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TIME AND AGE SPECIFIC MATING SUCCESS OF BUMBLEBEE (*Bombus terrestris* L.) REARED AT DIFFERENT PHOTOPERIODIC REGIMES

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Abstract

In this study, mating success of bumblebee, *Bombus terrestris* was recorded by five age groups of queens viz. 3, 5, 7, 9, and 11 days old, and the males were two days older than each age group of queens. Newly emerged males and queens were collected from different colonies and they were reared under four photoperiods e.g., L0:D24, L8:D16, L16:D8, and L24:D0 for observing the mating activities. Three days old queens with 5 days old males showed the lowest mating success at all photoperiodic regimes. Most of the couples mated within first 15 minutes of pairing and mating success increased with increasing duration of mating exposure. At the L0:D24 and L8:D16 photoperiodic regimes 9 days old queens with 11 days old males had the highest mating success (65.4 ± 3.3 and 85.0 ± 2.3%, respectively) whereas at L16:D8 and L24:D0 photoperiodic conditions, the highest mating success occurred when 7 days old queens paired with 9 days old males (92.1 ± 1.7 and 76.3 ± 3.2%, respectively). These results indicate that rearing of bees at different light regimes, age of bees and duration of mating exposure had significant effect on mating success of *B. terrestris.*

Keywords: Age, *Bombus terrestris,* mating, photoperiod, time.

Introduction

Bumblebees, *Bombus terrestris* L. (Hymenoptera: Apidae) are primitively eusocial bees which have been shipped throughout the world in vast numbers for pollinaton of glass house crops (Goka *et al.,* 2001; Velthuis, 2002). Inseminated queens emerge from hibernation at the end of spring and the colony is founded by a single queen. At the end of summer, colonies produce males and queens that mate and the young queens leave the nest, and fly for several weeks in day light before they hibernate into the ground (Tasei and Aupinel, 1994). Now a days, mass rearing of bumblebees are very common, especially *B. terrestris* is extensively used for pollination. For commercial rearing, it is necessary to arrange mating of newly emerged queens and males. Readiness of mating depends on the sexual maturity of male and perfect overian development of queen. Halliday (1983) reported that the successful mating is the result of numerous interacting factors including male and female choice, male courtship behaviour, female responses to courtship, female

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reproductive or motivational status and mating competition. Bergstrom (1981) reported that volatile secretions from the cephalic labial glands of bumblebee males and the mandibular glands of queens played a significant role in producing sex pheromone which had great role for mating.

Philogene and McNeil (1984) stated that sperm migration and mating of insects was influenced by artificial light. Mating activity of the fruit fly, *Drosophila mercatorum* shows the daily rhythms of the mating activity under 12:12 light-dark (LD) cycles and several *Drosophila* species show the daily rhythms of male courtship under different LD cycles (Sakai and Ishida, 2001). Circadian rhythms also influence behaviour by transmitting intracellular signals to organize cellular metabolism, and intercellular signals to organize multicellular physiology and behaviour (Jackson *et al.,* 1998). Duchateau (1985) reported results on the impact of temperature, light and time of day for bumblebee mating. Photoperiods are positively correlateld with temperatures which change the timing of the life-history events of insects (Ishihara, 2000; Zhou, 2001). Therefore, this study was designed in confined conditions with altering photoperiods e.g., L0:D24, L8:D16, L16:D8, and L24:D0 to quantify whether light regimes have any stimulating effect on the different ages of bees for their mating success within different time frame.

Materials and Method

Artificially hibernated bumblebee queens were collected from the mass rearing system of the Department of Agricultural Biology, Kyungpook National University, Daegu, Korea. The study was conducted in the Laboratory of this Department during March 2004 to December 2006. *B. terrestris* colonies were developed at 28°C and 60% RH providing *ad. libitum* pollen of *Actinidia arguta* (Plench) and sugar solutions (1.5:1, w/v) as a diet. After emergence of new sexuals, queens were reared in small transparent plastic boxes measuring 16 x x 7 cm. Ten queens were kept in each box and provided with fresh pollen in a small petridish and sugar solutions (1.5:1, w/v) in a perforated tube. Males were reared in flight cages (40 x 30 x 30 cm) which had illumination facility. Fifty males were put in each cage which was supplied with fresh pollen and sugar solutions (1.5:1, w/v). The boxes of queens and cages of males were held in four separate rooms maintained at 25°C, 50% RH and photoperiodic regimes of L0:D24, L8:D16, L16:D8, and L24:D0 conditions until mating time. Photoperiodic treatments were scheduled with fluorescent white (L36/18-860, 300 Hz, λ = 460 nm) light at an intensity of 700 lux on the floor of the male cages and queen boxes. The intensity of light was measured with a digital lux meter and the photophase was regulated with timer. The mating success results were observed in 40 x 40 x 60 cm plastic cages. Sugar solutions and fresh pollen were supplied inside the cages. Mating was recorded in a room maintained at 25°C and 50% RH. During mating, the cages were illuminated with 200W mercury bulbs (GGY200, 60 Hz, λ = 580 nm) which were set 0.5 m above the mating cages. The intensity of light on the floor of the mating cages was 2300 lux. In each photoperiodic regime, five different age groups of queens were observed, having 3, 5, 7, 9, and 11 days old. The males were 2 days older than the queens in their respective age groups. Twenty queens and 40 males were released in each replication. Each age group reared in the same photoperiodic regime was replicated 12 times. The occurrence of copulation was recorded at 15, 30, and 45 minutes after pairing. Percent mating data were analyzed by analysis of variance (ANOVA) after arc-sine transformation. The mean values were then separated by Duncan's multiple range test (DMRT) and the standard errors (SE) have been shown on the figures. All the analyses were performed by SPSS Inc (PASW Statistics 17).

Results and Discussion

Fig. 1 shows the mating success of *B. terrestris* at 15 minutes exposure and entrained by 3, 5, 7, 9, and 11 days old queens reared at L0:D24, L8:D16, L16:D8, and L24:D0 photoperiodic conditions. Mating success data (Fig. 1a) of the bees reared at L0:D24 condition shows significant differences ($F_{4.59}$, 26.7, $p < 0.001$). Among the age groups, 3 days old queens showed the lowest mating success (29.2 ± 3.0%), whereas the highest mating success (60.4 ± 2.6%) was attained by the 9 days old queens. Fig. lb shows that the mating success by the bees reared at L8:D16 condition revealed significant variations ($F_{4.59}$, = 57.9, $p < 0.001$).

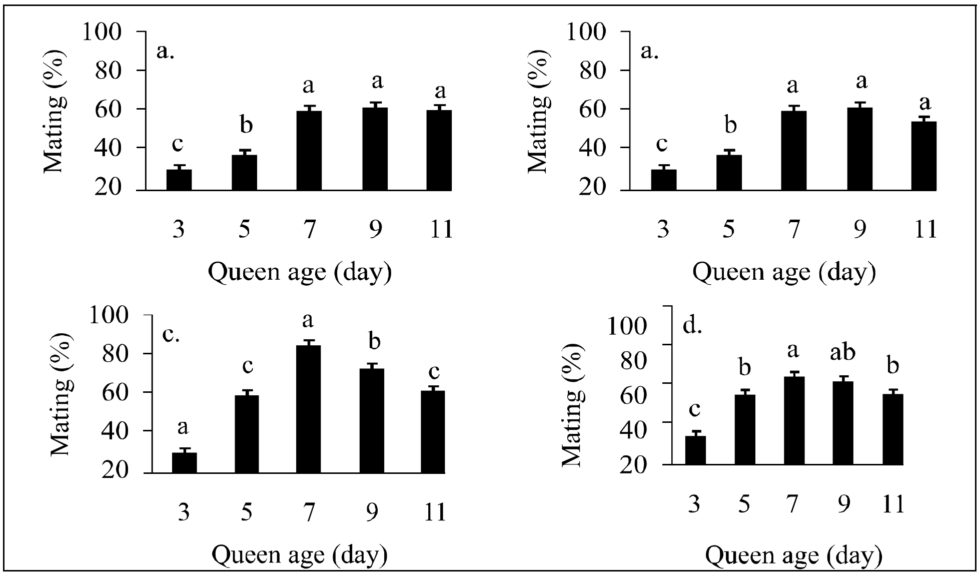

Fig. 1. Effect of age on the mating success (mean ± SE%) of *B. terrestris* within 15 minutes exposure when reared at a) L0:D24, b) L8:D16, c) L16:D8, and d) L24:D0 condition. Bars with no common letter (s) are significantly different (DMRT, $p \leq 0.05$).

The highest mating success (80.4 ±2.5%) was recorded by 7 days old queens and the lowest result (33.3 ± 2.7%) was found by the 3 days old queens. There were significant differences among the mating success rates of the bees reared at L16:D8 condition ($F_{4,59}$= 70.5, p <0.001). The highest (85.0 ± 2.2%) and lowest (30.0 ± 2.5%) mating success rates were performed by 7 and 3 days old queens, respectively (Fig. lc). The different age groups of bees reared at L24:D0 condition showed significant variation in their mating success rates ($F_{4.59}$ = 22.4, p < 0.001). The observed mating rates varied from 36.3 ± 2.9 to 70.0 ± 2.8 % and 7 days old queens showed the highest mating success.

Mating success of *B. terrestris* at 30 minutes exposure is presented in Fig. 2. Mating data of the bees reared in L0:D24 condition (Fig. 2a) showed significant differences ($F_{4.59}$ = 25.1, p < 0.001) and the highest (62.9 ± 2.7%) and lowest (30.4 + 2.7%) mating success rates were recorded by 9 and 3 days old queens, respectively. Fig. 2b shows that the mating success of the bees that were reared at L8:D16 condition revealed significant variations ($F_{4.59}$= 65.4, p < 0.001) on the basis of their age groups. The highest (82.9 ± 2.7%) and lowest (35.0 ± 2.4) mating success rates were recorded by 7 and 3 days old queens, respectively.

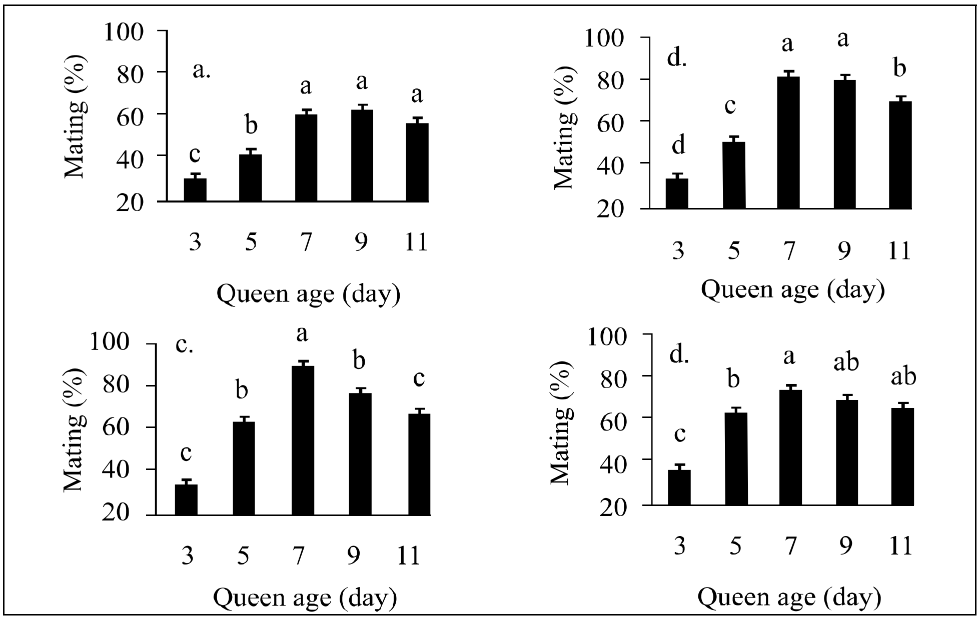

Fig. 2. Effect of age on the mating success (mean ± SE%) of B. *terrestris* within 30 minutes exposure when reared at a) LO:D24, b) L8:D16, c) L16:D8 and d) L24:DO condition. Bars with no common letter (s) are significantly different (DMRT, p ≤ 0.05)

The age groups of bees had significant effect ($F_{4.59}$ = 64.3, p <0.001) on the mating success when they were reared at L16:D8 condition (Fig. 2c), and their success rates ranged from 33.3 ± 2.7 to 89.6 ± 1.3%. Mating success rates by different age groups of bees reared at L24: DO condition also showed significant differences ($F_{4.59}$ = 22.9, p < 0.001) and the performed matings differed from 38.3 ± 2.8 to 74.2 ± 3.2%.

Mating success results of the different age groups of bees after 45 minutes of exposure is presented in Fig. 3. Fig. 3a shows that the mating success of the bees reared at L0:D24 condition were significantly different ($F_{4.59}$ = 22.7, p < 0.001). The highest (65.4 ± 3.3%) and lowest (32.9 ± 2.7%) mating success rates were attained by 9 and 3 days old queens, respectively. Fig. 3b shows that there was significant difference ($F_{4.59}$ = 65.7, p < 0.00 1) among the mating success of the bees reared at L8:D16 condition. The recorded mating success rates ranged from 36.3 * 2.6 to 85.0 ± 2.3%. The mating success rates of the different age groups of bees reared at L16:D8 condition varied from 35.4 ± 2.7% to 92.1 ± 1.7% which were significantly different ($F_{4.59}$ = 71.3, p <0.001). The different age groups of bees reared at L24:D0 condition showed significant differences in their mating success ($F_{4.59}$ = 27.5, p < 0.00 1). The observed mating success rates varied from 39.2 ± 3.0 to 76.3 ± 3.2 % and 7 days old quees showed the highest mating success.

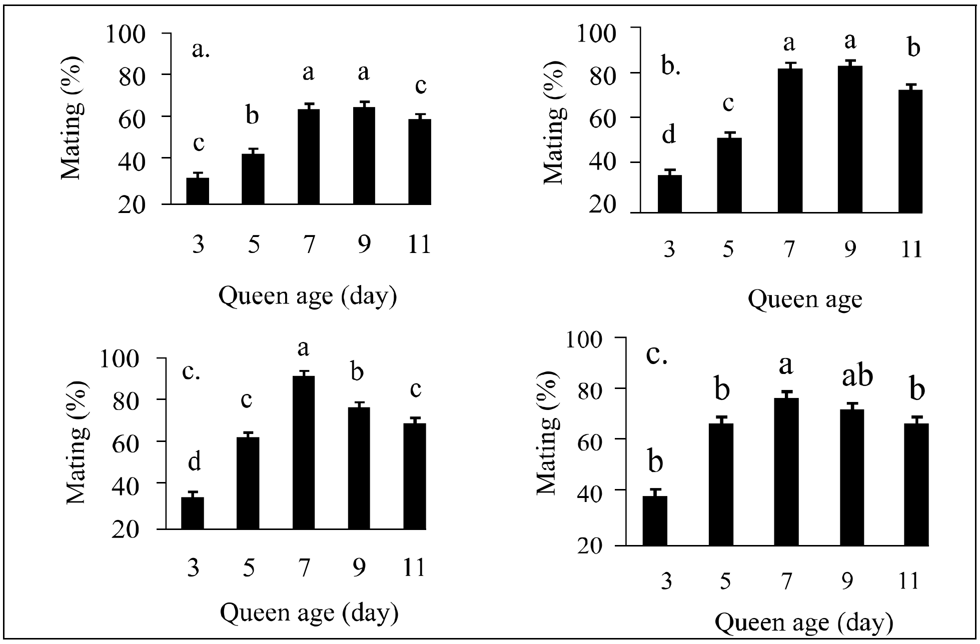

Fig. 3. Effect of age on the mating success (mean ± SE%) of *B. terrestris* within 45 minutes exposure when reared at a) L0:D24, b) L8:D16, c) L16:D8, and d) L24:D0 condition. Bars with no common letter (s) are significantly different (DMRT, p ≤ 0.05).

Different light regimes, age of bees, and duration of mating exposure showed a strong effect on the mating success of *B. terrestris.* The highest mating success (92.1 ± 1.7%) resulted at L16:D8 pre-mating conditions when queens were 7 days old and males 9 days old. These results were in agreement with Tasei *et al.* (1998) who reported that 6 days old queens were effective for mating. They also stated that the age groups for mating ranging between 6 and 27 days for males and between 2 and 11 days for queens, and showed that the chances of the queens mating dropped dramatically after 11 days. Duvoisin *et al.* (1999) found that the mean value of the age of mating was 6.1 days for queens and 12.1 days for males. It was suggested by Gretenkord (1997) that the readiness of queen to mate seemed to be the greatest at around 6 days of age. Our present study is in agreement of Duchateau (1985) who reported that aging queens become gradually less receptive to mating. She suggested that this might be due to a decrease in queen's sensitivity to the male pheromones. She also suggested that temperature, light or time of the day are important factors influencing mating success. Jung *et al.* (2001) recorded the highest mating rate of *B. ardens* by 7 day old queens. They found that 80% of queens mated within 4 hours at L14:D10 photoperiodic regime. Lee *et al.* (2002) conducted a study of bumblebee, *B. ignitus* mating in the field and in indoor conditions. They observed that the mating success in the field were 80%, and 60% in indoors. Our study suggests that 7 days old queens with 9 days old males living in long day cycle or 9 days old queens with 11 days old males reared in short day cycle have significantly higher success rates of mating compared to bumblebees of the same age, kept in constant light or dark cycle.

Light regimes influence the sexual maturity and pheromone production of black cutworm moth (Gemeno and Haynes, 2001), ovarian maturation of *Locusta* (Tanaka *et al.*, 1993) and reduction of pre-mating period in Heteroptera (Wang and Millar, 2000; Hel *et al.*, 2004). Barth *et al.* (1997) reared *Drosophila melanogaster* at different photoperiodic regimes and found that light regimes had a positive effect on mating. Photoperiod had a significant role in the mating success in moths (Seth *et al.*, 2002). So far, the discussion had concentrated the effects of light regimes on the mating behaviour. The present results showed that mating status of *B. terrestris* was affected by the combined effect of photoperiodism, the age of bees and exposure time. Queens of 7 days old and 9 days old males reared at long day, and queens of 9 days old and 11 days old males reared at short day photoperiodic regimes reached the best receptivity for mating. All the age groups of bees reared at different photoperiodic regimes showed higher receptivity for mating within first 15 minutes of exposure and mating success slightly increased with increasing exposure time. So, the findings of this study will contribute information to the commercial breeders for effective rearing of bumblebees.

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