Original article (Orijinal araştırma)

Current genetic status of honey bees in Anatolia in terms of thirty polymorphic microsatellite markers[1]

Anadolu'da bulunan bal arılarının otuz polimorfik mikrosatellit belirteçleri açısından güncel genetik durumları

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Abstract

Turkey, having three phytogeographical floristic regions, is a natural bridge among three continents. A lot of subspecies and ecotypes of honey bees have been reported within Turkey. However, hybridization due to informal cultivation and uncontrolled migratory beekeeping practices are thought to affect the genetic diversity of local honey bee populations, and this may result the loss of allele combinations resulting from long evolutionary processes. Numerous identification and conservation studies on honey bee subspecies have been conducted in many countries to determine the loss of genetic variability. On this basis, genetic causes and phylogenetic relationships of four common honey bee subspecies [*Apis mellifera anatoliaca* Maa, 1953, *Apis mellifera* carnica Pollmann, 1879, *Apis mellifera caucasica* Pollmann, 1889, *Apis mellifera syriaca* Skorikov, 1829 (Hymenoptera: Apidae)] from five provinces (Artvin, Düzce, Hatay, Kırklareli and Muğla) selected based on their importance in apicultural activities were studied using 30 microsatellite loci in 2018. The genetic distances of populations ranged from 0.30 to 0.70. Genetic variation was 8.96% among the populations, 44.9% among the individuals within the populations and 46.1% for all individuals. Further genetic researches on the honey bee populations will be of advantage for anticipating potential future problems.

Keywords: *Apis mellifera*, bottleneck, genetic variation, microsatellite

Öz

Üç fitocoğrafik floristik bölgeye sahip olan Türkiye, üç kıta arasında doğal bir köprüdür. Bugüne kadar Türkiye sınırları içerisinde birçok bal arısı alt türü ve ekotipi bildirilmiştir. Ancak, kayıt dışı yetiştiricilik ve kontrolsüz göçer arıcılık uygulamalarına bağlı melezleşmenin yerel bal arısı popülasyonlarının genetik çeşitliliğini etkilediği düşünülmektedir ve bu, uzun evrimsel süreçlerden kaynaklanan allel kombinasyonlarının kaybıyla sonuçlanabilir. Bal arısı alttürleri üzerinde genetik değişkenliğin kaybını önlemek amacıyla birçok ülkede tanımlama ve koruma çalışmaları yapılmıştır. Bu temelde, 2018'de arıcılık faaliyetlerindeki öneminden dolayı seçilen beş ilden (Artvin, Düzce, Hatay, Kırklareli ve Muğla) dört yaygın bal arısı ırkının [*Apis mellifera anatoliaca* Maa, 1953, *Apis mellifera* carnica Pollmann,1879, *Apis mellifera caucasica* Pollmann, 1889, *Apis mellifera syriaca* Skorikov, 1829 (Hymenoptera: Apidae)] genetik açıdan durumları ve filogenetik ilişkileri otuz mikrosatellit lokusu kullanılarak güncellenmeye çalışılmıştır. Popülasyonlar arası genetik mesafe 0.30 ile 0.70 arasında değişmiştir. Genetik varyasyonlar, popülasyonlar arasında %8.96, popülasyonlardaki bireyler arasında %44.9 ve tüm bireyler arasında %46.1 olarak hesaplanmıştır. Bal arısı ile ilgili daha fazla genetik araştırma, gelecekteki potansiyel sorunlardan kaçınmak için avantajlı olacaktır.

Anahtar sözcükler: *Apis mellifera*, darboğaz, genetik varyasyon, mikrosatelit

Introduction

Honey bee (*Apis mellifera* L., 1758) belonging to (Apidae) Hymenoptera are (essential pollinators in both nature and agriculture (Ryabov et al., 2014; Tantillo et al., 2015; Amakpe et al., 2018; Ellis et al., 2018; McMenamin et al., 2018) for producing of food (Poposka et al., 2018; Sforcin et al., 2017). Honey bees are also a model organism for neurobiology, development, social behavior and epigenomics (Güder et al., 2017). Honey bees adapt to many environmental conditions all over the world (Agra et al., 2018; Nawrocka et al., 2018) and they have 29 subspecies (De la Rua et al., 2003; Bouga et al., 2011; Chahbar et al., 2013; Oleksa & Tofilski, 2015; Ilyasov et al., 2016). According to Ruttner (1988), the classification and distinction of the *A. mellifera* subspecies using morphometric analyses suggested that the honey bee originally evolved in Africa and Europe, but also speciated in the Middle East (Ruttner, 1988). Also, recent analysis of single nucleotide polymorphisms strongly supported the hypothesis that honey bees originated in Africa (Whitfield et al., 2006). The history of spread and isolation of subpopulations resulted in notable variation in morphological traits (Nawrocka et al., 2018) and early morphometric analyses classified these into M, A, C and O lineages, which owe their origin to the glacial history of Europe (Ellis et al., 2018).

Turkey is at the junction of Africa, Asia and Europe where different honey bee subspecies scattered and adapted to those different climatic and floristic conditions (Kekeçoğlu & Soysal, 2010) that cover three phytogeographic regions: Euro-Syberian, Mediterranean and Iran-Turanian (Bouga et al., 2011). Subspecies [*Apis mellifera anatoliaca* Maa, 1953, *Apis mellifera carnica* Pollmann,1879, *Apis mellifera caucasica* Pollmann, 1889, *Apis mellifera meda* Skorikov, 1829, *Apis mellifera syriaca* Skorikov, 1829 (Hymenoptera: Apidae)] and ecotypes of the subspecies within Turkeys have been reported (Ruttner, 1988, Smith et al., 1997; Kandemir et al., 2000, 2006; Palmer et al., 2000; Bodur et al., 2007; Özdil et al., 2009; Fontana et al., 2018). There are important distinct ecotypes of *A. mellifera anatoliaca* such as Muğla and Düzce (Yığılca) bees in Turkey. Muğla honey bee is the well-known ecotype of *A. mellifera anatoliaca* spreading around the Aegean Sea coast in the west of Turkey (Ivgin Tunca & Kence, 2011; Akyol et al., 2014). Also, Düzce Province seems to have maintained characteristics of *A. mellifera anatoliaca.* Caucasian bee is in the northeastern region of Turkey (Kambur & Kekeçoğlu, 2018), especially in Artvin (Kence et al., 2009), there are also in neighboring countries, Georgia and Russia (Nikolova et al., 2015; Ghassemi-Khademi et al., 2018). According to Ruttner (1988), honey bee from Croatia is *A. mellifera carnica*, nonetheless there are *A. mellifera carnica* in many European and Balkan countries (Bouga et al., 2011). Many colonies from Kırklareli-Thrace have been reported to show similar morphometric and allozyme properties with *A. mellifera carnica (*Kandemir et al., 2000, 2005). The investigation of the genetic origin of Thrace honey bees in Turkey is ongoing (Ünal & Özdil, 2018). *Apis mellifera syriaca* from northern Syria is spatially in distinct from *A. mellifera anatoliaca* in Hatay-Turkey, and question remains concerning the level of introgression between them (Alburaki et al., 2013). In this respect, Anatolia has nearly 20% of the global honey bee genetic diversity. Hybridization and introgression because of commercial beekeeping manipulations affects the genetic variability of local honey bee populations, hence it can lead to the loss of combinations of alleles that have resulted from long periods of adaptive evolution (Bouga et al., 2011; Ellis et al., 2018). To counteract this process, numerous conservation efforts for the protection of native honey bees are being established across Europe. Honey bee subspecies have been routinely identified (Parejo et al., 2018). At first, identification was based on morphometrics and this method had been practiced for a long time. Molecular techniques have begun to be used with the developments in molecular technology as well as morphometric methods in the identification of honey bee subspecies (Bouga et al., 2011; Meixner et al., 2013). Although, numerous molecular markers such as RFLP, mitochondrial DNA analysis and allozyme analysis have been used in the studies of honey bee population genetics (Kandemir & Kence, 1995; Smith et al., 1997; Kekeçoğlu et al., 2009; Özdil et al., 2009), nowadays, the SSR (simple sequence repeat) loci have been widely used (Bodur et al., 2007; Liu et al., 2016; Rahimi et al., 2016; Haddad et al., 2018; Hassett et al., 2018; Yu et al., 2019).

Considering the increasing importance of the conservation of local honey bee genetics, this work aimed to determine the current genetic status and phylogenetic relationships among common honey bee subspecies in Turkey using microsatellites.

Materials and Methods

Sample collection

The colonies were obtained from different beekeepers in 2018 from five provinces selected based on their significance in apicultural activities in Turkey. A representative sampling of each province was randomly performed, resulting in 5 to 15 apiaries sampled per province. So, 30 colonies were determined for each of the five locations where the most common of the four-known species of honey bees in Turkey (Table 1). All samples were stored in collection tubes with pure ethanol at +4ºC until used for DNA extraction.

Table 1. Coordinates and altitudes of the locations where samples collected

Locations	Coordinate	Altitude (m)
Muğla (*A. mellifera anatoliaca*)	37º12′N 28º21′E	656
Hatay *(A. mellifera syriaca)*	36º12′N 36º9′E	85
Kırklareli (*A. mellifera carnica*)	41º44′N 27º13′E	203
Artvin (*A. mellifera caucasica*)	41º10′N 41º49′E	240
Düzce (*A. mellifera anatoliaca*)	40º50′N 31º9′E	146

DNA extraction and microsatellite amplification

Bees were taken from storage in alcohol using sterile tweezers were kept in bi-distilled water for 5 min to remove the alcohol, then dried on the blotting paper for 3 h. The body segments of the bees were carefully separated and the thorax of the bees taken from the same hives was collected in the same 5 ml tubes. Cold nitrogen was poured over the thorax samples and very quickly crushed with sterile glass rods. A little manipulated CTAB method described by Doyle (1990) was used for total DNA extractions from the thorax of worker bees. Quantities and qualities of DNAs were determined using BioDrop spectrophotometer. Also, DNA molecules were checked whether they were in one piece (broken or not broken) in a 2% agarose gel. DNAs were stored at −20ºC until needed.

Thirty microsatellite loci (described previously by Solignac et al., 2003) were used in the study PCR assays were conducted with 2 µl of each template DNA in a total reaction volume of 40 µl.The PCR reaction mix contained 0.25 mM dNTP mix, 2.0 mM $MgCl_2$, 1.5 units of Taq DNA polymerase and 0.075 mM each primers. The thermal cycling for PCR were 94ºC for an initial denaturation for 5 min; 35 cycles of 94ºC for 30 s, 30 s at the primer specific annealing temperature, and 72ºC for 45 s; and a final 72ºC for 5 min. The PCR products were detected on 2% agarose gel and were evaluated using fragment analysis in AATI fragment analyzer to determine the repeat sequence lengths of the microsatellite loci precisely.

Statistical analyses

The lengths of fragments obtained were scored with PROSize2.0. N, number of loci; N_P, number of polymorphic loci; N_A, number of observed alleles; N_E, number of effective alleles; H_E, expected heterozygosis; H_O, observed heterozygosis; F_{Is}, coefficient of inbreeding; HW, Hardy Weinberg equilibrium; and F-statistics were calculated by Popgene v.1.32 (Yeh et al., 1997). PIC values that give the information about the usefulness of a marker were determined by using a microsatellite toolkit (Park, 2001). Also, diagnostic alleles which are described by Garnery et al. (1998) as the allele occurred in relatively high proportions in a population and are either absent or in very low frequencies in all others were determined

for the bee colonies studied. Null alleles, also known as non-amplifying alleles, which are commonly encountered in population genetic studies, were estimated using ML-NullFreq (Kalinowski & Taper, 2006). Theta (H), G-W index (M value) (Garza & Williamson, 2001), allelic size range (ASR), and total heterozygosis, analysis of molecular variance (AMOVA) which are to determine the percentages of variation sources (Excoffier et al., 1992) were performed by using Arlequin v.3.11 (Excoffier et al., 2007). In addition, Bottleneck 1.2.02 (Piry et al., 1999) was used by comparing the decline in allele number versus heterozygosity to estimate the signatures of mutation-drift equilibrium. Because of more than 20 polymorphic loci and the high number of individuals, two-phase mutation model (TPM) was chosen with sing rank test (to calculate how many loci with heterozygosity deficiency or heterozygosity excess), standardized differences test (for the genetic signature of bottlenecks in the honey bee populations studied), and also Wilcoxon test (to determine whether heterozygosity deficiency or excess). The allele frequency distribution was established to see whether it is approximately L-shaped (as expected under mutation-drift equilibrium) or not (recent bottlenecks provoke a mode shift). Microsatellite alleles were classified into 10 frequency classes, which allowed us to check whether distribution followed normal L-shaped form where alleles with low frequencies (0.01 to 0.1) are the most abundant (Luikart et al.,1998).

Identification of the genetic structure of the populations was obtained by the clustering method of the software Structure v. 2.3.4. (Pritchard et al., 2000). This method assumes that there are K populations, each of which is characterized by allele frequencies at each locus. In the analysis, a burn-in of 100,000 iterations and an MCMC (Markov Chain Monte Carlo algorithm) of 500,000 iterations was applied for $2 \leq K \leq 8$ to estimate the most probable number of clusters. The most optimum number of clusters (ΔK) was determined in the Structure Harvester (Earl & vonHoldt, 2012) by calculating the distribution of the ΔK statistic as described by Evanno et al. (2005). Genetix v.4.05 (Belkhir et al., 1996-2004) was used for factorial correspondence analysis (FCA). FCA is based on the principle of examining genotypic data in a three-dimensional plane to reveal the relationships among individuals in the populations studied. In this analysis, the logic of linear transformation of the genotypes of each individual is used to draw a diagram in multiple dimensions. Thanks to the drawn diagram, each individual was compared to another individual and it was ensured that individuals were grouped together by forming a common class.

Results and Discussion

The descriptive statistics of genetic polymorphism showed that the 30 microsatellite loci used in this study were suitable for revealing heterozygosis. It is evident from the data presented in Table 2 that the means of the total heterozygosity, number of alleles and allele size range detected by the loci are quite high. The highest and lowest total heterozygosity was found at loci ap001 (0.92) and ap068 (0.62), respectively. Although, the locus with the highest number of alleles (22) was ap243, the highest value (41) for allele size range was found at loci ap001 (Table 2). When all loci in the populations are evaluated together, based on high theta (H) values, the changes in the repeat number of the microsatellite loci are high.

336

Table 2. Some descriptive statistics of genetic variations of 30 microsatellite loci in the honey bee populations studied

Locus	H_T	N_A	ASR	Theta (H)
ac006	0.80	12	13	2.41±1.03
ap238	0.86	18	21	3.09±1.34
a007	0.85	12	19	3.40±0.30
ap001	0.92	21	41	4.83±3.09
ac306	0.88	18	28	4.15±1.51
ap243	0.89	22	27	4.19±1.30
ab024	0.71	6	5	1.86±0.16
ap289	0.84	13	16	2.70±0.84
ap273	0.67	4	5	1.58±0.07
a088	0.73	5	8	1.98±0.14
ap218	0.73	10	9	1.81±0.61
ap068	0.62	5	5	1.90±0.26
ap226	0.85	11	18	2.63±0.93
ap049	0.74	10	12	1.74±0.44
ap223	0.82	17	21	2.95±0.85
ap249	0.81	8	10	2.31±0.43
ap288	0.71	12	16	1.73±0.28
hbc1605	0.90	21	33	3.39±1.08
ab124	0.78	17	32	2.51±1.11
hbc1601	0.73	8	12	2.03±0.34
a028	0.78	12	16	2.12±0.67
hbc1602	0.90	19	22	4.65±1.41
ap043	0.49	9	14	1.63±0.10
a113	0.81	13	29	2.52±0.42
a107	0.86	8	7	3.56±0.35
a014	0.86	8	7	3.77±0.31
a079	0.66	4	3	1.74±0.06
ap019	0.88	10	9	4.24±0.24
a076	0.88	10	9	4.22±0.55
a043	0.88	9	8	4,27±0,31
Mean	0.79±0.10	11.73±5.14	15.83±9.58	2.12±0.36

H_T, Total heterozygosis; ASR, allelic size range; N_A, number of observed alleles; and Theta (H), molecular diversity indices.

All microsatellite loci were found polymorphic in this study, and also the values of genetic polymorphism indicators, N_P, N_A, N_E, ASR, PIC, H_E, G-W, theta (H) and F_{Is}, were generally high in Anatolian honey bee subspecies (Table 3). The highest values of the N_A, N_E, ASR, PIC, H_E and theta (H) were found in Kırklareli but the lowest in Düzce. The observed heterozygosity in the Artvin population was higher than in the other populations. The highest intragroup variations (F_{Is}) was found in the Muğla honey bee population and the lowest in the Artvin honey bee population. Purely one diagnostic allele that 117 bp allelic size for Ap288 was observed in only the Kırklareli population. Also, numerous null alleles were calculated in all populations.

Table 3. Main diversity parameters for all honey bee populations according to studied SSR loci

	Muğla	Hatay	Kırklareli	Artvin	Düzce	Mean
N	30	30	30	30	30	30
N_P	30	30	30	30	30	30
N_A	8.13±3.80	7.97±4.07	9.57±3.87	7.77±3.71	7.23±2.57	8.13±3.60
N_E	6.78	6.05	7.93	6.10	4.43	8.56
ASR	10.50±7.52	11.50±8.87	13.87±8.70	10.67±7.10	11.10±6.80	11.52±7.79
PIC	0.79	0.74	0.83	0.75	0.68	0.75
H_E	0.76±0.14	0.70±0.18	0.80±0.12	0.73±0.17	0.66±0.18	0.73±0.16
H_O	0.33±0.28	0.33±0.27	0.40±0.20	0.44±0.26	0.37±0.22	0.37±0.25
GW	0.82±0.21	0.75±0.22	0.74±0.22	0.75±0.20	0.71±0.25	0.75±0.22
Theta (H)	2.28	1.90	2.66	2.03	1.72	2.12±0.36
F_{is}	0.59	0.58	0.52	0.40	0.47	0.52
HW (p<0.01)	0.00	0.00	0.00	0.00	0.00	0.00

N, loci number; N_P, number of polymorphic loci N_A, number of observed alleles; N_E, effective allele number; ASR, allelic size range; PIC, polymorphic information content; H_E; expected heterozygosis; H_O; observed heterozygosis; G-W, Garza-Williamson index; Theta (H), molecular diversity indices; F_{is}, coefficient of inbreeding; and HW, Hardy Weinberg equilibrium.

Although none of the populations studied was genetically bottleneck according to the GW index (Table 3) and the normal L-shaped distribution (Figure 1), which is a typical property of a population in equilibrium, none of the populations studied was found in HW balance (Table 3). However, differential results were estimated using the Bottleneck program. Namely, the sign test showed that there were statistical differences between expected heterozygosity excess and observed heterozygosity excess in the Muğla (0.04 to <0.05) and Düzce (0.01 to <0.05) populations. Statistically important heterozygosity deficiency in the Düzce population and heterozygosity excess in the Muğla and Artvin populations were estimated using by Wilcoxon's signed-rank test. Moreover, the Muğla, Hatay and Düzce populations were to be genetic bottleneck according to standardized differences test (Table 4).

Table 4. Bottleneck analysis using standardized differences test at two-phase mutation model

Statistical tests		TPM				
		Muğla	Hatay	Kırklareli	Artvin	Düzce
Sign test	EHE	18.03	18.06	17.87	17.85	17.79
	HD	7.00	14.00	10.00	10.00	19.00
	HE	23.00	16.00	20.00	20.00	11.00
	P	0.04	0.28	0.28	0.27	0.01
Standardized differences test	T2	2.83	-1.89	1.44	1.05	-6.62
	P	0.00	0.03	0.08	0.15	0.00
Wilcoxon's signed rank test	HD (P)	0.99	0.56	0.94	0.95	0.02
	HE (P)	0.00	0.44	0.07	0.05	0.99
	HDE (P)	0.00	0.89	0.13	0.10	0.03

TPM, two-phase mutation model; EHE, expected number of loci with heterozygosity excess; HD, one tail heterozygosity deficiency; HE, one tail heterozygosity excess; HDE, two tails for heterozygosity excess or deficiency; and T2: standardized differences test. Positive values of the T2 are indicative of gene diversity excess caused by a recent reduction in effective population size, while negative values are consistent with a recent population expansion without immigration or immigration of some private (unique) alleles in the population.
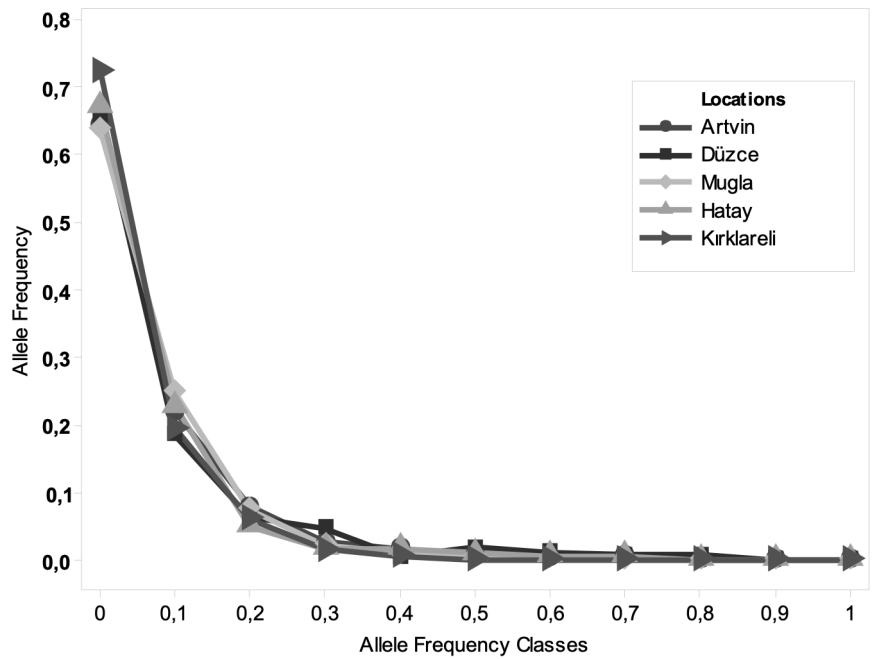
Figure 1. L-shaped mode-shift graph showing lack of recent genetic bottleneck in honey bee subspecies.

Genetic differences

The pairwise F_{ST} values developed by Weir & Cockerman (1984) were calculated to determine the genetic differences among populations (Table 5). Also, Nei's original measures of genetic identity and genetic distance were estimated (Table 6). The population pairwise F_{ST} values for the honey bee populations studied ranged from 0.04 to 0.16. The lowest and the highest pairwise F_{ST} value were determined between Kırklareli and Artvin (0.04) and, Hatay and Düzce (0.16), respectively. The genetic distances among populations (Nei, 1972) were ranged from 0.70 (Hatay and Muğla) to 0.30 (Kırklareli and Artvin).

Table 5. Pairwise F_{ST} values for honey bee populations studied

	Muğla	Hatay	Kırklareli	Artvin	Düzce
Muğla	*				
Hatay	0.12	*			
Kırklareli	0.06	0.07	*		
Artvin	0.10	0.09	0.04	*	
Düzce	0.13	0.16	0.10	0.10	*

Table 6. Nei's original measures of genetic identity (above diagonal) and genetic distance data (below diagonal) (Nei, 1972)

	Muğla	Hatay	Kırklareli	Artvin	Düzce
Muğla	*	0.49	0.66	0.52	0.57
Hatay	0.69	*	0.69	0.64	0.50
Kırklareli	0.41	0.35	*	0.74	0.64
Artvin	0.63	0.44	0.29	*	0.58
Düzce	0.55	0.68	0.44	0.52	*

AMOVA revealed the distribution of genetic among populations and within populations. Sources of total genetic variation were 8.96% among the populations, 44.9% among the individuals within the populations and 46.1% for all individuals. Also, the statistical significance of the differences between the populations was tested by permutation test. It was determined that among individuals (F_{It} 0.54), among the populations (F_{St} 0.09) and within populations (F_{Is} 0.49) genetic differences were significant ($p < 0.05$). Only a small part of the total genetic diversity was caused by the differences among the populations (9.00%) but this was statistically significant. These results coincided with the pairwise F_{ST} values (Table 5).

Clustering Analysis

The genetic structures of the honey bee populations were determined based on Bayesian clustering analysis by using Structure v. 2.3.4. According to the result of this analysis, ΔK value was calculated by the Structure Harvester and found to be four (ΔK = 4). These results indicated the phylogenetic relationships were best expressed in four clusters according to the 30 microsatellite markers (Figure 2) in the five populations studied. The honey bee populations in Muğla, Hatay and Düzce were clearly separated from each other. Interestingly, two populations (Kırklareli and Artvin) which were the most far to each other geographically were clustered in the almost same color. The honey bee population in Kırklareli Province was more heterogeneous than the other populations, and share almost all colors reflecting the common genetic similarities with the other bee populations.
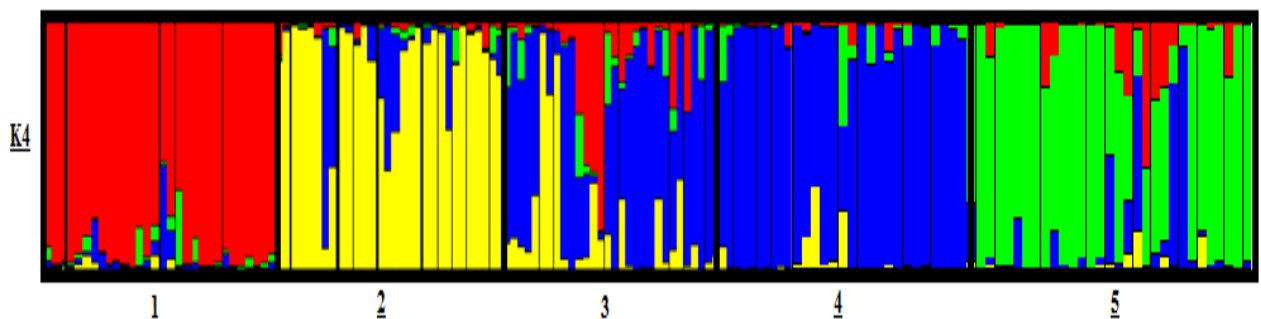

Figure 2. Genetic structure analysis of the examined five honey bee populations. K is the number of groups (populations: 1, Muğla; 2, Hatay; 3, Kırklareli; 4, Artvin; and 5, Düzce). Each color corresponds to one cluster, and the length of the colored segment represents the individual's membership coefficient in the cluster according to cluster analysis.

Factorial correspondence analysis revealed the phylogenetic relationships among populations on a three-dimensional plane (Figure 3). Figures 2 and 3 illustrated similar clustering. The results of FCA showed that honey bee populations were in four main groups: the first was Muğla (western Anatolia), the second was Hatay (southeast Anatolia), the third was Kırklareli (north western Anatolia, Thrace Region) and Artvin (northeast Anatolia), and the fourth was Düzce (central Anatolia). The Hatay population was clearly separated from the other natural populations and have the highest proportional differences (32.6%) on the x-axis. The populations of Muğla and Düzce, which are the ecotypes of the Anatolian bee, were found closer to each other.

Nearly two decades ago, microsatellite studies on honey bee populations generally focused on European and African honey bee subspecies (Franck et al., 1998, 2001), and then studies published for island populations and Mediterranean honey bee populations (Franck et al., 2001; Bodur et al., 2007; Dall'Olio et al., 2007). Previous studies using different methods showed the presence of five honey bee subspecies and different ecotypes in Turkey (Ruttner, 1988; Smith et al., 1997; Kandemir et al., 2000, 2006; Palmer et al., 2000; Bodur et al., 2007; Özdil et al., 2009; Güder et al., 2017). More recently, microsatellites have been used for determining the genetic structure of honey bee populations in many different regions of the world (Liu et al., 2016; Hassett et al., 2018).
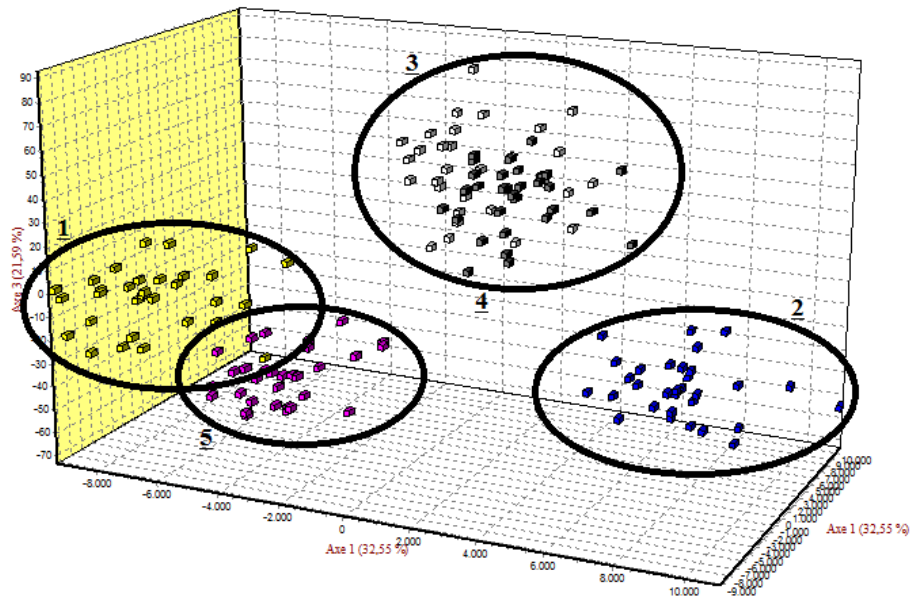
Figure 3. Factorial Correspondence Analysis of the honey bee populations studied (populations: 1, Muğla; 2, Hatay; 3, Kırklareli; 4, Artvin; and 5, Düzce).

Different numbers of microsatellite loci have been used in many studies in both Turkey and other countries (Bodur et al., 2007; Cánovas et al., 2011; Alburaki et al., 2013; Ilyasov et al., 2016; Ghassemi-Khademi et al., 2018; Hassett et al., 2018). In the current study, we used 30 microsatellite loci in order to determinate of the current status of the honey bee populations and all loci studied were suitable.

The allele number is an indicator for the adequacy of sample size to measure of genetic variation (Mielnik-Sikorska et al., 2013). The number of observed alleles present at each locus and in each population were more variable in this study. Average number of alleles the honey bee populations ranged from 7.23 (Düzce) to 9.57 (Kırklareli). The mean number of alleles considering all loci for all of the honey bee populations studied was estimated as 8.13, which is higher than the estimated values from previous microsatellite studies (Ivgin Tunca, 2009; Bodur et al., 2007).

In the current study, the gene diversity value ranged from 0.66 to 0.80. These values indicate the gene diversity among the honey bee populations of Turkey and the results are very close to Middle East, North and West Mediterranean honey bee populations. Lebanon honey bees including Middle Eastern honey bee populations were studied and the gene diversity for those populations was estimated to be 0.65 (Franck et al., 2000a). Also, Bodur et al. (2007) and Ivgin Tunca (2009) found gene diversity values between 0.54, 0.68 and 0.59, similar to Middle East and North Mediterranean honey bee populations. Also, Mediterranean honey bee gene diversity was reported ranging from 0.39 to 0.68 (Franck et al., 2000b). Dall'Olio et al. (2007) studied the genetic variability of *Apis mellifera ligustica* Spinola, 1806 (Hymenoptera: Apidae) at eight polymorphic microsatellite loci and reported the gene diversity for North Mediterranean honey bees ranged from 0.53 to 0.64.

The pairwise F_{ST} values for the populations studied ranged from 0.04 to 0.16. Bodur et al. (2007) estimated pairwise F_{ST} values ranged from 0.00 to 0.18 for Turkish honey bee populations using nine different microsatellite loci. Also, Ivgin Tunca (2009) was found F_{ST} ranged from -0.07 to 0.35 for 18 populations. F_{ST} values were determined for lineages by many studies (Franck et al., 2000a, 2001; Garnery et al., 1998; Dall'Olio et al., 2007). This study suggested that the moderate level of genetic differentiation was observed in Turkish honey bee populations considering the wide range of pairwise F_{ST} values.

The population from Kırklareli had the highest level of polymorphism with N_A, N_E, PIC, H_E and H_O, whereas the values were the lowest in Düzce (Table 3). Intragroup variation (0.59) in Muğla was considered to be the highest because of having large number of honey bees then that of the other locations. (Table 3). According to gene diversity (H_E) and effective allele numbers for the Kırklareli honey bee population, the higher values were observed among populations that showed the allelic richness. According to previous SNP results, Thrace honey bee (Kırklareli and Edirne) and Anatolian honey bee had different genetic composition. They were clustered separately compared to samples from other Anatolia Region and also, they were closer to European honey bees (Ivgin Tunca et al., 2012; Kence et al., 2012).

According to the results of our research, there are alterations in the genetic structures of the populations in the provinces where *A. mellifera caucasica* queen bees have been intensively sold. *Apis mellifera caucasica* queen bees, produced in the province of Artvin, are especially preferred in areas with cooler climates such as Kırklareli. The fact that the results for Kırklareli province and Artvin Province were clustered together, indicate the negative effects of this commercial queen bee trade. This situation can be seen as a good example of the genetic effect of commercial Caucasian queen bees produced in Artvin and sold to regions especially in the cold climate zone.

The deviations from HWE were determined for all population levels, all of them showed significant deviations ($p < 0.01$) in favor of homozygotes for the Artvin population (F_{Is}). Heterozygote deficiency is thought to result from the negative effects of the breeding model in a protected and closed area (Ministry of Agriculture and Rural Affairs of Turkey, Official Gazette 2004/25668).

Numerous colonies brought to Muğla Province by migratory beekeepers during pine honey production periods increases the bee populations of this province. Therefore, increased intragroup variation can occur, which leads to an increase in the genetic diversity. A migratory apiculture model poses a risk for genetic resources. However, as this activity usually does not occur in the swarming seasons, its effects are less than that of commercial queen breeding. Although the genetic effects of migratory beekeeping activity are not yet felt, the risk associated with the activity should be considered. According to our results, the reason for the clustering of the Muğla and Düzce populations close to each other was that they both consist of Anatolian bees. Since Anatolian bees are used extensively in these two provinces, the negative effects of commercial queen breeding are not yet felt. The consequences of the Hatay Province indicate that the bee populations there are quite different from the other provinces in our study. Syrian bees that dominate this region are very different from our other working groups in terms of genetic origin.

Genetic diversity needs to be taken into consideration during the planning of conservation programs if these are to be successful. Decreasing genetic diversity, which is one of the main causes of honey bee colony losses worldwide, clearly demonstrates the importance of conserving gene resources. With unique allele frequencies of honey bees in Anatolia, the region has the potential to be a source of honey bee genetic diversity for the world. As a transition zone between Africa, Asia and Europe, Turkey is a country that needs to be emphasized in terms of conservation of genetic resources.

There have been many studies on the genetic structure of honey bee populations. The results of the current study parallel the scientific literature and our results support the increased genetic variation in honey bee populations in recent years in Turkey. Ecological factors and geographic features that can vary widely in Anatolia, constitute the infrastructure of observed genetic diversity in honey bee populations in Turkey. The enormous diversity of floral sources resulting from these characteristics is highly effective in maintaining genetic diversity. However, adversities such as the migratory beekeeping model, improper breeding programs and uncontrolled commercial queen bee distribution pose a risk to the genetic diversity and sustainability of local honey bee ecotypes.

Our results on the status of honey bee populations using microsatellite markers in major beekeeping regions of Turkey should be taken into consideration during conservation, honey bee breeding and queen breeding programs. Realization of national beekeeping activities according to the results of scientific researches will be highly effective in minimizing the problems experienced in beekeeping evident in recent years. Further scientific investigation on the genetic structure of the honey bee populations will be of great advantage for avoiding potential future problems. In addition, commercial queen breeding and other beekeeping activities in the area should be conducted in accordance with sustainable ecological models. Increasing and supporting the scientific studies on these issues will accelerate the success to be achieved. The measures mentioned above should be implemented as soon as possible for maintaining the sustainability of honey bee genetic resources in Anatolia.

Acknowledgement

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