Assessing ecological impacts of *Bombus terrestris* (L.) naturalized in Hokkaido, northern Japan.

（北海道で野生化したセイヨウオオマルハナバチの生態影響評価）

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Table of Contents

CHAPTER 1

General Introduction

Effects of invasive species on biodiversity and introduced pollinator bees as biological agents

Species introduction, extinctions and range changes are natural components of dynamics of ecological communities over evolutionary time (Lodge 1993). Human activities over the short term, however, have greatly accelerated these processes (Lodge 1993). In recent years, the number of both accidental and intentional introductions of non-native species has ever increased, as a consequence of expanding human transport and commerce between continents (Mack et al. 2000). Although most introduced species perish en route to a new region or soon after release (Lodge 1993; Mack & Lonsdale 2001), a few of them wreak the devastating impacts on native ecosystems via predation, competition and/or parasitism (Vitousek et al. 1996). Therefore, biological invasions, compound with other human-induced changes in global environment such as habitat destruction and climate change, are currently thought to be one of the proximate causes of biodiversity loss worldwide (Kareiva 1996; Czech et al. 1997; Mack et al. 2000; Millennium Ecosystem Assessment 2005).

Terrestrial invertebrates have been often successful invaders of new regions around world, owing to their rapid reproduction and dispersal ability (e.g. Moller 1996; Chapman & Bourke 2001). Among them, the majority have probably been accidentally introduced along with human activities. One of examples of a highly successful invasive species is the red imported fire ant, *Solenopsis invicta* Buren, which was accidentally

introduced into the southern USA from South America in the 1930s (Callcott & Collins 1996; Vinson 1997) and has replaced its ecological equivalents, presumably by competitive exclusion (Morris & Steigman 1993; Porter & Savignano 1990; Holway et al. 2002). The FDA estimates that more than US$5 billion is spent annually on medical treatment, damage, and control in infested areas by *S. invicta*.

By contrast, some species are intentionally introduced as biological agents such as predatory arthropods and pollinators. Introduced bees, including the honeybee *Apis mellifera* (L.) and the alfalfa leaf-cutter bee *Megachile rotundata* (Fabr.), are generally appreciated to be beneficial for their roles in the pollination of crops and wild plants and, in the case of the honeybee, for production of honey. Because of these economic benefits, these introduced bees have not been regarded as potentially damaging to the environment until 1980s (Goulson 2003b).

The European large earth bumblebee, *Bombus terrestris* (L.) (Photo 1-1) is also initially considered to be undoubtedly only beneficial because of its pollination services and has been intentionally introduced as a pollinator for various crops worldwide (Velthuis & van Doorn 2006). In recent years, however, grave concerns over the potential ecological impacts of using both non-native bumblebee species (e.g. Dafni & Shmida 1996; Goka et al. 2001; Goulson et al. 2002a) and subspecies (e.g. Ings et al. 2005, 2006) for crop pollination have been raised, because these introduced biological agents have also been recognized to have the potential to damage native ecosystems (Howarth 1991).

The world major commercial pollinator species, *Bombus terrestris*

Bombus terrestris is a widely distributed native of Europe and adjacent territories,

2

including southern Scandinavia, England, most of Scotland, the north coast of Africa, major Mediterranean Islands, and some Atlantic Islands such as Madeira and the Canary Islands (Estoup et al. 1996; Chittka et al. 2004; Velthuis & van Doorn 2006). This species became available through commercial insectaries as a valuable pollinator for greenhouse crops, particularly tomato (*Solanum lycopersicum* L.) in the late 1980s. The benefits to farmers include reduced costs and labors from not having to pollinate by hand mechanically with electronic vibrators or chemically hormone treatments, increased fruit yields, improved fruit quality leading to higher sale price, and little or no use for pesticides (Velthuis & van Doorn 2006). Its introductions have been also performed to enhance the pollination of more than 25 different crops other than tomato, including red clover, cranberry, blueberry, kiwifruit, almond, apple, pear and sweet pepper (Velthuis & van Doorn 2006). Today, *B. terrestris* is used for commercial pollination at least in 36 countries including not only its Eurasian area of distribution but also East Asia (Japan, South Korea, China) and South America (Chile), and current worldwide sales are estimated at 900,000 colonies per year (Velthuis & van Doorn 2006).

Naturalization of *Bombus terrestris* and its impacts on native ecosystems, with particular references to New Zealand, Tasmania and Israel

Case studies in three regions, New Zealand (MacFarlane & Gurr 1995), Australian continental island of Tasmania (Semmens et al. 1993) and Israel (Dafni & Shmida 1996), are summarized below as references to the potential impacts of *B. terrestris* on Japanese native ecosystems.

New Zealand

Bombus terrestris, along with five other *Bombus* species native to the U.K., was introduced into the South Island of New Zealand in 1885, as a pollinator of clover crops (Macfarlane & Gurr 1995). Both natural and deliberate introductions were made of at least four species into farming areas and resulted in the eventual spread of *B. terrestris* over much of New Zealand at a rate of 90 km per year after it was introduced there (Macfarlane & Gurr 1995; Stout & Goulson 2000; Goulson & Hanley 2004). *Bombus terrestris* was recorded to visit more than 400 plant species, of which only 19 were native to New Zealand (Goodwin & Steiner 1997). Donovan (1980) proposed that competition between introduced *B. terrestris* and native bee species was probably negligible because of the low densities and the marked specialization for different flowers.

Bombus terrestris established in New Zealand has been regarded as an important pollinator of crops and wild plants (Macfarlane & Gurr 1995), and no one has considered it other than a great benefit. Although these studies on *B. terrestris* in New Zealand are often quoted as an evidence of its successful naturalization (e.g. Goka et al. 2001; Hingston et al. 2002; Matsumura et al. 2004b), the ecological impacts of this species have not been considered.

Tasmania, Australia

Bombus terrestris arrived in Horbart, Tasmania in 1992, perhaps through the accidental transportation in cargo from New Zealand (Semmens et al. 1993). The established population of *B. terrestris* probably originated from a single, inseminated queen (Buttermore et al. 1998) and has spread at approximately 12.5 km per year to occupy a

substantial portion of the island until 2001 (Buttermore 1997; Stout & Goulson 2000; Hingston et al. 2002). *Bombus terrestris* is observed foraging on a wide rage of native plants (Semmens 1996; Hingston 2006; Hingston et al. 2006). One study reported that *B. terrestris* foraged on 175 species of flowering plant, of which 156 were introduced and 19 were native (Semmens 1996), and another reported that the species foraged on 66 native plant species from 21 families (Hingston & McQuillan 1998). *Bombus terrestris* established in Tasmania exhibited overlap in floral resources with native pollinators, including bees and nectivorous birds and, therefore, was suggested the potential to compete with them (Hingston & McQuillan 1998). Indeed, competitive displacement of two native bees by *B. terrestris* has been reported (Hingston & McQuillan 1999).

Some studies have reported that *B. terrestris* foraged in Tasmanian native vegetation (Hingston & McQuillan 1998; Hingston et al. 2002) and also that the *B. terrestris* colony found in the Tasmanian National Park was successful in reproduction (Hingston et al. 2006). On the other hand, doubts have been expressed regarding the ability of *B. terrestris* to invade native vegetation in Tasmania because it occurs predominantly in urban and agricultural areas (Stout & Goulson 2000; Goulson et al. 2002c). In addition, the species richness, diversity, and abundance of native flower-visiting insects did not differ significantly across sites according to the presence or absence of *B. terrestris* (Goulson et al. 2002c).

Although some studies in Tasmania showed overlaps in floral resources between *B. terrestris* and native bees, the ecological impacts of *B. terrestris* on native bees and vegetations is still controversial.

Israel

Bombus terrestris is naturally distributed in the northern territories of Israel and its distribution range has been expanding southwards since 1960 to Mt. Carmel (Dafni & Shmida 1996; Dafni 1998). Dafni and Shmida (1996) reported that after the invasion of the species in 1978 and a following large fire on Mt. Carmel in 1989 the composition of the native flora and fauna drastically changed, and that the numbers of honeybees and native solitary bees declined in the burnt area. Since commercial rearing of *B. terrestris* for tomato pollination has started in Israel in 1991, an annual supply of some several thousand colonies is estimated (Dafni 1998). Therefore, researchers claimed that the invasion of *B. terrestris* was a threat to the biodiversity of these solitary bees and warned against commercial production and use (Dafni & Shmida 1996; Dafni 1998). However, Israel was invaded naturally from its northern areas and no feral establishment has occurred in southern areas where large-scale releases from greenhouse have been made since 1991.

The history of introduced *Bombus terrestris* and its impacts on native ecosystems in Japan

Introductions and current status of *Bombus terrestris* in Japan

Bombus terrestris colonies were first commercially introduced into Japan for pollination of tomato in greenhouse in 1991 (Ono 1998). Importation of *B. terrestris* colonies has been increasing annually, and approximately 70,000 colonies were used in 2004 (Kunitake & Goka 2006). An estimate is that approximately 250,000 colonies have been imported over the last 12 years, with the value of the bumblebee-pollinated tomato, totaling approximately 50 billion yen annually (Goka & Japanese Bumblebee

Companies Association 2003).

A naturally occurring nest of *B. terrestris*, the first evidence of naturalization in
Japan, was found in the Hidaka region of southern Hokkaido, northern Japan in 1996
(Washitani 1998). This region is adjacent to the agricultural area where commercial
colonies of *B. terrestris* have been mass introduced from more than 10 years
(Matsumura et al. 2004b). Since then, the number of foraging individuals and feral nests
of *B. terrestris* detected in the field has been increased over a wide range of Hokkaido
(Japanese Society of Conservation Ecology 2005; Yokoyama & Nakajima 2005;
Laboratory of Conservation Ecology 2007).

Suggested impacts of Bombus terrestris on native ecosystems in Japan

From the beginning of its introduction, the naturalization and the ecological impacts of
B. terrestris affecting the native bumblebee species and bumblebee-pollinated plants
were warned (Washitani et al. 1997; Goka 1998; Washitani 1998; Goka et al. 2001). The
four types of such impacts on native ecosystems by the invasion of *B. terrestris* were
concerned: (1) interspecific competition with native bumblebees, either for floral
resources or for nest sites; (2) mating interference and hybridization with closely related
species of native bumblebees; (3) transmission of parasites or pathogens to native
bumblebees; and (4) effects on pollination of native flora.

Competition with native species for floral resources and nest sites

Interspecific competition is defined that one species suffer a reduction in fecundity,
survivorship or growth as a result of resource exploitation or interference by another
species (Begon et al. 1996). If two competing species coexist in a stable environment,

they do so as a result of niche differentiation. If, however, there is no such differentiation, then one competing species will eliminate or exclude the other. Thus, competitive exclusion occurs when the niche of the superior competitor completely overlaps with that of inferior competitors. An invasive species, which overlaps niche with native species and has a competitive advantage, may cause suppression or even extinction of native species (Mack et al. 2000). In case of flower-visiting bees, competition for two types of requisites is mainly assumed, that is, foods (nectar and pollen) and nest sites (Thorp 1996).

Bombus terrestris is known to be highly polylectic species (Prys-Jones & Corbet 1991; Semmens 1996). This species was reported to visit a wide range of plants for nectar and/or pollen in the Hidaka region: 100 species across 26 families, of which 40 were native and the high niche overlap in flower visits between *B. terrestris* and native bumblebees was suggested the occurrence of competition for flower resources (Matsumura et al. 2004b).

Bombus terrestris nests are usually constructed in abandoned underground rodent nests in the native ranges (Sladen 1912; Alford 1975; Prys-Jones & Corbet 1991) and also in Japan (Matsumura et al. 2004a, b; Nakajima et al. 2004). Particularly high similarity in nest habitat selection between *B. terrestris* and two native species, *B. hypocrita sapporoensis* Cockerell and *B. diversus tersatus* Smith in southern Hokkaido also suggested the occurrence of competition for nest sites (Matsumura et al. 2004a, b; Nakajima et al. 2004). Nest usurpation by queens commonly occurs in bumblebees (Sladen 1912; Alford 1975; Richards 1978; Paxton et al. 2001). Ono (1997) reported that, in laboratory experiments, *B. terrestris* queens succeeded in usurping the nest of the native bumblebees within the artificial nest boxes. Furthermore, *B. terrestris* queens

are suggested to have marked advantage in occupying suitable nest sites over native queens because of their early emergence from hibernation (Matsumura et al. 2004b).

If *B. terrestris* is similar in ecological niche with native species, it will be advantageous at competition for limited resources because of ecological release from natural enemies regulating its populations. Thus, native bumblebees will decline or even locally extinct through competition. Kawahara (2004) reported that *B. hypocrita sapporoensis* decreased in Koshimizu-cho, east Hokkaido where *B. terrestris* has established. A quantitative survey conducted in 2003 using window traps within an approximately 270 km^2 area of windbreak forest in the Chitose River Basin, central Hokkaido, demonstrated that the distributions of *B. terrestris* and *B. ardens sakagamii* Tkalců were mutually exclusive (Inari et al. 2005). Subsequent investigation in 2004, however, showed that the local abundance of *B. terrestris* was not associated negatively with the number and body size of two native bumblebees, *B. hypocrita sapporoensis* and *B. aridens sakagamii*, and the authors could not demonstrate any evidence of competitive interaction between them (Nagamitsu et al. 2006).

Mating interference and hybridization with closely related species of native bumblebees

Reproductive and genetic interference may be caused by mating between the introduced and native species. Under the laboratory conditions, *B. terrestris* can easily mate with the consubgeneric native species, *B. hypocrita hypocrita* Pérez and *B. ignitus* Panfilov (Ono 1997; Goka 1998). A rare case was found where *B. terrestris* and *B. h. sapporoensis* produced hybrids (Goka 1998). Conversely, the development of embryo was observed to terminate in all cases of interspecific mating between those species

(Goka 1998). Kondo et al. (submitted) ascertained by analyzing the DNA sequence of spermatozoa stored in queen's spermatheca that wild queens of *B. hypocrita sapporoensis* and *B. hypocrita hypocrita*, mated with males of *B. terrestris* at relative high frequency. Most bumblebee species are monandry and even known polyandrous bumblebee species mate at very low frequencies (Estoup et al. 1995; Schmid-Hempel & Schmid-Hempel 2000). If the low mating frequency is also applied to Japanese native bumblebees, the mating with *B. terrestris* may induce the sterility of these native queens or considerable reduction in their fecundity.

Transmission of parasites or pathogens to native bumblebees

Bombus terrestris is a host to an impressive diversity of parasitic organisms in their native ranges (Shykoff & Schmid-Hempel 1991a; Durrer & Schmid-Hempel 1995) as well as in the established populations in New Zealand (Donovan 1980; Macfarlane & Gurr 1995), Tasmania (Allen et al. 2007) and Japan (Goka et al. 2000, 2001, 2006; Niwa et al. 2004). Several mite species (e.g. *Garmaniella* spp., *Hypoaspis* spp. and *Parasitellus* spp.) have phoretic life and live in bumblebee nests where they feed on pollen stores, debris and nest microfauna (Schmid-Hempel 1998). On the other hand, the insect parasitoids, nematodes, and internal microparasites sometimes induce changes in bee foraging behavior (Husband & Sinha 1970; Shykoff & Schmid-Hempel 1991b; Otterstatter et al. 2005), or significantly damage or even kill individuals and colonies (e.g. Müller & Schmid-Hempel 1992a; Schmid-Hempel & Loosli 1998; Brown et al. 2003).

The tracheal mite *Locustacarus buchneri* was found in commercial colonies of *B. terrestris* and the reimported colonies of *B. ignitus* that was commercialized in a

Europe company's factory (Goka et al. 2000, 2001, 2006). A laboratory experiment showed that the mite detected in *B. terrestris* could easily infest the native bumblebees (Niwa et al. 2004). In fact, *L. buchneri* mite infestation of European genotype was detected in natural populations of native bumblebees (Goka et al. 2001, 2006). In addition, the microsporidian *Nosema bombi* was discovered in imported *B. terrestris* (Niwa et al. 2004). These reports suggest that transportation of commercial colonies might cause spread of the parasites and pathogen of different origins into the introduced areas. Although it is difficult to predict the ecological impacts that these parasites will have on native bumblebees, cross-infestation by them may cause more serious damage to native bumblebees because there is no history of relationships between native hosts and alien parasites.

Effects on pollination of native flora

Reproductions of native flowering plants may be potentially affected by *B. terrestris* in several ways. First, the reduction of the long-tongued native bumblebees by competitive exclusion will cause reproductive failure of long-tubed flowers that are pollinated predominantly or exclusively by these bumblebees. Effective pollination requires a match between the morphology of the flower and that of pollinator. If there is a mismatch, then floral rewards may be wasted without efficient transfer of pollen (McDade & Kinsman 1980).

Second, *B. terrestris* has the potential to disrupt pollinator services by nectar robbing, i.e. sucking nectar from a hole by biting on the corolla of flowers with deep corolla tubes (Inouye 1980; Prys-Jones & Corbet 1991). Negative effects include a decrease in the reproductive success of plants (Irwin & Brody 1998, 1999; Navarro

2001), attributed to the infliction of damage to floral tissues by robbers (McDade & Kinsman 1980) or the reduction of attractiveness of robbed flowers to legitimate pollinators (Heinrich 1979; Irwin & Brody 1999; Navarro 2001; Newman & Thomson 2005). In Hokkaido, *B. terrestris* was observed to rob some native plant species: *Vicia cracca* L. (Matsumura et al. 2004b), *Corydalis ambigua* Cham. et Schltdl. (Kenta et al. 2007) and *Polygonatum odoratum* (Mill.) Druce var. *maximowiczii* (Fr. Schm.) Koidz. (M.N. Inoue, personal observation). Dohzono et al. (submitted) demonstrated that robbing *B. terrestris* queens reduced the seed set of *C. ambigua* in the Hidaka region.

Scope and outline of the thesis

Of four types of impacts of *B. terrestris* warned by the ecologists (Washitani et al. 1997; Goka 1998; Washitani 1998; Goka et al. 2001), interspecific mating, cross-infection of parasites and reduction in seed production of flowering plants were partly demonstrated in the natural habitats. Abundance of the introduced species, the high levels of niche overlaps, and decline of native bumblebees, which have been shown by the censuses in three regions of Hokkaido, suggested that competition exclusion is now in progress. However, there is no indisputable evidence that the establishment of *B. terrestris* has caused a decline or even local extinction of native bumblebees by competitive exclusion. In addition, the species ecology and the factors potentially limiting the population growth of *B. terrestris* in Japan are still poorly understood, with a few notable exceptions (e.g. Matsumura et al. 2004a, b; Nakajima et al. 2004). This is mostly because finding feral nests of bumblebees is relatively difficult. Since the artificial rearing method of bumblebees has established in 1980s, investigations of nest activities of bumblebees have been conducted by using either raised colonies in the laboratory or

commercial colonies (e.g. Duchateau & Velthuis 1988; Müller & Schmid-Hempel 1992a, b; Goulson et al. 2002a, b). However, it is not obvious whether, under these conditions, bumblebees act as they do in entirely natural circumstances.

Here, I attempt to assess the ecological impacts on native bumblebee population through competitive exclusion by field surveys and investigation of feral nests of *B. terrestris* in Hokkaido. The possibilities of competitive exclusion are enhanced when niche overlaps between the invader and native species, the common resources are limited, and the invader has a competitive advantage. In this thesis, I assess the possibilities of competitive exclusion of native bumblebees by *B. terrestris* by: (1) examining potential niche overlaps in flower resources and nest sites; (2) evaluating resources limitation for bumblebees; and (3) estimating competitive ability of *B. terrestris*, that is, the reproductive ability per successful colony and the potential population growth at local scale. In addition, I assess potential ecological impacts of *B. terrestris* on the mutualistic pollination systems in the maritime grassland in the Notsuke Peninsula as a model of a conservationally important region. The composition of this thesis and the contents of each chapter are as follows.

In Chapter 2, I examine potential niche overlaps in flower resources between *B. terrestris* and native bumblebees in terms of morphological characteristics, seasonal cycles of flight activity, foraging habitat use, and flower visits. I also investigate nesting habitat selections among the species to infer the possibility of competition for nest sites. Based on these data of niche overlaps, I evaluate potential competition for these resources between them. In addition, I estimate the amount of available flower resources for bumblebees and nest usurpation frequency among queens, and then discuss the causes of the decline of a certain native bumblebee species.

In Chapter 3, I perform a detailed investigation of *B. terrestris* feral nests collected in the study area and assess the colony growth potential and the reproductive ability of *B. terrestris* colonies.

In Chapter 4, I investigate the size selection on *B. terrestris* queens through hibernation and nest founding and quantify the demographic parameters during solitary phase. By combining the quantitative information on the potential productivity of new queens per nest (Chapter 3), I attempt to evaluate the potential population growth of *B. terrestris*.

In Chapter 5, I assess the ecological impacts of *B. terrestris* newly invading into the maritime grassland in a preserved area, which is one of the restricted distribution ranges of a rare native species, *B. florilegus* Panfilov. I examine potential niche overlaps between *B. terrestris* and native bumblebees and investigate a feral nest of *B. terrestris* found in the maritime grassland.

In Chapter 6, I finally discuss the ecological impacts on native ecosystems and the potential population growth of *B. terrestris* as well as future implications for effective management of its established population.

Photo 1-1 A worker of *Bombus terrestris* foraging the native plant species, *Stachys japonica* var. *intermedia* in the Iburi region, southern Hokkaido.

CHAPTER 2

Displacement of Japanese Native Bumblebees by the Recently Introduced *Bombus terrestris* in Northern Japan

Introduction

Bombus terrestris is naturally distributed in Europe and adjacent territories, including England, most of Scotland, the north coast of Africa, southern Scandinavia, major Mediterranean Islands, and some Atlantic Islands (Madeira and the Canary Islands) (Estoup et al. 1996; Chittka et al. 2004; Velthuis & van Doorn 2006). Since *B. terrestris* became commercially available as a valuable pollinator of greenhouse crops in the late 1980s, this species has been shipped throughout the world in vast numbers (Goka et al. 2001; Hingston et al. 2002). The invasive potential of *B. terrestris* has been suggested by its successful naturalization in New Zealand (Donovan and Wier 1978; Donovan 1980; MacFarlane & Gurr 1995; Goulson & Hanley 2004), Israel (Dafni & Shmida 1996; Dafni 1998), Chile (Ruz & Herrera 2001), and Tasmania, Australia (Semmens et al. 1993; Semmens 1996; Hingston & McQuillan 1998; Stout & Goulson 2000; Hingston et al. 2002; Stout et al. 2002; Hingston 2005, 2006; Hingston et al. 2006).

In Japan, *B. terrestris* has been used for pollination of greenhouse crops, particularly tomato, since 1991 (Ono 1998). Importation of *B. terrestris* colonies has been increasing annually, and approximately 70,000 colonies were used in 2004 (Kunitake & Goka 2006). Since the introduction of *B. terrestris* to Japan in 1991, researchers have warned about the strong potential impacts on native bumblebee species and mutualistic pollination systems (Kato 1993; Washitani & Morimoto 1993;

16

Washitani et al. 1997). Unfortunately, the warnings are becoming real. In 1996 a naturally occurring nest, which was the first evidence of naturalization of *B. terrestris* in Japan, was found in Monbetsu in the Hidaka region of Hokkaido, northern Japan, close to the agricultural area where *B. terrestris* was mass introduced (Washitani 1998). Since then, increasing numbers of *B. terrestris* queens emerging from hibernation have been recorded around the area, and this species had become dominant in the bumblebee fauna by 2003 (Matsumura et al. 2004b). Monitoring in the Hidaka region revealed that *B. terrestris* foraged on the flowers of approximately 100 species, of which 40 were native, suggesting potential competition for floral resource with native bumblebees (Matsumura et al. 2004b). A quantitative survey conducted in 2003 using window traps showed that the distributions of *B. terrestris* and certain native bumblebee species were mutually exclusive (Inari et al., 2005). Subsequent investigations in 2004, however, showed that the local abundance of *B. terrestris* was not associated negatively with the number and body size of native bumblebees, and evidence of competition between introduced and native bumblebees could not be demonstrated (Nagamitsu et al. 2006).

Mass infestation of *B. terrestris* queens was observed in 2003 in the Iburi region of southern Hokkaido, about 10 km away from a large source of bumblebees introduced for greenhouse tomato cultivation (Yokoyama et al. 2004). Although some *B. terrestris* colonies have been used in greenhouses in this area, the numbers are negligible compared with those used in the adjacent Hidaka region. The *B. terrestris* observed in the Iburi region appear to be naturalized and this species could have adverse effects on native flower-visiting insects through competition, either for floral resources (nectar and pollen) or for nest sites.

In this chapter, I assessed whether and in what ways native bumblebee species

were affected by the invasion of this new potential competitor, *B. terrestris*, in the Iburi region. I investigated potential niche overlaps between *B. terrestris* and native bumblebees in terms of morphological characteristics that are related to the range of flower use (Inoue & Yokoyama 2006), seasonal cycles of flight activity, foraging habitat use, and flower visits. I also investigated nesting habitat preferences among the species to infer the possibility of competition for nest sites. Based on the data, I evaluated potential competition for these resources and discussed the mechanisms for displacement of native bumblebee species by *B. terrestris*.

Materials and Methods

Study area

The study was carried out 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). The landscape of this region is mainly composed of rice paddies (Photo 2-1 (a)) and other cultivated farmland, surrounded by windbreak tree belts of *Fraxinus mandshurica* Rupr. var. *japonica* Maxim. and *Alnus japonica* (Thunb.) Steud. Wetland (Photo 2-1 (b)). There are also some patchy stands of *Larix leptolepis* Gord. plantation. The vegetation on the edge of the farmlands is dominated by several non-native herbaceous plants: *Taraxacum officinale* Weber in spring; *Trifolium pratense* L., *Trifolium repens* L., and *Solidago gigantea* Ait. var. *leiophylla* Fern. in summer; and *Aster novi-belgii* L. in autumn.

Monitoring *Bombus terrestris* and native bumblebees

A monitoring area of approximately 7.5 km² (42°35'–37'N, 141°53'–56'E) was set out in

the study area to include the representative habitats. According to calculations based on a 1:25,000 topographic map (issued by Geographical Survey Institute in 2002), the percentages of each habitat are 80.0 % of rice paddies, 6.4 % of other cultivated farmlands, 1.7 % of riverbed, 6.6 % of windbreak tree belt, and 5.3 % of residential areas.

The abundance of bumblebee species was surveyed in the monitoring area from May to September in 2003, 2004, and 2005. In censuses conducted along roads, riverbeds, and farmland ridges, I recorded the date, time, location, habitat types, bumblebee species, caste (queen, worker, or male), and plant species whose flowers were visited. I captured *B. terrestris* individuals as many as possible, because another major aim of this research is to suppress the naturalized population of this species. The investigation effort was 85.7 person-h in 2003, 101.3 in 2004, and 91.4 in 2005.

Foraging niche overlap between *Bombus terrestris* and native bumblebees
Measurement of morphological characters

To demonstrate potential overlap of flower resource use between *B. terrestris* and native bumblebees, morphological characteristics that affect the range of usable flowers were compared (Inoue & Yokoyama 2006). Native bumblebees were collected with insect nets and anaesthetized with carbon dioxide to measure the following body parts (after Inoue & Yokoyama 2006): head width (HW), head length (HL), prementum length (PL), glossa length (GL), maximum thorax width (TW), and maximum abdomen width (AW). I then marked each bumblebee with a colored paint dot on its thorax to avoid recapture, and released it at the point of capture. Measurements of body parts were made on native bees collected from the Iburi and Hidaka regions during 1998–2005. *Bombus terrestris*

19

captured from the same regions in 2003 were stored at −20 °C, and their body parts were measured in the laboratory.

Habitat use, flower resources, and active season

The study of foraging niche overlaps between *B. terrestris* and native bumblebees was conducted in early and late May and once monthly from June to September 2004. Three transects (T1, T2, T3), covering 13,590 m^2 in total, were placed within the study area. T1 and T3 included several habitat types, whereas T2 contained only wooded habitat (see Table 2-1 for transect details). Habitats were classified into the following five types (modified from Svensson et al. 2000): (1) farmland boundary (Fb), a grass-dominated strip between two farmland patches or between farmland and other open ground; (2) road boundary (Rb), a grass-dominated strip between a drivable road and farmland or other open ground; (3) other open ground (Op), mainly uncultivated open ground with abundant grass, tussocks, and stones (this habitat includes a grass-dominated strip along the riverbed of the Irisikabetsu River); (4) woodland boundary (Wob), a herbaceous vegetation strip between a windbreak belt and farmland, mainly consisting of grass, herbs, and shrubs; and (5) woodland (Wo), deciduous woodland with undergrowth mainly consisting of dwarf-shrubs and herbs.

Bumblebees were counted using the transect method (Banaszak 1980; Teräs 1983; Saville et al. 1997), and all bees in an area extending 1.5 m on either side of each transect were observed. For each observation, I noted the bumblebee species, the habitat type, and the plant species where the bee was foraging. T1 and T2 were surveyed twice, but no observations were made along T3 in early May. Each transect was inspected three times from late May to September. Walking speed during a transect inspection was

approximately 1.5 km h^{-1}. Inspections were conducted at three times of day: morning (08:00–10:00), noon (11:00–14:00), and afternoon (15:00–17:00).

Estimation of flower resources available for bumblebees

The two forage resources for bees are flower nectar and pollen. Nectar is mainly used as an energy source for adult bees, and pollen is the principal constituent of food for developing larvae. To estimate the amount of these resources available for bumblebees, the number of flowers/inflorescences of each flowering plant species that bees were observed foraging on was counted once a month from May to September 2005. This investigation was conducted along T1 because this transect was representative of the habitat condition in the study area where *B. terrestris* was extensively naturalized. Based on the floral architecture of the plant species, flowers were counted as racemes or panicles (e.g., *Barbarea vulgaris* R. Br. and *S. gigantea* var. *leiophylla*), heads (e.g., *Trifolium* spp.), capitulae (e.g., *T. officinale*), spikes (e.g., *Vicia villosa* Roth), or individual flowers (e.g., *Symphytum officinale* L. and *Impatiens textori* Miq.). Observations of bumblebees were also carried out every 3–8 days.

Nest sites and nest usurpation

Nest sites of *B. terrestris* and native bumblebees were found during the 2003–2005 censuses. In the course of excavating or investigating *B. terrestris* nests, both live and dead queens were found in and around the nests. Bumblebee queens vary greatly in their time of emergence from hibernation, even within species. When searching for nest sites, late-emerging queens will often find that suitable sites are already occupied by other queens. In this situation, they may attempt to take over an established nest (Sladen

1912; Alford 1975; Goulson 2003a). Live and dead queens found in and around the nests were presumably the successive usurpers and foundress queen. The numbers of live and dead queens were counted as an indicator of competition for nest sites. Foundresses could be distinguished from newly emerged queens based on the reduced body hair and damaged edges of wings.

Statistical analysis

Principal component analysis (PCA) was used to analyze the morphological variation within and among the native bumblebee species and *B. terrestris*. PCA was performed with Excel Statistics version 1.1 (Social Survey Research Information Co. Ltd., Tokyo, Japan).

The null hypothesis that the use of a particular habitat type occurs in proportion to its availability when all habitat types are considered simultaneously was tested with χ^2 goodness-of-fit analysis (Neu et al. 1974). Observations in each habitat type were summed for this analysis. When significant results were obtained, each habitat type was tested with a Bonferroni Z-test (Z score = 2.58 at P = 0.05) to see whether its use diverged significantly from availability (level of significance chosen following Neu et al., 1974).

The similarity of flower resource use between *B. terrestris* and native bumblebees (i.e., the plant species visited during a given study period in a given habitat) was calculated according to Horn (1966). The niche overlap index was calculated for queens, workers, and males of each bumblebee species as a whole, ranging from zero in the case of no overlap to one in the case of full overlap between species.

Results

Temporal pattern of abundance of *Bombus terrestris* and native bumblebees

During the three years, *B. terrestris* (subgenus *Bombus* s. str.) and six native species, *B. hypocrita sapporoensis* (*Bombus* s. str.), *B. ardens sakagamii*, *B. hypnorum koropokkrus* Sakagami et Ishikawa (*Pyrobombus*), *B. schrencki albidopleuralis* Skorikov (*Thoracobombus*), *B. pseudobaicalensis* Vogt, and *B. diversus tersatus* (*Diversobombus*), were recorded in the monitoring area (Table 2-2, Photo 2-2). *Bombus terrestris* was found to be the most abundant, comprising 52.5 % of the observations in 2003, 68.5 % in 2004, and 74.2 % in 2005. In contrast, the number of observations of *B. hypocrita sapporoensis* and its proportion among all species markedly decreased from 1.75 individuals person-h^{-1} (23.3 %) in 2003 to 0.62 (5.9 %) in 2004 and 0.84 (8.2 %) in 2005. *Bombus diversus tersatus* showed a similar trend, whereas *B. pseudobaicalensis* showed no noticeable change over the three years. Only small numbers of *B. ardens sakagamii*, *B. hypnorum koropokkrus,* and *B. schrencki albidopleuralis* were observed during the entire monitoring period.

The number of *B. terrestris* queens witnessed per year increased over the course of the study from 7.27 individuals person-h^{-1} in 2003 to 15.31 in 2005. Whereas, *B. hypocrita sapporoensis* queens decreased from 8.24 individuals person-h^{-1} in 2003 to 0.13 in 2004 and 0.06 in 2005 (Fig. 2-1). In 2004 this number was 4.45 individuals person-h^{-1}; for two days in early spring of that year, collaborated with citizen volunteers on a *B. terrestris* exclusion project (Kojima 2006). During this project 484 of 603 observed *B. terrestris* queens were captured with an effort of 21.4 person-days (C. Matsumura, personal communication), and this high pressure of capture may have

23

caused the reduction of *B. terrestris* in 2004.

Foraging niche overlap between *Bombus terrestris* and native bumblebees

Morphological variations

Morphological measurements were made on 417 individuals of six native bumblebee species and 720 *B. terrestris* (Table 2-3). PC1 accounted for all measured characters with positive coefficients. PC2 largely accounted for PL and GL (positive coefficients) and for HW, TW, and AW (negative coefficients). These two axes represented 77 % and 16 % of the total variation, respectively, and thus 93 % of the total variation was expressed by PC1 and PC2 (Table 2-4, Fig. 2-2). *Bombus diversus tersatus* and *B. hypocrita sapporoensis* were clearly distinguished from other native species based on the six morphological characters measured: the former had a long proboscis and a narrow body, whereas the latter had a short proboscis and a wide body. Two pairs of consubgeneric species, *B. ardens sakagamii* and *B. hypnorum koropokkrus* and *B. schrencki albidopleuralis* and *B. pseudobaicalensis*, largely overlapped with each other with regard to morphological characteristics. Compared with these native bumblebee species, *B. terrestris* had a shorter proboscis and a wider body; its morphology overlapped considerably with *B. hypocrita sapporoensis* and partly with *B. ardens sakagamii* and *B. hypnorum koropokkrus*.

Seasonal cycles of flight activity

Bombus terrestris and five native bumblebee species were divided into two groups (Fig. 2-3). The first group consisting of only *B. ardens sakagamii* has a short life cycle and ends its yearly activity before midsummer. The second category consists of *Bombus*

terrestris and the other four native species. These bumblebees are long-lived, lasting into October, being slow to develop to their full strength. *Bombus terrestris* queens first appeared in late May, and a new generation of newly emerged queens was observed in August and September. Workers of this species emerged in June, and have the longest flight season. *Bombus hypocrita sapporoensis* and *B. schrencki albidopleuralis* workers started to appear in June and were continuously observed until late August. *Bombus pseudobaicalensis* and *B. diversus tersatus* workers became active in July and were observed until late September. *Bombus hypnorum koropokkrus* was excluded from the analysis because only two individuals were observed during the three-year investigation.

Foraging habitat use

Individual bumblebee species showed significant associations with some of the five habitat types (χ^2-squared = 9.49, df = 4, P < 0.01, Fig. 2-4). *Bombus terrestris* and *B. pseudobaicalensis* were observed in all habitats except woodland. They used woodland boundary and woodland significantly less than expected according to availability (Table 2-5) and foraged selectively in open habitats. *Bombus terrestris* showed a significant preference for road boundary that was not favored by native species. Conversely, *B. schrencki albidopleuralis* was exclusively observed in woodland. *Bombus hypocrita sapporoensis* and *B. diversus tersatus* were observed in all five habitats, but the latter foraged significantly less in the open habitats, except for other open ground.

Flower visits

I recorded 44 plant species visited by *B. terrestris* and/or native bumblebee species

(Table 2-6). *Bombus terrestris* visited flowers of 26 plant species, including 17 native and nine non-native species. The non-native plant species were far more frequently visited, accounting for 86.8 % of recorded visits. *Bombus hypocrita sapporoensis* showed similar plant preferences. *Bombus pseudobaicalensis* was also found predominantly on non-native legumes, such as *Trifolium* spp., whereas two of the four common native bumblebee species, *B. schrencki albidopleuralis* and *B. diversus tersatus*, were found more frequently on native flowers (63.6 % and 59.5 %, respectively). Compared with the other species, *B. diversus tersatus* tended to forage on flowers with a deep corolla or gullet-shaped flowers.

Niche overlap in flower resource use

Horn's (1966) niche overlap index was calculated for flower resource use based on seasonal flight activity, foraging habitats, and flower visits (Table 2-7). *Bombus ardens sakagamii* and *B. hypnorum koropokkrus* were excluded from this analysis because of very low frequencies of observation. Niche overlaps with *B. terrestris* varied greatly among the other native bumblebee species. *Bombus pseudobaicalensis* (0.60) and *B. hypocrita sapporoensis* (0.48) showed large overlaps, whereas *B. diversus tersatus* showed a relatively small overlap (0.26) and *B. schrencki albidopleuralis* showed no overlap (0.00).

Estimation of flower resources for bumblebees

A total of 23 plant species were recorded along T1 in 2005, including 10 non-native species. *Barbarea vulgaris* (n = 27,397 in total) and *T. officinale* (n = 59,958) were major flower resources for bumblebees in May and June, and *T. repens* (n = 125,525)

and *T. pratense* (n = 59,690) were important flower resources from July to September. The total number of flowers/inflorescences was approximately 300,000 along T1, and 551 bumblebees were observed: 333 *B. terrestris*, 29 *B. hypocrita sapporoensis*, 124 *B. pseudobaicalensis*, one *B. schrencki albidopleuralis*, and 64 *B. diversus tersatus*.

Nest sites of *Bombus terrestris* and native bumblebees

During the three-year study, I found 30 *B. terrestris* nests, eight *B. hypocrita sapporoensis* nests, seven *B. pseudobaicalensis* nests, and one *B. diversus tersatus* nest in the study area. Most *B. terrestris* nests were constructed in abandoned underground rodent nests with entrance tunnels in ridges between rice paddies or other cultivated farmland or in dry riverbed sites. The eight *B. hypocrita sapporoensis* nests and one *B. diversus tersatus* nest were also found underground in similar microhabitats. In contrast, all seven nests of *B. pseudobaicalensis* were aboveground, covered with grass litter or a moss layer.

Nest usurpation in the *Bombus terrestris* nests

Eighteen of the 30 *B. terrestris* nests were collected, and the numbers of live and dead queens (excluding newly emerged queens) were counted. Only one extra queen was found in one nest in 2003 (mean number of queens: 1.33 ± 0.58, range: 1–2) and four dead queens were in and around one nest in 2004 (mean: 1.75 ± 1.50, range: 1–4). In 2005, however, the average number of queens found in and around the nests including the apparent foundress was 3.45 ± 2.25 (range: 0–7).

Discussion

Interspecific interactions such as competition may affect species survival and the stability of ecological communities. In general, two or more species vying for a limited common resource in a community are separated by differences in temporal activity patterns, habitat use, or foraging behavior, and competition leads to a partitioning of these resources to minimize niche overlap (Schoener 1974). For example, visitors to the same flowers tend to forage during different portions of the season (Ginsberg 1983) or at different times of the day (Paton 1993). When niche overlap occurs, however, competition may cause a reduction in fecundity, survivorship, or growth of one or both species. Thus, a newly invading species may restructure the ecological community; if the invading species has a competitive advantage, this may cause suppression or even extinction of native species (Mack et al. 2000).

The present study indicated that the native bumblebee species in the study area coexist by partitioning seasonal cycles of foraging activity, foraging and nesting habitats, and flower resources (Table 2-8). *Bombus ardens sakagamii* and *B. hypnorum koropokkrus* have short flight seasons, with the annual generation ending before midsummer, when other species are at the peak of their activities (Matsuura 1995). Of the other four species with long flight seasons, *B. hypocrita sapporoensis* and *B. diversus tersatus* were clearly differentiated by morphological characteristics (Fig. 2-2) and showed distinct flower visiting patterns (Table 2-7). *Bombus pseudobaicalensis* and *B. schrencki albidopleuralis*, belonging to the same subgenus, have markedly similar morphological characteristics (Fig. 2-3) and use similar nesting sites (Sakagami & Katayama 1977; Matsuura 1995; Matsumura et al. 2004a, b), but they differ significantly in foraging habitat use: *B. pseudobaicalensis* foraged throughout the open

28

habitats, whereas *B. schrencki albidopleuralis* was almost exclusively found in woodlands. These relationships among native bumblebees were likely formed over a long period of coexistence and evolution.

Bombus *terrestris*, however, has no history of coexistence with Japanese native bumblebees. Demonstration of niche overlap in this study suggested that *B. terrestris* may compete for flower resources and nest sites with *B. hypocrita sapporoensis*, for flower resources with *B. pseudobaicalensis*, and for nest sites with *B. diversus tersatus*. During the three years of monitoring, *B. pseudobaicalensis*, which shares only flower resources with *B. terrestris*, showed no noticeable reduction in its abundance (Table 2-2). The great abundance of non-native plants (e.g. *Taraxacum officinale*, *Barbarea vulgaris* and *Trifolium* spp.) in the study area can provide sufficient primary food supplies for all bumblebees throughout their active season. Such plants species tend to grow in high density in disturbed sites and may decrease effects of flower resource competition. It has been reported that mass flowering by tropical plant species buffers native bee species from resource competition with introduced species (Wilms et al. 1996; Minckley et al. 2003). The abundance of non-native plants in the study area may also prevent competition for flower resources between *B. terrestris* and *B. pseudobaicalensis*.

Even though *B. terrestris* was captured and removed during the survey, this study revealed an increase in the number of *B. terrestris* queens accompanied by a marked reduction in *B. hypocrita sapporoensis* queens in spring (Fig. 2-1). Most *B. terrestris* nests found in this study were constructed in abandoned underground rodent nests on the edge of farmland, as reported in previous studies (Sladen 1912; Alford 1975; Donovan & Wier 1978; Prys-Jones & Corbet 1991). The native species that

declined in abundance during this study, *B. hypocrita sapporoensis* also use abandoned rodent nests in similar microhabitats (Sakagami & Katayama 1977; Matsumura et al. 2004a, b; Nakajima et al. 2004; Takamizawa 2005; this study). Nakajima et al. (2004) observed that *B. terrestris* and *B. hypocrita sapporoensis* simultaneously used the same nesting hole. In addition, the average number of live and dead queens (including foundresses) found in *B. terrestris* nests increased between 2003 and 2005 (Table 2-8). These facts indicate that availability of nest sites is limiting and competition for nest sites among queens has become stronger, accompanied by an increase of *B. terrestris* queens. In the Canadian study, 95 usurpations were recorded, most of which involved nests of the same species, but 17 % involved takeovers of nests by different but closely related species (Richards 1978). This behavior was explained by a lack of available nest sites. Ono (1997) also reported that, in laboratory experiments, when *B. terrestris* queens were introduced into nest boxes containing incipient colonies of the consubgeneric species *B. ignitus* and *B. hypocrita hypocrita*, the *B. terrestris* queens succeeded in usurping the *B. ignitus* nest. Furthermore, the appearance of *B. terrestris* queens in spring is earlier than that of *B. hypocrita sapporoensis* (Matsumura et al. 2004b). Therefore, *B. terrestris* may occupy available nest sites before the native species appear in spring, resulting in a shortage of suitable nest sites. In fact, eight *B. terrestris* and seven *B. hypocrita sapporoensis* nests were found in 2003 but no *B. hypocrita sapporoensis* nest was found in the monitoring area since then. By contrast, seven and 15 *B. terrestris* nests were in 2004 and 2005, respectively. This suggests that *B. hypocrita sapporoensis* has been unable to establish nests and thus has decreased in number.

All the previous instances of *B. terrestris* naturalization were reported from

regions with no native bumblebees, and the species' naturalization in Japan is the first instance observed in the range of native bumblebees. In this study, I demonstrated that the strong invasive activity of *B. terrestris* was associated with decreased abundances of the native species of bumblebee. I was also able to distinguish interspecific competition between *B. terrestris* and the native bumblebees for particular resources. The findings suggest that competition for nest sites would be the major ecological mechanism for the displacement of native species, rather than competition for flower resources. In addition, the rapid expansion of *B. terrestris* and the large reduction in the number of *B. hypocrita sapporoensis* queens in spring indicates that introduced *B. terrestris* may cause local extinction of native bumblebees. *Bombus hypocrita sapporoensis* also decreased in other regions of Hokkaido where *B. terrestris* has established (Kawahara 2004). Therefore, methods to control *B. terrestris* populations in the wild urgently need to be established, and the effect of *B. terrestris* on native bumblebee communities has to be monitored over a long time.

Table 2-1 The proportion (%) of habitat types in each transect where bumblebee species were counted from May to September 2004.

Transect	Habitat type (%)[1]					Transect Total (m)[2]	N[3]
	Fb	Rb	Op	Wob	Wo		
T1	33	14	36	17	0	2230	17
T2	0	0	0	0	100	770	17
T3	20	4	0	76	0	1530	15
Habitat total (m)[4]	1042	373	803	1542	770	-	-
Habitat area (m^2)[5]	3126	1120	2408	4626	2310	-	-
Total length (m)[6]	17100	6225	13648	23887	13090	-	-

[1] Fb, farmland boundary; Rb, road boundary; Op, other open ground; Wob, woodland boundary; Wo, woodland.
[2] Total length of transects
[3] Number of inspections in each transect during the season
[4] Total length of transects in each habitat
[5] Total area of each habitat
[6] Total length of each habitat inspected during the season

Table 2-2 Total number of observations of foraging individuals of each species for the duration of this experiment, expressed as a percentage of the total number of observations of all species in 2003, 2004, and 2005. The effort toward investigation was 85.7 person-hours in 2003, 101.3 in 2004, and 91.4 in 2005.

Species[1]	2003			2004			2005		
	No.	No. person-h^{-1}	Percentage	No.	No. person-h^{-1}	Percentage	No.	No. person-h^{-1}	Percentage
Bt	339	3.96	52.5	730	7.21	68.5	700	7.66	74.2
Bhs	150	1.75	23.3	63	0.62	5.9	77	0.84	8.2
Bhk	3	0.04	0.5	4	0.04	0.4	2	0.02	0.2
Bas	10	0.12	1.5	8	0.08	0.7	3	0.03	0.3
Bsa	5	0.06	0.8	3	0.03	0.3	3	0.03	0.3
Bp	87	1.02	13.5	215	2.12	20.2	129	1.41	13.7
Bdt	51	0.60	7.9	43	0.42	4.0	29	0.32	3.1
Total	645	7.55		1066	10.52		943	10.31	

Bumblebee species are arranged in ascending order of proboscis length.

[1] Bt, *Bombus terrestris*; Bhs, *B. hypocrita sapporoensis*; Bas, *B. ardens sakagamii*; Bhk, *B. hypnorum koropokkrus*; Bsa, *B. schrencki albidopleuralis*; Bp, *B. pseudobaicalensis*; and Bdt, *B. diversus tersatus*.

Table 2-3 The mean ± SD (mm) of body size for bumblebee species and their castes.

Species	Caste	N	HW	HL	PL	GL	TW	AW
Bt	Q	268	5.5±0.2	5.3±0.3	3.5±0.3	7.8±0.7	7.9±0.5	9.5±0.5
	W	214	4.0±0.3	3.8±0.3	2.8±0.3	5.4±0.6	4.8±0.5	6.0±0.6
	M	238	4.4±0.2	4.2±0.3	3.2±0.3	6.1±0.4	5.4±0.4	6.9±0.5
Bhs	Q	24	5.5±0.1	5.2±0.2	3.9±0.2	7.2±0.3	7.8±0.2	9.8±0.4
	W	26	4.0±0.2	3.8±0.3	2.8±0.5	5.3±0.4	5.1±0.4	6.3±0.6
	M	2	4.4±0.1	4.1±0.0	2.8±0.3	5.7±0.1	5.6±0.1	7.2±0.2
Bhk	Q	0						
	W	24	3.5±0.2	3.7±0.2	2.7±0.2	5.3±0.4	4.6±0.2	5.7±0.4
	M	2	4.1±0.1	4.2±0.3	2.9±0.2	6.7±0.4	5.1±0.2	6.6±0.9
Bas	Q	3	4.6±0.2	5.1±0.0	3.6±0.1	7.7±0.4	6.6±0.1	8.2±0.2
	W	37	3.8±0.2	4.2±0.3	3.1±0.3	6.5±0.5	4.9±0.4	6.1±0.8
	M	7	4.0±0.3	4.3±0.4	3.3±0.4	6.9±0.5	5.0±0.4	6.5±0.6
Bsa	Q	13	4.6±0.1	5.0±0.1	3.7±0.3	8.7±0.4	6.5±0.3	8.3±0.5
	W	32	3.6±0.3	4.0±0.5	3.0±0.2	6.3±0.7	4.3±0.6	5.4±0.6
	M	3	2.6±0.1	3.8±0.3	3.0±0.1	6.4±0.2	4.5±0.2	5.7±0.2
Bp	Q	40	4.7±0.1	5.2±0.2	4.1±0.3	8.9±0.4	6.5±0.4	7.9±0.3
	W	59	3.7±0.3	4.1±0.3	3.1±0.2	6.7±0.7	4.6±0.5	5.4±0.6
	M	13	3.8±0.2	4.3±0.2	3.1±0.3	6.9±0.6	4.8±0.3	5.9±0.4
Bdt	Q	44	5.0±0.1	6.0±0.2	4.7±0.4	12.6±0.5	7.0±0.3	8.8±0.4
	W	64	3.7±0.2	4.4±0.4	3.4±0.3	8.6±1.1	4.3±0.5	5.3±0.6
	M	24	3.8±0.1	4.4±0.2	3.5±0.2	9.3±0.4	4.8±0.3	6.1±0.4

Q, queen; W, worker; M: male.
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 2-2.

Table 2-4 Correlation coefficients of morphological characters in 1,137 individuals with the first two factors of principal component analysis. Together, PC1 and PC2 account for 94 % of the total variance in morphology.

Morphological character	Correlation coefficients	
	PC1	PC2
HW	0.42	−0.38
HL	0.44	0.12
PL	0.36	0.52
GL	0.35	0.60
TW	0.43	−0.33
AW	0.43	−0.32
Eigenvalue	4.61	0.98
Percent variance explained	77	16

Symbols as in Table 2-3.

Table 2-5 Results of Bonferroni Z-tests for avoidance of or preference for each habitat type. The signs indicate whether the class was preferred (+), avoided (−), or used in proportion to its availability (=). Significance was set at $P = 0.05$ (Z-score = 2.58; see Neu et al. 1974).

| Species | Habitat type | | | | |
	Fb	Rb	Op	Wob	Wo
Bt	=	+	=	−	−
Bhs	=	=	+	−	=
Bsa	−	−	−	−	+
Bp	=	=	+	−	−
Bdt	−	−	+	−	=

Symbols as in Tables 2-1 and 2-2.

Table 2-6 Plant species visited by bumblebees in 2004.

Family	Plant species	Habitat	Bumblebee species						
			Bt	Bhs	Bhk	Bas	Bsa	Bp	Bdt
Actinidiaceae	*Actinidia polygama*	Wo							2
Apiaceae	*Heracleum lanatum* subsp. *asiaticum*	Wo					1		
Asclepiadaceae	*Metaplexis japonica*	Rb	1						
Asteraceae	*Aster novi-belgii**	Rb,Fb,Wob,Fp	42					6	2
Asteraceae	*Bidens forndosa**	Wob	4						
Asteraceae	*Cacalia hastata*	Wo					1		1
Asteraceae	*Cirsium pendulum*	Rb	1						
Asteraceae	*Crisium kamtschaticum*	Wo				2	4		8
Asteraceae	*Inula britannica* subsp. *japonica*	Fb	1						
Asteraceae	*Rudbeckia laciniata**	Wob,Wo	36				13	4	
Asteraceae	*Solidago gigantea* var. *leiophylla**	Rb,Wob,Wo	64	7			11	2	3
Asteraceae	*Solidago virgaurea* var. *asiatica*	Wob,Wo	1				1		3
Asteraceae	*Stenactis annuus*	Op	1						
Asteraceae	*Taraxacum officinale**	Rb,Fb,Op	17	4				2	1
Balseminaceae	*Impatiens noli-tangere*	Wob							1
Boraginaceae	*Symphytum officinale**	Fb,Wob	4			2		2	4
Brassicaceae	*Barbarea vulgaris**	Fb,Op	12	5					
Commelinaceae	*Commelina communis*	Op							1
Convolvulaceae	*Calystegia japonica*	Rb,Wob							2
Fabaceae	*Amphicarpaea edgeworthii* var. *japonica*	Wo							2
Fabaceae	*Trifolium pratense**	Fb,Rb,Op,Wob,Wo	44	3		1	2	56	73
Fabaceae	*Trifolium repens**	Fb,Rb,Op,Wob,Wo	73	17	2		2	35	3
Fabaceae	*Vicia villosa*	Rb,Wob	5	5				9	
Geraniaceae	*Geranium thunbergii*	Op,Wo					1	1	
Hydrangeaceae	*Deutzia crenata*	Rb	1						
Hydrangeaceae	*Hydrangea paniculata*	Wob	1						
Hydrangeaceae	*Staphylea bumalda*	Wo		1					
Iridaceae	*Iris ensata* var. *spontanea*	Wob							1
Lamiaceae	*Clinopodium chinense* var. *parviflorum*	Wob	3						
Lamiaceae	*Elsholtzia ciliata*	Fb,Rb,Op,Wob,Wo	7					8	12
Lamiaceae	*Lamium album* var. *barbatum*	Wo					3		1
Lamiaceae	*Scutellaria strigillosa*	Wob							1
Lamiaceae	*Stachys japonica* var. *intermedia*	Wob	2					2	6
Oleaceae	*Ligustrum obtusifolium***	Rb,Wob	1						1
Onagraceae	*Oenothera biennis**	Wo							1
Papaveraceae	*Corydalis ambigua*	Wo				1			
Polygonaceae	*Persicaria longiseta*	Fb,Op	1					1	
Ranunculaceae	*Anemone flaccida*	Wo					1		
Rosaceae	*Filipendula multijuga*	Wob	1						
Rosaceae	*Rubus crataegifolius*	Wob	2				3	2	4
Rosaceae	*Rubus parvifolius*	Wo				2	3		
Rosaceae	*Sanguisorba tenuifolia* var. *alba*	Wob	5						1
Rosaceae	*Spiraea salicifolia*	Wob	11					4	5
Ruscaceae	*Polygonatum odoratum*	Wo					1		

*Non-native plant species
** Cultivated forms of native plant
Symbols as in Tables 2-1 and 2-2.

Table 2-7 Horn's (1966) index of niche overlap calculated based on seasonal patterns of activity, habitat preference, and flower visits.

	Bt	Bhs	Bsa	Bp	Bdt
Bt		0.48	0.00	0.60	0.26
Bhs			0.12	0.39	0.12
Bsa				0.00	0.21
Bp					0.49
Bdt					

Symbols as in Table 2-2.

Table 2-8 Attributes of bumblebee ecology in Hokkaido based on data from this study.

Species	Subgenus	Sample size	Tongue length[1] Q	W	M	Active period	Habitat	Nesting site[2]	Distribution in Hokkaido[2]
Bt	*Bombus*	341	11.3±0.7	8.2±0.8	9.2±0.6	Long	Open	Underground	
Bhs	*Bombus*	42	11.0±0.4	7.9±0.6	8.5±0.4	Long	Open	Underground	Widespread, common[a,b,c]
Bhk	*Pyrobombus*	2		8.0±0.6	10.3±0.4	Short		Underground[b]	Local[a,b,c]
Bas	*Pyrobombus*	11	11.3±0.4	9.6±0.7	10.2±0.7	Short		Variable[b]	Local[a,b,c]
Bsa	*Thoracobombus*	44	12.4±0.5	9.3±0.6	9.4±0.2	Long	Wooded	Surface	Local[a,b,c]
Bp	*Thoracobombus*	134	12.9±0.5	9.7±0.8	10.0±0.8	Long	Open	Surface	Widespread, common[a,b,c]
Bdt	*Diversobombus*	148	17.3±0.7	12.0±1.3	12.8±0.5	Long	Variable	Underground	Widespread, common[a,b,c]

[1] Tongue length is sum of prementum and glossa lengths.
[2] References: [a] Ito 1991; [b] Matsuura 1995; [c] Washitani et al. 1997.
Blank indicates lack of data.
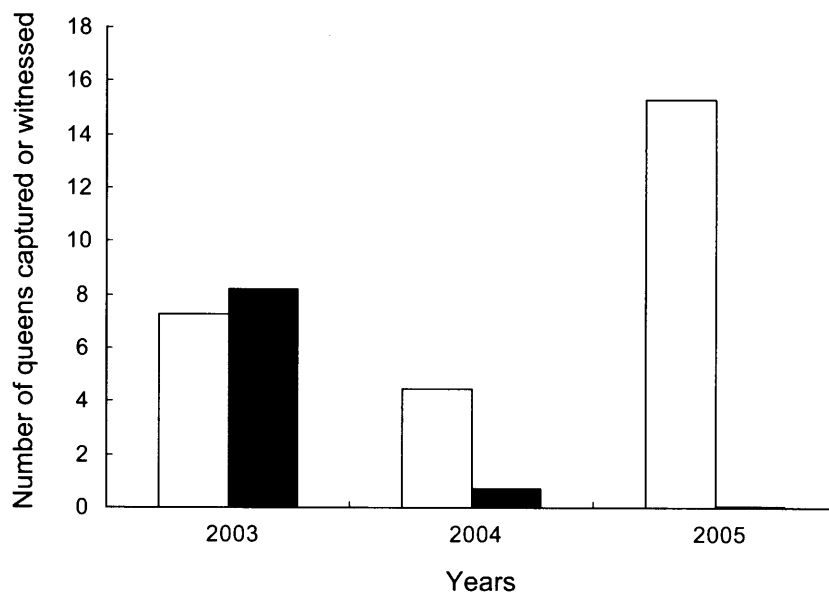Symbols as in Tables 2-2 and 2-3.

Fig. 2-1 The numbers of post-hibernating queens of *Bombus terrestris* (white) and *B. hypocrita sapporoensis* (black) captured or witnessed per person-hour from 2003 to 2005 in the study area. See text for details regarding the low number of *B. terrestris* queens in 2004.
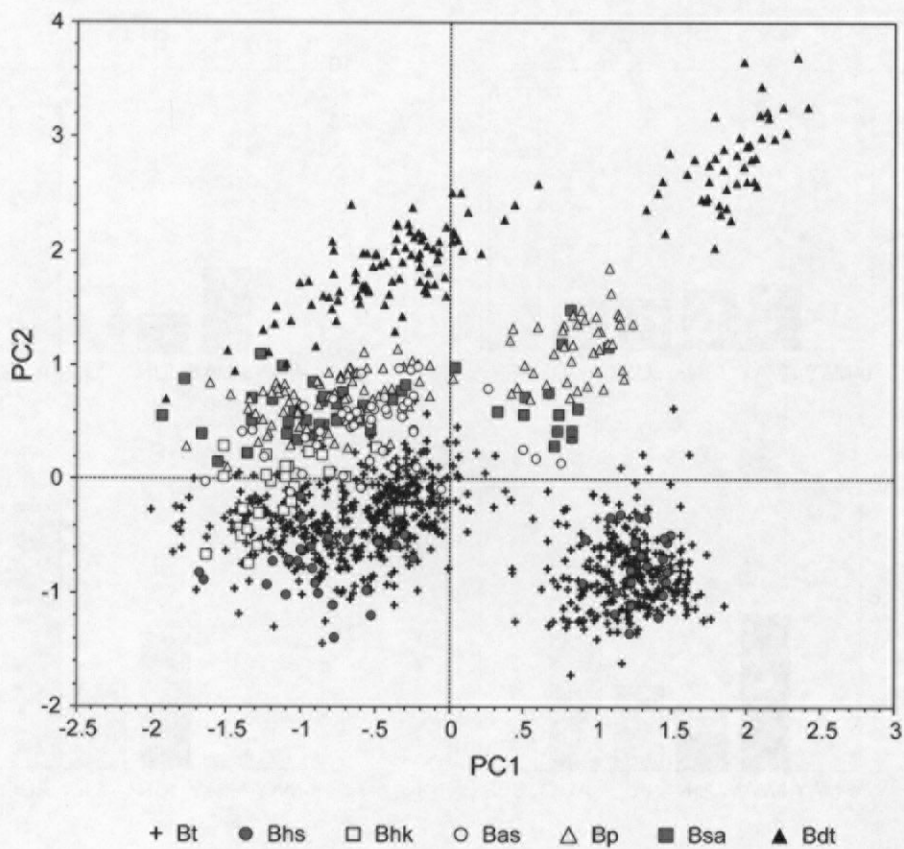
Fig. 2-2 Scatter diagram obtained from the first two principal components (PC1 and PC2) of 720 *B terrestris* and 417 native bumblebees.

Bt, *Bombus terrestris*; Bhs, *B. hypocrita sapporoensis*; Bas, *B. ardens sakagamii*; Bhk, *B. hypnorum koropokkrus*; Bp, *B. pseudobaicalensis*; Bsa, *B. schrencki albidopleuralis*; Bdt, *B. diversus tersatus*.
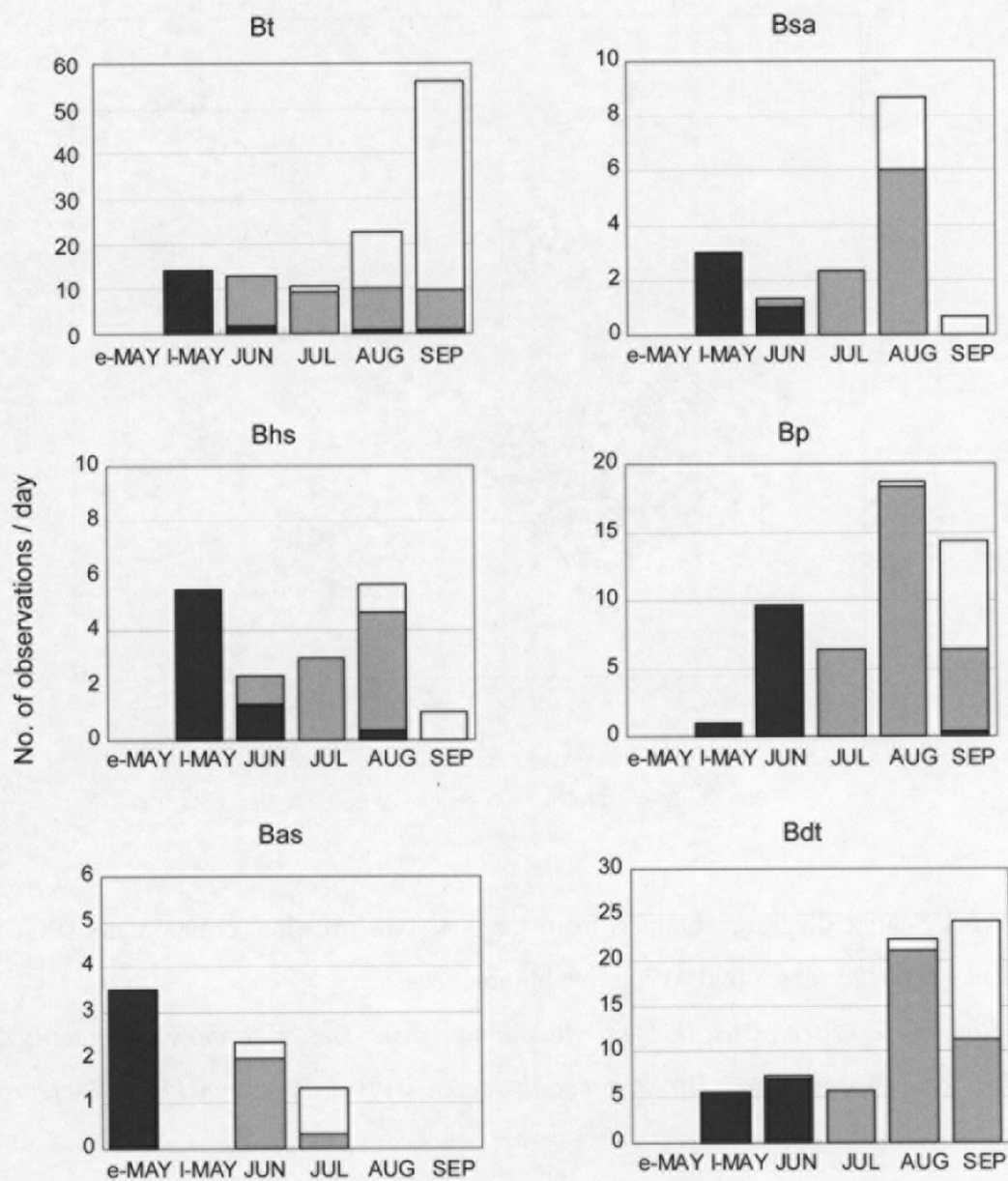
Fig. 2-3 Seasonal changes in the numbers of *Bombus terrestris* and five native bumblebees.

Black columns, queens; shaded columns, workers; white columns, males.
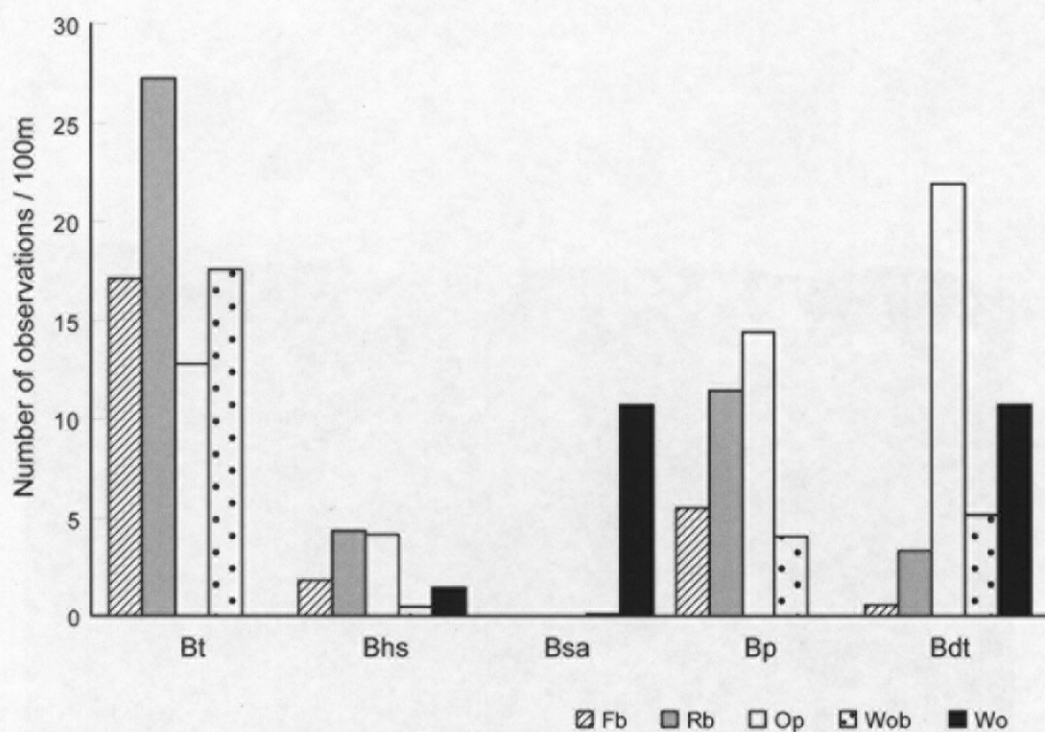
Symbols as in Fig. 2-2.

Fig. 2-4 The numbers of each bumblebee species observed per 100 m in five habitat types between 2003 and 2005.

Fb, farmland boundary; Rb, road boundary; Op, other open ground; Wob, woodland boundary; Wo, woodland.

Symbols as in Fig. 2-2.

Photo 2-1 The landscapes of the study area in the Iburi region, southern Hokkaido. Rice fields (a) and windbreak tree belts (b) are representative of the habitat condition in the study area.

Photo 2-2 Six native bumblebee species observed in the Iburi region, southern Hokkaido.