THE SUITABILITY OF NATIVE *Bombus terrestris dalmatinus* (Hymenoptera: Apidae) QUEEN FOR MASS REARING

Fehmi Gurel, Ayhan Gosterit

Akdeniz University, Faculty of Agriculture,
Department of Animal Science, 07059 Antalya, Turkey,
e-mail: fgurel@akdeniz.edu.tr

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Summary

We examined the suitability of mass rearing native *Bombus terrestris dalmatinus* queens in the laboratory. A total of 50 naturally mated and aestivated *B. t. dalmatinus* queens were collected in autumn, from the Mediterranean coastal region. The queens were allowed to start a colony in the laboratory under standard conditions (28°C, 60% R.H.). The first generation colonies were thus obtained. Newly emerged young queens and males were collected from these colonies and mated in a cage. After mating, queens were put into artificial hibernation at 4°C for 45 days. They were then allowed to start a colony following the same procedure. The second generation colonies were thus obtained. The timing of colony initiation, the number of egg cells in the first brood, the timing of first worker emergence and the total number of queens appear to be similar in both generations. The average number of workers in the first and second generation colonies were 71.90 ± 13.30 and 121.10 ± 22.70 respectively. Colony production ratio was also lower in the first generation colonies (46%) than in the second generation colonies (74.1%). Results show that this native population is suited to mass rearing. Colony development characteristics can be improved by increasing breeding techniques and selection.

Keywords: bumble bees, *Bombus terrestris dalmatinus*, Mediterranean population, mass rearing, colony development.

INTRODUCTION

Bumble bees play an important role in the pollination of a wide variety of field, forage and fruit crops, particularly greenhouse crops (Banda and Paxton 1991). There are about 250 species of bumble bees (Williams 1998). Currently five species of bumble bees are reared commercially on a large scale. The main commercially reared species is the Eurasian *Bombus terrestris* L. This species has wide distribution, produces large colonies and adapts quite well to artificial conditions (Velthuis and Doorn 2006). The natural range of *B. terrestris* covers the whole of Europe, coastal North Africa, and in West and Central Asia (Estoup et al. 1996, Widmer et al. 1998). There are about ten geographically separate subspecies. Many subspecies of *B. terrestris* were used in the early years of commercial rearing. Recently, however, *Bombus terrestris dalmatinus* (Dalla Torre) is the most reared subspecies for commercial pollination and has been used outside its natural distribution area. It is estimated that the current worldwide sales of *B. t. dalmatinus* has reached approximately 900,000 colonies per year (Velthuis and Doorn 2006).

In Turkey, native *B. terrestris* populations occur throughout most of the country (Özbek 1997) and belong to the subspecies of *B. t. dalmatinus* (Yeninar et al. 2000, Velthuis and Doorn 2006). This subspecies of bumble bee, forages in a wide range of habitats from sea level to 1500 m altitudes. It forages on all the major types of native vegetation in Turkey (Gurel et al. 2008). Because it is widespread, there are probably several ecotypes of this subspecies, each adapted to specific ecological conditions. These ecological conditions range from the Mediterranean coastal areas to the high mountain conditions of the central Anatolian area (Yeninar et al. 2000). In fact, this subspecies shows great ecological flexibility, particularly in terms of life cycle and diapause response (Estoup et al. 1996, Dafni 1998). For example, in Mediterranean and Aegean coastal regions, native *B. t. dalmatinus* queens have a summer diapause (aestivation) emerging in autumn while in inland areas and mainly in Europe they have a winter diapause (hibernation) emerging in spring (Gurel et al. 2008).

Although colony development patterns of native *B. t. dalmatinus* queens collected from Aegean and Mediterranean coastal regions were determined under laboratory conditions (Yeninar et al. 2000, Gosterit and Gurel 2005), these studies could not precisely reflect the potential performance of *B. t. dalmatinus* for mass rearing. There is considerable variation among *B. terrestris* colonies. Various colony characteristics and the diapause history of founder queens should also lead to these differences (Beekman and van Stratum 1998, 2000). Bumble bee producers today have reared colonies under standardized breeding conditions. These producers have developed their own rearing systems which have become independent from field collected queens (Velthuis and Doorn 2006). However commercial breeders have not published any comparative data. Therefore, the aim of this study is to determine the potential performance of *B. t. dalmatinus* for mass rearing, by following the same procedure as the commercial companies.

MATERIAL AND METHODS

Rearing procedures of bumblebee

A total of 50 naturally mated and aestivated *B. t. dalmatinus* queens were collected from the Mediterranean coastal region. The queens were collected in the field (on the flowers of *Arbutus unedo* L.) during autumn 2006 near Antalya, Turkey. To stimulate egg laying, each queen was anesthetized with CO_2 once for 30 min and placed separately in the small starting box with callow *B. terrestris* workers (Gurel and Gosterit 2008). The queens were allowed to start a colony in the laboratory under standard conditions (28°C, 60 % relative humidity, red light, food - pollen and sugar water- ad libitum). After all workers of the first brood emerged, the colonies were transferred to a larger rearing boxes and obtained the first generation colonies. Hygienic conditions were maintained inside the boxes. Newly emerged young queens and males were collected from first generation colonies and placed in a cage in the laboratory for mating. To avoid inbreeding effects, young queens were mated with males from different colonies. After mating, queens were put into artificial hibernation at 4°C for 45 days and then they were transferred to the laboratory and allowed to start the second generation colonies following the same procedure as described above.

Observations

Colony development was tracked by direct daily observation. In each generation, the colony initiation (the number of days from the introduction of the queen into the starting box until the laying of the first eggs), the number of egg cells in first brood, the timing of first worker emergence, the number of workers in first brood, the timing of second and third brood, the timing of gyne (young queen) production, the switch and competition points, the total number of workers, males and gynes produced by each colony, egg laying ratio, colony production ratio and progeny queen production ratio were recorded by periodic observations. During the observations, the dead bees in the colonies were counted and noted in order to determine the total number of workers, males and queens produced. In this study, a minimum of ten workers was the criteria used to define a colony. Queens that produced fewer than ten workers were not considered to be colony- producers. The timing of switch point, competition point and gyne production were counted from the day of first worker emergence (beginning of eusociality). The timing of gyne production was calculated by subtracting the developmental time of gyne (30 days) from the date of the emergence of the first gyne. The switch point (date of first haploid egg laid) was calculated by subtracting the developmental time of male (25 days) from the date of the emergence of the first male. We defined the competition point observing one or more of the following events: worker egg laying, worker egg eating, multiple egg cups remaining open simultaneously or clear signs of egg cup destruction (Duchateau and Velthuis 1988, Beekman et al. 1998, Cnaani et al. 2000). The progeny queen production ratio was calculated as follows: the number of queens that produced a progeny queen / the number of queens that produced a colony x 100. The results were analyzed using the MINITAB program. Descriptive statistics relating to traits were given. Groups were compared using variance analysis. The egg laying, colony production and progeny queen production ratio were compared using the t-test.

RESULTS AND DISCUSSION

The percentage of queens which laid eggs varied from 64% to 81.4% and that of queens which produced colonies from 46% to 74.1% (Table 1). The percentage of colony production of queens was much lower in the first generation than in the second generation ($Z = -2.36$, $P < 0.05$). The egg laying ratio of queens was also lower in the first generation than in the second generation, however, the difference was not significant. The progeny queen production ratio of the colonies obtained from the first generation colonies was significantly lower than in the second generation colonies ($Z = -3.25$, $P < 0.01$).

The timing of colony initiation, the number of egg cells in the first brood, the timing of first worker emergence and the total number of queens (gynes) were similar for the two groups of colonies (Table 2). The number of workers in the

Table 1

Comparison of the egg laying, colony production and progeny queen production ratio between first and second generation queens.

Colony foundation performances	First generation queens	Second generation queens
Egg laying ratio (%)	64.0 (n=50)	81.4 (n=27)
Colony production ratio (%)	46.0 **A** (n=50)	74.1 **B** (n=27)
Progeny queen production ratio (%)	30.4 **a** (n=23)	80.0 **b** (n=20)

Different letters on the same line indicate significant differences
(t-test levels: a, b: P<0.01; A, B: P<0.05)

first brood, the total number of workers and the total number of males were significantly different for two groups of colonies. The second generation colonies that were obtained from laboratory reared queens produced more than twice as many males and approximately 60% more workers than those from the first generation colonies that were obtained from field collected queens. Timing of second and third brood, timing of first gyne production, switch and competition points were also significantly different for two groups of colonies.

The second generation queens started second and third brood earlier than the first generation queens. The second generation queens also had earlier switch and competition points. Second generation queens that were reared in the laboratory started to make their first cells after an average of 8.32 ± 1.63 days. The time between the last cells of the first brood and new cells of the second brood was 24.05 ± 1.99 days. After a pause of 15.40 ± 1.02 days, the queens started to build cells for the third brood.

Table 2

Comparison of the colony characteristics (mean ± SE) between first and second generation colonies

Colony characteristics	First generation colonies	Second generation colonies
Colony initiation (days)	7.30 ± 0.95 (n=32)	8.32 ± 1.63 (n=22)
Number of egg cells in first brood	4.37 ± 0.25 (n=32)	4.24 ± 0.55 (n=22)
First worker emergence (days)	44.83 ± 2.81 (n=24)	39.81 ± 1.96 (n=20)
Number of workers in first brood	5.04 ± 0.63 **a** (n=24)	11.19 ± 0.91 **b** (n=20)
Timing of second brood (days)	36.65 ± 1.86 **a** (n=23)	24.05 ± 1.99 **b** (n=20)
Timing of third brood (days)	24.86 ± 2.01 **a** (n=22)	15.40 ± 1.02 **b** (n=20)
Timing of gyne production (days)	56.30 ± 11.08 **a** (n=7)	10.63 ± 2.91 **b** (n=16)
Switch point (days)	29.07 ± 5.59 **a** (n=15)	4.95 ± 1.71 **b** (n=21)
Competition point (days)	53.00 ± 3.51 **a** (n=16)	25.45 ± 2.80 **b** (n=20)
Total number of workers	71.90 ± 13.30 **A** (n=24)	121.10 ± 22.70 **B** (n=20)
Total number of males	30.50 ± 10.60 **a** (n=15)	70.90 ± 8.25 **b** (n=21)
Total number of queens	10.00 ± 3.85 (n=7)	12.69 ± 2.40 (n=16)

Different letters indicate significant differences between means (t-test levels: a, b: P<0.01; A, B: P<0.05)

DISCUSSION

In year-round rearing of bumble bees, rapid colony initiation by the queen and high success (colony production) rate are the major criteria used to reduce production costs and obtain good colonies. The results presented here clearly show that Mediterranean *B. t. dalmatinus* populations have a high colony production and rapid colony initiation. Another important colony characteristic for the use of bumble bees in pollination is the number of workers that are produced. In this study the average number of workers in a colony obtained from field collected queens (first generation) and laboratory reared queens (second generation) were 71.90 ± 13.30 and 121.10 ± 22.70 respectively. Similarly, Yeninar et al. (2000) reported an average of 150 workers per colony and Gosterit and Gurel (2005) reported an average of 167 workers per colony obtained from field collected *B. t. dalmatinus* queens. Duchateau and Velthuis (1988) also reported an average of 137 workers in the early switching colonies and 284 workers in the late switching colonies obtained from field collected *B. terrestris* queens.

Our results revealed several similarities and differences between the field collected (first generation) and laboratory reared (second generation) *B. t. dalmatinus* queens. The timing of colony initiation, the number of egg cells in the first brood, the timing of first worker emergence and the total number of queens (gynes) appear to be similar in both generations. Our observations suggest that native Mediterranean *B. t. dalmatinus* populations tend to produce less gyne than commercial colonies. This argument has been supported by some studies which have found that wild caught queens from the Mediterranean region did not produce an appreciable number of gynes under laboratory conditions (Yeninar et al. 2000, Gosterit and Gurel 2005). Similarly, Ings et al. (2006) found that commercial colonies produced significantly more gynes than native colonies under identical field conditions. The high gyne production of commercial colonies may be due to the short diapause (Beekmam and van Stratum 1998, Duchateau et al. 2002) and/or queen production process. This is because commercial companies are likely to minimize diapause length (Ings et al. 2006) and have selected for high gyne producing colonies to increase turnover.

Several important differences were found between the two generations. They are the colony size, the colony production ratio, and the switch and competition points. Second generation queens that had been reared in the laboratory produced more and better colonies than the field collected queens. A possible reason for these differences is that at the moment of capturing and handling, field collected queens are more sensitive to disturbances than laboratory reared ones (Yeninar et al. 2000). Another possible explanation is that the diapause durations of queens (queens collected after natural aestivation in the field) is not known and it is not known whether the queens started laying eggs or had founded a colony in the field at the moment of capturing.

CONCLUSIONS

B. t. dalmatinus have been commercially raised for 20 years. When commercial rearing of *B. t. dalmatinus* started in Belgium and Holland, Western European bumble bee producers collected queens from local populations in Turkey (in the Aegean and Mediterranean regions), Greece, and the Balkans in order to set up breeding stocks. But whether the commercial companies have been selecting these stocks is not well known. Our results indicate that Mediterranean *B. t. dalmatinus* adapts quite well to artificial conditions. It is not considered inferior quality for mass rearing. Our data clearly show that colony development characteristics (such as colony size and colony production ratio) of this native population can be improved even more by increasing breeding techniques and selection.

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OZNACZANIE PRZYDATNOŚCI RODZIMYCH MATEK *Bombus terrestris dalmatinus* (Hymenoptera: Apidae) DO HODOWLI MASOWEJ

Gurel F., Gosterit A.

S t r e s z c z e n i e

W pracy badano przydatność rodzimych matek *Bombus terrestris dalmatinus* do masowej hodowli w warunkach laboratoryjnych. Ogółem, 50 naturalnie skojarzonych matek *B. t. dalmatinus* będących w stanie snu letniego odłowiono w regionie przybrzeżnym Morza Śródziemnego jesienią. Matki pozostawiono, aby mogły założyć rodzinę w laboratorium w standardowych warunkach (28°C, 60% wilgotność względna), w ten sposób uzyskując pierwsze pokolenie rodzin. Świeżo wyklute młode matki i samce odłowiono z rodziny i skojarzono w klateczce. Po kojarzeniu, matki wprowadzono w stan sztucznej hibernacji w temperaturze 4°C przez 45 dni, po czym ponownie pozwolono im założyć rodziny wg poprzednio zastosowanej procedury, uzyskując w ten sposób drugie pokolenie rodzin. Czas założenia rodziny, liczba komórek jajowych w pierwszym wylęgu, czas pojawienia się pierwszej robotnicy oraz całkowita liczba matek okazały się być podobne w obu pokoleniach. Przeciętna liczba robotnic w rodzinach pierwszego i drugiego pokolenia wyniosła odpowiednio, 71,90 ± 13,30 oraz 121,10 ± 22,70. Wskaźnik produktywności rodzin był również niższy w rodzinach z pierwszego pokolenia (46%) niż z drugiego pokolenia (74.1%). Uzyskane wyniki wskazują, że badana rodzima populacja nadaje się do masowej hodowli oraz, że parametry rozwoju rodziny mogą zostać poprawione poprzez zwiększenie liczby technik hodowli i selekcję.

Słowa kluczowe: trzmiele, *Bombus terrestris dalmatinus*, populacja Morza Śródziemnego, hodowla masowa, rozwój rodziny.