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Full Length Research Paper

Genetic diversity of honey bee (*Apis mellifera* L.: Hymenoptera: Apidae) populations in Turkey revealed by RAPD markers

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The honeybee, *Apis mellifera* L. is an ecologically and economically important insect species. Recent honey bee losses causing decline of bee diversity is found alarming for the pollination of both wild plant biodiversity and crop production. Therefore, determination of genetic diversity of honey bee populations is essential and will provide a valuable resource for conservation purposes. Twenty Random Amplified Polymorphic DNA (RAPD) primers were used to assess the genetic diversity in 720 worker bees collected from 360 colonies of 25 provinces in Turkey. Ten out of twenty primers produced 105 reproducible, bright bands, all were polymorphic. Mean genetic diversity values ranged between 0.035 and 0.175, coefficient of gene differentiation (G_{ST}) values were estimated as 0.060 to 0.441, and the private band patterns reflected a high level of genetic variation. Analysis of Molecular Variance (AMOVA) partitioned the total genetic variation as 60% within, 40% among populations. The Mantel test did not reveal significant correlation between the genetic and geographic distances. First three Eigen values of principle coordinate analysis explained 63% of total variation, 27, 21, and 15% for the first, second and third respectively. The cluster analysis showed that the honey bees of Thrace region of Turkey and an island at a short distance were clustered together. The other two populations from southeastern Anatolia which belong to African lineage according to mitochondrial DNA analysis formed a separate cluster and rest of the populations which belong to north Mediterranean branch (C lineage) formed the third cluster. The results showed that genetic variability of honey bee populations from Turkey are determined using RAPD markers and provide information for future management and conservation plans.

Keywords: Honeybee, *Apis mellifera*, RAPD markers, genetic diversity, Turkey.

INTRODUCTION

The honey bee (*Apis mellifera L.*: Hymenoptera: Apidae) is speciated in Africa (Whitfield, 2006), naturally distributed to Europe and Asia, and introduced into America and Australia by humans. Based on morphometric, behavioral and biogeographical studies, 26 subspecies have been identified (Ruttner, 1988; Sheppard et al., 1997; Sheppard and Meixner, 2003; Engel, 2004; Arias and Sheppard, 2005). Five subspecies, *A. m. anatoliaca*, *A. m. caucasica, A. m. meda, A. m. syriaca,* and *A. m. carnica* (Ruttner, 1988; Kandemir et al., 2000) are found in Turkey, where apicultural activity has been carried out since 1300 B.C. as Hittite civilization was located in Bogazkoy, Central Anatolia (Akkaya and Alkan, 2007). The discrimination of the five honey bee subspecies in Turkey has been done by using morphometric, allozymic (Kandemir et al., 2000) and microsatellite (Bodur et al., 2007) analyses. In those studies, Southeastern Anatolian and Thracian populations were found to be divergent units. Mitochondrial DNA (mtDNA) analyses indicate that nearly all Turkish honey bees belong to C (Northern Mediterranean)

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identified by restriction site and sequence polymorphism of mtDNA (Ozdil et al., 2009; Solorzano et a., 2009; Kandemir et al., 2006). Furthermore, African mtDNA haplotypes were detected in Hatay in Southeastern Anatolia region and they clustered together with *A. m. meda* (Kandemir et al., 2006).

Up to date, many studies on honey bees have been conducted by using morphometry (Ruttner et al., 2000; Nazzi, 1992), allozyme variations (Kandemir et al*.,* 2000; 2005; Arias et al., 2006), Restriction Fragment Length Polymorphism (RFLP) (Suazo and Hall, 2002a; Szalanski and McKern, 2007), Amplified Fragment Length Polymorphism (AFLP) (Suazo and Hall, 1999; Smith et al., 2003), mtDNA (Palmer et al., 2000; Meixner et al., 2000), Random Amplified Polymorphic DNA (RAPD) (Suazo et al., 1998; Hunt and Page, 1992), microsatellite (Solignac et al., 2003; Bodur et al., 2007) and Single Nucleotide Polymorphism (SNP) (Whitfield et al., 2006) in order to determine variation and infer phylogenetic relationships of honey bee populations.

In various other studies it was found that African honey bee populations have very high genetic variation, compared to European populations (Garnery et al., 1998.), in bee populations of Turkey it is in between (Bodur et al., 2007). It has been also shown that genetic diversity in honey bee colonies increase colony fitness (Mattila and Seeley, 2007; Mattila et al., 2008).

Recent honey bee losses causing decline of bee diversity is found alarming and threatening the pollination of both natural biodiversity and crop production. Therefore, determination of genetic variation of honey bee populations with different markers is essential and will provide a valuable resource for selection for desired traits that may be tolerant to disease causing agents and ectoparasites and conservation purposes. Genetic variation in animal populations in general found at different levels when different markers are being used depending on the nature of the marker. Specifically in honey bee populations it is observed that allozyme markers indicate low level of variation due to haplo-diploidy. RAPD markers differ from others as being dominant and represent the whole nuclear genome. Genetic differentiation could be effectively determined by RAPD markers in different populations it is well justifiable to test it in other populations like other markers that were investigated in many different populations.

RAPDs are genetic markers that require no knowledge of the sequence. Random primers are used in RAPD and very useful for determination of genetic variation and genetic basis of behavioral characters (Williams et al., 1990; O'Donnell, 1996; Waldschmidt et al., 2002), linkage mapping (Fondrk et al., 1993; Hunt and Page, 1994), paternity analyses (Page et al., 1995), and the identification of Quantitative Trait Loci (Page et al., 1995; Hunt et al., 1998). However, the technique has some limitations especially for weakly amplified bands. Nevertheless, Suazo et al. (1998) had screened 700 primers in order to determine the differences between African and European honeybee populations and found five different band patterns each specific for the old world European, new world European, south African and new world African honeybees. Ivanova et al. (2007) demonstrated genetic variation among honey bees in two different mountainous regions and Thrace regions of Bulgaria and Turkey using RAPD markers. Genetic differentiation of Iranian honeybee (*A. m. meda*) populations was also studied with RAPD analysis (Kence et al., 2005). The objective of this study was to determine and compare the genetic variation of honey bee populations collected from 25 different regions in Turkey which are at least 300 km away from each other representing different populations.

MATERIALS AND METHODS

Animal material and PCR protocols

A total of 720 honey bee workers from 360 colonies (>250 colonies from hobbyist beekeepers and 110 colonies from sideliner), two per colony taken from inside the hives from 25 provinces in Turkey were collected (Figure 1 and Table 1). Total DNA was extracted from each individual worker using Fermentas 512 DNA purification kit. A total of twenty primers were screened (OPA-OPB series); ten primers (OPA7, OPB1-OPB9 Operon Tech., Alameda, CA) which illustrated bright (staining intensity), reproducible bands were tested on honey bees (Figure 2). RAPD PCR protocol was done according to Hunt and Page (1992). Amplification products were resolved by electrophoresis in 1.2% agarose gel with 0.8 X TBE buffer. After electrophoresis, gels were stained with ethidium bromide solution (10 µ/l ml) and visualized under UV.

Data analysis

Polymorphic and monomorphic bands were scored as present (1) or absent (0) for RAPD analyses. Percent of polymorphic bands, expected heterozygosity (*He*), gene diversity (Nei, 1973), Shannon's information indices (*I*) (Lewontin, 1972) are being used in ecology as a measure of species diversity whereas in genetics to estimating genetic variability as calculates according to the following equation, where *pi* stands for the proportion of the *i*th allele in the population:

$$I = -\sum p_i \ln p_i$$

The coefficient of gene differentiation (G_{ST}), and gene flow (*Nm*) were calculated using POPGENE 1.31 software (Yeh et al., 1999). The total band patterns, and Analysis of Molecular Variance (AMOVA), were carried out using Genalex6 software program (Peakall and Smouse, 2006). UPGMA tree was constructed based on Roger's (1972) original distance using TFPGA v.1.3 (Miller, 1997). Principle Coordinate Analysis (PCA) and Mantel test were performed using NTSYS v.2.20 software program (Rohlf, 2000).

RESULTS

Ten RAPD primers amplified 105 bands, all of which were polymorphic when all populations were considered.

Figure 1. Locations of the populations studied.

Table 1. Honey bee sample information, mean heterozygosity (*He*) and Shannon's index values (*I*).

Locations	Latitude	Longitude	N	Regions	Mean *He*	*I*
Hatay	36°14'N	36°10'E	30	Southern Anatolia (Mediterranean Region)	0.127	0.200
Antalya	36°52'N	30°45'E	30			
Aydın	37°51'N	27°51'E	29	Western Anatolia (Aegean Region)	0.134	0.213
Izmir	38°25'N	27°08'E	30			
Manisa	38°38'N	27°30'E	29			
Muğla	37°15'N	28°22'E	30			
Usak	38°68'N	29°40'E	30			
Artvin	41°14'N	41°44'E	30	North Anatolia (Black Sea Region)	0.112	0.178
Sinop	42°1'N	35°11'E	28			
Trabzon	41°0'N	39°45'E	27			
Beysehir	37°41'N	31°33'E	30	Central Anatolia	0.136	0.183
Nevsehir	38°33'N	34°40'E	30			
Sivas	39°43'N	36°58'E	29			
Yozgat	39°51'N	34°47'E	18			
Konya	37°52'N	32°35'E	30			
Kayseri	38°45'N	35°30'E	25			
Bilecik	40°05'N	30°05'E	30	Northwest Anatolia	0.136	0.217
Kırklareli	41°44'N	27°15'E	30	(Thrace Region)		
Bingol	38°53'N	40°29'E	28	East Anatolia	0.105	0.172
Kars	40°40'N	43°05'E	29			
Van	38°30'N	43°0'E	30			

Table 1. Contd.

Bitlis	38°20'N	42°03'E	28			
Şanlıurfa	37°13'N	38°76'E	30	South East Anatolia	0.035	0.073
Bozcaada	39°49'N	26°03'E	30	Marmara Region	0.128	0.212
Gökçeada	40°10'N	25°50'E	30	(Islands)		

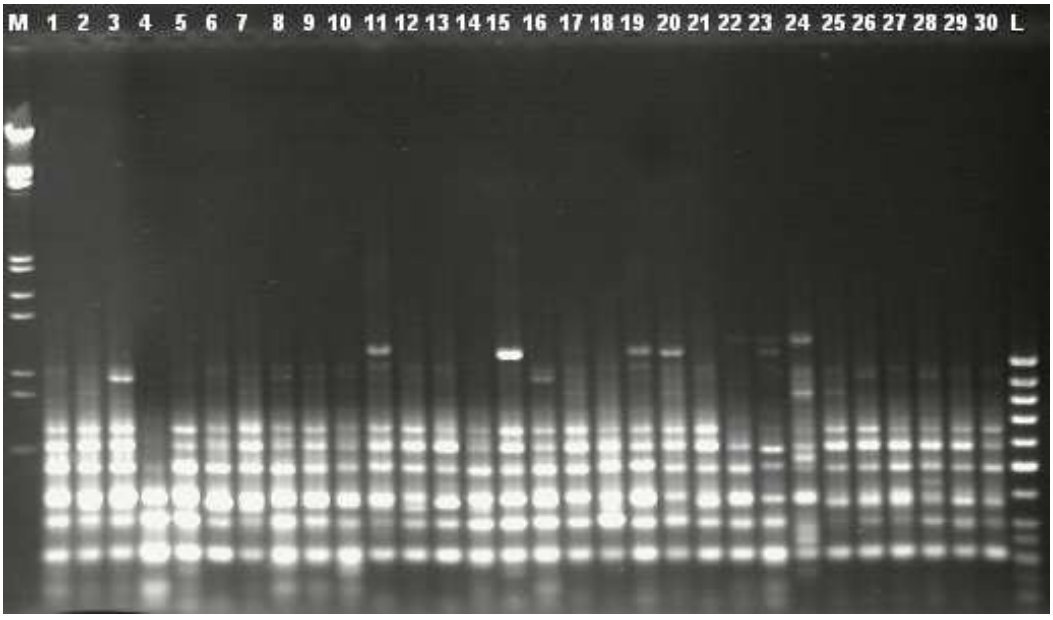

Figure 2. Banding pattern obtained by OPB-2 primer in Kırklareli honey bee population (M: Lambda DNA/EcoRI+HindIII; L: 100 bp DNA Ladder).

The percentage of polymorphic loci ranged between 37.14 and 64.76%. The highest and lowest percentages were calculated in Antalya and Hatay populations, respectively. The proportion of polymorphic bands, expected heterozygosity (*He*), Shannon's information index (*I*) values, and their standard errors are given in Table 2. The expected heterozygosity levels for honey bee populations ranged between 0.035 (Şanlıurfa) and 0.175 (Antalya). Gene diversity and Shannon's index values for all populations were estimated to be 0.187 and 0.305, respectively, the latter ranged from 0.073 (Şanlıurfa) to 0.271 (Antalya). The highest number of bands was observed in Bozcaada and Antalya populations as 72 and 70, whereas the lowest number (39 bands) obtained in Hatay population (Table 2). Aydın, Gökçeada, Muğla, and Şanlıurfa each have one different private band; 3000 (OPB-5), 2027 (OPB-7), 3530 (OPB-1) and 1632 bp (OPB-2), respectively.

Gene diversity (H_T) in total population and magnitude of differentiation among populations (G_{ST}) was 0.188 and 0.352, respectively. Pairwise G_{ST} values were given in Table 3. While the highest pairwise G_{ST} was observed between Trabzon and Şanlıurfa (0.441), the lowest was detected between Bilecik and Muğla populations (0.060). Analysis of Molecular Variance (AMOVA) indicated that 60% of within-population variation and 40% among-populations variation. The Mantel test did not reveal significant correlation between the genetic and geographic distances.

According to Roger's (1972) original genetic distances, Trabzon and Kırklareli were the most distant (0.202); Şanlıurfa and Hatay were the least distant populations (0.069). Dendogram illustrated that Kırklareli and Bozcaada known as an ecotype of *A. m. carnica* subspecies group were clearly diverged from all other populations. Within large cluster, Hatay and Şanlıurfa belonged to *A. m. syriaca* formed a cluster together apart from other populations and Artvin is standing by itself which represents subspecies *A. m. caucasica* (Figure 3). First three Eigen values of PCA explained 63% of total variation (27, 21 and 15% of first, second, and third Eigen values, respectively). Figure 4 shows the individuals of

Table 2. Observed number of bands, percentage of polymorphic bands, expected heterozygosity (*He*), Shannon's information index (*I*) values and their standard errors for populations studied.

Populations	Obs.# bands	Polymorphic bands (%)	*He*	*I*
Antalya	70	64.76	0.175	0.271±0.0267
Artvin	43	40.00	0.098	0.155±0.0233
Aydın	64	60.95	0.144	0.228±0.0249
Beysehir	61	57.14	0.172	0.262±0.0274
Bilecik	64	60.95	0.109	0.184±0.021
Bingol	60	56.19	0.113	0.185±0.0227
Bitlis	58	54.29	0.121	0.197±0.0231
Bozcaada	72	61.90	0.128	0.212±0.0219
Gökçeada	69	59.05	0.129	0.212±0.0224
Hatay	39	37.14	0.079	0.129±0.0209
Izmir	65	61.90	0.169	0.261±0.0277
Kars	54	51.43	0.101	0.164±0.0225
Kayseri	50	44.76	0.116	0.183±0.024
Kırklareli	65	56.19	0.136	0.217±0.0248
Konya	60	54.29	0.129	0.208±0.0233
Manisa	53	46.67	0.107	0.172±0.0232
Muğla	57	54.29	0.137	0.216±0.025
Nevsehir	46	40.95	0.083	0.136±0.0211
Sinop	46	40.95	0.121	0.188±0.0254
Sivas	67	58.10	0.156	0.245±0.0258
Trabzon	59	49.52	0.119	0.191±0.0237
Usak	65	58.10	0.114	0.189±0.0219
Van	46	43.81	0.084	0.140±0.0204
Yozgat	59	53.33	0.164	0.250±0.0272
Şanlıurfa	44	41.90	0.035	0.073±0.009

populations on plot with different colours. Kırklareli and Antalya honey bee populations also well separated from the other populations.

DISCUSSION

RAPD analysis in honey bee populations of Turkey has indicated that the expected heterozygosity (*He*) levels increases from northern to southern and eastern to western Anatolia. The Shannon's index value (*I)* also showed similar pattern. At the same time, *I* values of islands (Bozcaada and Gökçeada), Thrace and western Anatolian populations were higher than that of the eastern, northern, southeastern, and central Anatolia.

When comparison was made at population level, Antalya population had the highest gene diversity and Shannon's index, second to that were observed for the Aegean island populations, Gökçeada and Bozcaada. The highest genetic diversity in Antalya population is most likely resulted from having migratory beekeepers into this region because of favorable climatic and vegetative conditions for overwintering and extensive queen bee breeding. The observation of high genetic diversity in two island populations may be due to queen bee import and bee transfer during human settlements from many other provinces. The theory of island biogeography predicts that island populations have smaller genetic diversity than that of mainland populations arguing that higher inbreeding and extinction rates in islands (Losos and Ricklefs, 2010). However, the honey bee populations are mostly human manipulated by queen bee importation thus introducing different genotypes. That is more likely the factor increased genetic variation in two island populations.

Hatay and Şanlıurfa populations known to contain African mitochondrial haplotypes (Smith et al., 1997; Kandemir et al., 2006) and contain African genes based on microsatellite analysis (Bodur et al., 2007) clustered together also with RAPD markers. These findings show that Hatay and Şanlıurfa populations are more closely related to each other and to the African honey bees than to rest of the honey bee populations of Turkey.

Each one of Aydın, Gökçeada, Muğla, and Şanlıurfa populations had one different private band. In a previous study, Suazo et al. (1998) screened 700 RAPD primers in order to discriminate African and European honey bee populations and found specific banding pattern for

Table 3. Pairwise population coefficients of gene differentiation. $G_{ST.}$

Location	Antalya	Artvin	Aydin	Beysehir	Bilecik	Bingol	Bitlis	Bozcaada	Gokceada	Hatay	Izmir	Kars	Kayseri	Kirklareli	Konya	Manisa	Mugla	Nevsehir	Sinop	Sivas	Trabzon	Usak	Van	Yozgat
Artvin	0.234	***																						
Aydin	0.145	0.231	***																					
Beysehir	0.166	0.185	0.193	***																				
Bilecik	0.202	0.095	0.193	0.145	***																			
Bingol	0.216	0.132	0.154	0.142	0.115	***																		
Bitlis	0.203	0.192	0.185	0.216	0.161	0.167	***																	
Bozcaada	0.211	0.246	0.305	0.206	0.257	0.273	0.315	***																
Gokceada	0.248	0.216	0.274	0.224	0.19	0.235	0.188	0.280	***															
Hatay	0.275	0.147	0.228	0.202	0.142	0.132	0.196	0.321	0.280	***														
Izmir	0.178	0.118	0.139	0.118	0.088	0.107	0.134	0.244	0.16	0.138	***													
Kars	0.250	0.177	0.288	0.159	0.107	0.14	0.254	0.282	0.319	0.209	0.149	***												
Kayseri	0.208	0.243	0.247	0.140	0.179	0.214	0.265	0.274	0.327	0.258	0.169	0.199	***											
Kirklareli	0.230	0.292	0.300	0.212	0.287	0.279	0.315	0.193	0.358	0.346	0.24	0.259	0.284	***										
Konya	0.198	0.172	0.206	0.179	0.120	0.179	0.177	0.226	0.213	0.231	0.13	0.206	0.171	0.262	***									
Manisa	0.189	0.228	0.185	0.221	0.186	0.194	0.209	0.279	0.279	0.26	0.182	0.188	0.247	0.288	0.221	***								
Mugla	0.203	0.124	0.188	0.126	0.060	0.153	0.166	0.237	0.183	0.186	0.094	0.126	0.173	0.233	0.134	0.154	***							
Nevsehir	0.234	0.26	0.269	0.110	0.190	0.226	0.308	0.304	0.314	0.313	0.158	0.214	0.15	0.259	0.203	0.299	0.147	***						
Sinop	0.197	0.185	0.181	0.121	0.152	0.155	0.223	0.270	0.209	0.259	0.121	0.168	0.186	0.274	0.192	0.182	0.09	0.142	***					
Sivas	0.185	0.250	0.224	0.149	0.213	0.241	0.226	0.265	0.205	0.298	0.151	0.262	0.262	0.308	0.236	0.219	0.172	0.253	0.181	***				
Trabzon	0.302	0.269	0.27	0.236	0.27	0.193	0.293	0.351	0.253	0.317	0.192	0.292	0.352	0.352	0.307	0.257	0.253	0.366	0.236	0.274	***			
Usak	0.201	0.206	0.223	0.169	0.152	0.219	0.224	0.277	0.227	0.265	0.14	0.232	0.192	0.326	0.124	0.23	0.13	0.205	0.159	0.18	0.329	***		
Van	0.193	0.169	0.175	0.174	0.096	0.16	0.215	0.308	0.249	0.218	0.094	0.172	0.205	0.328	0.166	0.228	0.125	0.213	0.167	0.225	0.311	0.155	***	
Yozgat	0.197	0.214	0.196	0.174	0.191	0.200	0.238	0.226	0.202	0.226	0.129	0.217	0.211	0.280	0.171	0.243	0.196	0.225	0.198	0.179	0.248	0.202	0.170	***
Sanlıurfa	0.334	0.216	0.282	0.263	0.139	0.209	0.300	0.420	0.395	0.157	0.166	0.219	0.316	0.403	0.264	0.356	0.205	0.376	0.33	0.393	0.441	0.311	0.227	0.310

populations of different origins. The primer 539 had produced a banding pattern specific for East European honeybees and also found at high frequencies in New world European but absent in neotropical African bees. The primers, 652 and 691, produced bands which were specific for African population, whereas the bands produced by primers 694 and 514 were found at low frequencies in African, but at high frequencies in European populations (Suazo et al., 1998). In present study OPB-1 and OPA-7 primers seem to have specific band pattern in all populations except Hatay and Şanlıurfa. This is important as these two populations belong to A lineage, whereas the others belong to C lineage. Dendogram obtained with Roger's distance illustrated that Kırklareli and Bozcaada which are known as an ecotype of *A. m. carnica* subspecies group are distantly separated from all other populations. Artvin is standing by itself which represents subspecies *A. m. caucasica*, Hatay and Şanlıurfa formed a cluster together apart from other populations and belong to *A. m. syriaca*.

Thus, the RAPD markers used were effective to discriminate those known three races. Rest of the populations from Anatolia, for instance Antalya distinctly grouped with Aydın indicates they are genetically close to each other but different from others. PCA also indicated that Antalya and Kırklareli honey bee populations distinguished from other populations. It should be noted that Trabzon is a distinct population and deserve further investigation. Muğla bees which is an ecotype of *A. m. anatoliaca* with a life cycle that they are adapted to interact with *Marchalina hellenica* and produce pine honey, found close to Sinop bees which is another ecotype of A. m. anatoliaca. Remaining populations of Anatolian bee have gene pools of different levels of genetic

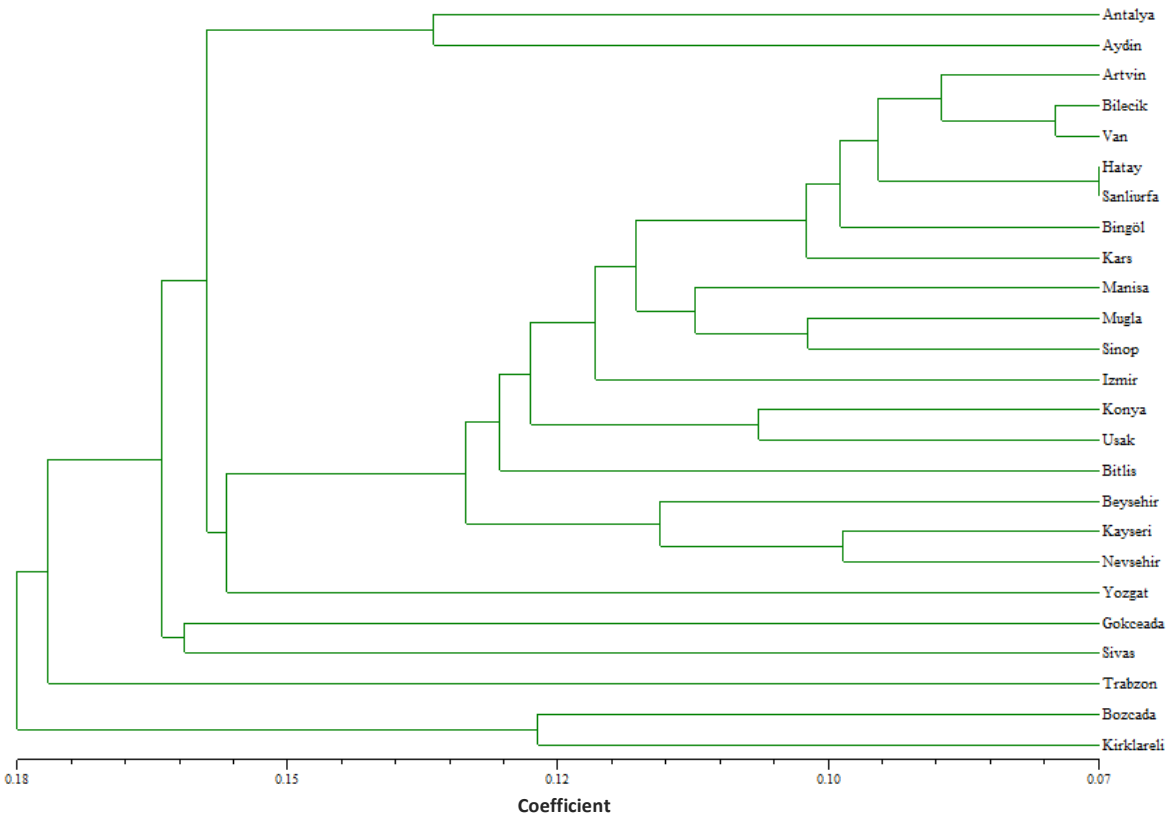

Figure 3. UPGMA dendrogram based on Roger's (1972) original genetic distances.

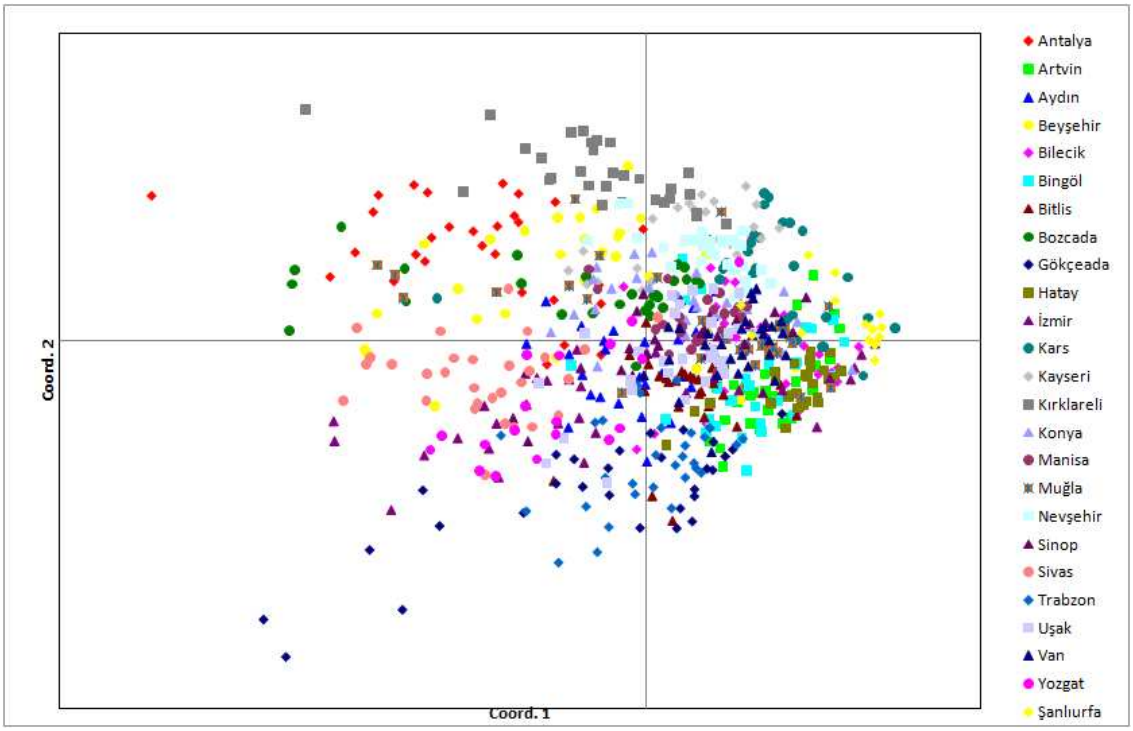

Figure 4. Principal coordinate analysis for honey bee population variation of Turkey based on RAPD data.

dissimilarities. In another study, genetic variation was demonstrated using RAPD in two different mountainous regions of Bulgaria and Thrace regions of Turkey where, three populations obtained from Thrace region of Turkey and one population (Plovdiv) from Bulgaria were clustered together indicating they belong to same subspecies, *A.m. carnica* (Ivanova et al., 2007). When the results of current study compared to that of Ozdil et al. (2006) who studied sixteen honeybee populations in Turkey using 20 RAPD primers reported a lower percentage of polymorphism, average heterozygosity, average population differentiation, and a higher gene flow value for all populations. Kence et al. (2005) reported a moderate level of genetic variation in Iranian (*A. m. meda*) populations by RAPD analyses and when genetic diversity in Iranian and Turkish honey bee populations was compared it was found that the genetic diversity for Turkish honey bee populations was higher than Iranian populations.

The data presented here showed that RAPD markers were effective in discriminating honeybee populations, separating A and C lineages and detecting the variability levels among populations. These results should be considered in conservation plans, particularly with regard to moving of colonies between regions and most importantly introducing bees of foreign origin and distributing queen bees from one center to all over the country which will homogenize the gene pools of the populations. Establishing new conservation areas in Hatay, Kırklareli and Muğla is suggested to preserve genetic diversity which is an essential resource for future selection programs.

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REFERENCES

Akkaya H, Alkan S (2007). Beekeeping in Anatolia from the Hittites to the present day. J. Apic. Res., 46(2): 120-124.

Arias MC, Sheppard WS (2005). Phylogenetic relationships of honey bees Hymenoptera:Apinae:Apini) inferred from nuclear and mitochondrial DNA sequence data. Mol. Phylogenet. Evol. 37: 25-35.

Arias MC, Rinderer TE, Sheppard WS (2006). Further characterization of honey bees from the Iberian Peninsula by allozyme, morphometric and mtDNA haplotype analyses, J. Apic. Res., 45: 188-196.

Bodur C, Kence M, Kence A (2007). Genetic structure of honeybee, *Apis mellifera* L. (Hymenoptera: Apidae) populations of Turkey inferred from microsatellite analysis. J. Apic. Res., 46(1): 50–56.

Engel MS (2004). Geological history of the bees (Hymenoptera: Apoidea). Revista de Tecnologia e Ambiente 10(2): 9-33.

Fondrk MK, Page RE, Hunt G (1993). Paternity analysis of worker honeybees using random amplified polymorphic DNA. Naturwissenschaften 80: 226-231.

Hunt GJ, Page RE (1992). Patterns of inheritance with RAPD molecular markers reveal novel types of polymorphism in the honeybee. Theor. Appl. Genet., 85: 15-20.

Hunt GJ, Page RE (1994). Linkage analysis of sex determination in the honey bee. Mol. Genet. Genom., 244: 512-518.

Hunt G, Guzman-Novoa E, Fondrk MK, Page JR, Robert E (1998). Quantitative trait loci for honeybee stinging behavior and body size. Genetics, 148: 1203-1213.

Ivanova E, Ivgin R, Kence M, Kence A (2007). Determination of genetic variation and differetiation in honeybees of Turkey and Bulgaria. 1st Balkan Countries Beekeeping Congress and Exhibition Abstracts p47. 29 March-1 April 2007, İstanbul, Türkiye

Kandemir I, Kence M, Kence A (2000). Genetic and morphometric variation in honeybee (*A. mellifera* L.) populations of Turkey. Apidologie, 31: 343-356.

Kandemir I, Kence M, Kence A (2005). Morphometric and electrophoretic variation in different honey bee (*Apis mellifera* L.) populations. Turk. J. Vet. Anim. Sci., 29: 885-890.

Kandemir I, Kence M, Sheppard WS, Kence A (2006). Mitochondrial DNA variation in honey bee (*Apis mellifera* L.) populations from Turkey. J. Apic. Res., 45(1): 33-38.

Kence M, Jabbari FH, Tunca İR (2005). Genetic comparison of İranian honeybee (*Apis mellifera* L.) populations by RAPD analysis. İnternational Beekeeping Congress:Beekeeping for Sustainable Livelihoods and Rural Development Abstracts, p. 23. 13-18 November 2005, Bangalore, İndia.

Lewontin RC (1972). The apportionment of human diversity. Evol. Biol., 6: 381-398.

Losos JB, Ricklefs RE (2010). The theory of island biogeography revisited. Princeton University Press, Princeton, USA, p. 476.

Mattila HR, Seeley TD (2007). Genetic diversity in honey bee colonies enhances productivity and fitness. Scieince, 317: 362-364.

Mattila HR, Burke KM, Seeley TD (2008). Genetic diversity within honeybee colonies increases signal production by waggle-dancing foragers. Proceedings of the Royal Society of London, B 275: 809-816.

Meixner MD, Arias MC, Sheppard WS (2000). Mitochondrial DNA polymorphisms in honey bee subspecies from Kenya. Apidologie, 31: 181-190.

Miller MP (1997). Tools for population genetic analyses (TFPGA) 1.3: A Windows program for the analysis of allozyme and molecular population genetic data. Computer software distributed by the author. Department of Biological Sciences, Nothern Arizona University, Flagstaff, AZ.

Nazzi F (1992a). Morphometric analysis of honey bees from an area of racial hybridization in northeastern Italy, Apidologie, 23: 89-96.

Nei M (1973). Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA, 70: 3321-3323.

O'Donnell S (1996). RAPD markers suggest genotypic effects on forager specialization in a eusocial wasp. Behav. Ecol. Sociobiol., 38: 83–88.

Ozdil F, Yıldız MA, Hall HG (2009). Molecular characterization of Turkish honey bee populations (*Apis mellifera*) inferred from mitochondrial DNA RFLP and sequence results. Apidologie, 40: 570-576.

Ozdil F, Yıldız MA, Meydan H, Gençer HV (2006). Genetic structure of Turkish honeybee populations based on RAPD and mtDNA RFLP markers. Proceedings of The Second European Conference of Apidology (edited by Viladimir Vesely and Dalibor Titera) p.53. 10-14 September, 2006. Prague, Czech Republic.

Page RE Jr, Robinson GE, Fondrk MK, Nasr NE (1995). Effects of worker genotypic diversity on honey bee colony development and behavior (*Apis mellifera* L.). Behav. Ecol. Sociobiol., 36: 387-396.

Palmer MR, Smith DR, Kaftanoglu O (2000). Turkish honeybees: genetic variation and evidence for a fourth lineage of *Apis mellifera* mtDNA. J. Hered., 91: 42-46.

Peakall R, Smouse PE (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol., 6: 288-295.

Rohlf FJ (2000). NTSYS-pc numerical taxonomy and multivariate analysis system, version 2.20e. Exeter Publication, New York.

Rogers JS (1972). Measures of genetic similarity and genetic distance. in "Studies in Genetics *VII*". University of Texas Publication no. 7213, Austin, pp. 145-154.

Ruttner F (1988). Biogeography and Taxonomy of Honeybees.

Springer-Verlag. Berlin Heidelberg, Germany, p. 284.
Ruttner F, Elmi MP, Fuchs S (2000). Ecoclines in the Near East along 36° N Latitude in *Apis mellifera* L. Apidologie, 31: 57-165.
Sheppard WS, Arias MC, Meixner MD, Grech A (1997). *Apis mellifera ruttneri*, a new honey bee subspecies from Malta. Apidologie, 28: 287–293.
Sheppard WS, Meixner MD (2003) *Apis mellifera pomonella*, a new honey bee subspecies from Central Asia. Apidologie, 34: 367-375.
Smith DR (1991). Mitochondrial DNA and honeybee biogeography. *In* Smith, D R(ed) *Diversity in the genus Apis*. Boulder Co.;Westview, pp. 131-176.
Smith DR, Slaymaker A, Palmer M, Kaftanoğlu O (1997). Turkish honeybees belong to the east Mediterranean lineage. Apidologie, 28: 269-274.
Smith DR, Palmer MR, Otis G, Damus M (2003). Mitochondrial DNA and AFLP markers support species status of *Apis nigrocincta*. Insectes Soc., 50: 185-190.
Solignac M, Vautrin D, Loiseau A, Mougel F, Baudry E, Estoup A, Garnery L, Haberl M, Cornuet JM (2003). Five hundred and fifty microsatellite markers for the study of the honeybee (*Apis mellifera* L.) genome. Mol. Ecol., 3(2): 307-311.
Solorzano CD, Szalanski AL, Kence M, McKern JA, Austin JW, Kence A (2009) Phylogeography and Population Genetics of Honey Bees (*Apis mellifera*) From Turkey Based on COI-COII Sequence Data. Sociobiology, 53(1): 237-246.
Suazo A, Hall HG (1999) Modification of the AFLP protocol applied to honeybee (*Apis mellifera* L.) DNA. Biotechniques, 26(4): 704-709.
Suazo A, Hall HG (2002a). A locus with PCR-RFLP alleles characteristic of African and European honeybee (*Apis mellifera* L.) (Hymenoptera: Apidae) groups of subspecies. Ann. Entomol. Soc., 95(1): 115-124.
Suazo A, McTiernan R, Hall HG (1998). Differences between African and European honey bees (*Apis mellifera*) in random amplified polymorphic DNA (RAPD). J. Hered., 89: 32-36.
Szalanski AL, Mckern AJ (2007). Multiplex PCR-RFLP Diagnostics of the Africanized Honey Bee (Hymenoptera: Apidae). Sociobiology 50(3): 939-945.
Waldschmidth AM, Marco-Junior P, Barros EG, Campos LAO (2002). Genetic analysis of *Melipona quadrifasciata* Lep. (Hymenoptera: Apidae, Meliponinae) with RAPD markers. Braz. J. Biol., 62(4B): 923-928.
Whitfield CW, Behura SK, Berlocher SH, Clark EG, Johnston JS, Sheppard WS, Smith DR, Suarez AV, Weaver D, Tsutsui ND (2006). Thrice out of Africa: ancient and recent expansions of the honey bee, *Apis mellifera*. Science, 314(5799): 642–645.
Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990). DNA Polymorphism Amplified by abitrary primers are used as genetic markers. Nucleic Acids Res., 18: 6531-6535.
Yeh FC, Yang R, Boyle T (1999). POPGENE 1.32: Microsoft Windows based freeware for population genetic analysis. University of Alberta, USA.