

Effects of alien invasion by *Bombus terrestris* L. (Apidae) on the visitation patterns of native bumblebees in coastal plants in northern Japan

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Abstract When alien pollinator species enter a native community of pollinators in which resource partitioning has been established, the pollination network between plants and pollinators may be modified through the interactions between the pollinators over the use of floral resources. We observed the floral-use patterns of native (*Bombus hypocrita* and *B. deuteronymus*) and alien (*B. terrestris*) bumblebee species in a coastal grassland in northern Japan. We analyzed the factors determining resource partitioning patterns. *B. hypocrita* tended to visit flowers with shallow or wide open corollas, such as *Rosa rugosa*, whereas *B. deuteronymus* visited flowers with complex or deeper corollas, such as *Lathyrus japonicus*. Given the wider floral preference of *B. terrestris*, floral use by the alien bumblebees consistently overlapped with that of native bumblebees. The visitation of *B. terrestris* to *R. rugosa* flowers was positively correlated with that of *B. hypocrita*. These bumblebee species frequently used similar floral resources, in part because of the large overlap in the seasonality of their foraging activity. The visitation frequency of *B. deuteronymus* to *L. japonicus* flowers was independent of the visitation frequency of other bumblebee species. The major visitation periods of the bumblebees to *L. japonicus* flowers reciprocally differed between *B. deuteronymus* and *B. terrestris*, suggesting phenological resource partitioning between these species. Our study suggests that phenological niche partitioning is more common in specialized flowers (*L. japonicus*) than in generalized flowers (*R. rugosa*).

Keywords Alien pollinator invasion · *Bombus terrestris* · Floral visitation frequency · Floral resource partitioning

Introduction

The coexistence of species within a community is often controlled by resource partitioning based on the morphological differences among species (e.g., Schoener 1974). Several studies have reported that coexisting bumblebee species differ distinctly in proboscis length (Heinrich 1976; Pyke 1982; Graham and Jones 1996). The study by Heinrich (1976) indicated that overlaps in floral-resource utilization were reduced by the morphological variation among bumblebee species. Furthermore, a study by Inouye (1978) showed that the removal of competing species of bumblebees rapidly changed the flower utilization behavior of the remaining species. This indicates that the foraging pattern of bumblebees can be determined by competitive interactions despite potential preferences in flower utilization. However, bumblebee communities sometimes consist of several species with similar proboscis lengths (Ranta et al. 1981; Ranta and Vepsäläinen 1981). The coexistence of multiple species could be attributed to spatio-temporal heterogeneity in floral resources (Ranta and Vepsäläinen 1981).

When a new species enters a bumblebee community in which resource partitioning has been established, new linkages between plants and pollinators may be formed through modifications to the foraging behavior of individual species via competition for floral resources. Recently, the impact of introduced bumblebees on native ecosystems has been of concern. Many researchers have identified the possibility that an abundance of alien bumblebees may disrupt plant–pollinator interactions among native species

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(Goulson 2003; Traveset and Richardson 2006; Dohzono and Yokoyama 2010; Dafni et al. 2010). However, the effect of alien bumblebees on the fitness of native bumblebees and their pollination service to plants is not consistent. A few studies have reported that native bumblebee species were replaced by an alien species (Madjidian et al. 2008). In one case, the seed production of native plants was reduced by changes in the foraging behavior of native bumblebees due to the depletion of floral nectar by alien bumblebees (Dohzono et al. 2008). In contrast, the effect of alien bumblebees on the foraging efficiency of native bumblebees was not clear in greenhouse experiments with different combinations of species (Nagamitsu et al. 2007a). Furthermore, changes in the foraging behavior of native bumblebees do not necessarily cause negative effects on the pollination success of native plants if alien bumblebees act as effective pollinators (Madjidian et al. 2008).

The main objective of this study was to reveal the niche overlap between alien and native bumblebee species in a natural coastal grassland in northern Japan. We accomplished this by observing the foraging behavior of two common native bumblebees, *Bombus hypocrita sapporoensis* Cockerell (hereafter *B. hypocrita*) and *B. deuteronymus deuteronymus* Schulz (hereafter *B. deuteronymus*), and one alien species, *B. terrestris* L. (hereafter, *B. terrestris*), as well as recording the abundance of bee-pollinated flowers in two plant communities throughout the flowering season for 4 years. In addition, we compared proboscis lengths among the bumblebees to elucidate the relationship between morphological traits and floral use in these bumblebee species. We hypothesized that the effect of alien species on the foraging behavior of native species is larger when they are morphologically similar to native species.

Methods

Study area

The field survey was conducted in a coastal sand dune grassland located on Ishikari Beach (43°13′–17′N, 141°19′–23′E) in Hokkaido, northern Japan. The study area was 25 km in length and 100–200 m in width, and divided into northern and southern sections by the mouth of the Ishikari River. On the landward side, the grassland is replaced by natural deciduous forests dominated by *Quercus dentata* (Fagaceae). More than 50 plant species grow in the grassland, including several non-native species (see dominant species in Table 2). The flowering season extends from May to October. The dominant species and species compositions were similar in the northern and southern areas of the grassland. In the northern area, cultivated fields and remnant patches of deciduous forest were inland of the coastal

vegetation, whereas the coastal grassland of the southern area was situated close to a residential area and some reclaimed land. *B. terrestris* was first sighted on Ishikari Beach in 2006 (Nishikawa and Shimamura, unpublished data). Preliminary observations recorded in 2008 suggested that *B. terrestris* was more abundant in the southern than in the northern area.

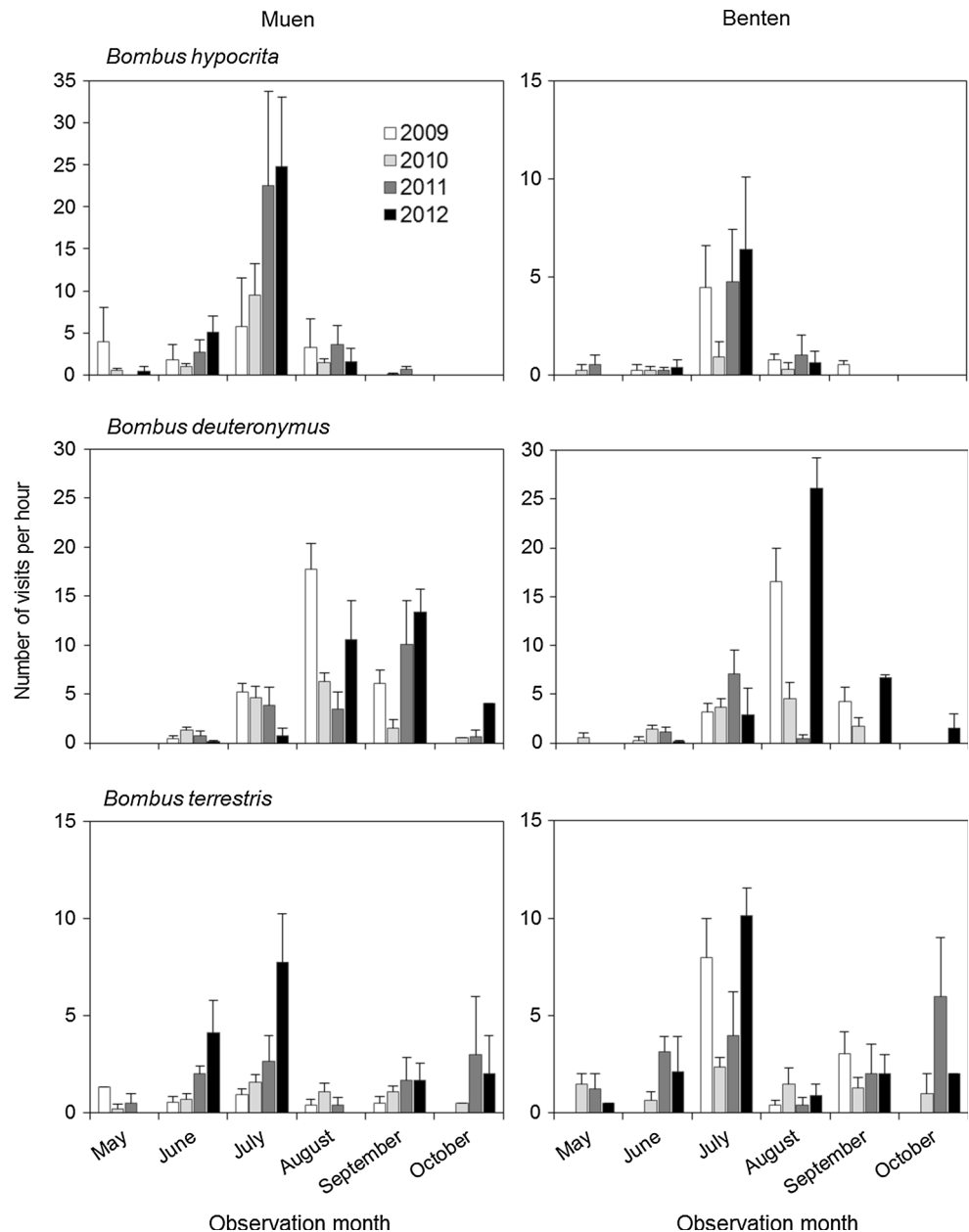
Frequency of bumblebee visits

Two observation sites, each 150 m × 200 m, were established in the coastal grassland; namely Muen (in the northern area) and Bente (in the southern area). The distance between the sites was about 7 km. The visitation frequency of all bumblebee species to native flowers was investigated at each site at 5–7 day intervals from late May to early October for 4 years (2009–2012). To observe bumblebee visits, a fixed trail was set at each observation time. The trail was designed to connect major flowering patches in accordance with flowering conditions. Two observers recorded the number of visits and the species of bumblebee that visited the flowers between 10:00 and 13:00 along two strips flanking the trail, each 2 m wide. The order in which sites were observed was rotated at each observational day to control for the dependence of pollinator activity on time and/or temperature. The observation period in each census was 30–120 min per observer, depending on the flowering conditions. Sequential visits to multiple flowers by a single bumblebee were counted as one visit. The caste of individual bumblebees was recorded, but this information was not included in the analysis because it was difficult to differentiate the queens and workers of some species in the field. Male bumblebees were excluded from the analysis because the males of only two species were recorded, and at extremely low frequencies. Discrimination of *B. deuteronymus* from *B. pseudobaicalensis* Vogt (hereafter *B. pseudobaicalensis*) was difficult in the field because of their morphological similarity, so observations for the two species were pooled. Of 57 individuals captured for identification, 81 % were *B. deuteronymus*. This suggests that *B. deuteronymus* was more abundant than *B. pseudobaicalensis* in the study area. Therefore, we treated the observed bumblebees of similar appearance as *B. deuteronymus*.

Flowering phenology

Fifteen native plant species were visited by bumblebees at the study sites (see Table 2). To determine the flowering periods and quantify the seasonal changes in these floral resources, we recorded the number of flowers for each plant species at every observation session over 4 years (2009–2012). At each site in 2009 and 2010, two quadrats

Fig. 1 Mean number of flower visits monthly to 15 native plant species by three bumblebee species (*Bombus hypocrita*, *B. deuteronymus*, and *B. terrestris*). The values are based on observations from May to October over 4 years (2009–2012) at Muen and Benten. The number of visits in each month is expressed per hour (mean \pm SE)



(each 5 m \times 5 m) were arbitrarily established on patches of species with typical floral densities, and the total number of open flowers was counted. Then, in 2011 and 2012, two 150 m \times 1 m belt transects were established where all 15 plant species could be observed at each location, and the total number of open flowers was recorded to quantify floral resources more precisely.

Morphology of bumblebee species

Three common bumblebees (*B. hypocrita*, *B. deuteronymus*, and *B. terrestris*) were captured on the flowers of *Rosa rugosa*, *Vicia japonica*, *Lathyrus japonicus* and/or

Calystegia soldanella at each site on the 2nd, 7th, 9th, 14th, and 30th of July 2010. They were preserved as dried specimens at room temperature until morphological measurements were performed. We measured proboscis length (total of prementum length and glossa length) and head width of the bumblebees, because the combination of these characters is closely related to the shape of flower that the bumblebees can utilize, i.e., the corolla tube length and width of corolla mouth (Inoue and Kato 1992). Before measurements were taken, the proboscis was uncoupled from the head and soaked in warm water for a few minutes to soften the muscle. Between 14 and 20 workers of each species, for which the glossa could be extended to its full

length, were sampled from the specimens. The proboscis length was measured with a caliper under a stereoscopic microscope (MZ125, Leica, Wetzlar, Germany).

Data analysis

Based on the total bumblebee counts (for which workers and queens were pooled), our analyses focused on the three most common species: *Bombus terrestris*, *B. hypocrita*, and *B. deuteronymus*.

To test seasonal differences in the three bumblebee species' activity, we compared their visitation frequencies to the 15 most common flower species (see Table 2) throughout the flowering seasons using a generalized linear mixed model (GLMM), postulating a negative binomial error distribution with a logarithmic link function. In the GLMM, the visit number to the common plants was set as a response variable, and bumblebee species (*B. terrestris*, *B. hypocrita*, and *B. deuteronymus*), observation week (week number in active period), interaction between bumblebee species and week, and site (Muen or Benten) were included as fixed factors. Year (2009–2012) was included as a random factor to control for the effects of variation in bumblebee abundance among years. The observation period (30–240 min) in each measurement was included as an offset term. In this model, the Akaike's information criterion (AIC) was calculated for the full model and all possible subsets of variables. The combination of variables that minimized the AIC value was then selected as the best-fit model. Next, visitation frequencies to the plant species with the longest flowering periods (*Rosa rugosa* and *Lathyrus japonicus*) were compared separately using the same GLMM, where the response variable was the visit number to either flower species. Because these plant species have different floral morphologies, i.e., shallow corolla in *R. rugosa* and tubed corolla in *L. japonicus*, we assessed the differences in floral choice among bumblebee species

in addition to the seasonal trend of foraging activity owing to the long flowering periods.

To test the difference in foraging patterns to common flowering species among bumblebee species, a contingency table analysis was performed for the total number of flower visits by the three bumblebee species to the five most common flowering plant species (*R. rugosa*, *V. japonica*, *L. japonicus*, *C. soldanella*, and *S. virgaurea*, see Table 2). The observed contingency table was compared to the expected values under random visits by Pearson's Chi squared test for the 4 years overall (2009–2012), and by Fisher's exact test for each year at each site.

Furthermore, similarities in floral-resource usage between pairs of bumblebee species was evaluated by the index of niche overlap (Colwell and Futuyma 1971) based on the floral use for 15 plant species as follows:

Niche overlap between bumblebee species *i* and *h*

$$= 1 - 0.5 \sum_{k=1}^{15} (P_{ik} - P_{hk}), \quad (1)$$

where

$$P_{ik} = \frac{\text{Individuals of species } i \text{ visiting plant species } k}{\text{Total individuals of species } i}$$

We analyzed the factors affecting *B. hypocrita* visits to *R. rugosa* flowers and *B. deuteronymus* visits to *L. japonicus* flowers. For the analyses, we used observation data from the beginning of June to the end of September, because the visitation of bumblebees, especially of the native species, to *R. rugosa* or *L. japonicus* flowers was generally rare in May and October (see Fig. 1). To assess the effect of alien bumblebees on the native bumblebees, we constructed models to explain the visitation patterns of individual bumblebee species using GLMMs, postulating a negative binomial error distribution with a logarithmic link function. In the GLMMs, the number of visits to *R. rugosa* flowers by *B. hypocrita* or visit number to *L. japonicus*

Table 1 Observed bumblebee species and their frequencies during the active period (May to October) over 4 years (2009–2012) in each site

<i>Bombus</i> species	Observed number										
	Muen					Benten					Total (%)
	2009	2010	2011	2012	Total (%)	2009	2010	2011	2012	Total (%)	
<i>B. hypocrita</i>	99	121	166	129	515 (42.6)	31	11	36	30	108 (13.6)	623 (31.1)
<i>B. deuteronymus</i> (including <i>B. pseudobaicalensis</i>)	222	132	71	105	530 (43.9)	152	84	51	166	453 (57.1)	983 (49.1)
<i>B. diversus</i>	3	0	0	0	3 (0.2)	0	0	0	0	0 (0)	3 (0.2)
<i>B. ardens</i>	0	0	0	4	4 (0.3)	0	0	0	0	0 (0)	4 (0.2)
<i>B. hypnorum</i>	0	1	1	0	2 (0.2)	0	0	0	0	0 (0)	2 (0.1)
<i>B. terrestris</i>	20	37	39	59	155 (12.8)	67	39	60	66	232 (29.3)	387 (19.3)

Table 2 Growth form and corolla length of plant species visited by bumblebees and total number of visits during the active period (May to October) over 4 years (2009–2012) by the three common bumblebee species (*Bombus hypocrita sapporoensis*, *B. deuteronymus deuteronymus*, and *B. terrestris*)

Plant species	Family	Growth form	Corolla length (mm)	Number of visits			
				<i>B. hypocrita</i>	<i>B. deuteronymus</i>	<i>B. terrestris</i>	Total
<i>Rosa rugosa</i>	Rosaceae	Shrub	Shallow	284	44	207	535
<i>Vicia japonica</i>	Leguminosae	Herb	10–12	3	483	9	495
<i>Lathyrus japonicus</i> subsp. <i>japonicus</i>	Leguminosae	Herb	25–30	26	339	91	456
<i>Calystegia soldanella</i>	Convolvulaceae	Herb	46–51	255	35	23	313
<i>Solidago virgaurea</i> subsp. <i>leiocarpa</i>	Asteraceae	Herb	6–8	1	59	31	91
<i>Elaeagnus umbellata</i>	Elaeagnaceae	Shrub	5–6	30	0	3	33
<i>Arabis stelleri</i> var. <i>japonica</i>	Brassicaceae	Herb	7–9	8	0	10	18
<i>Linaria japonica</i>	Scrophulariaceae	Herb	15–18	6	7	1	14
<i>Polygonatum humile</i>	Liliaceae	Herb	1.5–2	5	0	3	8
<i>Dianthus superbus</i> var. <i>superbus</i>	Caryophyllaceae	Herb	20–30	1	2	5	8
<i>Hieracium umbellatum</i>	Asteraceae	Herb	4–5	0	6	1	7
<i>Glehnia littoralis</i>	Apiaceae	Herb	Exposed	4	0	1	5
<i>Ampelopsis glandulosa</i> var. <i>heterophylla</i>	Vitaceae	Liana	Exposed	0	3	1	4
<i>Rubus parvifolius</i>	Rosaceae	Shrub	Shallow	0	2	1	3
<i>Ligustrum obtusifolium</i>	Oleaceae	Shrub	7–9	0	3	0	3

Table 3 Result of GLMM conducted for the number of flower visits to the major native plants (15 spp.) by alien (*Bombus terrestris*) and native bumblebee species (*B. hypocrita* and *B. deuteronymus*)

Variable	Coefficient	SE	z value	p value
Intercept (<i>B. terrestris</i> , site = Bente)	−3.555	0.197	−18.070	<0.001
Week	−0.005	0.007	−0.630	0.530
<i>B. hypocrita</i>	0.290	0.131	2.210	<0.05
<i>B. deuteronymus</i>	−0.047	0.121	−0.390	0.698
Site (Muen)	0.245	0.051	4.830	<0.001
<i>B. hypocrita</i> × week	−0.035	0.011	−3.210	<0.01
<i>B. deuteronymus</i> × week	0.068	0.009	7.600	<0.001

The best-fit models selected by AIC are shown
 Full model (=best-fit model) AIC = 5030

flowers by *B. deuteronymus* was set as a response variable. The number of visits to *R. rugosa* or *L. japonicus* flowers by *B. terrestris* (an alien species), number of visits by different native species (*B. deuteronymus* or *B. hypocrita*), their interaction, flower abundance (*R. rugosa* or *L. japonicus*), site (Muen or Bente), and month (June, July, August, and September) were included as fixed factors. For each plant species, the flower abundance on the observation day was expressed as a proportion of open flowers relative to the maximum flower number (100 %) during the observation period in each site and year. The month, from June to September in each year, was set as a representative of seasonal effects. Year (2009–2012) was included as a random factor. The observation period (30–240 min) of

each measurement was treated as an offset term. In each model, the best-fit model was selected based on the AIC value.

Proboscis length was compared among workers of the three bumblebee species using a generalized linear model GLM, postulating a gamma error distribution with a logarithmic link function. In the GLM, proboscis length was set as a response variable, and the bumblebee species (*B. terrestris*, *B. hypocrita*, and *B. deuteronymus*) and head width were included as fixed factors. The best-fit model was selected based on AIC value.

All statistical analyses were conducted using R ver. 3.1.2 (R Core Team 2014). The R package vcd (Meyer et al. 2015) was used for the contingency table analysis and

Table 4 Results of GLMMs conducted for the number of flower visits to (a) *Rosa rugosa* and (b) *Lathyrus japonicus* by alien (*Bombus terrestris*) and native bumblebee species (*B. hypocrita* and *B. deuteronymus*)

Variable	Coefficient	SE	z value	p value
(a) <i>Rosa rugosa</i> ^a				
Intercept (<i>B. terrestris</i>)	−4.428	0.157	−27.200	<0.001
<i>B. hypocrita</i>	0.061	0.101	0.610	0.543
<i>B. deuteronymus</i>	−1.642	0.148	−11.120	<0.001
Site(Muen)	0.342	0.095	3.610	<0.001
(b) <i>Lathyrus japonicus</i> ^b				
Intercept (<i>B. terrestris</i>)	−4.040	0.332	−12.190	<0.001
Week	−0.075	0.024	−3.200	<0.01
<i>B. hypocrita</i>	−1.060	0.402	−2.640	<0.01
<i>B. deuteronymus</i>	0.436	0.252	1.730	0.084
Site(Muen)	−0.271	0.080	−3.400	<0.001
<i>B. hypocrita</i> × week	−0.028	0.040	−0.700	0.483
<i>B. deuteronymus</i> × week	0.079	0.025	3.190	<0.01

The best-fit models selected by AIC are shown

^a Full model AIC = 2432, best-fit model AIC = 2429

^b Full model (=best-fit model) AIC = 1987

glmmADMB (Fournier et al. 2012) was used for the GLMM analysis.

Results

Bumblebee composition

Six native bumblebee species and one alien bumblebee species were recorded during the 4 years of observation (Table 1). The dominant species were *B. deuteronymus* (49 % of the total observed number throughout the study; this included some *B. pseudobaicalensis* individuals), *B. hypocrita* (31 %), and *B. terrestris* (19 %). The other species, *B. diversus tersatus* Smith, *B. ardens sakagami* Tkalců, and *B. hypnorum koropokkrus* Sakagami et Ishikawa, were observed only at Muen and at very low frequencies (<1 %). The total number of *B. hypocrita* individuals observed at Benten was less than at Muen over the years, whereas the number of *B. terrestris* individuals was higher at Benten. We focused, therefore, on *B. hypocrita*, *B. deuteronymus*, and *B. terrestris* in this study.

Plant species visited by bumblebees

Flowers of 15 native plant species (10 herbs, 4 shrubs, and 1 liana) were visited by the bumblebees (Table 2). Among these species, *Rosa rugosa*, *Vicia japonica*, *Lathyrus*

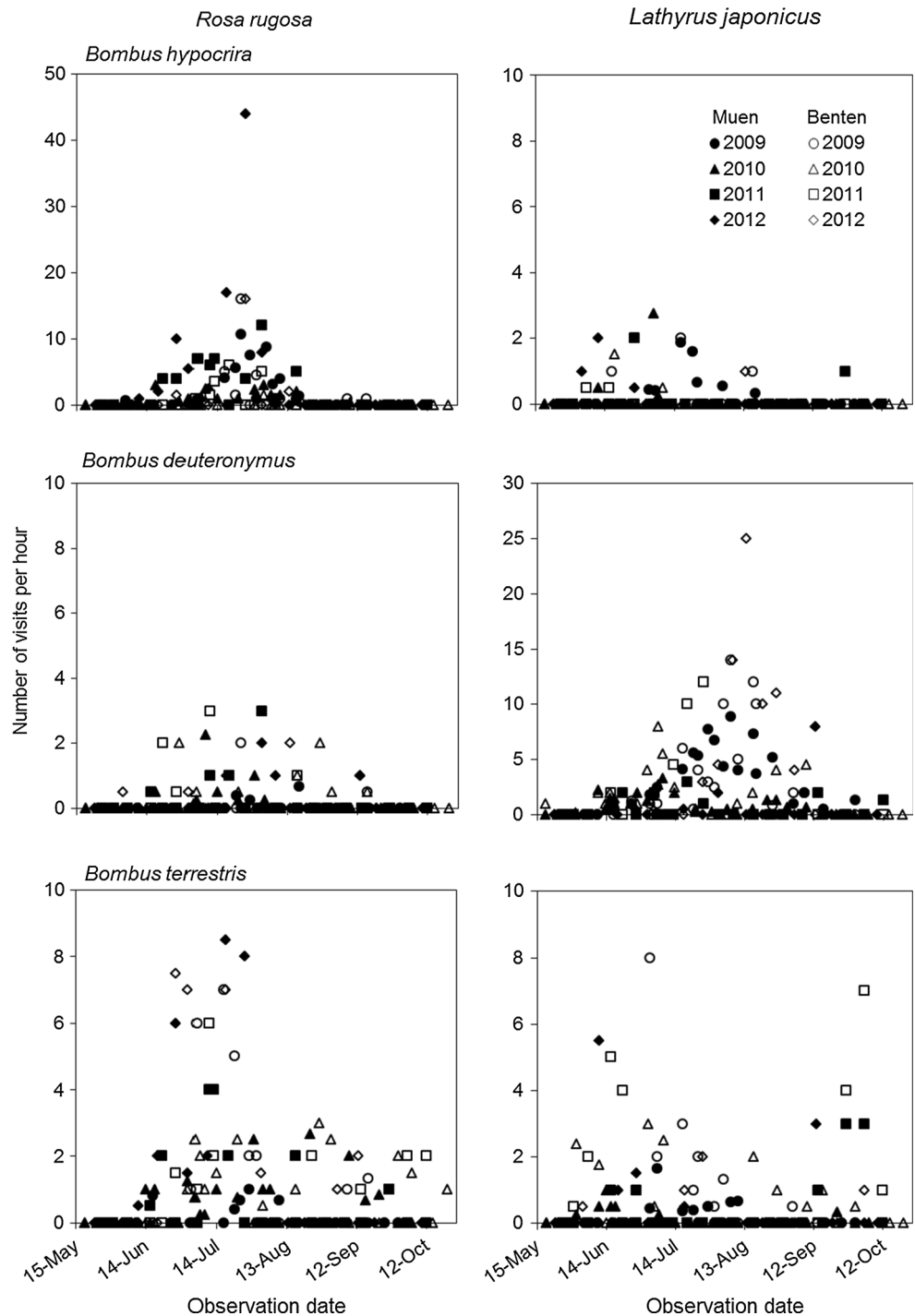
japonicus, *Calystegia soldanella*, and *Solidago virgaurea* were visited at high frequencies (>90 times in total) by the three common bumblebee species. Flowering of *R. rugosa* and *L. japonicus* lasted for 16–21 weeks, with peak flowering from mid-June to early July and subsequent sporadic flowering until early October (see “Appendix 1”). Other plant species had shorter flowering periods ranging from three to 13 weeks. Although several non-native plant species were present, such as *Trifolium pretense*, *Hypochaeris radicata*, and *Solidago gigantea* var. *leiophylla*, bumblebee visits to these flowers were rare; probably because of the small numbers of flowers on these species.

Seasonal trend in visitation frequency and floral-use pattern

In the GLMM conducted for the visitation frequency to the flowers of 15 common plant species, *B. hypocrita* showed significantly higher frequency than *B. terrestris* ($p < 0.05$), while there was no significant difference between *B. deuteronymus* and *B. terrestris* ($p = 0.70$; Table 3). However, a seasonal trend in visitation frequencies (detected as interaction between the visitation frequency and the week) differed significantly between *B. terrestris* and each of the native species; *B. hypocrita* ($p < 0.01$) and *B. deuteronymus* ($p < 0.001$; Table 3). These results indicate that the seasonal foraging activity significantly differed among the three bumblebee species. The appearance of *B. terrestris* at the study sites (normally from late May to early June, but early July at Benten in 2009) was timed almost identically to *B. hypocrita*, and peak foraging occurred in July in both species (Fig. 1). However, late in the season, *B. hypocrita* disappeared much earlier (by mid-September) than *B. terrestris* (by mid-October; Fig. 1). In contrast, the appearance of *B. deuteronymus* (normally from early to late June, but late May at Benten in 2009) was later than that of *B. terrestris*, and its peak foraging occurred in August. The disappearance of *B. deuteronymus* by mid-October was similar in timing to *B. terrestris* (Fig. 1). The visitation frequencies were higher at Muen than at Benten ($p < 0.001$; Table 3).

The GLMM conducted to assess the visitation frequency to *R. rugosa* flowers revealed that *B. deuteronymus* showed a significantly lower visitation frequency in comparison to *B. terrestris* ($p < 0.001$), while there was no significant difference between the visitation frequencies of *B. hypocrita* and *B. terrestris* ($p = 0.54$; Table 4a; Fig. 2). The seasonal factor (week) was excluded from the explanatory variables, indicating no seasonal trend in visitation frequency during the flowering period of *R. rugosa*. Visitation frequency was higher at Muen than at Benten ($p < 0.001$). In contrast, visitation frequency of *B. hypocrita* to *L. japonicus* flowers was significantly lower than that of *B.*

Fig. 2 Number of visits to *R. rugosa* and to *L. japonicus* flowers by three bumblebee species recorded in each observation day from May to October over 4 years (2009–2012) at Muen and Benten. The number of visits in each day is expressed per hour



terrestris ($p < 0.01$), while the visitation frequency of *B. deuteronymus* was marginally higher than that of *B. terrestris* ($p = 0.084$; Table 4b). Seasonal changes in visitation frequency were significantly different between *B. deuteronymus* and *B. terrestris* ($p < 0.01$), but did not differ between *B. hypocrita* and *B. terrestris* ($p = 0.48$; Table 4b). *B. deuteronymus* visits to *L. japonicus* were concentrated in the middle of the grassland’s flowering

season (mid-July to late August), while the number of *B. terrestris* visits was greater early and late in the season (Fig. 2). In contrast to *R. rugosa*, the visitation frequency to *L. japonicus* was lower at Muen than at Benten ($p < 0.001$; Table 4b).

Visitation frequencies to the five common plant species were significantly different among bumblebee species at both sites ($p < 0.001$; Table 5a, b). High frequencies of

Table 5 Contingency tables for the number of visits to the top five species of flower by three bumblebee species over the 4 years (2009–2012) at (a) Muen and (b) Benten

<i>Bombus</i> species	Plant species					Total
	<i>R. rugosa</i>	<i>L. japonicus</i>	<i>S. virgaurea</i>	<i>C. soldanella</i>	<i>V. japonica</i>	
(a) Muen ^a						
<i>B. hypocrita</i>	219	18	0	223	3	463
<i>B. deuteronymus</i>	20	125	37	29	299	510
<i>B. terrestris</i>	72	28	13	20	5	138
(b) Benten ^b						
<i>B. hypocrita</i>	65	8	1	32	0	106
<i>B. deuteronymus</i>	24	214	22	6	184	450
<i>B. terrestris</i>	135	63	18	3	4	223

^a $\chi^2 = 815.8$, $df = 8$, $p < 0.001$ by Pearson's Chi squared test

^b $\chi^2 = 522.9$, $df = 8$, $p < 0.001$ by Pearson's Chi squared test

Table 6 Results of GLMMs conducted for the visitation frequency of (a) *Bombus hypocrita* to *Rosa rugosa* flowers and (b) *B. deuteronymus* to *Lathyrus japonicus* flowers

Variable	Coefficient	SE	z value	p value
(a) <i>B. hypocrita</i> to <i>R. rugosa</i> ^a				
Intercept (Benten, June)	−5.154	0.516	−9.990	<0.001
Visit number of <i>B. terrestris</i>	0.105	0.040	2.660	<0.01
Site (Muen)	1.286	0.296	4.340	<0.001
Month (July)	1.004	0.397	2.530	<0.05
Month (August)	1.036	0.432	2.400	<0.05
Month (September)	−1.075	0.704	−1.530	0.127
(b) <i>B. deuteronymus</i> to <i>L. japonicus</i> ^b				
Intercept (Benten, June)	−4.066	0.273	−14.890	<0.001
Site (Muen)	−0.358	0.192	−1.860	0.063
Month (July)	1.364	0.277	4.920	<0.001
Month (August)	1.945	0.326	5.980	<0.001
Month (September)	0.593	0.341	1.74	0.082

The best-fit models selected by AIC are shown

^a Full model AIC = 470, best-fit model AIC = 464

^b Full model AIC = 545, best-fit model AIC = 540

visits were recorded for *B. hypocrita* to *R. rugosa* and *C. soldanella* flowers, *B. deuteronymus* to *L. japonicus* and *V. japonica* flowers, and *B. terrestris* to *R. rugosa* and *L. japonicus* flowers. These differences in floral usage patterns among bumblebee species remained largely consistent over the years of the study (“Appendix 2”).

Niche overlap based on the floral-use frequency varied highly between bumblebee species. The niche overlap between *B. hypocrita* and *B. deuteronymus* was low (0.16 ± 0.09 SD for Muen and 0.24 ± 0.15 for Benten), whereas that between *B. hypocrita* and *B. terrestris* was high (0.54 ± 0.08 for Muen and 0.62 ± 0.10 for Benten). The niche overlap between *B. terrestris* and *B. deuteronymus* was intermediate (0.34 ± 0.07 SD for Muen and 0.42 ± 0.15 for Benten). The trend of niche overlap was consistent over sites and years (“Appendix 3”).

Visitation frequency of native bumblebees

The visitation frequency of *B. hypocrita* to *R. rugosa* flowers was positively related to the visitation frequency of *B. terrestris* ($p < 0.01$), while the effects of the native species (*B. deuteronymus*) and flower abundance were excluded from the explanatory variables (Table 6a). *B. hypocrita* visits to *R. rugosa* flowers was higher at Muen ($p < 0.001$), and increased in July and August ($p < 0.05$ in both).

The visitation frequency of *B. deuteronymus* to *L. japonicus* flowers was independent of the frequencies of *B. terrestris* and *B. hypocrita*, and of flower abundance (Table 6b). The visitation frequency of *B. deuteronymus* to *L. japonicus* flowers was higher in July and August

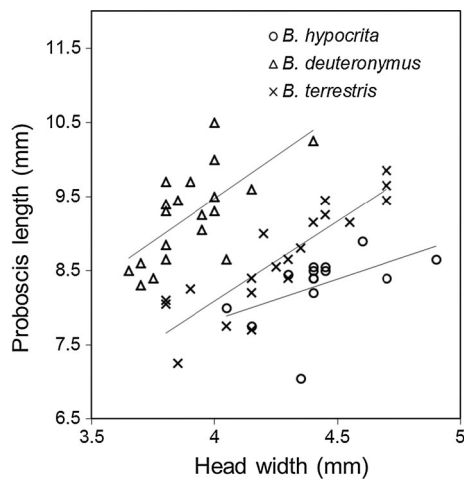


Fig. 3 Relationship between head width and proboscis length (total of prementum length and glossa length) of worker bees of *B. hypocrita*, *B. deuteronymus*, and *B. terrestris*

Table 7 Result of GLM conducted for the proboscis length of alien (*Bombus terrestris*) and native bumblebee species (*B. hypocrita* and *B. deuteronymus*)

Variable	Coefficient	SE	z value	p value
Intercept (<i>B. terrestris</i>)	1.197	0.124	9.672	<0.001
<i>B. hypocrita</i>	−0.076	0.017	−4.351	<0.001
<i>B. deuteronymus</i>	0.148	0.018	8.085	<0.001
Head width	0.225	0.029	7.771	<0.001

The best-fit models selected by AIC are shown

Full model (=best-fit model) AIC = 66

($p < 0.001$ in both months), and marginally lower at Muen ($p = 0.063$).

Morphological differences among bumblebee species

Worker bees showed considerable variation in head width and proboscis length among species (Fig. 3). Proboscis length in *B. hypocrita* was significantly shorter than in *B. terrestris* ($p < 0.001$), while that of *B. deuteronymus* was significantly longer than *B. terrestris* ($p < 0.001$; Table 7). Proboscis length was positively related to head width ($p < 0.001$; Table 7).

Discussion

In this study, we did not detect any negative effect of alien bumblebees on the foraging behavior of native bumblebees in the coastal grassland at the present time. However, we detected evidence of a consistent linkage between morphological similarity and niche overlap between bumblebee

species, including the alien species. This implies a possibility of changes in pollination networks in the future.

Floral-use patterns were markedly different between the two dominant native bumblebee species; *B. hypocrita* commonly visited flowers with shallow or widely open corollas (*R. rugosa* and *C. soldanella*), whereas *B. deuteronymus* tended to visit legume flowers with complex and deep corollas (*L. japonicus* and *V. japonica*; Tables 2, 5). The species-specific morphological traits of bumblebees, such as proboscis length, body mass, and wing length, often determine their ability to forage from specific flowers (Harder 1985). Although the interspecific variation in proboscis lengths was relatively small in this study, relative proboscis length was short in *B. hypocrita* and long in *B. deuteronymus* (Fig. 3). Low niche overlap between these native species might reflect the resource partitioning caused by the morphological differences, as indicated in previous studies (Heinrich 1976; Inouye 1980; Pyke 1982; Graham and Jones 1996). In contrast, the utilization of floral resources by alien bumblebees consistently overlapped with that by native bumblebees in this area. This is partly because *B. terrestris* is highly polylectic for floral use (Hingston and McQuillan 1998; Goulson and Darvill 2004; Matsumura et al. 2004). Moreover, the relative tongue length of *B. terrestris* was intermediate of two native species (Fig. 3). In particular, high niche overlap between *B. terrestris* and *B. hypocrita* might reflect the morphological similarity as shown by Nagamitsu et al. (2007b).

Previous studies have reported that native bees tend to avoid foraging in areas occupied by *B. terrestris* because of resource depletion (Dafni and Shmida 1996; Hingston and McQuillan 1999; Hingston 2007; Madjidian et al. 2008). However, there was no evidence in this study that native bumblebees avoid visits to *R. rugosa* flowers because of the visits of alien bumblebees. On the contrary, the visitation frequency to *R. rugosa* flowers was positively correlated between *B. hypocrita* and *B. terrestris* (Tables 4a, 6a; Fig. 2). Both bumblebee species commonly utilized similar floral resources during the same period in the study area. The positive correlation may reflect a similar colony life-cycle between *B. hypocrita* and *B. terrestris*, because the foraging activity of both species tended to be maximized in July, although the active period toward late season lasted longer in *B. terrestris* (Fig. 1).

The visitation frequency of *B. deuteronymus* to *L. japonicus* flowers was independent of the visitation frequency of other bumblebees and of flower abundance (Table 6b). The period of peak visitation frequency to *L. japonicus* flowers differed between bumblebee species; *B. deuteronymus* commonly visited *L. japonicus* flowers in the middle of the season, from July to mid-August, whereas

B. terrestris tended to visit early and late in the season (Table 4b; Fig. 2). Although further experimental study is necessary, this result implies that phenological niche partitioning is more common in specialized flowers (*L. japonicus*) than in generalized flowers (*R. rugosa*).

B. terrestris was more abundant at the Benten site than at the Muen site, whereas *B. hypocrita* showed the opposite trend (Table 1). The difference in bumblebee abundance cannot be explained by the site's floral resources, because the floral composition and flowering patterns are similar at both sites (see "Appendix 1"). Differences in the surrounding environments of coastal communities may be an important determinant of bumblebee species composition. The Benten site is adjacent to a residential area and reclaimed land, where the foraging and nesting habitats for bumblebees may be different from the Muen site, which is surrounded by cultivated fields and small fragmented forests. If the bumblebee abundances are attributable to the outside environment of focal plant communities, consideration of landscape features over entire foraging areas of individual bumblebee species is necessary to evaluate the impacts of alien species on native species (Goulson et al. 2010).

Although a similar floral-use pattern was observed between the sites (Table 5), probably because of the abundant floral resources in this grassland, the increased abundance of alien bumblebees is expected to have caused changes in the foraging pattern of native bumblebees. It may also affect the pollination service to the local plant communities (Dafni et al. 2010; Dohzono and Yokoyama 2010). However, replacement of native *B. hypocrita* by alien *B. terrestris* may not cause serious effects on the pollination service because of the similar morphological

characteristics and foraging behavior of these species (Nagamitsu et al. 2007b). On the other hand, Dohzono et al. (2008) reported that *B. terrestris* often robbed tubed flowers of nectar, resulting in decreased foraging by native bumblebees and reduced seed production in *Corydalis ambigua*. Although *L. japonicus* flowers were frequently visited by *B. terrestris*, nectar-robbing was comparatively low (<20 %, personal observation), and *B. terrestris* legitimately visited *L. japonicus* flowers in most cases, acting as legitimate pollinators. Generally, plant communities composed of generalist species visited by various pollinators, including short-tongued bumblebees, may be less susceptible to the invasion of alien pollinators.

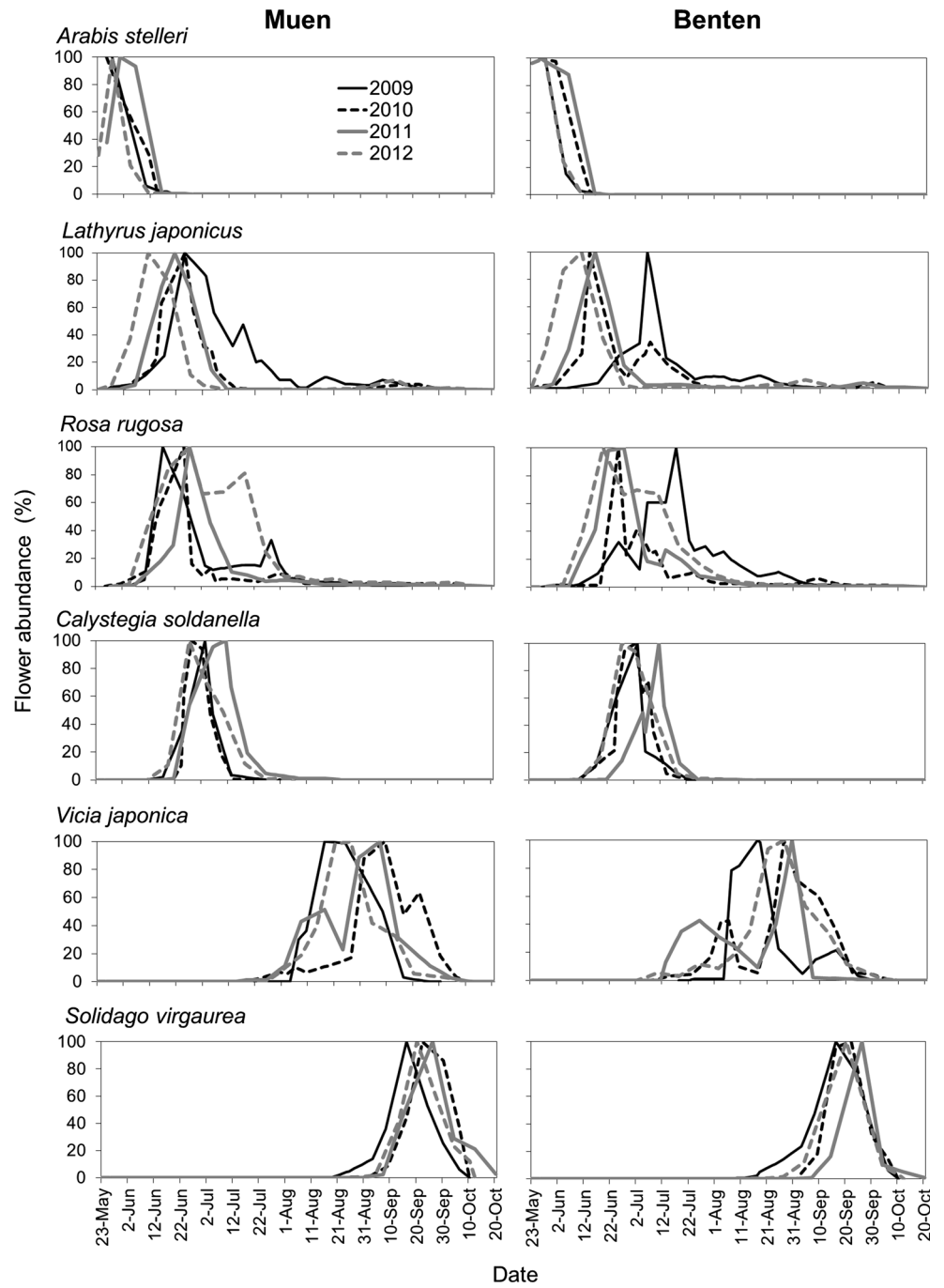
In this study, we observed that alien bumblebees utilize similar floral resources to native bumblebees with similar morphology. To clarify the resource use patterns of coexisting native and alien bumblebees, however, further experimental approaches are needed, including exclusion of alien bumblebees in the field. In addition, evaluation of the effects of foraging behavior changes in existing pollinators on the pollination success of native plants is required.

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Appendix 1

See Fig. 4.

Fig. 4 Flowering patterns of major species visited by bumblebees (>90 total visits; see Table 2) over 4 years (2009–2012) at Muen and Benten. The abundance of flowers is expressed as the relative number of open flowers in which the maximum flower number is set as 100 %



Appendix 2

See Table 8.

Table 8 Contingency tables for the number of visits to the flowers of the top five plant species among three bumblebee species (*B. hypocrita*, *B. deuteronymus*, and *B. terrestris*) at Muen and Benten during 4 years (2009–2012)

Year	<i>Bombus</i> species	Plant species					Total	Fisher's exact test
		<i>R. rugosa</i>	<i>L. japonicus</i>	<i>S. virgaurea</i>	<i>C. soldanella</i>	<i>V. japonica</i>		
<i>Muen</i>								
2009	<i>B. hypocrita</i>	50	7	0	7	0	64	$p < 0.001$
	<i>B. deuteronymus</i>	2	77	19	2	118	218	
	<i>B. terrestris</i>	6	2	4	2	2	16	
2010	<i>B. hypocrita</i>	29	4	0	75	2	110	$p < 0.001$
	<i>B. deuteronymus</i>	7	25	1	17	77	127	
	<i>B. terrestris</i>	21	5	0	7	3	36	
2011	<i>B. hypocrita</i>	50	3	0	111	1	165	$p < 0.001$
	<i>B. deuteronymus</i>	6	12	0	10	41	69	
	<i>B. terrestris</i>	16	9	3	5	0	33	
2012	<i>B. hypocrita</i>	90	4	0	30	0	124	$p < 0.001$
	<i>B. deuteronymus</i>	5	11	17	0	63	96	
	<i>B. terrestris</i>	29	12	6	6	0	53	
<i>Benten</i>								
2009	<i>B. hypocrita</i>	25	4	1	1	0	31	$p < 0.001$
	<i>B. deuteronymus</i>	2	71	14	0	65	152	
	<i>B. terrestris</i>	34	18	13	0	1	66	
2010	<i>B. hypocrita</i>	3	2	0	5	0	10	$p < 0.001$
	<i>B. deuteronymus</i>	7	42	0	1	32	82	
	<i>B. terrestris</i>	20	16	0	0	0	36	
2011	<i>B. hypocrita</i>	17	1	0	17	0	35	$p < 0.001$
	<i>B. deuteronymus</i>	8	29	0	5	9	51	
	<i>B. terrestris</i>	33	24	0	1	0	58	
2012	<i>B. hypocrita</i>	20	1	0	9	0	30	$p < 0.001$
	<i>B. deuteronymus</i>	7	72	8	0	78	165	
	<i>B. terrestris</i>	48	5	5	2	3	63	

p values of Fisher's exact tests are shown

Appendix 3

See Table 9.

Table 9 Niche overlaps for floral use between bumblebee species pairs at Muen and Bente in each of the 4 years (2009–2012)

Year	<i>Bombus</i> species	<i>B. hypocrita</i>	<i>B. deuteronymus</i>	<i>B. terrestris</i>
2009	<i>B. hypocrita</i>	–	0.089	0.582
	<i>B. deuteronymus</i>	0.174	–	0.304
	<i>B. terrestris</i>	0.669	0.389	–
2010	<i>B. hypocrita</i>	–	0.239	0.487
	<i>B. deuteronymus</i>	0.277	–	0.398
	<i>B. terrestris</i>	0.532	0.494	–
2011	<i>B. hypocrita</i>	–	0.249	0.452
	<i>B. deuteronymus</i>	0.434	–	0.396
	<i>B. terrestris</i>	0.536 0.574	–	
2012	<i>B. hypocrita</i>	–	0.079	0.624
	<i>B. deuteronymus</i>	0.076	–	0.271
	<i>B. terrestris</i>	0.730	0.218	–

Values below the diagonal are for Bente and those above the diagonal are for Muen

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