

CHAPTER 3

Colony Growth and Reproductive Ability of Feral Nests of the Introduced *Bombus terrestris* in Northern Japan

Introduction

Bombus terrestris has invaded the large parts of Hokkaido, northern Japan, since the introduction into Japan in 1991 (Yokoyama & Nakajima 2005; Laboratory of Conservation Ecology 2007). *Bombus terrestris* invasion still continues and causes the decline of native bumblebee species (Chapter 2: Inoue et al. 2008; Kawahara 2004). This species has been recorded predominantly in urban and agricultural areas in Hokkaido (Kadoya et al. unpublished). Currently, however, *B. terrestris* has been also found in the important regions for biodiversity conservation such as Mt. Taisetsu National Park in central Hokkaido (K. Terashima, K. Toba, S. Kadoya personal communication) and Notsuke-Furen Prefectural Natural Park in eastern Hokkaido (Chapter 5; Inoue et al. 2007). Therefore, effective conservation of such valuable ecosystems calls for proactive management. In spite of this, the species ecology and the factors potentially limiting population growth of *B. terrestris* in Japan is, with a few notable exceptions (Chapter 2: Inoue et al. 2008; Matsumura et al. 2004a, b), poorly understood. Demographic data of *B. terrestris* populations are considered to be indispensable to the development of conservation strategies.

Bumblebees have an annual colony cycle, mainly constituted of two parts: solitary phase and social phase. During the social phase when workers and subsequently sexuals are reared, the nest is the center from which all the activities of colony members

originate, such as social organization and reproduction. However, finding existing nests of bumblebees in the natural habitats is rather difficult, and usually only a small proportion of nests within the natural area can be located (Svensson & Lundberg 1977; Donovan & Wier 1978; Fussel & Corbet 1992). Researchers, therefore, have attempted to circumvent this difficulty in investigating natural colony activity by other means: starting colonies in artificial nest boxes in the laboratory and placing them in the field, or raising colonies entirely in the laboratory. However, it is not obvious whether, under these conditions, bumblebees grow their colonies and rear sexuals as they do in entirely natural circumstances. For example, those rearing methods brought about much the different results in sexual reproduction among the studies. A laboratory-based study (Müller & Schmid-Hempel 1992a) yielded a more male-biased sex investment ratio than colonies placed in the field (Müller & Schmid-Hempel 1992b), while the captive colonies allowed foraging in the field were less successful than laboratory colonies in new queen production (Müller et al. 1992).

In Chapter 2, I demonstrated the similarity of nest habitat selections between *B. terrestris* and some native species, which should lead to competition for nest sites. The current study comprises a more detailed investigation of 25 feral nests of *B. terrestris* in southern Hokkaido to examine colony growth and reproductive ability, which are the important components of fitness during social phase. Nest development in a single colony is difficult to observe continuously in the natural habitats, because a bumblebee nest is usually located underground. In order to evaluate colony development process, I assessed a number of nests excavated in different seasons from the natural habitats to reconstruct the seasonal development theoretically, according to the previous studies (e.g. Spradbery 1971; Archer 1981; Matsuura 1984). Since bumblebees are known to

vary largely in their colony size and productivity among colonies (Müller & Schmid-Hempel 1992a), I also attempted to investigate as many *B. terrestris* nests collected from the natural habitats as possible.

Materials and Methods

Study area

This 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 search and collection of *B. terrestris* nests are mainly conducted within the monitoring area. The details of the study area are given in the 'Materials and Methods' section of Chapter 2.

Investigation of nests

Collection of nests

Nests of *B. terrestris* were collected during the survey period from June and September in the years 2003–2006. A searching route was set out along the foot of the bank of cultivated fields, the road or the riverbank. To discover the nests, I walked slowly to search bees passing in and out.

When a nest was found, the nest traffic (i.e. number of foragers leaving and returning) was recorded for one or two hours. After the observation and capture of bees leaving or returning for a while, the nest was excavated from underground, and its entrance tunnel length and depth from the ground level were measured and mapped. All the bees were killed by using either ethyl acetate or freezing immediately after collection. Workers, either returning or leaving before excavation, or returning during or

after excavation, classified as foragers. The nest collected was stored at -20 °C in the laboratory until the dissection.

Nest constitution

Dissections of 25 nests were performed in the laboratory after removing the nest material from the top of the nests, according to the previous study by Sakagami and Katayama (1977). The numbers of the following components were recorded: workers, queens and males; eggs (E); larvae (L); pupal cells (P); post-emergence empty cocoons torn and crushed (Ct), intact (Ci) and provided with wax around the emergence hole (Cx); post-emergence cocoons with stored food with honey (Hh) and with pollen (Hp). Nest architecture was characterized by size (height, width and length) of the brood comb and wax nest envelop. Nest population was estimated by counting the number of adult bees (worker, queen and male) collected from the nest, excluding absent foragers. Colony size was defined as the sum of the number of all types of cocoons (Ct, Ci, Cx, Hh, Hp and P).

Diameters of all cocoons except for the ones classified as Ct were measured with a digital caliper. The numbers of new queen (gyne) and worker/males produced by the colony in the reproductive stage can be estimated from the numbers of cocoons in the two size classes with critical width of 11 mm because gyne cocoons are distinctly larger than worker/male cocoons in *B. terrestris* (Donovan & Wier 1978; Buttermore 1997). Cocoons of workers and males can not be discriminated mutually on the basis of size, so that I represented as a worker or male only when the sex of pupae was ascertained by dissection. The head width of individual adult bee was measured to examine differences in head width between nest bees and foragers.

According to Katayama and Takamizawa (2004), colony developmental stages were divided into the four stages; (1) 'newly founded', solitary stage of a founding queen; (2) 'growing', from the start of worker production before gyne production; (3) 'mature', after the first appearance of gynes and males; and (4) 'senescent', the end of sexual production and colony activity. In the present study, however, only the nests in the 'growing', 'mature' and 'senescent' stages were obtained.

Results

Nest site

A total of 37 *B. terrestris* nests were found during 2003–2006 in the study area (Fig. 3-1, Photo 3-1), two of which (Bt-5 and Bt-29) were located outside of the monitoring area (Table 3-1). Twenty four nests excavated were ascertained to be constructed in abandoned rodent nests or cavities with entrance tunnels underground in ridges between rice fields or other cultivated fields, or in a riverbank (Photo 3-2). These nests were covered with either dried plant fragment or wax nest envelopes, or with both (Photo 3-3). Three nests were found within artificial materials: the abandoned polyvinyl chloride pipe buried underground (Bt-6), and the drainpipes (Bt-16 and Bt-24). Bt-21 was situated on the old *B. terrestris* nest underground that might be built in the former year.

Nest traffic

Bombus terrestris workers in the study area emerged in June (Fig. 3-2). Nest traffic observed from the nest discovered increased in number from late July to August, and the highest traffic was observed in late August ($\bar{X} \pm \text{SD} = 113.8 \pm 44.0$, $n = 5$). The nest activities were ceased in September. Bt-22, which was excavated on 2 September, 2005,

was the most active nest, whose nest traffic was as frequent as 184 times per hour on 22 August, 2005.

Nest development

Twenty-five excavated nests were undoubtedly assigned to one of the three stages: seven nests to the ‘growing’ stage; 17 nests to the ‘mature’ stage; and one nest, Bt-25 without live bees, to the ‘senescent’ stage (Table 3-2). Bt-25 was heavily infested by lepidopteran insects and totally covered with webs and frass of moth larvae, and most of cocoons had been destroyed.

All the nests excavated in July in the study area were judged to be in the ‘growing’ stage. Of 19 nests collected in August and September, only two were in the ‘growing’ stage, while 17 were assigned to the ‘mature’ stage. The increased rate of cocoons in the ‘growing’ nests in July was much smaller than in the ‘mature’ nests with sexual cocoons excavated in August and September (Fig. 3-3).

Nest construction

Nests in the ‘growing’ stage

Bombus terrestris nests in the early stage of nest development (Bt-18, 19 and 20) contained only two or three batches (Fig. 3-4a, Photo 3-4). A brownish waxen honey pot built by a founding queen was individually observed in two nests (Bt-19 and Bt-20) but not in Bt-18. The first batch (“first brood”, Alford 1975) was shown batch code “1-1” in the figures of the three colonies. The next batches (“second and third brood”) were built on the first brood and subsequent broods were built on the second and third broods. Egg cells were built on the post-spinning cocoons in close contact with one another to make

a lump. Batches in the 'growing' nests were arranged clockwise in three- to four-fold volutions and their arrangements were relatively regular.

Foundresses were alive in all the 'growing' nests (Table 3-2). The numbers ($\bar{X} \pm \text{SD}$) of eggs, larvae and cocoons produced were 52.5 ± 44.9 , 96.8 ± 93.7 and 104.7 ± 99.1 ($n = 7$), respectively.

Nests in the 'mature' stage

The nests assigned to the 'mature' stage had the arrangement of the three- to five-fold volutions (Fig. 3-4b). The lower layers connected with each other vertically, but connection of each batches in upper layers, especially batches of sexuals, were much loosely connected. The representative composition of cocoon batches of Bt-38, which contained both gynes and males, are given in Table 3-3 (see Fig. 3-4b, Photo 3-5). In this nest, post-emergence batches (1-1-1-4, 2-1-2-8, 3-1, 3-2, 4-1-4-6) were used as food receptacles filled with honey (Hh) and pollen (Hp). Of 165 pupal cocoons, 64 and 52 appeared to emerge as gynes and males, respectively. The cocoon diameter of workers was 8.0 ± 0.8 mm ($\bar{X} \pm \text{SD}$, $n = 362$); that of gynes, 13.1 ± 0.4 mm ($n = 63$); and that of males, 9.9 ± 0.6 mm ($n = 52$). The cocoon diameter of workers tended to become bigger in the upper batches.

Foundresses were alive only in seven of 17 'mature' nests. The presence of the foundress in Bt-6 was not confirmed because the nest substances were scraped out from the polyvinyl chloride pipe. Of seven queenright nests, two produced only gynes and five produced both sexuals. Whereas, of nine queenless nests, three produced only males and one produced only gynes. Other five nests produced both sexuals.

The numbers ($\bar{X} \pm \text{SD}$) of eggs, larvae and cocoons produced in 17 nests were

145.7 \pm 142.2, 108.3 \pm 106.4 and 376.5 \pm 168.6, respectively. Bt-29, which contained 802 cocoons, was the largest colony in this study. Bt-22 was also developing larger at the excavation, and the total number of cocoons would have been over 1,000, if all eggs and larvae become adult bees. The mean number of gyne cocoons produced was 109.5 \pm 76.7 (n = 12) and the ratio of gyne cocoons to total cocoons was 26.9 \pm 14.1 %. Number of gynes produced was significantly larger in the nests with increasing colony size (R^2 = 0.513, P = 0.0012, Fig. 3-5).

Body sizes of queens, males, nest workers and foragers

The head width of workers was 3.9 \pm 0.4 mm (\bar{X} \pm SD, n = 2,017); that of gynes, 5.4 \pm 0.4 mm (n = 165); that of males, 4.4 \pm 0.2 (n = 75). Workers varied greatly in head width from 2.59 to 4.94 mm, with an overall coefficient of variance (CV) of 0.101. CV values for gynes and males were 0.066 and 0.046, respectively. Nest workers were significantly smaller than foragers (3.8 \pm 0.4 mm, n = 120 and 4.1 \pm 0.3 mm, n = 897, respectively; t test, P < 0.0001; Fig. 3-6). The smallest size class of workers, those with head width between 2.5 and 3.0 mm, were rarely found out of nests.

Discussion

Nest construction and architecture

As previously reported in Chapter 2 (Inoue et al. 2008), *B. terrestris* nests established in the study area were usually constructed in abandoned rodent nests or cavities underground with long entrance tunnels in ridges between cultivated farmlands or in dry riverbed sites. This result also confirmed the earlier observations reported for the native ranges (Sladen 1912; Alford 1975; Prys-Jones & Corbet 1991) and the introduced

regions (Donovan & Wier 1978; Matsumura et al. 2004a, b; Nakajima et al. 2004). In the present study, one nest of *B. terrestris* was found in the entirely same site in the previous year. Some post-hibernating queens are known to return to their maternal nest sites with the following year (Donovan & Wier 1978) and thought to build their new nests adjacent to the former nest sites (Frison 1930; Fussell & Corbet 1992). These facts indicate that bumblebee queens prefer to found their nests close to the former successful ones.

Nest architecture of *B. terrestris* showed the following features: (1) wax envelopes were often developed; (2) egg cells were usually built on the top of post-spinning cocoons and contiguous egg cells consisting of several component cells were frequently built; (3) batches loosely attached one another and irregularly arranged. These features are consistent with not only the reports of *B. terrestris* nests in the native ranges (e.g. Sladen 1912; Alford 1975), but also those for the native consubgenerics, *B. hypocrita hypocrita*, *B. hypocrita sapporoensis* and *B. ignitus* (e.g. Sakagami & Katayama 1977; Katayama & Takamizawa 2004). Bumblebees are known to be characterized differentially by their ecological features at the subgeneric level (Sakagami 1976). It is suggested that *B. terrestris* has similar nesting characteristics with the native consubgenerics, other than morphological characteristics, flower resource use and nest site selection (Chapter 2: Inoue et al. 2008).

Colony growth

Based on the data of foraging activities and colony growth pattern in the study area, the seasonal development of a success colony of *B. terrestris* can be described as follows: slow growth in the ‘growing’ stage, followed by rapid development in the ‘mature’ stage

and then the terminal decline after sexuals have been produced (Fig. 3-3). This pattern is consistent with the findings of the previous studies conducted by using colonies raised in the laboratory (Duchateau & Velthuis 1988) and these placed in the field (Goulson et al. 2002a).

As the *B. terrestris* nests developed larger in size during July to August, foraging activity of workers accordingly increased (Fig. 3-2). Worker cocoons also increased seasonally in size (Table 3-3), which in turn is reflected in an increase in mean size of adult workers (Harder 1985; Inoue & Kato 1992). Sexual production is known to be closely linked to food supply, and therefore foraging ability of the colony (Schmid-Hempel & Schmid-Hempel 1998; Pelletier & McNeil 2003). Queens are distinctly larger than males and store considerable fat reserves in their fat bodies as a source of energy during hibernation (Cumber 1949; Holm 1972), and thus gyne larvae need more food to develop than those of males. Therefore, queens are likely to be more costly to produce than males (Beekman & van Stratum 1998). A large amount of energy in the form of nectar is also required to produce gynes, though provision of pollen, as the protein source, is perhaps more important for sexual production (Beekman et al. 1998). Larger workers are known to be able to forage at lower temperatures (Heinrich & Heinrich 1983) and carry larger pollen/nectar loads (Goulson et al. 2002b) than smaller workers. Increases in both number and size of workers toward the reproductive stage contribute to supply enough food to feed more expensive gynes. Therefore, the late summer reproductive phase, at which colonies have to gather sufficient forage as provision for their gynes, is the time when foraging activity is most vital.

Reproductive success

The failure rate of bumblebee colonies is thought to be relatively high, especially during early developmental stage. For example, of 80 nests of *B. pascuorum* (Scopoli) in southern England, only 23 produced any gynes (Cumber 1953). During the newly founded stage, death of a foundress from predation (e.g. birds and spiders) or any accidents may be directly responsible for high rates of failure in nest founding. Changes in the scene around the nest sites by agricultural practices (e.g. mowing or weeding) may also disturb a foundress to return to its nest after foraging outside, as the cases observed for bumblebee workers (Taniguchi 1955; Miyamoto 1959; Nakajima et al. 2004). In addition, availability of a succession of suitable flowers is vital in the indirect way: Bowers (1985) found that colonies frequently died out if founded in particular subalpine meadows with a low availability of flowers.

During the reproductive stage, gyne production in *B. terrestris* nests was significantly positively correlated with overall productivity (Fig. 3-5), as found in the studies with various bumblebee species (Pomeroy 1979; Pomeroy & Plowright 1982; Duchateau & Veltuis 1988; Müller & Schmid-Hempel 1992b; Bourke 1997). The laboratory experiment by Pomeroy & Plowright (1982) showed that the number of workers affects the production of gynes, and that colonies with more workers produced more gynes. Large colonies with more workers may be at an advantage in food supply, nursing of offspring and defense from predators.

The early death of foundresses can also strongly influence production of sexulas. The male production may be ascribed either to death of the foundress or to loss of its ability to lay fertilized eggs (Owen et al. 1980). Therefore, the colony, in which the foundress die and workers start laying haploid eggs in early developmental stage,

was observed to produce only males (Owen et al. 1980). Three queenless colonies (Bt-4, 13 and 30) containing only males probably lost their foundresses before producing gynes, and these males were considered to be produced by worker egg-lying.

Some parasites can damage both individuals and colonies (e.g. Müller & Schmid-Hempel 1992a; Schmid-Hempel & Loosli 1998; Brown et al. 2003). In this study area, Bt-7, which was heavily infested by mite, was totally inactive to be collapsed before reproduction (Nakajima et al. 2004).

Reproductive ability of established *Bombus terrestris* population

Bombus terrestris is widely distributed in Europe and adjacent regions (e.g. Velthuis & van Doorn 2006). However, only a few records of *B. terrestris* feral nests were reported in the native ranges, though some authors (e.g. Sladen 1912; Free & Bulter 1959) described that the colony size of this species is relatively large among bumblebee species inhabiting in the same region. Two feral nests examined by Cumber (1949) in England contained 107–219 adults and pupae, including 11–13 gynes. In the feral nests in Poland transferred to nest boxes after the collection, the number of opened cocoons was 337.9 ± 114.6 ($n = 9$) (Wojtowski 1963). More detailed descriptions of *B. terrestris* feral nests were reported from the introduced regions. In New Zealand, *B. terrestris* produced 834.3 ± 329.7 cocoons ($n = 14$) in total and 140.6 ± 96.3 gynes ($n = 14$) (Donovan & Wier 1978). In Tasmania, Buttermore (1997) reported that *B. terrestris* produced 490.4 ± 354.4 cocoons ($n = 17$) in total and 96.8 ± 125.2 gynes ($n = 14$). More recently Hingston et al. (2006) reported for a large feral colony in southern Tasmania, containing 304 gynes and 939 worker/males.

Comparison between the nests reported overseas and those examined in the

present study is not so meaningful because the nests were not representatives of the region. However, it can be safely said that *B. terrestris* nests in this study are characterized by higher gyne proportion to total production (26.9 %) than the ones reported from the other regions. Similarly, *B. terrestris* produced more gynes than the consubgeneric native Japanese species (subgenus *Bombus* s. str.): *B. hypocrita hypocrita*, 383.8 ± 328.8 cocoons and 64.6 ± 81.5 gynes (Katayama & Takamizawa 2004), *B. h. sapporoensis*, 433 cocoons and 36 gynes (Sakagami & Katayama 1977), and *B. ignitus*: 399.8 ± 425.5 cocoons and 78.0 ± 146.0 gynes (Sakagami & Katayama 1977). Some recent studies showed that commercially imported *B. terrestris* outperformed native conspecifics in foraging activity (Ings et al. 2005, 2006) and in overall productivity (Gösterit & Gürel 2005; Ings et al. 2006). It is assumed that artificial selection on commercially reared colonies is responsible for the higher productive ability of gynes in *B. terrestris* nests in the study area than those reported from New Zealand and Tasmania, which might not experience such strong artificial selection.

In conclusion, high reproductive ability of *B. terrestris* nests revealed in the present study indicates the successful naturalization and high adaptive ability of *B. terrestris* in the new habitats of the region. The demographic parameters quantified during social phase and the potential gyne production estimated for a successful feral nest are useful in the evaluation of the potential population growth of *B. terrestris* in the region as attempted in the next chapter.

Table 3-1 Nest sites of *Bombus terrestris* found in this study.

Nest ID	Date found	Date collected	Site	Elevation from the ground level (cm)	Entrance tunnel length (cm)
Bt-3	04-Jun-03		Underground in a bank of an irrigation channel		
Bt-4	03-Jul-03	02-Aug-03	Rodent nest underground in a bank of an irrigation channel	-20	33
Bt-5	08-Jul-03	02-Aug-03	Rodent nest underground in a bank of the river	-15	70
Bt-6	04-Aug-03	23-Aug-03	Underground along a farm road between fields	-20	140
Bt-7	04-Aug-03		Underground in a bank of an irrigation channel		
Bt-8	05-Aug-03		Underground along a farm road between an irrigation channel		
Bt-9	05-Aug-03		Underground along a farm road between an irrigation channel		
Bt-10	27-Aug-03	28-Aug-03	Rodent nest underground in a bank between rice fields	-20	30
Bt-11	19-Jun-04		Underground in a bank of the river		
Bt-12	16-Jul-04	17-Jul-04	Rodent nest underground in a bank of the river	-25	57
Bt-13	22-Jul-04	19-Aug-04	Rodent nest underground in a bank of an irrigation channel	-20	45
Bt-14	22-Jul-04	07-Aug-04	Rodent nest underground along a farm road between fields	-11	11
Bt-15	07-Aug-04		Underground in a bank of an irrigation channel		
Bt-16	16-Aug-04		Drainpipe in a ridge between a rice field and an irrigation channel		
Bt-17	21-Aug-04	23-Aug-04	Rodent nest underground in a bank of an irrigation channel	-57	170
Bt-18	08-Jun-05	29-Jun-05	Rodent nest underground along a farm road between rice fields	-45	
Bt-19	14-Jun-05	15-Jul-05	Rodent nest underground in a bank of an irrigation channel	-30	50
Bt-20	01-Jul-05	08-Jul-05	Rodent nest underground in a bank of an irrigation channel	-45	145
Bt-21	28-Jul-05	09-Aug-05	Rodent nest underground in a bank of an irrigation channel	-40	80
Bt-22	30-Jul-05	02-Sep-05	Cavity underground in a ridge between a field and a farm road	-45	100
Bt-23	14-Aug-05	23-Sep-05	Rodent nest underground in a bank of the river	-20	48
Bt-24	14-Aug-05		Drainpipe in a ridge between a field and a farm road		
Bt-25	25-Aug-05	22-Sep-05	Underground cavity in a ridge of a farm road	-38	75
Bt-26	25-Aug-05	28-Aug-05	Rodent nest underground in a small bank between fields	-30	15
Bt-27	27-Aug-05	30-Aug-05	Rodent nest underground in a bank of the river	-25	85
Bt-28	30-Aug-05	09-Sep-05	Rodent nest underground in a bank of the river	-30	105
Bt-29	04-Sep-05	05-Sep-05	Cavity underground in a bank of the river	-70	180
Bt-30	05-Sep-05	06-Sep-05	Rodent nest underground in a bank of an irrigation channel	-45	70
Bt-31	05-Sep-05	06-Sep-05	Rodent nest underground in a bank of an irrigation channel	-50	80
Bt-32	24-Sep-05		Underground along a farm road between fields		
Bt-33	27-May-06		Underground in a bank of an irrigation channel		
Bt-34	02-Jul-06		Underground in a bank of the river		
Bt-35	03-Jul-06		Underground in a bank of the river		
Bt-36	07-Jul-06	08-Jul-06	Rodent nest underground in a bank of the river		
Bt-37	05-Aug-06	04-Sep-06	Rodent nest underground between a farm road and rice field	-40	80
Bt-38	25-Aug-06	02-Sep-06	Rodent nest underground in a bank of an irrigation channel	-40	140
Bt-39	31-Aug-06	31-Aug-06	Rodent nest underground in a bank of an irrigation channel	-40	80

Table 3-2 Colony size and productivity of the studied nests.

Stage	Nest ID	Nest size (cm)			Wax envelop	Adults ¹⁾				Egg	Larva	Cocoons ²⁾			NQ/total cocoons (%)
		Length	Width	Height		F	W	NQ	M			Total	Post NQ	Pre NQ ³⁾	
Growing	Bt-12	10.0	7.0	5.0	×	1	52	0	0	53	79	233	0	0	0.0
	Bt-18	4.0	3.5	2.5	×	1	1	0	0	2	13	1	0	0	0.0
	Bt-19	2.8	3.5	2.0	×	1	4	0	0	5	9	2	0	0	0.0
	Bt-20	5.0	3.5	2.0	×	1	3	0	0	4	8	20	0	0	0.0
	Bt-21	10.0	9.0	3.8	○	1	58	0	0	59	96	186	0	0	0.0
	Bt-28	10.0	10.0	7.0	○	1	84	0	0	85	116	142	0	0	0.0
	Bt-36	6.5	6.5	3.0	×	1	15	0	0	16	46	89	0	0	0.0
	Average					1.0	31.0	0.0	0.0	32.0	52.4	96.1	0	0	0.0
Mature	Bt-4	11.0	8.0	6.0	×	0	40	0	7	47	171	75	0	0	65
	Bt-5	20.0	13.0	8.0	×	1	119	0	0	120	168	236	0	108	0
	Bt-6	-	-	-	×	-	133	0	0	133	+	+	0	85+25*	2
	Bt-10	20.0	13.0	8.0	×	1	130	58	2	191	+	+	118	27	58
	Bt-13	11.0	10.0	5.5	○	0	79	0	27	106	70	86	0	0	97
	Bt-14	10.0	10.0	7.0	×	1	82	0	32	115	56	43	0	13	125
	Bt-17	19.0	17.0	7.0	○	1	176	0	0	177	170	177	0	73+88*	0
	Bt-22	25.0	19.0	7.5	○	0	294	0	0	294	501	289	0	274+21*	0
	Bt-23	-	-	-	○	0	40	0	0	40	11	10	100	0	26
	Bt-26	12.0	12.0	4.5	○	0	5	13	0	18	0	0	57	53	20
	Bt-27	12.5	12.5	5.0	○	1	96	0	1	98	167	18	0	43	42
	Bt-29	-	-	-	○	1	63	59	10	133	87	16	207	8	79
	Bt-30	11.5	14.0	6.0	○	0	107	0	0	107	173	220	0	0	83
	Bt-31	13.0	15.0	6.5	○	0	107	0	0	107	335	316	0	71+11*	0
	Bt-37	10.5	12.0	6.0	○	0	15	2	0	17	15	42	10	4	61
	Bt-38	14.5	14.5	7.0	○	1	180	0	0	181	243	63	0	64	52
	Bt-39	13.0	13.0	7.0	.	0	70	22	0	92	19	34	36	37	10
	Average					0.4	102.1	9.1	4.6	116.2	145.7	108.3	88.0	77.3	55.7
Senescent	Bt-25	-	-	-	×	0	0	0	0	0	-	-	-	-	-
Average		0.0				0.0				0.0				0.0	
		0.0				0.0				0.0				0.0	

1) 'F', 'W', 'NQ' and 'M' indicate 'foundress', 'worker', 'new queen' and 'male', respectively.

2) 'Post' and 'Pre' indicate 'post-emergence cocoon' and 'pre-emergence cocoon'.

3) * indicates large spinning larvae to new queens

Table 3-3 Batch composition in Nest Bt-38.

Batch		Cocoon diameter ¹⁾ (mm)										Contents ²⁾
Code	Size	5	6	7	8	9	10	11	12	13	14	
1-1	8		2	3	3							4Ci, 4Hh
1-2	13		2	6	5							10Ci, 1Cx, 2Hh
1-3	10			4	5							3P, 2Ci, 1Ct, 4Hh
1-4	3			1	2							1Ci, 2Hh
1-5	10				3	1						4Ci, 6Ct
1-6	4		2		2							3P, 1Ci
2-1	38	1	12	15	2							26Ci, 8Ct, 1Cx, 3Hh
2-2	13		2	5								3Ci, 6Ct, 2Cx, 2Hh
2-3	14		1	2	8	3						3P, 1Ci, 10Hh
2-4	40	1	10	12	8	2						4P, 16Ci, 7Ct, 5Cx, 8Hh
2-5	7		1	1	4	1						2P, 5Hh
2-6	21			3	14	4						1P, 5Ci, 14Hh, 1Hp
2-7	33		3	3	14	2						10P, 9Ci, 2Ct, 12Hh
2-8	13			3	5	5						4P, 9CHh
3-1	34	1	3	18	7							15Ci, 5Ct, 6Cx, 7Hh, 1Hp
3-2	15			9	2	3						1P, 4Ci, 1Ct, 8Hh, 1Hp
4-1	7		1		4	2						1P, 2Cx, 3Hh, 1Hp
4-2	16			5	10	1						2P, 4Ci, 2Cx, 7Hh, 1Hp
4-3	15		2	1	8	4						4P, 1Cx, 10Hh
4-4	32		2	7	14	2						3Ci, 7Ct, 13Hh, 9Hp
4-5	25	1		5	13	6						8Ci, 1Cx, 14Hh, 2Hp
4-6	48		6	14	19	4						11P, 6Ci, 2Ct, 3Cx, 26Hh
5-1	15					4	7		1	3		4Pq, 11Pm
5-2	36					9	11		5	10	1	16Pq, 20Pm
5-3	34				1	3	5	1	11	13		25Pq, 9Pm
5-4	20				2	5	1		4	10		14Pq, 6Pm
5-5	9						4		1	4		5Pq, 4Pm

¹⁾ Cocoon diameter of all or a part of old Ct is unknown.

²⁾ Ct, post-emergence cocoon torn and crushed; Ci, vacated but intact; Cx, empty but entirely or partly coated with wax; Hh, post-emergence cocoon with honey; Hp, with pollen. P, pupa; q, queen; w, worker; m, male.

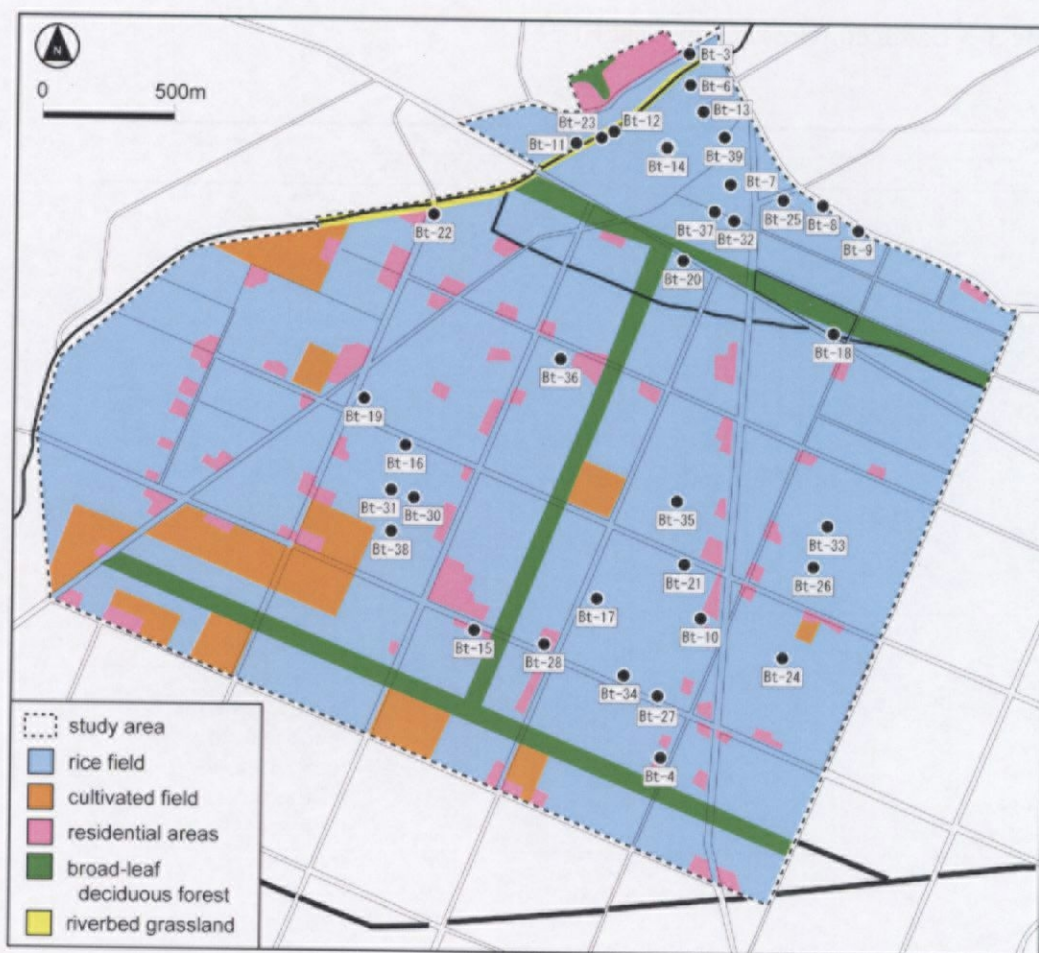


Fig. 3-1 Map showing nest sites found in the main study site, Mukawa-cho, southern Hokkaido. A total of 35 feral nests were found within this site and two were found in other sites of the study area. Nests are cited with abbreviations (ex. Bt-3 = *B. terrestris* Nest 3).

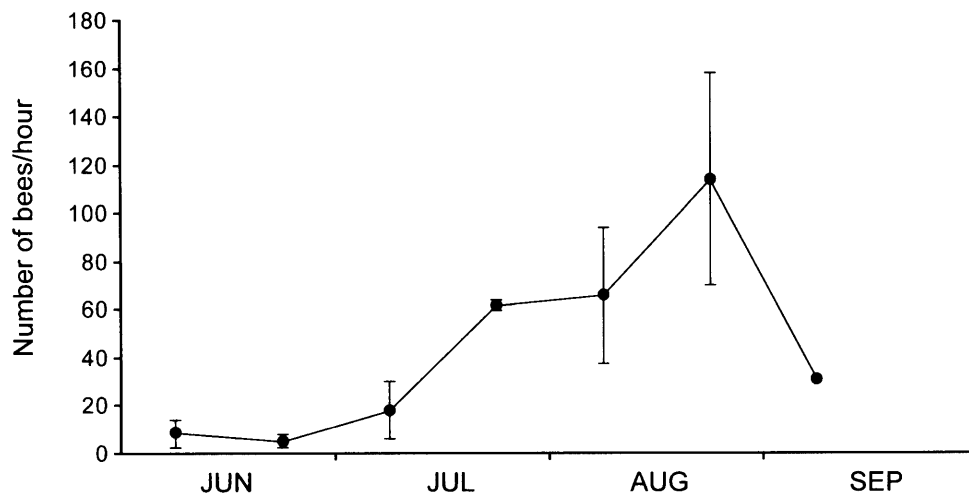


Fig. 3-2 Seasonal change of the nest traffic, that is, the number of foragers leaving and returning per hour in the *Bombus terrestris* colony at this discovery. Vertical line shows standard deviation.

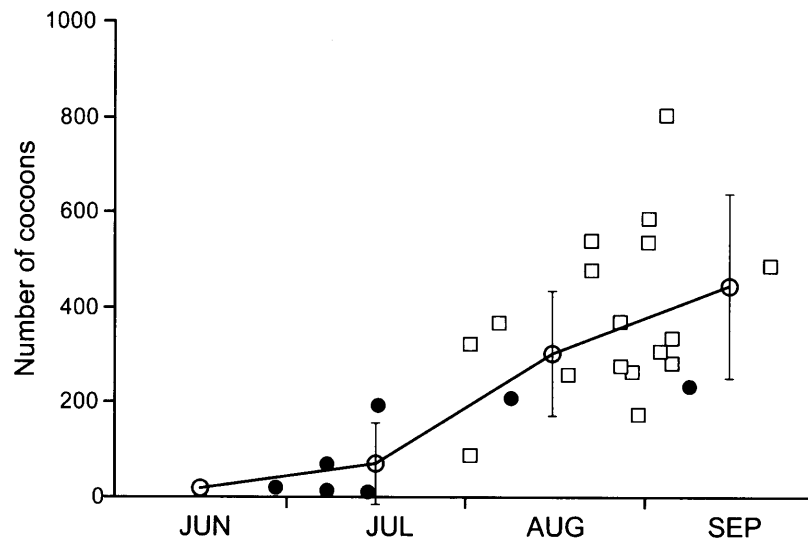


Fig. 3-3 Seasonal change in the number of cocoons in *Bombus terrestris* nests. Black circle, nests in the growing stage; white square, nests in the mature stage; open circle, monthly mean with standard deviation.

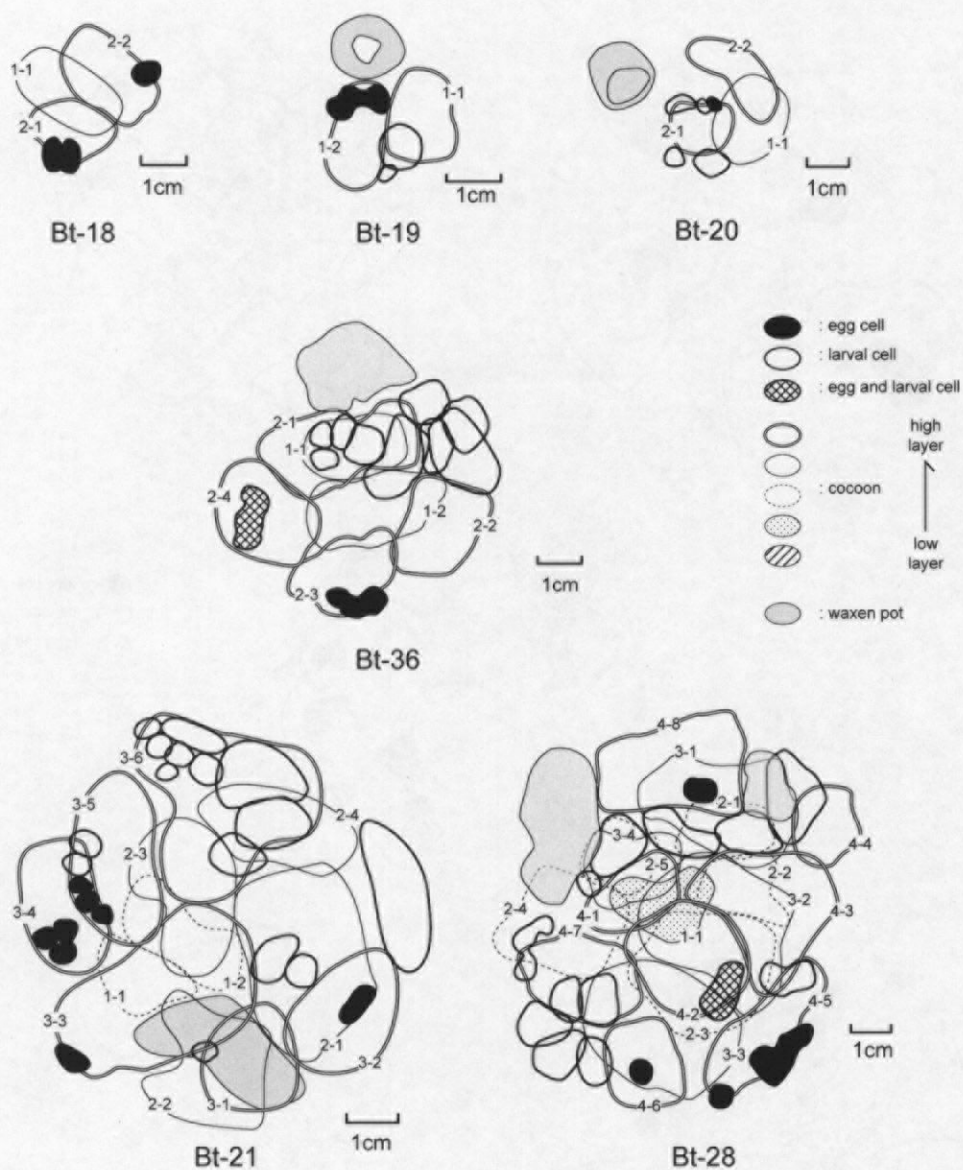


Fig. 3-4a Horizontal views of batch arrangement of *Bombus terrestris* nests in growing stage. Each batch (i.e. the group of immature born in the same egg cell) is designated as 1-1, 1-2, ... 2-1, 2-2, etc. upward from the nest bottom, and the sequence roughly corresponds to the order of ovipositions within the same layer.

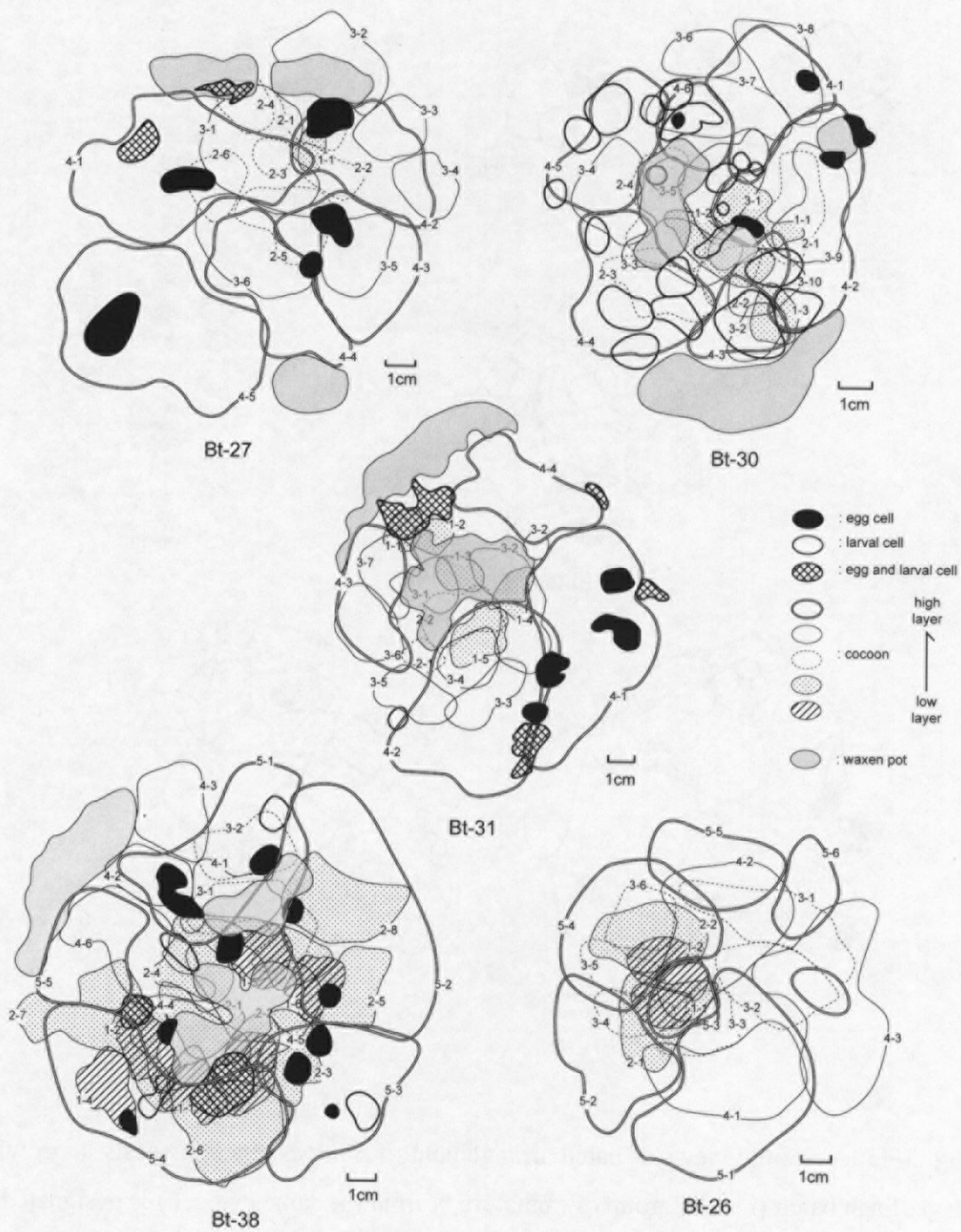


Fig. 3-4b Horizontal views of batch arrangement of *Bombus terrestris* nests in senescent stage.

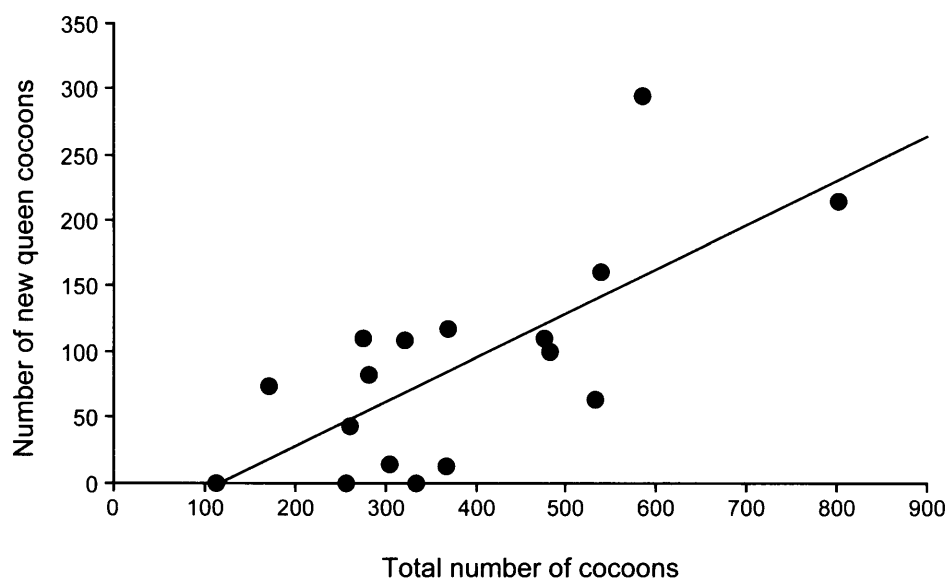


Fig. 3-5 Regression of number of new queens produced in the colony on colony size (regression analysis, paternal $r^2 = 0.513$; $F = 15.77$; $P = 0.0012$).

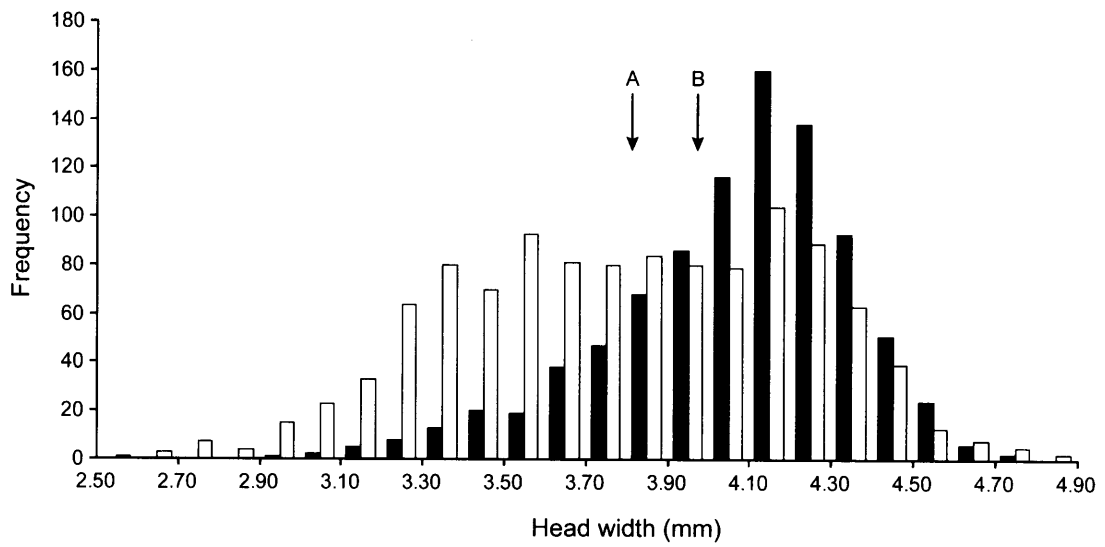


Fig. 3-6 Frequency distributions of head width of nest workers (white) and foragers (black). Mean head widths of nest bees (A) and foragers (B) are indicated by arrows.



Photo 3-1 The microhabitats of nest sites of *Bombus terrestris* in the study area. Bt-4 and Bt-20 were constructed in a bank of an irrigation channel; Bt-11, in a bank of the river; Bt-6, in the abandoned polyvinyl chloride pipe; Bt-16 and Bt-24, in drainpipes.



Photo 3-2 Three *Bombus terrestris* nest with the longest entrance tunnels in the study area. Bt-29, 180 cm long and 70 cm depth; Bt-17, 170 cm long and 57 depth cm; and Bt-18, 165 cm long and 45 cm depth.

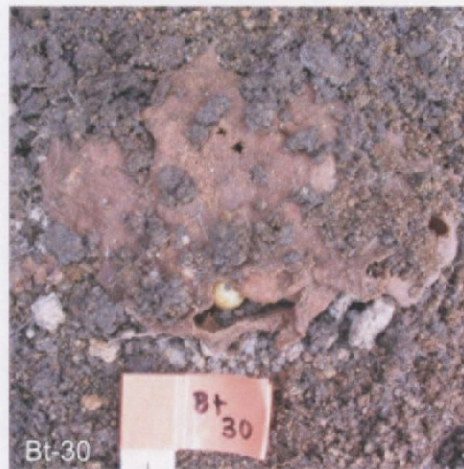


Photo 3-3 The nest materials of *Bombus terrestris* such as dried plant fragment (Bt-14, Bt-29) and wax nest envelop (Bt-30).



Photo 3-4 Batch arrangements of representative nests of *Bombus terrestris* in the early developmental stage.



Photo 3-5 Certain representative batch arrangements in Bt-38.