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Copulation Environment Favorable for colony development of the European Bumblebee, *Bombus terrersis*

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We investigated mating conditions about care temperature of queen before mating, mating period, and number of queen per mating cage to improve mating rate of *Bombus terrestris.* Among 19°C, 22°C and 25°C, care temperatures of queen before mating, queen cared at 19°C was more effective than those at 22°C and 25°C in death rate during care and mating periods, and colony development. In case of mating period, oviposition rate and preoviposition periods at queen mated during 3 days were 89.3% and 11.4 days, respectively, which was higher and earlier than those of queen mated during 5 days and 7 days. The rate of worker emergence, colony foundation and progeny-queen production at 3 days-mated queen were also 2.0-11.6% higher than those at queen mated during 5 days and 7 days. In number of queen per mating cage, the rate of worker emergence, colony foundation and progeny-queen production queen mated at mating cage with 10 queens and 30 males were 41.5%, 25.9% and 23.2%, respectively. These values correspond to 1.5-6.8 folds those queen mated at cage with 20 queens and 30 queens. Therefore, we supposed that care temperature favorable for *B. terrestris* queen was 19°C and the period need to mating was 3 days, and the number of queen per mating cage (55 × 45 × 65 cm) was 10.

Key words: Bumblebee, *Bombus terrestris*, Mating, Copulation, Care temperature, Mating cage

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Introduction

Bumblebees are an important pollinator of various greenhouse crops, particularly effective in pollinating for the night shade family, which includes tomato and eggplant (Buchmann and Hurley, 1978; Free, 1993). Introduction of bumblebees to greenhouses for pollination has become widespread in recent years, and the demand is increasing every year. The large earth bumblebee, *Bombus terrestris*, which is indigenous to Europe, has been artificially introduced throughout the world. Since 1988, *B. terrestris* in portable boxes have been available commercially from European company for crop pollination (Mitsuhata, 2000). Colonies of *B. terrestris* have already been imported into Korea, Japan, China, Taiwan, Maxico, Chile, Argentina, Uruguay, South Africa, Morocco and Tunisia for development in greenhouses (Dafni, 1996). In case of Korea, *B. terrestris* was firstly introduced in 1994. Bumblebees are eusocial insects and live in colonies of up to a few hundred individuals, and have generally one generation per year. Queens are the only caste to overwinter (enter diapause), and workers and males die during late summer and early autumn, respectively. In early spring queens that overwintered leave their hibernation sites. The queen builds up a store of pollen and lays her first batch eggs into the pollen mass after searching a suitable site to found a colony. As soon as the workers of the first brood have emerged they take over the foraging actives of the queen, who from now on spends her time predominantly on the laying of eggs. In the late summer, many males and new queens are produced and only mated queens hibernate and emerge in spring (Heinrich, 1979; Duchateau and Velthusis, 1988).

A decisive period in the life of bumblebees is mating. The nuptial flight of *B. terrestris* males consists of patrolling behavior during which secretions from the cephalic labial gland are deposited on marking spots. Males establish nuptial flight circuits by scent-marking plants and they attempt to copulate with young queens encountered on their flight-path (Svensson, 1980; Williams 1991). The active component of male pheromone secreted from the cephalic labial gland has been identified as farnesol. Young queens emit sex pheromones from mandibular glands (Honk *et al.*, 1978; Bergstrom, 1981). Djegham *et al.* (1994) produced a precopulatory ethogram for *B. terrestris* in a cage where no nuptial route could be established. They found that among the male and female behavioral sequences analyzed, antennal inspection by both sexes and queen mobility were key factors in the mating success. Other authors reported on the optimal age for mating in *B. terrestris* (Duchateau, 1985), and the sperm content and transfer of spermatheca (Röseler, 1973; Duvoisin *et al*, 1999) or vasa deferentia (Duchateau and Mariën, 1995). Tasei *et al.* (1998) reported that the relationship between aging, sexual behaviour and sperm prodction and transfer. Sperm length, sperm storge and mating system charavteristics, and bumblebee mating plug to prevents females from remating were also reported (Baer *et al.*, 2001, 2003).

Though *B. terrestris* has been selected as a reliable species for commercial mass-production of the bees, there are still some unsettled issues. Among these issues, mating environmental conditions must be the most principal factors in rearing of poikilothermal bumblebees. Despite abundant literature on bumblebee sexuality, there is no data available on the mating conditions of *B. terrestris* including care temperature of queen before mating, the period need to mating, and the number of queen per mating cage. Therefore this study was conducted to identify mating conditions most favorable for colony development of *B. terrestris.*

Materials and Methods

Origin of experimental insects

Experimental insects were CO_2-treated and artificial hibernated the 5 - 7th generation queens of *B. terrestris* reared in a controlled climates room (27 ± 1°C, 65% R.H. and continuous darkness). CO_2-narcosis was exposed to 99% CO_2 for 30 min daily during two consecutive days (Yoon *et al.*, 2003). For artificial hibernation, queens were hibernated for 5 months at 2.5°C to preserve them in a bottle filled with perlite and keep it around 80% R.H. (Yoon, 2003). After that, the queens were placed in flight cages for 3 days and then reared under the 27 ± 1°C, 65% R.H. (Yoon *et al.*, 2004).

Indoor rearing

The basic colony-rearing technique was followed as described in Yoon *et al.* (2002). The queens were reared in three types of plastic boxes each for colony initiation (8.0 × 11.0 × 8.0 cm: small box), colony foundation (15.5 × 16.5 × 11.0 cm: medium box), and colony maturation (22.0 × 27.0 × 14.0 cm: large box). Queens were first confined individually in small boxes for colony initiation and remained there until oviposition. To stimulate the egg-laying, two narcotized old *B. terrestris* worker 10 - 20 days aged after emergence was added to each queen (Yoon and Kim, 2002). When the adults emerged from the first brood, the nest was transferred to a medium box for colony foundation, and left there until the number of workers reached 50. The nest was thereafter moved to the big box for further colony development.

Forty percent sugar solution and pollen dough were provided *ad libitum*. The pollen dough was made from sugar solution and pollen (v : v = 1 : 1).

Colony development of *B. terrestris* queen at different care temperatures of queen before mating

To examine a suitable care temperature at queen before mating for colony development of *B. terrestris* queen, care temperature of queen were defined as 19°C, 22°C and 25°C. Care period is 5 days. Copulation room was maintained at 23 - 24°C, 14 L, 65% R.H., and the intensity of 2000 lux. For mating, thirty virgin queens and ninety males were introduced a wooden mating cage (55 × 45 × 65 cm) with wire mesh during one week. Queens used for the experiment were five-days-old and the male ten-days-old, which is the optimal age for mating (Duchateau, 1985; Tasei *et al.*, 1998; Yoon *et al.*, 1999). The number of queens allotted to this experiment was 30 and 5 replications. After mating, *B. terrestris* queens were hibernated for 5 months at 2.5°C and then reared in a controlled climates room (27 ± 1°C, 65% R.H. and continuous darkness) to investigate survival rate after hibernation and developmental ability of colony. The developmental ability of each colony was estimated by death rate during mating period, preoviposition period, and rate of oviposition, worker emergence, colony foundation and progeny-queen production. Colony foundation here indicates that more than 50 workers emerged in a colony. The queens that did not oviposit in 60 days were excluded from the number of oviposited colonies.

Colony development of *B. terrestris* queen at different mating periods

To investigate mating period favorable for colony development of *B. terrestris* queen, mating periods were defined as 3 days, 5 days and 7 days. Conditions of mating room were maintained at 23 - 24°C, 65% R.H., 14 L and the intensity of 2000 lux. The number of CO_2-treated queens allotted to this experiment was 35 and 2 replications. The developmental ability of each colony was estimated by oviposition rate, preoviposition period and rate of worker emergence, colony foundation and progeny-queen production.

Colony development of *B. terrestris* at the number of queen per mating cage

To examine the number of adequate queen per mating cage (55 x 45 x 65 cm) for colony development of *B. terrestris*, the number of queen per mating cage was defined as 10, 20 and 30. Conditions of mating room were maintained at 23 - 24°C, 65% R.H., 14 L and the intensity of 2000 lux. This experiment was conducted with CO_2-treated queens and 2 replications. The developmental ability of each colony was estimated by oviposition rate, preoviposition period, and rate of worker emergence, colony foundation and progeny-queen production.

Statistical analysis was done with Chi-square test and Tukey's pairwise comparison test (MINITAB Release 13 for Windows, 2000).

Results and Discussion

Comparison of colony development of *B. terrestris* at different care temperatures of queen before mating

Bumblebee reared at 27 - 28°C and until before mating, queens were kept at 25°C for 5 - 6 days. During this period, 15.0 - 20.0% queens died (Yoon H. J. unpublished). From this result, we investigated the care temperature of queen before mating to reduce death rate during care period of queens and improve colony development of *B. terrestris*. Among care temperatures of 19°C, 22°C and 25°C, the death rate during care periods and mating periods was low at 19°C as 6.4% and 18.9%, respectively, and low in order of 25°C (16.6% and 28.9%) and 22°C (17.2% and 31.1%) (Fig. 1). The death rate during care periods was significantly affected by care temperature of queen (Tukey's pairwise comparison test: F = 14.79, DF = 2, 12, p = 0.001) (Fig. 1). In *B. ignitus*, the death rate during care period at 25°C and mating period is 10.0 - 20.0%, and 8.3 - 39.1%, respectively. The later emergence of queen is, the higher death rate during care periods of queen and mating periods is (Yoon H. J. unpublished).

The survival rate and rate of weight loss after artificial hibernation were examined with the 6th generation queens produced from different care temperatures (Fig. 2). The survival rate after 1 month was 84.9 - 87.0%, 3 months was 63.2 - 67.9%. The survival rate after 5 months was high at 22°C as 52.1% and low in order of 19°C and 25°C

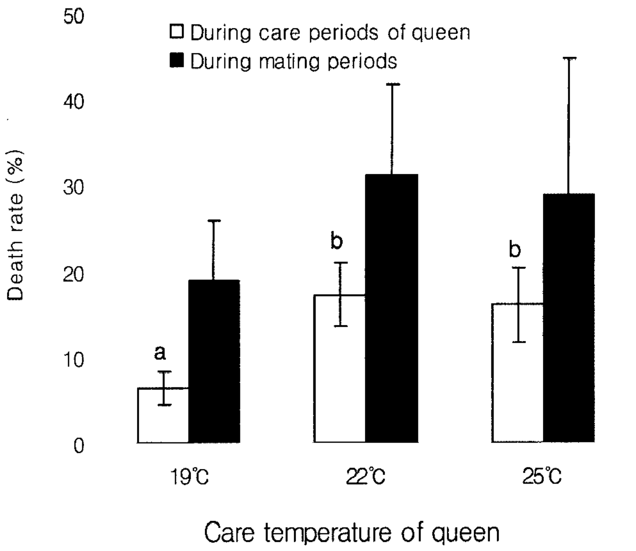

Fig. 1. Death rate during care period and mating period of *Bombus terrestris* queen in different care temperatures of queen before mating. There was significant difference in death rate during care period at different mating care temperatures of queen before mating at $p < 0.05$ by Tukey's pairwise comparison test.

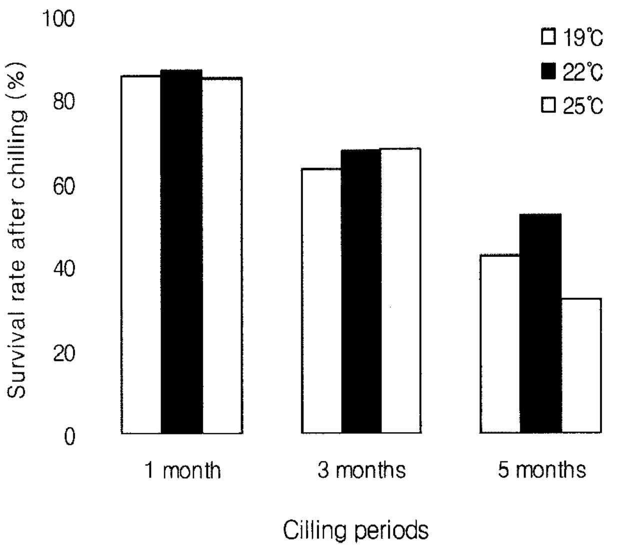

Fig. 2. Survival rate after artificial hibernation of *B. terrestris* at different care temperatures of queen before mating. There was no significant difference in survival rate after artificial hibernation of *B. terrestris* at different care temperatures of queen at $p < 0.05$ by Chi-square test.

as 42.6% and 32.1%, respectively. There is no statistically difference at the survival rate by care temperature of queen (Chisquare test: 1 month, $x^2 = 0.095$, DF = 2, 6, $p = 0.954$; 3 months, $x^2 = 0.837$, DF = 2, 6, $p = 0.837$; 5 months, $x^2 = 3.343$, DF = 2, 6, $p = 0.188$). The rate of weight loss after hibernation during 3 month is 12.8 - 14.6.0%, 5 months is 20.6 - 27.1% (Table 1). The longer

Table 1. Change of weight after artificial hibernation of *B. terrestris* at different care temperatures of queen before mating

Chilling periods (month)	Care temperatures of queen before mating	n	Change of weight in artificial hibernation		
			Prior (g)	After (g)	Rate of weight loss (%)
3	19°C	42	0.89±0.11	0.76±0.10	14.6
	22°C	31	0.78±0.14	0.68±0.09	12.8
	25°C	35	0.78±0.13	0.68±0.11	12.8
5	19°C	23	0.88±0.11	0.70±0.09	20.6
	22°C	16	0.87±0.11	0.64±0.07	27.1
	25°C	12	0.87±2.46	0.64±1.87	27.1

1) n means the number of surveyed.

hibernation time is, the higher the rate of weight loss after hibernation is. Hoem (1972) reported that the weight losses occurred during the first half of the hibernation period after which time the body weight increased. Significant, positive correlations were found between the body weight of the queens and the length of the period of survival during and after hibernation respectively. Alford (1969a) on average found 57% water in bumblebee queen following hibernation and a reduction of about 50% in both live weight and dried weight. The fat makes up an average of 34% of the total dry weight of the bumblebee queens prior to hibernation and 80% of this fat is absorbed during hibernation. A well developed fat body in a queen bumblebee will thus be of importance to safe hibernation (Arford, 1969b). Horber (1961) found an average weight loss during hibernation of 151.3 mg. Furthermore he proved that surviving queens were heavier than queens which died during hibernation. Hoem (1972) reported the weight of *B. terrestris* queens prior to hibernation varied from 400 mg to 1000 mg and after hibernation 300 mg to 900 mg. Similarly the weight of *B. lapidarius* queens prior to hibernation was 250 - 850 mg and after hibernation 250 - 750 mg.

Queens mated at different care temperatures was hibernated during 5 months at 2.5°C. After hibernation, we investigated oviposition rate and preoviposition period of *B. terrestris* queen. The oviposition rate of queen cared at 25°C was 60.0%, which was 1.7 - 3.5% higher than that of 19°C and 22°C, but there was no statistical difference (Chisquare test: $x^2=0.046$, DF=2, 6, $p=0.977$) (Fig. 3). The preoviposition period was 8.2 - 10.6 days and also no statistical difference (Tukey's pairwise comparison test: F=1.50, DF=2, 33, $p=0.238$) (Fig. 3). The rate of worker emergence of queen cared at 19°C was 29.2%, which was 7.5 - 9.2% higher than that of 22°C and 25°C

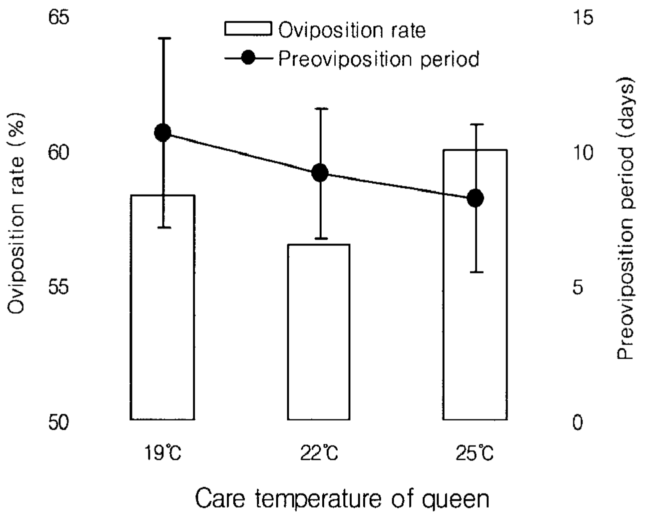

Fig. 3. Oviposition rate and preoviposition period of *B. terrestris* at different care temperatures of queen before mating. There were no significant differences in oviposition rate and preoviposition period of *B. terrestris* at different care temperatures at $p<0.05$ by Tukey's pairwise comparison test and Chi-square test.

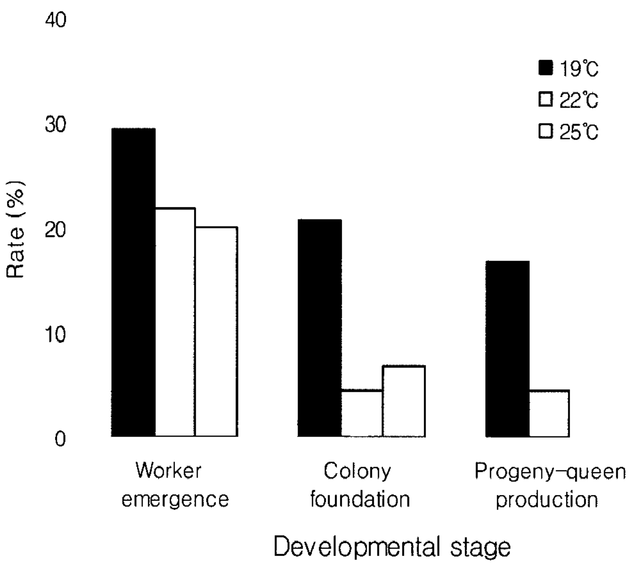

Fig. 4. Colony development of *B. terrestris* in different care temperatures of queen before mating. There was no significant difference in colony development of *B. terrestris* at different mating care temperatures at $p<0.05$ by Chi-square test.

although there is no statistically difference (Fig. 4). The rate of colony foundation and progeny-queen production were also the highest at 19°C as 21.7% and 16.7%, respectively, which were 14.1 - 16.4% and 12.3 - 16.0% higher than those of 22°C and 25°C (Fig. 4). But there was no significant differences in rate of colony foundation and progeny-queen production (the rate of colony foundation: $x^2=0.369$, DF=2, $p=0.165$, the rate of progeny-queen production: $x^2=4.140$, DF=2, $p=0.126$).

With above results, we supposed that 19°C at care temperature before mating was more effective than 22°C and 25°C in death rate during care periods and mating periods, and colony development of *B. terrestris* after artificial hibernation. Sauter and Brown (2001) reported that, despite potential selection on males for courtship behaviour, quantitative within-species variation in the precopulatory behaviors, there is little or no effect on whether copulation actually occurs. Rather, the reproductive status of females seems to be the most important determinant of copulation.

Colony development of *B. terrestris* at different mating periods

It was investigated appropriate mating periods for egg-laying characteristics and colony development of *B. terrestris*. In case of egg-laying characteristics, the rate of oviposition of queen mated during 3 days was 89.3%, which is 3.5 - 10.9% higher than that of queen mated during 5 days and 7 days (Fig. 5). But there is no significant difference in the oviposition rate at different mating periods. Among preoviposition periods of 3 days, 5 days and 7 days, queen mated during 7 days oviposited at 11.4 days, which is 3.0 - 3.6 days earlier than queen mated during 3 days and 5 days (Fig. 5). The preoviposition period was affected by the mating periods (Tukey's pairwise comparison test: F=3.36, DF=2, 46, $p=0.038$).

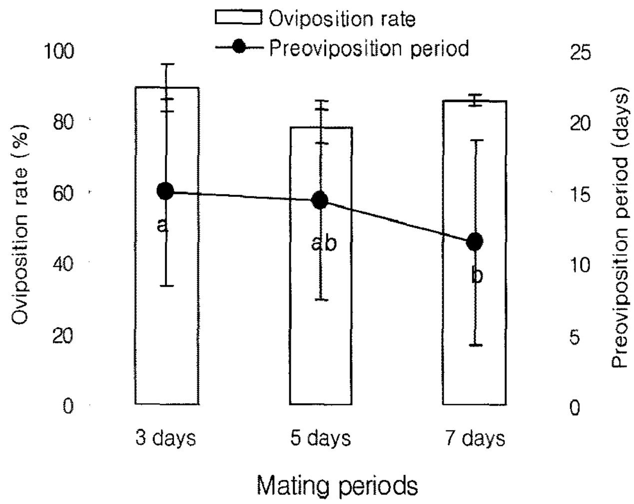

Fig. 5. Oviposition rate and preoviposition period of *B. terrestris* at different mating periods. There was significant difference in preoviposition period of *B. terrestris* at different mating periods at $p<0.05$ by Tukey's pairwise comparison test.

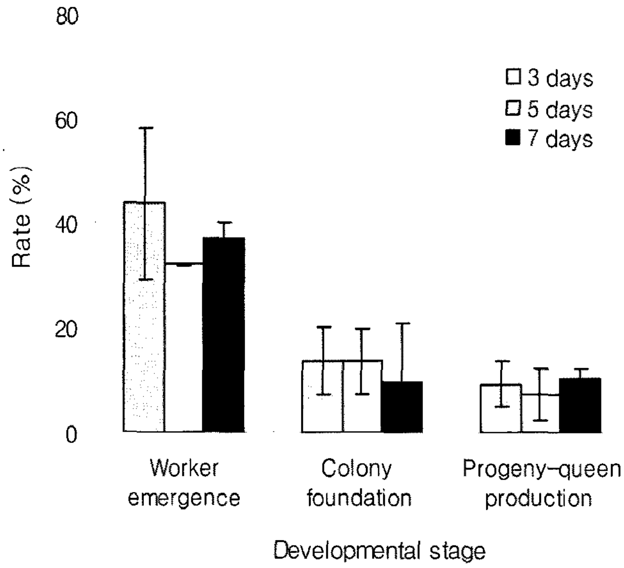

Fig. 6. Colony development of *B. terrestris* at different mating periods. There was no significant difference in colony development of *B. terrestris* at different mating periods at $p<0.05$ by Tukey's pairwise comparison test.

In the case of worker emergence rate, queen mated during 3 days was 6.7 - 11.6% higher than that of 5 days and 7 days although there was no statistical difference (F=0.53, DF=2, 3, $p=0.611$) (Fig. 6). The rate of colony foundation and progeny-queen production was also showed a similar tendency with worker emergence rate (Fig. 6).

Above results showed that 3 days was appropriate mating periods for colony development of *B. ignitus*. Generally, the European bumblebee, *B. terrestris* was mated during 7 days (Djegham *et al.*, 1994; Tasei *et al.*, 1998).

Colony development of *B. terrestris* at the number of queen per mating cage

We investigated appropriate the number of queen per mating cage for egg-laying characteristics and colony development of *B. terrestris*. The oviposition rate of queen mated at mating cage with 10 queens and 30 males, 20 queens and 60 males, and 30 queens and 90 males was 72.6 - 73.0% (Fig. 7). The preoviposition periods was 8.8-11.1 days and also no statistical difference (Tukey's pairwise comparison test: F=1.33, DF=2, 62, $p=0.273$) (Fig. 7). The rate of worker emergence, colony foundation and progeny-queen production of queen mated at mating cage with 10 queens and 30 males were 41.5%, 25.9% and 23.2%, respectively. These values correspond to 1.5 - 6.8 folds those at the other number of queen per mating cage. But there is no statistically differences (worker emergence

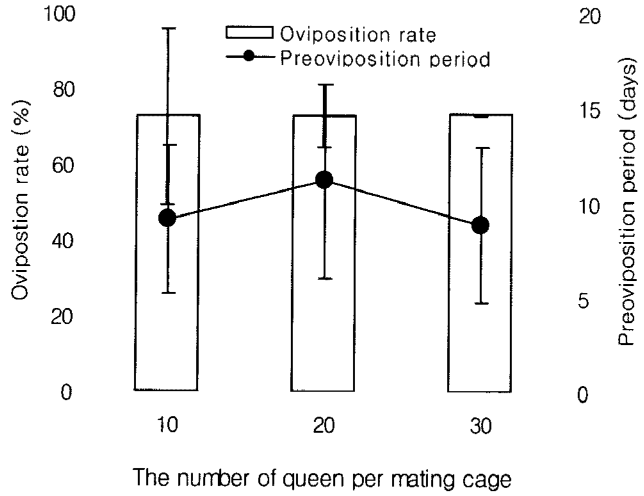

Fig. 7. Oviposition rate and preoviposition period of *B. terrestris* at the number of queen per mating cage. Mating cage size was 55 × 45 × 65 cm. There was no significant differences in oviposition rate and preoviposition period of *B. terrestris* at the number of queen per mating cage at $p < 0.05$ by Tukey's pairwise comparison test.

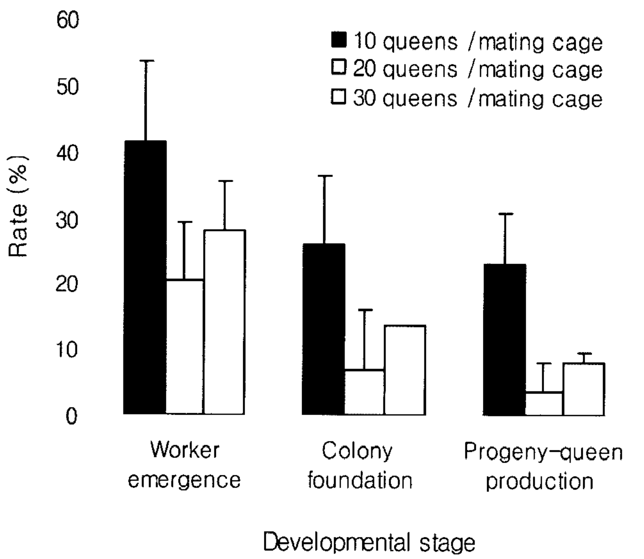

Fig. 8. Colony development of *B. terrestris* at the number of queen per mating cage. There was no significant difference in colony development of *B. terrestris* at the number of queen per mating cage at $p < 0.05$ by Tukey's pairwise comparison test.

rate: F = 0.30, DF = 2, 5, $p = 0.754$, colony foundation rate: F = 1.02, DF = 2, 5, $p = 0.426$, progeny-queen production rate: F = 1.67, DF = 2, 5, $p = 0.278$).

In above results, we supposed that the number of *B. terrestis* queen suitable per mating cage of 55 × 45 × 65 cm size was 10. In case of the bumblebee, *B. ignitus*, the number of queen suitable per mating cage of 55 × 45 × 65 cm size was 30 (Yoon *et al.* 2007). The reason which *B. terrestis* is needed bigger mating cage than *B. ignitus* is that *B. terrestis* is more active at mating. From those results, we think that the colony development of bumblebee was relative to the number of queen and male per density of mating cage. The nuptial flight of *B. terrestris* males consists of patrolling behavior during which secretions from the cephalic labial gland are deposited on marking spots (Svensson, 1980; Williams 1991). Young queens emit sex pheromones from mandibular glands (Honk *et al.*, 1978; Bergstrom, 1981). Djegham *et al.* (1994) found that among the male and female behavioral sequences analyzed, antennal inspection by both sexes and queen mobility were key factors in the mating success.

In view of the results so far archived, mating conditions favorable for colony development of *B. terrestris* were as follows. The care temperature of queen before mating favorable for *B. terrestris* queen was 19°C. The period need to mating *B. terrestris* queen was 3 days, and the number of queen suitable per mating cage of 55 × 45 × 65 cm was 10.

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