TÜBİTAK

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Effects of queen rearing period on reproductive features of Italian (*Apis mellifera ligustica*), Caucasian (*Apis mellifera caucasica*), and Aegean ecotype of Anatolian honey bee (*Apis mellifera anatoliaca*) queens

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Abstract: This study was conducted to determine the effects of rearing period on the quality characteristics of honey bee queens reared from Italian and Caucasian races and from the Aegean ecotype of Anatolian honey bee under the conditions of the Aegean region. Italian (I), Caucasian (C), and Aegean (A) queens were reared in April, June, and August of 2007 and 2008. The queens were weighed on the second day after emergence (WQ) and at the onset of oviposition (OWQ). The interval from emergence to the onset of oviposition (preoviposition period, POP) was determined in 3 genotype queen groups. The numbers of spermatozoa in the spermathecae (SN) were also measured after the queens started oviposition. The rearing period affected the weight of the queens at 2 days after emergence (WQ; $P < 0.01$) and at the onset of oviposition (OWQ; $P < 0.01$), the POP ($P < 0.01$), and the SN ($P < 0.01$). The differences in mean values of WQ, POP, and SN among genotypes were found to be statistically significant ($P < 0.05$, $P < 0.01$, and $P < 0.05$, respectively), whereas the OWQ was not affected by genotype. Our results showed that the quality of queens was affected by the rearing period, decreasing gradually from April to August.

Key words: *Apis mellifera ligustica, Apis mellifera caucasica,* Aegean ecotype, reproductive features, queen rearing periods

İtalyan (*Apis mellifera ligustica*), Kafkas (*Apis mellifera caucasica*) ırkları ve Anadolu arısı Ege ekotipi (*Apis mellifera anatoliaca*) ana arılarının üreme özellikleri üzerine yetiştirme dönemlerinin etkisi

Özet: Bu çalışmada, Ege Bölgesi koşullarında ana arı yetiştirme dönemlerinin Anadolu arısı Ege Ekotipi, Kafkas ve İtalyan ırkı ana arılarında üreme özellikleri üzerine etkileri belirlenmiştir. Bunun için 2007-2008 yılının Nisan, Haziran, Ağustos aylarında 3 genotipten yetiştirilen ana arılarda 2. gün canlı ağırlıkları, yumurtlama başlangıcı ağırlıkları, yumurtlama öncesi süreler ve sperm sayıları belirlenmiştir. Yetiştirme periyodu; ana arıların 2. gün canlı ağırlıklarını ($P < 0,01$), yumurtlama başlangıcı ağırlıklarını ($P < 0,01$), yumurtlama öncesi sürelerini ($P < 0,01$) ve sperm sayılarını ($P < 0,01$) etkilemiştir. Genotiplerin 2. gün ağırlıkları, yumurtlama öncesi süreleri ve sperm torbalarındaki sperm sayıları farklı bulunurken (sırasıyla $P < 0,05$, $P < 0,01$ ve $P < 0,05$), yumurtlama başlangıcı ağırlıkları farklı bulunmamıştır. Sonuçlar, yetiştirme periyodunun ana arı kalitesini etkilediğini, Nisan ayından Ağustos ayına doğru kalitenin azaldığını göstermiştir.

Anahtar sözcükler: *Apis mellifera ligustica, Apis mellifera caucasica*, Ege ekotipi, üreme özellikleri, ana arı yetiştirme dönemleri

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Introduction

Racial and environmental aspects are the fundamental factors affecting the reproductive features of queen honey bees. The main environmental factors determining the quality of a queen are season, climate, supply of food, age of grafted larvae, and condition of rearing colonies (1-6). The live weight of the queen, the length and volume of the queen cell, the number of ovarioles, the diameter of the spermatheca, and the number of spermatozoa in the spermatheca were reported by several authors (1,7,8) as the quality criteria of a queen.

Fresnaye (9) demonstrated that the rearing season and meteorological conditions influenced the rate of development and the age at which the queens reach sexual maturity. Shawer et al. (10) found that the body weight of virgin queens differed significantly depending on the rearing season. They also reported that queens produced in Egypt during May and August were heavier than those reared in other months. Similarly, Kaftanoğlu and Kumova (4) reported that commercial queen rearing in southern Turkey can be more successful and economical in April and May because of the abundance of nectar and pollen producing plants in the region.

The Anatolian honey bee (*Apis mellifera anatoliaca*, A), the widest honey bee group in Anatolia, comprises a number of ecotypes with different morphological and behavioral characteristics (11-17). The morphological structure and brood rearing pattern of the Aegean ecotype are different from those of other ecotypes of the Anatolian honey bee (16,18-22). It was reported that the Aegean ecotype had more reproduction activity (12,17,18,23,24) and more honey production (17,18) than those of other Anatolian ecotypes.

The Caucasian honey bee (*Apis mellifera caucasica*, C) is one of the original Anatolian honey bee races. This race is known to have a long tongue, extreme use of the propolis, a reluctance to swarm, and honey production. The third honey bee race examined in this research, the Italian honey bee (*Apis mellifera ligustica*, I), is known for its ability to adapt to different climatic conditions, its docility, and high honey yield. This race has been distributed to several countries during the last century. In Turkey, some beekeepers in the Aegean region have also recently preferred to use Italian queen bees.

Güler and Alpay (25) reported that the C, A, and I genotypes attained sexual maturity more rapidly during the period between 15 July and 15 August than the periods between 15 June and 15 July or 15 May and 15 June under the conditions of the central Anatolia region. They reported the mean values of the preoviposition periods of the A, I, and C genotypes as 12.1, 12.3, and 13.0 days and the mean values of the number of spermatozoa in the spermathecae as 5.80, 5.99, and 5.08 million, respectively. They also found that the queens reared in the period between 15 July and 15 August had more spermatozoa in their spermathecae than those reared in the periods between 15 May and 15 June or 15 June and 15 July.

Kahya et al. (26) observed the changes in body weight of Caucasian queens during pre- and postmating periods, weighing them at ages ranging from emergence to 40 days. They found that the weight of queens decreased continuously from emergence to mating. After mating, queens started to gain weight to oviposit, and they started oviposition when they exceeded their weight at emergence.

The objective of this study was to compare some reproductive traits of the Italian, Caucasian, and Aegean ecotype of Anatolian honey bee queens and to determine the effects of rearing period on reproductive features of queen bees under the subtropical conditions of the southern Aegean region in Turkey.

Materials and methods

The experiment was conducted in April, June, and August of 2007 and 2008 at the Apicultural Unit of Adnan Menderes University, Aydın, Turkey. Three genotypes, I, C, A, were used. The virgin queens were reared from each breeder colony (I, C, and A) using the single grafting method (27) in April, June, and August of both in 2007 and 2008. The larvae used for rearing the queens were less than 1 day old. A closed swarm box was used for starting the queen cells and 3 genotypes were also used as a starter colony. To eliminate the effect of a nursing colony, the genotype of the starter colony was changed according to the period and the year. Shaken into the swarm box, were 1 kg of young worker bees. From each genotype, 10 worker larvae were grafted. A frame containing 3 cell

bars, each with 10 grafted larvae in wax cell cups, was placed in the swarm box. The starter colony contained I (10 larvae), C (10 larvae), and A (10 larvae) genotypes. To eliminate the effect of location of larvae, the locations of the genotypes were changed for each rearing period. As a result, all genotypes were placed in the frame at upper, middle, and lower sections. This practice was also repeated the following year. The larvae remained in the starter hive for 24 h and then the cell bars were transferred to the finisher colony. The Aegean ecotype was used as a finisher colony. Mature queen cells were introduced into newly established Kirchainer mating nuclei 10 days after grafting. All queens emerged from the queen cells on the second day after the cells were introduced into the mating nuclei. In order to identify the emergence of queens, mating nuclei were inspected regularly. The virgin queens were weighed when they were about 2 days old. To determine the onset of oviposition dates, the mating nuclei were checked regularly for the first 5 days after emergence. When the queens started oviposition, they were weighed and then dissected to remove their spermathecae so that the spermatozoa could be counted.

The data were analyzed by one-way ANOVA using MINITAB (13.0) software.

Results

Weight of queens at different ages

The weight of 2-day-old queens varied depending on the rearing period. The mean weights of 2-day-old queens decreased from April to August. The queens reared in August were significantly lighter than the queens reared in April and June ($P < 0.05$, Table 1).

The queens reared from the C genotype were lighter than the queens reared from both the I and A genotypes at 2 days of age. Differences in weights of 2-day-old queens reared from the A, I, and C genotypes were found to be statistically significant ($P < 0.05$, Table 1).

The weight of queens at the onset of oviposition was affected by the rearing period ($P < 0.01$, Table 1). The differences in weight at the onset of oviposition between genotypes, however, were not found to be statistically significant (Table 1). The year factor also did not affect the weight of queens at the onset of oviposition (Table 1).

Preoviposition period

The interval from emergence to onset of oviposition was affected by the rearing period ($P < 0.01$, Table 2), genotype ($P < 0.01$, Table 2), and year

Table 1. Weights (mg) of Caucasian, Aegean, and Italian queens reared in April, June, and August of 2007 and 2008.

Factor	Weight at 2 days after emergence		Weight at onset of oviposition	
	N	Mean ± S.E.	N	Mean ± S.E.
Genotype				
Caucasian	50	168.38 ± 1.028 ^{Aa}	43	216.08 ± 1.314^{Aa}
Aegean	54	171.73 ± 0.984 ^{Ab}	51	219.84 ± 1.185^{Aa}
Italian	53	171.31 ± 0.989 ^{Aab}	53	218.85 ± 1.155^{Aa}
Year				
2007	74	170.51 ± 0.843 ^{Aa}	69	218.64 ± 1.029^{Aa}
2008	83	170.44 ± 0.791 ^{Aa}	78	217.87 ± 0.962^{Aa}
Period				
April	59	173.30 ± 0.933 ^{Aa}	58	222.22 ± 1.100^{Aa}
June	50	171.09 ± 1.020 ^{Aba}	47	218.22 ± 1.238^{ABb}
August	48	167.04 ± 1.046 ^{Bb}	42	214.33 ± 1.312^{Bb}

Different uppercase letters denote significant differences at the $P < 0.01$ level and different lowercase letters denote significant differences at the $P < 0.05$ level.

Table 2. Preoviposition periods (days) and the number of spermatozoa (×10^6) in Caucasian, Aegean, and Italian queens reared in April, June, and August of 2007 and 2008.

Factor	Preoviposition period (days)		Number of spermatozoa (×10^6)	
	N	Mean ± S.E.	N	Mean ± S.E.
Genotype				
Caucasian	46	12.46 ± 0.151 ^{Aa}	19	4.24 ± 0.599^{Aa}
Aegean	53	10.99 ± 0.138 ^{Bb}	19	4.43 ± 0.599^{Aab}
Italian	53	11.12 ± 0.137 ^{Bb}	19	4.44 ± 0.599^{Ab}
Year				
2007	71	11.68 ± 0.120 ^{Aa}	29	4.51 ± 0.489^{Aa}
2008	81	11.37 ± 0.112 ^{Ab}	28	4.23 ± 0.489^{Bb}
Period				
April	59	10.81 ± 0.129 ^{Aa}	18	4.69 ± 0.599^{Aa}
June	48	11.37 ± 0.145 ^{Ab}	21	4.28 ± 0.599^{Bb}
August	45	12.39 ± 0.151^{Bc}	18	4.15 ± 0.599^{Bb}

Different uppercase letters denote significant differences at the P < 0.01 level and different lowercase letters denote significant differences at the P < 0.05 level.

(P < 0.05, Table 2). The queens reared in both April and June started oviposition significantly earlier than the queens reared in August (Table 2). The Caucasian queens started oviposition significantly later than either the A or I queens (Table 2).

Number of spermatozoa in spermathecae

The rearing period (P < 0.01, Table 2), genotype (P < 0.05, Table 2), and year (P < 0.01, Table 2) affected the number of spermatozoa in the spermathecae of queens. The C queens had significantly less spermatozoa (P < 0.05) than either the A or I queens. The number of spermatozoa entering the spermathecae decreased from April to August. The highest mean number of spermatozoa was determined in the spermathecae of queens reared in April (Table 2).

Discussion

In the present study, the mean weight of queens and the mean number of spermatozoa were lower, and the preoviposition period was longer, than in the results of some other studies conducted in Turkey (5,6,25,28). This result could be due to the unfavorable climatic conditions in 2007 and 2008 during the queen rearing periods and the environmental and climatic differences among the regions.

In this study, we found that the weight after emergence (WQ) for the A genotype was similar to that of the I genotype, but it was different from the C genotype (P < 0.05). We found that there was no statistically significant difference between the mean weight at the onset of oviposition (OWQ) values of the genotypes (P > 0.05). However, the rearing period had a statistically significant effect on the OWQ (P < 0.01). It was determined that the queens reared in April were heavier than those reared in June or August. Kahya et al. (26) demonstrated that during the maturation period, from emergence to mating, queens lost weight and had similar weights at the time of mating, despite the weight differences at emergence. Kahya et al. (26) also added that the age at which the queens reach sexual maturity was not affected by the queen's weight at emergence.

In the present study, the shortest preoviposition period (POP) was recorded in April (10.81 days) and the longest in August (12.39 days). Güler and Alpay (25) found the longest POP between 15 June and 15 July (13.81 days) and the shortest between 15 July and 15 August (11.86 days). According to the results of the present study and the results of Güler and Alpay (25), it can be said that the POP is affected strongly by regional climatic conditions. Fresnaye (9) already mentioned that rearing season and

meteorological conditions had a significant influence on the age at which queens reach sexual maturity. Lensky and Demter (29) reported that the mating flights of queens and drones occurred at between 26 °C and 35 °C. Furthermore, Ruttner (30) stated that hardly any mating flights occurred in the morning, in spite of good weather conditions. In the present study, temperatures in the southern Aegean region usually ranged higher than 35 °C in July and August, especially during queen mating times; possible mating times in the region were therefore restricted due to the high temperatures. Therefore, it can be said that the POP was extended in August.

In the present study, we found that the POP of the C queens (12.46 ± 0.151 days) was 1.47 and 1.34 days longer than those of the A and I genotypes, respectively. These differences were found to be statistically significant ($P < 0.05$). Similarly, Güler and Alpay (25) found that the queens reared from the Italian and western Anatolia (Muğla) races had a shorter POP than other genotypes (*A. m. caucasica*, *A.m. carnica*, central Black Sea). The POP of the C queens in this study was longer than that found by Kahya et al. (26), but shorter than that found by Güler and Alpay (25). Because the Italian and Aegean ecotype of the Anatolian honey bee have adapted to a mild climate, their POPs could be shorter than those in a cold climate, as was the case with the Caucasian race.

The mean numbers of spermatozoa in the spermathecae of all of the queens from the 3 genotypes were lower than those reported by Güler and Alpay (25) and Kahya et al. (26). However, our findings, showing that the Italian queens had the highest number of spermatozoa and that the Caucasian queens had the lowest, are in agreement with the findings reported by Güler and Alpay (25). On the other hand, the mean number of spermatozoa of the A queens found in this study was similar to that reported by Koç and Karacaoğlu (22).

In the present study, queens reared in April had the highest spermatozoa number, and the number decreased in June and August. This result is in agreement with the findings reported by Koç and Karacaoğlu (22). Güler and Alpay (25) also stated that the highest number of spermatozoa was found in the third period (15 July-15 August), in which the mean temperature was 25 °C, despite the decrease in pollen flow.

The southern Aegean region, where this experiment was performed, has plenty of pollen sources in April. However, the quantity and variety of pollen decreases in June and August, depending on the increase in temperatures in the area. We suppose that high temperatures or decreasing pollen sources negatively affected not only the number of drones, but also the quality of their spermatozoa.

Consequently, our results demonstrated that April and June are ideal periods for rearing the best quality queens and that the Aegean queens had similar reproductive characters to the Italian queens under subtropical conditions.

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References

1. Woyke, J.: Correlation between the ages at which honeybee brood was grafted, characteristics of resultant queen and of insemination. Journal of Apicultural Research, 1971; 10: 45-55.
2. Weiss, K.: The influence of rearing conditions on queen development. In: Ruttner, F., Ed. Queen Rearing, Biological Basis and Technical Instruction. 1983; 83-177.
3. Rawash, I.A., El-Gayar, F.H., El-Helaly, M.S., Ibrahim, S.M.A.: Effect of larval age and number of cell cups on the quality of Carnio-Egyptian F1 hybrid of honey bee queens. Proc. Int. Conf. on Apic. in Trop. Clim., 1983; 320-326.
4. Kaftanoğlu, O., Kumova, U.: A study on the effects of rearing season on the quality of queen honey bees (*Apis mellifera* L.) raised under Çukurova region conditions. Turkish Journal of Veterinary and Animal Sciences, 1990; 16: 569-577.
5. Gençer, H.V., Shah, S.Q., Fıratlı, Ç.: Effects of supplemental feeding of queen rearing colonies and larval age on the acceptance of grafted larvae and queen traits. Pakistan Journal of Biological Sciences, 2000; 3: 1319-1322.
6. Koç, A. U., Karacaoğlu, M.: Effects of rearing season on the quality of queen honey bees (*Apis mellifera* L.) raised under the conditions of Aegean region. Mellifera, 2004; 4: 34-37.

7. Zhadanova, T.S.: Influence of nest temperature on quality of queens produced artificially. XXI. Int. Apim. Cong., Romania, 1967; 245-249.

8. Woyke, J.: Rearing conditions and the numbers of sperms reaching the queen's spermatheca. XXI. Int. Apim. Cong., Romania, 1967; 232-234.

9. Fresnaye, J.: Influence des variations de l'age de maturité sexuelle chez les reines d'abeilles (*Apis mellifica mellifica*) fécondées par insémination artificielle. Annales de l'Abeille, 1966; 9: 237-242.

10. Shawer, M.B., Abd El-Rahim, W.A., Abd El-Rahman, I.: Effect of rearing season on certain characters of Carniolan honeybee at Kafr El-Sheikh Governorate. J. Agric. Res. Tanta University, 1980; 6: 195-203.

11. Karacaoğlu, M.: Orta Anadolu Karadeniz geçit ve Ardahan izole bölgeleri arılarının bazı morfolojik özellikleri üzerinde bir araştırma. Doktora Tezi. Ankara Üniversitesi, Ankara, Türkiye. 1989.

12. Doğaroğlu, M., Özder, M., Polat, C.: Trakya bölgesi koşulları için en uygun bal arısı (*Apis mellifera* L.) genotipini belirleme çalışmaları. Türkiye Bilimsel Teknik Araştırma Kurumu Veterinerlik ve Hayvancılık Araştırma Grubu, 1992.

13. Karacaoğlu, M., Fıratlı, Ç.: Studies on characteristics of Anatolian honey bee ecotypes (*A. m. anatoliaca*) and their crosses: I. Morphological characters. Turk. J. Vet. Anim. Sci., 1998; 22: 17-22.

14. Genç, F., Dülger, C., Dodoloğlu, A., Kutluca, S.: Kafkas, Orta Anadolu ve Erzurum balarısı (*Apis mellifera* L.) genotiplerinin Erzurum koşullarındaki bazı fizyolojik özelliklerinin karşılaştırılması. Tr. J. Vet. Anim. Sci., 1999; 23: 645-650.

15. Gençer, H.V., Fıratlı, Ç.: Orta Anadolu ekotipleri (*A. m. anatoliaca*) ve Kafkas ırkı (*A. m. caucasica*) bal arılarının morfolojik özellikleri. Turk. J. Vet. Anim. Sci., 1999; 23: 107-114.

16. Güler, A., Kaftanoğlu, O.: Morphological characters of some important races and ecotypes of Turkish honeybees (*Apis mellifera* L.) - II. Turk. J. Vet. Anim. Sci., 1999; 23: 571-576.

17. Güler, A., Kaftanoğlu, O.: Determination of performances some important races and ecotypes of Turkish honey bees (*Apis mellifera* L.) under migratory beekeeping conditions. Turk. J. Vet. Anim. Sci., 1999; 23: 577-582.

18. Akyol, E.: Kafkas ve Muğla arılarının (*Apis mellifera* L.) saf ve karşılıklı melezlerinin morfolojik fizyolojik ve davranışsal özelliklerinin belirlenmesi. Doktora Tezi. Çukurova Üniversitesi, Adana, Türkiye. 1998.

19. Karacaoğlu, M., Uçak, A.: Güney Ege koşullarında farklı dönemlerde yetiştirilen ana arılar ile oluşturulan kolonilerin gelişimi. III. Ulusal Zootekni Kongresi, Ankara. 2002; 181-189.

20. Gençer, H.V., Karacaoğlu, M.: Kafkas ırkı (*Apis mellifera caucasica*) ve Kafkas ırkı ile Anadolu arısı-Ege ekotipi (*Apis mellifera anatoliaca*)'nin karşılıklı melezlerinin Ege bölgesi koşullarında yavru yetiştirme etkinlikleri ve bal verimleri. Yüzüncü Yıl Üniversitesi, Ziraat Fakültesi Tarım Bilimleri Dergisi (J. Agric. Sci.), 2003; 13: 61-65.

21. Karacaoğlu, M.: Anadolu arısı Ege ekotipi (*A. m. anatoliaca*) ve İtalyan arısı (A. *m. ligustica*) × Ege ekotipi melezi arılarının morfolojik özellikleri. Adnan Menderes Üniversitesi, Ziraat Fakültesi Dergisi, 2004; 1: 37-42.

22. Koç, A. U., Karacaoğlu, M.: Anadolu arısı Ege ekotipi (*Apis mellifera anatoliaca*) ana arılarında üreme özellikleri. Adnan Menderes Üniversitesi, Ziraat Fakültesi Dergisi, 2005; 2: 73-77.

23. Doğaroğlu, M.: Türkiye'de yetiştirilen önemli arı ırk ve tiplerinin Çukurova Bölgesi koşullarında performanslarının karşılaştırılması. Çukurova Üniversitesi, Ziraat Fakültesi Yıllığı, 1982; 13: 46–60.

24. Budak, M. E.: Ülkemizde çeşitli kurumlarca yetiştirilen ana arılar ile oluşturulan kolonilerin fizyolojik, morfolojik ve davranışsal farklılıklarının araştırılması. Doktora Tezi. Ankara Üniversitesi, Ankara, Türkiye. 1992.

25. Güler, A, Alpay, H.: Reproductive characteristics of some honeybee (*Apis mellifera* L.) genotypes. Journal of Animal and Veterinary Advances, 2005; 4: 864-870.

26. Kahya, Y., Gençer, H.V., Woyke, J.: Weight at emergence of honey bee (*Apis mellifera caucasica*) queens and its effect on live weights at the pre and post mating periods. Journal of Apicultural Research and Bee World, 2008; 47: 118-125.

27. Laidlaw, H. H.: Contemporary Queen Rearing. Dadant and Sons, Hamilton, Illinois, USA. 1985.

28. Dodoloğlu, A., Genç, A.: The effect of rearing and mating methods on some characteristics of queen honeybees (*Apis mellifera* L.). Turk. J. Vet. Anim. Sci., 1997; 21: 379-385.

29. Lensky, Y.: Demter, M.: Mating flights of the queen honey bee (*Apis mellifera*) in a subtropical climate. Comparative Biochemical Physiology, 1985; 81: 229-241.

30. Ruttner, H.: Maintaining queen during the mating period. In: Ruttner, F. Ed. Queen Rearing, Biological Basis and Technical Instruction. Apimondia Publishing House, 1983; 235-277.