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Original article (Orijinal araştırma)

The effects of altitude and rearing period on some characteristics of honey bee queens, *Apis mellifera caucasica* Gorbachev, 1916 (Hymenoptera: Apidae)[1]

Rakım ve yetiştirme döneminin ana arıların, *Apis mellifera caucasica* Gorbachev, 1916 (Hymenoptera: Apidae) bazı özellikleri üzerine etkileri

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Abstract

In the study, some characteristics of honey bee queens, *Apis mellifera caucasica* Gorbachev, 1916 (Hymenoptera: Apidae), reared at two different altitudes (140 m vs 920 m) and three rearing seasons (May, June-I, June-II) in 2019-2020 were examined in Ordu. These are larvae grafting, length of the queen cell, weight of queens at various periods, and spermathecae parameters of queens. One-day old 50 larvae were grafted at each period and altitude. The effect of period and period*altitude interaction on the weight at emergence of queen was significant (p<0.001) while the effect of altitude and year was nonsignificant. Weights at emergence of queens were lower in May at high altitudes and in June-II at low altitudes. The effects of year, period and altitude were significant (p<0.001) in the terms of the diameter and volume of the spermathecae, and the number of spermatozoa in the spermathecae. When the two-year data was evaluated together, the number of spermatozoa in the spermathecae was higher in the queen bees reared in May. It is recommended to pay attention to the period and altitude when pollen and nectar flow is intense in queen rearing in Ordu and similar climatic conditions.

Keywords: Honeybee, queen, rearing period, spermathecae, number of spermatozoa

Öz

Çalışmada, Ordu ilinde, 2019-2020 yıllarında iki farklı rakım (140 m ile 920 m) ve üç yetiştirme döneminde (Mayıs, Haziran-I, Haziran-II) yetiştirilen ana arıların, *Apis mellifera caucasica* Gorbachev, 1916 (Hymenoptera: Apidae), bazı özellikleri incelenmiştir. Bu özellikler; larva kabul oranı, yüksük uzunluğu, ana arı çıkış ağırlığı, ana arının yumurtlama başlangıcındaki ağırlığı, yumurtlamaya başladıktan sonraki 3. gün ağırlığı, spermateka çapı ve hacmi ve spermatozoa sayısıdır. Her dönem ve rakımda bir günlük yaşta 50'şer adet larva aşılanmıştır. Ana arı çıkış ağırlığı üzerine dönem ve dönem*rakım interaksiyon etkisi önemli (p<0.001), rakım ve yıl etkisi önemsiz bulunmuştur. Yüksek rakımda Mayıs döneminde, düşük rakımda ise Haziran-II döneminde ana arı çıkış ağırlıkları daha düşüktür. Spermateka çapı, spermateka hacmi ve spermatozoa sayısı bakımından yıl, dönem ve rakımın etkisi önemli bulunmuştur (p<0.001). İki yıl verileri birlikte değerlendirildiğinde spermatozoa sayısı Mayıs dönemi yetiştirilen ana arılarda daha yüksektir. Ordu ve benzer iklim koşullarında ana arı yetiştiriciliğinde polen ve nektar akımının yoğun olduğu dönem ve rakıma dikkat edilmesi önerilmektedir.

Anahtar sözcükler: Bal arısı, ana arı, yetiştirme dönemi, sperm kesesi, sperm sayısı

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Introduction

Beekeeping is an important animal breeding activity in terms of its role in economic, ecological, and rural development. In colony yield, environmental factors including climate, flora, and topography characteristics are effective together with the genetic structure of the colony and beekeeper practices. Furthermore, genetic capacity of the honey bee queen, *Apis mellifera* L., 1758 (Hymenoptera: Apidae) which is the most important individual of the colony, and genetic capacity of the drones with which it mates are the determinants of many physiological and behavioral characteristics that emerge in the colony. The queen bee, one of the individuals of the colony, plays a crucial role in ensuring the continuity of colonies. The low-quality queens will result in the honey yield and colony lifespan not being at the intended level, even if the other conditions are adequate. Quality of the queen is crucial for this reason. Morphological and physiological characteristics and health status of the queen are still being investigated by various studies (Delaney et al., 2011; Hatjina et al., 2014; Porporato et al., 2015; Amiri et al., 2017).

Rearing system, flora, season, larval age, condition, composition of the colonies used, feeding and characteristics of queen cells are seen more among the factors influencing the quality of queens (Woyke, 1971; Winston, 1987; Tarpy et al., 2011; Rangel et al., 2013; De Souza et al., 2019). The breeding quality of the queen and the drones which she mates with and whether a breeding program is implemented are the main factors. To explain the quality of the queen, the parameters such as the weight at emergence, live weight in various periods, the mating success and the frequency, the number of ovarioles, the ovary weight, the diameter of the spermathecae, the volume of the spermathecae and the number of spermatozoa in the spermathecae have been examined (Kahya et al., 2008; Büchler et al., 2013; Arslan et al., 2021; Facchini et al., 2021; Frost et al., 2021). It was reported that there were correlations between the weight at the emergence of a queen, its weight at different life stages and the condition of the colony, the various morphological and reproductive organ characteristics of the queen (Kahya et al., 2008; Delaney et al., 2011; Tarpy et al., 2012). It was found that the weight of the queen had a significant effect on the acceptance rate of the queen, starting to oviposition, rate of oviposition, diameter of the spermatheca, and the number of spermatozoa in the spermatheca (Akyol et al., 2008). Similarly, there is a correlation between the weight at the emergence of a queen, diameter of the spermathecae and amount of stored sperm (Dodoloğlu et al., 2004; Collins & Pettis 2013; Hatjina et al., 2014). A queen must have an efficient metabolism, a large spermatheca, and a large ovarium including more and longer ovarioles so that she can be considered as superior in terms of reproductive characteristics (Tarpy et al., 2000). The diameter of the spermatheca and volume of the spermatheca are affected by factors such as the genetic structure of the queen, rearing conditions, larval age, and season (Hatch et al., 1999; Güler & Alpay 2005; Uçak Koç & Karacaoğlu, 2011).

When the existence of colonies and amount of honey production are researched in our country, it is seen that the honey production per colony and production of other beekeeping products except honey are not at the level desired. Similarly, it is seen that it is insufficient when we compare the number of the queen breeders (143 ones) having production permits for rearing and commercial queens in our country and the queen production capacity of these enterprises (approximately 510 thousand units) with our colony existence (approximately 8.1 million colonies) and the annual estimated demand of queen (Burucu, 2021; Anonymous, 2022). The intensive rearing of queens starts primarily in the coastal provinces in the Mediterranean. On the other hand, the queen rearing continues to decrease by depending on the effects of season and flora in the inner and higher altitude areas.

The study aimed to examine the parameters of the acceptance rate of larvae, length of the queen cell, weight at the emergence of a queen, weight at the onset of oviposition, weight on the third day after oviposition, diameter of the spermatheca, volume of the spermatheca and the number of spermatozoa in the spermatheca of Caucasian queens *Apis mellifera caucasica*, Gorbachev, 1916 (Hymenoptera: Apidae), reared at two different altitudes and three rearing periods.

Materials and Methods

The study was conducted at a low altitude (Dedeli), which is at an altitude of 140 m (40°54'24" N and 37°50'06" E) and a high altitude (Sayaca), which is at an altitude of 920 m (40°53'24" N and 37°41'55" E) in Ordu province in two separate apiaries between 2019 and 2020. Ordu province is a region where the climate of the Black Sea is dominant. Although winters are cool and summers are warm, there is a possibility of precipitation in all months of the year. In the fields where this study was conducted in the province of Ordu, there are no predominant nectar and pollen sources during spring months. However, it is known that there are secondary or minor pollen sources. *Diospyros lotus* L. and *Trifolium repens* L. species are found in secondary amounts (Cınbırtoğlu, 2014). Additionally, different amounts of pollen and nectar source plants are found in the region during the spring and summer seasons. These include *Castanea sativa* Miller (Dominant-Dominant), *Laurocerasus officinalis* Roemer (Secondary-Iz), *Rhododendron ponticum* L. subsp. *ponticum* L. (Minor-Dominant), and *Vaccinium myrtillus* L. (Minor-Secondary) species (Cınbırtoğlu, 2021; Anonymous, 2023).

Queen rearing

Queen rearing was performed simultaneously in both apiaries during the periods of 10 May, 4 June (I) and 29 June (II). The standard procedure of rearing a queen was followed (Laidlaw, 1985). One-day old larvae were used in larvae grafting. 50 larvae in both apiaries for each period and a total of 600 larvae in two years were grafted. The queen cells prepared from beeswax and the frames with three-lathed were used in larvae grafting. A total of 65 honeybee colonies were used in this study, representing each season and altitude over a span of two years. As a source colony for larvae, one colony of Caucasian race *Apis mellifera caucasica*, Gorbachev, 1916 (Hymenoptera: Apidae) was used. For each altitude (140m and 920m) and season, one queenless starter colony (consisting of 2 frames with open brood, 4 frames with capped brood, and 2 frames with stored pollen and nectar), one queenright and double-storey finishing colony, five drone rearing colonies, and mating boxes were prepared. The mating nucleus was also prepared with young worker bees, which were shaken down equally every period, and they were distributed to the field in the trial apiary. Queen rearing colonies were regularly fed with sugar syrup at a rate of 1:1.

Methods

The acceptance rate of larvae (the number of larvae accepted / the number of larvae grafted *100) was determined 24 hours after larvae grafting. The accepted larvae were transferred to the finisher colonies. The length of the queen cell was measured with a digital caliper before placing it in the incubator (a day before the emergence of the queen). The emergence of the queens was observed in the incubator at each period (34ºC, 60% humidity). The larvae transfer success rate (The number of the queen at emergence/ The number of larvae grafted *100) was determined.

The electronic balance (WL-303L 0.001gr) was used to determine the weight at the emergence of a queen. The queens at the emergence were randomly distributed to the mating nuclei with a queen cage. The time to start oviposition of queens was determined by monitoring open-mated queens. The weight at the onset of the oviposition and weight on the third day after the oviposition of the queens were weighed. The characteristics of the spermatheca were determined in 15 queens randomly selected from among the queens reared in each period. The diameter of the spermatheca (mm) was measured under an ocular microscope with a micrometer (Olympus SZ61) without a tracheal net. The volume of spermathecae was calculated according to the measured diameter (calculated according to the formula of the sphere volume). After the spermathecae were discharged in a porcelain cup comprising 1 ml of 0.9% sodium chloride solution, the spermatozoa were dispersed by mixing it with a Pasteur pipette. Tap water was added to make up the mixture to 5 ml (Kaftanoğlu & Kumova, 1992; Genç, 1996; Carreck et al., 2013). The number of spermatozoa in the spermatheca was determined by the samples taken from this mixture under the microscope (Carl Zeiss Axio Scope A1) with the Thoma counting chamber (Kaftanoğlu & Kumova, 1992; Genç, 1996; Carreck et al., 2013).

Statistical analysis

The research was carried out in a factorial design (2*3*2) according to the plan of the randomized block design. Two-way ANOVA was used to analyze the data. The mathematical model of the design used in the study is;

$$Yijk=\mu+\alpha i+\beta j+(\alpha\beta ij)+\gamma k+eijk$$

these indicate

$Yijk$= measurement values,

μ= population mean,

αi= i-th altitude effect (A),

βj= j-th period effect (P),

$(\alpha\beta ij)$= altitude*period (A*P) interaction effect,

γk= k-th year effect (block effect),

$eijk$=P random error.

The differences between group means were determined by Duncan's multiple comparison test. In addition, whether the acceptance rate of larvae changed or not according to the periods was determined by the chi-square test of independence. The correlations among all parameters investigated such as the weight at the onset of the oviposition, the weight on the third day after oviposition, the diameter of the spermathecae, and the volume of the spermathecae were evaluated with the Pearson's correlation coefficient. The statistical package program of SPSS v.22.0 was used for all statistical calculations. It was considered that the research findings were significant at p<0.05 level by expressing as n, mean, and standard deviation.

Results and Discussion

The acceptance rate of larvae

The acceptance rate of larvae did not change according to altitude in May 2019. It was determined that acceptance rate of larvae changed according to the altitude in the June-I (χ^2_1 =6.002; p< 0.05) and June-II (χ^2_1=8.854; p< 0.05) periods. There was no difference in the acceptance rate of larvae according to the altitude for 2020 in all periods. Although there was no significant difference in the acceptance rate of larvae in June-I and June-II periods at low altitude in 2019 and 2020, it was found that there was a significant difference in the acceptance rate of the larvae in the 3rd rearing period in 2019-2020 (χ^2_1=6.529; p=0.011). The acceptance rate of larvae was higher in 2019 (86%) than in 2020 (64%). There was no statistical difference between the acceptance rate of larvae in 2019 and 2020 in all periods when queen rearing was conducted at high altitude. The acceptance rate of larvae (%) and the number of observation (n) for the length of the queen cell, weight at the emergence of the queens, weight at the onset of oviposition of the queen and weight on the third day after the oviposition of the queen were given in Table 1.

Table 1. The larvae acceptance rate

Periods	Acceptance rate of larvae (n, %) 2019		Acceptance rate of larvae (n, %) 2020	
	Low altitude (140 m)	High altitude (920 m)	Low altitude (140 m)	High altitude (920 m)
May	36 (72)	31 (62)	38 (76)	34 (68)
June-I	44 (88)	34 (68)	38 (76)	36 (72)
June-II	43 (86)	30 (60)	32 (64)	37 (74)

Güler & Alpay (2005) reported that there was no difference between the acceptance rate of larvae according to the queen genotypes, but the acceptance rate of larvae showed a significant difference according to the rearing periods in their study in Sivas province. The acceptance rate of larvae they reported for the genotype of Caucasian, *Apis mellifera caucasica*, Gorbachev, 1916 (Hymenoptera: Apidae) is similar to the values obtained in this study. The acceptance rate of larvae obtained from this study was similar to those reported by Gençer et al. (2000) at 73.47% and Cengiz et al. (2019) at 72.16%. Onk et al. (2016) found that there was a difference in the acceptance rate of larvae for the Caucasian queen according to the year and rearing period, and the values they obtained are higher than in this study. Dodoloğlu et al., (2004) reported that the acceptance rate of larvae was higher than in the current study.

The length of the queen cell

In the study, it was found that while the impact of the rearing period and year on the length of the queen cell was significant ($p< 0.001$), there was no interaction effect of altitude and period*altitude ($p>0.05$). The length of the queen cell was higher in the measurements made in 2019 than in the ones in 2020. In both years, the length of the queen cell was high and similar in May (23.99 mm) and June-II (23.89 mm) periods and had the lowest average in the June-I (21.27 mm) period ($p< 0.05$). The average length of the queen cells was found 23.19±2.46 mm at low altitude and was found at 22.75±3.02 mm at high altitude ($p>0.05$) (Table 2).

Table 2. The length of the queen cells by years and periods (mm)

Altitude	Year	Periods	n	The queen cell length (mm) (mean±SE)
Low	2019	May	35	23.12±1.62
		June-I	44	23.11±0.82
		June-II	43	24.70±2.61
	2020	May	35	25.04±1.63
		June-I	38	19.69±1.55
		June-II	32	23.51±1.79
	General	May	70	24.09±1.88^{A}
		June-I	82	21.52±2.10^{B}
		June-II	75	24.19±2.35^{A}
High	2019	May	31	22.48±0.87
		June-I	34	22.58±0.93
		June-II	28	26.76±2.30
	2020	May	29	25.39±2.80
		June-I	31	19.13±1.07
		June-II	35	20.95±1.59
	General	May	60	23.89±2.50^{A}
		June-I	65	20.94±2.00^{B}
		June-II	63	23.53±3.49^{A}

^{a,b} denote the significant differences between means in the same column ($p< 0.05$)

The weight at the emergence of the queens

In the study, it was found that the effect of period and period*altitude interaction upon weight at the emergence of the queen was significant ($p< 0.001$) while the impact of altitude and year was not significant ($p> 0.05$) (Table 3). The weight at the emergence of the queen obtained in the periods of May (218.4 mg) and June-I (222 mg) was higher than the period of June-II (213.5 mg) ($p< 0.05$). The mean weight at the emergence of the queen was found 218.5±19.61 mg at low altitude and was found 218.0±22.47 mg at high altitude (p>0.05). However, if the interaction effect is taken into account, queens with low emergence weight are raised at high altitudes in the June-I period. On the other hand, the low weight at emergence was obtained at low altitude in the period of June-II.

Table 3. Queen weight at the emergence according to years and periods (mg)

Altitude	Year	Periods	n	Queen weight at the emergence (mg) (mean±SE)
Low	2019	May	34	234.9±17.85
		June-I	43	221.4±13.37
		June-II	42	200.4±14.61
	2020	May	34	217.1±18.97
		June-I	38	219.4±19.49
		June-II	31	221.1±17.07
	General	May	68	226.0±20.35^{A}
		June-I	81	220.5±16.41^{AB}
		June-II	73	209.3±18.68^{C}
High	2019	May	29	197.9±14.86
		June-I	34	229.0±16.23
		June-II	28	216.7±18.18
	2020	May	28	222.0±22.08
		June-I	30	221.2±30.65
		June-II	35	219.8±17.82
	General	May	57	209.6±22.15^{C}
		June-I	64	225.3±24.33^{AB}
		June-II	63	218.5±17.91^{B}

^{A,B,} denote the significant differences between means in the same columns (p< 0.05).

The weight at the onset of oviposition of the queen

While the effect of the period, altitude, and period*altitude interaction on the weight at the onset of oviposition of the queen were significant (p< 0.001), there was no effect of the year (p>0.05). In the rearing of the queen bee, the highest weight at the onset of oviposition was obtained in May at a low altitude. Although the weight at the onset of oviposition of the queen was highest in the period of June-II in high altitude, there was no difference between the periods (Table 4). The average weight at the onset of oviposition of the queen was found to be 221.9±24.52 mg at low altitude and 217.7±22.16 mg at high altitude according to general average of altitudes (p< 0.05). The weight at the onset of the oviposition of the queen was determined 225.6 mg in May, 216.1 mg in June-I, and 218.9 mg in June-II according to the general average of the rearing periods (p< 0.05). The correlations between various characteristics of queens were investigated. It was obtained that there was a low significant correlation between the weight at the emergence of the queens and their weight at the onset of the oviposition (r = 0.167; p< 0.01).

Table 4. The weight at the onset of oviposition and the weight on third day after oviposition of the queens (mg)

Altitude	Year	Periods	n	The queen weight at the onset of oviposition (mg) (mean±SE)	The queen weight on the third day after the oviposition (mg) (mean±SE)
		May	33	245.6±12.53	234.9±14.87
	2019	June-I	43	211.5±26.66	221.0±10.47
		June-II	41	213.7±21.67	214.0±12.26
		May	33	223.3±25.22	231.8±13.57
Low	2020	June-I	37	221.1±23.59	222.2±17.32
		June-II	31	220.8±18.46	232.0±18.52
		May	66	234.5±22.75^{A}	233.3±14.21^{A}
	General	June-I	80	216.0±25.59^{B}	221.5±13.98^{BC}
		June-II	72	216.7±20.54^{B}	221.7±17.57^{BC}
		May	28	206.8±32.00	208.8±35.19
	2019	June-I	33	212.0±15.85	219.0±8.802
		June-II	28	219.1±18.02	225.5±20.75
		May	27	224.1±14.58	228.8±12.27
High	2020	June-I	30	221.0±23.98	233.1±16.67
		June-II	34	223.5±19.73	255.6±13.32
		May	55	215.2±26.41^{B}	218.4±28.32^{C}
	General	June-I	63	216.3±20.49^{B}	225.7±14.84^{B}
		June-II	62	221.6±18.97^{B}	225.5±16.87^{B}

^{A,B,C} denote the significant differences between means in the same columns (p< 0.05).

The weight on the third day after the oviposition of the queen

While the interaction effect of the year and the period*altitude was significant (p< 0.001) on the weight on the third day after the oviposition of the queen, there were no effects of both altitude and period (p> 0.05). It was found that the weight on the third day after the oviposition of the queen in 2020 was higher than the one in 2019. In rearing queen bee, the highest weight on the third day after oviposition was obtained in the period of May in low altitude. The lowest weight on the third day after oviposition was found in May in high altitude (Table 4). It was found that there was a positive correlation between the weight at the emergence of the queen bees and weight on the third day after oviposition (r = 0.487; p< 0.01).

In the study, it was determined that the interaction of the rearing period and altitude had a significant effect when the results were examined in terms of weight at the emergence, weight at the onset of oviposition and weight on the third day after oviposition of the queen bees. The weight at the emergence of the queen is an important criterion related to both colony productivity and various morphological and physiological characteristics of the queens (Akyol et al., 2008; Collins & Pettis, 2013; Hatjina et al., 2014). Nevertheless, it was reported that there was a change in the body weight of queen from the weight at emergence to the weight at the onset of oviposition in many studies (Kahya et al., 2008). It was recommended that body weight of the Carniolan queens *Apis mellifera carnica*, Pollmann, 1879 (Hymenoptera: Apidae), should be determined when they reached the age of about 2 days (Skowrenek et al., 2004). Uçak Koç & Karacaoğlu (2011) reported that the live weight on the second day of the queens, the weight at the onset of oviposition and the preoviposition period of queen bees were affected by the period of rearing. The values obtained in terms of weight at the emergence of the queens in our study were found to be higher than the mean values which Dodologlu et al. (2004); Onk et al. (2016) and Cengiz et al. (2019) obtained in their studies.

When evaluated in terms of the periods (218.4±22.66 mg, 222.6±20.36 mg and 213.5±18.84 mg, respectively) and altitudes (low altitude 218.5±19.61 mg and high altitude 218.0±22.47 mg), the values in our study were higher than those reported by Tarpy et al. (2011), Oztokmak & Ozmen Ozbakır (2017) and Akyol et al. (2008). As a result of our study, the weight at onset of oviposition of queens (225.6±26.25 mg, 216.1±23.40 mg and 218.9±19.91 mg according to the periods; 221.9±24.2 mg and 217.7±22.16 mg according to the altitudes) was higher than the values Kahya et al. (2008), Kumar & Mall (2016) reported in their studies, but similar results were obtained with some periods in the studies of Uçak Koç & Karacaoğlu (2011). Kahya et al. (2008) stated that the weight on the third day after oviposition reached 220.9 mg in a study about determining the queen's live weight at different periods and reproductive characteristics of the queens. The values which Kahya et al. (2008) are found in their study were lower than the values found in this study (the period and the altitude values ranged from 223.3±20.84 mg to 226.5±23.04 mg).

The diameter of the spermathecae

While the effects of the year ($p<0.001$), period and altitude were significant in terms of the diameter of the spermatheca ($p<0.05$), the effect of the interaction was insignificant ($p>0.05$). The diameter of the spermathecae mean was determined 1.159^{AB} mm in May, 1.143^{B} mm in June-I, and 1.163^{A} mm in June-II according to the general average of the rearing periods ($p<0.05$). The diameter of the spermathecae mean was found to be high in 2020. While the diameter of the spermathecae mean was high in the periods of May and June-II in low altitude, it was low in June-I. On the other hand, the diameter of the spermathecae was high in the period of June-II at high altitude. The diameter of the spermathecae mean was determined 1.158±7.121 mm at low altitude and 1.151±5.938 mm at high altitude according to general average of altitudes ($p<0.05$) (Table 5).

Table 5. The spermathecae traits of queens*

Altitude	Year	Periods	n	The diameter of the spermathecae (mm) (mean±SE)	The volume of the spermathecae (mm^3) (mean±SE)	The number of spermatozoa (million) (mean±SE)
Low	2019	May	15	1.136±6.240	0.774±0.126	3.682±0.934
		June-I	15	1.110±6.022	0.722±0.117	4.062±0.987
		June-II	15	1.119±6.946	0.742±0.138	3.892±0.970
	2020	May	15	1.207±3.232	0.927±.0726	5.179±0.594
		June-I	15	1.182±5.995	0.871±0.127	4.331±0.749
		June-II	15	1.219±5.121	0.952±0.123	3.892±0.819
	General	May	30	1.172±6.096	0.848±0.127	4.431±1.083
		June-I	30	1.144±6.980	0.791±0.142	4.186±0.890
		June-II	30	1.162±7.918	0.832±0.168	3.883±0.903
High	2019	May	15	1.122±6.535	0.740±0.128	3.675±0.961
		June-I	15	1.113±5.667	0.727±0.111	3.198±0.687
		June-II	15	1.133±6.386	0.767±0.132	3.703±1.115
	2020	May	15	1.168±3.578	0.836±0.075	3.822±0.930
		June-I	15	1.174±5.112	0.852±0.111	3.876±0.916
		June-II	15	1.191±3.138	0.887±0.069	3.627±0.898
	General	May	30	1.144±5.766	0.786±0.116	3.746±0.941
		June-I	30	1.142±6.193	0.787±0.132	3.521±0.868
		June-II	30	1.165±5.640	0.834±0.118	3.661±0.898

* Since the interaction effect was insignificant ($p>0.05$), the lettering was not given in the table.

Gençer & Fıratlı (1999) reported that the diameter of the spermatheca of the queens raised from 1-day-old larvae was 1.063±0.077 mm in their study in which some internal and external characteristics of queen bees reared from 1- and 2-day-old larvae were compared. Dodoloğlu & Genç (1997) reported that the average spermatheca diameter of the queens mating naturally in Erzurum was 0.929 mm in their study while Uçak Koç & Karacaoğlu (2004) reported the average spermatheca diameter of the queens reared under the conditions in Aydın was 1.121 mm in his study. The values determined in this study were higher than those obtained by Gençer & Fıratlı (1999), Dodoloğlu & Genç (1997) and Uçak Koç & Karacaoğlu (2004) in their studies. Cengiz et al. (2019) stated that the diameter of the spermatheca of queens reared from 1-day-old larvae was 1.04±0.018 mm. On the other hand, Arslan & Cengiz (2020) reported that the average diameter of the spermatheca of queen bees was 1.015±0.007 mm in their studies where the quality criteria of queens taken from different enterprises were evaluated. Also, the mean spermatheca diameter was reported as 1.044±0.071 mm. in the study of Arslan et al. (2021), in which the quality characteristics of the queen bee were examined. Ozmen Ozbakır (2021) reported that the diameter of the spermatheca of the queen bees reared from emergency cells, one-day-old and two-day-old larvae were 1.14 mm, 1.21 mm and 1.16 mm, respectively. In our study, the diameter of the spermatheca of the queen bees was higher than the average diameter of the spermatheca which Kahya et al. (2008), Uçak Koç & Karacaoğlu (2005), Dodoloğlu et al. (2004), Cengiz et al. (2019), Arslan & Cengiz (2020) and Arslan et al. (2021) reported in their studies.

The volume of the spermathecae

In terms of the volume of the spermathecae, while the impacts of the year, the period and the altitude were found to be significant (p<0.05), the effect of the interaction was insignificant (p>0.05) (Table 5). The diameter of the spermathecae mean was determined 0.820^{A} mm^3 in May, 0.789^{B} mm^3 in June-I, and 0.832^{A} mm^3 in June-II according to the general average of the rearing periods (p<0.05). The volume of the spermathecae was higher in 2020. At low altitude, while the volume of the spermathecae was high in the periods of May and June-II, it was low in the June-I period. The volume of the spermathecae was higher in the period of June-II in high altitude. The volume of the spermathecae mean was determined 0.822±0.148 mm^3 at low altitude and 0.802±0.122 mm^3 at high altitude according to general average of altitudes (P< 0.05).

The volume of the spermathecae obtained in this study was higher than the values which Gençer & Fıratlı (1999); Kahya et al. (2008) and Arslan et al. (2021) reported in their studies. Al-Ghzawi and Zaitoun (2008) reported that the volume of the spermathecae (0.82 mm3) in Syrian queens *Apis mellifera syriaca*, Buttel-Reepen, 1906 (Hymenoptera: Apidae), was smaller than the volume of the spermathecae in Italian queens *Apis mellifera ligustica*, Spinola, 1806 (Hymenoptera: Apidae), (0.89 mm3). In this study, there were similar values in the mean volume of the spermathecae obtained at low altitude. Uçak Koç & Karacaoğlu (2011) reported that the period of queen rearing did not affect the volume of the spermathecae. Kahya et al. (2008) found that the volume of the spermathecae differed according to the weight of the queen and there was more volume of the spermatheca in the heavy queens. In this study, there were low insignificant correlations between the weight at emergence, diameter of the spermathecae and volume of the spermathecae. Furthermore, the effect of the rearing period on the volume of the spermathecae was significant, unlike the study of Uçak Koç & Karacaoğlu (2011).

The number of spermatozoa in the spermathecae

While the effect of the year, altitude (p<0.001), and period (p<0.05) were significant in terms of the number of spermatozoa, the interaction effect was insignificant (p>0.05). The number of spermatozoa mean was determined as 4.161 million at low altitude and 3.640 million at high altitude according to general average of altitudes (p<0.001). When considering 2019 and 2020 together in terms of the number of spermatozoa, the period of May was higher in both low altitude (4.431±1.083 million) and high altitude (3.746±0.941 million). The number of spermatozoa in 2020 was higher at low altitude (4.467 million) than the one at high altitude (3.775 million) (Table 5). The number of spermatozoa in the spermathecae mean was determined as 4.115^{A} million in May, 3.892^{AB} million in June-I, and 3.781^{B} million in June-II according to the general average of the rearing periods ($p < 0.05$). There was no significant correlation between the weight at the emergence of the queen and characteristics of the spermatheca. A positive correlation was found between the diameter of the spermathecae and the volume of the spermathecae (r = 0.994; $p < 0.01$). There were low correlations between the number of spermatozoa in the spermathecae and the diameter of the spermathecae (r = 0.263; $p < 0.01$) and the volume of spermathecae (r = 0.265; $p < 0.01$). There were low correlations between the diameter of the spermathecae and the weight at the onset of oviposition (r = 0.116; $p < 0.05$), and there were also low correlations between the volume of spermathecae and the weight at the onset of oviposition (r = 0.117; $p < 0.05$).

Tarpy et al. (2011) noticed that the number of spermatozoa of the queens within the low-weight group was 2.80 million and the ones of the queens in the high weight group were 5.06 million while the average number of spermatozoa was 3.72 million in their study on the reproductive potentials of the queen and the mating success. While the values found in our study were partially similar to those of Tarpy et al. (2011), they were lower than the high-weight group of the queen in the same study. The number of spermatozoa was found to be 4.877 million in the study conducted by Kahya et al. (2008) on the determination of the live weights at different periods and reproductive characteristics of the queen. Cengiz et al. (2019) reported that the number of spermatozoa of the queens reared from one-day-old larvae was 4.44±0.429 million in their study. Dodologlu et al. (2004) determined the number of spermatozoa of the queens reared with the Doolittle method as 4.65±0.08 million in their study conducted by the Doolittle method and natural queen rearing. In addition, Arslan et al. (2021) reported that the mean number of spermatozoa was 4.454±0.177 million/queen in their study examined the quality characteristics of the queen. Dodologlu & Genç (1997) found the average number of spermatozoa of the queen bees mating naturally to be 4.625 million in their study in Erzurum. It is seen that the values in the studies carried out by other researchers are higher than the values found in our study. In the study in which Akyol et al. (2008) evaluated the queens as heavy, normal, and light according to their weights at emergence, the spermatozoa number of the queens was determined as 5.2 million/queen for the heavy group, 4.8 million/queen for the normal group and 4.2 million/queen for light group. The values found in our study were lower than those in the study of Akyol et al. (2008). The number of spermatozoa of the queen bees reared in the study of Uçak Koç & Karacaoğlu (2011) was higher than the number of spermatozoa obtained in this study.

Conclusion

As a result of the study, it was revealed that the rearing period and the altitude had effects separately and together on the quality parameters of Caucasian queens, *Apis mellifera caucasica*, Gorbachev, 1916 (Hymenoptera: Apidae). Especially in the conditions of Ordu province, it is recommended that the enterprises with commercial queen rearing and those engaged in beekeeping activities should pay attention to the altitude and the period when the flows of pollen and nectar are intense. In terms of the acceptance rate of larvae, June is recommended for the transfer of larvae in Ordu and the regions with similar conditions. In terms of the weight at the emergence of the queen, the weight at the onset of the oviposition, or the weight on the third day after oviposition, it is recommended that the transfer of larvae should be after 10 May at low altitudes, and at the beginning of June at higher altitudes. In the terms of the diameter of the spermathecae, while there were high and similar averages in May and June-II at low altitudes, high averages were obtained in June-II at high altitudes. In the terms of the characteristic of the number of spermatozoa, it is recommended that the transfer of larvae should be on a date after 10 May at both low and high altitudes.

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