Studies on the nesting biology of hairy footed flower bee, *Anthophora plumipes* (Hymenoptera: Apidae), with special reference to its utilization as a crop pollinator in protected culture

（ケブカハナバチの営巣生態に関する研究，とくに施設栽培における送粉昆虫としての利用について）

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Chapter 1

General Introduction

1.1. Pollination and pollinators

Pollination is process of transferring pollens in and between flowers leading to fertilization and successful seed and fruit production in plants. Pollination ensures a plant to produce full sized fruits and fertile seeds. Pollination occurs when pollen is travelled within flowers or carried from flower by pollinators. Pollination is a critical phenomenon in crop production, therefore pollinators are also essential for the development of seeds for many root and fiber crops as well as in forage, horticulture and orchard production. Pollinators are essential for transferring genes within and among several plant species (Kearns et al., 1998). Pollinators contribute society by increasing food security and improving livelihood, playing to conserve biological diversity in agriculture and natural ecosystem. It is estimated that about one third of all plants or plant products eaten by humans are directly or indirectly depend on bee pollination. The contribution of pollinators to the production of crops used directly for human food has been estimated at 153 billion dollar annually (Gallai et al., 2009) (Fig. 1.1), which is about 9.5% of the total value of human food production worldwide.

Pollination provides stability in the food web and maintenance the whole food chain of life. Pollination services are provided by both managed and native pollinators. Approximately 80% of all flowering plant species grown are pollinated by animals, including vertebrate and mammals, but the main pollinators are insects (Losey and Vaughan, 2006). In fact, pollinators such as bees, birds and bat affect 35% of the world's crop production, helping for increasing yields in leading food crops worldwide as well as many plant derived medicines (Klein et al., 2007). Among the world food production, cross pollinated crop like vegetables, oil-crops, fruits and nuts are highly depend on insect pollination for fruit set.

Bees are the most important insect pollinator for most of the flowering plants worldwide (Kearns et al., 1998). Bees have branched body hair, excellent foraging behaviours and abilities, and they reliance on floral resources for raising their offspring (Free, 1993). Bees transfer pollen from flower to flower and from plant to plant. Their foraging increases pollen movement for cross pollination (James and Pitts-Singer, 2008). Moving pollen optimizes seed production while the bees gain food resources in the form of pollen and/or nectar.

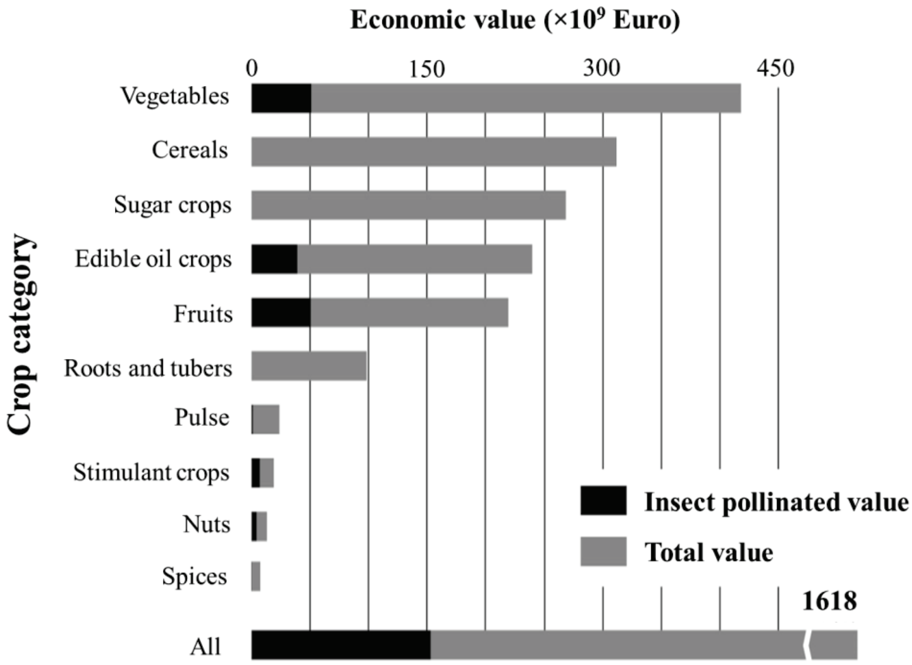


Fig. 1.1. Economic impact of insect pollination on agricultural production in the world (Source: Gallai et al., 2009)

1.2. Honeybees and pollination

Honeybees are the principal pollinator for most of the crops (Free, 1993). They are polylectic and pollinate many plant species, but it is becoming evident that reliance on them for all pollination may no longer be sufficient. Honeybees are not able to pollinate all flowers due to nectar chemistry, flowering phenology, floral morphology, and body size. Moreover, honeybees are becoming less attractive as commercial pollinators because they are difficult to maintain (Losey and Vaughn, 2006), and also declining (USDA National Agricultural Statistics Service, 2015). In the United States, managed honeybees have declined from over 4 million colonies in the 1970s to 2.74 million colonies in 2014 (USDA National Agricultural Statistics Service, 1977 and 2015) (Fig. 1.2), because of problems such as parasitic mites and pesticide misuses (Ellis and Munn, 2005; Matheson et al., 1996) and the problem of Colony Collapse Disorder (CCD). Due to CCD, 50–90% loss of bee colonies have been reported. Several reports show that a honeybee species, *Apis mellifera* may result in increasing the ecological risks, e.g., competition with non-*Apis* bees, increasing weeds through pollination, migrating pathogens and small insect pests (Roubik, 1989; Butz Huryn and Moller, 1995; Oldroyd et al., 2006).

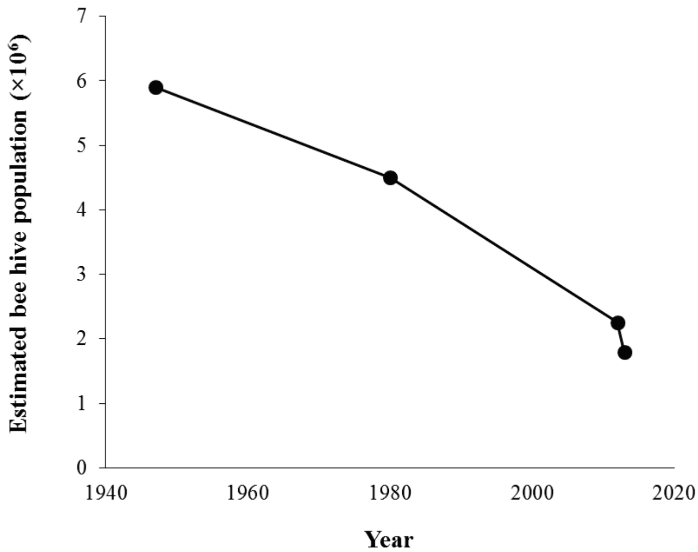


Fig. 1.2. Estimated honeybee hive population in USA from 1947 to 2013 (Based on the data USDA, 2014)

1.3. Wild bees and pollination

Wild bees significantly contribute for the pollination of several kinds of plant species (O'Toole and Raw, 1991; Kremen et al., 2002, Morandin and Winston, 2005, Greenleaf and Kremen 2006a; b; Winfree et al., 2007 and Winfree et al., 2008). They can efficiently pollinate plants which are not efficiently pollinated by managed pollinators. Even though several thousands of bee species have been reported so far (Michener, 2007), only 11 species has been managed by farmers for their pollination services (Parker et al., 1997; Batra, 1995). Worldwide decline of honeybees directs towards a need to learn more about alternative pollinators for our crops. *Apis* genus has been shown to be risky due to *Apis* specific parasites and pathogens which have led to massive declines in honeybee numbers. Biotic stress accompanied with climate change may cause further population declines and lead farmers and researchers to look for alternative pollinators. Recently, wild bees are receiving more attention for their pollination service due to the reduced availability of honey bee colonies (Delaplane and Mayer 2000; FAO, 2008). Some wild bees are more efficient pollinators than honey bees of specific crops (Delaplane and Mayer, 2000).

Crop plants those are more efficiently pollinated by wild bees include alfalfa, blueberries, and cranberries (Delaplane and Mayer, 2000). Bumble bees (*Bombus* spp.) are important blueberry and tomato pollinators (Banda and Paxton, 1991) because they have the ability to buzz-pollinate. Bumble bees also play an important role in natural landscapes (Hellwig and Frank, 2000), because they are able to pollinate certain flowers better than other bees due to their size and long tongue (Velthius and van Doorn, 2006). The alfalfa leafcutter bee (*Megachile rotundata)* and the alkali bee (*Nomia melanderi*) are efficient pollinators of alfalfa (Bohart, 1972; Cane, 2002). Alfalfa flowers need to be tripped to expose the stigma and to release the pollen. When leafcutter or alkali bees visit the flower they release the pressure on the interlocking keel petals which allow the fused reproductive column to snap upward depositing pollen on the bee (Frank, 2003). Alfalfa and Alkali bee are efficient pollinators of alfalfa (James and Pitts-Singer, 2008), because they forage from the centre of the flower causing it to trip.

Crop plants bloom for a short window of time. Many wild bees that contribute to pollination require forage sources outside of the crop bloom period (Tuell et al., 2008). Natural landscapes adjacent to crop fields provide floral resources for all seasons and are important to the sustainability of the wild bees. Creating areas of flowering plants will conserve pollinators and improve crop pollination (Tuell et al., 2008). Areas of floral resources also provide both wild and managed bees a place to escape from the pesticides applied crop lands (James and Pitts-Singer 2008). Most of the wild bees have a smaller foraging radius as compared to the honeybees, and their foraging distances frequently correlate with their body size (Gathmann and Tscharntke, 2002). So, it is important that foraging and nesting resources are to be close in proximity to one another.

Other wild bees, which are well-known for their pollination services to crops are, mason bees (*Osmia* spp) for pollination of orchard crops (Maeta, 1990; Bosch and kemp 2002; Maccagnani et al., 2003) and australian native bee (*Amegilla chlorocyanea)* (Hogendoorn et al., 2006) for tomatoes. Stingless bees are important pollinators of tropical plants (Heard, 1999), tomatoes (Hikawa and Miyanaga, 2009) and Strawberry (Maeta et al., 2012).

Stingless bees resemble those of honey bees in some extent, including their preference for a wide range of crop species which are making them attractive for commercial management.

It is reported that non-managed wild bees are responsible for an estimated 3 billion dollars in pollination to crops every year (Losey and Vaughan, 2006). The pollination provided by wild bees are considered free because investments of money and effort are not always necessary to benefit from their services. Wild bees are not as well studied as honeybees and little is known about their biology.

1.4. Evidences of pollinator decline

Worldwide, the number of flower visiting insect species is estimated to be around 150,000 (Buchman and Nabhan, 1997) in which bee accounts for 25,000 to 30,000 species. Though pollinators are known to provide essential services for well-functioning of the ecosystem, they are declining worldwide. Pollinators require a range of resources from their environment for nesting, foraging, reproduction and shelter. The loss of any one of these requirements can cause pollinators to become decline and extinct (FAO, 2008).

Decline of pollinators has been noted in many region of the world. Honeybee (*Apis mellifera*) colonies, both managed and wild bees are also declining because of pesticides and chemical misuse, disease and parasites, habitat loss, introduction of new plant species and habitat degradation (Allen-Wardell et al., 1998; Frankie et al., 1990; Kevan et al., 1993; Klein et al., 2003a, b; Kremen et al., 2002, 2003, 2004; O'Toole, 1993, 1994; UNEP 2010). Habitat destruction and fragmentation often shift the balance of nature in remaining habitat patches so that native organisms can't persist for long time. Changes in land-use pattern such as increasing agricultural intensification (Corbet et al., 1991) and landscape structure also affect pollinators. Climate change may potentially be one of the most severe threats to pollinator biodiversity (Hegland et al., 2008; Potts et al., 2010). Climate change may cause for the changes in the time of growth, flowering and maturity of crop plants, with consequent impacts on crop pollinators. Studies have shown that loss of bee pollinators resulted in loss of pollination services and causing for severe ecological and economic threats that could significantly affect the maintenance of crop production, food security, wild plant diversity, human welfare and wider ecosystem stability (Kevan, 1977; Ricketts, 2004; Potts et al., 2010; Kjohl et al., 2011).

1.5. Conservation of wild bees and their habitat management

A rapidly increasing human population will reduce the amount of natural habitats through an increasing demand for urbanization, food producing areas, and other land-use practices putting pressure on the pollination services delivered by wild bees. The efforts in many parts of the world to conserve and better manage wild bees are proposing innovative concepts in the conservation of biodiversity. Conservation of wild bees should mean conservation of plant species and vice-versa (Roubik, 1995). Population decline of either plant or pollinator bee species threatens the pollination of crops or wild flowers. When pollination systems are threatened by environmental changes natural biodiversity and agriculture system become highly vulnerable. Food plants, nest site and nesting materials for pollinating bees to be known for conservation and management their population. Food and nesting resources through habitat management and enhancement is the best way to support their populations (Shepherd et al., 2003). Management would be relatively easy to plan if all pollinator-plant relationships are known. Area with diverse floral resources that bloom over an extended time period has been shown to sustain or increase the diversity of wild bees (Vaughan and Black, 2006). A diversity of flowering plants will attract and maintain a higher diversity of bees and other pollinators. The conservation of existing flowering plants and cultivating native plants that provide nectar and larval food for pollinators is also important in conserving beneficial bee species (Blaauw and Isaacs, 2014). Natural areas that provide bare ground, dead trees and cavities and areas planted with floral resources are ideal habitats for pollinators. Wild bees may nest in the crop fields they help pollinate, but cultivation, irrigation and tilling practices can kill developing larvae. The selection of environmentally friendly pesticides is an important agricultural management practice to manage the population of pollinators. Providing suitable nesting habitat and floral resources will promote bee population and reproduction (Bohrt, 1972).

The abundance of natural habitat in the vicinity of an agricultural site has a significant, positive effect on the pollination service of wild bees (Kremen et al., 2004). Efforts to conserve and enhance wild bees will benefit many bee species. Habitat requirements for vital pollinators and habitat designations for endangered plants should be prioritized for sustainable agriculture and biodiversity of bees and host plants (Batra, 1995). Minimizing use of pesticides and chemicals also help the unwanted loss of pollinators.

1.6. Management of wild bees in enclosures

Management of wild bees for crop pollination has been practiced only for few crops which are poorly or inefficiently pollinated by honeybees. Recent declines in managed and honeybee populations have greatly increased interest in the current and potential role of wild pollinators in agricultural pollination. Honeybees and bumblebees have been used to pollinate plants in greenhouses for at least 60 years (Bohart, 1972), but only in the last 10 years some studies have been done on other species such as *Megachile rotundata* (Bohart, 1972), *Osmia* spp. (Bosch, 2002). Management of wild bees has been tried only for few crops which are poorly or inefficiently pollinated by honeybees. Wild bee management has been used for alfalfa, red clover, tomatoes and apples, but principally in the areas where their pollination by the honey bee presents particular problems.

Wild bees are diverse in appearance and behavior. Some wild bee females make their own nest and provision their offspring and some parasitize the nests of others and use the food provisioned by the host to rear her offspring. Nests are generally lined and partitioned with materials such as mud, leaves, plant resin, and glandular secretions. These lining protect the brood from desiccation, disease and excess moisture (Shepherd et al., 2003). Naturally or artificially created bare patches of undisturbed ground or persistent embankments may increase aggregation of ground nesting bees, such as alkali bees and sweat bees. Old wooden structures loose debris piles and thick underbrush may be attractive to carpenter bees and bumblebees as nest sites. Old pithy plant stems, hollow reeds or boards with drilled holes may be inviting to cavity nesting leaf cutter bee and mason bee (James and Singer 2008).

1.7. Objectives and hypothesis of this study

Thousands of bee species visit crop plants globally (Free, 1993) but few species have been managed. Habitat and biology remain unknown about individual species. Understanding the distribution of habitat and the resources is the important step to conserve and manage the bee population. Little is known about most wild bee species and efforts to understand their significance in pollinating wild plants and is critical to their conservation.

This study was carried out to examine the nesting and foraging activities of hairy footed nesting bee *Anthophora plumipes* in its natural habitat as well as under controlled conditions with the aim of utilizing it as a pollinator for wild and cultivated

Chapter 2

Nesting activities of *Anthophora plumipes* (Hymenoptera: Apidae) in their natural habitat

2.1. Introduction

The bee genus *Anthophora* is one of the largest solitary, soil nesting bees in the family Apidae, with over 450 species worldwide in 14 different subgenera (Michener, 2007). *Anthophora plumipes* (Pallas, 1972)*,* called 'hairy footed flower bee' (sometimes also called 'shaggy fuzzy foot bee') is one of the common species, is protandrous and univoltine. This bee is a polylectic and visits wide variety of flowering plants from early spring to mid-summer. Both male and female of *A. plumipes* are fed from a narrow range of characteristically deep throated flowers species in the plant families Labiatae, Primulaceae, Fumariaceae, Leguminaceae and Boraginaceae (Bond and Kirby, 1999; Batra, 1994; Stone et al., 1995; Roberts, 2010). Females build densely aggregated nests in old soft stone walls containing high amounts of sand or lime, mortar joints, south-facing cliffs and more rarely in the ground (Batra, 1994; Roberts, 2010). Their nests are also found in adobe walls of rural houses which are tolerated by the people because these bees are not aggressive. Females excavate their new nests on the same day they emerge from their natal nest and probably mate (Batra, 1994).

A. plumipes is widespread and common in Mediterranean region, and occurs in a variety of color forms from Northwest Europe, Central Asia, and Japan (Brooks 1983; Batra, 1994; Stone, 1993; Stone, 1995; Proschchalykin and Lelej, 2004; Neeman et al., 2006). It is one of the first bee species active during spring (Stone, 1993). This species has relatively stable flight activity and engaged in the early spring which is expected to be used as pollinators of spring flowering plants.

This study was carried out to elucidate the nesting activities and foraging behavior of *A. plumipes* bees under their natural habitats.

2.2. Materials and Methods

2.2.1. Nesting site and density of nests

The study was conducted in a natural habitat of *A. plumipes* bees located in Atagoyama Park, Izumo, Shimane Japan (Fig. 2.1) (Lat. 35° 26' 0", Long.132° 49' 0"). Two nesting sites inside the park were observed. Topography, vegetation, and soil texture were examined around the nesting sites. Nests within 15 square centimeters at the highly aggregated spot was selected for the observation of foraging and nesting activities of the bees.

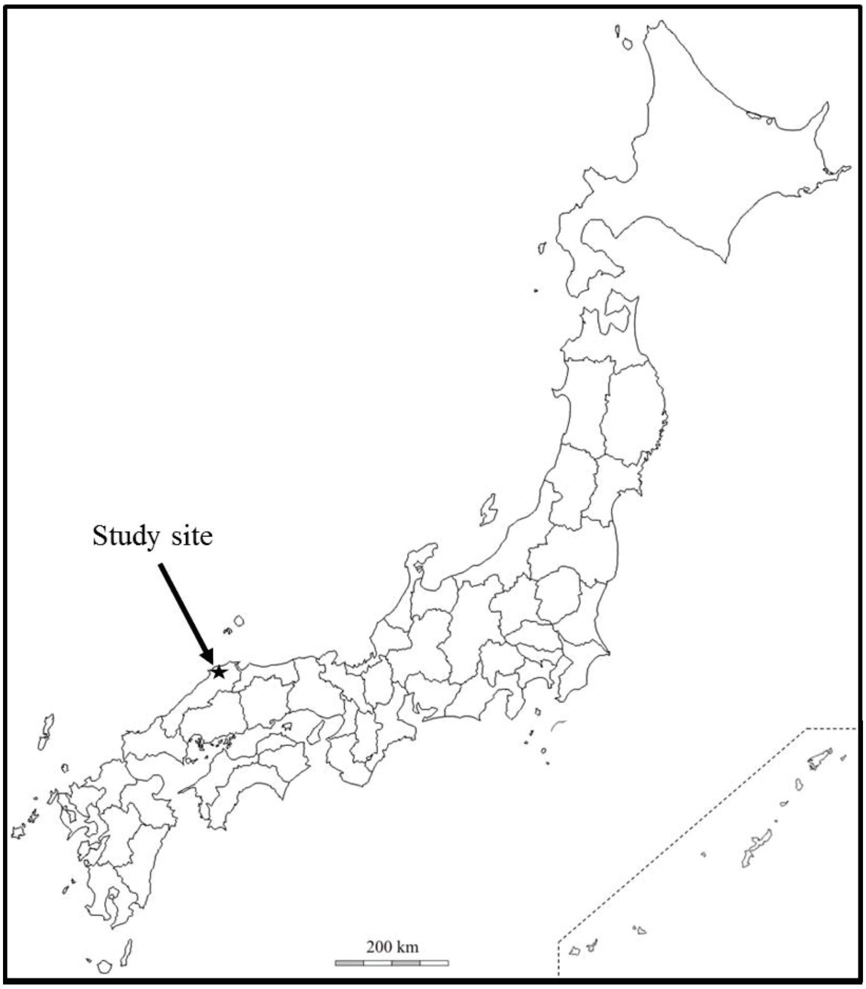


Fig. 2.1. Map of Japan showing the study site (Atayagoyama Park, Izumo, Shimane

2.2.2. Flying period and floral resources

Observation was carried out from April 9 to May 10, 2014. Bee was noticed whether starting nesting activities or not. On April 16, no tags and color mark were given. Daily foraging activities were observed from April 16 to May 10 2014, on the fine weather days. Nesting and diurnal activities was observed mainly in the site A, as number of working bees were higher in this site. Actively nesting females were observed from 8.30 a.m. to

16.30 p.m. Arrival of different bees to their nest and the presence or absence of pollen on their scopae was recorded. The number and duration of pollen and non-pollen foraging trips were observed throughout the observation period. Pollen and non-pollen foraging trips were expressed by the duration from leaving to returning of the bees to their nests with or without pollen, respectively. Days spent to complete one cell and to complete one nest were also recorded. Number of pollen flights for making one pollen ball was also recorded. Total number of nests made by one bee was also recorded.

2.2.3. Nest architecture and cell contents

Nineteen nests in the site A were excavated on July 2014 after completion of nesting activity. Nests were excavated and dimension of various parts of nests (i.e., entrance, burrow, provisioned cell and pre-chamber) were measured. Cell contents, including immature were also examined.

2.3. Results

2.3.1. Description of the nesting sites

At both sites (site A and B) nests of the bees were found below the roots of pine stumps (Fig. 2.2 A). The stumps were exposed and prevented from the rain. At both sites, the nests were densely aggregated (19 to 21 nests per 15 cm^2) (Fig. 2.2).

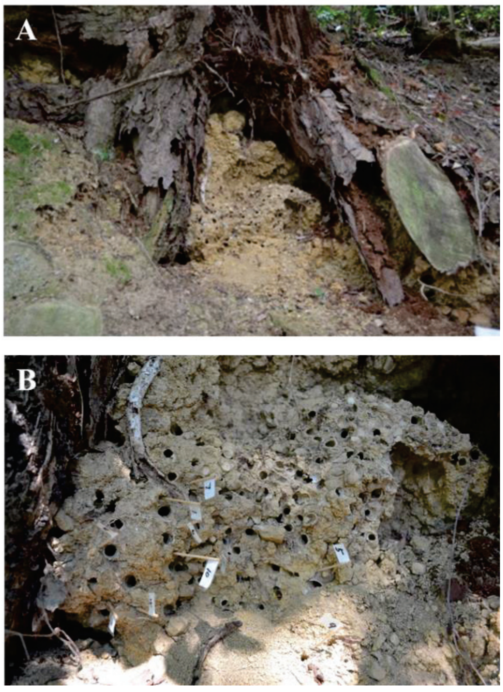


Fig. 2.2. Natural habitat of *Anthophora plumipes* used in this study (A) and their natural nest aggregation (B)

2.3.2. Flight period and floral resources

Foraging activities were observed only in the site A. Bees started flying from early April and lasted until middle of the May. Mating and nesting activities were observed from mid-April through mid-May. Adults of the bees commenced foraging activities from early morning and continued until the sunset in this study. Patrolling of *A. plumipes* males was noticed at the nesting site as well as around the flowers of the plants visited by females. Bees collected nectar and pollen mainly from *Rhododendron indicum*, *Rosa* spp., *Ranunculus japonicus.*

2.3.3. Foraging activities

Both males and females foraged the flower for nectar. Females actively foraged from the morning to the evening. Number of foraging trips per female in a day is shown in Fig. 2.3. Number of foraging trips per female per hour ranged from 0 to 3, and the highest number of trip was observed from 12:00 to 13:00.

The bees were observed for their pollen and nectar foraging activities. First pollen flight was observed on April 16, which lasted until May 10. In a total observation period of 22 hours, 105 flights were for pollen foraging and 58 were for nectar foraging. Duration of each foraging trip (pollen and non-pollen) of a single bee in three consecutive days are shown in (Fig. 2. 4). Duration of flight varied according to the type of work (Table 2. 1). Average flight duration for collecting pollen was 29.9 min (N=105, range: 7.2–60.7 min) and for non-pollen flight was 15.6 min (N= 58, range: 2.6–66.6 min). Average number of fights for making a pollen ball was 11 (N = 3, range: 9 to 14 pollen flights).

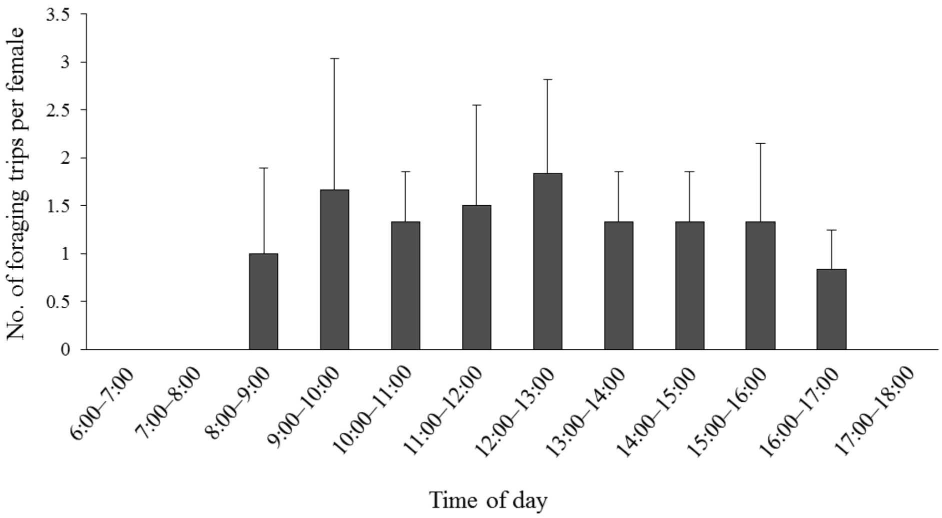


Fig. 2.3. Number of foraging trips per female every one hour in their natural habitat

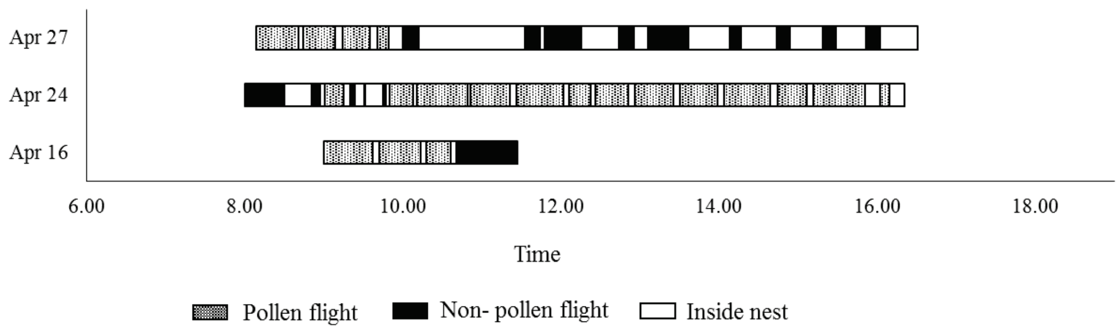


Fig. 2.4. Foraging activity observed in natural habitat of *A. plumipes* bees. The observation indicates the foraging trips by the same bee on different days.

2.3.5. Nest architecture and cell contents

Bees completed a nest in 2 to 12 days (Table 2. 1). Bees took an average of 8 hours to complete a cell. It was observed that the bees reused the previous year's nest. Nests of *A. plumipes* were excavated after one month of completion of making nests.

Table 2.1. Values on nesting activities by *A. plumipes* bees in their natural habitat

Nesting activity	Mean	N*	Range
Total nests made by a bee	1.7	11	1–3
Total cells made by a bee	7.9	7	2–17
Time to complete a nest (day)	7.4	10	2–12
Time to complete to make a cell (hour)	8	3	7.5–8.3
Number of pollen flight for making a pollen ball	11	3	9–14
Trip duration for collecting pollen (min)	29.9	105	7.1–60.7
Trip duration without pollen (min)	15.6	58	2.6–66.6
Time to seal a nest (min)	22.9	20	3.2–80.9
Time to release pollen in the nest (min)	6.4	105	2.1–13.8

* Number of observations

Photo of representative nest is shown in (Fig. 2. 5). Nst entrance was circular and the inner walls of the burrow were more or less smooth and were not coated with some detectable secretion. Cells in the nests were either in single or branched series (Fig. 2. 6). Each female made a maximum of 3 nests and a completed nest contained 2 to 17 cells. In general, one nest entrance was used by one bee; however two bees sharing the same entrance was also observed. Nests without burrow and cell cap was also observed. Pre-chamber similar to the shape of provisioned cells and filled with loose soil was observed, but burrow was remained unfilled. Provisioned cells were oriented downward together with pre-chamber. The cells contained either of pre-pupae, pollen ball, dead larvae, or was empty. Pre-pupa bent its body inwardly to place the dorsal body on the bottom wall and fecal pellets were attached on the innermost base wall, where broods were found. Average number of nests and cells per female was 1.7 (range: 1–3, N = 11) and 7.85 (range 2–17, N = 7), respectively. Number of female and male cells per bee ranged from 1–5 and 1–4, respectively. In all nests, male offspring were nearer to the entrance, indicating that male eggs are laid later. The brood cells were in shape with a relatively constricted mouth. The first brood cells were located 37.44 mm (range: 16–61 mm, N = 24) apart from the entrance. The inner wall of both provisioned cell and pre-chamber were coated white. The size and dimensions of various parts of the nest found in natural habitat are summarized in (Table 2. 2).

Table 2.2. Dimension of nests of *A. plumipes* bees in their natural habitat

Dimension (mm)	Mean	N*	Range
Cell length	19.2	43	11.9–21.5
Cell width	14.5	48	9.4–13.5
Diameter of neck	10.1	46	9–11.8
Cell plug thickness	2.3	49	1.6–3.5
Nest length	69.3	3	60–85
Length of burrow	12.3	3	11.5–13
Entrance diameter	14.25	2	14–14.5

* Number of observations

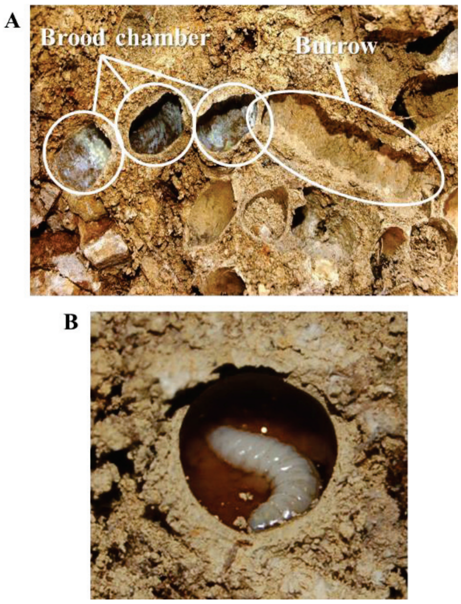


Fig. 2.5. Nests photo of *A. plumipes* bees in natural habitat

2.4. Discussion

A. plumipes bees started flying from early April and lasted until middle of the May in their natural habitat. First pollen flight was observed on April 16, which lasted until May 10 in this study. Both male and female bees were actively foraging nectar flight. Male bees do not provision nest but forage for own consumption (Batra, 1994; Neeman et al., 2006 and Tsuji and Kato, 2010). Foraging from different species of flower suggests that this bee is polylectic (Batra, 1994). Number of foraging trips per female per hour ranged from 0 to 3, and the highest number of trip was observed from 12:00 to 13:00 (3 trips). Females actively foraged from the morning to the evening. Average flight duration for collecting pollen was 29.93 min (N=105, range 7.15–60.66 min) and for non-pollen flight was 15.63 min (N= 58, range 2.58- 66.61 min). Batra (1994) reported duration of a pollen trip of *A. pilipes* ranging from 6–47 min under field conditions. Stone et al. (1999) reported that *A. pauperata* took longer time to collect pollen in the morning. Batra (1994) reported the same bee species visited 12–15 blueberry flowers per minute. In the study of Bond and Kirby (1999) it was 2.5 seconds per flower of *Vicia faba*, which was faster than bumble bee (*Bombus hortorum*). Average number of flights for making one pollen ball was 11 (N = 3, range 9 to 14 pollen flights).

The dense nest aggregation were found in hard clay walls in sloppy land under the root stumps of pine. Mated females of *A. plumipes* typically nest in old cob walls, in soft mortar joints in walls, and occasionally in the ground. In favored locations, the bee can easily nest in aggregations of hundreds (Roberts, 2010). Cane (1991) reported females might nest gregariously or singly beneath hardwood forests litter, in open sand, or in the walls of large earthen hole. Old nest were reused by offspring. To make the nests in the soil, the *A. plumipes* bees soften the soil by nectar same as in Anthophorid, *Amegilla dawsoni* which habitat in the desert use the nectar to moisten the soil to make soft (Alcock, 1999). This species is closely related to the *Anthophora abrupta* making unique sound in the ticking of the nest (Norden, 1984). This species is closely related to the *Anthophora abrupta* making unique sound in the ticking of the nest (Norden, 1984). Anthophoridae show a wide range of variation in their nesting sites and nesting strategies. Some members (for example, *Xylocopa* sp.) nest in woody plants (Stephen et al., 1969), and rest of them are ground nesting. Some of the ground nesting bees are gregarious in nesting strategies (*Anthophora abrupta,* Norden, 1984; *A. plumipes,* Batra, 1994; Stone, 1995; *Anthophora walteri*, Gonzalez and Chavez, 2004), but other species are true solitary, nesting individually well apart from each other. Some always dig a new nest every time (*A. pauperata,* Semida, 2000). It is also reported that some soil nesting bee reuse natal nests (*A. atriceps*, Kamel 1981, *Lassioglossum scitulum*, Miyanaga et al., 2000).

Single series and multiple series nests were found having linearly arranged cells. Cells without burrow were also observed. Roberts (2010) also reported the female *A. plumipes* nest consisted of a single burrow or a series of branching burrows. The barrel shaped cells are either isolated or in series in the burrows, and are made of earth or clay in such a way that they can be separated from the surrounding matrix (Michener, 2007). Tadauchi et al. (2005) reported soil nesting bees constructed nests containing several brood cells connected with the main burrow. The burrows were more or less perpendicular to the level of brood cell. The cells were made by clay soil which was used for making nest, and were situated at the end of burrows. A completed nest contained 2-17 cells. Compared to the findings of Roberts (2010), this study showed the high capacity to produce offspring. After completion of nesting activity this bee overwinter (Batra, 1994).

In this study, most of the brood cells were declined 30–40° downwards, and the cells were arranged linearly in single and multiple series. Downward declination by 30–40° of the brood cells of *Andrena* was also reported by Tadauchi et al. (2005). Semida (2000) reported each nest of *Anthophora pauperata* contained from 2–4 complete cells arranged vertically or semi-vertically at the bottom of long tunnel. Miyanaga et al. (1998) reported burrow of soil nesting bee *Lassioglossum mutilum* descended nearly vertically or slightly declined to the bottom end. Nest with 60–85mm in length having 14–14.5 mm in entrance diameter and 11.5–13mm burrow was observed. Tadauchi et al. (2005) reported 14–20 cm long burrow in the nests of *Andrena* bees. Miyanaga et al. (2006) reported 5.0–5.2 mm wide burrows of *Lassioglossum* bees. Tadauchi et al. (2005) has reported 5.5–6.0 mm entrance diameter in the nests of *Andrena* bees.

The inner walls of the burrow were more or less smooth, and were not coated with any detectable secretion. Bee secret dufour's gland lipids which is used to waterproof the cell walls (Cane, 1991 and Cane and Carlson1984).The dufor gland secretions in *Anthophora abrupta* is later eaten by the bee larvae (Norden et al., 1980). The components of white coat are reported to have secretions from the dufour gland of *A. pillipes* (Batra, 1994). In

Anthophora abrupta, nectar and pollen collected in brood chamber is added to the dufour gland secretion which smells like cheese (Norden et al., 1980). Secretions from the dufour gland are used for water proofing membrane brood (Batra, 1994). *Anthophora* secrets triglyceride, a clear oily secretion, is altered to diglyceride to form a soft and opaque waterproof membrane (Norden et al., 1980). *Anthophora* use a secretion from the dufour glands, using a high-energy state for the young, the food is easier to digest than pollen and royal jelly, believed to have similar functionality to the milk. According to (Norden et al., 1980) young *A. abrupta*, everything consumed food cache, a bite taken from the inner wall of shiny diglycerides brood, and how the food has been confirmed.

2.5 Conclusions

A. plumipes is a spring emerging bee, which forages in spring blooming crops in warm humid temperate climate. It is a gentle and gregarious bee tolerant to human activities. It forages for food and nest provisions. Nests were gregariously made in dry soils in horizontal cliffs. Nests were found in single series and multiple series.

Chapter 3

Nesting activities of *Anthophora plumipes* (Hymenoptera: Apidae) under the closed circumstance

3.1. Introduction

There is a debate that honey bees are best pollinator for most of the crops inside the green house and open field, and these bees are not able to pollinate all flowers due to nectar chemistry, flowering phenology, floral morphology, and body size. Due to higher costs and difficulty in maintenance, honey bees are becoming less attractive as commercial pollinators (Losey and Vaughn, 2006). Recent report of worldwide decline of honeybees have shown a need to study more about alternative pollinators for plants (Cox-Foster et al*.,* 2007). On the other hand, wild bees are receiving more attention for their pollination service due to the reduced availability of honey bee colonies. Some wild bees are more efficient pollinators than honey bees of particular crops.

Management of wild bees for crop pollination has been tried only for few crops which are poorly or inefficiently pollinated by honeybees. Recent declines in managed and feral honey bee populations have greatly increased interest in the current and potential role of wild pollinators in agriculture. Honey bees have been used to pollinate plants in greenhouses since 1910s such as *Megachile rotundata* (Bohart, 1972), *Osmia* spp. (Bosch, 2002), leafcutter bee (*M. rotundata)* and the alkali bee (*Nomia melanderi*) (Frank, 2003; James and Pitt-Singer, 2008). Bumble bees are another group of the popular pollinators inside greenhouse. However, usefulness of bumble bee colonies is limited because if colonies are not properly handled, the bees can invade natural habitats and compete with other native bees (Hingston and McQuillan, 1999; Delaplane and Mayer, 2000; Dohzone, et al., 2008).

Number of studies shows the usefulness of various species of the genus *Anthophora*. *A. plumipes* is highly recommended for management and development as a pollinator of spring blooming crops in warm, humid temperate climate (Batra, 1994). This species is known to be good pollinators of blueberries (Batra, 1994; Maeta et al., 1990). *A. plumipes* was also more effective to pollinate autumn-sown broad beans *(Vicia faba* major) compared to *Bombus hortorum*, *Bombus pascuorum* and *Apis mellifera* (Bond and Kirby*,* 1999).

However their pollinaton efficiency on crops under closed conditions remains unknown. Therefore, in this study the *A. plumipes* were collected and reared in a green house, with the purpose of developing this bee as an alternative pollinator under closed conditions.

In the USA, this species has been tried to use as pollinators for several crops, including fruit trees (Batra, 1994; Contance et al., 1999). Higher efficiency of *A. plumipes* has been observed to visit flowers of autumn-sown broad beans *(Vicia faba* major) than *Bombus hortorum*, *B. pascuorum* or *Apis mellifera*. Higher foraging activity of *A. plumipes* has also been reported compared to *B. pascuorum* (Bond and Kirby, 1999).

Although *A. plumipes* are shown to have good pollinators for several flowering plants under open field conditions, their behavior under closed conditions remained unknown. Therefore, in this study the *A. plumipes* were collected and reared in a green house, with the main purpose of developing this bee as an alternative pollinator under closed conditions. There were three considerations before starting this work: (1) whether this bee can forage under closed condition, (2) can adopt artificial nest, and (3) can reproduce in closed condition.

3.2. Material and methods

3.2.1. Collection of bees for the experiment

During the spring of 2010, 30 females and 4 male individuals were collected from the nest of at a Mountain of Hirata Park, Izumo, Japan (Lat. 35° 26' 0", Long.132° 49' 0") (Fig. 2.1). During the spring of 2011, 24 females and 9 males were collected from the same park. Nests were densely aggregated and nesting ground was beneath to the exposed roots of the pine stumps near to the road side.

3.2.2. Preservation of collected bees in the laboratory

In 2010, the collected bees were brought back to the University's laboratory and kept in an incubator at a temperature of 6ºC for about one month. Each bee were kept separately in plastic cups (5.5 cm in diameter and 4 cm in height) filled with sphagnum moss. Five among the 30 female individuals died during the incubation.

Bees collected in 2011 were also kept in an incubator at the same temperature (6ºC). Each bee was kept in a glass tube (10.0 cm long and 4.0 cm in diameter) closed by cotton.

3.2.3. Greenhouse condition

A small greenhouse (8.1 m in length, 4.5 m in width and 2.9 m in height) was used for the entire experiment (Fig. 3.1). The greenhouse was iron framed and netted by polyester. Black plastic was covered above the nests to cool down the bees during hot days. The greenhouse was located inside the premise of Shimane University, Matsue, Japan (lat. 35º29', elevation 170 m).

3.2.4. Nesting materials for the bees

In 2010, red clay soil blocks of different sizes were used as a nesting substrate for the bees. Clay soil was sieved and mixed with water before filling into the pots. Blocks were prepared 17 days before releasing the bees. Before the mud gets dried, entrance holes of 13 to 17.4 mm in diameter and 10 mm depth were made. The holes were made 5 to 8 cm apart. Seven soil blocks of various sizes were kept on a table inside the green house, among which six were prepared in soil pots and one in a plastic pipe. The plastic pipe was 16.5 cm wide and 34 cm long with five holes. Two soil blocks were 19 cm wide and 23 cm long with 18 holes, three blocks were 24 cm wide and 29 cm long with 23 holes, and one block was 22 cm wide and 43 cm long with 19 holes (Fig. 3.2A, 3.2B). Old nest soil was kept at the entrance ofnest.

In 2011, soil cylinders and soil blocks were used as the nesting material. Soil blocks were prepared 15 days before releasing the bees, as mentioned above for the previous year. Four kinds of soils: (1) house building clay (mixture of red and white colored soils) (2) soil collected from their natural nest, (3) red clay soil and (4) mixture of natural nest soil and house building clay, were used to make the soil cylinder to check the preference of soil for hole to attract the bees.

Fig. 3.1. Greenhouse used for this study (A) and flowers inside the greenhouse in 2010 (B)

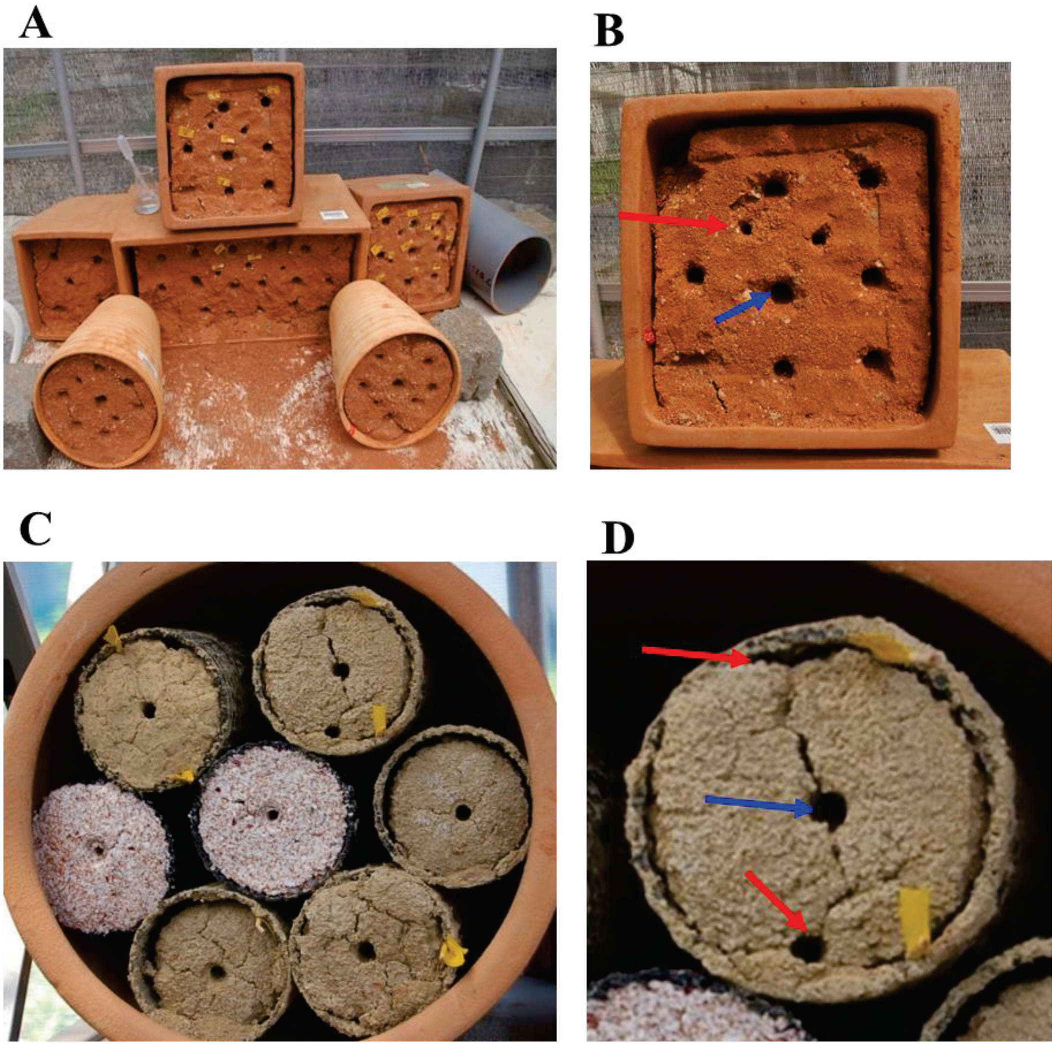


Fig. 3.2. Artificial nests: soil blocks (A, B), and soil cylinder (C, D). Blue arrows-artificial nests; red arrows-nests made by bees

3.2.5. Floral resources

Four types of flowering plants differing in floral structure were used to know the adjustability of bees in different flower condition in 2010 and 2011 (Table 3.1). During the first year (2010), Around 300 pots of various flowering plants were supplied as main food resources. Floral resources included 85 pots of phacelia [*Phacelia tanacetifolia* (Hydrophyllaceae)], 52 pots of borage [*Borago officinalis* (Boraginaceae)] and 38 pots of centaurea [*Centaurea cyanus* (Asteraceae)]. The flower pots were prepared and kept inside the greenhouse on April 30. Two pots of phacelia, 46 pots of borage and 39 pots of centaurea were added later on May 2 (Fig. 3.3). After the flowers matured, sweet clover [*Melilotus officinalis* (Fabaceae)] and giant catmint (*Nepeta grandiflora* (Lamiaceae) were supplied to save the bees from starvation. Bees visited giant catmint but did not collect the pollen, while they did not visit sweet clover. The greenhouse was opened after the shortage of floral resources.

In the second year (2011) phacelia, borage and red clover [*Trifolium pretense;* (Fabaceae)] were used as floral resources. On May 6, 166 pots of phacelia, 66 pots of borage and 80 pots of red clover were kept in inside the greenhouse.

Phacelia has hairy and coiling type flower and usually blue in color. A phacelia flower is about a centimeter long and has protruding whiskery stamens. The borage flowers are complete, perfect with five narrow and triangular-pointed petals. Flowers are most often blue in color, although pink flowers are sometimes observed. The flowers arise along scorpioid cymes to form large floral displays with multiple flowers blooming simultaneously, suggesting that borage has a high degree of geitonogamy. It has an indeterminate growth habit which may lead to prolific spreading. The flowers of centaurea were intense blue, pink and white in color. The flower produced in flower heads (capitula) 1.5–3 cm diameter, with a ring of a few large, spreading ray florets surrounding a central cluster of disc florets. The red clover flowers are dark pink with a paler base, 12–15 mm long, with a dense inflorescence.

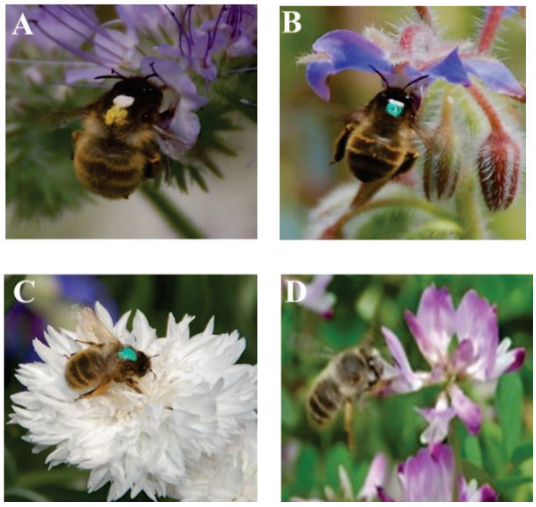


Fig. 3.3. Bees foraging on different flowers in the greenhouse: phacelia (A), borage (B), centaurea (C), and red clover (D)

Table 3.1. List of materials used in greenhouse in 2010 and 2011

Material type	Material	2010	2011
Flower	Phacelia	O	O
	Borage	O	O
	Centaurea	O	
	Red Clover		O
Nesting material	Soil blocks	O	O
	Soil cylinders		O

3.2.6. Releasing bees in the greenhouse

In 2010, after about one month of rearing in the incubator, the bees were liberated in a greenhouse in dark. Ten female bees were liberated in the greenhouse on April 30 at 6:00 pm with an additional liberation of 15 more females and four males on May 1. Among the liberated bees, 10 females and two males died, and 15 females and one male were active for a long period.

During the spring of 2011, a total of 34 female and 16 male individuals were liberated in the same greenhouse used for 2010. Among the liberated bees, 10 females and seven males were the offspring from 2010 and other 24 female and nine males were collected on April 8, 2011 from their natural habitat. Twelve females and five males were liberated on May 7 at 8.00 pm, 12 females and two males on May 9, 10 females and two males on May 13, and 7 males on May 19 at 9.00 am. Purpose to release bees at different time of the day was to know the effect of time of release on their behavior. Three females and two males died and 16 female and five males escaped from the greenhouse.

3.2.7. Marking bees and nest entrances

In the first year, after 12 days of liberation (May 12, 2010) inside the greenhouse, each females were marked with opaque color paints of various colors on the thorax for the identification. A bee was left unmarked, because this bee was not found at the time of marking. Markings were done with blue (B), blue and yellow (BY), green (G), red (R), red and blue (RB), red and green (RG), red and white (RW), red and yellow (RY), white (W), white and blue (WB), white and green (WG), white and red (WR), white and yellow (WY), yellow (Y) and yellow green (YG). For marking, females were captured by net and kept in a small vial for a few minutes in an ice box to make them inactive for short period. Marking were carried out at 7:40 am, and no bees were dead while marking. In the second year, bees were marked by the same way but done before liberation. For marking the entrance holes, an identification number tag was attached near each hole used by the working bees.

3.2.8. Collection and observation of pollen loads

In 2011, pollen load were collected from the bees and nests to identify the preference of flowering plant to collect the pollen. Pollen loads were collected by forcing the females to deposit the pollen from their pollen basket after capturing them and keeping in small vial for few minutes in an ice box. Pollen grains in the pollen balls collected by the bees and obtained inside the brood cells were observed under a compound microscope. Pollen balls in the nests were collected after nest excavation.

3.2.9. Foraging activity and relevant behaviors

Time of departure and arrival of bees from and to the nests

After marking bees and nests in 2010, foraging activities of fifteen actively nesting females were observed from around 5:25 am to 19:35 pm. Their time of departure and arrival time from and to their nests were recorded from 6:00 am to 6:00 pm for 5 days (May 13, 15, 16, 17, and 20). While observing arrival of each bee to their nests, the presence or absence of pollen on their scopae was also recorded.

Flight route of the bees in the blocks of different flowers

Some of the bees were followed to observe their route of flower visit. The route of visiting flower blocks at various positions inside the greenhouse was recorded chronologically.

Time spent for visiting each flower

Time interval of the bees spent to on a flower of each of the three species were recorded by using a stopwatch.

Way of collecting pollen and nectar

When bees visited flower, their activities were carefully observed for the ways of collecting pollen from different flowers.

Number of flights to make a pollen ball

Number of flight to make a pollen ball was expressed by the arrival of individual with pollen load to non-pollen load.

Preference to floral resources

In 2010, time spent by bees to visit flowers of phacelia, borage and centaurea was recorded. Time spent by different bee individuals to forage each of the phacelia, borage, centaurea and red clover inside the greenhouse was also recorded. Observation was made from marked individuals.

Time for digging nests and brood cells

Time spent inside the nests for digging nests and brood cells were recorded.

3.2.10. Behavior of the male bees

Patrolling, flying period and mating behavior of the males were observed during observation period.

3.2.11. Pollen availability in the flower

In 2011, each of the three flowers (phacelia, borage and red clover) was observed for the pollen availability at different time periodically on a day. For this purpose, a flower pot was randomly selected and the flowers bloomed at a time were picked up after counting to overcome the chance of repetition. A block of each of three flowering plants inside the green house was also checked for its pollen availability in a 0-4 scale basis. Where, '0' stands for no-flower or no-pollen and '4' for flowers with highest pollen load.

3.2.12. Nesting activities

In 2010, all of the marked bees were observed for the nesting activities. Nests were kept in the greenhouse until July 2010, after which nests were moved outside in the dark and brought back to the lab for observation of nest structures.

3.2.13. Nest architecture

For examination of nest architecture, a total of 25 nests were excavated from August 24 to November 25 in 2010. In 2011, 6 nests were excavated from August 7–18. Forceps, small hammer and spatula were used for the excavation. Nest structure, stage of brood, number of brood per nest, diameter of main burrow, depth of brood cell and size and dimension of brood cells were measured using digital Vernier calipers. Structure of male and female cells, nest and cell diameter, total number of nests and cell, were also recorded**.**

3.2.15. Distribution of sexes, male ratio, and number of eggs laid per female

After excavating the nests, each brood was kept in a sample tube (5 cm in height and 2 cm in diameter) and stored at room temperature until emergence of adults. The sexes were determined by their shape, size and color. Pre-pupae, pupae and adult found in the nest were also examined. After examining the sex of each brood, sample tubes were kept in an incubator at 12ºC during November and 8ºC during December**.**

3.2.16. Temperature, relative humidity and light intensity

Temperature, relative humidity and light intensity inside the green house during the examination of foraging activities were recorded by using thermometer, hygrometer and light meter, respectively, from 6:00 am to 6:00 pm at an interval of one hour.

During the study period, temperature ranged from 9.5ºC to 36.4ºC, humidity from 21 to 99% and light intensity from 125 to 1,980 lux. Details of the weather data is presented in (Fig. 3.4).

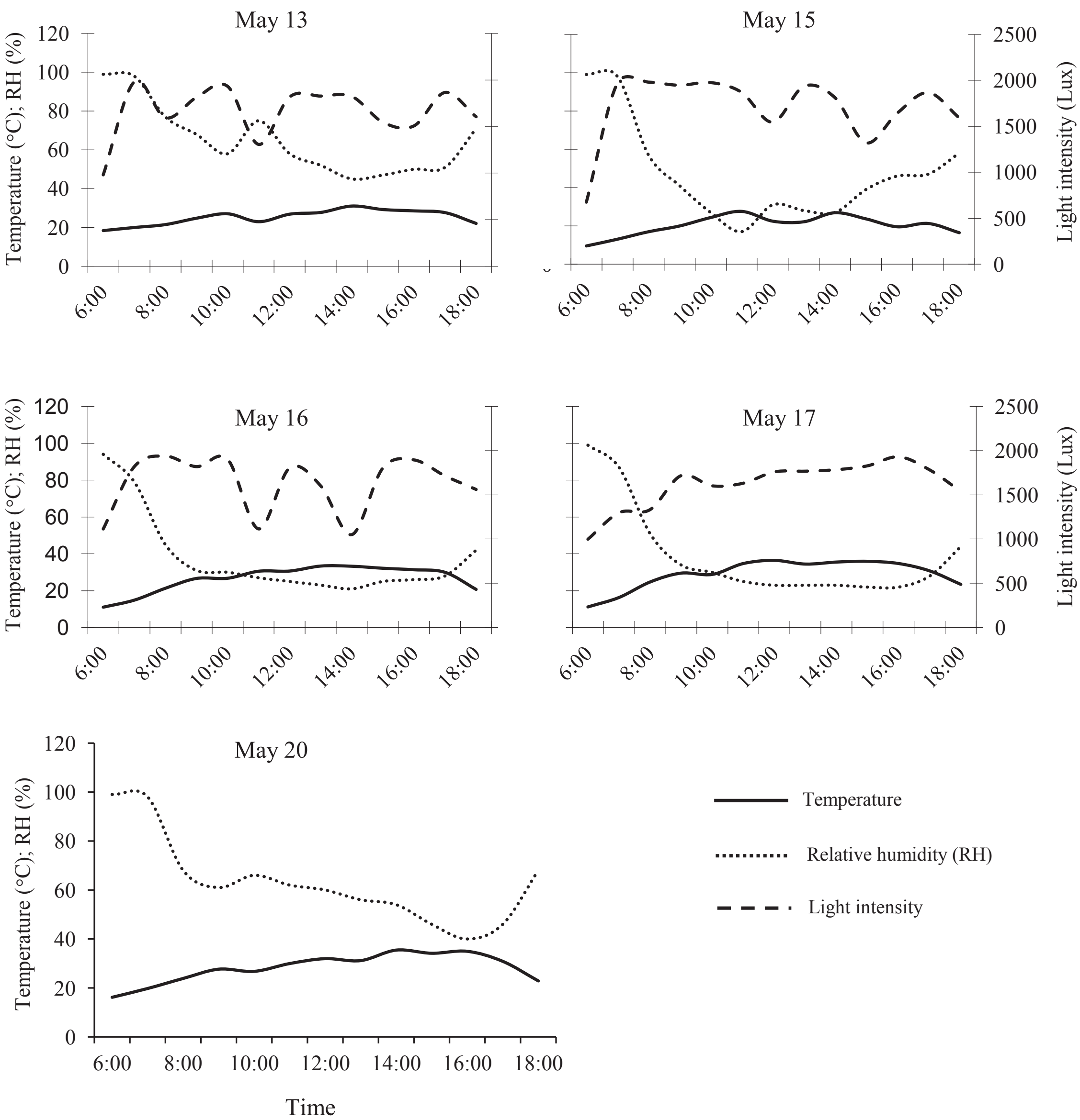


Fig. 3.4 Temperature, humidity and light intensity during the observation period from May 13 to May 20, 2010

3.3. Results

3.3.1. Acclimatization of bees in the greenhouse

Bees started finding nests from the next day of liberation in both of the years (2010 and 2011). Bees released in the evening started visiting flowers and finding nests right from the nest morning, and those released in the morning did just after an hour. Females flew to the hover in front of artificial soil blocks on the next day of liberation. Both males and females visited flowers and sucked the nectar. Some females flew over the ceiling and many were flying around the artificial nests.

3.3.2. Behavior of male bees after releasing

In 2010, out of four male bees released, two died and one disappeared after 2 days of release. Remaining one was observed for a period of 25 days in the greenhouse in 2010. In 2011, there were nine male bees were present inside the greenhouse for a long time.

Male bees released in the evening started chasing females from the next morning of liberation, and those of released in the morning did so just after one hour of liberation. Morning released males showed mating behavior after 3 hours of liberation. Males pounced on the foraging females on the foraging area, around the nest entrance and over the height of 1.5 to 2 m from the ground level. Duration of mating lasted for about one minute. Patrolling males frequently chased females in rapid circling and zigzag flights. Sometimes males were found sleeping inside the nest burrows, however it did not enter the burrow in search of females. Males did not participate in nest construction and provisioning activities. They only visited flowers for nectar for their own consumption.

3.3.3. Preference to artificial nests

In 2010, out of seven soil blocks provided, bees used 3 blocks for making nests. Of which, two blocks were of 24 cm in width and 29 cm in length and one was 19 cm in width and 23 cm in length. A total of 25 nests were built by 16 bees in those three blocks. Among a total of 65 drilled entrance holes, 21 were occupied by 12 bees and bees made others four entrance holes by themselves with sizes of 8.7, 9.7, 11.8 and 17.4 mm in diameter.

Bees used soil cylinders but did not use soil blocks for making nests in 2011. Among the soil cylinders filled with different kinds of soils, they used only the cylinder filled with the mixture of white clay and light clay soils. Although, the bees were attracted to all types of cylinders and soil blocks, they could not start burrowing rest of the materials. Rejection of soil blocks and some soil cylinders might be due to relatively harder soil materials.

3.3.4. Response of bees during and just after marking

In 2010, 16 bee individuals were successfully marked. One bee was weak after marking, later it was fed with sugar syrup, but could not survive. After marking, females easily recognized their nests. An identification number tag was given to each nest on first come basis.

3.3.5. Foraging activity and relevant behaviors

In this study, females visited flowers for pollen and nectar, whereas males visited only for nectar and chased females for mating. Females started foraging before males and foraged until later in the evening than the males (data not presented).

3.3.5.1. Number of foraging days

Foraging activities of a total of 15 differently colored females were observed for 5 days (May 13, 15, 16, 17 and 20). Among the total observed bees, five foraged for all of the 5 days. Other 5 bees foraged for 4 days, 2 bees foraged for 3 days and remaining 3 bees foraged for only one day (Fig. 3.5). Yellow green (YG) bee foraged only on May 13, because it had already completed its first nest and escaped thereafter. Other two bees, white (W) and red yellow (RY) also foraged only for a single day, as these bees were selecting site for nesting until the later days of observation period. On the first observation day (May 13), out of 13 foraging bees, only five made pollen flight. On the second day (May 15), out of 12 working bees, 9 made pollen flight. On the third day (May 16), 11 bees were active and 9 among them made pollen flight. On the fourth day (May 17), nine out of 10 active bees made pollen flight. On the fifth day (May 20), seven out of eight active bees collected pollen.

3.3.5.2. Foraging behavior from the morning to evening

Females started foraging from early in the morning until the late evening. They started foraging before 5:25 and continued until 19:35, however data from 6.00 to 18.00 are shown (Fig. 3.6 and Fig. 3.7). Besides visiting flowers, bees collected pollen throughout the day. On the first day, a few bees collected pollen after 10:00 am, and on the second day more bees started collecting pollen after 9:00 am. From the third observation day (May 16), bees started collecting pollen even during the early visits.

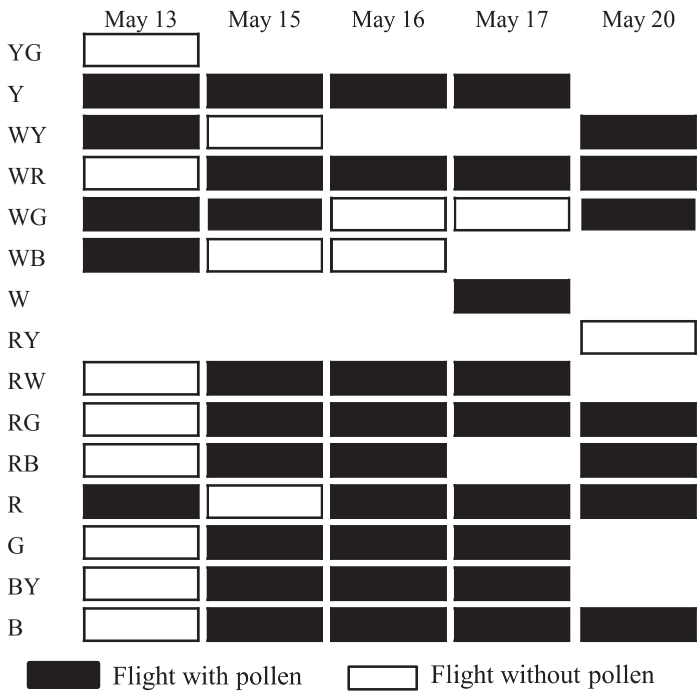


Fig. 3.5. Foraging by 15 bees returned with or without pollen from May 13–20, 2010

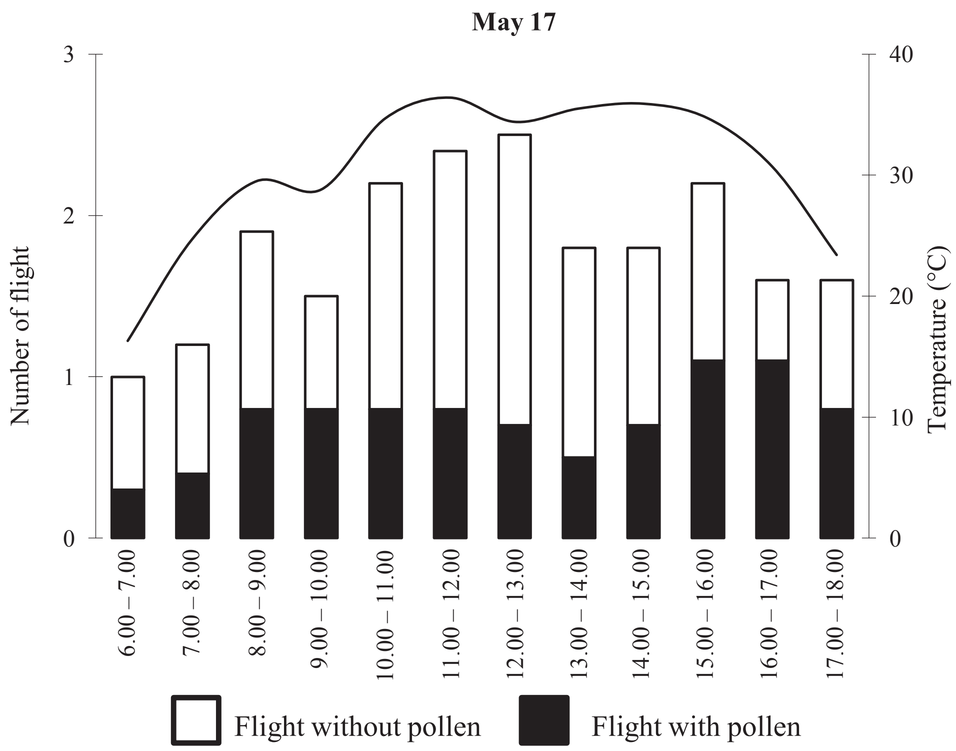


Fig. 3.6. Number of flights with pollen (black) and without pollen (white) on May 17, 2010

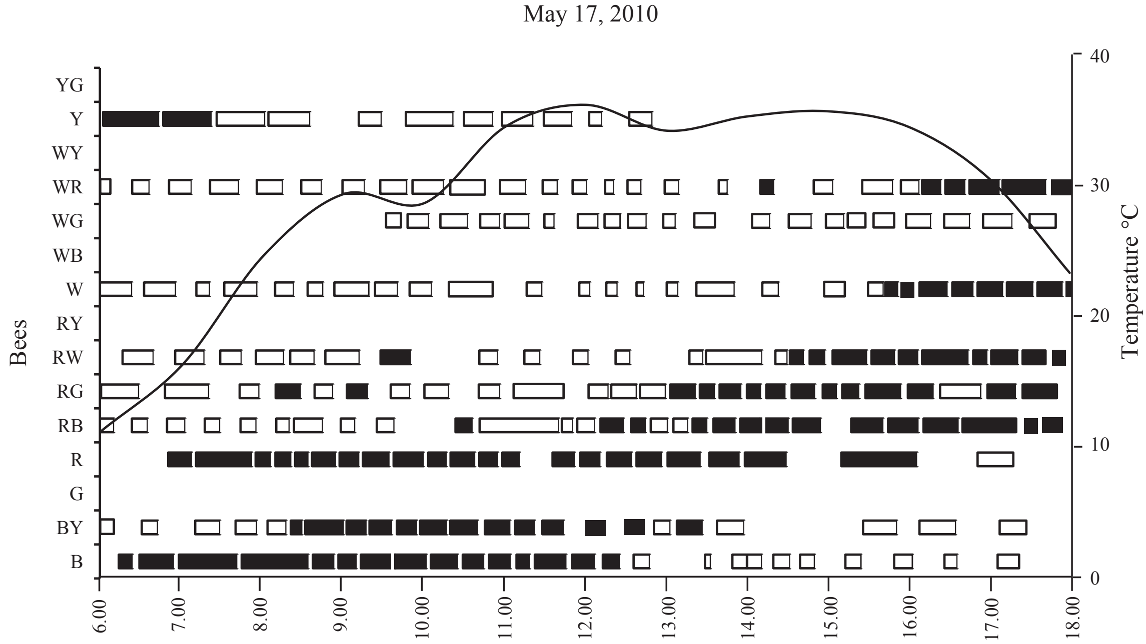


Fig. 3.7. Span of pollen (black boxes), non-pollen (white boxes) and inside nest (space between boxes) made by differently colored bees reared under greenhouse from 6:00 to 18:00 hrs on May 17, 2010. Short cases on the left indent denote bees with different color marks; B= blue, BY= blue and yellow, G= green, R= red, RB= red and blue, RG= red and green, RW= red and white, RY= red and yellow, W= white, WB= white and blue, WG= white and green, WR= white and red, WY= white and yellow, Y= yellow, YG= yellow and green.

3.3.5.3. Influence of light and temperature on foraging

Bees actively foraged even at a low temperature of 9.5ºC to a high of 36.4ºC (Fig. 3.7), showing a high stability of collecting pollen and nectar over a wide range of temperature. Foraging activity was commenced between 17–99% relative humidity and light intensity from 125–1,980 lux. Morning activity was observed even in a temperature less than 9.5ºC but pollen foraging flight was observed from 9.5ºC to 36.4ºC

3.3.5.4. Preference to flowers

In 2010, among the three flower species, bees preferred phacelia, followed by borage and centaurea (Fig. 3.8). Average foraging time for a single flower was 5.40, 6.95 and 7.31 sec for phacelia, borage and centaurea, respectively (Table 3.3). Relatively longer duration was observed when flowers had poor nectar reserve (data not presented). Of the total flower visiting duration, bees spent 51%, 33% and 16% time to phacelia, borage and centaurea, respectively. Bees visited phacelia from 6:30 am to 16:00 pm and borage until 16:00 pm on both of the observation days. On both of the days, bees did not visit centaurea from 10:30 am to 14:00 pm. High temperature during the middle of day might have caused drying of the pollen or poor nectar source making bees reluctant to visit centaurea flowers.

In 2011, phacelia, borage and red clover were used as the floral resources. Former two were selected on the basis of their higher preference in 2010. Preference to flowers by bees was analyzed by examining pollen grains in the pollen balls collected by the bees. Relative proportion of pollen grains of three flower species in the 38 pollen load collected from nine bees were 64%, 28% and 8% of phacelia, red clover and borage, respectively (Fig. 3.9). The relative proportion of pollen grains of phacelia ranged from 40–80%, red clover from 0–34% and borage from 5–22%. When the preference of three flowers between morning (9:00 am–10:00 am) and afternoon (15:00 pm–16:00 pm) was compared, phacelia pollen remained unchanged at both time periods, while proportion of borage was decreased and that of red clover was increased in the afternoon. The data much varied over the six days (May 20–25) (Fig. 3.10). Higher preference to phacelia in both of the years might be due to its higher pollen availability (Fig. 3.11) and number of bloomed flower over a day (Fig. 3.12) compared to other flowers. Visual observation showed higher nectar availability in borage flowers, therefore it can be suspected that bees visit phacelia for pollen and borage for nectar.

Table 3.3. Time (in sec) spent by bees per flower during n 2010 (N = 3 days)

Time	Phacelia	Borage	Centaurea
6.30-7.30	4.08	2.97	4.55
8.00-9.00	5.27	5.86	8.66
9.30-10.30	4.34	8.57	7.50
11.00-12.00	4.28	6.06	-
12.30-13.30	6.31	12.17	-
14.00-15.00	8.20	4.85	-
15.30-16.30	5.30	9.02	5.82
17.00-18.00	-	6.13	10.02
Average	5.40	6.95	7.31

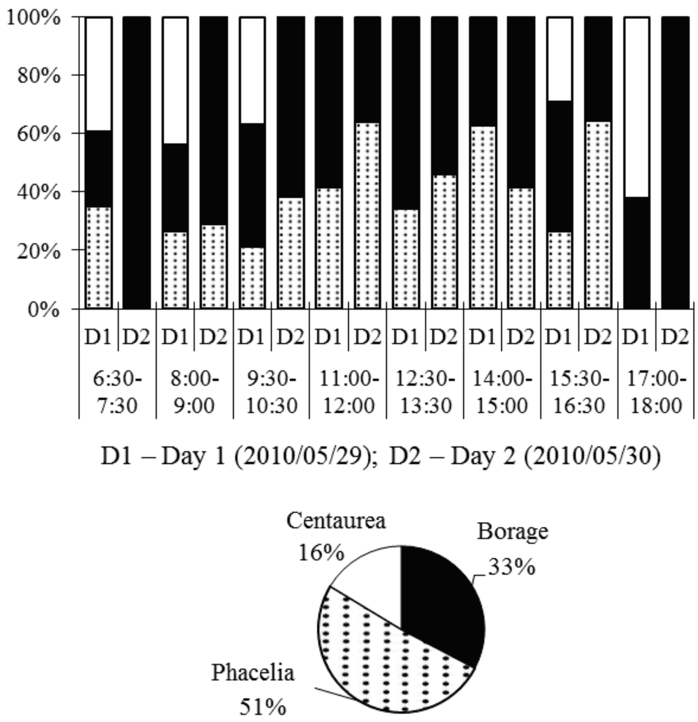


Fig. 3.8. Hourly pattern of relative proportion of foraging time among three different flowers at different time interval of the day (up) and average over the whole observations (below) during spring of 2010.

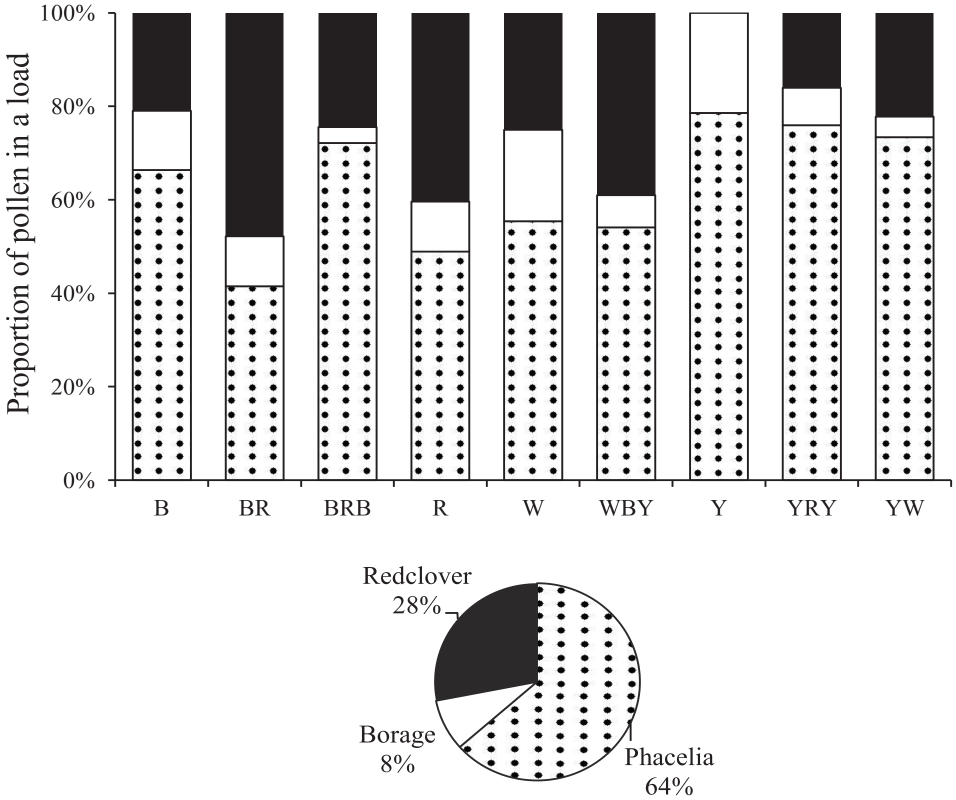


Fig. 3.9. Relative proportion of pollens of three different flowers in pollen loads collected by different bees (N = 1–9) (above) and average of all bees (below) during spring of 2011.

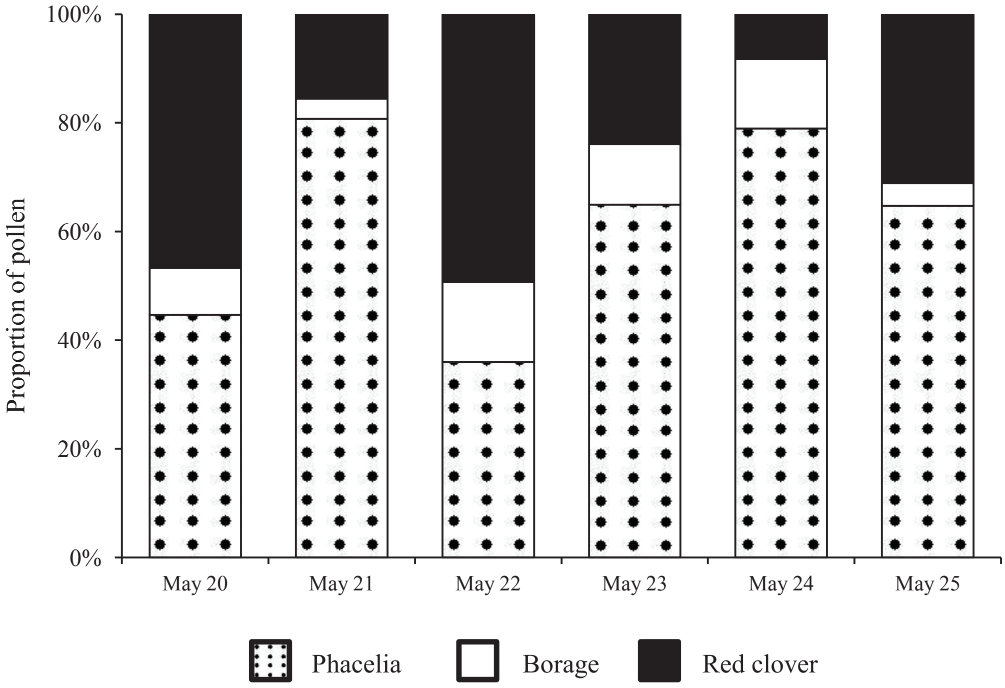


Fig. 3

.10. Relative proportion of pollens of three different flowers in pollen loads collected by nine bees corresponding to Fig. 3.10 at different dates. Each value is an average of 6 observations.

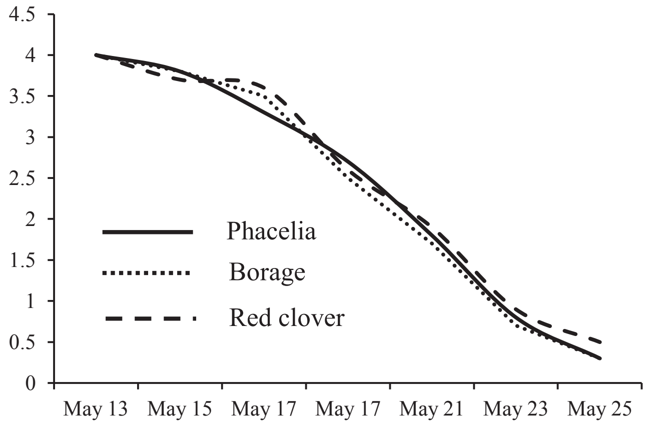


Fig. 3.11. Pollen availability in the flower from May 13–25, 2011, in a 0 (no pollen) to 4 (pollen at highest level) scale visual judgment basis. Each value is an average of 10 observations. Observation was taken from 9.00 to 10.00

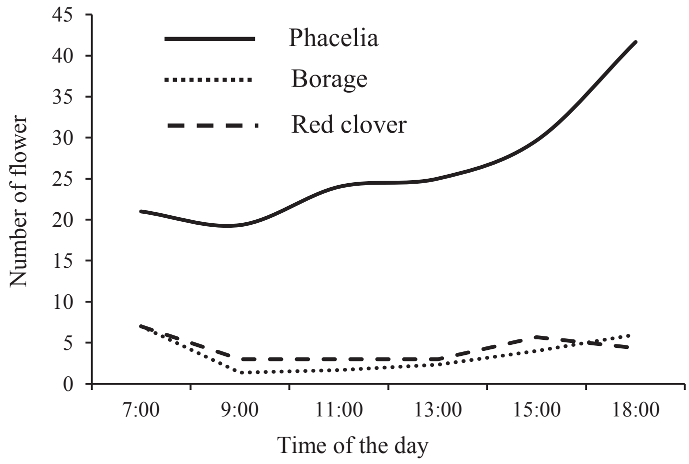


Fig. 3.12. Number of flower bloomed over a day (May 18, 2011

3.3.5.5. Ways of collecting pollen from different flowers

Bees started collecting pollen after 4 days of liberation. They used middle leg and fore leg to collect pollen from phacelia. While collecting, they touched anther by middle legs and rubbed on it with the help of fore leg and passed the pollen to the hind tibial scopae. In centaurea, they touched the upper part of flower and rubbed the forelegs and middle leg to detach the pollen and finally passed it to the hind tibial scopae. In borage, they landed on the flower and extended their tongue and held onto the petals with the anterior and middle legs. When they introduced the tongue into the corolla for collecting nectar, pollen was also collected. To collect pollen from red clover, bees opened the anther (which was tightly enclosed by the petals) using their tongue and legs. After opening, they collected pollen using forelegs and middle legs then transferred to hind tibial scopae.

3.3.5.6. Number of foraging flight in a day

Five bees (red-white, non-marked, yellow-green, white-yellow and red-green) started collecting pollen after four days of liberation (from May 3). Others four bees (white-red, blue-yellow, red and white-blue) started from May 4. Other five (red-blue, green, yellow, blue and white-green) started from May 5. After most of the bees started collecting pollen, number of flights during 2 hours each in the morning (10:00 am to 12:00 pm) and afternoon (14:00 pm to 16:00 pm) for four consecutive days from May 6–9 was observed. Number of flights made by different bees during this period is presented in Fig. 3.13. More detailed observation of foraging behavior of 15 females was taken from May 13 to May 20 in 2010 from 6:00 am to 18:00 pm. All of the 15 females were monitored during five observation period in the greenhouse. Number of foraging flights during this period is presented in Table 3.4. They completed 955 foraging trips, of which 581 were for nectar and 374 were for pollen foraging. Average number of foraging flight in a day inside the greenhouse was 17.3 per bee. Number of flight per bee per day for nectar ranged from 5–28 (N=54) and that for pollen flight ranged from 0–22.

Table 3.4. Number of flight made by each bee per day from 2010/05/13 to 5/20

Bee	Number of flight					
	May 13	May 15	May 16	May 17	May 20	Average
Blue	13	17	24	25	14	18.6
Blue/Yellow	16	16	16	22		17.5
Green	13	15	13	20		15.2
Red	18	12	22	24	21	19.4
Red/Blue	14	18	17		22	17.7
Red/Green	11	17	22	27	17	18.8
Red/White	12	15	25	24		19
Red/Yellow					23	
White					28	
White/Blue	14	10	13			12.3
White/Green	17	18	5	23	20	16.6
White/Red	8	19	25		20	18
Yellow	17	16	27	12	11	16.6
Yellow/Green	13					

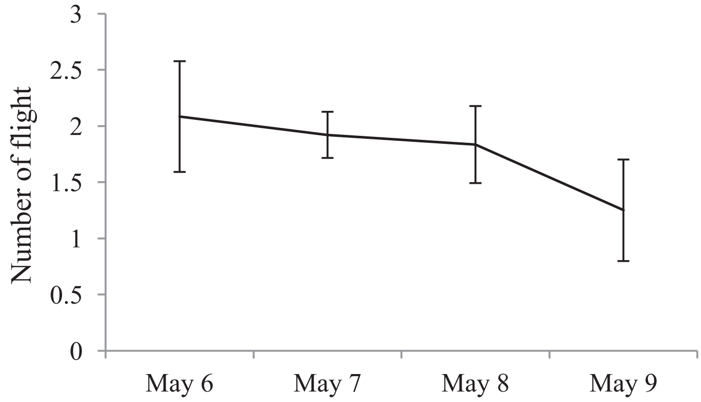


Fig. 3.13. Number of pollen flight per hour during nest burrowing period

3.3.5.7. Number of pollen flights to make a pollen ball

Generally, bees made pollen flights continuously until they prepared a pollen ball. After the pollen ball was prepared, they either took a rest or started making non-pollen flights (probably collected nectar only). In this study, to prepare a pollen ball, bees made an average of 15.6 flights (range: 12–22, N = 8). The number of flights under greenhouse was higher than under their natural habitat (Chapter 2). Smaller amount of floral resources under greenhouse might have caused for the longer time to collect pollen.

3.3.5.8. Characteristics of pollen ball

Three pollen balls were observed for morphological properties comprising two collected in 2010 and one in 2011. Average diameter of a pollen ball was 6.93 mm (range: 6.79–7.04 mm, N = 3). Thickness was 3.47 mm (range: 3.26–3.8 mm, N = 3). Among the two balls collected in 2010, one was white and another was violet in color. The pollen ball of 2011 was also violet in color. White and violet color of pollen ball indicate the dominancy of borage and phacelia pollens, respectively.

3.3.5.9. Time spent for various nesting tasks

Time spent for various nesting tasks by females of *A. plumipes* during 2010 and 2011 is shown in Table 3.5. Average duration for a pollen flight was 28.4 min per trip (range: 4.3–83.5 min, N = 374) and for a non-pollen flight was 25.9 min (range: 2.5–158.8 min, N = 581). Average duration of non-pollen flight during digging the nest burrow was 10.12 min (range: 7.46–15.02 min, N = 6). The average duration of nectar flight during sealing the nests was longer than that under field conditions 23.36 min (range: 16.14–28.36 min, N = 3). Average duration inside the nest to deposit pollen and nectar was 3.14 min (range: 1.10–5.04 min, N= 15). Similarly, for sealing the nest, it was 21.21 min (range: 15.3–28.16 min, N = 3). For digging a burrows, bees spent 6.32 min (range: 3.1–9.5, N = 7). Bees completed a nest in 3–7 days using the drilled hole. It took 3–16 days for completing a nest using the entrance hole dug by themselves. One nest contained 1–8 cells. After completing a nest, some bees started second nest and completed in three days. A nest with highest number of cells (8 cells) was completed in four days.

Table 3.5. Time spent for various nesting tasks by females of *A. plumipes* observed in greenhouse (2010 and 2011)

Activity	Duration (min)	Range	N*
Trips with pollen	28.37	14.32–46.35	9
Trips without pollen	25.9	13.08–72.76	9
Trips for digging burrows	11.28	7.27–15.77	8
Trips for sealing nests	22.96	16.14–24.39	3
While digging	7.78	4.52–10.5	6
While sealing	20.82	15.27–19.02	3
While releasing pollen	3.14	1.10–5.04	15

* Number of observations

3.3.6. Active period

In 2010, bees were active for 35 days (from April 30 to June 4). Of the released bees, 75% survived during this period. In 2011, due to poor floral resources, bees were reared only for 20 days (from May 7 to May 27). Survival rate in 2011 was also same (75%) to that of 2010.

3.3.7. Site selection for making nests

Bees were attracted to soil blocks quickly after releasing. They started checking drilled holes in the blocks after about 12 hours. Bees visited several holes frequently while selecting the site for nesting. The females left holes within a few seconds, but sometimes they dug for about 5 minutes. While the bees were not involved in digging, they were gathered around the soil blocks, flowers and ceiling of the greenhouse. Five, four, six and two female bees started to use drilled holes after two, three, five and 12 days of releasing, respectively. Four bees rejected drilled holes and started digging a new hole. Female bees occupying the holes for few hours or longer, entered very quickly after foraging. Sometimes, when bees were out for foraging for long time, some bees tried to enter their nests; later the occupiers were dragged or chased out upon arrival of the real owners. Usually, it took about few seconds to 2 min for dragging out the occupiers (white red colored bee drag out white blue bee in 2 minutes). To make escape of those occupiers, owner bees gripped their leg into her mandibles and made loud, long, high-pitched buzzing sound. After which, those bees did not try to reenter the hole, later they began to dig another hole by themselves. In another case, a bee (white-blue) used the nest already sealed by another bee (red).

In 2011, for selecting the nesting sites, bees visited regularly all kinds of soil cylinders, but could not use all. They visited soil cylinders made from both light and heavy soils for about 3–4 times, but made nest only in the cylinders made by mixing natural nest soil and house made soil of whitish clay colored soil. Among the soil types used, loose soil was much preferred than hard blocks.

3.3.8. Digging

Bees started to dig the artificial hole for making nest after the next day of liberation. While digging burrow, bees did not make pollen flight. This bee was observed making a unique sound of ticking during the mining of the nest, the vibration of this sound might have loosened the soil. While making burrow, this sound was heard frequently. Bee burrowed the soil in both clockwise and anti-clockwise direction. They dug the soil with tongue and throw it with the help of fore legs and hind legs.

3.3.9. Sealing of the nests

Entrance was sealed after completion of making nest. While sealing, they did not forage pollen. Bees showed chewing and licking behavior for sealing. They put wet soil in their mouth parts, shoved back, and loosened by their legs rotating in both clockwise and anticlockwise directions. After completion of the sealing, cells seemed shiny, smooth and wet. After accomplishing the sealing task, bees visited the sealed nest frequently for 3 to 4 times probably to confirm the seal. After confirmation, they either took a rest or immediately started second nest. Three bees (red, white and green-blue) started second nest immediately after finishing the first, while three other bees (yellow, blue-yellow and green) started second nest after 3 days. Bee tried to select the second nest near to the first nest unless the place was occupied by another bee. Entrances of two nests were found unsealed, one of which was due to the death of the bee before completion of the nest, while another was reopened. Number of brood individual was found near the mouth of nest structures.

3.3.10. Nest architecture

In 2010, 16 bees prepared 25 nests varying in size and number of cells (Fig. 3.14). A single female made 1–3 nests. Eight females made single nest, seven made two nests, and one made three nests. Among the 25 nests, 16 were single-series and 9 were multi-series type. The nests were aggregated 5–8 cm apart. Entrance of the nest was nearly circular. Dimension of the nests prepared by the bees in 2010 are summarized in (Table 3.6). The diameters of the necks of female and male cells were 9.48 mm (range: 7–10.6, N = 13) and 9.18–9.49 mm, respectively. Single series nests had 1–4 cells, and there were 4–8 cells in multi-series nests. Cells containing brood was sealed with cell-plugs having thickness of 2.83 mm (range: 1.5–4.5, N = 25) in males and 2.6 mm (range: 1.3–3.8, N = 8) in females. The brood cells in the nests were arranged linearly in the soil blocks and the first brood cells were located 37.44 mm apart from the entrance (range: 16–61 mm, N = 24). Nest entrance prepared artificially before releasing the bees was circular in shape with the diameter ranging from 15.45 mm (range: 10.8–20 mm, N = 65), while the diameter of the nest entrance made by the bees was 10.58 mm (range: 8.7–12.1 mm, N = 4). The average diameter of main burrow was 14.79 (range: 11–20 mm, N = 23) with a length of 37.14 mm (range: 19–50.9 mm, N=23) and the average length of burrow made by the bees was 24.5 (range: 12.5–29.5, N = 4). No lining was made on the wall of the burrows. In nine nests (nest numbers, 2, 3, 11, 16, 20, 21, 23, 24 and 25), brood cells were linearly arranged from the innermost to uppermost part of the single series nests. In 16 nests, there were two cell plugs filled with soil powder. A gap (hollow cavity) of 5.78 mm in length (range: 2–11, N = 16) was present between the burrows and cells between the plug. Thickness of the cell plug used to partition the hollow cavity was 3.35 mm (range: 1.8–8.1, N = 8). Length of the cell plug in hollow cavity was 14.22 mm (range: 10.9–20, N = 7). Diameter of the cell plug to partition hollow cavity was 12.96 mm (range: 10.2–14.7, N = 5) and coated white. The brood cell was oval shaped and the mouth was relatively narrower with a diameter of 9.48 mm (range: 7–10.6 mm, N = 13) in female cells and 9.18 mm (range: 7.0–10.5 mm, N = 31) in male cells. The average length of female brood cells was 18.31 mm (range: 16–20, N = 16), and it was 17.37 mm in male brood cells (range: 10.5–20, N = 53). The diameters of the female and male brood cells were 11.32 mm (range: 10.4–12.5, N = 17) and 11.45 mm (range: 9.7–12.5, N = 43), respectively. The content of cell was either of large larvae, pre-pupae, pupa, or adults, while egg was not found (Table 3.7). The inner surface of the cell having pollen and larvae was smooth with white coating. No white coating was found in the cells having pupae and adults; it was rather black coated on the tip. Inner wall of the brood had a white coat. In another case, after a bee (red) completed her nest, another (white-blue) bee reopened the sealed entrance and started nesting activities and pollen foraging. Cells in two branches made by two bees can be seen clearly in nest number 8 (Fig. 3.14).

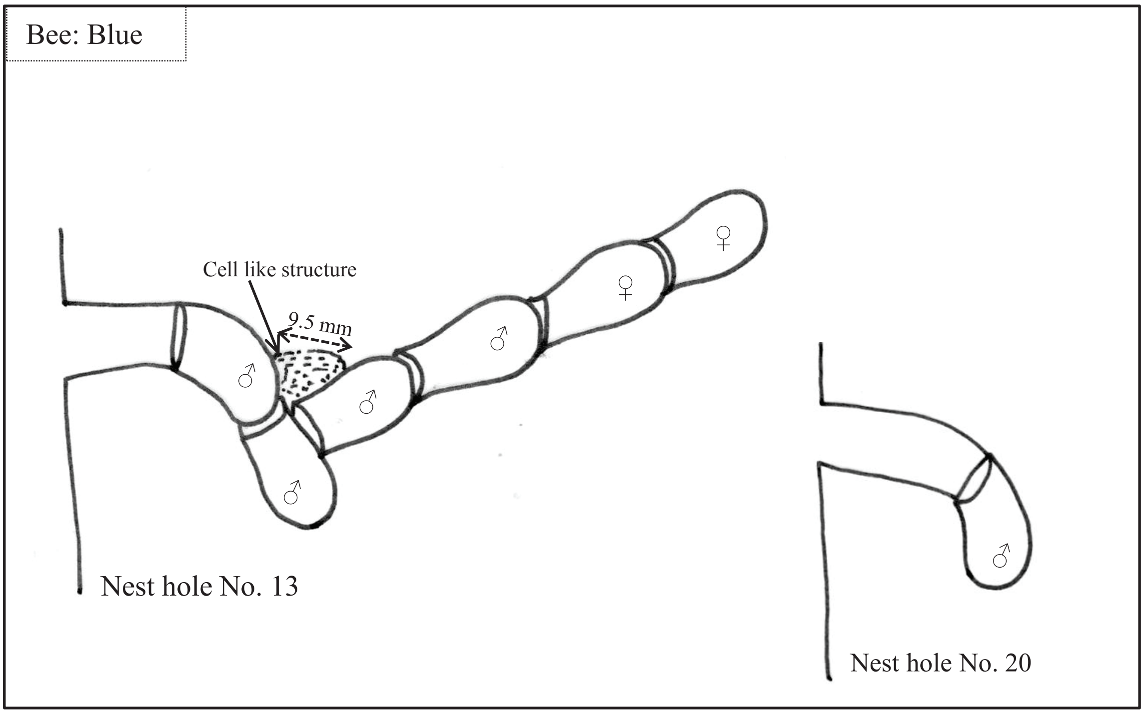
Table 3.6. Dimension of nests in soil blocks and soil cylinder (2010 and 2011)

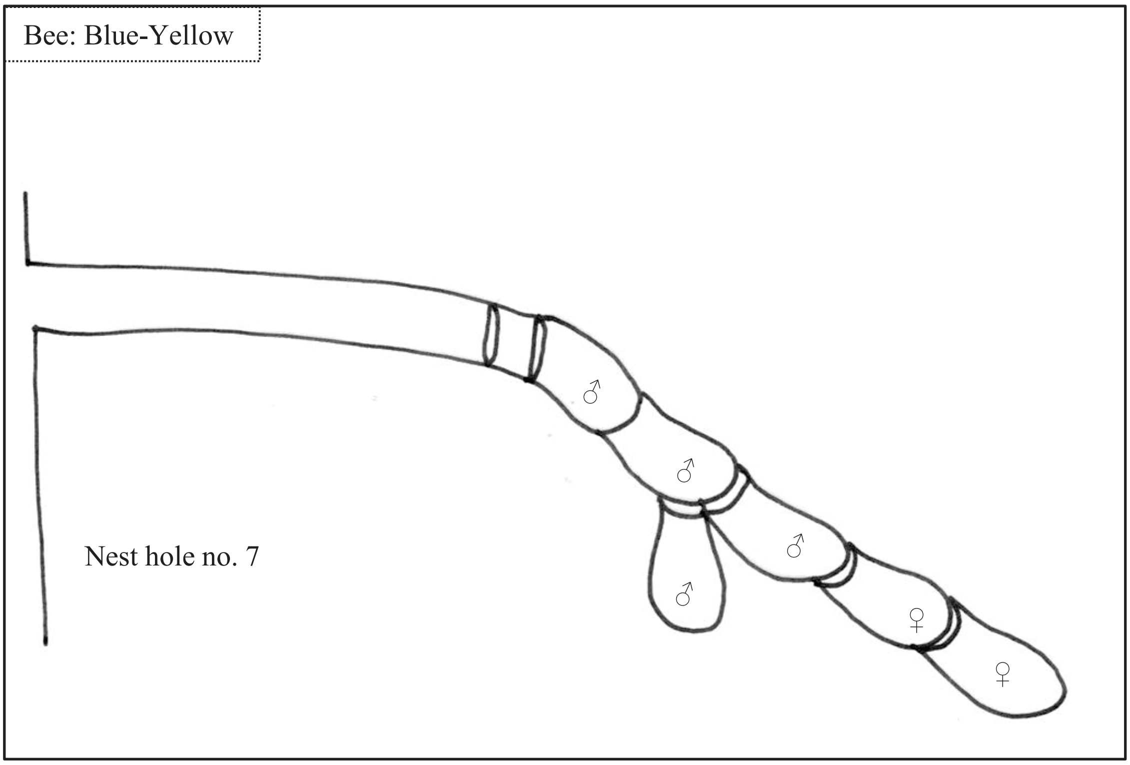
Dimension (mm)	Soil blocks (2010)			Soil cylinder (2011 only)		
	Average	Range	N*	Average	Range	N*
Nest entrance diameter	15.45	10.8–20	11	13	9–17	2
Bee made entrance diameter	10.58	8.7–12.1	4	9	9	4
Length of burrow	37.14	19–50.9	13	22.7	10.5–33	5
Diameter of burrow	14.79	11–20	18	11.5	10–12	4
Length of nest	75.22	43.5–106.5	14	52.88	41.0–67.5	4
Width of nest	32.21	13–60.5	14	32.63	27.5–38	4
Cell length (female)	18.31	16–20	16	19.05	17.7– 20.5	7
Cell length (male)	17.37	10.5–20	53	17.29	12–20.2	8
Cell diameter (female)	11.32	10.4–12.5	17	11.12	10.6–12.1	7
Cell diameter (male)	11.45	9.7–12.5	43	12.09	9.3–14.6	9
Cell plug thickness (female)	2.6	1.3–3.8	9	2.6	2.2–2.9	7
Cell plug thickness (male)	2.83	1.5–4.5	25	2.25	1.25–4.13	10
Diameter of neck (female)	9.48	7.0–10.6	13	9.01	6.9–8.9	7
Diameter of neck (male)	9.18	7.0–10.5	31	9.49	8.2–11.1	11
Diameter of pollen	6.93	6.79–7.07	2	6.94	8.2	1
Thickness of pollen	3.53	3.26–3.8	2	3.35	3.9	1

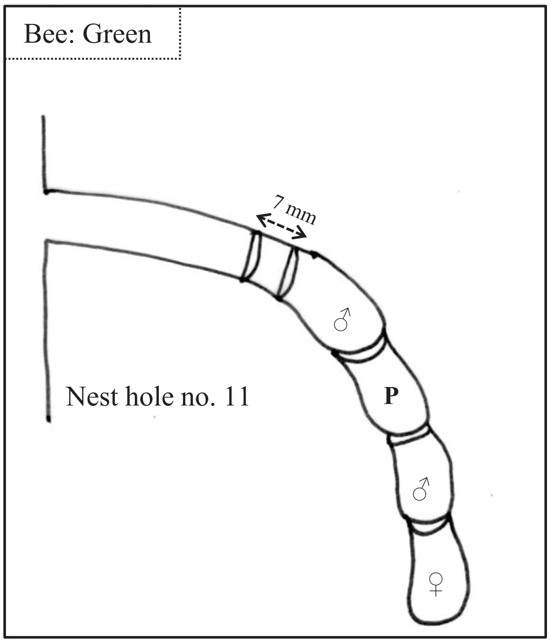
* Number of observation

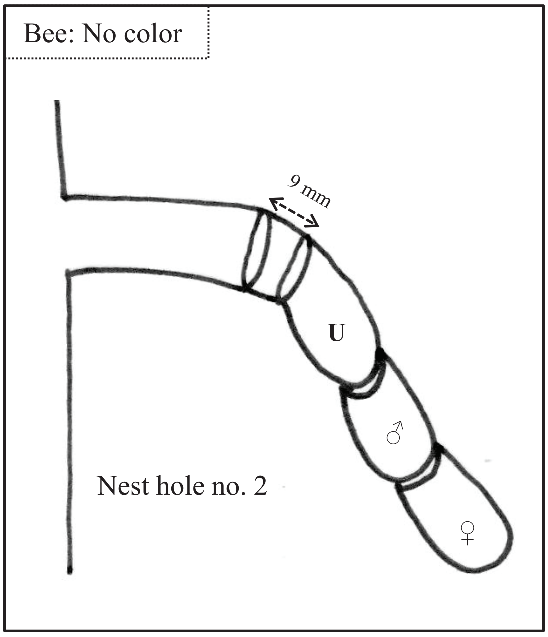
Table 3.7. Stage of broods in the cells during excavating nests in 2010

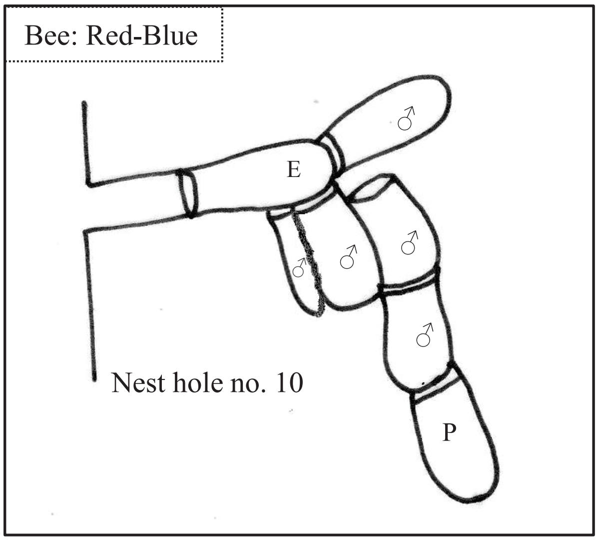
Date of nest excavation	No. of nest	Stage composition
August 24	1	Prepupae
August 25	1	Prepupae
September 8	6	Late pupae
September 9	4	Late pupae
September 10	3	Late pupae
October 13	1	Adult
November 22	4	Adult
November 25	5	Adult

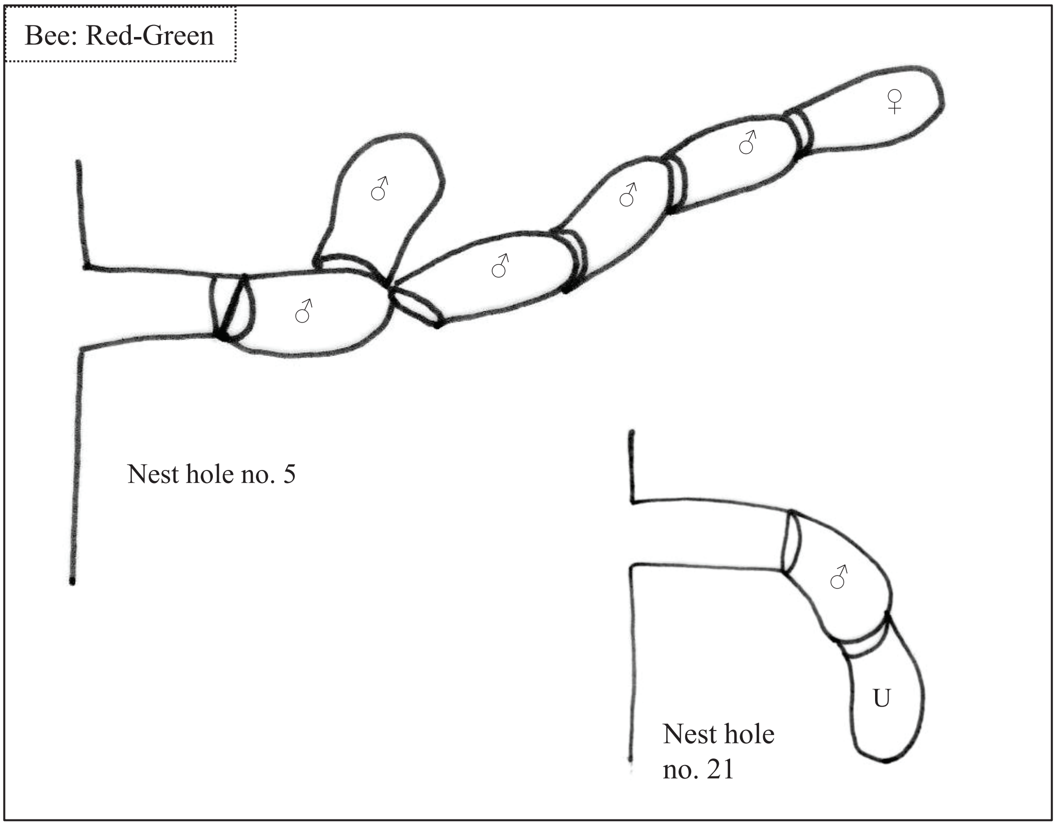

Bee: Blue
Cell like structure
9.5 mm
♂
♂
♂
♂
♀
♀
Nest hole No. 13
♂
Nest hole No. 20

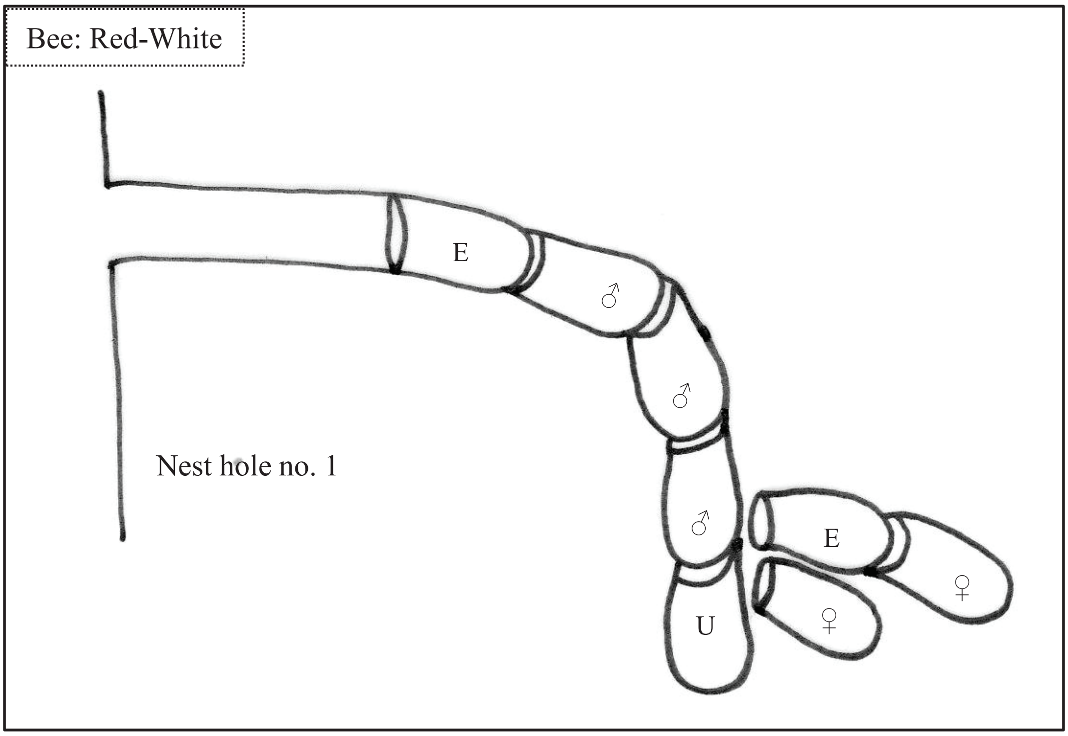

Bee: Blue-Yellow
♂
♂
♂
♂
♀
♀
Nest hole no. 7

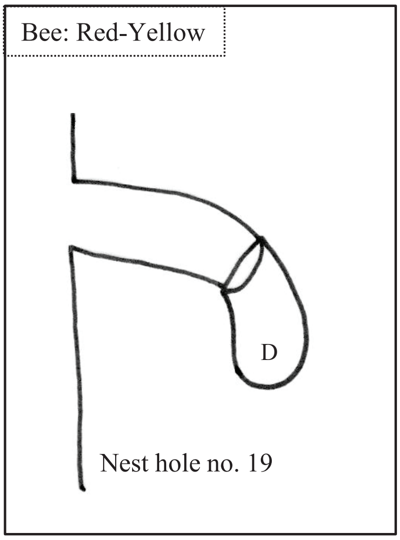

Bee: Green
7 mm
♂
P
♂
♀
Nest hole no. 11

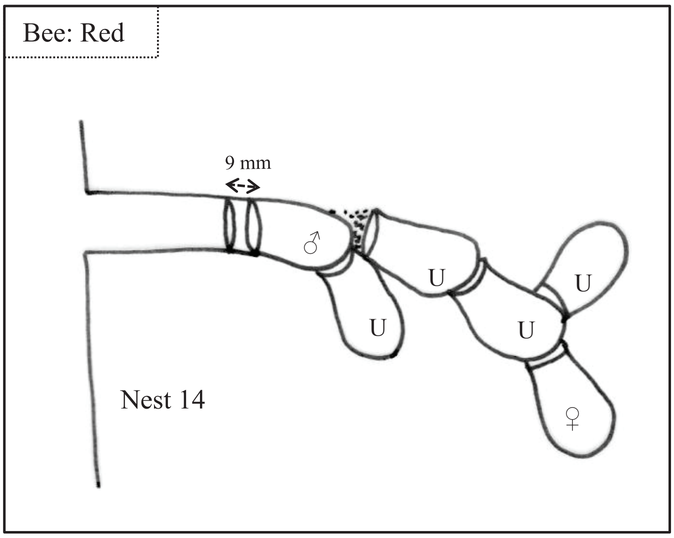

Bee: No color
9 mm
U
♂
♀
Nest hole no. 2

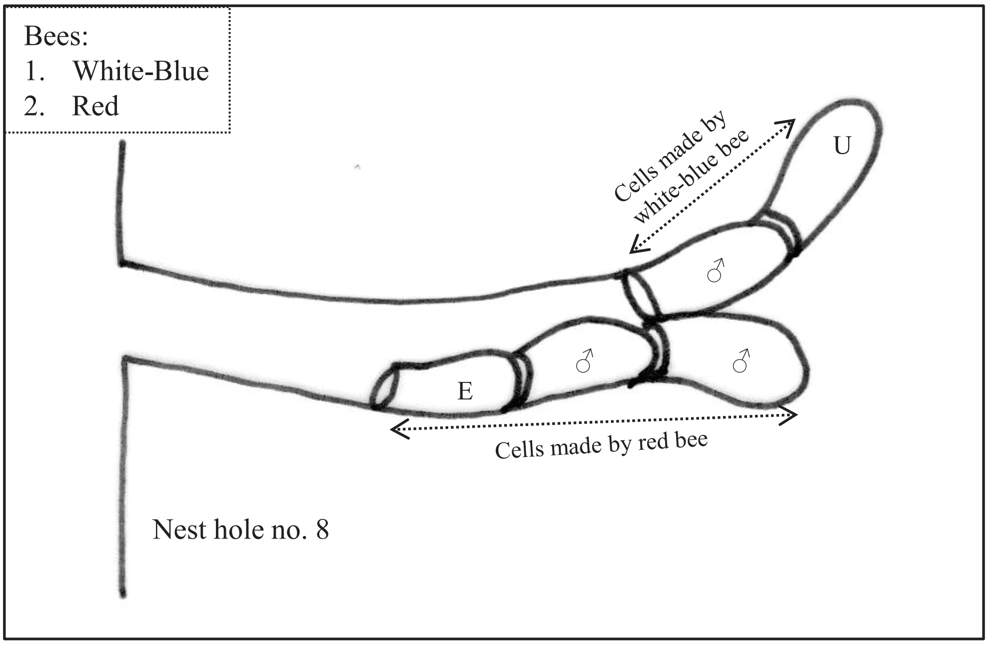

Bee: Red-Blue
♂
E
♂
♂
♂
♂
P
Nest hole no. 10

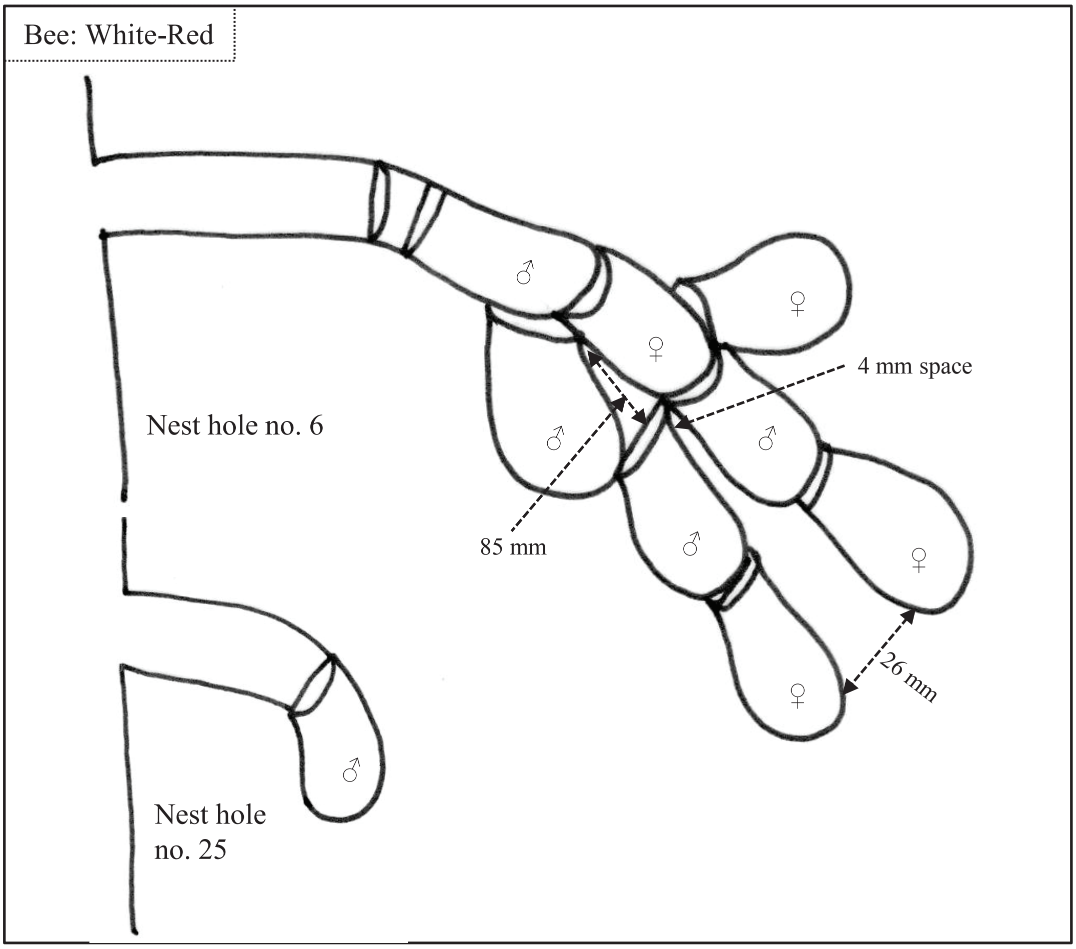

Bee: Red-Green
♂
♂
♂
♂
♂
♀
Nest hole no. 5
♂
U
Nest hole
no. 21

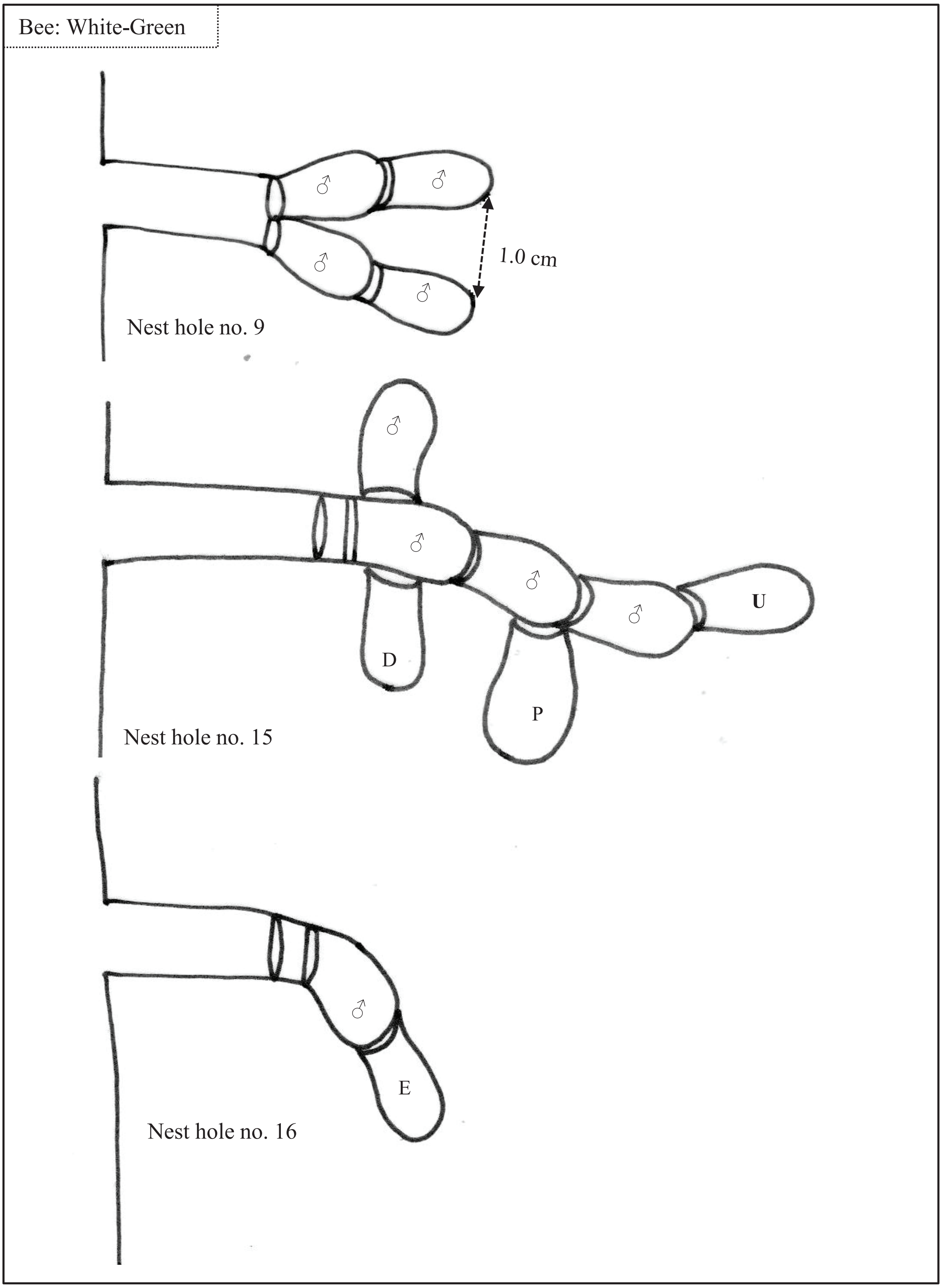

Bee: Red-White
E
♂
♂
♂
U
E
♀
♀
Nest hole no. 1

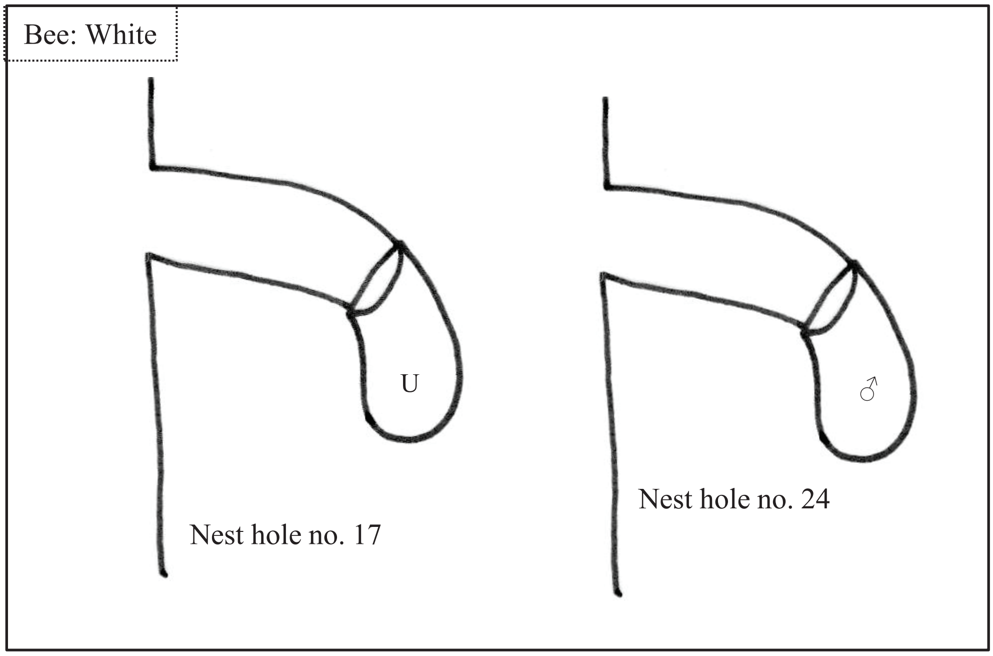

Bee: Red-Yellow
D
Nest hole no. 19

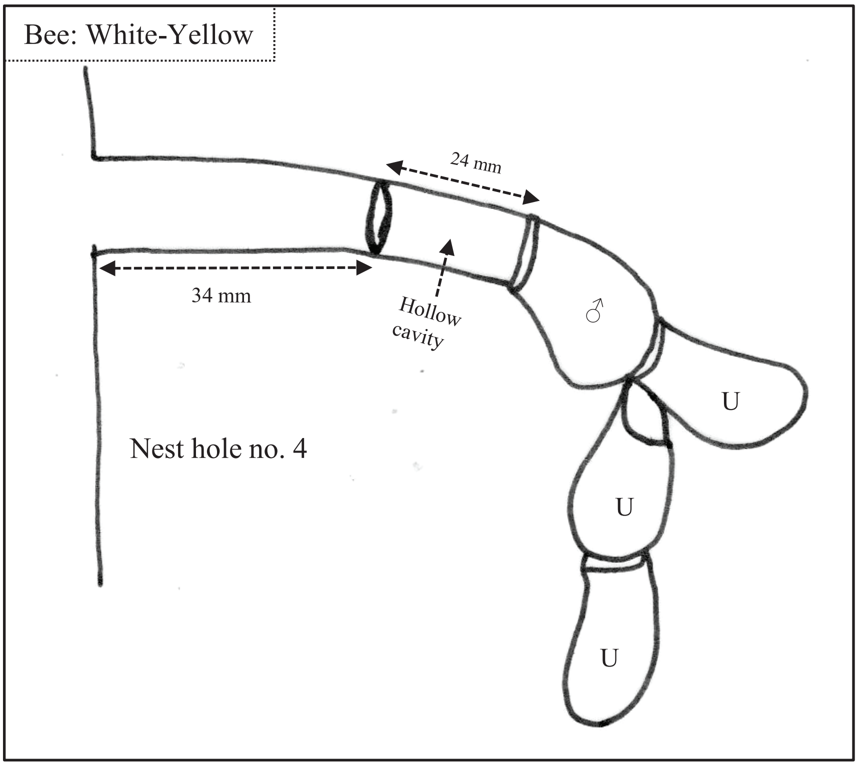

Bee: Red
9 mm
♂
U
U
U
U
♀
Nest 14


Bees:
1. White-Blue
2. Red
Cells made by white-blue bee
U
♂
♂
♂
E
Cells made by red bee
Nest hole no. 8


Bee: White-Red
Nest hole no. 6
♂
♀
♀
4 mm space
♂
♂
85 mm
♂
♀
26 mm
♀
♂
Nest hole
no. 25


Bee: White-Green
♂
♂
♂
♂
1.0 cm
Nest hole no. 9
♂
♂
♂
♂
U
D
P
Nest hole no. 15
♂
E
Nest hole no. 16


Bee: White
U
♂
Nest hole no. 24
Nest hole no. 17


Bee: White-Yellow
24 mm
34 mm
Hollow cavity
♂
U
U
U
Nest hole no. 4

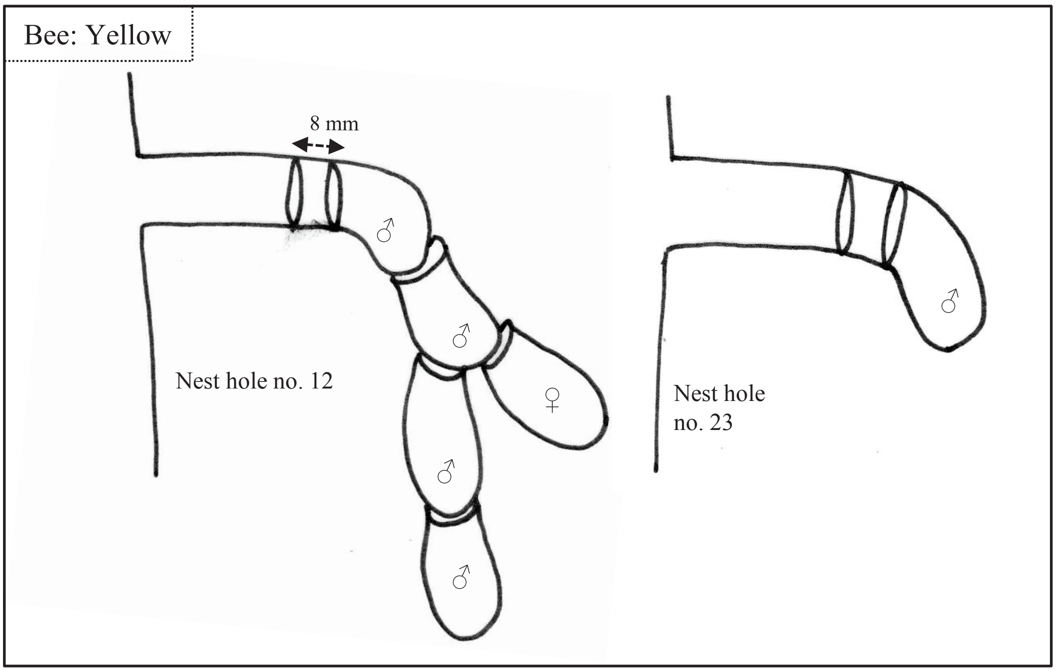


Fig. 3.14 . Sketches of the nests made by 16 bees in red clay soil blocks during 2010 in a greenhouse. Nest sketches show the relative position of each cell containing male (♂), female (♀), unidentified (U), empty (E) and pollen ball (P). Nest(s) in a box was/were made by a single bee.

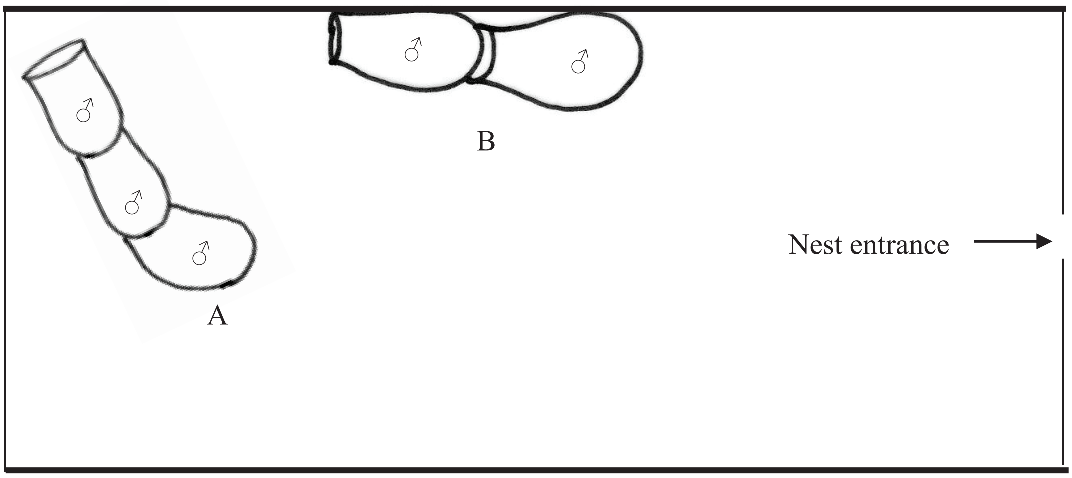


Fig. 3.15. Sketch of the positions of two nest series (A and B) in a soil cylinder inside a greenhouse during 2011 showing male (♂) larvae in the cells.

In 2011, among the 12 marked bees, eight bees made nests and other four only searched the site but did not make any nest. Among the eight nest making bees, four used artificially prepared holes and the other four made holes by themselves. One bee made entrance at the top of the cylinder, and the size of the nest was 9 mm in diameter (Fig. 3.15). Another bee made the entrance of the same size below the cylinder. Remaining two bees used entrance in the opposite side of the cylinder with diameter of the same size. The bees preferred dark areas for making nests. Among the eight nests, four were excavated for observation. A total of 14 cells were observed in four nests. All of the cells were male cells, probably due to the disturbance in pollen flight since pollen carrying bees was captured and forced to remove. Entrance in 2011 was circular in shape, similar to those observed in 2010.

In 2011, average length and width of nest was 52.88 mm (range: 41–67.5, N = 4) and 32.63 mm (range: 27.5–38, N = 4), respectively (Table 3.5). Diameters of the artificial and the bee made entrances were 13 mm (range: 9–17, N = 2) and 9 mm (N = 2), respectively. Similarly, length and diameter of the burrow was 22.7 mm (range: 10.5–33, N= 5) and 11.5 mm (range: 10–12, N= 4), respectively. Length and diameter of the male cell was 17.29 mm (range: 12–20.2, N = 8) and 12.09 mm (range: 9.3–14.6, N= 9), respectively. Diameter of neck was 9.49 mm (range: 8.2–11.1, N = 11). Cell plug thickness was 2.25 mm (range: 1.25–4.13, N = 10). Likewise, diameter of pollen was 6.94 mm and thickness was 3.35 mm.

3.3.11. Distribution of sexes, male ratio, and number of eggs laid per female

In 2010, a total of 16 females made a total of 25 nests having 93 cells containing 53 males, 16 females and 14 unidentified offspring. Average number of nests and cells per female was 1.6 (range: 1–3) and 5.8 (range: 1–9), respectively (Table 3.8). Number of female and male cells per bee ranged from 0–6 and 0–4, respectively.

Among the unidentified cells, eight contained dead larvae, six had dead pupae, five were empty, one had blackish dust and four cells contained only pollen balls having the size of 9 mm in diameter without egg. In all nests, male offspring were nearer to the entrance, indicating that male eggs are laid later (Fig. 3.14). Length of the larvae was 11.60 mm and that of pupae was 11.17 mm (Table 3.9).

Table 3.8. Number of cells, nests and brood of different bee individuals in 2010

Bee	Number of						
	Total nests	Total cells	Male cells	Female cells	Unidentified cells		
					Dead	Empty	Pollen ball
Blue	2	7	5	2	0	0	0
Blue/Yellow	1	6	4	2	0	0	0
Green	1	4	2	1	0	0	1
No color	1	3	1	1	1	0	0
Red	2	9	3	1	4	1	0
Red/Blue	1	7	4		1	1	1
Red/Green	2	8	6	1	1	0	0
Red/white	1	8	3	2	1	2	0
Red/Yellow	1	1	0	0	1	0	0
White	2	2	1	0	0	0	1
White /Red	2	9	5	4	0	0	0
White/Blue	1	6	5	0	1	0	0
White/Green	2	9	5	0	1	2	1
White/Yellow	2	4	1	0	3	0	0
Yellow	3	6	5	1	0	0	0
Yellow/Green	1	4	3	1	0	0	0
Total	25	93	53	16	14	6	4
Average	1.6	5.8	3.3	1.1	0.9	0.4	0.3

Table 3.9. Length of larvae and pupae (mm)

	2010			2011		
	Average	SD	N	Average	SD	N
Larvae	11.6025	1.58769	4	12.865	2.616391	4
Pupae	11.1775	0.69684	10	13.89	0.452548	2

Both males and females were developed into fully formed adults by the end of the summer, but remained overwintered in their sealed cells, and were ready to emerge in early spring. In the nests, survival rate of offspring was 83.1% (69 were live out of 83). In 2011, although it was difficult to dismantle the nests due to light textured soil, it was observed that each female made 2 to 5 cells. In both of the years, a large number of offspring in the nests indicated that this bee can reproduce efficiently even in the closed condition using different types of nesting materials.

3.4. Discussion

In this study, both male and female *A. plumipes* bees started nectar foraging after four minutes of releasing bee inside the greenhouse. Both males and females forages for nectar flight, male forages for own consumption and female for brood provision and food (Batra, 1994; Neeman et al., 2006; Tsuji and Kato, 2010).

Flowers of all plants provided in this study was preferred by this bee. *A. plumipes* bees have a tongue length of about 12–15 mm (Stone, 1995). The depth of nectar gland in phacelia, borage and centaurea were 4.49, 10.97 and 7.42 mm, respectively. Longer tongue length compared to the depths of nectar gland in the flowers of these plants indicated that this bee can access nectar easily. In a study of Maeta et al. (1990), a positive correlation between the length of tongue and the number of flower visit by *A. plumipes* bees.

Male showed patrolling behavior soon after liberation. Male chased the female around foraging area and entrance of the nest. Roberts (2010) reported male *A. plumipes* bees emerged earlier than females, and once females were emerged, started chasing and pouncing. Batra (1994) reported that males of this bee had started to pounce females next day of their emergence from the nest. Stone (1995) found the mating system of *A. plumipes* was strongly dependent on the male density. In another study, a solitary anthophorid bee (*Anthophora* sp.) in the Sinai Desert showed sexually dimorphic diurnal activity patterns (Willmer et al., 1994).

In this study, bee visited flowers for pollen even in first flight around 5:00 am. In a similar study of Batra (1994), female *A. plumipes* began foraging as early as at 5:03 (15 minutes before sunrise), and remained continuously active after sunset until 19:30. Stone (1995) reported first foraging trips of *A. plumipes* on comfrey flower (*Symphytum orientale*) were for nectar. In this study, pollen flight was observed from early in the morning and after sunset ranged from 0–22. Hogendoorn et al., (2007) reported blue banded bee (*Amegilla cingulata)* performed 9 pollen flights per day in the greenhouse.

Pollen foraging was observed under high variation of temperature (9.5 to 36.4°C) and light intensity (1,250 to 19,810 lux). *A. plumipes* is reported to have ability to regulate heat flow between thorax and abdomen (Stone, 1993). Batra (1994) observed that this bee foraged under adverse weather conditions for collecting high bush and low bush blueberry. Stability in pollen flight even at high temperature throughout the day is rare to other pollinators. Number of foraging flight per female was highest during 12:00 to 13:00 (temperature in the greenhouse was highest at this time) under the greenhouse indicating this bee can forage even under closed condition at high temperature.

The pollen foraging duration of this bee under greenhouse was 4.3 to 83.5 min which was similar to that under natural habitat condition (7.2 to 60.7 min) (Chapter 2). Batra (1994) reported duration of a pollen trip of this bee ranging from 6–47 min under field conditions. Stone et al. (1999) reported that *A. pauperata* took longer time to collect pollen in the morning. In this study, foraging duration per trip was longer when temperature was less than 10°C and more than 25°C. Longer foraging trip in the morning might be due to less pollen availability in the flower forcing bees to visit more flowers to make a full pollen load.

Nesting activity was also affected when the duration of foraging flight was longer. Bees took longer time for sealing nests under this condition. The nest sealing duration was clearly higher than that under natural habitat condition (Chapter 2), probably due to lower floral resources in the greenhouse.

Bee shows nest founding behavior soon after liberation. Nest construction usually starts after the females are emerged and mated (Alcock, 1999; Cane, 1995; Batra 1994; Miyanaga et al., 1999). For making nests in the soil blocks, this bee used nectar to moisten and soften the dry, hard red clay. In a similar study *A. abrupta* used nectar to make the soil soft, and collects water while digging a nest on the ground (Norden, 1984). Alcock (1999) and Tomkins et al., (2001) reported Anthophorid *Amegilla dawsoni* used nectar to moisten the soil in desert probably due to the lack of available water. In this study, with the same assumption, a water tray was kept near the nesting site, but none of the bees used it. This bee completed their nest in a shorter time as compared to that under their natural habitat (Chapter 2), probably due to limited space in the artificial nesting materials.

This bee showed a good offspring productivity under greenhouse. Floral resources and nesting materials provided in the study was well adapted by this bee indicating that this bee can be reared under controlled condition even in poor facilities.

3.5. Conclusions

Bees showed high adaptability to the closed condition. It easily made nests in soil blocks and soil cylinders, which are cheap, easy to handle and store during dormant periods. Judging from flight behavior, it was known that this bee can be used as a pollinator of crops inside the greenhouse in poor facilities. A short flower visiting period indicated this bee can be a good pollinator for greenhouse plants. The stable flower visiting over a wide range of temperature and brood production observed in the study indicated that the bee can be reared and managed easily under the controlled condition. Thus, *A. plumipes* could be a profitable candidate as a crop pollinator for various flowering plants under greenhouse conditions.

Chapter 4

Utilization of *Anthophora plumipes* (Hymenoptera: Apidae) for pollination of strawberries in greenhouse

4.1. Introduction

Cultivation of strawberry in greenhouse is increasing worldwide for getting better quality and higher value of fruits. In Japan, about 90% of the total strawberry production is carried out under protected structures (Takeda, 1999). Berry deformity is severe problem in commercial cultivation of strawberries in greenhouses. Strawberry flowers are hermaphrodite and self -compatible to a certain extent. Strawberry flowers contain many carpels and it is necessary that all of these carpels should contain a fertilized ovule (seed) in order to produce a well-formed berry. Berries are deformed at parts in which achenes are not fertilized. Therefore, for a successful pollination is required for seed formation and better quality of the fruits. Studies have shown that number of fertilized achenes and quality of strawberry fruit are directly related to the pollination (Nitsch, 1950; McGregar, 1976; Crane, 1990; Pratap, 2000). Since fertilization of strawberry has direct influence on the fruit quality, it is crucial that the flower inside greenhouses need agents for pollinate.

Pollination by using special devices (such as, electric toothbrush or hand brush) and manual pollination are commonly practiced for the pollination of strawberry in greenhouses (Wang and Zheng, 2001). Although these devices improve the pollination efficiency, they are expensive and have risks of damaging the flowers. Alternatively, bees have been used as pollinators for better quality fruit production under greenhouse.

Bees are considered as a perfect pollinator of many cross pollinated crops. Studies have shown *Apis mellifera* as an effective pollinator of strawberry inside greenhouse (Free, 1968; Chang et al., 2000; Jian et al., 2006; Zaitoun, et al., 2006) and in open fields (Albano et al., 2009; Chagnon et al., 1993). Several studies have been carried out with an aim of extending the range of appropriate pollinators for this crop. For greenhouse conditions, *Bombus* spp. is another widely used pollinator group (Free, 1968; Paydas, et al., 2000; Zaitoun et al., 2006). In Japan, several species of stingless bees, such as, *Nannotrigona testaceicornis*

Lepeletier and *Trigona minangkabau* have been successful tested for strawberry pollination inside greenhouses (Maeta et al., 1992; Kakutani et al., 1993; Roselino et al., 2009; and Malagodi-Braga and Kleinert 2004). In Brazil, *Tetragonisca angustula Latreille* was found effective for pollinating strawberry with a significant increase in overall strawberry production. Some Megachilidae, such as *Osmia rufa* L., *Osmia cornifrons* were also found to be effective pollinators for this crop, applicable in green house (Wilkaniec and Radajewska, 1997; Maeta et al., 2012).

As an approach for finding alternative pollinators for strawberry under greenhouse conditions, *Anthophora plumipes* (Pallas) (Anthophoridae) was used in this study. As explained in the previous chapters, this bee is widespread and common in Mediterranean Israel, NorthWest Europe, Central Asia and Japan. It is one of the first species active during the spring (Neeman, et al., 2006; Batra, 1994; Stone, 1995). In chapter 2, a high adaptability, well offspring production, and good pollinating behavior in some wild flowering plants with stable flight activity in the early spring inside the greenhouse was reported. In this chapter, foraging activities of *A. plumipes* bees on strawberries flowers in the greenhouse condition and effects on the quality and quantity of the fruits pollinated by *A. plumipes* are described.

4.2. Materials and methods

Two experiments were carried during 2013 and 2014. In 2013, two treatments, bee-pollination and no supplementary pollination (control) were maintained. While in 2014, three treatments, bee-pollination, hand-pollination, and no supplementary pollination (control) were maintained. The details are described in the following sections:

4.2.1. Collection of the bees

Adult *A. plumipes* bees were collected from their natural habitat located in Atogayama Park, Izumo (Chapter 2). The collected bees were kept in an incubator for about one month at 6ºC before liberating in the greenhouse. Same number of 25 female and six male bees were used for both year’s experiments.

4.2.2. Preparation of nesting materials

Soil blocks were provided as nesting materials. Details about the construction of soil blocks is explained in Chapter 3, section 3.2.4.

4.2.3. Experimental set-up

Both year's experiments were conducted in a small greenhouse (8.1 m long × 4.5 m wide × 2.9 m high) located at Shimane University, Matsue Japan (lat. 35º29', long. 133º 27' and elevation 170 m). For the 2013 experiment, Two hundred strawberry (*Fragaria ananassa var.* Hokowase) seedlings at 5-leaf stage were planted in the plastic pots (each pot was 35 cm wide and 40 cm deep) on 13th October, 2012. Of the 200 plants, 150 were allocated for the bee pollination and 50 plants were maintained for the control. To control the bees from visiting flowers, flower buds were covered with polyethylene exclusion bags (1×1 mm mesh) before releasing the bees. The exclusion bags permitted airflow into the flower. Bees were released in the greenhouse on April 15, 2013.

For the 2014 experiment, 250 seedlings were used **(**Fig. 4.1**).** Number of plants for three treatments were as follows: 150 (bee-pollination), 50 (hand-pollination) and 50 (control). Bees were released on April 15, 2014. The flowers bloomed before liberating bees in the greenhouse were picked. Most of the flowers used for the experiment were third and fourth clusters.

Fig. 4.1. Pots of strawberry plants inside a greenhouse (A), strawberry flowers covered with exclusion bags (B), strawberry flowers at full bloom stage (C), *A. plumipes* bees visiting strawberry flowers (D).

4.2.4. Fertilizer, disease and insect management

Pots were filled with soil mixed with composted poultry manure (2%, w/w). Ammonium sulfate (3 g/pot) was top dressed just before the flower initiation. No pesticide was applied for controlling disease and insects. Insects were hand-picked when observed.

4.2.5. Observation of foraging activities

Time spent by the bees for pollen and non-pollen flights was recorded. Number of flowers visited per unit of time by male and female bees and handling time for each successive visit were also recorded. Time spent by bees to visit a flower of different age was also recorded to see the effect of pollen and nectar availability.

4.2.6. Effect of number of flower visit on pollination

In both 2013 and 2014 experiments, 100 randomly selected flowers were bagged from the plants allocated for the bee-pollination plants. The bagged flowers were opened for single, double and triple visits. Time spend by bees for each visit was recorded. After a certain number of visits, the flower was bagged again to avoid further bee visit.

4.2.7. Measurement of fruit yield and quality parameters

Strawberries were harvested periodically when fruits were red. Number of fertilized and non-fertilized achenes were recorded to evaluate the pollination efficiency. After separating the seeds from the pulp, it was taken in a beaker filled with water. Submerged and floated seeds in the water were recorded as fertilized and non-fertilized achenes, respectively.

Fruit length and width were measured using Vernier calipers. Sugar content in fruit were analyzed using refractometer. In 2013, three-level deformity ranking was made based on the measurement of shapes and visual observations as follows: normal, slightly deformed, and highly deformed. In 2014, five-level deformity ranking was made as follows: quite few unfertilized achenes and not deformed (A), some unfertilized achenes but not deformed (B), many unfertilized achenes and slightly deformed (C), quite unfertilized achenes but slightly deformed fruits (D), and many unfertilized achenes and highly deformed (E).

4.2.8. Weather conditions

During the experiment, temperature inside the greenhouse ranged from 10.7 to 29.6ºC, relative humidity was from 21 to 77%, and solar radiation levels were between 1,082 to 1,830 lux.

4.2.9. Data analysis

Significance of three pollination treatments were evaluated by analyzing the data using ANOVA (Minitab version 14). Treatment means were compared using the least significant difference test at $p < 0.05$.

4.3. Results

4.3.1. Survival of bee

In 2014 experiment, bees were reared for 30 days in greenhouse. Relationship of flower availability and survival rate of bee is shown in (Fig. 4.2). Seventy percent bees were survived even in lower flower population.

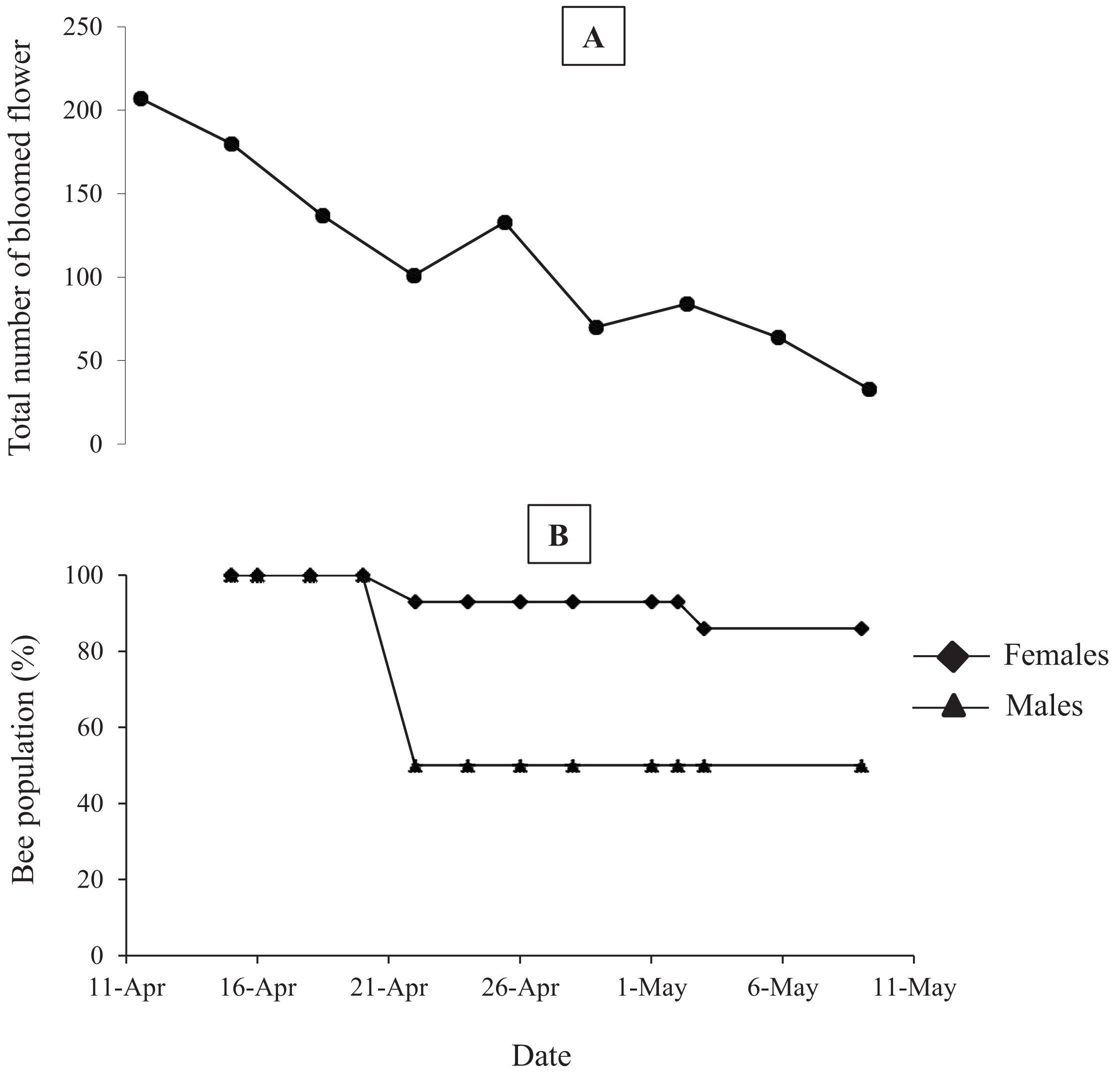


Fig. 4.2. Total number of bloomed flowers (A) and survival rate (%) of bees liberated (B) during the experimental period from 11 April to 11 May, 2013

4.3.2. Foraging behavior

In 2014, bees started foraging nectar from the strawberry flowers after 8.3 min and collecting pollen after four days of liberation in greenhouse. While visiting the flowers (Fig. 4.1 D), bee always landed on the top of flower with a circular movement either from right to left or left to right. The bees landed at the corner of flower disc for collecting nectar and walked to the whole pistils for collecting pollen. Both females and males successfully visited strawberry flowers (Table 4.1). The average time spent in the flower (handling time) by the male bees was 8.7 sec (±4.9) (n = 132) and that of the females was 11.0 sec (±6.9) (n = 340). The handling time slightly affected with the age of the flower with shorter time on the old flowers, but the difference was not significant (Fig. 4.3). The handling time slightly high with the repeated visit on same flower (Fig. 4.4). Male bees visited an average of 10.2 (±3.0) (n =28) flowers per minute, and that is females was 6.5 (±3.0) (n = 68) (Table 4.1) flowers per min and average duration of foraging trips for collecting pollen was 36.84 min and that for nectar was 33.01 min.

Table 4.1. Hourly flower handling and visiting patterns of males and females of *A. plumipes* bees on strawberry flowers in a greenhouse

Time	Handling time (sec/flower) ± SD (number of observations)		Number of flower visited / min ± SD (number of observations)	
	Male	Female	Male	Female
8:00-9:00	6.3 ±3 (8)	12.7 ±8.4 (6)	13.4 ±3 (9)	5.5 ±4 (6)
9:00-10:00	7.7 ±4 (15)	12.6 ±8.7 (27)	11.1 ±4 (4)	7.1 ±4 (17)
10:00-11:00	9.0 ±5.6 (35)	13.2 ±8.3 (29)	10.5 ±2 (9)	7.5 ±2 (6)
11:00-12:00	7.1 ±3.7 (25)	9.4 ±5.3 (68)	11.3 ±5 (3)	8.7 ±1 (9)
12:00-13:00	7.7 ±4.8 (39)	8 ±2.6 (43)	6.2 ±1 (2)	7.3 ±5 (9)
13:00-14:00	8.7 ±4.5 (4)	10.77 ±6.4 (47)	8.5 (1)	4.9 ±5 (4)
14:00-15:00	7.5 ±3.9 (2)	11 ±8.9 (61)	ND	5.2 ±2 (8)
15:00-16:00	15.6 ±9.3 (4)	10.5 ±6.3 (59)	ND	5.5 ±1 (9)
Mean	8.7 ±4.9 (132)	11 ±6.9 (340)	10.2 ±3 (28)	6.5 ±3 (68)

ND=not detecte

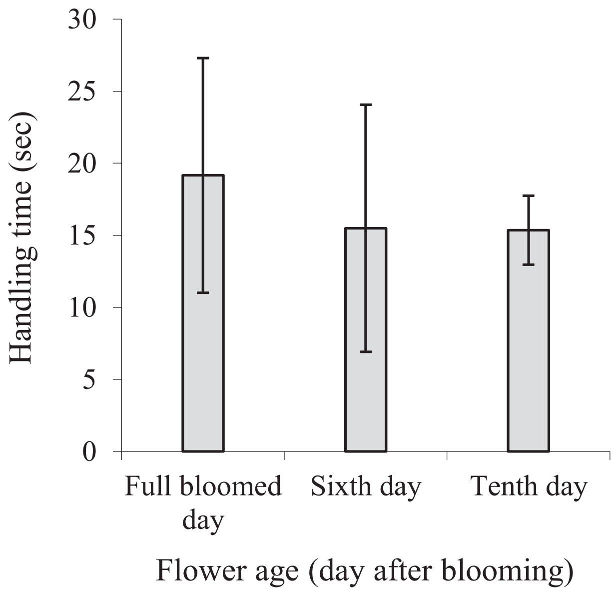


Fig. 4.3. Effect of flower age on handling time of *A. plumipes* bees in strawberry flowers

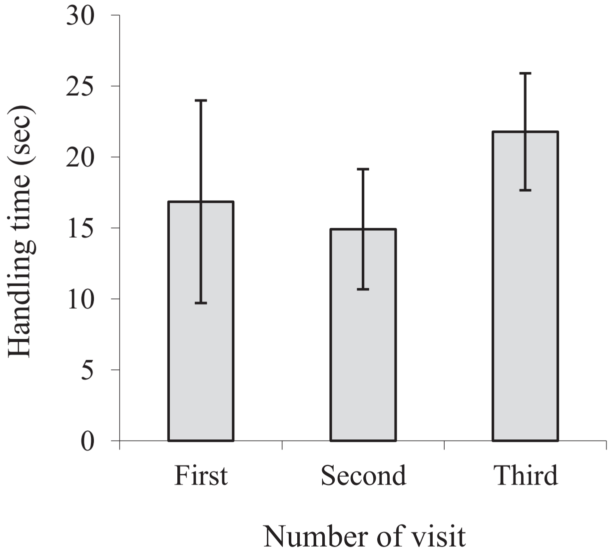


Fig. 4.4. Handling time at different consecutive visits by *A. plumipes* bees in strawberry flowers

4.3.3. Effect of pollination on seed fertilization

The rate of seed fertilization in different treatments is shown in (Fig. 4.5). The rate of seed fertilization in bee pollinated flowers was 59.9% which was significantly higher than that in the control (31%). No significant difference in seed fertilization rate was observed between bee and hand pollination.

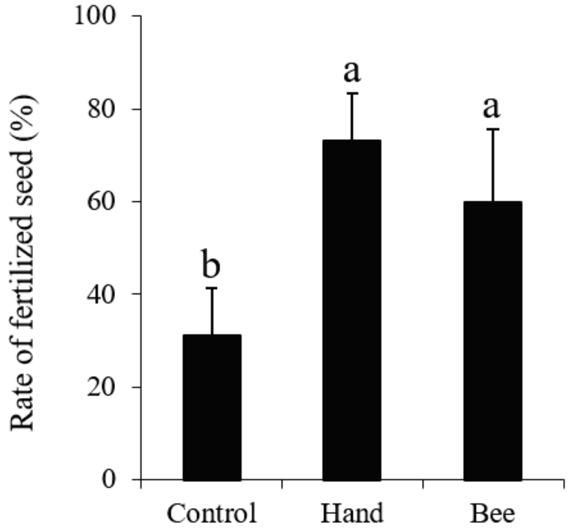


Fig. 4.5. Rate of fertilized seeds in control, hand-pollinated, and *A. plumipes* bee pollinated strawberry flowers. Values with the same letter are not significant at $p<0.05$ (least significant difference test).

4.3.4. Effect of flower visiting number on pollination

When flowers were opened to visit by bees for a controlled number of visits, even a single visit was able to produce 68% fertilized seeds in 2013 and 50% in 2014, and no further improvements in seed fertility were observed with further visits (Fig. 4.6).

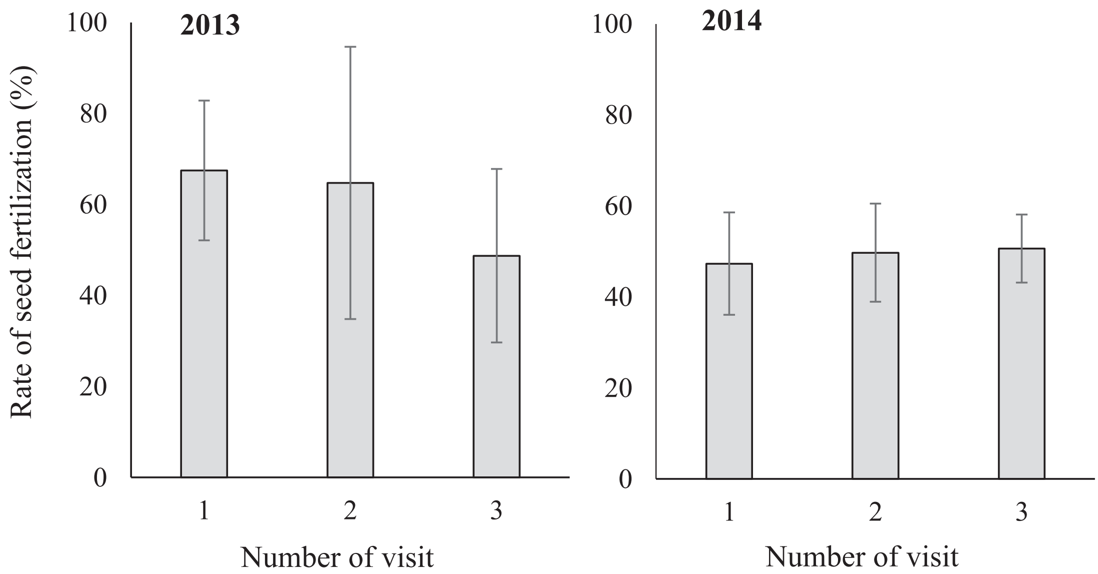


Fig. 4.6. Rate of seed fertilization as affected by number of flower visit by *A. plumipes* bees in 2013 and in 2014

4.3.5. Fruit yield and quality

In 2013, fruit size was bigger at the initial pickings, but almost same sized fruits were harvested during 2014 (Fig. 4.7). In 2013, the size of the fruit was closely affected by bee-pollination (Fig. 4.8). The average fruit weight in bee-pollinated and control treatments were 10.20 g (N = 52) and 6.83 g (N = 40), respectively. The proportion of big sized fruits (>20 g) was higher in with bee pollination, while the higher proportion of small sized fruits (<5 g) was observed in non-pollinated control. The sugar content in fruits were not significantly different among three treatments **(**Fig. 4.9**).** In the 2014 experiment, shape of the fruits varied among the three treatments (Fig. 4.10). The length/width ratio of almost all fruits (>95%) was 1.0 to 1.5 in hand and bee-pollinated fruits, while it was only 40% in control. As the length/width ratio between 1.0–1.5 is preferred high among the Japanese consumers, pollination seems crucial for not only increasing the fruit production, but also to achieve higher profit (Fig. 4.11).

Higher deformity was observed in control treatment both in 2013 (Fig. 4.12) and 2014 (Fig. 4.13 and Fig. 4.14). In 2013, in control treatment, more than 65% were highly deformed, 35% were slightly deformed and no normal fruits were observed. While, of the bee pollinated fruits, 44.23 % were normal, 42.69 % were slightly deformed and 11.53 % were highly deformed. Normal and slightly deformed fruits were marketable fruits. When the deformity was ranked in 5-level ranking in 2014, all of the fruits in control treatment were medium to highly malformed, and a large proportion of normally formed fruits was observed with hand-pollination and bee-pollination.

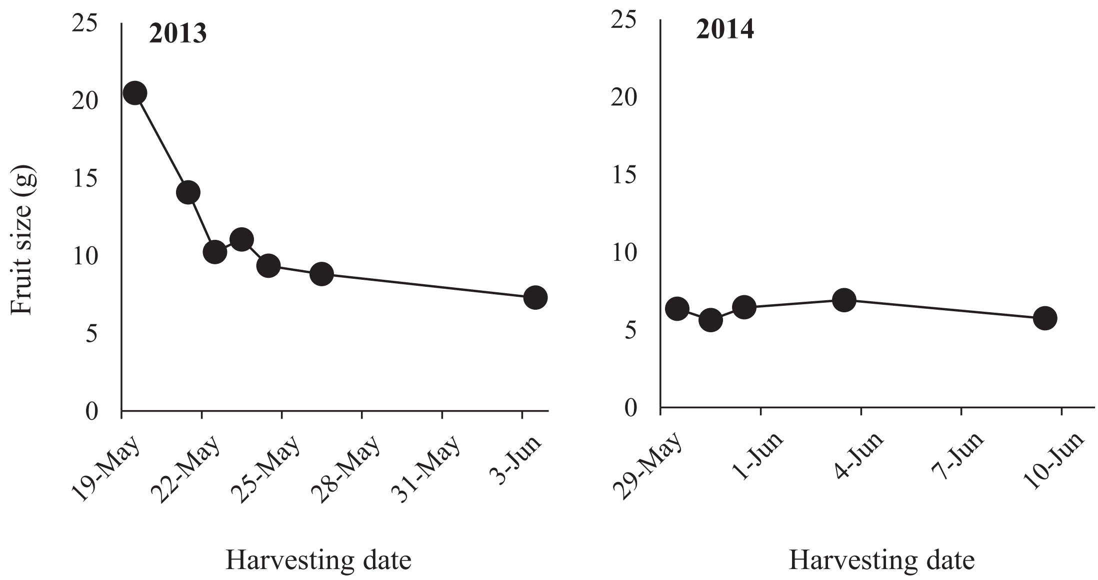


Fig. 4.7. Size of strawberry fruits over the harvesting period in 2013 and 2014

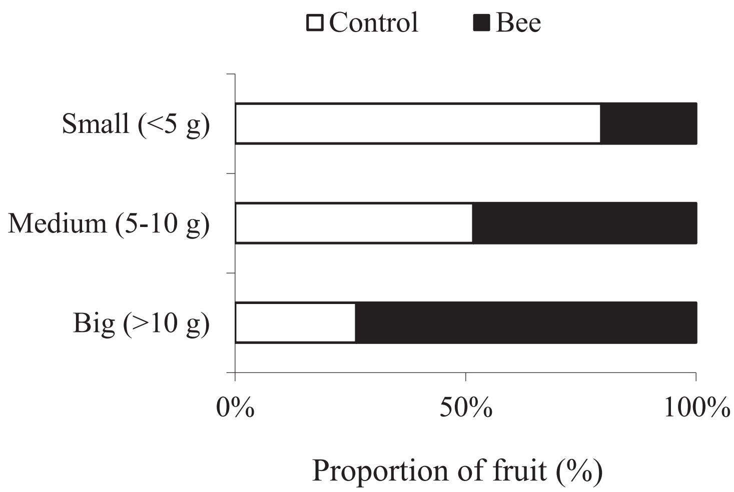


Fig. 4.8. Size of fruits in bee-pollinated and no supplementary pollinated strawberries in 2013

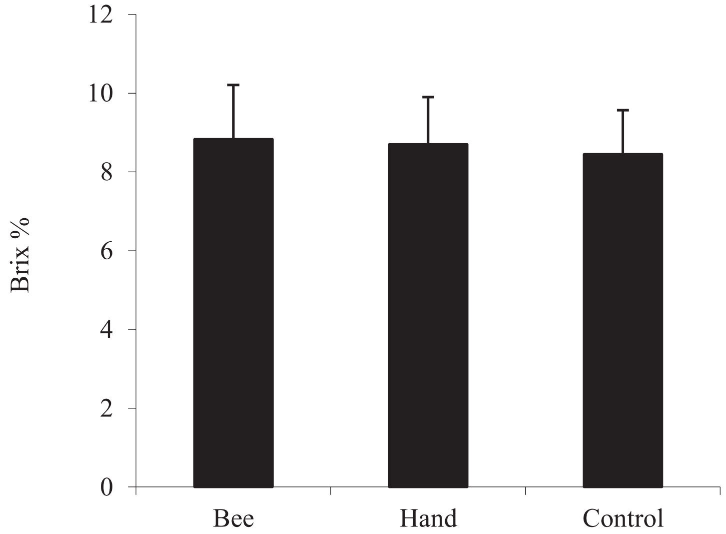


Fig. 4.9. Brix content in bee-pollinated, hand-pollinated and non-pollinated strawberry fruits in 2014

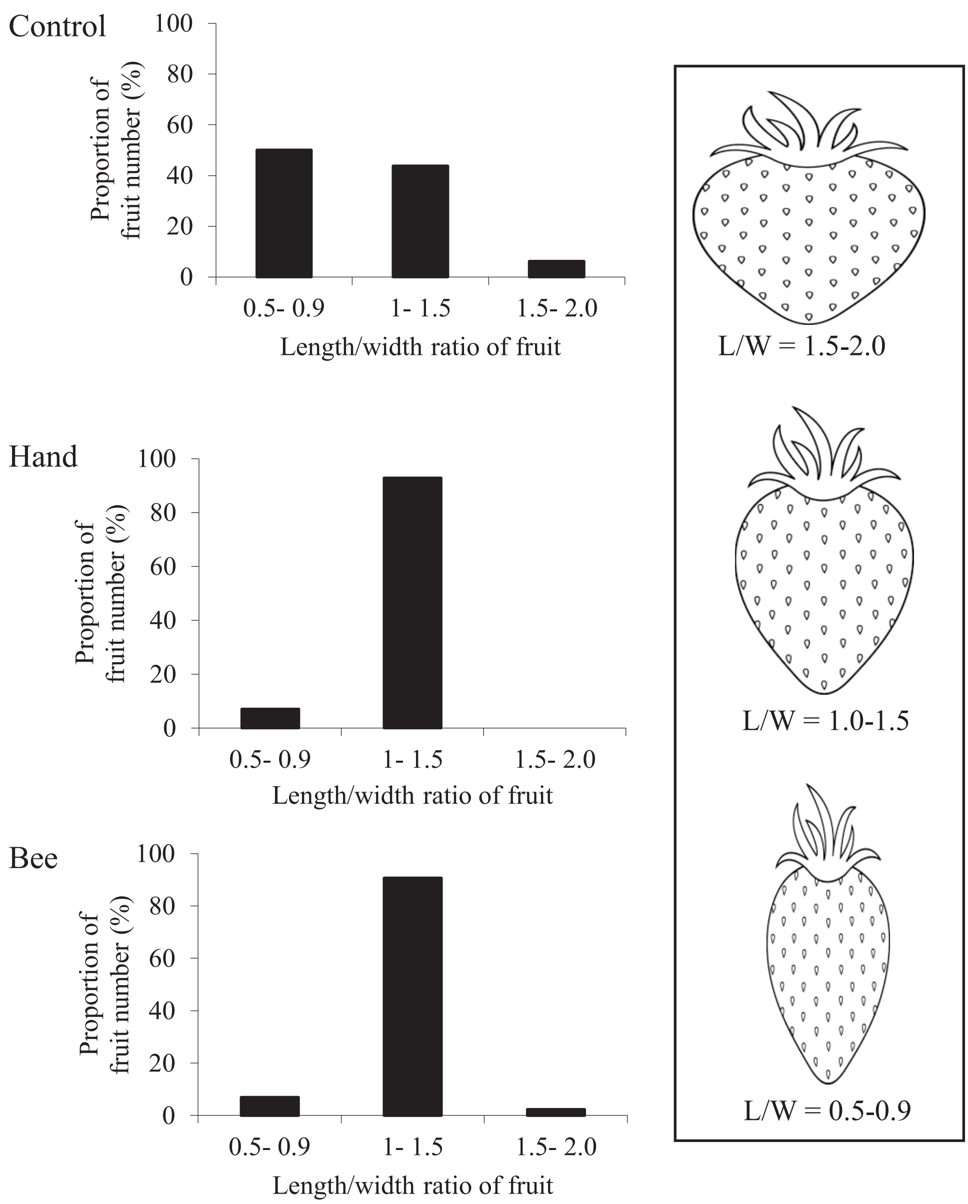


Fig. 4.10. Length/width ratio (L/W) of the fruits in bee-pollinated, hand-pollinated and no supplementary pollinated strawberry fruits in 2014

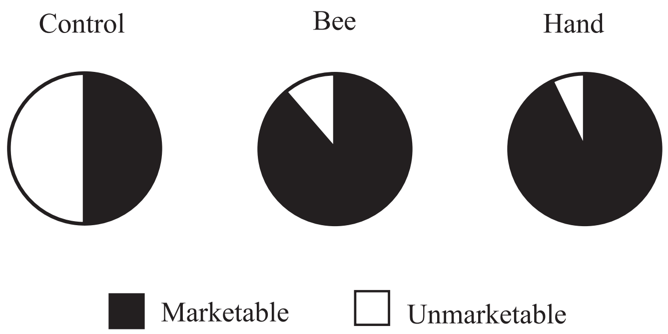


Fig. 4.11. Proportion of marketable and unmarketable fruits in bee-pollinated, hand-pollinated and no supplementary pollinated strawberry fruits in 2014

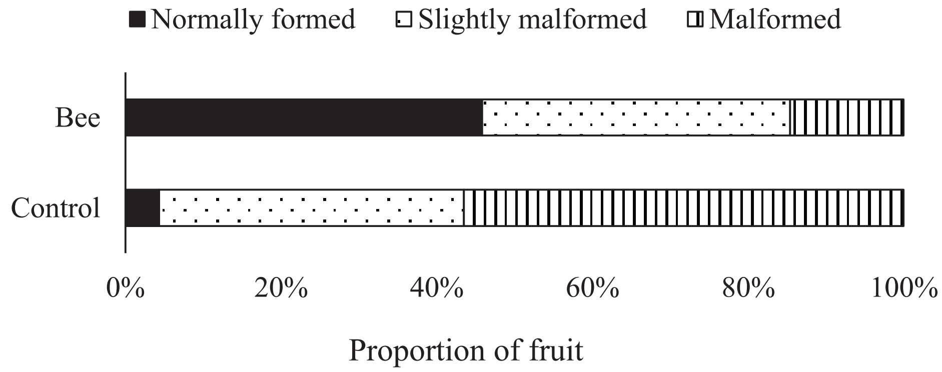


Fig. 4.12. Fruits of different deformity levels in bee-pollinated and no supplementary pollinated (control) strawberry fruits in 2013

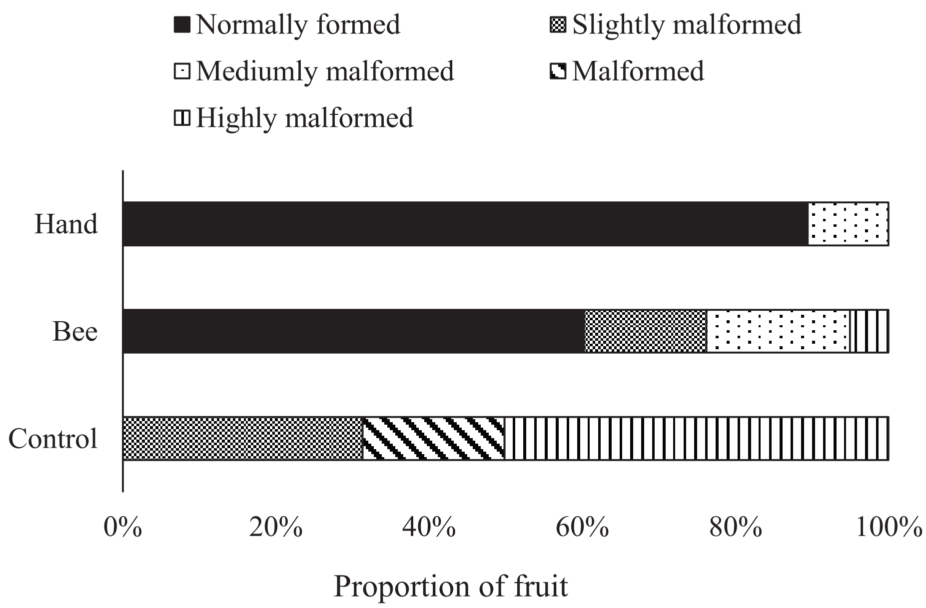


Fig. 4.13. Fruits of different deformity levels in bee-pollinated, hand-pollinated, and no supplementary pollinated strawberry fruits in 2014

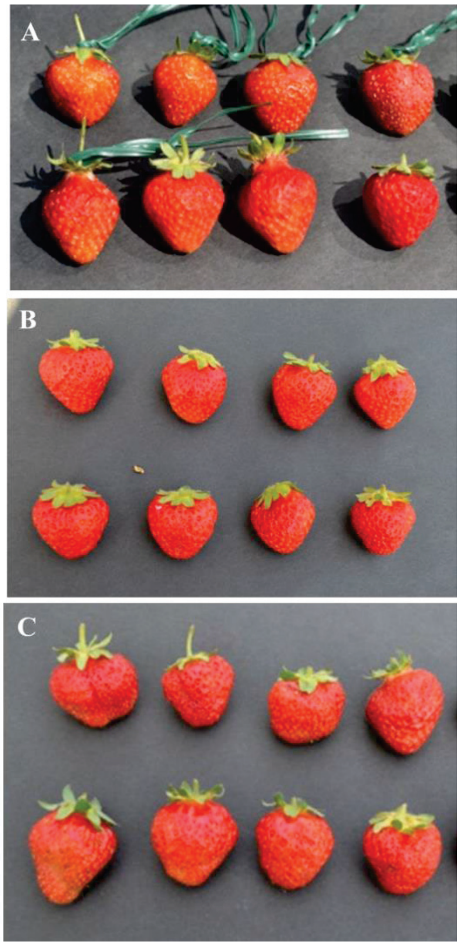


Fig. 4.14. Fruits obtained from the bee pollination (A), from hand-pollination (B), and from no supplementary pollination (C).

4.4. Discussion

A. plumipes pollinated strawberry flowers in the greenhouse as effectively as hand pollination. Both female and male bees visited the flowers efficiently suggesting this bee as a potential alternative pollinator for greenhouse strawberry. The seed fertility and quality were significantly higher in bee- and hand-pollinated flowers compared with the flowers which received no supplementary pollination. These results are similar to those reported for strawberry pollination by honeybees inside greenhouse conditions (Bond et al., 1999).

A. plumipes bees showed a high rate of adaptability in closed condition and started foraging and nesting activities soon after liberation, while no such adaptability to the closed condition is reported in honey bees, bumble bees, stingless bees, carpenter bees and *Osmia* spp. (Kakutani et al., 1993; Roselino et al., 2009; Maeta et al., 1992; Sadeh et al., 2007). Results suggested that even under an adverse weather condition, *A. plumipes* could be a suitable pollinator for in strawberry and other flowering plants.

Although, the handling time and foraging duration of *A. plumipes* was longer than that of *Osmia cornifrons* in a similar study (Maeta et al., 2012), the longer handling time in this study might be due to a small number of flowers during the active foraging period.

Flowers pollinated by bees make a better quality of fruit than self-pollinated and open-pollinated (Pratap, 2000; Kakutani et al., 1993; Roselino et al., 2009; Maeta et al., 2012). Also in this study, a higher quality of fruits by the pollination of bees was found. Higher rate of the fertilized seed and shape of the fruits pollinated by the bees suggested that the use of *A. plumipes* could be a good alternative for making higher profit from the greenhouse strawberries.

4.5. Conclusions

A. plumipes bees could be used as an alternative pollinator for greenhouse strawberry. For the sustainability of greenhouse strawberry production using *A. plumipes* as a pollinator, study on the reproductive ability of this bee under greenhouse condition is necessary when strawberry is the only floral resource.

General discussion

Recent declines in managed and feral honey bee populations have greatly increased interest in the current and potential role of wild pollinators in agricultural pollination. Understanding the distribution habitat and the resources is the important step to conserve and manage the bee population. Little is known about most wild bee species and efforts to understand their significance in pollinating wild plants and is critical to their conservation.

Anthophora plumipes is a vernal, univoltine and protandrous bee widespread and common throughout Mediterranean Israel and occurs in a variety of color forms from NW Europe across central Asia as far east as temperate Japan (Neeman, 2006). It is gregarious, gentle and tolerant to human activities. Both male and female bee forages on the characteristically deep throated flowers species. This study was carried out to examine the nesting and foraging activities of a soil nesting bee, *A. plumipes* in its natural habitat as well as in controlled conditions with the aim of utilizing it as an additional pollinator.

In chapter 2, nesting and foraging activities under natural condition is described. And In chapter 3, nesting and foraging activities of this bee for crop pollination under closed condition is described. It is revealed that *A. plumipes* is a spring emerging bee, which forages in spring blooming crops in warm humid temperate climate and nest is gregariously made in dry soils in horizontal cliffs. *A. plumipes* is a soil nesting bee. Several nesting materials with various designs were tested for *A. plumipes* in this study. Bees accepted a wide variety of artificial nesting materials from hard soil to a very light phenol-formaldehyde resin floral foam. Moreover, they accepted different nesting materials of different colors (red, black). Nesting female bees preferred dry nesting materials while building the nest. Well dried nesting material are attractive to bees and are easy to dig the holes for bees. Bees were attracted to the entrance holes of 9 mm in diameter and 15 cm in depth. Nests built in shorter cavities typically contained fewer cells. The size of the preferred entrance hole might have differed as per the size of the nesting female. Cavity in nest built in shorter length made fewer cells than longer length but longer than 15 cm may contain more female progeny but they are less attractive to bees and more difficult to drill and manage. Dense entrance holes also attracted the nesting females.

Bees started digging nests from the next day of liberation in both of the years (2010 and 2011) and start pollen collecting after four days of liberation in soil blocks and cylinder but in Phenol-formaldehyde resin floral foam bee start digging from 8 days of liberation and pollen collecting was started from 11 days of liberation. Females started foraging before males and foraged until later in the evening than the male. They started foraging before 5:25 and continued until 19:35, however data were recorded and presented only from 6.00 to 18.00 (Fig. 1.6 and 1.7). Besides visiting flowers, bees collected pollen throughout the day. Bees actively foraged even at a low temperature of 9.5ºC to a high of 36.4ºC, showing a high stability of collecting pollen and nectar over a wide range of temperature. Foraging activity was commenced between 17–99% relative humidity and light intensity from 1250–19810 lux. Morning activity was observed even in a temperature less than 9.5ºC but pollen foraging flight was observed from 9.5ºC to 36.4ºC. Stability in pollen flight even at high temperature throughout the day is rare to other pollinators. Bond (1999) observed high foraging activity of *A. plumipes* compared to that of other bees on *Vicia faba* at the temperature above 24.5ºC. In a study of Gonzalez et al. (2006), there was foraging flight of *A. walteri* up to 25.7ºC. Agreeing to the results of this study, Batra (1994) found starting of morning activity of *A. plumipes* at 10ºC when the nest site received only 150 lux (Batra 1994). *A. plumipes* is reported to have ability to regulate heat flow between thorax and abdomen. In a study, Stone (1993) reported this bee was able to fly at low ambient temperatures by tolerating thoracic temperatures as low as 25ºC, reducing the metabolic expense of endothermic activity. Activity of female is influenced by temperature (Stone, 1994). Average number of foraging flight in a day inside the greenhouse was 17.3. Number flight per bee per day for nectar ranged from 5–28 (N=54) and that for pollen flight ranged from 0–22 in soil blocks but in case of Phenol-formaldehyde resin floral foam average number of foraging flight in a day was 15.8 and average number of flight per bee for nectar was 8.4 (N= 8) and for pollen flight was 7.4 (N=4). Generally bees make pollen flights continuously until they prepare a pollen ball. In this study, to prepare a pollen ball, bees made an average of 15.6 flights (range: 12–22, N = 8) but 11flight was taken in field condition flight was many in closed condition may be due to poor availability of pollen in flower. Average duration for a pollen flight was 28.4 min per trip (range: 4.3–83.5 min, N = 374) in closed condition. In field, 29.93min and Batra (1994) also reported duration of a pollen trip of *A. pilipes* ranging from 6–47 min under field conditions. In this study, foraging duration per trip was longer when temperature was less than 10°C and more than 25°C. Longer foraging trip in the morning might be due to less pollen availability in the flower which made bees to visit more flowers to make a full pollen load (Fig. 1.19).

Average duration for a non-pollen flight was 25.9 min (range: 2.5–158.8 min, N=581) and in aqua foam field condition it was 15.63min. . Average duration of non-pollen flight during digging the burrow of nests was 10.12 (range: 7.46–15.02, N=6). The average duration of nectar flight during sealing the nests was longer than that under field conditions 23.36 (range: 16.14–28.36, N = 3) as Batra (1994) reported. In this study, bees were active for 35 days (from April 30 to June 4) in 2010 and 20 days in 2011 and 40 days in field condition. Batra, (1994) observed this bee active for relatively longer period under field conditions (from April 8 to June 11) in Shimane and Fukuoka Prefectures. Shorter active period in this study under closed conditions compared to field conditions might be due to limited floral resources in the former. In 2010, survival rate after 35 days was 75%. In 2011, due to poor floral resources, bees were reared only for 20 days (from May 7 to May 27) inside the same greenhouse used in 2010. Survival rate after 20 days was 75% which was same as 2010. After bees started making nests, they show high adaptability except accidental case.

A single female made 1–3 nests. Single series nests had 1– 4 cells, and there were 4–8 cells in multi-series nests. Bee completed making nest in 2–12 days and one cell was completed in 8 hours under controlled condition which was longer than field condition which was 3–7 days to complete nest and 6 hours to complete cell may be due to the poor source of floral resources. After completion of nesting activity this species overwinter.

Average number of nests and cells per female was 1.7 (range: 1–3, N = 11) and 7.85 (range 2–17, N = 7), under field condition and 1.6 (range: 1–3) and 5.8 (range: 1–9), under controlled condition respectively. Number of female and male cells per bee ranged from 1–5 and 1–4 under field condition and 0–6 and 0–4 under controlled condition respectively.

A completed nest contained 1–8 cells. Roberts (2011) reported 6–12 cells in a completed nest of *A. plumipes* under field conditions. In this study, most of the brood cells were declined 30–40° downwards, and the cells were arranged linearly in single and multiple series.

In chapter 4, I describe the utilization of this bee for pollination of strawberry under closed condition. In this study, *A. plumipes* was evaluated as an alternative pollinator for greenhouse strawberry. The fruits obtained from cross pollination by bees were compared to those from the self-pollinated control to evaluate the efficiency of this bee to be used as a pollinator for strawberry.

Previous studies suggested that *A. plumipes* have a very high foraging activity under various weather conditions. (Bond, 1999) reported a high pollination efficiency of this bee compared to honeybees inside greenhouse conditions. Also in this study, this bee showed a high rate of adaptability in closed condition and started foraging and nesting activities soon after liberation, while no such higher adaptability to the closed condition is reported in honey bees, bumble bees, stingless bees, carpenter bees and *Osmia* spp. (Sadeh et al., 2007; Kakutani et al., 1993; Maeta et al., 1992 and 2012).

However, the handling time and foraging duration was longer than that of *Osmia cornifrons* in a similar study of (Maeta et al., 2012). The longer handling time in this study might be due to a low number of flowers during the active foraging period.

Bee collected pollen more frequently during morning and noon hours this might be because the maximum number of pollen grains was presented by this crop during morning and noon hours which was also found in same study of (Maeta et al., 2012).

Fertilized seed is recognized as a direct indicator of pollination (Burd, 1994). In this study, a higher rate of fertilized seed was observed with bee pollination compared to non-pollinated control. The rate of fertilized seed ranged widely from less than 30% to 100% in this study. This variation might be due to the handling time or the age of flowers. Self-pollination as indicated by the percentage of fertilized seeds in bagged flower was highly variable among the flowers. A high variation in self-pollinated strawberry seeds was also observed in a similar study (Kakutani et al., 1993).

Studies have suggested that the flowers pollinated by bees had a better quality of fruit than self-pollinated and open-pollinated (Roselino et al., 2009; Kakutani et al., 1993; Pratap, 2000; Maeta et al., 2012). Also in this study, a higher quality of fruits by the pollination of bees was found. Both of the quality indicators in this study (proportion of the fertilized seed and shape of fruits) were ranked higher in the fruits pollination by bees. Commercial market requires production of good shaped fruits as ranked in this study, which can be achieved through the pollination of *A. plumipes* bees.

Just a single visit by *A. plumipes* with a handling time of at least 20 sec resulting into seed fertilization rate of 84.5% within 3 days of blooming suggested that *A. plumipes* can effectively pollinate strawberry flowers. Thus, this bee could be used as an alternative pollinator for greenhouse strawberry. However, further study is necessary on the reproduction ability when strawberry flowers are the only floral resource to know the sustainability of greenhouse strawberry production using *A. plumipes*.

Summary

Anthophora plumipes is univoltine and distributed throughout Western Europe to East Asia including Japan. Flying period is ca. one month from the middle of April to the end of May in south-western Japan. Both males and female bees prefer to visit flowers with a long corolla tube. Nests of the species are often found on the bare clayish slope at the margin of secondary forests . It is gregarious, gentle and tolerant to human activities. Previous studies have suggested that *A. plumipes* can be used as a manageable pollinator of various fruit crops. In order to evaluate their effectiveness as a pollinator of horticultural crops, foraging activity of the species under the closed condition was studied in the greenhouse at the campus of Shimane University, Matsue (lat. 35°22', alt.120m). Also, the life cycle and nesting activity of them was studied at the natural habitat near Hirata (lat. 35°26', atl.30m), Shimane, Japan.

To know the nesting biology of *A. plumipes* under their natural habitat, a study was carried out by examining the nesting and foraging activities in an urban park located at Hirata, Shimane, Japan (lat. 35°26', atl.30m). The bees were found flying for about 32 days. Number of foraging trips per female per hour ranged from 0 to 3, and the highest number of trip was observed from 12:00 to 13:00 (3 trips). Average flight duration for collecting pollen was 29.9 min and for non-pollen flight was 15.6 min. Average number of flights for making one pollen ball was 11. The dense nest aggregation was found in hard clay walls in sloppy land under the root stumps of pine. Single series and multiple series nests were found having linearly arranged cells.

To know the nesting behavior under controlled condition, this bee was reared in a greenhouse located at Shimane University, Matsue, Japan (lat. 35º29’, elevation 170 m) for five consecutive years (2010 to 2014). Several nesting materials of various designs were tested. They accepted different nesting materials (Soil blocks and soil cylinder) made from soils with different colors (red and grey). Nesting female bees preferred well dried nesting materials while building the nest. Four wild flowering plants, phacelia *(Phacelia tanacetifolia*), borage *(Borago officinalis)*, centaurea *(Centaurea cyanus)* and red clover *(Trifolium pretense)* and a cultivated flowering plant, strawberry (*Fragaria annanasa*), were used as floral resources. In the greenhouse, bees started digging nests from the next day of liberation, and started collecting pollen after 4 days. Besides visiting flowers, bees collected

pollen throughout the day. In addition, the bees actively foraged from a low temperature of 9.5ºC to a high of 36.4ºC. Foraging activity was commenced at 17–99% relative humidity and light intensity from 1,250–19,810 lux, showing a high stability over a wide range of temperatures and light intensity. Morning activity was observed even in a temperature less than 9.5ºC but pollen foraging flight was observed only from 9.5ºC to 36.4ºC. Stable foraging activities under a wide conditions indicated that this bee is a potential pollinator for greenhouse plants.

Under closed condition (in a greenhouse), bees made an average of 17.3 foraging flights per day per bee. They spent an average of 29.10 min per flight for collecting pollen, which is closer to the field condition for collecting pollen. Bees took 15.6 flights to prepare a pollen ball. A single bee prepared a maximum of 3 nests, having a maximum of 8 cells per nest. The nests were diverse in structure, with single and/or multi-series cells. A nest completed in 3 to 5 days. In this study, most of the brood cells were oriented 30–40° downwards, arranged linearly in both single and multiple series nests. Bees were active for 35 days in the greenhouse, which was almost similar to that under field condition.

Pollination efficiency of this bee for pollinating cultivated flowering plants (i.e., strawberry) was also examined. Bees took handling time of 11.02 sec flower^{-1} (N = 340), and visited 6.47 flowers min^{-1} (N = 68) for strawberry flower. Handling time differed by age of the flower and frequency of the visit. Seed fertilization and fruit quality (evaluated based on the shape and deformation) in bee-pollinated fruits was significantly higher than in fruits with no supplementary pollination and almost similar to those in hand-pollinated flowers. The bees visited strawberry flowers mostly for collecting nectar, however collection of pollen was not seen clearly. Frequent visit of bee for nectar seem to favor for the pollination of fruit. This bee can be used as an additional pollinator but cannot be reared for brood provision if strawberry is the only floral resource under the greenhouse conditions.

It is concluded that *A. plumipes* bees can be reared under the closed conditions and can be used as a promising alternative pollinator of different kinds of flowering plants under such conditions.

摘要

ハチ目ミツバチ科コシブトハナバチ属のケブカハナバチ *Anthophora*（*Anthophora*）*plumipes*（Pallas）はユーラシア大陸に広く分布する旧北区系の地中営巣性ハナバチで，日本には北海道と南西諸島を除く全土に分布する．成虫の活動期は春季（4 月中旬～5 月中旬）にあり，生活史は年 1 化性で推移する．いわゆる「長舌ハナバチ」の一群で，雌雄とも筒状の花冠をもつ顕花植物を主要餌資源として利用する．乾燥した土壌に好んで営巣することで知られ，表土が露出した日当たりのよい崖の法面などにしばしば密な営巣集団を形成する．人家周辺では建物の土壁などで巣が発見されることも多い．早春の不安定な天候下でも比較的安定した飛翔活動を行うことから，春季に開花期をもつ栽培作物の送粉者としての実用化が期待されている．米国では 1980 年代に日本から導入された個体群を用いてリンゴやブルーベリーでの送粉効果について継続的な調査が行われている．著者は施設栽培作物の送粉者として本種を利用するため，実験圃場に設置したビニールハウス内において本種を飼養し，完全な閉鎖環境下における本種の飼養技術を確立するとともに，栽培イチゴでの本種の送粉効果を明らかにした．また，比較のため，野外営巣地における本種の営巣生態についても明らかにした．以下にその結果を概説する．

１．野外におけるケブカハナバチの営巣生態

閉鎖環境に対する本種の順応性を評価する基礎として，営巣活動の指標となるメスあたりの完成巣数，時間あたりの採餌頻度や採餌所要時間，巣当たりの育房数などを野外で明らかにした．調査した出雲市・平田の営巣地におけるメスの採餌期間はほぼ 1 か月と推定された．営巣メスの 1 時間あたり採餌回数は最大 3 回で，そのピークは 12 時～13 時にあった．採餌所要時間の平均は花粉採餌が 29.9 分，花蜜採餌が 15.6 分であった．また，メスあたりの創設巣数は最大で 3 巣で，巣当たりの平均育房数は 7.9 個，自然状態における幼態の死亡率は 14.5％であった．

２．閉鎖環境におけるケブカハナバチの営巣生態

施設栽培作物の送粉者としてケブカハナバチの利用の可否を検討するため，島根大学構内の実験圃場に設置した小規模なビニールハウス内で本種を放飼し，完全な閉鎖的環境下でも正常な営巣活動を行うかどうかを検討した．花資源植物として，プラスチックポットに植えたヤグルマギク *Centaurea cyanus*（キク科），ボリジ ***Borago officinalis***（ムラサキ科），アンジェリカ *Phacelia tanacetifolia*（ハゼリソウ科）の 3 種を配置した．また，営巣基として粘土質の細土を水で練り，3 種の素焼きの植木鉢に充填して乾燥させた「土ブロック」と暗渠用メッシュパイプに土を充填した「ネストシリンダー」を配置した．営巣活動の指標となるメスあたりの完成巣数，時間あたりの採餌頻度や採餌所要時間，巣当たりの育房数などを野外の自然営巣集団と比較した．本種は小規模な閉鎖環境下においても，「土ブロック」を利用して速やかに巣を創設することが確認された．放飼開始から花粉採餌までに要した日数は，わずか 6 日間であった．また，本種は優れた体温調整機能を有しており，ハウス内でも気温が 10℃を下回るような低温から 30℃を超える高温まで，幅広い温度域で飛翔活動を行った．ハウス内における営巣メスの日あたりの採餌飛翔は平均 15 回で，創設した巣の数は最大で 3 巣に達した．メスあたりの創設巣数および生産子孫数，巣あたりの育房数および子孫死亡率はハウスと野外で有意差は認められなかった．閉鎖環境でも正常に飼養でき，幅広い活動条件を示す本種は，施設栽培の送粉者としても優れた特性を備えていることが示唆された．

３．ハウスイチゴにおける送粉効果

施設栽培作物における本種の送粉効果を明らかにするため，ハウスイチゴを用いた実証実験を島根大学構内の圃場に設置した小規模ビニールハウスで行った．ハウス内にプラスチックポットに植えたイチゴ（宝交早生）を配置し，本種の雌雄成虫を放飼した．ハウス内には営巣基として，上記した「土ブロック」を配置した．送粉効果の指標として，果実のサイズ，果実重，果実あたりの受精種子数，糖度，果形などを計測し，人工授粉区および無放飼区のそれらと比較した．また，ハチの花上滞留時間，単位時間あたりの訪花花数，有効訪花回数を測定した．無放飼区との比較では，受精種子数および果実重に放飼区および人工授粉区で有意な違いが認められた．果形については果実長と果実幅の比が 1.0-1.5 となるものを「正常果」とし，その割合を各処理区で比較すると，放飼区と人工授粉区では正常果の割合が無放飼区よりも有意に高い結果となった．果実の一部が委縮し，果形が変形しているものを「奇形果」とし，その出現頻度を処理区間で比較すると，無処理区ではその割合が極めて高くなる一方，放飼区と人工授粉区では低く抑えられた．これは受精種子率の違いに起因するものと考えられた．

一般に，本種のような飛翔力の強い中型のハナバチ類は採餌圏が広いため，閉鎖環境への順応は困難とされる．しかし，本研究から本種は小規模なビニールハウス内でも「土ブロック」などの営巣基を用いて，速やかに正常な営巣活動を行うことが明らかとなった．イチゴに対する送粉効果も高く，本種は施設栽培作物における新たな送粉昆虫として実用化が期待できることが示された．

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List of publications

1. Adhikari Devkota Radha and Ryoichi Miyanaga (2015). Utilization of hairy footed flower bee *Anthophora plumipes* (Hymenoptera: Apidae) for pollination of greenhouse strawberry. *Advances in Entomology*. January 15 2016 (From the works described in chapter 4)
2. Radha Devkota ADHIKARI・前田泰生・泉洋平・宮永龍一（2016）閉鎖環境下におけるケブカハナバチの営巣活動. 日本昆虫学会（From the work described in chapter 2 and 3）

List of presentations at scientific societies

September 14–16, 2014	Does *Anthophra plimipes* (Hymenoptera, Apidae) forage Strawberry flower? Paper presented in 74th Annual Meeting of Entomological Society of Japan, Hiroshima University, Higashi Hiroshima, Japan Adhikari, R. D. and Miyanaga, R.
January 27–29, 2012	Nesting biology and pollinating behavior of *Anthophora plumipes* (Hymenoptera, Apidae) under the closed conditions Poster presented in JSPS International Symposium on Pollinator Conservation, Kyushu University, Fukuoka, Japan Adhikari, R. D. and Miyanaga, R.
September 17–19, 2011	Nesting behavior of *Anthophora plumipes* Pallas in greenhouses (Hymenoptera, Apidae) Paper presented in 71st Annual Meeting of Entomological Society of Matsumoto, Japan Adhikari, R. D. and Miyanaga, R.
October, 2010	Nesting behavior of *Anthophora plumipes* (Pallas) in greenhouses (Hymenoptera, Apidae): implications for use as pollinators under the closed conditions. Poster presented in Annual Meeting of Japanese Society of Entomology Chugoku Region, Tottori, Japan Adhikari, R. D., Miyanaga, R. and Harada M.