



Bumblebees' flower preferences are associated with floral abundance and buzz frequency when buzz-pollinating co-flowering plants

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With 3 figures

Abstract: Buzz-pollination is used by some bees to expel pollen through vibrating flowers. Yet, little is known about the determinants influencing bee preferences among buzz-pollinated flowers. We studied five co-flowering, nectarless species of *Pedicularis* (Orobanchaceae) buzz-pollinated by bumblebees in an alpine meadow, to investigate bumblebees' flower preferences in response to fluctuations of floral abundance across five years. We also recorded and analyzed the buzzing frequencies produced by the three dominant bumblebee species. Our results indicate that *Bombus friseanus* and *B. lepidus* visited different *Pedicularis* flowers using similar buzz frequencies and displayed an abundance-dependent flower preference across years. These two bumblebee species had staggered phenologies with distinct timing of peak abundances across the five years. In contrast, *B. festivus* used lower fundamental buzz frequencies, had a constant flower preference across years, but used different buzz frequencies across *Pedicularis* species. Although the amount of pollen released after bumblebee visitation varied across *Pedicularis* species, we found that after a single visit all bumblebees deposited similar amounts of pollen on stigmas. Our study indicates that bumblebees' flower preferences is sometimes, but not always, modulated by floral abundance, and that at least one bumblebee species was observed to produce buzzes of different frequencies in different plant species. Competition for floral resources among bumblebees and for pollination services among co-flowering *Pedicularis* species may structure plant-pollinator interactions and affect species coexistence.

Keywords: buzz pollination; optimal foraging strategy; *Pedicularis*; pollen removal and deposition

1 Introduction

Sympatric species sharing food resources may use those resources differentially to reduce competition (Graham & Jones 1996; Temeles et al. 2017). Pollinator species have distinct flower preferences because of differences in innate preferences and flower handling capacities (Klumpers et al. 2019; Latty & Trueblood 2020). This includes differences in preferences for flower color, shape, scent, or reward types, and differences in the ability to drink nectar efficiently or release pollen from specific flowers. Different pollinator functional groups display distinct floral syndrome prefer-

ences, which may contribute to the partitioning of floral resource use (Fenster et al. 2004). However, in co-occurring species from the same functional group (e.g., *Bombus* spp.), floral syndromes are usually overall similar, and mechanisms determining partitioning of floral use, are thought to be typically influenced by factors such as handling skills, morphological constraints and floral abundance (Balfour et al. 2021).

Pollen is an essential resource for pollinators that is vital for bee larval development (Kriesell et al. 2017). Some flowering species can only release pollen grains when floral visitors apply vibrations to the flower (floral vibrations, floral

buzzes, or sonication) (Vallejo-Marín 2019). Examples of such buzz-pollinated flowers include some species of the pollen-only *Pedicularis* (Orobanchaceae) genus with narrow corolla beaks that open via terminal pores (poricidal flowers) (Corbet & Huang 2014; Macior 1968). The determinants influencing flower preference of pollinators when foraging among buzz-pollinated plants remain unclear.

Floral vibrations produced by bees differ among bee species in properties such as frequency, duration, and amplitude, which collectively affect how much pollen could be released and collected (Corbet & Huang 2014; De Luca et al. 2013; Rosi-Denadai et al. 2020). Among these properties, buzz frequency is easier to measure in the field compared to amplitude because it can be accurately recorded using an acoustic recorder (De Luca et al. 2020; Vallejo-Marín 2022). Although buzz frequency has a relatively minor effect on pollen release compared to amplitude (De Luca et al. 2013), recent work suggests that it could play an important role in pollen removal when it can be matched to the natural frequencies (i.e., frequencies at which flowers naturally vibrate when disturbed) of the coupled bee-flower system, inducing resonance (i.e., an amplification of the vibrations) (Nunes et al. 2021; Jankauski et al. 2022). Therefore, improvements of pollen extraction efficiency caused by adjustment of buzz frequency may potentially affect bee and plant fitness, although this has never been demonstrated. Individual bees vary in the fundamental frequency of their buzzes (e.g., Morgan et al. 2016) raising the possibility that they could alter this vibration property while visiting different flowers (Switzer & Combes 2017); besides, frequency does not appear to be entirely constrained by body size (Corbet & Huang 2014; De Luca et al. 2019). To date, little is known about buzz frequency variation among buzz-pollinating bee species visiting the same plant species as well as about intraspecific variation in bee species visiting different plant species within a community (but see Corbet & Huang 2014; Rosi-Denadai et al. 2020; Switzer & Combes 2017).

Field studies suggest that the flower preferences of buzz pollinators depend on context and are affected by flower abundance across years (Kemp et al. 2022; Switzer & Combes 2017). In this case, pollinators may improve pollen collection by visiting more flowers per foraging trip (Balfour et al. 2021). However, if pollinators have similar flower preferences which depend on floral abundance, strong interspecific competition may occur, especially when the level of floral resource is low, e.g., at the early flowering stage. Floral use partitioning may reduce such competition, e.g. through staggered bee phenologies (Duchenne et al. 2019; Paudel et al. 2019).

In this study, we aimed to detect variations in flower preferences among bumblebee species on co-flowering pollen-rewarding flowering species via buzz pollination, and whether bumblebees' flower preferences are associated with buzz frequencies and fluctuations in floral resources

across time. We monitored five co-flowering nectarless *Pedicularis* species (lousewort) in a natural alpine meadow, and recorded six bumblebee species (of which three were abundant) visiting flowers of the five *Pedicularis* species during the blooming period in five seasons from 2018 to 2022. We hypothesized that bumblebees' flower preferences are associated with fluctuations in floral resources across time. In addition, the amount of pollen removal from flowers and deposition on stigmas per visit should vary among *Pedicularis* species when they are visited by different species of bumblebees. Specifically, we asked the following questions: (i) Do different species of bumblebees vary in their buzz frequency, and does the same species produce different buzz frequencies while visiting different flowering species? (ii) Are bumblebees' flower preferences linked to fluctuations in floral resources, and if so, how could bumblebees achieve partitioning of floral resource use? And (iii) Does pollen removal and deposition efficiency depend on bumblebee species?

2 Materials and methods

2.1 Study site and species

Study system. This study was conducted in a natural alpine meadow in Shangri-La, northwest of Yunnan Province, China (27°37'40"N, 99°47'32"E, c. 3,379 m a.s.l.), that occupied an area of 120,000 square meters. We studied five perennial, exclusively pollen-rewarding flowering plant species of *Pedicularis* L., namely, *P. cephalantha* Franch ex Maximowicz, *P. rhinanthoides* Schrenk ex Fischer & C. A. Meyer, *P. monbeigiana* Bonati, *P. longiflora* Rudolph and *P. siphonantha* Don. The flowering periods of these species overlap and occur from late June to August (due to altitude etc.). *Pedicularis* species in the eastern Himalayan region are exclusively pollinated by bumblebees (Corbet & Huang 2014). The five nectarless, exclusively pollen-rewarding *Pedicularis* species studied here have a beak-like corolla, and the four longitudinally dehisced anthers are located in the twisted corolla beak (Fig. S1). These plants are entirely reliant on bumblebee pollination for reproduction.

Survey of bumblebees and *Pedicularis* flowers. We sampled bumblebees for five consecutive years, from 2018 to 2022. In each survey, we sampled selected walking transects (50 m in length and 2 m in width) weekly throughout the flowering season. The transects were set to include a representative diversity and abundance of floral resources (see details in Appendix S1). New walking transects were added to the field survey as floral resources fluctuated in abundance and space over the course of the flowering season. In total, 15 to 61 walking transects were established from 2018 to 2022, respectively (Table S1). We recorded all bumblebees visiting flowers by walking transects at a constant speed for about 10 minutes, from 9:30am to 4:30pm on warm and dry

days. For each bumblebee species, we estimated its abundance as the average number of individuals across all transects for each census day. All bumblebee individuals were caught with a sweep net for later identification (see details in Appendix S1).

To quantify the flowering resources available for bumblebees, we established five 2×2 m plots within each walking transect, homogeneously distributed every ten meters along the walking transects. The number of flowers of all five *Pedicularis* species within each plot was counted after each transect walk (Appendix S1). For each *Pedicularis* species, the mean number of open flowers per plot was calculated as a measure of floral abundance on each census day (Appendix S1).

Bumblebee's buzz frequency. Bee buzzing behavior in the field was examined at the flowering peak from July to August in 2020. Visiting bees were observed approaching the flowers. When the bees were about 5 cm away, we used a mobile phone (iPhone 11) to record a video of the bees visiting the flowers (De Luca et al. 2020). Recordings were saved as wave files (44.1 kHz sampling rate). The phone used a long stand to reduce noise interference and bee disturbance. We used Audacity v. 2.4.2 (<https://sourceforge.net/projects/audacity/>) to measure the fundamental frequency (hertz, Hz) of floral sonication, and the duration (seconds) of floral sonication vibrations. We measured peak frequency using the "Spectrum" function (Hamming window, FFT size = 8,192 Hz) (De Luca et al. 2019, 2013). Some bees would buzz a flower several times during a single visit, in which case we measured each individual buzz and used the mean values for that bee in subsequent analyses (De Luca et al. 2013). After extending the recorded waveform on the time axis, the pitch of each buzz was estimated by counting the major cycles in one-tenth of a second (Corbet & Huang 2014; De Luca et al. 2019). We recorded buzz frequency for the three main bumblebee species when visiting flower of each of the five flowering species (number of audio clips recorded: 80 for *B. festivus*, 146 for *B. friseanus* and 29 for *B. lepidus*).

2.2 Bumblebees' flower preferences and fluctuations in floral resources

We investigated the flower preference of each bumblebee species as a function of the relative floral abundance of the five *Pedicularis* species. The flower preference of a bumblebee species referred to the relative visiting frequency on each of the five flowering species across years, estimated as the total number of visits to each *Pedicularis* species in a year divided by the total number of visits to all five *Pedicularis* species in a year. The relative floral abundance for a *Pedicularis* species in a year referred to the total flower number of the species out of the total number of flowers from all five flowering species for all censuses within the year. We then investigated whether the preference for a *Pedicularis* flower for each bumblebee species changed with the fluctua-

tions in relative floral abundance of the flowering species across years. By using the recorded bumblebee abundance across the census days, we estimated the abundance dynamic curve for each of the bee species in each year from 2019 to 2022, except for 2018 due to the limited census days. We employed the Schoener index to assess the level of phenological overlap among pairs of bumblebees; Schoener index values range between 0 and 1, with higher values indicating a larger overlap (Schoener 1970).

2.3 Bumblebees' pollen removal and deposition efficiency

We assessed the amount of pollen released per visit as an indicator of the efficiency of pollen removal through buzz pollination by each bumblebee species. We quantified pollen deposition efficiency by counting the stigmatic pollen load of a flower following a single visit by a specific bumblebee species. For each plant species, fifty individual plants were bagged with fine-meshed nylon nets before flowering to exclude visits by bumblebees. The flowers were exposed to bumblebees on the day they opened. Flowers receiving a single visit by a bumblebee were marked and enclosed immediately; those flowers were fixed in 75% ethanol the next day for laboratory measurement of pollen removal and deposition (Appendix S1). For each bumblebee species, more than 30 flowers were harvested to estimate pollen removal and deposition efficiency (Appendix S1). In 2020, we observed 10 interaction types between the five *Pedicularis* species and the three main bumblebee species, data from these interactions (Table S2) were used for further analyses.

2.4 Statistical analysis

To investigate whether bees vary their buzz frequencies when visiting flowers from different *Pedicularis* species, we used separate linear mixed effects models (LMMs) for each bee species, in which buzz frequency was the response variable, plant species the explanatory variable, with bee ID as a random effect. LMMs were also used to compare the buzz frequency among different species of bumblebees, in which buzz frequency was the response variable, bee species the explanatory variable, with bee ID as a random effect. We chose not to use a single model with both bee and plant species because our dataset contained missing values (some interactions between bee-plant species pairs were never observed naturally occurring). We log-transformed buzz frequency to fit the assumptions of linear regression (Ramsey & Schafer 2002) and used a normal error distribution and verified that the residuals of the models had an approximately normal distribution. When the ANOVA (Type III Analysis with Satterthwaite's method) of the model was significant, a posthoc Tukey's test was used for mean comparisons between species.

To investigate whether bumblebees' flower preferences are influenced by the relative floral abundance of flowering

species, we used generalized linear models (GLMs) with a quasi-binomial error distribution and a logit-link function. Three GLM models were applied for each of the three main bumblebee species. Flower preference was used as a response variable, and the relative floral abundance and year were used as explanatory variables. We then plotted partial residuals of the GLM models using the *visreg* R-package (Breheny & Burchett 2017).

To compare pollen removal across bumblebee species and among different flowering species, we used GLMs with a gamma error distribution and an inverse-link function to accommodate that pollen removal data are continuous. The plant species was used as the response variable and pollen removal was used as the explanatory variable. To assess pollen removal between different bumblebee species visiting the same flowering species, we used GLMs with a gamma error distribution and an inverse-link function. The bumblebee species was used as the response variable and pollen removal was used as the explanatory variable. To investigate how pollen deposition on stigmas of flowers of a *Pedicularis* species differed among bumblebee species, we used generalised linear mixed effects models (GLMMs) with a quasi-Poisson error distribution and a log-link function. The bee species was used as the explanatory variable, the pollen depositions was used as the response variable, and bee ID as a random effect.

All models were analyzed in R v. 3.5.1 (R Core Team 2022) using the packages *glmmTMB* for GLMMs, *MASS* for GLMs, and *lme4* and *lmerTest* for LMMs. Significance of terms in GLMs and GLMMs was estimated using the function “Anova” (Type II Wald chisquare tests) from the package *car*.

3 Results

A total of 1,686 interactions were recorded between the six bumblebee species and the five *Pedicularis* species from 37 censuses over the five years (Table S1, S3). Among these interactions, *B. festivus*, *B. friseanus*, *B. lepidus*, *B. securus*, *B. impetuosus*, and *B. longipennis* were identified as visitors to *Pedicularis* flowers in the meadow. Our investigations indicated that on a given census day, the ratio of overall visits to the five pollen-rewarding flowers out of total visits of all co-flowering species by the bumblebees could reach 68% (Table S1). *B. lepidus* and *B. friseanus* were the most abundant bees in the meadow, followed by *B. festivus*; they accounted for 40.60%, 37.65% and 21.74% out of all the recorded visits, respectively. The other three bee species were recorded to visit the five *Pedicularis* species at a very low frequency (a total of 15 times in five years; Table S3; Fig. S2A) and were thus removed from further analysis. In addition, the relative floral abundance of the five *Pedicularis* species fluctuated substantially across the five years (Fig. S2B; Table S4). For example, the relative abundance of *P. siphonantha* was only 5.6% in 2019 but over 50% in the other years.

3.1 Bumblebee's buzz frequency

Buzz frequency significantly differed among the three bumblebee species (Fig. 1A, Table S5). *B. friseanus* and *B. lepidus* had the highest buzz frequency while *B. festivus* had the lowest frequency (Fig. 1A). Individuals of *B. friseanus* and *B. lepidus* did not alter their buzz frequency when visiting different flowering species (Table S5; Fig. 1C, D). In contrast, *B. festivus* used a lower buzz frequency when visiting *P. longiflora* flowers compared to those of other species (mean buzz frequency: 296 Hz for *P. rhinanthoides*, 286 Hz for *P. cephalantha*, 266 Hz for *P. longiflora*; Fig. 1B; Table S5).

3.2 Bumblebee's flower preferences and fluctuations in floral resources

The three main bumblebee species displayed different patterns in flower preferences among the flowering species as a function of relative floral abundance. Flower preference in *B. friseanus* and *B. lepidus* increased significantly as a function of floral abundance ($\chi^2 = 17.44$, $p < 0.001$ for *B. friseanus* and $\chi^2 = 23.63$, $p < 0.001$ for *B. lepidus*; Fig. 2A, B). However, flower preference in *B. festivus* did not change with fluctuations in relative floral abundance ($\chi^2 = 0.0001$, $p = 0.99$; Fig. 2C). Results indicated that across the five years, the foraging preference of *B. festivus* to flowers of *P. cephalantha* and *P. rhinanthoides* (accounting for 90% of its total visits) was maintained despite the fluctuations in relative floral abundance of the five pollen-rewarding plants (Fig. S3; Table S3). However, *B. friseanus* and *B. lepidus* substantially changed their foraging preferences across years (Fig. S3); both bee species mainly foraged on the most abundant flowers in each year (Fig. S2, S3). Moreover, we did not find the flower preferences was influenced by year for the bees ($\chi^2 = 0.001$, $p = 1$ for *B. festivus*, $\chi^2 = 0.35$, $p = 0.987$ for *B. friseanus* and $\chi^2 = 0.50$, $p = 0.974$ for *B. lepidus*).

Results revealed that the time of peak abundance of *B. friseanus* and *B. lepidus* was substantially staggered between the two species in most of the years (Fig. S4), indicating a limited phenological overlap (Table S6). However, the peak abundance of *B. festivus* overlapped with either that of *B. friseanus* or *B. lepidus* (Fig. S4), showing a greater phenological overlap compared to that between *B. friseanus* and *B. lepidus* (Fig. S4; Table S6).

3.3 Bumblebees' pollen removal and deposition efficiency

For each of the *Pedicularis* species, there were no significant differences in pollen deposition when the flower was buzz-pollinated by different bumblebee species ($\chi^2 = 2.42$, $df = 1$, $p = 0.120$ for *P. cephalantha*, $\chi^2 = 0.96$, $df = 2$, $p = 0.619$ for *P. longiflora*, $\chi^2 = 1.78$, $df = 1$, $p = 0.183$ for *P. rhinanthoides* and $\chi^2 = 0.13$, $df = 1$, $p = 0.719$ for *P. siphonantha*; Fig. S5). However, our results indicated that for each of the bumblebee species, pollen removal per visit significantly

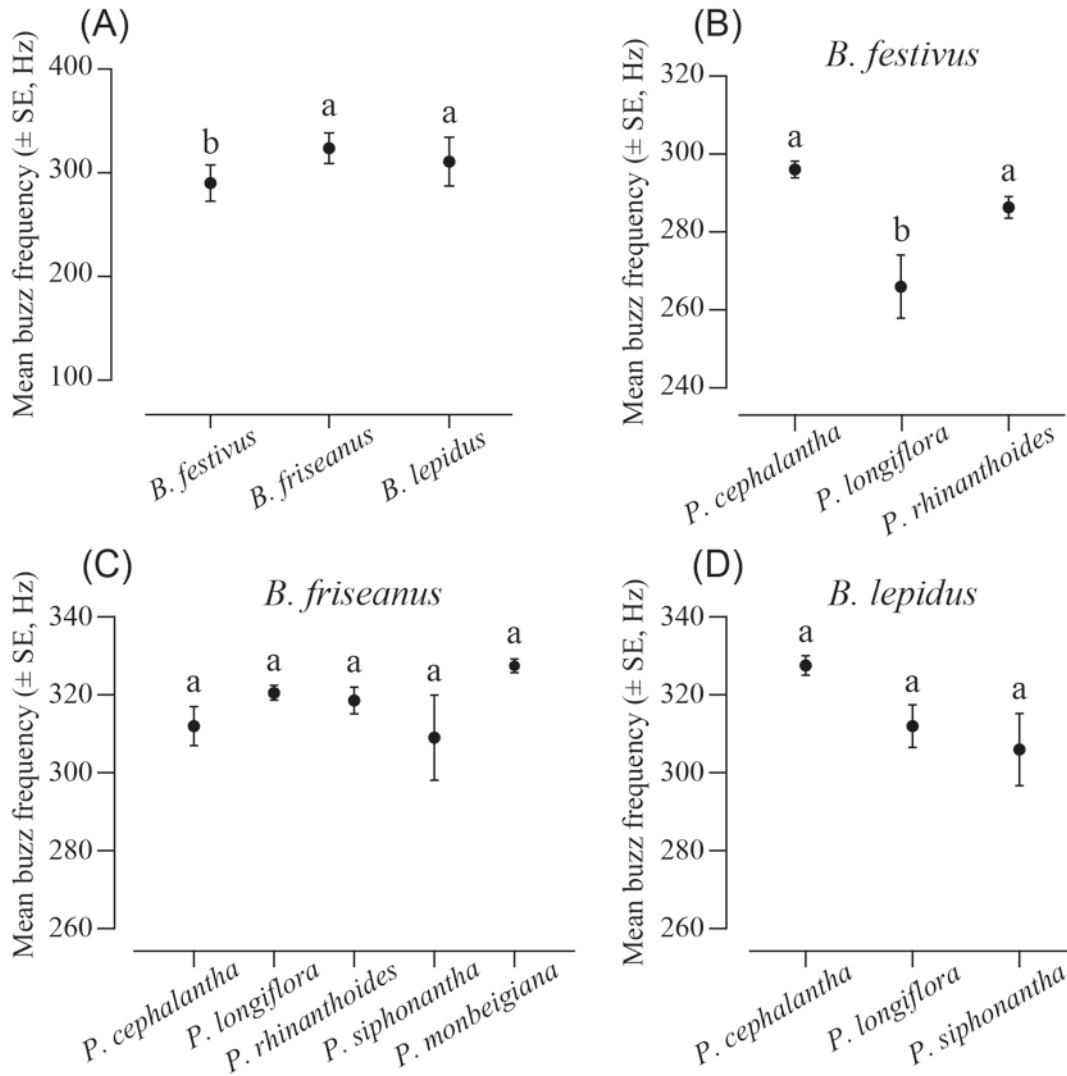


Fig. 1. Buzz frequency of the three main bumblebee species when visiting different *Pedicularis* species. (A) Mean buzz frequencies for the three bumblebee species; Buzz frequencies of (B) *B. festivus*, (C) *B. friseanus* and (D) *B. lepidus* while visiting different *Pedicularis* flowers. Different letters in each panel indicate statistically significant differences as assessed with a Tukey's test.

differed when visiting different plants ($\chi^2 = 5283.8$, $df = 4$, $p < 0.001$ for *B. friseanus*, $\chi^2 = 1750.6$, $df = 1$, $p < 0.001$ for *B. lepidus* and $\chi^2 = 1592.1$, $df = 2$, $p < 0.001$ for *B. festivus*; Fig. S6). For the *Pedicularis* species excluding *P. siphonantha*, the pollen removal per visit significantly differed when visited by different bumblebee species ($\chi^2 = 6.08$, $df = 1$, $p = 0.014$ for *P. cephalantha*, $\chi^2 = 8.689$, $df = 2$, $p = 0.013$ for *P. longiflora*, $\chi^2 = 5.719$, $df = 1$, $p = 0.017$ for *P. rhinanthoides* and $\chi^2 = 0.001$, $df = 1$, $p = 0.974$ for *P. siphonantha*; Fig. S6). Regardless of the bumblebee species, the amount of pollen removal was highest in flowers from *P. rhinanthoides*, followed by *P. cephalantha*; and the lowest were from *P. siphonantha*, *P. longiflora* and *P. monbeigiana* ($\chi^2 = 9528.6$, $df = 4$, $p < 0.001$; Fig. S7).

4 Discussion

Morphological matching plays important roles in determining the flower preferences of bees when foraging flower nectar (Klumpers et al. 2019). However, little is known about factors influencing bees' flower preferences when collecting pollen through buzz pollination. We have shown here that in buzz-pollinating different obligate pollen-rewarding *Pedicularis* species, *B. friseanus* and *B. lepidus* used a similar buzz frequency and displayed a flower preference that changed in response to fluctuations in relative floral abundance. Conversely, *B. festivus* mostly visited *P. rhinanthoides* and *P. cephalantha* regardless of their relative abundance in the community, and using similar buzz frequencies, while

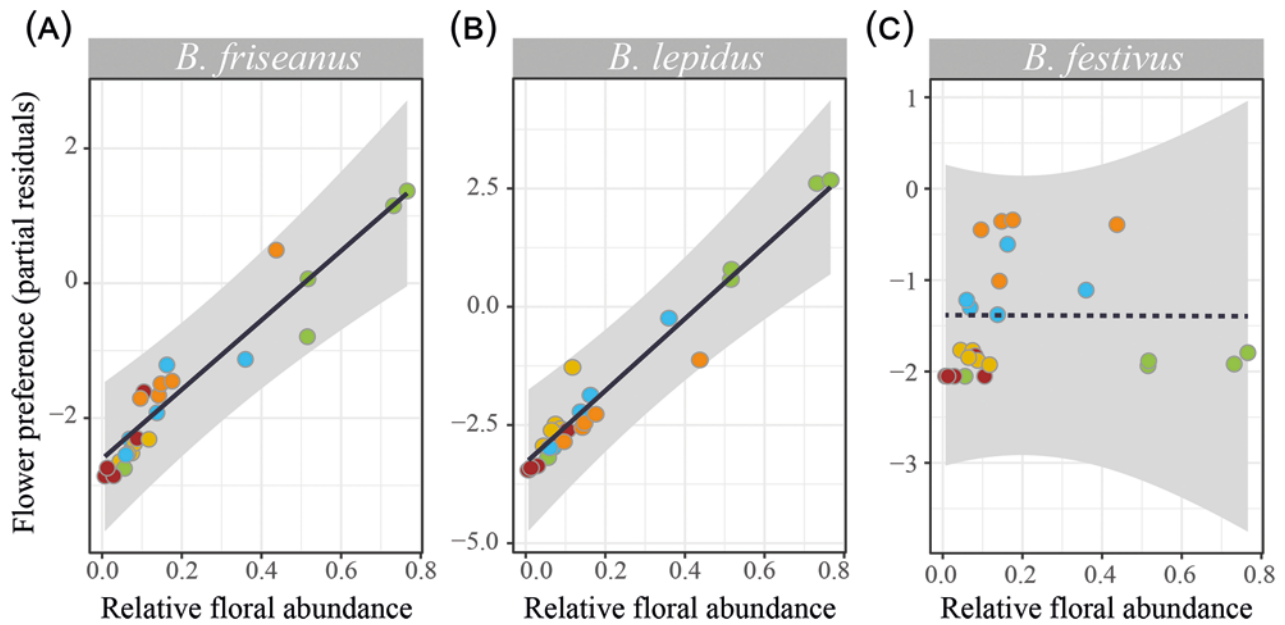


Fig. 2. Partial residuals plots showing the flower preference of (A) *B. festivus*, (B) *B. friseanus* and (C) *B. lepidus* in response to relative floral abundance of the *Pedicularis* species over the five studied years. Plain lines show significant correlations, while the dashed line shows a non-significant correlation; the shaded areas show: 95% confidence intervals. The different colors refer to different *Pedicularis* species, namely, green dots for *P. siphonantha*, orange for *P. cephalantha*, blue for *P. rhinanthoides*, brown for *monbeigiana*, yellow for *P. longiflora*, respectively. Y-axes show partial residuals, i.e. deviations of the observed preference values from the predicted model values in (A) and (B), partial residual values depend on relative floral abundance, where negative partial residuals are found at low relative floral abundance (indicating that the preference to the corresponding flowering species in that year is lower than expected), while positive partial residuals are found at high relative floral abundance. The flower preference of a bumblebee species is determined by dividing the number of visits to each *Pedicularis* species in a year by the total visits to all five *Pedicularis* species. The relative floral abundance of a *Pedicularis* species within a year is calculated based on the total count of its flowers compared to the total number of flowers from all five flowering species across all censuses conducted in that year.

using a lower buzz frequency when foraging on *P. longiflora*. The different flower preferences among bumblebees when buzz-pollinating co-flowering plants are potentially associated with bees' vibration properties (or strategies).

Individuals of *B. friseanus* and *B. lepidus* used similar buzz frequencies to release pollen grains from different flowering species, and they preferred to visit the most abundant floral resources independently of the species of *Pedicularis*. Such an abundance-dependent foraging strategy may enhance the efficiency of their net energy intake. Bumblebees focusing on abundant flowers may avoid searching across a wider area (Pyke 1984), which enables them to minimize energy expenditure associated with long flights and extensive searching (Balfour et al. 2021). However, studies have shown that there may be a trade-off between the suitability of a resource and its availability (Balfour et al. 2015), and thus, some pollinators could prefer plants with greater net energetic benefit but with lower abundance at the cost of increased foraging time (Balfour et al. 2021). For example, individuals of *B. festivus* did not respond positively to floral abundance, which might hint that they used a strategy to increase net energy intake per visit instead of foraging from the most-abundant flowers. This argument is supported by the fact that the preferred

flowers of *B. festivus* (*P. rhinanthoides* and *P. cephalantha*) provided more pollen than flowers of the other three lousewort species. Curiously, when visiting the rarer *P. longiflora*, which offers fewer rewards, they displayed a different buzz frequency. Whether this change in buzz frequency in *B. festivus* as a function of the plant species visits arises due to the capacity to adjust buzz frequency by individual bees or through sorting of different bee individuals on different flowering species remains to be established. It is unclear whether an optimal buzz frequency is required for a bumblebee to pollinate a specific flower. Corbet & Huang (2014) reported that the ratio of buzz-to-wingbeat frequency produced by bumblebees was changed according to variation in floral traits (e.g., galea length) among *Pedicularis* species, suggesting a link between the vibration properties of bees and the specific flowering species they visited. Yet, change in buzz frequency alone may not result in increased efficiency of pollen transfer via floral vibration because bumblebees may depend on all the vibration properties (e.g., frequency, duration, and amplitude) to release pollen (De Luca et al. 2013; Papaj et al. 2017; Switzer et al. 2019; Vallejo-Marín 2019). For instance, bees can increase pollen collection from a flower by using multiple or longer vibrations (Harder

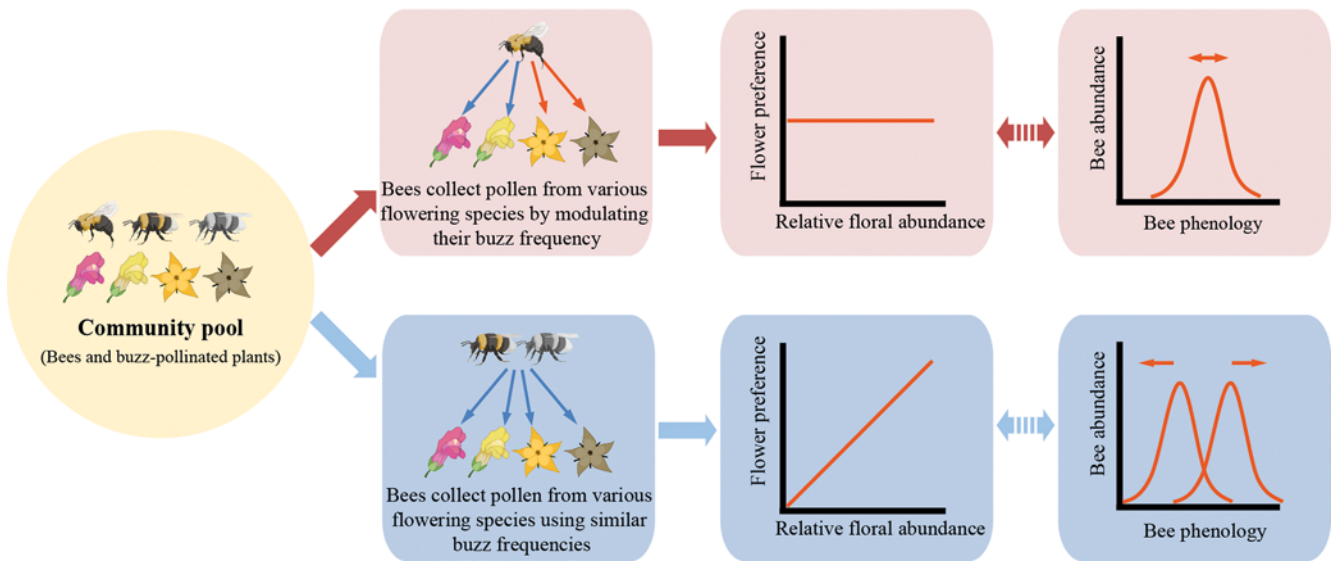


Fig. 3. A conceptual framework based on results of the present study showing the interactions between bumblebees' buzz-pollination strategy and flower preferences on co-flowering buzz-pollinated species in a community leading to partitioning of floral resources use. Bumblebees may have two patterns of flower preference: (i) They use distinct buzz frequencies to expel pollen from flowers of different plant species. This could drive a constant flower preference towards the most rewarding flowering species independently of its relative floral abundance. In addition, the specific flower preference enabled by a specialist buzzing behavior contributes to partitioning of floral resources use with co-occurring bees with no strong selection for diverging phenologies. (ii) They collect pollen using similar buzz frequencies among bee species and to expel pollen from different flowering species, and their flower preferences are driven by the relative floral abundance. In this scenario with a generalist behavior, staggered phenologies among bee species are expected to be selected to achieve partitioning of floral resource use and reduce competition among bee species.

1990; Kawai & Kudo 2009; De Luca et al. 2013). In our study, two bumblebee species, *B. friseanus* and *B. lepidus* were found to use similar buzz frequencies to expel pollen from different flowering species, suggesting that bee modulations of buzz frequency are either not achievable or not beneficial. Comparatively, variations in buzz frequency for *B. festivus* when visiting different flowers suggests that at the species level, there could be associations between buzz frequency and the flowering species visited. Investigations on the determinants and species-level plasticity in vibration strategies among buzz-pollinating bumblebee species when visiting flowers with different architectures would be useful to understand the mechanisms shaping plant-pollinator interactions in buzz-pollinated systems.

Abundance-dependent foraging strategies like those found in *B. friseanus* and *B. lepidus* are important in structuring plant-pollinator interactions in a community, and theoretically enhance the pollination success of the abundant species by transferring higher intraspecific to interspecific ratio of pollen (Vázquez et al. 2009; Verdú & Valiente-Banuet 2011). However, individuals of the two bumblebee species may strongly compete for floral resources due to their similar flower preferences. In our study meadow, the staggered phenologies between *B. friseanus* and *B. lepidus* may reduce resource competition and partition the use of the most abundant floral resources, ultimately contributing

to enhancing the stability of pollinator-plant interactions (Duchenne et al. 2019; Paudel et al. 2019). We found that *B. festivus* has a different fundamental buzz frequency compared to the other two bumblebee species and uses different buzz frequencies when foraging on distinct plant species. Its flower preference is different from the other two bumblebee species but is constant towards high pollen-rewarding flowering species across years independently of their floral abundance. This flower preference contributes in segregating its ecological niche from that of the other two bumblebee species despite their overlapping phenologies. This specialized interaction of bumblebee and flower may function as an additional mechanism in partitioning of floral use among the co-occurring bumblebee species (Pauw 2013).

The five *Pedicularis* plants co-flowered within a relatively short period (about two months) in this alpine meadow. As the only few exclusively pollen-rewarding plants, they may be key for early development of bumblebee colonies (Kriesell et al. 2017). Our results show that the different bumblebee species deposited the same amount of pollen per visit but differed in pollen removal efficiency, which could affect male fitness in flowering species, selecting for the *Pedicularis* species to favor visitation by particular bumblebee species. Illustrating the link between floral traits and flower vibration properties, and its influence on pollen release efficiency through bee's buzz pollination may be

helpful to understand the mechanism on how such selection occurs (Corbet & Huang 2014), however, it needs further investigation.

Our study suggests a potential framework of how different pollen foraging mechanisms (e.g., different buzz frequencies) might shape preferences of buzz-pollinators for different flowering species (Fig. 3). If the capacity to remove pollen from different flowering species depends on the type of buzzes bees can produce, bees might choose flowering species not only based on their relative abundance but also on what types of buzzes are most effective at removing pollen from each flowering species. If bumblebees can expel pollen from different flowering species using a similar buzz frequency, they might display an abundance-dependent flower preference. Conversely, when bumblebee species produce different buzz frequencies and these differentially affect how different flowering species expel pollen grains, bees might adopt a reward-dependent flower preference triggering a constant preference to flowering species with high pollen reward, even when at low abundance. The cost of strong interspecific competition for floral resources raised by similar flower preferences is expected to be reduced by segregated phenologies among the bees. The processes structuring the interactions between bumblebees and their buzz-pollinated flowers may enable the coexistence of diverse bumblebee species and co-flowering plant species in a community.

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Figure S1–S7, Table S1–S6, Appendix S1