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Author(s)	Shibata, Akari; Kudo, Gaku
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1 **Floral abundance and bee density affect species-specific foraging patterns of alpine bumble**
2 **bees**

3
4 **Akari Shibata^{1,2}, Gaku Kudo²**

5 1. *Corresponding author:*

6 *Akari Shibata,*

7 aka.11mbbc28@gmail.com

8 2. *Faculty of Environmental Earth Science, Hokkaido University, Sapporo, Hokkaido, 060-*
9 *0810 Japan*

10

11

12 **Abstract**

13

14 In response to the qualitative and quantitative changes in floral resources, bumble bees flexibly
15 forage multiple plant species throughout the growing season. During the short summer in an alpine
16 ecosystem, the activity of worker bees is maximized in the middle of the season, when the
17 competition for floral resources may be intense.

18 We predicted that the foraging patterns of bumble bees are affected by both relative floral
19 abundance and interactions between bumble bee species. We recorded the floral abundance of
20 individual plant species and the foraging frequency of bumble bees in an alpine meadow during the
21 major flowering period over 3 years.

22 Two bumble bee species were common during the major flowering period. Although they
23 tended to visit abundant floral species, the shorter-tongued species (*Bombus hypocrita*) showed a
24 more diverse and flexible floral choice than the longer-tongued species (*Bombus beaticola*). The
25 degree of floral use overlap between two bumble bee species tended to decrease when the foraging
26 density of the longer-tongued species was high.

27 These results indicated that multiple bumble bee species are able to coexist when certain bee
28 species can flexibly change targeting flowers in response to the temporal variations in flowering

29 species and the density of competing bee species. The extent of foraging flexibility is related to the
30 morphological traits of bee species and availability of floral resources.

31

32 **Keywords:** *Bombus*, floral choice, flowering phenology, pollination, seasonal dynamics, visitation
33 frequency

34

35 **Introduction**

36

37 Bumble bees (*Bombus* spp.) are the most common and effective pollinators in cool-temperate, alpine,
38 and subarctic ecosystems (Heinrich 1979; Bingham and Orthner 1998; Kudo 2016). They are
39 eusocial insects with an annual life cycle, and their colony growth and reproductive success depend
40 on the availability of floral resources (Schmid-Hempel and Schmid-Hempel 1998; Pelletier and
41 McNeil 2003; Inari et al. 2012). Reflecting the annual cycle of colony development, the activity of
42 worker bees is maximized in the middle of summer. In the short growing season of alpine
43 environments, floral visits by worker bees drastically increase after mid-July (Kudo 2016). Intensive
44 floral use by worker bees may cause the partitioning of floral resources among bee species, but the
45 pattern of interspecific resource partitioning was not consistent in the previous studies (Goulson
46 2010; Miller-Struttman and Galen 2014).

47 Interspecific competition for resource acquisition is one of the most important factors
48 affecting community structure (Schoener 1974; Schaffer et al. 1979). Foraging patterns of bumble
49 bees were surveyed several times in the previous studies in order to evaluate the existence of
50 resource competition (Heinrich 1976a; Inouye 1978; Pyke 1982; Thomson 2004). Foraging bees
51 obtain nutrients from nectar and/or pollen, and the floral use period of multiple species can overlap.
52 When floral resources are abundant, the bees can share the preferable floral species; otherwise, they
53 have to shift to other available floral species to reduce the competition. One of the mechanisms for
54 resource partitioning is morphological matching between floral shape and tongue length of bees in
55 which bumble bee species having different tongue length can coexist due to small foraging niche
56 overlap (Heinrich 1976a; Pyke 1982; Inoue and Yokoyama 2006; Pyke et al. 2012). However, this
57 pattern was not always clear. Some studies reported that bumble bee species with similar tongue
58 length coexisted irrespective of large niche overlap (Ranta E 1980; Goulson et al. 2008). Therefore,
59 resource partitioning between bumble bee species needs to be explained not only by morphological
60 matching but also by species composition and abundance of bumble bees and floral resources in
61 each site.

62 Previous studies have shown that bumble bees tend to visit abundant floral species, which is

63 termed ‘frequency-dependent foraging’ (Smithson and Macnair 1996). However, the pattern of floral
64 choice is also affected by the preference and foraging experience of individual bees (Heinrich 1979;
65 Dukas and Real 1993; Raine and Chittka 2005). They potentially prefer floral species with the
66 corolla shape matching their body shape and those with more floral rewards. After learning which
67 floral species possess specific traits associated with floral rewards, individual bees tend to have their
68 own flower constancy (Heinrich 1976b; Gegeer and Thomson 2004). In addition, the relative
69 importance of floral resources for bee colonies changes depending on the stage of colony
70 development. Pollen, which contains proteins and lipids, is used for larva development, and nectar
71 is used as energy source for adult bees also (Tasei and Aupinel 2008; Nicolson 2011). The
72 composition of pollen and nectar varies among different floral species (Somme et al. 2015). In
73 natural plant communities, available floral species are replaced quickly and bee abundance is also
74 variable as the season progresses. When the density of foraging bees is high, individual bees may
75 behave in a more generalist manner having wider floral diet (Fontaine et al. 2008). Therefore,
76 seasonal changes in abundance and diversity of floral species and species-specific population
77 dynamics of bumble bees across the season should be taken into account when trying to understand
78 the competitive situation of bumble bees. However, analytical studies on the foraging patterns of
79 bumble bees responding to the seasonal dynamics of floral resources are scarce.

80 In this study, we evaluated the factors affecting foraging patterns of bumble bees in an alpine
81 meadow on Mt. Asahidake in Hokkaido, northern Japan. Several bumble bee species having
82 different body size and tongue length, such as *Bombus beaticola moshkarareppus*, *B. diversus*
83 *tersatus*, *B. hypnorum koropokkrus*, *B. hypocrita sapporoensis*, and *B. yezoensis*, were recorded in
84 the alpine zone of this mountain area (Kudo 2014). This provided us an opportunity to analyze the
85 effects of floral dynamics and bee density on foraging patterns of bumble bees in the field. We used
86 data on foraging frequency of worker bees and floral abundance throughout the active foraging
87 season during 3 years. We addressed the following questions: (1) How extent do species composition
88 of bumble bees and availability of floral resources vary as the season progresses in the alpine
89 meadow? (2) Do bumble bees tend to visit floral species with large abundance at any given time?
90 (3) Does the floral resource partitioning between bee species occur intensively at the time of high

91 bee density? If so, does the pattern of resource partitioning reflect the morphological difference
92 between the bee species?

93

94 **Materials and Methods**

95

96 **Study site and survey**

97 This study was conducted in an alpine meadow (1,610–1,745 m a.s.l.) on Mt. Asahidake in the
98 Taisetsu Mountains, Hokkaido, northern Japan (43°39'49"N, 142°51'15"E). In this area, the treeline
99 is located at around 1,600 m a.s.l., and snowmelt progresses gradually from early June to late July.
100 The mean annual temperature at 1,700 m a.s.l. is -1.9°C , ranging from -16.1°C in January to 12.5°C
101 in August (average of the period 2002–2018; G. Kudo, unpublished data). The flowering of alpine
102 plants usually occurs from early June to the middle of September. We recorded visitation frequency
103 of bumble bees, flowering phenology of insect-pollinated plant species, and the amount of floral
104 resources along the 2.6 km trail, which was divided into four transects (Fig. S1). Observation was
105 conducted at approximately 10-day intervals throughout the active season of worker bees (from mid-
106 July to early September) in 2015 and 2016 (in total, ten surveys each summer). Furthermore,
107 additional observations were conducted three times at the peak of the season in 2018.

108

109 **Bee observation**

110 We recorded the number of bumble bees that were visiting the flowers as well as the number of
111 plant species foraged by bumble bees within about 5 m on both sides of the trail. On each
112 observation day, one to three observers walked slowly along the trail for about 30 min in each
113 transect. In total, 37, 36, and 12 observations were made in 2015, 2016, and 2018, respectively.
114 Species and castes of bumble bees were identified in the field. Observations were conducted under
115 conditions of no rain, air temperature higher than 9°C , and with wind speed lower than 6 m/sec.

116

117 **Floral abundance and flowering phenology**

118 We recorded the number of open flowers within 2×2 m quadrats. We arbitrarily set quadrats on
 119 floral patches in order to cover every insect-pollinated plant species that was flowering on each
 120 day of the investigation. In total, around 35 (ranging 8 to 48) quadrats were set depending on the
 121 flowering conditions of individual plant species in each survey. The flowering species and
 122 abundance largely change across the season (Kudo 2019; Fig. 2a). Because a half of the transect
 123 was still covered with snow in early season, we conducted the survey only at the snow-free area.
 124 In late season, flowering periods of the most plant species had finished and the amount of
 125 flowering species was small. As a result, the number of quadrats was small early and late in the
 126 season. Therefore, flower density and diversity were evaluated at floral patch scale in this study.
 127 Average number of flowers per 4 m² (total flower number of each species divided by the number
 128 of quadrats) was used as an index of floral abundance at community scale. Instead of counting the
 129 actual number of florets, we used the number of capitula for Asteraceae species, number of small
 130 units of umbels for Apiaceae species, and number of inflorescences for *Spiraea* species. Plant
 131 species that were visited by two dominant bee species, *Bombus hypocrita sapporoensis* and/or
 132 *Bombus beaticola moshkarareppus*, at a frequency of >1% of total visits in 2015 and 2016 were
 133 defined as major plant species (Table 1).

134

135 **Data analyses**

136 In order to assess the diversity of flowering plant species (floral diversity) and the diversity of
 137 plant species visited by bumble bees (foraging diversity), the Simpson's diversity index D
 138 (Simpson 1949) was calculated as follows:

$$139 \quad D = 1 - \sum_{k=1}^s \left(\frac{n_i(n_i-1)}{N(N-1)} \right),$$

140 where s is the number of plant species, n is the total flower number of plant species i , and N is total
 141 number of flowers in all plant species.

142 For the evaluation of floral use overlap between two dominant bumble bee species (j and
 143 k), the Morisita's niche overlap index C between j and k (Morisita 1959) was calculated as follows:

$$144 \quad C = \frac{2 \sum_i^n p_{ij} p_{ik}}{\sum_i^n p_{ij} [(n_{ij}-1)/(N_j-1)] + \sum_i^n p_{ik} [(n_{ik}-1)/(N_k-1)]},$$

145 where p is the proportion of flower number of plant species i to total number of flowers visited by
146 bee species j or k , n is the number of bee species j or k that visited plant species i , and N is the total
147 number of bee species j or k .

148 In order to evaluate the relationship between total floral abundance and total number of
149 observed bumble bees in each survey of 2015 and 2016, we performed generalized linear model
150 (GLM) postulating negative binomial error distribution with log-link function. Total floral
151 abundance available for each bee species was expressed as the cumulative flower number of the
152 plant species visited by the focal bee species at least once. Total number of bumble bees was set as
153 the response variable, and total floral abundance, bee species, and year were set as the explanatory
154 variables with inclusion of their interactions.

155 In order to evaluate the floral preference of bumble bees among major plant species, we
156 used GLMs to evaluate the relationships between relative floral abundance of specific plant
157 species and foraging frequency to the top five most-visited floral species by worker bees. Relative
158 floral abundance of a specific plant species in each survey time was calculated by dividing the
159 flower number of the species by the cumulative flower number of the top five plant species. GLMs
160 postulating binomial error distribution with logit-link function were performed independently for
161 each plant species. The foraging frequency was set as the response variable, and relative floral
162 abundance was set as the explanatory variable. Survey data including the plant species visited by
163 >5 worker bees were used for the analyses.

164 We used generalized linear mixed models (GLMMs) to test the effects of floral diversity,
165 another bee species, and own species on floral use patterns of two dominant bee species, *B.*
166 *hypocrita* and *B. beaticola*. GLMMs postulating beta error distribution with logit-link function
167 were performed using the glmmTMB package in R (Mollie et al. 2017). Foraging diversity was set
168 as the response variable, and floral diversity, number of heterospecific worker bees, and number of
169 conspecific worker bees in each survey were set as the explanatory variables. Survey data
170 including the plant species visited by >10 conspecific worker bees were used for the analyses. The
171 number of bees was log-transformed to reduce overdispersion. We classified the survey periods
172 into three colony developing stages in each bumble bee species, i.e., stage-1: early stage of colony

173 development in which only small number of worker bees were observed; stage-2: middle stage of
174 colony development in which the number of worker bees increased or remained at high level;
175 stage-3: late stage of colony development in which the number of worker bees decreased, and
176 males and new queen bees appeared. For each GLMM model, colony stage nested by year was set
177 as random factor in the GLMMs.

178 In order to test the effects of heterospecific and conspecific worker bees and floral diversity
179 on floral use overlap between two dominant bee species, *B. hypocrita* and *B. beaticola*, we
180 performed GLMM postulating beta error distribution with logit-link function. In the GLMM, niche
181 overlap index was set as the response variable, and floral diversity, the number of *B. hypocrita*
182 workers, and the number of *B. beaticola* workers foraging in each survey were set as the
183 explanatory variables. Survey data including >10 worker bees of each species were used for the
184 analyses. The number of bees was normalized to reduce overdispersion. Survey period was
185 divided into 10-day intervals starting from June 20th in order to reflect seasonal changes in the
186 species composition of flowers and foraging bees, and divided survey period nested by year was
187 set as random factor in the GLMMs. The best-fit model was selected based on AIC values in all
188 GLMs and GLMMs. All analyses were conducted using R 3.5.1 (R Core Team 2018).

189

190 **Results**

191

192 **Composition of bumble bee species**

193 In total, 1680 bees were recorded foraging flowers during 3 years (396, 1004, and 280 individuals
194 in 2015, 2016, and 2018, respectively). Among five recorded *Bombus* species, *B. beaticola*
195 (48.6%) and *B. hypocrita* (43.8%) were the most dominant species, followed by *B. yezoensis*
196 (3.9%), *B. hypnorum* (3.3%), and *B. diversus* (0.4%). The two most dominant species, *B. beaticola*
197 and *B. hypocrita*, were recorded almost throughout the whole observation period, while the other
198 three species were recorded only during a short period or occasionally. Overall, the highest number
199 of worker bees was recorded in the middle of summer (late July to mid-August), and males and
200 new queen bees appeared after mid-August (Fig. 1). We recorded species-specific yearly variation

201 in colony development, which was estimated from the appearance of worker and reproductive
202 bees. The timing of colony development of *B. hypocrita* seemed to be late in 2016, while the
203 observed number of *B. beaticola* workers was very low in 2018.

204

205 **Composition of floral species and flowering phenology**

206 Throughout the 3-year survey, we recorded 37 flowering insect-pollinated plant species belonging
207 to 14 families. Among them, 18 species were shrubs, including 13 ericaceous species. Snowmelt
208 proceeded gradually across the area, and flowering of individual species occurred in response to the
209 time of snowmelt in individual habitats (Fig. 2a). Three *Phyllodoce* species, two *Gentiana* species,
210 *Solidago virgaurea*, and *Spiraea betulifolia* continued to flower for long periods at regional scale.
211 The amount of *Phyllodoce* flowers highly varied from year to year (Fig. 2b).

212

213 **Floral visits of bumble bee species**

214 In total, we recorded 17 plant species that were visited by bumble bees. In 2015 and 2016, the most
215 visited species was *Phyllodoce aleutica* (27.8%), followed by *Gentiana triflora* (26.7%) and
216 *Phyllodoce caerulea* var. *yezoensis* (18.1%). Workers of *B. hypocrita* and *B. beaticola* visited 13
217 and 16 plant species throughout the whole study period, respectively (Table 1). A big difference in
218 the floral use patterns between the two bee species was that the flowers with shallow corollas were
219 more frequently visited by *B. hypocrita* than by *B. beaticola* (the ratio of visits on cup-shaped
220 flowers was 0.40 (=161/400) in *B. hypocrita* and 0.07 (=44/620) in *B. beaticola*). Workers of both
221 of these bee species showed a high diversification of floral use, i.e., Simpson's diversity indices for
222 *B. hypocrita* and *B. beaticola* were 0.68 and 0.61 in 2015, and 0.82 and 0.74 in 2016, respectively.

223 Total number of workers was positively correlated with the total floral abundance in both
224 species ($z=5.18$, $P<0.001$ for *B. hypocrita* and $z=3.35$, $P<0.001$ for *B. beaticola*; Fig. 1, Fig. 3).
225 There was no significant difference in the number of *B. hypocrita* workers between years ($z=-1.74$,
226 $P=0.08$) and interaction between explanatory variables was excluded by the AIC mode selection.
227 The number of *B. beaticola* workers was significantly increased in 2016 ($z=2.79$, $P<0.01$), and the
228 significant interaction between total floral abundance and year was detected ($z=-2.78$, $P<0.01$). We

229 recorded positive correlations between relative floral abundance and foraging frequency in *Sieversia*
230 *pentapetala*, *Phyllodoce aleutica*, and *Peucedanum multivittatum* for *B. hypocrita* workers, and in
231 *Phyllodoce aleutica*, *Phyllodoce caerulea* var. *yezoensis*, and *Gentiana triflora* for *B. beaticola*
232 workers (Fig. 4a). In *B. hypocrita* workers, foraging frequency to *P. caerulea* var. *yezoensis* was
233 independent of the floral abundance. They choose other floral species than *P. caerulea* var. *yezoensis*
234 late in the flowering season.

235 In the GLMM for the foraging diversity of *B. hypocrita*, worker bees tended to visit more
236 plant species when floral diversity was high ($z=1.93$, $P=0.054$; Fig. 5), while the numbers of
237 conspecific (*B. hypocrita*) and heterospecific bees (*B. beaticola*) were excluded by the AIC model
238 section (Table S1). In the GLMM for the foraging diversity of *B. beaticola*, all explanatory variables
239 were excluded by the AIC model section (Table S1), indicating that the foraging pattern was
240 independent of both of floral diversity and bee density (Fig. 5).

241 The degree of floral use overlap between *B. hypocrita* and *B. beaticola* decreased in the
242 middle season of 2016 compared to that in the middle season of 2015 (Fig. 6). The GLMM result
243 showed that the floral use overlap was negatively correlated with the number of *B. beaticola* workers
244 ($z=-3.55$, $P<0.001$), while floral diversity and the number of *B. hypocrita* workers were excluded
245 by the AIC model selection (Table S1).

246

247 **Discussion**

248

249 Two common bumble bees, *B. hypocrita* and *B. beaticola* visited similar plant species, while the
250 foraging pattern was different between them. *Bombus hypocrita* tended to visit diverse flowers more
251 frequently than *B. beaticola*. Furthermore, resource partitioning was detected under the condition of
252 high bee density. In the following sections, we discussed the mechanisms underlying these findings
253 considering the differences in morphological traits, colony cycle, and floral choice between the
254 investigated bee species.

255

256 **Seasonal trends of bumble bees and flowering plants**

257 In the present research, the two dominant species, *B. hypocrita* and *B. beaticola*, were continuously
258 recorded during the summer, indicating that these species are residents of the alpine site. *Bombus*
259 *hypocrita* is the most common bumble bee species in Hokkaido, distributed from the coastal
260 grasslands to the alpine areas, and all castes (overwintered queens, workers, new queens, and males)
261 were observed in the investigated alpine site, suggesting the existence of sedentary populations in
262 this alpine environment. *Bombus beaticola* is the second dominant species in the Taisetsu Mountains
263 and this species nests in alpine areas, but overwintered queens are rarely observed in early summer
264 (Tomono and Sota 1997; Kudo 2014). This indicates that queen bees may overwinter below
265 timberline and migrate to the alpine site for nesting. In contrast, other bee species were sporadically
266 recorded at low frequency in the alpine site. These less abundant species may be residents of lower
267 elevations and visit the alpine site for foraging when floral resources are abundant. Bumble bees are
268 able to fly long distances (>1.5 km) for foraging across diverse plant communities (Walther-Hellwig
269 and Frankl 2000; Kreyer et al. 2004; Osborne et al. 2008; but see also Geib et al. 2015). Therefore,
270 alpine meadow can be a foraging site also for bumble bees inhabiting at lower elevations (Tomono
271 and Sota 1997).

272 Although *B. hypocrita* workers were recorded most frequently during the 3 survey years, *B.*
273 *beaticola* workers were scarce in 2018. The same trend was also observed in other sites in this
274 mountain region (personal observation by A. Shibata and G. Kudo). One possible reason for this is
275 the occurrence of an unusual severe frost period in mid-June of 2018, during which the mean daily
276 temperature was 5°C lower than that in the usual year and freezing night time temperatures persisted
277 for a week. Such cold weather conditions might restrict survival of overwintered queens, migration
278 to alpine area or establishment of colonies in *B. beaticola*. In 2015 and 2016, colony development
279 was presumed to be successful for both species. Positive correlations between floral abundance and
280 the number of worker bees in both species indicated that colony development was correlated with
281 the progress of flowering during these years. Such a species-specific population dynamics of bumble
282 bees between years was reported previously in this mountain region (Kudo 2014), indicating that
283 the factors affecting population dynamics differ among bumble bee species. Further studies are
284 required to reveal the mechanism of colony development and to predict population dynamics of

285 bumble bees.

286 The flowering of 17 plant species commonly foraged by bumble bees progressed sequentially
287 during the summer, and species richness and floral abundance reached maximum levels in the
288 middle of summer. The flowering of individual species lasted for several weeks at regional scale.
289 Spatiotemporally diverse flowering patterns in alpine ecosystem is created by the existence of
290 snowmelt gradient by which flowering of alpine plants persists continuously at landscape scale
291 (Kudo 2016, 2019). Plant species inhabiting snowbeds, such as *Phyllodoce* spp. and *Sieversia*
292 *pentapetala*, continued to flower for long periods across the investigated sites owing to the
293 difference in snowmelt time, although the flowering period in each population was relatively short
294 (10–14 days). In contrast, flowering of several plant species, such as *Gentiana* spp., *Solidago*
295 *virgaurea*, and *Spiraea betulifolia*, progresses successively within individuals, resulting in longer
296 flowering periods in each population. The floral abundance and foraging frequency of *Phyllodoce*
297 spp. and *Gentiana triflora* were high in the investigated site, and their peak flowering periods were
298 different: flowering of *Phyllodoce* spp. commonly occurred about 10–14 days after the snowmelt,
299 while *G. triflora* was the latest bloomer in the snow meadow. Thus, these species are important floral
300 resources for bumble bees in this area. The flowers of *Phyllodoce* spp. were abundant in 2016, which
301 might have allowed bees to succeed in colony growth, resulting in larger number of foraging worker
302 bees. A large yearly variation in the abundance of *Phyllodoce* flowers was previously observed in
303 the same region (Kasagi and Kudo 2003; Kameyama and Kudo 2009; Kudo 2014). Therefore, the
304 quantity and quality of floral resources vary greatly from year to year in the alpine environment,
305 which may affect the development of bumble bee colonies.

306

307 **Effects of floral abundance and diversity on foraging patterns**

308 The workers of both dominant bee species tended to forage on abundant floral species. However,
309 the trends and foraging patterns were different between the species. *Bombus hypocrita* workers
310 frequently foraged on both shallow and deep corolla flowers, whereas *B. beaticola* workers mostly
311 foraged on deep corolla flowers. Furthermore, *B. hypocrita* workers had a more general floral use
312 pattern when floral diversity was high, whereas the species richness of visited flowers through the

313 season was higher in *B. beaticola* workers. Target floral species are often determined by the extent
314 of morphological matching between flowers and bees (Heinrich 1976a; Inoue and Yokoyama 2006).
315 *Bombus hypocrita* workers have larger body size, shorter tongue length, and larger head width (body
316 size=14.22±1.96 SE mm; proboscis length=7.82±0.83 mm; head width=3.97±0.43 mm) than those
317 of *B. beaticola* workers (11.04±0.95 mm; 8.03±0.63 mm, and 3.30±0.21 mm) (Kinota 2012).
318 Although the difference in proboscis length was small between them, the ratios of proboscis length
319 to head width of *B. hypocrita* and *B. beaticola* workers are 1.97 and 2.43, respectively. Not only the
320 proboscis length but also the combination of morphological traits can affect the floral choice in
321 bumble bees (Inoue and Kato 1992). Therefore, the ratios of proboscis length to head width may be
322 an important determinant factor of floral preference. *Bombus hypocrita* workers avoided foraging
323 on flowers with narrow and deep corollas, resulting in lower species richness of visited flowers
324 throughout the season. *Bombus hypocrita* workers preferred cup-shaped and urceolate-shaped
325 flowers, whereas *B. beaticola* workers did not prefer cup-shaped flowers. The abundance of cup-
326 shaped flowers (*S. pentapetala* and *Peucedanum multivittatum*) was relatively high in the
327 investigated site. Such a floral composition might result in higher foraging diversity for shorter-
328 tongued *B. hypocrita*. In addition, *B. hypocrita* workers have larger intraspecific size variation than
329 *B. beaticola* workers. Generally, bumble bee workers have large intraspecific size variation (Johnson
330 1986; Goulson et al. 2002). A previous study reported that larger workers of *B. terrestris* with longer
331 tongues tended to visit deeper flowers more frequently than smaller workers did (Peat et al. 2005).
332 Because of their high size variability, *B. hypocrita* workers might be able to forage on various floral
333 species.

334

335 **Effects of bee density on foraging pattern**

336 The number of different bee species was independent of the foraging diversity in both bee species,
337 whereas the degree of floral use overlap decreased with the increase in the number of *B. beaticola*
338 workers. Under conditions of high density of foraging bees on August the 1st, 2016 (the number of
339 *B. beaticola* workers was 160), *B. hypocrita* workers visited *S. pentapetala* more frequently than
340 they visited *Phyllodoce* flowers, which were frequently foraged on by *B. beaticola* workers (Fig. 7).

341 In contrast, when the density of foraging bees was low on July the 30th, 2015 (the number of *B.*
342 *beaticola* workers was 39), workers of both bee species predominantly visited *Phyllodoce* flowers.
343 Therefore, *B. hypocrita* workers were more flexible than *B. beaticola* workers in choosing target
344 flowers. Individual bumble bees commonly change target flowers in response to the availability of
345 floral resources (Heinrich 1979; Chittka et al. 1997; Wiegmann et al. 2003). These results suggest
346 that resource level of *Phyllodoce* flowers decreases as the number of foraging workers increases,
347 and the competition for the floral resource should be intensify, resulting in a shift to subsequently
348 preferred floral species in short-tongued *B. hypocrita*.

349 The stage of colony development also affects floral choice of worker bees via changes in the
350 nutrition demand of colonies. Nutritional compositions of pollen and nectar differ among different
351 floral species (Somme et al. 2015), and bees collect pollen and nectar for different purposes
352 (Nicolson 2011). During the survey on August the 1st, 2016, when the number of *B. hypocrita*
353 workers was still increasing, pollen resources might have been highly required for their larvae
354 growth (Fig. 1). In contrast, during the same period, the number of *B. beaticola* workers reached
355 maximum level, indicating that the demand for pollen resources might have decreased. Although
356 *Phyllodoce* flowers provide both pollen and nectar to bumble bees, *S. pentapetala* flowers provide
357 only pollen because of low nectar production. Seasonal variations in colony development of each
358 bee species might have resulted in different floral use patterns in *B. hypocrita* and *B. beaticola*.

359 In this study, we found that the relative floral abundance and foraging bee frequency affected
360 the foraging patterns of bumble bee workers, and that *B. hypocrita* had a more diverse and flexible
361 floral choice than that of *B. beaticola*. This difference might reflect the differences in morphological
362 traits and/or colony developmental cycles. When a competition for floral resources exists between
363 bumble bees, the possibility of resource partitioning can be a key issue for the coexistence of
364 multiple bumble bee species. The intensity of competition depends on the both of the availability of
365 floral resources and bee densities, and the possibility of resource partitioning highly depends on the
366 flexibility of floral usage of competing bee species. Therefore, coexistence mechanism of multiple
367 bee species is highly situation-dependent.

368
369

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371

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375

376 **Contribution of authors**

377

378 AS and GK designed the study. AS and GK collected field data, analyzed the data, discussed, and
379 wrote the manuscript.

380

381 **References**

382

383 Bingham RA, Orthner AR (1998) Efficient pollination of alpine plants. *Nature* 391:238–239.

384 <https://doi.org/10.1093/nar/26.4.1134>

385 Chittka L, Gumbert A, Kunze J (1997) Foraging dynamics of bumble bees: Correlates of

386 movements within and between plant species. *Behav Ecol* 8:239–249.

387 <https://doi.org/10.1093/beheco/8.3.239>

388 Dukas R, Real LA (1993) Effects of recent experience on foraging decisions by bumble bees.

389 *Oecologia* 94:244–246. <https://doi.org/10.1007/BF00341323>

390 Fontaine C, Collin CL, Dajoz I (2008) Generalist foraging of pollinators: Diet expansion at high

391 density. *J Ecol* 96:1002–1010. <https://doi.org/10.1111/j.1365-2745.2008.01405.x>

392 Gegear RJ, Thomson JD (2004) Does the flower constancy of bumble bees reflect foraging

393 economics? *Ethology* 110:793–805. <https://doi.org/10.1111/j.1439-0310.2004.01010.x>

394 Geib JC, Strange JP, Galen A (2015) Bumble bee nest abundance, foraging distance, and host-

395 plant reproduction: Implications for management and conservation. *Ecol Appl* 25:768–778.

396 <https://doi.org/10.1890/14-0151.1>

- 397 Goulson D (2010) *Bumblebees: behaviour, ecology, and conservation*. Oxford University Press
- 398 Goulson D, Lye GC, Darvill B (2008) Diet breadth, coexistence and rarity in bumblebees.
399 *Biodivers Conserv* 17:3269–3288. <https://doi.org/10.1007/s10531-008-9428-y>
- 400 Goulson D, Peat J, Stout JC, et al (2002) Can alloethism in workers of the bumblebee, *Bombus*
401 *terrestris*, be explained in terms of foraging efficiency? *Anim Behav* 64:123–130.
402 <https://doi.org/10.1006/anbe.2002.3041>
- 403 Heinrich B (1979) *Bumblebee Economics*. Harvard University Press, Cambridge
- 404 Heinrich B (1976a) Resource partitioning among some eusocial insects: bumblebees. *Ecology*
405 57:874–889. <https://doi.org/10.2307/1941054>
- 406 Heinrich B (1976b) The foraging specializations of individual bumblebees. *Ecol Monogr* 46:105–
407 128. [https://doi.org/10.1016/S0003-3472\(89\)80111-3](https://doi.org/10.1016/S0003-3472(89)80111-3)
- 408 Inari N, Hiura T, Toda MJ, Kudo G (2012) Pollination linkage between canopy flowering, bumble
409 bee abundance and seed production of understorey plants in a cool temperate forest. *J Ecol*
410 100:1534–1543. <https://doi.org/10.1111/j.1365-2745.2012.02021.x>
- 411 Inoue MN, Yokoyama J (2006) Morphological variation in relation to flower use in bumblebees.
412 *Entomol Sci* 9:147–159. <https://doi.org/10.1111/j.1479-8298.2006.00162.x>

413 Inoue T, Kato M (1992) Inter- and intraspecific morphological variation in bumblebee species, and
414 competition in flower utilization. In: Hunter M, Ohgushi T, Price P (eds) Effects of resource
415 distribution on animal-plant interaction, Academic P. San Diego, pp 393–40

416 Inouye DW (1978) Resource partitioning in bumblebees: Experimental studies of foraging
417 behavior. *Ecology* 59:672–678. <https://doi.org/10.2307/1938769>

418 Johnson RA (1986) Intraspecific resource partitioning in the bumble bees *Bombus ternarius* and *B.*
419 *pennsylvanicus*. *Ecology* 67:133–138. <https://doi.org/10.2307/1938511>

420 Kameyama Y, Kudo G (2009) Flowering phenology influences seed production and outcrossing
421 rate in populations of an alpine snowbed shrub, *Phyllodoce aleutica*: Effects of pollinators
422 and self-incompatibility. *Ann Bot* 103:1385–1394. <https://doi.org/10.1093/aob/mcp037>

423 Kasagi T, Kudo G (2003) Variations in bumble bee preference and pollen limitation among
424 neighboring populations: Comparisons between *Phyllodoce caerulea* and *Phyllodoce*
425 *aleutica* (Ericaceae) along snowmelt gradients. *Am J Bot* 90:1321–1327.
426 <https://doi.org/10.3732/ajb.90.9.1321>

427 Kinota K (2012) Metric comparison among the bumblebee species of Hokkaido on labial length,
428 head width, body- and antennal length. *J Hymenopterological Soc Japan* 20:37–52

429 Kreyer D, Oed A, Walther-Hellwig K, Frankl R (2004) Are forests potential landscape barriers for
430 foraging bumblebees? Landscape scale experiments with *Bombus terrestris* agg. and
431 *Bombus pascuorum* (Hymenoptera, Apidae). Biol Conserv 116:111–118.
432 [https://doi.org/10.1016/S0006-3207\(03\)00182-4](https://doi.org/10.1016/S0006-3207(03)00182-4)

433 Kudo G (2016) Landscape structure of flowering phenology in alpine ecosystems: significance of
434 plant–pollinator interactions and evolutionary aspects. In: Kudo G (ed) Structure and
435 Function of Mountain Ecosystems in Japan., Ecological Research Monographs. Springer,
436 Tokyo, pp 41–62

437 Kudo G (2014) Vulnerability of phenological synchrony between plants and pollinators in an
438 alpine ecosystem. Ecol Res 29:571–581. <https://doi.org/10.1007/s11284-013-1108-z>

439 Kudo G (2019) Dynamics of flowering phenology of alpine plant communities in response to
440 temperature and snowmelt time: Analysis of a nine-year phenological record collected by
441 citizen volunteers. Environ Exp Bot 103843.
442 <https://doi.org/10.1016/j.envexpbot.2019.103843>

443 Miller-Struttmann NE, Galen C (2014) High-altitude multi-taskers: bumble bee food plant use
444 broadens along an altitudinal productivity gradient. Oecologia 176:1033–1045.

445 <https://doi.org/10.1007/s00442-014-3066-8>

446 Mollie EB, Kasper K, Koen J van B, et al (2017) glmmTMB balances speed and flexibility among
447 packages for zero-inflated generalized linear mixed modeling. *R J* 9:378–400.
448 <https://doi.org/10.3929/ethz-b-000240890>

449 Morisita M (1959) Measuring interspecific association and similarity between communities. *Mem*
450 *Fac Sci Kyushu Univ Ser E* 3:65–80

451 Nicolson SW (2011) Bee food: the chemistry and nutritional value of nectar, pollen and mixtures
452 of the two. *African Zool* 46:197–204. <https://doi.org/10.3377/004.046.0201>

453 Osborne JL, Martin AP, Carreck NL, et al (2008) Bumblebee flight distances in relation to the
454 forage landscape. *J Anim Ecol* 77:406–415. [https://doi.org/10.1111/j.1365-
455 2656.2007.01333.x](https://doi.org/10.1111/j.1365-2656.2007.01333.x)

456 Peat J, Tucker J, Goulson D (2005) Does intraspecific size variation in bumblebees allow colonies
457 to efficiently exploit different flowers? *Ecol Entomol* 30:176–181.
458 <https://doi.org/10.1111/j.0307-6946.2005.00676.x>

459 Pelletier L, McNeil JN (2003) The effect of food supplementation on reproductive success in
460 bumblebee field colonies. *Oikos* 103:688–694. <https://doi.org/10.1034/j.1600->

461 0706.2003.12592.x

462 Pyke GH (1982) Local geographic distributions of bumblebees, near Crested Butte, Colorado:

463 competition and community structure. *Ecology* 63:555–573.

464 <https://doi.org/10.2307/1938970>

465 Pyke GH, Inouye DW, Thomson JD (2012) Local geographic distributions of bumblebees, near

466 Crested Butte, Colorado: competition and community structure revised. *Entomol Soc Am*

467 41:1332–1349. <https://doi.org/10.2307/1938970>

468 R Core Team (2018) R: A language and environment for statistical computing. R Foundation for

469 Statistical Computing

470 Raine NE, Chittka L (2005) Comparison of flower constancy and foraging performance in three

471 bumblebee species (Hymenoptera: Apidae: Bombus). *Entomol Gen* 28:81–89

472 Ranta E LH (1980) Resource partitioning in bumblebees: the significance of differences in

473 proboscis length. *Oikos* 35:298–302

474 Schaffer WM, Jensen DB, Hobbs DE, et al (1979) Competition, foraging energetics, and the cost

475 of sociality in three species of bees. *Ecology* 60:976–987. <https://doi.org/10.2307/1936866>

476 Schmid-Hempel R, Schmid-Hempel P (1998) Colony performance and immunocompetence of a

477 social insect, *Bombus terrestris*, in poor and variable environments. *Funct Ecol* 12:22–30.
478 <https://doi.org/10.1046/j.1365-2435.1998.00153.x>

479 Schoener TW (1974) Resource partitioning in ecological communities. *Science* (80-) 185:27–39

480 Simpson EH (1949) Measurement of diversity. *Nature* 163:688. <https://doi.org/10.1038/163688a0>

481 Smithson A, Macnair MR (1996) Frequency-dependent selection by pollinators: Mechanisms and
482 consequences with regard to behaviour of bumblebees *Bombus terrestris* (L.) (Hymenoptera:
483 Apidae). *J Evol Biol* 9:571–588. <https://doi.org/10.1046/j.1420-9101.1996.9050571.x>

484 Somme L, Vanderplanck M, Michez D, et al (2015) Pollen and nectar quality drive the major and
485 minor floral choices of bumble bees. *Apidologie* 46:92–106. [https://doi.org/10.1007/s13592-](https://doi.org/10.1007/s13592-014-0307-0)
486 [014-0307-0](https://doi.org/10.1007/s13592-014-0307-0)

487 Tasei J, Aupinel P (2008) Nutritive value of 15 single pollens and pollen mixes tested on larvae
488 produced by bumblebee workers (*Bombus terrestris*, Hymenoptera : Apidae). *Apidologie*
489 39:397–409. <https://doi.org/10.1051/apido:2008017>

490 Thomson D (2004) Competitive interactions between the invasive European honey bee and native
491 bumble bees. *Ecology* 85:458–470. <https://doi.org/10.1890/02-0626>

492 Tomono T, Sota T (1997) The life history and pollination ecology of bumblebees in the alpine

493 zone of central Japan. Japanese J Entomol 65:237–255

494 Walther-Hellwig K, Frankl R (2000) Foraging distances of *Bombus muscorum*, *Bombus*

495 *lapidarius*, and *Bombus terrestris* (Hymenoptera, Apidae). J Insect Behav 13:239–246.

496 <https://doi.org/10.1023/A:1007740315207>

497 Wiegmann DD, Wiegmann DA, Waldron FA (2003) Effects of a reward downshift on the

498 consummatory behavior and flower choices of bumblebee foragers. Physiol Behav 79:561–

499 566. [https://doi.org/10.1016/S0031-9384\(03\)00122-7](https://doi.org/10.1016/S0031-9384(03)00122-7)

500

501

Table 1. Plant species visited by bumble bees, the number of observed bees for each bee species and caste (Q: queen, W: worker, M: male), total number of observed bees, and visited plant species number in 2015 and 2016. The bold numbers indicate major plant species for workers of *Bombus hypocrita* and *B. beaticola*.

Plant species	Family	Flower shape/ Inflorescence type	Observed bee No.														
			<i>B. beaticola</i>			<i>B. diver sus</i>			<i>B. hypno rum</i>			<i>B. hypocrita</i>			<i>B. yezoensis</i>		
			Q	W	M	Q	W	M	Q	W	M	Q	W	M	Q	W	M
<i>Rhododendron aureum</i>	Ericaceae	funnel/ simple		3						2		4					
<i>Phyllodoce caerulea</i>	Ericaceae	urceolate/ unflowered	2	19	1			1				22					
<i>Vaccinium ovalifolium</i>	Ericaceae	urceolate/ unflowered	1	2													
<i>Vaccinium vitis-idaea</i>	Ericaceae	campanulate/ simple		1													
<i>Ledum palustre</i>	Ericaceae	cup/ simple						1				1					
<i>Siwersia pentapetala</i>	Rosaceae	cup/ unflowered		21	1			1				88					
<i>Phyllodoce caerulea</i>																	
var. <i>yezoensis</i>	Ericaceae	urceolate/ unflowered		190	3			7		1		59					
<i>Spiraea betulifolia</i>	Rosaceae	cup/ compound		15				18				40				2	

505 **Figure legends**

506

507 **Fig. 1**

508 Seasonal trends of the frequency of observed bees (histogram) and floral abundance (line) in 2015,
509 2016, and 2018 for (a) *Bombus hypocrita* and (b) *B. beaticola*.

510

511 **Fig. 2**

512 (a) Flowering phenology and (b) flower number of 17 plant species visited by bumble bees in 2015
513 and 2016.

514

515 **Fig. 3**

516 Relationships between total floral abundance and total observed number of *Bombus hypocrita* and
517 *B. beaticola* workers. Solid and broken regression lines represent 2015 and 2016, respectively.

518

519 **Fig. 4**

520 Relationship between relative floral abundance and relative foraging frequency of worker bees in
521 (a) *Bombus hypocrita* and (b) *B. beaticola* for top five most-visited floral species. The lines indicate
522 the prediction by GLM.

523

524 **Fig. 5**

525 Relationship between floral and foraging diversity of *Bombus hypocrita* and *B. beaticola* workers.
526 A positive correlation was detected only for *B. hypocrita* ($P=0.05$ by GLMM).

527

528 **Fig. 6**

529 Relationships between the number of *Bombus beaticola* workers and floral use overlap index
530 between *B. hypocrita* and *B. beaticola* workers. The line indicates the prediction by GLMM.

531

532 **Fig. 7**

533 Foraging frequency of *Bombus hypocrita* and *B. beaticola* workers observed in each floral species
534 on two observation days; low bee density on July 30th, 2015 (top) and high bee density on August
535 1st, 2016 (bottom). Floral species are *Gaultheria miqueliana* (GM), *Gentiana nipponica* (GN),
536 *Penstemon frutescens* (PF), *Peucedanum multivittatum*, (PM), *Phyllodoce aleutica* (PA),
537 *Phyllodoce caerulea* var. *yezoensis* (PCY), *Sieversia pentapetala* (SP), *Spiraea betulifolia* (SB).

538

539

540 **Fig. S1**

541 Map of the 2.6 km trail used for studying an alpine meadow on Mt. Asahidake in the Taisetsu
542 Mountains, Hokkaido, northern Japan. The trail was divided into four transects (T1, T2, T3, and
543 T4).

Fig 1

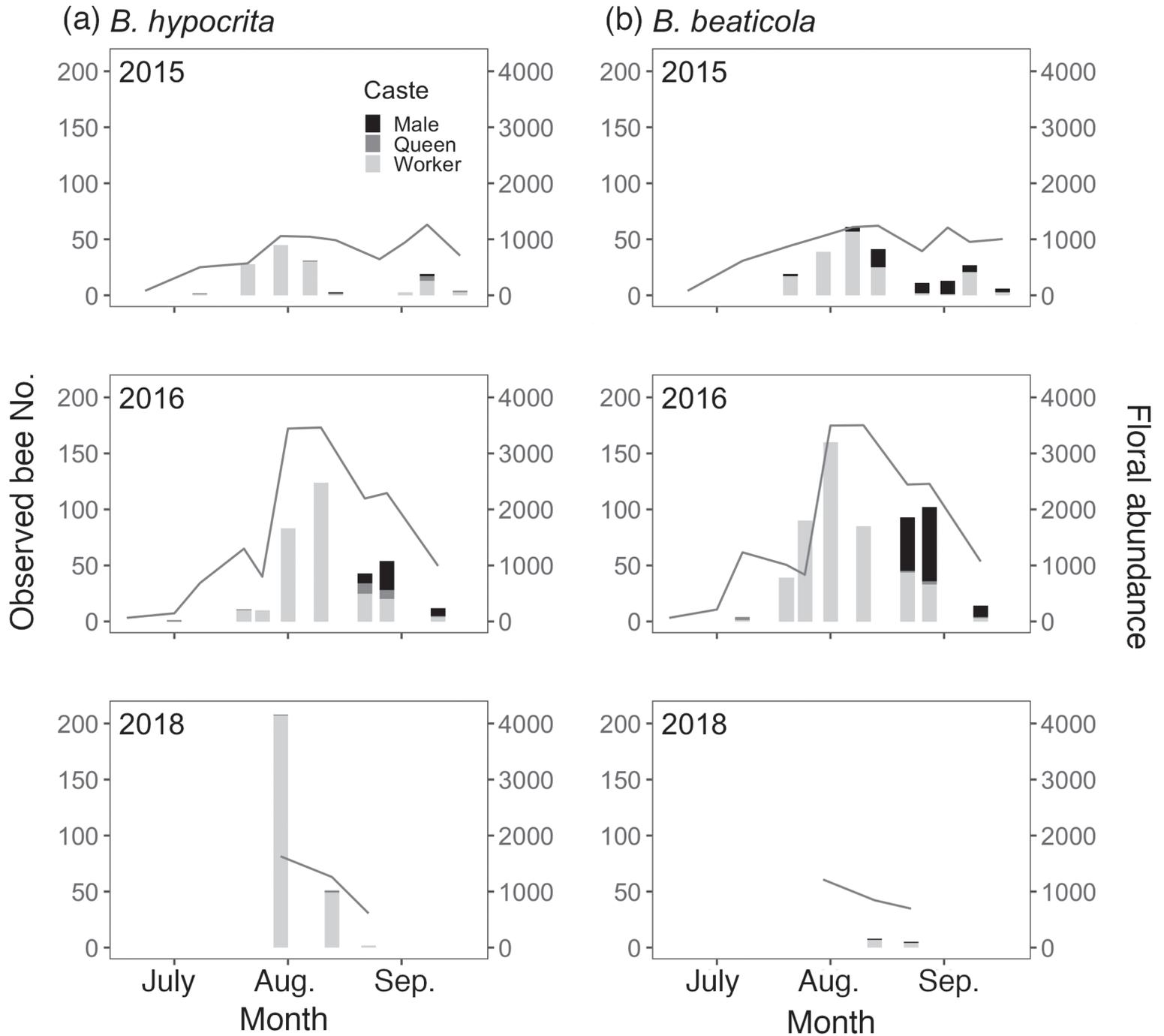


Fig 2

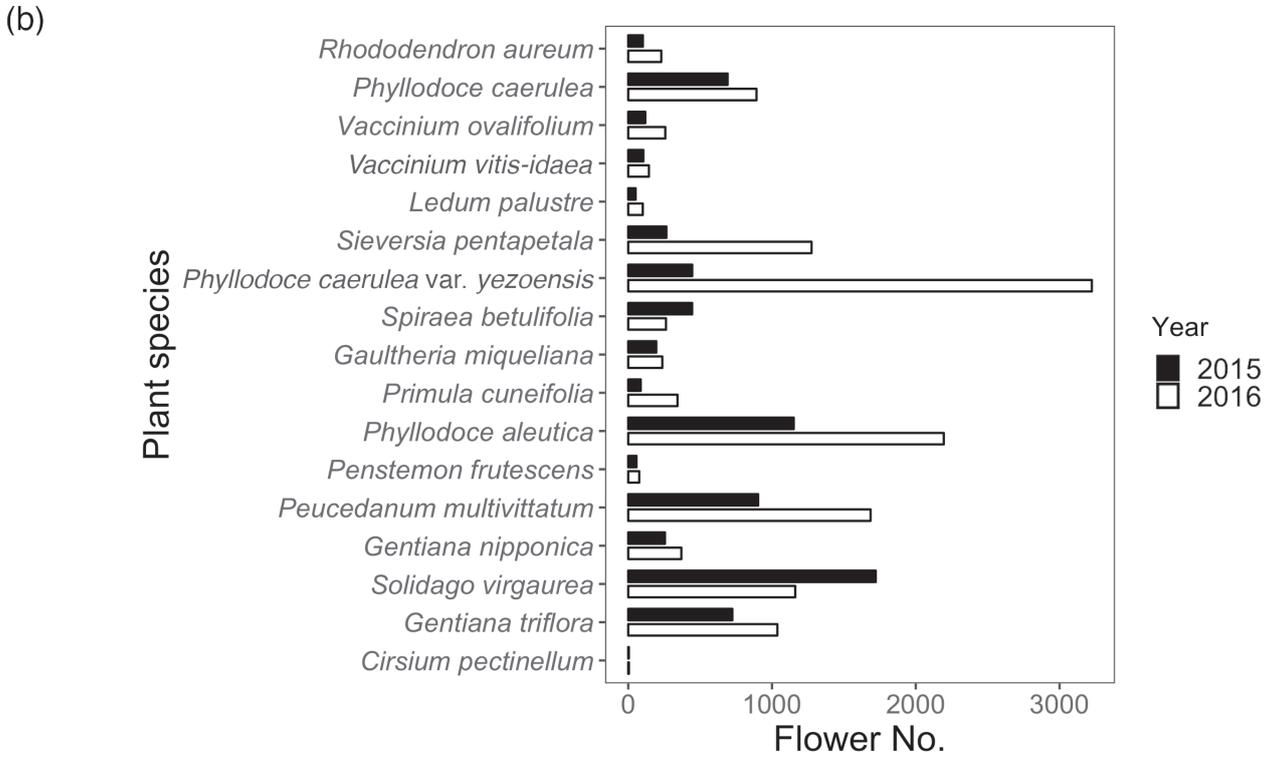
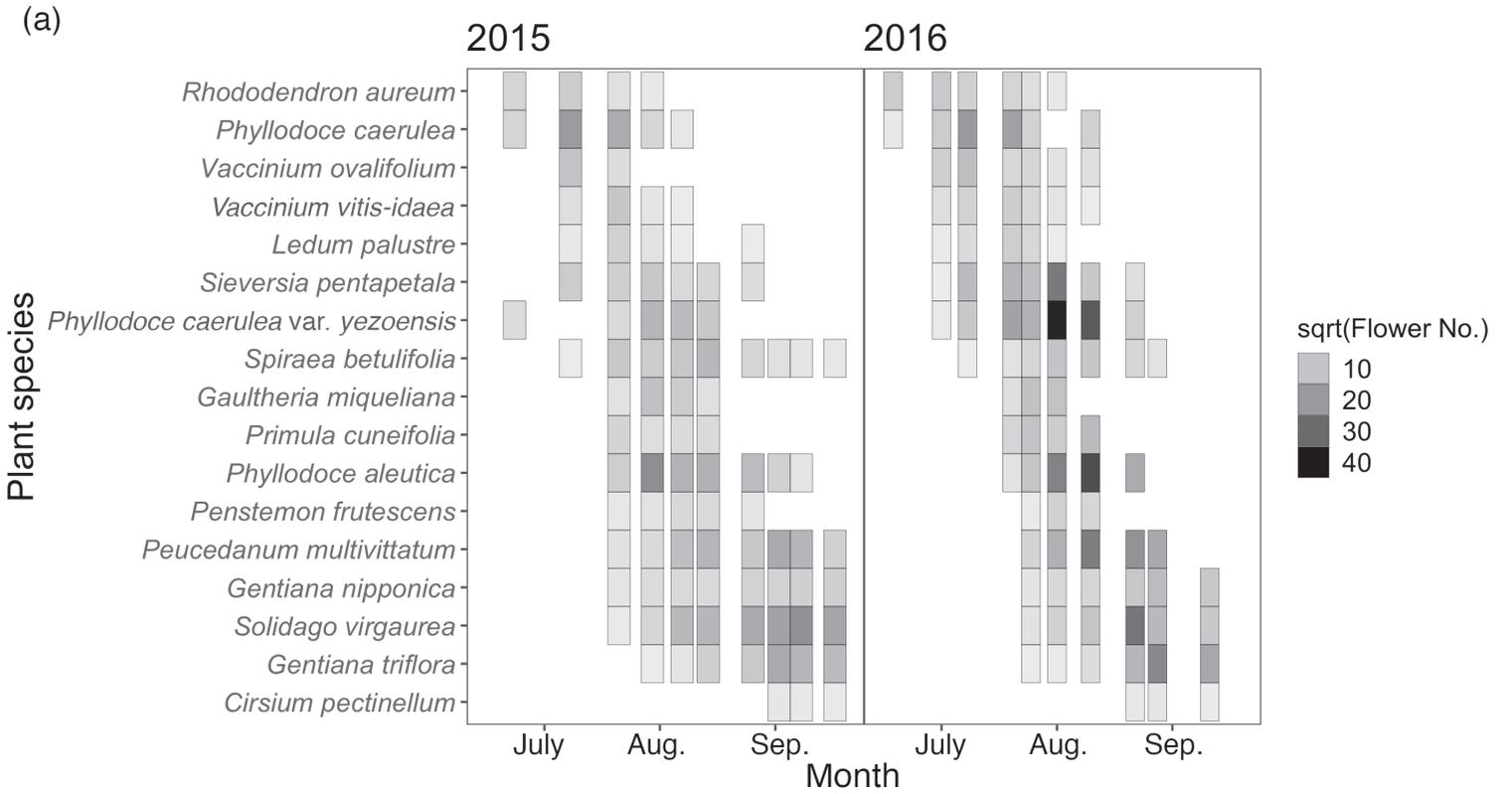


Fig 3

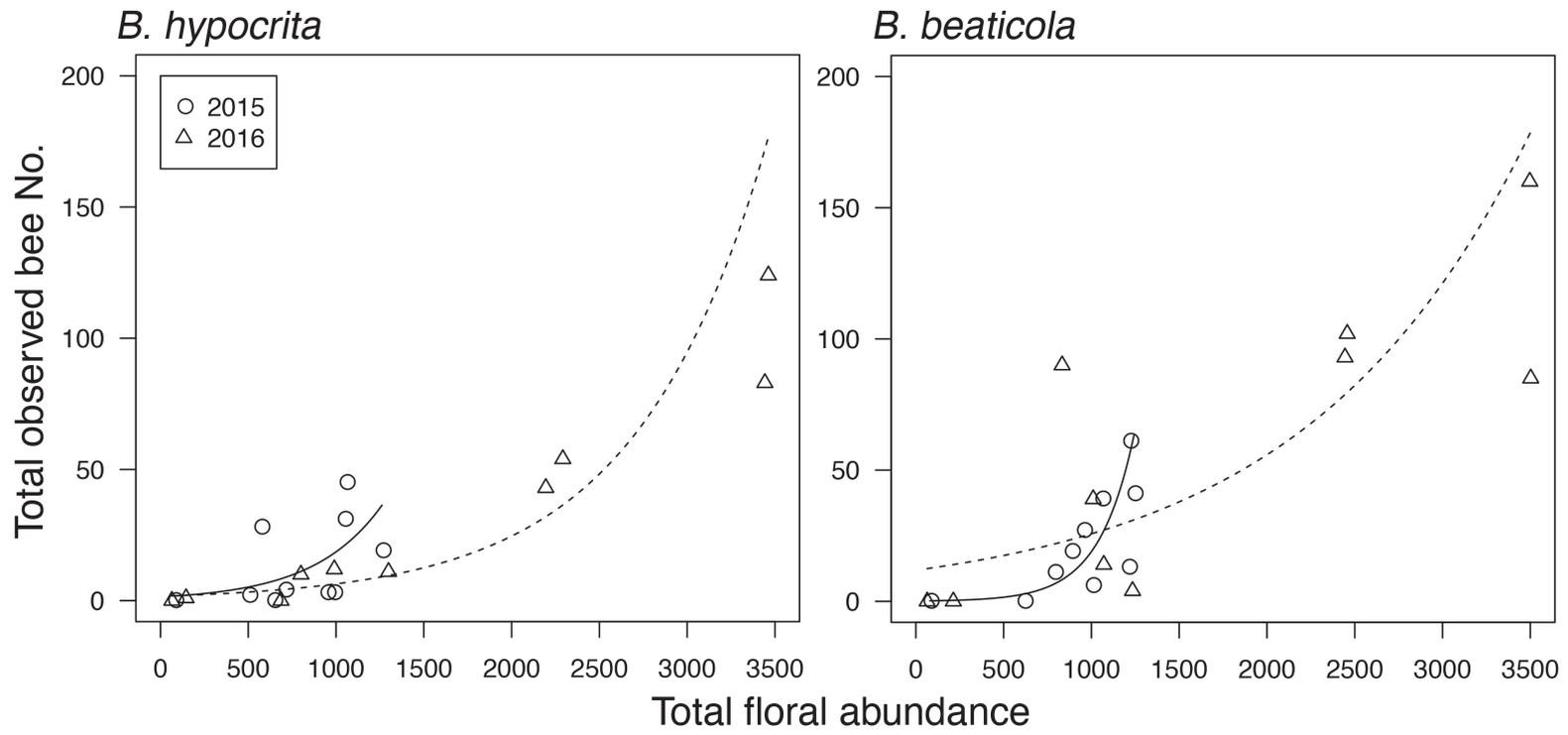


Fig 4

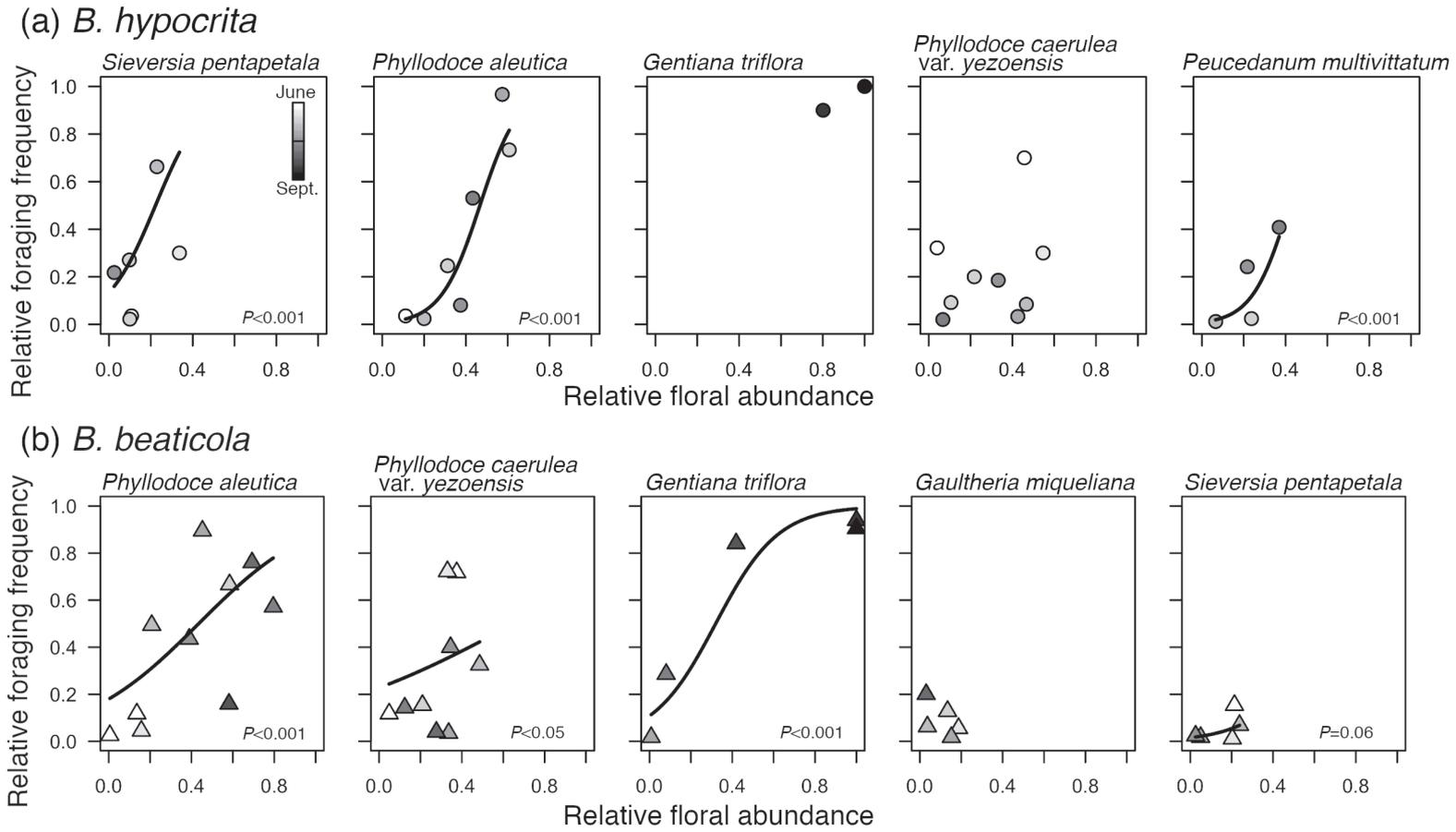
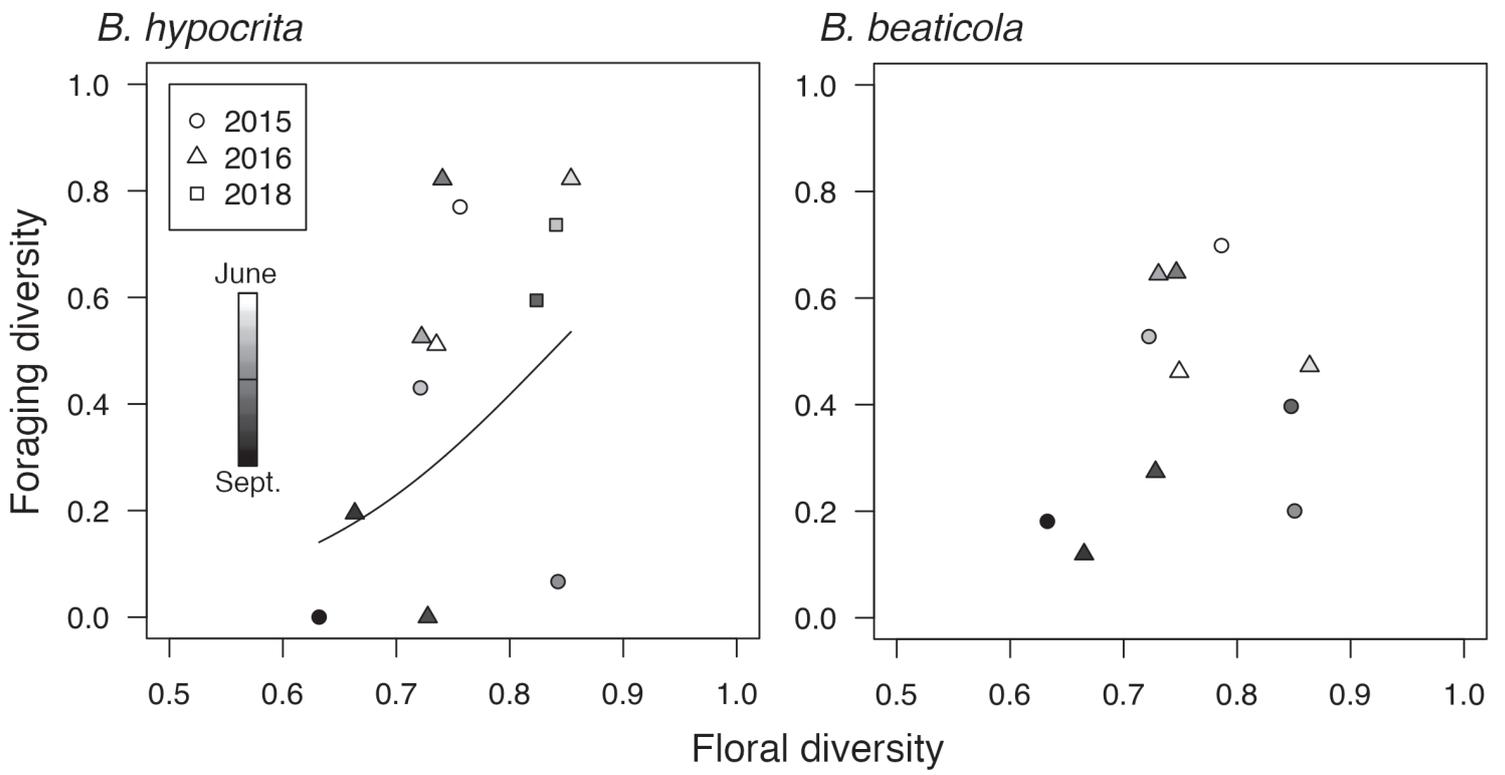


Fig 5



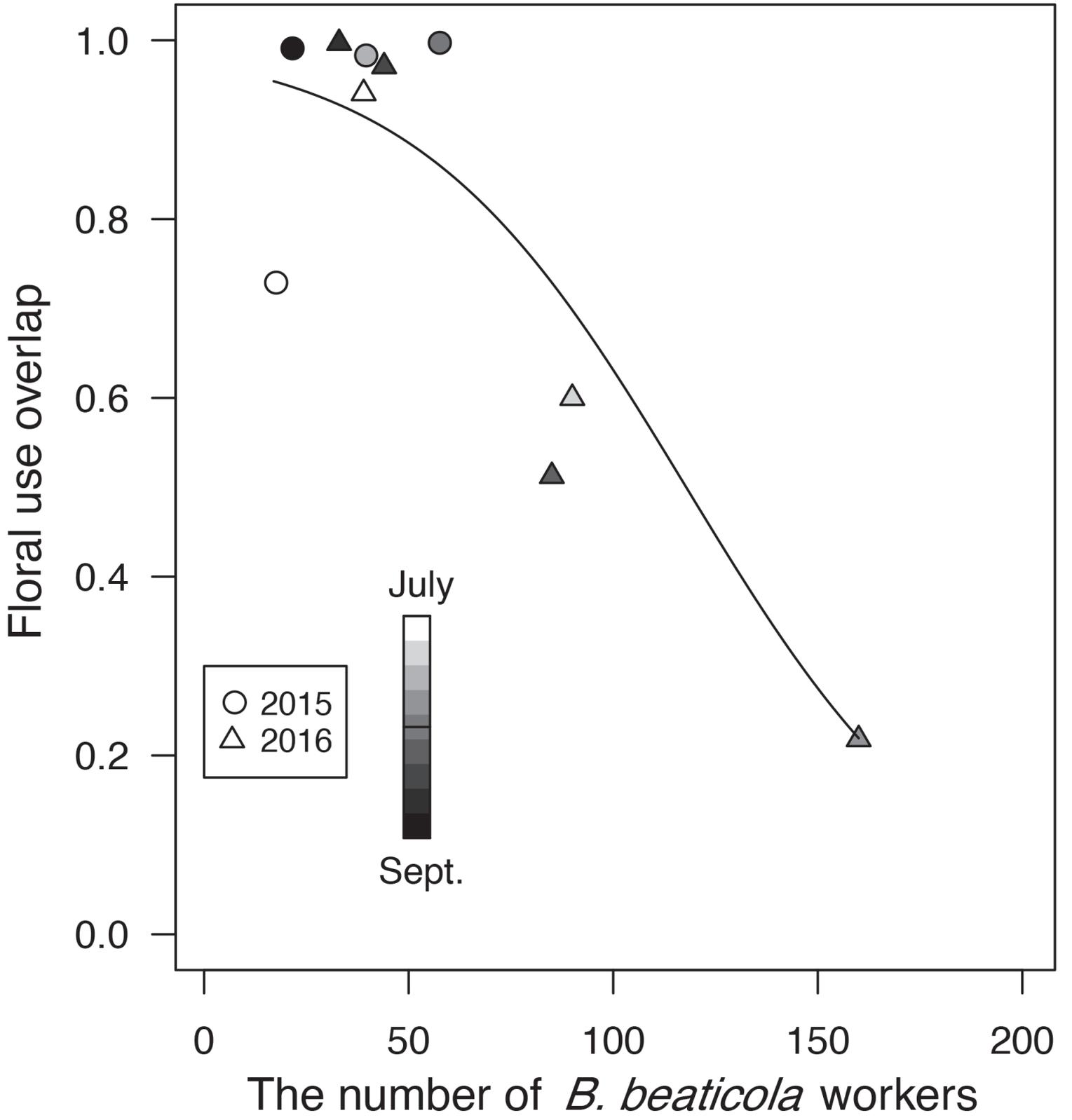
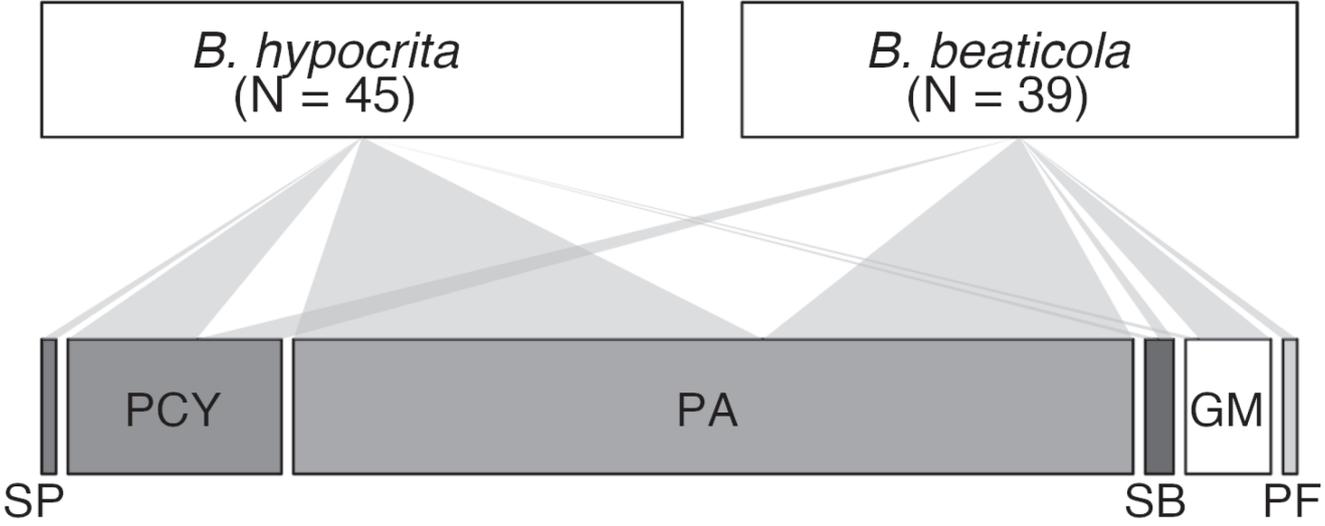
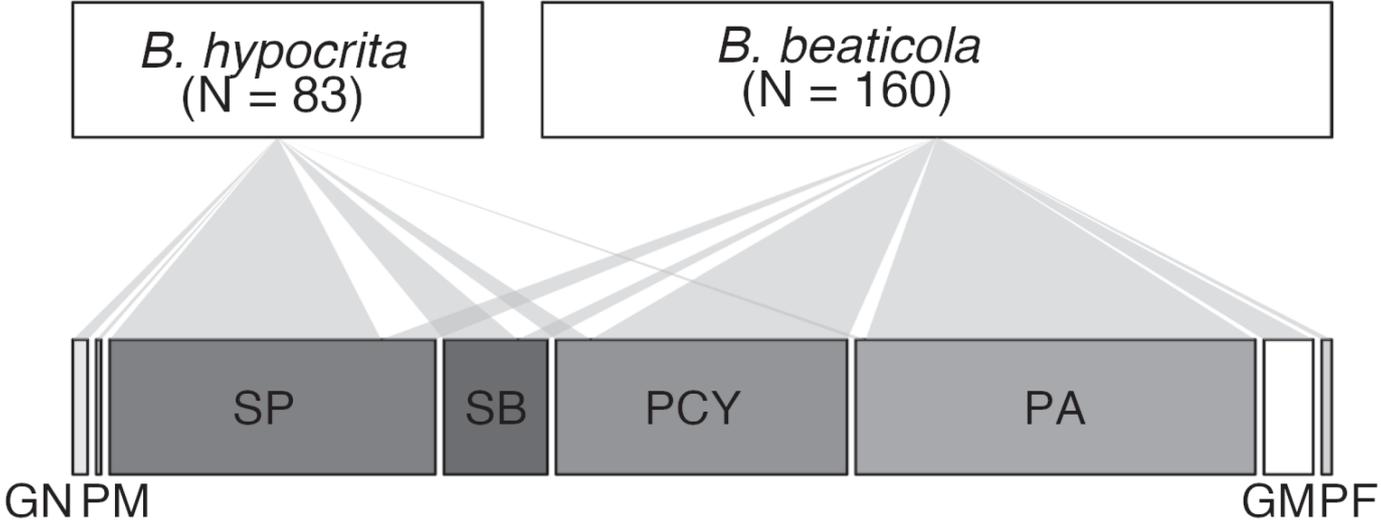


Fig 7

July/ 30th/ 2015



August/ 1st/ 2016



Article title: Floral abundance and bee density affect species-specific foraging patterns of alpine bumble bees
Journal name: Arthropod-Plant Interactions
Author names: Akari Shibata, Gaku Kudo
Affiliation: Graduate School of Environmental Science, Hokkaido University
E-mail address: aka.11mbbc28@gmail.com

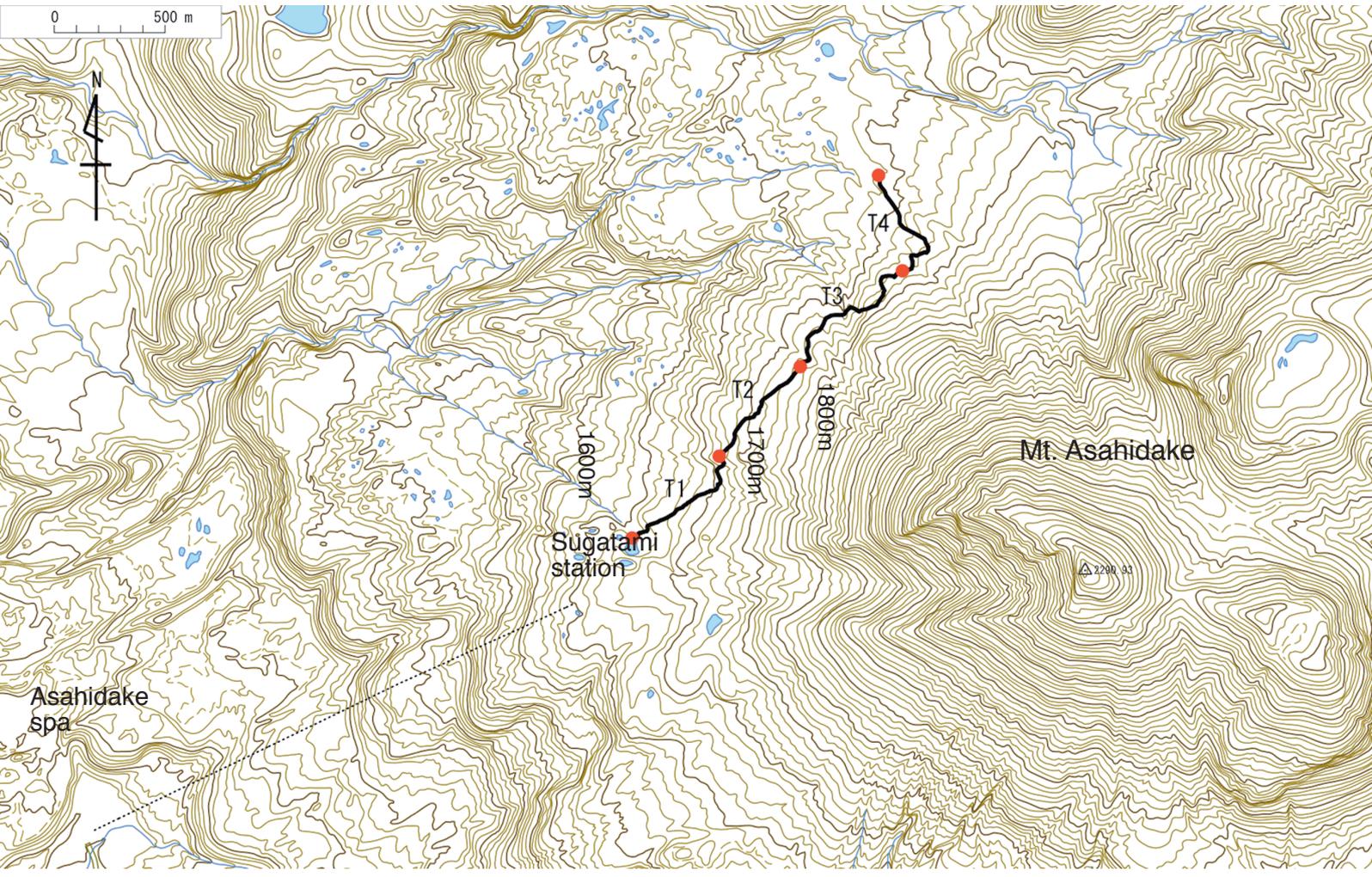


Table S1 Comparison of models explaining (a) foraging diversity in *Bombus hypocrita* and *B. beaticola* and (b) floral use overlap.

Model (explanatory variables)	AIC	ΔAIC
(a) Foraging diversity		
<i>B. hypocrita</i>		
floral diversity	-8.0	0.0
<i>B. hypocrita</i> worker No. + floral diversity	-7.2	0.8
other bee species worker No. + floral diversity	-6.6	1.4
null	-6.6	1.4
other bee species worker No. + <i>B. hypocrita</i> worker No. + floral diversity	-6.3	1.7
<i>B. hypocrita</i> worker No.	-5.4	2.6
other bee species worker No.	-5.3	2.7
other bee species worker No. + <i>B. hypocrita</i> worker No.	-4.3	3.7
<i>B. beaticola</i>		
null	1.8	0.0
<i>B. beaticola</i> worker No.	2.7	0.9
floral diversity	2.9	1.1
other bee species worker No.	3.8	2.0
<i>B. beaticola</i> worker No. + floral diversity	4.2	2.4
other bee species worker No. + <i>B. beaticola</i> worker No.	4.4	2.6
other bee species worker No. + floral diversity	4.8	3.0
other bee species worker No. + <i>B. beaticola</i> worker No. + floral diversity	6.1	4.3
(b) Floral use overlap		
<i>B. beaticola</i> worker No.	-16.7	0.0
<i>B. hypocrita</i> worker No. + <i>B. beaticola</i> worker No.	-15.7	1.0
<i>B. beaticola</i> worker No. + floral diversity	-14.7	2.0
<i>B. hypocrita</i> worker No. + <i>B. beaticola</i> worker No. + floral diversity	-13.8	2.9
<i>B. hypocrita</i> worker No.	-11.8	4.9
<i>B. hypocrita</i> worker No. + floral diversity	-10.4	6.3
null	-10.0	6.7
floral diversity	-8.6	8.1