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ORIGINAL ARTICLE

Intraspecific and interspecific resource partitioning between bumblebee workers and males related to nectar quantity and quality

Xiao-Fang Jin[1,2], Zhong-Ming Ye[3], Yong-Deng He[3], Chun-Feng Yang[3], Michael Orr[1,4], Arong Luo[1], Paul Williams[5] and Chao-Dong Zhu[1,6,7]

[1]*Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, Beijing, China;* [2]*College of Life Sciences, Wuhan University, Wuhan, China;* [3]*State Key Laboratory of Plant Diversity and Specialty Crops, Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan, China;* [4]*Entomologie, Staatliches Museum für Naturkunde Stuttgart, Stuttgart, Germany;* [5]*Natural History Musuem, Cromwell Road, London, UK;* [6]*College of Biological Sciences, University of Chinese Academy of Sciences, Beijing, China and* [7]*State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing, China*

Abstract Bumblebees are important pollinators for many natural and agricultural systems in temperate regions. Interspecific and intraspecific variation in floral resource preferences have been proposed to influence bumblebee community structure. In particular, sexual dimorphism is a major source of intraspecific niche variation. Although interspecific resource partitioning is well studied, few studies have explored the intraspecific dynamics between workers and males. Here, we report a study on a total of 11 528 workers and 2220 males of 14 bumblebee species recorded over 5 years in the Hengduan Mountains of Southwest China. We first compared the potential for interspecific and intraspecific competition between workers and males using visitation records and resource partitioning indices (overlap index). We then evaluated the influence of nectar traits on flower preference, including nectar volume and the levels of hexose, sucrose and 10 essential amino acids (EAAs). We found that the niche overlap between intraspecific workers and males was higher than that between different species, and temporal overlap alone did not strongly determine diet overlap. Males of most species preferred flowers with high levels of EAAs and hexose, whereas workers of some species preferred flowers with high nectar volume and sucrose levels. This study suggests that there is floral resource partitioning among bumblebee species, and between workers and males, which may play a key role in alleviating interspecific and intraspecific competition. These findings also provide a useful guide for which kinds of plants might be most valuable for bumblebees, especially the understudied males, in this biodiversity hotspot.

Key words bumblebees; castes; male bees; nectar traits; niche overlap; resource partitioning

Introduction

Bumblebees are important pollinators for both natural and agricultural systems (Potts *et al.*, 2010; Garratt *et al.*, 2014), and are distributed nearly worldwide and are especially abundant in temperate climates (Williams,

Correspondence: Zhong-Ming Ye, State Key Laboratory of Plant Diversity and Specialty Crops, Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan 430074 China. Email: yezhongming@wbgcas.cn

1998; Williams & Osborne, 2009). Most bumblebees produce annual colonies, initiated by a single overwintered fertilized queen, that can grow to include dozens to hundreds of individuals, depending on the species (Crone & Williams, 2016). Near the end of the season, the colony reaches a "switching point" where it produces the next generation of queens and males (Crone & Williams, 2016). Throughout their activity period, workers provide the majority of labor for growing and sustaining colonies (Jandt & Dornhaus, 2009), whereas males are believed to focus on meeting their own energetic needs once leaving the nest (Belsky *et al.*, 2020; Zhao *et al.*, 2021). Workers, equipped with pollen baskets on their hind legs, collect both nectar and pollen to provision the brood, whereas males, lacking these pollen baskets, are restricted to simply eating these resources (Michener, 2000). The long activity period of bumblebees makes them important pollinators for a wide variety of flower species, and even male bees can act as important pollinators (Oatevik *et al.*, 2010; Fliszkiewicz *et al.*, 2011; Ogilvie & Thomson, 2015).

Bumblebees obtain most of their nutrients from floral pollen and nectar, and these floral resources vary among plant species in their quality, quantity and availability (Roulston & Cane, 2000; Nicolson *et al.*, 2007; Moerman *et al.*, 2017). Hence, many researchers have studied bumblebee resource preferences and their potential consequences on community structure (Pelletier & McNeil, 2003; Roulston & Goodell, 2011). Interspecific competition for resource acquisition is considered one of the main drivers impacting community structure (Schoener, 1974; Schaffer *et al.*, 1979; Vellend, 2016). Resource partitioning alleviates interspecific competition, theoretically resulting in species coexistence in communities (Chesson, 2000). For example, an early hypothesis on bumblebee community structure suggested that 4 dominant species could coexist at a single site, with one long-, one medium- and one short-tongued species, as well as one final short-tongued nectar-robbing species, because bumblebees with different tongue lengths can theoretically partition floral resources to reduce interspecific competition (Inouye, 1977; Pyke, 1982). The tongue of a bumblebee specifically refers to the combined lengths of the glossa and prementum, and is a key morphological trait that significantly influences their foraging behavior and floral preferences (Heinrich, 1979). Bumblebees with long tongues are better at feeding on flowers with deep corolla tubes, whereas short-tongued species typically forage on flowers with shallower corolla tubes (Wood *et al.*, 2021).

Previous empirical studies have predominantly focused on interspecific rather than intraspecific resource partitioning, such as that observed between different castes (e.g., workers and males) of bumblebees (see Ranta & Lundberg, 1981; Miller-Struttmann & Galen, 2014). Intraspecific variations in traits and behavior, including the distinct floral preferences of castes, could complicate matters (Bolnick *et al.*, 2011; Zwolak, 2018). Sexual dimorphism is a large source of individual niche variation, including plant–pollinator interactions (Roswell *et al.*, 2019). In particular, male and female bees can exhibit different preferences for and impacts on the plants that they interact with (Roswell *et al.*, 2019).

The relative composition of floral resources is a key driver of floral preferences in pollinators. Nectar is an essential resource for all pollinators, containing sugars, amino acids, proteins and minerals (Nicolson *et al.*, 2007). Three sugars are predominant in nectar (glucose, fructose and sucrose) and constitute the most vital carbohydrate source for adult bees (Gardener & Gillman, 2002; Paoli *et al.*, 2014). Although the content of amino acids in nectar is much lower than that of sugars, they are also almost the only source of essential amino acids (EAAs) for adults (Petanidou *et al.*, 2006; Stabler *et al.*, 2015). Additionally, pollen is crucial for bumblebees as it provides the primary source of protein, lipids and micronutrients necessary for larval development and overall colony health (Vaudo *et al.*, 2016). Although bumblebees are generally considered a relatively behaviorally homogeneous group, distinct variations in their life history traits among different species can significantly influence their floral preferences (Sakagami, 1976; Heinrich, 1979). Moreover, the differing roles of workers and males could result in divergent objectives when visiting flowers (workers collect both nectar and pollen for offspring, whereas males solely collect nectar and pollen for themselves), thereby leading to differences in their floral preferences (Goulson, 2010). Therefore, discerning the patterns of resource partitioning between workers and males from different species is imperative for understanding and maintaining healthy bumblebee communities.

In this study, we conducted a 5-year field investigation within a meadow on Hengduan Mountain in Yunnan Province, China, where more than 14 coexisting bumblebee species and 120 flowering plant species are present. The relatively short flowering season forces numerous species to coexist, making it an ideal setting for testing potential competition dynamics. We hypothesized that the interspecific and intraspecific resource utilization between workers and males would be differentiated by their preferences for nectar nutrients. Specifically, the main questions of the study were: (i) is the intraspecific resource partitioning between workers and males weaker than the interspecific resource partitioning; and (ii) how do nectar nutrients affect resource utilization in workers and males of different species? By disentangling these dynamics in this complex montane environment, we can forecast how changes in floral resources will alter the dynamics of the community.

Materials and methods

Study area

The field survey was conducted in an alpine meadow in Shangri-La County (27°37′40″N, 99°47′32″E; 3379 m a.s.l.), north-western Yunnan Province, China. The study area consisted of approximately 12 ha in a small valley basin surrounded by mountains. Approximately 120 entomophilous plant species grow in the meadow, including more than 86 species visited by bumblebees (Ye *et al.*, 2024). The flowering season of the plants extends from June to September. According to the China Meteorological Data Service center (1981–2010), the climate is seasonal, with an annual mean temperature of 6.3 °C, a minimum average monthly (January) temperature of −2.3 °C and a maximum average monthly (July) temperature of 13.9 °C. The annual mean precipitation is 651.1 mm, mainly concentrated in June–September (accounting for 71% of annual precipitation).

Bumblebee sampling

Bumblebees were collected at the site for 5 consecutive years, from 2018 to 2022. Each survey was conducted along walking transects (50 m in length and 2 m in width) selected to represent the diversity and abundance of floral resources throughout the flowering season. We performed surveys weekly during the study period for each year. From 9:30 a.m. to 4:30 p.m. on warm and dry days favorable to bee activity, we recorded all bumblebees encountered and the flowers that they visited by walking along the transects at a constant speed for 10 min. Each transect was walked once for each survey day. In total, 1822 transects were walked across the 5 years, including 86 transects in 2018 over 5 weeks, 254 transects in 2019 over 12 weeks, 795 transects in 2020 over 10 weeks, 289 transects in 2021 over 8 weeks and 398 transects in 2022 over 7 weeks. Sampling involved collecting all bumblebees with sweep nets, and then all bees were cooled in an ice chest to slow their movement and allow identification with a hand lens. Each bumblebee was identified to species and social caste. To minimize the impact of our study we did not collect queens, as we sampled the same meadow for 5 consecutive years. For more details on the sampling methodology, see Ye *et al.* (2024). We accounted for potential variations in hair color and other characters used for bumblebee identification in accordance with the method described by Williams *et al.* (2009). The bees were released after being identified, except for those to be taken to the laboratory for verification of identification and morphological measurements. In total, 6465 (47.03%) individuals were captured and taken back to the laboratory, and 7283 (52.97%) individuals were released in the field. For bees that could not be identified via morphology, their DNA was extracted from a middle leg stored in 100% pure ethanol for identification by cytochrome *c* oxidase subunit I (*COI*) barcoding (Huang *et al.*, 2015). All specimens are presently housed at the Wuhan Botanical Garden, Chinese Academy of Sciences, but they will be transferred to the collection of the Institute of Zoology, Chinese Academy of Sciences, Beijing, China.

In total, 14 *Bombus* species were recorded: *Bombus friseanus* Skorikov, *Bombus lepidus* Skorikov and *Bombus festivus* Smith were the predominant species, followed by *Bombus impetuosus* Smith, *Bombus securus* Frison, *Bombus minshanicola* Bischoff, *Bombus nobilis* Friese, *Bombus prshewalskyi* Morawitz, *Bombus graham* Frison, *Bombus avanus* Skorikov, *Bombus hengduanensis* Williams, Ren & Xie, *Bombus remotus* Tkalcu, *Bombus infrequens* Tkalcu and *Bombus turneri* Richards (Table 1). Only *B. festivus*, *B. friseanus*, *B. impetuosus*, *B. lepidus*, *B. minshanicola* and *B. securus* were used for this study (Fig. S1), as insufficient males were collected for the other species. According to tongue length classification (Goulson *et al.*, 2005), *B. friseanus*, *B. lepidus* and *B. minshanicola* were categorized into the short length group (≤8 mm), *B. festivus* and *B. impetuosus* were classified into the medium length group (8–9 mm), and *B. securus* was placed in the long length group (≥9 mm). The proportion of males was calculated as the number of males divided by the sum of the number of males and workers of each species for each year.

Flower nectar traits

Nectar samples were collected during the peak blooming of each plant species visited by the bumblebees. In total, the nectar traits of 51 plant species were collected (volume, sugar composition and amino acid contents), as the nectar volumes of the other plant species were insufficient for collection. The day before nectar collection, the flowers were bagged with a 1-mm mesh to prevent pollinator visits. The following morning, nectar was retrieved from the newly opened flowers using

Table 1 Numbers of workers and males of all species surveyed per year.

	2018		2019		2020		2021		2022		Total	
Species	Worker	Male	Worker	Male	Worker	Male	Worker	Male	Worker	Male	Worker	Male (%)
Bombus friseanus	240	10	456	8	4 671	220	660	17	1 275	128	7 302	383 (4.98%)
Bombus lepidus	243	69	99	34	1 083	403	198	32	678	456	2 301	994 (30.17%)
Bombus festivus	76	20	90	11	141	261	259	30	456	452	1 022	774 (43.10%)
Bombus impetuosus	61	0	32	0	259	4	81	0	186	6	619	10 (1.59%)
Bombus securus	9	1	19	0	57	2	55	0	12	2	152	5 (3.18%)
Bombus minshanicola	7	3	9	1	10	36	2	0	2	11	30	51 (62.96%)
Bombus nobilis	4	0	8	0	30	0	1	0	19	0	62	0 (0%)
Bombus prshewalskyi	3	0	2	0	2	0	0	0	6	0	13	0 (0%)
Bombus hengduanensis	0	0	1	0	2	0	0	0	2	2	5	2 (28.57%)
Bombus infrequens	0	0	1	0	4	0	0	0	0	0	5	0 (0%)
Bombus grahami	0	0	0	0	7	0	1	0	0	0	8	0 (0%)
Bombus remotus	0	0	0	0	1	0	2	0	0	0	3	0 (0%)
Bombus avanus	0	0	0	0	0	0	2	1	3	0	5	1 (16.67%)
Bombus turneri	0	0	1	0	0	0	0	0	0	0	1	0 (0%)

Male (%) represents the proportion of males of each species.

microcapillary tubes with an inner diameter of 0.1–0.5 mm, depending on flower size. In the field, we determined the nectar volume by measuring the length of the fluid column (L) in the tubes, and then calculated the volume as $L \times \pi \times (\text{diameter}/2)^2$. For nutritional analyses, we collected nectar in a clean Eppendorf tube (0.2 mL), which was first kept in liquid nitrogen and transported to the laboratory, then frozen at −80 °C for storage. Sugar composition was quantified via high-performance liquid chromatography (HPLC), and the content of 10 EAAs for bees was detected by ultra-HPLC tandem mass spectrometry (UHPLC-MS/MS) analysis (Stabler *et al.*, 2015). Further details on the measurement of flower nectar traits are given in Appendix S1.

Flower pollen

We quantified pollen rewards by measuring the total number of pollen grains per flower (pollen number). To achieve this, we collected anthers from nearly open flowers and stored them in 70% alcohol. Eight flowers were sampled from each plant species. The samples were then sonicated for 10 min to release the pollen from the anthers, which is especially important for poricidal flowers in which the pollen is normally hidden. The resulting mixture was filtered through a 0.1-mm mesh to remove most plant debris and then centrifuged at a low speed to obtain a concentrated pollen suspension. The total number of pollen grains per flower was counted for subsamples of 20 μL and then averaged and multiplied by the volume dilution, which differed among the plant species.

Data analysis

To compare the diet breadth of the workers and the males of each species, we estimated the potential visited plant species using rarefaction analysis, calculating the abundance- and incidence-based rarefaction of species based on the number of individuals (a measure of completeness) with the package iNEXT (Hsieh *et al.*, 2022). We pooled the 5 years of plant–bumblebee interactions into a network for the rarefaction analysis.

We calculated the niche overlap in flower usage between caste pairs of the 6 bumblebee species (hereafter termed species_caste) for each year separately, following the methodology of Goulson *et al.* (2008). We set the overlap indices as 4 groups: intraspecific workers and males (intra_WM); interspecific workers (inter_W); interspecific males (inter_M); and interspecific workers and males (inter_WM).

The niche overlap between bee species_caste j and h is $1 - 0.5\sum_k (P_{jk} - P_{hk})$, where

$$P_{jk} = \frac{No.species\_caste\ j\ visiting\ plant\ species\ k}{Total\ no.\ species\_caste\ j}.$$

In addition, we used "chordDiagram" in the R package circlize (Gu, 2014) to visualize the pooled bee–plant networks based on the relative proportion of plants visited by each bumblebee species and caste.

As workers and males have differing life spans, which may influence their floral host use (Ranta & Lundberg, 1981; Miller-Struttmann & Galen, 2014), we calculated the degree of temporal overlap between workers and males from each bumblebee species with Schoener's index (SI) (Schoener, 1970). We also set up the temporal overlap indices using the 4 groups described in niche overlap section above (intra_WM, inter_W, inter_M and inter_WM).

To assess whether intraspecific resource competition between workers and males is stronger than interspecific competition, we compared the difference in niche overlap indices among the groups (inter_W, inter_M, inter_WM and intra_WM) by using a Bayesian linear mixed model (LMM), with the group as a fixed effect and combination type (the combination of paired species and caste) as a random factor. We then used the "hypothesis" function in the R package brms (Bürkner, 2017) to conduct multiple comparison procedures after performing the LMM analysis. The Bayesian framework employed in this method effectively addresses both type-I statistical errors and type-S (sign) errors that could arise in multiple comparisons (Gelman and Tuerlinckx, 2000). To explore the relationship between niche overlap and the proportion of males for each group (inter_W, inter_M, inter_WM and intra_WM), we fitted 4 Bayesian LMMs. For each bumblebee species, we averaged the niche overlap index values between the focal species and the other species within the same group, representing the mean niche overlap for each focal species. This averaged value was set as the response variable. The proportion of males of the focal species was set as the fixed effect, whereas the combinations of species and year were set as random effects in these models. Furthermore, to assess the impact of temporal overlap on niche overlap, we constructed 4 Bayesian LMMs for each group by setting niche overlap as the response variable, temporal overlap as the fixed effect and combinations of the species from which the overlaps were obtained as random effects. All LMMs were constructed with the function "brm" in the R package brms (Bürkner, 2017) and run with 4 Markov chain Monte

Table 2 Rarefaction analyses results using the package iNEXT.

Species	*N*	Observed	Estimated	SE	LCL	UCL	SC (%)
Fri_W	7302	60	74.08	10.42	60.00	94.51	99.82
Fri_M	383	17	21.16	7.25	17.00	35.37	98.70
Fes_W	2301	47	61.39	18.46	47.00	97.57	99.48
Fes_M	994	27	47.15	18.74	27.00	83.88	98.89
Lep_W	1022	47	83.06	23.66	47.00	129.43	98.14
Lep_M	774	26	42.65	17.96	26.00	77.85	98.71
Imp_W	619	21	41.22	28.59	21.00	97.26	98.55
Imp_M	10	4	5.80	2.44	4.00	10.58	82.00
Sec_W	152	18	26.94	8.46	18.00	43.51	96.07
Sec_M	5	3	3.80	1.11	3.00	5.98	73.33
Min_W	30	9	21.08	10.13	9.00	40.93	83.56
Min_M	51	5	6.96	2.73	5.00	12.30	96.16

The sampling effort and an estimation for the potential plant species visited by workers (W) and males (M) of each bumblebee species is presented. Abbreviations: *N*, sample size; Observed, observed species richness of plant species visited for each bumblebee species; Estimated, estimated species richness of the potential plant species visited for each bumblebee species; SE, logical variable for calculating the bootstrap standard error; SC, sample coverage estimate; LCL and UCL, lower and upper confidence limits of sample coverage, respectively. The abbreviations for bumblebee species are given in the legend to Fig. 1.

Carlo (MCMC) chains with 10 000 iterations and a warm-up of 3000 runs. Model convergence was checked by visually inspecting parameter traces, ensuring a scale reduction factor (R) of ≤ 1 and an effective sampling size (of more than 5000).

To explore the influence of nectar traits, including nectar volume, contents of hexose (including glucose and fructose) and sucrose, total content of EAAs and the total number of pollen grains per flower, on resource utilization in workers and males of different species, canonical correspondence analysis (CCA) was performed using the R package vegan (Oksanen *et al.*, 2022). Nectar traits and pollen number served as explanatory variables, whereas bumblebee–plant networks were used as response variables. All factors had a variation inflation factor (VIF) of <10 in the model, and thus we assumed robustness to collinearity (Anderson, 2003).

All the data analyses were performed in R 4.2.1 (R Core Team, 2022).

Results

In total, 13 748 bumblebees were recorded for 14 species, including 11 528 workers and 2220 males across the 5 years of sampling (Table 1). Each species and its castes (workers and males) demonstrated clear plant-resource preferences (Fig. 1; Table S1). The workers and males within *B. impetuosus*, *B. lepidus* and *B. minshanicola* largely preferred the same plants. For example, *Polygonum macrophyllum* D.Don was the plant most used by *B. lepidus* and *B. minshanicola*, and *Phlomis atropurpurea* Dunn was the plant most used by *B. impetuosus* (Fig. 1; Table S1). Whereas the workers and males within *B. festivus*, *B. friseanus* and *B. securus* differed in their most used plants (Fig. 1; Table S1). The males of *B. festivus* and *B. friseanus* mostly used *Ligularia pleurocaulis* (Franch.) Hand.-Mazz., and the males of *B. securus* most often used *Halenia elliptica* D.Don (Fig. 1; Table S1). The workers of *B. festivus*, *B. friseanus* and *B. securus* most often visited *Pedicularis cephalantha* Franch. ex Maxim., *P. atropurpurea*, and *H. elliptica* (Fig. 1; Table S1).

According to the potential maximum richness values estimated via rarefaction, the males of all species visited fewer plant species than the workers did (Table 2). Thus, males exhibited narrower diet breadths than workers. There were clear differences in diet breadth among species. For example, the estimated maximum breadths of diet of the workers of *B. friseanus* (74.08 ± 10.42), *B. lepidus* (61.39 ± 18.46) and *B. festivus* (83.06 ± 23.66) were wider than in the other species (Table 2). However, regarding another potential confounding factor, it seems unlikely that the duration of activity of each species explains the differences in diet breadth among species. For example, *B. lepidus* had a relatively short activity window but exhibited the third widest breadth of diet (Fig. S2; Table 2).

The proportion of males observed for each species was ranked as *B. minshanicola* (62.96%), *B. festivus*

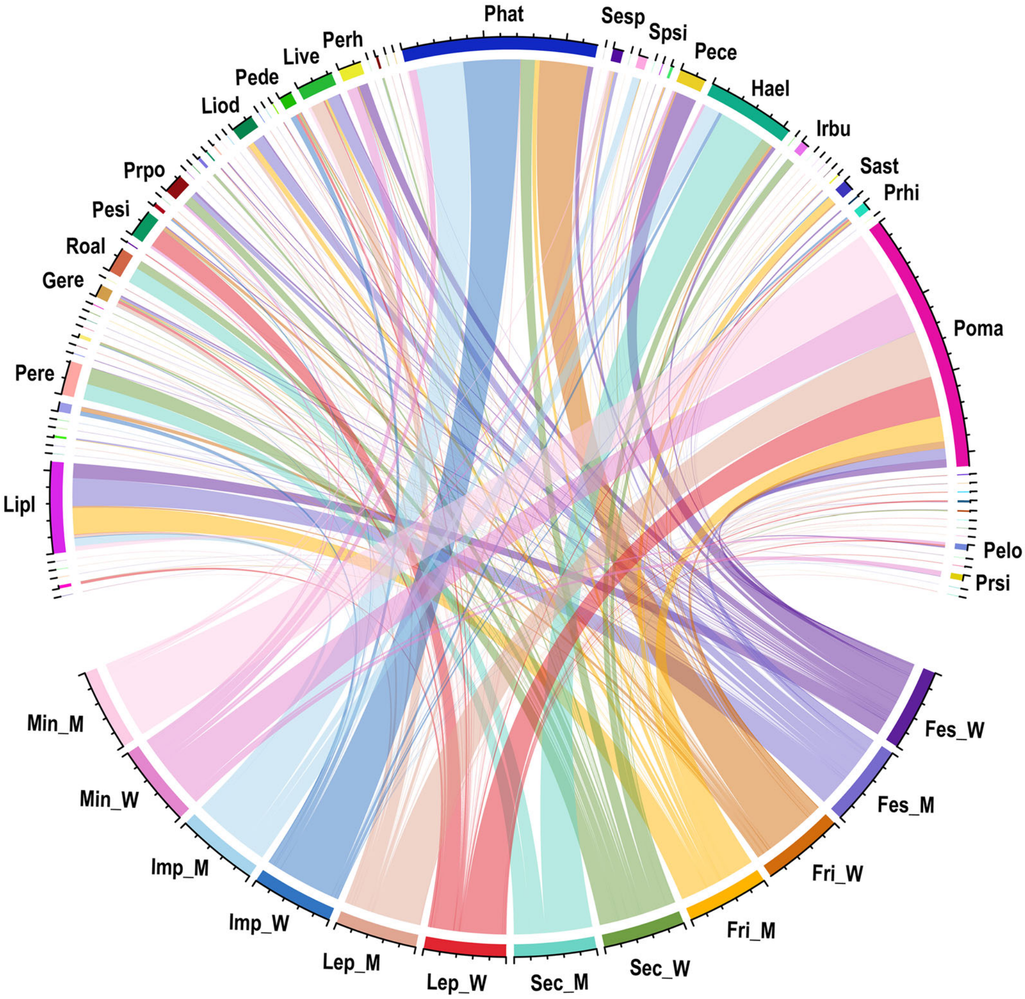

Fig. 1 A chord diagram shows the bipartite interactions between bumblebees (males and workers) and plants. The top half is composed of 86 species of plants, whereas the bottom half consists of bumblebees and their workers and males. The chord thickness corresponds to the relative proportion of plants visited by each bumblebee species and caste. Plant species are represented by the combination of the first 2 letters of the genus and the specific epithet. Bumblebees are denoted by combining the first 3 letters of their species name and their castes (M, males; W, workers). Fri_W and Fri_M represent the workers and males of *Bombus friseanus*, respectively. Lep_W and Lep_M represent the workers and males of *Bombus lepidus*, respectively. Fes_W and Fes_M represent the workers and males of *Bombus festivus*, respectively. Imp_W and Imp_M represent the workers and males of *Bombus impetuosus*, respectively. Sec_W and Sec_M represent the workers and males of *Bombus securus*, respectively. Min_W and Min_M represent the workers and males of *Bombus minshanicola*, respectively. With the large number of species, only the names of species with high interaction frequencies are displayed. The plant species names, represented by abbreviations here, are detailed in Table S1.

(43.10%), *B. lepidus* (30.17%), *B. friseanus* (4.98%), *B. securus* (3.18%) and *B. impetuosus* (1.59%) (Table 1). In addition, *B. minshanicola* and *B. festivus* both exhibited higher proportions of males in 2020 and 2022 (Fig. S3). None of the niche overlap indices of the 4 groups (intraspecific workers and males, interspecific workers, interspecific males, and interspecific workers and males) exhibited obvious relationships with the proportion of males, with the following slope coefficients (95% credible intervals): inter_M, −0.01 (−0.42, 0.41); inter_W, 0.01 (−0.11, 0.13); inter_WM, −0.07 (−0.24, 0.11); and intra_WM, 0.34 (−0.18, 0.83) (Table S3).

The niche overlap indices between intraspecific workers and males were higher than between other groups (Fig. 2; Table S2). For groups of interspecific males and intraspecific workers and males, niche overlap showed no relationship with temporal overlap, with the following slope coefficients (95% credible intervals): inter_M,

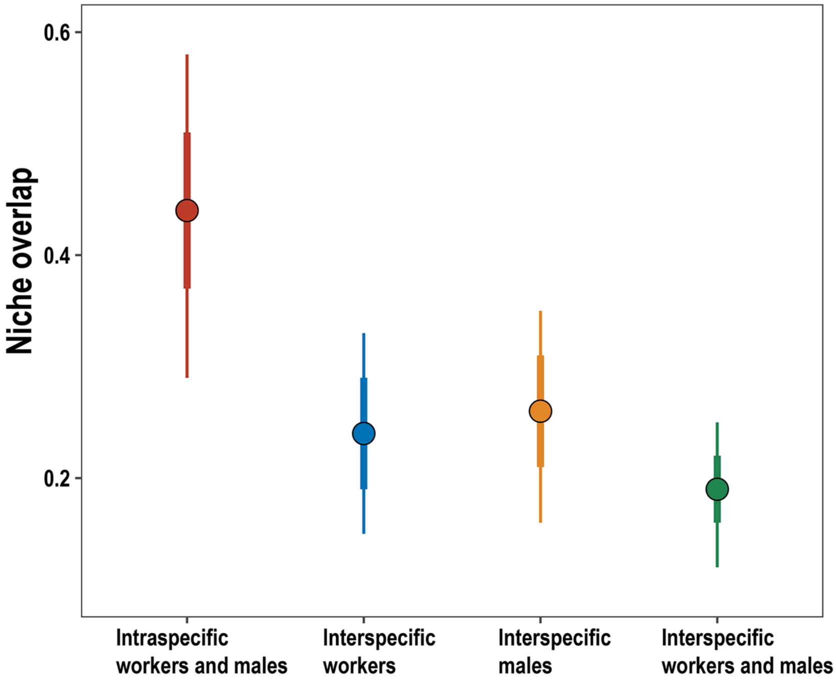

Fig. 2 Niche overlap compared for intraspecific workers and males, interspecific males, interspecific workers, and interspecific workers and males. The circles and lines show the model-predicted mean, 95% credible intervals (thin lines) and ±1 SD of the posterior mean (thick lines) for each group.

0.16 (−0.09, 0.42) and intra_WM, 0.12 (−0.35, 0.60) (Fig. 3A, C; Table S4). Conversely, for groups of interspecific workers and males and interspecific workers, the niche overlap exhibited a positive relationship with temporal overlap, with the following slope coefficients (95% credible intervals): inter_WM, 0.21 (0.06, 0.36) and inter_W, 0.32 (0.12, 0.52) (Fig. 3B, D; Table S4).

The results of the CCA showed that the first 2 axes together accounted for 91.71% of trait variation, whereas the first and second axes separately accounted for 72.91% and 18.80% of the variation, respectively. All of the nectar traits measured affected the floral preference of bumblebees, depending on the caste and species (Table S5). Males of most species and workers of *B. festivus* and *B. lepidus* preferred flowers with high hexose and EAA contents (Fig. 4). Specifically, the plants most visited by males were *P. macrophyllum*, *L. pleurocaulis*, *Ligularia vellerea* (Franch.) Hand.-Mazz., and *Ligularia odontomanes* Hand.-Mazz. (all high in hexose and amino acids; Table S6). In contrast, workers of *B. friseanus*, *B. impetuosus* and *B. securus* preferred to visit flowers with large nectar volumes and high sucrose contents (Fig. 4). However, the total number of pollen grains per flower had no significant effect on the visiting preferences of either workers or males (Fig. 4; Table S5).

Discussion

It is evident from our results that the trend of workers and males of the same species sharing their most important plants indicates that intraspecific niche overlap is strong among workers and males. However, the level of intraspecific niche overlap between workers and males was not related to the proportion of males. In addition, males of most species preferred flowers with high levels of EAAs and hexose, whereas workers of some species preferred flowers with high nectar volume and sucrose.

In our study, we found that the foraging niche overlap between workers and males within species was higher than that between species, indicating that resource partitioning was more pronounced between different species than within the same species. This may be ecologically significant, as it aligns with a classic hypothesis suggesting that when intraspecific competition is more pronounced (with low intraspecific resource partitioning), it helps buffer species against competitive exclusion, thereby promoting community stability (Tilman, 1982; Adler *et al.*, 2007). We propose that the resource partitioning patterns observed here originate in part from the fact that several species prefer similar resources across castes. The workers and males of *B. impetuosus*,

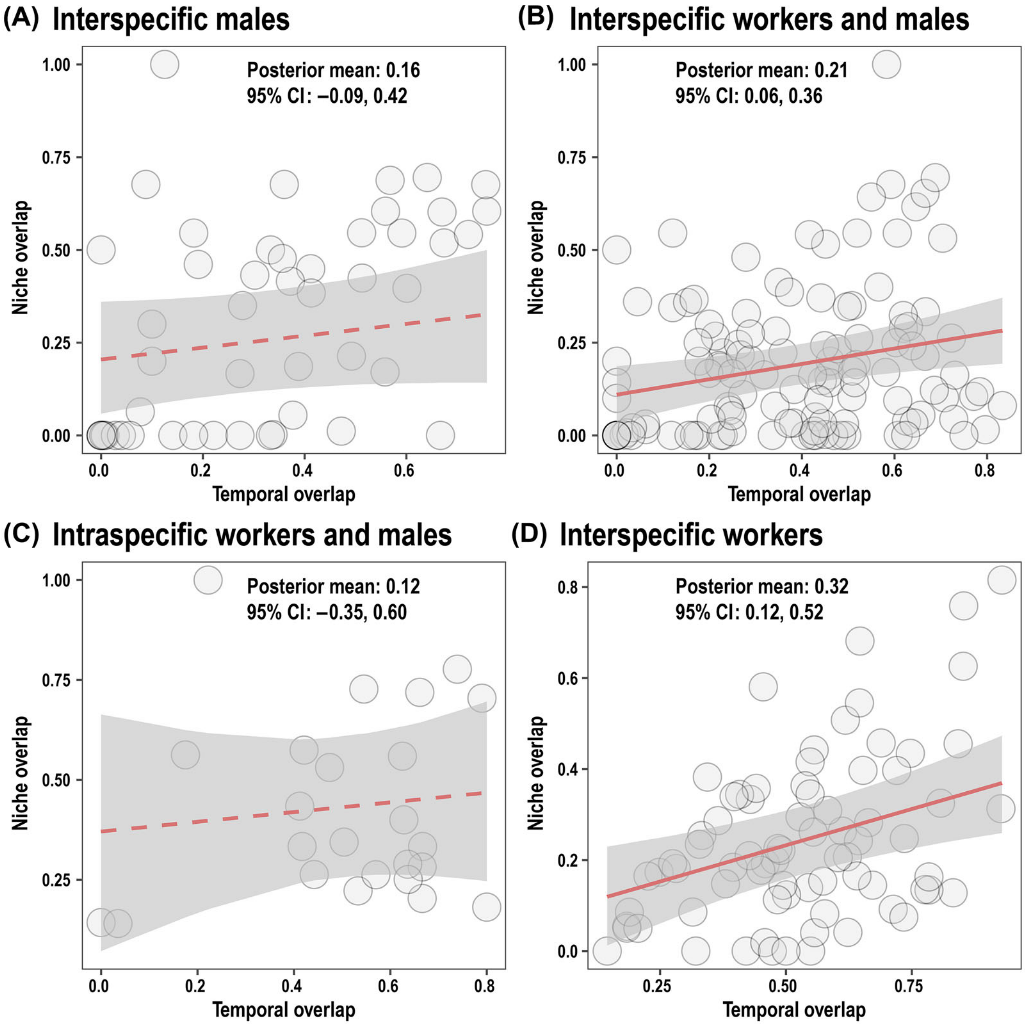

Fig. 3 Relationship between niche overlap and temporal overlap for: (A) interspecific males; (B) interspecific workers and males; (C) intraspecific workers and males; and (D) interspecific workers. The gray shaded area represents the 95% credible intervals (95% CIs) and there is a statistically significant relationship if the 95% CIs of the slope do not include 0.

B. lepidus and *B. minshanicola* foraged on the same most-used plants (*P. macrophyllum* and *P. atropurpurea*; Table S1). In contrast, some other species exhibited more distinct resource partitioning among castes, which may reflect an adaptation to minimize competition and optimize resource use. For instance, the workers and males of other bumblebee species (e.g., *B. friseanus* and *B. securus*) focused on different major plants (Table S1). As *B. friseanus* is one of the most common species in the study area, it may be that its abundance induced higher intraspecific competitive pressures. Interestingly, *B. securus* is relatively rare, yet workers and males appear to partition resource use, suggesting that rarity is not the only driver involved. Perhaps the long tongue of *B. securus* (Williams *et al.*, 2009) may limit other species from their preferred resources naturally, such that they can spread their efforts across flowers and avoid intraspecific competition more easily; the other species are notably short- or medium-tongued (Williams *et al.*, 2009). Still, it is challenging to thoroughly sample the complete breadth of diet of rare species, and this may limit our interpretations for those rarer species.

Our findings indicate that temporal overlap alone does not strongly determine diet overlap. Specifically, for groups of interspecific workers and males and interspecific workers, niche overlap exhibited a positive relationship with temporal overlap. As a consequence of their activity periods, the temporal availability of flowers

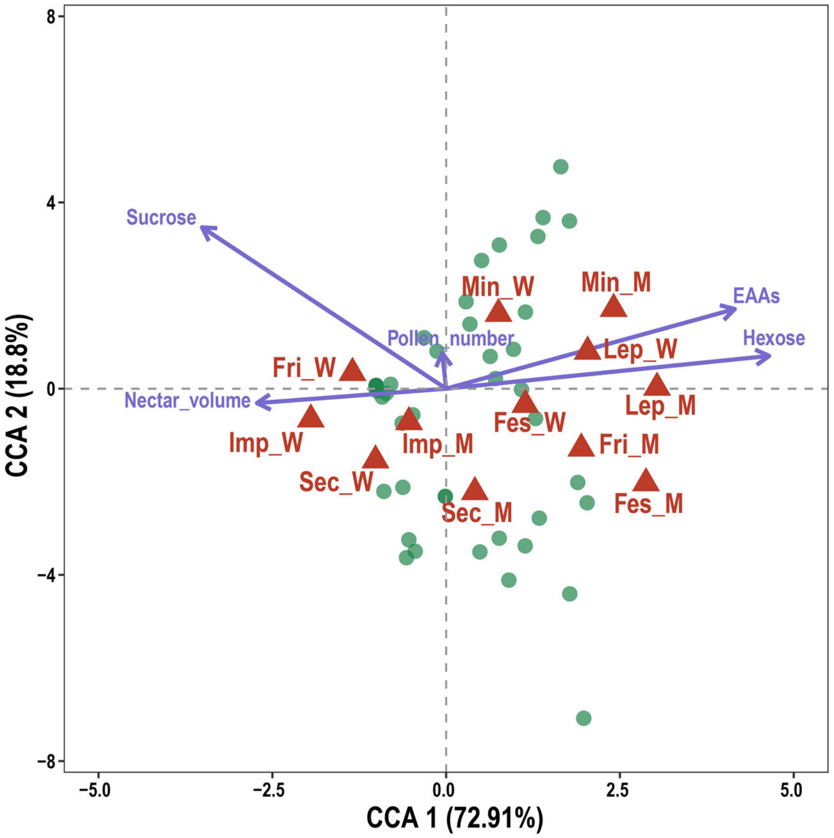

Fig. 4 Canonical correspondence analysis (CCA) biplot shows the preferences of bumblebees and their workers and males for nectar traits. Nectar traits, including nectar volume and the contents of hexose, sucrose and essential amino acids (EAAs) are indicated by the blue open-headed arrows pointing in the direction of increasing values. Bumblebees are represented by red triangles with letters, and plant species are represented by green dots. For detailed CCA results, refer to Table S5. The abbreviations for bumblebee species names are detailed in Fig. 1.

differs between castes (Goulson, 2010). Essentially, workers as a group can access flowers for the entire season, or nearly so, whereas males can only access resources near the end of bloom (Heinrich, 1979). Therefore, the niche overlap between workers may naturally be higher than that of their corresponding males (Ranta & Lundberg, 1981). However, the niche overlap between intraspecific workers and males and interspecific males showed no relationship with temporal overlap, indicating that phenological differentiation is not the sole factor driving resource partitioning between interspecific or intraspecific workers and males. Additionally, the flowering period of plants and the duration of activity of bumblebee species in the alpine meadow under study is relatively short, from June to September. Therefore, the phenological differentiation between bumblebee species and their castes was less pronounced than in more mesic environments (Fig. S2; Heinrich, 2004). Temporal similarity, a restricted flight season and consequent similarities in environmental parameters (temperature, precipitation, etc.) may ultimately explain why phenology did not dominate floral use.

Prior research suggests that bumblebees structure their foraging preferences based on nutritional needs (Miller-Struttmann *et al.*, 2015; Vaudo *et al.*, 2016; Hass *et al.*, 2019; Cameron & Sadd, 2020), and this could be based on numerous benefits from specific compounds. For instance, complementary nutrients can reduce or negate the effects of harmful plant compounds on larvae (LoCascio *et al.*, 2019; McAulay & Forrest, 2019). Different food resources may also be important for different purposes in colony building and maintenance; as workers rather than males are responsible for these colony-related duties, we might expect this to influence which castes use which resources (Heinrich, 1979; Jandt & Dornhaus, 2009; Brown & Brown, 2020). Consequently, as we see here, workers might use more flower species and prefer flowers with high levels of various types of rewards, necessary for their larger repertoire of tasks compared with males: the survival of offspring, feeding of the queen and larvae, and ongoing colony construction (Carnell *et al.*, 2020). This may explain why the workers collectively used more flower species throughout the season than the males in this study, although we cannot fully rule out the role of a lower sample size for males than for females.

Within castes, some trends in nutritional preferences also emerged, and prioritizing the planting of preferred species could bolster bumblebee populations. We found that the foraging preferences of the workers of *B. friseanus*, *B. impetuosus* and *B. securus* were affected by both nectar volume and sucrose content. This may be because workers prioritize higher nectar volume to increase per-visit returns, whereas high sucrose content is indicative of superior energy resources that better support colonies. Stabler *et al.* (2015) proposed that, like honeybees (Paoli *et al.*, 2014), bumblebee workers prioritize their carbohydrate intake over the ingestion of dietary EAAs. In contrast, the males of most species preferred flowers with high levels of hexose and EAAs. In terms of sugars, hexose may be easier to absorb and digest, making it more convenient for males to utilize (Brown & Brown, 2020). Moreover, males choose flowers with high levels of EAAs because nectar is their primary or only nitrogen source, whereas the workers would also take nitrogen from pollen (Heinrich, 1979). Interestingly, we found that the quantity of pollen per flower did not affect the visitation rates of worker and male bees. We propose that the primary pollen source for bumblebees at our study site was from 5 *Pedicularis* species, which did not provide any nectar for pollinators (Huang *et al.*, 2024). Consequently, most workers likely visited other floral species primarily for nectar, similar to the behavior observed in males. In fact, the 5 *Pedicularis* species were excluded from this analysis because they did not produce nectar and the males hardly foraged on them. Our findings indicate that the pollen quantities of these nectar-producing plants did not significantly influence the flower preferences of worker and male bees. From these findings, we can make recommendations on what types of plants to cultivate to bolster the health of bumblebees, but future studies remain necessary to understand how nutritional preferences change across scales. However, the nutritional ecology of adult males remains relatively poorly understood, and more research is required on this front (Woodard & Jha, 2017; Carnell *et al.*, 2020).

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Disclosure

The authors declare that they have no conflicts of interest associated with this work.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1 Measurement of flower nectar traits.

Fig. S1 The 6 selected bumblebee species and their visited flowers.

Fig. S2 The duration of seasonal activity of males (M) and workers (W) of each species per year (2020−2022).

Fig. S3 The male ratio of each species per year (2018−2022).

Table S1 The top 70% visits of each bumblebee species to specific plant species, separated by caste (workers and males).

Table S2 Coefficients from Bayesian linear mixed model (LMM) for the niche overlap across caste combinations.

Table S3 Coefficients from Bayesian linear mixed models (LMMs) for the relationship between niche overlap and male ratio.

Table S4 Coefficients from Bayesian linear mixed models (LMMs) for the relationship between niche overlap and temporal overlap.

Table S5 Results of canonical correspondence analysis (CCA) for the influence of nectar volume, concentrations of hexose (including glucose and fructose) and sucrose, total concentrations of essential amino acids (EAAs), and pollen number on the preference of the castes from different species.

Table S6 Nectar traits and pollen number of the specific plant species which were the top 70% visits of each bumblebee species shown in Table S1.