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### Authors

Liang, Huan  
Zhao, Yan-Hui  
Rafferty, Nicole E  
[et al.](#)

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# Functional Ecology

## RESEARCH ARTICLE

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

Huan Liang<sup>1</sup>, Yan-Hui Zhao<sup>1</sup>, Nicole E. Rafferty<sup>2,3\*</sup>, Zong-Xin Ren<sup>1</sup>, Li Zhong<sup>1</sup>, Hai-Dong Li<sup>4</sup>, De-Zhu Li<sup>5</sup>, Hong Wang<sup>1\*</sup>

<sup>1</sup> Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, PR China

<sup>2</sup> Department of Evolution, Ecology, and Organismal Biology, University of California, 900 University Ave., Riverside, CA 92521, USA

<sup>3</sup> Rocky Mountain Biological Lab, Crested Butte, CO 81224, USA

<sup>4</sup> State Key Laboratory of Integrated Management of Pest Insects and Rodents in Agriculture, Institute of Zoology, Chinese Academy of Sciences, Beijing, PR China

<sup>5</sup> Plant Germplasm and Genomics Center, Germplasm Bank of Wild Species, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, PR China

**\*Corresponding authors:** Prof. Hong Wang and Prof. Nicole E. Rafferty

Kunming Institute of Botany, Chinese Academy of Sciences

132 Lanhei Road, Kunming, Yunnan 650201, CHINA

Tel: +86-871-6522 3534

Fax: +86-871-6521 7791

Email: wanghong@mail.kib.ac.cn and nicole.rafferty@ucr.edu

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## **CONFLICT OF INTEREST**

The authors have no conflict of interest to declare.

## **AUTHORS' CONTRIBUTIONS**

HW, DZL and YHZ conceived the ideas and designed methodology; HL, ZXR and ZL collected the data; HL, NER, YHZ and HDL analysed the data; HL, HW and NER led the writing of the manuscript. All authors gave final approval for publication and agree 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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DR HUAN LIANG (Orcid ID : 0000-0003-3782-3724)

MR YAN-HUI ZHAO (Orcid ID : 0000-0002-7569-4591)

DR ZONG-XIN REN (Orcid ID : 0000-0001-7265-065X)

DR HAI-DONG LI (Orcid ID : 0000-0002-0789-7346)

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## RESEARCH ARTICLE

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

Huan Liang<sup>1</sup>, Yan-Hui Zhao<sup>1</sup>, Nicole E. Rafferty<sup>2,3\*</sup>, Zong-Xin Ren<sup>1</sup>, Li Zhong<sup>1</sup>, Hai-Dong Li<sup>4</sup>, De-Zhu Li<sup>5</sup>, Hong Wang<sup>1\*</sup>

<sup>1</sup> Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, PR China

<sup>2</sup> Department of Evolution, Ecology, and Organismal Biology, University of California, 900 University Ave., Riverside, CA 92521, USA

<sup>3</sup> Rocky Mountain Biological Lab, Crested Butte, CO 81224, USA

<sup>4</sup> State Key Laboratory of Integrated Management of Pest Insects and Rodents in Agriculture, Institute of Zoology, Chinese Academy of Sciences, Beijing, PR China

<sup>5</sup> Plant Germplasm and Genomics Center, Germplasm Bank of Wild Species, Kunming

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Institute of Botany, Chinese Academy of Sciences, Kunming, PR China

**\*Corresponding authors:** Prof. Hong Wang and Prof. Nicole E. Rafferty

## 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 2428 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-bumble 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

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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

## 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, Fründ & Schaefer 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 (Vázquez *et al.*, 2007; Krishna *et al.* 2008). Niche-based processes, such as forbidden links and trait matching, assume that the ecological traits of species constrain their interaction patterns (Maruyama *et al.* 2014; Maglianesi *et al.* 2014). As species are not independent but rather co-exist 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 *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 (Webb *et al.*, 2002; Ives & Godfray, 2006; Rezende *et al.*, 2007).

A growing body of literature has incorporated one or a few mechanisms to explain species

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

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 colors, flowers that are larger, and those that are bilaterally symmetrical (Rodriguez *et al.* 2004; Raine & Chittka 2007). 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, Dornhaus & Papaj 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; Szigeti *et al.* 2020).

Bumblebees (Apidae: *Bombus*) are of both great ecological importance for wildflower

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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 worldwide (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: 1) 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. 2) 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. 3) 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.

## **MATERIALS AND METHODS**

### **Study system**

The study was conducted within the field station of the Lijiang Alpine Botanic Garden, Yulong Snow Mountains, Lijiang, northwest Yunnan, China (27°0'8.84" N, 100°10'49.86" E, ca. 3240 m a.s.l.). At approximately the same elevation (3233 ± 38.5 m, mean ± SD), we selected five subalpine meadows (sites A-E, named according to the distance from the field



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station; Fig. 1) that were  $0.78 \pm 0.38$  km apart and  $0.26 \pm 0.23$  ha in area. Although the meadows were separated by *Pinus-Quercus* forests, according to a radio-tracking study of bumblebee flight distances, *Bombus terrestris* workers can fly a maximum of 2.5 km from the nest (Hagen, Wikelski & Kissling 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 1m  $\times$  1m quadrats were established at 10 m intervals along each transect. In total, we sampled 24 fixed quadrats at each site, and 120 quadrats total for all five sites.

We conducted 6 censuses of plant-bumblebee interactions from July 12 to September 2 in 2016. Census intervals ranged from 9-11 days because some weather conditions (e.g., heavy rain) prevented bumblebee observation. Each census was carried out over two days, the first day for sites A-C which were located near the field station, the second day for sites D and E (Fig.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 one second (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, 15:00-18:00, and ensured there was equal sampling effort at each site during each period. In total, the sampling time was approximately 10800 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).

#### **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 labeled.

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Bumblebees exhibit striking inter- and intraspecific variation in color pattern (Fig. S1), which makes it difficult to identify individuals by coat patterning alone. In addition, taxonomic data on the bumblebee species of southwest China is 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 (Fig. S2). Then, DNA barcoding (CO1 gene) was performed for 190 specimens which consisted of 5-20 specimens in each morphogroup. Finally, the sequences were aligned with the NCBI database to obtain species-level identities, and a subset of specimens were validated by taxonomists (Paul H. Williams of the Natural History Museum, UK and Jian Yao of the Institute of Zoology, Chinese Academy of Sciences).

### **Flower and bumblebee abundance, phenology, and functional traits**

#### *Abundance of flowers*

For each forb species, flower abundance data at each census was summed across all 120 quadrats. Mean flower abundance was calculated as the average value of six censuses. As plant abundance was not available for ten 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 ten species were in fact rare in these subalpine meadow communities.

#### *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 bumble bee foraging bouts (more than 400 records) which were recorded

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during the whole field investigation. Information concerning which plant species were visited by bumble bees 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.

#### *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 (color, size, height, symmetry), or filtering pollinators (flower tube depth, nectar accessibility, stamen location; Fornoff *et al.* 2017); correlations of plant traits are given in Fig. 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.

#### **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 (Fig. 2-3). 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 1000 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, Hines & Williams 2007) to constrain the plant and bumblebee tree, respectively. All the plant and bumblebee sequences are available on NCBI, and their GenBank accession numbers are listed in Dryad Digital Repository.

#### **Construction of plant-bumblebee interaction network**

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Based on our field sampling across all sites and time points, we constructed a quantitative interaction network using the R package *bipartite* (Dormann, Gruber & Fründ 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  $\geq 2$  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)  $\chi^2$  tests were used to evaluate goodness-of-fit of the models. Models were fitted using R package *nnet* (Venables & Ripley 2021).

### **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 analyzing 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, Ives & Waller (2017), we added each factor as both fixed and random terms to test if they can explain phylogenetic patterns. As each factor was added separately, multicollinearity did not interfere. Before

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analyzing, 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  $\lambda$  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 49999 Monte-Carlo permutations based on the permutation model 6 for better control of type I errors (Ter Braak, Cormont & Dray 2012). The analysis was conducted using the R package *ade4* (Dray & Dufour 2007).

To determine if traits matched at the individual level, we built generalized linear mixed-effects models (GLMM), with each of two trait combinations analyzed separately: individual bumblebee tongue length or body size as predictor variables, and mean flower tube depth or tube opening width as response variables (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, Johnson & Schielzeth 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.*, 2020) and *effects* (Fox & Weisberg 2018). Because interaction frequencies of each bumblebee individual were not recorded, we could not use fourth-corner analysis, which requires a

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matrix **L** (interaction frequency), for our individual-level data.

## **RESULTS**

### **Plant-bumblebee interactions**

In total, we observed 2428 interactions between 29 forb species and 12 bumblebee species across all five sites and six censuses (Fig. 2-3). Except for *Trifolium repens*, the forb species are native to Lijiang Yulong Snow Mountains. All the bumblebees fell into 8 subgenera and were classified into short, medium, and long-tongued categories (Table 1, Fig. 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. impetuous* and *B. remotus*). This possible new species is supported by the investigation of this group by Williams *et al.* (unpublished data).

### **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.

### **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.

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When we reanalyzed 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).

### **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 (Fig. 4), with a modularity value of 0.245 and Z-score of 39.14 (null model:  $0.034 \pm 0.005$ , 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 (Fig. 4, Fig. 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 (Fig. 2 O)

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but also their bodies (Fig. 2 N) into the flower tubes and obtain the nectar at the bottom (Fig. S6).

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

## 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., Rafferty & Ives 2013; Rohr & Bascompte 2014; Martín González *et al.* 2015), 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



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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, Arene & Lavergne 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 co-exist (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, Carneiro & Freitas 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, Stang & Klinkhamer 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, García & Pérez 2008) and may have obscured any phylogenetic signal. Another explanation is that there is phylogenetic signal in parts but not all the phylogenies.

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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 (Ranta & Lundberg 1980; Harder 1985), other studies have come to the opposite conclusion (Inouye 1980; Pyke, Inouye & Thomson 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, Inouye & Thomson 2012). Selection pressure may favor trait variation within species and adaptive generalization in short-tongued species to gain access to a variety of flowers (Suzuki, Dohzono & Hiei 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.

#### **SUPPORTING INFORMATION**

Additional supporting information: "Sampling completeness", "Methods of measuring functional traits", Figure S1-S6, Table S1-S2.

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## AUTHORS' CONTRIBUTIONS

HW, DZL and YHZ conceived the ideas and designed methodology; HL, ZXR and ZL collected the data; HL, NER, YHZ and HDL analysed the data; HL, HW and NER led the writing of the manuscript. All authors gave final approval for publication and agree 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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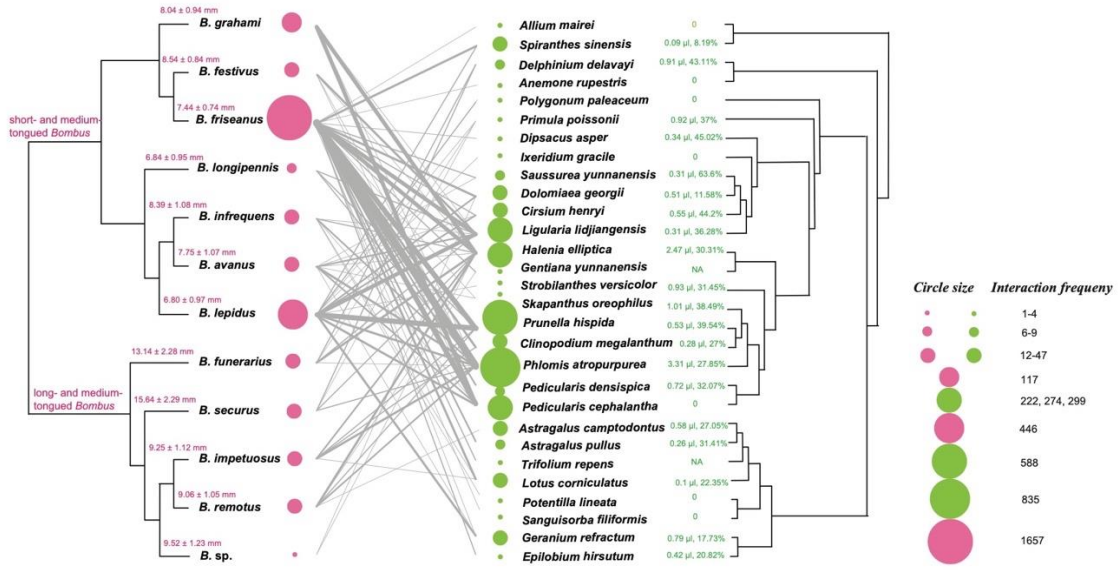
## FIGURES AND TABLES



**Figure 1.** Map and photo of study sites. The left map shows relative location of the LABG field station and five study sites (sites A-E). The right photo shows a view of site A.



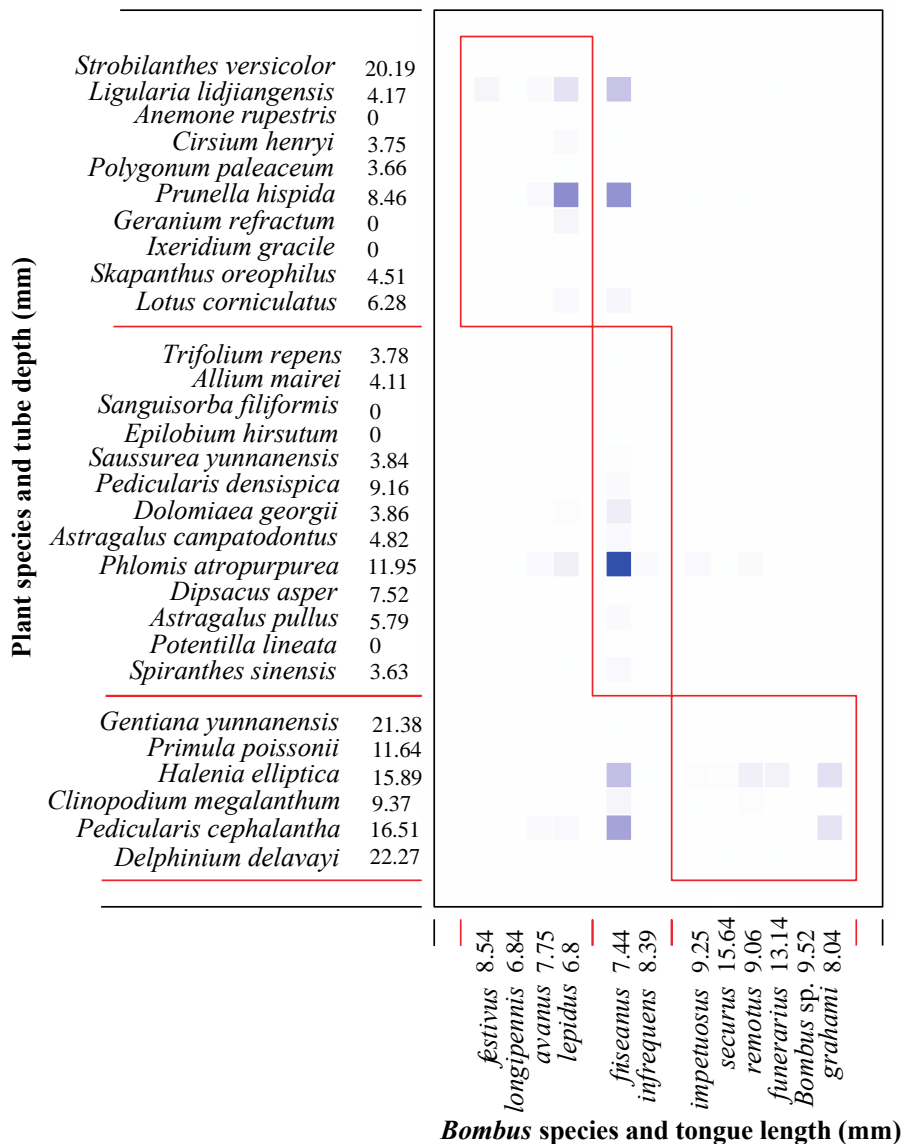
**Figure 2.** Different bumblebee species visiting wildflowers from 15 families and 27 genera in the study system. The four most frequently visited species are shown in A-D: *Pedicularis cephalantha*, *Prunella hispida*, *Phlomis atropurpurea*, *Halenia elliptica*. Other plants are: (E) *Pedicularis 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 photo N. Photo A by Robert A. Raguso, D and L by Zong-Xin Ren; P by Hai-Dong Li. All other photos by Huan Liang.



**Figure 3.** Phylogenetic relationships for 12 *Bombus* species (left) and 29 plant species (right).

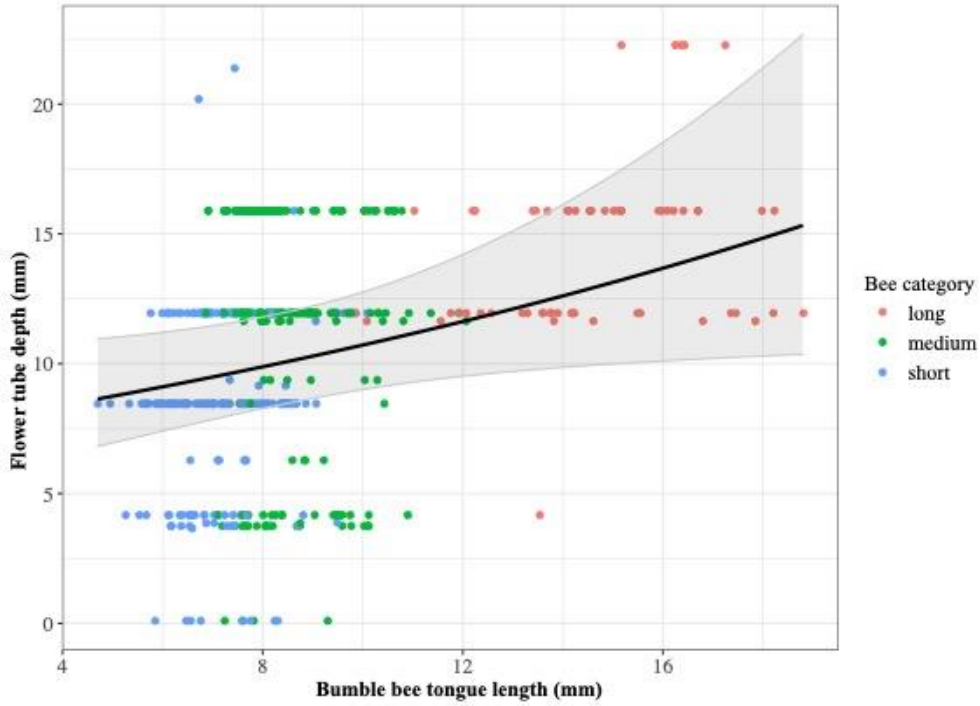
The middle grey links are interactions between bumblebees and plants, line thicknesses are proportional to the number of interactions. Circle sizes (pink for bumblebees, green for plants) and corresponding interaction frequencies are given.

Tongue length (mean ± 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 (μl) and sugar concentration (%) are given for each plant species.



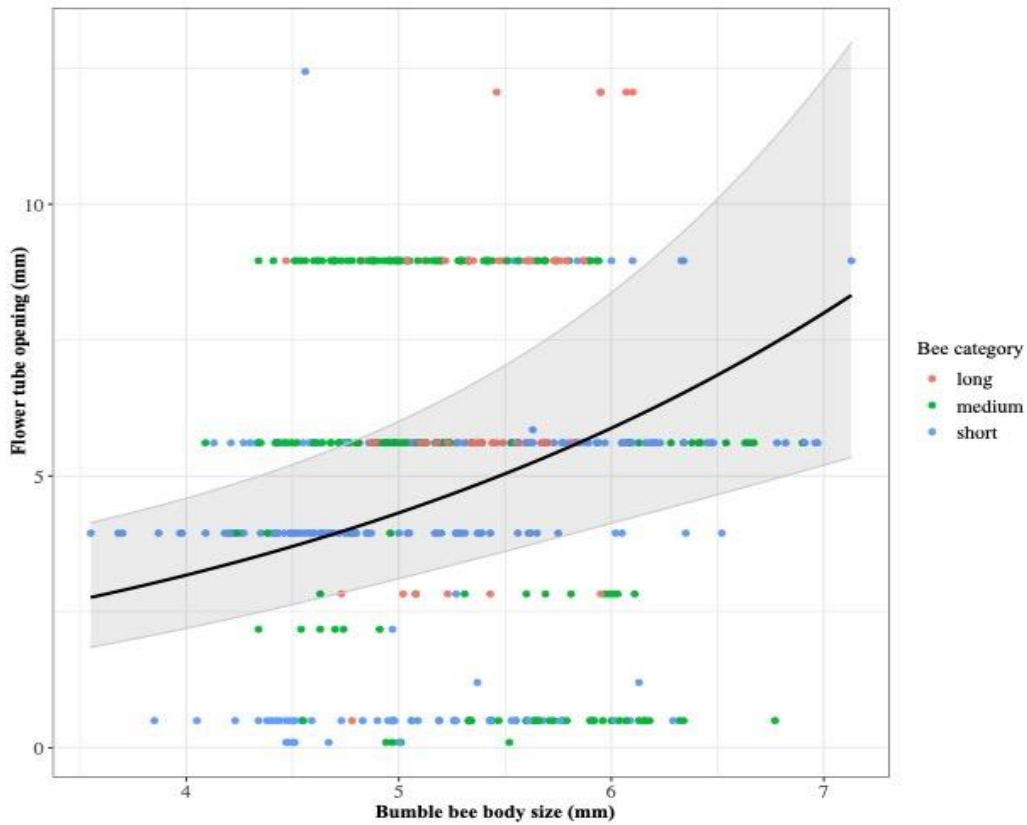
**Figure 4.** Module structure of the 29 plant and 12 bumblebee species interaction network.

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).



**Figure 5.** Relationship between tongue lengths of individual bumblebees and tube depths of visited flowers. Points represent each of the 463 bumble bee individuals, different colors 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 bumble bees and tube openings of visited flowers. Points represent each of the 463 bumble bee individuals, different colors represent different tongue length categories. 95% CI are marked with polygon. Black solid fitted line represents significant relationship ( $P < 0.001$ ).

**Table 1.** *Bombus* species identified in the study sites, their subgenera, number of samples for tongue length measurement (Nt), tongue length (mean  $\pm$  SD), tongue length category and total number of specimens collected (Ns).

<i>Bombus</i> Species	Subgenus	Nt	Tongue Length	Tongue Length	Ns
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			(mm)	Category	
<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
<b><i>Bombus</i> sp.</b>	<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> Skorikox	<i>Melanobombus</i>	156	7.44 ± 0.74	short	1657
<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

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.** 14 plant and 3 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 $\lambda$	Blomberg's $K$	Residual Phylogenetic Variance	Decrease in Phylogenetic Attraction
Plants	pollen grain quantity	numeric	399-256350	0.000	0.791	1.021	1.35%
	pollen size	$\mu\text{m}^3$	739-313959	1.354**	0.950*	1.022	1.26%
	nectar volume	$\mu\text{l}$	0-3.31	0.000	0.871	<b>0.372***</b>	<b>64.06%</b>
	sugar concentration	%	0-63.6	0.000	0.972	<b>0.476**</b>	<b>54.01%</b>
	flower tube depth	mm	0-22.27	1.347**	1.072*	0.884	14.59%
	inflorescence area	$\text{mm}^2$	12.95-2363.77	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 color	1/0	peak/or not	— <sup>(b)</sup>	—	0.982	5.12%
	mean abundance	numeric	0.1-4984.67	0.000	0.675	1.215	-17.39%
	peak flowering time	days <sup>(a)</sup>	10-60	0.000	0.728	1.025	0.97%

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	flowering duration	days	10-60	0.000	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**	<i>1.057</i>	<i>-2.13%</i>

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\*  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ . 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 10-60, referring to the time of each census 1-6.

(b): Flower color 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  $K = 1.012$ ,  $P = 0.014$ ).