averaged across five males and five females (specified in Appendix 2). In total, we obtained bill length estimates for 180 of the 181 hummingbird species (99% of the species; Appendix 2). Data on floral corolla length was based on measurements in the field. Apart from a few cases (~1% of the species), data on floral corolla length was collected at the same locality as the network. For plant species present in more than one network, if data on floral corolla

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length was collected in several localities (~19% of the species), we calculated species averages

across localities. In total, we obtained floral corolla length estimates for 962 plant species (76% of

the species; Appendix 3). As a supplement to bill and corolla length, we attempted to gather data on

bill and corolla curvature, as these traits may also match and the combination of bill length and

curvature may better predict resource specialization than bill length alone (Maglianesi et al., 2014;

Sonne et al., 2019). We were only able to gather a comprehensive dataset for bill curvature (99% of

the species; Appendix 2), which we used in supplementary analyses to validate our main focus on

bill length. The trait data can be downloaded from DRYAD [Link to be inserted upon acceptance of

the manuscript].

Measuring resource specialization

For each hummingbird species within the 93 hummingbird-plant networks, we calculated resource

specialization using two metrics, one reflecting niche overlap and one reflecting niche breadth. As a

measure of niche overlap, we used the complementary specialization index d' (Blüthgen et al.,

2006). We used this index as it is robust to variation in sampling effort, more than other measures of

resource specialization (Fründ et al., 2016). The index derives from Shannon's entropy and

quantifies the extent to which observed interaction frequencies differ from random encounter

probabilities of species, as derived from species' total interaction frequencies (Blüthgen et al.,

2006). This follows the assumption that if species specialize on specific interaction partners, these

preferences should be captured as deviations from random encounters given by partner availability

(Blüthgen et al., 2006). The index d' ranges between 0 and 1 for extreme generalization and extreme

specialization, respectively (Blüthgen et al., 2006). As a measure of hummingbird niche breadth, we

used the proportional generality index. The proportional generality index is a quantitative version of

proportional resource use (normalised degree in binary networks), making it suitable for

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290   comparisons between networks (Cusser et al., 2019). A proportional generality value at or close to

291   zero indicates a narrow niche breadth (i.e. a specialized species), whereas higher values indicate a

292   broader niche breadth. Note that the proportional generality index may be larger than one. For each

293   hummingbird species in each network, we calculated species-level specialization d' and

294   proportional generality using the *bipartite* package in R (Dormann et al., 2008).

295

296   *Biogeographical regions*

297   The datasets were separated into four major biogeographical regions: Lowland South America,

298   Andes, North & Central America, and the Caribbean (Fig. 1). This separation is based on the

299   division of hummingbirds into biogeographical regions, as extant hummingbirds supposedly

300   originated in lowland South America ~ 22 million years ago, then expanded into the Andes (~ 16

301   million years ago) and north of the Isthmus of Panama (~ 12 million years ago), arriving in the

302   Caribbean region more recently ~ 5 million years ago (McGuire et al., 2014). Our 'Lowland South

303   America' region includes all networks south of the Isthmus of Panama, excluding networks located

304   in the Andean mountains. The 'Andes' region includes all networks within the Andean mountains

305   as defined by Rahbek et al. (2019). The 'North & Central America' region includes all networks

306   located on the mainland north of the Isthmus of Panama. Finally, the 'Caribbean' region includes all

307   networks located on oceanic islands in the Caribbean Basin, excluding the continental island of

308   Trinidad located south of Bond's line, which for biogeographical reasons was included in the

309   'Lowland South America' region (Carstensen et al., 2013). The 93 hummingbird-plant interaction

310   networks were distributed as follows: 41 in Lowland South America, 22 in North & Central

311   America, 21 in the Andes, and nine in the Caribbean (Fig. 1). We observed most hummingbird

312   species in the Andean networks (76 species), followed by Lowland South America (55 species),

313   North & Central America (53 species) and the Caribbean island networks (12 species). With respect

314   to hummingbird-visited plant species, we recorded 641 species in Lowland South America, 367

315   species in the Andes, 233 species in North & Central America, and 65 species in the Caribbean.

316

317   *Statistical analyses*

318   First, we explored whether the hummingbird clades and plant families were distributed randomly

319   across the four biogeographical regions. To examine whether differences in hummingbird clade and

320   plant family distribution were statistically different between the four biogeographical regions, we

321   used Fisher's Exact Test followed by False Discovery Rate (FDR) adjusted pairwise comparisons

322   between all regions for both hummingbird clades and plant families. Significance levels were

323   calculated with the function 'fisher.test' in R (R Development Core Team, 2016) using Monte Carlo

324   simulations with 10,000 replicates. For plants, to focus on the main families, only the five most

325   frequent families in each region were chosen, which across the four regions gave a total of 11

326   families (plus the category 'others' including the rest of the plant families).

327   Second, we used one-way ANOVA tests followed by FDR adjusted multiple comparisons to

328   examine whether functional traits (hummingbird bill length, floral corolla length) and hummingbird

329   resource specialization (complementary specialization d', proportional generality) varied between

330   biogeographical regions and hummingbird clades. When performing the one-way ANOVAs

331   between hummingbird clades, the comparison was based on all observed species within each clade,

332   irrespective of regional affinities. We only used the seven most species-rich clades in our dataset,

333   excluding the species-poor clades Topazes (three species) and *Patagona* (one species). When

334   performing the one-way ANOVAs between biogeographical regions, the comparison was based on

335   all species observed within each region, i.e. we allowed species to be affiliated with multiple

336   biogeographical regions (only 13 hummingbird species, i.e. ~7%, occurred in two regions; four

337   species, i.e. ~2%, in three regions, and no species occurred in four regions). For all analyses, we

338 log-transformed bill and corolla length. Supplementary one-way ANOVAs showed that bill

339 curvature varied significantly between hummingbird clades and regions (for details, see Fig. S3 in

340 Supporting Information), but there was no indication that this caused clade and regional differences

341 in resource specialization (compare Figs. 2-3 with Fig. S3). The ANOVA tests were run using the

342 'anova' function in R. We also constructed linear-mixed effect models (LMMs) using the *nlme*

343 package in R (Pinheiro et al., 2019), with the aim to control for clades as a random intercept (when

344 examining how regions differ in traits and specialization level) and regions as a random intercept

345 (when examining how clades differ in traits and specialization level). However, these models did

346 not converge due to singularities (see Bates et al., 2015), likely caused by hummingbird clades

347 being non-randomly distributed across regions (see the Results of Fisher's Exact Test).

348   Third, we investigated whether the length of hummingbird bills was associated

349 positively with: (a) the mean corolla length of the flowers they visit, and (b) their degree of resource

350 specialization (complementary specialization d', proportional generality) averaged across networks.

351 We tested these potential associations with linear mixed-effects models. For each response variable,

352 we built two sets of models both with bill length as a fixed effect: one considering hummingbird

353 clades and another considering biogeographical regions as random intercepts because models

354 including both random effects simultaneously did not converge. When using clades as a random

355 factor, mean corolla length and resource specialization were estimated for each species irrespective

356 of regional affinity. When using regions as random factor, mean corolla length and resource

357 specialization were estimated for each species within each region. For each of these analyses, we

358 constructed both unweighted and weighted models, the latter weighted with the square root number

359 of plants visited (when predicting mean corolla length) and the square root number of networks

360 (when predicting mean resource specialization; Maglianesi et al., 2014). The weighting procedure

361 gave higher weight to hummingbird species that visited more plants and occurred in more networks,

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362 respectively. We used this weighting procedure as estimates of mean corolla length and mean

363 resource specialization may be more reliable when based on larger sample sizes, however, it may

364 also bias the results towards frequent and geographically widespread species. Thus both weighted

365 and unweighted LMMs were constructed. To estimate the significance of bill length in the LMMs,

366 we used the function 'anova' from the *car* package in R (Fox & Weisberg, 2019). We estimated the

367 proportion of variance explained by bill length in the LMMs as marginal R^2, and the proportion of

368 variance explained by both fixed and random factors as conditional R^2 with the function

369 'r.squaredGLMM' in the MuMIn package (Nakagawa et al., 2017; Barton, 2020).

370 In addition to the LMMs, we estimated Pagel's lambda λ to assess how well evolutionary

371 relatedness explains the distribution of bill length, mean corolla length and resource specialization

372 among hummingbird species (Pagel, 1999). Subsequently, to account for the non-independence of

373 the species-level data in our regression analyses, we repeated these using phylogenetic least squares

374 regression (PGLS) with the function 'pgls' in the package *caper* (Orme et al., 2018). In these

375 analyses, we derived the expected co-variances among all species using the maximum clade

376 credibility tree of McGuire et al. (2014). To reconcile our trait dataset with the McGuire et al.

377 (2014) phylogeny we: (1) excluded species included in our networks but not sampled in the tree, (2)

378 dropped species that McGuire et al. (2014) demonstrated to be paraphyletic or polyphyletic with

379 respect to other taxa included in our networks, and (3) used the mean bill / corolla length and

380 specialization value for species pairs that represent recent taxonomic splits only represented by a

381 single tip in the McGuire et al. (2014) phylogeny. These amendments reduced our dataset to 155

382 species (bill vs. corolla length) and 158 species (bill vs. specialization) in the PGLS regressions. As

383 additional analyses, we repeated the unweighted / weighted and PGLS regressions separately for

384 each of the seven hummingbird clades (excluding Topazes and *Patagona* with only three and one

385 species, respectively) and for each of the four biogeographical regions. Finally, we constructed

386   supplementary LMM and PGLS regressions to examine whether bill curvature provides additional

387   explanatory power in predicting resource specialization. These analyses showed negligible effects

388   of bill curvature (for details, see Table S1).

389   **Results**

390   *Hummingbird clade and plant family distribution across regions*

391   There was a clear difference in the distribution of hummingbird clades between the four regions (P

392   < 0.05; Fig. 1). Notably, the Brilliants and Coquettes dominated the Andean networks and appeared

393   in very low numbers outside of the Andes. In the other three regions, Emeralds were the most

394   species-rich group, along with Hermits (Lowland South America), Mountain Gems and Bees (North

395   & Central America) and Mangoes (Caribbean). Likewise, there were clear differences in plant

396   family distribution between the four regions ($P < 0.05$; Fig. 1). The Ericaceae dominated the

397   Andean networks, and were well represented in North & Central America, but had few species in

398   the Lowland South American and the Caribbean networks. The Bromeliaceae dominated in the

399   Lowland South American networks, and were well represented in the Andean and North & Central

400   American networks, but were poorly represented in the Caribbean. The Caribbean networks were

401   dominated by the Rubiaceae, which were also well represented in the other regions (Fig. 1).

402

403   *Comparing traits and resource specialization between clades and between regions*

404   The hummingbird clades differed significantly in bill length and the length of the flowers visited

405   (bill length: $F_{6, 165} = 25.29$, $P < 0.001$; corolla length: $F_{6, 165} = 12.64$, $P < 0.001$; Fig. 2a). The

406   Hermits and Mountain Gems had the longest bills, although the bill length of Mountain Gems was

407   not significantly longer than those of Mangoes and Brilliants. The shortest bills were those of the

408   Coquettes and Bees, whereas Emeralds had bills of intermediate length, only overlapping with those

409   of Mangoes. The Hermits and Mountain Gems visited the longest flowers, although the flowers

410   visited by Mountain Gems were not significantly longer than those visited by Brilliants and

411   Mangoes. The Coquettes and Bees visited the shortest flowers, although the Coquettes' flowers

412   were not significantly shorter than those visited by Emeralds and Mangoes (Fig. 2a). Hummingbird

413   resource specialization d' largely differed among clades in accordance with differences in bill /

414   corolla length: the Hermits and Mountain Gems were the most specialized clades (i.e., species in

415   these clades were most exclusive in their resource use), significantly more specialized than the Bees

416   and Emeralds, with intermediate levels of specialization shown by Mangoes, Brilliants and

417   Coquettes ($F_{6, 169} = 4.29$, $P < 0.001$; Fig. 2b). The hummingbird clades showed similar level of

418   proportional generality, i.e. niche breadth did not differ significantly between clades ($F_{6, 169} = 1.19$,

419    $P = 0.32$; Fig. 2c).

420   Hummingbird bill length was similar across regions ($F_{3, 191} = 1.15$, $P = 0.33$), but there

421   were regional differences in the corolla length of hummingbird-visited flowers ($F_{3, 575} = 10.15$, $P <$

422   0.001): North & Central American flowers were the longest, whereas the Caribbean flowers were

423   significantly shorter than flowers in all mainland regions (Fig. 3a). There were also regional

424   differences in hummingbird resource specialization, both when measured as d' ($F_{3, 192} = 2.82$, $P <$

425   0.05) and proportional generality ($F_{3, 192} = 13.35$, $P < 0.05$). Notably, the Caribbean hummingbirds

426   were more generalized than mainland hummingbirds, both in terms of niche overlap d' and

427   proportional generality (Fig. 3b-c).

428

429   *Associations between traits and between traits and resource specialization*

430   In LMMs across all hummingbird species, when including region as a random factor, bill length

431   was strongly and positively associated with mean corolla length (Table 1; Fig. 4b), weakly related

432 to mean resource specialization when measured as d' (Table 1; Fig. 4d), but unrelated to mean

433 proportional generality (Table 1; Fig. 4f). When including clade as a random factor, hummingbird

434 bill length was also strongly positively associated with mean corolla length (Table 1; Fig. 4a), but

435 bill length was not associated with neither measure of resource specialization (Table 1; Fig. 4c, Fig.

436 4e). The species-level variation in bill length, mean corolla length of visited flowers, and both

437 measures of resource specialization displayed a phylogenetic signal that was significantly greater

438 than zero ($P < 0.001$ in all cases). In particular, closely related hummingbird species are likely to be

439 similar in bill length ($\lambda = 0.97$), more so than the corolla length of visited flowers ($\lambda = 0.59$), with

440 weaker phylogenetic signals for resource specialization: proportional generality ($\lambda = 0.51$) and d' (λ

441 $= 0.23$). When accounting for this phylogenetic non-independence using PGLS, we continued to

442 detect a strong positive association between bill length and mean floral corolla length ($\beta = 0.83$, R^2

443 $= 0.32$, $P < 0.001$), but there was no association between bill length and mean resource

444 specialization, both measured as d' ($\beta = 0.05$, $R^2 = 0.00$, $P = 0.70$) and proportional generality ($\beta =$

445 0.01, $R^2 = 0.00$, $P = 0.94$).

446   Analysing the individual hummingbird clades separately showed that most clades had

447 a moderate to strong positive association between bill length and mean corolla length, although

448 relationships for some clades were weak and non-significant (Fig. S1a). Only Brilliants showed a

449 weakly positive association between bill length and mean resource specialization d' (Fig. S1b). For

450 all other clades, there were no significant associations between bill length and both measures of

451 resource specialization (Fig. S1b).

452   For all four regions analysed separately, there was a strong positive association

453 between hummingbird bill length and the mean corolla length of their flowers (Fig. S2a). The

454 association between bill length and resource specialization d' was non-significant for all four

455 regions when using unweighted regressions and PGLS, but weakly positive for Andes and Lowland

456   South America when using weighted regressions (Fig. S2b). For all regions, there was no

457   association between bill length and proportional generality, i.e. niche breadth (all fits had $P > 0.05$).

458

459   **Discussion**

460   We demonstrate that morphological trait-matching is a recurrent phenomenon in hummingbird-

461   plant networks throughout the Americas, i.e. the length of a hummingbird's bill correlates with the

462   corolla length of the flowers they visit (Fig. 4a-b). This result was repeated within all

463   biogeographical regions and within most hummingbird clades (Fig. S1-S2). Trait-matching was also

464   evident when comparing between clades, as clades dominated by long-billed hummingbirds (e.g.

465   Hermits) generally visited longer flowers and *vice versa* for clades with shorter bills (Fig. 2a). If we

466   had local floral trait measurements for all hummingbird-visited plants and local measurements of

467   hummingbird traits (rather than from museum specimens), these recurrent patterns of trait-matching

468   may have been even stronger. When comparing between clades, traits also largely co-varied with

469   resource specialization d', e.g. the long-billed Hermits were also the most exclusive in their resource

470   use (Fig. 2b). However, there was no difference in niche breadth between clades (Fig. 2c) and

471   morphological traits did not generally mediate resource specialization, as bill length was only a

472   weak predictor of both measures of resource specialization (Fig. 4c-f; bill curvature also had

473   negligible effects on resource specialization, see Table S1). Notably, bill length was associated with

474   resource specialization only within the Brilliants and within the regions of Lowland South America

475   and the Andes (Fig. S1-S2). Taken together, these results demonstrate the strong influence of

476   biogeographical and evolutionary histories on recurrent patterns of morphological trait-matching,

477   and the weak relationships between hummingbird bill length and resource specialization.

478   The recurrent patterns of trait-matching show that hummingbirds feed on flowers

479   approximately similar in length to their bills, even though long-billed hummingbirds are able to

22

access nectar from flowers with shorter corollas. This may be because long-billed hummingbirds

minimize competition with short-billed hummingbirds by primarily feeding on flowers inaccessible

to short-billed hummingbirds (Maglianesi et al., 2015; Sonne et al., 2020). It may also be related to

nectar production, as longer flowers often offer more nectar than shorter flowers (Stiles, 1981;

Buzato et al., 2000; Ornelas et al., 2007; Dalsgaard et al., 2009), making it energetically optimal for

longer-billed hummingbirds to feed on flowers with longer corollas, up to a given threshold

(Montgomerie et al., 1984). From the plant's perspective, it may also be an advantage to attract

hummingbirds with matching traits, thereby increasing pollination efficiency (Montgomerie et al.,

1984). Therefore, there is a clear association between these functional traits of hummingbirds and

plants. Similar patterns have been reported for other plant-pollinator systems, such as hawkmoths

and other insect pollinators visiting flowers approximately similar in length to their proboscises

(Stang et al., 2009; Sazatornil et al., 2016; Klumpers et al., 2019). Interestingly, on average, species

in all hummingbird clades visited flowers with slightly longer corollas than their bills (Fig. 2a),

reflecting that hummingbirds extend their tongues while drinking nectar (Hainsworth, 1973;

Montgomerie et al., 1984). The Bees and the Coquettes, the two clades with the shortest bills,

visited flowers considerably longer than their bills (Fig. 2a). This may be because short-billed

hummingbirds benefit from exploring flowers with longer corolla, as these offer more nectar than

short-corolla flowers fitting their bills. Therefore, short-billed hummingbirds may have evolved the

ability to extend their tongues proportionally longer than long-billed hummingbirds (Hainsworth,

1973).

Floral corolla length was on average longer than hummingbird bill length across all

mainland regions, but not in the Caribbean islands where floral corolla length was shorter than on

the mainland (Fig. 3a). This result is consistent with reports that the Caribbean hummingbirds often

feed on shorter insect-syndrome flowers (Dalsgaard et al., 2009; Lehmann et al., 2019) and have a

504   more generalized feeding behaviour than mainland hummingbirds, both in terms of floral niche

505   overlap and niche breadth (Fig. 3b-c). Although the majority of the plants visited by Caribbean

506   hummingbirds are either endemic or native to the region (~33% endemic and ~55% native in our

507   dataset), their opportunism also makes Caribbean hummingbirds more likely to incorporate

508   introduced plants into their feeding niche (~12% in our dataset; Maruyama et al., 2016). As all

509   except one of the Caribbean hummingbirds are endemic to the region (Dalsgaard et al., 2018), these

510   Caribbean *vs.* mainland patterns are in agreement with the idea that many plants and pollinators on

511   oceanic islands have evolved towards generalism (Olesen et al., 2002; Olesen & Valido, 2003;

512   Traveset et al., 2015), but may also reflect the more recent colonization history and limited trait

513   evolution among Caribbean hummingbirds (Dalsgaard et al., 2018). Despite the distribution of plant

514   families and hummingbird clades differing significantly across regions (Fig. 1), functional traits and

515   the degree of resource specialization were similar across mainland regions, but the Caribbean

516   islands had both shorter flowers and hummingbirds with more generalized niches (Fig. 3). Traits

517   and resource specialization (niche overlap d') also largely co-varied between hummingbird clades

518   (Fig. 2), illustrating the influence of biogeographical and evolutionary histories in both functional

519   traits and floral specificity.

520   Despite the generally consistent trait-matching and associated level of resource

521   specialization observed between clades, and between mainland *vs.* islands (Figs. 2-3), trait-

522   matching did not generally translate into trait-mediated resource specialization for individual

523   species, as bill length and bill curvature were only weak predictors of resource specialization (Fig.

524   4c-f; Table 1, Table S1). While the diversity of hummingbird traits within a community influences

525   overall community-level specialization and partitioning of interactions (Maruyama et al., 2018),

526   additional mechanisms appear to operate at the species-level (Tinoco et al., 2017; Simmons et al.,

527   2019). Notably, while functional traits may constrain species into their fundamental niche of

528   possible pairwise interactions (Junker et al., 2013), a hierarchy of multiple mechanisms determines

529   the realized niche, i.e. which of the possible pairwise interactions are realized (Junker et al., 2013).

530   In hummingbird-plant communities, trait-matching determines which interactions are possible

531   (Sonne et al., 2020), but other mechanisms – such as hummingbird abundance and local conditions

532   related to resource availability and competition with other floral visitors – determine how often

533   these interactions are realized (Tinoco et al., 2017; Dalsgaard et al., 2018; Simmons et al., 2019).

534   Thus, there is no general relationship between bill morphology and level of resource specialisation

535   (Fig. 4c-f; Table 1, Table S1), illustrating that morphological and ecological specialization can be

536   disassociated (Ollerton et al., 2007; Armbruster, 2017). The only exceptions to this trend are the

537   weak associations we found between bill length and resource specialization within the Brilliants and

538   within Lowland South America and the Andes. Here, hummingbirds and plants have had the longest

539   co-evolutionary history (McGuire et al., 2014) and have experienced more benign conditions during

540   the Quaternary to evolve more specialized associations (Dalsgaard et al., 2011; Sonne et al., 2016).

541   A prime example of this is the association between the Andean species of *Passiflora* and the Sword-

542   billed Hummingbird (*Ensifera ensifera*), an iconic long-billed species within the Brilliants

543   (Abrahamczyk et al., 2014). The Sword-billed Hummingbird has by far the longest bill of any

544   hummingbird species, uses very long-corolla flowers, and tends to be ecologically specialized (Figs.

545   4, S1-S2). In line with that, long-tubed *Passiflora*, and other plants specialized on hummingbird-

546   pollination, tend to have evolved longer corolla flowers when compared to related plants pollinated

547   by most other groups of pollinators (Abrahamczyk et al., 2014; Pauw, 2019). Nevertheless, even

548   these specialised systems with tightly matching traits may have been dynamic over evolutionary

549   time (Abrahamczyk et al., 2017) and generally long-billed and short-billed hummingbirds show

550   similar degrees of resource specialization (Fig. 4c-f).

551   In conclusion, we demonstrate the influence of biogeographical and evolutionary

552   histories on recurrent patterns of trait-matching in hummingbird-plant associations, and weak

553   effects of functional traits on resource specialization. These findings indicate that morphological

554   traits can be used to predict resource utilization, not only at the level of resource type (e.g.,

555   nectarivore, frugivore, granivore, and others; Pigot et al., 2020), but even at the level of specific

556   species or functional groups of resources. Thus, our macroecological study reveals that trait-

557   matching rules are generally good predictors of interaction partners across trophic levels, whereas

558   the degree of resource specialisation is less predictable by morphological traits but is highly

559   dependent on the biogeographical, ecological and evolutionary context. These findings reveal that

560   functional traits have great potential, but also key limitations, as a toolkit for understanding trophic

561   interactions in ecological communities.

562

References

564   Abrahamczyk, S., Souto-Vilarós D., & Renner, S.S. (2014). Escape from extreme specialization:

565   passionflowers, bats and the sword-billed hummingbird. *Proc. R. Soc. B.*, 281, 20140888.

566   https://doi.org/10.1098/rspb.2014.0888

567   Abrahamczyk, S., Poretschkin, C. & Renner, S.S. (2017). Evolutionary flexibility in five

568   hummingbird/plant mutualistic systems: testing temporal and geographic matching. *J.*

569   *Biogeogr.*, 44, 1847–1855. https://doi.org/10.1111/jbi.12962

570   Armbruster, W. S. (2017). The specialization continuum in pollination systems: diversity of

571   concepts and implications for ecology, evolution and conservation. *Funct. Ecol.*, *31*, 88–100.

572   https://doi.org/10.1111/1365-2435.12783

573   Barton, K. (2020). MuMIn: Multi-model inference. *R package version 1.43.17*. https://cran.r-

574   project.org/web/packages/MuMIn/index.html

575   Bascompte, J. & Jordano, P. (2007). Plant-animal mutualistic networks: the architecture of

576   biodiversity. *Ann. Rev. Ecol. Evol. Syst.*, 38, 567–593.

577   https://doi.org/10.1146/annurev.ecolsys.38.091206.095818

578   Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models

579   Using lme4. *J. Stat. Softw.*, 67, 1. https://www.jstatsoft.org/v067/i01

580   Bleiweiss, R. (1998). Origin of hummingbird faunas. *Biol. J. Linn. Soc.*, 65, 77–97.

581   https://doi.org/10.1111/j.1095-8312.1998.tb00352.x

582   Blüthgen, N., Menzel. F. & Blüthgen, N. (2006). Measuring specialization in species interaction

583   networks. *BMC Ecol.*, 6, 9. https://doi.org/10.1186/1472-6785-6-9

584   Buzato, S., Sazima, M. & Sazima, I. (2000). Hummingbird-pollinated floras at three Atlantic forest

585   sites. *Biotropica*, 32, 824–841. www.jstor.org/stable/2663920

586   Carstensen, D.W., Dalsgaard, B., Svenning, J.-C., Rahbek, C., Fjeldså, J., Sutherland, W.J. &

587   Olesen, J.M. (2012) Biogeographical modules and island roles: a comparison of Wallacea and

588   West Indies. *J. Biogeogr.*, 39, 739–749. https://doi.org/10.1111/j.1365-2699.2011.02628.x

589   Cotton, P.A. (1998) Coevolution in an Amazonian hummingbird-plant community. *Ibis*, 140, 639–

590   646. https://doi.org/10.1111/j.1474-919X.1998.tb04709.x

591   Cusser, S., Neff, J.L. & Jha, S. (2019) Landscape context differentially drives diet breadth for two

592   key pollinator species. *Oecologia*, 191, 837–886. https://doi.org/10.1007/s00442-019-04543-5

593   Dalsgaard, B., Martín González, A.M., Olesen, J.M., Ollerton, J., Timmermann, A., Andersen, L.H.

594   *et al.* (2009). Plant–hummingbird interactions in the West Indies: floral specialisation

595   gradients associated with environment and hummingbird size. *Oecologia*, 159, 757–766.

596   https://doi.org/10.1007/s00442-008-1255-z

597   Dalsgaard, B., Magård, E., Fjeldså, J., Martín González, A.M., Rahbek, C., Olesen, J.M., *et al.*

598   (2011). Specialization in hummingbird-plant networks is associated with species richness,

599   contemporary precipitation and quaternary climate-change velocity. *PLoS ONE*, 6, e25891.

600   https://doi.org/10.1371/journal.pone.0025891

601   Dalsgaard, B., Kennedy, J.D., Simmons, B.I., Baquero, A.C., González, A.M.M., Timmermann, A.

602   *et al.* (2018). Trait evolution, resource specialization and vulnerability to plant extinctions

603   among Antillean hummingbirds. *Proc. R. Soc. B.*, 285, 20172754.

604   https://doi.org/10.1098/rspb.2017.2754

605   Dormann, C., Gruber, B. & Fründ, J. (2008). Introducing the bipartite package: analysing ecological

606   networks. *R news*, 8, 8–11. https://www.r-project.org/doc/Rnews/Rnews_2008-2.pdf

607   Eklöf, A., Jacob, U., Kopp, J., Bosch, J., Castro-Urgal, R., Chacoff, N.P. *et al.* (2013). The

608   dimensionality of ecological networks. *Ecol. Lett.*, 16, 577–583.

609   https://doi.org/10.1111/ele.12081

610   Feinsinger, P. & Colwell R.K. (1978). Community organization among Neotropical nectar-feeding

611   birds. *Am. Zool.,* 18, 779–795. https://www.jstor.org/stable/3882536

612   Fleming, T.H., & Muchhala, N. (2008). Nectar-feeding bird and bat niches in two worlds:

613   pantropical comparisons of vertebrate pollination systems. *J. Biogeogr.*, 35, 764–780.

614   https://doi.org/10.1111/j.1365-2699.2007.01833.x

615   Fox, J. & Weisberg, S. (2019). An R Companion to Applied Regression, Third Edition. Thousand

616   Oaks, CA: Sage.

28

617 Fründ, J., McCann, K.S., & Williams, N.M. (2016). Sampling bias is a challenge for quantifying

618   specialization and network structure: lessons from a quantitative niche model. *Oikos*, 125,

619   502–513. https://doi.org/10.1111/oik.02256

620 Hainsworth, F.R. (1973). On the tongue of a hummingbird: its role in the rate and energetics of

621   feeding. *Comp. Biochem. Physiol.*, 46A, 65–78. https://doi.org/10.1016/0300-9629(73)90559-

622   8

623 Jordano, P., Bascompte, J. & Olesen, J.M. (2003). Invariant properties in coevolutionary networks

624   of plant–animal interactions. *Ecol. Lett*., 6, 69–81. https://doi.org/10.1046/j.1461-

625   0248.2003.00403.x

626 Junker, R.R., Blüthgen, N., Brehm, T., Binkenstein, J., Paulus, J., Schaefer, M.H. *et al.* (2013).

627   Specialization on traits as basis for the niche-breadth of flower visitors and as structuring

628   mechanism of ecological networks. *Funct. Ecol.*, 27, 329–341. https://doi.org/10.1111/1365-

629   2435.12005

630 Klumpers, S.G., Stang, M., & Klinkhamer, P.G. (2019). Foraging efficiency and size matching in a

631   plant–pollinator community: the importance of sugar content and tongue length. *Ecol. Lett*.,

632   *22*, 469–479. https://doi.org/10.1111/ele.13204

633 Lehmann, L.J., Maruyama, P.K., Bergamo, P.J., Maglianesi, M.A., Rahbek, C., Dalsgaard, B.

634   (2019). Relative effectiveness of insects versus hummingbirds as pollinators of Rubiaceae

635   plants across elevation in Dominica, Caribbean. *Plant Biol.*, 21, 738–744.

636   https://doi.org/10.1111/plb.12976

637 Lessard, J.P., Belmaker, J., Myers, J.A., Chase, J.M., Rahbek., C. (2012). Inferring local ecological

638   processes amid species pool influences. *Trends Ecol. Evol.*, 27, 600–607.

639   https://doi.org/10.1016/j.tree.2012.07.006

640   Maglianesi, M.A., Blüthgen, N., Böhning-Gaese, K. & Schleuning, M. (2014). Morphological traits

641   determine specialization and resource use in hummingbird-plant networks in the Neotropics.

642   *Ecology*, 95, 3325–3334. https://doi.org/10.1890/13-2261.1

643   Maglianesi, M.A., Böhning-Gaese, K. & Schleuning, M. (2015). Different foraging preferences of

644   hummingbirds on artificial and natural flowers reveal mechanisms structuring plant–pollinator

645   interactions. *J. Anim. Ecol.*, 84, 655–664. https://doi.org/10.1111/1365-2656.12319

646   Martín González, A.M, Dalsgaard, B., Nogués-Bravo, D., Graham, C.H., Schleuning, M.,

647   Maruyama, P.K. *et al.* (2015). The macroecology of phylogenetically structured

648   hummingbird-plant networks. *Global Ecol. Biogeogr.,* 24, 1212–1224.

649   https://doi.org/10.1111/geb.12355

650   Maruyama, P.K., Vizentin-Bugoni, J., Oliveira, G.M., Oliveira, P.E. & Dalsgaard, B. (2014).

651   Morphological and spatio-temporal mismatches shape a Neotropical savanna hummingbird-

652   plant network. *Biotropica,* 46, 740–747. https://doi.org/10.1111/btp.12170

653   Maruyama, P.K., Vizentin-Bugoni, J., Sonne, J., Martín González, A. M., Schleuning, M., Araujo,

654   A.C. *et al.* (2016). The integration of alien plants in mutualistic plant–hummingbird networks

655   across the Americas: the importance of species traits and insularity. *Divers. Distrib.* 22, 672–

656   681. https://doi.org/10.1111/ddi.12434

657   Maruyama, P.K., Sonne, J., Vizentin-Bugoni, J., Martín González, A.M., Zanata, T.B.,

658   Abrahamczyk, S., *et al*. (2018). Functional diversity mediates macroecological variation in

659   hummingbird-plant interaction networks. *Global Ecol. Biogeogr.*, 27, 1186–1199.

660   https://doi.org/10.1111/geb.12776

661   McGill, B., Enquist, B.J., Weiher, E. & Westoby, M. (2006). Rebuilding community ecology from

662   functional traits. *Trends Ecol. Evol.,* 21, 178–185. https://doi.org/10.1016/j.tree.2006.02.002

McGuire, J.A., Witt, C.C., Remsen, Jr. J.V., Corl, A., Rabosky, D.L., Altshuler, D.L., Dudley, R.
 (2014). Molecular phylogenetics and the diversification of hummingbirds. *Curr. Biol.*, 24,
 910–916. https://doi.org/10.1016/j.cub.2014.03.016

Montgomerie, R.D., Eadie, J., Harder, L.D. (1984). What do foraging humming birds maximize?
 Oecologia, 63, 357–363. https://doi.org/10.1007/BF00390665

Nakagawa, S., Johnson, P.C.D., Schielzeth, H. (2017). The coefficient of determination R^2 and
 intra-class correlation coefficient from generalized linear mixed-effects models revisited and
 expanded. *J. R. Soc. Interface*, 14, 20170213. https://doi.org/10.1098/rsif.2017.0213

Nilsson, L.A, (1988). The evolution of flowers with deep corolla. *Nature*, 334, 147–149.
 https://doi.org/10.1038/334147a0

Olesen, J.M., Eskildsen, L.I., Venkatasamy, S. (2002). Invasion of pollination networks on oceanic
 islands: importance of invader complexes and endemic super generalists. *Divers. Distrib.*, 8,
 181–192. https://doi.org/10.1046/j.1472-4642.2002.00148.x

Olesen, J.M. & Jordano, P. (2002). Geographic patterns in plant–pollinator mutualistic networks.
 Ecology, 83, 2416–2424. https://doi.org/10.1890/0012-
 9658(2002)083[2416:GPIPPM]2.0.CO;2

Olesen, J.M. & Valido, A. (2003). Lizards as pollinators and seed dispersers: an island
 phenomenon. *Trends. Ecol. Evol.*, 18, 177–181. https://doi.org/10.1016/S0169-
 5347(03)00004-1

Ollerton, J., Killick, A., Lamborn, E., Watts, S. & Whiston, M. (2007). Multiple meanings and
 modes: on the many ways to be a generalist flower. *Taxon*, 56, 717–728.
 https://doi.org/10.2307/25065855

685 Orme, D., Freckleton, R., Thomas, G., Petzoldt, T., Fritz, S., Isaac, N., Pearse, W. (2018). caper:

686   Comparative Analyses of Phylogenetics and Evolution in R. R package version 1.0.1.

687   https://CRAN.R-project.org/package=caper.

688 Ornelas, J.F., Ordano, M., De-Nova, A.J., Quintero, M.E. & Garland, T.Jr. (2007). Phylogenetic

689   analysis of interspecific variation in nectar of hummingbird–visited plants. *J. Evolution. Biol.*,

690   20, 1904–1917. https://doi.org/10.1111/j.1420-9101.2007.01374.x

691 Pagel, M. (1999) Inferring the historical patterns of biological evolution. *Nature*, 401, 877–884.

692   https://doi.org/10.1038/44766

693 Pauw, A. (2019). A bird's-eye view of pollination: biotic interactions as drivers of adaptation and

694   community change. *Ann. Rev. Ecol. Evol. Syst.*, *50*, 477–502. https://doi.org/10.1146/annurev-

695   ecolsys-110218-024845

696 Phillips, R.D., Peakall, R., Niet, T. van der, Johnson, S.D. (2020). Niche perspectives on plant–

697   pollinator interactions. *Trends Plant Sci.*, 25, 779–793.

698   https://doi.org/10.1016/j.tplants.2020.03.009

699 Pigot, A.L., Sheard, C., Eliot, T.M., Bregman, T.P., Freeman, B.G., Roll, U., Seddon, N., Trisos,

700   C.H., Weeks, B.C., Tobias, J.A. (2020). Macroevolutionary convergence connects

701   morphological form to ecological function in birds. *Nat. Ecol. Evol.* 4, 230–239.

702   https://doi.org/10.1038/s41559-019-1070-4

703 Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team (2019). nlme: linear and nonlinear

704   mixed effects models. R package version 3.1-140, https://CRAN.R-

705   project.org/package=nlme.

706 Rahbek, C., Borregaard, M.K., Colwell, R.K., Dalsgaard, B., Holt, B.G., Morueta-Holme, N.,

707 Nogues-Bravo, D., Whittaker, R.J., Fjeldså, J. (2019). Humboldt's enigma: what causes

708 global patterns of mountain biodiversity? *Science*, 365, 1108–1113.

709 https://doi.org/10.1126/science.aax0149

710 R Core Team (2016). *R: A language and environment for statistical computing.* R Foundation for

711 Statistical Computing, Vienna, Austria.

712 Rezende, E.L., Lavabre, J.E., Guimarães, P.R., Jordano, P. & Bascompte, J. (2007). Non-random

713 coextinctions in phylogenetically structured mutualistic networks. *Nature*, 448, 925–928.

714 https://doi.org/10.1038/nature05956

715 Sazatornil, F.D., Moré, M., Benitez-Vieyra, S., Cocucci, A.A., Kitching, I.J., Schlumpberger, B.O.,

716 Oliveira, P.E., Sazima, M., Amorim, F.W. (2016). Beyond neutral and forbidden links:

717 morphological matches and the assembly of mutualistic hawkmoth–plant networks. *J. Anim.*

718 *Ecol.*, 85, 1586–1594. https://doi.org/10.1111/1365-2656.12509

719 Schleuning, M., Neuschulz, E.L., Albrecht, J., Bender, I.M.A., Bowler, D.E., Dehling, M.D. *et al.*

720 (2020). Trait-based assessments of climate-change impacts on interacting species. *Trends*

721 *Ecol. Evol.*, 35, 319–328. https://doi.org/10.1016/j.tree.2019.12.010

722 Simmons, B.I., Vizentin-Bugoni, J., Maruyama, P.K., Cotton, P.A., Marin-Gomez, O.H., Lara, C. *et*

723 *al.* (2019). Abundance drives broad patterns of generalisation in hummingbird-plant

724 pollination networks. *Oikos*, 128, 1287–1295. https://doi.org/10.1111/oik.06104

725 Sonne, J., Martín González, A.M., Maruyama, P.K., Sandel, B., Vizentin-Bugoni, J., Abrahamczyk,

726 S. *et al.* (2016). High proportion of smaller-ranged hummingbird species coincides with

727 ecological specialization across the Americas. *Proc. R. Soc. B.,* 283, 20152512.

728 https://doi.org/10.1098/rspb.2015.2512 Sonne, J., Zanata, T.B., Martín-González, A.M.,

729   Cumbicus Torres, N.L., Fjeldså, J., Colwell, R.K. *et al.* (2019) The distributions of

730   morphologically specialized hummingbirds coincide with floral trait matching across an

731   Andean elevational gradient. *Biotropica*, 51, 205–218. https://doi.org/10.1111/btp.12637

732   Sonne, J., Vizentin-Bugoni, J., Maruyama, P.K., Araujo, A.C., Chávez-González, E., Coelho, A.G.

733   *et al.* (2020). Ecological mechanisms explaining interactions within plant–hummingbird

734   networks: morphological matching increases towards lower latitudes. *Proc. R. Soc. B.*, 287,

735   20192873. https://doi.org/10.1098/rspb.2019.2873

736   Stang, M., Klinkhamer, P.G.L., Waser, N.M., Stang, I., Meijden, E. van der. (2009). Size-specific

737   interaction patterns and size matching in a plant–pollinator interaction web. *Ann. Bot.*, 103,

738   1459–1469. https://doi.org/10.1093/aob/mcp027

739   Stiles, F.G. (1981). Geographical aspects of bird-flower coevolution, with particular reference to

740   Central America. *Ann. Miss. Bot. Gard.,* 68, 323–351. https://doi.org/10.2307/2398801

741   Temeles, E.J. & Kress, W.J. (2003). Adaptation in a hummingbird-plant association. *Science,* 300,

742   630–633. https://www.jstor.org/stable/3834361

743   Thompson, J.N. (2005). The geographic mosaic of coevolution. University of Chicago Press.

744   Tinoco, B.A., Graham, C.H., Aguilar, J.M., Schleuning, M. (2017). Effects of hummingbird

745   morphology on specialization in pollination networks vary with resource availability. *Oikos*,

746   126, 52–60. https://doi.org/10.1111/oik.02998

747   Traveset, A., Olesen, J.M., Nogales, M., Vargas, P., Jaramillo, P., Antolín, E. *et al.* (2015). Bird–

748   flower visitation networks in the Galápagos unveil a widespread interaction release. *Nat.*

749   *Comm.* 6, 6376. https://doi.org/10.1038/ncomms7376

750   Vizentin-Bugoni, J., Maruyama, P.K. & Sazima, M. (2014). Processes entangling interactions in

751   communities: forbidden links are more important than abundance in a hummingbird–plant

752   network. *Proc. R. Soc. B.,* 281, 20132397. https://doi.org/10.1098/rspb.2013.2397

753   Weinstein, B.G. & Graham, C.H. (2017). Persistent bill and corolla matching despite shifting

754   temporal resources in tropical hummingbird-plant interactions. *Ecol. Lett*., 20, 326–335.

755   https://doi.org/10.1111/ele.12730

756   Wolf, L.L., Stiles, F.G. & Hainsworth, F.R. (1976). Ecological organization of a tropical, highland

757   hummingbird community. *J. Anim. Ecol.*, 45, 349–379. https://doi.org/10.2307/3879

758   Zanata, T.B., Dalsgaard B., Passos F.C., Cotton P.A., Roper J.J., Maruyama P.K. *et al.* (2017).

759   Global patterns of interaction specialization in bird–flower networks. *J. Biogeogr.*, 44, 1891–

760   1910. https://doi.org/10.1111/jbi.13045

Table 1. Linear mixed-effects models (LMMs), analysing the association between hummingbird bill length and (a) mean corolla length of visited flowers, and mean resource specialization, measured both as (b) complementary specialization d' and (c) proportional generality. For each response variable, we built two sets of models: one considering hummingbird clades and another considering biogeographical regions as random intercepts, with bill length as a fixed effect. For each of these analyses, we constructed both unweighted and weighted models, the latter weighted with the square root number of plants visited (when predicting mean corolla length) and the square root number of networks (when predicting mean specialization d' and mean proportional generality). Unweighted models are in bold. We estimated the proportion of variance explained by bill length in the LMMs as marginal R^2, and the proportion of variance explained by both bill length and random factors as conditional R^2. We also report standardized coefficient estimates as well as corresponding P-values and standard errors.

| Model	Random factor	R^2 marginal	R^2 conditional	coefficient	Std Error
(a) Corolla length	**clade**	**0.45**	**0.45**	**0.80****	**0.07**
	clade	0.11	0.11	0.87**	0.08
	region	**0.41**	**0.50**	**0.78****	**0.06**
	region	0.11	0.13	0.86**	0.07
(b) Specialization d'	**clade**	**0.01**	**0.10**	**0.13^{NS}**	**0.11**
	clade	0.00	0.01	0.09^{NS}	0.12
	region	**0.04**	**0.04**	**0.25***	**0.09**
	region	0.00	0.02	0.18^{NS}	0.10
(c) Proportional generality	**clade**	**0.00**	**0.02**	**0.07^{NS}**	**0.12**
	clade	0.00	0.01	0.14^{NS}	0.14
	region	**0.00**	**0.43**	**0.10^{NS}**	**0.10**
	region	0.00	0.10	0.10^{NS}	0.11

**P<0.001, *p < 0.05, ^{NS} P > 0.05

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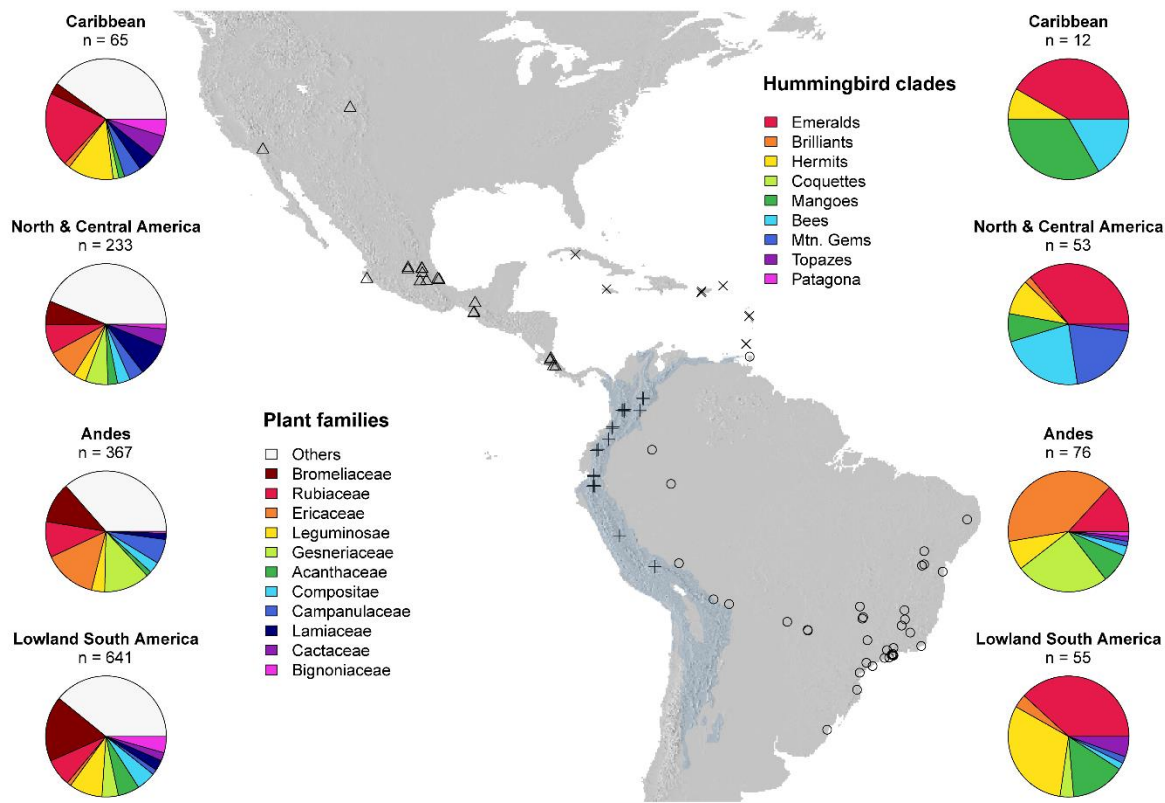
Figure 1. The distribution of 93 hummingbird-plant interaction networks across four

biogeographical regions (O = Lowland South America, + = Andes, Δ = Central & North America,

× = Caribbean). The pie charts visualize the distribution of all nine hummingbird clades (right) and

the 11 most frequently visited hummingbird-plant families (left) within each biogeographical

region. The category 'others' includes the rest of the plant families visited by hummingbirds.

Within a given pie chart, the size of a clade/family reflects the number of species observed in the

networks within a given region. Fisher's exact test showed that all regions differed in respect to

plant family distribution ($P < 0.05$ for all pairwise comparisons). For hummingbirds, all mainland

regions differed significantly in clade distribution ($P < 0.05$), but the Caribbean was not

significantly different from North & Central America and Lowland South America ($P > 0.05$), the

two regions from where hummingbirds colonized the Caribbean (Dalsgaard et al., 2018).
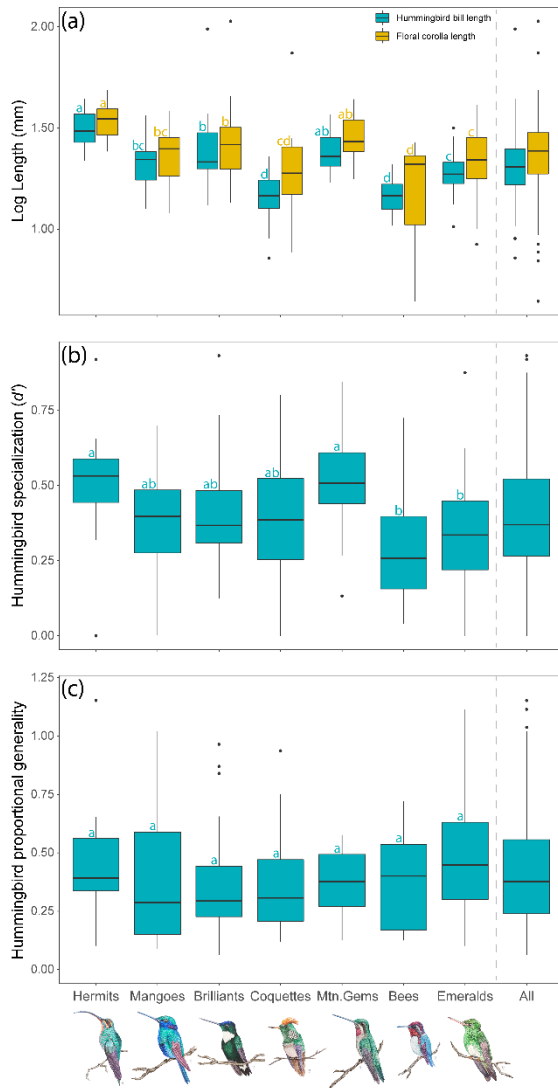
Figure 2. Hummingbird clade specific differences in (a) hummingbird bill length (turquoise) and

mean corolla length of visited flowers (yellow), and (b) mean hummingbird specialization d', and

(c) mean hummingbird specialization measured as proportional generality. This comparison was

based on all species within each clade, irrespectively of regional affinities. Boxes indicate the first

and third quartiles (Q1 and Q3), horizontal lines inside boxes are medians, vertical lines indicate

Q1/Q3 + 1.5 x interquartile ranges (IQR), and circles are outliers. Different letters represent

statistical difference ($P < 0.05$) according to one-way ANOVAs with multiple post-hoc

comparisons using the fFalse Discovery Rate (FDR). For comparison, on the right in both figures

we show boxplots across all species. Hummingbird paintings by Katrine Hansen.
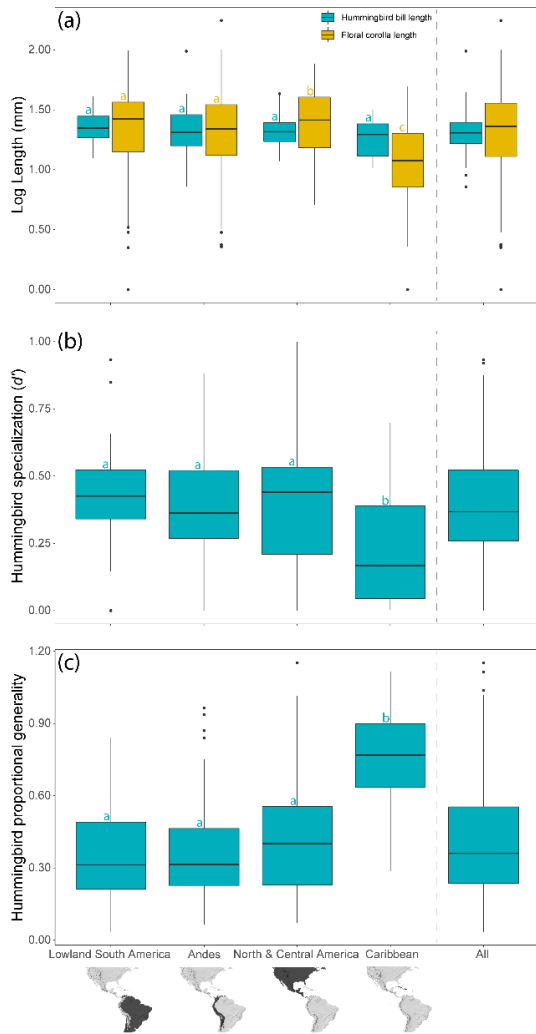
Figure 3. Differences in (a) hummingbird bill length (turquoise) and mean floral corolla length (yellow), (b) mean hummingbird specialization d', and (c) mean hummingbird specialization measured as proportional generality between biogeographical regions: Lowland South America, Andes, Central & North America, and the Caribbean. The comparison was based on the species pool for each region, as extracted in the networks within each region. Boxes indicate the first and third quartiles (Q1 and Q3), horizontal lines inside boxes are medians, vertical lines indicate Q1/Q3 + 1.5 x interquartile ranges (IQR), and circles are outliers. Different letters represent statistical difference ($P < 0.05$) according to one-way ANOVAs with multiple post-hoc comparisons using the False Discovery Rate (FDR). For comparison, on the right in both figures we show boxplots across all species.

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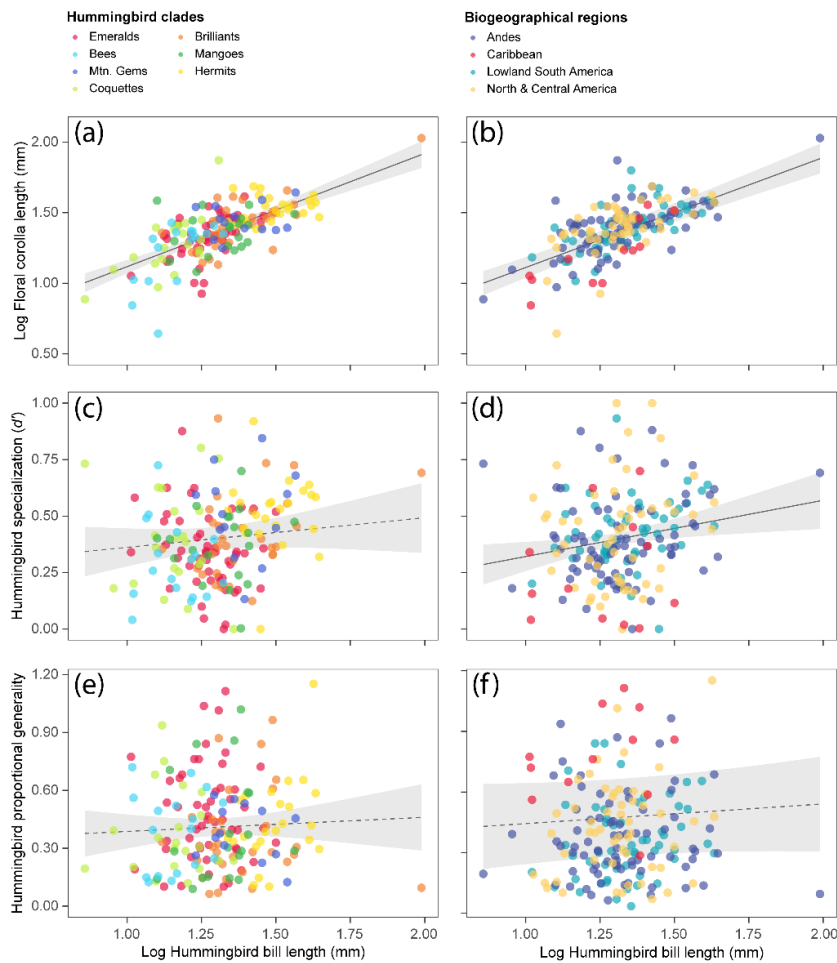
Figure 4. Associations between hummingbird bill length and (a, b) mean floral corolla length, (c, d) mean hummingbird specialization d' and (e, f) mean hummingbird specialization measured as proportional generality, when using hummingbird clades as a random factor (clades in different colours: a, c and e; n = 172 and n = 177) and when using biogeographical regions as a random factor (regions in different colours: b, d and f; n = 191 and n = 195). The black lines (with grey 95% confidence intervals) represent the overall fits of unweighted linear-mixed effects models; the dotted line represents a non-significant fit. Note that a few hummingbird species were recorded in more than one region and, thus, appear more than once in the analyses including regions as a random factor (b, d and f). See supplementary Figure S1 for individual plots for each hummingbird clade and Figure S2 for individual plots for each biogeographical regions.