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The investigation of quality features of Turkish commercial honey bee queens (*Apis mellifera* L.) in different seasons

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The quality of queen bees plays a crucial role in colony losses and overall productivity. However, there is a lack of studies utilizing commercially available queen bees with a substantial sample size. This study aims to address this research gap by assessing the quality attributes of queens sold in Türkiye and investigating the influence of season and breeds on these attributes. Anonymously obtained from 15 different queen producers across five regions in three different seasons, queens were evaluated, based on reproductive criteria. In addition to these queen producers, we also included queens from Apiculture Research Institute, in which this study was conducted. Weight features and dissection characteristics were found to be influenced by the time period and ecotypes. This study examined the average diameter of the spermatheca, with measurements of 1.148 mm in April-May, 1.109 mm in July, and 1.115 mm in September. The average overall sperm count recorded was approximately 4.086 million. It is considered that this number is adequate and could be further improved by applying elements such as optimal location selection, drone cultivation, and correct and thorough implementation of the production method. While this study revealed multiple advantages of Yığılca Ecotype queen bees, the findings did not establish a substantial advantage over other breeds to recommend their widespread usage in beekeeping enterprises. To make definitive suggestions, it is crucial to conduct similar research over multiple years and assess colony growth in regions favored by migratory beekeepers during winter. The findings of this study provide valuable information about the quality of available queens from different regions and ecotypes in different seasons, aiding beekeepers in making informed decisions when purchasing queen bees. Additionally, the research offers valuable insights for queen bee producers, assisting in the timing of queen bee rearing operations based on specific regions and ecotypes.
***Keywords**:* Dissection, breeds, migratory beekeeper, sperm count, season

INTRODUCTION

Beekeeping is a substantial source of income, especially for individuals living in rural areas, and an integral part of the agricultural industry. The honey bee, *Apis mellifera*, holds the most ecological and economic position among the bee species classified under the *Apis* genus (Güler, 2017). The only sexually mature female in a colony of honey bees, known as the queen bee, is the only one who lays eggs. Consequently, every individual in the colony is her offspring (Winston, 1987; Okuyan and Akyol, 2018). During mating, queen bee gathers sperms from male bees (drones), store them in her sperm sac (spermatheca), and uses these sperms to fertilize a large number of eggs. The queen bee ensures the colony's growth and continuity by laying eggs, inhibiting the development of the female worker bees' reproductive organs, and maintaining order and unity within the colony through the release of pheromones (Kocher and Grozinger, 2011). A young queen bee's pheromones, due to their increased quanity and effectiveness, more perceptible to other members of the colony (Rangel *et al*., 2016).
As the queen bee ages, the amount of sperm stored in the spermatheca diminishes, resulting in an increased rate of sterile egg-laying. This is anticipated to increase the number of drones while simultaneously decreasing the number of worker bees (Szabo, 1993; Lee *et al*., 2019). To minimize the likelihood of this undesirable outcome, it is crucial to replace queen bees frequently. This necessity is further heightened when beekeepers aim to achieve continuous nectar flow from various locations within a single season. In this practice

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known as migratory beekeeping, the demand for queen bees to continuously lay eggs necessitates queen bee replacement (Akyol *et al*., 2008b).

In order to establish a swarm of colonies with the desired qualities, it is crucial to select and ensure the quality of breeding queen bees. The term "quality" actually refers to the reproductive potential of the queen bee (Amiri *et al*., 2017). The reproductive capacity of a queen bee cannot be determined only by the number of sperms. Various factors, such as the method used for the production of the queen bees, their age, body weight, sperm viability, number of mating drones, diameter and volume of the spermatheca, weight of the right-left ovary, number of ovarioles, etc., influence queen bee's reproductive potential (Facchini *et al*., 2021).

In modern beekeeping, both in Türkiye and globally, the issue of quality is encountered. Studies pertaining to "bee quality" globally including Türkiye are typically featuring small sample sizes from a certain region. In contrast, this study draws from a large number of samples acquired from diverse time periods and geographic regions. The data was collected concurrently from the commercial beekeeping operations from whom the bees were purchased for more accurate interpretation of the data. The objective of this study is to demonstrate the effects of season and breed on the quality features of bees, as well as to assess the current state of bees sold in Türkiye based on the qualities to be evaluated.

MATERIALS AND METHODS

Sample Collection: The research was conducted in the Turkish province of Ordu from April to December 2021. In this study, samples were collected from 15 beekeeping enterprises with an annual production capacity of at least 3000 units. Additionally, samples were also obtained from the apiary of the Apiculture Research Institute, where the study was conducted. The businesses were chosen based on their location and the bee species they maintained. During early spring, summer, and fall, a total of 480 honey bees were acquired, 10 per period and 30 per firm. The samples were taken on the days when honey bee sales began in the region. In each phase, honey bees were evaluated based on the quality attributes of honey bees in focus.

The queen bees were taken from the businesses anonymously and without a specified address. This was intended to prevent the manipulation of material collected from businesses. The period between the samples' departure from the company and their arrival in the laboratory was equalized.

Measuring the weight: The weight features of the queen bee (mg) and the weight of the right ovary (mg) were measured using a highly sensitive scale of the brand Presica XB 220A with a sensitivity of 0.001 g. The weight of the right ovary was measured after the fresh material was dried by placing it on both sides on drying paper.

Dissection Methods: Under a stereomicroscope, the ovaries and testes were removed by dissection. The queen bees were attached to a Petri plate lined with paraffin using insect needles from their thorax, abdomen, and legs. Internal organs were isolated from the abdominal walls, and the digestive tracts were removed (Dade, 1962; Carreck *et al*., 2013).

The spermatheca was extracted by sliding it between the thumb and index finger, and its dimensions were examined using a stereo microscope (Olympus SZ61).

In accordance with the studies conducted by Collins (2005) and Gençer and Kahya (2010), we employed a spectrophotometer (Perkin-Elmer Lambda 25 UV/VIS) for the measurement of sperm counting. The total quantity of sperm extracted with the Harbo syringe was transferred to a quartz cuvette containing 1 mL of modified Kiev solution (100 mL of pure water with 0.3 g D+glucose, 0.41 g potassium chloride, 0.21 g sodium bicarbonate, and 2.43 g trisodium citrate-2 hydrate). After ensuring the uniform dispersion of sperm in the modified Kiev solution using a micropipette, absorbance values at 260 nm wave length were collected using a spectrophotometer, and the sperm count was calculated using these data. The absorbance readings of 12 samples of semen-solution mixtures with varying concentrations were assessed with a spectrophotometer, and then the sperm count was performed using a Thoma counting chamber under a light microscope to translate the absorbance readings into sperm count. The absorbance values were measured with a spectrophotometer (Gençer and Kahya, 2010).

Statistical analysis: Study data expressed mean and standard deviation (SD). The data were analyzed by Two-way Repeated Measures ANOVA with period as a within-subjects factor and Breed as a between-subjects factor. As a multiple comparison test, Tukey's HSD test was used. The sperm count was analyzed using the Kruskal-Wallis test, and multiple comparisons were performed using the Dunn-Bonferroni test. The computations and interpretations utilized a 5% level of statistical significance. Minitab 19.2 (Minitab Inc., PA, USA) statistical software was used for all calculations.

RESULTS

Considering weight as a factor, we observed no significant seasonal impact on the Yığılca Ecotype (YE), Caucasus (C-CR**)**, and Black Sea Ecotype (BSE) bees. However, a distinct lag was noted in the Carniolan (C) bees during the transition from April-May to July, while the Italian and Anatolian bees exhibited a decline in September ($p < 0.05$, Table 1)

In April-May, the difference between the weight averages of the Carniolan and Yığılca Ecotype was statistically significant, whereas in July, the difference between the Anatolian-Italian-Carniolan and Yığılca Ecotype-Caucasus-Black Sea Ecotype groups was also statistically significant. In addition, the average weight of Carniolan bees in July lagged behind all other groups during the same time period ($p < 0.05$, Table 1). In September, there was a statistically significant difference between the average weights of Anatolian bees, Yığılca Ecotype bees, and Black Sea Ecotype bees ($p < 0.05$, Table 1).

In July, the Black Sea Ecotype had the heaviest queen bees, with an average weight of 206.560 milligrams, while the Carniolan breed had the lightest queen bees, with an average weight of 161.020 milligrams (Table 1).

In terms of the weight of the right ovary, it has been shown that all breeds are statistically substantially affected by the period factor ($p < 0.05$, Table 2). Other than the Black Sea Ecotype, the average changes in the right ovary between the April-May and July periods were statistically significant ($p < 0.05$, Table 2). The weight of the right ovary changed significantly between July and September in the Anatolian and Carniolian groups, and between April-May and September in the Italian, Anatolian, Yığılca Ecotype, and Black Sea Ecotype groups ($p < 0.05$, Table 2). In April-May, the weight of the right ovary of the queen bees of the Caucasus and Carniolian breeds was found to be less than the averages of the Yığılca Ecotype, Italian, and Black Sea Ecotype, whereas in July, the Black Sea Ecotype was found to be statistically higher than the other breeds, and the Carniolian was found to be statistically lower than all other breeds and ecotypes except the Italian and Caucasian bees ($p < 0.05$, Table 2). In September, it was determined that the average weight of the right ovary of the Anatolian bee was significantly less than that of the Black Sea Ecotype and Carniolian ($p < 0.05$, Table 2).

The time period factor significantly influenced the average spermatheca diameter of Italian and Carniolan queen bees ($p < 0.05$, Table 3). The change of the Carniolan bee during the transition to the July-September period and the change of the Italian bee between the April-May-July period transitions were statistically significant ($p < 0.05$, Table 3).

There was no statistically significant difference in the spermatheca diameter between breeds from April to May ($p > 0.05$, Table 3). During the months of July and September, however, the average spermatheca diameters of Carniolan and Italian bees were statistically smaller than those of the Caucasian and Black Sea Ecotypes ($p < 0.05$, Table 3).

The Black Sea Ecotype in September (1.183 mm) and the Carniolan breed in July (1.061 mm) were found to have the

Table 1. Descriptive statistical values and statistical analysis results for queen bee weight (mg).

Breeds/	April-May			July			September			Total		
Ecotypes	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
C-MR	30	187.487^{Ba}	10.852	30	161.023^{Cb}	17.611	30	183.557^{ABa}	18.742	90	177.356	19.783
I-MR	30	197.010^{ABa}	17.390	30	183.257^{Bab}	23.956	30	179.857^{ABb}	23.513	90	186.708	22.832
A-CAR	30	189.447^{ABa}	15.660	30	181.947^{Bab}	13.412	30	172.597^{Bb}	13.833	90	181.330	15.777
YE-YR	30	202.563^{Aa}	12.099	30	198.940^{Aa}	18.137	30	188.220^{Aa}	18.771	90	196.574	17.527
C-CR	30	194.110^{ABa}	10.329	30	190.970^{Aa}	14.861	30	184.833^{ABa}	13.853	90	189.971	13.574
BSE-BSR	10	197.410^{ABa}	14.557	10	206.560^{Aa}	15.634	10	194.920^{Aa}	15.193	30	199.630	15.465
Total	160	194.329	14.409	160	184.686	22.169	160	182.632	18.683	480	187.215	19.339
P	Period:0.000 - Breed:0.000 - Period x Breed Int.:0.000											

*In the same column, there is a statistically significant difference among breeds that do not share the common capital letter ($p < 0.05$). In the same row, there is a statistically significant difference among races that do not share the common lowercase letter ($p < 0.05$).
*C-MR (Carniolan-Mediterranean region), I-MR (Italian-Mediterranean region), A-CAR (Anatolian-Central Anatolia region), YE-YR (Yığılca Ecotype-Yığılca region), C-CR (Caucasus-Camili region), BSE-BSR (Black Sea Ecotype-Black Sea region).

Table 2. Descriptive statistical values and statistical analysis results for right ovary weight (g).

Breeds/	April-May			July			September			Total		
Ecotypes	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
C-MR	28	0.027^{Ca}	0.005	27	0.016^{Cb}	0.005	28	0.025^{Aa}	0.005	83	0.023	0.007
I-MR	30	0.034^{ABa}	0.005	29	0.022^{BCb}	0.006	26	0.024^{ABb}	0.006	85	0.027	0.008
A-CAR	30	0.032^{BCa}	0.011	29	0.024^{Bb}	0.009	27	0.018^{Bc}	0.005	86	0.025	0.010
YE-YR	27	0.037^{ABa}	0.009	30	0.026^{Bb}	0.006	30	0.021^{ABb}	0.006	87	0.028	0.010
C-CR	28	0.027^{Ca}	0.005	30	0.020^{BCb}	0.005	28	0.022^{ABab}	0.005	86	0.023	0.006
BSE-BSR	10	0.042^{Aa}	0.007	9	0.037^{Aab}	0.011	10	0.029^{Ab}	0.004	29	0.036	0.009
Total	153	0.032	0.009	154	0.023	0.008	149	0.022	0.006	456	0.026	0.009
P	Period:0.000 - Breed:0.000 - Period x Breed Int.:0.000											

*In the same column, there is a statistically significant difference among breeds that do not share the common capital letter ($p < 0.05$). In the same row, there is a statistically significant difference among races that do not share the common lowercase letter ($p < 0.05$).
*C-MR (Carniolan-Mediterranean region), I-MR (Italian-Mediterranean region), A-CAR (Anatolian-Central Anatolia region), YE-YR (Yığılca *Ecotype-Yığılca region), C-CR (Caucasus-Camili region), BSE-BSR (Black Sea Ecotype-Black Sea region).

Table 3. Descriptive statistical values and statistical analysis results for spermatheca diameter (mm).

Breeds/	April-May			July			September			Total		
Ecotypes	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
C-MR	28	1.118^{Aab}	0.071	26	1.061^{Bb}	0.111	28	1.131^{Aa}	0.077	82	1.104	0.091
I-MR	30	1.140^{Aa}	0.152	29	1.082^{Bab}	0.065	26	1.065^{Bb}	0.112	85	1.097	0.119
A-CAR	30	1.139^{Aa}	0.071	29	1.117^{ABa}	0.040	27	1.105^{ABa}	0.062	86	1.121	0.060
YE-YR	27	1.183^{Aa}	0.081	30	1.114^{ABa}	0.056	30	1.110^{ABa}	0.069	87	1.134	0.076
C-CR	28	1.165^{Aa}	0.051	30	1.147^{Aa}	0.061	28	1.142^{Aa}	0.058	86	1.152	0.057
BSE-BSR	10	1.141^{Aa}	0.052	9	1.159^{Aa}	0.036	10	1.167^{Aa}	0.070	29	1.155	0.054
Total	153	1.148	0.092	153	1.109	0.074	149	1.115	0.081	455	1.124	0.084
P	Period:0.000 - Breed:0.000 - Period x Breed Int.:0.000											

*In the same column, there is a statistically significant difference among breeds that do not share the common capital letter ($p < 0.05$). In the same row, there is a statistically significant difference among races that do not share the common lowercase letter ($p < 0.05$).
*C-MR (Carniolan-Mediterranean region), I-MR (Italian-Mediterranean region), A-CAR (Anatolian-Central Anatolia region), YE-YR (Yığılca Ecotype-Yığılca region), C-CR (Caucasus-Camili region), BSE-BSR (Black Sea Ecotype-Black Sea region).

Tablo 4. Descriptive statistical values and statistical analysis results for sperm count (million).

Breeds/	April-May			July			September			Total			p*
Ecotypes	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	
C-MR	28	5.051^{Aa}	0.909	27	2.345^{Cc}	0.706	28	3.908^{Bb}	1.469	83	3.785	1.541	0.000
I-MR	30	5.455^{Aa}	1.038	29	2.950^{Cb}	0.653	26	3.383^{Bb}	1.352	85	3.967	1.520	0.000
A-CAR	30	4.857^{Aa}	1.341	29	3.695^{BCb}	0.787	27	3.902^{Bb}	0.901	86	4.165	1.154	0.001
YE-YR	27	5.222^{Aa}	0.707	30	4.935^{ABa}	0.871	30	4.808^{Aa}	1.275	87	4.980	0.992	0.237
C-CR	28	2.844^{Ba}	1.120	30	4.123^{Bb}	0.590	28	3.800^{Bb}	1.111	86	3.601	1.099	0.000
BSE-BSR	10	2.552^{Ba}	0.589	9	5.480^{Ab}	1.097	10	3.597^{Ba}	0.694	29	3.821	1.445	0.000
Total	153	4.555	1.459	154	3.747	1.224	149	3.955	1.279	456	4.086	1.366	
p*		0.000			0.000			0.004					

*In the same column, there is a statistically significant difference among breeds that do not share the common capital letter ($p < 0.05$). In the same row, there is a statistically significant difference among races that do not share the common lowercase letter ($p < 0.05$).
*C-MR (Carniolan-Mediterranean region), I-MR (Italian-Mediterranean region), A-CAR (Anatolian-Central Anatolia region), YE-YR (Yığılca Ecotype-Yığılca region), C-CR (Caucasus-Camili region), BSE-BSR (Black Sea Ecotype-Black Sea region).

largest and smallest average spermatheca diameters, respectively (Table 3).

Except for the Yığılca Ecotype, all populations of the breeds have been significantly impacted by the time factor ($p < 0.05$, Table 4). Between April-May and July, a significant change in the mean sperm count of Italian, Anatolian, and Caucasian honey bee queens was detected ($p < 0.05$, Table 4). The variation in the mean sperm count of Carniolan queens was statistically significant throughout all periods, but the variation in the mean sperm count of Black Sea Ecotype queens was only statistically significant in July ($p < 0.05$, Table 4).

During the months of April and May, the Caucasian and Black Sea Ecotypes had a lower mean sperm count than other breeds ($p < 0.05$, Table 4). In July, the Black Sea Ecotype surpassed the other breeds, excluding Yığılca Ecotype, by a statistically significant margin, while the Carniolan and Italian bees have fallen behind the other breeds, excluding the Anatolian bee, by a statistically significant margin ($p < 0.05$, Table 4).

There was a statistically significant difference ($p < 0.05$, Table 4) between the general average of Yığılca Ecotype and other Breeds/Ecotypes.

The Black Sea Ecotype had the highest average sperm count in July (5.488 million), while the Carniolan breed had the lowest average sperm count (2.345 million). (Table 4).

DISCUSSION

The physical qualities of the queen bees, such as their weight, play a significant role in determining the queen bee's quality following emergence and mating (Hatjina *et al*., 2014). Several factors, such as their genotype, the season in which they are raised, the age at which they are transferred to the colony, and the status of the colony in which they were produced, might influence the weight of the queen bees (Skowronek *et al.*, 2004). Skowronek *et al.* (2004) discovered that queen bees lose weight rapidly between emergence and mating flight, but swiftly regain it once they begin laying eggs. In addition, there are substantial positive correlations between the weight at emergence, the weight during mating, and the weight at egg-laying ($r = 0.607$ and $r = 0.465$, respectively). In a further study, $r = 0.695$ was found between the weight during emergence and the weight at the start of egg laying (Ozmen, 2004). In agreement with previous research

(Szabo *et al*., 1987; Güler *et al*., 1999; Al-Ghzawi and Zaitoun, 2008), this study measured the weight of commercially available queen bees at the time of sale (when they have begun laying eggs) and discovered that the production season and genotype significantly affect the weight of the queen bees.

During the autumn, Güler (2017) categorized mated queen bees as high-quality if their weight was at least 220 mg, medium-quality if their weight was between 200 and 220 mg, and low-quality if their weight was less than 200 mg. 187.215 mg was the average weight of the queen bees utilized in the study. Consequently, the queen bees were deemed to be of poor quality based on their weight when compared to other period and breed averages. Other groups, excluding the Black Sea Ecotype and the Yığılca Ecotype, almost always fell below the desired weight level during all time periods, which is believed to be a significant indicator of probable problems in colony management.

In addition to parameters such as mating and the time of collection, the amount and length of a queen bee's ovaries can significantly affect its quality (Shehata *et al*., 1981; Amiri *et al.,* 2017). According to Tarpy *et al.* (2000), in order for a queen bee to be deemed of high quality, its ovaries must be of enough size and fully functional.

The study results indicated a statistically significant decrease in the weight of the right ovary of queen bees of all breeds during the transition from April-May to July. The average weight of ovaries during the period of April-May was 32.5 mg, whereas the average weight during the periods of July and September was 23 mg and 22.5 mg, respectively. In their research, Gregorc and Škerl (2015) discovered a strong correlation ($r = 0.5243$) between the weight of the ovaries and the weight of queen bees collected from Slovenian queen bee producers. Dolasevic *et al.* (2019) discovered a strong correlation between the weight of the fertilized queen bee and the weight of the ovaries ($r = 0.666$). Our findings confirm this association.

Previous studies on ovary weight have reported significant variations in their findings. For instance, Dolasevic *et al*. (2019) reported the highest average value of 123.9 mg, whereas Haarmann *et al*. (2002) reported the lowest average value to date of 47.9 mg. These divergent results can be attributed to the utilization of different measurement techniques. In some research, the ovaries are weighed fresh, without the use of drying paper, whereas in others, they are dried on paper for a specified amount of time prior to weighing. In this study, the ovaries were weighed after being placed on drying paper on both sides. As outlined in the methods section, the histology procedure used for ovarian counting prevented proper drying of the ovaries. As a result, the average ovary weight of 52 mg found in this study cannot be compared to that of earlier research.

According to some research, the diameter of the spermatheca of a "high-quality" queen bee should be at least 1.2 millimeters (Carreck *et al*., 2013; Hatjina *et al*., 2014). In our study, the average diameter of the spermatheca was 1.148 mm in April-May, 1.109 mm in July, and 1.115 mm in September. In their research of the Çukurova Region, Kaftanoğlu and Kumova (1990) determined the average precipitation to be 1.17, 1.159, 1.137, 1.148, 1.166, and 1.190 millimeters in April, May, June, July, August, and September, respectively. Similar to our findings, it is observable that the significantly high average April-May values diminish during the summer months. In addition, the Caucasian breed (1.152 mm), which has the largest average of all breeds, including the Black Sea Ecotype, in terms of spermatheca diameter, was determined to be the breed with the largest spermatheca diameter by Güler *et al* (1999). Arslan *et al.* (2021) discovered an average spermatheca diameter of 1.044 mm in their investigation of the qualitative parameters of queen bees gathered from several queen bee-producing firms in the Mediterranean Region. They reported that the result they obtained aligned with the criteria established for the Mediterranean Region during early spring. Similarly, the average diameter of 1.124 mm found in our research is within the permitted range. In our study, we found that the Caucasian breed exhibited the maximum average spermatheca diameter, measuring 1.165 mm (April-May), which is consistent with the findings of Kaftanoğlu and Kumova (1990) and Güler *et al.* (1999). This number is interesting since it is lower than the reference value of 1.2 mm given by Carreck *et al.* (2013) and Hatjina *et al.* (2014). In our study, the Caucasian breed exhibited the maximum average spermatheca diameter, measuring 1.165 mm (April-May), which is consistent with the findings of Kaftanoğlu and Kumova (1990) and Güler *et al*. (1999). Interestingly, this value is lower than the reference value of 1.2 mm proposed by Carreck *et al*. (2013) and Hatjina *et al*. (2014). However, considering the findings from other studies conducted in the Mediterranean region, including Kahya (2017) with a value of 1.1 mm, Arslan and Cengiz (2020) with 1.015 mm, Dolasevic *et al*. (2019) with 1.237 mm for spermatheca diameter, and Tarpy *et al*. (2012) with 0.94 mm^3, Ahmed *et al*. (2018) with 1.20 mm^3, and Al-Ghzawi and Zaitoun (2008) with 0.86 mm^3 for spermatheca volume, it is evident that the reference value of 1.2 mm is inappropriate for Turkish breeds and ecotypes in the Mediterranean region. Using such a reference value would unjustly classify even the highest quality queen bees reared in this region as low quality. The evaluation of queen bee quality in numerous studies has emphasized the relationship between spermatheca diameter and sperm count. This association has consistently been reported as favorable and strong (Woyke, 1966; Bieńkowska *et al*., 2008), with Akyol *et al*. (2008a) calculating a correlation coefficient of $r = 0.97$. Interestingly, Hatjina *et al*. (2014) observed that certain European studies conducted in Bulgaria, Greece, and Slovenia did not include sperm counts; instead, they relied solely on measuring spermatheca size, assuming that a larger spermatheca would correspond to higher sperm counts.

However, it is worth noting that previous studies have revealed that spermathecae are not entirely filled, with an average fill level of approximately 47% (Tarpy *et al*., 2012; Amiri *et al*., 2017). In line with these findings, Arslan *et al*. (2021) observed a negative correlation (r = -0.068) between spermatheca diameter and sperm quantity in their study, which assessed various quality features of queen bees obtained from commercial businesses.

In our study, the association between spermatheca diameter and sperm count generally exhibited a positive trend, albeit at a low level, with considerable variability. Notably, the Italian honey bee exhibited distinct correlation levels during different periods, such as April-May, July, and September (0.477, -0.163, 0.485). Interestingly, although there was no statistically significant change in spermatheca diameter during the transition from April-May to July, there was a significant 46% reduction in sperm count. These findings highlight the influence of other environmental factors and underline the limitations of assuming spermatheca diameter as a sole predictor for sperm count.

Considering Hatjina's observations, the incomplete filling of spermathecae reported in previous studies, and the negative correlation found by Arslan *et al*., it is evident that relying solely on spermatheca diameter may not provide an accurate estimation of sperm count. Further investigations considering additional factors are necessary to comprehensively evaluate queen bee quality.

Our analysis revealed no statistically significant shift in inter-period sperm count among the different bee breeds. However, a notable observation was the lower sperm counts recorded in Caucasian and Black Sea Ecotype queen bees during the months of April and May, with an average count of 4.555 million. A plausible explanation for this finding is the limited availability of drones in the Black Sea region during these months, likely due to seasonal conditions. The reduced presence of drones for mating during this period may contribute to the lower sperm counts observed in queen bees of these breeds. Consequently, relying solely on morphological characteristics may not accurately reflect the quality of queen bees during April and May in the Black Sea region.

Previous studies, such as those conducted by Mattila and Seeley (2007) and Bratu *et al*. (2022), have demonstrated that queens mated with a larger number of drones yield colonies with superior reproductive characteristics compared to those mated with a smaller number of drones. Additionally, research by Boes (2009) and Bratu *et al*. (2022) has shown that the number and quality of drones raised by a colony are influenced by seasonal variations, food availability, and colony strength, with peak drone production occurring in June and July. Therefore, considering the impact of seasonal variations on the availability of drones for mating is crucial for a comprehensive evaluation of queen bee quality during this period. Future studies should aim to assess both morphological and reproductive parameters to gain a more accurate understanding of queen bee quality, considering the regional dynamics of seasonal changes.

In contrast, breeds such as Carniolan, Italian, Anatolian, and Yığılca Ecotypes exhibited the highest sperm counts during April and May, as observed in our study's inter-period comparisons. This finding aligns with previous research highlighting the significance of rearing queen bees during the spring months in semi-arid regions of the Mediterranean (Al-Ghzawi and Zaitoun, 2008). The higher sperm counts observed in these breeds during April and May can be attributed to the favorable conditions for queen bee rearing during this period. Abundant pollen and nectar flow provide the necessary resources for optimal queen bee development and mating. However, as the season progresses, and the availability of pollen and nectar decreases, potentially affecting the overall quality and reproductive capacity of queen bees reared during those periods.

These findings underscore the importance of considering timing and environmental factors when evaluating queen bee quality. The months of April and May emerge as critical periods for rearing high-quality queen bees in the Mediterranean region. Understanding the influence of seasonal variations on the reproductive performance of different bee breeds can inform beekeeping practices and enhance the overall success of queen bee rearing programs in these regions. In addition, Koç and Karacaoğlu (2004) revealed that April and May are the optimal months for queen bee production. July's rise in the sperm count of Black Sea Ecotype and Caucasian bees can be ascribed to an increase in sunny days, rising temperatures, and the resulting rise in the drone population. The considerable fall in sperm count among bee breeds can be ascribed to exposure to high temperatures and insufficient nectar-pollen flow. This study discovered that in September, the average breed was approaching each other. In general, a fall in nectar-pollen quantities and the accompanying decrease in the drone population by worker bees are additional factors affecting sperm count (Al-Ghzawi and Zaitoun, 2008). According to Koç and Karacaoğlu (2004), the months of August and September are unsuitable for queen bee raising.

The average overall sperm count obtained in this study was 4.086 million, which falls within the range of previous research assessing the fertilization status of queen bees. Woyke (1962) stated that a fertilized queen bee should possess more than three million sperm, while Delaney *et al*. (2011) classified queen bees with less than five million sperm as "insufficiently fertilized." Studies investigating the quality parameters of queen bees from different regions reported varying sperm counts. For instance, Hatjina *et al.* (2014) observed sperm counts ranging between 0.80 and 6.01 million in 2006 and between 3.34 and 6.69 million in 2008 for

Slovenian queen bees. Other studies have reported similar findings, including Tarpy *et al*. (2012) with 4.37 million, Güler and Alpay (2005) with 5.61 million, Arslan *et al*. (2021) with 4.454 million, Uçak (2001) with 3.625-8.125 million, Delaney *et al*. (2011) with 3.99 million, and Kaftanoğlu and Kumova (1990) with 3.820 million.

Based on our findings from commercially available queen bees in Türkiye, the average overall sperm count fell within the range observed in previous studies. These reference values can be useful for evaluating the fertilization status of queen bees and ensuring their reproductive potential. It is worth noting that factors such as optimal location selection, drone cultivation, and the correct implementation of production methods can potentially enhance the sperm count.

Conclusion: The findings of this study revealed notable variations in the weight and sperm count of queen bees belonging to the Caucasian breed and Black Sea Ecotype during the months of April and May. In this time period, the queen bees exhibited weights ranging from 194 mg to 197 mg, while their corresponding sperm counts were measured at 2.8 million and 2.5 million, respectively. This once again underlines the potential pitfalls of relying solely on morphological characteristics, when assessing the quality of queen bees. During the months of April and May, when queen bee sales are at their highest, it is recommended that early purchases be made from the Mediterranean Region, while Yığılca Ecotype and Anatolian queen bees can be appraised in the beginning of May.

In the regions where Caucasian and Black Sea Ecotype queen bees are produced, a large increase in sperm count was noted in July, associated with an increase in the drone population. July is the optimal month for businesses that prefer Caucasian queen bees, according to the report.

It is also noteworthy that the Yığılca Ecotype, which outperforms other breeds in terms of sperm count in September, has averages that are greater than acceptable levels and does not exhibit statistical change over time. However, the study concludes that the Yığılca Ecotype's considerable advantages over other breeds are insufficient to promote it to beekeeping businesses on its own. In order to make such a recommendation, it is believed that comparable research should be repeated in different years and colony growth should be evaluated in places where migratory beekeepers commonly spend the winter.

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