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Determination of colony characteristics and suitability for mass production of native *Bombus terrestris* L. population in the Central Black Sea coast region

Salim AKTÜRK[1,*], **Ayhan GÖSTERİT**[2], **Gökhan AKDENİZ**[1], **Samet OKUYAN**[1], **Ahmet Emir ŞAHİN**[1]

[1]Apiculture Research Institute, Ordu, Turkey

[2]Department of Animal Science, Faculty of Agricultural Sciences and Technologies, Isparta University of Applied Sciences, Isparta, Turkey

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Abstract: In this study, two successive generations of queens belonging to a native *Bombus terrestris* population in the Central Black Sea Region of Türkiye were reared under controlled laboratory conditions to examine its developmental characteristics and suitability for mass production. The first generation was formed by a hundred *Bombus terrestris* queens which were caught from native fauna in Ordu Province, Türkiye. Young queens and males produced at the end of the life cycle in different colonies of first generation were mated in laboratory conditions. Mated queens were placed in a refrigerated incubator for their two months diapause period. After the diapause period, all queens were raised under the same conditions, and second generation colonies were reared. Some characters such as first egg-laying time, the timing of switch point, the timing of competition point, and total numbers of individuals produced in colonies of the first and second generation were determined. In these characteristics, the total numbers of workers and queens were found to be statistically significant between generations ($p < 0.05$). According to the findings of this study, some of the features such as first egg-laying time, the timing of switch point, and the number of young queens produced may be unsatisfactory for mass production.

Key words: *Bombus terrestris*, life cycle, native population, diapause, mass production

1. Introduction

Insects are the most important organisms that undertake the task of pollination in nature. An important part of the pollination of agricultural products consumed by humans, which is considered to be valuable on a global scale, is provided by insects [1-3]. The most effective insect groups in the pollination of flowering plants are bees with approximately 25 thousand species [4,5].

Honey bees and bumblebees, which have been reared commercially by humans, are commonly used for pollination. Honey bees are known as the most effective pollinator in nature since they can form large populations and are cultivated in almost every country in the world. Bumblebees, on the other hand, constitute the most important group after honey bees in pollination and are widely used especially in the greenhouses. They are indispensable especially for greenhouse tomato production due to their features such as increasing the quantity and quality of the products, reducing the labor force, and limiting the use of chemical pesticides [6-8].

The use of bumblebees, whose importance in pollination has been known since the beginning of the 20th century, has been increasing in the last 30 years [9,10]. This increase was realized with the understanding that bumblebees could be used instead of mechanical methods and plant growth regulators. Thus, mass production of bumblebees became widespread in Europe [8]. The features of bumblebees such as superior foraging abilities, working at low light temperature intensity, not being aggressive, having a large body size and not tending to go out of the greenhouse have made them stand out from honey bees for pollination in the greenhouses [11,12].

Among the 250 species of bumblebees described, *Bombus terrestris, Bombus lucorum, Bombus ignites, Bombus occidentalis* and *Bombus impatiens* are commonly reared commercially [8]. *Bombus terrestris* is the most commercially cultivated species among the bumblebee species due to its easier cultivation and more crowded colony population. Another reason why this species is preferred more is that it has a very widespread area [13,14].

Usage of commercial *Bombus terrestris* colonies for pollination has become widespread in the Mediterranean and Aegean Regions of Türkiye where greenhouse cultivation is carried out extensively. Thus, scientific studies in the past have mostly focused on the native populations in these regions. On the other hand, *Bombus*

* Correspondence: slmaktrk51@gmail.com

terrestris species are seen in a wide range of habitats from sea level to 1500 m in Türkiye [15,16].

This study aimed to determine the developmental characteristics, and suitability for mass production of the population by breeding *Bombus terrestris* queens native to Central Black Sea Region under controlled laboratory conditions.

2. Materials and methods

2.1. Origin of bees, experimental design, and rearing of colonies

The research was carried out in the Bumble Bee Research Laboratory at Apiculture Research Institute (Ordu, Türkiye) in 2020. The study was carried out under standard laboratory rearing conditions (27–28 °C; 50% RH). Control of temperature and humidity is provided by air conditioners and humidifiers in the laboratory [17,18]. The research material was composed of a hundred *Bombus terrestris* queens, which were caught in the wild fauna of Ordu Province (41.1338, 37.6824, at an altitude of 10–30 m). Bumble bees were collected in the month of March which they terminate their diapause period in the nature. They were caught from the flowers of the *Lamium amplexicaule* L. during the collection trips made to the specified region between 05 and 08 March 2020. Ventilated tubes were used as a trap for collecting the queens. Collected queens were transferred to the laboratory on the same day. Queens and colonies were fed with fresh pollen stored in –20 °C and sugar solutions (50 Brix). Frozen pollen was thawed in room temperature prior to feeding it to colonies. Nutrients were purchased from the local businesses and all colonies were fed ad libitum with the same food throughout the study [2,19]. Sugar syrup and pollen were replaced with fresh ones at 4-day periods. In the study, queens of wild populations were placed into individual starting boxes (8 cm × 8 cm × 6 cm) shown in Figure 1, and first generation colonies were reared. After all of the workers in the first brood had emerged, the colonies were transferred to larger plastic boxes (20 cm × 30 cm × 25 cm). At the end of the colony life cycle, queens and males, collected from different colonies, were mated in mating cages (30 cm × 30 cm × 30 cm) shown in Figure 2 in an illuminated mating room at 23 ± 1 °C and 50 ± 5% RH [20]. Mated queens were placed in a refrigerated incubator so that they could pass their diapause (artificial hibernation) period at +2.5 °C for about 2 months [21,22]. At the end of the diapause period, queens were reared under similar rearing conditions and colonies belonging to the second generation were reared.

2.2. Obtained and analysis of data

Colony development was continuously monitored, and the parameters of colony formation were evaluated using the data that was periodically gathered. These parameters are as follows: egg-laying rate, colony production rate, male and young queen production ratio of colonies, time of first egg-laying, switch point, competition point, marketable colony production rate. The egg-laying rate is the rate of laying eggs among queens placed in the starter boxes. Colony production rate is the proportion of the queens that produced at least 10 workers. The time of first egg-laying is the time elapsed from placing the queens in the starting boxes to observing the first egg. The switch point is determined by deducting twenty-five days from the interval between the start of the social phase and the first male emergence. This deduction was

Figure 1. Starting boxes.

Figure 2. Mating cage.

made in order to account for the time spent during the males' larval development [23-25]. The competition point is the time interval between the onset of the social phase and the beginning of conflict between the queen and the workers [26]. The marketable colony production rate is the proportion of the queens that produced at least 50 workers [16]. To determine the change in the colony population, the number of workers in the colony was counted and recorded every week from the first workers' emergence. The boxes were cleaned to keep them clean.

2.3. Statistical analysis

Descriptive statistical values of each characteristic related to colony development were determined. It was tested whether the data showed normal distribution and square root transformation was applied to the data that did not show normal distribution. Generations were compared with a one-way analysis of variance (ANOVAs) in terms of development traits. Percentage values of colonies in the first and second generation were compared using two proportions *z* tests.

3. Results

In the study, the average number of workers, males, and queens in the first generation was found 87.64 ± 9.48, 164.0 ± 16.6, and 10.31 ± 3.84 and the second generation was found 155.6 ± 13.93, 113.0 ± 18.67, and 106.0 ± 16.01, respectively (Table 1). While there was no significant difference between the generations in terms of the total number of males, the difference between the first and second generation was statistically significant in terms of the characteristics of the total number of workers and queens ($p < 0.05$) (Table 1).

The queens of the first generation started to lay eggs 16.15 ± 2.49 days after they were transferred to the starting boxes, and the egg-laying time of the queens of the second generation was determined as 17.08 ± 1.81 days. In this study, no difference was observed between generations in terms of egg-laying time ($p < 0.05$). The switch and competition point were calculated as 12.53 ± 2.35 and 33.09 ± 1.77 days, respectively, in the first generation, 11.00 ± 3.79 and 35.06 ± 1.58 days in the second generation. There was no significant difference between the generations in terms of switch point and competition point ($p < 0.05$).

According to obtained data, 39% of the queens of the first generation laid eggs, and 36% (producing at least 10 worker bees) formed a colony. In the second generation, the egg-laying rate was 52% and the colony production rate was 32%. While there was no colony producing only queens in the first generation, the rate of queen production in the second generation was found to be 31%. Queen and male production rates were found to be 44% and 50% in the first and second generations, respectively, as shown in Table 2.

There was no difference between generations (32%) in the marketable colony production rate. The population growth characteristics were not statistically different between first and second generations ($p > 0.05$), except for the ratio of only queen-producing colonies ($p < 0.05$).

4. Discussion

In this study, the number of workers and virgin queens was found to be greater in colonies that were produced by the queens reared in the laboratory conditions compared to colonies produced by the queens collected from the nature. This discrepancy may have resulted from some factors, about which we lack adequate amount of information, impacting the gathered queens prior to collection. These factors can be listed as the colony formation status of the queen at the time of collection, the available nutrition sources in the nature. In addition, the reactions of queens when they are brought to the laboratory may be another reason for the difference between generations.

Furthermore, in the first generation the average number of queens produced (10.31 ± 3.84 was found to be lower in comparison with commercial queens studied in previous work [27]. Commercial queens were shown to produce approximately twice as many young queens

Table 1. Developmental characteristics of colonies belonging to generations.

Characteristics	First generation		Second generation		p value
	n	x̄ ± SEM	n	x̄ ± SEM	
Egg-laying time (days)	39	16.15 ± 2.49	26	17.08 ± 1.81	0.789
Switch point (days)	34	12.53 ± 2.35	12	11.00 ± 3.79	0.739
Competition point (days)	33	33.09 ± 1.77	15	35.06 ± 1.58	0.492
Total number of workers	39	87.64 ± 9.48[a]	16	155.6 ± 13.93[b]	0.000
Total number of males	37	164.0 ± 16.6	12	113.0 ± 18.67	0.109
Total number of queens	16	10.31 ± 3.84[a]	13	106.0 ± 16.01[b]	0.000

**Means followed by different letters (a, b) in the same row are statistically different (p < 0.05).*

Table 2. Some traits for queens and colonies in the first and second generation.

Traits	First generation	Second generation
Egg-laying rate (%)	39	52
Colony production rate (%)	36	32
Produced only queen (%)	0[a]	31[b]
Male and young queen production rate (%)	44	50
Marketable colony production rate (%)	32	32

**Values followed by different letters (a, b) in the same row are statistically different (p < 0.05).*

as queens collected from the nature [27]. These results are in agreement with the results of previous studies on the native population of the Mediterranean Region [28]. Similarly, Ings et al. [29] reported that commercial *Bombus terrestris* colonies exposed to similar field conditions produced more queens than native population colonies. According to Matsumura et al. [30], the number of queens produced in commercial *Bombus terrestris* colonies used in Japan is 4.4 times higher than in native population colonies. During the initial years of commercial breeding, companies collected queens from the native populations of the region including Türkiye, Greece, and the Balkans to create their breeding stocks. It can be said that selections made during a breeding period of approximately 25–30 years are a possible factor in the differentiation of some developmental characteristics such as the number of young queens.

In the studies that Gösterit and Gürel [27], Yeninar et al. [31], Gürel et al. [32] examined the populations of the Mediterranean Region, they calculated the egg-laying time as 8.0, 2.4, 5.9 days, respectively. The discrepancy between the results of our study and those of that study (16.52 days) might be attributable to the different climatic conditions. It is known that *Bombus terrestris* populations can fit two life cycles in a year in some provinces such as Antalya and Adana in the Mediterranean Region of Türkiye, while they may be limited to a single life cycle in the Black Sea Region. The switch point, which is the stage in which the queen starts to lay unfertilized (haploid) eggs instead of fertilized (diploid) eggs, varies according to many factors [26]. The point of competition is where the influence and superiority of the founding queen are lost and the workers begin to lay eggs [33,34]. Both characteristics are critical stages of the life cycle in *Bombus terrestris* colonies. When the switch and competition points—which could be regarded as the two of the most crucial quality criteria in the mass production of *Bombus terrestris*—are compared between the populations studied in different regions, it has been found that the switch point is generally reached earlier in the Central Black Sea population (switch – competition points: Gösterit and Gürel [27] 18.9 and 37.3 days, Yeninar et al. [31] 22.8 and 28.6 days, Gösterit and Başkar [28] 16.3 and 38.6 days).

Marketable colony production rate (%) is an important criterion in mass production. Even if the conditions are completely suitable, the fact that most of the queen bees recruited for laying cannot form high-quality colonies and that the colony production rate suitable for pollination is mostly between 25% and 35% [35] coincides with our result (%32).

5. Conclusion

In this study, the developmental characteristics of the population were investigated by breeding queens of a native *Bombus terrestris* population in the Central Black Sea Region of Türkiye. The studies can be a guide in terms of accurately predicting the possible impact of a probable hybridization on ecology in the future, determining and protecting genetic resources as a result of the widespread use of greenhouse cultivation and *Bombus terrestris*.

It is worth noting that the queens we assessed may be unsatisfactory for mass production in comparison with the commercial queens in terms of some of the quality parameters. These parameters, which have a very important place in commercial production, can be listed as egg-laying period, switch point and the number of young queens produced. When the findings are examined, it is thought that this population can be made much more valuable by selection in terms of features that are important for mass production.

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