Türk. entomol. derg., 2021, 45 (4): 463-474
DOI: http://dx.doi.org/10.16970/entoted.982406

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Determination of the wing morphology differentiation of old and recent honey bee samples from western Turkey using geometric morphometrics[1]

Batı Anadolu eski ve yeni bal arısı örneklerinin kanat morfolojisi farklılaşmasının geometrik morfometri kullanılarak belirlenmesi

Mustafa KÖSOĞLU[2*] **Rahşan İVGİN TUNCA[3]** **Neslihan ÖZSOY[2]**
Yahya Tuncay TUNA[4] **Erkan TOPAL[2]** **Belgin GÜNBEY[5]**

Abstract

In this study, old and recent honey bee, *Apis mellifera* L., 1758 (Hymenoptera: Apidae) specimens were compared using geometric morphometrics. The old honey bee samples were collected from different apiaries in Edirne, Balıkesir, Çanakkale, Denizli and Muğla Provinces, and Gökçeada (an Aegean island) in Turkey in 1987-1988 under a project of the Aegean Agricultural Research Institute and the recent samples were collected in the same locations in 2017. The mean values determined for each region were grouped using Mahalanobis distances, and the results were summarized on dendrogram. While the old samples constituted one group, the recent samples constituted another one. When the results of the discriminant function analysis were compared, it was observed that overall old and recent samples were statistically different from each other ($P < 0.0001$). The evaluation of both groups has revealed that the recent population of Gökçeada was different from morphologically the other mainland populations in the current situation. However, the Thrace (Edirne) honey bee specimens were different from the Anatolian (Çanakkale, Denizli, Balıkesir, Muğla) and island (Gökçeada) specimens in the past according to dendrogram relationships.

Keywords: *Apis mellifera*, geometric morphometrics, Gökçeada, honey bee, Thrace, Turkey

Öz

Bu çalışmada eski ve yeni bal arısı, *Apis mellifera* L., 1758 (Hymenoptera: Apidae) örnekleri geometrik morfometrik yöntemlerle morfolojik olarak karşılaştırılmıştır. Eski bal arısı örnekleri, Ege Tarımsal Araştırma Enstitüsü tarafından yürütülen bir proje kapsamında, 1987-1988 yıllarında Edirne, Balıkesir, Çanakkale, Denizli, Muğla illeri ve Gökçeada Adası'ndaki farklı arılıklardan toplanmıştır ve 2017 yılında aynı lokasyonlardan alınan güncel bal arısı örnekleri ile çalışılmıştır. Her bölge için belirlenen ortalama değerler Mahalanobis mesafesi kullanılarak gruplandırılmış ve sonuçlar dendrogram üzerinde özetlenmiştir. Eski örnekler bir grubu oluştururken, güncel örnekler bir diğerini oluşturmuştur. Eski ve güncel örnekler için diskriminant fonksiyon analizi sonuçları karşılaştırıldığında, geçmiş ve şimdiki genel örneklerin istatistiksel olarak birbirinden farklı olduğu gözlenmiştir ($P < 0.0001$). Her iki grubun değerlendirmesi, Ada'nın (Gökçeada) mevcut popülasyonunun, mevcut durumda diğer anakara popülasyonlarından morfolojik olarak farklı olduğunu ortaya koymuştur. Öte yandan Trakya (Edirne) bal arısı popülasyonunun, dendrograma göre geçmişte Anadolu (Çanakkale, Denizli, Balıkesir, Muğla) ve Ada (Gökçeada) bal arısı örneklerinden farklı olduğu belirlenmiştir.

Anahtar sözcükler: *Apis mellifera*, geometrik morfometrik, Gökçeada, bal arısı, Trakya, Türkiye

[1] This study was supported by Republic of Turkey ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policies (TAGEM), Ankara, Turkey, Grant Project No: TAGEM/HAYSÜD 15/06/01/02.
[2] Apiculture Research Center, Aegean Agricultural Research Institute, 35661, Menemen, Izmir, Turkey
[3] Muğla Sıtkı Koçman University, Ula Ali Koçman Vocational School, Department of Plant and Animal Breeding, Apiculture Program, 48640, Ula, Muğla, Turkey
[4] Tekirdağ Namık Kemal University, Agriculture Faculty, Department of Animal Science, 59030, Tekirdağ, Turkey
[5] Republic of Turkey Ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policies (TAGEM), 06800, Ankara, Turkey
* Corresponding author (Sorumlu yazar) e-mail: mustafakosoglu@gmail.com
Received (Alınış): 13.08.2021 Accepted (Kabul ediliş): 09.12.2021 Published Online (Çevrimiçi Yayın Tarihi): 19.12.2021

Introduction

The previous studies conducted in Turkey revealed that there are different honey bee subspecies and ecotypes in Turkey (Kandemir et al., 2006; Bodur et al., 2007; Tunca, 2009; Tunca & Kence, 2011). Bodenheimer (1941) defined the honey bees, *Apis mellifera* L., 1758 (Hymenoptera: Apidae) morphologically in Anatolia and reported that there were different honey bee subspecies from western to eastern of Turkey. Despite the low level of economic efficiency of local bee races and subspecies in Turkey, they adapt to the specific conditions of their geographical regions, resistant to various diseases, and are able to produce in extreme environmental conditions and maintain the reproductive ability. For the existence of these genetic resources, it is necessary to reveal their superior characteristics and to benefit from these qualities both today and for future. This situation can be revealed with current methods (Ertuğrul et al., 2000; Kence, 2006).

The studies on genetic variation in different populations have been conducted for a long time (Smith, 2002). It was reported that there was a risk of losing the genetic differences of the bees in Turkey before fully characterizing them. The debates on the subject about the threat of the genetic diversity of the honey bees have recently gained importance in Turkey. Consequently, morphological and genetic studies have been conducted to examine the effects of migratory beekeeping (Kükrer, 2013; Kambur et al., 2018). In order to morphologically distinguish honey bee samples, there has been a transition from the standard to geometric morphometrics methods (Tofilski, 2008; Turan, 2011; Koca, 2012; Koca & Kandemir, 2013). The honey bees identified by Ruttner (1988) in the Middle East have been now analyzed by way of geometric morphometrics, which is more reliable to distinguish the honey bee subspecies (Koca & Kandemir, 2013).

The geometric morphometric method can help to distinguish shape differences more clearly despite the body size being more readily affected by the environmental conditions, as differences in the, shape of the body mostly originate from genetic differences (Kence, 2006). As a result, the data obtained from geometric morphometry gives more reliable classification of honey bees than standard morphometrics (Kence, 2006; Koca & Kandemir, 2013; Kambur et al., 2018). Nowadays, both geometric morphometric and DNA-based studies are used to determination of evolutionary lineages or subspecies and in evaluating of genetic structure within honey bee subspecies (Barour & Baylac, 2016; Zammit-Mangion et al., 2017; Alattal et al., 2019; Henriques et al., 2020).

In this study, honey bee samples collected from western Turkey 30 years ago were compared with recent bee samples collected in 2017 from local beekeepers from the same regions. The aim of the study is to reveal possible changes in populations with the possible effects of climate change, production activities and migratory beekeeping over this period. In a previous project conducted by the Aegean Agricultural Research Institute in 1987-1988, the number of colonies was around 2.8 million in Turkey. Now there are approximately 8 million colonies in Turkey based on the 2018 data FAO (FAO, 2018). This study was conducted to determine if there is any change in the wing morphology between the old and recent honeybee samples.

Materials and Methods

Honey bee samples examined were composed of two groups; old and recent samples (Table 1). All of the old and recent samples were obtained from the same apiaries located in Edirne, Balıkesir, Çanakkale, Denizli, Muğla and Gökçeada. The old samples had been collected in 1986 (Öztürk et al., 1992) and deposited as a collection at the Aegean Agricultural Research Institute. The recent samples represented were collected from the same apiaries and regions as for the old ones. For each location, 50 wing samples were used (300 old and 300 recent samples; 600 as total).

Table 1. Areas (number of sites and apiaries) where measured samples (50 bees each, 600 total) were collected

Old samples (1987-1988)			Recent samples (2017)		
Area	Sites	Apiaries	Area	Sites	Apiaries
Balıkesir	2	6	Balıkesir	4	7
Çanakkale	7	33	Çanakkale	6	6
Denizli	6	13	Denizli	3	6
Edirne	3	8	Edirne	4	10
Muğla	9	74	Gökçeada	1	6
Gökçeada	1	11	Muğla	7	19

All samples were collected from local beekeepers that were not in contact with migratory beekeepers. Six hundred honey bee samples were measured in this study. At least 20 samples from each hive were collected from local beekeepers. The forewings of worker bees were dissected and prepared on slides and their high-resolution photos were taken under microscope (BAB-STR 45) for geometric morphometric analysis. Twenty landmarks on the right-side forewings were digitized according to Bookstein's landmark definition (Bookstein, 1990) (Figure 1). Data files (tps) were prepared using tpsUtil 1.40 and landmarks were digitized on the images using tpsDig 2.11 (Rohlf, 2008a; Rohlf, 2008b). In order to assess the variation among honey bee samples, procrustes ANOVA test, canonical variate analysis (CVA), and discriminant function analysis (DFA) were performed with MorphoJ version 1.06d program (Klingenberg, 2011). A UPGMA cluster analysis was performed on Mahalanobis distances of data to show the clustering among honey bee populations using NTSYS-PC (2.2) (Rohlf, 2000).

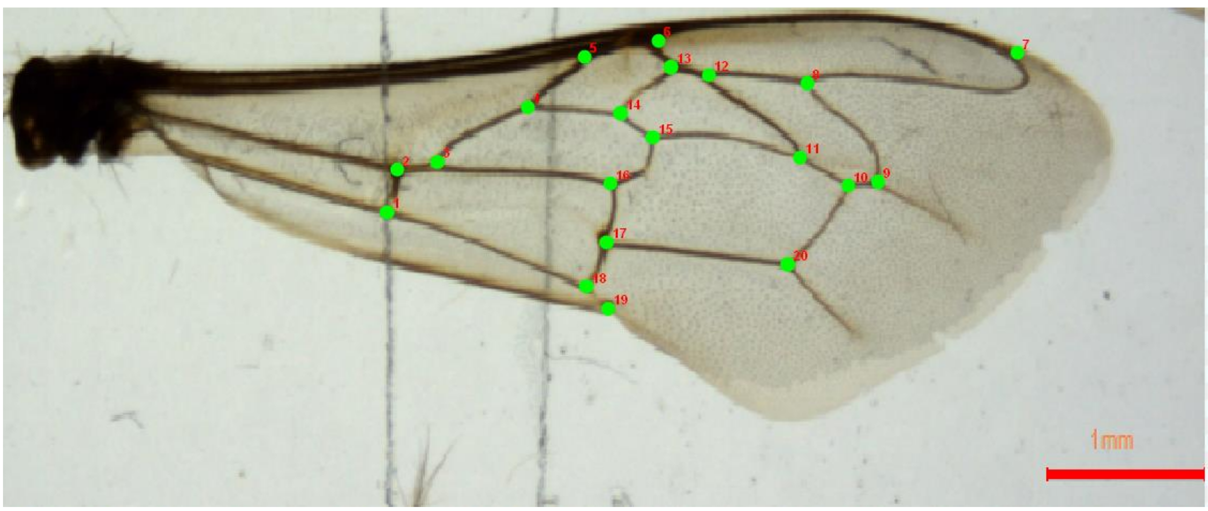

Figure 1. Location of landmarks on *A. mellifera* worker the fore wing.

Results

Old (1987-1988) honey bee samples - 300 wings were analyzed from the old samples. Procrustes ANOVA test applied to assess the population differences showed significant shape differences between locations (P < 0.0001) but not significant in terms of centroid size (Table 2).

Table 2. Procrustes ANOVA for old honey bee samples

Centroid size					
Effect	SD	MS	df	F	P (param.)
Individual	12373	2475	5	1.77	0.118
Residual	410518	1396	294		

Shape, procrustes ANOVA							
Effect	SD	MS	df	F	P (param.)	Pillai trace	P (param.)
Individual	0.0205	0.000114	180	5.34	< 0.0001	1.55	< 0.0001
Residual	0.225	0.0000213	10584				

CVA indicated that the total shape variation was explained by five axes as 41.8, 25.6, 13.8, 11.3, and 7.47%, respectively. The first three axes explained 81.3% (cumulative) of the total variation among the honey bee groups. Edirne (Thrace), Gökçeada (Aegean island) and Balıkesir (Anatolia) each of them formed a separate group, while other populations including Muğla, Denizli and Çanakkale populations formed one group according (Figure 2).

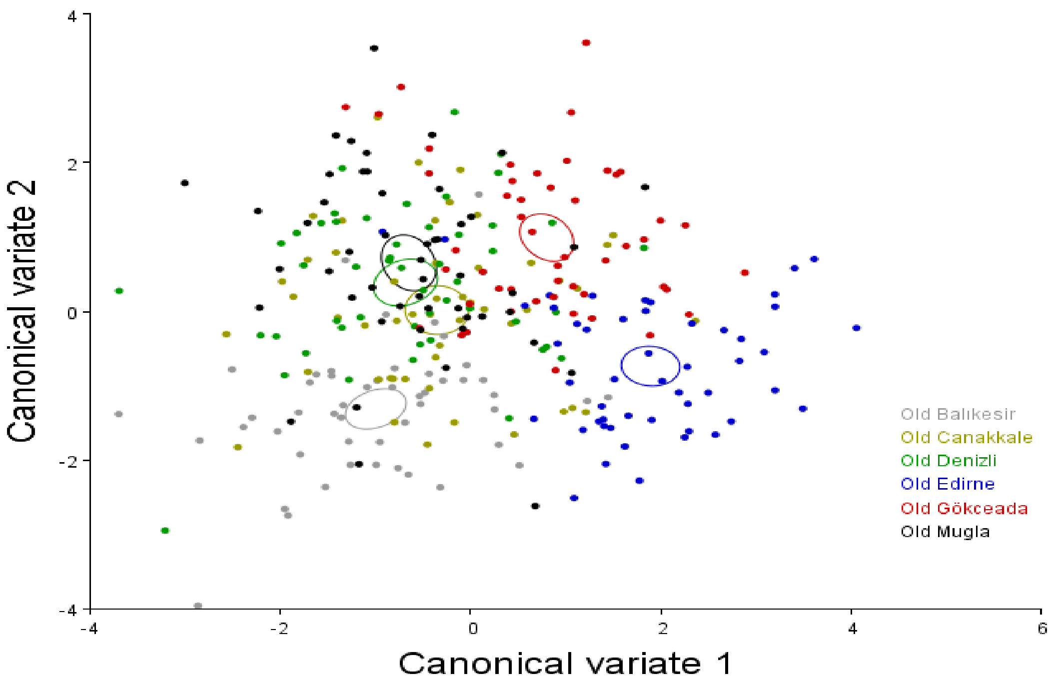

Figure 2. Distribution of the form differences generated by 20 landmarks in the old worker bee wing samples on the first two axes (canonical variate analysis).

DFA showed that differences both means of procrustes and Mahalanobis distances and also true allocation of the population comparisons between old Çanakkale-old Denizli, old Çanakkale-old Muğla, old Denizli-old Muğla were not statistically significant (P > 0.0001). Comparisons old Balıkesir-old Çanakkale, old Balıkesir-old Denizli pairs were also not significant (P > 0.0001) according to the t-square calculated from Mahalanobis distances (Table 5).

Recent (2017) honey bee samples - 300 worker bee wing samples were analyzed and compared by locations. In the procrustes ANOVA test, the shape and centroid were estimated from total variation based on size (Table 3).

Table 3. Procrustes ANOVA for recent honey bee samples

Centroid size							
Effect	SS	MS	df	F	P (param.)		
Individual	25855	5171	5	5.34	0.0001		
Residual	284472	968	294				
Shape, procrustes ANOVA							
Effect	SS	MS	df	F	P (param.)	Pillai trace	P (param.)
Individual	0.0125	0.0000694	180	3.8	< 0.0001	1.52	< 0.0001
Residual	0.190	0.0000180	10584				

Procrustes ANOVA test showed that there were statistically significant differences between locations in terms of shape (P < 0.0001). Three main clusters were observed on CVA distribution diagram. Gökçeada and Edirne populations were different from other populations collected from the west part of Anatolian populations (Figure 3).

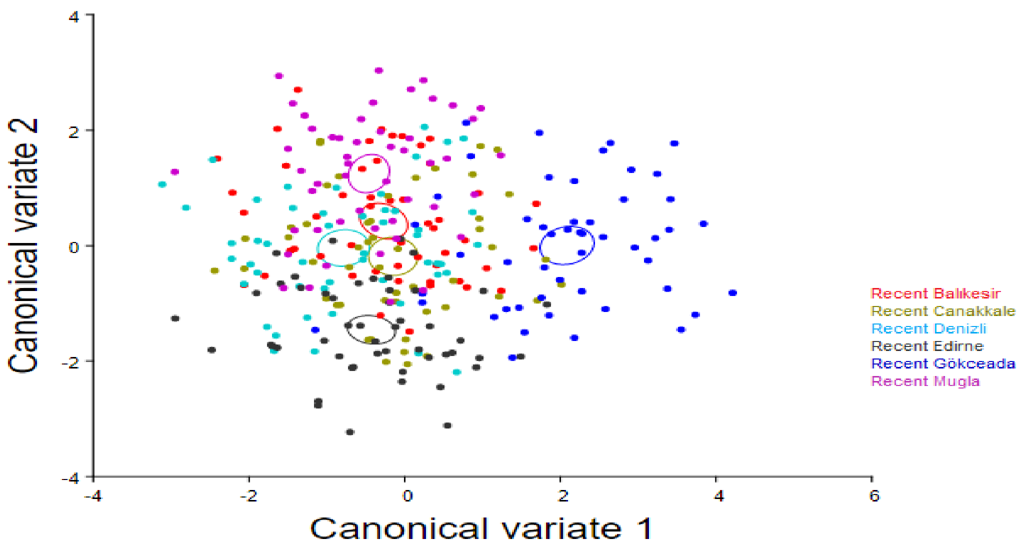

Figure 3. Distribution of form differences generated by 20 landmarks in recent worker bee wing samples on first two axes (canonical variate analysis).

With CVA of the six populations, the total shape variation was explained by five axes as 38.0, 27.1, 18.2, 11.4 and 5.34%, respectively. The first three axes explained 83.3% (cumulative) of the total variation. DFA showed that differences both means of procrustes and Mahalanobis distances and also true allocation of the population comparisons between the recent populations of Balıkesir-Çanakkale and Çanakkale-Denizli were not statistically significant (P > 0.0001) (Table 5).

Old (1987-1988) and recent (2017) honey bee samples - procrustes ANOVA test revealed that there are significant differences between the locations of the old and recent samples in terms of both centroid size and shape (P < 0.0001) (Table 4).

Table 4. Procrustes ANOVA for old versus recent honey bee samples

Centroid size					
Effect	SS	MS	df	F	P (param.)
Individual	1301016	118274	11	100	< 0.0001
Residual	694990	1181	588		

Shape, procrustes ANOVA							
Effect	SS	MS	df	F	P (param.)	Pillai	P (param.)
Individual	0.0556	0.000140	396	8.36	< 0.0001	2.21	< 0.0001
Residual	0.415	0.000019	21168				

The total shape variation was explained by eleven axes. The first three axes explained 70.5% (cumulative) of the morphological variation of populations (first three axes: 39.6,19.2 and 11.8%). The CVA from populations also showed that old and recent populations were clustered distinctly from each other. While old and recent populations were in two groups on the plot, it was also observed the differences the populations represented within their groups (old or recent) (Figure 4). According to DFA, there were significant differences between population both in procrustes and Mahalanobis distances. (Table 5).

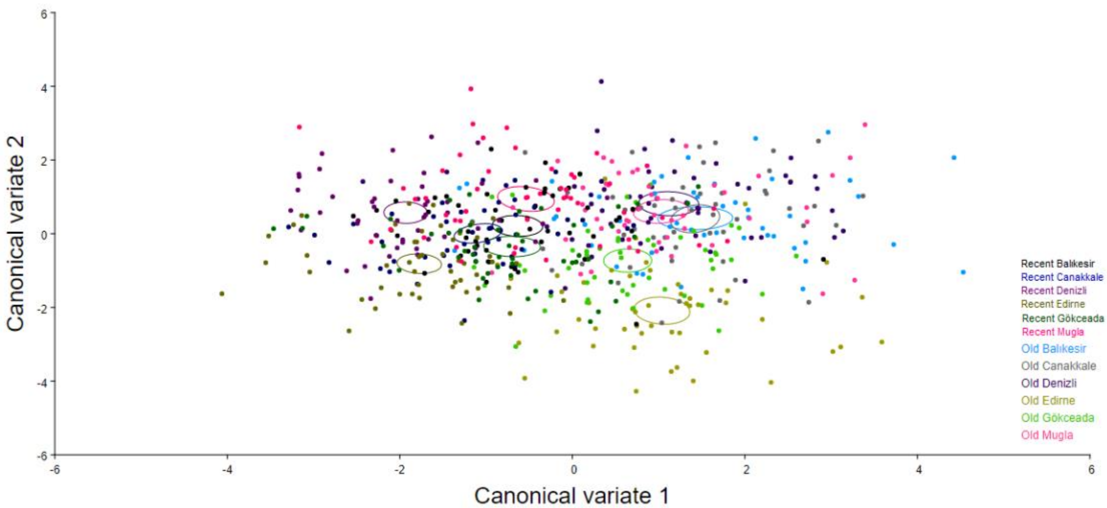

Figure 4. Distribution of the form differences generated by 20 landmarks in the old and recent worker bee wing samples on the first two axes (canonical variate analysis).

Comparisons recent Balıkesir-recent Muğla and recent Denizli-recent Edirne were not significant for procrustes distances (P > 0.0001). Comparisons old Çanakkale-old Denizli, Old Çanakkale-old Muğla, Old Denizli-old Muğla, recent Balıkesir-recent Çanakkale, recent Çanakkale-recent Denizli, recent Çanakkale-recent Edirne were not significant in terms of discriminant function and cross-validation (P > 0.0001). In the evaluation of the results regarding the old and recent bee samples simultaneously, the old and recent populations were grouped into two main clusters on the dendrogram (Figure 5). All old populations were placed on the one main branch. The important point is that old Edirne population was located from different branch from other old populations. However, the recent honey bee populations from Western Anatolia and Thrace specimens were clustered together, and Gökçeada population was separated from them. Evaluation of both groups shows that there are wing morphological differences in populations from past to present. According to analysis, Gökçeada population is separated from recent populations but in the past, it was clustered with Western Anatolia (Figure 5).

While the statistical differences among the old honey bee populations for Balıkesir-Denizli were not significant in the past, recent populations had significant differences for both locations according to Mahalanobis distances. However, the differences between the old Balıkesir and Muğla were significant in the past but this difference was not observed between both populations according to procrustes distances (Table 5).

Table 5. Discriminant function analysis for old, recent and combined honey bee populations

Comparison	Procrustes distance	Procrustes distance (P)	T-square (P)	Mahalanobis distance	T-square (value)	T-square (P)
Old Balıkesir-old Mugla	1.39×10^{-2}	<0.0001	<0.0001	2.67	178	<0.0001
Old Balıkesir-old Canakkale	1.14×10^{-2}	<0.0001	<0.0001	2.35	138	0.0009
Old Balıkesir-old Denizli	0.98×10^{-2}	<0.0001	<0.0001	2.45	150	0.0003
Old Balıkesir-old Edirne	1.69×10^{-2}	<0.0001	<0.0001	3.36	282	<0.0001
Old Balıkesir-old Gökceada	1.84×10^{-2}	<0.0001	<0.0001	3.52	309	<0.0001
Old Canakkale-old Denizli	0.15×10^{-2}	0.017	0.324	1.59	63.3	0.329
Old Canakkale-old Edirne	1.29×10^{-2}	<0.0001	<0.0001	2.83	200	<0.0001
Old Canakkale-old Gökceada	1.26×10^{-2}	<0.0001	<0.0001	2.63	173	<0.0001
Old Canakkale-old Mugla	0.72×10^{-2}	0.043	0.012	2.03	103	0.0172
Old Denizli-old Edirne	1.44×10^{-2}	<0.0001	<0.0001	3.46	299	<0.0001
Old Denizli-old Gökceada	1.26×10^{-2}	<0.0001	<0.0001	2.94	215	<0.0001
Old Denizli-old Mugla	0.92×10^{-2}	0.002	0.002	2.28	130	0.0017
Old Edirne-old Gökceada	1.22×10^{-2}	<0.0001	<0.0001	2.95	217	<0.0001
Old Edirne-old Mugla	1.43×10^{-2}	<0.0001	<0.0001	3.58	321	<0.0001
Old Gökceada-old Mugla	1.27×10^{-2}	<0.0001	<0.0001	2.90	210	<0.0001
Recent Balıkesir-recent Canakkale	0.70×10^{-2}	0.009	0.15	1.73	74.8	0.155
Recent Balıkesir-recent Denizli	0.95×10^{-2}	<0.0001	<0.0001	2.70	182	<0.0001
Recent Balıkesir-recent Edirne	1.16×10^{-2}	<0.0001	<0.0001	3.14	246	<0.0001
Recent Balıkesir-recent Gökceada	0.97×10^{-2}	<0.0001	<0.0001	2.99	224	<0.0001
Recent Balıkesir-recent Mugla	0.60×10^{-2}	0.117	<0.0001	2.30	133	0.0013
Recent Canakkale-recent Denizli	0.63×10^{-2}	0.037	0.002	2.25	126	0.0024
Recent Canakkale-recent Edirne	0.78×10^{-2}	<0.0001	0.005	2.23	125	0.0027
Recent Canakkale-RecentGökceada	1.11×10^{-2}	<0.0001	<0.0001	2.90	210	<0.0001
Recent Canakkale-recent Mugla	0.81×10^{-2}	<0.0001	0.001	2.41	146	0.0004
Recent Denizli-recent Edirne	0.80×10^{-2}	0.001	<0.0001	2.79	195	<0.0001
Recent Denizli-recent Gökceada	1.39×10^{-2}	<0.0001	<0.0001	3.21	257	<0.0001
Recent Denizli-recent Mugla	0.86×10^{-2}	<0.0001	0.004	2.32	134	0.0011
Recent Edirne-recent Gökceada	1.40×10^{-2}	<0.0001	<0.0001	4.18	436	<0.0001
Recent Edirne-recent Mugla	1.18×10^{-2}	<0.0001	<0.0001	3.52	310	<0.0001
Recent Gökceada-recent Mugla	1.16×10^{-2}	<0.0001	<0.0001	4.04	408	<0.0001

Table 5. Continued

Comparison	Procrustes distance	Procrustes distance (P)	T-square (P)	Mahalanobis distance	T-square (value)	T-square (P)
Recent Balıkesir-old Balıkesir	1.53×10^{-2}	<0.0001	<0.0001	2.97	220	<0.0001
Recent Balıkesir-old Canakkale	1.40×10^{-2}	<0.0001	<0.0001	3.41	290	<0.0001
Recent Balıkesir-old Denizli	1.18×10^{-2}	<0.0001	<0.0001	3.02	228	<0.0001
Recent Balıkesir-old Edirne	1.62×10^{-2}	<0.0001	<0.0001	3.53	311	<0.0001
Recent Balıkesir-old Gökceada	1.28×10^{-2}	<0.0001	<0.0001	3.10	240	<0.0001
Recent Balıkesir-old Mugla	1.60×10^{-2}	<0.0001	<0.0001	3.33	278	<0.0001
Recent Canakkale-old Balıkesir	1.57×10^{-2}	<0.0001	<0.0001	3.36	282	<0.0001
Recent Canakkale-old Canakkale	1.53×10^{-2}	<0.0001	<0.0001	3.86	373	<0.0001
Recent Canakkale-old Denizli	1.39×10^{-2}	<0.0001	<0.0001	3.87	373	<0.0001
Recent Canakkale-old Edirne	1.66×10^{-2}	<0.0001	<0.0001	4.11	423	<0.0001
Recent Canakkale-old Gökceada	1.52×10^{-2}	<0.0001	<0.0001	3.75	352	<0.0001
Recent Canakkale-old Mugla	1.80×10^{-2}	<0.0001	<0.0001	3.91	382	<0.0001
Recent Denizli-old Balıkesir	1.18×10^{-2}	<0.0001	<0.0001	3.95	390	<0.0001
Recent Denizli-old Canakkale	1.84×10^{-2}	<0.0001	<0.0001	4.65	540	<0.0001
Recent Denizli-old Denizli	1.65×10^{-2}	<0.0001	<0.0001	4.14	429	<0.0001
Recent Denizli-old Edirne	1.93×10^{-2}	<0.0001	<0.0001	4.83	584	<0.0001
Recent Denizli-old Gökceada	1.75×10^{-2}	<0.0001	<0.0001	4.47	500	<0.0001
Recent Denizli-old Mugla	2.06×10^{-2}	<0.0001	<0.0001	4.45	496	<0.0001
Recent Edirne-old Balıkesir	1.91×10^{-2}	<0.0001	<0.0001	3.93	386	<0.0001
Recent Edirne-old Canakkale	1.92×10^{-2}	<0.0001	<0.0001	4.86	592	<0.0001
Recent Edirne-old Denizli	1.79×10^{-2}	<0.0001	<0.0001	4.97	616	<0.0001
Recent Edirne-old Edirne	1.57×10^{-2}	<0.0001	<0.0001	4.40	485	<0.0001
Recent Edirne-old Gökceada	1.71×10^{-2}	<0.0001	<0.0001	4.25	452	<0.0001
Recent Edirne-old Mugla	2.13×10^{-2}	<0.0001	<0.0001	4.24	449	<0.0001
Recent Gökceada-old Balıkesir	1.67×10^{-2}	<0.0001	<0.0001	3.43	293	<0.0001
Recent Gökceada-old Canakkale	1.51×10^{-2}	<0.0001	<0.0001	3.61	326	<0.0001
Recent Gökceada-old Denizli	1.37×10^{-2}	<0.0001	<0.0001	3.76	354	<0.0001
Recent Gökceada-old Edirne	1.60×10^{-2}	<0.0001	<0.0001	4.31	465	<0.0001
Recent Gökceada-old Gökceada	1.21×10^{-2}	<0.0001	<0.0001	3.13	245	<0.0001
Recent Gökceada-old Mugla	1.65×10^{-2}	<0.0001	<0.0001	3.66	334	<0.0001
Recent Mugla-old Balıkesir	1.46×10^{-2}	<0.0001	<0.0001	3.40	288	<0.0001
Recent Mugla-old Canakkale	1.26×10^{-2}	<0.0001	<0.0001	2.94	217	<0.0001
Recent Mugla-old Denizli	0.98×10^{-2}	<0.0001	<0.0001	2.18	119	0.0045
Recent Mugla-old Edirne	1.60×10^{-2}	<0.0001	<0.0001	3.56	317	<0.0001
Recent Mugla-old Gökceada	1.30×10^{-2}	<0.0001	<0.0001	3.02	228	<0.0001
Recent Mugla-old Mugla	1.43×10^{-2}	<0.0001	<0.0001	2.59	167	<0.0001

Permutation tests using the T-square statistic is equivalent to tests using Mahalanobis distances.

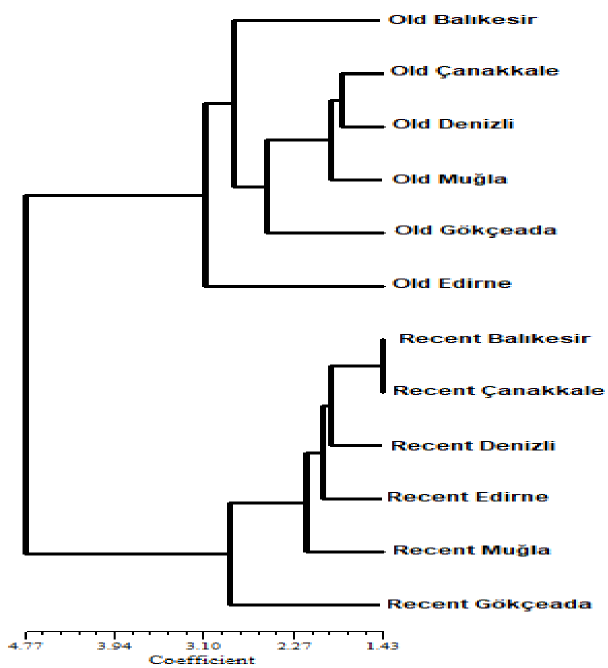

Figure 5. Dendrogram of old and recent honey bee populations.

Discussion

Morphological characters have been used to determine the insect populations with both traditional and geometric morphometrics (Zhou et al., 2018; Power et al., 2019). For more than 10 years, geometric morphometrics have been widely used in determine the variation of honey bee populations (Francoy et al., 2008; Tofilski, 2008; Francoy et al., 2009; Kandemir et al., 2009; Özkan & Kandemir, 2010; Santoso et al., 2018). The geometric morphometrics has enabled the construction of more meaningful clusters for the old and recent honey bees' specimens. The populations represented in the same locations from the past to the present have also revealed the differences from each other in this study.

The results of this study showed that there has been change in honey bee populations since 1987. Results for past and recent populations are consistent with previous studies (Kandemir et al., 2000; Palmer et al., 2000; Koca & Kandemir, 2013). In the recent honey bee populations, the significant differences were not observed pairs of Western Anatolia populations especially Balıkesir, Çanakkale, Muğla, Denizli and Edirne. The possible explanation of this is that these populations could be influenced by each other through the selling the queen bees and colonies during migratory beekeeping activities.

While there was a difference between old Balıkesir samples and old Muğla samples, no difference was observed between Balıkesir and Muğla recent samples. A reason for this, could be that pine honey production areas have moved northward due to climate change over recent years. Especially in Balıkesir, pine honey production areas have been expanding recently. This may be the reason that the producer of the region obtains queen bees from the colonies that are successful in pine honey production and chooses the colonies that are similar to Muğla bees in creating new colonies. While there is no difference between the old Çanakkale-old Denizli and recent Çanakkale-recent Denizli samples, the difference in comparing the old and recent samples explains that the change is holistic. This is also evident in the analysis results. Overall, there is a difference when all old and recent sampling locations are compared, and this includes the island population from Gökçeada.

Gökçeada population is different from other Anatolian and Thrace populations in present. It should be mentioned that Gökçeada bee registered officially by Republic of Turkey Ministry of Agriculture and Forestry in 2018 (Official Gazette 16/05/2019/30776). It showed that the island honey bee population can be maintained as long as there is no new introduction to the gene pool of the island from mainland. However, that the population may contract in the long term due to the inbreeding in island populations. The government agencies should development an alternative plan for this situation.

Overall data revealed that the bees from Thrace, Western Anatolia and Gökçeada are different from the past to the present and this change is significant (based on DFA). Even if there are some changes in the genetic material due to the intense migratory beekeeping and particularly due to the preference of queen bees of different genotypes from outside the region, so it is possible that the changes occurred gradually. Hence, both government institutions and beekeeper associations have to implement their strategic action plans for conservation of local bee populations as soon as possible. The observed changes in Turkish bee populations were reported in a previous study (Kambur & Kekeçoğlu, 2018).

As a result, although honey bees, whose lives are completely dependent on nature, can survive for thousands of years, it is known that beekeepers turn to new sources and want to increase production using different genotypes that are not suited to their regions in the long term. The effects of these choices made by beekeepers and their relationship with climate change have emerged as issues that should be studied and researched further. According to the results of this analysis, although the reason for the changes in the populations from the past to the present is possibly queen bee changes or migratory beekeeping, these two factors are actually a consequence of other factors. Yield or colony losses due to poor colony management led to the need for queen bee changes and migratory beekeeping. Also, uncontrolled sales of queens and colonies belonging to different subspecies and beekeeping activities conducted in different regions, especially during queen rearing seasons, can contribute to changes in the gene pools of populations. All problems on this subject can be solved with the joint decisions of producers, beekeeper associations and government institutions.

It is hoped that bees, whose lives are completely dependent on nature and have lived for thousands of years, will always continue their lives as an inseparable part of nature.

References

Alattal, Y., M. Alsharhi, A. Alghamdi & S. Fuchs, 2019. Characterization of Socotra (Yemen) honey bees (*Apis mellifera*) using morphometric and genetic markers. Bulletin of Insectology, 72 (2): 281-285.

Barour, C. & M. Baylac, 2016. Geometric morphometric discrimination of the three African honey bee subspecies *Apis mellifera intermissa*, *A. m. sahariensis* and *A. m. capensis* (Hymenoptera, Apidae): Fore wing and hind wing landmark configurations. Journal of Hymenoptera Research, 52: 61-70.

Bodenheimer, F. S., 1941. Studies on The Honey Bee and Beekeeping in Turkey. Merkez Zirai Mücadele Enstitüsü. Ankara, 60 pp.

Bodur, Ç., M. Kence & A. Kence, 2007. Genetic structure of honey bee, *Apis mellifera* L. (Hymenoptera: *Apidae*) populations of Turkey inferred from microsatellite analysis. Journal of Apicultural Research, 46 (1): 50-56.

Bookstein, F. L., 1990. “Introduction to Methods for Landmark Data, 216-225”. In: Proceedings of the Michigan Morphometrics Workshop 1988 (Eds. F.J. Rohlf & F. L. Bookstein). University of Michigan Museum of Zoology, Special Publication No. 2, Ann Arbor, MI, USA, 396 pp.

Ertuğrul, M., N. Akman, G. Dellal & T. Goncagül, 2000. “Hayvan gen kaynaklarının korunması ve Türkiye hayvan gen kaynakları, 285-300”. Türkiye Ziraat Mühendisliği V. Teknik Kongresi (17-21 Ocak 2000) Ankara, 1133 s (in Turkish).

FAO, 2018. FAOSTAT Live Animals in 2018. (Web page: http://www.fao.org/faostat/en/#data) (Date accessed: June 2021).

Francoy, T. M., D. Wittmann, M. Drauschke, S. Müller, V. Steinhage, M. A. Bezerra-Laure, D. De Jong & L. S. Gonçalves, 2008. Identification of Africanized honey bees through wing morphometrics: two fast and efficient procedures. Apidologie, 39 (5): 488-494.

Francoy, T. M., D. Wittmann, V. Steinhage, M. Drauschke, S. Müller, D. R. Cunha, A. M. Nascimento, V. L. C Figueiredo, Z. L. P. Simões, D. De Jong, M. C. Arias & L. S. Gonçalves, 2009. Morphometric and genetic changes in a population of *Apis mellifera* after 34 years of Africanization. Genetics and Molecular Research, 8 (2): 709-717.

Henriques, D., J. Chavez-Galarza, S. G. J. Teixeira, H. Ferreira, J. C. Neves, T. M. Francoy & M. A. Pinto, 2020. Wing geometric morphometrics of workers and drones and single nucleotide polymorphisms provide similar genetic structure in the Iberian honey bee (*Apis mellifera iberiensis*). Insects, 11 (2): 89.

Kambur, M. & M. Kekeçoğlu, 2018. Türkiye bal arısı (*Apis mellifera* L.) alttürlerinde genetik çeşitlilik kaybı. Anadolu Tarım Bilimleri Dergisi 33 (1): 73-84 (in Turkish with an abstract in English).

Kandemir, İ., M. Kence & A. Kence, 2000. Genetic and morphometric variation in honey bee (*Apis mellifera* L.) populations of Turkey. Apidologie, 31 (3): 343-356.

Kandemir, I., M. Kence, W. S. Sheppard & A. Kence, 2006. Mitochondrial DNA variation in honey bee (*Apis mellifera* L.) populations from Turkey. Journal of Apicultural Research, 45 (1): 33-38.

Kandemir, I., M. G. Moradi, B. Ozden & A. Ozkan, 2009. Wing geometry as a tool for studying the population structure of dwarf honey bees (*Apis florea* Fabricius 1876) in Iran. Journal of Apicultural Research and Bee World, 48 (4): 238-246.

Kence, A., 2006. Türkiye bal arılarında genetik çeşitlilik ve korunmasının önemi. Uludağ Arıcılık Dergisi, 6 (1): 25-32 (in Turkish with an abstract in English).

Klingenberg, C. P., 2011. MorphoJ: An integrated software package for geometric morphometrics. Molecular Ecology Resources, 11 (2): 353-357.

Koca, A. Ö., 2012. Ortadoğu'da Yayılış Gösteren *Apis mellifera* L. (Hymenoptera: Apidae) Alt Türlerinin Geometrik Morfometri Yöntemleriyle Analizi. Ankara Üniversitesi, Fen Bilimleri Enstitüsü, (Unpublished) Doktora Tezi, Ankara, Türkiye, 167 s (in Turkish with an abstract in English).

Koca, A. Ö. & İ. Kandemir, 2013. Comparison of two morphometric methods for discriminating honey bee (*Apis mellifera* L.) populations in Turkey. Turkish Journal of Zoology, 37 (2): 205-210.

Kükrer, M., 2013. Genetic Diversity of Honey Bee Populations in Turkey Based on Microsatellite Markers: A Comparison Between Migratory Versus Stationary Apiaries and Isolated Regions Versus Regions Open to Migratory Beekeeping. Middle East Technical University, (Unpublished) MSc Thesis, Ankara, Turkey, 70 pp.

Özkan, A. & I. Kandemir, 2010. Discrimination of western honey bee populations in Turkey using geometric morphometric methods. Bee World, 87 (2): 24-26.

Öztürk, A. İ., İ. Alataş, A. Settar, Y. Boduroğlu & B. Uyguner, 1992. Ege Bölgesi Arı Populasyonlarında Bazı Morfolojik Özelliklerin Saptanması. Ege Tarımsal Araştırma Enstitüsü Müdürlüğü, İzmir, Tagem Proje Raporu, 104 s (in Turkish).

Palmer, M. R., D. R. Smith & O. Kaftanoğlu, 2000. Turkish honey bees: genetic variation and evidence for a fourth lineage of *Apis mellifera* mtDNA. The Journal of Heredity, 91 (1): 42-46.

Power, K., E. D'Anza, M. Martano, S. Albarella, F. Ciotola, V. Peretti, & P. Maiolino, 2019. Morphological and morphometric analysis of the Italian honeybee (*Apis mellifera ligustica*) spermatozoa: A preliminary study in Campania region. Nucleus, 4: 1-61.

Rohlf, F. J., 2000. NTSYS-PC, Numerical taxonomy and multivariate analysis system, version 2.2. Department of Ecology and Evolution, State University of New York, Exeter Software, Stony Brook, NY, USA.

Rohlf, F. J., 2008a. tpsUtil, Version 1.40. Department of Ecology and Evolution, State University of New York, Stony Brook, NY, USA.

Rohlf, F. J., 2008b. tpsDIG, Version 2.11. Department of Ecology and Evolution, State University of New York, Stony Brook, NY, USA.

Ruttner, F., 1988. Biogeography and Taxonomy of Honey Bees. Springer, Verlag, Berlin. New York, 284 pp.

Santoso, M. A. D., B. Juliandi & R. Raffiudin, 2018. Honey bees species differentiation using geometric morphometric on wing venations. In IOP Conference Series: Earth and Environmental Science, 197 (1): 012015 (1-7).

Smith, R. D., 2002. Genetic diversity in Turkish honey bees. Uludag Bee Journal, 2 (3): 9-17.

Tofilski, A., 2008. Using geometric morphometrics and standard morphometry to discriminate three honey bee subspecies. Apidologie, 39 (5): 558-563.

Tunca, R. I., 2009. Determination and Comparison of Genetic Variation in Honey Bee (*Apis mellifera* L.) Populations of Turkey by Random Amplified Polymorphic DNA (RAPD) and Microsatellite Analyses. (Unpublished) Ph.D. Thesis, Middle East Technical University, Ankara, Turkey, 141 pp.

Tunca, R. I. & M. Kence, 2011. Genetic diversity of honey bee (*Apis mellifera* L.: Hymenoptera: Apidae) populations in Turkey revealed by RAPD markers. African Journal of Agricultural Research, 6 (29): 6217-6225.

Turan, H., 2011. Trakya Bölgesi balarısında *(Apis mellifera* L*.)* Geometrik Morfometrik Çalışmalar. Namık Kemal Üniversitesi, (Unpublished) Yüksek Lisans Tezi, Tekirdağ, Türkiye, 77 s (in Turkish with an abstract in English).

Zammit-Mangion, M., M. Meixner, D. Mifsud, S. Sammut & L. Camilleri, 2017. Thorough morphological and genetic evidence confirm the existence of the endemic honey bee of the Maltese Islands *Apis mellifera ruttneri*: recommendations for conservation. Journal of Apicultural Research, 56 (5): 514-522.

Zhou, S., X. Zhu, X. Xu, J. Gao & B. Zhou, 2018. Multivariate morphometric analysis of local and introduced populations of *Apis cerana* (Hymenoptera: Apidae) on Hainan Island, China. Journal of Apicultural Research, 57 (3): 374-381.