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Reproductive Characteristics of Some Honeybee (*Apis mellifera* L.) Genotypes

[1]Ahmet Guler and [2]Hasan Alpay
[1]Faculty of Agriculture, Department of Animal Science,Ondokuz Mayis University,
55139 Kurupelit, Samsun[2]State Control Laboratory, Tokat-Turkey

Abstract: Queen honeybees of different races such as *A. m. carnica*, *A. m. ligustica*, *A. m. caucasica* (NEA-TKV) and *A. m. caucasica* (NEA-Camili) races and west Anatolia (Muðla) and central Black Sea (Tokat) region genotypes were raised in central Anatolia (Sivas) from May to Aug in 2001. The acceptance ratio of larvae, the mating ratio, the preoviposition period, the volume of spermatheca and the number of spermatozoa in per spermatheca were investigated. In this study the significant ($p<0.05$ and $p<0.01$ respectively) differences were found among genotypes in terms of preoviposition periods and the volume of spermatecha. However, periods had significant ($p<0.01$, $p<0.01$, $p<0.05$ and $p<0.01$ respectively) effects on the acceptance rate of larvae, preoviposition period, volume of spermatheca and the number of spermatozoa. This result indicated that environment or climatic conditions had greater effects on the reproductive physiological characters of the queens than the genotypic structure. The preoviposition period of the *A. m. carnica* was (15.04±0.23 day) longer than those of the other genotypes. Queens could mate at the average temperatures at around 18-19°C. There were significant positive correlations between the temperature and the number of spermatozoa, whereas there were negative correlations between temperature and the volume of spermatheca, between the number of spermatozoa and the volume of spermatheca, between the preoviposition period and the number of spermatozoa in the per spermatheca.

Key words: Honey bees, *Apis mellifera*, subspecies, genotype, reproductive, Turkey

INTRODUCTION

The queen alone has significant effect on the colony performance. However, besides racial character, a number of other factors such as seasons, climatic conditions, physiological factors of the queen and drone, behaviour of workers and other predators towards queen effect the queen quality[1-6].

Ruttner[7] stated that with the heritable there are numerous non-heritable factors which are involved in making performance a reality as the conditions under which the queens are reared and the mating process are very important.[8]demonstrated that sexual maturity was attained more rapidly in June and July than in earlier months due to the photoperiod. Güler *et al.*,[9] observed that the volume of spermatheca and the preoviposition period of queens reared from different genotypes were significantly different and Caucasian had the largest volume (0.97±0.03 mm^3) of spermatheca than the other genotypes. Genotypes adapted to the mild climate had short preoviposition period than those adapted to the upland steppe climate conditions. Woyke[10] observed that the conditions before mating, as early as the grafting time of the larva, influenced the result of insemination. A smaller number of spermatozoa entered the smaller spermatheca despite a surplus of semen in the oviducts and plenty of space in the spermatheca. Woyke and Jasinski[11] stated that the rearing conditions, as well as the insemination conditions, influenced the number of spermatozoa entering the spermatheca. Queens kept at 34 °C after insemination had 808 thousand more spermatozoa in the spermatheca than those kept at 24 °C or in colonies. Woyke and Jasinski[12] found that the highest number of spermatozoa entering the spermatheca were obtained when queens 5-10 day-old inseminated. Older queens could be inseminated, but significantly fewer spermatozoa entered their spermatheca. Ruttner[13] recorded that the most flight could be observed after a period of bad weather, at which time flight would occur at temperatures even lower than 20 °C. Szabo *et al.*[14] found that there was a close relationship between maximum daily temperature and time of oviposition. A large number of queens mated at temperatures below 25 °C. The mean preoviposition periods were observed previously by[9,14-16] as 10.4±0.5, 10.33±0.68, 10.6±0.1 and 10.7±0.2 days, respectively.

Although many studies have been done on the rearing conditions and the reproductive characteristics of honeybee queens, there are no enough information on the comparative reproductive physiology of honeybee

Corresponding Author: Dr. Ahmet Güler, Ondokuz Mayýs University, Faculty of Agriculture, Department of Animal Sciences. Tel: (90) 362 4576020

queens from different subspecies or genotypes. Therefore, the aim of the present study was to investigate the reproductive physiological characteristics of the queens reared from important different subspecies and genotypes in the central Anatolia (Sivas) Region.

MATERIAL AND METHODS

The study was carried out in central Anatolia region (Sivas-Turkey) conditions between 15 May and 15 August 2001. Six honeybee genotypes were used such as *A. m. carnica*, *A. m. ligustica*, *A. m. caucasica (north east Anatolia*-TKV), *A. m. caucasica (north east Anatolia*-Camili), central Black Sea (Tokat) and west Anatolia (Muðla) genotypes. Queens of these subspecies and genotypes were provided from private breeders and were introduced to the colonies which had 5 standard Langstroth frames in the 2000.

Four days (72 h) before being grafted larvae, the queens were confined on empty building combs. Each month 6 starter-finisher colonies were prepared and total of 1080 one-day old larvae were transferred from 6 genotypes during 3-month periods. Starter or nursing colonies were prepared and fed using method described by Ruttner,[7], Laidlaw and Page,[17].The grafted cell were placed in the queenless starter-finisher colonies for ten days[10,7]. Two days before the onset of emergence or ten days after grafting the queen cells were removed from the starter-finisher colonies and 30 queen cells from each genotype were introduced into the 2 standard Langstroth sized frames nucleus (an approximately 4500-5000 worker bees) colonies for emergence and open mating. To observe the mating ratio and the preoviposition period, each period 15 queens from each genotype were remarked and the nucleus were inspected daily and the onset of oviposition in laying queens were recorded. The volume of spermatheca and the number of spermatozoa in the spermatheca were determined using the method described by[14,16,18,19]. Also, from each genotype 15 and total of 90 colonies were weighted weekly to determine the nectar secretion during the experiment.

The data were analysed in two way ANOVA using SPSS statistical program and the differences between means were compared by Duncan's Multiple comparison test for genotypes and periods (SAS)[20].

RESULTS

Acceptance rates of grafted larvae (%): The acceptance rates of grafted larvae from genotypes by starter colonies are presented in Table 1. From total of 1080 larvae 818 were accepted and the mean of accepted larvae was found as 75.83±1.41%. The differences among genotypes in acceptance rates were not significant ($p>0.05$). The acceptance rates were affected by periods ($p<0.01$).

The mating ratio of queen (%): There were no significant ($p>0.05$) differences among genotypes in terms of mating ratios. The highest mating ratio was obtained from west Anatolia (77.80 %) and the lowest from Carniolan genotype (51.23 %). Periods had no significant effect on the mating ratio of queens (Table 2).

Onset of oviposition (day): The mean time for oviposition was 12.89±0.14 days. There were significant ($p<0.001$) differences among genotypes and periods in terms of preoviposition period. The onset of oviposition was longest in queens from *A. m. carnica* (15.04±0.23 day) than those of reared from other genotypes. The oviposition periods were found as 12.92±0.23, 13.81±0.15

Table 1: Means and standard errors (X ± S x) for acceptance rate (%) of larvae from genotypes in starter colonies. NEA (north east Anatolia), WA (west Anatolia), CBS (Central Black sea)

	Periods				
Genotypes	No. Larvae	I	II	III	Means
Ligustica	180/142	78.33±5.58	85.00±4.89	73.33±5.99	78.89±3.05
Carnica	180/136	83.33±5.04	81.67±5.24	63.33±6.52	76.11±3.19
NEA (Camili)	180/141	73.33±5.99	85.00±4.83	73.33±5.99	77.22±3.13
NEA (TKV)	180/125	60.00±6.63	86.67±4.60	71.67±6.10	72.78±3.33
WA	180/142	78.33±6.39	83.33±5.04	63.33±6.52	71.11±3.39
CBS	180/132	66.67±5.58	86.67±4.60	71.67±6.10	78.89±3.05
Means	1080/818	73.33±2.33b**	84.72±1.89a	69.44±2.43b	75.83±1.41
Average Temperature (°C)		18.16	21.52	25.00	
Weight of Colony (g day^{-1})		214	487	170	

I-(15 May-15 June), II (15 June-15 July), III (15 July-15 August); values within rows with different letters differ significantly (**P<0.01).

Table 2: Means and standard errors (S) for mating rate (%) of queens from genotypes. NEA (north east Anatolia), WA (west Anatolia), CBS (central Black sea)

	Periods				
Genotypes	No. Queen	I	II	III	Means
Ligustica	45/29	53.30	80.00	60.00	64.43±7.22
Carnica	45/23	46.70	60.00	46.67	51.11±7.54
NEA (Camili)	45/25	40.00	60.0	66.67	55.56±7.49
NEA (TKV)	45/28	53.33	73.33	60.00	62.22±7.31
WA	45/35	80.00	86.67	66.67	77.80±6.27
CBS	45/31	53.33	80.00	73.33	68.89±6.98
Means	270/171	12.92±0.23b**	13.81±0.15a	11.86±0.26c	12.89±0.14
Average Temperature (°C)		18.16	21.52	25.00	
Weight of Colony (g/day)		214	487	170	

I-(15 May-15 June), II (15 June-15 July), III (15 July-15 August).

Table 3: Means and standard errors (S) for onset of oviposition (day) in genotypes. NEA (north east Anatolia), WA (west Anatolia), CBS (central Black sea)

	Periods				
Genotypes	No. Queen	I	II	III	Means
Ligustica	45/29	12.25±0.49	12.83±0.32	11.56±0.29	12.28±0.23 b
Carnica	45/23	14.71±0.61	15.22±0.22	15.14±0.40	15.04±0.23 a
NEA (Camili)	45/25	14.00±0.45	14.50±0.42	11.45±0.71	13.04±0.45 b
NEA (TKV)	45/28	12.88±0.40	14.00±0.38	12.11±0.56	13.07±0.30 b
WA	45/35	12.17±0.39	13.08±0.15	11.00±0.38	12.11±0.23 b
CBS	45/31	12.38±0.50	13.83±0.27	11.09±0.48	12.48±0.31 b
Means	270/171	12.92±0.23b**	13.81±0.15a	11.86±0.26c	12.89±0.14
Average Temperature (°C)		18.16	21.52	25.00	
Weight of Colony (g/day)		214	487	170	

I-(15 May-15 June), II (15 June-15 July), III (15 July-15 August); values within rows with different letters differ significantly (**p<0.01).

Table 4: Means and standard errors (S) for volume of spermatheca (mm^3) in genotypes. NEA (north east Anatolia), WA (west Anatolia), CBS (central Black sea).

	Periods				
Genotypes	No. Queen	I	II	III	Means
Ligustica	29	0.766±0.04	0.911±0.03	0.704±0.04	0.807±0.03 ab**
Carnica	23	0.783±0.06	0.770±0.05	0.706±0.06	0.754±0.03 bc
NEA (Camili)	25	0.792±0.03	0.797±0.03	0.642±0.04	0.728±0.03 c
NEA (TKV)	28	0.770±0.02	0.868±0.05	0.800±0.06	0.818±0.03 ab
WA	35	0.827±0.03	0.838±0.03	0.706±0.02	0.793±0.02 abc
CBS	31	0.906±0.02	0.890±0.04	0.742±0.05	0.842±0.03 a
Means	171	0.810±0.02 a°*	0.852±0.02 a°	0.715±0.02 b°	0.793±0.01
Average Temperature (°C)		18.16	21.52	25.00	
Weight of Colony (g/day)		214	487	170	

I-(15 May-15 June), II (15 June-15 July), III (15 July-15 August); values within rows with different letters differ significantly (*p<0.05, **p<0.01).

and 11.86±0.26 days for I, II and III periods, respectively (Table 3).

Volume of spermatheca (mm^3/queen): Both genotypes and periods had significant effect on the volume of spermatheca. The queens reared from central Black Sea (Tokat) genotype had highest (0.842±0.029 mm^3) and queens from north east Anatolia (Camili) had lowest (0.728±0.025 mm^3) volume of spermatheca. The volume of spermatheca were 0.810±0.015, 0.852±0.017 and 0.715±0.018 mm^3 in I., II. and III. periods, respectively.

The number of spermatozoa in per spermatheca (million/queen): The mean number of spermatheca was 5.61±0.10 million. The differences in the number of spermatheca in genotypes were not statistically significant (p>0.05). However, the number of spermatozoa of queens reared in different periods was significantly different. The queens reared in the third period (15 July-15 Aug) had highest (6.36±0.24 million) number of

Table 5: Means and standard errors (S) for number of spermatozoa (million) in per spermatheca of genotypes. NEA (north east Anatolia), WA (west Anatolia), CBS (central Black sea)

	Periods				
Genotypes	No. Queen	I	II	III	Means
Ligustica	29	5.47±0.11	5.67±0.25	6.89±0.38	5.99±0.19
Carnica	23	4.87±0.23	6.01±0.33	5.94±0.49	5.64±0.23
NEA (Camili)	25	4.45±0.30	4.79±0.29	5.63±0.25	5.08±0.18
NEA (TKV)	28	5.23±0.11	5.16±0.14	6.25±0.62	5.53±0.22
WA	35	5.27±0.09	5.24±0.21	6.99±0.43	5.80±0.20
CBS	31	5.39±0.14	5.05±0.22	6.38±0.97	5.61±0.36
Means	171	5.16±0.08b**	5.32±0.10b	6.36±0.24a	5.61±0.10
Average Temperature (°C)		18.16	21.52	25.00	
Weight of Colony (g/day)		214	487	170	

I-(15 May-15 June), II (15 June-15 July), III (15 July-15 August); values within rows with different letters differ significantly (**p<0.01).

Table 6: Correlation coefficients (r) for some characters.

Characters	No. Queen	Acceptance Rate of Larvae	Preoviposition Period	Volume of Spermatheca	Number of Spermatozoa
Preoviposition Period	171	-	-	-	-0.585
Volume of Spermatheca	171	-	-	-	-0.479*
Average Temperature.(°C)	171	-0.197	-0.340	-0.538*	+0.742**
Hive Weight (g/day)		+0.744**	+0.568*	+0.729**	-0.630**

*P<0.05, **P<0.01

spermatozoa than those of reared in the first and second periods of experiment (Table 5).

DISCUSSION

In this study the significant differences were found among genotypes in terms of preoviposition periods and the volumes of spermatecha. However, periods had significant effect on the acceptance rate of larvae, preoviposition period, volume of spermatheca and the number of spermatozoa. This result indicated that environment or climatic conditions had greater effects on the reproductive physiological characters of the queens than the genotypic structure.

The highest acceptance rate of larvae (84.72±1.89 %) was in the second period. The average temperature of the periods were 21.52, 18.16 and 25.00 °C, respectively. It might be possible to say that the lowest and the highest temperature had negative effect on the acceptance rate of larvae. Also, the daily colony hive weight positively affected the acceptance rate of larvae.

The highest number (73.33±4.69 %) of queens mated in the second period during which the temperature was 21.52°C. Also the pollen flowing and the nectar secretion were rich in that period. However, in the first period in which the number of queens mated was lowest, the temperature was lower and pollen flowing and nectar secretion was poor when compared to the second period. Also there was high beebird (*Merops apiaster* L.) population in that period. Therefore it might be said that many factors as temperature, pollen and particularly nectar flow, photoperiod, the number of drones, the sexual maturity effect the natural mating of the queens. Also, we may conclude from the result that queens could mate in a average temperature near 18-19°C. Similar result was reported previously by[13], who stated that the most active flight could be observed after a period of a bad weather, at which time flight would occur at temperature even lower than 20 °C. Jung[21] observed a mating success of 82-100% in periods of good weather, whereas in cooler and wetter periods it was only 59 %. Lensky and Demter[22] stated that mating flight of queens and drones were at 26-35 °C and flight activity of queens was reduced between 15 and 20 °C. Fresnaye[8] reported that sexual maturity was attained more rapidly in June and July than in earlier months due to long photoperiods. In this study, a total of 171 queens out of 270 (63.33 %) mated. The mean percentage of mating was lower in present study than those of reported by[2,9,23].

The mean preoviposition period of all groups was found as 12.89±0.14 days, whereas the mean preoviposition period of queens reared from *A. m. carnica* was 15.04±0.23 days. This indicated that the queens of *A. m. carnica* genotype mated 2 days later than those of the others genotypes. In the three periods the latest mating queens were observed in *A. m. carnica* genotype. High variations were found between the genotypes and the season regarding preoviposition periods. This result might be explained by the differentiation of sexual maturity of genotypes and the effects of environmental

factors as temperature, nectar and pollen sources. The longest preoviposition period was recorded in the second period in which the temperature was 21.52 °C and the shortest was in the third period in which the temperature was 25.00 °C. We did not find relationship between temperature and preoviposition period. However, the preoviposition period was longer in the low temperatures. Fresnaye[8] reported that big differences occurred between queens inseminated at different period of the year. The queens reared from Ligustica and west Anatolia genotypes had shorter preoviposition period than those of the others and the first egg laying queens were observed in these genotypes. These two genotypes adapted to mild climate. This result indicated that genotypes coming from mild climates become early sexual maturity. In the present study, the mean preoviposition period (12.89±0.14 days) was longer than those of reported by Crane [9,14,15,16,23] 10.4±0.5; 10.6±0.1; 10.33±0.68, 11.5±0.3 , 10.07±0.2 days, respectively. One of the reasons for belated preoviposition period was the severe climatic conditions of central Anatolia (Sivas) region. Since there was a high variation between the average minimum (10.45, 11.53 and 15.67 °C, respectively) and maximum (25.44, 27.02 and 31.76 °C, respectively) temperatures of these periods in the region. A significant negative relationship (r=-0.585) exists between the preoviposition period and the number of spermatozoa in per spermatheca. We infer from the result that the decrease in the preoviposition period caused the increase the number of spermatozoa in the natural mating.[24] found that most of the queens mated were younger than 14 days old. He also found that the younger queens mated often with a greater number of drones than did the older ones. [25]observed that, the larger the eggs in the ovaries, the smaller the volume of semen brought home by the queen from the mating flight. Woyke and Jasinski[12] recommended that the instrumental insemination of queen should be 5-14 days. Older queen can be inseminated, but fewer spermatozoa enter their spermatheca.

A high variation was observed among genotypes in terms of the volume of spermatheca. Central Black Sea (Tokat) genotype had the largest and north east Anatolia (Camili) genotype had the smallest volume of spermatheca. Carniolan and Ligustica genotypes had small volume of spermatheca as well. The mean volume of spermatheca was lower (0.793±0.011 mm^3) in the present study than those of found by[25] in one day old larvae group (1.093 mm^3) and by[9] (0.91±0.01 mm^3), but similar to found by[23] (0.768 mm^3). These differences might be resulted from regional conditions and genotypes. A large volume of spermatheca was recorded in the first and second periods during which the average daily temperature were 18.16 and 21.52 °C, respectively. With increasing temperature the volume of spermatheca decreased. This was also reflected by the negative correlation between temperature and volume of spermatheca (r=-0.513). This result can not be explained only by high temperature. Another reason might be unfavourable flow of pollen sources. In the central Anatolia region pollen sources decreased through the end of season.

Although there was a high variation among genotypes in terms of the volume of spermatheca, there was no any differences among genotypes regarding the number of spermatozoa in per spermatheca. It was expected that the queens having the large volume of spermatheca had the large number of spermatozoa, but such an increase had not been shown. In contrast to this expectation negative relationship was found between the volume of spermatheca and the number of spermatozoa (r=-0.479). These results indicated that the number of spermatozoa in the spermatheca did not depend on the volume of spermatheca. Woyke[10]showed that the relationship between the size of the spermatheca and its content of spermatozoa was closer when queens were inseminated with the largest amount of semen (2x8 mm^3). Woyke and Jasinski[11] observed that the rearing conditions as well as the insemination conditions influenced the number of spermatozoa entering the spermatheca. This results indicated that queens were able to have large number of spermatozoa during the natural mating if they mated in favourable conditions such as temperature over 25°C, rich nectar secretion and the higher number of drones.

The highest number of spermatozoa was found in the third period (6.36±0.24 million) in which the average temperature was 25°C and the average temperature during the day was 31.76 °C. Despite the decrease in pollen flow, the number of drones was highest in this period. The difference among the mean number of spermatozoa in different periods was higher than 1 million (Table 5). A high positive relationship was found between temperature and the number of spermatozoa (r=0.756). So, the number of spermatozoa increased linearly with the increase of temperature during the periods. This result suggested that increase in temperature positively affected the reproductive physiology of queen and drone. Woyke and Jasinski[11] observed that the rearing conditions, as well as the insemination conditions influenced the number of spermatoz oa entering the spermatheca. Queen kept at 35 °C after insemination had 808 thousand more spermatozoa in the spermatheca than those of kept at 24 ° Cor in colonies. Vesely[26] also found higher mean number of spermatozoa in the spermatheca of queens kept

at 34 °C than those of kept at room temperature. In the present study the highest number of spermatozoa entering the spermatheca (6.36±0.24 million) was obtained when the average temperature of the day was 25.00 °C or over. The mean number of spermatozoa (5.61±0.10 million) in per spermatheca of queens, was similar to those of reported by Woyke[10] in one day old larvae and instrumental insemination with 2x8 mm^3 groups, but lower than those of queens reared from egg and higher than those of reported by[9,11,12,23]. Therefore, it could be said that there was no unfavourable case on the content of spermatozoa of queens reared in the central Anatolia (Sivas-Turkey) region.

CONCLUSIONS

This experiment showed us that the abundant nectar secretion positively affected all reproductive characteristics of queen honeybee. Under the conditions of the central Anatolia (Sivas) region prolific queens can be produced in June, July and August, while these periods are unfavourable in the some other regions of country.

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