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Colony traits of native *Bombus terrestris dalmatinus* from the Western Black Sea Region of Turkey: comparison with commercial colonies

Batı Karadeniz Bölgesi doğal *Bombus terrestris dalmatinus* arılarının koloni özelliklerinin ticari koloniler ile karşılaştırılması

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ABSTRACT

The present experiment was conducted to determine the developmental characters of the native bumblebee *Bombus terrestris dalmatinus* that occurs in the Western Black Sea region of Turkey, where commercial colonies have not been used as pollination agents. The colony traits of this native population were compared with the traits of commercial colonies of *B. terrestris*. A total of 200 queens, 100 naturally diapaused ('native' population) and 100 artificially diapaused ('commercial' population), were allowed to found colonies. There were no differences in egg laying and colony founding success between the native and commercial queens. Queens collected from the field commenced egg-laying earlier than commercial queens, and produced less egg cells and workers in the first brood. Colonies founded by commercial queens produced more gynes (82.11 ± 9.32) than colonies founded by native queens (32.85 ± 3.97). Native and commercial colonies also differed in their patterns of production of males and gynes.

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ÖZ

Bu çalışma ticari bombus arısı kolonilerin kullanılmadığı Batı Karadeniz Bölgesi'nde doğal olarak yayılış gösteren *Bombus terrestris dalmatinus* arılarının koloni gelişim özelliklerinin belirlenmesi amacıyla gerçekleştirilmiştir. Bu doğal populasyon koloni gelişim özellikleri bakımından ticari *B. terrestris* kolonileri ile karşılaştırılmıştır. Araştırmada diyapoz dönemini doğal koşullarda geçiren 100 adet ('doğal' populasyon) ve laboratuvar koşullarında geçiren 100 adet ('ticari' populasyon) olmak üzere toplam 200 adet ana arı kullanılmış ve bu ana arıların koloni oluşturması sağlanmıştır. Doğal ve ticari populasyon ana arıları arasında yumurtlama ve koloni oluşturma başarısı bakımından farklılıklar önemli bulunmamıştır. Doğadan toplanan ana arılar ticari populasyon ana arılarına göre daha erken yumurtlamaya başlamış ve birinci kuluçka döneminde daha az yumurta hücresi ve işçi arı üretmişlerdir. Ticari ana arılar tarafından oluşturulan koloniler (82.11 ± 9.32 adet) doğal populasyon ana arıları tarafından oluşturulan kolonilere göre (32.85 ± 3.97 adet) daha fazla sayıda genç ana arı üretmişlerdir. Doğal ve ticari koloniler arasında erkek ve genç ana arı üretimi stratejisi bakımından farklılık belirlenmiştir.

1. Introduction

Bumblebees are general pollinators in modern agriculture. They are particularly valuable in greenhouse tomato production because of their pollination efficiency, which decreases pollination labour costs and increases the quality and quantity of the crop (Dasgan et al. 2004; Velthuis and van Doorn 2006). They are also among the most abundant of insects visiting flowers in the Mediterranean, continental Europe, and several Atlantic islands (Estoup et al. 1996; Chittka et al. 2004).

Currently, about 250 species of true bumblebees have been identified (Williams 1998). Turkey, which represents a bridge between Europe and Asia, provides a gene pool for bumblebees. In Turkey, 50 species of bumblebees are recognised (Ozbek 1997). The most widespread species of the native fauna is *Bombus terrestris*, which is also the species most commonly reared as a pollination agent. This species is common in the Aegean, Marmara, Black Sea and Mediterranean regions of the country (Gosterit and Gurel 2005; Gurel and Gosterit 2009). Nine subspecies of *B. terrestris* have been described in the world. *B. t. dalmatinus* Dalla Torre 1882, which is naturally distributed in Turkey (Rasmont et al. 2008), forages on a large

number of flowering plants at altitudes ranging from sea level to 1500 m (Gurel et al. 2008).

B. t. dalmatinus follows an annual life cycle but is partially bivoltine in warmer areas such as the Mediterranean (Schmid-Hempel et al. 2007). It is a eusocial insect that lives in colonies composed of a founder queen, workers, males and the immature brood (Hartfelder et al. 2000). Diapaused queens find a nest site in which they lay eggs from which the first workers are produced. These workers forage for pollen and nectar and help the queen expand the colony. Generally, colonies produce reproductive young queens (gynes) and males towards the end of their life. The young queens enter diapause after mating and the mother queen, her workers, and the males die (Alford 1975). There are considerable differences between different local populations in the time of diapause termination of queens and in the timing of the production of sexuals (Gurel et al. 2008).

Annually, more than one million bumblebee colonies are commercially produced worldwide and exported widely (Velthuis and van Doorn 2006). About two hundred thousand commercially produced *B. terrestris* colonies are used in Turkey for greenhouse pollination. However, intensive use of commercial colonies could have negative impacts on native ecosystems (Goka et al. 2001; Kanbe et al. 2008; Hingston and McQuillan 1998; Kenta et al. 2007; Inoue et al. 2008; Yoon et al. 2011). In addition, wild bumblebee populations may decline in agricultural areas as a result of increased pesticide use, decreased plant diversity, and habitat loss (Allen-Wardel et al. 1998). Therefore, it is important to determine the developmental patterns of native bumblebee populations to assist development of pollination services and to improve conservation strategies. The goal of this study was to determine the developmental patterns of native *B. t. dalmatinus* colonies in the Western Black Sea region (Turkey), where commercial colonies have not yet been used. Colony traits of this native population were also compared with traits of commercial *B. terrestris* colonies under controlled laboratory conditions.

2. Materials and Methods

2.1. Origin of queens and colony rearing

A total of 200 queens were used; 100 naturally diapaused wild queens of *B. t. dalmatinus* were collected from the Western Black Sea region of Turkey (40°54′N, 31°01′E and 400–450 m above sea level; the 'native' group) and 100 artificially diapaused commercial queens of *B. terrestris* (the 'commercial' group) were obtained from a commercial supplier (Bio Group, Antalya-Turkey). Queens from the wild population were collected in one day on the flowers of *Arbutus unedo* L. from same location in November 2014.

All queens were transferred to bumblebee research laboratory in cages containing a sugar solution, placed singly into starting boxes and allowed to found a colony in climate-controlled room (27–28 °C and 50% RH). A newly emerged *B. terrestris* worker was added to each queen to stimulate egg laying (Gurel and Gosterit 2008). These young workers were replaced with new young individuals each week until the first worker hatched. The nests were transferred to larger boxes when the population reached about ten workers. Queens and their colonies were provided with sugar solution (50 Brix) and fresh frozen pollen *ad libitum* (Velthuis and van Doorn 2006).

2.2. Observations

Nests were checked twice a week to reduce the effects of stress factor and the following development traits were recorded: colony initiation time (date of egg laying of the queen); number of egg cells in the first brood, first worker emergence time (i.e., the beginning of the social phase; Duchateau and Velthuis 1988), number of workers produced in the first brood, gyne production time, "competition point" (the time when worker oviposition, oophagy and egg-cup destruction were observed; Duchateau and Velthuis 1988; Bloch and Hefetz 1999), the "switch point" when the first unfertilized egg was laid (Bourke 1997; Duchateau et al. 2004), and the total number of individuals (workers, males and gynes) produced by each colony. The methods reported by Gosterit (2011) were followed to determine the proportion of queens that laid eggs and successfully founded colonies (produced more than 10 workers) and the proportion of colonies that reached sufficient size for pollination (50 or more workers before the production of sexuals), and to record other colony characteristics. The timing of gyne production, switch point and competition point were counted from the beginning of the social phase. The timing of gyne production was calculated by subtracting 30 days (gyne developmental time) from the time of hatching of the first offspring queen. To calculate the time of the switch point, the male developmental time (25 days) was subtracted from the time of hatching of the first male (Duchateau and Velthuis 1988).

2.3. Data analysis

All data were subjected to square root transformation for normality before undergoing analysis. Two sample t-tests and Mann-Whitney U-tests were applied to compare the colony traits of the two groups. Data expressed as proportions were compared using two-proportion z-tests. Analyses were performed using Minitab Statistical Software (Version 16.2.4).

3. Results and Discussion

There were no significant differences with respect to egg laying and colony founding success between native (field-collected) and commercial queens (Table I). However, there were significant differences between the two groups with respect to some of the colony development traits (Table II). Native queens commenced egg laying earlier, but produced less egg cells and workers in the first brood than did commercial queens ($P<0.01$, Mann-Whitney U-test). It was possible that, native queens had already started egg laying or founded colony when they were caught. This probability might be a reason for shorter colony initiation time. The timing of first worker emergence ($P<0.01$, Mann-Whitney U-test) and the timing of gyne production ($P<0.01$, two sample t-tests) in colonies founded by native and commercial queens were also different.

Table 1. The percentages (%) of native and commercial queens that laid eggs, founded colonies (≥10 workers), and whose colonies reached sufficient size for pollination (≥50 workers).

	Laid eggs	Founded colonies	Reached size for pollination
Native queens	88.00	64.00	47.00
Commercial queens	80.00	60.00	42.00
P-value	0.123	0.560	0.477

Table 2. Developmental traits of colonies founded by native and commercial queens.

Developmental traits	Colonies founded by:				
	Native queens		Commercial queens		
	n	Mean ± S.E	*n*	Mean ± S.E	P-value
Colony initiation time (days)	88	7.53 ± 0.70	80	11.06 ± 0.54	0.000
Number of egg cells in first brood	88	4.65 ± 0.21	79	5.87 ± 0.26	0.000
First worker emergence (days)	72	35.14 ± 1.30	61	37.57 ± 1.11	0.013
Number of workers in first brood	66	13.67 ± 0.89	60	19.78 ± 1.22	0.000
Timing of gyne production (days)	42	24.21 ± 2.07	57	16.49 ± 1.25	0.008
Switch point (days)	57	16.33 ± 2.64	51	13.51 ± 2.23	0.400
Competition point (days)	61	38.67 ± 1.20	57	30.84 ± 1.60	0.000
Total number of workers	64	135.13 ± 8.19	60	142.50 ± 8.73	0.539
Total number of males	59	89.05 ± 7.40	54	96.56 ± 9.73	0.540
Total number of gynes	40	32.85 ± 3.97	56	82.11 ± 9.32	0.000
Timing of size for pollination (days)	47	66.21 ± 1.57	42	62.50 ± 1.15	0.223

The most striking difference between the two groups of colonies was in the total number of gynes produced. Colonies founded by commercial queens produced more than twice the number of gynes than colonies founded by native queens (P<0.01, two sample t-tests). The difference between the two groups in the time of the competition point was significant (P<0.001, Mann-Whitney U-test,) but the timings of the switch point and of reaching the size for pollination were similar (P>0.05, Mann-Whitney U-test).

Patterns of production of gynes and males by the colonies are shown in Fig. 1. Significant differences were found between the groups in the gynes and males production strategies. While 50 out of 60 colonies headed by commercial queens produced both gynes and males, only 37 of 64 colonies headed by native queens produced both males and gynes (P<0.01). The percentages of colonies that produced only male sexuals were 34.37% and 6.67% in native and commercial groups, respectively (P<0.01). However, differences in the percentages of colonies that produced only gynes were not significant (P>005).

Because the commercially produced colonies originated from populations that were genetically different those in other regions, queens and males that escape from greenhouses could transport foreign alleles to other native populations (Ings et al. 2005; Nagamitsu et al. 2010). As a consequence, invasion of commercial *B. terrestris* genotypes into new areas could have negative effects on native populations (Goulson 2003). Therefore, investigation of the colony development patterns of local populations before the introduction of commercially produced colonies is crucial for the characterisation and protection of natural genetic resources.

B. t. dalmatinus populations are native to nearly in all regions of Turkey (Ozbek 1997; Rasmont et al. 2008). Several studies have investigated colony developmental traits of native *B. t. dalmatinus* populations in the Mediterranean and Aegean

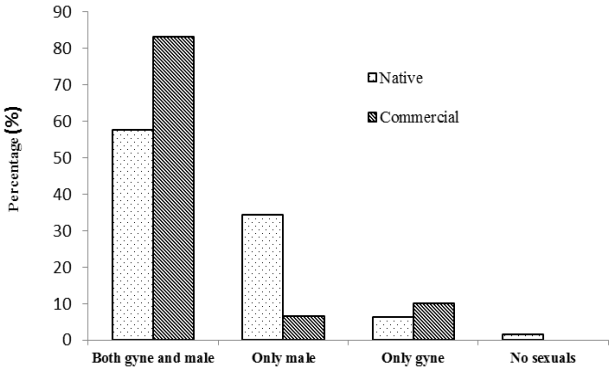

Figure 1. Gyne and male production patterns of colonies produced by native and commercial queens (%).

regions, where the greenhouse sector developed (Yeninar et al. 2000; Gosterit and Gurel 2005; Gurel and Gosterit 2009). The present study is the first to report these traits in a native and genetically pure population of *B. t. dalmatinus* from the Western Black Sea region, where commercial colonies have not yet been introduced for pollination.

In the present study, the percentages of native and commercial queens that laid eggs, founded colonies of more than 10 workers, and produced colonies of an adequate size for pollination (>50 workers) did not differ. In our previous study (Gurel and Gosterit 2009), the egg laying and colony production ratios of native *B. t. dalmatinus* queens from the Mediterranean region were 64% and 46%, respectively. Gosterit and Gurel (2005) also reported that 91% of the queens from Mediterranean populations and 82% of the commercial queens laid eggs.

There is marked local variability among colonies of *B. terrestris* in the production of reproductives (young queens and males) (Alaux et al. 2005; Lopez-Vaamonde et al. 2009; Gosterit 2011). Here, we showed that native and commercial colonies also exhibited different patterns. In particular, the patterns of production of young queens (gynes) of the two colony groups differed. Possibly, the commercial companies have been selecting for breeding colonies that produce the most queens and males for year-round rearing. High rates of gyne production by commercial colonies have also been reported by other researchers (Gosterit and Gurel 2005; Ings et al. 2005).

We collected the field-population queens from the flowers of *A. unedo* in November 2014. In fact, *A. unedo* is a determinative flowering plant in the life cycle of wild *B. terrestris* and the emergence of the queens from diapause overlaps with the flowering of this plant (Rasmont et al. 2005; Gurel et al. 2008). It is interesting that the queens emerged from diapause immediately before the winter season in the Western Black Sea region. This suggests that the times of emergence from diapause are similar in the Black Sea and Mediterranean populations of *B. t. dalmatin*us. This similarity also extends to reports of bivoltinism in these *B. terrestris* populations (Estoup et al. 1996; Rasmont et al. 2005).

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