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Species morphology better predicts plant–hummingbird interactions across elevations than nectar traits

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1 **Running head:** morphological vs nectar traits in pollination networks

2

3 **Article title:** Species morphology better predicts plant-hummingbird interactions across
4 elevations than nectar traits

5

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19

20 **Abstract**

21 Species traits greatly influence interactions between plants and pollinators where floral nectar is
22 the primary energy source fostering this mutualism. However, very little is known about how
23 nectar traits mediate interactions in pollination networks compared to morphological traits. Here,
24 we evaluated the role of morphological and nectar traits in shaping plant-hummingbird
25 interaction networks along an elevation gradient. For this, we assessed patterns in floral
26 phenotypic traits and network properties of plant species across elevations in Costa Rica. We also
27 analysed whether plant species with generalised flower traits are ecological generalists and how
28 morphological trait matching vs nectar traits affect interactions. We found marked variation in
29 floral phenotypic traits and flower abundance of hummingbird-visited plant species across ten
30 sites along the elevation gradient. We did not find evidence for a relationship between flower
31 morphology and nectar traits or between morphological and ecological generalisation of plant
32 species. Plant-hummingbird interaction frequency increased when the lengths of hummingbird
33 bill and flower corolla were similar, indicating morphological matching, whereas nectar traits
34 were unrelated to interactions. While nectar may play a difficult-to-detect secondary role within
35 plant-hummingbird networks, our results reinforce the idea that morphological matching is an
36 important factor structuring ecological communities.

37

38 **Keywords:** elevation | flower morphology | hummingbirds | interactions | nectar | pollination

39 **1. Introduction**

40 A main goal of community ecology is to identify the mechanisms shaping species interaction
41 networks, such as those including antagonistic or mutualistic relationships. In pollination
42 systems, morphological traits of the mutualistic partners greatly influence interactions since they
43 facilitate or restrict the access to floral resources (e.g. [1–3]). In particular, trait matching has
44 been found to structure plant-pollinator networks [4–6], including both *trait complementarity*
45 (corresponding morphology) and *exploitation barriers* (non-matching traits) [7]. However, the
46 quantity and quality of floral nectar is also expected to influence interactions, since sugar is the
47 primary energy source offered by plants to pollinators. Floral nectar is particularly important for
48 vertebrate pollinators, such as hummingbirds, due to their highly energetic lifestyle [8,9]. Further,
49 many pollinators, including hummingbirds, can quickly learn which flowers are more rewarding
50 and change their foraging patterns based on nectar availability [10,11]. Whereas the influence of
51 species morphological traits on plant-hummingbird interactions is relatively well studied [12,13],
52 that of nectar along with species morphology (e.g. corolla and bill length) has been largely
53 neglected. We fill this gap by simultaneously evaluating the role of morphological trait matching
54 and floral nectar in mediating plant-hummingbird interactions.

55 Phenotypic floral traits, including both flower morphology (e.g. corolla length) and nectar
56 (e.g. nectar volume), may vary markedly among plant species along environmental gradients and
57 influence to each other [14-17]. Despite increasing research, it is not clear yet how this variation
58 influences interactions in pollination networks. Analysing how phenotypic traits affect species
59 interactions at different elevations may contribute to a better mechanistic understanding of
60 pollination networks. For example, nectar traits may vary across elevations [16,17], which may
61 influence the foraging decisions of pollinators about which flowers to visit [18]. As nectar is
62 almost always hidden inside the flower, and olfactory signals do not seem to be used by

63 hummingbirds [19], questions remain as to how hummingbirds choose the flowers they visit and
64 if their foraging maximises their chance of finding nectar. Although nectar traits exert discernible
65 effects on pollinators [20], variation in the volume and concentration, combined with the overall
66 difficulty in collecting this information (especially as compared to morphological traits), has
67 hindered our ability to detect their relative influence on interactions. Some studies have found a
68 positive relationship between corolla length and nectar production [15,21], which means that
69 flower morphology could be used as a proxy for nectar. Therefore, analysing how flower
70 morphology is related to nectar traits may substantially improve our knowledge about plant-
71 pollinator interactions and facilitate future research in community ecology.

72 From the plant perspective, both morphological and nectar traits can contribute to deter
73 visitation by certain pollinators whilst attracting a reduced set of others, which are potentially
74 more effective. For example, plant species with long-corolla flowers, which often have copious
75 nectar that is difficult to access, may be visited by a less diverse pollinator assemblage than those
76 with short, easily accessible, but less energetically profitable flowers [1,22-24]. Thus,
77 phenotypically specialist plants tend to be visited by a small set of pollinators owing to
78 morphological barriers and are thus ecological specialists [7,24,25] (but see [26]). As a result,
79 plants with specialised floral phenotypes often occupy a less central position in a network [27],
80 meaning that they interact with few pollinator species compared to generalist plants. Thus,
81 phenotypic specialisation is usually thought to be associated with ecological specialisation of
82 plant species in pollination networks. However, this relationship between both views of plant
83 specialisation (phenotypic and ecological), in particular considering floral morphology as well as
84 nectar traits of plant species is poorly understood [24]. From the pollinator perspective, we can
85 expect that long-billed hummingbird species visit plants with corresponding long-corolla flowers

86 (trait complementarity) more frequently because they can feed more efficiently (see [34]), which
87 is consistent with optimal foraging theory [33].

88 Ecological specialisation and centrality are key properties structuring mutualistic networks and
89 these vary across ecological and environmental gradients [13]. For instance, ecological
90 specialisation has been found to be higher in species-rich communities commonly found at low
91 and mid elevations where species benefit from more finely partitioning the food resource niche
92 compared to highlands [3,28,29]. However, there is also evidence of more specialised use of food
93 resources by pollinators in tropical high Andes, which has been associated with greater richness
94 of morphological specialists and trait matching compared to mid elevations [30]. It is likely that
95 these elevation-driven changes in network properties are tightly connected to the interplay
96 between species traits and interactions. Analysing how network properties and floral traits of
97 hummingbird-visited plant species vary along an elevation gradient has been insightful for
98 investigating the factors mediating interactions (e.g. [31,32]). However, there is a lack of studies
99 linking this variation to species phenotypes, especially to floral nectar traits, which would allow a
100 deeper understanding on the mechanisms leading to species interactions.

101 Here, we evaluated the role of species morphology and nectar in shaping plant-hummingbird
102 interactions along an elevation gradient in Costa Rica (Fig.1). More specifically, we (1) assess
103 patterns in floral phenotypic traits and flower abundance of plant species along an elevation
104 gradient and the extent to which flower morphology predicts nectar traits; (2) assess the influence
105 of phenotypic traits and elevation on ecological specialisation and centrality of plant species in
106 the networks; (3) analyse the relative importance of species morphology and nectar traits in
107 shaping plant-hummingbird interactions across elevations. Addressing these aims will yield a
108 more thorough perspective on the mechanisms driving mutualistic interactions in ecological
109 communities.

110

111 **2. Materials and methods**

112 **(a) Study area and sampling design**

113 The study was conducted at 10 sites at different elevations ranging from 700 to 3100 m on the
114 Pacific slope of the Talamanca Mountain Range in central-southern Costa Rica. Along this
115 gradient we had 2-3 replicates at a similar elevation in four different bands (Appendix 1). This
116 area is a biodiversity hotspot with high levels of endemism [35] which forms the spine of the
117 Central American isthmus from central Costa Rica through western Panama. The average annual
118 temperature is 22-24°C in the lower sites and 10-12°C in the higher elevation sites, while the
119 range of annual precipitation is 2500-3000 and 3000-4000 mm, respectively. At each study site,
120 we established one 1.5 km long x 4 m wide transect along existing trails where we recorded
121 monthly data on species traits, flower abundance and plant-hummingbird interactions. Field data
122 collection started in June 2019, covering two entire sampling years for species interactions,
123 whereas it extended to February 2023 for species traits and flower abundance.

124

125 **(b) Data collection**

126 **(i) Species morphological traits**

127 We included in our analysis morphological traits of interacting species that have been reported to
128 affect plant–hummingbird interactions. For plants, we measured total corolla length (distance
129 from base to opening), corolla curvature and width of the corolla opening (length of the flower
130 opening in its lateral plane) [30,36,37]. Although we recorded a total of 144 plant species visited
131 by hummingbirds across all the study sites, we used a subset of 59 plant species in our analyses
132 for which we could quantify both morphological and nectar traits. From these species, we
133 measured a total of 1101 flowers from 562 plant individuals with a mean of 26.12 flowers and a

134 range of 7-100 flowers per species. For hummingbirds, we measured total bill length (distance
135 from tip to nostril) and curvature of 220 individuals belonging to 22 species (n = 10 per species)
136 [29,37]. We calculated flower and bill curvature by fitting them to the curvature of a circle,
137 according to the inverse radius method outlined by [37]. Measurements were taken from photos
138 of fresh flowers and mist-netted hummingbird individuals in the field, complemented with
139 museum specimens, and then processed with ImageJ software [38]. Hummingbird traits
140 correspond only to adult birds as determined from plumage and/or <10% of bill corrugations
141 [39], since some morphological traits such as bill length may not be fully developed in juveniles.
142 While some species in the studied communities are sexually dimorphic, the standard deviation of
143 a trait value within a species including measures of both sexes is much lower than the standard
144 deviation of a trait across males of different species [40]. Thus, intraspecific variation related to
145 sex is unlikely to influence our results.

146

147 **(ii) Nectar traits and flower abundance**

148 We quantified daily nectar production and concentration per flower for common plant species
149 visited by hummingbirds in the understory (see more details on the criteria for plant selection in
150 the next section). We sampled these nectar traits from flowers that were bagged with nylon
151 netting (~0.5-1 mm mesh size) prior to anthesis to prevent visitation by animal pollinators during
152 24 h. After this period, nectar was extracted with microcapillaries (10 and 15 μ l capacity),
153 obtaining its volume, and sugar concentration was measured by using a calibrated pocket
154 refractometer (REED R9500 Brix Refractometer; range concentration 0-32%, g sugar per 100 g
155 nectar). The observed Brix reading was corrected for temperature obtaining the final
156 concentration measurement. We included in our analysis mean values of nectar traits of plant
157 species with a minimum and mean sample size of 10 and 45 flowers, respectively, from at least

158 three individuals per species, totaling 2876 flowers from the 59 species for which morphological
159 traits were recorded. As some plant species had few flowering individuals in certain sites, we
160 pooled the data from plants that occurred at more than one site within the same elevation band,
161 i.e. we calculated mean nectar traits at the species level.

162 To assess patterns in hummingbird floral resources across elevations, in addition to
163 quantifying nectar, we counted open flowers in these plants, identified them to species level and
164 calculated the mean number of flowers per individual across species. Voucher specimens of plant
165 species were deposited at the Luis A. Fournier Herbarium (USJ) at the University of Costa Rica.
166 We recorded over 3098 flowers for hummingbird's resource abundance analyses.

167

168 **(iii) Plant-hummingbird interactions**

169 To record the interactions between plant and hummingbird species in the understory, we used
170 time-lapse cameras combined with automated computer vision review which significantly
171 increases the sampling efficiency in comparison to previous human-observer surveys [41]. Along
172 the sampling transects, we placed unattended cameras on flowers that fit the traditional
173 ornithophilous syndrome, i.e. those with tubular-red, -yellow, or -purple corollas, scentless and
174 no landing platforms [42-44], roughly in proportion to their abundance. Because hummingbird-
175 visited flowers do not always have these characteristics [45], we also considered plant species
176 that fit other syndromes (e.g. bat- or insect-pollinated flowers) known to be visited by
177 hummingbirds based on field observations and previous research (e.g. [9, 46-48]).

178 At each study site, six cameras were set to turn on at dawn and record an image every second
179 for three days following standardised protocols by [49], using a total of 60 cameras for the 10
180 sites. After this period, the cameras were set up to film another plants for another three days. This
181 sampling scheme resulted in a unique dataset with millions of images which we then processed

182 by using Deep Meerkat software [50]. This software extracts the images with hummingbirds to
183 be manually identified at the species level along with other relevant data, such as illegitimate
184 visits [51]. These visits, where the hummingbird did not access the flower through the corolla
185 entrance (i.e. acting as robber in a non-mutualistic relationship) were excluded from further
186 analyses. We recorded visitation of hummingbirds to 2239 plant individuals from the 59 species
187 for which we quantified both morphological and nectar traits; this resulted in an average of 5071
188 hours of videos per site totalising 50 715 hours across the 10 sites for the whole sampling. Based
189 on these camera data, we pooled the interactions between each plant and hummingbird species at
190 a given site across the monthly periods over the two years, thus calculating the total number of
191 pairwise interactions.

192

193 **(c) Statistical analysis**

194 **(i) Flower morphology as predictor of nectar traits across elevations**

195 We performed phylogenetic generalised least squares (PGLS) to analyse (i) the variation in floral
196 phenotypic traits (morphological and nectar traits) and flower abundance of plant species visited
197 by hummingbirds across elevation and (ii) the extent to which flower morphology predicts nectar
198 traits. PGLS fits a linear model while controlling for the non-independence between the samples
199 resulting from the phylogenetic structure in the data, which is commonly the case for the studied
200 traits [15,52]. We built a plant phylogeny to be included in these models with the *R* package
201 V.PhyloMaker [53] (see Appendix 2 for details).

202 *(i) Variation in floral phenotypic traits and flower abundance across elevation.* The univariate
203 PGLS included a given floral morphological trait (corolla length, curvature, opening) or nectar
204 trait (volume, concentration) or mean flower abundance per species as the response variable and
205 elevation as predictor. Nectar volume, corolla opening and flower abundance were log-

206 transformed prior to analyses. For example, nectar volume of species i is explained by elevation
207 of site j :

$$208 \quad \log(N \text{ volume}_i) \sim \text{elevation}_j + e_{ij} \quad (1)$$

209 where e_{ij} is a normally distributed error term following $N(0, \sigma^2 C_\lambda)$, where C_λ is the variance-
210 covariance matrix extracted from the plant phylogeny (C) to which the Pagel's λ transformation has
211 been applied (Appendix 2).

212 *(ii) Relationship between nectar traits and flower morphology.* The PGLS included a given nectar
213 trait, i.e. volume (log-transformed) or concentration per species, as the response variable and
214 floral morphological traits per species along with flower abundance as the predictors. Predictors
215 were normalised to zero mean and unit variance prior to analysis in order to bring them to a
216 common scale that allows for comparison [54]. For example, the model explaining nectar volume
217 variation is described as follows:

$$218 \quad \log(N \text{ volume}_i) \sim \text{flower length}_i + \text{flower curvature}_i + \text{flower opening}_i + \\ 219 \quad \text{flower abundance}_i + e_i \quad (2)$$

220 where i is the species index and e_i is a normally distributed error term following $N(0, \sigma^2 C_\lambda)$.

221 We ran these PGLSs with the packages nlme [55] and "ape" [56] and used maximum likelihood
222 to simultaneously estimate the regression parameters and the phylogenetic signal Pagel's lambda
223 [57] (Appendix 2).

224

225 **(ii) Morphological and nectar traits as predictors of network metrics across elevations**

226 We analysed how flower phenotypic traits (morphology and nectar) influenced two measures of
227 network structure: ecological specialisation and closeness centrality, which reflect the role and
228 position of plant species in the studied communities, respectively. These metrics are important
229 because they contribute to species coexistence and stability in ecological communities, hence,

230 they are key properties structuring mutualistic networks [13,58]. To calculate both metrics, we
231 built quantitative interaction matrices, where we included the interaction rate per day between
232 hummingbirds and plants at each site; this standardised measure is independent of differences in
233 sampling effort among plants. Interaction rate consisted of the total number of pairwise
234 interactions divided by the total number of hours of observation on the corresponding plant,
235 subsequently multiplied by 24, i.e. daily interaction rate [59]. From these matrices, we calculated
236 *ecological specialisation* with the index d' , which measures how strongly the interaction
237 frequency of a given species deviates from null-model expectations where it interacts
238 proportionally to their partner abundance [60]. The index d' is a sampling-robust metric derived
239 from Kullback–Leibler distances and ranges from 0 for a fully generalised to 1 for a completely
240 specialised species, respectively (Appendix 3). We also calculated *closeness centrality*, which
241 describes the position of a species in the network [61] as the inverse of the average shortest
242 distance of a focal plant species to all other plants [62]. Plants with high centrality values occupy
243 a central position in the network, which means they have shorter average distances to all other
244 species coflowering plants via shared pollinators (Appendix 3).

245 We tested the influence of floral traits on specialisation and closeness centrality across
246 elevations by performing two PGLS based on the plant phylogeny described above. In these
247 models, we included a given log-transformed network metric at the species level ($\log + 1$ index d'
248 or log-closeness centrality) as the response variable and floral traits (both morphological and
249 nectar traits) and elevation as predictors. All predictors were normalised to zero mean and unit
250 variance. As some plant species occurred in multiple sites, they have different network metrics
251 but the same morphological and nectar measures since it was not tractable to measure traits on a
252 per site basis. So, the model explaining specialisation of plant species i at site j is described by
253 the following equation:

254 $\log(d' + 1)_{ij} \sim \text{flower length}_i + \text{flower curvature}_i + \text{flower opening}_i$
 255 $N \text{ volume}_i + N \text{ concentration}_i + \text{elevation}_j + e_{ij} \quad (3)$

256 where N indicates nectar and e_{ij} is a normally distributed error term following $N(0, \sigma^2 C_\lambda)$.

257

258 **(iii) Morphological vs nectar traits as predictors of plant-hummingbird interactions**

259 We analysed the effect of species morphology on plant-hummingbird interactions by calculating
 260 the degree of mismatch between corresponding pairs of morphological traits (bill-corolla length
 261 and bill-corolla curvature) and considering a barrier to nectar access for each pairwise interaction.

262 For this, we calculated the absolute difference between the bill length or curvature of the
 263 hummingbird j and the corolla length or curvature of the plant i ; values close to 0 indicate strong
 264 trait matching. To simultaneously evaluate the effect of morphological and nectar traits on
 265 species interactions, we: (i) fitted a full generalised linear mixed model with negative binomial
 266 error distribution (GLMM) as suggested for overdispersed count data, where we included plant-
 267 hummingbird interaction frequency as the response variable with an offset term on the
 268 cumulative sampling hours for each plant species due to its influence in the dependent variable,
 269 and seven fixed effects and (ii) conducted automated selection to identify minimal adequate
 270 models from the full model that best explain the effect of morphological and nectar traits on
 271 interaction frequency. We used the package `glmmTMB` to perform the GLMM [63].

272 *(i) Full GLMM.* In addition to the offset term, we included in this model: morphological
 273 mismatches (bill-corolla length, barrier to nectar access and bill-corolla curvature) and nectar
 274 traits (volume and concentration). The two nectar traits were uncorrelated (Pearson test, $r = 0.07$,
 275 $p = 0.207$). Considering that a bill longer than a corolla is likely to differently influence the
 276 interaction compared to a bill shorter than a corolla, we included a binary variable depicting a

277 trait barrier and its interaction with bill-corolla length mismatch. We assigned this variable a
 278 value of 0 and 1 when bill length was longer and shorter than the corolla length, respectively. We
 279 also included in the model the total number of flowers per species at a given site as a fixed effect
 280 in order to detect whether there is a relationship between interaction frequency and flower
 281 abundance. To test whether the relationship between plant-hummingbird interaction frequency
 282 and predictor variables was context dependent, we included in the GLMM the interaction term
 283 between elevation and each of the fixed effects. Predictors were normalised to zero mean and unit
 284 variance prior to analysis in order to bring them to a common scale [54]. To account for variation
 285 among species and taxonomic relatedness among networks, we included in the model plant and
 286 hummingbird species nested within its genus, and study site as random intercept effects. The
 287 model is described by the following equation:

$$\begin{aligned}
 288 \quad & I_{ihj} \sim \text{NB}(\lambda_{ihj}, \phi); \\
 289 \quad & \log(\lambda_{ihj}) \sim ML_{ih} * barrier_{ih} + MC_{ih} + flower\ abundance_i + \\
 290 \quad & N\ volume_i + N\ concentration_i + elevation_j + offset(\log(sampling\ hours)) + \\
 291 \quad & |Pg_i + P_i + Hg_h + H_h| + e_{ihj} \quad (4)
 \end{aligned}$$

292 where I_{ihj} is the total number of interactions between plant species i and hummingbird species h
 293 on site j . M indicates mismatch in bill-corolla length (ML) and bill-corolla curvature (MC) and N
 294 indicates nectar. P_i and H_h are random effects of plant and hummingbird species, whereas Pg_i and
 295 Hg_h are random effects of plant and hummingbird genus, i.e. we accounted for phylogenetic
 296 relationships across plants and hummingbird species; all these random effects are normally
 297 distributed, centred on zero and with a unique variance per random effect.

298 (ii) *Minimal adequate models*. To identify the best fitting and most parsimonious models from
 299 the full GLMM, we performed automated selection procedure according to the corrected Akaike

300 information criterion (AICc) with the function dredge in the package MuMIn [64]. As we
301 compared the performance of the full GLMM to alternative simpler models, parameters in this
302 case were estimated with Maximum Likelihood approximation as suggested by [65] to prevent
303 biased fixed-effect parameter estimates in models with differing fixed effect structures. To fit
304 the selected models (those with ΔAICc to all other models <2), we used Restricted Maximum
305 Likelihood that produces unbiased estimates of variance and covariance parameters [65]. To
306 facilitate interpretation, we report the exponent of the model coefficients, i.e. the incidence rate
307 ratios (IRR), which describes the proportional change in incidence rate when increasing a given
308 predictor variable by one unit. An $\text{IRR} = 1$ implies that there is no effect of the predictor on the
309 interaction frequency, whereas values of $\text{IRR} < 1$ indicate a percentage decrease in the response
310 variable (negative relationship) and values of $\text{IRR} > 1$ denote an increase (positive relationship).
311 All statistical analyses were conducted in the *R* programming environment version 4.3.1 [66].

312

313 **3. Results**

314 **(i) Floral traits & network structure across elevations**

315 We found that both morphological and nectar floral traits as well as flower abundance varied
316 across the elevation gradient. Corolla length decreased with elevation ($\beta = -0.76, p = 0.002$),
317 whereas nectar volume per flower had a hump-shaped pattern ($\beta = 2.57, p < 0.001$) and flower
318 abundance was greater at the highest elevation ($\beta = 0.55, p < 0.001$), (Fig. 2). *Fuchsia paniculata*
319 (Onagraceae) and *Gaiadendron punctatum* (Loranthaceae) had the highest abundance with a
320 mean of 212 and 104 flowers per individual, respectively. Mean nectar volume per flower ranged
321 from 1 μl in *F. paniculata* to 18 μl in *Psammisia ramiflora* (Ericaceae) with a mean of $5.42 \mu\text{l} \pm$
322 $0.30 \mu\text{l}$ standard error for all hummingbird-visited plant species. Whereas mean sugar

323 concentration varied from 4% in *Goepertia indecora* (Marantaceae) to 25% in *Passiflora*
324 *vitifolia* (Passifloraceae) with a mean of $16\% \pm 0.32\%$. Neither nectar volume nor nectar
325 concentration were linked to morphological traits or flower abundance (Fig. S1, Appendix 4).
326 Regarding network properties, neither metrics (ecological specialisation and closeness centrality
327 of plant species) was related to either floral morphological or nectar traits, whereas closeness
328 centrality increased with elevation ($\beta = 0.48$, $p = 0.005$; Fig. S2, Appendix 4).

329

330 **(ii) Influence of flower morphology & nectar on interactions**

331 We recorded 6925 visits of 22 hummingbird species to 59 plant species (Appendix 5) with a
332 consistent effect of species morphology on interaction frequency. More specifically, the
333 automated model selection procedure provided five minimal adequate models where all of them
334 included mismatch in bill-corolla length as a predictor variable that was significantly and
335 negatively related to interaction frequency (Table 1, Fig. 3). Also the barrier and its interaction
336 with mismatch in bill-corolla length was significantly and negatively related to interaction
337 frequency in these models. Nectar volume was included in only two of the minimal adequate
338 models and in none of them it was significantly related to interaction frequency (Fig. 3). Thus,
339 hummingbirds visited less frequently plant species with flowers poorly matching their bill length
340 than with those similar in length, especially in the presence of a barrier (when the corollas were
341 longer than the bills). This relationship was context independent, that is, was not affected by
342 elevation since we did not find a relationship between plant-hummingbird interaction frequency
343 and morphological mismatch \times elevation in any of the five minimal adequate models.

344

345 **4. Discussion**

346 Our results show that both morphological and nectar traits, as well as flower abundance of plant
347 species visited by hummingbirds varied across elevations, with a decrease in corolla length and
348 nectar volume, and an increase in flower abundance at high elevations. We did not find evidence
349 that floral morphological traits or flower abundance predicted nectar traits or that the two
350 network metrics of plant species were related to flower morphology or nectar traits. From these
351 two network metrics, ecological specialisation remained similar along the elevation gradient,
352 unlike closeness centrality that increased with altitude. Further, interaction frequency consistently
353 increased across sites along the gradient when hummingbird bills and flower corollas had similar
354 lengths, i.e. with morphological matching, whereas nectar traits were unrelated to interaction
355 frequency. Together, our results reveal that species morphology better predicts patterns of
356 interactions in plant-hummingbird networks than nectar traits, supporting the idea that
357 morphological matching is a key mechanism structuring ecological communities [1,3,4,49].

358

359 **(a) Linking flower morphology to nectar traits across elevations**

360 **(i) Patterns in flower morphology, abundance and nectar traits**

361 Elevation negatively impacts net primary production of plants [67] hence, it is expected to
362 increase the cost of resources offered to pollinators (e.g. flowers and nectar) as a consequence of
363 temperature-mediated effects on photosynthesis [68]. In particular, nectar production may be
364 expensive for plants in terms of the amount of energy invested to the detriment of growth and/or
365 reproduction [69]; thus, plants are better adapted when they produce the smallest possible nectar
366 volume to attract their pollinators [70]. The increased number of flowers at higher elevations in
367 our study may reveal a plant strategy to increase attractiveness to hummingbirds. This may be
368 specially important for plant species such as *Fuchsia paniculata* (Onagraceae) and *Gaiadendron*
369 *punctatum* (Loranthaceae) which are not dominant in the high-elevation communities but had the

370 highest flower abundance. High flower production likely comes with a cost for a plant, that might
371 be offset by a reduced nectar production per flower at the highest elevation [71]. This is
372 consistent with our results where *F. paniculata* had the highest mean flower abundance per
373 individual and the lowest nectar volume per flower. The pattern consisting of plants producing
374 many flowers with little nectar calls for caution when using flower abundance as a single measure
375 to quantify resource availability for pollinators rather than using it in combination with nectar
376 measurements. Another conspicuous pattern in our study is a decrease in corolla length across
377 elevations, which is in line with [14, 16] and contrasts with [17]. Short-corolla flowers, together
378 with an increased flower abundance in highland communities suggests a trade-off between flower
379 number and size. A reverse pattern, i.e fewer and larger flowers in high-altitude plants visited by
380 insect and sunbird pollinators in a non-tropical region [17] is consistent with the idea of a flower
381 number/size trade-off (high cost of producing large flowers could be offset by a reduced flower
382 abundance). A promising avenue for future studies is to explore the existence of trade-offs in
383 plants embedded in pollination networks that may reveal a resource conservative strategy to cope
384 with extreme environmental conditions and how such trade-offs influence interactions. A hump-
385 shaped pattern in nectar volume we found across elevation poses an alternative explanation to
386 this strategy where the strong competition for pollinators in diverse communities at mid
387 elevations leads plant species to increase nectar reward, thus enhancing their attractiveness
388 [72,73].

389

390 **(ii) Relationship between flower morphology and nectar traits**

391 Some studies have found a weak relationship between nectar volume and corolla length [74,75]
392 whereas others report a positive correlation between both [15,21]. Our results revealed that
393 flower morphology is not a good predictor of nectar volume and concentration. Instead, flowers

394 in the highest elevation had on average reduced nectar volume, suggesting that nectar production
395 was more influenced by environmental conditions than by flower morphology in our study
396 system. The absence of predictive power of nectar traits on corolla length indicates that this
397 morphological trait does not constitute a reliable cue of high-reward flowers. In the compromise
398 of attracting pollinators for reproduction at the lowest possible cost, this lack of signalling may be
399 a convenient strategy for plants since, on the one hand, they force hummingbirds to probe many
400 flowers in search of food, ensuring the pollination of at least some of them. On the other hand,
401 high attractiveness (increased flower abundance at the high elevation) also promotes many flower
402 visits during a single plant visit, which may increase selfing and decrease pollen export [76]. A
403 bonanza-blank nectar secretion pattern, where some flowers produce abundant nectar and others
404 secrete little or no nectar, may benefit plants by reducing energy expenditures on nectar while
405 increasing the duration of hummingbirds' foraging bouts [77,78] and avoiding the negative
406 consequence of attractiveness since pollinators tend to abandon plants with high nectar variability
407 in their flowers [76,79]. Another advantage of uninformative floral signal-reward for plants is
408 that they may be less detected by nectar thieves, thereby reducing antagonistic relationships [80].

409

410 **(b) Linking floral phenotypic traits to network structure across elevations**

411 **(i) Patterns in network properties**

412 Although interaction networks are overall sensitive to changes in the local distribution of
413 mutualistic partners along altitudinal gradients [31,81], some authors have reported the absence
414 of elevation-driven changes in network metrics (eg. [82]). We found that ecological specialisation
415 of plant species was not related to elevation, suggesting that the level of pollinator niche
416 partitioning remained similar along the gradient. Instead, closeness centrality increased with
417 elevation, which indicates that plant species occupy more central positions in the network, i.e.

418 they are well connected to the other co-flowering plants via shared pollinators. Central plants are
419 often the most abundant and generalist ones [58,83]. Generalisation has been proposed to make
420 plant species less susceptible to the loss of any particular pollinator species allowing them to face
421 pollinator resource fluctuation or scarcity [84]. Thus, generalist plant species positioned centrally
422 in the network may have a great advantage in highland ecosystems with highly variable and
423 unpredictable environmental conditions affecting their pollinators [85].

424

425 **(ii) Relationship between phenotypic and ecological specialisation**

426 Plant species can have phenotypically specialised flowers, e.g. with long-corolla, that impose
427 restricted access to nectar reward, thereby narrowing their pollination niche [26] as only certain
428 pollinators may be able to use these flowers, e.g. long-billed hummingbirds [47,86]. Contrary to
429 our expectations, we did not find increased ecological specialisation or reduced centrality in
430 interacting plant species with morphologically specialised flowers. These results may be related to
431 the fact that hummingbirds can extend their tongue beyond the bill tip and thereby can access nectar
432 from flowers longer than their bill. Thus, short-billed hummingbirds can overcome morphological
433 barriers and visit plant species with specialised morphology, especially because long-corolla
434 flowers may have high nectar volume, as found in some studies [15,21]. Therefore, phenotypic
435 specialisation in flowering plant species does not necessarily mean that they are ecologically
436 specialised or occupy a peripheral position in the network [22]. Hummingbird tongue might be an
437 important trait in mediating interactions with flowering plants. Hence, we highlight the need to
438 consider this trait in future studies, for which empirical data are urgently needed, in order to
439 improve knowledge on how species morphology contributes to the realisation of plant-
440 hummingbird interactions.

441

442 **(c) Untangling the role of flower morphology and nectar on species interactions**

443 Studies simultaneously addressing the role of flower morphological and nectar traits on plant-
444 pollinator interaction networks are remarkably scarce (but see [18,87]). A recent study based on
445 experimental data, suggests that visitation rates of long-billed hummingbirds to long-corolla
446 flowers might be influenced more by nectar properties than by flower morphology [20]. We
447 found the opposite; interaction frequency increased when the hummingbird's bill and flower
448 corolla had similar length, indicating morphological matching, whereas nectar traits were
449 unrelated to interaction frequency, which is consistent with [87]. The match between pairs of
450 corresponding morphological traits not only improves the access by pollinators to floral rewards
451 but also benefits the partners by increased pollinator feeding efficiency and plant pollination
452 success [5,6,88]. In plant-hummingbird interactions, the match in bill-corolla length in particular,
453 is a major factor influencing visitation of hummingbirds to plants at both local [3,49] and large
454 scale [13,29]. Notably, the influence of trait matching on interactions did not depend on the
455 environmental context since it was consistent across elevation. In a broad sense, our study
456 supports the idea that morphological matching is an important factor shaping pollination
457 networks promoting pollinator species coexistence, whereas floral nectar appears to play a
458 secondary role in structuring these networks.

459

460 **Data accessibility**

461 Data and scripts to analyse the data are available at Dryad [89].

462 Supplementary material is available online [90].

463

464 **Declaration of AI use**

465 We have not used AI-assisted technologies in creating this article.

466

467 **Authors' contributions**

468 M.A.M.: conceptualisation, data curation, formal analysis, investigation, methodology, project
469 administration, supervision, writing – original draft. E.Br.: conceptualisation, data curation,
470 formal analysis, investigation, methodology, project administration, visualisation, writing –
471 review and editing. N.C.-E., K.Z. and A.C.J.: investigation, writing – review and editing. E.Ba.
472 and F.F.: conceptualisation, data curation, methodology, writing – review and editing. C.H.G.:
473 conceptualisation, funding, supervision, validation, writing – review and editing. All authors gave
474 final approval for publication and agreed to be held accountable for the work performed therein.

475

476 **Conflict of interest declaration**

477 We declare we have no competing interests.

478

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493

494 **Footnotes**

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752 **Table 1.** Model selection and averaging results of the full generalised linear mixed model to test
753 the relationship between plant-hummingbird interaction frequency and predictor variables along
754 an elevation gradient in Costa Rica. These predictors are species morphology (mismatch in bill-
755 corolla length, barrier (B) and its interaction term, and mismatch in bill-corolla curvature) and
756 nectar volume. Shown are incidence rate ratios calculated as the exponent of coefficients (e^β)
757 from minimal adequate models ($\Delta AICc < 2$) for each predictor variable and the level of
758 significance in bold (indicated with asterisks * $p < 0.05$ and *** $p < 0.001$). For example, an IRR
759 of 0.55 for the predictor mismatch in bill-corolla length indicates that an increase in this variable
760 decreases the interaction frequency by 45%. Marginal R^2 is shown as a goodness of fit statistic
761 for each of the models.

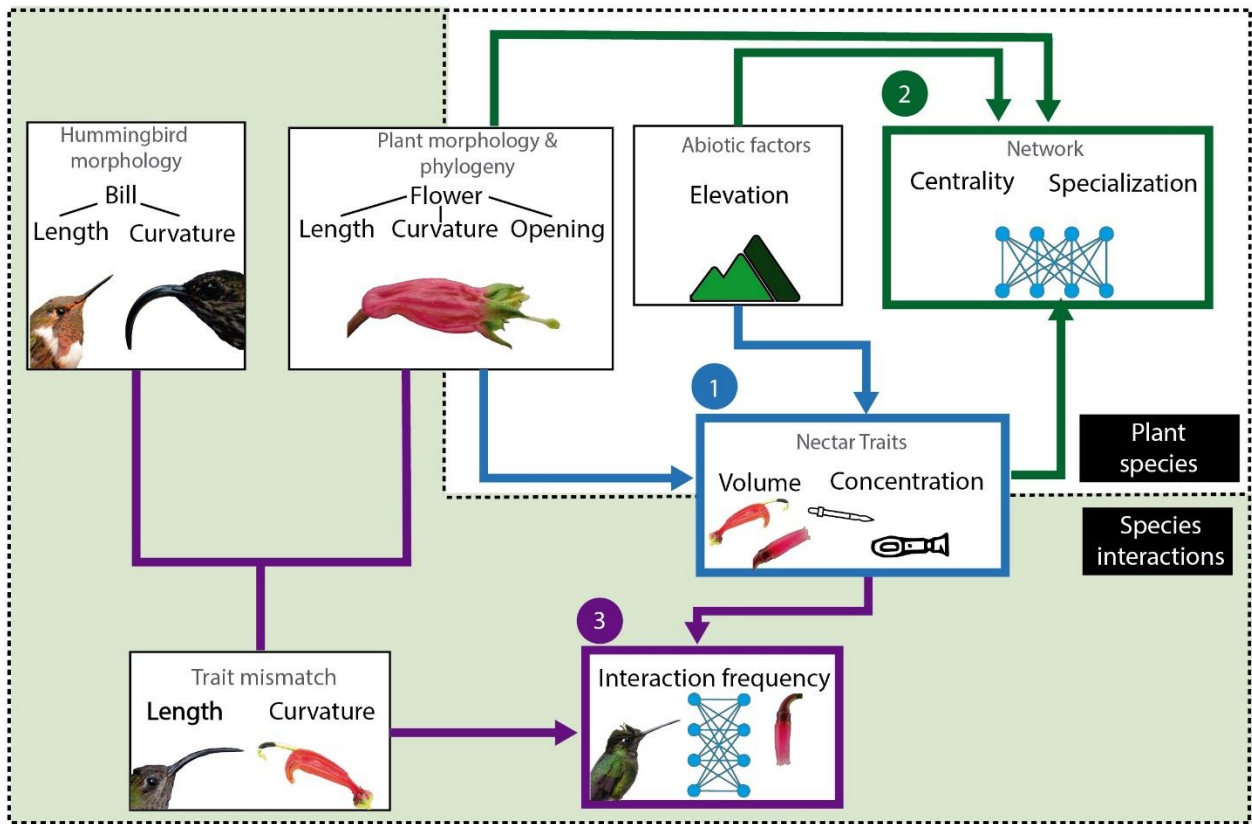
Model	Mismatch Length	Barrier	Mismatch Length \times B	Mismatch Curvature	Nectar Volume	R^2_m
1	0.73	0.49*	0.59*	0.83	1.16	0.17
2	0.7	0.51*	0.64			0.15
3	0.74	0.47*	0.56*	0.81	1.16	0.18
4	0.55***	0.64				0.11
5	0.71	0.50*	0.63		1.12	0.16

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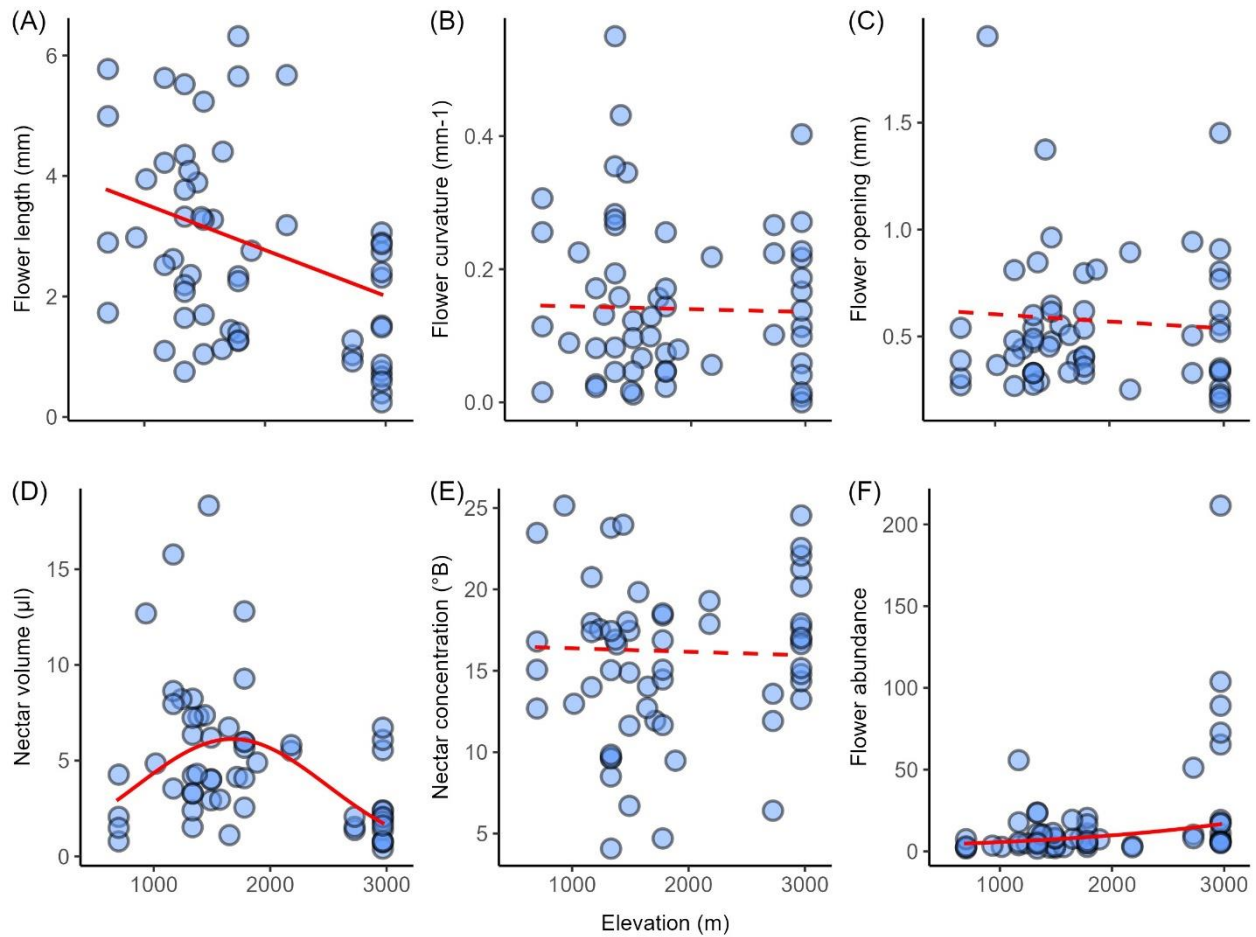


766

767 Figure 1. Conceptual diagram showing the relationships between factors influencing plant-
 768 hummingbird mutualistic interactions along an elevation gradient addressed in our study. We aim
 769 to (1) assess patterns in floral phenotypic traits and flower abundance of plant species across
 770 elevations and how they are related to each other (blue); (2) analyse the influence of floral
 771 phenotypic traits and elevation on ecological specialisation and centrality of plant species in the
 772 networks (green); (3) analyse the relative importance of species morphology and nectar traits in
 773 driving interactions (purple). Aims 1 and 2 (white background) focus on analysis regarding plant
 774 species, whereas aim 3 (green background) focuses on the species interactions. To facilitate
 775 interpretation, we used the same colour for the aims here and for the figures in the results section.

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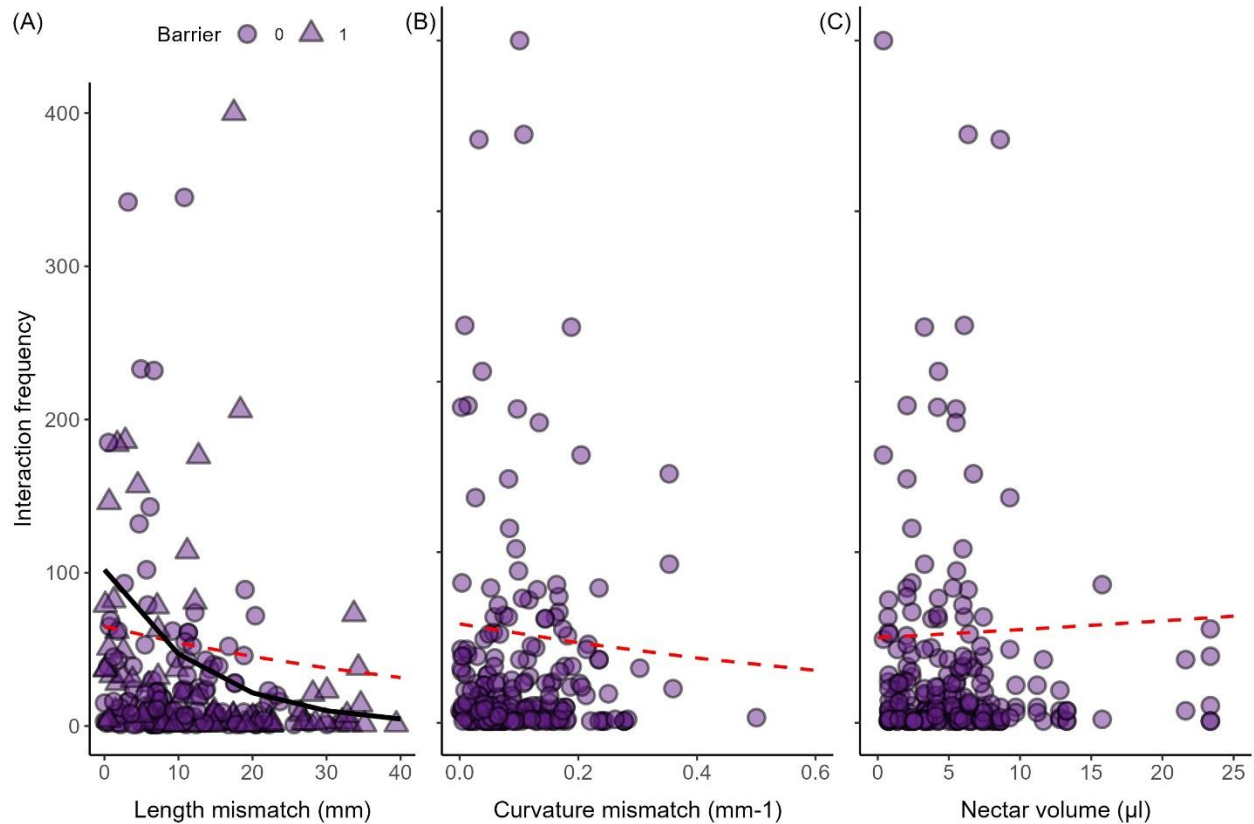
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778
 779 Figure 2. Variation in floral phenotypic traits and flower abundance of plant species visited by
 780 hummingbirds along an elevation gradient in Costa Rica. Dots represent mean values of floral
 781 morphological traits (A-C) and nectar traits (D-E) across flowers and individuals of plant species
 782 and total number of flowers per plant species (F) across transects and sampling periods at a given
 783 study site. As nectar volume, corolla opening and flower abundance were log-transformed, we
 784 report back transformed mean values for these variables. We used the predicted values from
 785 univariate phylogenetic generalised least squares (average estimates across 1000 models) to fit a
 786 trend line in each plot, where solid lines depict a statistically significant association between
 787 floral traits or flower abundance and elevation ($P < 0.05$) and dashed lines represent non
 788 statistically significant associations. Due to the hump-shaped relationship between nectar volume

789 and elevation, we added an additional quadratic term of nectar volume to the phylogenetic
790 models (i.e. a fitted polynomial model).

791



792

793 Figure 3. Averaging results of model selection from generalised linear mixed models to test the
 794 relationship between plant-hummingbird interaction frequency and predictor variables along an
 795 elevation gradient in Costa Rica. Automated selection provided five minimal adequate models
 796 where all of them included mismatch in bill-corolla length and barrier to nectar access as
 797 predictor variables, and four of them included the interaction term between plant-hummingbird
 798 interaction frequency and barrier (A); two of these models included mismatch in bill-corolla
 799 curvature (B) and other two models included nectar volume (C), (Table 1). Circles represent total
 800 interaction frequency between one plant and one hummingbird species at a given study site. For
 801 mismatches in bill-corolla length, we used triangles to depict when interactions occurred in the
 802 presence of a barrier. We used the average of the predicted values to fit a trend line in each plot,
 803 where solid lines depict a statistically significant association between interaction frequency and a

804 given predictor ($P < 0.05$) and dashed lines represent non statistically significant associations. In
805 A, the red line represents the predicted values for mismatch in bill-corolla length with barrier = 0,
806 and the black line with barrier = 1.
807