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MORPHOMETRIC CLASSIFICATION OF HONEYBEE POPULATIONS (*APIS MELLIFERA* L.) ALONG THE SOUTHEAST BORDER OF TURKEY

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Abstract

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The purpose of this study was to classify and compare the honeybee populations of Turkey, Syria and Iran based on their morphometric traits. Worker honey bees were sampled from populations of Van, Hakkari, Şırnak, Mardin, Şanlıurfa, Kilis and Hatay in Turkey; Lattakia, İdlib, Aleppo, Ar-Raqqah and Dayr az-Zawr in Syria and Urmia, Maku and Khoy in Iran, the settlements along the common border in the Southeast of Turkey. In the summer of 2008, 38 apiaries along the border in 3 countries were visited and 3340 worker honey bee specimen collected from 167 colonies. Univariate analysis showed that significant differences for all characters were found among honeybee samples from 15 localities ($P<0.001$). Discriminate analysis correctly classified 92.2% of the colonies and 56.3% of the individuals to their actual localities. According to colony averages; three overlapping clusters of Van-Hakkari-Şırnak-Iran, Şanlıurfa-Kilis and Hatay-Syria were formed separately. Mardin group showed a distinct cluster in the analyses. When Turkey-Syria-Iran groups were analyzed using the individual data, 71.4% of the 3340 worker honeybees were assigned to actual groups. Upon analysis of this percentage, it was found that 64.6%, 80.7% and 91.8% of individuals were assigned to their country groups of Turkey, Syria and Iran, respectively.

Key words: honeybee, *Apis mellifera* L., morphometry, discriminant analysis

Introduction

Because of the high adaptation ability of honeybees to different ecological conditions, they spread all over the world. Turkey has wide range of climates and habitats rise from geographic variation and exist many honeybee subspecies and ecotypes with different morphological, physiological and behavioral aspects.

Taxonomy and classification studies of honeybees have used morphometry, geometric morphometry, biochemical and molecular techniques. Because of morphometric studies, the western honeybee subspecies (*Apis mellifera* L.) were classified into four main groups: West Mediterranean and Northeast Europe (M branches), Central and Southeast Europe (C branches), Neareast (0 branches) and Tropic Africa (A branches). Near Eastern subspecies including Anatolian (*A. m. anatoliaca*), Caucasian (*A. m. caucasica*) and Iranian (*A. m. meda*) honey bees are the members of Oriental branch "O" (Ruttner, 1988; Kauhaussen-Keller et al., 1997). The molecular techniques also confirmed this morphometric classification (Arias and Sheppard, 1996; Franck et al., 2000). However, some diversity has been reported that these evolutionary lineages for example, *A. m. intermissa* and *A. m. sahariensis* subspecies are classified in A lineage (Cournuet and Garnery, 1991). The subspecies *A. m. anatoliaca* and *A. m. caucasica*, *A. m. meda*, *A. m. syriaca*, *A. m. adami*, *A. m. cypria* and *A. m. armeniaca* that were classified in the oriental 'O-branch' have been included in the evolutionary C lineage (Smith et al., 1997; Franck et al., 2000).

The subspecies in Turkey are *A. m. caucasica* in the northeast, *A. m. meda* in the southeast and *A. m. anatoliaca* throughout the rest of the country. However, *A. m. syriaca* would also have existed in Hatay province. *A. m. anatoliaca* and *A. m. meda* could play important role in the evolution of honeybees Ruttner, 1988, 2000; Smith et al., 1997; Palmer et al., 2000). According to Ruttner (1988), the honeybees from Southeast Anatolia of Turkey were not Syrian honeybees (Bodenheimer, 1942). The honeybees of the region from Van

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Lake to Mediterranean corner have been claimed that they are one of six Iranian honeybee ecotypes (*A. m. meda*). Morphometric classification of honeybees in Syria showed that *A. m. meda* existed in North and Northeast Syria and *A. m. syriaca* in the south region of the country. *A. m. meda* from Syria was distinct from *A. m. meda* samples from Turkey, but very close to *A. m. meda* samples from Iraq (Ftayeh et al., 1994). Recent findings explained that mtDNA markers of *A. m. syriaca* have different haplotypes and situated geographically in area of contact between the A and O lineages, at the northeast of Syria local populations still exist (Daraa, Quneitra, and Al-Hasakah) and also C lineage is clearly dominant (Alburaki et al., 2011).

Honeybees along the southeast border of Turkey contact with Anatolian, Iranian and Syrian honeybees but this interaction and hybridization limits are not known exactly. The main aim of this study was to classify and compare honeybee populations of Turkey, Syria and Iran based on the morphometry.

Material and Methods

Worker honey bees were collected from populations of Van, Hakkari, Şırnak, Mardin, Şanlıurfa, Kilis and Hatay in Turkey; Lattakia, İdlib, Aleppo, Ar-Raqqah and Dayr az-Zawr in Syria; Urmia, Maku and Khoy in Iran, located along the common border in the Southeast of Turkey. In the summer of 2008, 38 apiaries along the border in 3 countries were visited and 3340 worker honey bee specimen collected from 167 colonies. Sampling localities were chosen from more traditional and stationary beekeeping areas where queen replacement has not been practiced (Table 1). Young worker bees were collected from the brood areas, killed with chloroform and fixed in 70% ethanol until morphological measurements were carried out. Twenty worker honeybees were selected randomly from each sample and dissected for measurements. This study was summarized from Gonca Özmen Özbakır's PhD thesis.

Thirty-two morphological characters were measured for each worker bee. These included the following: 1. Tongue length (TL), 2. Length of hairs on tergite 5 (HL), 3. Width of tomentum (a), 4. Width of the dark stripe (b), 5. Tergite 3, longitudinal (T3), 6. Tergite 4, longitudinal (T4), 7. Femur length (FeL), 8. Tibia length (TiL), 9. Metatarsus length (MetL), 10. Metatarsus width (MetW), 11. Forewing length (FWL), 12. Forewing width (FWW), 13. Length of cubital vein a (CVA), 14. Length of cubital vein b (CVB), 15. Angle A4, 16. Angle B4, 17. Angle D7, 18. Angle E9, 19. Angle G18, 20. Angle J10, 21. Angle J16, 22. Angle K19, 23. Angle L13, 24. Angle N23, 25. Angle O26. Secondary characters calculated from the main characters (index and sum): 26. T3+T4, longitudinal (T3+T4), 27. Hind leg length (HLL), 28. Forewing index (FWI), 29. Cubital index (CI), 30. Cubital index percentage (CI%), 31. Tomentum index (TI) and 32. Metatarsal index (MTI). Body parts (tongue, right forewing and right hind leg) were mounted on projector slides and the morphological characters were measured with Leica Z16APO macroscope in accordance with perivious researches Alpatov (1929); Goetze (1940, 1959) and Ruttner et al. (1978).

Descriptive statistics were calculated for 15 locations. Morphological characters of individuals at each location were examined with univariate analysis (ANOVA) and different group means of each location were determined by Duncan's multiple range test. In 3 countries (Turkey, Syria and Iran) at 15 groups, 25 morhopological characters (without secondary characters) were examined from individual and colony data with multivariate analysis (MANOVA). Wilk's lambda statistic was used to test for significance of differences between vectors of means of the characters entered into the discriminant functions. Mahalanobis distances (D^2) were calculated between all 15-group centroids using a pooled variance covariance matrix. To classify honeybees according to morphological traits, the colony and individual data were used in discriminant analysis. All statistical analyses were performed using SPSS v.15 for Windows.

Table 1
Sampling locations and sample size

Location	Van	Hakkari	Şırnak	Mardin	Şanlıurfa
Worker, n	140	240	240	360	380
Colony, n	7	12	12	18	19
Location	Kilis	Hatay	Idlib	Lattakia	Aleppo
Worker, n	260	580	280	200	80
Colony, n	13	29	14	10	4
Location	Raqqah	Dayr az-Zawr	Urmia	Maku	Khoy
Worker, n	100	100	200	100	80
Colony, n	5	5	10	5	4

Results

Univariate analysis showed significant differences for all characters among honeybees from 15 localities (P<0.001). Honeybees from Kilis group are smaller for TL, HL, FWL, FWW, HLL and T3+T4 than other groups. The highest values were found for TL in Hakkari, HL in Maku, FWL, FWW and T3+T4 in Khoy, HLL in Şırnak and TI in Mardin honey bees. According to Duncan's multiple range test, TL, FWL and FeL traits showed the most variation among groups whereas the angle G18 showed the least (P<0.05).

When individual data were examined with multivariate analysis, Wilk's Lambda statistic was important for the groups (P<0.001). According to Mahalanobis distance, Hakkari and Şırnak group centroids were found the nearest morphologically. Other close groups were Hatay-Lattakia, Van-Hakkari, Idlib-Lattakia, respectively. However, Mahalanobis distance between Kilis and Maku group centroids were found the farthest (P<0.01). Kilis-Urmia, Kilis-Khoy, Idlib-Urmia, Idlib-Maku and Idlib-Khoy group centroids being far from each other means that honey bees from these groups are morphologically dissimilar. Discriminant analysis of the 25-morphometric variables performed to individuals yielded 14 canonical discriminant functions. The first and second functions explained 66.7% and 13.5% of the total variation, respectively. Discriminant analysis correctly classified 56.3% of the individuals to their localities. Among the total of 3340 honeybees, 1880 honey bees were assigned to their actual groups while 1460 honey bees were scattered. Distribution of 3340 honeybees to 15 groups according to canonic discriminant functions is given in Figure 1.

When univariate analysis was performed to 15 locality and 167 colony means, all morphological traits were significant except CVB (P<0.001). According to Duncan's multiple range test, the colony group means were highly different for angle E9, in other words, the number of different groups were high for angle E9. On the other hand, for the traits T3, FeL, TiL, MetW, FWL, T3+T4 and angle B4, smaller number of different groups were occured (P<0.05). When colonies were examined with multivariate analysis, the Wilk's Lambda statistic was found significant for groups (P<0.001). The discriminant analysis of the colony means of 25 morphometric characters showed that the 92.2% of samples were correctly classified to their preassigned localities (Table 2). Van, Mardin, Lattakia, Aleppo, Raqqah, Dayr az-Zawr and Khoy colonies were assigned to actual groups by 100%. Distribution of 167 colonies to 15 groups according to canonic discriminant functions is given in Figure 2. Distribution of colonies in Figure 2 showed that Hatay-Syria clustered together with Sanliurfa-Kilis groups, whereas Van-Hakkari-Sirnak-Iran groups clustered together and Mardin group formed a separate cluster.

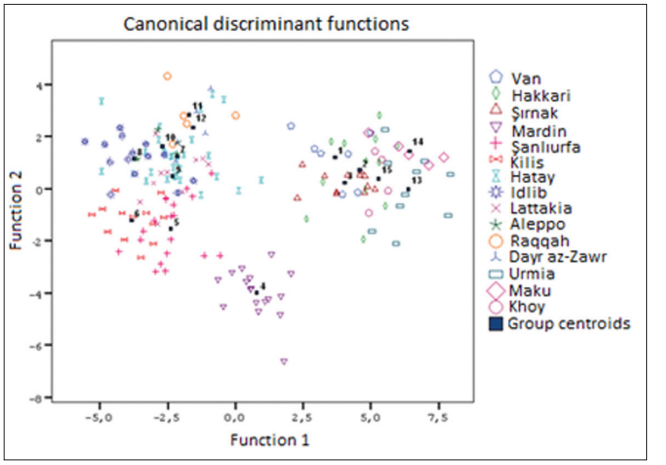

Fig. 2. Distribution of colonies

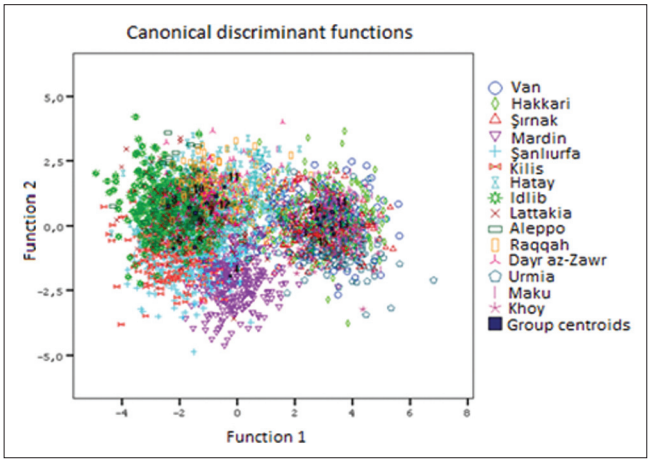

Fig. 1. Distribution of individuals

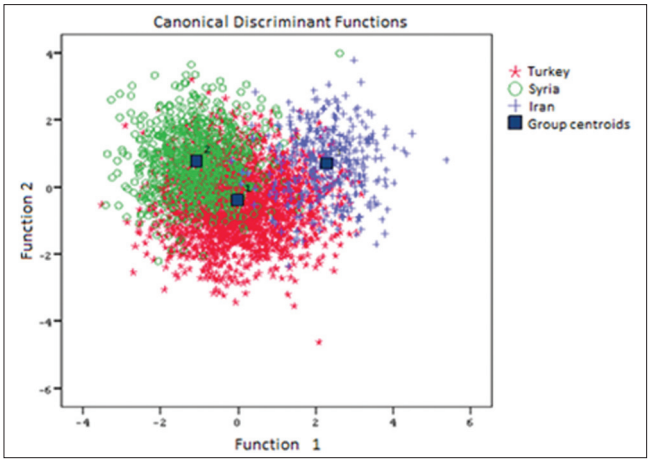

Fig. 3. Distribution individuals to country groups

Honeybees from Turkey, Syria and Iran were examined morphologically and analyzed using univarite and multivariate analysis techniques. Descriptive statistics for morphometric characters of country groups were given in Table 3. Univariate analysis showed that all morphologic variables were significant except CVB and TI ($P<0.001$). According to Duncan's multiple range test HL, b, TiL, FWL, FWW, CVA, HLL, FWI, CI, angles B4, E9, G18, J10, N23 were different for all country groups ($P<0.05$).

When country groups were examined using multivariate analysis, Wilk's Lambda statistic was important for the groups ($P<0.001$). According to Mahalanobis distances between Turkey and Syria group centroids were found the closest ($D^2=0.953$) whereas Syria and Iran group centroids were found the farthest ($D^2=0.953$), Mahalanobis distances between Turkey and Iran group centroids were found as middle value ($D^2=2.63$). Discriminant analysis of the 25-morphometric characters showed that individuals were correctly classified by 71.4% to their localities (Table 4).

Honeybees from Turkey were classified in their actual groups in Syria and Iran by 64.6%, 23.1% and 12.2%, respectively. However, discriminant analysis correctly classified 80.7% of the individuals to Syria and 91.8% to Iran. Distribution of individuals to 3 country groups according to canonic discriminant functions is given in Figure 3.

Discussion

The result of this study showed that the honeybees from southeast border of Turkey were more similar to Syrian honeybees than to Iranian honeybees. Neighbor sampling sites and

Table 2
Classification results of discriminant analysis applied to colony means (n, %)

Locality	Classified as															n
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	%
Van (1)	**7**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100
Hakkari (2)	0	**11**	1	0	0	0	0	0	0	0	0	0	0	0	0	12
	0.0	**91.7**	8.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100
Şırnak (3)	0	1	**11**	0	0	0	0	0	0	0	0	0	0	0	0	12
	0.0	8.3	**91.7**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100
Mardin (4)	0	0	0	**18**	0	0	0	0	0	0	0	0	0	0	0	18
	0.0	0.0	0.0	**100**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100
Urfa (5)	0	0	0	0	**17**	1	0	0	1	0	0	0	0	0	0	19
	0.0	0.0	0.0	0.0	**89.5**	5.3	0.0	0.0	5.3	0.0	0.0	0.0	0.0	0.0	0.0	100
Kilis (6)	0	0	0	0	0	**12**	1	0	0	0	0	0	0	0	0	13
	0.0	0.0	0.0	0.0	0.0	**92.3**	7.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100
Hatay (7)	0	0	0	0	0	0	**27**	0	1	1	0	0	0	0	0	29
	0.0	0.0	0.0	0.0	0.0	0.0	**93.1**	0.0	3.4	3.4	0.0	0.0	0.0	0.0	0.0	100
İdlib (8)	0	0	0	0	0	0	1	**12**	1	0	0	0	0	0	0	14
	0.0	0.0	0.0	0.0	0.0	0.0	7.1	**85.7**	7.1	0.0	0.0	0.0	0.0	0.0	0.0	100
Lattakia (9)	0	0	0	0	0	0	0	0	**10**	0	0	0	0	0	0	10
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**100**	0.0	0.0	0.0	0.0	0.0	0.0	100
Aleppo (10)	0	0	0	0	0	0	0	0	0	**4**	0	0	0	0	0	4
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**100**	0.0	0.0	0.0	0.0	0.0	100
Raqqah (11)	0	0	0	0	0	0	0	0	0	0	**5**	0	0	0	0	5
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**100**	0.0	0.0	0.0	0.0	100
Dayr az-Zawr (12)	0	0	0	0	0	0	0	0	0	0	0	**5**	0	0	0	5
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**100**	0.0	0.0	0.0	100
Urmia (13)	0	0	0	0	0	0	0	0	0	0	0	0	**7**	3	0	10
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**70**	30	0.0	100
Maku (14)	0	0	0	0	0	0	0	0	0	0	0	0	1	**4**	0	5
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20	**80**	0.0	100
Khoy (15)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**4**	4
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**100**	100

Table 3
Descriptive statistics ($\overline{X} \pm \sigma_{\overline{X}}$) for country groups

Variable	Turkey	Syria	Iran
	n=2200	n=760	n=380
TL, mm	6.35±0.004	6.34±0.005	6.46±0.005
HL, mm	0.18±0.000	0.16±0.000	0.22±0.000
FWL, mm	8.67±0.005	8.53±0.007	8.98±0.007
FWW, mm	2.98±0.002	2.91±0.002	3.12±0.003
T3+T4, mm	4.35±0.002	4.35±0.004	4.45±0.005
HLL, mm	7.72±0.005	7.76±0.006	7.84±0.009
CI	2.42±0.008	2.36±0.012	2.50±0.018
TI	2.68±0.010	2.72±0.013	2.68±0.015
MTI	57.74±0.040	57.04±0.061	57.11±0.104

Table 4
Classification results of discriminant analysis for country groups (n, %)

Locality	Classified as			n
	Turkey	Syria	Iran	%
Turkey	1422	509	269	2200
	64.6	23.1	12.2	100
Syria	131	613	16	760
	17.2	80.7	2.1	100
Iran	27	4	349	380
	7.1	1.1	91.8	100

sample sizes were effective on this result. However, Syrian and Iranian honeybee samples were clustered separately. Mahalanobis distances also confirmed these results. Syrian and Iranian honeybee group centroids were distant whereas honeybees of Southeastern Turkey were closer to Syrian than Iranian. Especially, Hatay samples were clustered together with Lattakia-Idlib-Aleppo-Ar-Raqqah-Dayr az-Zawr honey bees from Syria and Kilis-Sanlıurfa honeybees were close this cluster. Van-Hakkari-Sirnak honeybees were clustered with Urmia-Maku-Khoy honeybees from Iran in respect to morphological traits. Mardin group was clustered separately but more similar to Hatay-Kilis-Şanlıurfa and Syria groups. HL and HLL measurement means were low and other morphological trait means were high but relatively similar to Syrian samples compared to previous findings (Ruttner, 1988; Ftayeh et al., 1994). Our measurements for T3+T4 and TI were high, HL was low, TL, FWL, HiL, MTI, CI were similar to Iranian honeybees which accorded with the previous findings (Ruttner et al. 1985; Ruttner, 1988).

Conclusions

Measurement sensitivity, extensive colony movement, trade of commercial honeybee queens, natural selection and mating system of honeybees might affect and make difficult to classify and define honeybee subspecies with morphometry. There is a still contradiction to classification of honeybees for this sampling area. North and northwest of Syria and southeast of Turkey honeybees from Van Lake to Hatay province may be classified as *A. mellifera meda* or ecotypes of it. The results of this study showed that neighbor groups were similar and clustered together but Syria and Iran groups showed diversity morphologically. To explain interactions with *A. m. anatoliaca*, *A. m. syriaca* and *A. m. meda* honeybee subspecies, further morphometric and genetic studies may be conducted in the inner and wider areas beyond borders.

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