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# The relationship between the internal and external morphological parameters of honeybee queens (Hymenoptera: Apidae) and the determination of morphological variation

**G. Özmen Özbakır#**

Harran University, Faculty of Agriculture, Department of Animal Science, Şanlıurfa, Türkiye

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## Abstract

This study was carried out to determine the crucial external and internal morphological characteristics for evaluating queens in three rearing periods (May, June, and July). Data of 65 queens reared from the local honeybees of the Sanliurfa (*Apis mellifera* L.) and Anatolian (*Apis mellifera anatoliaca*) honeybee colonies were used. Discriminant and principal component analyses (PCA) were done for thirty-one external and internal morphological characteristics of queens. The highest weight of the queen at emergence was determined in May for the Sanliurfa queens and in June for the Anatolian queens. The averages in ovary weight and spermathecae diameter of queens were found to be non-significant according to groups and periods. The number of ovarioles of the queens was different according to rearing period. Using the left basitarsus width variable, the Sanliurfa and Anatolian queens were classified correctly to their pre-assigned groups using discriminant analysis (73.8%). According to the result of PCA applied to all variables of queens, nine components explained 81.68% of the total variation. The seven variables in the first principal component were the left basitarsus length, the right hindleg length, the left hindleg length, the right basitarsus length, the left tibial length, the right tibial length, and the left basitarsus width. The forewing and the hindwing characteristics were included in the second principal component, and the number of ovarioles was included in the third principal component. The study shows that genotypes can be discriminated using the hindleg variables, in particular, as well as the internal and external morphological parameters of the queens.

**Keywords**: *Apis mellifera,* morphometry, ovariole number, principal component analysis, queen honeybee
#e-mail: gozmenozbakr@harran.edu.tr

## Introduction

In the honeybee colony, the queen is the only individual that lays fertile eggs and is the mother of all individuals in the colony. Therefore, the genotype of the queen and the drones she mates with is the most important factor affecting colony performance. The many factors that explain the quality of the queen include environmental conditions and practices of the beekeeper, as well as the genetic history of the queen, its physiological development from the larval stage, mating success, and resistance to diseases and pests. Physiological and anatomical characteristics such as high body weight, large spermatheca size, the high number of spermatozoa in the spermathecae, the high number of ovarioles, and many features that appear in the colony such as swarming, defence, and hygiene behaviours are considered for the expression of 'quality' or 'good' of the queen (Winston, 1987; Amiri *et al*., 2017; Fine, 2020; Mattiello *et al*., 2022).

Queen weight is an important feature that correlates with both colony productivity and internal–external morphological characteristics of the queen (Tarpy *et al*., 2012; Collins & Pettis, 2013; Hatjina *et al*., 2014; Mattiello *et al*., 2022). Queen weight has a significant effect on acceptance ratio of queens, the onset of oviposition, diameter of spermatheca and laying rate, and number of spermatozoa in the spermatheca (Akyol *et al*., 2008). In recent years, studies on inadequate queens (in terms of internal and external characteristics)

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and their role in colony losses have attracted attention (van Engelsdorp *et al*., 2008; Amiri *et al*., 2017). The relationship between the morphological features of the queen (such as head, thorax, wing, leg, and reproductive organ characteristics) were also used to explain queen quality. Queen ovary weight and number of ovarioles, spermatheca diameter and volume, and spermatozoid number in the spermatheca have been investigated by many researchers (Gregorc & Smodis Skerl, 2015; Al-Sarhan *et al*., 2019; Walsh *et al*., 2021). For example, a significant correlation was found between thorax width, stored sperm count, and mating frequency (Delaney *et al*., 2011). Larval age (0- and 2-days old) affected the queens' wet weight, thorax width, and diameter of the spermatheca, but no difference was observed in terms of head width (Tarpy *et al*., 2011). Different larval ages and supplementary feeding did not affect the wing lengths of the queens (Mahbobi *et al*., 2012). In a recent study, weak correlations were found between the morphological and reproductive traits of the queen, while high and positive phenotypic correlations with queen size were reported. Morphological and reproductive characteristics were not found to be related (Facchini *et al*., 2021). Since the determination of most morphological and reproductive organ characteristics of queens is time-consuming, expensive, and impractical, it is recommended that non-destructive features be included in commercial queen rearing programs (Frost *et al*., 2021).

In the current study, honeybee queens were examined in terms of internal and external morphological characteristics, with the aim of evaluating them according to queen rearing periods. The internal and external morphological characteristics of Anatolian queens (*Apis mellifera anatoliaca*) were compared with queens reared in local Sanliurfa honeybee colonies. Evaluations of many characteristics were made to elucidate the relationships between the internal and external morphological characteristics of the queens to benefit breeding studies and to determine the criteria that commercial queen producers can benefit from.

## Materials and methods

Queen rearing was carried out between May and July in 2019 in Sanliurfa, located in the southeast of Turkey, in the Apiculture Research and Application Unit of Harran University (37°11'05'' N and 38°59'40'' E). The live material for the study consisted of the local honeybee, *Apis mellifera* of Sanliurfa in the apiary; Anatolian (*Apis mellifera anatoliaca*) colonies were purchased from an apiary where queens are reared as a breed. The standard queen rearing process of Laidlaw (1985) was followed. Two larval source colonies were selected from both genotypes. Twenty, one-day-old larvae were transferred to grafting frames of two bars carrying queen cell cups, which were made from beeswax. The same larval source colonies were used for each genotype group and queen rearing was repeated in May, June, and July.

The queens were weighed (mg) at emergence (Radwag PS 750. R2; 0,001g), and released back to their own colonies. After oviposition, the queens were kept at -20 °C and dissected one by one under a stereomicroscope to determine the external and internal morphological characteristics (Leica S8 APO with LAS Software). The queens were also weighed before the dissection. The spermatheca was removed and its diameter (mm) was measured without the tracheal net. The wet ovary was weighed (mg). The number of right and left ovarioles was determined under the microscope using the real-time counting method. External morphological characteristics of the queens; head width and length (mm), thorax width and length (mm), right and left forewing length (mm), right and left forewing width (mm), right and left hindwing length (mm), right and left hindwing width (mm), right and left hamuli numbers, right and left hindleg length (femur+tibia+basitarsus length) (mm), and left and right hindleg basitarsus width (mm) were measured according to the method of Ruttner (1988).

Multivariate analysis of variance was applied to the morphological and reproductive organ characteristics of the queens reared in two different genotype groups over the three trial periods. According to the queen rearing periods and genotype groups, stepwise discriminant analysis was performed to determine the correct classification rates of queens into original groups in terms of all variables. A principal component analysis was also carried out to determine which variables explain the total variation in terms of external and internal morphological characteristics of queens. Bonferroni multiple comparison tests were used to compare means. The correlations between characters were found by the Pearson correlation method. Statistical analyses were performed using SPSS (v21).

## Results and Discussion

The highest grafting acceptance rate (90%) was obtained in the Anatolian group in May and June. The mating success of queens decreased from May to July. Similarly, the pre-oviposition period of the queens was prolonged in July. The averages of weight at the emergence of the queens were different according to the rearing periods ($P$ <0.05). While the average weight at the emergence of the queens in May (185.2 mg) and June (182.9 mg) was similar, the average in July (173 mg) was low. The highest average weight at emergence of the queens was determined in May (189.5 mg) for the Sanliurfa group and in June (183.2 mg) for the Anatolian group. Group and period interaction was insignificant in terms of the emergence weight of the queens

($P$ >0.05). The averages of fresh ovary weight of the queens were not different according to queen groups and rearing periods ($P$ >0.05). The ovariole number of the queens was different according to rearing period ($P$ <0.05), but the group and rearing period interaction was not significant ($P$ >0.05). The number of ovarioles of the queens in May (335.4) and June (349.7) was different; July (340) was similar to June. On the contrary, group and period interaction was important in terms of the number of right ovarioles ($P$ <0.05). The multiple comparison test in terms of the number of right ovarioles indicated that the general averages of June and July were similar; May (164.0) was different from June (176.3) and July (177.1) for the Sanliurfa queens ($P$ <0.05). In July, the difference between the averages of the number of right ovarioles for Anatolian (177.1) and Sanliurfa queens (164.8) was different ($P$ <0.05). The means of the diameter of spermathecae of the queens were not different according to genotype groups and periods ($P$ >0.05). Some characteristics of the queens are given in Table 1 according to groups and periods (mean ± standard error, SE).

**Table 1** Some external and internal characteristics of queens (mean ± standard error) from Sanliurfa (*Apis mellifera*) and Anatolian (*Apis mellifera anatoliaca*) colonies

| Characters | Group | Periods | | | | | | N | General |
|---|---|---|---|---|---|---|---|---|---|
| | | n | May | n | June | n | July | | |
| **Weight at emergence (mg)** | Sanliurfa | 12 | 189.5 ± 3.19 | 11 | 182.6 ± 3.35 | 7 | 176.0 ± 4.81 | 30 | 182.7 ± 2.07 |
| | Anatolian | 13 | 181.0 ± 3.06 | 12 | 183.2 ± 3.19 | 10 | 170.1 ± 3.49 | 35 | 178.1 ± 1.88 |
| | General | 25 | 185.2 ± 2.21$^{a}$ | 23 | 182.9 ± 2.30$^{a}$ | 17 | 173.0 ± 2.72$^{b}$ | 65 | 180.4 ± 1.40 |
| **Weight at dissection (mg)** | Sanliurfa | 12 | 215.5 ± 3.54 | 11 | 209.4 ± 3.70 | 7 | 191.8 ± 4.63 | 30 | 205.6 ± 2.30 |
| | Anatolian | 13 | 218.0 ± 3.40 | 12 | 213.0 ± 3.54 | 10 | 190.2 ± 3.88 | 35 | 207.0 ± 2.08 |
| | General | 25 | 216.7 ± 2.45$^{a}$ | 23 | 211.2 ± 2.56$^{a}$ | 17 | 191.0 ± 3.02$^{b}$ | 65 | 206.3 ± 1.55 |
| **Wet weight of ovary (mg)** | Sanliurfa | 12 | 48.7 ± 1.51 | 11 | 50.3 ± 1.58 | 7 | 48.4 ± 1.98 | 30 | 49.1 ± 0.98 |
| | Anatolian | 13 | 47.3 ± 1.45 | 12 | 47.9 ± 1.51 | 10 | 45.6 ± 1.66 | 35 | 46.9 ± 0.89 |
| | General | 25 | 48.0 ± 1.05 | 23 | 49.1 ± 1.09 | 17 | 47.0 ± 1.29 | 65 | 48.0 ± 0.66 |
| **Right ovarioles number** | Sanliurfa | 12 | 164.0 ± 3.12$^{a}$ | 11 | 176.3 ± 3.26$^{b}$ | 7 | 177.1 ± 4.08$^{b}$ | 30 | 172.5 ± 2.03 |
| | Anatolian | 13 | 169.3 ± 2.99 | 12 | 171.8 ± 3.12 | 10 | 164.8 ± 3.42$^{a}$ | 35 | 168.6 ± 1.83 |
| | General | 25 | 166.6 ± 2.16 | 23 | 174.0 ± 2.25 | 17 | 170.9 ± 2.22 | 65 | 170.5 ± 1.36 |
| **Left ovarioles number** | Sanliurfa | 12 | 166.7 ± 3.56 | 11 | 177.6 ± 3.72 | 7 | 172.7 ± 4.66 | 30 | 172.3 ± 2.31 |
| | Anatolian | 13 | 170.7 ± 3.42 | 12 | 173.5 ± 3.56 | 10 | 165.4 ± 3.90 | 35 | 169.9 ± 2.10 |
| | General | 25 | 168.7 ± 2.47 | 23 | 175.6 ± 2.57 | 17 | 169.0 ± 3.04 | 65 | 171.1 ± 1.56 |
| **Number of ovarioles** | Sanliurfa | 12 | 330.7 ± 5.50 | 11 | 354.0 ± 5.74 | 7 | 349.8 ± 7.20 | 30 | 344.8 ± 3.57 |
| | Anatolian | 13 | 340.1 ± 5.28 | 12 | 345.4 ± 5.50 | 10 | 330.2 ± 6.02 | 35 | 338.5 ± 3.24 |
| | General | 25 | 335.4 ± 3.81$^{b}$ | 23 | 349.7 ± 3.97$^{a}$ | 17 | 340.0 ± 4.69$^{ab}$ | 65 | 341.7 ± 2.41 |
| **Diameter of spermatheca (mm)** | Sanliurfa | 12 | 1.10 ± 0.019 | 11 | 1.15 ± 0.020 | 7 | 1.19 ± 0.025 | 30 | 1.14 ± 0.012 |
| | Anatolian | 13 | 1.14 ± 0.018 | 12 | 1.15 ± 0.019 | 10 | 1.15 ± 0.021 | 35 | 1.15 ± 0.011 |
| | General | 25 | 1.12 ± 0.013 | 23 | 1.15 ± 0.014 | 17 | 1.17 ± 0.016 | 65 | 1.15 ± 0.008 |
| **Head width (mm)** | Sanliurfa | 12 | 3.71 ± 0.026 | 11 | 3.65 ± 0.027 | 7 | 3.71 ± 0.034 | 30 | 3.69 ± 0.017 |
| | Anatolian | 13 | 3.68 ± 0.025 | 12 | 3.68 ± 0.026 | 10 | 3.66 ± 0.028 | 35 | 3.67 ± 0.015 |
| | General | 25 | 3.70 ± 0.018 | 23 | 3.66 ± 0.019 | 17 | 3.68 ± 0.022 | 65 | 3.68 ± 0.011 |
| **Head length (mm)** | Sanliurfa | 12 | 3.52 ± 0.034 | 11 | 3.39 ± 0.035 | 7 | 3.49 ± 0.044 | 30 | 3.47 ± 0.022 |
| | Anatolian | 13 | 3.47 ± 0.032 | 12 | 3.39 ± 0.034 | 10 | 3.41 ± 0.037 | 35 | 3.42 ± 0.020 |
| | General | 25 | 3.50 ± 0.023$^{a}$ | 23 | 3.39 ± 0.024$^{b}$ | 17 | 3.45 ± 0.029$^{ab}$ | 65 | 3.44 ± 0.015 |
| **Thorax width (mm)** | Sanliurfa | 12 | 4.59 ± 0.037$^{b}$ | 11 | 4.53 ± 0.039 | 7 | 4.62 ± 0.049 | 30 | 4.58 ± 0.024 |
| | Anatolian | 13 | 4.43 ± 0.036$^{a}$ | 12 | 4.58 ± 0.037$^{b}$ | 10 | 4.54 ± 0.041 | 35 | 4.52 ± 0.022 |
| | General | 25 | 4.51 ± 0.026 | 23 | 4.56 ± 0.027 | 17 | 4.58 ± 0.032 | 65 | 4.55 ± 0.016 |
| **Thorax length (mm)** | Sanliurfa | 12 | 4.78 ± 0.042 | 11 | 4.65 ± 0.044 | 7 | 4.83 ± 0.055 | 30 | 4.75 ± 0.027 |
| | Anatolian | 13 | 4.68 ± 0.040 | 12 | 4.75 ± 0.042 | 10 | 4.74 ± 0.046 | 35 | 4.72 ± 0.025 |
| | General | 25 | 4.73 ± 0.029 | 23 | 4.70 ± 0.030 | 17 | 4.78 ± 0.036 | 65 | 4.74 ± 0.018 |

$^{a,b}$ Different superscripts in the same row and column for each variable represent statistically significant means ($P$ <0.05)

In the study, the weight of the queens at emergence differed according to the rearing periods and the general average was found to be similar to the queens from the Anatolian bee, Muğla ecotype, in the medium weight group (Akyol *et al*., 2008) and the queens reared from one-day old larvae (Okuyan & Akyol, 2018). In a previous study, similar results were obtained in the averages of the emergence weight of the queens reared from 1-day old larvae (Ozmen Ozbakır, 2021). The live weight of the queens varied between 171–223 mg in the queens obtained from commercial queen rearing enterprises (Arslan *et al*., 2021). The results of the current study were found to be lower than the average weights at the emergence of Italian queens (Faccini *et al*., 2021) and Caucasian queens (Kahya *et al*., 2008). Although ovary weight is a trait that is examined to express the reproductive potential of the queens, it could vary in terms of the number of egg cells at different developmental levels in the ovarioles (Kahya *et al*., 2008). Ovary mass is expressed as one of the important parameters affecting the body mass of the queens (Presern & Smodis Skerl, 2019). However, Yi *et al*. (2020) reported that the queens reared from eggs have a higher weight at the emergence, queen cell length, number of ovarioles, and shorter development time than older worker larvae. In the current study, there was no significant difference between the ovary weight of the Anatolian and Sanliurfa queens, and the highest value was obtained in June. The ovary weight averages in the study were similar to the values obtained by Gilley *et al*. (2003) for the queens reared from 0-day-old larvae and the values which Kahya *et al*. (2008) reported for the light queen group, but were lower than the values reported by Gregorc & Smodis Skerl (2015). In terms of the right ovariole numbers, the difference between the averages of Anatolian and Sanliurfa queens was found to be significantly different in July. The highest number of ovarioles of queens was obtained in June; the lowest value was obtained in May, which was similar to the average of July. The number of ovarioles was similar to the values reported by Kahya *et al*. (2008), Jackson *et al*. (2011), Gregorc & Smodis Skerl (2015), and Walsh *et al*. (2021) but higher than those reported by Faccini *et al.* (2021). In the current study, the spermathecal diameter of the queens was similar to the values reported in many studies (Akyol *et al*. 2008; Kahya *et al*., 2008; Arslan *et al*., 2021) but was lower than those reported by Faccini *et al*. (2021).

The head width, head length, thorax width, and the thorax length of the queens is given in Table 1. According to rearing period, head length of the queens was different in May and June ($P$ <0.01). However, the group and period interaction were found to be significant in terms of the thorax width and thorax length ($P$ <0.05). As a result of the multiple comparison tests of thorax width, the difference between the May and June averages was significant for the Anatolian group ($P$ <0.05); the June and July averages were similar. While the difference between Anatolian and Sanliurfa queens' thorax width averages in May was significant ($P$ <0.05), it was not significant for other rearing periods. As a result of the multiple comparison tests of thorax length, May and July averages were similar in the Sanliurfa queen group, while in the Anatolian group, the July average was similar to that of May and June ($P$ >0.05).

The characteristics of both right and left forewing and hindwing in Sanliurfa and Anatolian group queens were examined in three rearing periods. The characteristics of right forewing width ($P$ <0.05) and right hindwing width ($P$ <0.05) were different according to the genotype groups. There was no significant difference in all wing characteristics according to the queen rearing period. The group and period interaction were significant only in terms of the number of hooks on the right hindwing ($P$ <0.05). The averages of the three rearing periods were similar in terms of hook number on the right hindwing of the Anatolian queens. The May average was different from June and July for the Sanliurfa queens ($P$ <0.05). The difference between Anatolian and Sanliurfa group averages in May was significant ($P$ <0.05), but not in the other periods. The characteristics of the right and left forewing and hindwing of Anatolian and the Sanliurfa queens (mean ± SE) are given in Figures 1 and 2.

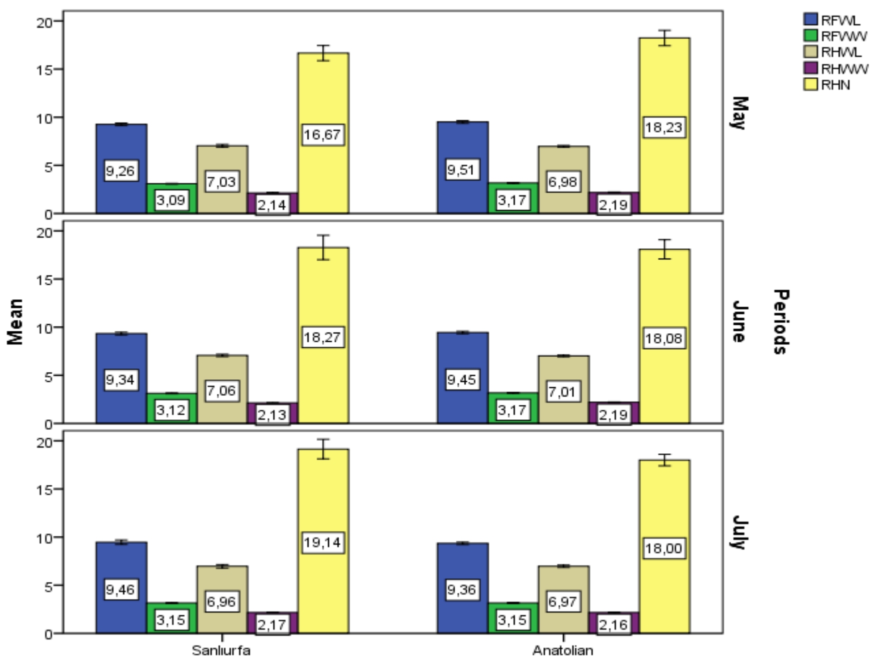


**Figure 1** Right forewing and hindwing characters of Anatolian and Sanliurfa queens (mm), (mean ± standard error). Right forewing length (RFWL), right forewing width (RFWW), right hindwing length (RHWL), right hindwing width (RHWW), right hamuli number (RHN)

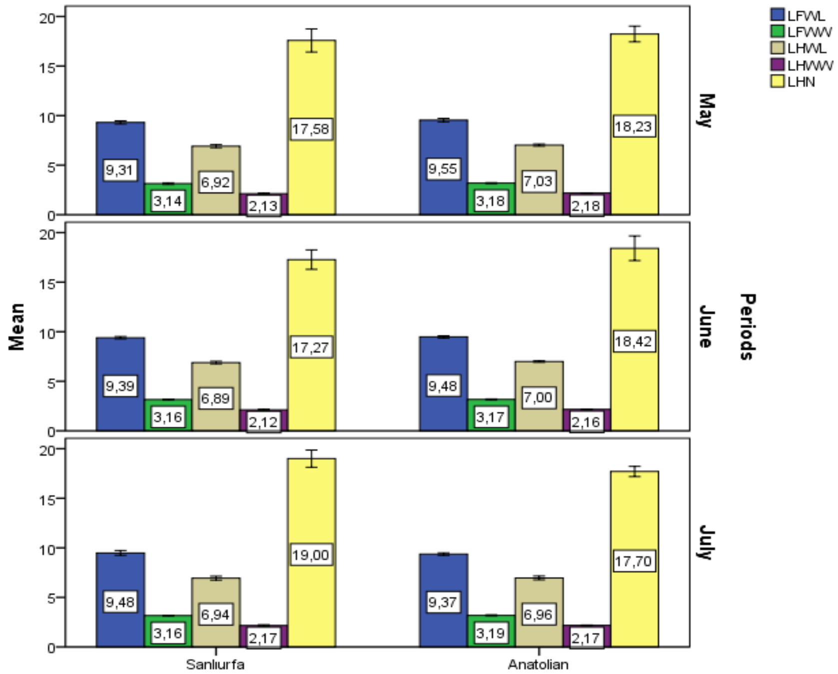


**Figure 2** Left forewing and hindwing characters of queens (mm), (mean ± standard error). Left forewing length (LFWL), left forewing width (LFWW), left hindwing length (LHWL), left hindwing width (LHWW), and left hamuli number (LHN)

The right hindleg and the left hindleg characteristics of queens are given in Figures 3 and 4. Only the left basitarsus width (*P* <0.01) was different according to the queen genotype group. The group and period interaction was significant in terms of right tibial length, right basitarsus length, left tibial length, and right hindleg length of the queens (*P* <0.05). In terms of right tibial length, the difference between the general averages of Anatolian and Sanliurfa queens was significant (*P* <0.05). The overall averages of the rearing periods were similar. The difference between the right tibia length averages of Anatolian and Sanliurfa queens in both May and June was significant in the pairwise comparison of rearing periods (*P* <0.05). As a result of the multiple comparison test, the difference between the general averages of the right basitarsus length of Anatolian and Sanliurfa queens was not significant. In terms of general averages of the rearing periods, June was similar to both May and July. However, the difference between the mean of May and July in the Sanliurfa group was significant (*P* <0.05). The averages were similar for all rearing periods in the Anatolian group. The difference between the right basitarsus length averages of Anatolian and Sanliurfa queens in May was significant (*P* <0.05). As a result of the multiple comparison test, the difference between the general averages of the left tibial length of Anatolian and Sanliurfa queens was not significant. June and July were found to be similar in terms of the general averages of the rearing periods. The May average of the left tibial length was different from June and July in the Sanliurfa queens (*P* <0.05). The averages were similar in the Anatolian queens for all rearing periods. The difference between the left tibial length averages of Anatolian and Sanliurfa queens in May was significant (*P* <0.05). As a result of the multiple comparison test, the difference between the general averages of the right hindleg length of Anatolian and Sanliurfa queens was not significant. In the Anatolian group, May and June averages were similar. The difference between the right hindleg length averages of Anatolian and Sanliurfa queens in May was significant (*P* <0.05).

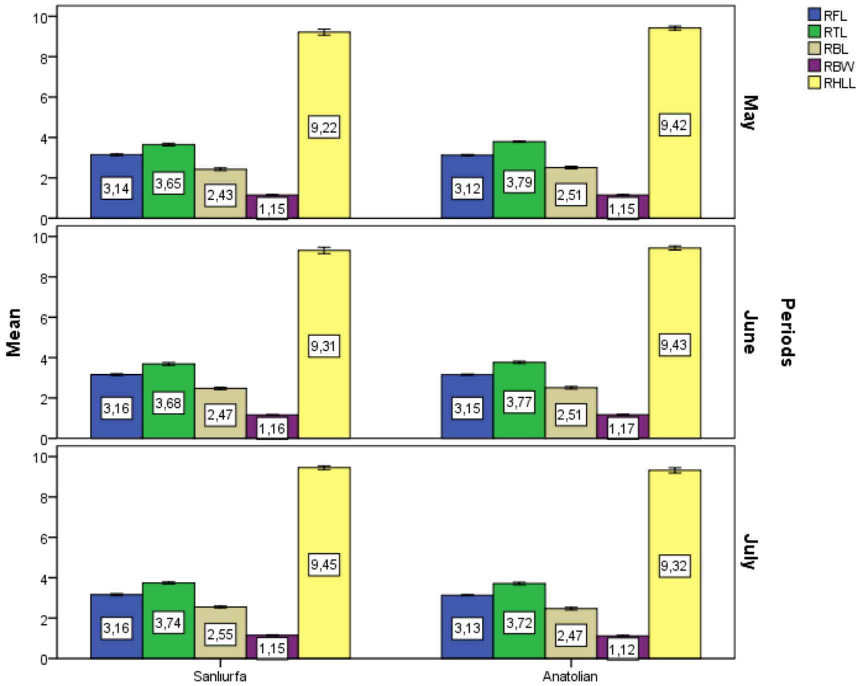


**Figure 3** Right hindleg characters of queens (mm), (mean ± standard error). Right femur length (RFL), right tibial length (RTL), right basitarsus length (RBL), right basitarsus width (RBW), right basitarsus length (RBL), and right hindleg length (RHLL)

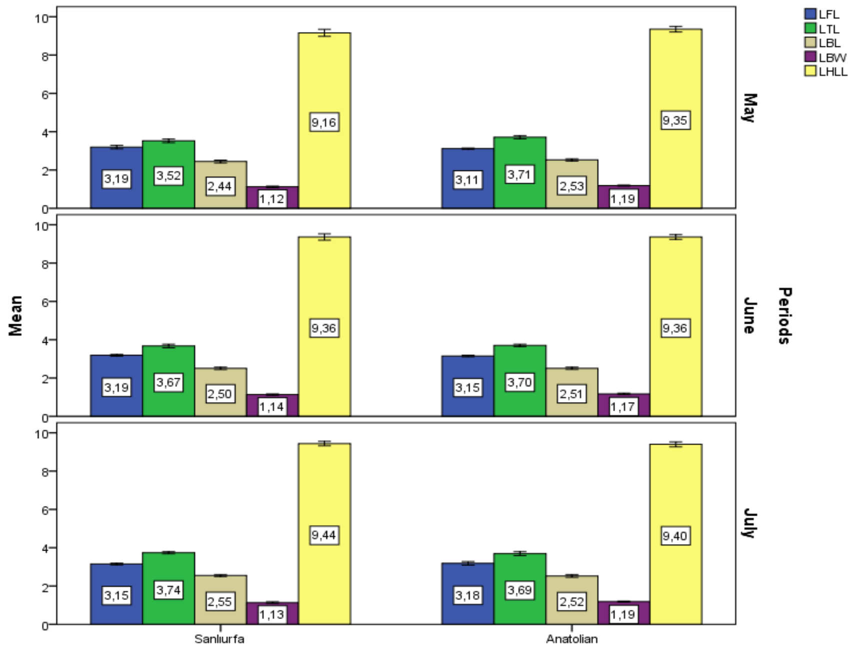


**Figure 4** Left hindleg characters of queens (mm), (Mean ± standard error). Left femur length (LFL), left tibial length (LTL), left basitarsus length (LBL), left basitarsus width (LBW), left basitarsus length (LBL), and left hindleg length (LHLL)

The group × period interaction was found to be significant for the characteristics of the thorax width and the thorax length, the number of hooks on the right wing, the right tibial length, the right basitarsus length, the left tibial length, and the right hindleg length of the queens. The means of head width and length obtained in the current study were found to be similar to those reported by Mahbobi *et al*. (2012), Okuyan & Akyol (2018), and Faccini *et al*. (2021). The thorax width averages were higher than those reported by Okuyan & Akyol (2018) and Rangel *et al*. (2013) and similar to the results of the study by Faccini *et al*. (2021). The averages of the right forewing length and the right forewing width were lower than the results of the study by Okuyan &

Akyol (2018) and Faccini *et al*. (2021) and similar to the results of the study by Al-Sarhan *et al*. (2019). According to the results of a previous study (Ozmen Ozbakır, 2021), the right forewing and the hindwing characteristics were similar to the results of the current study, while the right hindleg length was found to be higher in the current study.

A stepwise discriminant analysis was applied in terms of the external and internal morphological characteristics of the queens; the Wilk's Lambda test statistic was significant ($P$ <0.01). The first canonical discriminant function accounted for 100% of the total variation of queen groups (Function 1; eigenvalue = 0.214; % of variance = 100%; canonical correlation = 0.420). The Sanliurfa and Anatolian group queens were classified correctly to their original groups (73.8%) by using only the left basitarsus width variable. Eight Sanliurfa queens were misclassified as Anatolian group and nine Anatolian queens were misclassified as Sanliurfa group. When the stepwise discriminant analysis was performed for all variables of the queens for the three rearing periods, the Wilk's Lambda test statistic was significant ($P$ <0.01) for the first discriminant function of May ($P$ <0.01), June ($P$ <0.05), and July ($P$ <0.01). The first canonical discriminant function accounted for 100% of the total variation in each queen rearing period. By using four variables at four steps in May, the right tibial length, the weight of queen at the emergence, the right forewing width, and the right femur length (Function 1; eigenvalue = 2.609; % of variance = 100%; canonical correlation = 0.850); by using one variable at one step in June, the right hindwing length (Function 1; eigenvalue = 0.207; % of variance = 100%; canonical correlation = 0.414); by using five variables at five steps in July, the left hamuli number, the left basitarsus width, the number of right ovarioles, the right femur length, and the number of ovarioles (Function 1; eigenvalue = 5.117; % of variance = 100%; canonical correlation = 0.915), the queens were correctly classified 96%, 69.6%, and 100% of instances in May, June, and July, respectively. One Sanliurfa queen was misclassified in the Anatolian group in May; three Sanliurfa queens were misclassified in the Anatolian group, and four Anatolian queens were misclassified in the Sanliurfa group in June (Table 2). Gençer *et al*. (2017) stated that it is possible to discriminate Caucasian and Italian queens according to morphological characteristics. They found statistically significant differences between the two subspecies in all hindleg characteristics; except for the width of the right forewing, the differences in wing characteristics were not found to be significant. They also reported that the standard morphometry data set correctly classified 89.8% of queens according to their own group. The findings of this study agree with those of Gençer *et al*. (2017).

**Table 2** Predicted group membership according to external and internal morphological characters of queens (n, %)

| Periods | Groups | Predicted Group Membership (n, %) | | Total (n, %) | P |
|---|---|---|---|---|---|
| | | Sanliurfa | Anatolian | | |
| **May** | Sanliurfa | 11 (91.7) | 1 (8.3) | 12 (100) | 0.000 |
| | Anatolian | 0 (0) | 13 (100) | 13 (100) | |
| **June** | Sanliurfa | 8 (72.7) | 3 (27.3) | 11 (100) | 0.049 |
| | Anatolian | 4 (33.3) | 8 (66.7) | 12 (100) | |
| **July** | Sanliurfa | 7 (100) | 0 (100) | 7 (100) | 0.000 |
| | Anatolian | 0 (100) | 10 (100) | 10 (100) | |

For May, June, and July, 96%, 69.6%, and 100% of originally grouped cases were correctly classified

The possibilities of using many internal and external morphological characteristics of queens in different rearing periods in the evaluation of queens were also examined using factor analysis. According to the result of the principal component analysis, nine components explained 81.68% of the total variation. The hindleg characteristics in PC1, the wing characteristics in PC2, and the number of ovarioles of queens in PC3 explained the variation (Table 3). It was determined that 28.6% of the total variation could be explained by the first component, 11.17% by the second component, and 10.47% by the third component. Seven variables were in the first principal component group of all the morphological characteristics of the Anatolian and Sanliurfa queens. These variables were the left basitarsus length, the right hindleg length, the left hindleg length, the right basitarsus length, the left tibial length, the right tibial length, and the left basitarsus width, respectively. When the principal component analysis was performed only for the external morphological characteristics of both queen groups, seven variables explained 79.69% of the total variation. The first seven components explaining the total variation in the analysis were the right hindleg length, the right forewing length, the right tibial length, the left forewing length, the left hindleg length, the right basitarsus length and the left basitarsus length. It was determined that 34.62% of the total variation was explained by PC1, 12.11% by PC2, and 9.49% by PC3 in terms of only the external morphological characteristics of the queens. The number of ovarioles, which is the major component, explained 60.03% of the total variation when PCA was performed only for the reproductive organ characteristics. When the weight of queens at emergence was included in the analysis with the reproductive organ characteristics, the number of ovarioles as the major component explained 50.4% of the total variation while the second major component, which was the number of ovarioles in the left ovary, explained 20.05% of the remaining variation (Figure 5).

**Table 3** Rotated component matrix in terms of all morphological variables of queen bees

| Variables | Component | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| **Left basitarsus length** | 0.830 | | | | | | | | |
| **Right hindleg length** | 0.827 | | | | | | | | |
| **Left hindleg length** | 0.821 | | | | | | | | |
| **Right basitarsus length** | 0.806 | | | | | | | | |
| **Left tibia length** | 0.798 | | | | | | | | |
| **Right tibia length** | 0.723 | | | | | | | | |
| **Left basitarsus width** | 0.581 | | | | | | | | |
| **Right hindwing width** | | 0.840 | | | | | | | |
| **Left hindwing width** | | 0.776 | | | | | | | |
| **Right forewing width** | | 0.722 | | | | | | | |
| **Left forewing length** | | 0.717 | | | | | | | |
| **Right forewing length** | | 0.693 | | | | | | | |
| **Left forewing width** | | 0.678 | | | | | | | |
| **Left hindwing length** | | 0.674 | | | | | | | |
| **Number of ovarioles** | | | 0.910 | | | | | | |
| **Right ovarioles number** | | | 0.851 | | | | | | |
| **Left ovarioles number** | | | 0.696 | | | | | | |
| **Left femur length** | | | | 0.856 | | | | | |
| **Right femur length** | | | | 0.775 | | | | | |
| **Right hindwing length** | | | | 0.578 | | | | | |
| **Diameter of spermathecae** | | | | | 0.754 | | | | |
| **Left hamuli number** | | | | | 0.622 | | | | |
| **Right hamuli number** | | | | | | | | | |
| **Weight of ovary** | | | | | | | | | |
| **Head length** | | | | | | 0.902 | | | |
| **Head width** | | | | | | 0.840 | | | |
| **Thorax width** | | | | | | | 0.900 | | |
| **Thorax length** | | | | | | | 0.751 | | |
| **Weight at the dissection** | | | | | | | | 0.921 | |
| **Weight at the emergence** | | | | | | | | 0.882 | |
| **Right basitarsus width** | | | | | | | | | 0.908 |

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.

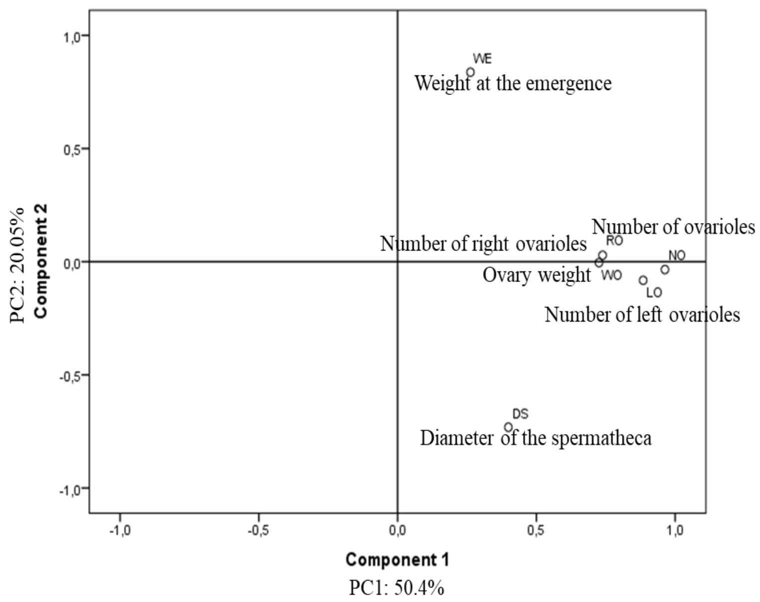


**Figure 5** Component plot in rotated space for queen weight at emergence and internal morphometric characteristics

The correlations between the weight of queens at emergence and the reproductive organ characteristics of the queens were low and insignificant. There were significant correlations at different levels between all external morphological characteristics of queens as expected. When the correlations between external and internal morphological characteristics of the queens were examined, the correlation between ovary weight and left forewing width (r = 0.409, $P$ <0.01) and the number of hooks on the left hindwing (r = 0.269, $P$ < 0.05) was significant. Correlations were also determined between ovary weight and left forewing width (r = 0.420, $P$ <0.01) and between ovary weight and left basitarsus width (r = -0.359, $P$ <0.01). The correlations between the number of ovarioles and the number of hooks on the right hindwing (r = 0.407, $P$ <0.01) and left hindleg length (r = 0.406, $P$ <0.01) and the left tibia length (r = 0.379, $P$ <0.01) were prominent. There was a correlation between the diameter of spermatheca and the number of ovarioles in the left ovary (r = 0.371, $P$ <0.01), ovary weight (r = 0.292, $P$ <0.05), the number of hooks on the right hindwing (r = 0.378, $P$ <0.01). It was determined that there were moderately significant correlations between the number of hooks on the right and left hindwing and the characteristics of all reproductive organs. However, there is no study to support the relationship between the number of hooks on the wing and the external and internal morphological characteristics of the queens. There was no correlation between the ovary weight and the weight of queens at emergence. A significant correlation was determined between the ovary weight and the characteristics of the left forewing width and the hook numbers on the left wing. As in this study, there was no results reporting a correlation between the number of hooks and the reproductive organ characteristics of the queens.

The weight and size of queens are affected by larval grafting age, nutrition, rearing period, colony conditions, and genetic factors and they are related to reproductive physiology. It has been reported that the queens with a high reproductive capacity and longevity have greater body weight, more mating success, and more sperm storage and ovary capacity. There is also variation in the weight of the queens in different life periods (Kahya *et al*., 2008; Tarpy *et al*., 2011; Yi *et al*., 2020). De Souza *et al*. (2019) stated that the queen's reproductive potential can be improved via dietary supplementation, and the queen's reproductive potential affects the productivity and health of honeybee colonies. Aamidor *et al*. (2021) emphasized the significance of the queen’s social environment and nutritional status on her reproductive capacity. The production of high-quality queens is one of the finest achievements in apiculture, requiring exceptional expertise (Dolasevic *et al*., 2020). Gençer *et al* (2018) examined 31 morphological characteristics in queens from different races and the ecotypes of the same races. As a result of discriminant analysis, queens were classified in their own breeder station (89.9%), in their own location (91.1%), and in their own race (94.9%) according to their morphological characteristics. They reported that queens from different genotypes could be discriminated reliably according to morphological characteristics. Therefore, many factors should be examined together and considered to evaluate queen quality.

## Conclusions

According to the results of this study, Anatolian and Sanliurfa queens, which are assumed to be different genotypic groups, can be classified separately even though they had similar averages in terms of the morphological characteristics. The hindleg, the wing characteristics, and the number of ovarioles of the queens stood out in the evaluation. The hindleg characteristics in particular were discriminative for queen genotype groups. It is possible to measure the hind leg and the wing characteristics of queens without damaging the queen, but studies are needed to determine more precise correlations for rapid estimation of the queen's reproductive capacity. The variability is determined by the level of correlation between the queens' morphological characteristics and reproductive characteristics. Therefore, we can focus on finding new evaluations that will also define the queen’s reproductive capacity.

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**Conflict of interest declaration**

The author declares there is no conflict of interest.

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