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Morphometric Divergence of Anatolian Honeybees Through Loss of Original Traits: A Dangerous Outcome of Turkish Apiculture

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Abstract

Five honeybee subspecies exist naturally in Anatolia. Unfortunately, owing largely to migratory beekeeping and lack of control mechanisms against requeening, the native honey bee subspecies located in Anatolia are facing extinction. Beekeeping activities, especially migratory beekeeping jeopardizes the presence of the naturally evolved indigenous subspecies of Anatolia. The present study examined morphological deformation in three *Apis mellifera* (L.) subspecies (*A. m. caucasica*, *A. m. carnica, A. m. syriaca*) and two ecotypes of *A. m. anatoliaca* (Muğla and Yığılca) that have been kept all together in a long-term breeding program at the common apiary. Worker bee samples representing each honeybee subspecies and ecotype were collected from the common apiary, and also from their original locations. To demonstrate the potential hybridization effect on variations of the Anatolian native honeybee subspecies and ecotypes, the geometric morphometric method was applied on the samples of honeybees that had been kept together in the same apiary since 2008. The findings showed that the honeybee population of the common apiary and those from their native settings formed two different configurations on the scatter plots. Hybridization and promiscuous mating among the different honeybee races maintained in the common apiary may have led to the loss of a valuable combination of morphometric traits. Hence, there is an urgent need for an active monitoring system and a ban on queen trading and migratory practices as well as for periodic testing of registered apiaries to identify ongoing variations in the gene pool.

Introduction

Twenty-seven subspecies of *Apis mellifera* (L.) have been identified based on morphometric characteristics and grouped into four evolutionary lineages: African lineage (A), Western and Northern European lineage (M), Southeastern European lineage (C), and Near and Middle Eastern lineage (O) (Garnery et al., 1993; Sheppard & Meixner, 2003; Cánovas et al., 2008; Miguel et al., 2011). These subspecies have also been described as 'geographic races' (Ruttner, 1998 Sheppard et al., 1997; Engel, 1999; Sheppard & Meixner, 2003; De la Rùa et al., 2005). At least four of these subspecies are known to be present naturally in Anatolia (Turkey) (Ruttner, 1988; Smith, 1997; Kandemir et al., 2000; Palmer et al., 2000; Bodur et al., 2007).

Preliminary studies on the distributional patterns of the Anatolian honeybee population were conducted by Buttel-Reepen (1906) along limited areas of the Aegean and Marmara regions. These were followed by Bodenheimer (1942) who employed morphological characteristics to divide Turkey into seven zones. Maa (1953) was the first taxonomist to name the subgenus of *A. m. anatoliaca* using morphometric characteristics. Findings similar to those of Bodenheimer (1942) were reported by Adam (1983), who identified four honeybee races and many ecotypes of Anatolian honeybees. He stressed that there were morphological differences between the bee populations of the Black Sea coast and the Mediterranean coast of Anatolia (Adam, 1983), and this finding also revealed the traits of the Syrian bee (*A. m. syriaca*). However, Ruttner (1988) determined that *A. m. anatoliaca* was found throughout

all of Turkey including the European part, with the exception of the northeast and the southeast corners of the country, where *A. m. caucasica* and *A. m. meda*, respectively, were identified. Ruttner's analysis of honeybee samples from southeast Anatolia showed that they were not *A. m. syriaca* as generally assumed previously, but were the Iranian bee, *A. m. meda.*

Many surveys based on alloenzyme and mtDNA variation that have reviewed the honeybee biodiversity of Anatolia support the findings of Ruttner (1988), Kandemir and Kence (1995) and Kandemir et al. (2000, 2006). Recent studies on Thracian honeybees have revealed the existence of *A. m. carnica* in the European part of Turkey (Smith et al., 1997; Palmer et al., 2000; Kandemir et al., 2006) and of *A. m. syriaca* in Hatay Province (Palmer et al., 2000; Kandemir et al., 2006). Both morphometric and mtDNA study results have shown five subgenera of *A. mellifera* spread throughout Turkey, including *A. m. anatoliaca, A. m. caucasica, A. m. meda, A. m. carnica* and *A. m. syriaca* (Ruttner, 1988; Smith et al., 1997; Palmer et al., 2000; Kandemir et al., 2006; Özdil & İlhan, 2012).

In addition to those subspecies, various local ecotypes, which have not been adequately studied to date, such as the Muğla and Yığılca bees have also been identified in some isolated local areas of Turkey (Kekeçoğlu, 2010; Kekeçoğlu & Soysal, 2010a; Bouga et al, 2011). The Muğla bee is an ecotype of *A. m. anatoliaca* which has adapted its life cycle by foraging on the scale insect *Marchalleina hellenica* on pine trees (Güler & Kaftanoğlu, 1999a, b). The Yığılca bee is also considered to belong to ecotypes marked by larger size and darker color, respectively, while it differs from other bee species with regard to length of wings and legs. It rears its brood in the total brood area in early spring. In addition, it has a greater capacity for honey production than *A. m. caucasica* or *A. m. anatoliaca* (Kekeçoğlu, 2010; Gösterit et al., 2012, 2016).

Turkey is a geographically disjunctive region covering three continents, Asia, Europe and Africa. Due to its rich topography and climatic variation, it presents a wide range of habitats in a relatively small geographical area. Thanks to its geographical and biological diversity, a large amount of genetic variation can be observed in the honeybees of Turkey. However, within the last 15-20 years, migratory beekeeping has become widespread in Turkey (Güler, 2010). The extensive practices of migratory beekeeping and commercial breeding can promote gene flow between bee races. As a consequence, genetic homogenization has emerged as a threat to the Turkish honeybee populations. If protective policies are not enacted by the Ministry of Agriculture against intensive queen breeding and migratory beekeeping, the gene pool of the Turkish honeybee populations will be homogenized, as has already occurred in Greece (Bouga et al., 2005).

The recent anxiety over the loss of biodiversity requires an international effort in order to develop strategies for conservation and sustainable use affecting biodiversity. It must be recognized that each honeybee race has peculiar characteristics for dealing with environmental conditions, stress and diseases (Tozkar et al., 2015). If one takes into consideration that global climate changes and the increasing use of pesticides provide unfavorable conditions to certain races, some races may cease to exist despite their unique traits. Therefore, it is highly crucial to preserve the genetic resources of the Anatolian honeybee races and ecotypes. Four out of the five Anatolian honeybee lineages found around the Mediterranean region, in particular, carry different traits of production capacity and hygiene behavior as well as resistance against climate change, disease, and pesticide residues (Garnery et al., 1993; Cánovas et al., 2008; Miguel et al., 2011; Tozkaret al., 2015). Nevertheless the genetic hybridization caused by migratory beekeeping modifies the genetic pool of the local honeybee populations, leading to the loss of their unique genetic and morphological traits. Because of migratory beekeeping, and especially commercial breeding, Turkish native honeybee subspecies and ecotypes have been exposed to introgressive hybridization with Caucasian bees (Güler, 2010). Future honeybee conservation efforts should therefore be aimed at reducing the introduction of alien subspecies into isolated areas already occupied by native honeybees.

The objective of this study was to prove the effect of the hybridization on honeybee biodiversity. To this aim, colonies representing all the native honeybee races of Turkey, *A. m. anatoliaca, A. m. caucasica, A. m. carnica* and *A. m. syriaca,* were maintained all together over a period of seven years in common apiary in central Anatolia. Specimens from the common apiary were then compared with samples from isolated areas where mating takes place in Yığılca, Muğla, Artvin, Kırklareli and Hatay, and where hybridization among the standing populations is avoided.

Material and Methods

The sampling areas and the five symbols representing the native honeybee subspecies of Turkey are shown in Fig 1.

The private research apiary in central Anatolia, was established approximately seven years ago. Since 2008, colonies representing Turkish native honeybee subspecies and ecotypes (*A. m. caucasica, A. m. carnica*, *A. m. syriaca* and the Muğla and Yığılca ecotypes of *A. m. anatoliaca*) have been maintained all together in that apiary. Between April and June 2015, honeybee worker samples representing the native honeybee subspecies and ecotypes of Turkey were collected from the common apiary and also from isolated locationsin Artvin (*A. m. caucasica*), Düzce (Yığılca ecotype), Muğla (Muğla ecotype), Kırklareli (*A. m. carnica*) and Hatay (*A. m. syriaca*). In each apiary, ten worker bees were obtained from each colony. Honeybee worker samples were collected from the colony by uncovering the top of the hive and shaking the honey combs through clean cloths. Samples of adult workers were transported to the laboratory in small plastic vials containing 96% ethanol, and then stored at 4°C. The localities and geographical coordinates based on GPS (Magellan Explorist 110) are given in Table 1.

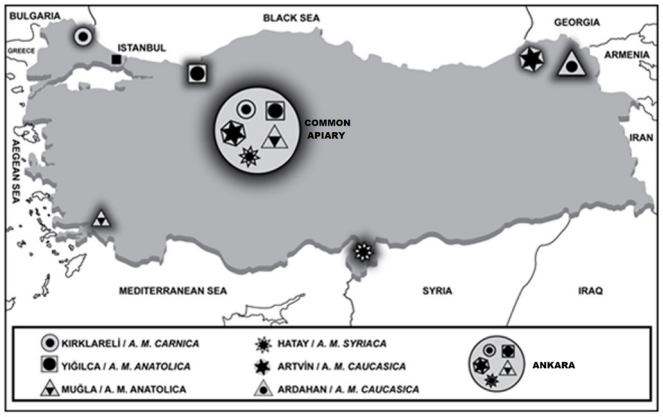

Fig 1. The distribution of native honeybee subspecies of Turkey and sampled provinces indicated by different symbol

Extraction techniques

All of the specimens were kept in 96% ethanol until dissection of the wings using forceps. The right forewings of the honeybees were cut at the wing base and then transferred to 70%, 50%, and 20% ethanol solutions, respectively, for gradual hydration. Distilled water was used to flatten the wings. The head segments of the worker bees were kept in 30% alcohol for the proboscis measurements, while the rest of the body segments were stored in 70% lactic acid for 24 h in order to soften the tissues for better resolution. After the dissection process, the right forewings and legs were placed in petri dishes filled with distilled water, and then fixed on the surface of microscope slides fastened on a 7.5 × 2.5 cm slide frame with special transparent tape (3M Scotch® Magic™ Mat tape, 19 mm × 33 m).

Geometric and standard morphometric techniques

Images of the wings were obtained using a video camera (Bs200Pro, BAB Imaging Systems, BAB Ltd, 1993) mounted on a microscope (BAB STR45) with a 1 × objective. Photographs were downloaded to the Basic Research picture program (BAB Bs200Pro Image Analysis Systems) and all files were saved. The wing images were scaled and rotated, and then19 landmarks located at vein intersections (Fig 2) were digitized and the two-dimensional X and Y Cartesian coordinates were recorded using the Bs200Pro program. In addition to the Cartesian coordinates of the 19 landmarks, mathematical parameters among the landmark coordinates (angle, length, and index) were evaluated. Characteristics of the hind legs and proboscis were determined via measurements made with BAB Bs200Pro software (Kambur, 2017).

Table 1. Geographical coordinates of sampling locations. N = number of colonies sampled.

	Sampling honeybee subspecies	Region	City-Provinces	Apiary	N	Altitude (m)	Geographical coordinates
Isolated Native regions	**Artvin** (*A.m.caucasica*)	Northeast Anatolia (BlackSea)	Artvin Borçka	Karşıköy	20	598	45°89’57”N 37°07’27”E
				Balcı-Naznara	20	701	45°76’41”N 37°07’36”E
				Macahel	20	460	41°10’14”N 41°04’35”E
	Düzce-Yığılca (Yığılca ecotype of *A. m. anatoliaca*)	Northwest Anatolia	Düzce (Yigilca)	Hoşafoğlu	15	329	36°16’27”N 36°53’36”E
				Kırık	15	317	45°30’ 63”N 36°50’41”E
				Redifler	15	401	45°35’ 75”N 37°72’39”E
	Muğla (Mugla ecotype of *A. m. anatoliaca*)	Aegean	Muğla (Bodrum)	Ortakent	15	18	40°29’51”N 35°31’40”E
				Çömlekçi	15	54	41°12’48”N 35°35’42”E
				Yalı-Alazeytin	15	181	40°29’40”N 35°54’52”E
	Kırklareli (*A. m. carniaca*)	Thrace region	Kırklareli	Igneada	20	300	41°52’34”N 27°59’ 28”E
				Demirkoy-Şişlioba	25	203	41°58’45”N27°54’58”E
	Hatay (*A. m. syriaca*)	Southeast Anatolia	Hatay	Erzin	20	253	36°12’ 39”N 36°52’56”E
				Antakya	25	205	30°09’ 28”N 36°13’30”E
Common Apiary	*A. m. caucasica* *A. m. syriaca* *A. m. carniaca* Muğlaecotype and Yığılcaecotype of *A. m. anatoliaca*	Central Anatolia	Ankara	Center (common apiary)	210	871	39°54’56” N 32°52’10”E

Common Apiary: All colonies represented five different honeybee subspecies of Turkey have been maintained altogether in the common apiary since 2008.

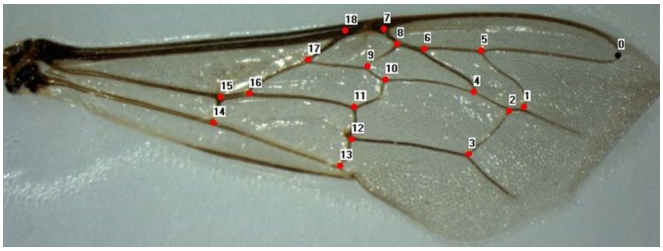

Fig 2. Right forewing of Anatolian honey bee with 19 landmarks plotted in the vein junctions.

Statistical analysis

Data obtained from the X and Y coordinates were analyzed via geometric morphometrics and angle, length and index values via standard morphometrics using the SPSS 15.0.1 package (SPSS, 2005). Stepwise discriminant function analysis (DFA) was performed based on the spectral decomposition of a covariance matrix of the pooled results of each locality. Canonical variant analyses (CVA) and cross validation tests were conducted to check the accuracy of the equations in identifying the colonies. A dendrogram showing the relationships among the honeybee populations was constructed according to the pooled results of each honeybee population via the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) using the PYHLIP 3.67 package (Felsenstein, 1993). Multivariate (MANOVA) and univariate (ANOVA) statistical analyses were applied on the dataset of the morphometric characters for comparison of the groups.

Results

Morphometric differences in native ancestor honeybee populations

Honeybee colonies from isolated native populations were evaluated by using the geometric morphometric method. The discrimination of five native honeybee populations, Artvin (*A. m. caucasica*), Muğla (Muğla ecotype of *A. m. anatoliaca*), Hatay (*A. m. syriaca*), Kırklareli (*A. m. carnica*) and Düzce (Yığılca ecotype of *A. m. anatoliaca*) was carried out by discriminant function analyses (DFA). Four statistically significant canonical vectors were extracted from the matrix. The scatter plot generated via canonical variant analysis (CVA) revealed that the colonies from the native areas had formed non-overlapping distant clusters, except for the Kırklareli population (Fig 3). The CVA indicated the existence of great and highly significant differences among the native honeybee subspecies (Wilks' lambda=0.06, $F_{(4,236)}$= 47.437 p <0.0001). The first three vectors discounted 95.4% of the among-group variations together. The first and second axis explained 65.6% and 20.4% of total variation, respectively. The contribution of different variables to the canonical coordinates were assessed using the standardize coefficients, and X14 was the variable with the highest loading on the first canonical axis, whereas the second canonical axis included Y1, Y2, Y3, Y4, Y5, Y6, Y7, Y8, Y9, Y10 and Y11 variables contributing to the separation of the groups (Fig 3).

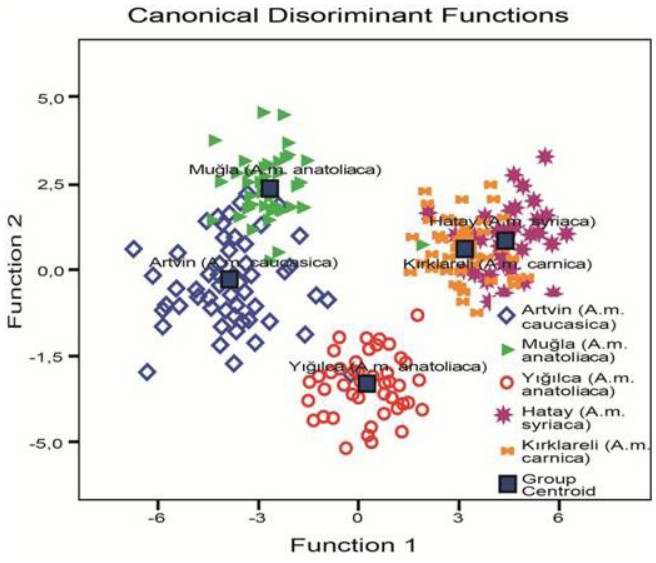

Fig 3. The discrimination of native honeybee race and ecotypes from isolated area on two dimensional scatter plot by using geometric morphometric.

Table 2. Predicted group membership of honeybee samples from isolated areas including native Anatolian honeybee races.

**Locations (isolated area)	**Artvin** (*A.m.caucasica)*	**Muğla** (Muğla ecotype)	**Yığılca** (Yığılcaecotype)	**Hatay** (*A.m.syriaca*)	**Kırklareli** (*A.m.carnica*)	Total
Artvin (*A.m.caucasica*)	56 (93%)	4 (6.7 %)	2 (3.3 %)	0	0	60
Muğla (Muğla ecotype)	3 (6.7 %)	41 (91.1 %)	0	1 (2,2 %)	0	45
Yığlca (Yığılca ecotype)	0	0	45 (100%)	0	0	45
Hatay (*A.m.syriaca*)	0	0	0	45 (100%)	0	45
Kırklareli (*A.m.carnica*)	0	0	0	3 (6.7 %)	42 (93.3 %)	45

Number of coloniesand percent classifications are in parentheses were given in each column.
** indicate collection sites which are isolated area 93.8% of original grouped cases correctly classified.

Cross validation tests correctly identified 93.8%of native colonies from isolated original locations using geometric morphometrics. If only the bee colonies from Artvin are taken into consideration, four of 60 identifications were incorrectly identified as the Muğla ecotype (6.7%) and two as the Yığılca ecotype (3.3%) of *A. m. anatoliaca*. The most common errors in the identifications were made in the honeybee colonies from Artvin and Muğla provinces.Cross validation tests were able to correctly identify 100% of the honeybees from the Yığılca and Hatay populations and 93.3% of the honeybee samples from Kırklareli (Table 2).

Geometric morphometric differences in common apiary honeybee populations

Discrimination of the honeybee populations of the common apiary was carried out based on DFA. Higher eigenvalues for four canonical functions were extracted from the matrix resulting from the product of the among-group by the within-group covariance matrix. The first two canonical discriminant functions calculated using the data from the honeybee colonies of the common apiary were able to explain 87.5% of the among-group variation. The first and second canonical discriminant functions explained 59.8% and 27.7% of the total variation, respectively. Function one included Y0, Y1, Y2, Y3, Y4, Y5, Y6, Y7, Y8, Y9, Y10, Y11 and Y18, and function two included X0, X1, X2, X3, X4, X5, X6, X7, X9, X10, X11, X12, X13, X14, X15, X16, Y14, Y15 and Y16. The CVA scores of the right forewings of the common apiary honeybee colonies showed that there was more overlapping among the honeybee populations and thus, there were no clear borderlines among the groups. Samples from the common apiary where all Turkish honeybee subspecies had been kept together in the same area showed that significant alterations of wing formation and loss of homogenic characteristics had occurred and new heterogeneous groups had been formed (Fig 4).

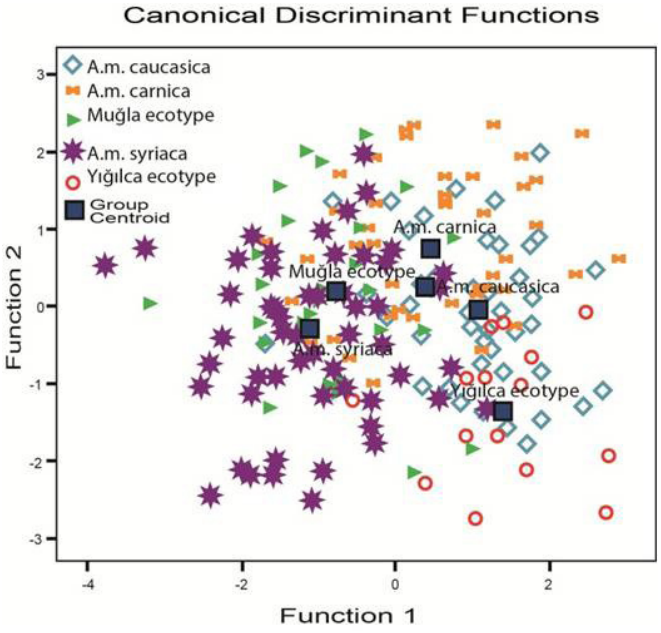

Fig 4. The discrimination of honeybee colonies from common apiary on two dimensional scatter plot by using geometric morphometric.

The canonical discriminant function data generated via geometric morphometrics were able to identify and assign the honeybee populations of the common apiary to their original populations based on wing-shape data. As a result, 54.4% of the honeybee colonies were correctly classified. Correct classification rates of the bee populations were: *A. m. caucasica* 51.1%, *A. m. carnica* 55.6%, *A. m. syriaca* 61.7% and Yığılca ecotype of *A. m. anatoliaca* 50 % (Table 3).

The honeybee colonies from the common apiary were not easily assigned to groups, possibly because significant morphological detailswere fragmentary, damaged, or obscured. They created an overlapping homogeny group on the scatter plot and showed fewer differences (Fig 4, Table 3). In contrast, the differences between the native ancestor honeybee populations from isolated locations were found to be significant for all of the variables except X0 and Y12 ($p < 0.05$).

The discriminant function analysis of the two groups (native ancestor and common apiary population) was also supported by the UPGMA clustering based on the matrix of Mahalanobis distances. As shown on the first UPGMA dendrogram (Fig 5a), the native ancestor population of Kırklareli (KIR), naturally covering *A. m. carnica,*was grouped with Muğla and Hatay, whereas on the second UPGMA dendogram (Fig 5b) of the common garden apiary, *A. m. carnica* (CAR) was clustered with *A. m. caucasica* (CAU)

Table 3. Predicted group membership of honeybee colonies from common apiary covered all honeybee races of Turkey.

Subspecies in Common Apiary	*A.m.caucasica*	*A.m.carnica*	Muğla ecotype	*A.m.syriaca*	Yığılca ecotype	Total
A.m.caucasica	23 (51.1 %)	10 (22.2 %)	4 (8.9 %)	2 (4.4 %)	6 (13.3 %)	45
A.m.carnica	4 (8.9 %)	25 (55.6 %)	8 (17.8 %)	5 (11.1 %)	3 (6.7 %)	45
Muğla ecotype	3 (10 %)	5 (16.7 %)	11 (36.7 %)	9 (30 %)	2 (6.7 %)	30
A.m.syriaca	2 (3.3 %)	5 (8.3 %)	13 (21.7 %)	37 (61.7 %)	3 (5 %)	60
Yığılca ecotype	8 (26.7 %)	3 (10 %)	2 (6.7 %)	2 (6.7 %)	15 (50 %)	30

Regarding geometric morphometric 54.4% of original grouped cases correctly classified.

and the Yığılca ecotype of *A. m. anatoliaca* (YIGE). The configuration differentiation between the first and second UPGMA dendrograms showed alteration of the morphometric construction due to hybridization (Figs 5a and b).

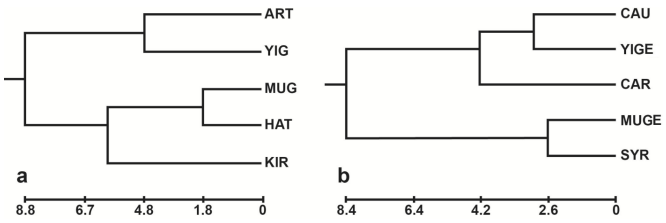

Fig 5. UPGMA dendogram constructed from Mahalonobis Distance **a:** Native Area ART: Artvin, YIG: Yığılca, MUG: Muğla, HAT: Hatay, KIR: Kırklareli; **b:** Common apiary where all native honeybee subspecies were maintained altogether; CAU: *A. m. caucasica,* SYR: *A. m. syriaca,* CAR: *A. m. carnica*, MUG: Muğla ecotype of *A. m. anatoliaca* and YIG: Yigilca ecotype of *A. m. anatoliaca.*

Comparison of honeybee populations in native areas with those in the common apiary

Honeybee colonies from both the native areas and the common apiary were evaluated together by DFA, CVA analysis and cross validation tests. Native honeybee populations from Artvin, Kırklareli, Hatay and Muğla formed distinct clusters completely separated from the samples of the common apiary along the first CV axis (Lambda = 0.002, chisq. = 3600.428, df = 280, p< 0.000). The Yığılca honeybee ecotype of *A. m. anatoliaca* and the common apiary were further separated along the second CV axis (Wilks'lambda = 0.14, chisq = 2399.686, df = 243, p<0.000). Three main clusters occurred in canonical variant analysis (CVA) (Fig 6). As shown in Fig 6, all the common apiary honeybee populations formed a completely overlapping group on the plot, whereas they would normally be expected to coincide with their own native ancestor honeybee populations.

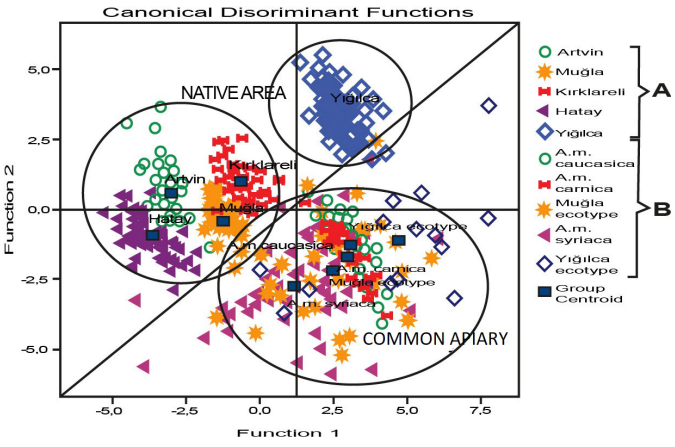

Fig 6. Two dimensional clustering by Discriminant Function Analysis of Colony Means of honeybee subspecies from 5 isolated native area, Artvin and Ardahan (*A. m. caucasica*), Kirklareli (*A. m. carnica*), Muğla (Muğla ecotype of *A. m. anatoliaca*), Hatay (*A. m. syriaca*), Düzce (Yığılca ecotype of *A. m. anatoliaca*) and common apiary (*A. m. caucasica, A. m. syriaca, A. m. carnica*, Mugla and Yigilca ecotype of *A. m. anatoliaca*). A: isolated native area, B: common apiary.

In addition, cross validation tests were also performed to determine the group affinity of the colonies. The ancestors of the populations of Yığılca, Muğla, Artvin, Kırklareli and Hatay (Figs 6 and 7) were known, while the colonies of the common apiary were maintained in the same area and the honeybees could have come into contact with each other. Interbreeding between the subspecies can cause hybridization, morphometric deformation and emergence of novel morphometric characteristics.

The Mahalanobis squared distance among the populations was used to construct a UPGMA dendogram based on the matrix of the Mahalanobis distance. As shown on the dendrogram, the native ancestor honeybees from isolated areas and the common apiary honeybees formed two distinct main clusters. Except for the native Muğla ecotype of *A. m. anatoliaca*, all ancestor honeybee populations constituted first main clusters, while the honeybees of the common apiary were located on a second cluster. Furthermore, the first main cluster was divided into two groups revealing Artvin as one cluster and Yiğilca, Kirklareli, and Hatay as a second cluster. Muğla was distinguished from the first main cluster (A), and the second main cluster (B) included the common apiary honeybees, *A. m. carnica, A. m. caucasica, A. m. syriaca,*the Yığılca and Muğla ecotypes of *A. m. anatoliaca* and the native Muğla honeybee population (Fig 7).

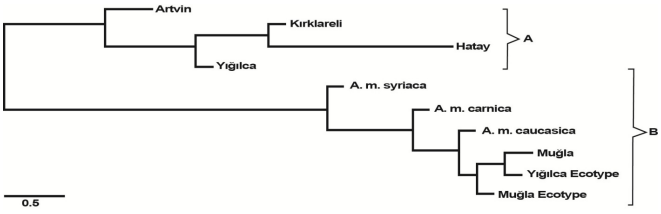

Fig 7. The classification of the honeybee population based on UPGMA dendogram. A: isolated native area, B: common garden apiary.

Standard morphometric

In addition to geometric morphometrics, standard morphometric analyses were used to determine whether quantifiable morphometric alteration could have occurred in any wing characteristics of the pure honeybee populations due to hybridization as a result of interbreeding in the common apiary. A total of 19 landmarks consisting of nine lengths, 17 angles and four index characters were evaluated on the right forewing, leg and proboscis. The results of 37 morphometric characteristics were accurately determined and recorded (Tables 4 and 5). Findings for the forewing showed that there were significant differences between the native ancestors and the hybrid common apiary honeybees according to morphological characteristics, with the exception of WL, TU, TB, B, C and D length. Although the highest discodial shift (1.71 mm) was seen in the native Artvin honeybees (*A. m. caucasica*), after the native colonies had been relocated and kept all together in the common apiary, the discodial shift (1.65 mm) of *A. m. caucasica* in the common apiary was decreased

due to the morphometric modifications occurring in the large and small spatial scales. It can also be seen that the N23 values of *A. m. caucasica* (87.30 mm) and the Yığılca ecotype of *A. m. anatoliaca* (89.95 mm) in the common apiary were lower than those of the native ancestors in the Yığılca and the other stationary populations. The A4 value of theYığılca ecotype in the common apiary was higher than that of the Yığılca stationary population as well.

The cubital index (CI) is an important wing characteristic for *A. m. carnica.* The highest CI value was found in Kırklareli Province, covering the native ancestors of the *A. m. carnica* race. The CI value of the native Kırklareli population was greater than that of *A. m. carnica* hybridized in the common apiary. The lowest CI values were those of the native Hatay population (Table 4).

Table 4. Means ± Stdoflengths and indexcharacters determined by standard morphometric method by using 19 landmark.

Length and ındex characters	Abv.	ISOLATED NATIVE AREA Mean±Std. Dev.				COMMON APIARY Mean±Std. Dev.					
		Artvin *caucasica* (N=60)	Kırklareli *carnica* (N=45)	Muğla Ecotype (N=45)	Hatay *Syriaca* (N=45)	Yığılca Ecotype (N=45)	*A.m. caucasica* (N=45)	*A.m. carnica* (N=45)	Muğla ecotype (N=30)	*A.m. syriaca* (N=60)	Yığılca ecotype (N=30)
Wing length	**WL**	**9,81±0,30**	**9,57±0,21**	**9,51±0,32**	**9,54±0,29**	**9,74±0,27**	**9,86±0,20**	**9,68±0,19**	**9,39±0,18**	**9,39±0,23**	**9,81±0.15**
Wing width	**WW**	**3,24±0,14**	**3,15±0,07**	**3,18±0,15**	**3,17±0,09**	**3,31±0,07**	**3,27±0,08**	**3,23±0,07**	**3,16±0,09**	**3,14±0,11**	**3,34±0.06**
Femur length	**FL**	**3,21±0,15**	**3,18±0,11**	**3,15±0,11**	**3,23±0,13**	**3,21±0,13**	**3,32±0,13**	**3,25±0,12**	**3,25±0,10**	**3,21±0,10**	**3,33±0,07**
Tibia length	**TL**	**3,19±0,13**	**3,14±0,09**	**3,10±0,13**	**3,11±0,10**	**3,10±0,21**	**3,30±0,12**	**3,19±0,12**	**3,18±0,10**	**3,17±0,10**	**3,26±0,10**
Basit. length	**BL**	**2,09±0,08**	**2,04±0,08**	**2,01±0,09**	**2,01±0,09**	**2,05±0,09**	**2,13±0,09**	**2,06±0,08**	**2,01±0,08**	**1,98±0,09**	**2,15±0,09**
Basit. length	**BW**	**1,21±0,07**	**1,17±0,06**	**1,16±0,07**	**1,14±0,08**	**1,23±0,08**	**1,24±0,06**	**1,20±0,05**	**1,19±0,05**	**1,19±0,05**	**1,27±0,06**
Proboscis length	**PL**	**6,44±0,50**	**6,56±0,31**	**6,44±0,43**	**5,96±0,63**	**6,77±0,20**	**6,67±0,32**	**6,50±0,40**	**6,68±0,17**	**6,56±0,17**	**6,89±0,24**
Radial length	**RADL**	**3,58±0,13**	**3,43±0,08**	**3,45±0,09**	**3,51±0,09**	**3,58±0,10**	**3,58±0,07**	**3,53±0,08**	**3,46±0,09**	**3,45±0,09**	**3,64±0,06**
Length A	**A**	**0,51±0,05**	**0,52±0,06**	**0,52±0,06**	**0,53±0,06**	**0,57±0,05**	**0,54±0,05**	**0,57±0,04**	**0,53±0,06**	**0,51±0,07**	**0,54±0,03**
Length B	**B**	**0,27±0,04**	**0,26±0,04**	**0,24±0,02**	**0,24±0,04**	**0,25±0,03**	**0,27±0,03**	**0,27±0,03**	**0,26±0,03**	**0,27±0,04**	**0,23±0,03**
Length C	**C**	**0,91±0,05**	**0,88±0,04**	**0,88±0,03**	**0,89±0,04**	**0,93±0,04**	**0,91±0,03**	**0,93±0,04**	**0,88±0,03**	**0,88±0,04**	**0,92±0,03**
Length D	**D**	**1,99±0,08**	**1,93±0,05**	**1,92±0,05**	**1,92±0,06**	**2,02±0,06**	**1,99±0,05**	**1,95±0,05**	**1,92±0,05**	**1,90±0,07**	**1,99±0,05**
Inner wing length	**IWL**	**4,56±0,15**	**4,44±0,10**	**4,43±0,0,9**	**4,45±0,11**	**4,62±0,11**	**4,57±0,10**	**4,51±0,09**	**4,40±0,10**	**4,41±0,14**	**4,56±0,08**
Inner wing width	**IWW**	**2,07±0,08**	**1,99±0,05**	**1,95±0,05**	**1,98±0,06**	**2,05±0,06**	**2,06±0,06**	**2,04±0,05**	**1,99±0,06**	**1,98±0,07**	**2,08±0,05**
Cubital Index	**CI**	**1,92±0,41**	**2,00±0,46**	**2,16±0,34**	**2,20±0,49**	**2,26±0,43**	**2,00±0,36**	**2,10±0,32**	**2,04±0,32**	**1,95±0,45**	**2,30±0,34**
Pre cubital Index	**PCI**	**2,77±0,14**	**2,71±0,16**	**2,82±0,18**	**2,78±0,14**	**2,80±0,14**	**2,74±0,13**	**2,74±0,17**	**2,70±0,18**	**2,71±0,17**	**2,73±0,12**
Dum bel Index	**DUMBI**	**0,86±0,08**	**1,01±0,11**	**0,92±0,08**	**0,97±0,10**	**0,95±0,08**	**0,92±0,07**	**0,98±0,08**	**0,95±0,09**	**0,96±0,10**	**0,83±0,06**
Radial Index	**RI**	**1,73±0,07**	**1,67±0,05**	**1,64±0,05**	**1,68±0,05**	**1,73±0,05**	**1,74±0,04**	**1,73±0,05**	**1,68±0,04**	**1,69±0,05**	**1,74±0,04**
Discodial shift	**DiscS**	**0,34±0,10**	**0,23±0,11**	**0,28±0,08**	**0,29±0,12**	**0,26±0,17**	**0,15±0,12**	**0,21±0,11**	**0,29±0,14**	**0,34±0,13**	**0,25±0,13**
Disc length	**DiscsL**	**1,71±0,07**	**1,66±0,05**	**1,63±0,05**	**1,66±0,06**	**1,70±0,06**	**1,73±0,05**	**1,72±0,66**	**1,65±0,05**	**1,66±0,06**	**1,73±0,05**

Discussion

In this study, in order to evaluate the effect of hybridization on Turkish honeybee biodiversity due to migratory beekeeping, honeybee colonies representing three Turkish honeybee races and two ecotypes were placed in the common apiary where they were subjected to interaction studies. The hybridization effect was evaluated by examining the morphological deformation in the wings of worker bees. The results of CVA showed that populations from Düzce-Yığılca, Muğla, Artvin, Hatay and Kırklareli, respectively, were not known ancestors of the common apiary populations, which included *A. m. anatoliaca, A. m. meda, A. m. caucasica, A. m. syriaca* and *A. m. carnica* races. In previous studies, the distribution of the subspecies of *Apis mellifera* in the native areasof this study was found as: *A. m. anatoliaca* in Muğla and Yığılca, *A. m. caucasica* in Artvin, *A. m. carnica* in Kırklareli and *A. m. syriaca* in Hatay (Kence, 2006; Bodur et al., 2007; Kekeçoğlu et al., 2009; Kekeçoğlu & Soysal, 2010a).Thus, it was expected that each honeybee subspecies of the common apiary would be grouped with its own parental native honeybee population on the scatter plot (Fig 5); however, this was not the case.

Table 5. Mean ±Stdofangle characters determined by standardmorphometric method by using 19 landmarks.

Angles	Abv.	ISOLATED NATIVE AREA					COMMON APIARY				
		Mean±Std. Dev.					Mean±Std.Dev.				
		Artvin (N=60)	Kırklareli (N=45)	Muğla (N=45)	Hatay (N=45)	Yığılca (N=45)	*A.m. caucasica* (N=45)	*A.m. carnica* (N=45)	Muğla ecotype (N=30)	*A.m. syriaca* (N=60)	Yığılca ecotype (N=30)
Angle A1	A1	**22,32±4,24**	**23,68±5,17**	**22,37±4,02**	**20,52±2,60**	**20,63±2,98**	**23,17±5,61**	**24,38±4,98**	**21,52±5,40**	**21,09±5,52**	**24,91±5,86**
Angle A4	A4	**35,50±2,94**	**33,20±3,14**	**32,46±2,44**	**33,02±3,23**	**32,54±2,25**	**35,51±3,04**	**33,15±2,77**	**35,13±3,51**	**34,54±3,43**	**35,30±2,92**
Angle B3	B3	**80,96±4,06**	**79,43±3,80**	**79,69±3,92**	**79,08±4,77**	**78,86±5,43**	**79,68±4,26**	**79,36±5,04**	**78,16±4,64**	**79,57±4,76**	**81,27±3,65**
Angle B4	B4	**98,63±6,15**	**102,15±6,55**	**103,36±6,48**	**103,06±5,70**	**100,67±8,15**	**96,17±7,43**	**97,73±6,63**	**96,61±6,55**	**99,36±6,55**	**99,91±7,13**
Angle D7	D7	**101,78±3,64**	**99,62±3,14**	**101,69±3,29**	**99,96±3,87**	**100,06±3,45**	**99,28±3,30**	**99,75±3,16**	**99,44±3,21**	**99,96±4,05**	**103,28±2,82**
Angle E9	E9	**19,07±1,98**	**19,92±2,20**	**18,40±1,67**	**18,68±2,09**	**18,24±1,65**	**17,69±1,86**	**18,67±1,87**	**18,39±2,08**	**18,83±2,31**	**19,45±2,17**
Angle G7	G7	**25,69±17,29**	**24,09±1,40**	**23,57±1,26**	**25,87±15,69**	**28,60±23,82**	**24,09±1,07**	**25,22±1,18**	**24,33±1,06**	**24,33±1,31**	**23,87±0,94**
Angle G18	G18	**90,04±4,21**	**89,07±4,91**	**90,24±3,49**	**88,51±4,80**	**87,60±3,68**	**89,26±4,58**	**88,75±5,29**	**90,57±6,45**	**89,91±5,56**	**90,67±4,33**
Angle H12	H12	**14,13±2,07**	**18,14±2,53**	**15,46±2,44**	**14,72±2,88**	**15,56±2,07**	**15,88±3,03**	**16,16±3,41**	**16,45±3,13**	**17,29±3,65**	**14,50±2,97**
Angle J10	J10	**51,36±4,91**	**53,74±5,50**	**51,65±4,11**	**51,59±4,69**	**52,37±9,95**	**51,31±5,18**	**50,16±5,00**	**52,39±6,69**	**49,91±4,85**	**52,87±4,66**
Angle J16	J16	**85,89±6,07**	**89,22±6,01**	**88,65±3,77**	**87,78±5,20**	**89,37±5,28**	**85,47±4,49**	**86,64±5,37**	**88,25±6,09**	**87,52±6,63**	**80,75±3,52**
Angle K19	K19	**73,84±3,65**	**78,60±5,37**	**76,09±4,43**	**75,72±5,26**	**75,67±3,88**	**77,70±5,05**	**77,61±5,28**	**77,56±5,72**	**79,25±5,52**	**75,49±4,85**
Angle L13	L13	**13,77±1,68**	**11,93±1,86**	**13,33±1,32**	**12,48±1,52**	**15,08±15,96**	**24,22±38,11**	**13,63±1,52**	**13,23±2,15**	**13,02±1,89**	**13,92±1,71**
Angle M17	M17	**33,07±5,36**	**32,85±4,32**	**31,56±3,38**	**31,58±4,83**	**31,55±4,39**	**30,14±5,14**	**31,77±5,54**	**31,14±5,26**	**30,67±5,36**	**29,24±2,84**
Angle N23	N23	**87,30±5,91**	**91,95±5,73**	**88,67±3,70**	**90,17±4,81**	**89,95±5,40**	**85,23±5,20**	**85,37±5,13**	**86,70±5,18**	**86,38±7,86**	**82,72±3,55**
Angle O26	O26	**36,06±5,94**	**37,72±7,28**	**37,26±5,00**	**36,40±5,70**	**39,15±5,64**	**38,66±4,42**	**37,49±5,85**	**40,69±8,56**	**34,17±7,40**	**33,30±3,58**
Angle Q21	Q21	**36,51±1,77**	**38,29±17,06**	**36,44±1,76**	**37,96±1,87**	**35,70±1,75**	**40,67±22,33**	**43,36±26,37**	**47,46±32,74**	**36,52±3,92**	**36,86±1,80**

Common **Apiary**: All colonies represented five different honeybee subspecies of Turkey have been maintained altogether in common apiary since 2008.

The UPGMA dendogram showed that allof the Muğla native samples were classified in their real group, whereas the common apiary samples were not classified in their native ancestor groups although the samples originated from the native areas of Artvin, Hatay, Kırklareli, Yığılca and Muğla. In addition, the common apiary samples had different morphological characteristics than the native ancestor samples regarding A1, A4, B4, D7, J16, K19, L13, M17, N23, M17, O26, RADF, A, B, D, InnL, DumB, RADI and DisShıft. All native honeybee races had unique morphometric characteristics. After maintaining these bee subspecies all together in the common apiary, the occurrence of morphometric deformations was made possible, depending on the random mating among the bee races in common apiary. For example, although the CI value was 2.00 for the native bees of Kırklareli (*A. m. carnica),* for those in common apiary it was 2.10. The A4, A3 and B4 values of the common apiary samples were higher than those of the native bees, although the same samples from common apiary showed a similarity with the native samples. This variation was attributed to hybridization. Consistent with this idea is the observation that in Turkey, native honeybee subspecies and ecotypes have been exposed to introgressive hybridization with Caucasian bees (Güler, 2010). Future honeybee conservation efforts should therefore be aimed at reducing the introduction of alien subspecies into isolated areas already occupied by native honeybees.

By cross validation testing, the assignment of native honeybee colonies to their population of origin was 93.8%, whereas the assignment of the hybrid honeybee colonies in the common apiary was 54.4%. The overall success of group designation decreased when the common apiary populations were taken into account, as they overlapped to form one group. These results demonstrated that the low rates of correct classification of the common apiary colonies (Table 3) and the overlapping of different honeybee subspecies on the scatter plot (Fig 4) indicated that a gene flow between honeybee subspecies had occurred. Because all colonies representing three different subspecies and two ecotypes in Turkey had been maintained all together in the common apiary since 2008, the native subspecies had come into contact with each other resulting in different formations of new heterogeneous groups and loss of homogenic characteristics due to random mating of honeybee subspecies.The mating system of the honeybee is considered to lead to one of the most extreme form of panmixia in the animal kingdom because it is based on the aggregation of thousands of males from many colonies at drone congregation areas, which virgin queens visit in order to mate repeatedly with tens of drones. Controlling queen mating is extremely difficult within a small area (Koeniger & Koeniger, 2000; De la Rùa et al., 2009). Gene flow between indigenous honeybee subspecies maintained in the same area is therefore probable. It is recognized that native honeybee subspecies and ecotypes might have lost their characteristics because of the hybridization caused by migratory beekeeping, commercial queen bee usage, and uncontrolled mating (Ruttner, 1988; Rinderer et al., 1990; Moritz, 1991; Kauhausen-Keller et al., 1997).

Migratory beekeeping, in which beekeepers move from north to south and from east to west following the blooming of the target honey plants, is very common and predominant in Turkey. This means that the hives have to be moved from one place to another with the goal of ultimately collecting good-quality honey in reasonable quantities. Beekeepers transport their hives extensively during the year, up to distances of 2000-4000 km (Sıralı, 2002). The natural distribution of honeybee races and ecotypes has been affected seriously by this migratory beekeeping practice. In many regions of Turkey, the native honeybee races and local ecotypes have been destroyed or hybridized (De la Rùa, 2009; Güler, 2010). In order to solve this problem, the movement of migratory beekeepers into certain districts in the seven geographic regions of Turkey should be legally regulated. Yet, there is not one regulation or restriction related to the routes that the beekeepers use. Urgent regulation of routes used for migratory populations and legal geographical boundaries related to migratory beekeeping should be determined in order to prevent the gene flow between migratory populations and local populations. This is a crucial issue for the conservation of the current biodiversity of honeybees. To preserve the current diversity and genetic structure as much as possible, extensive isolated areas should be established in order to reduce the effect of migratory beekeeping. Moreover, rules on the commercial trade of queen bees should be determined in order to prevent the loss of genetic diversity and the homogenization of the genetic structure of the honeybee population. First of all, the most important guarantee for the preservation of the genetic resources of the Turkish honeybee races or ecotypes having different genetic composition is the registration of all the distinct natural Turkish honeybee races and ecotypes. At present, the only standard of identification and registration is that of the Caucasian bee race (*A. m. caucasica*), recorded under the declaration of the Registration of Native Animal Races and Ecotypes (Official Gazette, 2004). (No: 2004/39, Official Gazette No. 25668, 12 December 2004, http://rega.basbakanlik.gov.tr/eskiler/2004/12/20041212.htm. This registration standard includes biochemical and molecular markers of DNA in addition to the morphological characteristics. Unfortunately, to date, there have been no registration studies establishing standards forthe Anatolian (*A. m. anatoliaca*) honeybee. Some scientific research based on morphometric, biochemical, and genetic analyses carried out for this purpose has reported the identification of ecotypes in isolated areas and other regions (Asal et al., 1995; Kandemir & Kence, 1995; Güler & Kaftanoğlu, 1999a,b; Kandemir et al., 2000, 2006; Kekeçoğlu, 2009, 2010a,b); however, legal protection has still not been established.

Naturally-evolved, region-specific species can be threatened with extinction (Mooney & Cleland, 2001) through genetic pollution, potentially causing uncontrolled

hybridization, introgression and genetic swamping. These processes can lead to homogenization or replacement of local genotypes as a result of either a numerical and/or fitness advantage of the introduced plant or animal (Aubry et al., 2005). Non-native species can threaten native plants and animals with extinction by hybridization and introgression, either through purposeful introduction by humans or through habitat modification, bringing previously isolated species into contact. These phenomena can be especially detrimental for rare species coming into contact with more abundant ones. Interbreeding between the species can cause a 'swamping' of the rarer species' gene pool, creating hybrids that supplant the native stock. The extent of this phenomenon is not always evident from outward appearance alone. Although some degree of gene flow occurs in the course of normal evolution, hybridization with or without introgression may threaten a rare species' existence (Rhymer & Simberloff, 1996). These patterns are in accordance with the findings of this study.

Morphometric, alloenzymatic and genetic studies have shown that there are five subgenera of *A. mellifera* throughout Turkey, including *A. m. anatoliaca, A. m. meda, A. m. caucasica, A. m. syriaca* and *A. m. carnica* (Ruttner, 1988; Smith et al., 1997; Palmer et al., 2000; Kandemir et al., 2006). In the present study, geometric morphometrics correctly identified the majority of all native honeybees except the Kırklareli natural *A. m. carnica*. The CVA scatter plot showed that except for the Kırklareli, all native honeybee populations formed non-overlapping distant clusters. In the cross-validation test, some colonies from the native Kırklareli were assigned to the native Hatay population. The cubital index is an important wing characteristic for *A. m. carnica* (Rutner, 1988). In general, the highest CI value was found in Kırklareli province in the native ancestors of the *A. m. carnica* race (Table 4). Although the sampling area in Kırklareli was declared to be the origin of the *A. m. carnica*, some hybridization was observed due to lack of a sufficient legal regulatory mechanism. In previous studies, Kırpık et al. (2010) and Güler (2010) reported similar results for the native *A. m. caucasica* population. As for the native Kırklareli population, opinions differ regarding identification of the Thracian honeybees in the European part of Turkey. Some apicultural scientists have described Thracian bees as ecotypes of *Apis mellifera anatoliaca* and others as those of *Apis mellifera carnica* (Adam, 1983; Ruttner, 1988; Smith, 1997).

Bees, as the main pollinators of food crops, represent a critical natural resource which needs to be carefully exploited and managed. In recent years, however, hybridization caused by migratory beekeeping has modified the genetic pool of the local honeybee population, leading to the loss of their unique genetic and morphological traits (De la Rùa et al., 2009; Güler, 2010). The present study has emphasized the effect of this hybridization on the diversity of Turkish honeybees throughout all specific regions. Studies on the morphometric divergence of Anatolian honeybees through loss of their original traits have been quite limited (Kence, 2006; Güler, 2010; Kekeçoğlu & Soysal, 2010b). Hence, the findings of this study provide a significant contribution to the current program for the conservation of native Turkish honeybee gene resources.

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