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Effects of Rearing Periods on Some Reproductive Characteristics of Caucasian (*Apis mellifera caucasica*) Queen Bees

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Abstract: This research was carried out to determine some reproductive characteristics such as larval acceptance rate, length of queen cell, weight at emergence, mating rate and pre-oviposition period of Caucasian race honey bee queens *(Apis mellifera caucasica*). It was conducted in the province of Ardahan in the months of June, July and August between the years of 2013-2015. In this study, Caucasian race bee colonies were used. The larvae were grafted by the Doolittle's method were given to the starter colonies which were prepared without the queen. In terms of larva acceptance rate, the difference between the years was found to be insignificant, while the difference between the months was found significant (P<0.01). The average length of the queen cells obtained from the research colonies was determined as 30.11 ± 0.039 mm, while the average length of the queen cells between the years of 2013-2015 were found to be 33.43 ± 0.056 mm, 25.87 ± 0.050 mm and 32.53 ± 0.051 mm respectively. In the study, the average weight at emergence of queen bees was determined as 203.34 ± 1.97 mg, while the averages between the years of 2013-2015 were detected to be 214.42 ± 3.68 mg, 188.37 ± 2.75 mg and 207.25 ± 2.83 mg respectively. The effect of both months and years on the weight at emergence was found significant (P<0.01). The effect of years on pre-oviposition period was found to be insignificant (p> 0.05) while the effect of months was found to be significant (P<0.01).

Keywords: *Apis mellifera caucasica*, Queen Bee rearing periods, Reproduction characteristics.

Kafkas Irkı (*Apis mellifera caucasica*) Ana Arılarının Bazı Üreme Özellikleri Üzerine Yetiştirme Dönemlerinin Etkisi

Öz: Bu araştırma Kafkas ırkı ana arılarının (*Apis mellifera caucasica*) larva kabuloranı, kapalı yüksük uzunluğu, ana arıçıkış ağırlığı, çiftleşme oranı ve yumurtlama öncesi süre gibi bazı üreme özelliklerini belirlemek amacıyla yapılmıştır. Araştırma, Ardahan ilinde, 2013, 2014 ve 2015 yıllarının Haziran, Temmuz ve Ağustos aylarında yürütülmüştür. Bu çalışmada Kafkas ırkı arı koloniler kullanılmıştır. Doolitle yöntemiyle transfer edilen larvalar ana arısız olarak hazırlanan başlatıcı kolonilere verilmiştir. Larva kabul oranı bakımından yıllar arasındaki farklılık önemsiz bulunurken, aylar arasındaki farklılık önemli bulunmuştur (P<0.01). Araştırma kolonilerinden elde edilen kapalı ana arı yüksüklerinin ortalama uzunluğu 30.11 ± 0.039 mm belirlenirken, 2013, 2014 ve 2015 yıllarına gore ortalaması ise sırası ile 33.43 ± 0.056 mm, 25.87±0.050 mm ve 32.53±0.051 mm olarak belirlenmiştir. Araştırmada ana arıların ortalama çıkışağırlığı 203.34 ± 1.97 mg olarak tespit edilirken, 2013, 2014 ve 2015 yıllarına gore ortalamaları ise sırasıyla 214.42 ± 3.68 mg, 188.37±2.75 mg ve 207.25±2.83 mg olarak tespit edilmiştir. Ana arı çıkış ağırlıklarına üzerine hem yılların ve hem de ayların etkisi önemli bulunmuştur (P<0.01). Ana arıların yumurtlama öncesi sure üzerine yılların etkisi önemsiz bulunurken (P>0.05), ayların etkisi önemli bulunmuştur (P<0.01).

AnahtarKelimeler: *Apis mellifera caucasica,* Ana arı yetiştirme dönemleri, Üreme özellikleri.

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INTRODUCTION

Caucasian bee race has a great importance in bee rearing in Turkey and the world regarding being one of the most productive 4 bee races recognizedin the world. In bee rearing, the queen bee is the most important individual in the colony and has the first-degree liability for the future of the colony. Therefore, the profit to be made from a colony by a beekeeper depends on the quality of the queen bee that manages the colony (1). Many environmental factors affect the quality of the queen bee in queen bee rearing. These factors include the age of the transferred larvae, origin of the larvae, young worker bees and food supplies of starter and finisher colonies, and mating of the queen bee with a sufficient number of male bees (1-3).

One of the factors to produce a queen bee of high quality is the age of larvae used for implantation. In a study where eggs and 1, 2, 3 or 4-day old larvae were implanted; a reduction was found in the body weights of queen bees, the size of spermatheca diameters, and the number of ovarioles depending on the increase of the implanted larva age (4).

It has been reported that the queen bees reproduced from eggs had higher body weights at emergence than the ones reproduced from 1-day larvae, however the difficulty of the process and the low acceptance rates made the method of larva transfer superior in queen bee rearing (5). In most of the studies conducted on the queen bee's body weight at emergence, it has been reported that the body weight at emergence can be considered a quality and selection criterion (6-10).

Breeding value of the queen bee is not only related to its age but also the period it is bred and the methods used for its breeding. An ideal breeding period for queen bees with high breeding value is the swarming season. In a study, it has been stated that the queen bees bred in the swarming season had higher body weights at emergence (11). The periods when nectar and pollen are sufficient, the number of sexually mature male bees is abundant and climatic conditions are convenient for bees continuously flying in regions where apiculture is performed are indisputably more convenient for breeding queen bees of good quality. Despite all this, it is recommended to feed the breeding colonies constantly (12-14).

Pre-oviposition period of queen bees is the expression of the period, which is stated in terms of days, between the emergence of the queen bee and the date they start to lay eggs, and the length of this period varies depending on environmental and genetic factors. It is reported that the period of starting to lay eggs of queen bees varies between 4 and 22 days depending on seasons (15). In studies conducted on the queen bees' period of starting to lay eggs, this period has been reported to be an average of 10.36 and 12.30 days (10, 16). It has been stated that mating rates of bred queen bees depend on climatic conditions, natural enemies, the topographic structure of the region, the color of mating hives and their array in the apiary (17).

The purpose of this study was to analyze the Caucasian queen bees bred in the city of Ardahan, which is an isolated region, regarding some reproduction characteristics such as larva acceptance rates, the length queen cells, body weights of queen bees at emergence, mating rates and periods before laying eggs.

MATERIALS and METHODS

The study was conducted in the city of Ardahan in three separate periods in the months of June, July and August in the years of 2013, 2014, and 2015. Caucasian bee race (*Apis mellifera caucasica*) colonies were used for the study. Larvae transferred

through Doolittle method were distributed to starter colonies prepared without queen bees (2). 0-24-hour old larvae taken from the breeding colony were implanted on the royal jelly diluted at the rate of 1:1. In each period, 3 starter colonies were prepared, and 20 larva transfers were made to each of them. During the experiment, colonies breeding queen bees were constantly fed with sugar syrup at a rate of 1:1. 60 larvae (3 x 20) in each year and a total of 180 larvae in 3 periods (3 x 60) were transferred. The queen cells sealed by the bees (queen cell cups) were encaged and transferred to an incubator with an internal temperature of 33 ± 1 °C and proportional moisture of 60-65% for emergence (18, 19). Queen bees completing their emergences in the incubator were weighed with a sensitivity of 0.001 and their emergence body weights were determined. Queen bees with an emergence body weight less than 180 mg have been out and a total of 60 queen bees were randomly distributed to mating boxes with three frames every year. Queen bees distributed to mating boxes for naturally-occurring mating were controlled twice a day every day after the sixth day and their dates of laying eggs and mating rates were determined.

Measurements related to larva acceptance rate, the length queen cells, body weights of queen bees at emergence, mating rates and pre-oviposition period were performed, and reproduction characteristics of the Caucasian race were determined in this study (10, 20).

Statistical Analysis

During the statistical analysis of the obtained data; larva acceptance rates and mating rates were compared according to Kruskal-Wallis test; on the other hand, the average queen cell lengths, body weights at emergence and the pre-oviposition periods of queen bees were compared according to one-way analysis of variance. LSD test was used for comparison of mean values. All the statistical analyses were performed with the SPSS 16.0 (2007) software package (21).

RESULTS

Table 1 illustrates larva acceptance rates of Caucasian queen bees according to different years and months. The difference between years and months in terms of larva acceptance rates were statistically significant ($P<0.01$). Larva acceptance rate was found to be higher in June than July and August. As the result of the Kruskal-Wallis test which has been done to determine whether the acceptance queen bee larvae show a meaningful difference or not, according to the years and months, the larva rate is statistically significant ($P<0.05$). The queen bee emergence rate of the accepted larva was determined to be 100.00% according to years and months.

Sealed queen bee cells produced through controlled larva transfer every year were measured. The average length of closed queen bee cells of the experiment colonies was 33.43 ± 0.056 mm in 2013; on the other hand, this average value was determined to be 25.87 ± 0.050 mm in 2014 and 32.53 ± 0.051 mm in 2015 (Table 2). It was determined that the queen bee cells in 2013 and 2015 were significantly longer than the cells in 2014. The effect of both years and months on the queen bee cell length was found to be significant in the analysis of variance applied to queen cell length values ($P<0.01$). Average Sealed Queen Cell Lengths decreased during the period from June to August.

Table 1. Larva acceptance and emergence rates of Caucasian queen bees according to different years and months (%).
Tablo 1. Kafkas ırkı ana arıların farklı yıllara ve aylara göre larva kabul ve çıkış oranları (%).

Years	Months	Inoculated Larva (item)	Accepted Larva (item)	Larva Acceptance Rate (%)	Larva Acceptance P	Queen Bee Emergence Rate (%)	Average Yearly Larva Acceptance Rate (%)
2013	June	40	38	95.00[a]	0.001	100	83.33
	July	40	33	82.50[b]		100	
	August	40	29	72.50[c]		100	
2014	June	40	35	87.50[a]		100	75.83
	July	40	30	75.00[b]		100	
	August	40	26	65.00[c]		100	
2015	June	40	37	92.50[a]		100	80.83
	July	40	32	80.00[b]		100	
	August	40	28	70.00[c]		100	
General		360	288	80		100	79.99

	Years	N	Mean Rank	Chi-square	df	P
Larva Acceptance Rate	2013[a]	60	110.50	17.900	2	0.001
	2014[b]	60	70.50			
	2015[c]	60	90.50			
	Months					
	June[a]	60	150.50	161.100	2	0.001
	July[b]	60	90.50			
	August[c]	60	30.50			
	Years* Months					0.000

a, b, c: The differences between the rates in the same column with different letters are significant (P<0.01)

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Table 2. The average length of sealed queen cell and emergence weight of Caucasian queen bees according to different years and months.
Tablo 2. Kafkas ırkı ana arıların farklı yıllara ve aylara göre ortalama yüksük uzunluğu ve çıkış ağırlığı.

Years	Sealed Queen Cell (item)	Average Sealed Queen Cell Lengths (mm)				
		June $\overline{X} \pm S_{\overline{X}}$	July $\overline{X} \pm S_{\overline{X}}$	August $\overline{X} \pm S_{\overline{X}}$	Yearly Average $\overline{X} \pm S_{\overline{X}}$	P
2013	60	36.10 ± 0.045[a]	34.80 ± 0.054[a]	29.40 ± 0.107[b]	33.43 ± 0.056[a]	0.001
2014	60	29.40 ± 0.029[a]	26.10 ± 0.039[b]	22.10 ± 0.081[c]	25.87 ± 0.050[b]	0.000
2015	60	36.30 ± 0.035[a]	32.20 ± 0.041[b]	29.10 ± 0.084[c]	32.53 ± 0.051[a]	0.000
General	180	33.93 ± 0.047	31.03 ± 0.054	26.87 ± 0.068	30.61 ± 0.039	
		Average Body Weight at Emergence (mg)				P
2013	60	225.35 ± 3.11[a]	222.65 ± 2.87[a]	195.25 ± 8.89[b]	214.42 ± 3.68[a]	0.001
2014	60	206.10 ± 2.66[a]	187.90 ± 2.34[b]	171.10 ± 5.06[c]	188.37 ± 2.75[b]	0.000
2015	60	224.20 ± 2.55[a]	210.95 ± 2.27[b]	186.60 ± 4.98[c]	207.25 ± 2.83[a]	0.000
General	180	218.55 ± 1.95	207.17 ± 2.36	184.32 ± 3.93	203.34 ± 1.97	

a, b, c: The averages in the same row and column are different (P < 0.01).

Body weights at the emergence of queen cells bred in different years were determined as mg. The average body weights at emergence in experiment colonies according to years were respectively 214.42 ± 3.68 mg, 188.37 ± 2.75 mg, and 207.25 ± 2.83 mg (Table 2). As a result of the analysis of variance applied to the body weights of queen bees at emergence, the effect of both years and months on the body weights at emergence was found to be significant (P<0.01). Average Body Weight at Emergence decreased during the period from June to August.

Table 3. The values related to the matingrates (%) of Caucasian queen bees according to different years and months.
Tablo 3. Kafkas ırkı ana arıların farklı yıllara ve aylara göre çiftleşme oranlarına (%) ilişkin değerler.

Years	Queen Bee N	June	July	August	$\overline{X}$
2013	60	80.00[a]	70.00[b]	75.00[ab]	75.00
2014	60	75.00[a]	65.00[b]	70.00[ab]	70.00
2015	60	80.00[a]	70.00[b]	75.00[ab]	75.00
General	180	78.33	68.33	73.33	73.33

	Years	N	Mean Rank	Chi-square	df	P
Mating Rates	2013[a]	60	107.17	40.315	2	0.001
	2014[b]	60	57.17			
	2015[a]	60	107.17			
	Months					
	June[a]	60	143.83	137.610	2	0.001
	July[b]	60	37.17			
	August[c]	60	90.50			
	Years* Months					0.000

a, b: The averages in the same row and column are different (P < 0.01).

According to Table 3, queen bee mating rates, 20 queen bees for every 3 periods and therefore 60 queen bees at total were delivered to mating boxes every year. A total of 180 queen bees were assessed within three years, and 132 of them were fertilized.

In the conducted rate test, the effect of years and months on mating rate were found statistically significant (P<0.01).The data related to the average pre-oviposition periods of queen bees were 11.55 ± 0.45, 13.85 ± 0.49, 12.40 ± 0.39 days in 2013, 11.35 ± 0.47, 15.60 ± 0.63, 12.50 ± 0.58 days for 2014 and 11.70 ± 0.42, 14.40 ± 0.52, 13.10 ± 0.40 days for 2015 for the months of June, July and August respectively (Table 4). The average pre-oviposition period of the Caucasian race was 12.93 ± 0.18 days. While the effect of years on the pre-oviposition period of queen beeswas insignificant (P>0.05), the effect of months on the pre-oviposition period of queen bees was significant (P<0.01). The longest pre-oviposition period was determined in July; whereas the shortest period was found in June.

Table 4. Pre-oviposition period (day) of queen bees bred in different times.
Tablo 4. Farklı dönemlerde yetiştirilen ana arıların yumurtlama öncesi süreleri (gün).

Years	Queen Bee (n)	June $\overline{X} \pm S_{\overline{X}}$	July $\overline{X} \pm S_{\overline{X}}$	August $\overline{X} \pm S_{\overline{X}}$	$\overline{X}$	P
2013	45	11.55 ± 0.45[b]	13.85 ± 0.49[a]	12.40 ± 0.39[b]	12.60 ± 0.28	0.002
2014	42	11.35 ± 0.47[b]	15.60 ± 0.63[a]	12.50 ± 0.58[b]	13.15 ± 0.40	0.000
2015	45	11.70 ± 0.42[c]	14.40 ± 0.52[a]	13.10 ± 0.40[b]	13.06 ± 0.29	0.000
General	132	11.53 ± 0.25	14.62 ± 0.32	12.67 ± 0.27	12.93 ± 0.18	

a, b, c: The averages in the same row differ from each other (P<0.01).

DISCUSSION and CONCLUSION

The general mean of larva acceptance rate of caucasian colonies was 79.99% in this study. The larva acceptance rate was lower than a larva acceptance rate of 95.00% reported by Cengiz et al (22) for Caucasian colonies whereas it was found to be higher than the larva acceptance rate of 71.67% stated by Arslan and Hangir (23) for *Apis mellifera caucasica* and it showed similarity with the average larva acceptance rate of 78.32% reported by Şahinler and Kaftanoğlu (24) for *Apis m.caucasica.*

The difference observed in the implantation performance values of the study in terms of months is thought to be arising from the fact that honeybees focus more on honey storage instead of breeding offspring with the change of seasons.

The average sealed queen bee cell lengths in the colonies analyzed in the study were respectively found to be 33.43, 25.87 and 32.53 mm in the years of 2013, 2014, and 2015. The obtained results showed that colonies constructed queen cells with different lengths in different years. The difference between queen cell lengths showed that colony condition, climate, and flora affected the constructed queen cell lengths. The average queen cell length of 30.11 mm obtained from the study according to years showed similarity with the value of 30.82 mm obtained from colonies queenless for *Apis mellifera ligustica, Apis mellifera carnica,* and Buckfast bees (25).

In a study (6) where the average value of queen body weight obtained from experiment colonies through controlled reproduction methods was 201.27 mg, the average values reported for the queen bees bred from 0, and 1 day old larvae were found to be between 209 ± 2.40 mg and 189 ± 1.00 mg. According to obtained results, the effect of both years and months on the body weight at emergence was significant ($P<0.01$). Body weights of queen bees at emergence and colony condition decrease or increase depending on the climate and flora.

132 of 180 queen bees in total transferred to mating boxes mated and the average mating rate was 73.33%. While the highest mating rate was 78.33% in June, the lowest mating rate was 68.33% in July. The mating rate of 73.33% obtained from the study was higher than the average mating rate of 71.60% reported by Kaftanoğlu et al. (12) for *Apis mellifera L.* but it was lower than the average mating rate of 75% determined Güler et al. (26) for *A. m. caucasica* and *A. m. anatoliaca*. The obtained results are thought to be arising from the difference of breeding regions. As a matter of fact, it has been reported that weather conditions, the number of male bees in mating regions and the age of queen bee affect the mating success of sexually mature queen bees (27, 28).

No difference was observed between years in terms of the average periods before laying eggs among queen bees bred through controlled methods and allowed to mate naturally. Generally, A tendency of laying eggs earlier was found in June in all years. It is thought to be related to the climate of the region.

The average pre-oviposition period of the Caucasian race was 12.93 ± 0.18 days. This obtained result shows similarity with values reported to be an average of 12.15 ± 0.39 days among bees bred through controlled methods and an average of 12.36 ± 0.43 days for queen bees obtained from natural queen cells by a study previously conducted in the region of Erzurum (29) for Apis *mellifera L.,* and the value reported to be an average of 11.80 ± 0.533 days received through single implantation conducted by Yazıcı and Kırmızıbarak (30) for Caucasian colonies in the region of Ardahan.

In conclusion, the larva acceptance rates, queen cell lengths, body weights of queen bees at emergence and pre-oviposition period varied according to the breeding periods of Caucasian queen bees bred in different years and different months in the city of Ardahan; on the other hand, no significant difference was observed in terms of mating rate. Since the city of Ardahan is a region where Caucasian bees are isolated, the queen bees bred in this region are in great demand from other regions. Generally speaking, even though a low level

of activity was observed in characteristics such as larva acceptance rates and body weights of queen bees at emergence in August when queen bee breeding is not possible in many regions, it can be asserted that this period is convenient for queen bee breeding under the conditions of Ardahan. However, if body weights at emergence are considered a reliable index regarding queen bee quality according to these results, it is more appropriate to perform queen bee breeding in the months of June and July in the city of Ardahan for a reproduction of higher quality.

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