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Light intensity improves the copulating behavior and colony initiation of bumblebee, *Bombus terrestris* (Hymenoptera: Apidae)

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Abstract

Impact of mating tendency was investigated for *Bombus terrestris* under different light intensities (400, 800 and 1200 lux) with different age groups of daughter queens and males (3DQ-5DM, 5DQ-7DM, 7DQ-9DM, 9DQ-11DM) under controlled laboratory standard conditions. Percent mating, queens' survival after diapause and successful colony initiation were observed. Mating rate was more at light intensity of 1200 lux with more than 83%. However, it was around 56% at 400 lux and 68% at 800 lux suggesting 1200 lux light intensity to be the best light intensity condition for improving success rate of mating. Mating tendency of more than 80% was achieved with 7-days old queens with 9-days old males with constant dark-treated queens. Among light intensity of 400, 800 and 1200 lux average copulation duration was highest (24.05 ±1.51 min) at 400 lux. Among mating of 3,5, 7 and 9 days old queen with 5,7,9 and 11 days old males average copulation duration was highest (24.4±1.62 min) when mating of 7 days old queen with 9 days old male was done. These results represent the positive influence of light intensity to mating success rate, age-group for maximum copulating pairs and their possible impact on future generations.

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Introduction

Solitary social insects especially bumblebees has one generation per year and queens which mate in late summer seek out for suitable hibernation sites. In such social insects, mating strategies are of special interest influencing the production of next season generation and their sex ratio (Boomsma and Ratnieks, 1996). Mating success is the result of numerous genetic and environmental factors including mate choice, courtship behavior, female reproductive or motivation and mating competition (Halliday, 1983). Environmental factors affecting such behavioral patterns may include temperature, relative humidity, food and light (Duchateau, 1985). Readiness of mating also depends on the sexual maturity of both partners and production of sex pheromones by the both partners also triggers this phenomenon (Bergstrom, 1981) which can be utilized to enhance this behavior for captive breeding of such social bees.

European bumblebee, *Bombus terrestris* is the species of choice for the majority of bumble bee rearing industry because of its ease to acclimatize the laboratory rearing conditions, handling and readily available in Europe where technology evolved (Velthuis and Doorn, 2006). Relatively high labor costs and often low success rate were some barriers for its commercial rearing. Colony initiation, queen rearing, mating and breaking of diapause are the major stages in the commercial rearing of bumblebees (Hughes, 1996;Velthuis and Doorn, 2006). It is biologically and economically very important pollinators of agriculture crops both in the field conditions and in greenhouses (Kevan *et al.,* 1991; Dogterom *et al.,* 1998; Morandin *et al.,* 2001a, b; Velthuis and Doorn, 2006; Winter *et al.,* 2006; Ali *et al.*, 2014; Umer *et al.*, 2014).

Mating behaviors differ somewhat in the enclosed laboratory rearing facilities where males fail to establish scent marks for female attractiveness in natural field conditions. This may restrict the male precopulatory behavior on female mate choice in the laboratory inside the flight cages (Sauter and Brown, 2001) considered very important factor including searching, guarding and patrolling by bumblebee males for mating success in nature (Duvoisin *et al.*, 1999). Bumblebee females are selective in their mating behavior and may reject males in the laboratory (Duvoisin *et al.*, 1999) as well as in the field (Kindl *et al.*, 1999). Analysis of precopulatory mating behaviors observed antennal inspection by both sexes and queen mobility as the key factors in the mating success (Djegham *et al.*, 1994).

Bumblebee queens are generally monandrous (Schmid- Hempel and Schmid-Hempel, 2000) and the best mating age for queens ranged between 5 and 11 days while the males between 6 and 27 days (Tasei *et al.*, 1998) which may male more than once. Bumblebee queens are selective in their mating behavior and reject many males in the laboratory (Duvoisin *et al.*, 1999) and field (Kindl *et al.*, 1999). Perfect mating or copulating age in *B. terrestris* observed previously has been reported 7-day old queens with 9-day old males with 93% mating success (Kwon *et al.*, 2006). The copulation may last for around 23-30 minutes in *B. ignitus* and *B. terrestris*, respectively (Yoon *et al.*, 1999; Duvoisin *et al.*, 1999; Ptacek, 2001; Brown *et al.*, 2002). After which the males generally block the reproductive duct of female with sticky substance to inhibit further mating of female bees to retain its sexual genetic characters for next generation (Duvoisin *et al.*, 1999; Brown *et al.*, 2002).

Light intensity is considered an important factor influencing the mating behavior in bumblebees. An intensity of 1000 lux was found more effective than that of 100 or 2000 lux in *B. ignitus* queens (Yoon *et al.*, 2007). However, the impact of age on mating pair and their future possible impact due to specific illumination for this important biological process need to be explored further which make the present study to perform under controlled conditions of rearing with different age groups mating under different light intensities. We were interested to find out the most suitable illumination conditions which may help to increase not only the mating percentage but also to enhance their possible productivity in next generation.

Materials and methods

Laboratory rearing of bumblebee colonies

Sixteen European bumblebee (*Bombus terrestrisdalmatinus* (Dalla Torre)) hives were imported from Biobest®, Belgium and reared under growth room maintaining 25±2°C temperature and 60±10% relative humidity in red light. Fresh pollens were purchased locally from a beekeeper of mix flora, refrigerated below -15°C for their utilization on daily basis as per need of the colony with fresh sugar solution (1.5:1, w/v) changed daily. Observations were recorded under red light with intensity not more than 10 lux (Kwon *et al.*, 2006). Colony development, changes in numbers of workers with their larvae, pupae and adults were recorded on daily basis. After development of reproductive cocoons, they were keenly observed for their possible development.

Collection and separation of reproductive progeny

Newly emerged daughter queens and males were separated daily and kept separately in small plastic cages (16 x 11 x 7 cm) provided with sugar solution (1.5:1, w/v) and fresh pollens daily marked with hive number and date of emergence for further experimentation of mating for different age groups. A sum of 420 queens was used for this study. Males were reared in illuminated flight cages (30 x 30 x 30 cm) provided with fresh pollen and sugar solution (1.5:1, w/v) on daily basis.

Impact of different light intensities

Survived queens and males of 2 days old than queen were paired. Mating session of one hour for the copulation occurrence and withdrawal of mating couples after 5-10 minutes of observation to make the mating successful and later shifted in small plastic boxes for further observation of mating time. Transparent plastic mating cages (75x75x75cm^3) were used for mating observations under three different light intensities of 400, 800 and 1200 lux on the mating floor of these plastic cages. Arrangements of mercury bulbs were adjusted to achieve the required light intensities for their possible impact on pre-mating.

Age group and light influence on mating tendency

There were three replicates of twenty daughter queens and forty 2-days older males in each treatment as previously suggested by Kwon *et al.* (2006). Four different pairs of queens and males were made as 3-days old queens to mate with 5-days old males, 5-days old queens with 7-days old males, 7-days old males with 9-days old males and 9-days old queens with 11-days old males.

Age group and light influence on colony initiation of mated queens

Queens mated were provided with fresh pollens and sugar solution to store and meet their diet needs before going into hibernation of 2 x ½ month in refrigerator around 2.5 degree centigrade (Yoon *et al.*, 1999). After hibernation period, the queens were introduced into flight cages of 40×40×60 cm and provided with pollen grain and sugar solutions as their diet source. The cages were kept for one week at 28°C (Duchateau and Marien, 1995), 50% RH and a photoperiodic regime of L8:D16.

Queen in starter boxes were provided with an artificial pupa and honey cup with two small size workers as helper to the queen in initiation of egg lying (Kwon *et al.*, 2006). The pupa was horizontally fixed on hard drawing paper with paraffin so that it could not roll and queen could sit for oviposition. The artificial pupa and anaesthetized worker were replaced once a week till the queen laid eggs on the cocoon or on the hard drawing paper until the workers of the first brood emerged.

Observation of colony parameters

From the day after the introduction of the activated queens to the small starter boxes, the colonies were observed on daily basis and the following parameters were noted.

a. Colony initiation: number of days between first pupa supplied and first egg laid

b. Number of egg cells in first brood

c. Colony foundation: number of days between first pupa supplied and worker emergence from the first laid egg

d. First brood size: number of workers produced from egg cells of the first brood.

Statistical analysis

Data of colony initiation period, number of egg cell in first brood, first worker emergence time and number of workers in first brood were analyzed using analysis of variation and means were compared with Fisher's Least Significant Difference (LSD) test. All analysis was performed using SPSS programs (NorusICE, SPSS Inc. 2006).The means are presented as value ± SE using MS Excel.

Results

Effect of different light intensities on mating percentage and colony initiation of Bombus terrestris queens

We investigated the illumination favorable for mating of *B. terrestris* and their effect on colony initiation. Fig. (1) show that among illuminations of 400, 800 and 1200 lux, the highest percentage of mating (83%) was observed at 1200 lux. Other intensities show comparatively lower mating percentage with 68% at 800 lux and 57% at 400 lux. Mean copulation duration was observed 24.05 ±1.51 min at 400 lux, 22.98±1.27 min at 800 lux and 23.39±1.49 min for mating under light intensity of 1200 lux (Fig.2). These copulation durations at three different light intensities, however, remained non-significant due to the overlapping among them. At light intensity of 400 lux minimum copulation duration of 6 minutes and maximum copulation duration of 44 minutes was observed. Minimum copulation duration of 9 minutes and maximum copulation duration of 58 minutes was observed at 800 lux. It was about 7 minutes and 47 minutes respectively at 1200 luxwhich, too, showed overlapping of the observed copulating duration under these three light intensities (Fig. 3).

Table 1. Impact of different light intensities on colony initiation parameters under controlled environmental conditions.

Light Intensity (lux)	Colony Initiation%	Colony initiation period (d)	No. of egg cell in first brood	First worker emergence (d)	No. of workers in first batch
400	43	10.16±0.64 a	1.50±0.17 b	27.83±0.81 a	7.50±0.51 a
800	45	7.33±0.81 b	1.33±0.16 b	25.00±1.18 a	7.00±0.56 a
1200	60	8.33±0.68 ab	2.33±0.25 a	25.66±0.88 a	7.5±0.59 a

Data expressed as Mean ±S.E.

Mean followed by different letter are significantly different (P≤0.05)

*(d) = number of days required.

Table 2. Impact of mating age on colony initiation parameters of *B. terrestris* under controlled environment conditions.

Mating pairs age group	Colony initiation %	Colony initiation period(d)	No .of egg cell in first brood	First worker emergence (d)	No. of workers in first batch
3DQ-5DM	62.5	13.00±0.92 a	1.40±0.17 a	32.20±1.15 a	6.40±0.52 a
5DQ-7DM	54.5	12.20±0.87 ab	1.40±0.17 a	29.60±0.69 ab	7.60±0.65 a
7DQ-9DM	45.0	9.60±0.52 bc	1.60±0.17 a	27.80±0.68 bc	8.20±0.60 a
9DQ-11DM	40.9	7.40±0.72 c	1.40±0.17a	24.80±0.75 c	6.20±0.60 a

Data expressed as Mean±S.E.

Mean followed by different letter are significantly different (P≤0.05)

*(d) = number of days required

*DQ = age of the queen in days

*DM= age of male in days.

Colony initiation was more profound by the queen mated at 1200 lux (60%) than other mated queen at higher light intensities of 800 lux and 400 lux with 45% and 43%, respectively showing non-significant behavior for light (Table 1). However, queens mated at 800 lux took short time as colony initiation period (7.33±0.81 days) which is statistically non-significant for 1200 lux (8.33±0.68 days) but significant at 400 lux (10.16±0.64 days) (Table 1). Number of egg cells in first brood was the highest (2.33±0.25) for queens mated at 1200 lux which was statistically significant from egg cell at 400 and 800 lux (1.50±0.17; 1.33±0.16). First worker emerged bit late after (27.83±0.81 days) by mated queens at 400 lux than (25.00±1.18 and 25.66±0.88 days) by mated queens at 800 and 1200 lux, respectively with non-significant number of first batch of workers emergence which was around seven for these three light treatments.

Impact of different age of queen and male on mating percentage and colony initiation of Bombus terrestris queen

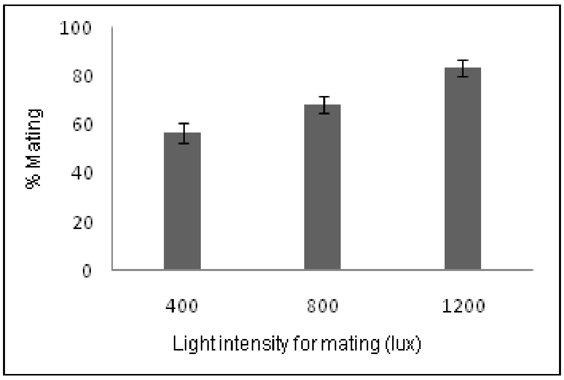

Fig. 1. Mating percentage of *Bombus terrestris* queen under different light intensity.

Four different age groups of queens and male were used to observe the mating propensity at different age of queen and male. Queen of age 3, 5, 7, 9 days old and male of age 5, 7, 9, 11 days old were used for mating purpose. Fig. (4) show highest percentage of mating (82%) was observed when 7DQ and 9DM were used and the lowest mating percentage (37%) was observed with 3DQ and 5DM. Mean copulation duration of 24.4±1.62 min was observed for mating pair of 7DQ with 9DM, 22±1.38 min of 9DQ with 11DM, 20.1±1.38 min of 5DQ with 9DM and the lowest of 19.7±1.73 min was observed for 3DQ with 5 DM, respectively (Fig.5). Fig. (6) show quite variation in minimum and maximum copulation duration for different mating pairs of different age groups. Minimum copulation duration of 5 minutes and maximum copulation of 33 minutes was observed for mating of 3DQ with 5DM. It was 4 and 37 minutes for mating of 5DQ with 7DM, 5 and 57 minutes during mating of 7DQ with 9DM and minimum copulation duration of 5 minutes and maximum copulation duration of 43 minutes was observed during mating of 9DQ with 11DM. In all pairs of different age group, pair with 7DQ with 9 DM took maximum time of mating with 57 minutes.

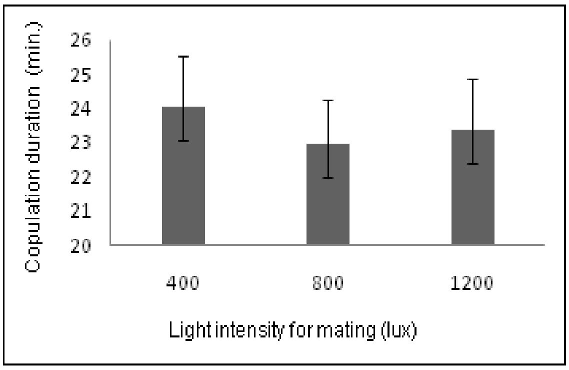

Fig. 2. Impact of different light intensity on copulation duration of *Bombus terrestris*.

Colony initiation percentage was 63% for 3DQ, 55% for 5DQ, 45% for 7DQ and 41% for 9 days old mated queens (Table 2). Significant difference was observed for colony initiation period with shortest time of (7.40±0.72 days) was observed for 9DQ and longer colony initiation period of (13.00±0.92 days) for 3DQ. Number of egg cell in first brood was non-significant for all the age groups of mated queens. Worker emergence took 24.80±0.72 days for 9DQ and 32.20±1.15days for 3DQ which was about a week earlier and show significant difference in worker emergence time. First brood size of 8.20±0.60 workers was observed for 7DQ followed by 7.60±0.65 for and for 5DQ and for 3DQ and 9DQ, it was 6.40±0.52 and 6.20±0.60, respectively that show no variation statistically among mated queens of different age group.

Discussion

Like other abiotic factors, light plays an important role in different biological processes like migration of sexual maturity of sperms and ovarian development and mating of insects (Philogene and McNeil, 1984; Tanaka *et al.*, 1993). It also affects the normal circadian rhythms which may disturb mating courtship behavior under influence of intracellular signals to organize cellular physiology and behavior (Jackson *et al.*,1998; Sakai and Ishida, 2001). Mating is a critical period in the indoor rearing of bumblebee. Successful mating of bumblebee depend on numerous factors including male and queen choice, courtship behavior of male, queen response to courtship and reproductive status of queen(Halliday,1983). Under natural conditions, queens of bumblebee emerged from cocoons flew in daylight for maturation or copulation (Free and Butler, 1959). Such influence affects their mating or courtship behavior under controlled laboratory conditions as tested in present study.

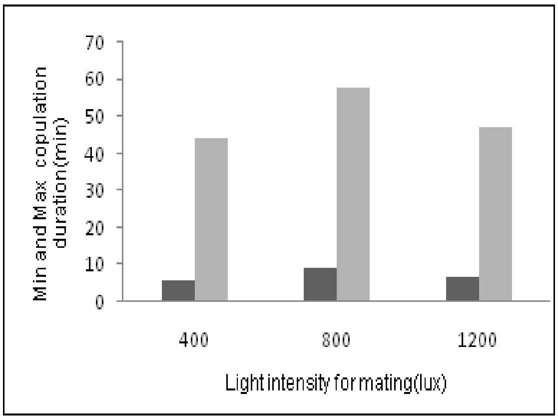

Fig. 3. Maximum and minimum copulation duration of *Bombus terrestris* under different light intensity.

Light illumination of 1200 lux was favorable for mating of *B. terrestris* daughter queens with higher percentage of colony initiation than other conditions tested which is close to previously observed favorable illumination intensity of 1000 lux (Hyung *et al.*, 2007) recommended for colony development of *B. terrestris* during mating periods. High light illumination at 2000 lux was also observed effective for mating but the low temperature of 20°C might resulting the light source as alternate of temperature need more suitable and improved behavior (Djegham *et al.*, 1994).

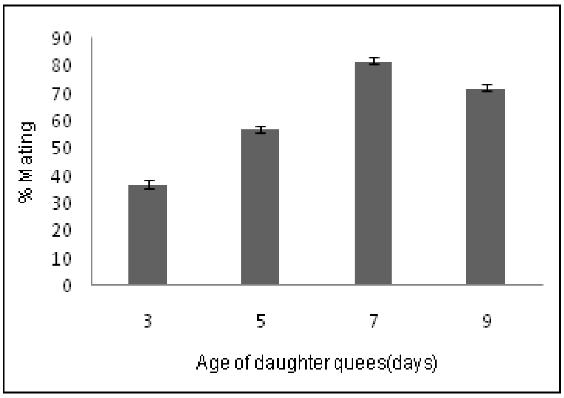

Fig. 4. Effect of queen age on the mating percentage of *Bombus terrestris*.

Age of the queen and male, duration of mating exposure and light condition in mating room have a strong effect on the mating success of *B. terrestris*. (Amin and Kwin, 2011). Mating was observed maximum when 7DQ mated with 9DM with mean copulation duration of 24 minutes. There exist variation with 10 day old queens and 15 day old males with mean copulation duration of 37 minutes in *B. terrestris* (Duvoisin *et al.*, 1999). Tasei *et al.* (1998) reported 6 day old queens effective for mating. He also defined different age groups for mating as ranging between 6 and 27 days for males and 2 and 11 days for queens showing the decreased chances of the queens mating dramatically after 11 days. Duvoisin *et al.* (1999), on the other hand, stated that the mean age of mating was 6.1 days for queens and 12.1 days for males with sperm content of their spermatheca increased from emergence until day 6. Readiness of queens to mate seemed to be the greatest at around 6 days of age (Gretenkord, 1997). The highest mating rate recorded for *B. ardens* was 7 day old queens (Jung *et al.*, 2001). Such variation in different *Bombus* species may occur due to their genetic and physiological characters; however, most of them recorded 6-7 days old queen to be more receptive for mating to achieve the highest mating success rate in laboratory rearing. The study suggests that 7 day old queens with 9 day old males living in dark conditions have higher successful rate of mating.

Significant difference was observed in colony initiation period of queen when we consider appropriate light intensity for mating and impact of age on mating of *B. terrestris* queen. But no significant difference was observed in the brood size of first batch in both the experimental conditions which shows that some other factors like genetic inheritance of queen and diapauses duration also play an important role in different parameters of colony development (Duchateau *et al.*, 2004).

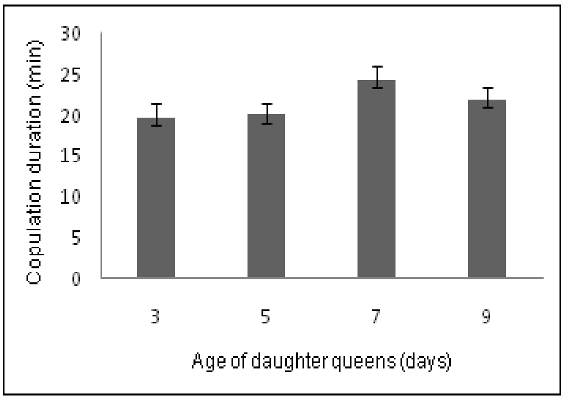

Fig. 5. Copulation duration of *Bombus terrestris* queen at different age group.

For commercial rearing of this important pollinating species, abiotic as well as biotic factors are considered very important at each and every step to enhance the rate of higher colonies in next generation. This helps in better economic returns and availability of bumblebee colonies to the growers at economical rate. Long term breeding and rearing program of such bees are crucial for enclosed farming systems like high tunnel growers, hydroponics and even high value cultivated fruit crops. Establishment of such an industry can help the farmers to have access for these bees at very economical rates, delays in import and their availability in required time period.

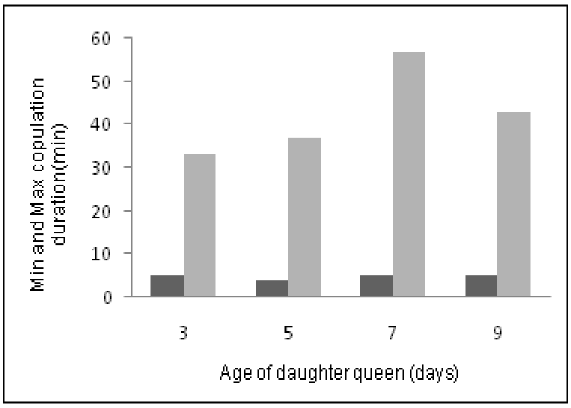

Fig. 6. Impact of different age groups on the maximum and minimum copulation duration of *Bombus terrestris* queen.

Conclusion

The study suggests that the light intensity during mating period and queen and male age are very important factors that affect mating success of *Bombus terrestris*. The mating or copulation rate desired to obtain high number of mated queen is necessary for commercial breeding of these important pollinators. Seven days younger queen were more attractive to copulate with nine days old male bumblebees under controlled light conditions. Such studies help to utilize time in maximum and avoid any losses.

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References

Amin MR, Kwin VJ. 2011. Time and age specific mating success of bumblebee (*Bombus terrestris* l.) reared at different photoperiodic regimes. Bangladesh Journal of Agriculture Research **36,** 13-20.

Ali M, Saeed S, Sajjad A, Bashir MA. 2014. Exploring the best native pollinators for pumpkin (*Cucurbita pepo*) production in Punjab, Pakistan. Pakistan Journal of Zoology **46,** 531-539.

Bergstrom G, Svensson BG, Appelgren M, Groth I. 1981. Complexity of bumble bee marking pheromones: Biochemical, ecological and systematical interpretations. In:Biosystematics of Social Insects (Howse P. E. and Clement J.-L., Eds), Vol. 19.Academic Press, London and New York, 175-183 P.

Boomsma JJ, Ratnieks FLW. 1996. Paternity in eusocial Hymenoptera. Philosophical Transaction of the Royal Society of London **351,** 947-975.

http://dx.doi.org/10.1098/rstb.1996.0087

Brown MJF, Baer B, Schmid-Hempel R, Schmid- Hempel P. 2002. Dynamics of multiple-mating in the bumble bee *Bombus hypnorum.* Insectes Sociaux **49,** 315-319. http://dx.doi.org/10.1007/PL00012654

Djegham Y, Verhaeghe JC, Rasmout P. 1994. Coupulation of *Bombus terrestris* L. (Hymenoptera: Apidae) in captivity. Journal of Apiculture Research **33,** 15-20.

Dogterom MH, Matteoni JA, Plowright RC. 1998. Pollination of greenhouse tomatoes by the North American *Bombus vosnesenski* (Hymenoptera: Apidae). Journal of Economic Entomology **91,** 71-75. http://dx.doi.org/10.1093/jee/91.1.71

Duchateau MJ, Velthuis HHW, Boomsma JJ. 2004. Sex ratio variation in the bumblebee *Bombus terrestris*. Behavioral Ecology **15,** 71-82. http://dx.doi.org/10.1093/beheco/arg087

Duchateau MJ, Mariën J. 1995. Sexual biology of haploid and diploid males in the bumble bee *Bombus terrestris*. Insectes Sociaux **42,** 255–266. http://dx.doi.org/10.1007/BF01240420

Duchateau MJ. 1985. Analysis of some methods for rearing bumblebee colonies. Apidologie **16,** 225- 227.

Duvoisin N, Boris B, Schmid-hemple P. 1999. Sperm transfer and male competition in a bumblebee. Animal Behavior **58,** 743-749.

Free JB, Butler CG. 1959. Bumblebees. Collins, London.

Gretenkord C. 1997. Laboratory breeding of the dark earth bumblebee *Bombus terrestris* L. (Hymenoptera: Apidae) and toxicological studies under semi-field conditions Laborund. Ph. D. Thesis, Rheinische Friedrich Wilhelms Univ. Bonn.

Halliday TR. 1983. The study of mate choice. In: Bateson P. (Ed.), Mate choice, Cambridge University Press, Cambridge, 3-32 P.

Hughes MJ. 1996. Commercial rearing of bumble bees. In:Matheson A (Ed): Bumble Bees for Pleasure and Profit. First ed., IBRA, Cardiff, 40-47 P.

Hyung JY, Sam EK, Kyeong YL, Sang BL, Park IG. 2007. Mating conditions favorable for improving mating rate of the Bumblebee, *Bombus ignites*. International Journal of Industrial Entomology **15,** 107-114.

Jackson FR, Zhang X, Mcneil GP. 1998. Oscillating molecules and circadian clock output mechanisms. Molecular Physiology **3,** 381-385. http://dx.doi.org/10.1038/sj.mp.4000431

Jung JK, Mah YI, Kim JS. 2001. Mating rate of *Bombus ardens* (Hymenoptera: Apidae) queens with day time and age. Korean Journal of Apiculture **16,** 37-44.

Kevan PG, Straver WA, Offer O, Laverty TM. 1991. Pollination of greenhouse tomatoes by bumblebees in Ontario. Proceeding of the Entomological Society of Ontario **122,** 15-19.

Kindl J, Hovorka O, Urbanova K, Valterova I. 1999. Scent marking in male premating behavior of *Bombus confuses*. Journal of Chemical Ecology **25,** 1489-1500. http://dx.doi.org/10.1023/A:1020824529729

Kwon YJ, Amin MR, Suh SJ. 2006. Mating propensity of *Bombus terrestris* reared in different photoperiodic regimes. Apidologie **37,** 679-686. http://dx.doi.org/10.1111/j.1748-5967.2006.00041.x

Kwon YJ, Than KK, Suh SJ. 2006. New method to stimulate the onset of *Bombus terrestris* (Hymenoptera: Apidae) rearing: using worker helpers in the presence of frozen pupae. Entomological Research **36,** 202-207.

http://dx.doi.org/10.1111/j.1748-5967.2006.00041.x

Morandin LA, Laverty TM, Kevan PG. 2001a. Effect of bumblebee (Hymenoptera: Apidae) pollination intensity on the quality of greenhouse tomatoes. Journal of Economic Entomology **94,** 172-179.
http://dx.doi.org/10.1603/0022-0493-94.1.172

Morandin LA, Laverty TM, Kevan PG. 2001b. Bumblebee (Hymenoptera: Apidae) activity and pollination levels in commercial tomato greenhouses. Journal of Economic Entomology **94,** 462-467.
http://dx.doi.org/10.1603/0022-0493-94.2.462

Norusich MJ. 2006. Upper Saddle River, N. J. Prentice Hall SPSS Inc.

Philogene B, Mcneil JN. 1984. The influence of light on the non diapause related aspects of development and reproduction in insects. Photochemistry and Photobiology **40,** 753-761.
http://dx.doi.org/10.1111/j.1751-1097.1984.tb04648.x

Ptacek V. 2001. Some biological aspects of bumble bee (*Bombus*, Hymenoptera) management. Acta Horticulturae **561,** 279-286.

Sakai T, Ishida N. 2001. Circadian rhythms of female mating activity governed by clock genes in *Drosophila*. Proceeding of National Academy of Sciences **98,** 9221-9225.
http://dx.doi.org/10.1073/pnas.151443298

Sauter A, Brown MJF. 2001. To copulate or not? The importance of female status and behavioral variation in predicting copulation in a bumblebee. Animal Behaviour **62,** 221-226.
http://dx.doi.org/10.1006/anbe.2001.1742

Schmid-Hempel R, Schmid-Hempel P.2000. Female mating frequencies in Bombus spp. from central Europe. Insectes Sociaux **47,** 36-41.
http://dx.doi.org/10.1007/s000400050006

Tanaka S, Hakomori T, Hasegawa E. 1993. Effect of day length and hopper density on reproductive traits in a Japanese population of the migratory locust, *Locusta migratoria* L. Journal of Insect Physiology **39,** 571-580.
http://dx.doi.org/10.1016/0022-1910(93)90039-T

Tasei JN, Moinard C, Himpens B, Guyonnaud S.1998. Relationship between age, mating and sperm production in captive *Bombus terrestris*. Journal of Apiculture Research **37,** 107-113.

Umer AAS, Munir A, Imran M, Nasir M, Shafqat S, Imran B. 2014. Distribution of bumblebee, *bombus haemorrhoidalis* Smith, and its association with flora in lower northern Pakistan. Pakistan Journal of Zoology **46,** 1045-1051.

Velthuis HHW, Doorn AV. 2006. A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. Apidologie **37,** 421-451.

Winter K, Adams L, Thorp R, Inouye D, Day L, Ascher J, Buchmann S. 2006. Importation of non-native bumble bee's into North America: potential consequences of using *Bombus terrestris* and other non-native bumble bees for greenhouse crop pollination in Canada, Mexico and the United States. A white paper of the North American Pollinator Protection Campaign (NAPPC).

Yoon HJ, Kim SE, Lee KY, Lee SB, Park IG. 2007. Mating conditions favorable for improving mating rate of the bumblebee, Bombus *ignitus*. International Journal of Industrial Entomology **15,** 107-114.

Yoon HJ, Mah YI, LEE MY, Park IG. 1999. Studies on ovary development and mating of bumblebee, *Bombus ignitus* Smith. Korean Journal of Apiculture **14,** 35-42.