




Evolutionary and ecological factors structure a plant–bumblebee network in a biodiversity hotspot, the Himalaya–Hengduan Mountains

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Funding information

the Yunling Scholarship of Yunnan Provincial Government, Grant/Award Number: YLXL20170001; the China Scholarship Council, Grant/Award Number: 201804910805; Strategic Priority Research Program of the Chinese Academy of Sciences, Grant/Award Number: XDB31000000; the Joint Fund of the National Natural Science Foundation of China–Yunnan Province, Grant/Award Number: U1502261; the National Natural Science Foundation of China, Grant/Award Number: 31700361 and 32071670

Handling Editor: Alison Brody

Abstract

1. Species interactions, such as those between plants and pollinators, are known to be shaped by both evolutionary history and ecological factors. However, little is known about how multiple factors (e.g. phylogeny, phenology, abundance and functional traits) interactively affect interaction patterns.
2. Using a plant–bumblebee network comprising 2,428 interactions between 29 plant species and 12 bumblebee species in the Himalaya–Hengduan Mountains, we tested for phylogenetic signal and whether phylogenetic pattern was explained by abundance, phenology and 13 plant and bumblebee functional traits. We also tested whether trait matching in two pairs of plant–bumblebee traits explained interaction frequencies at both species and individual levels.
3. The network showed significant phylogenetic signal; closely related bumblebees tended to visit similar sets of plants, but not vice versa. Among all the measured factors, nectar volume and sugar concentration, rather than other phylogenetically constrained factors, were most important for explaining phylogenetic pattern (64% and 54% respectively). Although long–tongued bumblebee species tended to interact with long–tubed flowers, trait matching did not predict short–tongued bee interaction frequencies. Despite this, trait matching was apparent at the level of individual bees, reflecting intraspecific variation in tongue length and body size. Different selection pressures may exist within this bumblebee community, resulting in specialized, co–evolved traits in long–tongued species and adaptive generalization in short–tongued species to gain access to a variety of flowers.
4. Together, these findings contribute to our understanding of phylogenetic trait–based structure in species interaction networks. In particular, this study provides new evidence for the importance of nectar rewards in structuring interaction patterns in pollination mutualisms. The results also demonstrate that trait matching may occur at the individual level, despite not being detectable at the species level, and underline the necessity of taking intraspecific trait variation into account in studies of community structure.

KEYWORDS

bumblebees, functional traits, Himalaya–Hengduan Mountains, phylogenetic signal, plant–pollinator interactions, trait matching

1 | INTRODUCTION

Investigating the potential evolutionary and ecological mechanisms underlying species interaction patterns in networks is critical for understanding the determinants of community assembly and how species and communities may respond to disturbances (Bascompte & Jordano, 2007; Dormann et al., 2017). Network structure results directly from individual interactions of species, which are driven by a combination of neutral and niche–based processes. Neutral hypotheses presume that species with higher abundances have more interaction partners and higher interaction frequencies than rare species (Krishna et al., 2008; Vázquez et al., 2007). Niche–based processes, such as forbidden links and trait matching, assume that the ecological traits of species constrain their interaction patterns (Maglianesi et al., 2014; Maruyama et al., 2014). As species are not independent but rather coexist in a community, the above two processes are influenced by community structure (species composition, richness and relative abundance), which is directly affected by species' phenotypes or historical biogeographical processes (Vázquez, Blüthgen, et al., 2009). Phylogenetically related species often display more similar phenotypes; thus, evolution can play a role in shaping network structure by imprinting a phylogenetic signal (Ives & Godfray, 2006; Rezende et al., 2007; Webb et al., 2002).

A growing body of literature has incorporated one or a few mechanisms to explain species interaction patterns (Sazatornil et al., 2016; Vázquez, Chacoff, et al., 2009), and found that when incorporating multiple determinants, network structure could be more fully explained (Dormann et al., 2017; Stang et al., 2007). However, we still lack knowledge about how multiple factors interactively affect network structure in a phylogenetic context, that is, to what extent species interaction patterns correlate with abundance, phenology and functional traits after accounting for phylogenetic effects (Ives, 2018; Li & Ives, 2017). This is important because phylogenetically based approaches inform our understanding of community assembly processes and can help predict community changes (Ives & Godfray, 2006; Peralta, 2016; Rafferty & Ives, 2013).

A typical flower is a multi–sensory advertisement that uses a variety of visual, olfactory and tactile stimuli to attract and reward pollinators in return for the service of pollen transfer (Raguso, 2004). Previous studies have shown that bumblebees exhibit innate preferences for flowers of certain colours, flowers that are larger and those that are bilaterally symmetrical (Raine & Chittka, 2007; Rodríguez et al., 2004). Via associative learning, bees may prefer floral signals that are associated with high rewards in a community (Schiestl & Johnson, 2013). Trait matching, the morphological fit between shape and length of floral

tubes and pollinator mouthparts, has been frequently recognized as key in some specialized systems (Sazatornil et al., 2016; Sonne et al., 2020). For example, length and curvature of hummingbird bills and flower tubes closely matched, structuring their interaction patterns (Maglianesi et al., 2014). However, it remains unclear whether trait matching applies to generalist pollination systems (e.g. plant–bumblebee networks), where multidimensional traits may affect bipartite interactions (Leonard et al., 2011), and pollinators with small body size may be able to enter flower tubes, thus weakening the degree of overlap between corresponding traits. Moreover, previous studies have assessed trait matching via discrete or mean trait values assigned at the species level, neglecting the possibility that plant–pollinator interactions are based on continuous traits that vary within species, thereby structuring interactions at the individual level (Bolnick et al., 2011; Szegeti et al., 2020).

Bumblebees (Apidae: *Bombus*) are of both great ecological importance for wildflower pollination and great economic importance for crop pollination, due to their general foraging patterns and adaptation to cold environments (Woodard, 2017). Wild bumblebee populations are experiencing declines due to multiple interacting factors, such as habitat loss and climate change (Goulson et al., 2015). However, plant–bumblebee interaction networks have seldom been studied in the mountains around the Qinghai–Tibetan Plateau, the principal hotspot for bumblebee species diversity world–wide (Funamoto, 2019). Bumblebees act as the only hub pollinator in comprehensive networks and are highly connected to many other species (50% of forb species at subalpine meadows and more at higher elevations; Fang & Huang, 2012; Zhao, 2016). Some species–rich plant groups (e.g. *Pedicularis* spp.) in this region are entirely dependent on bumblebees for reproduction (Liang et al., 2018).

Here, we used an approach that incorporates the phylogenies of both plants and bumblebees along with abundance, phenology and 13 floral and bumblebee functional traits to address the questions: (a) Is there phylogenetic signal in this plant–bumblebee network? Specifically, we asked whether closely related bumblebee species were more likely to visit the same plant species or vice versa. (b) To what extent are plant–bumblebee interactions explained by multiple ecological factors (functional traits, abundance and phenology) while accounting for phylogenies? We predicted that ecological factors that show significant phylogenetic signal themselves would explain interaction patterns. (c) Do traits between bumblebees and flowers match at both the species and individual levels? As species exhibit intraspecific trait variation, we predicted that trait matching would explain interaction patterns at the individual level.

2 | MATERIALS AND METHODS

2.1 | Study system

The study was conducted within the field station of the Lijiang Alpine Botanic Garden, Yulong Snow Mountains, Lijiang, north-west Yunnan, China (27°0'8.84''N, 100°10'49.86''E, c. 3,240 m a.s.l.). At approximately the same elevation ($3,233 \pm 38.5$ m, mean \pm SD), we selected five subalpine meadows (sites A–E, named according to the distance from the field station; Figure 1) that were 0.78 ± 0.38 km apart and 0.26 ± 0.23 ha in area. Although the meadows were separated by *Pinus–Quercus* forests, according to a radiotracking study of bumblebee flight distances, *Bombus terrestris* workers can fly a maximum of 2.5 km from the nest (Hagen et al., 2011), so bumblebees are considered free to forage between the five meadows. Within each meadow, we established three parallel transects (2 m \times 30 m) that were separated from one another by 20–40 m according to the size of meadows. Eight 1 m \times 1 m quadrats were established at 10-m intervals along each transect. In total, we sampled 24 fixed quadrats at each site, and 120 quadrats in total for all five sites.

We conducted six censuses of plant–bumblebee interactions from 12 July 2016 to 2 September 2016. Census intervals ranged from 9 to 11 days because some weather conditions (e.g. heavy rain) prevented bumblebee observation. Each census was carried out over 2 days, the first day for sites A–C which were located near the field station and the second day for sites D and E (Figure 1). We varied the first meadow sampled at each census and sampled subsequent meadows in haphazard order to reduce bias. During each census, flower numbers of every forb species in the quadrats were recorded, and flower numbers in the Asteraceae family were counted as inflorescence numbers. Two observers walked along the transects at a slow and steady pace to collect bumblebees once the bees were observed contacting either anthers or stigmas for more

than 1 s (i.e. potential pollinators; Gibson et al., 2011). In case there was daily temporal niche differentiation among bumblebee species, we divided the daytime into four periods—7:00–9:00, 9:00–12:00, 12:00–15:00 and 15:00–18:00—and ensured there was equal sampling effort at each site during each period. In total, the sampling time was approximately 10,800 min (360 min \times 6 censuses \times 5 sites). Sampling completeness of the full network as gauged by the Chao 2 estimator was 45.37% (see Supporting Information ‘Sampling completeness’ for details; Chacoff et al., 2012).

2.2 | Bumblebee collection and identification

Bumblebees were collected along the transects on sunny days. The time at which each bumblebee was collected and the identity of the flower species it was visiting were recorded. Each bee was put into a centrifuge tube upon collection and was later pinned and labelled.

Bumblebees exhibit striking inter- and intraspecific variation in colour pattern (Figure S1), which makes it difficult to identify individuals by coat patterning alone. In addition, taxonomic data on the bumblebee species of south-west China are lacking. We therefore identified bumblebee specimens by combining morphological characteristics and DNA sequences. Reference specimens of more than 100 bumblebee species were studied in the National Animal Collection Resource Center, Institute of Zoology, Chinese Academy of Sciences to distinguish species by morphological characteristics (Figure S2). Then, DNA barcoding (CO1 gene) was performed for 190 specimens which consisted of five to 20 specimens in each morpho-group. Finally, the sequences were aligned with the NCBI database to obtain species-level identities, and a subset of specimens was validated by taxonomists (Paul H. Williams of the Natural History Museum, UK and Jian Yao of the Institute of Zoology, Chinese Academy of Sciences).



FIGURE 1 Map and photograph of study sites. The left map shows relative location of the LABG field station and five study sites (sites A–E). The right photograph shows a view of site A

2.3 | Flower and bumblebee abundance, phenology and functional traits

2.3.1 | Abundance of flowers

For each forb species, flower abundance data at each census were summed across all 120 quadrats. Mean flower abundance was calculated as the average value of six censuses. As plant abundance was not available for 10 plant species (which were not growing in quadrats but were observed interacting with bumblebees during transect walking sampling), their mean abundance values were given as 0.1 during analysis (for comparison, the plant species with the lowest abundance in the quadrats was *Dolomiaea georgii*, which had a mean abundance of 1.33). Furthermore, the species represent eight different families and are distributed across the plant phylogeny. Based on our non-systematic observations, these 10 species were in fact rare in these subalpine meadow communities.

2.3.2 | Phenology

Quadrat data along with field observations were used together to estimate flowering phenology. For each species, floral abundances were recorded every 10 days for all 120 quadrats. For plant species which were scattered and not in the quadrats, we estimated their phenology from bumblebee foraging bouts (more than 400 records) which were recorded during the whole field investigation. Information concerning which plant species were visited by bumblebees at each census was applied as well. Two indices, peak flowering time and flowering duration, were used for evaluating flower phenology. For bumblebees, peak abundance time was inferred from bee collection at every census.

2.3.3 | Functional traits

For bumblebee traits, we measured 17–156 specimens of each species (Table 1). The measured bee traits (tongue length and body size) influence access to floral resources (Harder, 1985). For each forb species, all traits were measured on at least 30 flowers or inflorescences of different individual plants, and all measured plant morphological traits are known to be important for rewarding (nectar volume, sugar concentration, pollen size and quantity), attracting (colour, size, height, symmetry) or filtering pollinators (flower tube depth, nectar accessibility, stamen location; Fornoff et al., 2017); correlations of plant traits are given in Figure S3. Detailed methods for measurements of all traits can be found in Supporting Information: 'Methods of measuring functional traits'. A full list of all measured factors and their ranges/levels is given in Table 2.

2.4 | Construction of plant and bumblebee phylogenetic trees

Of 52 forbs in five meadows, 29 species (belonging to 15 families and 27 genera) were observed interacting with bumblebees (Figures 2 and 3). We obtained genetic sequences (ITS, matK, rbcL) for these 29 plant species from an iFlora database of the Germplasm Bank of Wild Species, Kunming Institute of Botany. Sequences were aligned using MAFFT, followed by manual refinement using Geneious version 8. We estimated a maximum likelihood phylogeny with RAxML in the website CIPRES, using the GTRGAMMA model and 1,000 bootstrap replicates. For bumblebees, we used the CO1 sequences to construct a phylogenetic tree in the same way. We used APG IV (Chase et al., 2016) and a *Bombus* genus-level phylogeny (Cameron et al., 2007) to constrain the plant and bumblebee tree respectively.

<i>Bombus</i> species	Subgenus	N_t	Tongue length (mm)	Tongue length category	N_s
<i>B. securus</i> Frison	<i>Megabombus</i>	42	15.64 ± 2.29	Long	12
<i>B. funerarius</i> Smith	<i>Orientalibombus</i>	48	13.14 ± 2.28	Long	32
<i>B. impetuosus</i> Smith	<i>Thoracobombus</i>	27	9.25 ± 1.12	Medium	26
<i>B. remotus</i> Tkalcu	<i>Thoracobombus</i>	43	9.06 ± 1.05	Medium	47
<i>Bombus</i> sp.	<i>Thoracobombus</i>	17	9.52 ± 1.23	Medium	2
<i>B. grahami</i> Frison	<i>Alpigenobombus</i>	58	8.04 ± 0.94	Medium	117
<i>B. friseanus</i> Skorikov	<i>Melanobombus</i>	156	7.44 ± 0.74	Short	1,657
<i>B. festivus</i> Smith	<i>Melanobombus</i>	62	8.54 ± 0.84	Medium	20
<i>B. lepidus</i> Skorikov	<i>Pyrobombus</i>	73	6.80 ± 0.97	Short	446
<i>B. avanus</i> Skorikov	<i>Pyrobombus</i>	38	7.75 ± 1.07	Short	45
<i>B. infrequens</i> Tkalcu	<i>Pyrobombus</i>	30	8.39 ± 1.08	Medium	15
<i>B. longipennis</i> Friese	<i>Bombus</i>	21	6.84 ± 0.95	Short	9

TABLE 1 *Bombus* species identified in the study sites, their subgenera, number of samples for tongue length measurement (N_t), tongue length (mean ± SD), tongue length category and total number of specimens collected (N_s)

Note: *Bombus* sp. is the new species we found in this study. For species with fewer than 30 specimens, we measured all available specimens and additional specimens from another study at the same field sites (unpublished data).

TABLE 2 Fourteen plant and three bumblebee ecological factors, their individual phylogenetic signal and the extent to which they explain the phylogenetic attraction pattern in the full plant–bumblebee interaction network

	Ecological factor	Units/coding	Range/levels	Pagel's λ	Blomberg's K	Residual phylogenetic variance	Decrease in phylogenetic attraction
Plants	Pollen grain quantity	numeric	399–256,350	0	0.791	1.021	1.35%
	Pollen size	μm^3	739–313,959	1.354**	0.950*	1.022	1.26%
	Nectar volume	μl	0–3.31	0	0.871	0.372***	64.06%
	Sugar concentration	%	0–63.6	0	0.972	0.476**	54.01%
	Flower tube depth	mm	0–22.27	1.347**	1.072*	0.884	14.59%
	Inflorescence area	mm^2	12.95–2,363.77	1.34**	0.926	1.01	2.42%
	Flower height	cm	7.6–115	1.021	0.832	1.031	0.39%
	Stamen location	1/0	Inside/outside	1.357***	1.5***	0.971	6.18%
	Nectar accessibility	1/0	Open/concealed	1.357***	1.265**	0.859	17.00%
	Flower symmetry	1/0	Radial/bilateral	1.357***	1.405***	0.912	11.88%
	Flower colour	1/0	Peak/or not	– (b)	–	0.982	5.12%
	Mean abundance	numeric	0.1–4,984.67	0	0.675	1.215	–17.39%
	Peak flowering time	days ^(a)	10–60	0	0.728	1.025	0.97%
	Flowering duration	days	10–60	0	0.761	1.02	1.45%
Bumblebees	Tongue length	mm	6.8–15.64	2.048**	1.129*	0.971	6.18%
	Intertegular span	mm	4.52–5.94	1.766*	1.223*	1.025	0.97%
	Peak abundance time	days	10–60	2.055***	1.406**	1.057	–2.13%

Note: Significant results are given in bold; values for factors that did not reduce the residual variance are given in italics. (a) Phenology was coded from 10 to 60, referring to the time of each census 1–6. (b) Flower colour was classified into four binomial categories (UV, blue, yellow and red), so phylogenetic signal was calculated individually. Only yellow showed significant phylogenetic signal (Blomberg's $K = 1.012$, $p = 0.014$).

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

All the plant and bumblebee sequences are available on NCBI, and their GenBank accession numbers are listed in Dryad Digital Repository.

2.5 | Construction of plant–bumblebee interaction network

Based on our field sampling across all sites and time points, we constructed a quantitative interaction network using the R package *BIPARTITE* (Dormann et al., 2008). The strength of each interaction was the number of bumblebees collected from each plant species. Because each module includes species groups which are taxonomically or functionally related (e.g. convergent phenology or phenotype; Olesen et al., 2007), network modularity was quantified. We used the most common algorithm *QuanBiMo* in *BIPARTITE* to detect modularity (Dormann & Strauss, 2014). As the algorithm is a stochastic process and results may vary among runs, we retained the optimal modular configuration after 30 independent runs (the modules with highest values; Dormann & Strauss, 2014). A null model comparison was then used to standardize the observed modularity value to a Z-score by the functions *r2dtable* and *vaznull*; values ≥ 2 indicate significant modularity (Dormann & Strauss, 2014). To evaluate the probability of module membership

based on flower tube length, we used multinomial logistic regressions with module identity as the response variable and tube length as the predictor variable (Morente-López et al., 2018). Likelihood ratio (LR) χ^2 tests were used to evaluate goodness-of-fit of the models. Models were fitted using R package *NNET* (Venables & Ripley, 2021).

2.6 | Statistical analysis

To test for phylogenetic signal in the plant–bumblebee interaction network and determine which of the 17 factors (abundance, phenology and functional traits) exhibit phylogenetic signal, we used the function *communityPGLMM* in the R package *PHYR* to construct phylogenetic generalized linear mixed models (PGLMMs). These models can incorporate both hierarchical and phylogenetic covariance matrices, and flexibly combine phylogenies and traits to provide an integrated, quantitative framework for analysing ecological networks (Ives, 2018). If ecological factors are responsible for the phylogenetic signal between plants and bumblebees, then incorporating information about these factors should remove the phylogenetic residual variation. Following the methods of Li et al. (2017), we added each factor as both fixed and random terms to test if they can explain phylogenetic patterns. As each factor was added



FIGURE 2 Different bumblebee species visiting wildflowers from 15 families and 27 genera in the study system. The four most frequently visited species are shown in (a)–(d): *Pedicularis cephalantha*, *Prunella hispida*, *Phlomis atropurpurea* and *Halenia elliptica*. Other plants are as follows: (e) *Pedicularis densispica*, (f) *Lotus corniculatus*, (g) *Astragalus camptodontus*, (h) *Astragalus Pullus*, (i) *Saussurea yunnanensis*, (j) *Ligularia lidjiangensis*, (k) *Dolomiaea georgii*, (l) *Dipsacus asper*, (m) *Delphinium delavayi*, (n) *Strobilanthes versicolor*, (o) *Gentiana yunnanensis*, (p) *Primula poissonii*. Note that bumblebees can get into flower tubes and obtain nectar at the bottom as shown by the red arrow in photograph (n). Photograph (a) by Robert A. Raguso, (d) and (l) by Zong-Xin Ren and (p) by Hai-Dong Li. All other photographs by Huan Liang

separately, multicollinearity did not interfere. Before analysing, we log-transformed continuous factor data and then Z-transformed factor values to have means of 0 and standard deviations of 1, allowing coefficients in the models to be interpreted as effect sizes.

To investigate if individual ecological factors that may account for phylogenetic patterns in the network showed phylogenetic signal themselves, we used two metrics, Pagel's λ and Blomberg's K . The analyses were conducted using the R packages `PHYTOOLS` (Revell, 2012) and `PICANTE` (Kembel et al., 2010) respectively.

To investigate if trait matching at the species level explained plant–bumblebee interactions, we used fourth-corner analysis, which was originally used to assess species trait responses to

environmental variation but can be extended to network data to test if morphological trait matching explains interaction patterns (Dray et al., 2014; Maglianesi et al., 2014). In our analysis, we used species-level mean trait values to construct three matrices: a plant–bumblebee interaction matrix (**L**), a flower trait matrix (**R**) and a bumblebee trait matrix (**Q**). The fourth-corner approach combines matrices **L**, **R** and **Q** into a single matrix describing plant and bumblebee trait associations (fourth-corner matrix). Significance was tested using 49,999 Monte Carlo permutations based on the permutation model 6 for better control of type I errors (Ter Braak et al., 2012). The analysis was conducted using the R package `ADE4` (Dray & Dufour, 2007).

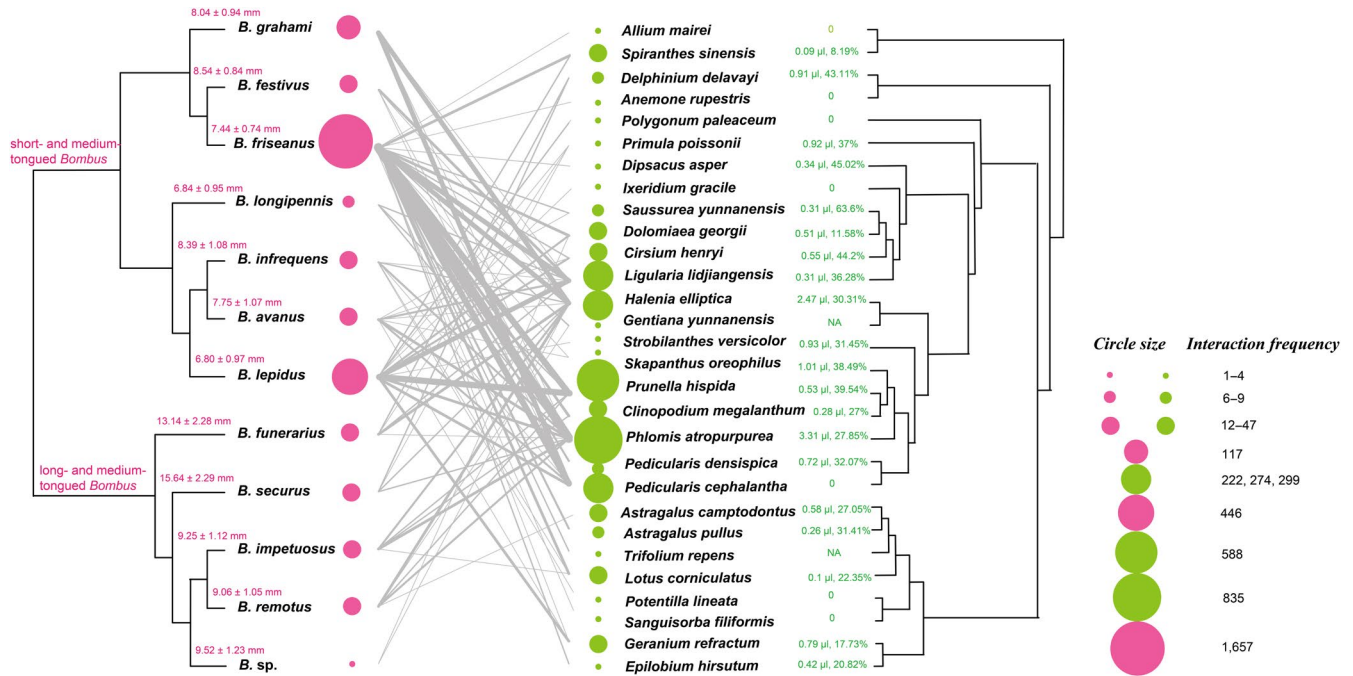


FIGURE 3 Phylogenetic relationships for 12 *Bombus* species (left) and 29 plant species (right). The middle grey links are interactions between bumblebees and plants, line thicknesses are proportional to the number of interactions. Circle sizes (pink for bumblebees and green for plants) and corresponding interaction frequencies are given. Tongue length (mean \pm SD) is given for each bumblebee species. As closely related bumblebees tend to visit similar sets of plants because of nectar traits, mean values of nectar volume (μ l) and sugar concentration (%) are given for each plant species

To determine if traits matched at the individual level, we built generalized linear mixed-effects models (GLMM), with each of two trait combinations analysed separately: individual bumblebee tongue length or body size as predictor variable, and mean flower tube depth or tube opening width as response variable (Anderson & Johnson, 2009; Szigeti et al., 2020). We included bumblebee species identity as a random effect. Because response variables were gamma-distributed, we used gamma distributions in the models (Nakagawa et al., 2017). In total, we used individual trait data from 463 bumblebees ($n = 11$ –100 individuals per species) in this analysis, conducted with the R packages LME4 (Bates et al., 2021) and EFFECTS (Fox & Weisberg, 2018). Because interaction frequencies of each bumblebee individual were not recorded, we could not use four-corner analysis, which requires a matrix L (interaction frequency), for our individual-level data.

3 | RESULTS

3.1 | Plant–bumblebee interactions

In total, we observed 2,428 interactions between 29 forb species and 12 bumblebee species across all five sites and six censuses (Figures 2 and 3). Except for *Trifolium repens*, the forb species are native to Lijiang Yulong Snow Mountains. All the bumblebees fell into eight subgenera and were classified into short-, medium- and

long-tongued categories (Table 1; Figure S4). Two specimens of bumblebees were identified as a new species (*Bombus* sp.), as they show distinct differences in CO1 sequences, morphological characters and male genitalia structure with their sister groups (*B. impetuosus* and *B. remotus*). This possible new species is supported by the investigation of this group by Williams et al. (unpublished data).

3.2 | Phylogenetic patterns in plant–bumblebee interactions

The full interaction network showed significant phylogenetic signal ($p < 0.001$), meaning that there was a strong interaction between the plant and bumblebee phylogenies. In addition, bumblebee interactions showed significant phylogenetic signal ($p < 0.001$), indicating that closely related bumblebees were more likely to visit the same plant species. However, plants did not show significant phylogenetic signal ($p = 1$); thus, closely related plant species did not tend to attract the same bumblebee species.

3.3 | Combining ecological factors to explain phylogenetic patterns

Among all the factors, two individual plant traits (nectar volume and sugar concentration) explained most of the variance among species

and therefore significantly reduced the phylogenetic signal (signal was reduced by 64.06% and 54.01% respectively; Table 2). Twelve factors related to both plants and bumblebees reduced the residual variance to different degrees, but not significantly. The remaining two factors (mean abundance of plants and peak abundance time of bumblebees; italicized in Table 2) did not reduce the residual variance, which means they could not individually explain phylogenetic patterns of plant–bumblebee interactions. Although nectar volume and sugar concentration were not available for *Gentiana yunnanensis* and *Trifolium repens*, this did not affect the conclusions. When we reanalysed a subnetwork excluding these two species, the results were still significant, with phylogenetic signal reduced by 59.08% (nectar volume) and 52.17% (sugar concentration).

Tests for phylogenetic signal in individual factors showed that the two factors (nectar volume and sugar concentration), which explained plant–bumblebee phylogenetic attraction patterns, did not show significant signal themselves (Table 2). However, six plant traits (pollen size, flower tube depth, inflorescence area, stamen location, nectar accessibility and flower symmetry) and three bumblebee factors (tongue length, body size and peak abundance time) showed significant phylogenetic signal in one or both metrics (Table 2).

3.4 | Trait matching of bumblebees and plants

Given phylogenetic patterns showed that closely related bumblebees were more likely to visit the same plant species, and bumblebee tongue length and body size were phylogenetically conserved (Table 2), it is plausible that the two traits could explain interaction patterns. However, neither trait significantly reduced the phylogenetic signal in the PGLMM residuals. On one hand, the module analysis showed that the matrix was divided into three modules (Figure 4), with a modularity value of 0.245 and Z-score of 39.14 (null model: 0.034 ± 0.005 , mean \pm SD), which indicate significant modularity. Multinomial logistic regressions showed that modularity was explained by flower tube depth (LR test: $\chi^2 = 14.89$, $df = 2$, $p = 0.0006$). Long-tongued bumblebees tended to visit long-tubed flowers (Figure 4; Figure S5).

On the other hand, fourth-corner analysis of species-level trait matching showed that, except for significant matching between flower height and tongue length ($p = 0.024$), none of the trait pairs matched ($p > 0.05$, Table S1). In this subalpine bumblebee community, long-tongued bumblebees accounted for only 1.81% of the total number, whereas short-tongued bees dominated, accounting for 88.84% of total bee abundance. Short-tongued bees tended to generalize across tube depths; for example, the short-tongued *B. friseanus* interacted with 80% of the plant species in our study sites (23 of 29 species). The five most long-tubed flowers have tube openings that are significantly wider than the bees' intertegular span (t tests: $p < 0.01$; Table S2). Thus, bumblebees can put not only their heads (Figure 2o) but also their bodies (Figure 2n) into the flower tubes and obtain the nectar at the bottom (Figure S6).

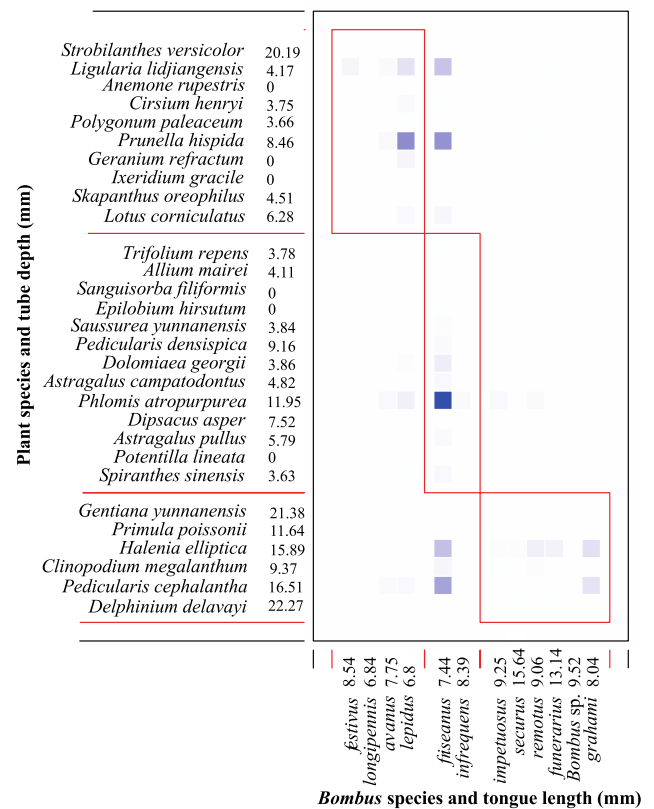


FIGURE 4 Module structure of the 29 plant and 12 bumblebee species interaction network. Intensity of blue shading represents the interaction frequency. Tube depth and tongue length for each plant and bumblebee species are shown alongside their names. Overall, long-tongued bumblebees tended to visit long-tubed flowers (the right module), but short-tongued bees generalized across tube depths, especially *Bombus friseanus* (the left and middle modules)

However, at the level of individual bees, we found evidence for significant positive relationships between both bumblebee tongue length and flower tube depth (GLMM: $\chi^2 = 4.76$, $p = 0.029$, Figure 5) and bumblebee body size and flower tube opening width (GLMM: $\chi^2 = 16.54$, $p < 0.001$, Figure 6), which suggests matching between these two trait pairs.

4 | DISCUSSION

To our knowledge, this is the first study to incorporate multiple evolutionary and ecological factors to explain plant–bumblebee interaction patterns in a biodiversity hotspot, the Himalaya–Hengduan Mountains. Given the important ecological role of bumblebees as pollinators and the fact that a new species was identified during our investigation, additional research in this biodiversity hotspot is needed, particularly as climate change may affect bumblebee distributions (Kerr et al., 2015) and disrupt trait matching between bumblebees and flowers (Miller–Struttman et al., 2015). Consistent with previous studies of plant–pollinator networks (e.g. Martín González

FIGURE 5 Relationship between tongue lengths of individual bumblebees and tube depths of visited flowers. Points represent each of the 463 bumblebee individuals, different colours represent different tongue length categories. 95% CI are marked with polygon. Black solid fitted line represents significant relationship ($p = 0.029$)

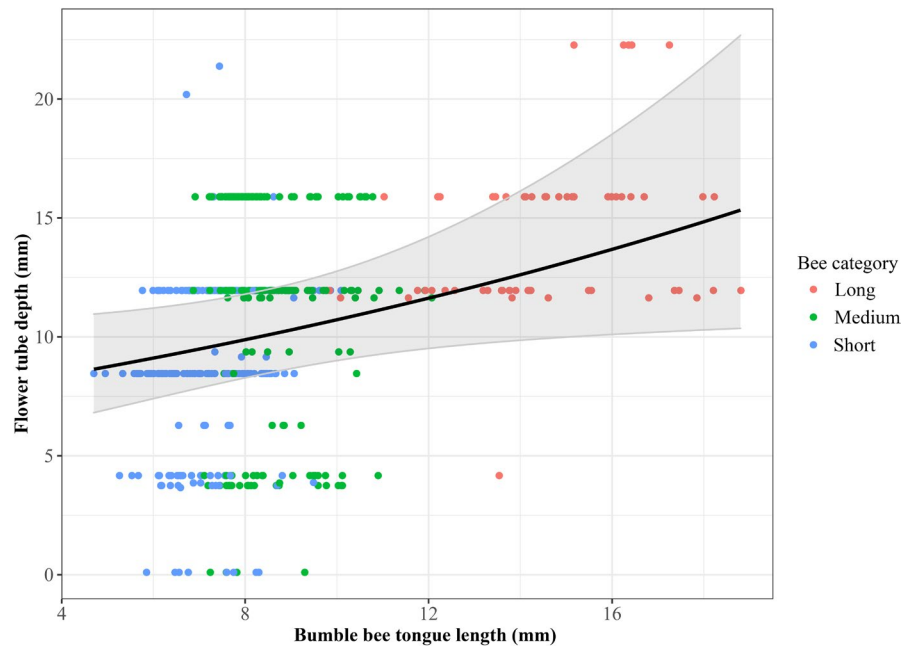
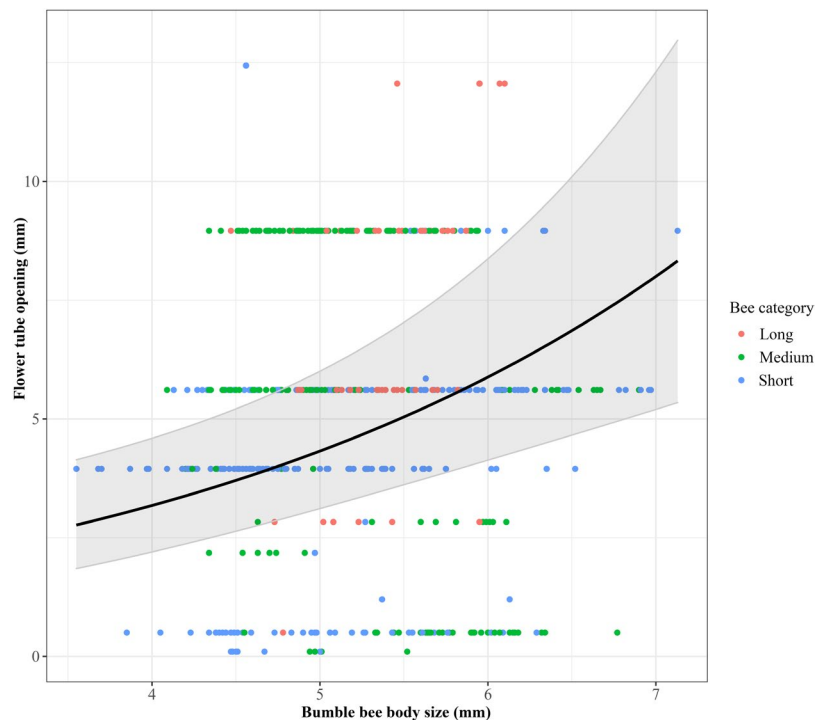


FIGURE 6 Relationship between body sizes of individual bumblebees and tube openings of visited flowers. Points represent each of the 463 bumblebee individuals, different colours represent different tongue length categories. 95% CI are marked with polygon. Black solid fitted line represents significant relationship ($p < 0.001$)



et al., 2015; Rafferty & Ives, 2013; Rohr & Bascompte, 2014), we found phylogenetic signal in this plant–bumblebee network. Our work is unique in showing that nectar traits, rather than other phylogenetically constrained factors, were responsible for this interaction pattern. We also highlight that trait matching may occur at the individual level, despite not being detectable at the species level, and underline the necessity of taking intraspecific trait variation into account in studies of community structure.

Bumblebees showed stronger phylogenetic signal than plants, and the reasons may be twofold. Firstly, we conducted a ‘partial network’ sampling approach in this study, which means other pollinators

of plants, such as honeybees and hawk moths, were not included. This may weaken the phylogenetic signal of plants when considering bumblebees as the only functional pollinator group. Secondly, phylogeny may have played a role in bumblebee interaction patterns, because traits are conserved and do not change rapidly over time, leading to a pattern of ‘taxonomic niche phylogenetic signal’ (Polidori et al., 2011). In contrast, closely related plant species in this system may vary in their traits to attract different bumblebees (i.e. divergent evolution), or distantly related plant species may have converged on traits to attract similar bumblebees (i.e. convergent evolution and pollination syndromes), which is referred to as ‘functional niche

phylogenetic signal' (Ibanez et al., 2016). Bee community assembly at high altitudes seems to be dominated by environmental filtering (Hoiss et al., 2012), and their mobility may limit direct competition for resources and allow more similar species to coexist (Harmon-Threatt & Ackerly, 2013). Competition might be a more important driver within the plant community as the phylogeny is not clustered, and traits are not conserved (Wolowski et al., 2017). In another partial network, however, phylogenetically related plants tended to share similar hummingbird pollinators (Martín González et al., 2015). Interspecific competition and resource partitioning among closely related pollinators may be higher in such specialized systems than in our generalized plant-bee system.

Closely related bumblebees tended to visit the same plants, not because they have similar tongue length and body size, but because those plants share similar nectar rewards. Nectar resources supply important energy for bumblebees and other pollinators, especially in subalpine and alpine regions where the environment is cold, and bees need energy to warm up quickly (Heinrich, 1979). In this study, both nectar volume and sugar concentration were important in explaining why closely related bumblebees tended to visit similar sets of plants, providing new evidence for the importance of nectar rewards as determinants of bumblebee flower choice and thus the structure of interaction networks (Klumpers et al., 2019). Meanwhile, the fact that these two factors did not themselves show significant phylogenetic signal could reflect the biotic filtering process (pollination niche), which may influence their distribution in phylogenies (Joffard et al., 2019). Additionally, microbial communities (e.g. yeasts) can affect standing crops of nectar (Herrera et al., 2008) and may have obscured any phylogenetic signal. Another explanation is that there is phylogenetic signal in parts but not all the phylogenies.

Trait matching, which can decrease handling time and thus increase interaction effectiveness, has been shown to be an important determinant of interaction patterns in various mutualistic systems (e.g. plant-hawkmoth, plant-hummingbird interactions; Maruyama et al., 2014; Sazatornil et al., 2016). For bumblebees, although some studies have found long-tongued bees, instead of short-tongued bees, tend to feed on a larger number of plant species because they have access to nectar in a greater variety of flowers (Harder, 1985; Ranta & Lundberg, 1980), other studies have come to the opposite conclusion (Inouye, 1980; Pyke et al., 2012). Our study supports the latter idea, which is probably a result of co-evolution between extremely long tongues and flower tubes for more efficient foraging (Sazatornil et al., 2016; Serrano-Serrano et al., 2017). Short-tongued bumblebees, however, had significant trait matching at the individual rather than species level. As short-tongued bumblebees overwhelmingly dominated, intraspecific competition was probably high (Pyke et al., 2012). Selection pressure may favour trait variation within species and adaptive generalization in short-tongued species to gain access to a variety of flowers (Suzuki et al., 2007). Altogether, trait matching was not apparent in our overall plant-bumblebee network but occurred at the species level (long-tongued bees) and at the individual level, highlighting the importance of considering

trait variation within species in studies of community ecology (Des Roches et al., 2018; Smith, 2019).

In the end, as none of the factors we examined explained all the phylogenetic signal, future studies may need to include other potentially important factors, such as flower scents and macronutrient composition/ratios in nectar and pollen, which are 'honest' indicators for attracting and rewarding bumblebees (Knauer & Schiestl, 2015; Vaudo et al., 2016). Additionally, further studies that investigate plant-bumblebee interactions at larger spatiotemporal scales and comparative studies that consider other generalized plant-pollinator systems would help to determine the generality of the patterns found here.

ACKNOWLEDGEMENTS

The authors sincerely thank Wan-Qin Chen, Dan-Dan Wang, Zheng Li, Xiao-Peng Guo and Chu Tao for assistance in fieldwork; Paul H. Williams and Jian Yao for help in bumblebee identification; De-Tuan Liu and Zhi-Kun Wu for plant identification; Anthony R. Ives, Fei Zhao and Zhe Chen for helpful data analysis suggestions; and Zhi-Rong Zhang and the Germplasm Bank of Wild Species for providing plant genetic sequences. They are grateful to all the staff of Yunnan Lijiang Forest Ecosystem Observation and Research Station for accommodation and support. This study was supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (XDB31000000), the Joint Fund of the National Natural Science Foundation of China-Yunnan Province (U1502261), the Yunling Scholarship of Yunnan Provincial Government (YXL20170001), the China Scholarship Council (CSC, 201804910805) and the National Natural Science Foundation of China (31700361, 32071670).

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHORS' CONTRIBUTIONS

H.W., D.-Z.L. and Y.-H.Z. conceived the ideas and designed the methodology; H.L., Z.-X.R. and L.Z. collected the data; H.L., N.E.R., Y.-H.Z. and H.-D.L. analysed the data; H.L., H.W. and N.E.R. led the writing of the manuscript. All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

DATA AVAILABILITY STATEMENT

Data are available from the Dryad Digital Repository <https://doi.org/10.5061/dryad.sf7m0cg34> (Liang et al., 2021).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Liang, H., Zhao, Y.-H., Rafferty, N. E., Ren, Z.-X., Zhong, L., Li, H.-D., Li, D.-Z., & Wang, H. (2021). Evolutionary and ecological factors structure a plant-bumblebee network in a biodiversity hotspot, the Himalaya-Hengduan Mountains. *Functional Ecology*, *35*, 2523–2535. <https://doi.org/10.1111/1365-2435.13886>