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Colony Developmental Characteristics of the Bumblebee Queen, *Bombus ignitus* by the First Oviposition Day

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It was investigated whether developmental characteristics of foundation queens of *Bombus ignitus* collected in the 4 localities in Korea would be affected by the first oviposition days of them. The first ovipostion day was classified as 1 – 4 days (immediate early), 5 – 6 days (early), 7 – 10 days (delayed early), 11 – 20 days (medium), 21 – 40 days (late), and above 41 days (very late). The queen that had the early first oviposition day, *i.e.*, laid eggs so early after starting to be raised indoors, showed much higher rate of colony foundation and progeny-queen production and much shorter period of colony foundation and worker emergence. Besides, the numbers of worker and progeny-queen emerged from the queen that had the early first oviposition day were higher than those of the queen that had the late first oviposition. In results, the queen that had the early first oviposition day could make colony stronger and could make colony formation period shorter, therefore, the first oviposition day of foundation queen was proved to be a criterion for the selection of super colonies when *B. ignitus* is raised indoors.

Key words: Bumblebee, *Bombus ignitus*, First oviposition day, Colony development

Introduction

The introduction of bumblebees for effective pollination of various greenhouse crops, especially for tomatoes, has recently become widespread and the demand is increasing every year. Thus there has been increasing interest in commercial use of the insects for pollination (Free, 1993). Recent advances in commercial rearing of the European bumblebee (*Bombus terrestris*) made it possible to package bumblebee for crop pollination (de Ruijter, 1997; Masahiro, 2000). Bumblebees are distributed world widely including alpine, cool temperate and even arctic environments of the northern continents (Williams, 1989). Until now 239 species are listed but still synoptic revision is undergoing (Williams, 1998).

We chose *Bombus ignitus* out of seven Korean native bumblebees tested, because the species showed the best results both in artificial multiplication and in pollinating ability (Yoon *et al.*, 1999). Now we are studying an artificial year-round mass rearing of *B. ignitus* selected as the most reliable native species in crop pollination (Yoon *et al.*, 2002; Yoon and Kim, 2003). Bumblebee colonies are short-lived and new colonies start each year. The young queens that have mated with males in late summer hibernate and emerge in spring. 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. After workers from the first batch emerge, the queen can spend more time on oviposition because the workers start to forage two- or three-days after emerging. In the late summer, many males and new queens are produced and only mated queens hibernate and emerge in spring (Heinrich, 1979). Sometimes we experienced that the early oviposited queens that lay egg at 2 – 3 days after rearing start from post-hibernating queens (no pollen gathering) were observed to make colony stronger than late oviposited queens that lay eggs after 2 – 3 months when foundation queens collected in field from April to May were reared in artificial conditions. Duchateau (1991) reported that failure of the foundress queen in first brood rearing seemed to influence further colony development. Therefore, this study was conducted to identify whether colony development of collected *Bombus ignitus* queens would be

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affected by the first oviposition days of them.

Materials and Methods

Collection of the post-hibernated queens

The post-hibernated queens of the Korean native bumblebee, *Bombus ignitus*, that had not started brood rearing (no pollen gathering) were collected in 4 localities including Jeong-Sun, Korea from late April to late May in 2000. The queens collected in the field were confined in round plastic containers (7.5 cm in diameter and 5.5 cm in depth; two or three queens per container), and supplied with 50% honey solution until they moved into the nest initiation boxes. The duration from collection up to the start of rearing was within one day.

Indoor rearing

The basic colony-rearing technique followed that used by Yoon *et al.* (2002). The collected queens were reared in three types of cardboard (1.5 mm thick) boxes each for nest initiation (10.5 × 14.5 × 6.5 cm: small box), colony foundation (21.0 × 21.0 × 15.0 cm: medium box), and colony maturation (24.0 × 27.0 × 18.0 cm: large box). Each box had a wire net window on its lid for ventilation. The sizes of these windows were 5.5 × 6.5 cm, 7.0 × 14.0 cm and 10.0 × 20.0 cm, respectively. Queens collected in the field were first confined individually in small boxes for colony initiation and remained there until oviposition. 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.

Fifty percent sugar solution and pollen dough were provided *ad libitum*. The pollen dough was made from 50% sugar solution and fresh pollen collected from an apiary (v : v = 1 : 1).

Distribution and developmental characteristics of the post-hibernated queens of *B. ignitus* by the first oviposition day

To investigate developmental characteristics of *B. ignitus* by the preoviposition period, we classified 169 foundation queens indoor-reared by the first oviposition with six groups (Fig. 1). As shown in Fig. 1, the first oviposition day was classified as six groups: 1 – 4 days (immediate early), 5 – 6 days (early), 7 – 10 days (delayed early), 11 – 20 days (medium), 21 – 40 days (late), and above 41 days (very late). The numbers of queens allotted to each experimental group were 44 in IE (immediate early), 53 in E (early), 23 in DE (delayed early), 20 in M (medium), 11 in L (late), and 18 in VL (very late), respectively. The developmental ability of each colony was estimated by rate of colony foundation and progeny-queen foundation, production of progeny, and period up to first adult emergence. Colony foundation here indicates that more than 50 workers emerged in a colony. Period up to first adult emergence designates the duration from the first oviposition to the first adult-emergence.

Statistical analysis was done with a one-way ANOVA test, Chi-square test and Tukeys pairwise comparison test (MINITAB Release 13 for Windows, 2000). The Chi-square test was used to compare the rates of colony development of *B. ignitus* reared by first oviposition day. Tukeys pairwise comparison test was used to examine the

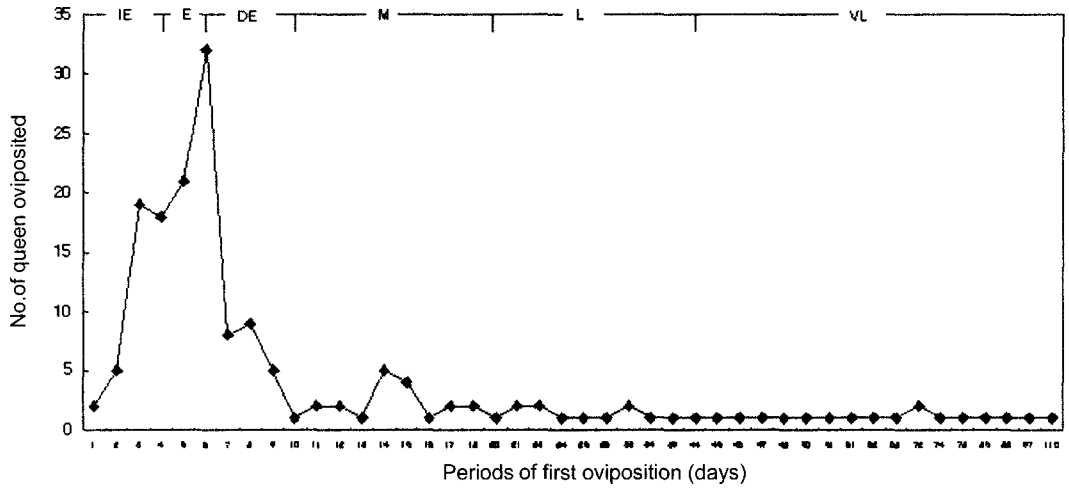

Fig. 1. Distribution of foundation queens of *B. ignitus* by the first oviposition day. Abbreviations: IE, immediate early (1 – 4 days); E, early (5 – 6 days); DE, delayed early (7 – 10 days); M, middle (11 – 20 days); L, late (21 – 40 days); VL, very late (over 41 days).

durations until colony foundation and first adult emergence, as well as the number of adults produced.

Results and Discussion

Distribution of foundation queens of *B. ignitus* by the first oviposition day

To elucidate relationship between the preoviposition period and developmental characteristics of *B. ignitus*, we assorted 169 foundation queens by the first oviposition (Fig. 1). Two out of 169 queens oviposited in one day after rearing, and the dispersion of data presents the largest peak in six days, which were oviposited 32 queens. The 71% of total oviposition laid egg within 10 days after rearing after rearing start from post-hibernating queens. Also we confirmed that one or two queens rarely oviposited when 100 days was passed. It is regarded that the late emerged progeny-queen in nature hibernated late so that diapause break resultantly was completed late. Ono (1999) mentioned that field collected queen had constructed egg-cup with wax extruded from between the plates of her abdomen and pollen mostly within one week and then, laid eggs. And also, queens of B. hypocrita stat nesting in May, suggesting that Type-1 colonies survive through July, while Type-2 and Type-3 colonies maintain activity in autumn. B. ignitus has Type-2 and Type-3 colonies that continue activity for 3.5 – 5 months (Asada and Ono, 2000).

Developmental characteristics of the post-hibernated queens of *B. ignitus* by the first oviposition day

We investigated relationship between days need to the first oviposition and developmental characteristics of *B. ignitus* to classify six groups. In case of rates of colony foundation, the foundation queens reared at IE group showed the best performance as 95.4% among other groups and decreased in the order of E, DE, M, L and VL group (Table 1). The rates of colony foundation of the groups oviposited within 10 days was over 90% and it was improved 8.2 – 8.6 fold than the value at VL group, oviposited above 41 days. The rates of colony foundation of *B. ignitus* were statistically affected by the first oviposition day (Chi-squared test: $x^2 = 76.19$, $p < 0.001$). In periods of colony foundation, durations up to colony foundation of queens reared at DE group, 49.1 ± 4.4 days, was the shortest among other groups. It was 11 – 19 days shorter than the L and VL group-rearing and prolonged in the order of IE, E, DM, VL and L group. The duration up to colony foundation was significantly affected by preoviposition period (Tukeys pairwise comparison test, F = 4.37, df = 3, $p = 0.001$) (Table 1). Rate of progeny-queen production was also compared by the first oviposition. As a result, the shorter the preoviposition period is, the higher the rate of progeny-queen production is. For example, the rates of progeny-queen production of the foundation queens reared at IE group showed the best performance as 79.5% among other groups and decreased in the order of E, DE, M, L and VL group (Table 1). Though the rates of progeny-queen production of the groups oviposited within 20 days was over 50%, those of L and VL group which oviposited after 20 days were under 30%. There was statistically significant relationship between rate of progeny-queen and the first oviposition day (Chi-squared test: $x^2 = 76.19$, $p < 0.001$) (Table 1). With above shown results, we confirmed the rates of colony foundation, progeny-queen production and periods of colony foundation of the colony that the preoviposition period is short, are higher and

Table 1. Colony development of foundation queens of *B. ignitus* by the first oviposition day

First oviposition day[a]	n[b]	Rate of colony foundation (%)[b]	n[b]	Periods of colony foundation (days)[b,c]	n[b]	Rate of progeny-queen production (%)[b]
IE	44	95.4	35	53.5 ± 5.4 a	44	79.5
E	53	94.3	43	53.7 ± 6.6 a	53	62.3
DE	23	91.3	16	49.1 ± 4.4 a	23	73.9
M	20	60.0	9	54.3 ± 9.9 a	20	50.0
L	11	45.5	2	68.3 ± 7.8 b	11	27.3
VL	18	11.1	2	60.0 ± 2.8 ab	18	22.2

[a]Duration up to first oviposition after rearing of foundation queen. For abbreviation, see legend to Fig. 1.
[b]n means the number of colony surveyed.
The figures stand for means ± SD. Means followed by different letters in the same column are significantly different at $p < 0.001$ by Tukey's pairwise comparison test.
[c]Statistical analysis: Rate of colony foundation; Chi-square test, $x^2 = 76.19$, $p < 0.001$.
Periods of colony foundation; Tukey's pairwise comparison test, F = 5.36, $p < 0.001$.
Rate of progeny-queen production; Chi-square test, $x^2 = 25.49$, $p < 0.001$.

Table 2. Duration up to adult emergence from foundation queens of *B. ignitus* by the first oviposition day

First oviposition day[a]	First adult emergence (days)					
	n[b]	Worker[c]	n[b]	Male[c]	n[b]	Queen[c]
IE	41	25.2 ± 3.0 a	40	70.6 ± 10.1	32	83.1 ± 9.0
E	50	24.5 ± 4.1 a	44	71.3 ± 8.1	31	84.4 ± 11.5
DE	20	22.0 ± 2.3 a	18	68.4 ± 7.0	15	80.6 ± 10.8
M	14	26.9 ± 7.6 ab	12	68.5 ± 10.8	10	91.9 ± 22.1
L	4	31.5 ± 6.1 ab	3	68.3 ± 9.1	3	82.7 ± 32.3
VL	5	31.6 ± 11.5 b	5	64.6 ± 11.2	3	82.3 ± 17.6

[a]For abbreviation, see legend to Fig. 1.
[b]n means the number of colony surveyed.
[c]The figures stand for means ± SD. Means followed by different letters in the same column are significantly different at $p < 0.001$ by Tukey's pairwise comparison test.

shorter than those of the colony that it is long. Duchateau (1991) mentioned that a quick start of the hibernated queen contributes to a later switch, and that such a quick start may also depend on the rearing method. And also, the quick start of colonization is the key point of colony management (Hannan *et al.*, 1997).

Table 2 shows the durations up to first worker, male and queen emergence of *B. ignitus* indoor-reared by the first oviposition day. The durations up to first worker emergence at groups (IE, E and DE) oviposited within 10 days are 22.0 – 25.2 days, which was 1.7 – 4.9 days shorter than E group-rearing and 6.6 – 9.6 days shorter than L and VL group-rearing. The period of first worker emergence was significantly different at $p < 0.01$ by Tukeys pairwise comparison test. In case of first male emergence, that of VL group was 64.6 days, and this estimate was about 4 – 8 days shorter than that of other groups but there was no statistical significance in durations up to first male emergence by preovposition period. For queens reared at M group, the periods up to first queen emergence was prolonged 7.5 – 11.3 days compared to those at other groups. But the duration up to first queen emergence did not differ by the first oviposition day.

The number of adults produced from foundation queens by the first oviposition day is shown in Table 3. In case of the number of worker produced from foundation queen, the number of worker produced at queens oviposited within 10 days (IE, E and DE) was 159 – 181 and that of VL group was 42, which was less over 100 than those of IE, E and DE. The number of worker produced from foundation queen was significantly increased as the first oviposition day is earlier (Tukeys pairwise comparison test, F = 5.71, df = 5, $p = 0.001$). The number of male produced from foundation queen also showed the same tendency as the number of worker produced from it, and the numbers were significantly different at $p < 0.05$. The queen reared at IE group produced their progeny queen well comparing with those at other groups although there was no statistical difference. Particularly, IE group produced 38.8 queens, which corresponded to 5.7 – 11.1 fold of those at L and

Table 3. Number of adults produced from foundation queens of *B. ignitus* and longevity of foundation queen by the first oviposition day

First oviposition day[a]	Number of adults produced						n[b]	Longevity of foundation queen (days)[c]
	n[b]	Worker[c]	n[b]	Male[c]	n[b]	Queen[c]		
IE	42	159.4 ± 56.8 a	41	562.8 ± 201.1 a	35	38.8 ± 50.2	33	90.0 ± 24.7
E	50	175.6 ± 68.5 a	50	537.0 ± 193.5 a	33	26.3 ± 23.8	50	87.8 ± 22.0
DE	21	181.1 ± 52.2 a	21	620.7 ± 201.6 a	17	35.6 ± 51.5	18	90.4 ± 23.6
M	11	141.4 ± 52.9 a	11	532.5 ± 303.9 a	10	35.1 ± 38.2	6	93.3 ± 28.7
L	5	106.4 ± 95.4 ab	5	285.3 ± 158.2 ab	2	3.5 ± 3.5	9	88.6 ± 35.7
VL	5	42.4 ± 41.8 b	5	105.2 ± 117.1 b	4	6.8 ± 10.2	8	105.2 ± 35.2

[a]For abbrevation, see legend to Table 1.
[b]n means the number of colony surveyed.
[c]The figures stand for means ± SD. Means followed by different letters in the same column are significantly different at $p < 0.001$ for number of worker and at $p < 0.05$ for number of male by Tukey's pairwise comparison test.

VL group. Longevity of foundation queens was not significantly affected by the preovipostion period either.

In view of the results so far archived, the queen that had the early first oviposition day showed much higher rate of colony foundation and progeny-queen production and much shorter at periods of colony foundation and worker emergence. And also, the numbers of worker and progeny-queen emerged from the queen that had the early first oviposition day were a bit higher than those of the queen that had the late first oviposition. The queens oviposited after 40 days in indoor-rearing of artificial hibernated *B. terrestris* were regarded as non-diapause (Beekman *et al.*, 1996), and *B. ignitus* queen treated with CO_2 almost oviposited within 20 days (Yoon and Kim, 2002). And also, in this study, colony development of queens laid eggs within 20 days were better than those of queens oviposited after 20 days. With the fact like this, discard queens oviposited after 20 days seems to be economic in year-round mass rearing of bumblebees.

In conclusion, the present results indicate that the queen that had the early first oviposition day could make colony stronger and could make colony formation period shorter, therefore, the first oviposition day of foundation queen was proved to be a criterion for selecting superior colonies when raising *B. ignitus* indoors.

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