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The effect of queen removal on sexual production in the bumble bee, *Bombus terrestris* (Hymenoptera: Apidae)

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Abstract: We analyzed the effects of queen removal on male and queen production in the bumble bee, *Bombus terrestris*. In total, 43 *Bombus terrestris* colonies were reared under standard laboratory rearing conditions. Before the competition point, when the colonies comprised 35-40 workers and many diploid larvae, we removed the queens from 20 randomly selected colonies to test the effects of queenlessness. The timing of gyne production in queenless colonies (25.6 ± 1.5 days) was significantly earlier than in queenright colonies (32.8 ± 3.2 days). Although queen removal had a significant effect on the timing of gyne production, there was no significant difference between the timing of the switch point, or the number of gynes and males produced in the queenright and queenless colonies.

Key words: Bumble bee, *Bombus terrestris*, male and queen production, queenlessness

Bombus terrestris (Hymenoptera: Apidae) arılarında ana arı uzaklaştırmasının genç ana ve erkek arı üretimi üzerine etkisi

Özet: Bu çalışmada, bombus arısı kolonilerinde kurucu ana arı uzaklaştırmasının genç ana ve erkek arı üretimi üzerine etkileri incelenmiştir. Toplam 43 adet *Bombus terrestris* kolonisi standart yetiştirme koşullarında yetiştirilmiştir. Ana arısızlığın etkisini belirlemek için rekabet noktasından önce, çok sayıda diploit larvası ve 35-40 adet işçi arısı bulunan 20 koloni rasgele seçilmiş ve bu kolonilerin ana arıları alınmıştır. Genç ana arı üretim zamanı ana arısız kolonilerde (25,6 ± 1,5 gün) ana arılı kolonilerden önemli derecede daha erken gerçekleşmiştir (32,8 ± 3,2 gün). *B. terrestris* kolonilerinde ana arı uzaklaştırılmasının genç ana arı üretim zamanı üzerine etkisi önemli bulunmasına rağmen, dönüşüm noktası ve kolonilerde üretilen toplam erkek ve ana arı sayısı üzerine etkisi önemli bulunmamıştır.

Anahtar sözcükler: Bombus arısı, *Bombus terrestris*, erkek ve ana arı üretimi, anasızlık

Introduction

Bombus terrestris L. is an important pollinator of greenhouse crops and is reared on a large scale under controlled conditions (Beekman et al., 2000). Current worldwide sales of *B. terrestris* have reached some 900,000 colonies per year and demand increases every year. This species produces large colonies and adapts quite well to artificial conditions (Velthuis and Doorn, 2006). *B. terrestris* has been used as a pollinator of tomato, pepper, and eggplant in Turkey since 1998 (Aytekin et al., 2002). In 2008, approximately 60,000 commercially produced *B. terrestris* colonies were

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used for the pollination of 3000 ha of greenhouse area. The commercial rearing of *B. terrestris* is currently not fully developed in Turkey. In recent years Turkish companies have imported queens rather than colonies and produced colonies from these queens.

Colony initiation, queen rearing, mating, and breaking of diapause are the major stages in the commercial rearing of bumble bees (Hughes, 1996; Velthuis and Doorn, 2006). Although the development of production techniques has increased rapidly since 1988, maximizing the quality and profitability of artificially reared colonies remains a problem in commercial production (Hughes, 1996). One of the major problems of breeding bumble bees is the production of young queens in sufficient numbers to meet the demands of a rapidly increasing market for colonies (Hughes, 1996; Velthuis, 2002). *B. terrestris* colonies vary greatly in the number of workers, males, and queens produced, even if colonies are maintained under similar laboratory conditions (Beekman et al., 1998; Velthuis, 2002). Many factors influence male and queen production, such as the split sex ratio, worker to larva ratio, food quality and quantity, and diapause history of the founding queen (Duchateau and Velthuis, 1988; Ribeiro et al., 1996; Duchateau et al., 2004).

B. terrestris colonies develop through 2 major phases. During the first phase the queen is dominant and is the only egg layer, and all female larvae develop into workers. In the second phase the social environment in the colony changes dramatically; some workers start to lay eggs and most of the female larvae develop into young queens. This phase is termed the competition phase and is characterized by several changes in the colony: the start of worker egg laying, the occurrence of mutual egg-eating by queen and workers, and direct aggressive encounters between workers and queens. The onset of the competition phase is strongly correlated with the onset of gyne (new queen) production and marks the end of the colony life cycle (Duchateau and Velthuis, 1988; Bloch, 1999; Cnaani et al., 2000; Alaux et al., 2006). Nevertheless, queen and male production occur under several different social conditions—not only in the presence of the queen, but also in the absence of the queen (Cnaani et al., 1997; Alaux et al., 2005).

The aim of the present study was to determine a way to enhance gyne production for bumble bee mass rearing. We expected that queen removal might result in an earlier switch to queen production, as a vigorous queen produces a pheromone that inhibits gyne production and a decrease in this pheromone triggers gyne production (Cnaani et al., 2000; Alaux et al., 2005). We also sought to determine if modification of the colony social structure could change the production of new queens (gynes), which is important for the establishment of new colonies. Before the competition point we removed the queens from some colonies and compared queenright and queenless colonies in order to determine the effect of queen removal on gyne and male production.

Materials and methods

Colony rearing

Queens were obtained from our laboratory-reared colonies. The diapause length of all queens was about 8 weeks. To stimulate egg laying, each queen was anesthetized with CO_2 once for 30 min and placed separately in a small starting box with 1 newly emerged *B. terrestris* worker (Gurel and Gosterit, 2008). Callow workers were changed every week until the first workers emerged. All queens and colonies were kept in captivity under standard laboratory rearing conditions (28 ± 1 °C and 60 ± 5% relative humidity) and were fed ad-libitum the same pollen and sugar syrup. When the workers of the first brood emerged the colonies were transferred to larger plastic boxes.

Experimental design and observations

In total, 43 *B. terrestris* colonies were reared in a climate-controlled room. Before the competition point, when the colonies comprised 35-40 workers and many diploid larvae, we removed the queens from 20 randomly selected colonies (queenless group). The date of egg laying after queens were placed in nest boxes, the number of egg cups in the first brood, the date of emergence of the first worker, the number of workers in the first brood, the date of initiation of the second and the third broods, the switch point, the competition point, the total number of workers, the total number of queens (gynes), and the total number of males produced by each colony were recorded.

During observations dead bees were removed from colonies and noted in order to calculate the total number of bees produced by each colony. The timing of the switch point, competition point, and gyne production were determined from the day of emergence of the first worker (beginning of eusociality). The timing of gyne production was calculated by subtracting the developmental time of the gyne (30 days) from the date of emergence of the first gyne. The switch point (date the first haploid egg was laid) was calculated by subtracting the developmental time of males (25 days) from the date of emergence of the first male. The initiation of the competition phase was determined by one or more of the following events: worker oviposition, observation of oophagy, observation of clear signs of egg-cup destruction, and observation of 2 or more open egg cups during the previous 2 days (Duchateau and Velthuis, 1988; Bloch and Hafetz, 1999). Descriptive statistics relating to traits were given and groups were compared using the Mann-Whitney U test. The chi-square test was used to compare the percentage of colonies in the queenless and queenright groups that produced males and gynes.

Results

Colony development

Before queen removal (competition point) the average pattern of colony development in the 43 *B. terrestris* colonies was determined. Queens started laying eggs 17. 7 ± 1.4 days after they were placed in nest boxes. On average, the first brood produced 3.8 ± 0.2 egg cells and 8.2 ± 0.6 workers emerged in the first brood. The time between the last cells of the first brood and new cells of the second brood was 21.4 ± 1.3 days. After a pause of 19.2 ± 0.9 days the queens started to build the cells for the third brood.

The effect of queen removal on the production of sexuals

There was no significant difference in the number of males and queens produced (Figure 1); however, the average number of workers produced per colony was significantly different ($P < 0.05$, Mann-Whitney U test). The average number of workers in queenright and queenless colonies was 126.6 ± 6.5 and 102.3 ± 6.7, respectively. In queenless colonies the switch to male production occurred no earlier than in queenright colonies (25.0 ± 2.4 days, n = 14 and 28.4 ± 2.4 days, n = 18, respectively, Figure 2); however, the timing of gyne production and the competition point in queenless colonies (25.6 ± 1.5 days and 32.9 ± 1.4 days, respectively) occurred significantly earlier than in queenright colonies (32.8 ± 3.2 days and 40.0 ± 2.1 days, respectively; $P < 0.05$ and $P < 0.01$, respectively, Mann-Whitney U test).

In the queenright group 8 colonies (35%) produced only males, 4 colonies (17%) produced only gynes, 10 colonies (44%) produced both gynes and males, and 1 colony (4%) did not produce reproductives, while in the queenless group 4 colonies

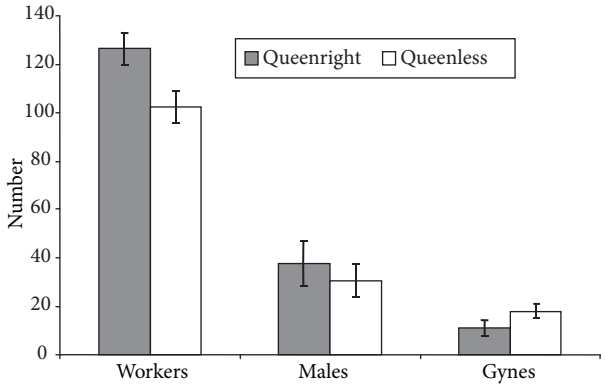

Figure 1. Comparison of the number of workers, males, and gynes produced in queenright and queenless *B. terrestris* colonies.

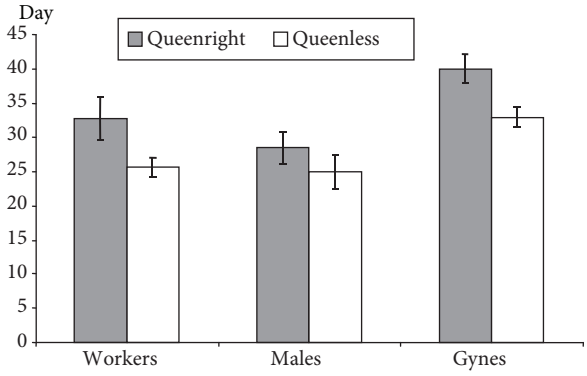

Figure 2. Timing of gyne production, the switch point, and the competition point in queenright and queenless *B. terrestris* colonies.

(20%) produced only males, 6 colonies (30%) produced only gynes, and 10 colonies (50%) produced both gynes and males. The percentage of colonies that produced both gynes and males was similar in both groups (Table).

Discussion

Social insects with an annual life cycle generally produce males and gynes towards the end of the colony cycle (Macevicz and Oster, 1976); however, even if colonies are reared under identical conditions, the exact timing of the switch from the ergonomic to reproductive phase varies between colonies (Müller et al., 1992; Beekman et al., 1998). Sexual production in the bumble bee is strongly male biased. Thus, one of the limiting factors for bumble bee rearing is the number of queens produced. In the present study we investigated the effects of queen removal on male and queen production in the bumble bee *B. terrestris*.

We showed that the timing of gyne production was under queen control. Queen removal had a significant effect on the timing of gyne production, as *B. terrestris* queens produce a pheromone that inhibits the development of larvae into gynes. This pheromone may act indirectly by causing changes in worker behavior, as in honey bees, or it may act directly on the larvae themselves (Cnaani et al., 1997). Although queenless colonies began gyne production significantly earlier than queenright colonies, the difference between the total number of gynes in queenright (11.1 ± 3.0) and queenless (18.0 ± 3.0) colonies was not significant. As expected, the average numbers of workers produced by the 2 types of colonies were significantly different, because the queen is the only individual that can lay diploid eggs (female eggs) and queen removal suppresses the production of diploid eggs.

The competition point in queenless colonies occurred earlier than in queenright colonies, but queen removal did not affect the number of males or the switch point, both of which are related to the time of first male production. This strong correlation between the competition point and the onset of queen rearing has been observed previously (Duchateau and Velhuis, 1988; Bloch, 1999; Cnaani et al., 2000). Lopez-Vaamonde et al. (2007) also reported that queenlessness induced significantly earlier onset of queen production. Results of the present study show that queen removal did not have a direct influence on the number of gynes or males produced, although queenless colonies began gyne production significantly earlier than queenright colonies. In general, our results agree with those of Lopez-Vaamonde et al. (2003)—reproductive activity in *B. terrestris* workers does not reduce the production of new queens. Our findings suggest that queen removal from colonies alone is not a satisfactory method for queen production in mass rearing. To enhance queen production it would be useful to investigate combining queen removal with transferring diploid eggs from queenright colonies to queenless colonies.

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Table. Percentage of colonies that produced males and gynes in queenright and queenless *B. terrestris* colonies.

Production of sexuals	n	Queenright	n	Queenless	χ^2	P
Males only (%)	23	35	20	20	0.543	0.461
Gynes only (%)	23	17	20	30	0.377	0.539
Males + gynes (%)	23	44	20	50	0.015	0.903
No sexuals (%)	23	4	20	0	0.005	0.944

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