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Research Article

Comparison of Development Patterns of Imported and Native *Bombus terrestris* L. (Hymenoptera: Apidae) Colonies in the Mediterranean Coastal Region

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Abstract: This study was carried out to compare the colony development patterns of native and commercially imported *Bombus terrestris* colonies, which were used in greenhouses for pollination in the Mediterranean coastal region. Total of 57 queens, 23 of which were collected from the field and 34 obtained from the commercial company, were used. 91% of the queens collected from the field and 82% of the queens obtained from the commercial company laid eggs. The colony foundation ratio of the queens was determined as approximately 57%. The colonies founded by the queens taken from the commercial company produced 60.8 ± 12.70 queens, while the colonies founded by the queens collected from the field did not produced queens. The numbers of workers and males produced, switch and competition points in the social phase of the colonies founded by the queens collected from the field were determined to be an average of 167.2 ± 34.90 bees, 98.6 ± 21.50 bees, 18.9 ± 2.37 days and 37.3 ± 2.68 days, while those in the colonies founded by the queens taken from the commercial company were 225.3 ± 30.90 bees, 156.0 ± 42.60 bees, 25.0 ± 3.69 days and 50.8 ± 4.44 days, respectively.

Key Words: Bumblebee, *Bombus terrestris*, colony development patterns

Akdeniz Sahil Bölgesindeki Yerel ve İthal Edilen Ticari *Bombus terrestris* L. (Hymenoptera: Apidae) Kolonilerinin Gelişim Özelliklerinin Karşılaştırılması

Özet: Bu çalışmada Akdeniz sahil bölgesinde bulunan yerel ve örtü altı yetiştiricilikte tozlaşmayı sağlamak için ithal edilen ticari *Bombus terrestris* arılarının koloni gelişim özelliklerinin karşılaştırılması amaçlanmıştır. Araştırmada 23 adedi doğadan toplanan ve 34 adedi ticari bir firmadan alınan toplam 57 adet ana arı kullanılmıştır. Doğadan toplanan ana arıların % 91'i ticari firmadan alınan ana arıların ise % 82'si yumurtlamıştır. Ana arıların koloni oluşturma oranı yaklaşık % 57 olarak belirlenmiştir. Ticari firmadan alınan ana arıların oluşturduğu koloniler ortalama 60,8 ± 12,70 adet ana arı üretmişken doğadan toplanan ana arıların oluşturduğu koloniler hiç ana arı üretmemiştir. Üretilen işçi arı sayısı, erkek arı sayısı, sosyal fazdaki dönüşüm ve rekabet noktası doğadan toplanan ana arıların oluşturduğu kolonilerde sırasıyla ortalama 167,2 ± 34,90 adet, 98,6 ± 21,50 adet, 18,9 ± 2,37 gün ve 37,3 ± 2,68 gün, ticari firmadan alınan ana arıların oluşturduğu kolonilerde ise 225,3 ± 30,90 adet, 156,0 ± 42,60 adet, 25,0 ± 3,69 gün ve 50,8 ± 4,44 gün olarak belirlenmiştir.

Anahtar Sözcükler: Bombus arısı, *Bombus terrestris*, koloni gelişim özellikleri

Introduction

In recent years, the commercial use of insects for pollination has become prevalent (1). There is a steadily growing interest in bumblebees due to their pollination efficiency and the increasing quality and quantity of crops, especially in greenhouses (2,3). Bumblebees, of which 239 species have been determined, are used for the pollination of 25 different cultivated plants in more than 30 countries (4-6). In comparison to other bumblebee species, *Bombus terrestris* has the most year round reared species, because rearing is easier and their colony population is larger than the others. The year round rearing of this species was achieved in the Netherlands and Belgium due to the efforts of commercial companies approximately twenty years ago.

Some companies in Turkey import bumblebee colonies in cooperation with companies that practice year round rearing of *Bombus terrestris* in the Netherlands and Belgium. Only one company imports queens rather than colonies, and produces colonies from these queens and markets them. Approximately 20,000-25,000 *Bombus terrestris* colonies are used for pollination in greenhouses

in Turkey. It is estimated that the number of bumblebee colonies will reach 100,000 in the next ten years if the greenhouse areas in Turkey are taken into account.

The commercial year round rearing of *Bombus terrestris* has not yet been fully developed in Turkey. Early queen producing in the colonies, mating, managing diapause and the founding of new colonies are major problems during the year round rearing of bumblebees. Significant variations among the colonies are seen in the production time and number of queens, males and workers (7,8). Approximately 50 bumblebee species were determined by a few studies in Turkey (9-11). The most common species in Turkish natural flora is *Bombus terrestris*. This species is widely observed in the Mediterranean, Black Sea, Marmara and Aegean coastal regions. It is estimated that there are many bumblebee species, subspecies and ecotypes in these regions, which vary in ecology and habitat. Differences were also observed in the life cycles and colony development patterns of native *Bombus terrestris* queens collected from different regions. In the Mediterranean and Aegean coastal regions *Bombus terrestris* queens emerge from diapause in autumn (September-November), whereas in inland regions and in Europe they emerge from diapause in spring (February-March). Kaftanoğlu et al. (12), Yeninar (13), Yeninar and Kaftanoğlu (14), Gürel et al. (6) and Yeninar et al. (15) determined the colony development patterns of native *Bombus terrestris* collected from Aegean and Mediterranean coastal regions. However, no study has been performed to determine the colony development patterns of the imported colonies, which are used in the greenhouse sector in the Mediterranean Region.

This study aimed to compare the colony development patterns of *Bombus terrestris* queens taken from a commercial company and collected from the Mediterranean Region under the same environmental conditions. It has been reported that *Bombus terrestris* is widely distributed, has high adaptability, can take the place of the natural pollinators, can be hybridized by the local species and may assist in spreading of diseases and parasites (16). For this reason, their potentially harmful effects on the environment are being observed carefully (17). In East Mediterranean countries and islands two main taxa at subspecific level were recognized: *dalmatinus* Dalla Torre in the North of Italy, the former Yugoslavia and the whole Balkan Peninsula and *lucoformis* Kruger in Anatolia. However, these taxa are poorly differentiated and their correlation characters are particularly inconspicuous. It is difficult to draw a definite borderline and to separate these two subspecies. By 1996 there had been high imports of *Bombus terrestris* colonies into Turkey from different companies of which the origin of the colonies were not well known, but possibly these were the latest generations of the queens collected from the Balkan Peninsula and Agean Arcipelago (18). In this study, colony development patterns of native *Bombus terrestris* populations in the Mediterranean Region were determined before hybridizing. If these populations are thought to be used in breeding, the results will be used as a guide.

Materials and Methods

A total of 57 *Bombus terrestris* queens, which had emerged from diapause and had yet to establish a nest, were used in this study. 23 of these queens were collected from the *Arbutus unedo* L. and *Arburtus andhrance* L. in the Mediterranean coastal region in December, 2001. The other 34 queens were taken from a commercial company in Antalya, which imports queens and sells the colonies to the farmers. CO_2 was given to all queens in order to stimulate them. Subsequently each queen was placed separately in the starting box. One newly emerged *Bombus terrestris* worker and its pupa were put into the starting box together with the queen (19). These workers were substituted every five days until the first workers emerged. This study was carried out in a dark rearing room (28-30 °C, 60 - 65% R.H.) (20).

During the experiment, the queens and colonies were fed ad libitum with a sugar syrup (1:1 sugar / water) and fresh frozen pollen collected from *Sinapis arvensis, Cistus* spp. and *Papaver rhoeas* by honeybees (*Apis mellifera* L.). The pollen used for feeding contained approximately 20-22% crude protein (21). The nests were checked every day and the syrup and pollen were replaced when necessary.

A daily note was made of the queens: which laid eggs, or did not lay eggs or died. After the first workers emerged, the colonies were put into the larger rearing boxes. The number of workers and egg cells in the first, second and third broods, the time of the queens' first egg laying period, the time of emergence of the first worker,

switch and competition points in the social phase, total number of workers, males and queens produced, numerical sex ratio and the point at which a total of 50 workers was reached were determined in colonies by periodical observations. During the observations, the dead bees in the colonies were counted and noted in order to determine the number of workers, males and queens.

The time of the switch point and numerical sex ratio were calculated for all colonies as follows:

- Switch point = (time of first male emerging – time of first worker emerging) - development period of the male,
- Numerical sex ratio = the number of males / (the number of males + the number of queens)

The competition point between queens and workers was recognized by workers' behavior, such as egg robbing, egg laying and attacking the founder queen and by founder queen behavior such as oophagy.

The results were evaluated using the MINITAB (22) statistical program. Descriptive statistics relating to traits were given and the groups were compared using variance analysis.

Results

It is necessary that one healthy queen and a minimum of 50 workers exist in the *Bombus terrestris* colonies used for pollination in the greenhouses. In this study, the queens that reared healthy workers were accepted as though they had founded colonies. Colonies that produced 50 or more workers were evaluated as big colonies, and those with less than 50 workers as small colonies. 91% of the queens taken from the commercial company and 82% of the queens collected from the field laid eggs. 57% of the queens used in this study founded colonies. However, 34% of queens founded big colonies, which can be used in greenhouses. The egg laying and colony foundation ratios of the queens are shown in Table 1.

The number of workers and the produced egg cells observed in the colonies in first, second and third broods are shown in Table 2. The differences between the produced egg cells and number of workers in the first, second and third broods in the colonies, which were founded by the queens collected from the field and queens taken from the commercial company, was not significant. However, when examined at the colony level, the number of workers and produced egg cells are different in the first, second and third broods. The number of workers produced in the colonies in the second brood was 3 times and in the third brood 15 times higher than the first brood.

The other colony development patterns, which were observed in the colonies founded by the queens collected from the field and taken from the commercial company, are shown in Table 3. The queens collected from the field began egg laying on an average of 8.0 ± 0.65 days and the queens taken from the commercial company on an average of 9.4 ± 0.93 days. The average timing of the switch and the competition points in the social phase were 18.9 ± 2.37 days and 37.3 ± 2.68 days in the colonies founded by the field collected queens, and 25.0 ± 3.69 days and 50.8 ± 4.44 days in the colonies founded by the queens taken from the commercial company, respectively. While the observed differences in the switch points between the colonies founded by the queens from both groups were not significant, the observed

Table 1. Egg laying and colony foundation ratio of the queens.

	Egg laying						Not egg laying		Total
	Big colony		Small colony		No colony				
	n	%	n	%	n	%	n	%	n
Field	8	35	5	21	8	35	2	9	23
Com. company	11	32	8	23	9	27	6	18	34
Total	19	34	13	23	17	30	8	13	57

Table 2. The numbers of workers and produced egg cells in the first, second and third broods in colonies.

	Field		Com. company		Total	
	n	$\bar{X} \pm S\bar{x}$	n	$\bar{X} \pm S\bar{x}$	n	$\bar{X} \pm S\bar{x}$
Egg cells in 1st br.	13	4.9 ± 0.32	17	4.4 ± 0.23	30	4.6 ± 0.20
No. of workers in 1st br.	13	10.1 ± 0.84	17	8.4 ± 1.07	30	9.2 ± 0.99
Egg cells in 2nd br.	10	6.8 ± 0.90	15	8.9 ± 0.71	25	7.9 ± 0.58
No. of workers in 2nd br.	10	32.0 ± 6.82	10	42.0 ± 7.05	20	37.0 ± 4.91
Egg cells in 3rd br.	10	42.8 ± 5.19	15	38.6 ± 4.22	25	40.7 ± 3.23
No. of workers in 3rd br.	10	125.1 ± 27.10	10	175.0 ± 32.60	20	150.0 ±21.80

Table 3. Some colony development characteristics in the colonies from field and commercial company.

	Field		Com. company		Total	
	n	$\bar{X} \pm S\bar{x}$	n	$\bar{X} \pm S\bar{x}$	n	$\bar{X} \pm S\bar{x}$
Colony initiation (day)	21	8.0 ± 0.65	28	9.4 ± 0.93	49	8.7± 0.55
First worker emerging (day)	13	37.5 ± 1.67	17	35.7 ± 1.93	30	36.6 ± 1.30
First male emerging (day)	8	73.6 ± 4.01	12	65.2 ± 6.79	20	69.4 ± 4.39
*First queen emerging (day)	-	-	10	89.7 ± 6.33	10	89.7 ± 5.72
Switch point (day)	7	18.9 ± 2.37	7	25.0 ± 3.69	14	21.9 ± 2.27
*Competition point (day)	8	37.3 ± 2.68	11	50.8 ± 4.44	19	44.0 ± 3.21
Total number of workers	10	167.2 ± 34.90	11	225.3 ± 30.90	21	196.3 ± 23.50
Total number of male	7	98.6 ± 21.50	10	156.0 ± 42.60	17	127.3± 26.90
*Total number of queens	-	-	9	60.8 ± 12.70	9	60.8 ± 12.70
*Total number of individuals	10	236.2 ± 52.20	11	417.1 ± 39.40	21	326.7 ± 37.40
Numerical sex ratio	-	-	8	0.6 ± 0.09	8	0.6 ± 0.09
Time to reach 50 workers	6	61.5 ± 1.18	7	71.9 ± 4.31	13	66.7 ± 2.74

* Concerning these characteristics, significant differences were determined between the colonies founded by the queens collected from the field and those taken from the commercial company ($P < 0.05$).

differences in the competition points were significant ($P < 0.05$).

The colonies founded by the queens taken from the commercial company produced an average of 60.8 ± 12.70 queens. However, it was surprising that no queens were produced in the colonies founded by the queens collected from the field. A few queen pupae were seen in one colony only, but no adult queen emerged from them. In the colonies founded by the queens taken from the commercial company, a minimum of 9 and a maximum of 120 queens were produced. 6 out of 8 colonies founded by the queens collected from the field produced males only, 2 out of 11 colonies founded by the queens taken from the commercial company produced males only, one of them produced only queens and 8 of them produced both.

The colonies founded by the queens taken from the commercial company produced an average of 156.0 ± 42.60 males. Numerical sex ratio was determined to be an average of 0.6 ± 0.09 in these colonies. The colonies founded by the queens collected from the field produced an average of 98.6 ± 21.50 males. As no queens were produced in these colonies, the numerical sex ratio was not calculated. The difference between the number of males produced in the colonies founded by the queens collected from the field and those taken from the commercial company were not significant.

Colony life span and total pollen consumption in the colonies founded by the queens collected from the field were determined to be an average of 97.7 ± 2.52 days and 340.9 ± 61.90 g and in the colonies founded by the queens taken from the commercial company, an average

of 89.3 ± 7.13 days and 481.6 ± 46.80 g, respectively. The development period of workers was determined to be an average of 27.9 ± 0.75 days for all colonies.

Discussion

During the rearing of *Bombus terrestris*, the egg laying stage of the queens is the most important stage for colony foundation, since the food quality, the queen quality and environmental factors have an influence on the success of colony foundation and egg laying of the queens (7,8). The colony foundation ratio of the queens used in this study was determined to be approximately 57%. This foundation ratio resembles that given by Yeninar (13) and Gürel et al. (6) (51.2% and 50.3%, respectively). 50% of the colony foundation ratio in *Bombus terrestris* was accepted as successful by Heinrich (7). In commercial rearing, one of the main criteria is how many out of 100 queens can establish colonies of marketable quality (including a minimum of fifty workers). In our analysis, this value was found to be 35% for queens collected from the field and 32% for queens taken from the commercial company. Although accurate information is not available, this ratio is estimated to be less than 50% in the commercial companies.

The colony initiation time and the numbers of egg cells, males and workers produced in the first, second and third broods in the colonies founded by the queens collected from the field and taken from the commercial company resemble the parameters determined for *Bombus terrestris* (15,19,20,23). Switch points and competition points in the colonies founded by the queens collected from the field were seen to be earlier than in the colonies founded by the queens from the commercial company. Switching from worker production to male and queen production in the early stages is not a desirable trait in colonies for greenhouse use.

In this study, the colonies founded by the queens taken from the commercial company produced an average of 60.8 ± 12.70 queens. This value is in harmony with that of Gretenkord and Drescher (19). Beekman et al. (24) reported that the colonies produced an average of 128 queens. This is the reason why the number of produced queens varies between colonies and this variation is influenced by a large number of factors. Yeninar and Kaftanoğlu (14) and Yeninar et al. (15) reported that the colonies faunded by the queens collected from the field produced an averaged of 31.3 ± 4.61 and 27.8 ± 9.1 queens, respectively. However, it was surprising that no queen was produced in the colonies founded by the queens collected from the field in our study. This is a disadvantage for the production of breeder queens. On the other hand, in nature, *Bombus terrestris* produce queens in colonies in nature for the continuation of their species. The results of the experiments showed that the native population has a lesser tendency for queen production and there is a large variation in queen production.

Significant variations were seen in the numbers and times of queen, male and worker production (7,8). While some colonies produce only males or queens, some colonies can produce both. Many factors such as worker intensity in the nest, worker / larva ratio, diseases and harmful, environmental conditions inside and outside the nest, food availability and the quality of the founder queen have influences on male and queen production time, their number and the colony life cycle. Generally, when the worker population of the colony reaches its highest point, male and queen production begins, yet males and queens can also be produced at the beginning of the colony development. Pollen quality also has an effect on queen and male production (25). In this study, however, as all colonies were fed with the same pollen, no influence was expected with regard to pollen quality.

As a result, it is desirable to have strong, long lasting colonies, which will produce queens and males in the later stages for pollination in the greenhouses. The colonies producing the most males and queens can be selected as breeding colonies for year round rearing. It is presumed that the commercial companies have been making selection over the last 15 years. No differences were found between the egg laying and colony foundation of queens from the native population and those from commercial companies. Selection is thought to have an influence on the colony size and observed differences in the number of queens.

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