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Differences in Pollen Resource Usage and Foraging Periods between the Exotic Bumblebee *Bombus terrestris* and the Native *B. pseudobaicalensis* and *B. hypocrita sapporoensis* in Hokkaido, Japan

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Abstract

Bombus terrestris Linnaeus is an exotic bumblebee species that has recently been naturalized in Hokkaido, Japan. Some studies have suggested that the naturalization of this species has caused a decline in the native bumblebee populations. The present study focuses on differences in plant resource usage between coexisting exotic (*B. terrestris*) and native (*B. pseudobaicalensis* and *B. hypocrita* subsp. *sapporoensis*) bumblebee species in the Ishikari lowland region of Hokkaido. We observed the workers of the three species that came to forage in the investigation area during an approximately five-month period. In addition, we captured some of the bees carrying pollen to analyze the pollen grains attached to their body surfaces. The pollen grains were identified at the genus or species level. Compared with the two native species, *B. terrestris* workers were relatively abundant during the investigation period. This species also exhibited a relatively long duration of foraging activity and a larger foraging range than the other two native species. The patterns of pollen resource usage were generally different among the three species, suggesting that these species have different flower preferences. Cluster analysis based on use of pollen species by each worker revealed that the cluster compositions overlapped among the three species in July, but this overlap disappeared in August. The usage in pollen species tended to differ through the season.

Key words: *Bombus terrestris*, exotic species, flower resources, pollen analysis, interspecific competition

Introduction

The impact of exotic species on local ecosystems has recently attracted much attention. There is much evidence that the presence of exotic bees in an area leads to population declines or extinction of native species through predation, interspecific competition, or the introduction of harmful parasites into native species in the ecosystem (Goka *et al*. 2001, Goulson *et al*. 2003). *Bombus terrestris* Linnaeus is one of such exotic species and causes ecological problems around the world. This bumblebee was introduced into Japan in 1991 as a pollinator for greenhouse-grown tomato plants (Ono 1998). Since then, some individuals of the species have escaped from greenhouses and have begun to naturalize. This naturalization was first confirmed in 1996, when a colony of this species was found in the Hidaka region, southern Hokkaido, which is near the main area of the introduction (Washitani 1998, Kawahara 2004, Matsumura *et al*. 2004). Some reports have demonstrated that the naturalized colonies of this species have expanded in Hokkaido since its introduction (Inari *et al*. 2005, Yokoyama *et al*. 2009, Goka 2010). *Bombus terrestris* is characterized by a long season of activity (Sladen 1912, Prys-Jones and Corbet 1991) and an extensive foraging range (Walther-Hellwig and Frankl 2000, Ne'eman *et al*. 2002, Knight *et al*. 2005).

Bombus terrestris has a relatively short tongue and can decrease the pollination efficiency of flowering plants by nectar robbing (Prys-Jones and Corbet 1991, Kenta *et al*. 2007). This species was also shown to have negative effects on some particular flowering plants (Kenta *et al*. 2007). In addition, relatively large body size of *B. terrestris* is probably effective to take over nests of other bumblebee species (Richards 1978). Thus, once *Bombus terrestris* is introduced into a new area, it has the potential to become the dominant species. Some studies have suggested that the naturalization of this bumblebee is responsible for the observed decline in the populations of native bumblebees (Kawahara 2004, Inoue *et al*. 2008). In a controlled experiment, interspecific competition between this exotic bumblebee and other native bumblebees was not observed (Nagamitsu *et al*. 2007a). However, the exotic species showed exclusive distribution in the natural environment (Nagamitsu *et al*. 2007b). A removal experiment in the natural environment suggested that there is interspecific competition between the exotic bumblebee and other native bumblebees (Nagamitsu *et al*. 2010).

Nevertheless, it is not clear whether *B. terrestris* always becomes dominant in every locality in which it has been introduced. It is possible that *B. terrestris* exhibits different patterns of plant resource use from

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other native bumblebee species, thereby avoiding interspecific competition for pollination resources. Kenta *et al*. (2007) conducted an experiment to examine the impact of *B. terrestris* on the pollination success of several native plant species. However, Inoue *et al*. (2008) reported that *B. terrestris* and other native bumblebee species (*B. pseudobaicalensis* and *B. hypocrita* subsp. *sapporoensis*) visited exotic plant species far more frequently than native plant species. Due to the high abundance of exotic plant species in Hokkaido, it is important to evaluate the use of exotic plant resources in order to clarify the relationship between coexisting bumblebee species in the natural environment of Hokkaido. We, therefore, conducted a study focusing on plant resource usage among exotic and native bumblebee species in an environment where exotic plants are dominant. We evaluated the patterns of pollen resource use among bumblebee species by identifying the pollen grains attached to the bodies of workers.

Materials and Methods

The investigation was conducted in Chitose City, in the lowlands of Ishikari region, Hokkaido. A private garden (approximately 400 m^2) in this City was chosen as investigation area to observe and capture bumblebees efficiently. In the investigation area, 27 species (belonging to 15 families) of blooming flowers were observed during the investigation period (Table 1). All of the species except *Commelina communis*, *Hosta montana* and *Spiraea salcifolia* were ornamental or exotic plants. In this garden, the three bumblebee species surveyed in this study frequently came to forage during the investigation period. There were no greenhouses that employed *B. terrestris* colonies within a radius of approximately 5 km around the garden. According to some reports, the foraging ranges of this species are less than 625–758 m from the colony (Darvil *et al*. 2004, Knight *et al*. 2005). Therefore, all of the *B. terrestris* foraging in this area probably came from naturalized colonies (Yokoyama *et al*. 2004, Nagamitsu *et al*. 2007b). The landscape of this region consists mainly of grasslands, cultivated farmland and natural forests. Several exotic herbaceous plants are dominant in the grasslands and at the farmland edges, including *Taraxacum officinale*, *Trifolium pretense*, *Tr. repens* and *Solidago canadensis* var. *scabra*. There are also some patches of *Sp. salcifolia* in the grasslands. The natural forests are mainly composed of deciduous broad-leaved trees, including *Quercus crispula*, *Q. dentata*, *Q. serrata*, *Acer mono*, *Tilia japonica* and *Betula ermanii*.

Table 1. Blooming flowers from June to November 2010 within a radius of approximately 1 km around the investigation area

Family	Plant species
Apiaceae	*Petroselinum neapolitanum*
Asteraceae	*Anthemis nobilis*
Asteraceae	*Chrysanthemum × morifolium*
Asteraceae	*Echinacea purpurea*
Asteraceae	*Erigeron annuus*
Asteraceae	*Helianthus annuus*
Asteraceae	*Solidago canadensis* var. *scabra*
Asteraceae	*Taraxacum officinale*
Asteraceae	*Tegates* sp.
Boraginaceae	*Symphytum officinale*
Caryophyllaceae	*Silene alba*
Commelinaceae	*Commelina communis* *
Cucurbitaceae	*Cucurbita maxima*
Ericaceae	*Rhododendron brachycarpum*
Fabaceae	*Trifolium pretense*
Fabaceae	*Trifolium repens*
Lamiaceae	*Lavandula* sp.
Lamiaceae	*Nepeta cataria*
Liliaceae	*Hosta montana* *
Liliaceae	*Lilium auratum*
Malvaceae	*Althaea rosea*
Onagraceae	*Oenothera erythrosepala*
Plantaginaceae	*Antirrhium majus*
Ranunculaceae	*Anemone hupehensis* var. *japonica*
Rosaceae	*Fragaria × ananassa*
Rosaceae	*Sorbaria sorbifolia*
Rosaceae	*Spiraea salicifolia* *

* Native species.

The investigation period began on June 15 and ended on November 7, 2010. The investigation was conducted one to five days per week. Each day, two observation sessions were conducted; one in the morning (from 09:00 to 12:00) and one in the afternoon (from 15:00 to 18:00). During each session, all bumblebee individuals coming to forage in the area were counted. All of the individuals carrying pollen grains were caught as soon as they appeared, and they were anesthetized using carbon dioxide gas. During this anesthetization, one pollen load in a corbicula of each individual was collected. Pollen grains of every observed blooming plant species were also collected directly from the plants during the investigation period within a radius of approximately 1 km around the area for use as reference specimens (Table 1). Only the workers caught in July and August were used for the pollen analysis because all three bumblebees appeared only during these two months.

After collecting pollen samples, two types of slide-mounted pollen specimens were prepared, namely, slide specimens of bumblebee-carried pollen (bumblebee slides) and those of flower-derived pollen (flower slides). First, each pollen sample was softened in 100% acetic acid for one night. After removing the acetic acid, the pollen samples were dissolved in acetolysis solution (sulfuric acid: acetic anhydride = 1: 9) for approximately 3 minutes at 100ºC and rinsed twice with distilled water. The samples were sequentially stored in several solutions in the following order: 70%, 80%, 90%, 95%, 99.5% and 100% ethyl alcohol, a mixture (benzene: ethyl alcohol = 1: 1) and 100% benzene. Subsequently, the samples were treated with silicon oil for approximately 20 hours at 70°C. A portion of each sample was randomly placed onto each microscope slide so that more than 50 pollen grains were mounted, although some specimens contain fewer than 50 pollen grains. The pollen grains were sealed in paraffin wax, and slide-mounted specimens were prepared. All of the pollen grains on the bumblebee slides were observed under a microscope, and almost all pollen grains were identified at the species level (some of the grains were identified at the genus level) by comparing them with the flower slides containing previously identified pollen species. The number of pollen grains was also counted, and the proportions of plant taxa were then calculated.

The difference in the number of observed workers amongthe three bumblebee species was examined using a Kruskal-Wallis test. A three-sample chi-square test was conducted (according to the number of days when the workers of each bumblebee species were observed) to determine the differences in the durations of appearance among the bumblebee species as a whole. For further examination, a two-sample Kolmogorov-Smirnov test based on the average number of observed workers per day was applied three times (for every pair of species), and the results were revised using the Bonferroni correction. The difference in the diversity of plant species among the bumblebee species were analyzed by one-way ANOVA. The bumblebee species was utilized as an independent variable, whereas the number of pollen species utilized by each worker and the Simpson's Diversity Index of plant species diversity carried by each worker were applied as dependent variables. To clarify the differences in the pattern of pollen resource use among the bumblebee species, cluster analysis was conducted according to Ward's method. Dissimilarities among the workers were examined with the squared Euclidean distance based on the pollen compositions of each worker, and the pollen species representing each cluster were then determined by averaging the pollen grain compositions of the workers.

Results

During this period, 169 workers of *B. terrestris* came to forage, whereas the workers of *B. pseudobaicalensis* and *B. hypocrita sapporoensis* were less numerous; there were 33 *B. pseudobaicalensis* workers and 18 *B. hypocrita sapporoensis* workers (Fig. 1). The number of observed workers were, however, not significantly different among the three bumblebee species (Kruskal-Wallis test, H = 0.37, p = 0.83). The number of days when the *B. terrestris* workers were observed (50 days) was more than three times that of the two native bumblebee species *B. pseudobaicalensis* (13 days) and *B. hypocrita sapporoensis* (10 days; Fig. 1), and the result of the chi-square test was significant (X^2_{cal} = 40.79, p < 0.001). The number of observed *B. terrestris* workers gradually decreased until September (like the two other species), but the number again increased from mid-September. *Bombus terrestris* continued to forage as late as the end of November, whereas the two native bumblebee species disappeared as early as the end of August (Fig. 1). Thus, *B. terrestris* showed the longest duration of foraging activity, due to its earlier appearance and remarkably late disappearance. The result of the Kolmogorov-Smirnov test revealed that there was no significant difference between *B. terrestris* and *B. hypocrita sapporoensis* (X_0^2 = 3.12, p = 0.42) or between *B. pseudobaicalensis* and *B. hypocrita sapporoensis* (X_0^2 = 5.89, p = 0.10). However, a significant difference was detected between *B. terrestris* and *B. pseudobaicalensis* (X_0^2 = 9.84, p = 0.01) (p = 0.04 after Bonferroni correction). One queen of *B. terrestris* and one worker of *B. diversus* subsp. *tersatus* were also observed during the investigation period.

In July and August, 65 workers of *B. terrestris*, 20 workers of *B. pseudobaicalensis* and 18 workers of *B. hypocrita sapporoensis* were observed (Fig. 1), and the workers caught in these two months were used for the pollen analysis, including 39 workers of *B. terrestris*, 11 workers of *B. pseudobaicalensis* and 10 workers of *B. hypocrita sapporoensis* (Tables 2–4). The results of pollen analysis showed remarkable differences in the number of pollen resources used among the three bumblebee species. *Bombus terrestris* used the pollen of 17 plant species in total, whereas *B. pseudobaicalensis* and *B. hypocrita sapporoensis* foraged from 10 and six plant species, respectively (Tables 2–4). Thus, *B. terrestris* exhibited the largest foraging range among the three bumblebee species. The results of one-way ANOVA revealed that the number of pollen species and the Simpson's Diversity Index of each worker were significantly influenced by the bumblebee species ($F = 3.20$, $p < 0.05$ and $F = 7.62$, $p < 0.05$, respectively). Cluster analysis based on the pollen composition showed that bumblebee workers could be divided into six clusters, i.e., clusters A–F (Fig. 2). The analysis showed that cluster A was characterized by *Trifolium* sp., cluster B by *Echinacea purpurea*, cluster C by *Antirrhium majus*, cluster D by *Althaea rosea*, cluster E by *Symphytum officinale* and cluster F by *Lavandula* sp. and other minor plants (Table 5). The results of cluster analysis revealed that there were different tendencies among the three bumblebee species in terms of cluster composition. The composition of *B. terrestris* included all clusters, while only clusters A and F appeared for *B. pseudobaicalensis* and *B. hypocrita sapporoensis*. In *B. terrestris*, five clusters appeared in July, but only cluster B, which was not observed in the compositions of the two native bumblebees, appeared in late August. For *B. pseudobaicalensis*, cluster A was mainly observed, and one worker belonging to cluster F appeared only in early July. In the case of *B. hypocrita sapporoensis*, by contrast, two workers of cluster A appeared only in early July, after which cluster F was mainly observed (Fig. 3).

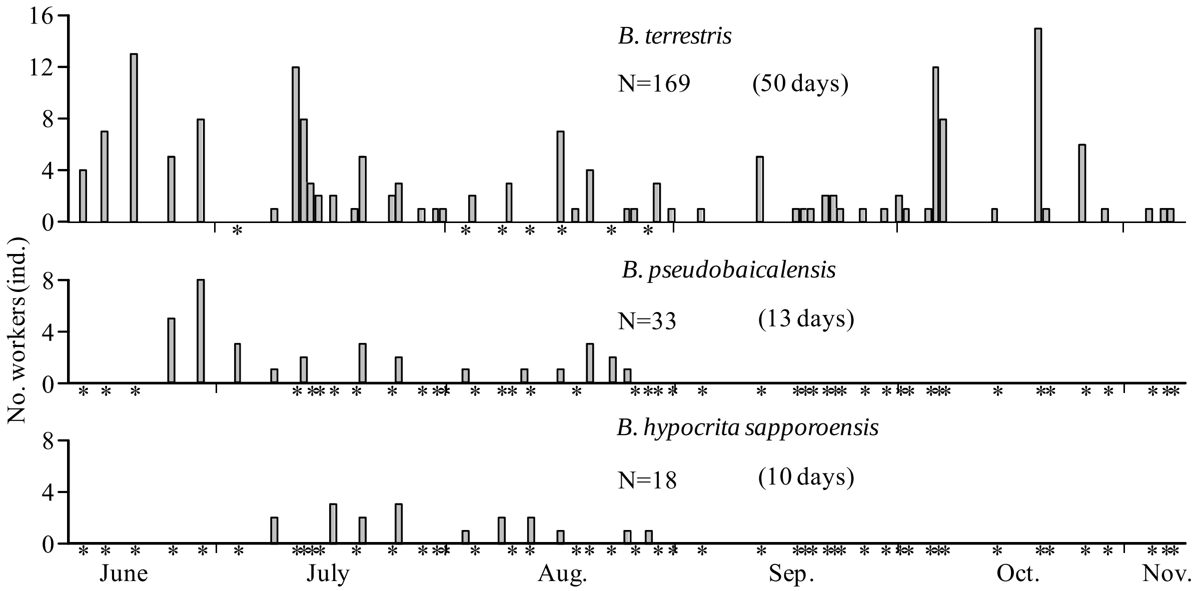

Fig. 1. Seasonal changes in the numbers of workers of each species during the investigation period.
The number of observed workers is indicated, and the days of appearance are shown in parentheses.
* No workers observed.

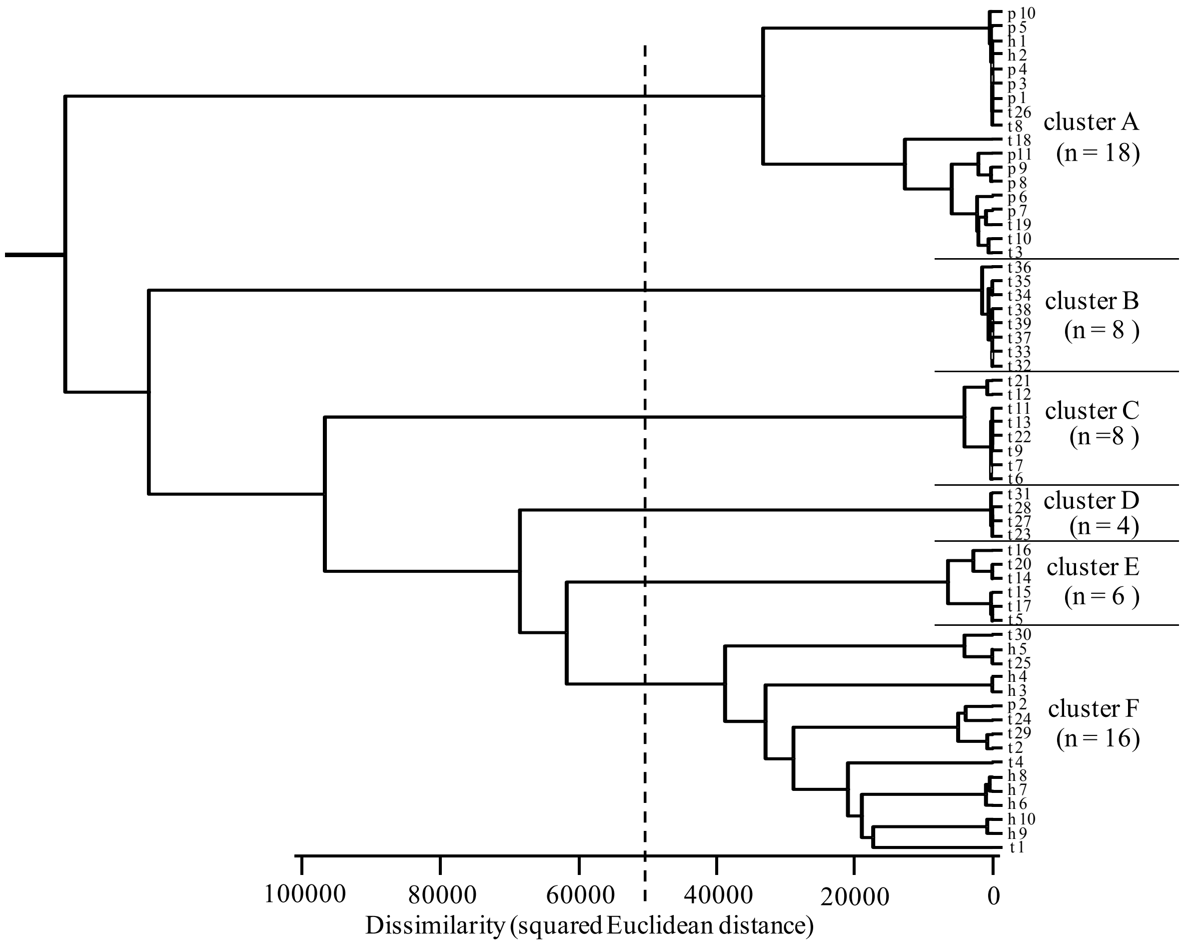

Fig. 2. Results of cluster analysis based on the composition of pollen carried by each bumblebee worker. Worker denominations are the same as Tables 2–4. The numbers of workers belonging to each cluster are shown in parentheses.

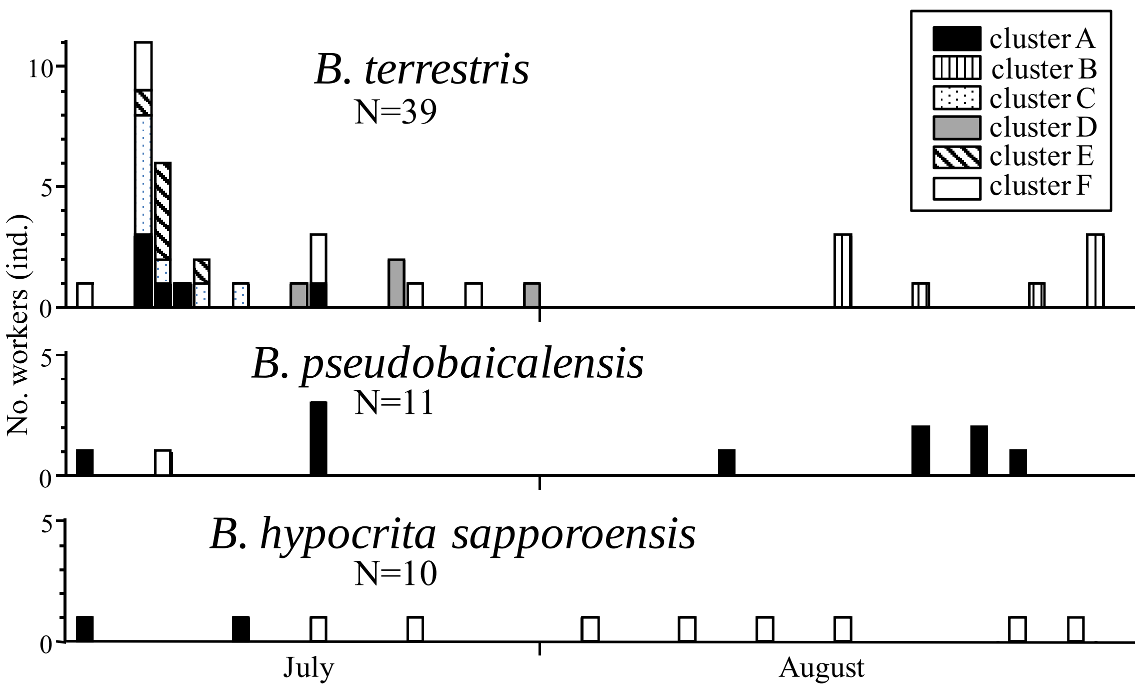

Fig. 3. Seasonal changes in cluster composition of workers of each species.

Table 2. Composition of pollen collected from *B. terrestris* workers
(N=39, foraged from 17 plant species)

Plant species	Pollen composition (%)*																	
	July																	
	8	11	11	11	11	11	11	11	11	11	11	11	12	12	12	12	12	12
	t1	t2	t3	t4	t5	t6	t7	t8	t9	t10	t11	t12	t13	t14	t15	t16	t17	t18
Petroselinum neapolitanum	0	0	53.1	0	0	0	0	0	0	34.0	0	0	5.0	0	0	0	0	87.0
Anthemis nobilis	85.4	0	0	0	0	0	0	0	0	2.1	0	0	0	0	0	0	0	0
Chrysanthemum × morifolium	0	1.9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Echinacea purpurea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Erigeron annuus	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tegates sp.	0	0	0	0	0	0	0	0	0	0	6.2	0	0	0	0	0	0	0
Symphytum officinale	0	0	0	0	100	11.6	1.2	0	1.3	0	0	0	0	60.0	91.8	54.8	100	0
Silene alba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cucurbita maxima	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	35.7	0	0
Rhododendron brachycarpum	0	0	0	0	0	0	1.2	0	0	0	0	0	0	0	0	0	0	0
Trifolium sp.	0	0	46.9	0	0	0	10.6	100	0	63.8	0	15.4	0	25.0	0	0	0	0
Lavandula sp.	0	77.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nepeta cataria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hosta sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Althaea rosea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oenothera erythrosepala	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Antirrhium majus	14.6	3.6	0	0	0	88.4	87.1	0	96.0	0	93.8	61.5	95.0	0	0	0	0	0
Anemone hupehensis var. *japonica*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unidentified	0	17.3	0	0	0	0	0	0	2.7	0	0	23.1	0	15.0	8.2	9.5	0	13.0
	(50)	(151)	(147)	(**)	(**)	(129)	(85)	(**)	(75)	(94)	(65)	(195)	(119)	(180)	(98)	(126)	(**)	(23)

The number of analyzed pollen grains is shown in parentheses.
* t1–39: Worker's denominations.
** Pollen grains of only one plant species were observed.

													August							
13	14	14	16	19	20	20	20	24	24	25	28	31	16	16	16	20	26	29	29	29
t19	t20	t21	t22	t23	t24	t25	t26	t27	t28	t29	t30	t31	t32	t33	t34	t35	t36	t37	t38	t39
10.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	93.4	96.6	89.8	84.0	70.3	98.6	98.2	100
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21.1	58.1	2.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4.3	0	0	0	1.8	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
63.2	34.8	30.6	0	0	0	0	100	0	0	0	40.0	0	0	0	0	1.2	0	0	0	0
0	0	0	0	0	54.5	0	0	0	0	100	0	0	0	0	0	0	17.1	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.8	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.2	0	0	0
0	0	0	0	100	0	0	0	100	100	0	0	89.6	0	0	5.9	11.1	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.2	0	0	0	0
0	0	66.7	97.5	0	45.5	0	0	0	0	0	0	10.5	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.5	0	0	0	0
5.3	7.1	0	2.5	0	0	100	0	0	0	0	60.0	0	6.6	3.4	0	0	5.7	1.4	0	0
(171)	(155)	(108)	(120)	(**)	(99)	(**)	(**)	(**)	(**)	(**)	(240)	(134)	(183)	(29)	(254)	(81)	(158)	(74)	(165)	(**)

Table 3. Composition of pollen collected from *B. pseudobaicalensis* workers (N=11, foraged from 10 plant species)

Plant species	Pollen composition (%)*										
	July					August					
	8	20	25	25	25	12	20	20	21	23	23
	p1	p2	p3	p4	p5	p6	p7	p8	p9	p10	p11
Petroselinum neapolitanum	0	34.1	0	0	0	31.0	19.0	0	0	0	0
Anthemis nobilis	0	0	0	0	0	0	0	0	0	0	0
Chrysanthemum × morifolium	0	0	0	0	0	0	0	0	0	0	0
Echinacea purpurea	0	0	0	0	0	14.3	16.5	0	0	0	0
Erigeron annuus	0	0	0	0	0	0	0	0	0	0	0
Tegates sp.	0	0	0	0	0	0	0	0	0	0	0
Symphytum officinale	0	0	0	0	0	0	0	0	0	0	0
Silene alba	0	0	0	0	0	0	0	0	0	0	15.6
Cucurbita maxima	0	0	0	0	0	9.5	2.5	16.1	14.3	0	0
Rhododendron brachycarpum	0	0	0	0	0	0	0	0	0	0	0
Trifolium sp.	100	0	100	100	91.8	26.2	53.2	40.3	54.0	85.4	37.8
Lavandula sp.	0	42.9	0	0	0	0	0	0	0	0	0
Nepeta cataria	0	0	0	0	0	0	0	0	0	0	0
Hosta sp.	0	0	0	0	0	0	0	0	0	0	0
Althaea rosea	0	23.8	0	0	8.2	0	0	0	0	0	13.3
Oenothera erythrosepala	0	0	0	0	0	19.0	6.3	0	0	0	0
Antirrhium majus	0	0	0	0	0	0	0	29.0	20.6	5.2	6.7
Anemone hupehensis var. *japonica*	0	0	0	0	0	0	0	0	0	0	20.0
Unidentified	0	0	0	0	0	0	2.5	14.5	11.1	9.4	6.7
	(**)	(91)	(**)	(**)	(49)	(84)	(79)	(62)	(63)	(96)	(45)

The number of analyzed pollen grains is shown in parentheses.
* p1–11: Worker's denominations.
** Pollen grains of only one plant species were observed.

Table 4. Composition of pollen collected from *B. hypocrita sapporoensis* workers (N=10, foraged from 6 plant species)

Plant species	Pollen composition (%)*									
	July				August					
	16	20	22	25	3	8	12	16	25	28
	h1	h2	h3	h4	h5	h6	h7	h8	h9	h10
Petroselinum neapolitanum	0	0	0	0	0	0	0	0	0	0
Anthemis nobilis	0	0	0	0	0	0	0	0	0	0
Chrysanthemum × morifolium	0	0	0	0	0	0	0	0	0	0
Echinacea purpurea	0	0	0	0	0	0	0	0	0	0
Erigeron annuus	0	0	0	0	0	0	0	0	0	0
Tegates sp.	0	0	0	0	0	0	0	0	0	0
Symphytum officinale	0	0	0	0	0	0	0	0	0	0
Silene alba	0	0	0	0	0	0	0	0	0	0
Cucurbita maxima	0	0	0	0	2.9	9.1	7.1	21.8	77.9	64.1
Rhododendron brachycarpum	0	0	85.2	94.1	0	0	0	0	0	0
Trifolium sp.	95.6	97.8	0	0	0	0	0	0	0	0
Lavandula sp.	0	0	0	0	0	11.4	28.6	11.5	0	0
Nepeta cataria	0	0	0	0	0	0	0	0	0	0
Hosta sp.	0	0	0	0	0	0	0	0	0	0
Althaea rosea	0	2.2	11.5	5.9	0	0	0	0	3.9	25.6
Oenothera erythrosepala	0	0	0	0	0	0	0	0	0	0
Antirrhium majus	0	0	0	0	0	0	0	0	0	0
Anemone hupehensis var. *japonica*	0	0	0	0	0	56.8	64.3	66.7	0	0
Unidentified	4.4	0	3.3	0	97.13	22.7	0	0	18.2	10.3
	(113)	(136)	(61)	(51)	(104)	(44)	(14)	(87)	(77)	(39)

The number of analyzed pollen grains is shown in parentheses.
* h1–10: Worker's denominations.

Table 5. Plant species representing each cluster

Pollen species	Pollen grain composition (%)*					
	A (n=18)	B (n=8)	C (n=8)	D (n=4)	E (n=6)	F (n=16)
Petroselinum neapolitanum	13.0	0	0.6	2.0	0	1.9
Echinacea purpurea	1.7	**91.1**	0	13.3	0	0
Symphytum officinale	1.2	0	2.1	0.5	**77.2**	0
Cucurbita maxima	2.4	1.0	0	0.5	6.0	11.8
Rhododendron brachycarpum	0	0	0.1	0	0	11.2
Trifolium sp.	**69.6**	0.2	7.1	11.0	9.9	2.5
Lavandula sp.	0	2.1	0	0.3	0	**20.2**
Althaea rosea	1.3	2.1	0	**56.0**	0	5.0
Antirrhium majus	3.5	0	**86.1**	14.4	0	4.0
Anemone hupehensis var. *japonica*	1.1	0.3	0	0.2	0	11.7
Others	6.2	3.2	4.3	2.0	6.9	**31.9**
Total	100	100	100	100	100	100

The number of workers belonging to each cluster is shown in parentheses.
*A–F: Cluster name.

Discussion

In this study, *Bombus terrestris* exhibited a relatively long duration of appearance and a large number of foraging workers (Fig. 1), which suggests that this species has become the dominant species of the bumblebee community in the investigation area. However, since the number of workers is influenced by colony size and the distance of a colony from the investigation area, the results should be interpreted carefully. The days of appearance were significantly different among the three species. Considering this fact and the results of the Kolmogorov-Smirnov test, the duration of foraging activity of *B. terrestris* may have been longer than that of the other two species. This suggestion is in line with the results of Inoue *et al.* (2008).

The results of ANOVA suggest that the number of pollen species used by workers was different among the three bumblebee species. The difference in the cluster compositions among the three bumblebee species suggests that the patterns of pollen resource use were different among the species (Fig. 3). This difference may have resulted from differences in flower preference. In July, clusters A and F appeared in the panels of all bumblebee species, which indicates that the pollen resources overlapped among the three species during this period. However, the cluster compositions differed among the species in August. The three bumblebee species may have used different pollen resources as a result of interspecific competition. Some studies have suggested that *B. terrestris* competes for flower resources with *B. pseudobaicalensis* and *B. hypocrita sapporoensis* (Nagamitsu *et al.* 2007b, Inoue *et al.* 2008, Nagamitsu *et al.* 2010). Considering the overlap in pollen usage in July and the fact that *B. terrestris* exhibited a relatively long duration of foraging activity and a large foraging range, the competition with *B. terrestris* may decrease the abundance of native bumblebees if pollen resources are limited. The patterns of pollen resource use are also influenced by the diversity of flowering plants. For example, *Echinacea purpurea*, which represents cluster B (Table 5), began to bloom in August and may have caused the appearance of cluster B in the case of *B. terrestris*. Therefore, further studies should also consider changes in plant diversity to help elucidate the competition for plant resources between exotic and native bumblebees. This study was performed in an environment where exotic plants are dominant. Future studies should be conducted in the environments where exotic and native plants coexist. In the present study, the patterns of pollen resource use were successfully evaluated by analyzing the pollen composition. However, pollen resources must also be quantified. As the nectar of flowering plants is an important resource for bumblebees, studies concerning nectar are needed to evaluate the ecological interactions concerning plant resources among bumblebee species.

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