

CHAPTER 4

Evaluation of the Potential Population Growth of the Introduced *Bombus terrestris* in Northern Japan

Introduction

As the social phase is an important step in determining fitness (Chapter 3), the stages of hibernation and colony initiation are also critical steps during the solitary phase with high probabilities of failure (e.g. Cumber 1953). Under natural conditions, queens hibernate in small cavities (hibernacula) and hibernation usually lasts from 6 to 9 months, depending on the species (Alford 1969, 1975, Photo 4-1). To survive such a long period with a harsh condition, bumblebee queens entering hibernation need considerable fat reserves in their bodies as a source of energy, which are lost more than 10 % in weight during the first period of 100 days of hibernation (Holm 1972). Thus, weight and body size prior to entering hibernation has an important effect on hibernation survival of queens in the laboratory experiments (Holm 1972; Owen 1988; Beekman et al. 1998). Furthermore, one of the major factors in regulation body temperature is body size, because convective heat loss is proportional to body surface area (Digby 1955; Church 1960). Therefore, large queens are likely to be advantage in hibernation than small queens.

In the following spring, competition may ensue between post-hibernating queens over nest sites during nest establishment and the early provisioning phase because of a shortage of suitable nest sites for queens (Sladen 1912; Alford 1975; Richards 1978; Paxton et al. 2001). Before workers first emerge, a foundress is at risk of

losing its nest to usurpers. In addition to such usurpation disputes, bumblebee queens may encounter predators such as birds and spiders (Goulson 2003a), or parasites which are responsible for high rates of failure in colony founding (Macfarlane & Griffin 1990; Shykoff & Schmid-Hempel 1991a; Brown et al. 2003).

Here, I performed the investigation of the natural selection on *B. terrestris* queens during the solitary phase and quantified the success rates of queens in hibernation and subsequent nest founding as demographic parameters, which are also important components of fitness. First, the size distributions of queens were compared to examine the selection on body size of queens through hibernation and nest founding. Second, the success rate in hibernation was estimated by the assumption of size-dependent survivorship, and the success rate in nest founding was indirectly estimated by the proportion of pollen-carrying queens to total post-hibernating queens.

In general, the success of invasive species is often attributed to escape from biotic forces, especially from natural enemies which may regulate populations in their native environment (Keane & Crawly 2002; Wolfe 2002). Therefore, biotic factors (e.g. predators and pathogens) responsible for mortality of queens, which are difficult to assess especially where the mortality is not high, were not addressed here. By combining the quantitative information on the increase rate of *B. terrestris* population (Chapter 2), the potential productivity of new queens per a nest (Chapter 3) and the success rates in hibernation and colony founding (this chapter), I attempted to evaluate the potential population growth of *B. terrestris* in the introduced area.

Materials and methods

Measurements

Queens were collected in Atsuma-cho and Mukawa-cho in the Iburi region of southern Hokkaido, Japan (42°34'–41'N, 141°50'–58'E, 5–100 m above sea level), described in detail in the “Materials and Methods” section of Chapter 2. I categorized them into the following four stages of life cycle: (1) new queens produced in feral nests during August to October; (2) post-hibernating queens in spring without pollen during May to June; (3) post-hibernating queens in spring with pollen; and (4) foundresses, which have produced their offspring. Because queens, which have discovered a suitable nest location, collect pollen to make a brood clump and to nourish the developing brood (Alford 1975), pollen-carrying queens in the corbiculae are judged to have started nest foundation.

New queens and foundresses were sampled from the feral nests found in the study area during 2003–2006 (Chapter 3). Damaged edges of wings and a shabby, greasy appearance could distinguish the foundresses from newly emerged queens. I also uncapped queen pupal cocoons and sampled only well-developed queens from excavated nests (with fully formed, hard and black compound eyes). Since pre-emergent developing queens and emerged queens were of similar size (Harris & Beggs 1995), they are categorized as new queens. Post-hibernating queens were captured using an insect net and asphyxiated with ethyl acetate in spring during 2003–2004.

Each collected bee was kept at -20 °C freezer to preserve and, subsequently, its maximal head width (HW) was measured in the laboratory with a digital caliper. HW is a common measure of intraspecific size variation in bees (Paxton & Tengö 1996; Richards 2000) and positively correlates to the other morphological characters (Chapter

2: Inoue et al. 2008; Inoue & Kato 1992; Inoue & Yokoyama 2006).

Estimation of demographic parameters

I assumed the size-dependent success rate during hibernation following a simple equation:

$$SR = 1 - \exp(-a(x - b)), \quad a > 0 \quad (1)$$

where x is head width and a determines mean of exponential distribution and b is threshold head width value below which success rate becomes zero. I assumed a priori b was 4.8 which was the minimum head width category observed in post-hibernating queens. Parameter a was estimated from the head width distribution between autumn and spring stages, so as to minimize the absolute difference between weighted mean of head width of autumn stage by estimated success rate by the equation and mean of head width observed at the spring stage.

I calculated the success rate of nest founding as the proportion of pollen-carrying queens to total post-hibernating queens.

Results

Queen size distribution

Queen size varied significantly among four queen stages (one-way ANOVA, $F_{3, 59.69} = 21.20$, $P < 0.0001$, Fig. 4-1). Post-hibernating queens without pollen loads (5.53 ± 0.14 , $n = 725$) and ones with pollen loads (5.57 ± 0.13 , $n = 97$) were significantly larger than new queens ($\bar{X} \pm SD = 5.44 \pm 0.26$, $n = 357$) (Tukey's HSD test, $P < 0.001$).

Although there was no significant differences between the two post-hibernating queen groups (Tukey's HSD, $P = 0.099$), the frequency distributions of head width (Fig. 4-1) showed that carrying-pollen queens tended to be larger than queens without pollen. Foundresses ($\bar{X} \pm \text{SD head width} = 5.63 \pm 0.11$, $n = 14$) were significantly larger than post-hibernating queens (t test, $P = 0.0099$) and under-represented in the small size classes (Fig. 4-1).

Head width of new queens largely varied within individual colony and differed significantly among nests (one-way ANOVA, $F_{8, 51.40} = 33.95$, $P < 0.0001$, Fig. 4-2) and years (one-way ANOVA, $F_{3, 61.99} = 19.03$, $P < 0.0001$, Table 4-1). New queens tended to become larger during 2003–2006. Post-hibernating queens also showed similar tendencies to new queens even though only two-year measurements were conducted.

Estimation of success rate in queens

Success rate of new queens during hibernation was estimated to be 44.0 % by the assumption of size-dependent survivorship. 11.8 % of post-hibernating queens carried pollen loads with them.

Discussion

Queen size and fitness

In bumblebees, adult body size is known to be influenced by the total amount of food with which any particular larva will be provided: the more food an individual consumes during the larval stage, the larger it is as an adult (Plowright & Jay 1968; Sutcliffe & Plowright 1988, 1990). Because of the different accessibility to food in a larva cell (Sladen 1912; Cumber 1949; Free & Butler 1959; Alford 1975), bumblebees exhibit

large variation in body size within a colony (Alford 1975; Goulson et al. 2002b), and thus within the species (e.g. Brian 1957; Ranta & Lundberg 1980; Inoue & Kato 1992; Inoue & Yokoyama 2006). The size variation among workers contributes to the division of tasks (Goulson et al. 2002b; Chapter 3) and a wide range of flower resource supply to a nest (Inoue & Kato 1992; Goulson 2003a). On the other hand, large queens have been thought to be at an advantage for hibernation (Holm 1972; Owen 1988), foraging efficiency (Harder 1983) and thermoregulation (Heinrich & Heinrich 1983). Therefore, it is suggested that these incidences may be forces tending to select for large body size or size dependent success in bumblebee queens (Plowright & Lavery 1984).

As my results showed, body size of queens largely varied within and among colonies (Fig. 4-2). Sexual production is known to be closely linked to food supply, and thus foraging ability of the colony (Schmid-Hempel & Schmid-Hempel 1998; Pelletier & McNeil 2003). Although genetic factors should be considered as referred to the size variation in workers by Cumber (1949), the colony conditions such as number and size of workers influence on not only quantity (Chapter 3) but also quality (i.e. body size) of queens.

Queen size differently affected their fitness at different stages of the life cycle. During hibernation period, the size-dependent selection considerably has an effect on survival of new queens (Fig. 4-1). Large bees maintain higher and more stable abdominal temperatures than small bees of the same species (Heinrich & Heinrich 1983) because the cost of thermoregulation per unit weight generally decreases with increasing weight. The amount of fat in the abdomen was also positively correlated with body size and weight (Holm 1972). Therefore, the hibernation mortality of small queens is considered to be higher than large queens.

Although there was no significant difference in size distribution among post-hibernating queens, large queens also tended to be more successful in nest founding, as found in other bumblebee species (Owen 1988; Vogt et al. 1994). Post-hibernating queens consume nectar and pollen to nourish their ovaries, and then each bee seeks out a suitable nest site before founding a nest (Cumber 1949; Free & Butler 1959; Alford 1975). For the higher and more stable abdominal temperatures of large queens (Heinrich & Heinrich 1983), large queens can accelerate their ovary development faster than small bees (Heinrich & Vogt 1993; Vogt et al. 1994), and thus large queens initiate nests earlier than small queens. The under-representation of small queens in the foundress suggests that large queens may have an advantage over small queens in encroaching nests from small queens or maintaining residency of nests, or in both. As mentioned in Introduction, however, size-independent mortality such as predators and parasites is also considered to be cause failure of colony initiation.

Potential population growth of *Bombus terrestris*

For the appropriate management of established *B. terrestris* populations, quantification of the demographic parameters limiting its population growth throughout the life cycle is indispensable. However, it is difficult to examine the queen mortality during hibernation because bumblebees usually hibernate underground beneath a layer of litter at depth of 2-15 cm, depending on the species (Alford 1969, 1975). In addition, available nest sites of bumblebees within the area, which would predominantly contribute to the carrying capacity, are difficult to be located. In Chapter 3, I evaluated the potential productivity of *B. terrestris* in the study area and showed that approximately 110 queens per colony were produced in a successful nest. To

demonstrate that the high productivity of the species contributes to the population growth, I attempted to estimate potential population growth of *B. terrestris* by estimating success rates in hibernation and nest founding and compared with the increase rate of *B. terrestris* population shown in Chapter 2 (Inoue et al. 2008).

The success rate of founding queens of *B. terrestris* is multiplied by the success rates in hibernation (44.0 %) and nest founding (11.8 %). In combination with the result in Chapter 3, which evaluated the potential productivity of gyne, 5.7 of 110 queens (5.2 %) produced in a single nest potentially initiate nest foundation in the following year. In this study, I did not examine the success rate of *B. terrestris* in reproduction. Cumber (1953) demonstrated that of 80 nests of *B. pascuorum* in southern England, 23 produced any gynes. If this success rate of nests (approximately 30 %) is assumed, 1.8 queens (1.6 %) per colony (i.e. per successful founded queen) will succeed in reproduction in the following year.

Bombus terrestris population during 2003–2005 in the study area increased approximately twice (Chapter 2; Inoue et al. 2008), and thus its average increase rate is estimated to be approximately 1.5 per year. The potential reproductive success rate per colony, that is 1.8 queens, is coincided well with the population growth rate observed in the study area. Inferring from the increase in usurpation among queens (Inoue et al. 2008: Chapter 2), *B. terrestris* population in the study area has become almost saturated. Therefore, the population growth rate calculated here is assumed to be the value under the condition where strong density effect operated. In this study, I estimated the success rate of queens in nest founding when density effect operates to compare the population increase rate surveyed in the study area. If competition for nest sites is small, post-hibernating queens may have an opportunity to found a nest. The potential intrinsic

rate of natural increase of *B. terrestris* is thus estimated to be approximately 20 per year by excluding success rate in nest founding (11.8 %) under density effect. Consequently, at larger spatial scales, such populations, which grow large enough, will provide sufficient numbers of emigrants for further spread.

Table 4-1 Variation in mean \pm SD (mm) of *Bombus terrestris* queens for four stages of the adult life cycle.

Stage	Head width (mm)			
	2003	2004	2005	2006
New queens	5.31 \pm 0.26 (n=124)	5.49 \pm 0.29 (n=19)	5.49 \pm 0.22 (n=179)	5.61 \pm 0.23 (n=35)
Post-hibernating queens without pollen	5.50 \pm 0.15 (n=292)	5.55 \pm 0.13 (n=435)		
Post-hibernating queens with pollen	5.54 \pm 0.15 (n=49)	5.61 \pm 0.10 (n=48)		
Foundresses	5.71 \pm 0.07 (n=2)	5.57 \pm 0.07 (n=2)	5.68 \pm 0.10 (n=7)	5.51 \pm 0.06 (n=3)

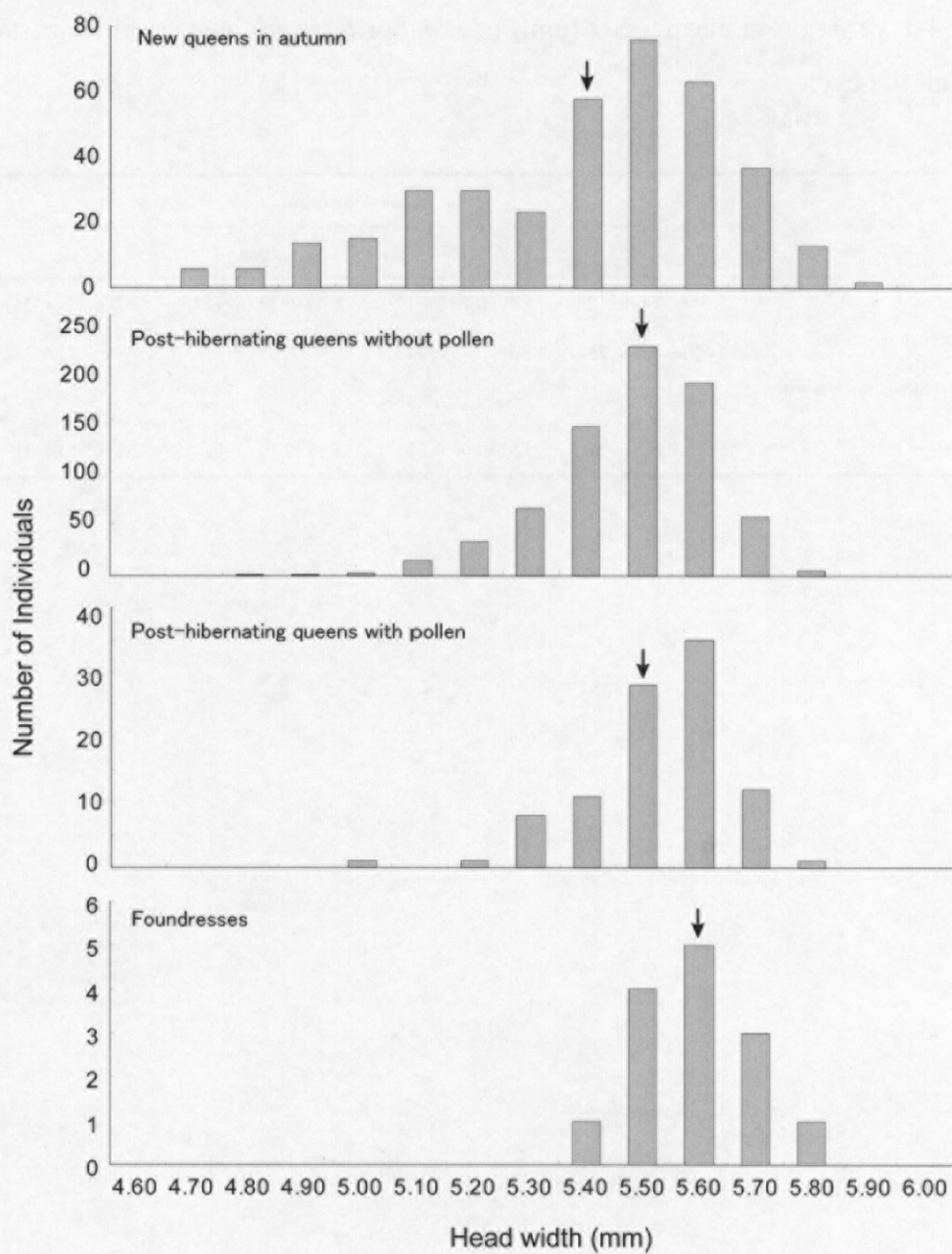


Fig. 4-1 Size distributions (head width in millimeters) of new queens, post-hibernating queens without/ with pollen, and foundresses collected in study area. Head widths of queens are indicated by arrows.

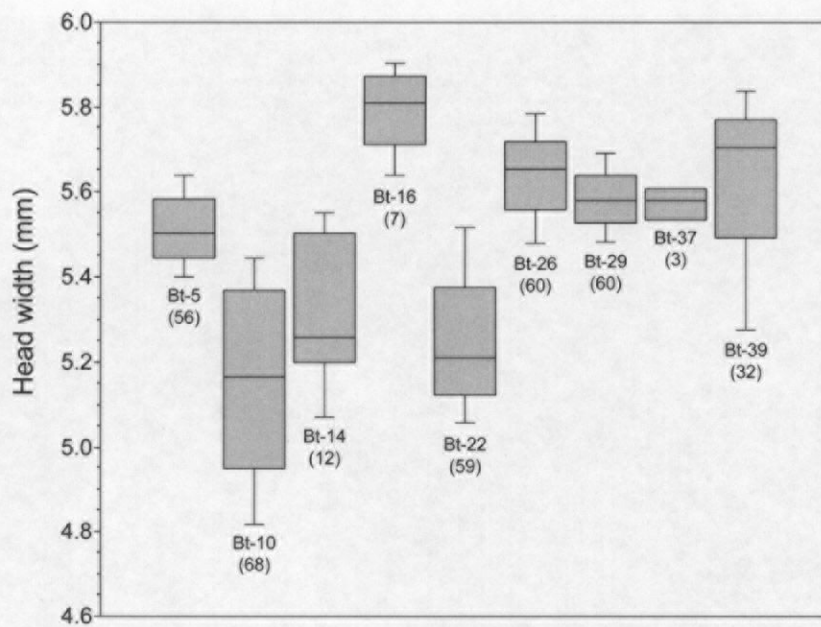


Fig. 4-2 Variation in head width of new queens sampled from the feral nests in the study area. Vertical lines, means; horizontal lines, ranges; horizontal bars, standard deviations. Sample size is indicated in parentheses.



Photo 4-1 A hibernating queen of *Bombus terrestris* found underground at a depth of 13 cm in the west facing slope under the deciduous trees (*Quercus serrata* Murray) in the study area (21 November, 2004).

CHAPTER 5

Conservation Ecological Study of *Bombus terrestris* Invading into a Preserved Area of the Notsuke Peninsula, Northern Japan

Introduction

The introduction of invasive alien species to a previously unoccupied region is considered to be a key threat to the integrity of native ecosystems worldwide (Czech et al. 1997; Mack et al. 2000; Sala et al. 2000). A number of documented cases have ascertained that some invasive species caused radical changes in abundances of native organisms, including local extinctions in both terrestrial and aquatic environment (e.g. Porter & Savignano 1990; Rhymer & Simberloff 1996; Boettner et al. 2000).

Bombus terrestris has been widely established in a wide range of Hokkaido, northern Japan (Yokoyama & Nakajima 2005; Laboratory of Conservation Ecology 2007), and is suggested to cause the decline of certain native species (Chapter 2: Inoue et al. 2008; Kawahara 2004). The invasiveness of *B. terrestris* has shown to be associated with superior competitive ability (Chapter 2), high reproductive ability (Chapter 3) and the potential population growth (Chapter 4). These characteristics are responsible for the attainment of numerical dominance in the region where *B. terrestris* has invaded and are thought to encourage further range expansion of this species.

The invasions of *B. terrestris* into the Notsuke and the Nemuro Peninsula, eastern Hokkaido were recently reported (Yokoyama et al. 2006; Inoue et al. 2007; Nakatani & Nakamura 2007). These regions are characterized by the following features. First, they are the restricted distribution ranges of a rare native species, *B. florilegus*

(Sakagami & Ishikawa 1969; Nakatani 1999b), which has been considerably declined in its number (Nakatani 1999b; Matsuura 2004). *Bombus florilegus* has marked morphological traits of short broad face and a relatively short tongue, features associated with its habit to collect nectar from flowers with rather short corolla tube or open flowers (Matsuura 1995; Washitani et al. 1997). It was reported that its colony size is the smallest among native bumblebees, producing only 10-30 workers and up to 20 sexuals (Matsuura 2004). *Bombus florilegus* and *B. terrestris*, belonging to the same subgenus (*Bombus* s. str.), are considered to have similar morphological and ecological features, and thus are likely to compete for flower resources and nest sites.

The second prominent feature in these regions is the species-rich maritime grassland extending along the coast. The development and persistence of the maritime grassland is associated with the agricultural practices such as grazing of domestic animals (Notsuke Peninsula Nature Center 2007). Due to the recent farmland exploitations, the maritime grasslands have dramatically declined in Hokkaido (Nakatani 1999a). The maritime grassland in the Notsuke Peninsula, however, offers a rich variety of vegetation associations, with a high herbaceous plant species richness that comprised by many species endemic to northern maritime regions (Takamizawa 2005; Notsuke Peninsula Nature Center 2007). A large number of these native plants are supposed to be pollinated predominantly or exclusively by bumblebees.

Hence, it is assumed that the invasion of *B. terrestris* into the Notsuke Peninsula affects negatively on both native bumblebees, including *B. florilegus*, and bumblebee-pollinated plants. In the present chapter, I conducted a census by recording flower visits in the Notsuke Peninsula and assessed potential niche overlaps in flower resources and habitat selections between introduced and native bumblebees. I also

investigated a feral *B. terrestris* nest found in the maritime grassland during the survey.

Materials and Methods

Study site

The study was carried out in the maritime grassland of the Notsuke Peninsula, Betsukai-cho in the Notsuke region of eastern Hokkaido, Japan (Fig. 5-1). The vegetation of this region is mainly composed of coastal grassland (characterized by *Thermopsis lupinoides* and *Rosa rugosa*), freshwater marsh (*Iris ensata* Thumb. var. *spontanea* (Makino) Nakai and *Lobelia sessilifolia* Lamb.), salt marsh (*Salicornia europaea* L. and *Triglochin maritimum* L.) and mixed woodland of conifers and broadleaf deciduous trees (dominated by *Abies sachalinensis* (F. Schmidt) Mast. and *Quercus crispula* Blume) at 3-5 m above sea level. The entire area of the Notsuke Peninsula is comprised in Notsuke-Furen Prefectural Natural Park of Hokkaido and most of it is covered by native vegetation (Notsuke Peninsula Nature Center 2007). Some non-native plants such as *Taraxacum officinale*, *Trifolium pratense* and *T. repens* occur along the roads, or around the margins of open spaces used for activities related to fishery.

Flower resource use and habitat selection in *Bombus terrestris* and native bumblebees

Nine investigation sites (S1-S9) were placed within the study area, with 1.7-2.6 km apart from each other along the main road (Fig. 5-2, Photo 5-1). S1, S7, S8 and S9 were in the coastal grassland adjacent to the coastal sand dune. S2, S3, S5, and S6 were in the coastal grassland and only S4 was close to the woodland. S2 and S3 were located on the

opposite side of the road and differently consisted of the dominant plants in June: the vegetation at S2 was dominated by *Taraxacum* spp. (*T. officinale*, *T. shikotanense* Kitam and their hybrids); and the vegetation at S3 was dominated by *Thermopsis lupinoides*. Bumblebees visiting flowers of approximately 15 m² at the individual site were observed between 9 and 13 June in each of S1-S8, and between 3 and 9 August in each of S1 and S3-S9, 2007. Bumblebee species and caste (worker, queen, or male) were recorded for 15 minutes at twice of a day: morning (08:30-12:30) and afternoon hours (12:30-15:30).

Based on the data, the index for similarity of flower visit, habitat selection and seasonal patterns of flight activity among bumblebee species was calculated according to Horn (1966). The niche overlap index was calculated for workers, queens, and males, as described in Chapter 2. *Bombus hypnorum koropokkrus* were excluded from the analysis because of very low frequencies of observation.

Measurement of morphological characters

The workers of *B. terrestris* and two native species, *B. hypocrita sapporoensis* and *B. florilegus*, were captured using an insect net and immediately asphyxiated with ethyl acetate in August during the study. These consubgeneric species are considered to overlap each other in body size relating to foraging activities. Each collected bee was kept at -20 °C freezer to preserve and subsequently the size of the following body parts were measured with a digital caliper: head width (HW), head length (HL), prementum length (PL), glossa length (GL), maximum thorax width (TW), and maximum abdomen width (AW) (Chapter 2: Inoue et al. 2008; Inoue & Yokoyama 2006).

Investigation of a nest

A nest of *B. terrestris* found on 6 August 2007 (43°34'48.3"N, 145°20'40.6"E) was excavated on 8 August and placed in a -20 °C freezer to kill remaining bees and preserve the nest. Subsequently, the nest was dissected and the numbers of each colony component was recorded, according to Chapter 3: egg (E), larvae (L), pupal cocoon (P), post-emergence vacated cocoon (C), honey stored cocoon (Hh) and pollen stored cocoon (Hp). Colony size was defined as the sum of the number of all types of cocoons (P, C, Hh and Hp). Diameter of the cocoons was measured with a digital caliper to distinguish new queen (gyne) or worker/male on the basis of size (Chapter 3). The head width of all adult bees collected was also measured.

Results

Flower use and foraging habitat

I recorded a total of 554 bumblebees: 14 *B. terrestris* and 540 native bumblebees, consisting of six species, 182 *B. hypocrita sapporoensis*, 21 *B. florilegus*, 5 *B. hypnorum koropokkrus*, 162 *B. schrencki albidopleuralis*, 133 *B. pseudobaicalensis*, 20 *B. diversus tersatus*, and 17 *B.* (subgenus *Megabombus*) *yezoensis* Matsumura (Table 5-1, Photo 5-2). Irrespective of the species, bumblebees frequently foraged *Thermopsis lupinoides* and *Taraxacum* spp. in June, and *Geranium yezoense* Franch. et Sav. var. *pseudopalustre* Nakai, *Vicia japonica* A. Gray, *V. villosa* Roth and *Rosa rugosa* (for pollen) in August (Photo 5-3). The non-native plants such as *Taraxacum* spp. and *Trifolium* spp. were less frequently foraged by bumblebees, including *B. terrestris*, than native plants.

Bombus terrestris was observed at five sites but more frequently at S7 close to

its nest (Fig. 5-2). *Bombus hypocrita sapporoensis* was observed at all sites but tended to decrease from S7 toward the peninsula extremity. *Bombus pseudobaicalensis* was also found at all sites but less in S4. On the other hand, *B. florilegus* was found only at the peninsula extremity (S6, S7 and S8), and *B. schrencki albidopleuralis* also showed the same tendency. *Bombus diversus tersatus* was restricted in S4, and *B. yezoensis* was also observed mostly at S4 and S9.

The degree of niche overlaps in flower resources with *B. terrestris* varied from species to species of native bumblebees: relatively large overlaps with *B. florilegus*, *B. pseudobaicalensis* and *B. schrencki albidopleuralis* (0.33-0.40); small overlap with *B. hypocrita sapporoensis* (0.18); and no overlap with *B. diversus tersatus* and *B. yezoensis* (0.00) (Table 5-2). Niche overlap also varied greatly among native bumblebees. *Bombus hypocrita sapporoensis* and *B. florilegus* showed small overlaps with *B. diversus tersatus* and *B. yezoensis* (0.00 to 0.30), and *B. pseudobaicalensis* also showed small overlap with *B. diversus tersatus* (0.11). *Bombus schrencki albidopleuralis* overlapped largely with *B. pseudobaicalensis*, *B. diversus tersatus* and *B. yezoensis* (0.57 to 0.65). Niche overlap was also large between *B. diversus tersatus* and *B. yezoensis* (0.63).

Morphological variations

Morphological measurements were made on 15 *B. terrestris*, 9 *B. hypocrita sapporoensis* and 9 *B. florilegus* (Table 5-3). The morphology was quite similar among the three species, but *B. terrestris* had relatively longer glossa than two native species, while *B. florilegus* had relatively wider body than other two species.

Nest size

A colony of *B. terrestris* was found within the coastal grassland vegetation, close to the observation site S7 (Photo 5-4 (a), (b)). This nest was constructed underground at a depth of 40 cm with a 100 cm entrance tunnel and covered with dried plant fragment that was presumably an abandoned rodent nest. The nest was approximately 10 cm width, 15 cm long and 6 cm height, and arranged in three-fold volutions. No wax envelop was observed.

A total of one foundress, 129 workers, 362 cocoons, 85 eggs and 280 larvae were collected from the nest (Photo 5-4 (c)). Cocoons were assigned to 226 pupal cocoons and 136 post-emergence cocoons, including ones used for 61 honey receptacles and six pollen receptacles. All the cocoons, of which diameter was $8.6 \pm 0.8\text{mm}$ ($\bar{X} \pm \text{SD}$, range = 5.2 - 10.9 mm, $n = 334$), were determined as workers. The head width of foundress was 5.6 mm and that of workers was $3.8 \pm 0.4\text{mm}$ (2.9 - 4.5 mm, $n = 122$).

Discussion

Potential ecological impacts on *Bombus florilegus*

Bumblebees are generally considered to coexist by partitioning flower resources on the basis of proboscis length and flower corolla morphologies because there are close positive correlations between them (e.g. Brian 1957; Heinrich 1976; Inouye 1980; Pyke 1982; Barrow & Pickard 1984). The measurements of body parts in this study showed that *B. terrestris* has similar morphological characteristics with *B. hypocrita sapporoensis* and *B. florilegus* (Table 5-3). Comparing with the data in the previous study by Inoue et al. (Chapter 2: 2008, Table 2-3), *B. terrestris* also partly overlaps with *B. schrencki albidopleuralis* ($\bar{X} \pm \text{SD}$ glossa length = 6.3 ± 0.7 mm) and *B.*

pseudobaicalensis (6.7 ± 0.7 mm). On the other hand, there are little overlaps between *B. terrestris* and *B. diversus tersatus* (9.3 ± 0.4 mm, Table 2-3 in Chapter 2: Inoue et al. 2008) / *B. yezoensis* (11.3 ± 1.4 mm, unpublished data). Therefore, it is assumed that *B. terrestris* potentially competes for flower resources with four native species, *B. hypocrita sapporoensis*, *B. florilegus*, *B. schrencki albidopleuralis* and *B. pseudobaicalensis*.

The niche overlaps with *B. terrestris* were relatively large between *B. florilegus*, *B. schrencki albidopleuralis* and *B. pseudobaicalensis* but small with *B. hypocrita sapporoensis* (Table 5-2). In the Notsuke Peninsula, *B. terrestris* has just invaded (Inoue et al. 2007) and thus was restricted its distribution at a low density during the census, comprising only 2.5 % of all observations. Therefore, niche overlaps between *B. terrestris* and native bumblebees, especially *B. hypocrita sapporoensis*, are smaller in the Notsuke Peninsula than in the Iburi region (Chapter 2: Inoue et al. 2008).

The *B. terrestris* nest was found within the distribution range of *B. florilegus*. Since these two species are subterranean nesters (Chapter2: Inoue et al. 2008; Matsumura et al. 2004a, b; Matsuura 1995, 2004), competition for nest sites may be intense. Inoue et al. (Chapter 2: 2008) ascertained that the two native species, which overlap in nest sites with *B. terrestris*, have rapidly declined in their numbers after the invasion of the species. If *B. terrestris* continues to increase in this region, competition may cause a reduction or even local extinction of *B. florilegus*.

Establishment in the native ecosystem

Bombus terrestris has been recorded predominantly in urban and agricultural areas in Hokkaido (Kadoya et al. unpublished) and mostly visited non-native plants (Chapter 2:

Inoue et al. 2008, Kawahara 2004), even though this species exhibited the ability to forage on many species of both non-native and native plants (Matsumura et al. 2004b). The present study, however, demonstrated that *B. terrestris* visited more frequently on native plants than on non-native plants in the study region. At the time of nest excavation, the *B. terrestris* nest has similarity in productivity as records in the ‘mature’ nests of *B. terrestris* (Chapter 3). Since the foundress was still alive and much food was stored in the nest, this colony would be able to reproduce sexuals successfully if not excavated. Flower visits to the native plants and the colony foundation in the Notsuke Peninsula indicates the successful naturalization of *B. terrestris* in native vegetation in Japan.

In addition to *B. hypocrita sapporoensis* and *B. florilegus*, *B. terrestris* also overlaps in nest sites with *B. diversus tersatus* and *B. yezoensis* (Nakajima et al. 2004; Matsuura 2004). These long-tongued species are important pollinators of plants with deep corollas such as *Iris setosa* and *I. ensata* var. *spontanea*, characteristic of the maritime grassland (Washitani et al. 1997). The reduction of the long-tongued species will cause reproductive failure of these long-tubed flowers, of which pollination exclusively depend on these bumblebee species. The invasion of *B. terrestris* poses an additional threat to biodiversity of the maritime grassland.

Implication for suppression of *Bombus terrestris*

The Notsuke Peninsula is separated from Shari-cho, the closet area where *B. terrestris* has already naturalized, by approximately 70 km (Niwa et al. 2001; Kawahara S., personal communication), and is about 120 km away from Kushiro-cho, where the commercial colonies of *B. terrestris* was used for some agricultural crops (from the

statistical data of Ministry of Agriculture, Forestry and Fisheries of Japan). Although very little is known about the dispersal ability of bumblebees, bumblebee queens were recorded passing across the Gulf of Finland with 80 km long distance (Mikkola 1984). It is reasonable to suppose that *B. terrestris* in the Notuske Peninsula has invaded from Shari-cho. For the effective management of its growing populations, extermination is needed not only in such valuable regions but also in the surrounding regions where potential source populations of *B. terrestris* are established.

Table 5-1 Plant species visited by bumblebees in 2007.

Family	Plant species	Bumblebee species ¹⁾							
		Bt	Bhs	Bf	Bhk	Bsa	Bp	Bdt	Be
Compositae	<i>Achillea ptarmica</i> var. <i>macrocephala</i>					1			
Compositae	<i>Crisium kamtschaticum</i>		4			11	9		
Compositae	<i>Taraxacum</i> spp. ²⁾		61	6	1	7	6		
Geraniaceae	<i>Geranium yesoense</i> var. <i>pseudopalustre</i>	3	59	6	2	60	18	4	
Labiatae	<i>Scutellaria strigillosa</i>		1	1		1	1		
Labiatae	<i>Stachys japonica</i> var. <i>villosa</i>		1			2	1	1	
Leguminosae	<i>Lathyrus japonicus</i>		3			7	5		
Leguminosae	<i>Thermopsis lupinoides</i>	1	44		2	31	22	9	10
Leguminosae	<i>Trifolium pretense</i> *					5	1	6	1
Leguminosae	<i>Trifolium repens</i> *	1					1		
Leguminosae	<i>Vicia japonica</i>		4	3		10	28		
Leguminosae	<i>Vicia villosa</i>	2	1	3		10	25		
Ranunculaceae	<i>Thalictrum minus</i> var. <i>hypoleucum</i>						1		
Rosaceae	<i>Rosa rugosa</i>	1	4			14	11		6
Rosaceae	<i>Rubus parvifolius</i>	3				1	2		
Rosaceae	<i>Sanguisorba tenuifolia</i> var. <i>alba</i>	3		2		1			
Ruscaceae	<i>Polygonatum humile</i>					1	2		

*Non-native plant species

¹⁾ Bt, *Bombus terrestris*; Bhs, *B. hypocrita sapporoensis*; Bf, *B. florilegus*; Bhk, *B. hypnorum koropokkrus*; Bsa, *B. schrencki albidopleuralis*; Bp, *B. pseudobaicalensis*; Bdt, *B. diversus tersatus*; and Be, *B. yezoensis*.

²⁾ *T. officinale*, *T. shikotanense* and their hybrids

Table 5-2 Horn’s (1966) index of niche overlap calculated based on seasonal patterns of activity, habitat preference, and flower visits.

	Bt	Bhs	Bf	Bsa	Bp	Bdt	Be
Bt		0.18	0.34	0.33	0.40	0.00	0.00
Bhs			0.33	0.50	0.51	0.26	0.30
Bf				0.45	0.48	0.00	0.00
Bsa					0.65	0.57	0.57
Bp						0.11	0.34
Bdt							0.63
Be							

Species symbols as in Table 5-1.

Table 5-3 The mean \pm SD (mm) of body size for bumblebee workers.

Species	<i>N</i>	HW	HL	PL	GL	TW	AW
Bt	15	4.0 \pm 0.3	3.8 \pm 0.3	2.8 \pm 0.3	5.8 \pm 0.7	5.1 \pm 0.5	6.0 \pm 0.6
Bhs	9	4.0 \pm 0.3	3.8 \pm 0.3	2.8 \pm 0.3	5.1 \pm 0.5	5.1 \pm 0.5	6.3 \pm 0.7
Bf	9	4.0 \pm 0.1	3.9 \pm 0.1	2.5 \pm 0.2	5.1 \pm 0.4	5.3 \pm 0.3	6.5 \pm 0.4

HW, head width; HL, head length; PL, prementum length; GL, glossa length; TW, maximum thorax width; AW, maximum abdomen width.
Species symbols as in Table 5-1.

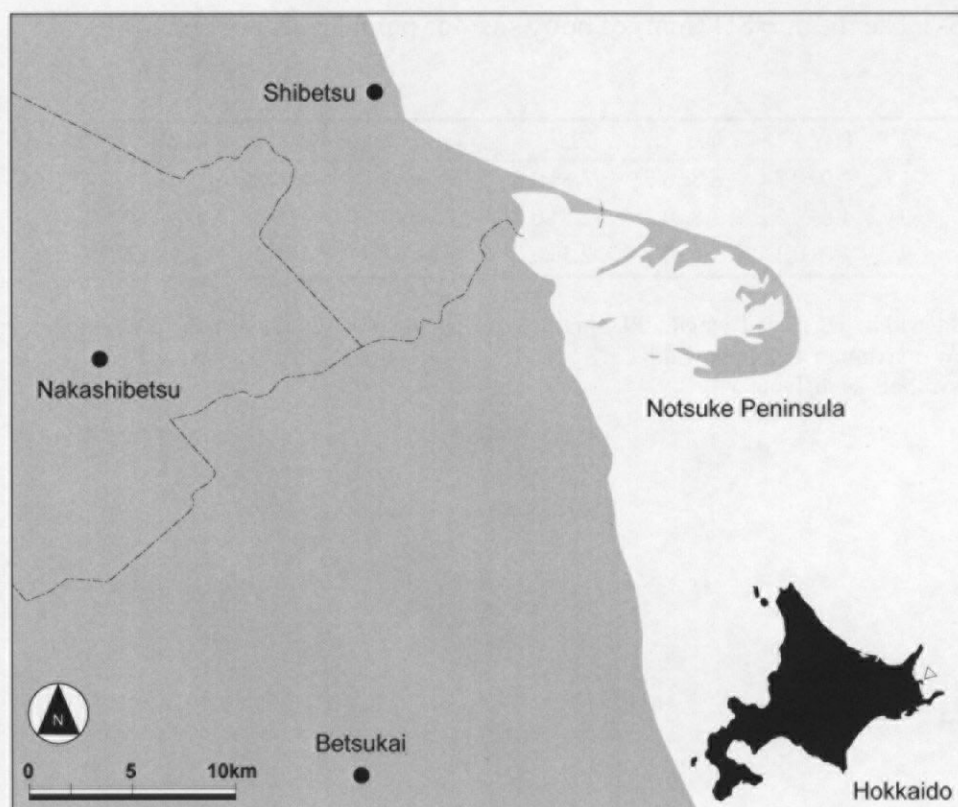


Fig. 5-1 The location of the study site, the Notsuke Peninsula.

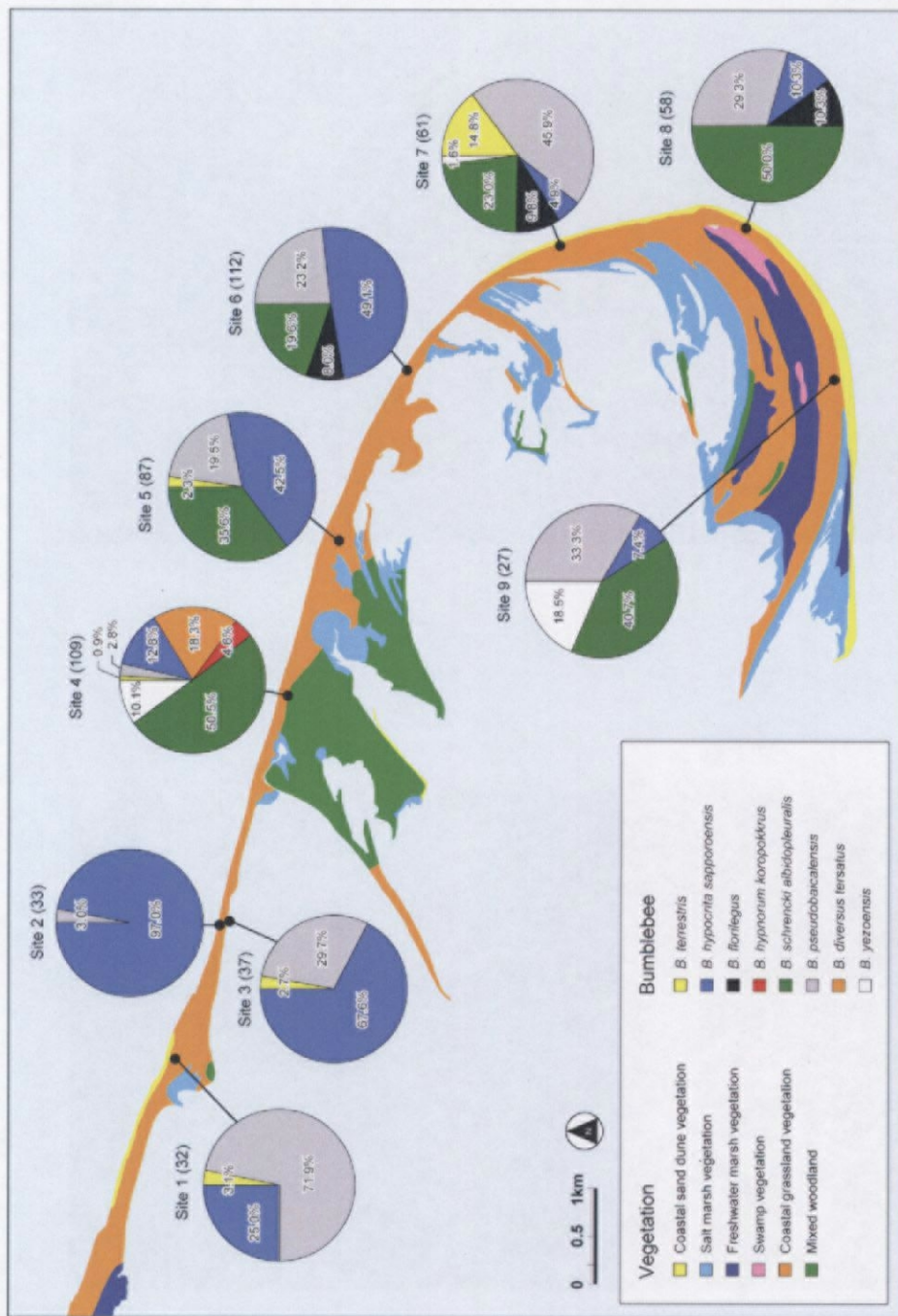


Fig. 5-2 The flower visits of *Bombus terrestris* and seven native bumblebee species at each observation site. Numerals in the parentheses indicate number of bumblebees observed at each sites. Vegetation types were modified from Notsuke Peninsula Nature Center (2007).



Photo 5-1 The landscapes of the study sites in the Notsuke region, eastern Hokkaido. S5 in June (a) and S3 in August (b) are representative of the habitat condition in the study area.



Photo 5-2 Six native bumblebee species in the Notsuke Peninsula, eastern Hokkaido. *Bombus hypocrita sapporoensis* and *B. florilegus* visiting *Taraxacum* spp.; *B. schrencki albidopleuralis*, *B. diversus tersatus*, and *B. yezoensis*, *Thermopsis lupinoides*; *B. pseudobaicalensis*, *Lathyrus japonicus*.



Photo 5-3 Native plants visited by bumblebees in the Notsuke Peninsula, eastern Hokkaido.



Photo 5-4 The nest of *B. terrestris* found in the Notsuke Peninsula, eastern Hokkaido. The nest was located in the coastal grassland vegetation (a) and the diameter of its entrance was approximately 2 cm (b). The nest contained worker cocoons, eggs and larvae (c).