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The Flight of the Bumblebee Queen, *Bombus terrestris,* After Diapause Termination Affects to Oviposition and Colony Development

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It was investigated whether or not flight has any effects on oviposition and colony development of the artificially hibernated *Bombus terrestris* queen and CO_2-treated queen. Flight periods were defined as 0 days (control), 1 day, 3 days and 6 days. The weights of queens after flight were 1.5 – 8.9% lower than those before flight depending on the flight periods. The oviposition and colony development of artificially hibernated and CO_2-treated *B. terrestris* queen were affected by the flight. Among flight periods tested, in particular, the queens start to flight for 3 days showed better flight effect than those other flight periods in the colony development, rate of colony foundation, rate of progeny-queen production, the number of worker and queen produced. But, the longer the flight periods is, the worse the oviposition and colony development of the queens hibernated artificially and CO_2-treated are (*i.e.*, the 6 days-flight queen).

Key words: Bumblebee, *Bombus terrestirs*, Flight, Oviposition, Colony development

Introduction

Bumblebees are an important pollinator of various greenhouse crops, particularly effective in pollinating for the night shade family, which includes tomato and eggplant. (Michener, 1962; Buchmann and Hurley, 1978; Free, 1993). Bumblebees provide farmers the opportunity to decrease the labor costs of pollination and promise a good yield both in quantity and in quality (Iwasaki, 1995).

Bumblebee colonies undergo 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 overwinterd 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).

Flying insects produce extreme amounts of heat as a by-product during the contractions of their thoracic flight muscles (Heinrich, 1989). Before flight, metabolic heat may serve to warm up the thoracic muscles until the minimum lift-off temperature is reached (Heinrich, 1974b; Stone and Willmer, 1989; Esch and Goller, 1991). Social bees and wasps are also able to use the heat produced in their flight muscles for brood incubation and for active regulation of nest temperatures (Heinrich, 1974a; Seeley and Heinrich, 1981; Schltze-Motel, 1991). After diapause was terminated, *Bombus terrestris* queens were kept in the mating cage for one week after which they were moved to small boxes (one queen per box) and transferred to the climate room (Beekmen *et al.*, 1996).

We chose *Bombus ignitus* out of seven Korean native bumblebees, because the species showed the best result 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*, because the species is the most reliable native bumblebee in crop pollination (Yoon and Kim, 2003; Yoon *et al.*, 2004). In this study, we conducted to identify whether or not oviposition and colony development of artificial hibernated queen and CO_2-treated queens would be affected by flight.

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Materials and Methods

Origin of experimental insects

Experimental insects were artificially hibernated- and CO_2-treated $4^{th} - 7^{th}$ generation queens obtained from *Bombus terrestris* colonies year-round reared in a controlled climates room (28°C, 65% R. H. and continuous darkness). For artificial hibernation, queens were hibernated for 4 months at 2.5 to preserve them in a bottle filled with perlite and keep it around 80% R. H. And also, to break diapause with CO_2 treatment, queens at 13 days after eclosion were exposed to 99% CO_2 for 30 min daily during two consecutive days (Yoon *et al.*, 2003).

Indoor rearing

The basic colony-rearing technique was followed as described in Yoon *et al.* (2002). The 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 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.

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).

For mating, newly emerged queens and males were collected from the colonies and maintained in a box until mating and one six-day-old virgin queens were placed in flight cages (55.0 × 65.0 × 40.0 cm) in mating room (uncontrolled photoperiod and 24 – 25°C) with one ten-day-old virgin males from other colonies during one week.

Flight effect

To examine effect of flight on oviposition and colony development of *B. terrestrs*, the following environmental conditions were provided. Flight periods were defined as 0 days (control), 1 day, 3 days and 6 days. Flight was performed at flight cages (55.0 × 65.0 × 40.0 cm) in mating room of uncontrolled photoperiod and 24 – 25°C. During flight periods, fifty percent sugar solution and pollen dough were provided *ad libitum* into flight cage. Queens finished flight were weighed and confined individually in small boxes for colony initiation. The numbers of queens allotted to flight experiment for artificial hibernated- and CO_2-treated *B. terrestris* were 40.

The developmental ability of each colony was estimated by rate of oviposition, 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. The queens that did not oviposit in 60 days were excluded from the number of oviposited colonies.

Statistical analysis was done with Chi-square test and Tukeys pairwise comparison test (MINITAB Release 13 for Windows, 2000). The Chi-square test was used to compare colony development of *B. terrestris* by flight periods. Tukeys pairwise comparison test was used to examine the durations until colony foundation and first adult emergence, as well as the number of adults produced.

Results and Discussion

Changes in body weight before and after flight of bumblebee queen

In order to know the change in body weight before and after flight of artificially hibernated *B. terrestris*, we mea-

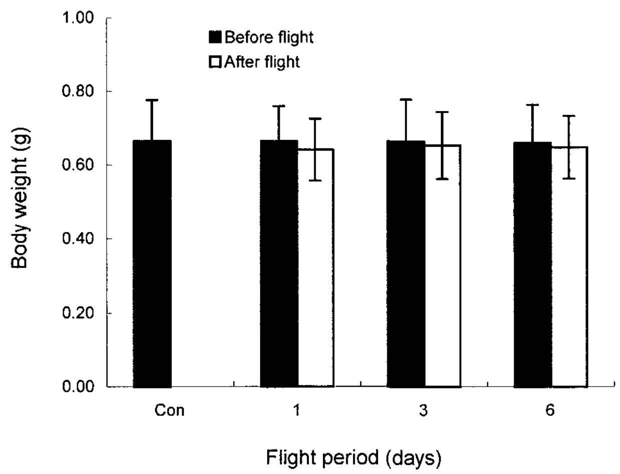

Fig. 1. Changes in body weight before and after flight of artificially hibernated *Bombus terrestris* queen. Queens were hibernated for 4 months at 2.5. There were no significant differences in changes of body weight at flight periods of artificial hibernated bumblebee queen at $p < 0.05$ by Tukey's pairwise comparison test.

sured weight of queen (Fig. 1). The weights of queens before flight of 0 day (control), 1 day, 3 days and 6 days were 0.664 g, 0.663 g, 0.663 g and 0.659 g, respectively (Tukey's pairwise comparison test: F = 0.02, df = 3, 216, $p = 0.491$). But queen weights after flight during 1 day, 3 days and 6 days were 0.641 g, 0.653 g and 0.648 g, respectively, which was 1.5 – 3.3% lower than weight of queen before flight although sugar solution and pollen dough were provided ad libitum during flight periods. However, statistical analysis did not support the difference of body weight by flight periods on the basis of Tukey's pairwise comparison test (F = 0.24, df = 2, 133, $p = 0.788$). Queens drain the fat body during hibernation and some queens with a slightly less developed fat body may be expected to be so weak after the long period of hibernation that they are unable to continue normal development during the spring (Hoem, 1972).

Changes in body weight before and after flight of CO_2-treated queen was also investigated (Fig. 2). As like changes in body weight before and after flight of artificially hibernated queen, similarly weights of CO_2-treared queens after flight during 1 day, 3 days and 6 days were 0.804 g, 0.762 g and 0.746 g, respectively, which was 2.5 – 8.9% lower than weight of queen before flight. In case of weights of CO_2-treated queen after flight, the longer the flight period is, the lower the weight of queen is, but there were no significant difference in changes of body weight at flight periods at $p < 0.05$ by Tukey's pairwise comparison test (F = 1.20, df = 2, 51, $p = 0.310$). And also, weight of CO_2-treated queens, 0.82 – 0.83 g, was heavier than that of after artificial hibernated queens, which was 0.64 – 0.69 g (Figs. 1 and 2). Alford (1969a) on average found 57% water in bumblebee queens following hibernation and a reduction of about 50% in both live weight and dried weight. Reserves of fat and glycogen but not of protein-are stored in the fat body of the queens during the period prior to hibernation and there reserves serve as a source of energy during hibernation. 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 (Alford, 1969b). A well-developed fat body in a queen bumblebee will thus be of importance to safe hibernation.

Flight effect on oviposition and colony development of artificially hibernated bumblebee queen

We investigated relationship between flight and developmental characteristics, rates of oviposition, colony foundation and progeny-queen production, of artificially hibernated bumblebee queen (Fig. 3). In the flight periods of 0 days, 1 days, 3 days and 6 days under 24 – 25 and 65% R. H., the oviposition rate of the 1 day-flight queen showed the best performance as 70.0% among other regimes and decreased in the order of the 3 days-, 0 day- and 6 days-flight queen, 65.0%, 60.0% and 50.0%, respectively, but there was no significant differences in oviposition rate of artificial hibernated bumblebee queen in different flight periods at $p < 0.05$ by Chi-square test ($x^2 = 5.547$, df = 3, $p = 0.136$) (Fig. 3). In case of the colony foundation rate, the 3 days-flight queen showed the best performance as 22.5% among other regimes, which was

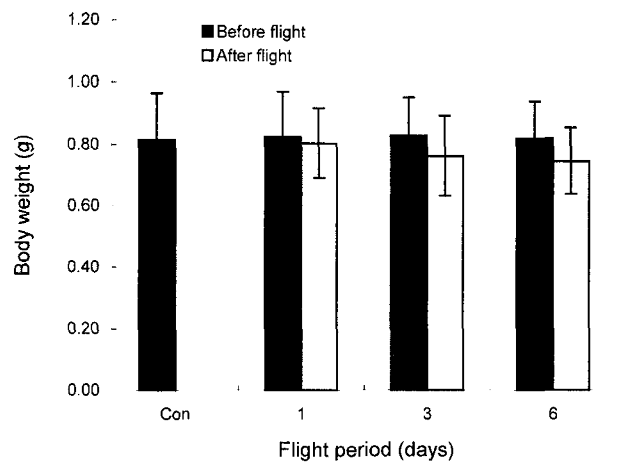

Fig. 2. Changes in body weight before and after flight of CO_2-treated *Bombus terrestris* queen. Queens at 13 days after eclosion were exposed to 99% CO_2 for 30 min daily during two consecutive days. There were no significant differences in changes of body weight at flight periods of CO_2-treated bumblebee queen at $p < 0.05$ by Tukey's pairwise comparison test.

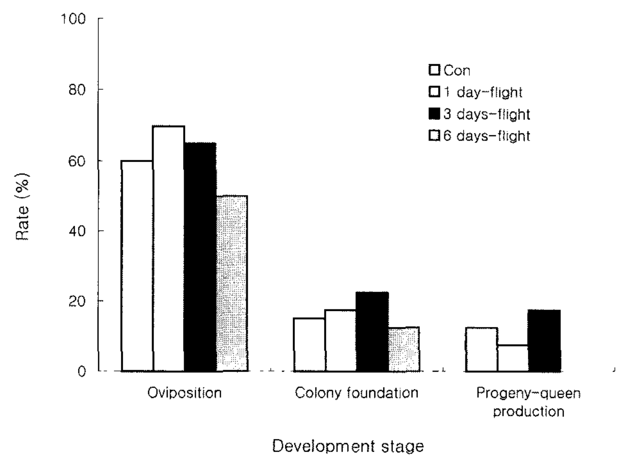

Fig. 3. Comparison of colony development of artificial hibernated *B. terrestris* queen at different flight periods. For the statistical analysis, a Chi-square test was used for each developmental stage: $x^2 = 5.547$, $p > 0.05$ (N.S.) for oviposition; $x^2 = 0.669$, $p > 0.05$ (N.S.) for colony foundation; and $x^2 = 7.871$, $p > 0.05$ for progeny-queen production. Forty queens were allotted for each experimental regime.

1.3 – 1.8 fold higher than those of other flight periods, but colony foundation rate of artificially hibernated queen was not affected by the flight periods ($x^2 = 0.669$, df = 3, $p = 1.559$) (Fig. 3). Rate of progeny-queen production was also compared depending on the flight periods, As shown in Fig. 3, the rate of progeny-queen production of the 3 days- flight queen was 17.5% and this value was also 1.4 17.5 fold higher than those at the 0 day, 1 day and 6 days- flight and there was statistically significant differences in progeny-queen production rate of artificial hibernated bumblebee queen in different flight periods at $p < 0.05$ by Chi-square test ($x^2 = 7.871$, df = 3, $p = 0.049$). With above results, we supposed that colony development of artificial hibernated bumblebee queen was affected by the flight. Specifically, the 3 days-flight queen showed the best results in colony development, rate of colony foundation, rate of progeny-queen production.

Table 1 shows the duration up to preoviposition, colony foundation, and adult emergence from artificially hibernated queen at different flight periods. Duration up to preoviposition at the 0 day and 1 day-flight queen was 8.9 days and 8.4 days, respectively and these were 1.4 – 2.5 days shorter than those of the 3 days- and 6 days- flight but no significant difference was detected between flight periods regimes at $p < 0.05$ by Tukey's pairwise comparison test (F = 0.70, df = 3, 98, $p = 0.555$). In case of duration up to colony foundation, that of the 3 days- flight queen was the shortest (50.8 ± 6.2 days) among other flight periods. It was 3.0 3.8 days shorter than those of the 1 day- and 6 days- flight and prolonged in order of the 0 day, 6 days and 1 day-flight. However, the preoviposition period was not affected by the flight periods (F = 0.52, df = 3, 22, $p = 0.672$). The period up to first worker emergence did not differ among the 0 day, 1 day and 3 days- flight queen except the 6 days-flight queen (29.8 days); it was about 27 days. The period of first worker emergence was not statistical difference (F = 0.92, df = 3, 52, $p = 0.439$). The period up to first male emergence at the 6 days-flight queen was also 2.9 – 5.8 days longer than those at other flight periods, but there was no statistical difference between them (F = 0.22, df = 3, 33, $p = 0.882$). Besides, the period of first emergence queens was not either affected by the flight periods although the period of queens emergence at the 0 day- flight queen was 4.9 8.1 days shorter than those of the 1 day- and 6 days-flight (F = 0.79, df = 2, 12, $p = 0.476$).

The number of workers and males produced at the 0 day-, 1 day-, 3 days- and 6 days-flight was surveyed with

Table 1. Duration up to preoviposition, colony foundation, and adult emergence from queens of artificial hibernated *B. terrestris* at different flight periods

Flight periods (day)	n[a]	Preoviposition (days)[b]	n[a]	Colony foundation (days)[b]	First adult emergence (days)[b]					
					n[a]	Worker	n[a]	Male	n[a]	Queen
0	24	8.9 ± 4.1	6	51.3 ± 8.6	15	27.2 ± 4.4	8	53.4 ± 16.0	5	68.2 ± 12.8
1	28	8.4 ± 3.8	6	54.5 ± 5.4	16	26.9 ± 3.2	10	52.1 ± 17.4	3	76.3 ± 5.0
3	30	10.3 ± 5.4	7	50.8 ± 6.2	15	26.8 ± 3.9	11	55.0 ± 19.6	7	73.1 ± 7.5
6	23	10.9 ± 10.4	5	53.8 ± 5.4	10	29.7 ± 7.7	8	57.9 ± 21.3	0	-

[a]n means the number of colony surveyed.
[b]The figures stand for means ± SD. There were no significant in duration up to preoviposition, colony foundation, and adult emergence from queens of artificial hibenated *B. terrestris* in different flight periods factor at $p < 0.05$ by Tukey's pairwise comparison test.

Table 2. Number of adults produced from queens of artificial hibernated *B. terrestris* at different flight periods

Flight periods (day)	Number of adults produced						n[a]	Longevity of foundation queen (days)[b]
	n[a]	Worker[b]	n[a]	Male[b]	n[a]	Queen[b]		
0	6	104.0 ± 23.3	6	175.7 ± 56.6	5	19.2 ± 11.0	12	74.9 ± 15.5
1	6	84.2 ± 8.2	4	152.5 ± 28.4	3	12.3 ± 9.9	16	67.2 ± 16.5
3	8	104.4 ± 26.6	8	159.7 ± 47.6	7	39.1 ± 28.4	13	79.5 ± 20.9
6	5	87.6 ± 23.3	3	131.6 ± 18.2	0	-	10	77.6 ± 19.2

[a]n means the number of colony surveyed.
[b]The figures stand for means ± SD. There were no significant in number of adults produced from queens of artificial hibenated *B. terrestris* in different flight periods factor at $p < 0.05$ by Tukey's pairwise comparison test.

more than 50 workers emerged colonies (Table 2). The numbers of worker at the 0 day- and 3 days-flight was about 104, which was 16 – 20 numbers more than those at the 1 day- and 6 days-flight but there was no statistical difference at $p < 0.05$ by Tukey's pairwise comparison test ($F = 1.42$, df = 3, 21, $p = 0.265$). In case of the number of males produced at different flight periods, the 0 day- flight queen was 175.7 ± 56.6 numbers and these values were 16 – 44 numbers more than those among other flight periods, but there was no statistical difference between them at $p < 0.05$ ($F = 1.00$, df = 3, 19, $p = 0.416$). In case of the number of queens produced, which is an important point in year-round rearing of bumblebee, the 3 days - flight queen produced 39.1 ± 28.4 numbers, which corresponded to 2.0 – 3.2 fold of those of the 0 day-flight and 6 days-flight queen not produced progeny-queen at all. However, the number of queen produced from artificially hibernation queen was not detected statistical difference by the flight period ($F = 2.43$, df = 2, 13, $p = 0.127$). Longevity of foundation queens of the 3 day-flight was 79.5 ± 20.9 days, which is 1.9 – 12.3 days longer than those of the other regimes though there was no significant difference by the flight periods ($F = 1.30$, df = 3, 47, $p = 0.285$).

In view of the results so far achieved, the flight in the artificial hibernation queen, specifically the 3 days-flight queen, exerted a greater stimulation on colony development, preoviposition period, rate of colony foundation, rate of progeny-queen production and the number of worker and queen produced. But the longer the flight periods is, the worse the colony development of the artificially hibernation queen is (*i. e.*, the 6 days-flight queen). Beekmen *et al.* (1996) reported that after diapause was terminated, *B. terrestris* queens were kept in the mating cage for one week after which they were moved to small boxes.

Flight effect on oviposition and colony development of CO_2-treated bumblebee queen

The relationship between flight and developmental characteristics of CO_2-treated *B. terresris* queen was investigated (Fig. 4). In the flight periods of 0 days, 3 days and 6 days, the oviposition rate of the 0 dayflight queen showed the best performance as 86.7%, which was 11.2 – 22.3% higher than those of the 3 days- and 6 days-flight and decreased in the order of the 3 days- and 6 days-flight, 75.5% and 64.4%, respectively, and there was statistically significant differences in oviposition rate of CO_2-treated bumblebee queen in different flight periods at $p < 0.05$ by Chi-square test ($x^2 = 6.016$, df = 2, $p = 0.049$) (Fig. 4). In case of the colony foundation rate, the 3 days-flight queen showed the best performance as 22.5%, which was 1.8 – 2.7 fold higher than those of the other flight periods, but colony foundation rate of CO_2-treated queen was not affected by the flight periods ($x^2 = 4.398$, df = 2, $p = 0.111$) (Fig. 4). Rate of progeny-queen production was also compared depending on the flight periods, As shown in Fig. 4, The rate of progeny-queen production of the 3 days-flight queen was 24.4% and this value was also 2.2 – 3.6 fold higher than those at the 0 days- and 6 days-flight and there was statistically significant differences in progeny-queen production rate of CO_2-treated queen in different flight periods at $p < 0.05$ by Chi-square test ($x^2 = 6.370$, df = 2, $p = 0.041$). With above results, we supposed that colony development of CO_2-treated bumblebee queen was affected by the flight and particularly, the 3 days-flight queen showed better effect than other flight periods in colony development, rate of colony foundation, rate of progeny-queen production. The result was similar to that of the artificially hibernation queen (Fig. 3).

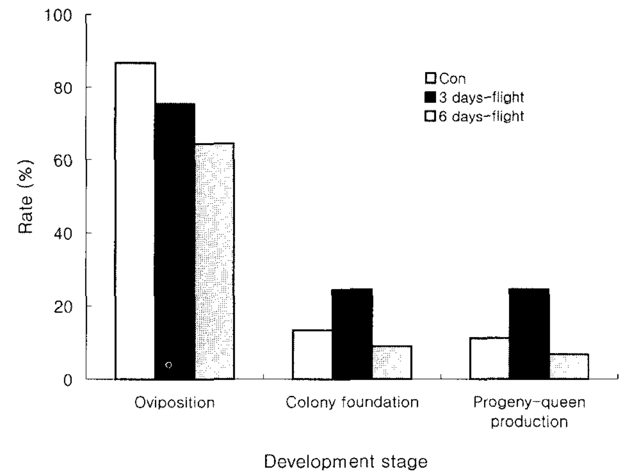

Fig. 4. Comparison of colony development of CO_2-treated *B. terrestris* queen at different flight periods. For the statistical analysis, a Chi-square test was used for each developmental stage: $x^2 = 6.016$, $p < 0.05$ for oviposition; $x^2 = 4.398$, $p > 0.05$ (N.S.) for colony foundation; and $x^2 = 6.370$, $p > 0.05$ for progeny-queen production. Forty queens were allotted for each experimental regime.

Table 3 shows the duration up to preoviposition, colony foundation, and adult emergence from CO_2-treated queen at different flight periods. Duration up to preoviposition at the 6 days-flight was 6.6 days and these were 4.1 – 5.0 days shorter than those of the 0 day- and 3 days-flight and statistically significant difference was detected between flight periods regimes at $p < 0.05$ by Tukeys pairwise comparison test ($F = 5.82$, df = 3, 98, $p = 0.044$). In case of duration up to colony foundation, the preoviposition period was not affected by the flight periods ($F = 0.01$, df = 2, 13, $p = 0.991$). The period of first worker emergence was also not statistical difference ($F = 0.47$, df = 2, 26, $p = 0.632$). The period up to first male emergence at the 3 days-flight queens was 3.0 – 12.1 days longer than those

Table 3. Duration up to preoviposition, colony foundation, and adult emergence from queens of CO_2-treated *B. terrestris* at different flight periods

Flight periods (day)	n[a]	Preoviposition (days)[b]	n[a]	Colony Foundation (days)[b]	First adult emergence (days)[b]					
					n[a]	Worker	n[a]	Male	n[a]	Queen
0	39	11.6 ± 7.1 a	5	58.2 ± 12.3	9	31.6 ± 6.0	16	50.8 ± 13.6	6	69.5 ± 17.1
3	32	10.7 ± 5.8 a	7	57.4 ± 9.5	14	29.4 ± 4.8	14	62.9 ± 18.0	10	79.7 ± 13.0
6	27	6.6 ± 4.8 b	4	57.8 ± 6.6	6	30.3 ± 5.4	7	59.9 ± 20.4	3	67.6 ± 5.7

[a]n means the number of colony surveyed.
[b]The figures stand for means ± SD. Means followed by different letters in the same column are significantly different at $p < 0.05$ by Tukey's pairwise comparison test.

Table 4. Number of adults produced from foundation queens of CO_2-treated *B. terrestris* at different flight periods

Flight periods (day)	Number of adults produced						n[a]	Longevity of foundation queen (days)[b]
	n[a]	Worker[b]	n[a]	Male[b]	n[a]	Queen[b]		
0	5	92.4 ± 28.3	5	187.8 ± 77.6	6	51.8 ± 47.5	2	86.0 ± 26.6
3	9	89.0 ± 9.9	9	217.0 ± 90.7	9	51.8 ± 33.9	54	101.7 ± 27.5
6	4	78.5 ± 8.1	4	141.3 ± 28.1	3	34.7 ± 35.7	67	98.7 ± 18.8

[a]n means the number of colony surveyed.
[b]The figures stand for means ± SD. There were no significant in number of adults produced from queens of CO_2-treated *B. terrestris* in different flight periods factor at $p < 0.05$ by Tukey's pairwise comparison test.

at other flight periods, but there was no statistical difference between them ($F = 2.08$, $df = 2, 34$, $p = 0.141$). Besides, the period of first emergence queens was not either affected by the flight periods although the period of queens emergence at the 3 days-flight queen was 12 days longer than those of the 0 day- and 6 days-flight ($F = 1.64$, $df = 2, 16$, $p = 0.225$).

The number of workers and males produced at the 0 day-, 3 days- and 6 days-flight queens was surveyed with more than 50 workers emerged colonies (Table 4). The numbers of worker, which is an important point in evaluating the capability of pollination, on at the 0 day-flight was about 92.4 ± 28.3, which was 3.4 – 13.9 numbers more than those at the 3 days- and 6 days-flight but there was no statistical difference at $p < 0.05$ by Tukey's pairwise comparison test ($F = 0.83$, $df = 2, 15$, $p = 0.454$). In case of the number of males produced at different flight periods, the 3 days-flight queen was 217.0 ± 90.7 numbers and these values were 29 – 76 numbers more than those at other flight periods, but there was no statistical difference between them at $p < 0.05$ ($F = 1.30$, $df = 2, 15$, $p = 0.301$). In case of the number of queens produced, which is an important point in year-round rearing of bumblebee, the 0 day- and 6 days-flight queen produced 51.8 numbers, which corresponded to 1.5 fold of that of the 6 days-flight queen. However, the number of queen produced from CO_2-treated queen was not detected statistical difference by the flight period ($F = 1.88$, $df = 2, 43$, $p = 0.164$). Longevity of foundation queens of the 3 days-flight was 101.7 ± 27.5 days, which is 3.0 – 15.7 days longer than those of the other regimes though there was no significant difference by the flight periods ($F = 1.88$, $df = 2, 43$, $p = 0.164$).

In view of above results, the flight in CO_2-treated queen he artificial hibernation queen, specifically the 3 days-flight queen, have better positive effect on colony development, rate of colony foundation, rate of progeny-queen production and the number of worker and queen produced. As like in the artificially hibernation queen, colony development in the 6 days-flight of CO_2-treated queen was worse than that of non-flight queen.

In conclusion, the present results indicate that queen weights after flight were lower than weight of queen before flight although statistical analysis did not support the difference at flight periods. The oviposition and colony development of artificially hibernated queen and CO_2-treated *B. terrestris* queen was affected by the flight. Particularly, the 3 days-flight queen showed the best results in colony development, rate of colony foundation, rate of progeny-queen production, the number of worker and queen produced. But the longer the flight periods is, the worse the colony development of the artificially hibernated and CO_2-treated queens is (*i.e.*, the 6 days-flight queen).

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