

Foraging habitats and floral resource use by colonies of long- and short-tongued bumble bee species in an agricultural landscape with kabocha squash fields

Teruyoshi Nagamitsu · Sa-aya Tsukuba ·
Fumi Ushirokita · Yasuo Konno

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Abstract Bumble bees pollinate and forage on flowers of crop and wild plants in agricultural landscapes. These interactions may depend on landscape patterns and bumble bee traits. We studied the abundance, colony density, and foraging range in long-tongued *Bombus diversus* Smith and short-tongued *B. hypocrita* Pérez, and evaluated their visits to flowers of wild plants and cultivated kabocha squash (*Cucurbita maxima* Duchesne). In forests in a farmland, *B. hypocrita* workers were trapped more frequently in the canopy. Full-sibs determined by nuclear microsatellite genotypes among workers collected in the farmland showed higher colony density and a larger foraging radius in *B. hypocrita* (30.8 km⁻² and 848 m) than in *B. diversus* (8.3 km⁻² and 723 m), respectively. Regarding wild plants, workers more frequently visited shallow flowers in *B. hypocrita* and deep flowers in *B. diversus*. These results suggest that bumble bees with different traits forage on different wild flowers in different habitats. Squash flowers were visited by both bumble bee species at similar frequency in the latter period of colony growth when males and new queens appeared. Composition of full-sib workers visiting squash and wild flowers did not depend on the number of collected workers of individual colonies, indicating that foraging on squash flowers was not associated with colony growth. Thus, growth and reproduction of bumble bee colonies may be supported by various wild plants and cultivated squash, respectively.

Keywords *Bombus diversus* · *Bombus hypocrita* · Colony density · *Cucurbita maxima* · Foraging range

Introduction

Pollination services from wild animals are critical to both wild plants and agricultural crops (Burd 1994; Klein et al. 2007). There is growing concern of a decline in the abundance and diversity of pollinators, probably due to human disturbance and habitat fragmentation (Winfrey et al. 2009). The reduced pollination services in small populations of wild plants in fragmented habitats often result in low reproductive success (Aguilar et al. 2006; Dauber et al. 2010). Agricultural intensification is likely to jeopardize wild pollinators and their subsidizing effect on pollination services (Klein et al. 2007). The need to reduce the impacts of modern agriculture on biodiversity in rural landscapes has been recognized in Europe (Bignal 1998). Recently, the Environmental Stewardship Scheme, in which farmers are paid to manage their land for the benefit of pollinators, has been adopted in England (Butler et al. 2007), and is expected to promote the creation of suitable habitats for pollinating insects. In Japan, traditional management of farms and secondary forests has been evaluated as reservation of biodiversity and pollination service (Putra and Nakamura 2009).

In agricultural landscapes, semi-natural habitats, such as non-cropped field margins, can provide floral and nesting resources for bumble bees (Kells et al. 2001), which are important pollinators for both wild plants and agricultural crops (Goulson 2003). To supply the resources for bumble bees, a seed mixture of flowering plants was sown on the field margins, which increased the abundance of foraging bumble bees (Carvell et al. 2004). The difference in the

T. Nagamitsu (✉)
Forestry and Forest Products Research Institute (FFPRI),
Tsukuba 305-8687, Japan
e-mail: nagamit@ffpri.affrc.go.jp

S. Tsukuba · F. Ushirokita · Y. Konno
Obihiro University of Agriculture and Veterinary Medicine,
Obihiro 080-8555, Japan

treatments of the field margins (Pywell et al. 2005) and the compositions of the seed mixtures (Pywell et al. 2006) led to different effects on the bumble bee abundance (Carvell et al. 2007). The landscape structure and farming practices also affected the abundance and diversity of foraging bumble bees (Heard et al. 2007; Rundlof et al. 2008).

In contrast to the emphasis of semi-natural habitats for resource use by bumble bees, the availability of mass flowering crops had substantial effects on bumble bee density (Westphal et al. 2003). Floral resources of mass flowering crops positively affected the abundance of bumble bees, probably because the size of their colonies increased (Herrmann et al. 2007). Furthermore, mass flowering of oil seed rape improved the gain of the nest weight (Westphal et al. 2009). However, farming schemes deployed to enhance farmland biodiversity had little effect on colony growth (Goulson et al. 2002).

Both agricultural crops in arable fields and wild plants in semi-natural habitats within foraging ranges could supply floral resources to bumble bees and increase the local density of their colonies (Knight et al. 2009). Floral resource distribution usually differs between agricultural crops and wild plants: flowers of diverse wild plants seem to be temporally stable and spatially scattered, whereas a monoculture of agricultural crops has the opposite pattern. Such resource heterogeneity in seasonal and landscape scales offers indirect benefits between wild plants and agricultural crops mediated by bumble bees: mass flowering crops in arable fields are pollinated by bumble bees, which are supported by stably flowering wild plants in semi-natural habitats, and vice versa (Keitt 2009). These interactions depend on the temporal and spatial patterns of resource use by various bumble bee species, which have different traits in foraging behavior and feeding morphology (Goulson 2003).

In this study, to reveal the foraging of pollinators on wild and crop plants in rural landscapes, two bumble bee species with different traits were investigated in an agricultural area including kabocha squash fields, where pollination services are required for fruit production (Julier and Roulston 2009). First, the abundance and seasonality were observed using window traps, which are useful to monitor pollinators in farmlands (Nagamitsu et al. 2007; Howlett et al. 2009), to describe the colony life cycles. Next, foraging workers were collected at wild and crop flowers to record their flower visitation, and full-sibs among the foraging workers were identified by nuclear microsatellite genotypes to estimate the colony density and foraging range (Darvill et al. 2004; Nagamitsu and Yamagishi 2009). Finally, flower visitation by workers of individual colonies was examined to evaluate effects of floral resource use on colony growth.

Materials and methods

Study area

An area of 6 km² with sides 2 and 3 km long was located in a farmland in the Tokachi plain, Hokkaido, Japan (42°53'N, 143°8'E, altitude 90 m; Fig. 1a). In this area, two rivers run northwards, and there were a forest belt and fragmented forests along the rivers (Fig. 1a). Most of the area was covered with arable fields of potato, wheat, maize,

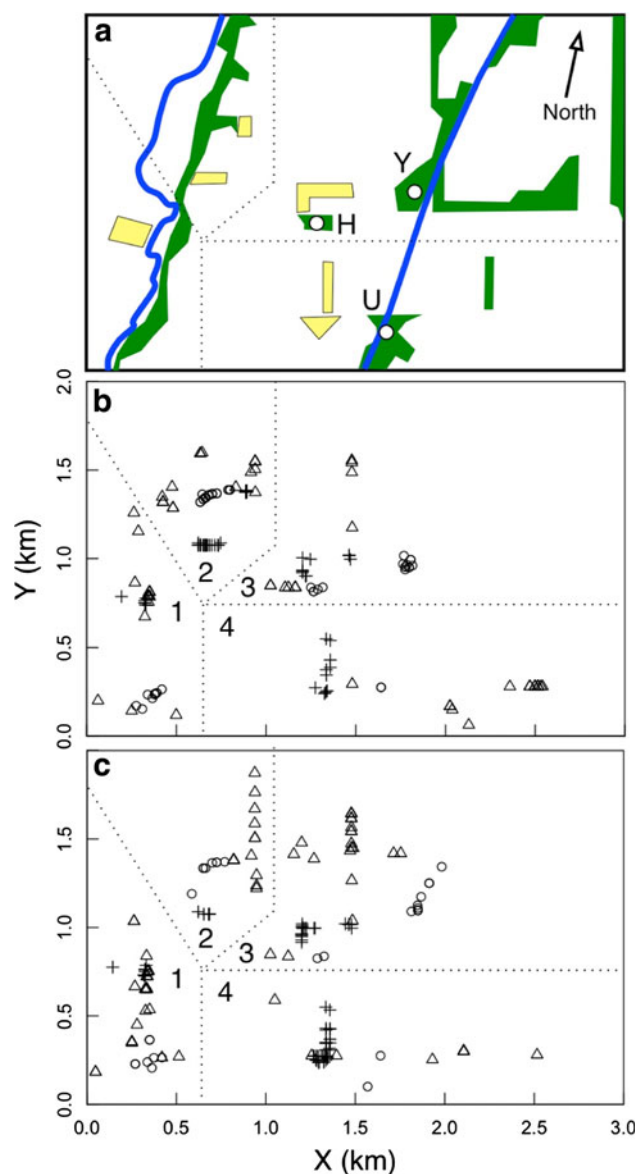


Fig. 1 Study area (a): blue lines, yellow areas, and green areas indicate rivers, squash fields, and forests, respectively; open circles with H, U, and Y letters indicate window trap sites; dotted lines are borders separating the study area into four areas, and locations of collected foragers of *Bombus diversus* (b) and *B. hypocrita* workers (c): crosses, triangles, and circles indicate collected locations at farms, field margins, and forests, respectively

and sugar beet. Among such crops, squash (*Cucurbita maxima* Duchesne) flowers were frequently visited by bumble bees. There were four patches of squash fields, and around them, four areas including field margins and forests were separated with regard to spatial heterogeneity in the study area (Fig. 1a). There were some residential areas where horticultural plants were cultivated.

Window traps

In the study area, three sites in the forest fragments surrounded by farms were selected for trapping bumble bees to monitor their abundance in the farmland (Fig. 1a). The forests were dominated by *Ulmus davidiana* Planch., *Alnus japonica* (Thunb.) Steud., and *Fraxinus mandshurica* Rupr. Site H was located in an isolated forest surrounded by arable fields, and sites Y and U were in fragmented forests along one of the rivers (Fig. 1a). In each site, two sets of two window traps, one in the canopy (7.5–13.7 m height above the ground) and the other in the understory (0.9–1.3 m), were located 10–20 m from each other. The trap design and trapping procedure were described by Nagamitsu et al. (2007). Trapping was conducted for 19 weeks from 5 June to 16 October 2008 and for 21 weeks from 23 April to 17 September 2009.

Bumble bees collected from these traps every week were preserved in 70 % ethanol and identified by species and sex. The head width of trapped bees was measured using a digital caliper (CD-20, Mitutoyo Corporation, Kawasaki). On the basis of a gap in the head width, trapped females were discriminated into queens and workers.

Sampling foraging workers

Foraging workers of two abundant bumble bee species, *Bombus diversus tersatus* Smith and *B. hypocrita sapporoensis* Cockerell, were collected using hand nets in the study area. The two species use similar nest sites, such as underground abandoned rodent nests (Sakagami and Katayama 1977), but have tongues (glossa and prementum) of different lengths (12.0 mm in *B. diversus* and 8.1 mm in *B. hypocrita* on average in Hokkaido) (Inoue et al. 2007; Nagamitsu et al. 2007).

When foraging workers were collected, the locations and plant species of visited flowers were recorded to evaluate the foraging habitats and plants. The locations of visited flowers were categorized into three types: Farm, including squash fields; Margin, including semi-natural habitats, such as meadows between arable fields and irrigation channels, wind-break trees, or roads; and Forest, including a forest belt along the rivers and forest patches surrounded by arable fields. The plant species of visited flowers were classified into three types: Crop, cultivated

squash; Wild S, wild plants with shallow (cup- or dish-shaped) flowers with nearly exposed nectar; and Wild D, wild plants with deep (bilateral or tubular) flowers with nectar concealed by petals.

Collection was conducted from 0700 to 1600 hours for 46 days, when weather conditions were suitable for foraging of bumble bees, from 27 June to 13 September 2008. Foraging workers were not collected in 2009 when the abundance of *B. diversus* was low. To incorporate temporal heterogeneity in the flowering phenology of wild plants into the following data analysis, the duration of collection in 2008 was divided into period 1 (from 27 June to 10 August), period 2 (from 11 to 19 August), and period 3 (from 21 August to 13 September) of squash flowering, in which the number of collected workers was similar. Collected workers were preserved in 99 % ethanol at -20°C .

Genotyping nuclear microsatellites

Total DNA was extracted from flight muscle in the thorax of preserved workers using DNeasy Tissue Mini Kit (Qiagen, Hilden). The genotypes of nuclear microsatellites were determined at nine loci: B11, B124, and B126 (Estoup et al. 1995); B100 and B132 (Estoup et al. 1996); BT02, BT08, BT10, and BT26 (Funk et al. 2006) in *B. diversus* and at 11 loci: B10, B11, B124, and B126 (Estoup et al. 1995); B96, B100, and B132 (Estoup et al. 1996); BT01, BT10, BT22, and BT26 (Funk et al. 2006) in *B. hypocrita*. Multiplex polymerase chain reaction (PCR) was performed for each set of three or four of the loci in 12 μl of a mixture containing 6 μl of Qiagen Multiplex PCR Master Mix (Qiagen), 0.2 μM of each primer, and 1 μl of the template DNA solution using a GeneAmp 9700 thermal cycler (Applied Biosystems, Foster City, USA) programmed for 15 min at 95°C , followed by 21–27 cycles, which were optimized to each primer set, consisting of 30 s at 94°C , 90 s at 60°C , and 60 s at 72°C , and finished by 30 min at 60°C . The size of the PCR product was measured using an ABI PRISM 3100-Avant Genetic Analyzer with POP4 in 36-cm capillaries and GeneScan Analysis software (Applied Biosystems, Foster City, USA).

Data analysis

Whether the number of workers collected each year from each trap differed between years (2008 and 2009), among sites (H, Y, and U), and between heights (canopy and understory) was examined for each bumble bee species using a generalized linear mixed model (GLMM). The GLMM included a response variable following a Poisson distribution and a linear predictor consisting of fixed effects of the three factors and random effects of the 12 traps with a logarithmic link function. Differences in the

proportion of *B. diversus* foragers in collected workers among the three habitat types (Farm, Margin, and Forest) or among the three plant types (Crop, Wild S, and Wild D) were examined using a GLMM. The GLMM included a response variable following a binomial distribution and a linear predictor consisting of a fixed effect of one of the two factors and random effects of 12 combinations of the four areas and the three periods with a logistic link function. The maximum likelihood estimates of parameters of the fixed effects and statistical significance of their differences on the basis of Wald-*t* statistics were obtained using glmmML in R 2.9.0 (R Development Core Team 2009). Significance levels α were corrected by the Bonferroni method for multiple comparisons.

Full-sib groups among collected workers were estimated from their multilocus genotypes using Colony 2.0 (Wang 2004). The rates of allele dropout and genotyping error were set to 0.0078 and 0.0175, respectively, according to differences in 265 pairs of genotypes obtained from the repetition of genotyping for the same worker. Because a single monoandrous queen usually founds her colony in the subgenera (*Diversobombus* and *Bombus* s. str.) of the studied species (Schmid-Hempel and Schmid-Hempel 2000; Kokuvo et al. 2009), each full-sib group was regarded as a single colony.

The foraging range was assessed on the basis of the frequency distribution of the distance between collected locations of full-sib pairs in each bumble bee species. Whether the frequency distributions differed between the bumble bee species was examined using ks.test (Kolmogorov-Smirnov test) in R 2.9.0.

The number of colonies was estimated assuming that the number of workers collected per colony followed a truncated negative binomial distribution, because the expected number of workers collected per colony was likely to vary among colonies, and because of supposing that colonies from which no worker was collected were never observed (Nagamitsu and Yamagishi 2009). The number of workers per colony follows a negative-binomial distribution:

$$P(x, \pi, n) = \binom{n+x-1}{n-1} \pi^n (1-\pi)^x \quad (1)$$

where $P(x, \pi, n)$ is the probability for the number of workers per colony $x = \{0, 1, 2, 3, \dots\}$ and a real number π between 0 and 1 and a positive integer n are the parameters of the distribution. The likelihood equation was defined as a product of the probability that more than zero workers were collected:

$$L(\pi, n) = \prod_{x=1}^m \left\{ \binom{n+x-1}{n-1} \pi^n (1-\pi)^x / (1-\pi^n) \right\}^{k_x} \quad (2)$$

where k_x is the number of colonies from which $x = \{1, 2, 3, \dots, m\}$ workers were collected. The maximum likelihood

estimates of π and n were obtained according to Eq. 2 using R 2.9.0. The estimated number of colonies was calculated from an equation:

$$N = \sum_{x=1}^m \frac{k_x}{1 - \hat{\pi}^{\hat{n}}} \quad (3)$$

where $\hat{\pi}$ and \hat{n} are the estimates of π and n , respectively.

To evaluate the relationship between crop flower use and colony size, whether the proportion of foragers visiting squash flowers in collected workers differed among colonies from which different numbers of foraging workers were collected was examined using a GLMM. The GLMM for each bumble bee species included a response variable following a binomial distribution and a linear predictor consisting of a fixed effect of the number of collected workers and random effects of individual colonies with a logistic link function. A parameter of the fixed effect was estimated, and its difference from 0 was examined using glmmML in R 2.9.0.

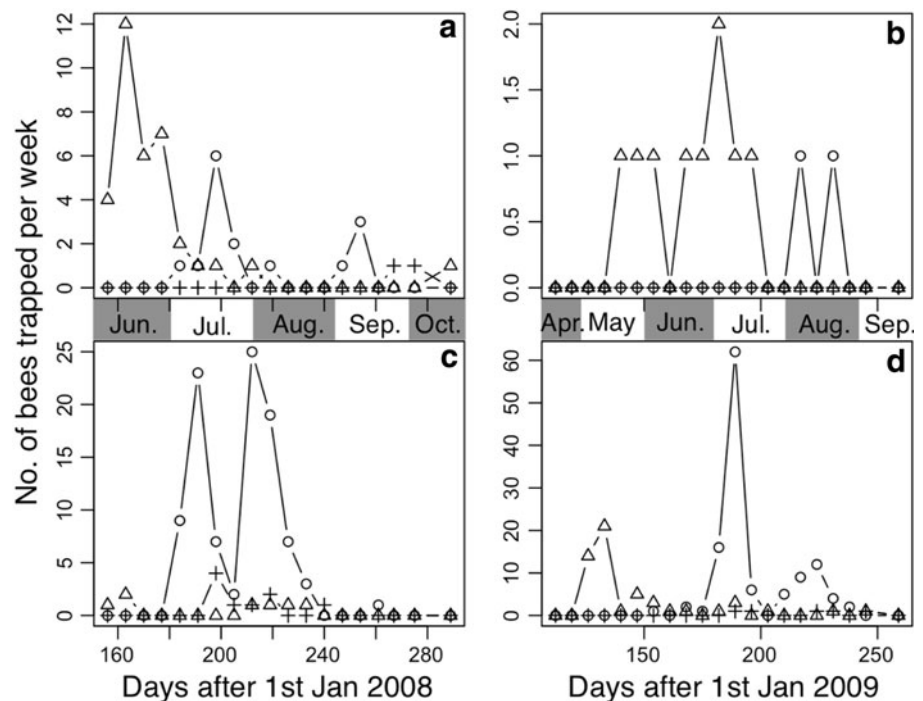
Results

Trapped bumble bees

In total, 407 bumble bees of eight species were trapped in 2008 and 2009. Among these species, *B. hypocrita* was the most abundant (60 queens, 216 workers, and 14 males), followed by *B. diversus* (44, 17, and 2); workers were more abundant than queens in the former, but vice versa in the latter. In addition to the two species, *B. terrestris* L. (6, 17, and 3), *B. ardens sakagami* Tkalcû (3, 5, and 5), *B. pseudobaicalensis* Vogt (3, 0, and 0), *B. hypnorum koropokkrus* Sakagami et Ishikawa (5, 2, and 1), *B. deuteronymus deuteronymus* Schulz (5, 0, and 0), and *B. schrencki* Skorikov (1, 0, and 0) were trapped. In *B. diversus*, overwintering queens were trapped in May, June, and July in both years, workers were trapped frequently in July and September but rarely in August in 2008, and new queens (gynes) and males appeared after September in 2008 (Fig. 2a, b). In *B. hypocrita*, overwintering queens were trapped in May and June, workers were trapped frequently in early July and early August but rarely in late July, and gynes and males appeared after July in both years (Fig. 2c, d).

Significantly more workers of *B. diversus* were trapped in 2008 (1.25 per trap) than in 2009 (0.17, $P = 0.007$), but more *B. hypocrita* workers were trapped in 2009 (10.0) than in 2008 (8.0), although the difference was not significant ($P = 0.103$). Among the three sites (Fig. 1a), similar numbers of workers of both species were trapped, and their differences were not significant

Fig. 2 Seasonal changes in weekly number of trapped bumble bees (*triangles* queen, *circles* worker, and *crosses* male) in 2008 (**a**) and 2009 (**b**) in *Bombus diversus* and in 2008 (**c**) and 2009 (**d**) in *B. hypocrita*



($P > 0.019 > 0.017 =$ significance level α corrected by the Bonferroni method in *B. diversus* and $P > 0.132$ in *B. hypocrita*). More workers of *B. diversus* were trapped in the understory (1.00 per trap) than in the canopy (0.42), although the difference was not significant ($P = 0.100$), but significantly more *B. hypocrita* workers were trapped in the canopy (11.5) than in the understory (6.5, $P < 0.001$).

Foraging workers

In total of 363 foraging workers collected in 2008, 340 workers were collected while visiting flowers of 21 plant species, and 23 workers were collected when they were not visiting any flowers (Table 1). Among the plant species, squash flowers (Crop) were the most frequently visited by the both bumble bee species (Table 1). Among the 20 wild plant species, 12 and 8 species were categorized into plant types with shallow flowers (Wild S) and with deep flowers (Wild D), respectively (Table 1). Among the Wild S type, *Solidago* flowers in the Margin habitat and *Hydrangea* flowers in the Forest habitat were often visited by *B. hypocrita*. Among the Wild D type, *Impatiens* flowers in the Forest habitat and *Trifolium* flowers in the Margin habitat were frequently visited by *B. diversus*. Among the collected workers, 144 workers were collected in field margins (Margin) scattered in the study area, and 131 and 88 workers were collected in several patches of squash fields (Farm) and forests (Forest), respectively (Table 1; Fig. 1b, c).

The proportion of *B. diversus* foragers in collected workers was significantly higher in the Forest habitat than

in the Farm habitat ($P < 0.009 < 0.017 = \alpha$; Fig. 3a), significantly lower in the Wild S flowers than in the Crop flowers ($P < 0.001 < \alpha$; Fig. 3b), and significantly higher in the Wild D flowers than in the Crop flowers ($P < 0.001 < \alpha$; Fig. 3b).

Colonies

Among the 184 and 179 collected foragers of *B. diversus* and *B. hypocrita* workers, 68 and 124 full-sib groups (colonies), respectively, were detected on the basis of their multilocus genotypes (Table 2). Negative binomial distributions with the estimated parameters ($\hat{\pi} = 0.37$, $\hat{n} = 1$ in *B. diversus* and $\hat{\pi} = 0.69$, $\hat{n} = 1$ in *B. hypocrita*) were fit to the frequency distributions of the number of foraging workers per colony. According to the parameters, the estimated numbers of colonies were 107.9 in *B. diversus* and 400.0 in *B. hypocrita* (Table 2).

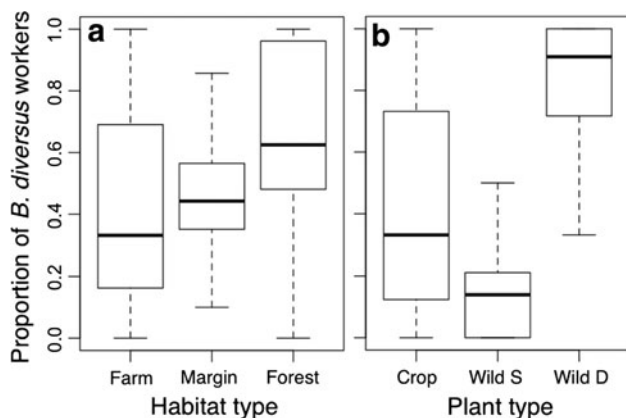
The frequency distributions of the distance between collected locations of full-sib workers were significantly different between the two species ($P < 0.001$; Fig. 4). The median, mean, and maximum distances were 0.272, 0.351, and 1.446 km in *B. diversus* and 0.560, 0.586, and 1.697 km in *B. hypocrita*, respectively.

The temporal distributions of foraging workers collected from individual colonies indicated that foraging of most colonies was active in August and that foragers of some colonies were collected repeatedly after their appearance (Fig. 5). In addition to the active period, foraging workers of *B. hypocrita* were frequently collected in early July and early September (Fig. 5). Visits to squash flowers were

Table 1 Number of foraging workers of *Bombus diversus* and *B. hypocrita* collected at three types of flowers: squash flowers (Crop) and wild plants with shallow flowers (Wild S), and deep

flowers (Wild D) in three types of habitats: squash fields (Farm), field margins (Margin), and forest patches (Forest) in 2008

Plant species	Flower type	<i>B. diversus</i>				<i>B. hypocrita</i>				Total
		Farm	Margin	Forest	Total	Farm	Margin	Forest	Total	
<i>Cucurbita maxima</i>	Crop	55			55	61	1		62	117
<i>Solidago gigantea</i>	Wild S		4	2	6		43	6	49	55
<i>Hydrangea paniculata</i>	Wild S				0		7	11	18	18
<i>Filipendula kamschatica</i>	Wild S				0		3	3	6	6
<i>Chelidonium majus</i>	Wild S			3	3			2	2	5
<i>Filipendula glaberrima</i>	Wild S				0			3	3	3
<i>Solanum nigrum</i>	Wild S	2	1		3				0	3
<i>Oenothera biennis</i>	Wild S		1	1	2	1			1	3
<i>Thalictrum minus</i>	Wild S			2	2				0	2
<i>Spiraea salicifolia</i>	Wild S				0			2	2	2
<i>Persicaria longiseta</i>	Wild S				0		1		1	1
<i>Dactylis glomerata</i>	Wild S				0			1	1	1
<i>Hieracium umbellatum</i>	Wild S		1		1				0	1
<i>Impatiens noli-tangere</i>	Wild D			34	34			2	2	36
<i>Commelina communis</i>	Wild D	1	9	2	12		15		15	27
<i>Trifolium pratense</i>	Wild D		21		21		3		3	24
<i>Symphytum officinale</i>	Wild D		12		12		1		1	13
<i>Lespedeza bicolor</i>	Wild D		8	2	10				0	10
<i>Aconitum sachalinense</i>	Wild D			7	7				0	7
<i>Lupinus polyphyllus</i>	Wild D				0		4		4	4
<i>Vicia amoena</i>	Wild D		2		2				0	2
Flying		8	2	4	14	3	5	1	9	23
Total		66	61	57	184	65	83	31	179	363

**Fig. 3** Box-and-whisker plots of the proportion of *Bombus diversus* foragers in both *B. diversus* and *B. hypocrita* workers collected in three types of habitats (a) and at three types of flowers (b)

observed in August and September, while visits to flowers of wild plants were continuously observed throughout the collection period (Fig. 5).

The proportion of foragers visiting squash flowers in collected workers did not depend on the total number of

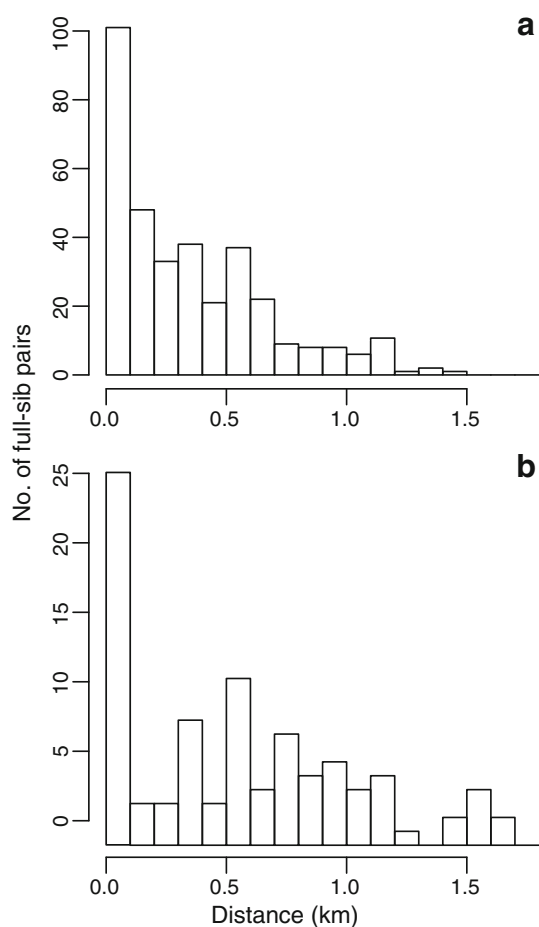
foraging workers collected from individual colonies in *B. diversus* ($P = 0.257$) and in *B. hypocrita* ($P = 0.292$; Fig. 6).

Discussion

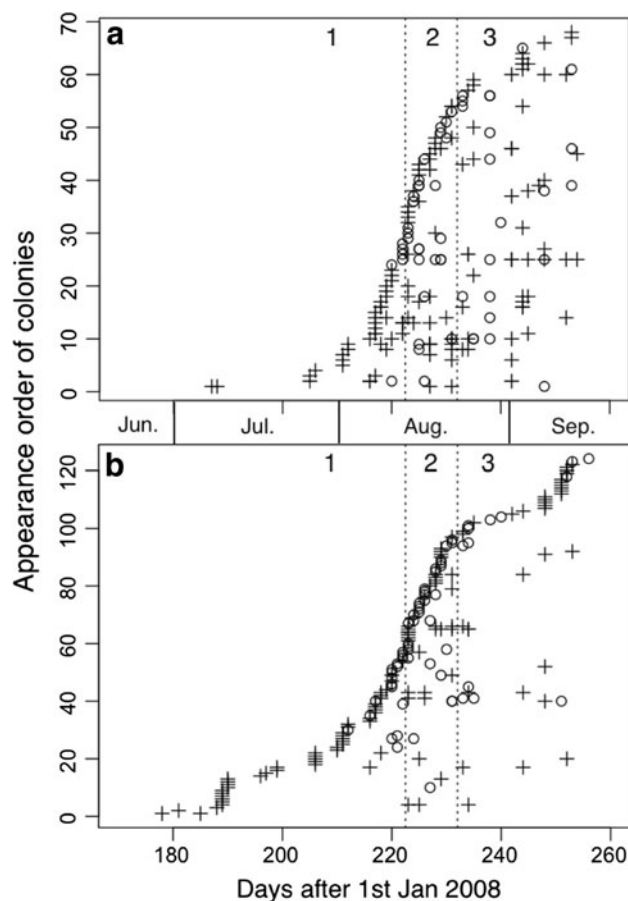
The two studied bumble bee species, long-tongued *B. diversus* and short-tongued *B. hypocrita*, differed in flower visitation as expected: *B. diversus* preferred deep flowers, and *B. hypocrita* preferred shallow flowers among wild plants (Fig. 3b). Coexisting bumble bees with different tongue lengths are known to partition floral resources according to floral morphology (Inouye 1980; Inoue and Kato 1992). The studied bumble bee species are often sympatric and frequently observed to visit flowers with different floral morphologies (Inoue et al. 2007; Ishii et al. 2008). In our study area, wild plants with both deep and shallow flowers were continuously blooming during the study periods as their species composition was changing (Table 1). These findings suggest that diverse wild plant species provide stable floral resources to bumble bee

Table 2 Frequency distributions of the observed and expected number of foraging workers collected per full-sib group in *Bombus diversus* and *B. hypocrita*

No. of full-sib groups	No. of foraging workers collected from each full-sib group															Total
	0	1	2	3	4	5	6	7	8	9	10	11	12	≥13		
<i>B. diversus</i>																
Observed	NA	27	17	7	5	5	3	1	1	0	0	1	0	1	68	
Expected	39.9	25.2	15.9	10.0	6.3	4.0	2.5	1.6	1.0	0.6	0.4	0.2	0.2	0.3	107.9	
<i>B. hypocrita</i>																
Observed	NA	92	21	4	3	3	1	0	0	0	0	0	0	0	124	
Expected	276.0	85.6	26.5	8.2	2.5	0.8	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	400.0	

**Fig. 4** Histograms of the distance between collected locations of full-sib pairs of foraging workers in *Bombus diversus* (a) and *B. hypocrita* (b)

species with different feeding traits. Kabocha squash (*C. maxima*) flowers were visited by both *B. diversus* and *B. hypocrita* workers (Fig. 3b). Resource use from squash flowers was also confirmed by squash pollen stored in *B. hypocrita* nests (Takeuchi et al. 2005). Although squash flowers are categorized into shallow flowers according to their morphology, plenty of nectar and pollen in the large flowers may attract both short- and long-tongued bumble

**Fig. 5** Temporal distributions of foraging workers collected from individual colonies in *Bombus diversus* (a) and *B. hypocrita* (b); circles and crosses indicate foragers visiting crop flowers and other foragers, respectively; dotted lines separate three collection periods

bees. Julier and Roulston (2009) also reported that flowers of *C. pepo* L. were visited by various bees including honey bees, bumble bees, and other solitary bees.

In addition to flower visitation, *B. diversus* and *B. hypocrita* differed in foraging habitats. Based on trapped workers in the canopy and understory layers, *B. hypocrita* preferred the canopy. The collection of foraging workers indicated that the proportion of *B. diversus* foragers in

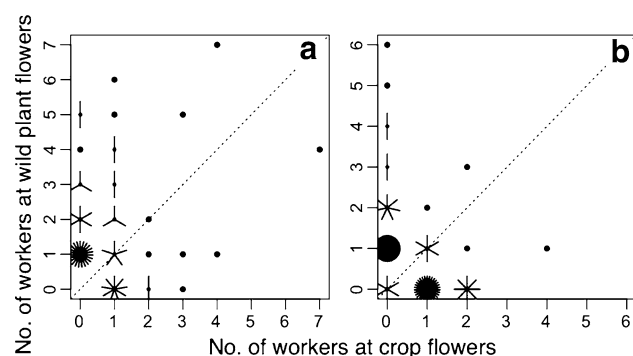


Fig. 6 Scatter plots between the number of foraging workers of individual colonies collected at flowers of wild plants and crops in *Bombus diversus* (a) and *B. hypocrita* (b); the number of bars radiated from points indicates the number of overlapped samples; dotted lines indicate unbiased flower visitation between wild plants and crops

collected workers was higher in forests than in squash fields (Fig. 3a). These results suggest that workers tend to forage in open, sunny habitats in *B. hypocrita* and in shrubby, shaded habitats in *B. diversus*. The two bumble bee species have similar nest sites, such as underground abandoned rodent nests (Sakagami and Katayama 1977), and similar distributional ranges including various vegetations and elevations in Hokkaido (Saito 1995). Thus, the difference in foraging habitats might result from variation in floral resources among the habitats, and the allocation of foragers into the different floral resources could result in different preferences in foraging habitats.

Genetic analysis of foraging workers revealed that *B. hypocrita* had higher colony density (Table 2) and a larger foraging range (Fig. 4) than *B. diversus*. In our study, the area where nests potentially existed was 13 km² within a 1 km radius from collection locations, and thus, the colony density is estimated to be 8.3 km⁻² in *B. diversus* and 30.8 km⁻² in *B. hypocrita*. Such densities are relatively low and average, respectively, in comparison with those of *B. pascuorum* (Scopoli) in farmland in the UK (19.7–51.6 km⁻²) (Knight et al. 2009). The foraging radius, which is half of the maximum distance between locations where full-sib workers were collected, was 723 m in *B. diversus* and 848 m in *B. hypocrita*. Such radii are relatively large in comparison to those of *B. terrestris* (625–758 m), *B. pratorum* (L.) (674 m), *B. lapidarius* (L.) (450 m), and *B. pascosum* (312–449 m) (Darvill et al. 2004; Knight et al. 2005; Nagamitsu and Yamagishi 2009). On the basis of the colony density and foraging range in our study area, most colonies were likely to access the foraging habitats of both crops and wild plants in the agricultural landscape (Fig. 1a).

Seasonal changes in the weekly number of trapped workers were bimodal in *B. hypocrita*, and the two peaks occurred at similar periods in both 2008 and 2009 (Fig. 2c,

d). These peaks in early July and early August were also found in the collection of foraging workers (Fig. 5b), which may reflect the foraging activity of the first and second broods of workers. In *B. diversus* in 2008, workers trapped in forests were abundant in July and September (Fig. 2a), but more foraging workers were collected in August in various habitats including farms and field margins. These results suggest that *B. diversus* workers shift their foraging habitats between forest and open habitats to follow floral resources, which was also observed in an agro-forestry landscape in Japan (Ushimaru et al. 2008). Both *B. diversus* and *B. hypocrita* visited squash flowers in August and September (Fig. 5), when colonies were likely to have grown already and produced males and new queens (Fig. 2). Therefore, floral resources from cultivated squash potentially facilitate the production of reproductive individuals of colonies.

The effects of resource use from crop flowers on colony growth were evaluated by comparing the flower visitation of foraging workers among different colonies. The composition of foraging workers visiting flowers of cultivated squash and wild plants did not depend on the number of workers collected from individual colonies, which might reflect the colony size, in *B. diversus* and *B. hypocrita* (Fig. 6). This result does not support the idea that resource use from crop flowers assists with colony growth. With regard to the later flowering period of cultivated squash, resource use from squash flowers is likely to facilitate the reproduction of mature colonies, which have been grown using floral resources from wild plants blooming in the early period. In another agricultural landscape, crop flowers blooming in the early period of colony growth, such as oil seed rape (*Brassica napus* L.), increased the colony size (Herrmann et al. 2007) and the nest weight (Westphal et al. 2009). Thus, the effects of resource use from mass flowering crops on colony performance are likely to depend on the matching between colony life cycle and flowering phenology of the crops. Both crops and wild plants could increase the colony density owing to improvement in colony growth and/or reproductive output (Knight et al. 2009). Our study demonstrates that cultivated squash attract both long- and short-tongued bumble bees and that they visited wild plant flowers with different morphologies in different habitats. These relationships suggest that floral resources from diverse wild plants in semi-natural habitats may support the growth of bumble bee colonies in agricultural landscapes and provide the pollination services to cultivated crops in arable fields.

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