DETERMINATION AND COMPARISON OF GENETIC VARIATION IN HONEYBEE (*Apis mellifera* L.) POPULATIONS OF TURKEY BY RANDOM AMPLIFIED POLYMORPHIC DNA AND MICROSATELLITE ANALYSES

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

RAHŞAN İVGİN TUNCA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY
IN
BIOLOGY

FEBRUARY 2009

Approval of the thesis

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ABSTRACT

DETERMINATION AND COMPARISON OF GENETIC VARIATION IN HONEYBEE (*Apis mellifera* L.) POPULATIONS OF TURKEY BY RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) AND MICROSATELLITE ANALYSES

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February 2009, 141 pages

We analyzed a total of 760 worker bees, two samples per colony, 390 colonies in 26 provinces in Turkey to determine and compare the genetic variation of Turkish honey bee (*Apis mellifera* L.) populations using 10 primers for RAPD and 6 microsatellite loci. Mean gene diversity levels ranged from 0.035 (Şanlıurfa) to 0.175 (Antalya) for RAPD and 0.449 (Muğla) to 0.739 (Artvin) for microsatellite markers. Private band patterns and alleles, pairwise F_{ST} values support that the Anatolian honey bees belong to C lineage except for Hatay and Şanlıurfa populations illustrated from previous findings of mitochondrial DNA studies. Genetic differentiation (G_{ST}) from RAPD data ranged from 0.060 (Bilecik and Muğla) to 0.395 (Gökçeada and Şanlıurfa). The genetic diversity (F_{ST}) for microsatellites ranged from -0.068 (Gökçeada and İzmir) to 0.347 (Konya and Muğla).

The results of the present research are in agreement to that of previous study in Turkish honey bee populations which used different microsatellite loci. That is the genetic variation was the highest in African, the lowest in European and intermediate in the Mediterranean honey bee populations. The data presented here indicate that in spite of extensive migratory beekeeping, there is still a large genetic differentiation among honey bee populations.

These results should be considered in establishment of conservation plans particularly in moving of colonies between regions. The most importantly introduction of bees with foreign origin and distribution queen bees from one center to all over the country which will homogenize the gene pool of the populations should be prevented.

Key words: Honeybee, *Apis mellifera* L., RAPD, microsatellite, genetic diversity, Turkey

ÖZ

TÜRKİYE BALARISI (*Apis mellifera* L.) POPULASYONLARININ RASTGELE ÇOĞALTILMIŞ POLİMORFİK DNA (RAPD) VE MİKROSATELİT ANALİZLERİ İLE GENETİK ÇEŞİTLİLİĞİNİN BELİRLENMESİ VE KARŞILAŞTIRILMASI

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Türkiye bal arısı (*Apis mellifera* L.) toplumlarında genetik çeşitliliğin belirlenmesi ve karşılaştırılması amacıyla 26 ilden 390 kolonide, koloni başına 2 örnek olacak şekilde toplam 760 işçi arı örneği, 10 RAPD öncül DNA' sı ve 6 mikrosatelit lokusu kullanılarak analiz edilmiştir. Ortalama gen farklılaşma düzeyleri RAPD analizi için 0.035 (Şanlıurfa) ile 0.175 (Antalya) ve mikrosatelit analizi için 0.449 (Muğla) ile 0.739 (Artvin) arasında değişmiştir. Toplumlara özgü band kalıpları ve alelleri, ikili F_{ST} değerleri, daha önceki mitokondrial DNA çalışmalarının sonuçlarında gösterildiği gibi Hatay ve Şanlıurfa populasyonlarının dışında Anadolu bal arılarının C soy hattına ait olduğunu desteklemektedir. RAPD verilerinden elde edilen genetik farklılaşma (G_{ST}), 0.060 (Bilecik and Muğla) ve 0.395 (Gökçeada and Şanlıurfa) arasındadır. Ayrıca, mikrosatelit verilerinden elde edilen genetik çeşitlilik (F_{ST}), -0.068 (Gökçeada and İzmir) ile 0.347 (Konya and Muğla) arasında değişmiştir.

Bu çalışmanın sonuçları, Türkiye bal arısı toplumlarında farklı mikrosatelit lokusları kullanılarak yapılmış olan çalışma ile uyumludur. Genetik çeşitliliğin Afrika' da yüksek, Avrupa' da düşük ve Akdeniz toplumlarında orta seviyede olduğu sonucuyla uyum içindedir. Burada sunulan veri, yoğun gezgin arıcılığa rağmen bal arısı toplumları arasında genetik farklılığın hala fazla olduğunu göstermiştir.

Bu sonuçlar koruma planlarının oluşturulmasında özellikle de kolonilerin bölgeler arası taşınmasında göz önünde bulundurulmalıdır. En önemlisi yabancı kökenli arıların girişi ve ana arıların bir merkezden bütün ülkeye dağıtılması, toplumların gen havuzunu tekdüzeleştireceği için engellenmelidir.

Anahtar kelimeler: Bal arısı, *Apis mellifera* L., RAPD, mikrosatelit, genetik çeşitlilik, Türkiye

To My Family

ACKNOWLEDGMENTS

I am greatly thankful to Dr. Meral Kence for her guidance, critism, encouragement, advice, and insightful comments throughout my research. I would like to thank Dr. Aykut Kence for his advice and critism.

I am thankful to thesis examining committee members, Dr. Zeki Kaya, Dr. Nihat Bozcuk, Dr. Musa Doğan and Dr. Ergi Deniz Özsoy for critical reading of the thesis and their valuable comments.

I also thank to my labmates Mr. Mehmet Kayım, Mr. Ayhan Altun, Dr.Tolga Kankılıç for their friendship and help in various ways during labwork and writing the thesis. I would also like to thank to Mr. Gün Köleoğlu, Mr. Yusuf Atağan, and Miss. Havva Dinç for their help during experiments.

I am greatly thankful to my lovely husband, my pretty daughter and my dear parents, for their patience, tolerance and supports. They always encouraged me to achieve my goals.

This study was supported by Faculty Development Program (ÖYP) of Middle East Technical University financed by DPT.

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CHAPTER 1

INTRODUCTION

1.1. Importance of Honey bees

Honey bees have been shown to be very important biological material for breeders and scientists due to their economic role in honey production and pollination, which provide continuity of the wild flora and increase the yield of cultivated plants. Honey bees are also social insects which makes them a model organism in order to understand the evolution of social organization.

Wilson (1982) set the criteria to determine the sociality of insects as cooperation among adults in nest construction and brood care, division of labor according to their reproduction and overlapping of at least two generations (Dietz, 1986).

Based on the natural selection of individuals, the evolution of sociality has been explained by four main theories; kin selection, group selection, mutualism, and polyandry. Brian (1983) suggested that these four theories tried to explain why haplodiploid mode of sex determination in Hymenoptera has been a successful starting point of evolution of sociality. Altruism and inclusive fitness, based on kin selection, has had a strong effect on social insects (Hamilton, 1964).

1.2. The Evolutionary History of A. mellifera L.

Honey bees are classified within Apinae subfamily of Apidea family. This subfamily contains special pollen collecting organs. The origin of *Apis* can be traced back to the early Oligocene based on the well preserved fossil of true *Apis* type (Culliney, 1983; Ruttner, 1988; Engel, 1998). On the other hand, the time of origin is estimated taking the Meliponini (Apidae) into account, known as stingless bees, as approximately between 100-300 Mya* (Michener, 1979; Ruttner, 1988; Camargo and Wittman, 1989; Arias and Sheppard, 2005). Previous data from Engel (1998) indicated that *Apis* diversity has slightly increased as only three species are currently known from the Oligocene, followed by another five species from the Miocene (not including *A. melisuga*), eventually leading up to the six species.

Ruttner (1988) and Engel (1998) studied wing venation of living and fossil honey bees in order to determine the differences among them and their close relatives. According to their results, *Bombus spp, A. mellifera + A. cerana, A. florea* and *Synapis*+A. armbrusteri + A. dorsata* compound clusters were formed. It was observed that wing venations of *Synapis + A. dorsata* have not greatly changed since the early Miocene. However, excluding their wing sizes, *A. armbrusteri + A. dorsata* have been found to completely overlap since late Miocene *(10-12 my).* An *A. florea* was assumed to have separated from *Synapis + A. armbrusteri + A. dorsata* and *A. mellifera + A. cerana* in the Oligocene (Engel, 1998).

Ruttner and Maul (1983) revealed that *A. cerana* and *A. mellifera* are still in an immature stage of speciation, have very similar characteristics and they do not have pre-mating barriers. They were isolated during the last glaciation and their recent distribution in the temperate zone shows a postglacial pattern (Ruttner, 1988; Schmitt, 2007). Thus, they have existed for nearly 50,000 years.

** Mya: Million years ago- *Synapis well preserved Apis type specimens were found in amber and come from the lower Miocene (22-25 my old)*

Engel and Schultz (1997) reanalyzed the genus *Apis* based on morphological characters and DNA sequence data with 6 taxa. According to reanalysis study, *A. mellifera and A. cerana* were considered most recently derived sister taxa. *A. mellifera + A. cerana* shared a common ancestor with *A. dorsata. A. dorsata + A. mellifera + A. cerana* shared a common ancestor with *A. florea.* Cameron *et al*. (1992) and Engel and Schultz (1997) revealed that *A. koschevnikovi* was combined with *A. cerana* and *A. adreniformis* was the sister taxon to only *A. florea.*

According to mtDNA-CoII sequences and allozyme differentiation among populations, several researchers proposed that *A. cerana – A. mellifera* group divergence occurred much earlier in evolutionary history of *Apis*, nearly 6-8 myr ago (Garnery *et al.*, 1991; Sheppard and Berlocher, 1989). Engel (1999) supported the proposal of the earlier group divergence using taxonomic analyses of fossil and living honey bees.

1.3. Classification and Geographical Distribution of Honey Bees

Honey bees are grouped into three major clusters based on morphometric analyses (Ruttner, 1988), nuclear and mt DNA analyses (Arias and Sheppard, 2005) and presently *A. dorsata*, *A. binghami*, and *A. laboriosa* are called giant bees. *A. andreniformis* and *A. florea* are called dwarf bees and cavity-nesting bees consist of *A. mellifera*, *A. cerana*, *A. koschevnikovi*, *A. nuluensis*, and *A. nigrocincta.* The first cluster is comprised of giant honey bees. It includes three species; *A. dorsata, A. binghami and A. laboriosa.* The genetic relationship between *A. dorsata and A. binghami* was not clearly solved as Ruttner (1988) proposed that *A. dorsata* is very uniform within its main area (South Asia) and included four subspecies; *A. d. binghami*, *A. d. breviligula*, *A. d. dorsata*, and *A. d. laboriosa.* However, the sequence variation of mtDNA RFLP separated *A. d. binghami* and *A. d. dorsata* into two different groups (Smith, 1991). Arias and Sheppard (2005) did not observe a clear distinction between *A. binghami* and *A. dorsata* based on ND2 gene sequence and interpreted this as an indication of either recent speciation or intraspecific variability although they first considered them as separate species.

In contrast, the *A. laboriosa* was well diverged from *A. dorsata.* The second cluster consists of dwarf honey bees including *A. florea* and *A. andreniformis*. They are distributed throughout southern and southeastern Asia and together the subgenus *Micrapis*, and are the most primitive of the living species of *Apis*, reflected by their small colony size, and simple nest construction. The last cluster was composed of cavity-nesting honey bees including *A. mellifera, A. cerana, A. koschevnikovi and A. nigrocincta.* The cavity- nesting honey bees are considered in two geographic groups. The first one is eastern group including species *A. cerana, A. koschevnikovi and A. nigrocincta.* Sequence data from both mtDNA and AFLP supported that *A. nigrocincta* is a species diverged from *A. cerana* (Smith *et al.,* 2003; Arias and Sheppard, 2005).

Western honey bee species include *Apis mellifera L*. distributed from eastern to northern Europe and Asia. It is variously called the Western, European or Common honey bee in different parts of the world (Ruttner, 1988). Based on morphometric, behavioral and biogeographical studies, 26 subspecies have been identified (Ruttner, 1988; Sheppard *et al.,* 1997; Sheppard and Meixner, 2003) (Table 1.1). Many researchers have studied the subspecies based on biogeography, morphology, genetics and behavior (Arias and Sheppard, 1996; Franck *et al.,* 2000a; Garnery *et al.,* 1993, 1995; Meixner *et al.,* 1993, 1994; Kandemir *et al.,* 2000; Bodur *et al.,* 2007). The western honey bee *A. mellifera* has been adapted to many kinds of climates ranging from tropical to cold temperate and from humid areas to semi deserts. According to Adam (1983), *A. m. anatoliaca* is classified within western honey bee and is known to have evolved characteristics in order to survive during long, hard winter conditions.

Table 1.1. Honey bee (*Apis mellifera* L.) subspecies according to their geographical distribution (Ruttner, 1992; Sheppard *et al.* 1997).

Near East Subspecies
Apis mellifera anatoliaca Maa (1953)
A. m. adami Ruttner (1975)
A.m. cypria Pollman (1879)
A. m. syriaca Buttel-Reepen (1906)
A. m. meda Skorikov (1929)
A. m. caucasica Gorbachev (1916)
A. m. armeniaca Skorikov (1929)
Tropical Africa Subspecies
Apis mellifera lamarckii Cockerell (1906)
A. m. yemenitica Ruttner (1975)
A. m. litorea Smith (1961)
A. m. scutellata Lepeletier (1836)
A. m. adansonii Latreille (1804)
A. m. monticolo Smith (1961)
A. m. capensis Escholtz (1821)
A. m. unicolor Latreille (1804)
West Mediterranean (West and North Europe and North Africa) Subspecies
Apis mellifera sahariensis Baldensperger (1924)
A. m. intermissa Buttel-Reepen (1906)
A. m. iberica Goetze (1964)
A. m. mellifera Linnaeus (1758)
A. m. major Ruttner (1978)
Central Mediterranean and Southeast Europe Subspecies
Apis mellifera sicula Montagano (1911)
A. m. ligustica Spinola (1806)
A. m. cecropia Kiesenwetter (1860)
A. m. macedonica Ruttner (1987)
A. m. carnica Pollman (1879)
A. m. rutnerii Sheppard *et al.* (1997)

According to Ruttner (1988) the origin of western honey bees can be traced to Asia and are scattered over Africa and Europe in four evolutionary branches. These branches are North African and west European branch (M), north mediterranean branch (C), south and central African branch (A) and later, Frank *et al*. (2000a) reported the presence of fourth, Middle Eastern branch (O).

The M branch is common in most of Europe from Spain to Urals. These honey bees have large and dark colored abdomen. The C branch consists of six subspecies distributed all over the Italian Peninsula, the Balkans, South Ukraine, Sicily and Crete. The C branch bees are smaller than M branch ones and their color may range from grey to yellow. They have shorter hairs and a high cubital index. A branch honey bees have short tongues, small wings, short legs and are very slender. The O branch honey bees are distributed in the Middle East, smaller and have light body color with wide metatarsi (Ruttner, 1998; Franck *et al*., 2000a).

1.4. Middle Eastern Honey bee subspecies

The Middle Eastern honey bee races are distributed from Caspian coast and high mountains of central Iran to the Black Sea coast, the Caucasian Alpine region and Anatolia. Ruttner (1988) had discriminated seven races as, *A. m. syriaca, A. m. adami, A. m. anatoliaca, A. m. meda, A. m. cypria, A. m. caucasica* and *A. m. armeniaca,* using multivariate statistical analysis of morphometric data. The Middle Eastern honey bee races have different characteristics; in the northern part, they are tall, dark and long haired varieties where as in the southern regions, they are small, yellow body colored and short haired. Furthermore, zoogeographic variations are demonstrated that *A. m. syriaca* and *A. m. cypria* are smaller and have yellow body color than northern races, but *A. m. adami* is as large as *A. m. caucasica* on the same latitude (Ruttner, 1988). Ruttner (1988) indicated that *A. m. macedonica* which is found in south eastern Europe, *A. m. sicula* have distribution in the central Mediterranean region and North Africa showed morphometric similarities with the western populations *of A. m. anatoliaca*. Owing to these similarities, Anatolia where

mainly *A. m. anatoliaca* is distributed seems to be the eastern genetic center of *A. mellifera* (Ruttner, 1988; Bodur *et al.*, 2007).

Up to date, 26 *A. mellifera* subspecies have been recorded in the world and five of them namely *A. m. anatoliaca, A. m. caucasica, A. m. meda, A. m. syriaca* and *A. m. carnica* are found within the borders of Turkey (Kandemir *et al.,* 2000). The beekeeping activity has been carried out in Anatolia since 1300 B.C. which addresses the Hittite civilization located in Boğazköy according to old tablets (Akkaya and Alkan, 2007).

A. m. anatoliaca is found in the western and central Anatolia. They are known as highly adaptive to extreme climatic conditions and poor nectar flows in their environment and with powerful reproductive abilities. The body color of *A. m. anatoliaca* is generally yellow but the color of the rings on abdomen is rather a smudgy orange turning to brown. Their abdomens and tarsi are broader; relative to their body size they have short legs and wings (Ruttner, 1988).

A. m. caucasica is distributed in northeastern Anatolia (Kandemir *et al.,* 2000) is also observed from whole Georgia to Azerbaijan. *A. m. caucasica* has the longest proboscis (tongue) among all *mellifera* bees. The color of their small body which is covered with short hair varies greatly from completely dark to narrow yellow stripes (Ruttner, 1988).

The distribution of *A. m. meda* extends from eastern Anatolia (Kandemir *et al.,* 2000) to Iran and Iraq. A. *m. meda* bees have broader metatarsi, slightly narrower forewings and a broader abdomen. Their scutellum color varies from yellow to dark (Ruttner, 1988).

A. m. syriaca is found in southeastern Anatolia (Kandemir *et al.,* 2000) and also observed in Israel, Jordan, Lebanon, and Syria. *A. m. syriaca* is the smallest honey bee among Middle East honey bee races, and their basitarsal and cubital index is shorter than those of oriental *mellifera* bees as well. They have slender abdomen, very short cover hair, brightly yellow scutellum and terga. They are very good nectar

collectors but because of their highly aggressive behavior, colony management is much more difficult (Ruttner, 1988).

The other subspecies *A. m. carnica* is distributed from Thrace in Turkey (Kandemir *et al.,* 2000) to Austria including Slovakia and part of Hungary, Romania, Crotia, Bosna Herzegovina, and Serbia. They have brown-grey body color which is relieved by lighter brown stripes. Their chitin is dark, but it is possible to find lighter colored or brown colored rings and dots on their bodies with short hair. Their abdomens are much slimmer and their tongues are very long (Rinderer, 1986).

Up to now, many studies have been completed in order to determine honey bee races by using morphometry (Kandemir *et al*., 2005; Arias *et al.,* 2006), Allozyme variations (Kandemir *et al*.,2005; Arias *et al.,* 2006), Restriction Fragment Length Polymorphism (RFLP) (Szalanski and McKern, 2007; Suazo and Hall, 2002), Random Amplified Polymorphic DNA (RAPD) (Suazo *et al.,* 1998; Hunt and Page, 1992; Ivanova *et al*., 2007), Amplified Fragment Length Polymorphism (AFLP) (Suazo and Hall, 1999; Smith *et al.,* 2003), mitochondrial DNA (mtDNA) (Palmer *et al.,* 2000; Smith *et al*., 2003), microsatellite (Garnery *et al.,* 1998; De la Rua *et al*., 2001; Bodur *et al.,* 2007), and Single Nucleotide Polymorphism (SNP) (Whitfield *et al.,* 2006).

1.5. Molecular Techniques and Their Applications on Honey bees

MtDNA and microsatellite markers are very useful to determine the phylogenetic relationships of the honey bee populations in the world (Meixner *et al.,* 2000; Ca'novas *et al.,* 2007). MtDNA markers are powerful discriminators at subspecies level and they confirmed the three evolutionary lineages proposed by Ruttner (1988) (Garnery *et al.,* 1992, Arias and Sheppard, 1996). Later, Frank *et al.* (2000a) reported the presence of the fourth lineage (O). Both nuclear and mitochondrial DNA polymorphism among *A. mellifera* subspecies have been determined by many researchers (Smith *et al.,* 1989, 1991; Garnery *et al.,* 1993, 1995; Franck *et al.,* 1998, 2000a, 2001).

In this research we used two different molecular methods, RAPD and microsatellite in order to determine the genetic variation of honey bee populations in Turkey. The relevant literature to some extent is presented below.

1.6. Random Amplified Polymorphic DNA (RAPD)

RAPD is one of the PCR techniques described by Welsh and McClelland (1990) and Williams *et al.* (1990), in which DNA fragments are randomly amplified by randomly designed primers (10 bases in length). RAPD does not require any specific knowledge of the DNA sequence of the target organism as the primers will bind randomly into complementary region in the whole sequence. RAPD analysis has been used for many insect populations in order to determine genetic variation and population structure.

O'Donnell (1996) studied with eusocial wasp *Polybia aequatorialis* in order to detect genotypic effects of forager specialization using RAPD methods. Swarm-founding wasp workers were used for the analysis and genotypic similarity detection. Although RAPD band patterns determined association with forager specializations which were found in two colonies, this pattern changed with the collection time in one colony. Also colony- level selection and variation in division of labor were the factors favoring the evolutionary maintenance of low relatedness in wasp and in other eusocial insects.

Taraves *et al.* (2001) worked with *Mellipona quadrifasciata* which is an important pollinator in several Brazilian ecosystems and this species has a great potential to become model organism for genetic studies because of its lack of sting and the possibility of making controlled crosses in laboratory. They used backcross progeny consisted of diploid drones and females in order to determine inheritance patterns of RAPD markers using 79 primers which yielded 527 bands with an average of 6.67 bands per primer. Three types of polymorphism were observed; band intensity, presence/absence, fragment length polymorphisms. Using these data, some primers were selected to verify the inheritance pattern of this marker in back cross progeny.

The genetic dissimilarity was evaluated between *M. q. quadrifasciata* and *M. q. anthidioides* in Brazilian *meliponinae* populations based on RAPD data (Waldschmidt *et al.,* 2002) and showed that these subspecies were distinguishable with RAPD markers.

RAPD markers were used in two other insect species, *Anopheles nuneztovari* in Colombia (Posso *et al.,* 2003) and *Aedes aegypti* populations in Brazil (Paduan *et al.*, 2006) in order to determine genetic variation and population structure and found ahigh gene diversity. RAPD analyses indicated significant correlation between genetic variation and geographic distances. In *Drosophila virilis* group, Mikholovsky *et al.* (2007) estimated RAPD variation and compared it with morphological, cyctological and hybridological data and found that *D. virilis* is equally distant from all the species of its group. In our laboratories, a RAPD analysis was carried out in order to determine the genetic variability among six silkworm (*Bombyx mori*) strains with different origins (Tunca *et al.,* 2007) showing that the genotypic diversity inferred from Shannon's index was higher than gene diversity for all populations, which suggests that diversity is distributed among lineages.

In honey bees, RAPD markers are used in determination of the genetic structure of different populations as well as in behavioral genetics, detection of Quantitative Trait Loci (QTL), linkage analysis, and paternity testing.

Hunt and Page (1992) demonstrated the inheritance pattern of RAPD markers in honey bees. 20 haploid drone and 12 diploid worker progeny of single queen artificially inseminated with single drone yielded four types of polymorphism; band presence/absence, band brightness polymorphism, fragment length polymorphism and heteroduplex band polymorphism. Although RAPDs are known as dominant markers, their results showed that heterodublex band formation gave rise to alleles which showed a small fragment length polymorphism, resulting in codominant markers.

Fondrk *et al.* (1993) have applied RAPD to paternity analysis of worker honey bees from a single colony containing a polyandrous queen and observed that subfamily membership of all individuals was clustered correctly. This illustrated that RAPD markers can be used for paternity analysis and also determination of population structure and genetic relationships.

Hunt and Page (1994a) constructed honey bee linkage map for black body color and malate dehydrogenase and also reported linkage analysis of sex determination in the honey bee using RAPD analyses (Hunt and Page, 1994b). Kraus and Hunt (1995) tested a model of coevolution of *A. mellifera* and its parasite, *Varroa jacobsoni*, and determined the origin of mites using RAPD markers.

Suazo *et al.* (1998) had screened 700 RAPD primers in order to determine the differences between African and European honey bee populations and discriminate them. The samples were clustered into four groups; old world European, new world European, South African and new world African and found one or two alleles that are specific markers for each group of bees.

Hunt *et al.* (1998) detected the Quantitative Trait Loci (QTL) for honey bee stinging behavior and body size using RAPD data and wings characters. They showed that several QTLs affect the intensity of colony stinging response and influence size of workers and drones. Chandra *et al.* (2001) also used RAPD markers and detected QTLs associated with reversal learning and latent inhibition in honey bee and concluded that learning behavior could be influenced by one locus with large effect.

Genetic variation was demonstrated using RAPDs in two different mountain regions and Thrace region of Bulgaria and Turkey (Ivanova *et al.,* 2007). Furthermore, genetic composition of the Iranian honeybee (*A. m. meda*) populations and genetic variation of Van Lake region honeybee populations was shown (Kence *et al.,* 2004; Tunca *et al.,* 2004). Genetic structure of 16 different honeybee populations of Turkey was determined using 20 different RAPD primers (Özdil *et al.,* 2006).

1.7. Microsatellites

Microsatellites (SSR) are short tandemly repeated sequences of DNA. In eukaryotes, according to Beckmann and Weber (1992), database analyses of the tandem repeats in genomic sequences illustrated that CA/TG repeats are the most common dinucleotide repeats, occurring about three times as often as AG/TC repeats and two times as frequently as AT repeats.

It is found that the types of microsatellites vary between species, GT type is being more abundant in mammals and *Drosophila,* whereas AT type in *Arabidopsis thaliana* and yeast and GA type in *Caenorhabditis elegans* (Schlötterer, 2000).

The draft sequence of the human genome showed that microsatellites hold 3% of the genome and the number of microsatellite loci are more than one million (Human Genome Consortium, 2001). While dinucleotide repeats are the most, trinucleotide repeats are the least frequent types in the human genome. Among the dinucleotides, (CA)n repeats are the most frequent, followed by (AT)n, (GA)n and (GC)n (Human Genome Consortium, 2001; Ellegren, 2004). It is also found that the mouse genome is repeat rich having two to threefold more microsatellites than human genome (Mouse Genome Consortium, 2002). In plants, AT-rich type dinucleotide repeats are the most common (Lagercrantz *et al.,* 1993).

Although, microsatellites are known as neutral markers (Arias *et al.,* 2006; Behura, 2006), many studies have shown that microsatellites have functional roles such as regulatory or coding elements on the genome in different species. According to studies of the functional role of the microsatellites, the microsatellite regions have been related to the upstream locations of same gene in different species. For instance; Braaten *et al.* (1988) found that mouse, rat and human have poly (TG) upstream of the rRNA transcription. Also, Cavener *et al.* (1988) showed that poly (CTGA) upstream of the same two genetic loci (Gld, Ted) was located in each of three *Drosophila* species.

Furthermore, microsatellites have a role in microbial gene expression (*i.e.* tetra-nucleotide repeats are present within the ORFs in genes coding for *Haemophilus influenzae* lipopolysaccharides,with variation in repeat number influencing protein production) (Van Belkum, 1999; Oliveira *et al.*, 2006).

1.7.1. Microsatellites in Insect genomes

Microsatellite composition and distribution vary in insect genomes. Archak *et al.* (2007) studied with five fully sequenced insect genome databases (*Drosophila, Anopheles, Bombyx, Tribolium,* and *Apis*) in order to determine the microsatellite compositions of insects. The most common repeat types were di- and tri-nucleotide repeats in *Drosophila* and *Anopheles*; however, tri- and tetra -nucleotide repeats were very ample in *Bombyx and Tribolium*. The shorter microsatellites and imperfect repeat types were very common in these insect genomes (Archak *et al.,* 2007). The higher number of compound microsatellite loci containing spreads of two or more different repeats was observed in *Apis* (6.12%). Archak *et al.* (2007) pointed out that the differences of those species as most of the microsatellites were present in exon regions and repeat types of microsatellites were trinucleotide except in *Apis* in which microsatellites were generally *AT* rich (Archak *et al.,* 2007).

1.7.2. Mutation Mechanism of Microsatellites

The evolutionary dynamics of microsatellites are influenced by many potential factors which are repeat number, sequence of the repeat motif, length of the repeat unit flanking sequence, interruptions in the microsatellite, recombination rate and transcription rate (Schlötterer, 2000).

The mutation mechanism of microsatellites has been focused on two models. One model includes unequal crossing over (UCO) from the recombination between homologous chromosomes that are imperfectly aligned (Eisen, 1999). But there is little evidence that recombination like UCO contributes to microsatellite evolution (Ellegren, 2004).

The distribution of genomic microsatellites is related with the site of recombination. Furthermore, the correlation between microsatellite density and recombination rate has not been found yet and also no evidence is available for the systematic differences in the rates (Ellegren, 2004).

Second model, the predominant one, is DNA slippage can occur during replication with a consequence of mispairing between the nascent and template strands. Later, the repeated DNA fragment is forced to "loop out" at the mismatch site. If this molecule is used for the production of new DNA strand, the microsatellite repeat number will change (Ellegren, 2004; Fan *et al.*, 2007). There are two important pathways for error correction which are exonucleolytic proofreading and post replication mismatch repair (Eisen, 1999).

1.7.3. Models of Mutation mechanisms for Microsatellite

There are four models of mutations generation of microsatellites. These are infinite allele model (IAM), stepwise mutation model (SMM), 'K' allele model (KAM) and two phase mutation model (TPM or MMM). SMM is the most common allele model for the microsatellite mutation.

1.7.3.1. Infinite allele model (IAM)

Infinite allele model (IAM) was proposed by Kimura and Crow (1964). Each new mutation randomly produces new allele and always results in a new allele state not previously encountered in the population (Estoup *et al.*, 2002; Oliveira *et al.*, 2006). According to this model, mutations change the length of repeats and IAM assumes that every mutation process leads to a novel allele that is not present in the population (Estoup *et al*., 1995). Therefore, IAM is also known as a nonhomoplasious model (Cornuet *et al*., 1999; Estoup *et al*., 2002). Wright's (1931) F_{ST} statistics is used for determination of inter population differentiation based on IAM. However, this model does not compatible with real mutation process based on microsatellite mutation studies (Fan *et al.*, 2007).

1.7. 3. 2. Stepwise Mutation Model

Stepwise mutation model was proposed by Ohta and Kimura (1973). The stepwise mutation model describes mutation of microsatellite by the loss or gain of single tandem repeat. SMM depends on the small changes in repeat number, equal probabilities of increasing and decreasing repeat number, unlimited allele size, independence of the rate and size of mutations from the repeat number (Fan *et al*., 2007). Therefore, any newly mutated allele may not be a new allele for the population and also alleles, more closely related in their length, have a more recent ancestor (Ellegren, 2004). SMM is in agreement with the strand-slippage replication mechanism currently accepted as the main mechanism for microsatellite mutations (Fan *et al.,* 2007).

1.7.3.3. Two phase (TP) model

Two phase model was proposed by Di Rienzo *et al*. (1994). Two phase mutation model (TPM) is also termed as generalized or multistep mutation model (MMM). This model states that mutations introduce a loss or gain X repeats (Estoup and Cornuet, 1999). This model is the extension of the SMM. There is positive correlation with variance in repeat number and multistep mutation. If the repeat number variance increases, the frequency of MMM will increase. Various organisms like primates showed TPM and the proportion of MMM in human dinucleotide repeats was determined to be 62.9% (Huang *et al.,* 2002).

1.7.3.4. K-alleles model (KAM)

The other mutation model is KAM proposed by Crow and Kimura (1970). The model assume that if there are exactly *k* possible alleles in a given locus then the probability of a given allele mutating into any other is $\mu/k-1$, where μ is the mutation rate (Estoup *et al.,* 2002; Oliveira *et al.,* 2006)

1.7.4. Homoplasy

Homoplasy is a concept that the given character present in two species is not derived from same character in common ancestral species, but the similarity is due to factors such a convergence, parallelism or reversion (Estoup and Cornuet, 1999). If homoplasy term is indicated in the gene level, it is identical in state, but not identical by descent. Except IAM, homoplasy is anticipated under SMM, TPM and KAM. Furthermore, homoplasy depends on evolutionary factors which are the mutation rate, effective population size, and the time of divergence between populations but this has not been formulized theoretically (Estoup *et al.,* 2002).

If homoplasy is evaluated under microsatellite fragment size (This is also called size homoplasy, SH), similar microsatellite fragments are identical in state but not identical by descent due to convergent mutations which have occurred in the lineages connecting the gene copies and their recent common ancestor. According to Estoup *et al.* (2002), population studied may influence homoplasy including high mutation rates and population size together with strong allele size constraints. Slatkin (1995) proposed a model that depend on traits with continuous distribution, number of base pairs or number of repeats, and groups individuals according to the number of repeats (Oliveira *et al.,* 2006). The significant amount of SH is anticipated at most of the microsatellite loci for the reason that;

(I)According to population and simulation studies, microsatellites evolve in stepwise way and fit SMM, TPM and KAM, although IAM cannot be always rejected (Shriver *et al.,* 1993; Estoup and Cornuet, 1999).

(II)Microsatellite mutation rate is very high per locus per generation in humans (Weber and Wong, 1993; Estoup and Cornuet, 1999)

(III) Selective constrictions act on the allele size range. This mechanism decreases the number of possible allelic states and supports the SH (Estoup and Cornuet, 1999; Estoup *et al.,* 2002).

1.7.5. Comparison of mutation models

Microsatellite mutations can be evaluated by using many mutation models. Mutations can be observed directly from pedigree analysis. Many studies illustrated that TPM and SMM were more realistic mutation models for microsatellites (Estoup *et al.,* 2002). According to Slatkin (1995) and Goldstein *et al.* (1995), IAM is not adequate for microsatellites because of their high mutation rates. Also the process of mutation conserves memory of ancestral allelic states.

Furthermore, many studies showed that different mutation models could fit to different organisms. Colson and Goldstein (1999) illustrated that allelic frequency distribution, detected from microsatellites in Sardinian human population, were more fit to the TPM. However, microsatellite variation was appropriated with SMM in three related species of *Drosophila* (Colson and Goldstein, 1999). Estoup *et al.* (1995) illustrated that IAM was a better model for microsatellites having repeats of two or three different length motifs in honeybee species.

1.7.6. Measures of Genetic Distances

Genetic distance is the gene difference between populations or species that is measured by numerical quantities. The genetic distance concept was first used by Sanghvi (1953) for evolutionary analysis but for the state of population differentiation, the similar concept was used Czekanowski (1909) and Pearson (1926). Later, Fisher (1936) and Mahalanobis (1936) improved these methods. Cavalli-Sforza and Edwards (1964; 1967) constructed the evolutionary tree of human populations in terms of gene frequency data. Genetic distance based on angular transformation of gene frequencies was used at Fisher's model. In 1972, Latter developed many measures that were closely related to Wright's (1951) fixation index. After that, Nei (1971, 1972 and 1978) proposed genetic distance methods using codon or gene substitutions per locus between two populations. In addition, many recent studies showed that microsatellite mutations followed SMM rather than IAM, which lead to development of new measures of genetic distance over older ones which were based on IAM.

X^2 distance of Shangvi's (1953), chord distance D_c of Cavalli-Sforza and Edwards (1967), Rogers' (1972) distance $D_{R,}$, Latter's (1972) *Fst* distance, Nei's (1972) standard genetic distance $D_{S,}$ Nei's (1973) minimum genetic distance, Nei *et al.'s* (1983) D_A, Reynolds' distance, Reynolds *et al.* (1983) (linearized F_{ST} for short divergence time) Goldstein *et al.*'s (1995) $(\delta\mu)^2$ distance, Slatkins' (1995) genetic distance R_{ST} (derived from F_{ST} values), Shriver *et al.'s* (1995) D_{SW} and Paetkau *et al.*'s (1995) likelihood genetic distance, D_{LR} can be used for determination of genetic differention of populations.

However, Takezaki and Nei (1996) constructed a computer simulation program for different conditions and different mutation models in order to evaluate which program gave the correct topology of tree. Allele frequencies were generated and Neighbour-Joining (*NJ*) and the Unweighted Pair Group Method with Arithmetic mean (*UPGMA*) trees were constructed. They replicated the data for 200 times and calculated the percentage of replication in which the correct topology was calculated. According to their simulation study, the correct tree topology can be assigned better with D_A and D_C distance in many different conditions examined. (Nei, 1987). *Ds* and $(\delta\mu)^2$'s expected values increase linearly with time under the IAM and the SMM, respectively.

However, the efficiencies of D_S and $(\delta\mu)^2$ are low for the correct tree topology, as D_S and $(\delta\mu)^2$ have a large sampling error (Takezaki and Nei, 1996). The general conclusion is that for the topology construction D_A and D_C are predominant measures than the other distance measures. However, for the branch length estimation, D_S and $(\delta\mu)^2$ are better than other measures (Takezaki and Nei, 1996). Among different genetic distance measures, D_S and D_{LR} gave the best result (Paetkau *et al.*, 1997).

1.7.7. Population studies based on microsatellite variations

The structures and differences of populations are detected using microsatellites variation. Microsatellites have been used for determination of genetic structure of honeybee populations.

Three African (*intermissa, scutellata,* and *capensis*) and four European (*mellifera, ligustica, carnica,* and *cercopia*) *A. mellifera* subspecies were studied with seven microsatellite loci (Estoup *et al.,* 1995). High genetic variation was observed between populations. The average heterozygosity and average number of alleles were significantly higher in African than in European subspecies. The study verified that *A. mellifera* evolved in three distinct and deeply differentiated lineages A, C, M previously detected by morphological and mitochondrial DNA studies (Estoup *et al.,* 1995).

Franck *et al.* (2000a) characterized Lebanese honeybee populations using eight microsatellite loci and mtDNA in honeybee samples A, M, and C lineages. Both microsatellite and mtDNA results supported the existence of O lineage. The western European honeybee subspecies profiles have been shown to be rather homogeneous from Spain to Scandinavia but Iberian honeybee populations appear to have some controversy. Samples from Iberian Pennisula were observed to be related with *A. m. intermissa* whereas some alleles and heterozygosity level showed interaction between *A. m. mellifera.* The study showed the nature of the hybrids without recent introgression from Africa and human affects (De la Rúa *et al.,* 2002)

Solignac *et al.* (2003) isolated 550 microsatellite loci from either genomic library of individuals belonging to the subspecies of *A. mellifera* or from BAC (Bacterial Artificial Chromosome) library. They performed cross priming test for three Asiatic *Apis* species and they optimized these microsatellite loci using M, C, and A lineages.

De la Rúa *et al.,* (2003) used microsatellites to determine genetic structure of Balearic honeybee populations and evaluate their evolutionary relationships of Iberian and African populations. The number of alleles and heterozygosity level

showed very low variation in island populations. Their results showed that ancestral populations should be conserved from threatening factors like queen bee importations. Furthermore, De la Rúa *et al.* (2006) determined the distribution of mitochondrial haplotypes of Azorian and Madeiran (Macaronesian archipelagos) honeybee populations and the population structure using microsatellites. The genetic variation in populations of Canarian islands was determined and compared with Morocco, Portugal and southern Spain continental honeybee populations. They concluded that the differences of the genetic structure of local populations are possibly the results of agricultural practices.

Dall'olio *et al.* (2007) determined the genetic variation of *A. m. ligustica* populations from Italian Peninsula and Sardinia using microsatellites. *A. m. mellifera*, *A. m. carnica* and the Buckfast breeding line were used to compare the Italian bees. High number of alleles and heterozygosity were detected but F_{IS} and F_{ST} values were very low. These results illustrated the absence of genetic structure within and among *A. m. ligustica* populations.

Bodur *et al.* (2007) studied twelve honeybee populations, eleven from Turkey and one from Cyprus and determined genetic structure of populations using microsatellites. They illustrated that the large number of rare alleles and highly differentiated populations is consistent with role of Anatolia as genetic center of Middle Eastern honeybees.

1.8. Purpose of the Study

The objective of this study was to determine and compare the genetic variation of honey bee populations in Turkey using RAPD markers for the first time and to expand on the previous study by collecting genetic data from six different microsatellite loci. Furthermore, at a larger scale to compare the genetic differentiation among these populations with European and African honey bee populations as well as phylogenetic relationships of those populations.

CHAPTER 2

MATERIALS AND METHODS

2.1. Biological Material

Specimens were collected by random sampling for all locations and honeybee colonies. A total of 760 honeybees, two samples per colony, 390 colonies in 26 provinces in Turkey. The map that demonstrates the sampling area is shown in Figure 2.1 and numbers of collected honeybees per location are given in table 2.1.

Figure 2.1. Map of the sampling area (http://earth.google.com)

Table 2.1. Honeybee samples size per location

Locations	Latitude	Longitude	N	Regions
Hatay	36°14'N	36°10'E	30	South Anatolia (Mediterranean region)
Antalya	36°52'N	30°45'E	30	
Aydın	37°51'N	27°51'E	30	West Anatolia (Aegean region)
İzmir	38°25'N	27°08'E	30	
Manisa	38°38'N	27°30'E	30	
Mugla	37°15'N	28°22'E	30	
Uşak	38°68'N	29°40'E	30	
Artvin	41°14'N	41°44'E	30	North Anatolia (Black sea region)
Sinop	42°1'N	35°11'E	30	
Trabzon	41°0'N	39°45'E	30	
Giresun	40°55'N	38°30'E	30	
Beyşehir	37°41'N	31°33'E	30	Central Anatolia
Nevsehir	38°33'N	34°40'E	30	
Sivas	39°43'N	36°58'E	30	
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	North West Anatolia (Marmara and Thrace Region)
Kırklareli	41°44'N	27°15'E	30	
Bingol	38°53'N	40°29'E	30	East Anatolia
Kars	40°40'N	43°05'E	30	
Van	38°30'N	43°0'E	30	
Bitlis	38°20'N	42°03'E	30	
Şanlıurfa	37°13'N	38°76'E	30	South East Anatolia
Bozcaada	39°49'N	26°03'E	30	Marmara Region (Islands)
Gökçeada	40°10'N	25°50'E	30	

Some of the collected honeybee samples were kept in -80 ^{O}C deepfreeze and others were kept in pure ethanol until DNA was isolated.

2.2. DNA Isolation and purification test

We used Fermentas 512 DNA purification kit for DNA isolation. For the isolation of DNA, bee thorax was used. Each thorax was put into a 1.5 ml tube which contained 200 µl TE buffer (Appendix A). Each sample was grounded in TE solution in order to prepare the samples for DNA isolation. DNA isolation protocol is given below.

- 400 µl lysis solution was added to each sample and samples were incubated at 65 ^{o}C for 5 minutes,
- After incubation, 600 µl chloroform was added to each sample,
- The samples were centrifugated at 10,000 rpm for 2 min,
- Before adding the precipitation solution, we diluted the 10X concentrated solution. In order to prepare precipitation solution, 720 µl sterile deionized water was mixed with 80 µl of supplied 10X concentrated solution.
- After centrifugation, we transferred upper aqueous phase containing DNA to the new tubes and added 800 µl of newly prepared precipitation solution.
- Mixture was inverted at room temperature for 2 minutes and centrifuged at 10.000 rpm for 2 minutes,
- We removed supernatant and dissolved the DNA pellet in 100 µl of 1.2 M NaCl solution. This stage was very important and we had to make sure that pellet was dissolved in the NaCl solution.
- 300 µl pure cold ethanol was added and DNA precipitates were conserved at -20 ^{o}C,
- Samples were centrifuged at 10.00 rpm for 4 minutes and pellets were washed with 70% cold ethanol,
- Alcohol was removed from all samples and DNA pellet was dissolved in 100 µl deionized water.

After DNA isolation, the DNA quantity was examined with spectrophotometer. 260 and 280nm were used to determine absorption of DNA and protein. DNAs were diluted in order to get 5 ng DNA per 1µl TE buffer or H_2O according to PCR protocols.

2. 3. RAPD amplification by PCR

A total of 10 primers of arbitrary sequences having high G+C content (60 %) from, OPA7, OPB1, OPB2, OPB3, OPB4, OPB5, OPB6, OPB7, OPB8 and OPB9 (Operon Tech. Alameda, CA. USA) were tested on honeybees (Table 2.2). These primers were used since they have been widely used for determination of genetic variation in different species. RAPD reactions were carried out on a final volume of 15 µl containing 25 ng DNA, 100 µM each of dNTP, 15 ng primer, 1U Taq DNA polymerase. Reactions were run for 35 cycles which consists of 94°C for 30 s (one cycle), 94°C 1 min, 35°C for 1min, 2 min ramp to 72°C, 72°C for 2 min (5 cycles), 94°C for 10 s, 35°C for 30s, 72°C for 30 s (30 cycles) and a last step at 72°C for 1 min.

Table 2.2. Primer name and sequences of RAPD primers

Primer Name	Sequences
OPA7	5'-GAA ACG GGT G-3'
OPB1	5'-GTT TCG CTC C-3'
OPB2	5-'TGA TCC CTG G-3'
OPB3	5-CAT CCC CCT G -3'
OPB4	5'-GGA CTG GAG T-3'
OPB5	5'-TGC GCC CTT C-3'
OPB6	5'-TGC TCT GCC C-3'
OPB7	5'-GGT GAC GCA G-3'
OPB8	5'GTC CAC ACG G-3'
OPB9	5'-TGG GGG ACT C-3'

2.3.1. Agarose Gel Electrophoresis

Amplification products were resolved by electrophoresis in 1.2 % agarose. Gels were run in 0.8 X TBE buffer (Appendix A). For the preparation of gel solution, we weight 1.8 gr agarose in 150 ml 0.8 X TBE buffer, waiting 2 minutes in order to boil the agarose in microwave. After gel got cold enough, the gel was poured into an electrophoresis gel tray and fit the well comb in gel tray. After the polymerization of gel, the well comb was removed. The gel was placed to the gel tank and enough gel buffer was added to cover the gel surface. We loaded each sample to each well. When the samples were loaded, loading buffers (Appendix A) was added to each sample and so a mixture of the sample and loading buffer were loaded. 5µl of λ DNA hind III/EcoRI was loaded into the first and last wells of the gel as standard DNA marker before running the gel. Double entering power supply was used in the electrophoresis and samples were run under 110 v/h stable electric field. After the electrophoresis procedure, the gels were stained with ethidium bromide solution (10µ/l ml) and visualized under UV.

2.4. Microsatellite amplification by PCR

Six *Apis mellifera* specific microsatellite loci, namely Ap001, Ap223, Ap243, Ap289, Ap019 and A76 were used, respectively in this study (Solignac *et al.*, 2003). These six loci were used for detection of genetic variation in Turkish honey bee populations and comparision of the variation of both European and African populations studied by Solignac *et al.* (2003). The core regions and primer sequences of these loci are given in (Table 2.3 and 2.4).

Table 2.3. Core sequences of microsatellite loci

Locus name	Core sequence
Ap001	$(CA)_8(TA)_{15}A_3(TA)_8$
Ap223	$(T)_5(C)_4A(T)_6(C_5)$
Ap243	$(TCC)_9$
A076	$(CT)_{32}$
Ap19	$(TC)_{11}$
Ap289	$(GA)_5$

Table 2.4. Sequences of forward and reverse primers for microsatellites

Locus	Forward primer	Reverse primer
Ap001	ACACGCGAACAATACAACA	ACTAATCGGCACGATGAAG
Ap223	TCGTACAACGTCGCGCAA	GCCGCTCGCCTGTATCTG
Ap243	AATGTCCGCGAGCATCTG	TGTTTACGAGAATTCGACGGG
A076	GCCAATACTCTCGAACAATCG	GTCCAATTCACATGTCGACATC
Ap019	CTCGTTTCTTCCATTGCG	CGGTACGCGGTAGAAAGA
Ap289	AGCTAGGTCTTTCTAAGAGTGTTG	TTCGACCGCAATAACATTC

PCR amplifications were carried out according to Estoup *et al.* (1995). Each PCR tube contained 25 µl of PCR solution containing 50 ng of template DNA, 400 nM of each primer, 75 µM of each 2'-deoxythymidine 5'-triphosphate (dTTP), 2'-deoxyguanidine 5'-triphosphate (dGTP) and 2'-deoxycytidine 5'-triphosphate (dCTP), 75 µM of 2'-deoxyadenosine 5'triphosphate (dATP), 20 µg/ml bovine serum albumin (BSA), 1x reaction buffer containing $(NH_4)_2SO_4$, 0.4 unit of Taq polymerase, and 1.2-1.5 mM $MgCl_2$. PCR started with a denaturation step of 3 minutes at 94 ºC and followed by 35 cycles each consisting of a 30 second denaturation segment at 94 ºC, a 30 second annealing segment at the optimum temperature, and a 30 second elongation segment at 72 ºC. The final elongation step was extended to 10 minutes in order to allow all the products to be fully extended. The forward primer of each marker was 5′-end-labelled with a fluorescent phosphoramidite (NED, 6-FAM or HEX). ROX 500 was used as size standard. ROX500™ Size Standard is designed for

sizing DNA fragments in the 35-500 nucleotides range and provides 16 single-stranded labeled fragments of 35, 50, 75, 100, 139, 150, 160, 200, 250, 300, 340, 350, 400, 450, 490 and 500 nucleotides. The annealing temperatures and $MgCl_2$ concentrations for each microsatellite locus are given in Table 2.5.

Table 2.5. $MgCl_2$ concentration (M), Annealing Temperatures (^oC) and Flourecent labeling of the microsatellite loci.

Locus	Labelled with	$MgCl_2$ concentration (M)	Annealing Tempratures(^oC)
Ap001	FAM	1.2mM	53
Ap223	NED	1.5mM	54
Ap243	HEX	1.5mM	51-54
Ap289	FAM	1.5mM	58-51
Ap019	NED	1.5mM	54
A76	HEX	1.5mM	51

2.4.1. Fragment analyses

Fluorescent labeled, HPLC purifity, oligonucleotide primers were used for the fragment analyses and fragment length detection was done by laser detection system. The system used to assignment of the fragment size, potential alleles, via fluorescent dye-labeled oligonucleotides. In this study, 6-carboxy-fluorescine (FAM), 6-carboxy-4, 7, 2', 4', 5', 7'-hexachlorofluorescein (HEX), and 2, 7', 8'-benzo-5'-fluoro-2', 4, 7-trichloro-5-carboxyfluorescein (NED) labeling were used (Table 2.5). All PCR products were done ABI 3130 X 16 capillaries sequencer systems and the intensity of emitted fluorescence is different for each dye. All PCR fragments gave several peaks contained allele size of fragments. In order to correct assignment of the peak, one can use the allele size range from literature or direct observation of peaks. GENEMAPPER^{TM} (v.4.0) software package was utilized for fragment analysis**.**

2.4.2. Genotyping

Following the fragment analysis, genotyping were done with the evaluation of the gel bands together with the result of the fragment analyses in order to minimize the error of misconstruction. The most common problems during genotyping process are; poor or non-specific amplification, and incomplete 3´ a nucleotide addition. This problem solved with applying suitable methods by aid of references guide of softwares (GENEMAPPER^{TM} v.4.0).

2.5. Statistical analyses

Polymorphic and monomorphic bands were determined and marker scored as present (1) or absent (0) for all individuals for RAPD analyses. For microsatellite analyses, allele sizes were determined and allele frequencies, pairwise F_{ST} measures, heterozygosity levels, gene diversity were estimated, Hardy Weinberg Equilibrium (HWE), linkage disequilibrium, assignment tests were done, genetic distance were calculated and used for construction of dendrogram.

2.5.1. Genetic Structure

Different test can be used to identify the genetic structure of honey bees.

Hardy-Weinberg Equilibrium Tests

Genepop v.4.0 (Raymond and Rousset 1995) and Arlequin v.3.11 (Excoffier *et al.*, 2006) were used to test the populations at HWE for microsatellites. Both softwares calculated *P*- values with the *Exact HW Test* of Haldane (1954), Markov Chain (MC) of Guo and Thompson (1992), and Weir (1996). Genepop uses the Complete Enumeration algorithm of Louis and Dempster (1987) and Markov Chain (MC) walk algorithm of Guo and Thomson (1992) and a form of *Metropolis-Hastings* algorithm (Metropolis *et al*., 1953; Hasting, 1970). Furthermore, Arlequin v.3.11 utilizes Markov-Chain random walk algorithm of Guo & Thompson (1992) and computes *P*-

values (Excoffier *et al.*, 2006). *P*-values give the significant deviation from HWE ($P< 0.05$).

Linkage Disequilibrium Test

Linkage disequilibrium (Lewontin and Kojima, 1960) also called as gametic phase disequilibrium (Crow and Kimura, 1970) occurs when the alleles at two or more loci co-occur more often than the expected on the basis of their frequencies. It usually involves two loci that are close together on a chromosome (Freeland, 2005). The null hypothesis for this option is that genotypes at one locus are independent from genotypes at the other locus. Pairwise linkage disequilibrium was performed with Genepop (Raymond and Rousset, 1995) 4.0 software program for 6 microsatellite loci. Probability test (Fisher's Exact Test) using Markov Chain algorithm of Raymond and Rousset (1995) were done after creating the contingency tables for all pairs of loci. *P* values for all pair of loci give level of significance about genotypic differences.

Fixation Index

Fixation indices, developed by Wright (1951) estimate the population differentiations from allele frequencies. F_{ST} is the correlation between two gametes drawn at random from each subpopulation and measures the degree of genetic differentiation of subpopulations (Wright, 1951) and ranges between 0 and 1. If two or more populations have the identical allele frequency, they will not differ from each other genetically therefore F_{ST} value equals to 0 (no differentiation). If the F_{ST} value is 1, the populations are fixed for different alleles in different populations (Wright, 1951). F_{ST} levels between 0 and 0.05 indicate little genetic differentiation; levels between 0.05 and 0.15 indicate moderate genetic differentiation; levels between 0.15 and 0.25 indicate great genetic differentiation and levels > 0.25 indicate very significant genetic differentiation (Wright, 1951; Hartl and Clark, 2007 625p).

In order to determine genetic differentiation of variation, different methods were developed. One of the methods used is *coefficient of gene differentiation*, G_{ST} developed by Nei (1973) and it ranges between 0 and 1. In the case the multiple alleles, G_{ST} is equivalent to the weighted average of F_{ST} for all alleles. G_{ST} highly depends on the gene diversity in total populations (H_T). G_{ST} value was computed with PopGene v.1.31 software (Yeh *et al.,* 1999) for RAPD results.

The pairwise F_{ST} values were calculated using Genepop (Raymond and Rousset, 1995) and tested for significance using FSTAT 2.9.3.2 (Goudet, 2002) with which multi-locus genotypes were randomised between pairs of samples (1530 permutations), and after Bonferroni corrections.

Both F_{IS} and F_{IT} are inbreeding coefficients used to describe the proportion of genetic variation within and total populations and to determine the deviation from HWE. F_{IS} measures the degree of inbreeding between related individuals. F_{IS} values reflect the deviation from heterozygosity level within subpopulations and calculated according to Wright (1978) and Weir & Cockerham (1984) whereas F_{IT} values indicate the heterozygosity level in total population (Freeman, 2005; Nei, 1987). F_{IS} can be positive and negative and range from -1 to +1. Also, F_{IT} can range from -1 to +1 depending on F_{IS} and F_{ST} values. The negative values demonstrate an excess of the heterozygotes, positive values will indicate deficiency of heterozygotes. F_{ST} can be calculated from allele frequencies alone but F_{IS} and F_{IT} can be determined from genotype frequencies. F_{IS} and F_{IT} were calculated with Arlequin and Genepop (Raymond and Rousset, 1995).

Shannon Diversity Index

Shannon index was adopted from community ecology and has been used to determine species richness (Shannon, 1949). Shannon index were calculated from RAPD data using PopGene v.1.31. (Yeh *et al.*,1999).

The formulation of Shannon's index;

$$H' = -\sum_{i=1}^{S} \frac{n_i}{N} \ln \frac{n_i}{N}$$

$$NH' = -\sum_{i=1}^{S} n_i (\ln n_i - \ln N) = -\sum_{i=1}^{S} n_i \ln n_i + \ln N \sum_{i=1}^{S} n_i$$

$$NH' - N \ln N = -\sum_{i=1}^{S} n_i \ln n_i$$

$$H_s = -\sum_{i=1}^{S} n_i \ln n_i$$

n_i: The number of individuals in species i; the abundance of species i

S: The number of species

N: The number of all individuals

p_i : The relative abundance of each species, calculated as the proportion of individuals of a given species to the total number of individuals in community n_i / N

So, final formulation of the index is

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

Analysis of Molecular Variance framework (AMOVA)

Analysis of Molecular Variance (AMOVA) estimates population differentiation directly from molecular data and testing hypotheses about such differentiation. Molecular marker data (for example, RFLP or AFLP, RAPD), direct sequence data, or phylogenetic trees based on this molecular data may be analyzed using this method (Excoffier *et al.*, 1992). It is possible to estimate genetic structure indices by using individuals' allele information and their allele frequencies (Excoffier *et al.*, 1992). Euclidian distance matrix was used to enter the information about differences

in allelic content between individuals. By using non-parametric permutation procedures the significance of the covariance components related to the different possible levels of genetic structure (within individuals, within populations, within groups of populations, among groups) is tested (Excoffier *et al.*, 1992). The permutation types differ for each covariance component. The kind of data and the genetic structure that is tested determine the number of hierarchical levels of the variance analysis and the kind of permutations to be done. Therefore, AMOVA test was performed for RAPD analyses in the Genalex 6.0 (Peakall and Smouse, 2006).

Gene Flow

The pairwise *Nm* for populations and *Nm* values for total population were estimated with Genepop software for microsatellites (Raymond and Rousset, 1995). This software provides a multi locus estimate of the effective number of migrants (*Nm*). The frequencies of private alleles observed only in one population were used in order to estimate the effective number of migrants per generation (Slatkin, 1995). *Nm* values give the information about genetic divergence between populations; if *Nm* value is smaller than 2, there is still considerable genetic differentiation among subpopulations (Hartl and Clarck, 2007). Furthermore, Popgene software was used to determine *Nm* value using RAPD data. The program calculates gene flow using G_{ST} or F_{ST} (Slatkin and Barton, 1989).

Genetic Distance

Genetic distance measurements were used for the construction of phylogenetic tree. For microsatellites, *Neighbor- Joining (NJ)* and *UPGMA* distance tree were used. PHYLIP v.3.68 and Popgene v.1.32 and TFPGA v.1.3 were used to estimate the Nei's standard genetic distance.

Nei's (1972) standard genetic distance (*Ds*)

$$D_S = -\ln\ [J_{XY}/\sqrt{J_X J_Y}]$$

$$J_X = \sum_j^r \sum_i^{m_j} x_{ij}^2/r$$

$$J_Y = \sum_j^r \sum_i^{m_j} y_{ij}^2/r$$

Jx and *Jy* are the average homozygosities over loci in populations *X* and *Y*, respectively

$$J_{XY} = \sum_j^r \sum_i^{m_j} x_{ij} y_{ij}/r$$

x_{ij} and y_{ij} are the frequencies of the ith allele at the *jth* locus in populations *x* and *y*, respectively.

Dendrograms were constructed using TFPGA v.1.3 (Miller 1997) from RAPD data and PHYLIP v.3.68 from microsatellite. Also NTSYS (v.2.20) software program (Rohlf, 2000) was used for consruction of *NJ* tree from both molecular data.

Assignment test

The assignment test calculates the log-likelihood of the genotype of each individual in its own population based on allele frequencies in that population (Halliburton, 2004; Paetkau *et al.,* 1995). GeneClass2 (Piry e*t al.,* 2004), Genalex6 (Peakall and Smouse, 2006) and Arlequin v.3.11 (Excoffier *et al.,* 2006) were used for calculation of log-likelihood value for both populations and individuals. Correct assignment value for populations was estimated using GeneClass2 program and also biplot of the respective log-likelihood values of individuals from two populations were constructed using Genalex6 softwares. Computations were checked with Arlequin

program. All programs used the formula of Paetkau *et al.* (1995) in order to compute log-likelihood values.

The log-likelihood can be calculated as follows,

$$Log(L) = Log(\Gamma(m+1)) - \sum_{i=1}^{k} Log(\Gamma(m_i+1)) + \sum_{i=1}^{k} m_i Log(p_i)$$

Γ is a gamma function

$$\Gamma(x+1) = x!,$$

m is the total number of genes to be assigned,
m_i is the number of copies of allele i in the to-be-assigned sample
p_i is the frequency of allele i in the reference population sample (Paetkau *et al*., 1995; Peakall and Smouse, 2006).

Consensus of Trees

Consensus trees are used to summarize the agreement between two or more trees. Majority rule consensus tree method was performed to construct the consensus tree. Two different genetic distance matrix data were used from RAPD and microsatellite and evaluated for 17 matching populations. PHYLIP 3.68 software program was used to construct consensus tree (Felsenstein, 2008).

Mantel Test

Mantel test is a statistical method using for evaluating the relationships between two distance matrices. It is widely used to compare genetic and geographical distances in population genetic studies (Mantel, 1967). The null hypothesis is that the determination of relationship between the two distance matrices could have been obtained by any random arrangement in the space of the observations through the study area (http://userwww.sfsu.edu/~efc/classes/biol710/spatial/spatial.htm).

Z statistic was calculated as

$$Z = \sum_{i=1}^{n} \sum_{j=1}^{n} A_{ij} B_{ij}$$

Where *A* and *B* are the distance matrices.

This value is commonly normalized via a standard normal transformation. This normalization is called *r* statistic (Matrix correlation).

$$r = \frac{\sum \sum stdA_{ij} stdB_{ij}}{n-1}$$

The significance can be tested using t statistic. Mantel test can be used to compare three or more distance matrices which is called Partial Mantel test. Both Mantel and Partial Mantel test were performed using NTSYS software program (v.2.20) (Rohlf, 2000). The distance matrices were attained from Nei's standard genetic distance matrix estimated from RAPD and microsatellites data and geographic distance matrix between the locations.

CHAPTER 3

RESULTS

3.1. RAPD Results

A total of 25 honey bee populations were studied for RAPD analysis using 10 primers (OPA-7, OPB-1, OPB-2, OPB-3, OPB-4, OPB-5, OPB-6, OPB-7, OPB-8, OPB-9). Amplification products are illustrated in figure 3.1 and Appendix B. After the evaluation of band patterns for all individuals, the data were computed using different softwares.

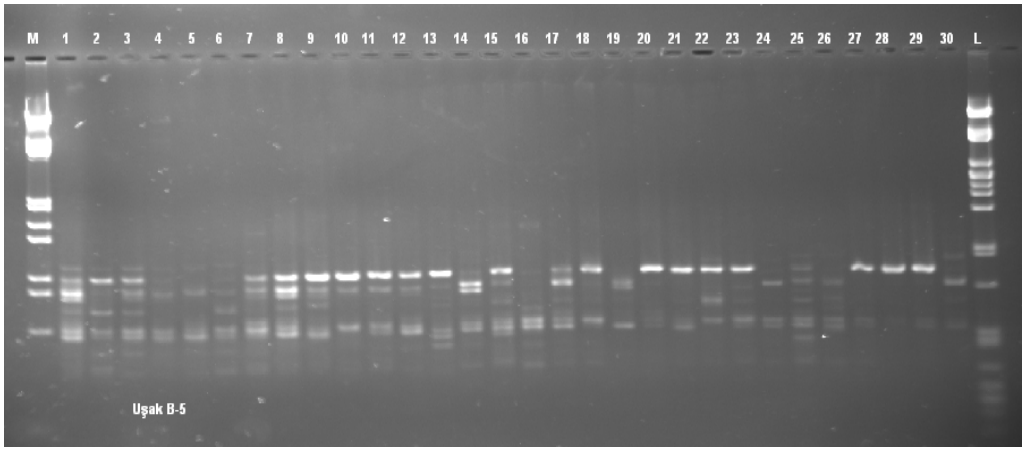


Figure 3.1. Banding pattern obtained by OPB-5 primer in Uşak honey bee population

Mean number of observed alleles per locus, mean number of effective alleles, percentage of polymorphic loci, expected heterozygosity (*He*), Shannon's information index (*H'*) values and their standard errors for populations were calculated (Table 3.1). The mean number of observed alleles ranged between 1.4 and 1.6, however, mean number of effective alleles ranged between 1.0 and 1.2. The highest number of observed alleles was detected in Antalya population (1.648), but the highest mean number of effective alleles was determined in Beyşehir population (1.286). Expected heterozygosity levels for Turkish honey bee populations ranged between 0.035 and 0.175 and the highest heterozygosity was observed in Antalya population (0.175), but the lowest heterozygosity value was detected in Şanlıurfa population (0.035). In total, 105 polymorphic loci were detected and the percentage of polymorphic loci was 100 % for all Turkish populations. The percentage of polymorphic loci was calculated also for each population and the highest and lowest percentage of polymorphic loci were observed in Antalya and Hatay populations, respectively (Table 3.1).

Mean gene diversity value for all populations was estimated as 0.187±0.1596 In addition, Shannon's index value for all populations was estimated to be 0.305± 0.2183 which ranged between 0.073 (Şanlıurfa) and 0.272 (Antalya).

Table 3.1. Mean number of alleles per locus, mean number of effective alleles, proportion of polymorphic loci, expected heterozygosity (*He*), Shannon's information index (*H'*) values and their standard errors for all populations.

Populations	Mean # of Obs. alleles	Mean # of Effective alleles	% polymorphic Loci	*He*	*H'*
Antalya	1.648±0.0480	1.291±0.0352	64.76%	0.175	0.271±0.0267
Artvin	1.40 ± 0.0492	1.159±0.0290	40.00%	0.098	0.155±0.0233
Aydın	1.610±0.0490	1.232±0.0320	60.95%	0.144	0.228±0.0249
Beyşehir	1.571±0.0497	1.286±0.0351	57.14%	0.172	0.262±0.0274
Bilecik	1.610±0.0490	1.162±0.0255	60.95%	0.109	0.184±0.0210
Bingöl	1.562±0.0499	1.175±0.0276	56.19%	0.113	0.185±0.0227
Bitlis	1.543±0.0501	1.185±0.0272	54.29%	0.121	0.197±0.0231
Bozcaada	1.619±0.0488	1.189±0.0259	61.90%	0.128	0.212±0.0219
Gökçeada	1.591±0.0494	1.191±0.0258	59.05%	0.129	0.212±0.0224
Hatay	1.371±0.0486	1.123±0.0249	37.14%	0.079	0.129±0.0209
İzmir	1.619±0.0488	1.278±0.0341	61.90%	0.169	0.261±0.0277
Kars	1.514±0.0502	1.160±0.0284	51.43%	0.101	0.164±0.0225
Kayseri	1.448±0.0499	1.182±0.0285	44.76%	0.116	0.183±0.0240
Kırklareli	1.562±0.0499	1.219±0.0316	56.19%	0.136	0.217±0.0248
Konya	1.543±0.0505	1.194±0.0266	54.29%	0.129	0.208±0.0233
Manisa	1.467±0.0501	1.170±0.0285	46.67%	0.107	0.172±0.0232
Muğla	1.543±0.0501	1.221±0.0316	54.29%	0.137	0.216±0.0250
Nevşehir	1.410±0.0494	1.129±0.0250	40.95%	0.083	0.136±0.0211
Sinop	1.499±0.0494	1.196±0.0303	40.95%	0.121	0.188±0.0254
Sivas	1.581±0.0496	1.252±0.0324	58.10%	0.156	0.245±0.0258
Şanlıurfa	1.419±0.0496	1.039±0.0591	41.90%	0.035	0.073±0.0090
Trabzon	1.495±0.0502	1.185±0.0278	49.52%	0.119	0.191±0.0237
Uşak	1.581±0.0496	1.174±0.0274	58.10%	0.114	0.189±0.0219
Van	1.438±0.0499	1.125±0.0231	43.81%	0.084	0.140±0.0204
Yozgat	1.533±0.0501	1.272±0.0347	53.33%	0.164	0.250±0.0272

Table 3.2. Mean gene diversity and Shannon's index value for geographic regions

	Mean *He*	Shannon's index
North Anatolia	0.112	0.178
South Anatolia	0.127	0.200
West Anatolia	0.134	0.213
East Anatolia	0.105	0.172
Southeastern	0.035	0.073
Central Anatolia	0.136	0.183
Thrace	0.136	0.217
Bozcaada	0.128	0.212
Gökçeada	0.129	0.212

Mean gene diversities for the 7 geographic regions of Turkey ranged between 0.035 (Southeastern Anatolia) and 0.136 (Thrace and Central Anatolia) (Table 3.2). Shannon's index values were determined between 0.073 (Southeastern Anatolia) and 0.217 (Thrace region).

Band patterns among Turkish honey bee populations were illustrated in figure 3.2 and table 3.3. The highest numbers of bands were observed in Bozcaada and Antalya populations as 72 and 70, whereas the lowest numbers of bands (39 bands) were observed in Hatay population. 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 (Table 3.3). Mean heterozygosity values and their distributions were illustrated in table 3.3 and figure 3.2. The mean heterozygosity was very high in Antalya and Beyşehir populations compared to others.

Based on RAPD results, gene diversity in total populations (H_T) was 0.188 and magnitude of differentiation among populations (G_{ST}) was 0.3517. Gene flow (Nm) based on G_{ST} was calculated as 0.9218 for all populations. Since gene flow value is smaller than 2, there is still considerable genetic differentiation among subpopulations.

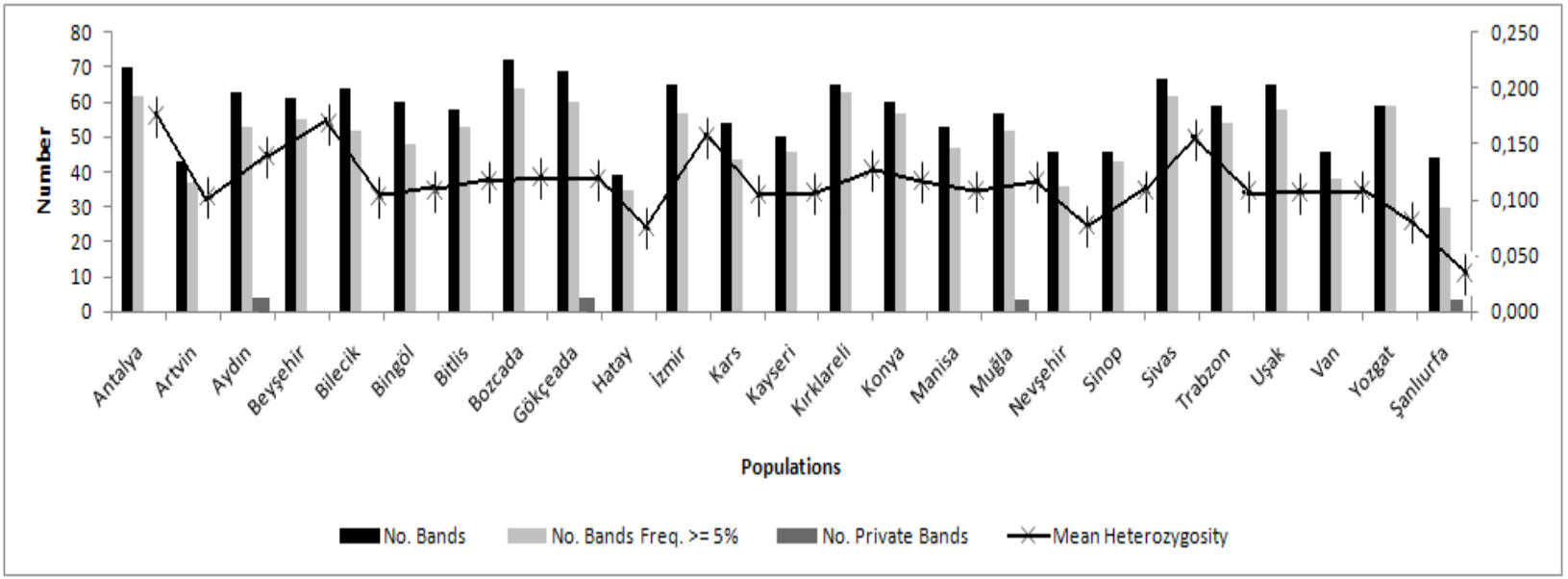


Figure 3.2. Band pattern across Turkish honey bee populations illustrating the number of bands, their frequencies, private bands and distribution of mean heterozygosities for all populations

Table 3.3. Number of bands, number of private bands and mean heterozygosity for Turkish honey bee populations

Population	Antalya	Artvin	Aydın	Beyşehir	Bilecik	Bingöl	Bitlis	Bozcaada
No. Bands	70	43	64	61	64	60	58	72
No. Private Bands	0	0	1	0	0	0	0	0
Mean Heterozygosity	0.175	0.098	0.144	0.172	0.109	0.113	0.121	0.128

Population	Gökçeada	Hatay	İzmir	Kars	Kayseri	Kırklareli	Konya	Manisa
No. Bands	69	39	65	54	50	65	60	53
No. Private Bands	1	0	0	0	0	0	0	0
Mean Heterozygosity	0.129	0.079	0.169	0.101	0.116	0.136	0.129	0.107

Population	Muğla	Nevşehir	Sinop	Sivas	Trabzon	Uşak	Van	Yozgat	Şanlıurfa
No. Bands	57	46	46	67	59	65	46	59	44
No. Private Bands	1	0	0	0	0	0	0	0	1
Mean heterozygosity	0.137	0.083	0.121	0.156	0.119	0.114	0.084	0.164	0.035

Table 3.4. Coefficient of Gene diversity, G_{ST} (below diagonal) and Gene Flow, *Nm* for populations (above diagonal)

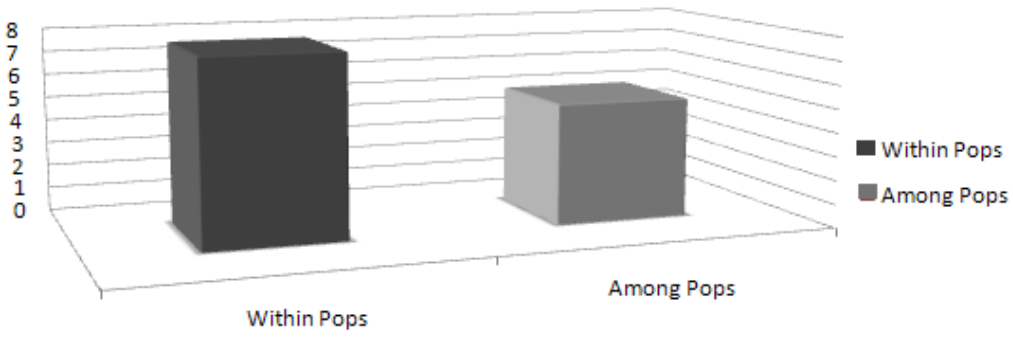
	Antalya	Artvin	Aydın	Beyşehir	Bilecik	Bingöl	Bitlis	Bozcaada	Gökçeada	Hatay	İzmir	Kars	Kayseri
Antalya	***	1.637	2.955	2.514	1.974	1.818	1.965	1.874	1.520	1.322	2.316	1.502	1.910
Artvin	0,234	***	1.669	2.197	4.763	3.277	2.105	1.531	1.812	2.914	3.741	2.329	1.556
Aydın	0.145	0.231	***	2.090	2.096	2.746	2.204	1.141	1.325	1.697	3.089	1.690	1.525
Beyşehir	0.166	0.185	0.193	***	2.953	3.022	1.815	1.929	1.730	1.980	3.734	2.654	3.072
Bilecik	0.202	0.095	0.193	0.145	***	3.832	2.610	1.443	2.131	3.013	5.169	4.162	2.300
Bingöl	0.216	0.132	0.154	0.142	0.115	***	2.502	1.329	1.631	3.299	4.170	3.067	1.835
Bitlis	0.203	0.192	0.185	0.216	0.161	0.167	***	1.088	2.153	2.048	3.244	1.472	1.388
Bozcaada	0.211	0.246	0.305	0.206	0.257	0.273	0.315	***	1.284	1.059	1.550	1.270	1.323
Gökçeada	0.248	0.216	0.274	0.224	0.190	0.235	0.188	0.280	***	1.286	2.635	1.066	1.031
Hatay	0.275	0.147	0.228	0.202	0.142	0.132	0.196	0.321	0.280	***	3.129	1.888	1.440
İzmir	0.178	0.118	0.139	0.118	0.088	0.107	0.134	0.244	0.160	0.138	***	2.855	2.453
Kars	0.250	0.177	0.288	0.159	0.107	0.140	0.254	0.282	0.319	0.209	0.149	***	2.016
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	***
Kırklareli	0.230	0.292	0.300	0.212	0.287	0.279	0.315	0.193	0.358	0.346	0.240	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	0.130	0.206	0.171
Manisa	0.189	0.228	0.185	0.221	0.186	0.194	0.209	0.279	0.279	0.260	0.182	0.188	0.247
Muğla	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
Nevşehir	0.234	0.260	0.269	0.110	0.190	0.226	0.308	0.304	0.314	0.313	0.158	0.214	0.150
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
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
Trabzon	0.302	0.269	0.270	0.236	0.270	0.193	0.293	0.351	0.253	0.317	0.192	0.292	0.352
Uşak	0.201	0.206	0.223	0.169	0.152	0.219	0.224	0.277	0.227	0.265	0.140	0.232	0.192
Van	0.193	0.169	0.175	0.174	0.096	0.160	0.215	0.308	0.249	0.218	0.094	0.172	0.205
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
Şanlıurfa	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

Table 3.4. Continued.

	Kırklareli	Konya	Manisa	Muğla	Nevşehir	Sinop	Sivas	Trabzon	Uşak	Van	Yozgat	Şanlıurfa
Antalya	1.672	2.020	2.148	1.965	1.634	2.033	2.210	1.155	1.984	2.089	2.041	0.996
Artvin	1.210	2.400	1.690	3.519	1.422	2.211	1.501	1.362	1.925	2.463	1.832	1.817
Aydın	1.644	1.928	2.197	2.162	1.361	2.263	1.733	1.353	1.746	2.364	2.053	1.274
Beyşehir	1.855	2.289	1.762	3.478	4.036	3.620	2.850	1.620	2.463	2.382	2.367	1.402
Bilecik	1.245	3.684	2.195	7.841	2.131	2.799	1.848	1.354	2.799	4.729	2.124	3.102
Bingöl	1.289	2.292	2.082	2.772	1.711	2.735	1.573	2.085	1.788	2.629	2.005	1.890
Bitlis	1.087	2.332	1.891	2.504	1.126	1.746	1.716	1.208	1.737	1.822	1.604	1.164
Bozcaada	2.094	1.710	1.293	1.609	1.146	1.351	1.388	0.926	1.308	1.121	1.708	0.692
Gökçeada	0.898	1.852	1.293	2.238	1.095	1.892	1.936	1.477	1.705	1.512	1.978	0.768
Hatay	0.942	1.665	1.420	2.191	1.097	1.431	1.181	1.077	1.385	1.790	1.716	2.676
İzmir	1.585	3.340	2.244	4.845	2.658	3.643	2.823	2.101	3.084	4.013	3.370	2.514
Kars	1.434	1.923	2.164	3.462	1.838	2.470	1.407	1.215	1.657	2.406	1.801	1.788
Kayseri	1.261	2.416	1.526	2.388	2.827	2.183	1.406	0.922	2.109	1.939	1.871	1.080
Kırklareli	***	1.407	1.239	1.642	1.432	1.322	1.122	0.912	1.033	1.023	1.289	0.742
Konya	0.262	***	1.759	3.221	1.963	2.107	1.623	1.128	3.546	2.515	2.421	1.396
Manisa	0.288	0.221	***	2.743	1.170	2.245	1.784	1.444	1.677	1.697	1.556	0.905
Muğla	0.233	0.134	0.154	***	2.894	5.036	2.411	1.476	3.346	3.487	2.049	1.936
Nevşehir	0.259	0.203	0.299	0.147	***	3.034	1.473	0.865	1.943	1.845	1.721	0.831
Sinop	0.274	0.192	0.182	0.090	0.142	***	2.257	1.616	2.650	2.501	2.027	1.014
Sivas	0.308	0.236	0.219	0.172	0.253	0.181	***	1.327	2.278	1.719	2.294	0.773
Trabzon	0.352	0.307	0.257	0.253	0.366	0.236	0.274	***	1.019	1.106	1.513	0.634
Uşak	0.326	0.124	0.230	0.130	0.205	0.159	0.180	0.329	***	2.718	1.975	1.107
Van	0.328	0.166	0.228	0.125	0.213	0.167	0.225	0.311	0.155	***	2.443	1.707
Yozgat	0.280	0.171	0.243	0.196	0.225	0.198	0.179	0.248	0.202	0.170	***	1.115
Şanlıurfa	0.403	0.264	0.356	0.205	0.376	0.330	0.393	0.441	0.311	0.227	0.310	***

Pairwise G_{ST} and *Nm* for populations were illustrated in tables 3.4. The highest G_{ST} was observed between Gökçeada and Şanlıurfa populations as 0.395. Pairwise G_{ST} for Trabzon and Nevşehir populations was 0.366. The lowest G_{ST} was detected between Bilecik and Muğla populations (0.060). The highest gene flow was observed between İzmir and Bilecik populations (5.169), the lowest value was between Trabzon and Şanlıurfa (0.634).

AMOVA indicated that within population variance was 60 % of the total variation, much larger than among populations' (40%) (Figure 3.3).



Source	df	Sum of squares	Estimation of Variance	%
Among Pops	24	3767,855	5,121	40%
Within Pops	704	5430,852	7,714	60%
Total	728	9198,708	12,835	

Figure 3.3. Results of Analysis of Molecular Variance

Nei's standard genetic distances (1972) and identities were calculated by using POPGENE program and illustrated in Table 3.5. Trabzon and Kırklareli were the most distant populations (0.175) whereas Şanlıurfa and Hatay were the closest populations (0.978).

Table 3.5. Nei’s genetic distances (below diagonal) and identities (above diagonal)

	Antalya	Artvin	Aydın	Beyşehir	Bilecik	Bingöl	Bitlis	Bzcada	Gkçada
Antalya	***	0.904	0.936	0.917	0.917	0.908	0.912	0.905	0.882
Artvin	0.101	***	0.918	0.930	0.976	0.964	0.942	0.917	0.929
Aydın	0.066	0.086	***	0.911	0.931	0.946	0.931	0.862	0.881
Beyşehir	0.087	0.073	0.094	***	0.945	0.946	0.906	0.909	0.898
Bilecik	0.087	0.025	0.071	0.056	***	0.967	0.950	0.907	0.937
Bingöl	0.097	0.037	0.055	0.056	0.033	***	0.947	0.897	0.916
Bitlis	0.092	0.060	0.072	0.099	0.051	0.054	***	0.869	0.934
Bzcada	0.099	0.087	0.148	0.095	0.098	0.109	0.140	***	0.885
Gkçada	0.125	0.073	0.127	0.108	0.065	0.088	0.069	0.122	***
Hatay	0.115	0.034	0.076	0.074	0.035	0.033	0.056	0.115	0.094
İzmir	0.094	0.041	0.062	0.057	0.031	0.040	0.053	0.119	0.068
Kars	0.112	0.049	0.086	0.061	0.029	0.040	0.089	0.107	0.129
Kayseri	0.093	0.079	0.103	0.056	0.057	0.073	0.102	0.111	0.145
Kırklareli	0.117	0.116	0.150	0.103	0.119	0.117	0.146	0.075	0.186
Konya	0.093	0.055	0.085	0.080	0.037	0.062	0.063	0.090	0.083
Manisa	0.079	0.070	0.067	0.096	0.057	0.061	0.071	0.108	0.109
Muğla	0.085	0.038	0.079	0.054	0.018	0.053	0.061	0.096	0.071
Nevşehir	0.094	0.073	0.098	0.036	0.051	0.066	0.106	0.108	0.114
Sinop	0.089	0.057	0.070	0.048	0.048	0.050	0.082	0.111	0.078
Sivas	0.094	0.102	0.107	0.071	0.086	0.104	0.098	0.127	0.089
Trabzon	0.162	0.094	0.119	0.110	0.100	0.065	0.120	0.165	0.101
Uşak	0.088	0.063	0.088	0.069	0.046	0.074	0.080	0.111	0.084
Van	0.073	0.042	0.055	0.062	0.023	0.042	0.065	0.111	0.082
Yozgat	0.105	0.085	0.093	0.089	0.077	0.083	0.109	0.105	0.090
Şanlıurfa	0.122	0.039	0.078	0.083	0.025	0.042	0.074	0.136	0.122

Table 3.5. Continued.

	Hatay	İzmir	Kars	Kayseri	Kırklareli	Konya	Manisa	Muğla
Antalya	0.891	0.910	0.894	0.911	0.889	0.911	0.924	0.918
Artvin	0.966	0.959	0.953	0.923	0.890	0.946	0.932	0.962
Aydın	0.926	0.940	0.918	0.902	0.860	0.918	0.934	0.924
Beyşehir	0.929	0.945	0.941	0.946	0.902	0.923	0.908	0.947
Bilecik	0.966	0.969	0.972	0.945	0.888	0.963	0.944	0.982
Bingöl	0.968	0.961	0.961	0.930	0.890	0.940	0.940	0.948
Bitlis	0.946	0.948	0.915	0.903	0.864	0.938	0.931	0.940
Bzcada	0.891	0.888	0.899	0.895	0.927	0.914	0.897	0.905
Gkçada	0.910	0.934	0.879	0.865	0.830	0.920	0.896	0.931
Hatay	***	0.956	0.948	0.925	0.872	0.930	0.927	0.945
İzmir	0.045	***	0.946	0.933	0.887	0.948	0.929	0.962
Kars	0.054	0.055	***	0.940	0.906	0.932	0.946	0.961
Kayseri	0.078	0.069	0.062	***	0.886	0.942	0.917	0.939
Kırklareli	0.137	0.120	0.098	0.121	***	0.891	0.888	0.903
Konya	0.072	0.053	0.070	0.059	0.115	***	0.924	0.952
Manisa	0.075	0.073	0.055	0.086	0.119	0.079	***	0.949
Muğla	0.056	0.038	0.040	0.062	0.101	0.048	0.051	***
Nevşehir	0.084	0.055	0.057	0.034	0.089	0.061	0.094	0.043
Sinop	0.081	0.047	0.052	0.063	0.118	0.070	0.059	0.029
Sivas	0.119	0.071	0.110	0.118	0.165	0.107	0.088	0.073
Trabzon	0.108	0.083	0.107	0.155	0.175	0.134	0.092	0.104
Uşak	0.080	0.054	0.075	0.063	0.148	0.039	0.076	0.043
Van	0.051	0.029	0.043	0.058	0.128	0.048	0.064	0.035
Yozgat	0.083	0.061	0.088	0.090	0.147	0.073	0.105	0.090
Şanlıurfa	0.027	0.043	0.041	0.077	0.133	0.064	0.087	0.048

Table 3.5. Continued.

	Nevşehir	Sinop	Sivas	Trabzon	Uşak	Van	Yozgat	Şanlıurfa
Antalya	0.910	0.915	0.910	0.851	0.915	0.930	0.900	0.885
Artvin	0.930	0.944	0.903	0.911	0.939	0.959	0.918	0.961
Aydın	0.906	0.933	0.898	0.888	0.916	0.946	0.911	0.925
Beyşehir	0.965	0.953	0.931	0.895	0.933	0.939	0.915	0.921
Bilecik	0.950	0.954	0.918	0.905	0.955	0.978	0.926	0.976
Bingöl	0.936	0.951	0.901	0.937	0.929	0.959	0.920	0.959
Bitlis	0.899	0.921	0.906	0.887	0.924	0.938	0.897	0.929
Bzcada	0.897	0.895	0.881	0.848	0.895	0.895	0.900	0.873
Gkçada	0.892	0.925	0.914	0.904	0.919	0.922	0.914	0.885
Hatay	0.919	0.922	0.888	0.898	0.923	0.950	0.921	0.978
İzmir	0.947	0.954	0.931	0.920	0.947	0.971	0.941	0.958
Kars	0.944	0.949	0.896	0.898	0.928	0.958	0.916	0.959
Kayseri	0.961	0.938	0.888	0.856	0.939	0.943	0.914	0.926
Kırklareli	0.914	0.888	0.847	0.839	0.861	0.879	0.863	0.875
Konya	0.940	0.932	0.898	0.875	0.961	0.953	0.930	0.938
Manisa	0.910	0.943	0.915	0.912	0.926	0.938	0.899	0.916
Muğla	0.957	0.971	0.929	0.900	0.957	0.965	0.914	0.953
Nevşehir	***	0.963	0.908	0.870	0.944	0.951	0.919	0.925
Sinop	0.038	***	0.929	0.915	0.949	0.954	0.918	0.918
Sivas	0.096	0.074	***	0.880	0.932	0.921	0.917	0.865
Trabzon	0.139	0.089	0.128	***	0.870	0.898	0.891	0.869
Uşak	0.057	0.052	0.071	0.139	***	0.960	0.918	0.928
Van	0.051	0.047	0.082	0.108	0.041	***	0.943	0.963
Yozgat	0.084	0.085	0.087	0.115	0.085	0.0586	***	0.904
Şanlıurfa	0.078	0.086	0.145	0.140	0.074	0.0374	0.102	***

Two main branches were observed in both *UPGMA* and *Neighbour Joining (NJ)* dendrogram based on Nei's (1972) standard genetic distances (Figure 3.4 and 3.5). For the *UPGMA*, first branch included Bozcaada and Kırklareli populations and the second branch consisted of other populations. However, Trabzon population clustered separately from other populations in the second branch (Figure 3.4). For *Neighbor Joining*, first branch included Bozcada, Kırklareli, Beyşehir, Kayseri and Nevşehir populations and the rest of the populations were included in the second branch (Figure 3.5).

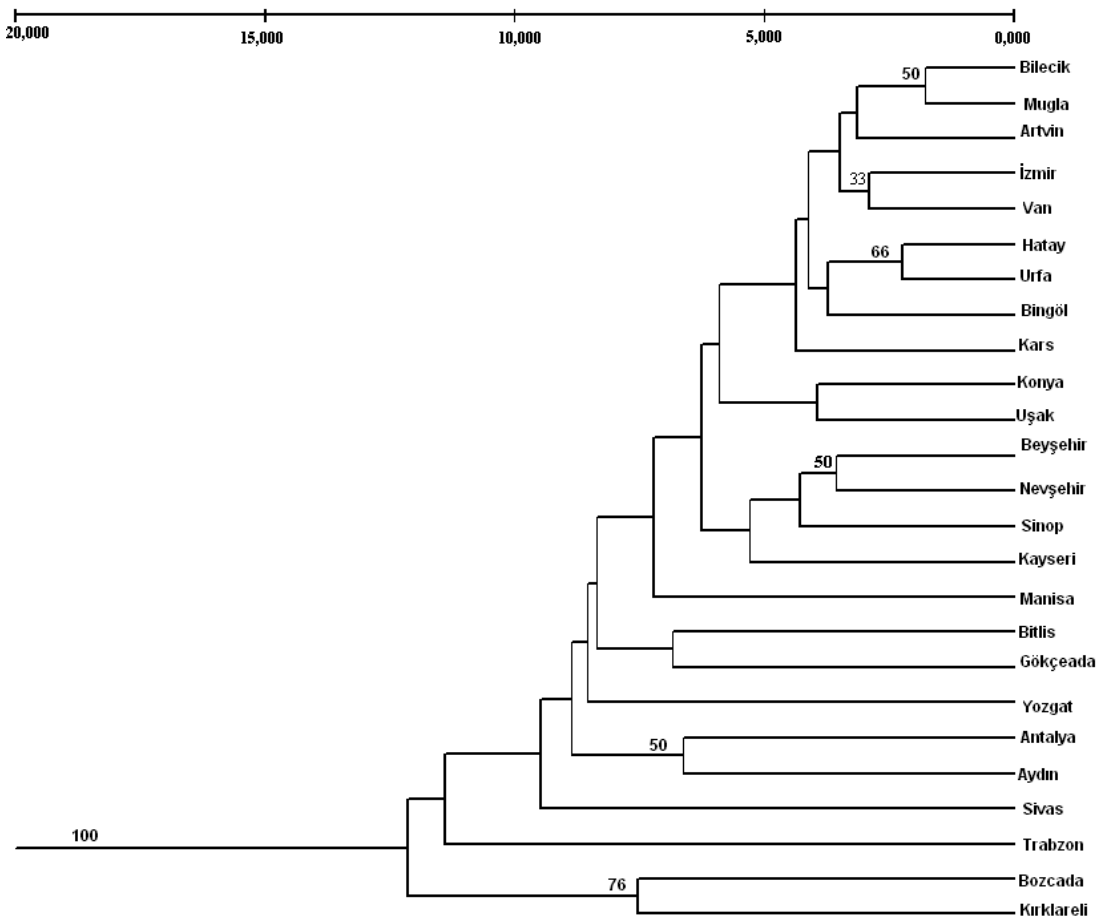


Figure 3.4. *UPGMA* dendrogram based on D_S .

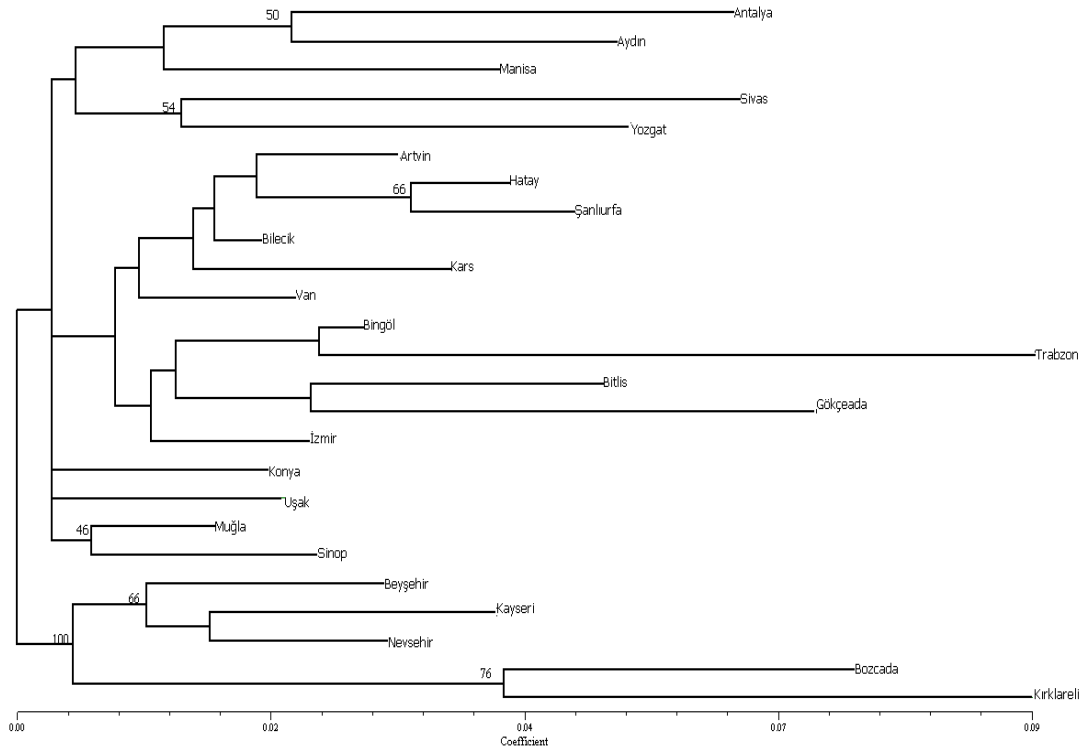


Figure 3.5. *Neighbor Joining* dendrogram based on D_S.

Mantel test was performed using genetic distance and geographic distance matrices. Matrix correlation value (*r*) was estimated 0.05849 from normalized Mantel statistic (*Z*). Without normalization of data, the raw Mantel statistic value (*Z*) was detected as 21.118.7243. The significance test including Mantel *t* test and P value were estimated to be 0.6587 and 0.7449 (>0.05), respectively. There is no correlation between genetic distance and geographic distance based on RAPD. Matrix comparison of distance matrices was illustrated in the scatter plot (Figure 3.6).

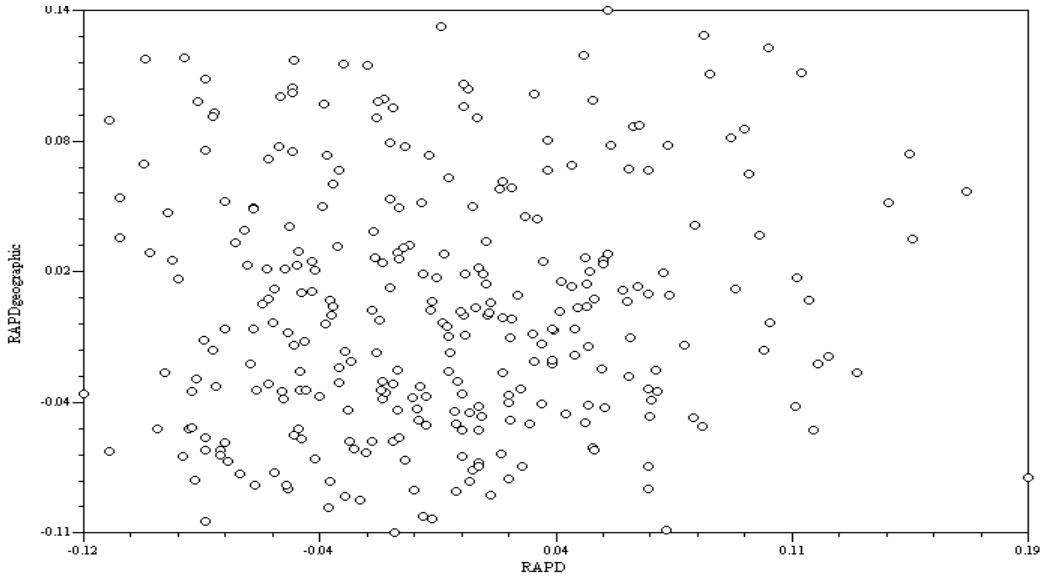


Figure 3.6. Correlation between genetic distance and geographic distances based on RAPD data

3.2. Microsatellite Results

The microsatellite analysis was completed using 6 microsatellite loci in 18 populations. ABI PRISM® 3130 Genetic Analyzer was used for fragment analyses and fragment sizes were determined with GeneMapper 4.0 software program. Fragment sizes for all loci are illustrated in figure 3.7. and Appendix C.

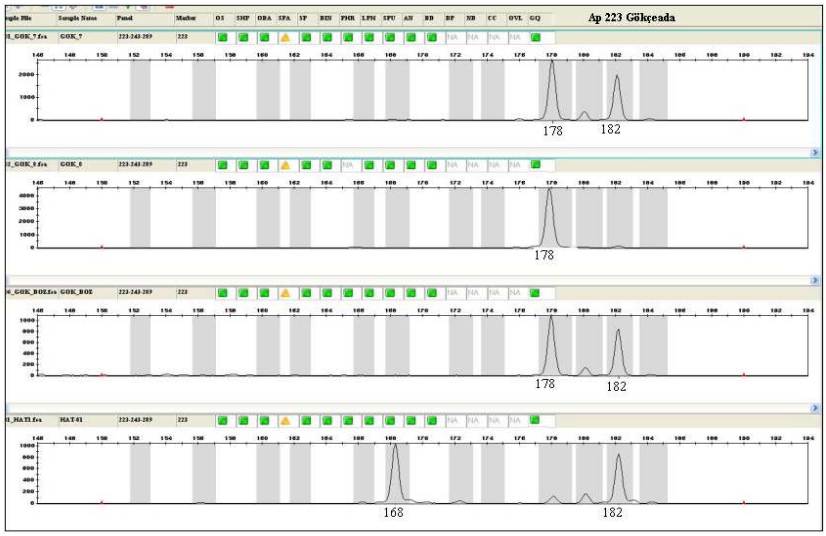


Figure 3.7. The alleles of Ap223 [$(T)_5(C)_4A(T)_6(C)_5$] locus

Observed and expected number of alleles, the number of private allele and heterozygosity level were given in figure 3.8.

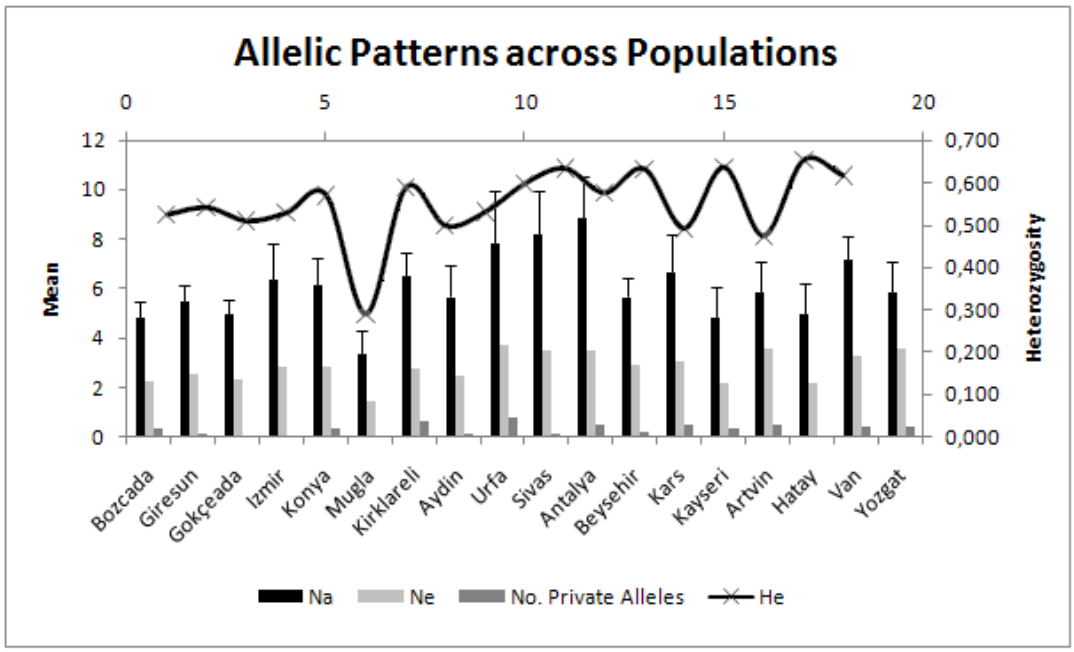


Figure 3.8. Alellic patterns across Turkish populations

The number of alleles and average number of alleles per locus were illustrated in table 3.6. In 520 worker bees from 18 honey bee populations, a total of 118 alleles were detected using 6 microsatellite loci. The highest number of alleles was observed in Ap001 locus with 32 alleles whereas the lowest number of alleles was detected in Ap243 locus with 10 alleles. The average number of alleles per locus ranged between 3.3 (Muğla) and 8.8 (Antalya).

Table 3.6. Number of alleles and average number of alleles per locus

	Ap223	Ap019	Ap001	Ap243	Ap289	A76	Average
Bozcaada	5	2	5	5	7	5	4.8
Giresun	5	6	5	3	8	6	5.4
Gökçeada	5	4	5	3	7	6	5.0
İzmir	6	6	7	3	13	3	6.3
Konya	4	7	9	3	9	5	6.2
Muğla	6	4	0	4	5	1	3.3
Kırklareli	9	5	8	4	9	4	6.5
Aydın	6	5	8	1	10	4	5.7
Şanlıurfa	9	3	17	4	5	9	7.8
Sivas	7	7	10	1	14	10	8.2
Antalya	5	8	15	4	12	9	8.8
Beyşehir	4	6	7	3	8	6	5.6
Kars	4	5	7	6	14	0	6.0
Kayseri	6	5	10	2	4	2	4.8
Artvin	4	4	8	3	5	11	5.8
Hatay	5	4	0	5	9	7	5.0
Van	8	5	9	4	10	7	7.2
Yozgat	6	7	11	3	5	3	5.8
TOTAL	12	13	32	10	26	25	6.0

Private alleles and their frequencies were shown in table 3.7. Private alleles mean that the alleles have been observed in only one population. The number of private alleles and their average frequencies were shown in Table 3.8. A total of 29 private alleles were detected from 14 populations. Private alleles were not observed in Ap223 locus. The highest number of private alleles was observed for A76 locus with 9 alleles.

Only one private allele was observed in Giresun, Beyşehir, Aydın and Sivas populations. Five private alleles were detected in Şanlıurfa population being the highest and average frequency of private alleles was 0.022. The highest average private allele frequency (0.065) was observed for Bozcaada population.

Table 3.7. Private allele and their allele frequencies of relevant populations

Populations	Locus	Allele	Frequency
Giresun	Ap019	126	0.017
Beyşehir	Ap019	132	0.036
Konya	Ap019	144	0.017
Antalya	Ap019	152	0.017
Yozgat	Ap001	205	0.056
Aydın	Ap001	214	0.019
Kayseri	Ap001	216	0.023
Artvin	Ap001	228	0.042
Yozgat	Ap001	241	0.056
Şanlıurfa	Ap001	243	0.017
Kayseri	Ap001	245	0.023
Kars	Ap243	211	0.053
Kars	Ap243	241	0.026
Şanlıurfa	Ap243	262	0.026
Bozcaada	Ap243	271	0.017
Kırklareli	Ap289	164	0.045
Konya	Ap289	174	0.026
Kırklareli	Ap289	178	0.023
Bozcaada	Ap289	192	0.065
Antalya	Ap289	206	0.045
Van	A76	210	0.025
Antalya	A76	220	0.045
Van	A76	221	0.025
Şanlıurfa	A76	227	0.017
Şanlıurfa	A76	229	0.017
Şanlıurfa	A76	230	0.017
Artvin	A76	259	0.020
Sivas	A76	260	0.023
Artvin	A76	263	0.040

Table 3.8. Number of private alleles and their average frequency

Populations	Number of private alleles	Average Frequency
Giresun	1	0.017
Beyşehir	1	0.036
Konya	2	0.022
Yozgat	2	0.056
Aydın	1	0.019
Kayseri	2	0.023
Artvin	3	0.034
Şanlıurfa	5	0.022
Kars	2	0.039
Bozcaada	2	0.041
Kırklareli	2	0.034
Van	2	0.025
Antalya	3	0.036
Sivas	1	0.023
Total	29	0.024

Diagnostic alleles can be defined as alleles having relatively high proportions in one population in comparison to all other populations. Diagnostic alleles for 18 Turkish honey bee populations were illustrated in table 3.9. Allelic frequencies of six microsatellite loci for each population were demonstrated in Appendix D.

Table 3.9. Diagnostic alleles for Turkish honey bee populations

Bozcaada	182 (Ap223);142(Ap19); 271 (Ap243)
Giresun	126 (Ap19)
Gökçeada	213(Ap001); 198(Ap289)
İzmir	207 (Ap076)
Konya	211 (Ap001);136, 144(Ap19);174,210(Ap289)
Muğla	138(Ap19)
Kırklareli	166,176,178(Ap223);140(Ap19);164,178,182 (Ap289)
Aydın	215 (Ap001)
Şanlıurfa	237,243(Ap001);262(Ap243); 227,229,230 (A76)
Sivas	260 (Ap076)
Antalya	168(Ap223);152 (Ap19);206(Ap289)
Beyşehir	132(Ap19)
Kars	229(Ap001);148(Ap19);211,241 (Ap243)
Kayseri	233(Ap001)
Artvin	217,228(Ap001);253 (Ap243);213,222(Ap289);209,259, 263(A76)
Hatay	253, 257(A76)
Van	128(Ap19); 210(A76)
Yozgat	205,215,223,241 (Ap001);216(Ap289);211(A76)

Observed heterozygosity (*Ho*), expected heterozygosity (*He*) and their P values were shown in Table 3.10. Muğla population for Ap001 and Ap289 loci, Aydın and Sivas populations for Ap243 loci, Kars population for A76 and Hatay population for Ap001 loci had only one allele. Therefore, these loci were not shown in the tables ($P<0.05$).

Table 3.10. Observed (*Ho*) and expected (*He*) heterozygosity for all populations

Bozcaada	Locus	Obs.Het. (*Ho*)	Exp.Het. (*He*)	P-value	St. Dev.
	Ap223	0.423	0.435	0.1218	0.00029
	Ap019	0.482	0.373	0.2826	0.00044
	Ap001	0.519	0.600	0.0225	0.00014
	Ap243	0.345	0.476	0.0003	0.00002
	Ap289	0.565	0.667	0.3442	0.00056
	A076	0.410	0.653	0.0001	0.00001
Giresun	Locus	Obs.Het. (*Ho*)	Exp.Het. (*He*)	P-value	St. Dev.
	Ap223	0.448	0.474	0.0538	0.00026
	Ap019	0.767	0.596	0.1609	0.00034
	Ap001	0.615	0.599	0.5124	0.00046
	Ap243	0.250	0.229	1.0000	0.00000
	Ap289	0.250	0.796	0.0000	0.00000
	A076	0.188	0.433	0.0167	0.00013
Gökçeada	Locus	Obs.Het. (*Ho*)	Exp.Het. (*He*)	P-value	St. Dev.
	Ap223	0.556	0.668	0.3701	0.00047
	Ap019	0.400	0.383	0.6266	0.00051
	Ap001	0.433	0.601	0.0359	0.00018
	Ap243	0.188	0.486	0.0075	0.00008
	Ap289	0.563	0.774	0.1249	0.00037
	A076	0.143	0.235	0.0033	0.00005
İzmir	Locus	Obs.Het. (*Ho*)	Exp.Het. (*He*)	P-value	St. Dev.
	Ap223	0.724	0.743	0.5816	0.00057
	Ap019	0.462	0.428	0.7698	0.00032
	Ap01	0.600	0.665	0.3798	0.00053
	Ap243	0.118	0.383	0.0034	0.00006
	Ap289	0.643	0.855	0.0100	0.00008
	A076	0.038	0.112	0.0195	0.00013
Konya	Locus	Obs.Het. (*Ho*)	Exp.Het. (*He*)	P-value	St. Dev.
	Ap223	0.636	0.673	0.3670	0.00046
	Ap019	0.800	0.676	0.0585	0.00024
	Ap001	0.577	0.678	0.1041	0.00031
	Ap243	0.461	0.384	1.0000	0.00000
	Ap289	0.684	0.835	0.0051	0.00006
	A076	0.107	0.271	0.0159	0.00012
Muğla	Locus	Obs.Het. (*Ho*)	Exp.Het. (*He*)	P-value	St. Dev.
	Ap223	0.619	0.648	0.2389	0.00039
	Ap019	0.148	0.143	1.0000	0.00000
	Ap243	0.476	0.495	0.0895	0.00022
	Ap289	0.684	0.509	0.5621	0.00046

Table 3.10. Continued.

Kirklareli	Locus	Obs.Het. (*Ho*)	Exp.Het. (*He*)	P-value	St. Dev.
	Ap223	0.724	0.736	0.0889	0.00024
	Ap019	0.440	0.520	0.1634	0.00038
	Ap001	0.772	0.812	0.0048	0.00006
	Ap243	0.360	0.379	0.5475	0.00048
	Ap289	0.455	0.530	0.4090	0.00029
	A076	0.350	0.640	0.0000	0.00001
Aydın	Locus	Obs.Het. (*Ho*)	Exp.Het. (*He*)	P-value	St. Dev.
	Ap223	0.643	0.610	0.0110	0.00010
	Ap019	0.739	0.541	0.3125	0.00044
	Ap001	0.461	0.647	0.0121	0.00010
	Ap289	0.640	0.800	0.2243	0.00024
	A076	0.047	0.431	0.0000	0.00000
Şanlıurfa	Locus	Obs.Het. (*Ho*)	Exp.Het. (*He*)	P-value	St. Dev.
	Ap223	0.731	0.775	0.1105	0.00032
	Ap019	0.296	0.268	1.0000	0.00000
	Ap001	0.933	0.930	0.5858	0.00033
	Ap243	0.310	0.389	0.2363	0.00051
	Ap289	0.310	0.572	0.0043	0.00006
	A076	0.172	0.395	0.0002	0.00002
Sivas	Locus	Obs.Het. (*Ho*)	Exp.Het. (*He*)	P-value	St. Dev.
	Ap223	0.731	0.728	0.7817	0.00031
	Ap019	0.920	0.697	0.0056	0.00007
	Ap001	0.521	0.804	0.0014	0.00004
	Ap289	0.607	0.853	0.0000	0.00000
	A076	0.210	0.448	0.0036	0.00008
Antalya	Locus	Obs.Het. (*Ho*)	Exp.Het. (*He*)	P-value	St. Dev.
	Ap223	0.629	0.621	1.0000	0.00000
	Ap019	0.533	0.552	0.2481	0.00025
	Ap001	0.750	0.858	0.0340	0.00010
	Ap243	0.238	0.302	0.2832	0.00051
	Ap289	0.409	0.816	0.0000	0.00000
	A076	0.325	0.648	0.0002	0.00002
Beyşehir	Locus	Obs.Het. (*Ho*)	Exp.Het. (*He*)	P-value	St. Dev.
	Ap223	0.600	0.598	0.0626	0.00023
	Ap019	0.750	0.699	0.0193	0.00012
	Ap001	0.480	0.695	0.0864	0.00023
	Ap243	0.481	0.498	0.7229	0.00045
	Ap289	0.600	0.855	0.0000	0.00000
	A076	0.076	0.217	0.0013	0.00003

Table 3.10. Continued

Kars	Locus	Obs.Het. (*Ho*)	Exp.Het. (*He*)	P-value	St. Dev.
	Ap223	0.703	0.522	0.1954	0.00042
	Ap019	0.862	0.617	0.0324	0.00020
	Ap001	0.478	0.623	0.2218	0.00046
	Ap243	0.736	0.645	0.0719	0.00026
	Ap289	0.600	0.856	0.0001	0.00001
Kayseri	Locus	Obs.Het. (*Ho*)	Exp.Het. (*He*)	P-value	St. Dev.
	Ap223	0.696	0.685	0.9754	0.00016
	Ap019	0.458	0.458	0.6441	0.00037
	Ap001	0.681	0.666	0.0277	0.00015
	Ap243	0.409	0.333	0.5371	0.00051
	Ap289	0.772	0.605	0.4231	0.00048
	A076	0.143	0.344	0.0019	0.00014
Artvin	Locus	Obs.Het. (*Ho*)	Exp.Het. (*He*)	P-value	St. Dev.
	Ap223	0.833	0.697	0.4494	0.00049
	Ap019	0.586	0.609	0.3274	0.00044
	Ap001	0.167	0.858	0.0000	0.00000
	Ap243	0.185	0.174	1.0000	0.00000
	Ap289	0.222	0.787	0.0000	0.00000
	A076	0.250	0.746	0.0000	0.00000
Hatay	Locus	Obs.Het. (*Ho*)	Exp.Het. (*He*)	P-value	St. Dev.
	Ap223	0.608	0.610	0.3252	0.00046
	Ap019	0.455	0.542	0.1320	0.00037
	Ap243	0.539	0.506	0.1659	0.00033
	Ap289	0.520	0.554	0.2980	0.00021
	A076	0.000	0.429	0.1424	0.00036
Van	Locus	Obs.Het. (*Ho*)	Exp.Het. (*He*)	P-value	St. Dev.
	Ap223	0.862	0.729	0.0412	0.00022
	Ap019	0.869	0.646	0.0022	0.00004
	Ap001	0.576	0.766	0.0193	0.00009
	Ap243	0.272	0.442	0.0279	0.00016
	Ap289	0.280	0.831	0.0000	0.00000
	A076	0.389	0.505	0.1006	0.00024
Yozgat	Locus	Obs.Het. (*Ho*)	Exp.Het. (*He*)	P-value	St. Dev.
	Ap223	0.643	0.566	1.0000	0.00000
	Ap019	0.875	0.706	0.1675	0.00035
	Ap001	0.556	0.934	0.0004	0.00002
	Ap243	0.133	0.246	0.2055	0.00037
	Ap289	0.285	0.769	0.0041	0.00005
	A076	0.181	0.679	0.0021	0.00005

Demorization step 1000000 P<0.005

Mean expected heterozygosities (gene diversity) ranged between 0.449 (Muğla) and 0.739 (Artvin). Mean expected heterozygosity for all populations was detected to be 0.588 (Table 3.11). The highest mean observed heterozygosity value was detected for Kars population (0.676) and the lowest observed heterozygosity was found for Artvin population (0.375). The mean observed heterozygosity for all populations was found 0.493.

Table 3.11. Mean observed (Ho) and expected heterozygosity (He) values and their standard deviations

	Mean *Ho*	Mean *He*
Bozcaada	0.457±0.0800	0.534±0.1230
Giresun	0.420±0.2303	0.521±0.1908
Gokceada	0.38±0.1793	0.524±0.197
İzmir	0.431±0.2876	0.531±0.273
Konya	0.544±0.2418	0.586±0.2127
Muğla	0.482±0.2389	0.449±0.2151
Kirklareli	0.517±0.1840	0.603±0.1585
Aydın	0.506±0.2255	0.606±0.1365
Şanlıurfa	0.458±0.3005	0.555±0.2511
Sivas	0.598±0.2109	0.706±0.1571
Antalya	0.48±0.1927	0.633±0.2051
Beyşehir	0.504±0.2135	0.624±0.2002
Kars	0.676±0.1450	0.634±0.2300
Kayseri	0.527±0.2362	0.506±0.1725
Artvin	0.375±0.2074	0.739±0.0934
Hatay	0.530±0.0632	0.528±0.0663
Van	0.542±0.2740	0.653±0.1524
Yozgat	0.446±0.2952	0.651±0.2321
Overall Mean	0.493±0.0657	0.588±0.0542

Mean expected and observed heterozygosities were calculated per loci for all populations (Table 3.12). The highest expected heterozygosity was detected for Ap289 (0.745), whereas the lowest was determined for Ap243 (0,350) locus. Gene diversities for total populations (H_T) ranged between 0.378 (Ap243) and 0.817 (Ap289). Mean observed heterozygosity value for A76 locus was detected the lower

than the other loci (0.174). The highest observed heterozygosity was observed for Ap223 loci as 0.656.

Table 3.12. Gene diversity per locus in total population (H_T), mean expected (*He*) and observed heterozygosity (*Ho*) per loci for all populations

Locus	H_T	Mean *He*	Mean *Ho*
Ap223	0.673	0.640	0.656
Ap019	0.556	0.514	0.647
Ap001	0.855	0.656	0.504
Ap243	0.378	0.350	0.300
Ap289	0.817	0.745	0.472
A76	0.543	0.405	0.174

Deviations from Hardy - Weinberg equilibrium were determined for all populations at 6 microsatellite loci, however, only significant deviations were given in Table 3.13 ($P<0.05$, 0.01 and 0.001). A total of 47 significant deviations of population locus combinations were detected out of 108 population locus combinations. All of the deviations were in favor of homozygotes except at Ap019 locus in Beyşehir, Sivas, and Van populations, at Ap001 locus in Kayseri and Ap223 in Aydın population.

Table 3.13. Significant deviations from Hardy-Weinberg Equilibrium

(** P<0.05, ** P<0.01, *** P<0.001)*

Populations	Locus	df	X^2	P
Bozcaada	Ap223	10	52.651	0.000***
Bozcaada	Ap001	10	30.118	0.001***
Bozcaada	Ap243	10	41.110	0.000***
Bozcaada	A76	10	34.488	0.000***
Giresun	Ap223	10	35.614	0.000***
Giresun	Ap289	28	132.05	0.000***
Giresun	A76	15	51.204	0.000***
Gökçeada	Ap001	10	18.743	0.044*
Gökçeada	Ap243	3	8.128	0.043*
Gökçeada	A76	15	84.105	0.000***
İzmir	Ap243	3	11.470	0.009**
İzmir	Ap289	78	112.78	0.006**
İzmir	A76	3	38.277	0.000***
Konya	Ap019	21	36.797	0.018*
Konya	Ap001	36	88.444	0.000***
Konya	Ap289	36	54.084	0.027*
Konya	A76	10	48.174	0.000***
Muğla	Ap223	15	45.087	0.000***
Kirklareli	Ap223	36	86.355	0.000***
Kirklareli	Ap001	28	69.347	0.000***
Kirklareli	A76	6	40.018	0.000***
Aydın	Ap223	15	26.814	0.030*
Aydın	Ap001	28	57.814	0.001***
Aydın	A76	6	33.413	0.000***
Şanlıurfa	Ap223	36	51.066	0.049*
Şanlıurfa	Ap289	10	23.145	0.010*
Şanlıurfa	A76	36	120.01	0.000***
Sivas	Ap019	21	47.390	0.001***
Sivas	Ap001	45	70.318	0.009**
Sivas	A76	45	67.322	0.017*
Antalya	Ap019	28	42.508	0.039*
Antalya	Ap001	105	188.12	0.000***
Antalya	Ap289	66	166.73	0.000***
Antalya	A76	36	76.501	0.000***
Beyşehir	Ap223	6	13.401	0.037*
Beyşehir	Ap019	15	38.324	0.001***
Beyşehir	Ap289	28	119.00	0.000***
Beyşehir	A76	15	108.05	0.000***
Kars	Ap243	15	26.070	0.037*
Kars	Ap289	91	116.68	0.036*
Kayseri	Ap001	45	113.82	0.000***
Kayseri	A76	1	4.954	0.026*
Artvin	Ap001	28	61.333	0.000***
Artvin	Ap289	10	54.080	0.000***
Van	Ap223	28	42.987	0.035*
Van	Ap019	10	29.038	0.001**
Van	Ap289	45	140.04	0.000***
Yozgat	A76	3	11.774	0.008**

Linkage disequilibrium test was performed for locus pairs in all populations. Linkage disequilibrium was observed in ten populations. The locus pairs and their P values were shown in table 3.14. Kırklareli, Artvin, and Van populations had 2 locus pair combinations of linkage disequilibrium.

Table 3.14. Significant linkage disequlibrium values *(P<0.05*) for locus pairs in populations.

Population	Locus Pair	P value
Bozcaada	Ap243 & Ap289	0.0338
Giresun	Ap019 & Ap289	0.0004
Gökçeada	Ap243 & A76	0.0343
Konya	Ap019 & Ap243	0.0213
Kirklareli	Ap223 & Ap019	0.0427
Kirklareli	Ap243 & A76	0.0171
Kayseri	Ap223 & Ap243	0.0310
Artvin	Ap019 & A76	0.0049
Artvin	Ap289 & A76	0.0047
Van	Ap019 & Ap001	0.0474
Van	Ap243 & Ap289	0.0443

dememorization = 1000; *# batches* = 1000, *iterations per batch* = 1000.

Table 3.15. P values of linkage disequilibrium test for all locus pairs over all populations

Locus pairs	X^2	df	P
Ap223 & Ap19	21.881	36	0.969
Ap223 & Ap1	20.234	30	0.910
Ap19 & Ap1	35.190	30	0.236
Ap223 & Ap243	30.699	32	0.532
Ap19 & Ap243	29.091	32	0.615
Ap1 & Ap243	11.426	26	0.994
Ap223 & Ap289	30.839	36	0.712
Ap19 & Ap289	33.351	36	0.595
Ap1 & Ap289	16.437	30	0.979
Ap243 & Ap289	37.533	32	0.230
Ap223 & A76	21.980	30	0.855
Ap19 & A76*****	48.585	30	0.017 *
Ap1 & A76	22.546	28	0.755
Ap243 & A76	36.560	28	0.129
Ap289 & A76	26.887	30	0.629

dememorization = 1000; *# batches* = 1000, *iterations per batch* = 1000.

*****P<0.05

Significant linkage disequilibrium was observed only between Ap019 & A76 locus pairs (Table 3.15).

The heterozygosity level within subpopulation *(F_{IS}),* the heterozygosity level in total populations (*F_{IT})* and the degree of genetic differentiation of subpopulations (*F_{ST}*) were given in table 3.16. Ap223 and Ap019 loci illustrated an excess of heterozygosity in all populations. However, other loci showed deficiency of heterozygotes in all populations according to F_{IS} values. F_{ST} values for four loci (Ap223, Ap289, Ap243, and Ap19) demonstrated moderate level of heterozygosity, while Ap001 and A76 illustrated significant genetic differentiation in all populations. Mean F_{ST} value was estimated as 0.162 which indicated a great genetic differentiation among populations. Mean gene flow (*Nm)* value was found as 1.29 which is smaller than 2, therefore, there still is considerable genetic differentiation among populations.

Table 3.16. F coefficients and *Nm* values for all populations

Locus	F_{IS}	F_{IT}	F_{ST}	*Nm*
Ap223	-0.047	0.026	0.070	3.3469
Ap019	-0.192	-0.084	0.090	2.5189
Ap001	0.206	0.410	0.257	0.7234
Ap243	0.117	0.205	0.100	2.2609
Ap289	0.343	0.423	0.121	1.8163
A076	0.476	0.642	0.316	0.5403
Mean	0.146	0.285	0.162	1.2903

The pair wise F_{ST} values were illustrated in table 3.17. According to Wright (1951), F_{ST} levels between 0 and 0.05 indicate little genetic differentiation: levels between 0.05 and 0.15 indicate moderate genetic differentiation; levels between 0.15 and 0.25 indicate great genetic differentiation and levels larger than 0.25 designate highly significant genetic differentiation (Hartl and Clark 2007).

The pair wise F_{ST} values ranged from -0.068 (Gökçeada and İzmir) to 0.347 (Konya and Muğla). Population pair F_{ST} values showed very significant variation in 16, great genetic variation in 10 population pairs while in 94 population pairs moderate and in 33 population pairs little genetic differentiation were observed. Multi locus genotypes belonging to each population pair were randomized by 15300 permutations. Goodness of fit test applied to F_{ST} data in order to find out the significant pairwise comparison after standard Bonferroni corrections (Table 3.17).

Table 3.17. Pairwise F_{ST} values and their significance test for all populations studied (**: $P < 0.01$; *: $0.01 < P < 0.05$)

	Bozcaada	Giresun	Gökçeada	İzmir	Konya	Muğla	Kırklareli	Aydın	Şanlıurfa	Sivas	Antalya	Beyşehir	Kars	Kayseri	Artvin	Hatay	Van	Yozgat
Bozcaada	-	**	*	**	**		**	**	**	**	**	**		**	**		**	
Giresun	0.059	-		*	**		**	*	**	*				**				
Gökçeada	0.076	0.049	-		*		*		**	*	*			*				
İzmir	0.076	0.054	-0.068	-	*		**		**	*				**				
Konya	0.099	0.075	0.072	0.031	-		**	**	**	**	**			**				
Muğla	0.293	0.304	0.291	0.318	0.347	-												
Kırklareli	0.099	0.117	0.101	0.084	0.107	0.314	-	**	**	**	**	*		**			*	
Aydın	0.073	0.015	0.047	0.034	0.062	0.305	0.106	-	**					*				
Şanlıurfa	0.095	0.094	0.084	0.066	0.096	0.303	0.072	0.076	-	**		**		**	*		**	
Sivas	0.094	0.028	0.058	0.040	0.045	0.297	0.101	0.015	0.069	-	*			**				
Antalya	0.060	0.042	0.055	0.039	0.053	0.249	0.065	0.020	0.038	0.031	-	**		**				
Beyşehir	0.087	0.061	0.057	0.037	0.028	0.344	0.126	0.046	0.107	0.046	0.058	-		**				
Kars	0.205	0.173	0.158	0.153	0.150	0.127	0.175	0.190	0.151	0.145	0.146	0.161	-					
Kayseri	0.125	0.076	0.048	0.053	0.118	0.294	0.107	0.063	0.069	0.070	0.041	0.099	0.184	-	*		**	
Artvin	0.126	0.096	0.133	0.108	0.098	0.291	0.078	0.083	0.109	0.071	0.050	0.109	0.142	0.112	-			
Hatay	0.062	0.090	0.084	0.071	0.094	0.292	0.046	0.075	0.016	0.098	0.032	0.104	0.138	0.064	0.100	-		
Van	0.081	0.037	0.046	0.031	0.019	0.283	0.082	0.027	0.080	0.022	0.029	0.024	0.108	0.071	0.038	0.085	-	
Yozgat	0.102	0.094	0.132	0.126	0.122	0.278	0.094	0.089	0.067	0.055	0.035	0.118	0.099	0.115	0.042	0.066	0.076	-

The *Nm* values give the information about genetic divergence or genetic similarity of subpopulations due to gene flow. If *Nm* value is smaller than 2, there is still considerable genetic differentiation among subpopulations. *Nm* values for total populations were detected to be 1.29. Hence, considerable genetic differentiation was observed among populations. The pairwise Nm values ranged from 0.294 (Bozcaada and Artvin) to 4.710 (İzmir and Gökçeada). *Nm* values for 48 pairs out of 153 population pairs were estimated to be larger than 2, so the genetic differentiation among those populations was small. Other pairwise Nm values were smaller than 2 indicating that the genetic differentiation between those populations was significant. Interestingly, pairwise *Nm* values between Artvin and all other populations were lower than 2 which indicate that there were considerable genetic differentiation among populations so was the case for Muğla and other population pairs. However, the pairwise *Nm* between Kırklareli and Gökçeada population was higher than 2, while that of between Kırklareli and Bozcaada population was smaller than this level. Hatay population had generally lower *Nm* values when it was compared with other populations except for Şanlıurfa and Kars (Table 3.18).

Table 3.18. *Nm* pairwise values

	Bozcaada	Giresun	Gökçeada	İzmir	Konya	Muğla	Kırklareli	Aydın	Şanlıurfa	Sivas	Antalya	Beyşehir	Kars	Kayseri	Artvin	Hatay	Van	Yozgat
Bozcaada	***																	
Giresun	0.965	***																
Gökçeada	1.295	1.607	***															
İzmir	2.027	1.680	4.439	***														
Konya	1.257	1.835	1.109	2.443	***													
Muğla	0.285	0.666	0.490	0.462	0.364	***												
Kırklareli	1.428	1.496	2.097	2.055	1.610	0.667	***											
Aydın	1.059	1.410	1.936	3.323	2.033	0.289	2.329	***										
Şanlıurfa	1.240	1.958	1.851	2.410	2.247	1.024	2.313	2.323	***									
Sivas	1.329	2.277	1.663	2.445	2.081	0.566	1.802	2.405	2.391	***								
Antalya	2.002	2.514	3.364	2.696	2.280	0.633	2.228	3.417	2.921	2.387	***							
Beyşehir	1.482	0.986	1.297	4.390	1.431	0.330	1.405	2.168	1.977	2.594	2.308	***						
Kars	0.617	1.062	0.659	0.940	1.271	1.304	1.541	0.722	1.389	1.359	1.347	0.887	***					
Kayseri	0.919	1.645	2.650	2.310	1.255	0.302	2.103	1.713	2.660	1.807	2.583	0.982	1.285	***				
Artvin	0.656	0.913	0.743	1.450	1.131	0.294	1.539	1.160	1.237	1.406	1.866	0.711	0.841	0.724	***			
Hatay	0.736	1.070	1.816	1.688	1.179	1.261	1.948	1.664	4.072	1.447	1.601	1.467	3.026	1.036	0.982	***		
Van	1.281	1.909	1.619	2.020	2.512	0.362	1.773	3.051	2.469	2.134	3.083	2.059	2.556	1.468	1.412	2.413	***	
Yozgat	0.684	0.631	0.775	1.020	0.842	0.315	1.077	1.094	1.721	1.842	2.078	0.680	2.118	0.617	0.854	0.741	1.127	***

Table 3.19 .Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

	Bozcaada	Giresun	Gökçeada	İzmir	Konya	Muğla	Kırklareli	Aydın	Şanlıurfa	Sivas	Antalya	Beyşehir	Kars	Kayseri	Artvin	Hatay	Van	Yozgat
Bozcaada	****	0.906	0.878	0.883	0.835	0.593	0.83	0.888	0.854	0.836	0.882	0.852	0.594	0.816	0.766	0.802	0.848	0.8
Giresun	0.099	****	0.904	0.901	0.866	0.654	0.801	0.959	0.85	0.929	0.922	0.878	0.633	0.874	0.823	0.76	0.916	0.816
Gökçeada	0.13	0.101	****	0.977	0.874	0.619	0.832	0.920	0.869	0.885	0.885	0.889	0.576	0.924	0.756	0.739	0.899	0.752
İzmir	0.125	0.105	0.024	****	0.930	0.592	0.858	0.938	0.892	0.912	0.911	0.919	0.557	0.922	0.8	0.758	0.924	0.76
Konya	0.18	0.144	0.135	0.073	****	0.502	0.804	0.900	0.841	0.898	0.875	0.921	0.542	0.825	0.796	0.715	0.93	0.74
Muğla	0.523	0.425	0.48	0.524	0.69	****	0.559	0.619	0.66	0.611	0.69	0.515	0.601	0.632	0.571	0.682	0.577	0.633
Kırklareli	0.187	0.222	0.184	0.153	0.218	0.581	****	0.831	0.872	0.797	0.851	0.762	0.58	0.825	0.815	0.864	0.814	0.772
Aydın	0.119	0.042	0.084	0.064	0.106	0.48	0.185	****	0.887	0.957	0.945	0.913	0.593	0.909	0.849	0.779	0.945	0.83
Şanlıurfa	0.158	0.163	0.141	0.114	0.174	0.416	0.137	0.120	****	0.873	0.914	0.817	0.624	0.897	0.793	0.888	0.847	0.854
Sivas	0.179	0.074	0.122	0.092	0.108	0.493	0.226	0.045	0.136	****	0.913	0.887	0.615	0.876	0.834	0.747	0.92	0.846
Antalya	0.126	0.081	0.122	0.093	0.134	0.371	0.162	0.056	0.09	0.091	****	0.86	0.624	0.915	0.855	0.858	0.903	0.855
Beyşehir	0.16	0.13	0.118	0.085	0.082	0.664	0.271	0.091	0.203	0.12	0.151	****	0.516	0.848	0.761	0.701	0.917	0.727
Kars	0.521	0.458	0.553	0.586	0.613	0.509	0.545	0.522	0.471	0.487	0.472	0.663	****	0.567	0.647	0.631	0.649	0.689
Kayseri	0.203	0.135	0.079	0.081	0.193	0.46	0.193	0.096	0.109	0.132	0.089	0.165	0.568	****	0.792	0.791	0.869	0.785
Artvin	0.266	0.195	0.28	0.223	0.228	0.561	0.204	0.163	0.232	0.182	0.157	0.273	0.436	0.233	****	0.786	0.876	0.815
Hatay	0.221	0.275	0.303	0.278	0.336	0.383	0.146	0.25	0.119	0.291	0.154	0.356	0.46	0.234	0.241	****	0.731	0.821
Van	0.165	0.088	0.106	0.079	0.073	0.55	0.206	0.057	0.166	0.083	0.102	0.086	0.433	0.14	0.132	0.313	****	0.777
Yozgat	0.224	0.204	0.285	0.275	0.301	0.457	0.259	0.186	0.158	0.167	0.156	0.319	0.372	0.242	0.205	0.197	0.252	****

Nei's genetic identity and genetic distance were illustrated in table 3. 19. Genetic distances (*Ds*) were estimated according to Nei (1973). Muğla and Konya were the most distant honeybee populations (0.69) whereas İzmir and Gökçeada were the closest populations (0.024). Neighbour Joining (*NJ*) and *UPGMA* phylogenetic trees were constructed using Nei's standard genetic distance (Figure 3.9- 10 and 3.11). In *NJ* tree, two main branches were observed. First one included Muğla and Kars populations while the second one consisted of other honeybee populations. In *UPGMA* tree, Kars honeybee population was separated from other populations by itself.

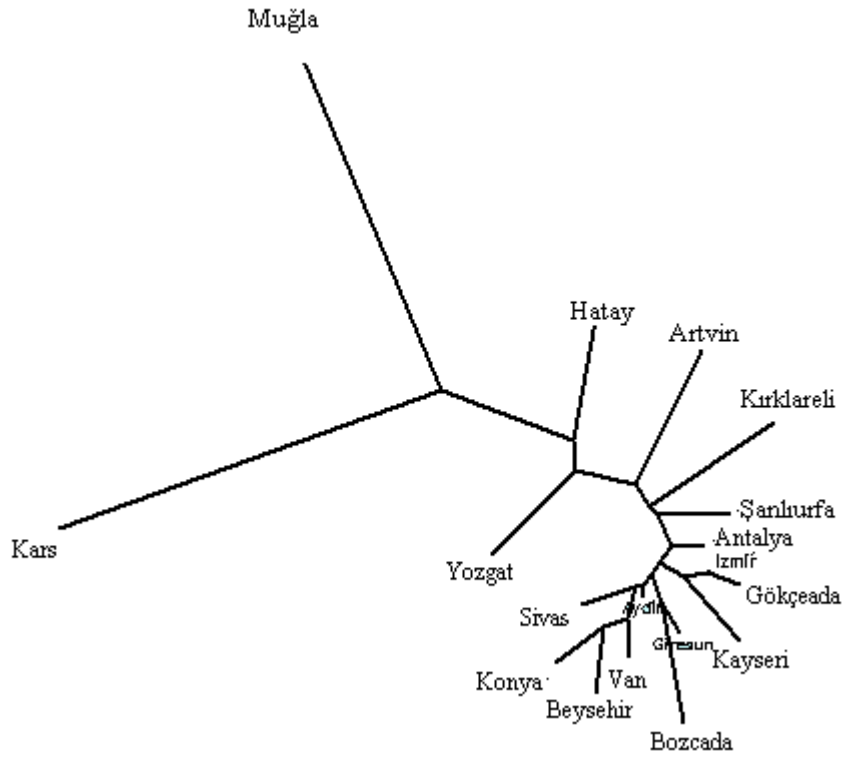


Figure 3.9. An unrooted *NJ* tree based on *Ds*.

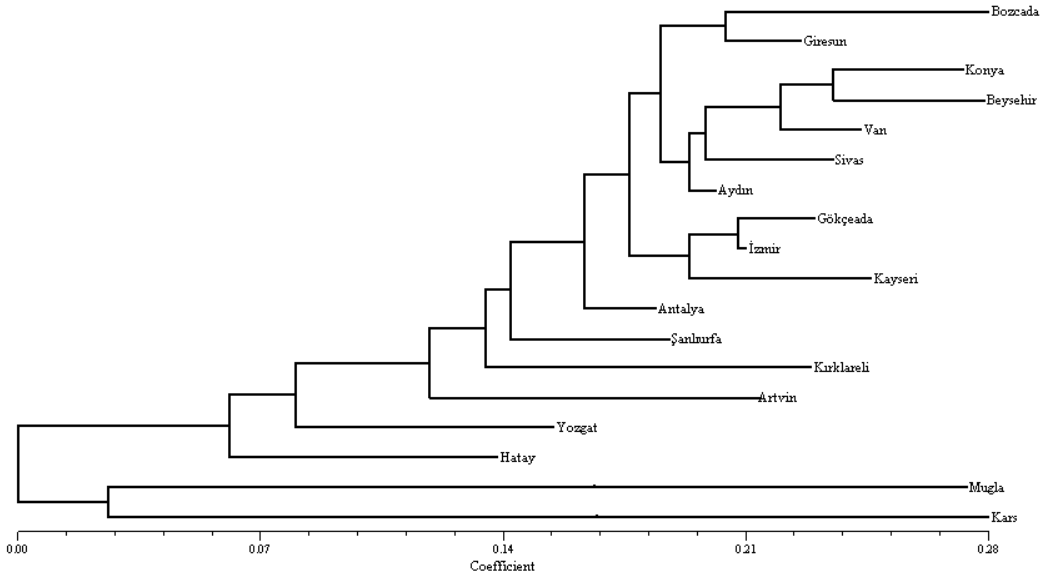


Figure 3.10. A rooted *NJ* tree based on *Ds*.

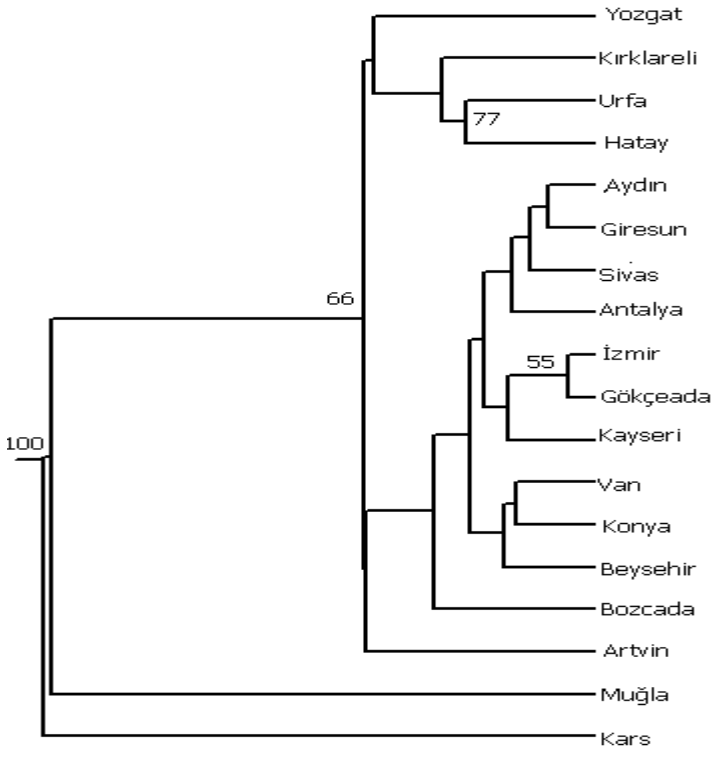


Figure 3.11. *UPGMA* tree based on *Ds*

The correlation between genetic and geographic distances was determined using Mantel test. The correlation (*r*) was estimated as 0.15325 from normalized Mantel statistic (*Z*). Without normalization of data, raw Z value was 3353.9440 and the significance tested by Mantel t-test and P value were calculated as 1.2848 and 0.9006 (>0.05), respectively. Therefore, there is no significant correlation between genetic distance and geographic distance based on microsatellite data. Matrix comparison of all data was illustrated as scatter plot (Figure 3.12).

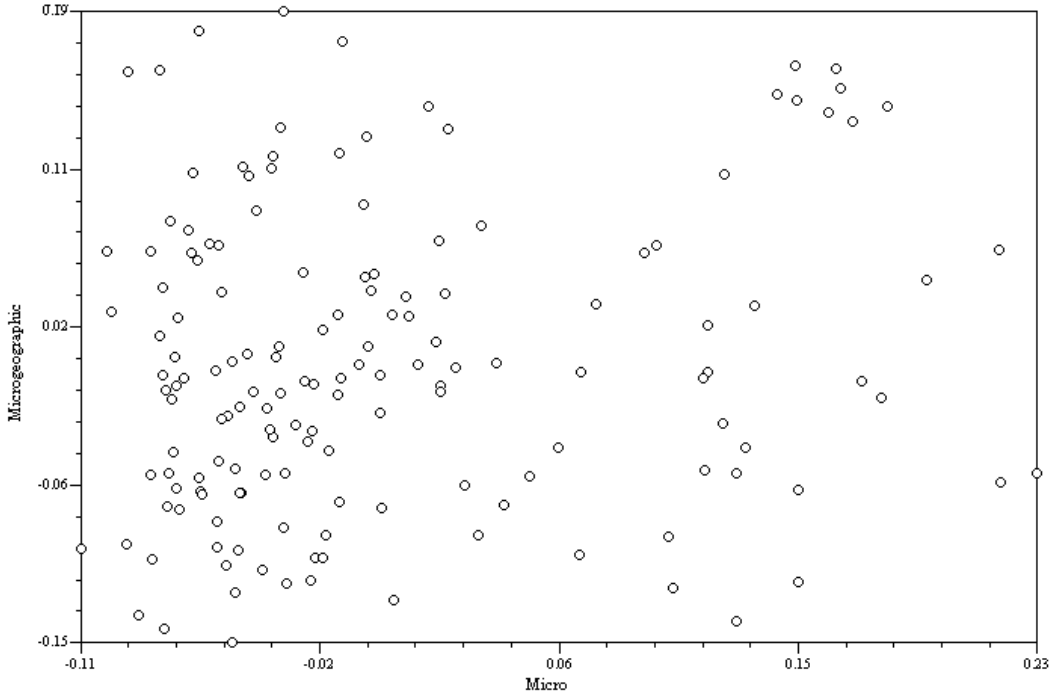


Figure 3.12. Matrix comparision of microsatellite genetic distance and geographic distance

Assignment test was performed in order to see the likelihoods of each individual belonging to populations. The percentage of correct assigned individuals in populations was given in table 3.20. İzmir, Aydın and Antalya populations had the lowest (47%), Bozcaada and Artvin had the highest correct assignment value (83%) for their individuals. The individuals from 15 populations illustrated correct assignment percentages higher than 50%. Mean correct assignment percentage was estimated as 64% for all populations.

Table 3.20. Percentage of correct assigned individuals (*N*: Total number of individuals within each population; *n*: number of individuals assigned to population).

	N	*N*	*%*
Bozcaada	30	25	83
Giresun	30	21	70
Gökçeada	30	16	53
İzmir	30	14	47
Konya	30	20	67
Muğla	28	18	64
Kirklareli	30	22	73
Aydın	30	14	47
Şanlıurfa	30	20	67
Sivas	28	14	50
Antalya	30	14	47
Beyşehir	30	17	57
Kars	30	24	80
Kayseri	25	19	76
Artvin	30	25	83
Hatay	30	22	73
Van	30	18	60
Yozgat	18	10	56

Log-likelihood assignment values of individuals for different population pairs were shown on scatter plots (Figure 3.13 and Appendix E). Accordingly, Muğla and Kars populations were separated from other populations.

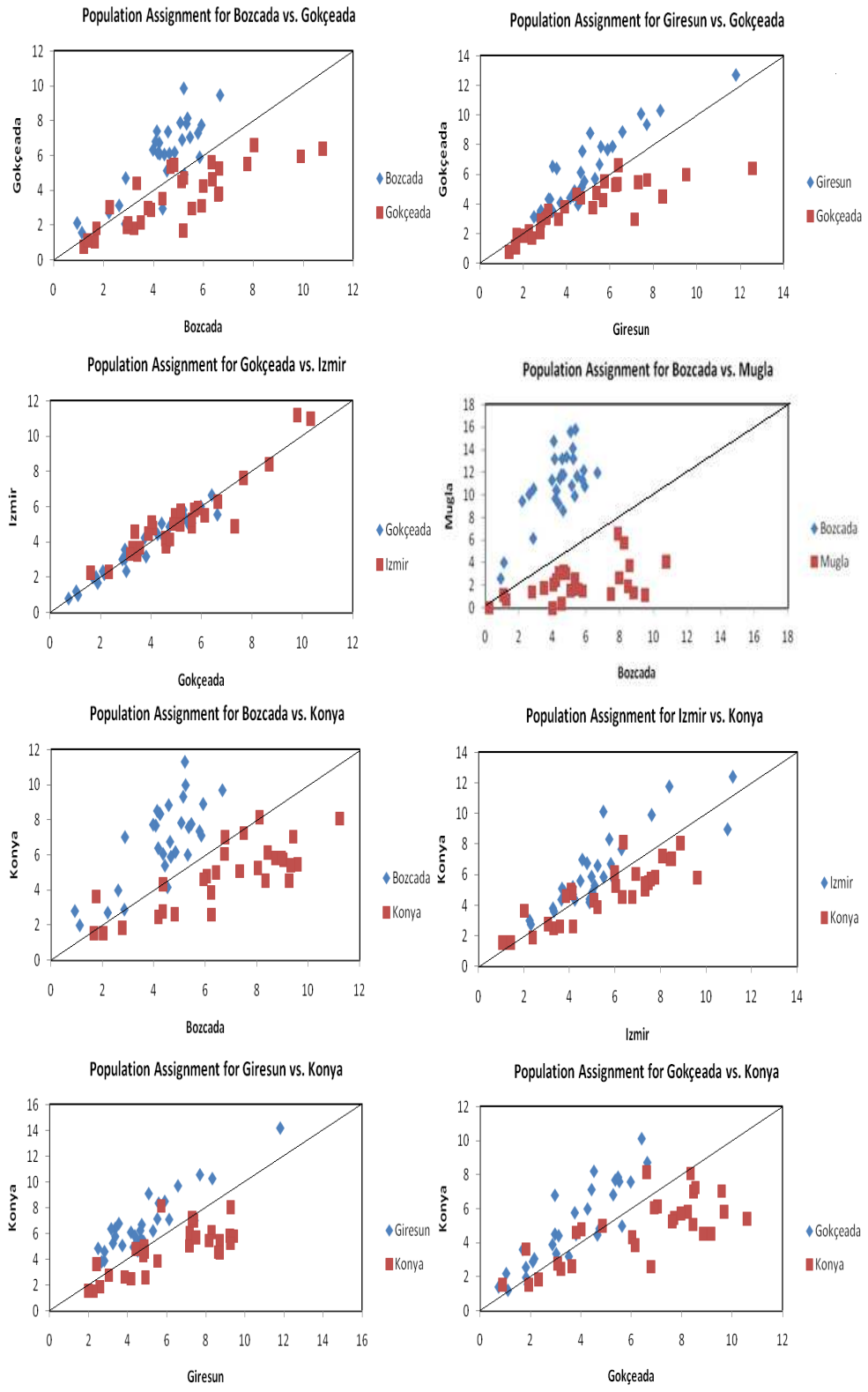


Figure 3.13. Scatter plots of Log-likelihood values of each individual drawn from each population, based on the allele frequencies in each population. Diagonal lines represent the one-to-one line in each case.

3.3. Assessment of two molecular methods

RAPD and microsatellite data were evaluated together. Seventeen populations that were common to both data sets were subjected to Mantel test in order to find out if there is congruency. On the other hand, Partial Mantel test was performed to determine the correlation between genetic and geographic distance obtained from RAPD and microsatellite markers.

The matrix correlation value (*r*) was estimated as -0.16592 from normalized Mantel statistic (*Z*). Without normalization of data, the raw mantel statistic value (*Z*) was detected as -0.1713. The significance test including mantel t test and P value was determined as -1.3183 and 0.0937 (> 0.05), respectively. Therefore, there is no significant correlation observed among RAPD and microsatellite genetic distance, and geographic distance. Scatterplot of genetic distance (Ds) and geographic distance is illustrated in Figure 3.14.

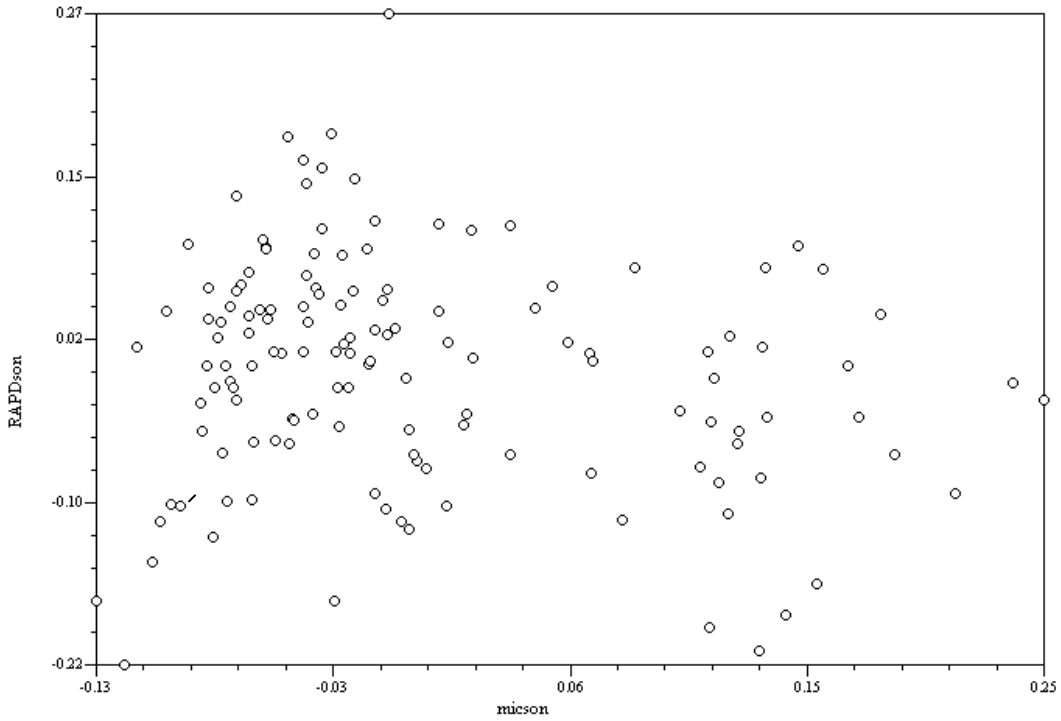


Figure 3.14. Scatterplot of genetic distance (Ds) and geographic distance

Consensus trees are being used to summarize the agreement between two or more trees (Felsenstein, 2008). Here, the consensus tree was constructed using distances from RAPD and microsatellite data. The consensus tree generated 3 main branches; one included Şanlıurfa, Hatay, Artvin, Kars and Muğla populations. In this branch Şanlıurfa and Hatay populations were clustered together. Second one consisted of Konya, Kayseri, Beyşehir, Kırklareli and Bozcaada populations. Within that branch Both Kırklareli–Bozcaada and Beyşehir–Kayseri populations were clustered together. The rest of the populations included in the third branch (Figure 3.15).

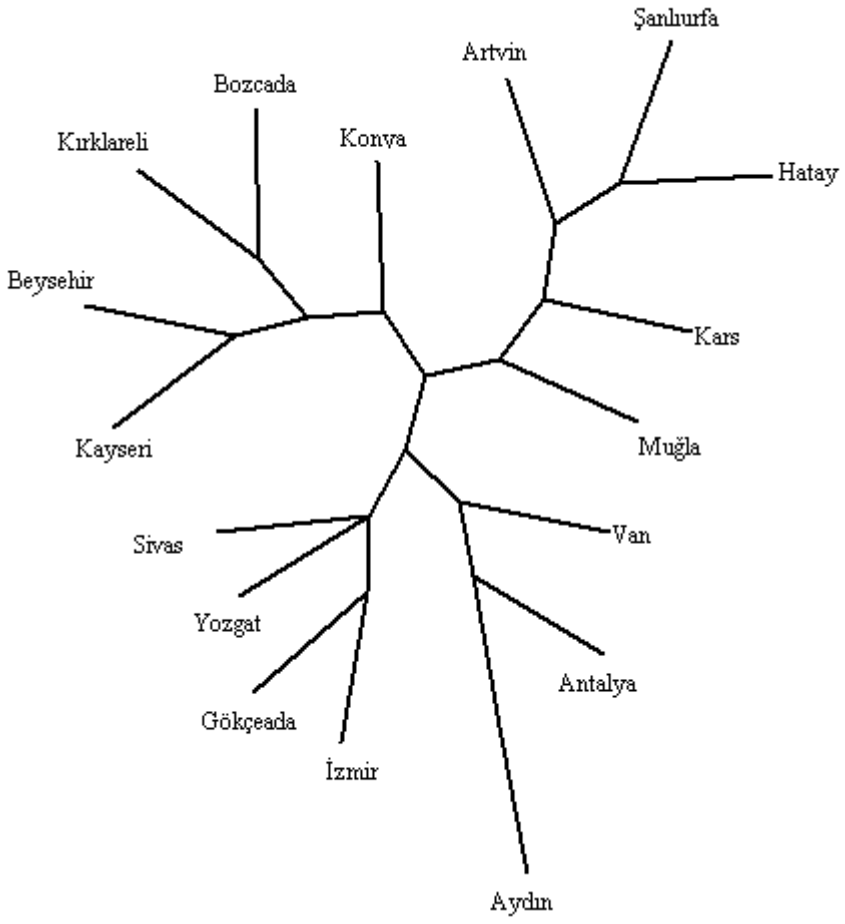


Figure 3.15. Consensus tree constructed using distances of RAPD and microsatellite data

CHAPTER 4

DISCUSSION

Five honey bee subspecies are found in Turkey, *A. m. caucasica* distributed in north eastern, *A. m. syriaca* in south eastern, *A.m. meda* in eastern, *A. m. anatoliaca* in Central Anatolia, and *A. m. carnica* in Thrace (Kandemir *et al.*, 2000; Bodur *et al.*, 2007). A large amount of variation in honey bee populations of Turkey was reported based on morphometry and allozymes (Kandemir *et al.*, 2000) and microsatellites (Bodur *et al.*, 2007). In the present investigation we employed two genetic markers, RAPD as dominant and microsatellites as codominant markers in order to determine differentiation of honey bee populations in Turkey.

RAPD analysis indicated that the expected heterozygosity (*He*) values ranged between 0.035 and 0.136 among geographic regions. We observed that expected heterozygosity values (*He*) increase from northern to southern and eastern to western Anatolia. The Shannon's index value (*H´)* also showed similar pattern. At the same time Shannon's index values of islands, Thrace and Central Anatolian populations were higher than that of the eastern, northern, and southeastern Anatolia.

The expected heterozygosities (Gene diversities) and Shannon's index values when compared at population level showed that Antalya population had the highest *He* (0.175), proportion of polymorphic loci (64.76%) and *H'* value (0.271). Meanwhile, the high *He* and *H'* values were also observed for the Aegean island populations, Gökçeada (*He*=0.128 and *H'*=0.212) and Bozcaada (*He*=0.129 and *H'*=0.212) which were higher than that of Kars, Hatay, Bilecik, Nevşehir, Uşak, Şanlıurfa, Artvin, Bingöl, Bitlis, Kayseri, Manisa, Trabzon and Van honey bee populations ranging between 0.035 and 0.114. The observation of high gene diversity in two

island populations may be due to queen bee import and bee transfer during human migrations.

Hatay and Şanlıurfa populations had the lowest genetic diversities and also these populations were clustered together in the dendrogram that was constructed using Nei's standard distance. This observation supports the results Bodur *et al.* (2007) obtained using a different set of microsatellites in which Hatay and Şanlıurfa populations also clustered together. These populations were shown to contain African mitochondrial haplotypes (Smith *et al.*, 1997; Kandemir *et al.*, 2006). These findings suggest that Hatay and Şanlıurfa populations are more closely related to the African honey bees than the rest of the Turkish honey bee populations.

The number of observed bands ranged between 39 (Hatay) and 70 (Antalya) and 4 private bands were observed for Aydın, Gökçeada, Mugla and Şanlıurfa populations. In a previous study, Suazo *et al.* (1998) screened 700 RAPD primers in order to determine the differences between African and European honey bee populations. Their analyses clustered the samples according to old world European, new world European, South African and new world African honey bee populations and showed that a band amplified by the primer 539 was specific for East European honey bee subspecies 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. Furthermore, the bands produced by the primers 694 and 514 were found at low frequencies in African, but at high frequencies in European populations (Suazo *et al.*, 1998).

In Turkish honey bee populations OPB-1 and OPA-7 primers seem to be having specific band pattern in all except Hatay and Şanlıurfa populations. This is important in that these two populations belong to A lineage, whereas the others belong to C lineage.

In the dendrogram obtained here, the northwestern honey bee populations, Kırklareli and Bozcaada were clustered together and different from all other populations. In addition, genetic variation was demonstrated using RAPD in two different mountain

and Thrace regions of Bulgaria and Turkey. In the dendrogram, three populations (Pinarhisar, Demirkoy, Derekoy) from Thrace region of Turkey and one population (Plovdiv) from Bulgaria were clustered together and the other two mountainous populations formed another cluster (Ivanova *et al.*, 2007).

In the current study, the proportion of polymorphic loci and mean heterozygosity levels were estimated as 100% and 0.122. H_T value was 0.188 and G_{ST} value was determined as 0.352. Gene flow (*Nm*) was estimated as 0.922 for all populations studied which indicate that there is still considerable differentiation among honey bee populations in Turkey. Özdil *et al.* (2006) studied sixteen honeybee populations using 20 RAPD primers and they estimated 92% polymorphism and 0.331 average heterozygosity, average population differentiation in total genetic diversity as 0.3299, coefficient of population differentiation as 0.2889, and the gene flow value for all populations as 1.2301. In addition, Tunca *et al.* (2004) illustrated the genetic variation based on RAPD in honey bee populations in Lake Van region (Van, Kars, Bitlis, Hakkari, Muş). They obtained a gene diversity value in total populations (H_T) as 0.196 and G_{ST} value among populations as 0.186. *Nm* for all populations was estimated at considerably high level, 2.039 in Lake Van region. Kence *et al.* (2004) reported medium level of genetic variation in Iranian (*A. m. meda*) populations by RAPD analyses. When genetic diversity in Iranian and Turkish honey bee populations compared here it is found that the genetic diversity for Turkish honey bee populations (0.3517) was higher than Iranian populations (0.2654).

We observed 118 alleles at 6 microsatellite loci in a total of 520 worker bees sampled from18 honey bee populations. The observed number of alleles per locus ranged between 10 and 32. We detected 32 alleles at Ap001 locus, in all populations, hovewer, Solignac *et al.* (2003) observed 20 alleles: l8 in bees belonging to M lineage (France) and 2 alleles in C lineage (Italy) and none in A lineage (Morrocco). In our study, we did not observe any allele in Hatay population which is reported to belong to A lineage by Kandemir *et al.* (2000) and Bodur *et al*. (2007). Similarly, these studies also revealed that Şanlıurfa population contains genes of A lineage and we observed 17 alleles in Şanlıurfa population. For Ap289 locus, we detected 26 alleles at all populations. For the same locus, Solignac *et al*. (2003) detected 25

alleles: 23 alleles for A lineage, 12 alleles for M lineage, and 3 alleles for C lineage. The lowest number of alleles was observed at Ap243 locus with 10 alleles only in our study. At this locus, Solignac *et al*. (2003) detected 4 alleles for M lineage populations, 2 alleles for C lineage, and none for A lineage. In contrast, we observed 5 alleles for Hatay and 4 alleles for Şanlıurfa populations. For Ap223 locus, we detected 12 alleles, whereas Solignac *et al.* (2003) observed 8 alleles: 4 alleles for M, 2 alleles for C and 6 alleles for A lineages. Finally, we observed 25 alleles at A76 and 13 alleles at Ap019; however, Chaline *et al*. (2002) detected 8 and 7 alleles, respectively, using 214 pupae in UK honey bee populations. The mean number of alleles considering all loci for all honey bee populations studied was estimated to be 6, whereas Bodur *et al*. (2007) estimated a little higher mean number of alleles at nine different loci for Turkish and Cyprus populations as 6.95.

In the current study, a total of 29 private alleles were detected for 14 populations out of 18, there were no private alleles in Ap223 locus. Among the populations studied, Şanlıurfa population had 5 private alleles that is being the highest number. Similarly, Bodur *et al.* (2005) detected 4 private alleles for Şanlıurfa population. The highest number of private alleles (9) was observed at A76 locus. When two A lineage populations are considered, the diagnostic alleles were 237 and 243bp at Ap001, 262 bp at Ap243, and 227, 229, 230 bp at A76 in Şanlıurfa, whereas, 253 and 257 bp. alleles at A76 locus in Hatay population. We did not detect any diagnostic alleles for A lineage at Ap223. Solignac *et al*. (2003) assigned Ap243, Ap223, and Ap001 as diagnostic loci but they did not detect any allele at Ap001 and Ap243 loci for A lineage; however, they observed 6 alleles at Ap223 locus in A lineage populations.

In the current research a total of 47 diagnostic alleles were detected in populations of C lineage comparable to 30 alleles Bodur *et al.* (2005) reported. Ap289 locus revealed 5 diagnostic alleles, thus we can assert Ap289 locus as diagnostic in contrast to Solignac *et al*. (2003).

The range of gene diversity levels for A lineages (Hatay and Şanlıurfa populations) was [0.528- 0.555] and for C lineages was [0.449- 0.739]. Bodur *et al*. (2007)

estimated gene diversity for Hatay population as 0.614 and a range between 0.542 and 0.681 for all populations.

Microsatellite studies on honey bee populations have generally focused on European and African honey bee subspecies (Frank *et al.,* 1998; 2001), whereas, recent studies have been published for island populations and Mediterranean honey bee populations (Dall'olio *et al.*, 2007; Franck *et al.*, 2001; Bodur *et al.,* 2007). Lebanon honey bees including Middle Eastern honey bee populations were studied using 8 microsatellite loci and the gene diversity for those populations was estimated to be 0.65 (Franck *et al.*, 2000a). Also, Bodur *et al*. (2007) found gene diversity values between 0.54 and 0.68 very similar to Middle Eastern populations. Mediterranean honey bee gene diversity value was reported ranging between 0.39 and 0.68 (Frank *et al.,* 2000b). Dall'olio *et al.* (2007) studied the genetic variability of *A. m. ligustica* at eight polymorphic microsatellite loci and reported the gene divesity for North Mediterranean honey bees between 0.528 and 0.637. The gene diversity value (0.588) we estimated is congruent with that of Middle East and North Mediterranean honey bee populations, if we disregard the use of different microsatellite loci.

Locus based gene diversity values ranged between 0.378 (Ap243) and 0.855 (Ap001). For Ap289 locus, gene diversity was estimated to be 0.817. Solignac *et al*. (2003) reported much lower gene diversity for C lineage as 0.14 and M lineage as 0.92, A lineage as 0.96. Accordingly, estimated gene diversity for Ap289 in honey bee populations of Turkey was greater than that of the results of Solignac *et al.* (2003) for C lineage, but smaller for M and A lineages. Ap243 revealed the smallest value (0.378) for gene diversity among the loci analyzed. At the same locus Solignac *et al*. (2003) estimated a higher gene diversity for M lineage (0.56) and lower gene diversity for C lineage (0.07). The gene diversity for Ap001 locus we calculated as 0.855 and the most frequent alleles were 211 and 213 bp. Solignac *et al.* (2003) estimated gene diversities as 0.93 and 0.26 for M and C lineages, and the most frequent alleles for the two lineages were 244 and 218 bp, respectively . The gene diversity for Ap223 was detected as 0.673 in our study. For same locus, the gene diversity value was estimated as 0.34 for M lineage, 0.07 for C, and 0.62 for A lineages by Solignac *et al.* (2003). The gene diversity estimations were 0.652 and

0.556 for A76 and Ap019 loci in the current study, but reported as 0.947 and 0.916 respectively by Chaline *et al.* (2002) in UK honey bee populations.

The most frequent alleles were determined for loci in our study and provide the comparision of three main lineages (A, M and C). Our study revealed that most frequent alleles were 168 and 182 bp for Ap223, 253 bp. for Ap243, and 182 and 184 bp for Ap289, 211, 213 and 215 bp for Ap001. According to Solignac *et al.* (2003); 169 and 178 bp for Ap223, 186 bp for Ap289, and 240 and 253 bp for Ap243 were the most frequent alleles and specifically, 253 bp in the C lineage was the most frequent allele. Furthermore, for Ap001, 220 bp was the most frequent alleles for A and M lineage and 216 bp was detected the most frequent allele for C lineage. For A76 and Ap019, most frequent alleles were 207 and 138 bp, respectively. Correspondingly, Chaline *et al*. (2002) demonstrated size ranges of A76 and Ap019 loci as 230-308 bp and 134-146 bp, respectively.

Tests for Hardy-Weinberg equilibrium revealed 47 significant deviations ($P<0.05$; 0.01 and 0.001) among 108 population-locus combinations. Among these 47 combinations, all but 5 of them were in favor of homozygotes (F_{IS}). When compared to Bodur *et al.* (2007), they observed 8 significant deviations ($P<0.05$) among 108 population-locus combinations and similarly with our results, all significantly deviated combinations were heterozygote deficit except for Ardahan-A24. In our case, all populations have heterozygote deficiency at all loci except Ap223 and Ap019 loci which have heterozygote excess. The heterozygote deficiency may be explained by inbreeding. Further tests by using different microsatellite loci are necessary.

According to Hartl and Clark (2007, p.283), F_{ST} levels between 0.00 and 0.05 indicate little genetic differentiation: levels between 0.05 and 0.15 indicate moderate; levels between 0.15 and 0.25 indicate great genetic differentiation and levels > 0.25 show very significant genetic differentiation. In our study, for a total of 153 pairwise F_{ST} comparisons among 18 populations: great genetic differentiation in 10, significant genetic differentiation in 16, moderate genetic differentiation in 94, and little genetic differentiation in 33 comparisons were detected. More specifically, the

lowest and the highest pairwise F_{ST} were detected for Gökçeada–İzmir (-0.068) and Konya–Muğla (0.347), respectively. Bodur *et al*. (2007) estimated pairwise F_{ST} values ranged between 0.0 and 0.183 for Turkish honey bee populations using nine different microsatellite loci. Additionally, F_{ST} values were determined for lineages by many studies (Frank *et al*., 2000a, 2001; Garnery *et al.*, 1998; Dall' Olio *et al.*, 2007). Franck *et al.* (2000a) revealed that the lineage pairwise F_{ST} values for A and M lineages were smaller than 0.1 whereas C lineage pairwise value was higher than 0.1 levels. Furthermore, Franck *et al.* (2001) illustrated that pairwise F_{ST} for A lineage changed between 0.01 and 0.12, for M lineage it was smaller than 0.1 and for C lineage F_{ST} value determined between 0.17 and 0.024. Among western European populations (Portugal, France, Spain, Sweden and Belgium), pairwise F_{ST} changed between 0.002 and 0.185 (Garnery *et al.*, 1998) and they obtained a lower genetic variability for populations of M lineage. Furthermore, Dall' Olio *et al.* (2007) estimated pairwise F_{ST} value as 0.0009 and 0.3221 for *A. m. ligustica* clustered in C lineage. Our findings suggest that a high level of genetic differentiation was observed in Turkish honey bee populations considering the wide range of pairwise F_{ST} values.

Gene flow values provide information on genetic divergence among subpopulations. 48 out of 153 *Nm* values were higher than 2, whereas rest of the pairwise *Nm* values were smaller than 2, so there is a considerable genetic divergence among populations. One surprising finding was that *Nm* pairwise for Antalya populations was higher than 2 except Muğla, Kars, Artvin and Hatay populations. This result illustrated high gene flow from other locations to Antalya or from there to other locations. There are several possible explanations for this result: migratory bee keeping in this region because of favorable climatic and vegetation conditions for wintering and also queen bee breeding activities are widely applied by many breeders or commercial companies. The distribution of queen bees may be explaining the high level of *Nm* values.

Contrary to expectations, the results revealed that number of migrants from Kırklareli, İzmir and Kayseri to Gökçeada was high. The possible explanations for this might be queen bee transfer and human migration. Although, there is no statistical or scientific information about the queen transfer or migrotary bee keeping

activity in Gökçeada, phenotype of island honey bees looks like carniolan bee (*A. m. carnica)* and some of the breeders bought queen bees from Kars nearly 10 years ago (County Agriculture Director, personal communication, 2009). Additionally, according to 2510 numbered "settlement law", human migration took place from Muğla, Isparta, Çanakkale (Biga), Erzurum, Bursa and Trabzon to Gökçeada in 1960. Last settlement (Şirinköy) was established with Turkish immigrants from Bulgaria at the end of the 2001 (Gökçeada Municipality, Personal Communication, 2009). Honey bee importation has been prohibited since 2004 by Gökçeada County Agriculture Directorate. In accordance with above information, we might explain the lowest pairwise F_{ST} value between Gökçeada and İzmir (-0.068) in such a way that Gökçeada honey bee gene pool seems to have been mixed with *carnica*, *caucasica* and Muğla honey bees. Also, the breeders from İzmir have transported their colonies during the spring and summer period from İzmir to vicinity of Kırklareli and Muğla. Also some of the breeders in İzmir bought c*aucasica* bees from Artvin. The gene pool of İzmir population may be affected by these activities which make the gene pools of Gökçeada and İzmir sharing same alleles.

Another important finding is that pairwise *Nm* values between Hatay and other populations were low except with Şanlıurfa population, and the low level of genetic divergence was observed between them. Once again, microsatellite allele frequencies also indicate that these two populations are genetically different from others. Similarly, Bodur *et al*. (2007) revealed that there was no genetic divergence between Şanlıurfa and Hatay populations.

Muğla and Kars honey bee populations had low gene flow and high F_{ST} value. Bodur *et al*. (2007) showed that gene flow into Muğla population was from Hakkari, İzmir, Kastamonu and Eskişehir populations and they observed lower Nm compared to other regions.

The estimated gene flow is not large to affect the differentiation of honey bee populations. In spite of controversial views on the reliability of the estimates of gene flow based on F_{ST} (Whitlock and McCauley, 1999) it is being used largely in population genetics.

Assignment test was also used to determine the level of genetic divergence among honey bee populations. The highest levels of correct assignment percentage were detected for Bozcada and Artvin (83%) populations. Izmir, Antalya and Sivas populations seem to be receiving gene flow due to high migration activity and their correct assignment percentages were 47% (İzmir, Antalya) and 50% (Sivas). Their F_{ST} and *Nm* value indicate that these locations were seriously affected from migratory bee keeping activities. Artvin honey bee population is shown to be divergent and isolated as a conserved area and that is supported with high correct assignment (83%). According F_{ST} value, Artvin population had significantly high level of genetic diversity and a low level of Nm. This is actually representing the unidirectional gene flow from Artvin population to other populations since it is known that there is no gene flow into Artvin (Camili) population. The correct assignment percentage for Kırklareli population was estimated to be 73%. Although F_{ST} value illustrated moderate level of genetic differentiation of Kırklareli population, it is obvious with the Nm value that it is getting migration from other populations. Hatay population indicated moderate level of genetic differentiation and the correct assignment percentage was calculated as 73%. The genetic differentiation of populations was supported with a low level of *Nm* value. However, Hatay honey bee population's gene pool seems to be affected by migration from Kars population. Although, low level of migration rate and high level of genetic differentiation were observed in Muğla population, the correct assignment percentage was determined as 64%. The possible explanation of this observation awaits further investigation .The high level of genetic differentiation and 80% assignment was observed in Kars population. Furthermore, gene flow must have been taking place from Urfa and Hatay to Kars population according *Nm* pairwise values.

Consensus tree was constructed using genetic distance obtained with molecular markers, RAPD and microsatellites. The differentiation of populations was demonstrated more efficiently with this constructed tree based on joined data sets, RAPD as dominant and microsatellite as codominant marker. 3 main branches were observed, Anatolian and Caucasian honey bee populations were placed on two separate branches while Kırklareli and Bozcada populations formed another group. One important point is that Hatay and Şanlıurfa populations were clustered together

and Artvin population which is *A. m. caucasica* was separated. Meanwhile, Muğla and Kars populations were very close to each other, because of the migratory beekeeping activities between Kars and Muğla regions.

Mantel and partial Mantel tests performed to both RAPD and microsatellite genetic distance and geographic distances revealed no correlation indicating the differences of allele frequencies were not affected by geographic distances among populations.

CHAPTER 5

CONCLUSION

Considering that Turkish honey bee populations cluster with European populations and that the only Turkish populations that carry African mitotypes are southeastern populations (Hatay and Şanlıurfa), the evidence that honey bee speciation occurred in Africa (Whitfield *et al*., 2006) and Anatolia being one of the major routes in the expansion of honey bees from Africa to Europe is reinforced. Turkish honey bee populations contain high diversity that may be due to the various climatic and phytogeographic characteristics in Anatolia. This points out the importance of Turkish honey bee diversity and that Anatolia probably was one of the earlier centers of honey bee diversification as earlier studies also suggested (Ruttner, 1988; Bodur *et al*., 2007).

At the moment, the *A. m. caucasica* population in Artvin is under protection by the Ministry of Agriculture and Rural Affairs of Turkey (Official Gazette 2004/25668). Kirklareli population is also regulated by the efforts of the local honey bee breeders association (Rıdvan Ulus, Personal Communication, 2008). However, without a general federal conservation plan, such localized efforts are bound to have little or no effect on the overall population.

Our results are concordant both with data collected by Bodur *et al.* (2007) and the results reported in Solignac *et al.* (1995) and Franck *et al.* (2000a) later two showed that the genetic variation was highest in African, the lowest in European and intermediate in Mediterranean honey bee populations.

The data presented here indicate that although the migratory beekeeping was extensive, but it was not extensive enough or severe as to wipe out a great deal of genetic variation.

The results we obtained will be valuable as a base for comparison with the samples from future generations, which should provide understanding of variation lost due to the recent bee losses and will reflect the pattern of recovery if it occurs.

These results should be considered in conservation plannings, particularly with regard to moving of colonies between regions and the most importantly introducing bees with foreign origin and distributing queen bees from one center to all over the country which will pollute the gene pool of the populations.

- To prevent loss of genetic diversity and pollution in honeybee;Strictly controling the queen bee trading
- Controlling migratory bee keeping activity and constructing migration routes as soon as possible
- Establishing new conservation areas for Hatay, Kırklareli and Muğla or private conservation areas and forming selection programs immediately for the subspecies found in these areas.

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APPENDIX A

SOLUTIONS

TE (Tris- EDTA Buffer)

Tris	10 mM
EDTA	1 mM
H2O	100 ml
(pH 8.0)	

10X TBE (Tris-Borate-EDTA) Electrophoresis Buffer

	per liter	Final 1X Concentration
Tris	108g	89mM
Boric Acid	55g	89mM
0.5M EDTA	40ml	2mM
(pH 8.0)		
H_2O	to 1 liter	

6X Loading Dye Solution

10mM Tris-HCl (pH 7.6), 0.03% bromophenol blue,
0.03% xylene cyanol FF, 60% glycerol, and 60mM EDTA.

APPENDIX B

RAPD RESULTS

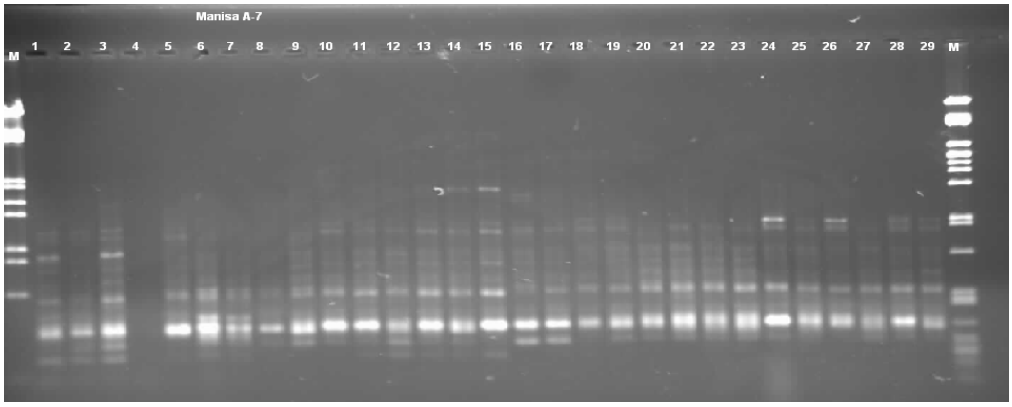


Figure B.1. Banding pattern obtained by OPA-7 primer in Manisa honey bee population

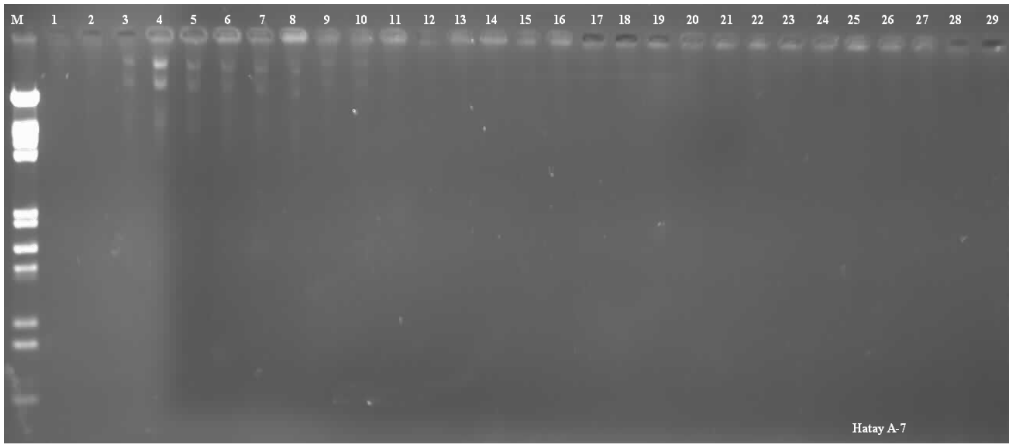


Figure B.2. Banding pattern obtained by OPA-7 primer in Hatay honey bee population

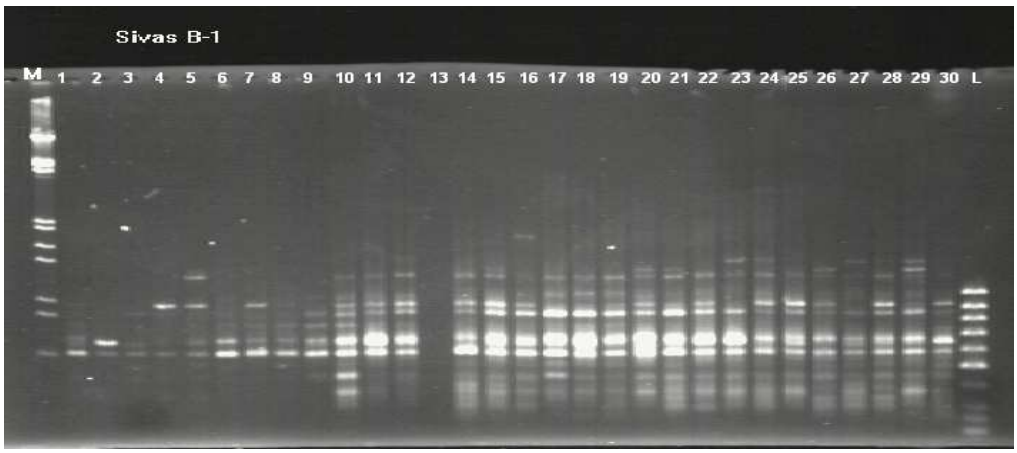


Figure B.3. Banding pattern obtained by OPB-1 primer in Sivas honey bee population

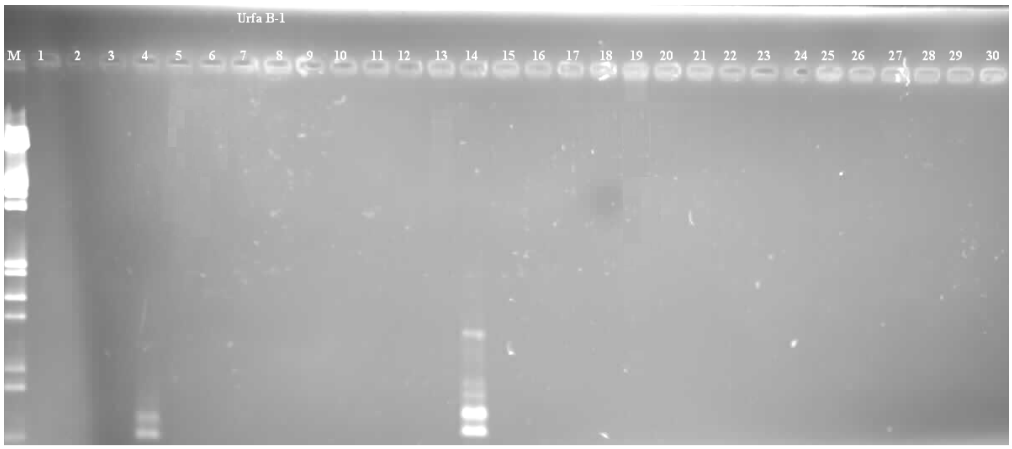


Figure B.4. Banding pattern obtained by OPB-1 primer in Şanlıurfa honey bee population

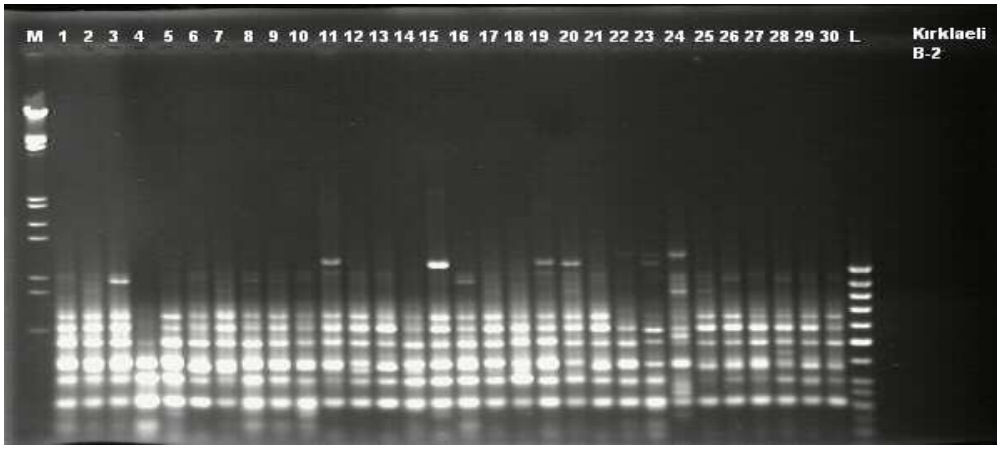


Figure B.5. Banding pattern obtained by OPB-2 primer in Kırklareli honey bee population

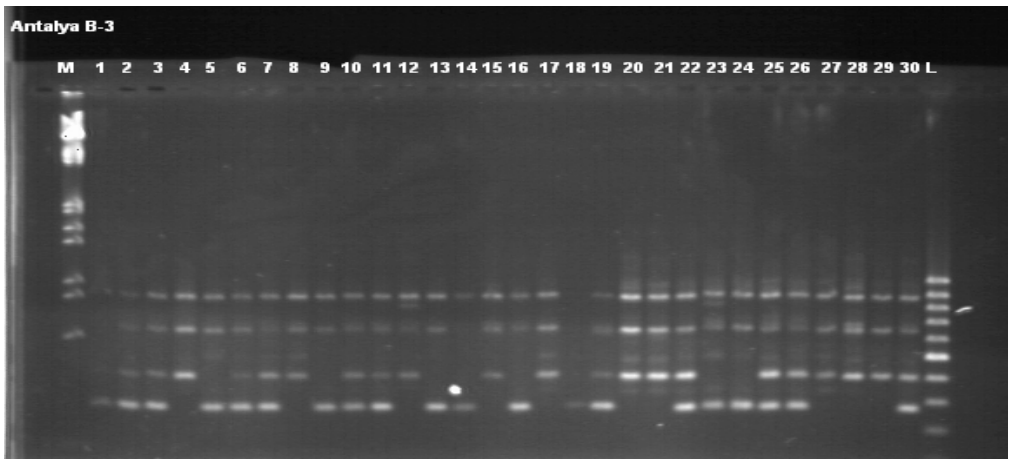


Figure B.6. Banding pattern obtained by OPB-3 primer in Antalya honey bee population

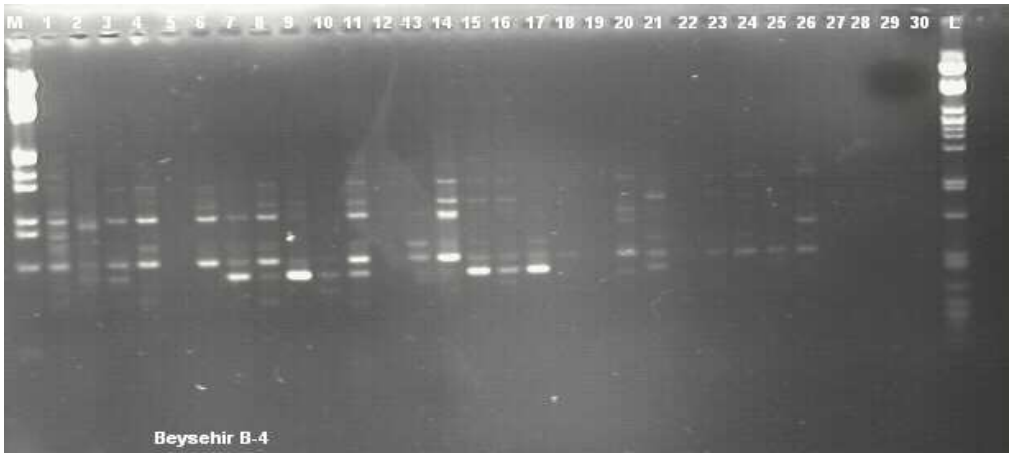


Figure B.7. Banding pattern obtained by OPB-4 primer in Beyşehir honey bee population

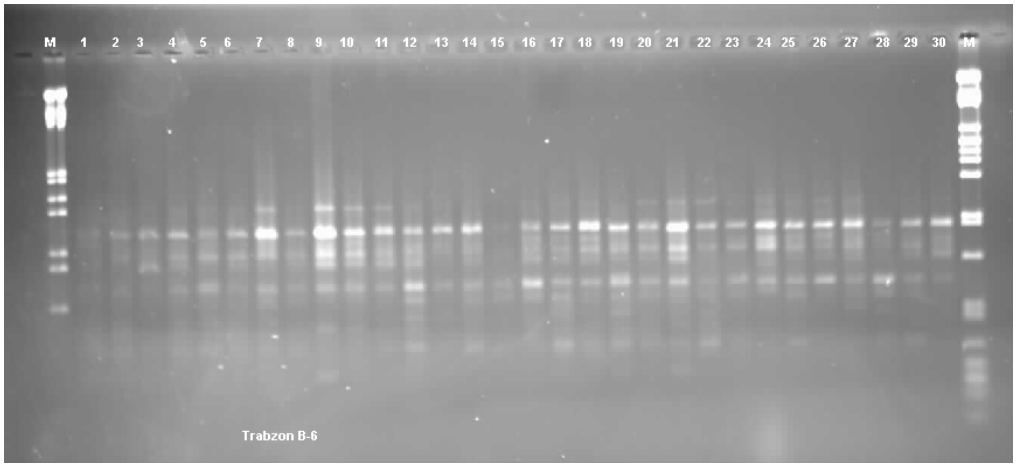


Figure B.8. Banding pattern obtained by OPB-6 primer in Trabzon honey bee population

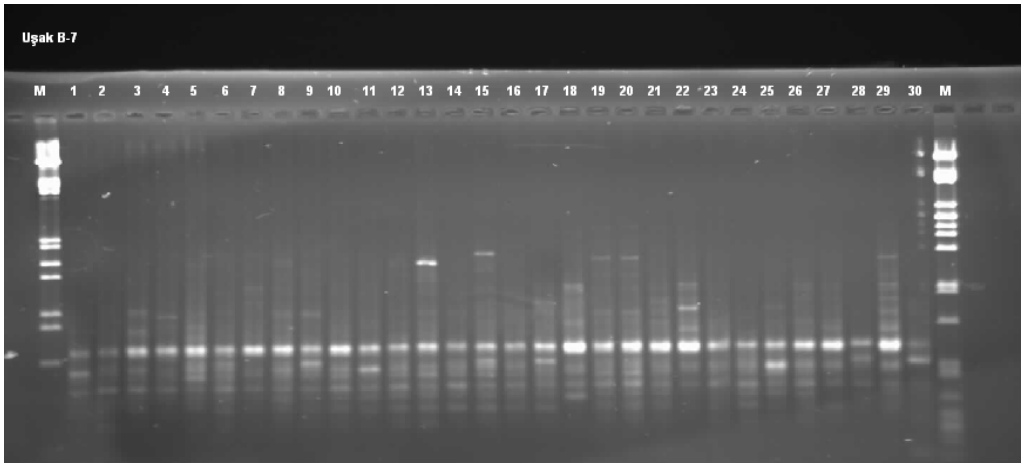


Figure B.9. Banding pattern obtained by OPB-7 primer in Uşak honey bee population

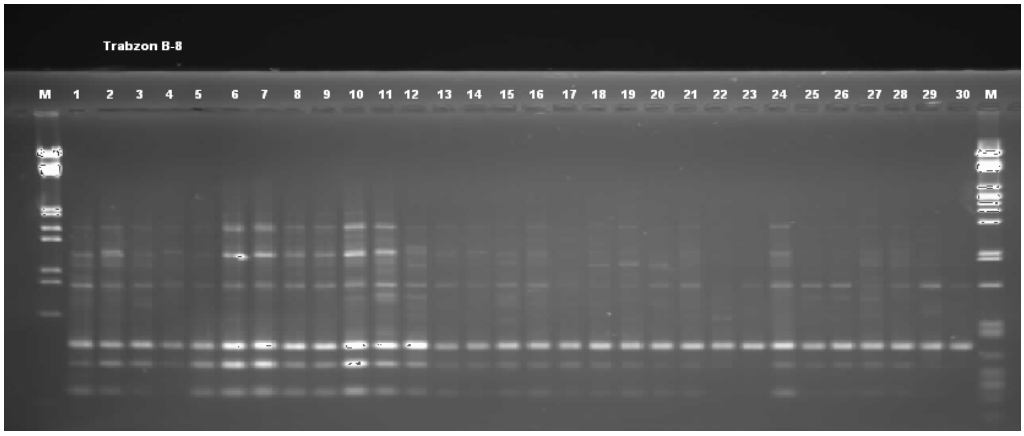


Figure B.10. Banding pattern obtained by OPB-8 primer in Trabzon honey bee population

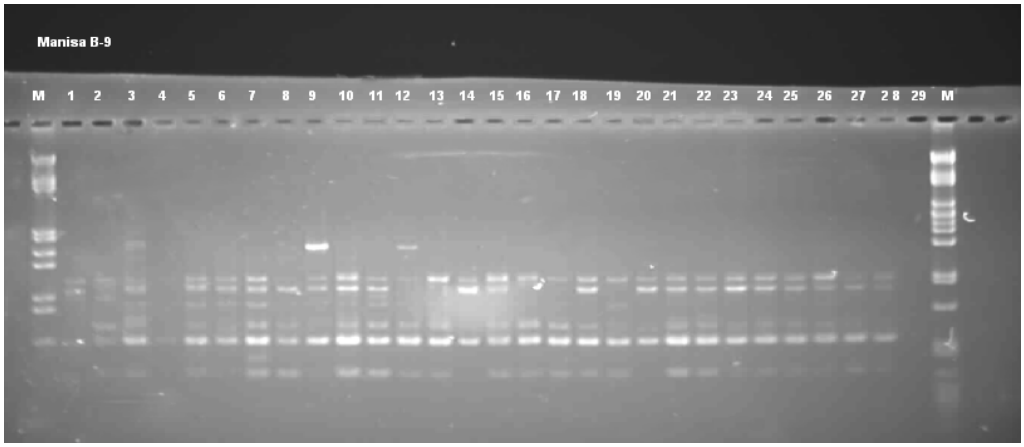


Figure B.11. Banding pattern obtained by OPB-9 primer in Manisa honey bee population

APPENDIX C

FRAGMENT ANALYSIS OF SAMPLES FOR MICROSATELLITE LOCI

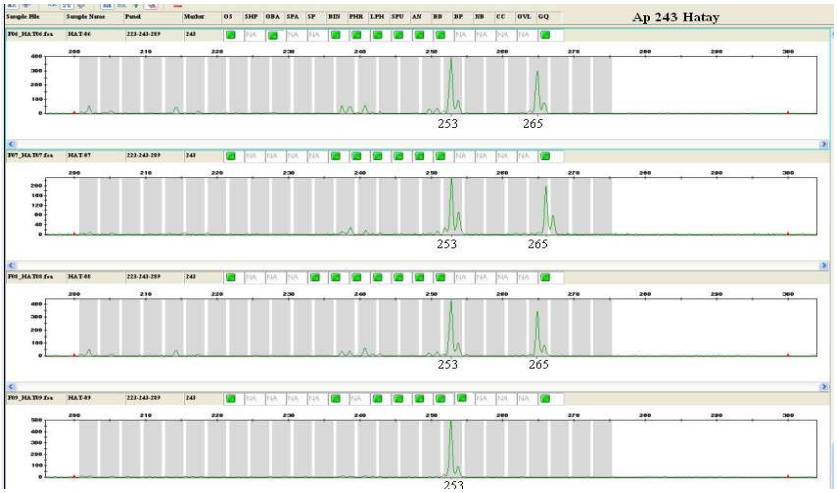


Figure C.1. The alleles of Ap243 [$(TCC)_9$] locus

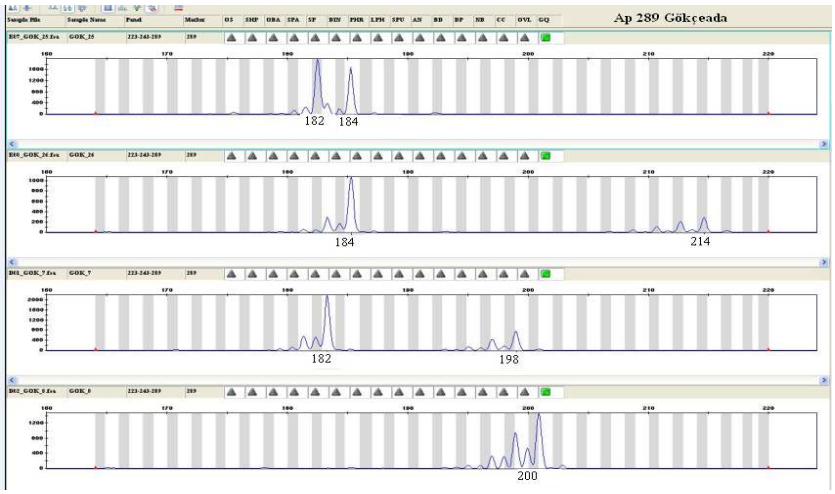


Figure C.2. The alleles of Ap289 [$(GA)_5$] locus

Figure C.3. The alleles of Ap001 $[(CA)_8(TA)_{15}A_3(TA)_8]$ locus

Figure C.4. The alleles of Ap019 $[(CT)_{12}\ ]$ locus

Figure C.5. The alleles of Ap076$[(CT)_{32}]$ locus

APPENDIX D

ALLELE FREQUENCIES OF MICROSATELLITE LOCI

Table D.1. Allele frequencies of Ap243 locus (*N*: number of alleles)

Locus: Ap243	202	211	238	241	253	256	262	265	268	271	*N*
Bozcaada	0.069	0.000	0.000	0.000	0.707	0.052	0.000	0.000	0.155	0.017	58
Giresun	0.000	0.000	0.089	0.000	0.875	0.000	0.000	0.000	0.036	0.000	56
Gökçeada	0.312	0.000	0.000	0.000	0.656	0.000	0.000	0.000	0.031	0.000	32
İzmir	0.206	0.000	0.000	0.000	0.765	0.000	0.000	0.000	0.029	0.000	34
Konya	0.000	0.000	0.000	0.000	0.769	0.038	0.000	0.000	0.192	0.000	26
Muğla	0.167	0.000	0.000	0.000	0.690	0.000	0.000	0.095	0.048	0.000	42
Kirklareli	0.020	0.000	0.100	0.000	0.780	0.000	0.000	0.100	0.000	0.000	50
Aydın	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	36
Şanlıurfa	0.000	0.000	0.000	0.000	0.842	0.000	0.026	0.053	0.079	0.000	38
Sivas	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	30
Antalya	0.071	0.000	0.000	0.000	0.833	0.000	0.000	0.024	0.071	0.000	42
Beyşehir	0.056	0.000	0.000	0.000	0.648	0.000	0.000	0.000	0.296	0.000	54
Kars	0.079	0.053	0.289	0.026	0.526	0.000	0.000	0.000	0.026	0.000	38
Kayseri	0.205	0.000	0.000	0.000	0.795	0.000	0.000	0.000	0.000	0.000	44
Artvin	0.000	0.000	0.074	0.000	0.907	0.000	0.000	0.019	0.000	0.000	54
Hatay	0.058	0.000	0.038	0.000	0.673	0.000	0.000	0.212	0.019	0.000	52
Van	0.000	0.000	0.068	0.000	0.727	0.000	0.000	0.023	0.182	0.000	44
Yozgat	0.100	0.000	0.033	0.000	0.867	0.000	0.000	0.000	0.000	0.000	30

Table D.2. Allele frequencies of Ap223 locus (*N*: number of alleles)

Locus:Ap223	156	160	162	166	168	170	172	176	178	180	182	184	*N*
Bozcaada	0.019	0.000	0.019	0.000	0.192	0.000	0.000	0.000	0.038	0.000	0.731	0.000	52
Giresun	0.000	0.000	0.034	0.000	0.172	0.000	0.000	0.000	0.034	0.000	0.707	0.052	58
Gökçeada	0.000	0.000	0.000	0.000	0.139	0.028	0.056	0.000	0.278	0.000	0.500	0.000	36
İzmir	0.000	0.000	0.000	0.000	0.259	0.000	0.034	0.000	0.259	0.034	0.362	0.052	58
Konya	0.000	0.000	0.000	0.000	0.364	0.000	0.000	0.000	0.205	0.000	0.409	0.023	44
Muğla	0.024	0.000	0.024	0.000	0.167	0.000	0.000	0.000	0.190	0.000	0.548	0.048	42
Kırklareli	0.052	0.017	0.000	0.017	0.241	0.000	0.017	0.017	0.414	0.017	0.207	0.000	58
Aydın	0.089	0.000	0.018	0.000	0.250	0.000	0.000	0.000	0.054	0.000	0.571	0.018	56
Şanlıurfa	0.077	0.019	0.038	0.000	0.250	0.038	0.135	0.000	0.038	0.000	0.385	0.019	52
Sivas	0.019	0.019	0.000	0.000	0.192	0.000	0.135	0.000	0.058	0.000	0.462	0.115	52
Antalya	0.000	0.000	0.000	0.000	0.463	0.000	0.019	0.000	0.093	0.000	0.407	0.019	54
Beyşehir	0.000	0.000	0.000	0.000	0.325	0.000	0.000	0.000	0.075	0.000	0.550	0.050	40
Kars	0.000	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.296	0.000	0.630	0.056	54
Kayseri	0.022	0.000	0.000	0.000	0.435	0.000	0.022	0.000	0.130	0.000	0.348	0.043	46
Artvin	0.042	0.000	0.000	0.000	0.292	0.000	0.000	0.000	0.271	0.000	0.396	0.000	48
Hatay	0.022	0.000	0.000	0.000	0.435	0.000	0.022	0.000	0.065	0.000	0.457	0.000	46
Van	0.052	0.000	0.017	0.000	0.207	0.017	0.000	0.000	0.224	0.017	0.431	0.034	58
Yozgat	0.036	0.000	0.000	0.000	0.179	0.036	0.071	0.000	0.036	0.000	0.643	0.000	28

Table D.3. Allele frequencies of Ap19 locus (*N*: number of alleles)

Locus: Ap19	126	128	130	132	134	136	138	140	142	144	148	150	152	*N*
Bozcaada	0.000	0.000	0.000	0.000	0.000	0.000	0.759	0.000	0.241	0.000	0.000	0.000	0.000	54
Giresun	0.017	0.250	0.017	0.000	0.000	0.100	0.583	0.000	0.033	0.000	0.000	0.000	0.000	60
Gökçeada	0.000	0.080	0.000	0.000	0.000	0.000	0.780	0.080	0.060	0.000	0.000	0.000	0.000	50
İzmir	0.000	0.077	0.000	0.000	0.000	0.019	0.750	0.038	0.096	0.000	0.019	0.000	0.000	52
Konya	0.000	0.117	0.117	0.000	0.000	0.133	0.533	0.067	0.017	0.017	0.000	0.000	0.000	60
Muğla	0.000	0.000	0.000	0.000	0.019	0.000	0.926	0.019	0.037	0.000	0.000	0.000	0.000	54
Kırklareli	0.000	0.020	0.000	0.000	0.000	0.020	0.640	0.280	0.040	0.000	0.000	0.000	0.000	50
Aydın	0.000	0.261	0.022	0.000	0.000	0.000	0.630	0.065	0.022	0.000	0.000	0.000	0.000	46
Şanlıurfa	0.000	0.056	0.000	0.000	0.000	0.000	0.852	0.093	0.000	0.000	0.000	0.000	0.000	54
Sivas	0.000	0.180	0.160	0.000	0.000	0.000	0.500	0.080	0.020	0.000	0.040	0.020	0.000	50
Antalya	0.000	0.167	0.000	0.000	0.033	0.000	0.650	0.033	0.033	0.000	0.050	0.017	0.017	60
Beyşehir	0.000	0.125	0.018	0.036	0.000	0.000	0.554	0.250	0.018	0.000	0.000	0.000	0.000	56
Kars	0.000	0.017	0.000	0.000	0.000	0.000	0.466	0.069	0.000	0.000	0.414	0.034	0.000	58
Kayseri	0.000	0.083	0.000	0.000	0.000	0.000	0.729	0.042	0.062	0.000	0.083	0.000	0.000	48
Artvin	0.000	0.259	0.017	0.000	0.000	0.000	0.552	0.000	0.000	0.000	0.172	0.000	0.000	58
Hatay	0.000	0.023	0.000	0.000	0.000	0.000	0.727	0.182	0.000	0.000	0.068	0.000	0.000	44
Van	0.000	0.283	0.000	0.000	0.000	0.000	0.522	0.043	0.000	0.000	0.109	0.043	0.000	46
Yozgat	0.000	0.031	0.188	0.000	0.000	0.031	0.500	0.062	0.031	0.000	0.156	0.000	0.000	32

Table D.4. Allele frequencies of Ap001 locus (*N*: number of alleles)

Locus: Ap001	205	207	209	211	213	214	215	216	217	219	220	221	223	*N*
Bozcaada	0.000	0.056	0.019	0.519	0.370	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	54
Giresun	0.000	0.000	0.000	0.462	0.442	0.000	0.019	0.000	0.038	0.000	0.000	0.000	0.000	52
Gökçeada	0.000	0.000	0.033	0.433	0.467	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000	60
İzmir	0.000	0.000	0.000	0.460	0.360	0.000	0.040	0.000	0.060	0.000	0.000	0.000	0.000	50
Konya	0.000	0.038	0.000	0.538	0.173	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	52
Muğla	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	00
Kırklareli	0.000	0.000	0.159	0.318	0.250	0.000	0.045	0.000	0.045	0.000	0.000	0.000	0.000	44
Aydın	0.000	0.000	0.038	0.385	0.462	0.019	0.000	0.000	0.038	0.000	0.000	0.000	0.019	52
Şanlıurfa	0.000	0.067	0.000	0.117	0.050	0.000	0.083	0.000	0.000	0.083	0.000	0.000	0.000	60
Sivas	0.000	0.000	0.043	0.348	0.261	0.000	0.065	0.000	0.000	0.000	0.000	0.065	0.022	46
Antalya	0.000	0.000	0.000	0.268	0.250	0.000	0.054	0.000	0.036	0.036	0.000	0.000	0.089	56
Beyşehir	0.000	0.060	0.000	0.360	0.420	0.000	0.080	0.000	0.020	0.000	0.000	0.000	0.000	50
Kars	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.000	0.022	0.000	0.000	0.000	46
Kayseri	0.000	0.000	0.045	0.068	0.545	0.000	0.023	0.023	0.000	0.023	0.000	0.000	0.000	44
Artvin	0.000	0.000	0.167	0.042	0.250	0.000	0.000	0.000	0.250	0.000	0.083	0.000	0.000	24
Hatay	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	00
Van	0.000	0.000	0.058	0.327	0.346	0.000	0.019	0.000	0.000	0.000	0.019	0.000	0.000	52
Yozgat	0.056	0.000	0.167	0.000	0.000	0.000	0.167	0.000	0.056	0.000	0.000	0.111	0.167	18

Table D.4. Continued.

Locus:Ap001	224	225	227	228	229	230	231	233	235	237	239	241	243	*N*
Bozcaada	0.000	0.000	0.000	0.000	0.037	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	54
Giresun	0.000	0.000	0.038	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	52
Gökçeada	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	60
İzmir	0.000	0.000	0.000	0.000	0.040	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	50
Konya	0.000	0.000	0.077	0.000	0.058	0.000	0.000	0.019	0.038	0.000	0.000	0.000	0.000	52
Muğla	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	00
Kırklareli	0.000	0.000	0.045	0.000	0.000	0.000	0.091	0.045	0.000	0.000	0.000	0.000	0.000	44
Aydın	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000	52
Şanlıurfa	0.000	0.017	0.100	0.000	0.100	0.000	0.017	0.083	0.067	0.133	0.017	0.000	0.017	60
Sivas	0.000	0.000	0.065	0.000	0.000	0.000	0.000	0.000	0.087	0.022	0.000	0.000	0.000	46
Antalya	0.018	0.000	0.000	0.000	0.054	0.000	0.000	0.000	0.018	0.036	0.018	0.000	0.000	56
Beyşehir	0.000	0.000	0.020	0.000	0.040	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	50
Kars	0.000	0.000	0.565	0.000	0.239	0.000	0.109	0.000	0.000	0.000	0.000	0.000	0.000	46
Kayseri	0.000	0.000	0.023	0.000	0.023	0.000	0.000	0.205	0.000	0.000	0.000	0.000	0.000	44
Artvin	0.083	0.000	0.000	0.042	0.000	0.083	0.000	0.000	0.000	0.000	0.000	0.000	0.000	24
Hatay	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	00
Van	0.000	0.038	0.058	0.000	0.115	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	52
Yozgat	0.000	0.056	0.056	0.000	0.056	0.000	0.000	0.000	0.000	0.000	0.000	0.056	0.000	18

Table D.4. Continued.

Locus : Ap1	245	247	249	251	255	257	*N*
Bozcaada	0.000	0.000	0.000	0.000	0.000	0.000	54
Giresun	0.000	0.000	0.000	0.000	0.000	0.000	52
Gökçeada	0.000	0.000	0.000	0.000	0.000	0.000	60
İzmir	0.000	0.000	0.000	0.000	0.020	0.000	50
Konya	0.000	0.000	0.000	0.038	0.000	0.000	52
Muğla	0.000	0.000	0.000	0.000	0.000	0.000	00
Kırklareli	0.000	0.000	0.000	0.000	0.000	0.000	44
Aydın	0.000	0.000	0.019	0.000	0.000	0.000	52
Şanlıurrfa	0.000	0.017	0.017	0.017	0.000	0.000	60
Sivas	0.000	0.000	0.000	0.000	0.000	0.022	46
Antalya	0.000	0.036	0.000	0.036	0.036	0.018	56
Beyşehir	0.000	0.000	0.000	0.000	0.000	0.000	50
Kars	0.000	0.000	0.022	0.022	0.000	0.000	46
Kayseri	0.023	0.000	0.000	0.000	0.000	0.000	44
Artvin	0.000	0.000	0.000	0.000	0.000	0.000	24
Hatay	0.000	0.000	0.000	0.000	0.000	0.000	00
Van	0.000	0.000	0.000	0.000	0.000	0.000	52
Yozgat	0.000	0.000	0.000	0.056	0.000	0.000	18

Table D.5. Allele frequencies of Ap289 locus (*N*: number of alleles)

Locus:Ap289	164	174	176	178	180	182	184	186	188	190	192	194	196	*N*
Bozcaada	0.000	0.000	0.000	0.000	0.000	0.522	0.000	0.022	0.109	0.000	0.065	0.000	0.000	46
Giresun	0.000	0.000	0.000	0.000	0.036	0.286	0.304	0.036	0.018	0.179	0.000	0.000	0.000	56
Gökçeada	0.000	0.000	0.000	0.000	0.000	0.281	0.375	0.000	0.000	0.000	0.000	0.000	0.000	32
İzmir	0.000	0.000	0.018	0.000	0.000	0.250	0.250	0.125	0.018	0.000	0.000	0.000	0.071	56
Konya	0.000	0.026	0.000	0.000	0.000	0.132	0.079	0.342	0.000	0.000	0.000	0.000	0.000	38
Muğla	0.000	0.000	0.000	0.000	0.000	0.263	0.658	0.000	0.026	0.000	0.000	0.000	0.000	38
Kırklareli	0.045	0.000	0.000	0.023	0.000	0.682	0.023	0.091	0.000	0.000	0.000	0.000	0.000	44
Aydın	0.000	0.000	0.000	0.000	0.000	0.280	0.320	0.120	0.060	0.000	0.000	0.000	0.120	50
Şanlıurfa	0.000	0.000	0.000	0.000	0.000	0.603	0.259	0.052	0.000	0.000	0.000	0.000	0.000	58
Sivas	0.000	0.000	0.000	0.000	0.000	0.125	0.321	0.107	0.125	0.000	0.000	0.018	0.018	56
Antalya	0.000	0.000	0.000	0.000	0.023	0.341	0.250	0.000	0.045	0.091	0.000	0.023	0.000	44
Beyşehir	0.000	0.000	0.000	0.000	0.000	0.059	0.118	0.235	0.176	0.059	0.000	0.000	0.235	34
Kars	0.000	0.000	0.020	0.000	0.000	0.220	0.280	0.100	0.000	0.020	0.000	0.000	0.000	50
Kayseri	0.000	0.000	0.000	0.000	0.000	0.386	0.500	0.000	0.000	0.000	0.000	0.023	0.000	44
Artvin	0.000	0.000	0.000	0.000	0.000	0.222	0.000	0.278	0.000	0.056	0.000	0.000	0.000	36
Hatay	0.000	0.000	0.000	0.000	0.000	0.660	0.080	0.080	0.000	0.000	0.000	0.020	0.000	50
Van	0.000	0.000	0.020	0.000	0.000	0.140	0.220	0.280	0.060	0.000	0.000	0.000	0.000	50
Yozgat	0.000	0.000	0.000	0.000	0.000	0.429	0.214	0.071	0.071	0.000	0.000	0.000	0.000	14

Table D.5. Continued.

Locus: Ap289	198	200	202	204	206	208	210	212	214	216	218	220	222	*N*
Bozcaada	0.000	0.000	0.239	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.022	0.000	0.022	46
Giresun	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.071	0.000	56
Gökçeada	0.125	0.094	0.031	0.000	0.000	0.000	0.000	0.000	0.031	0.000	0.062	0.000	0.000	32
İzmir	0.089	0.036	0.054	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.036	0.018	0.018	56
Konya	0.000	0.053	0.000	0.000	0.000	0.158	0.105	0.026	0.000	0.000	0.079	0.000	0.000	38
Muğla	0.026	0.026	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	38
Kırklareli	0.068	0.000	0.023	0.000	0.000	0.000	0.000	0.023	0.000	0.023	0.000	0.000	0.000	44
Aydın	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.020	0.020	0.000	0.020	0.020	0.000	50
Şanlıurfa	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.034	0.052	0.000	58
Sivas	0.036	0.000	0.018	0.054	0.000	0.000	0.000	0.018	0.018	0.089	0.036	0.000	0.018	56
Antalya	0.000	0.000	0.000	0.000	0.045	0.091	0.000	0.000	0.000	0.023	0.023	0.023	0.023	44
Beyşehir	0.000	0.000	0.059	0.059	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	34
Kars	0.020	0.020	0.000	0.000	0.000	0.060	0.040	0.020	0.020	0.020	0.120	0.000	0.040	50
Kayseri	0.000	0.000	0.000	0.000	0.000	0.091	0.000	0.000	0.000	0.000	0.000	0.000	0.000	44
Artvin	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.278	0.000	0.167	36
Hatay	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.080	0.020	0.020	0.020	50
Van	0.020	0.000	0.020	0.000	0.000	0.000	0.020	0.040	0.000	0.000	0.180	0.000	0.000	50
Yozgat	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.214	0.000	0.000	0.000	14

Table D.6. Allele frequencies of Ap076 locus (*N*: number of alleles)

Locus:A76	201	205	207	209	210	211	213	215	217	219	220	221	*N*
Bozcaada	0.000	0.155	0.534	0.138	0.000	0.103	0.069	0.000	0.000	0.000	0.000	0.000	60
Giresun	0.000	0.025	0.575	0.200	0.000	0.025	0.075	0.000	0.000	0.000	0.000	0.000	32
Gökçeada	0.000	0.036	0.875	0.036	0.000	0.018	0.018	0.000	0.000	0.000	0.000	0.000	56
İzmir	0.000	0.056	0.907	0.000	0.000	0.037	0.000	0.000	0.000	0.000	0.000	0.000	52
Konya	0.036	0.000	0.857	0.018	0.000	0.071	0.018	0.000	0.000	0.000	0.000	0.000	54
Muğla	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	20
Kırklareli	0.000	0.000	0.525	0.150	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000	40
Aydın	0.000	0.000	0.714	0.000	0.000	0.143	0.119	0.000	0.000	0.000	0.000	0.000	42
Şanlıurfa	0.000	0.000	0.759	0.052	0.000	0.069	0.000	0.034	0.000	0.000	0.000	0.000	58
Sivas	0.091	0.000	0.636	0.068	0.000	0.023	0.000	0.023	0.023	0.045	0.000	0.000	38
Antalya	0.000	0.000	0.477	0.045	0.000	0.136	0.136	0.000	0.045	0.023	0.045	0.000	40
Beyşehir	0.019	0.037	0.852	0.037	0.000	0.037	0.000	0.000	0.000	0.000	0.000	0.000	42
Kars	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0
Kayseri	0.000	0.000	0.833	0.167	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	42
Artvin	0.000	0.000	0.360	0.240	0.000	0.100	0.020	0.000	0.000	0.020	0.000	0.000	48
Hatay	0.063	0.000	0.313	0.063	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	8
Van	0.000	0.000	0.625	0.075	0.025	0.050	0.125	0.000	0.000	0.000	0.000	0.025	36
Yozgat	0.000	0.000	0.409	0.227	0.000	0.364	0.000	0.000	0.000	0.000	0.000	0.000	22

Table D.6. Continued.

Locus:A76	223	227	229	230	231	245	247	253	255	257	259	260	263	*N*
Bozcaada	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	60
Giresun	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.100	0.000	0.000	0.000	0.000	32
Gökçeada	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	56
İzmir	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	52
Konya	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	54
Muğla	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	20
Kırklareli	0.000	0.000	0.000	0.000	0.000	0.000	0.275	0.000	0.000	0.000	0.000	0.000	0.000	40
Aydın	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000	0.000	42
Şanlıurfa	0.000	0.017	0.017	0.017	0.017	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	58
Sivas	0.000	0.000	0.000	0.000	0.023	0.045	0.000	0.000	0.000	0.000	0.000	0.023	0.000	38
Antalya	0.023	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.068	0.000	0.000	0.000	0.000	40
Beyşehir	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.000	42
Kars	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	00
Kayseri	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	42
Artvin	0.000	0.000	0.000	0.000	0.000	0.060	0.080	0.020	0.000	0.040	0.020	0.000	0.040	48
Hatay	0.000	0.000	0.000	0.000	0.000	0.000	0.188	0.063	0.063	0.250	0.000	0.000	0.000	8
Van	0.075	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	36
Yozgat	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	22

APPENDIX E

SCATTER PLOTS OF LOG-LIKELIHOOD VALUES OF INDIVIDUALS

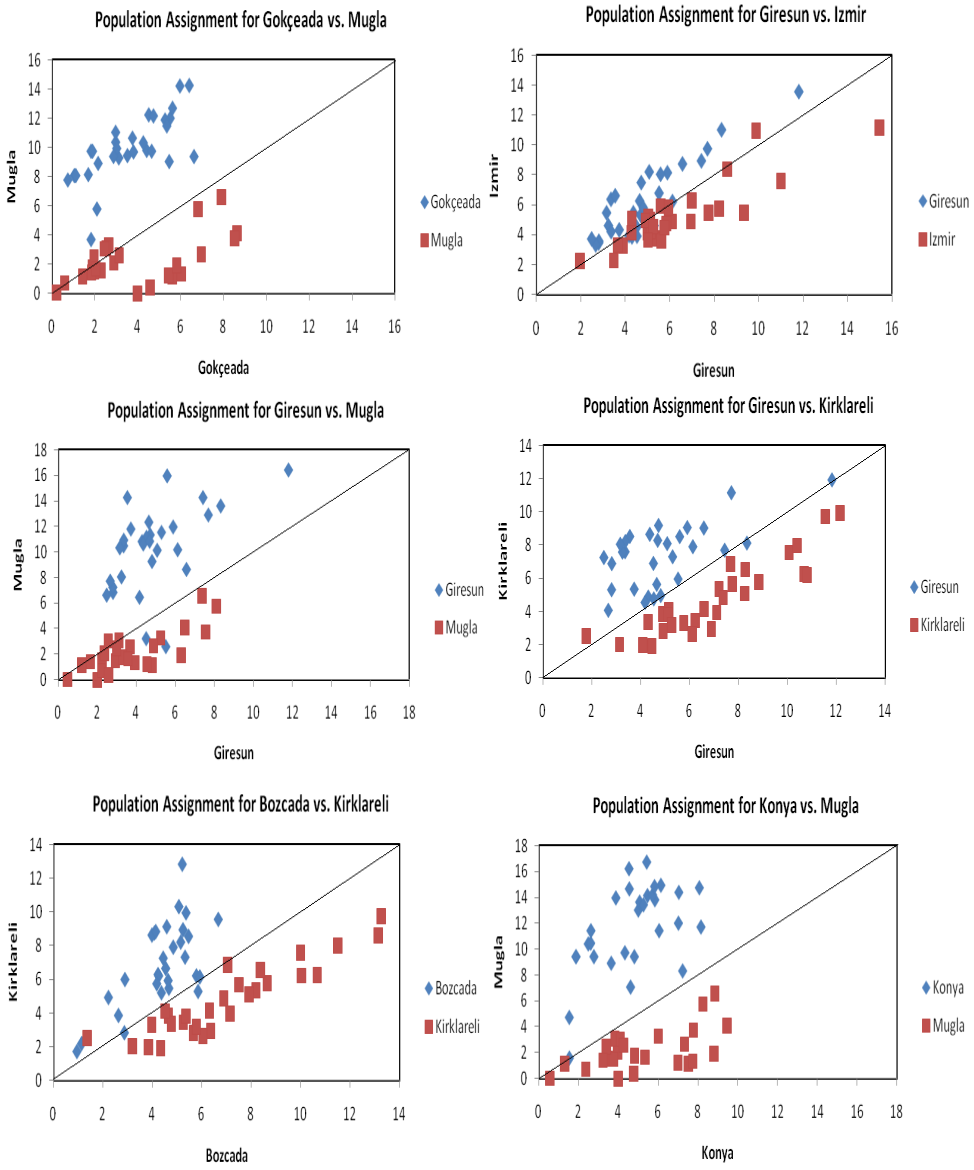


Figure E.1. Scatter plots of Log-likelihood values of individuals drawn from each population, based on allele frequencies in each population.

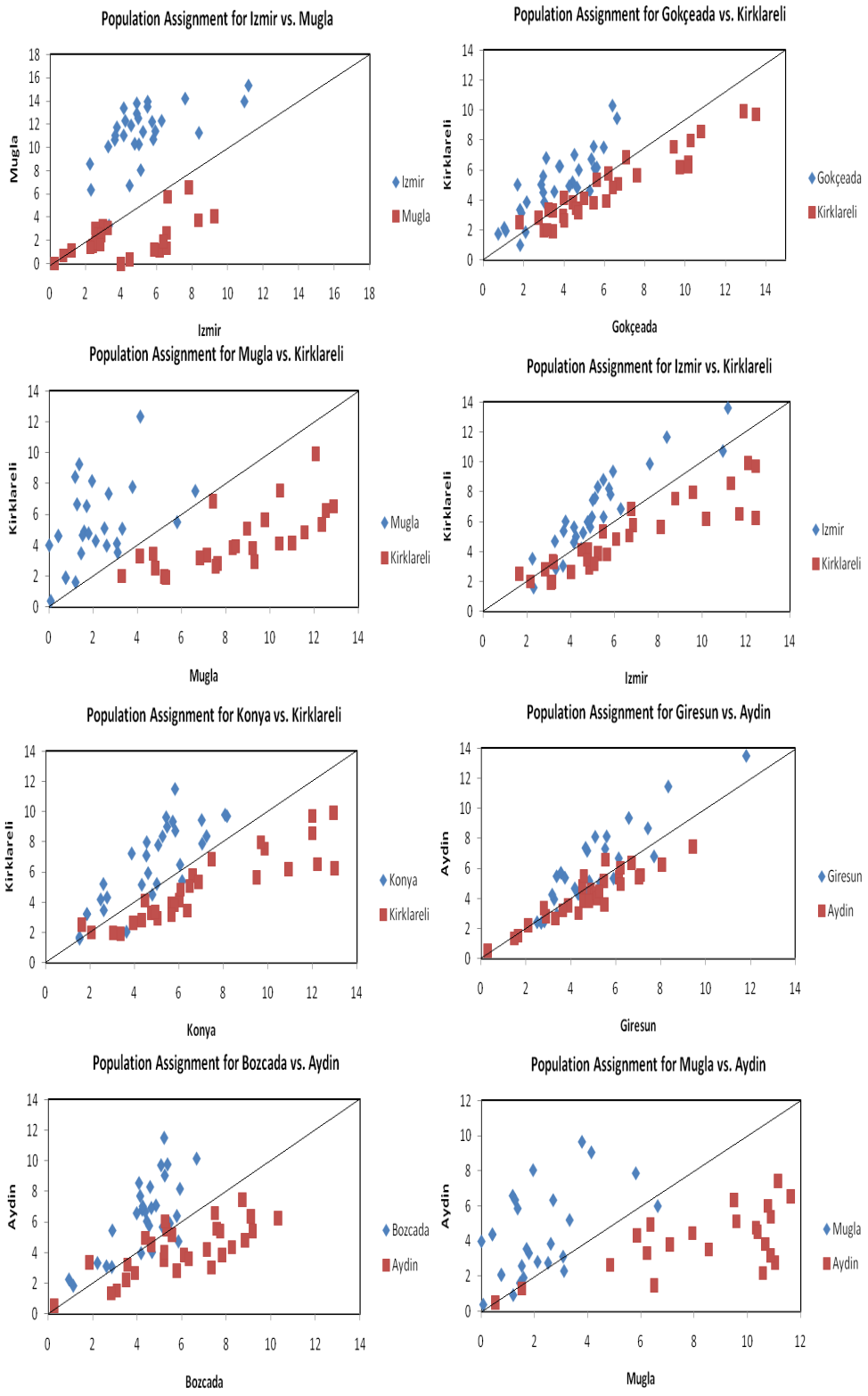


Figure E.1. Continued.

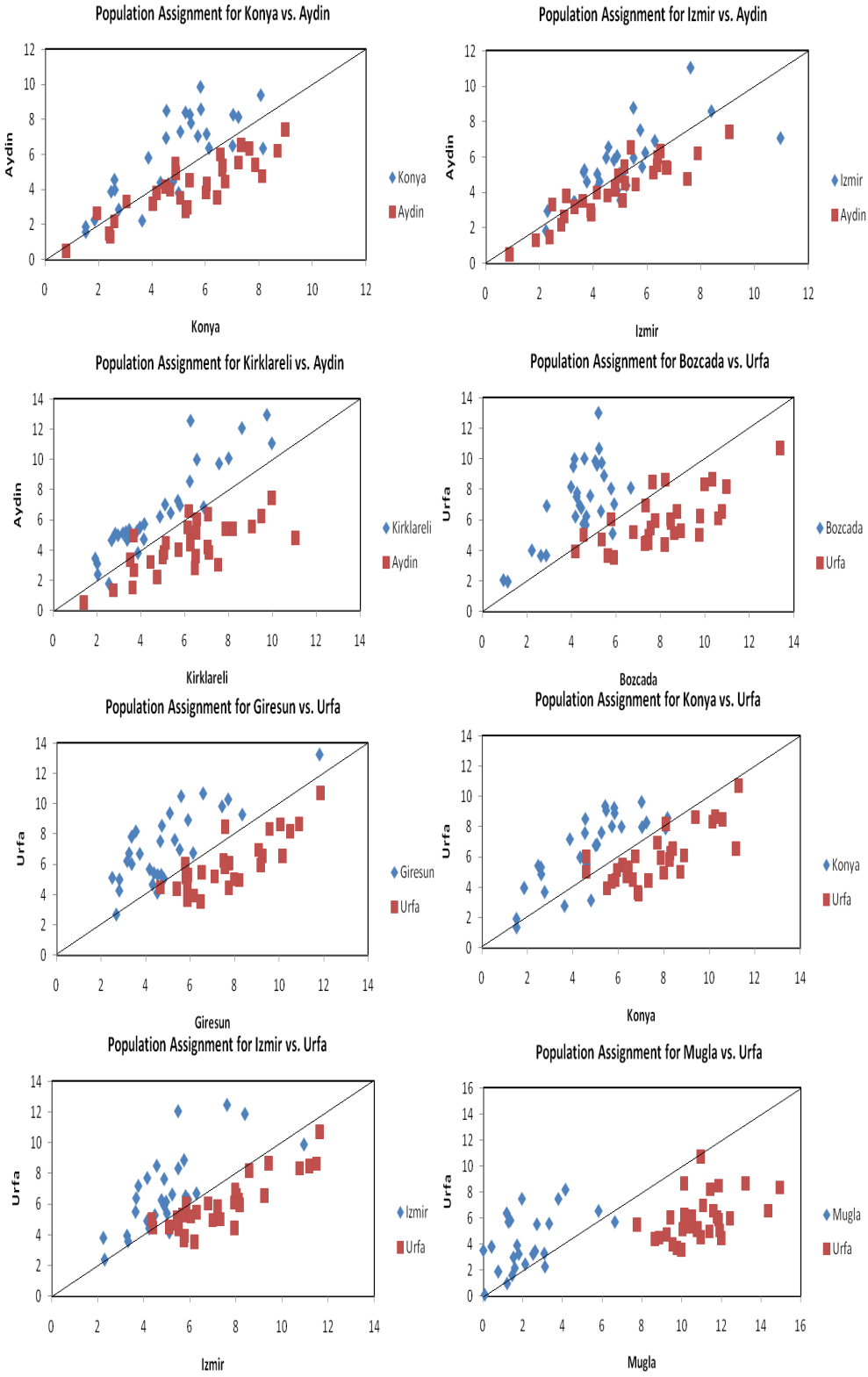


Figure E.1. Continued.

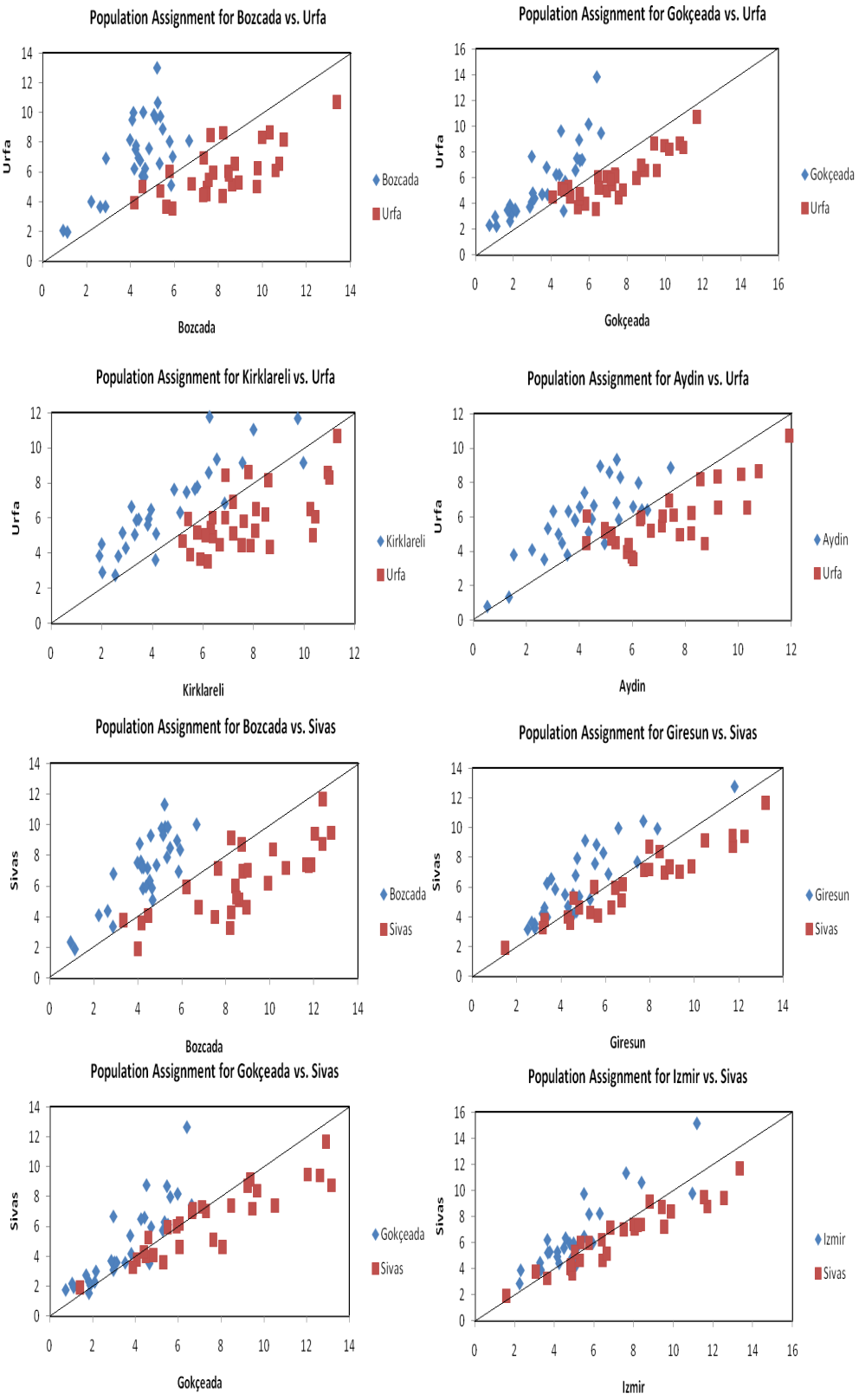


Figure E.1. Continued.

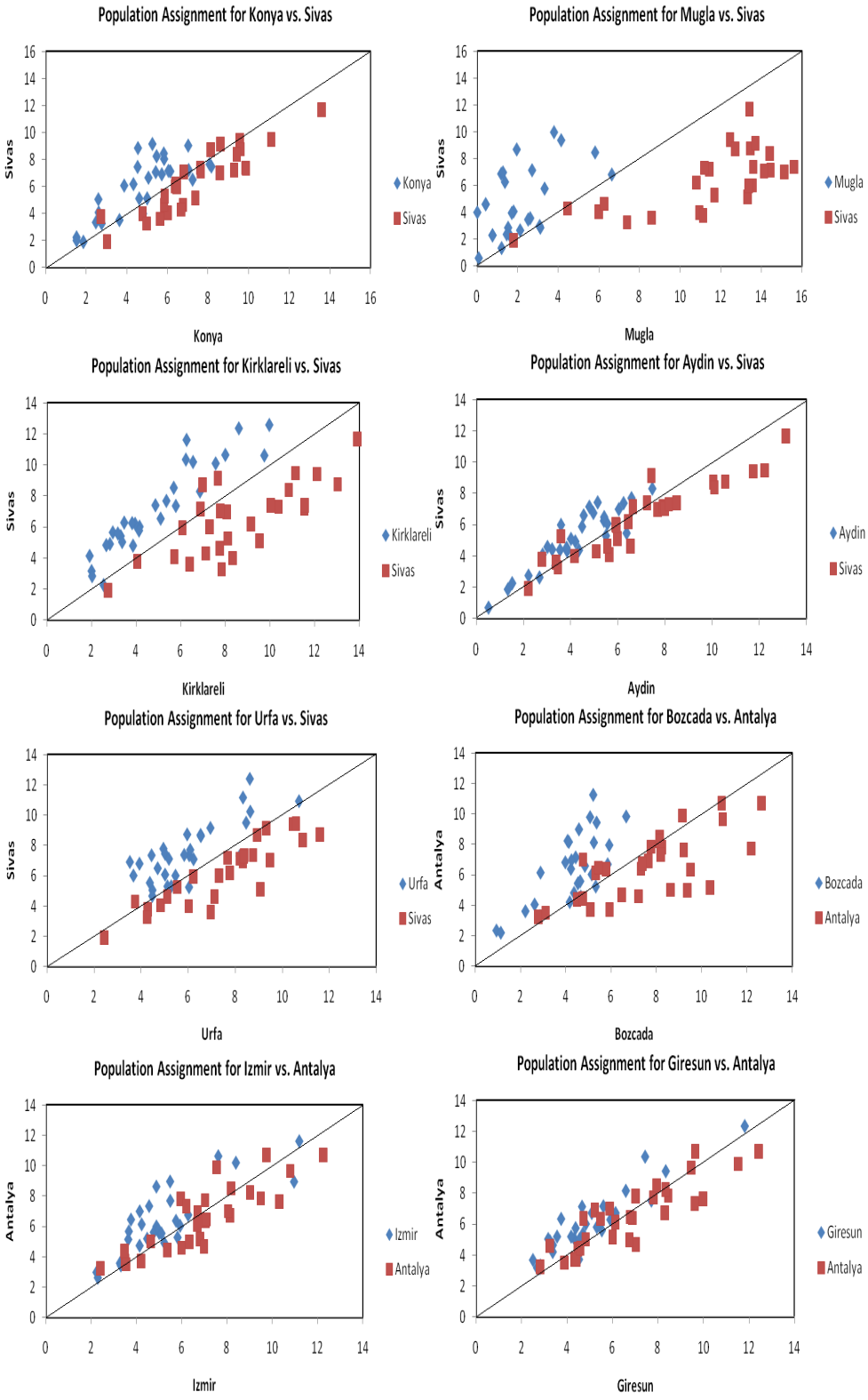


Figure E.1. Continued.

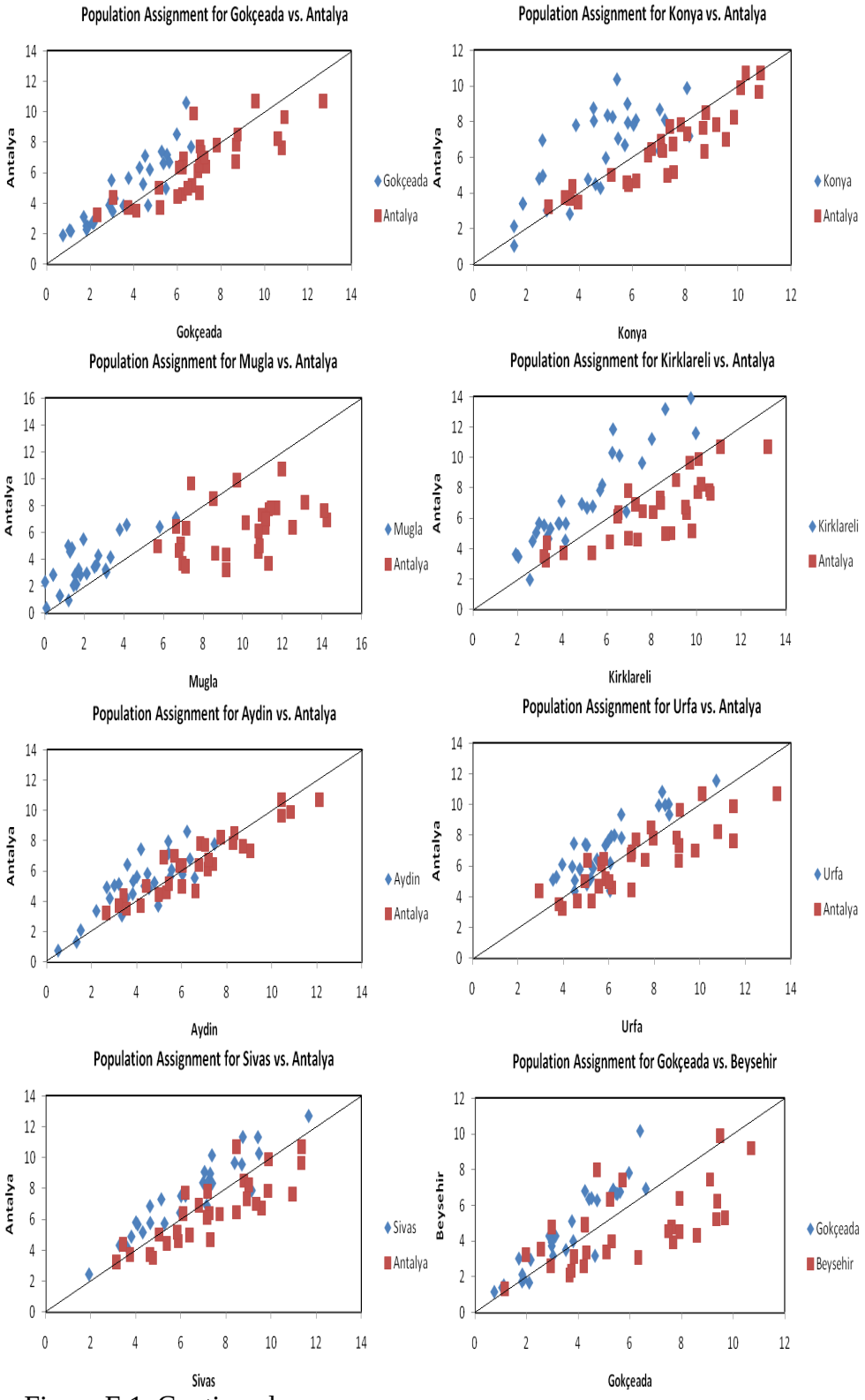


Figure E.1. Continued.

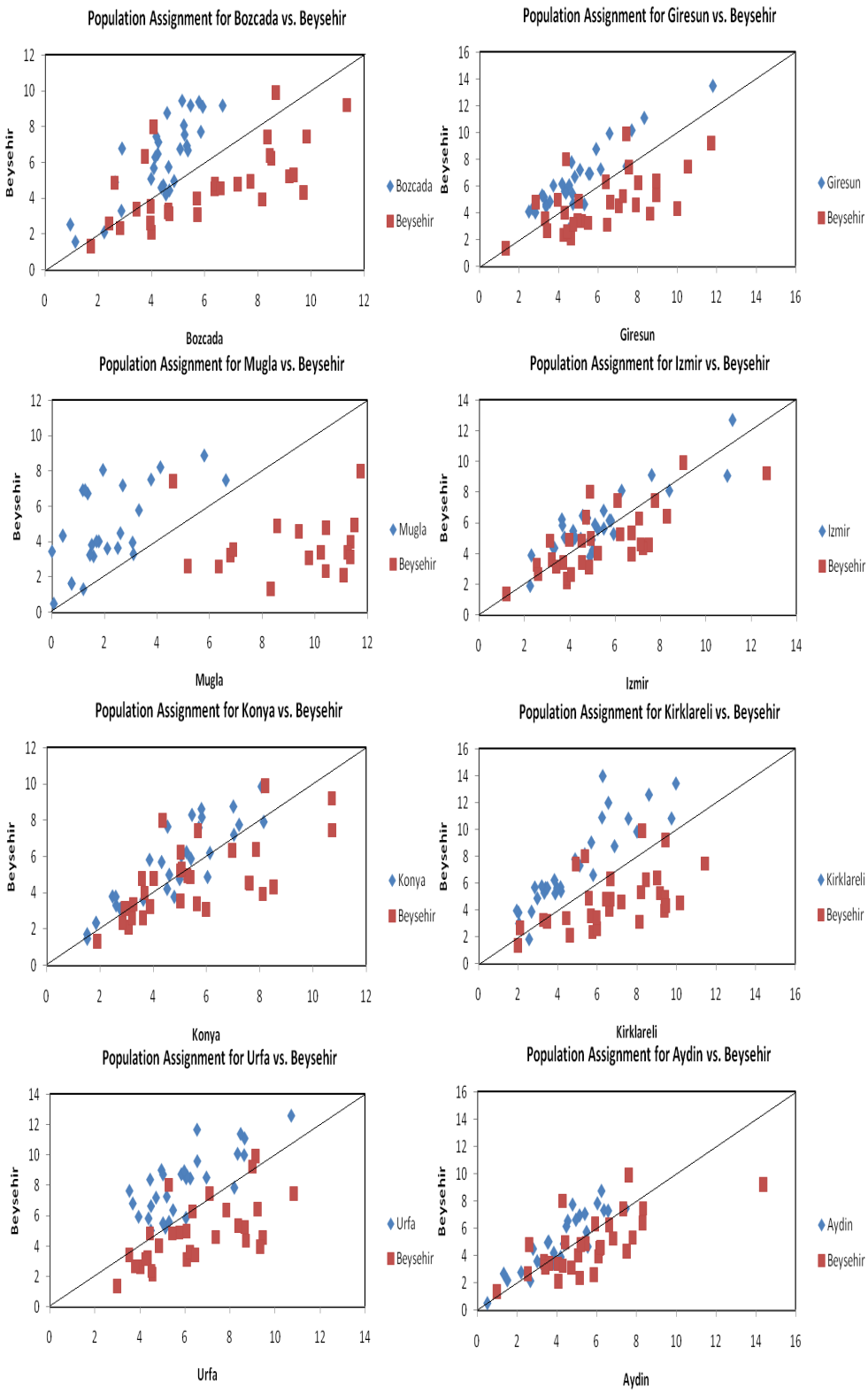
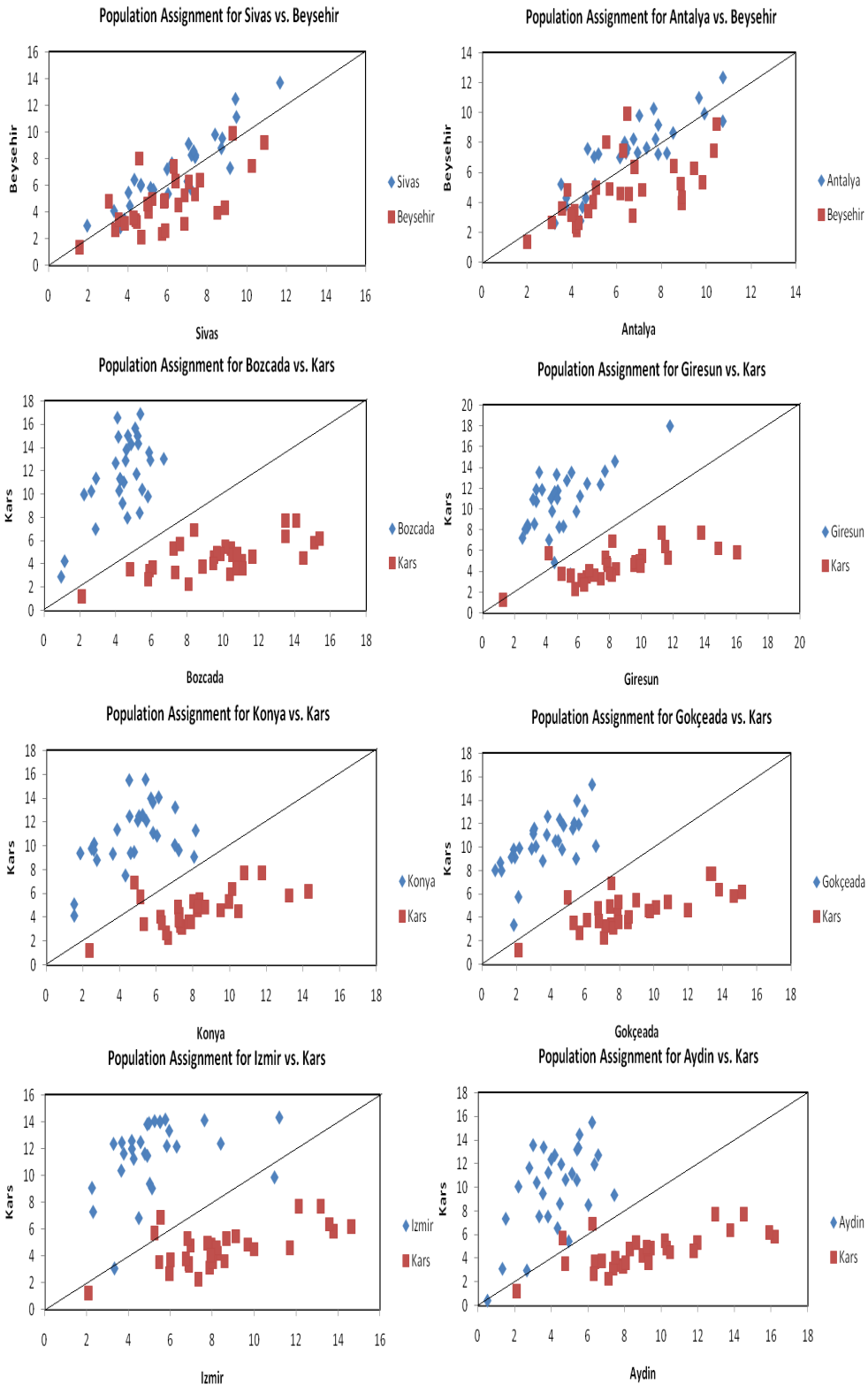


Figure E.1. Continued.



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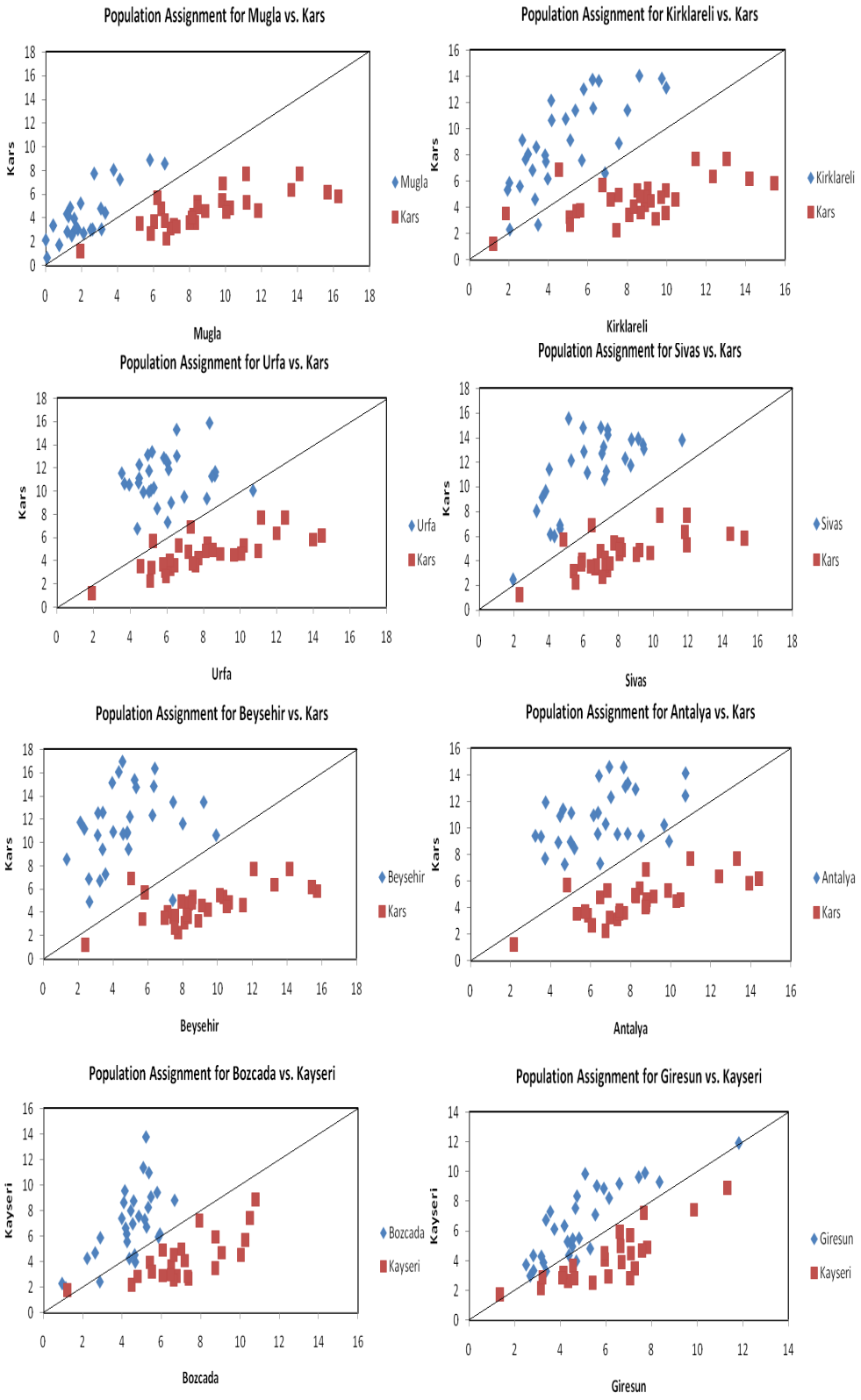
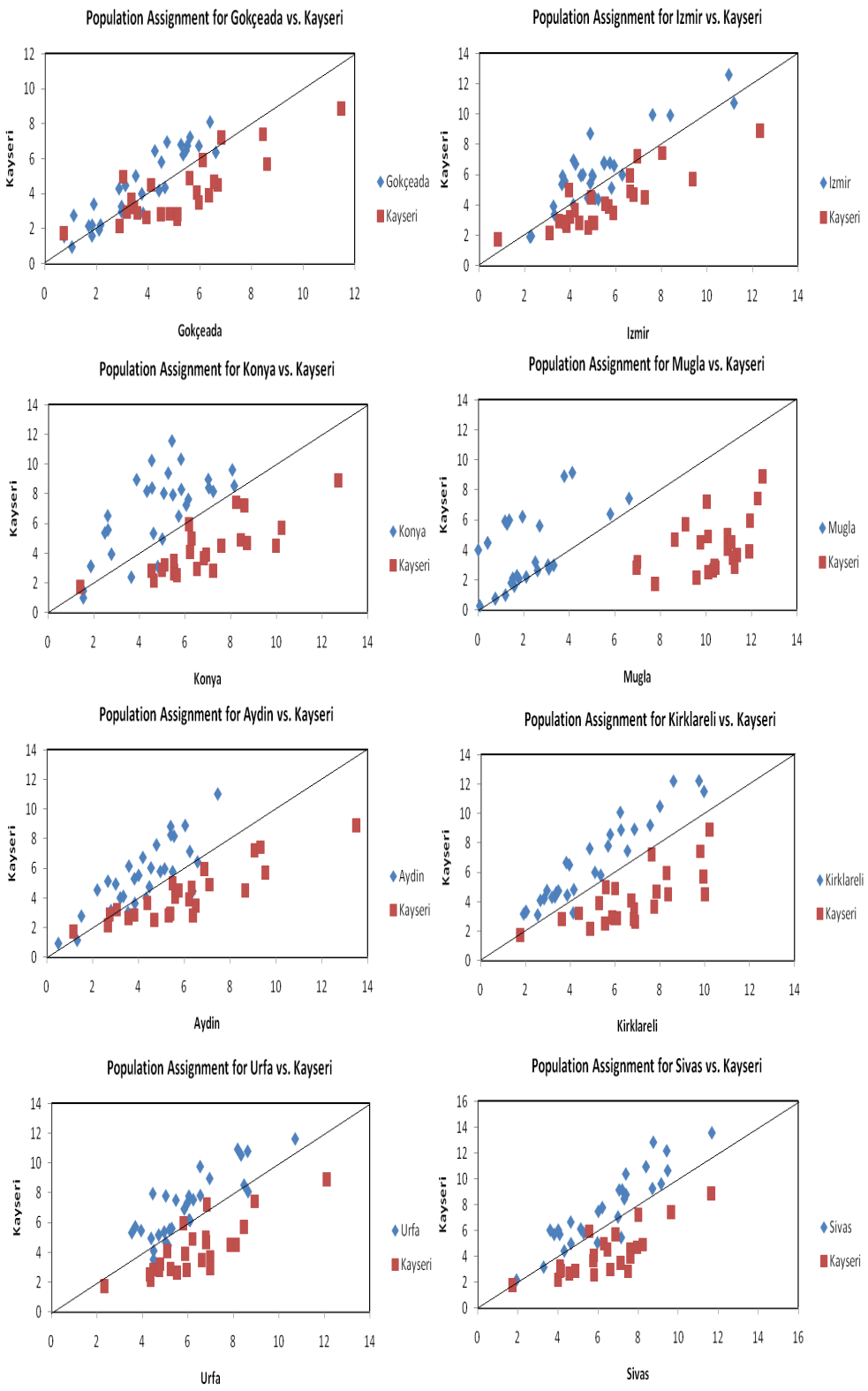


Figure E.1. Continued.



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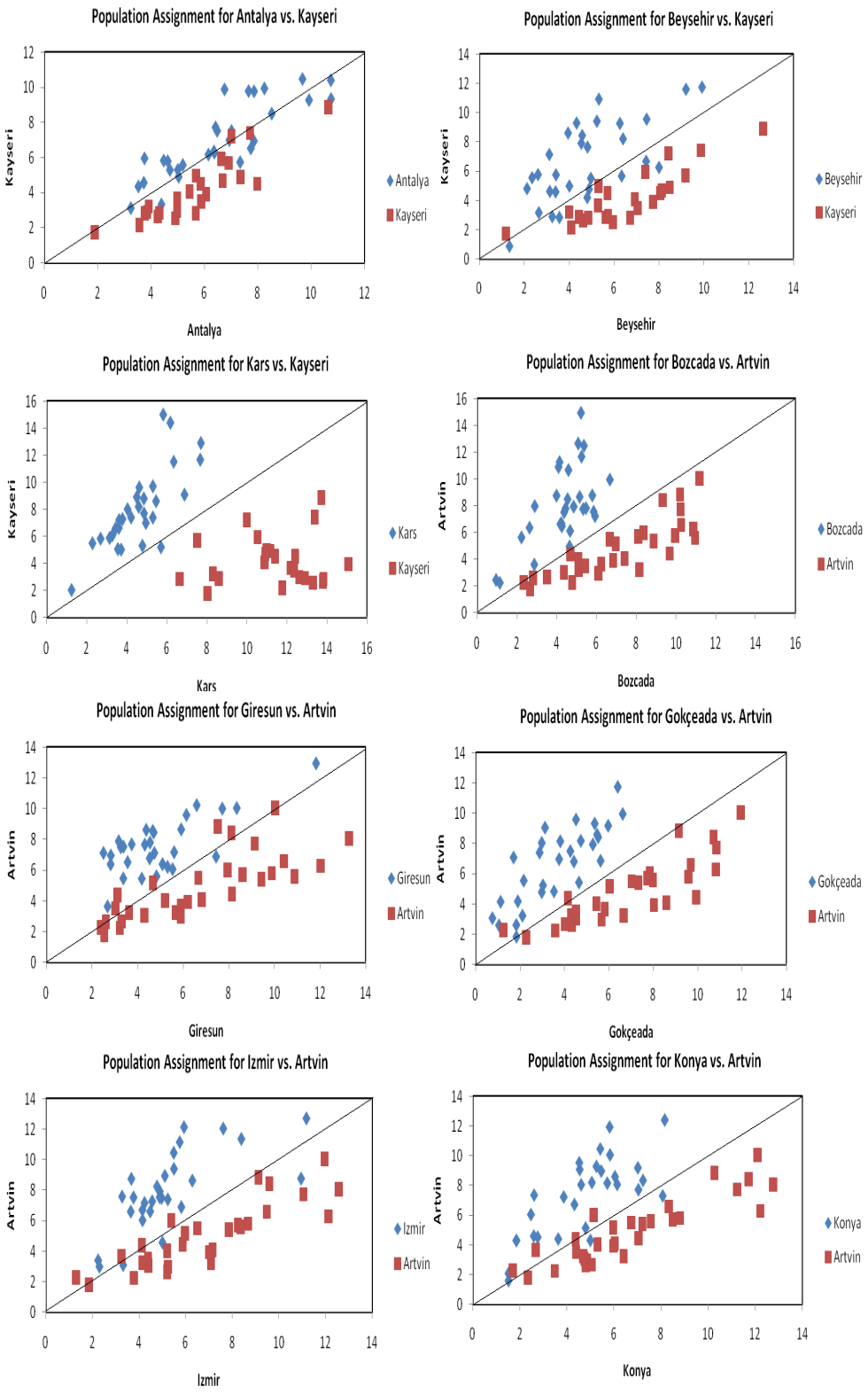


Figure E.1. Continued.

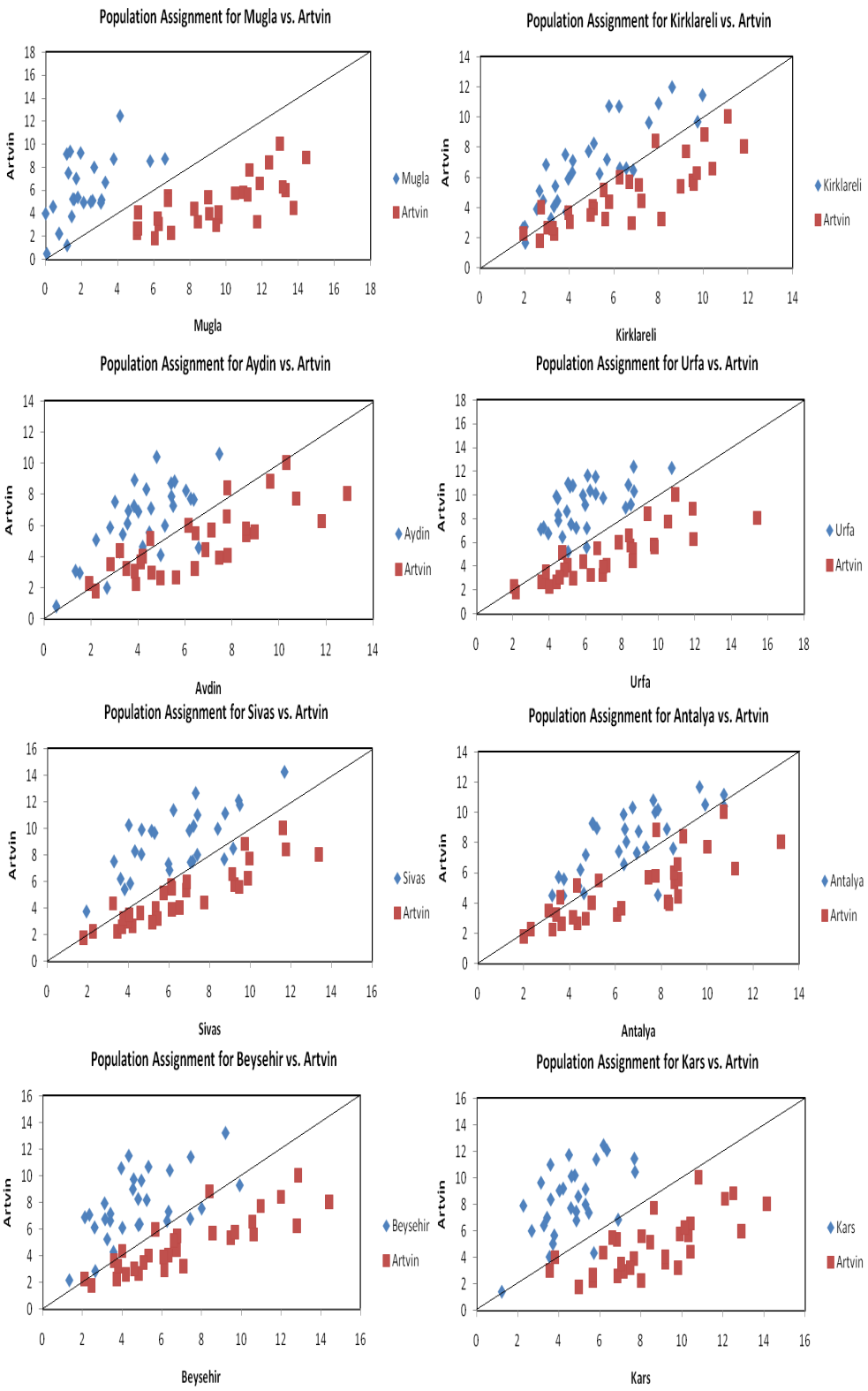
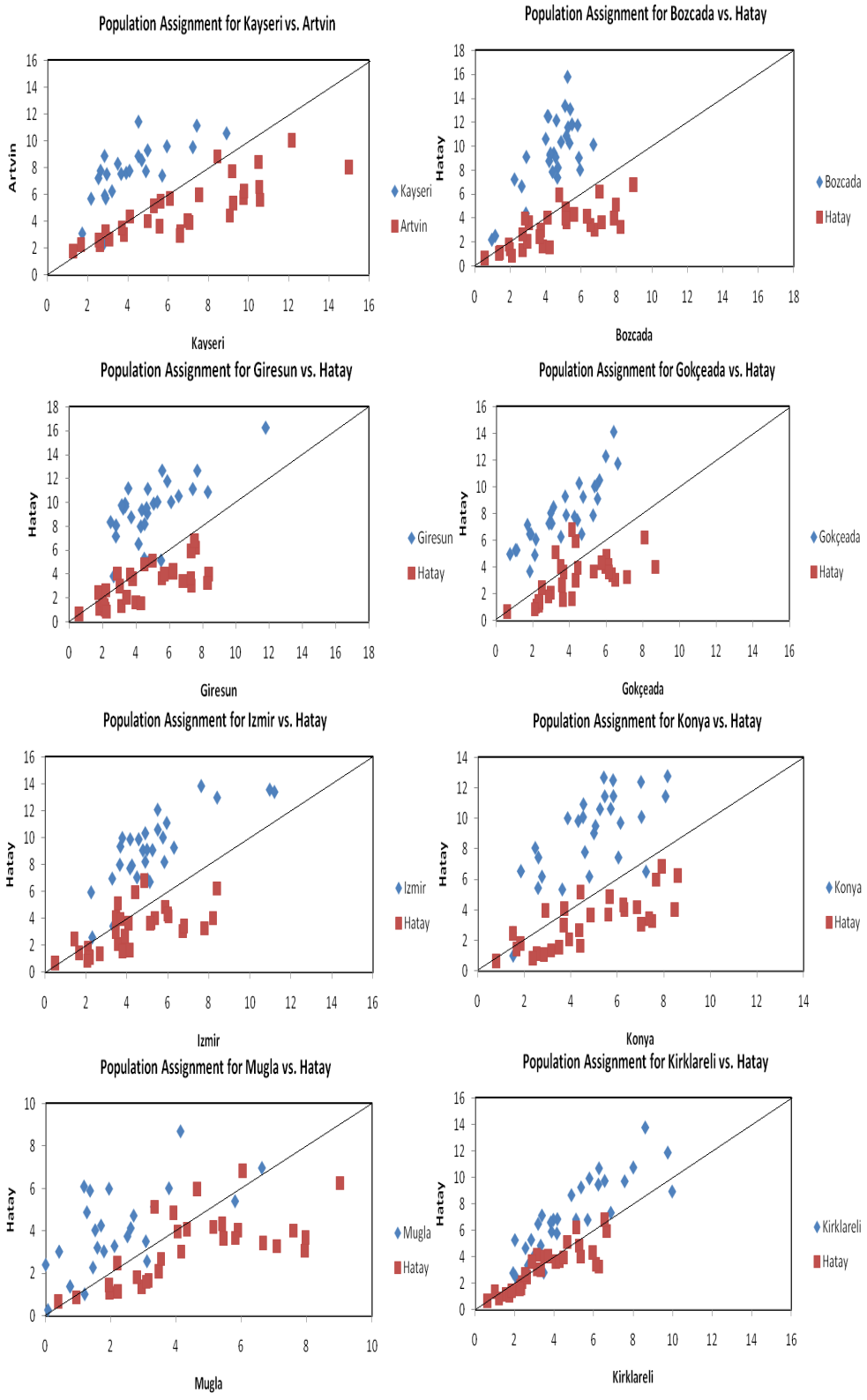


Figure E.1. Continued.



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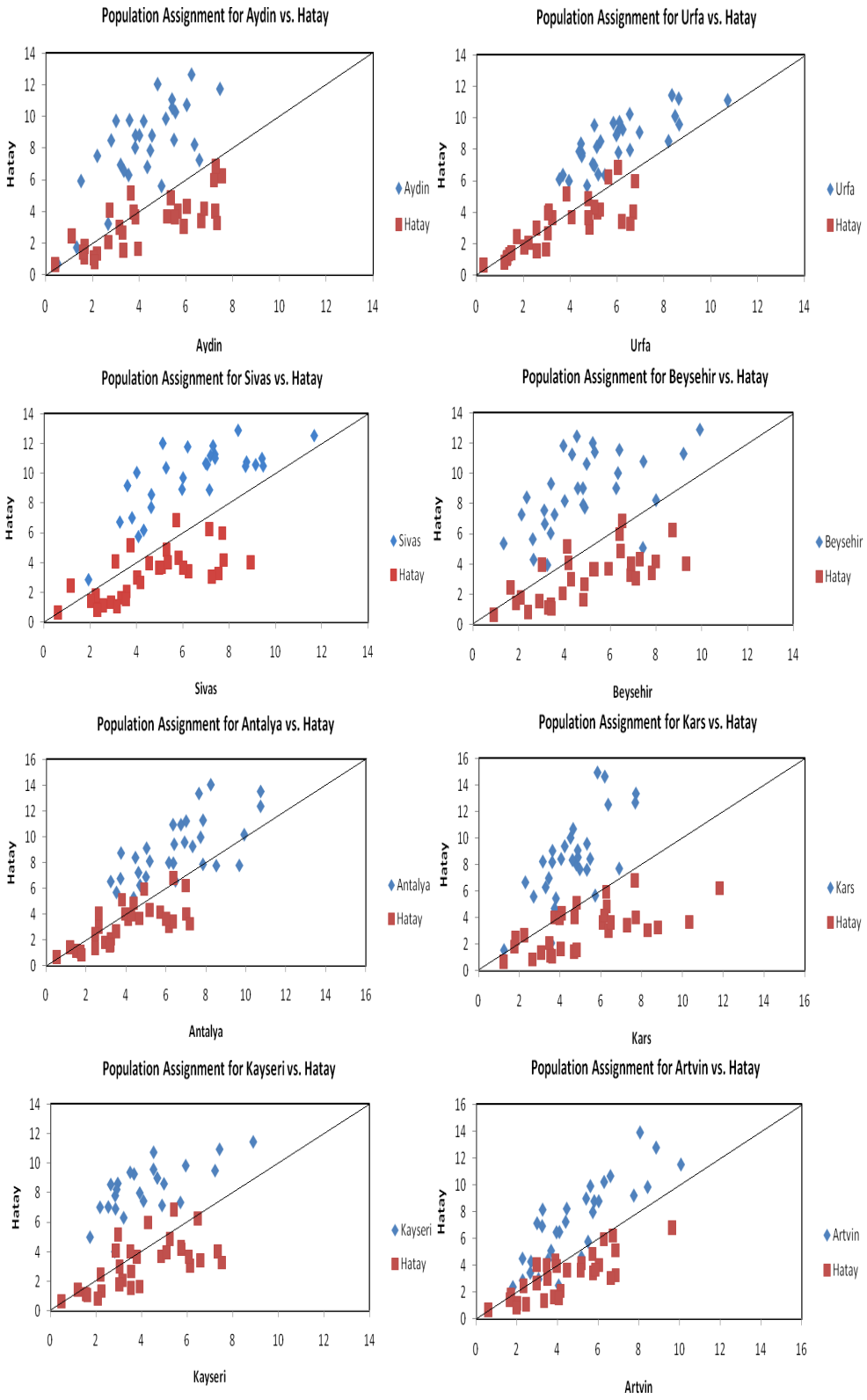

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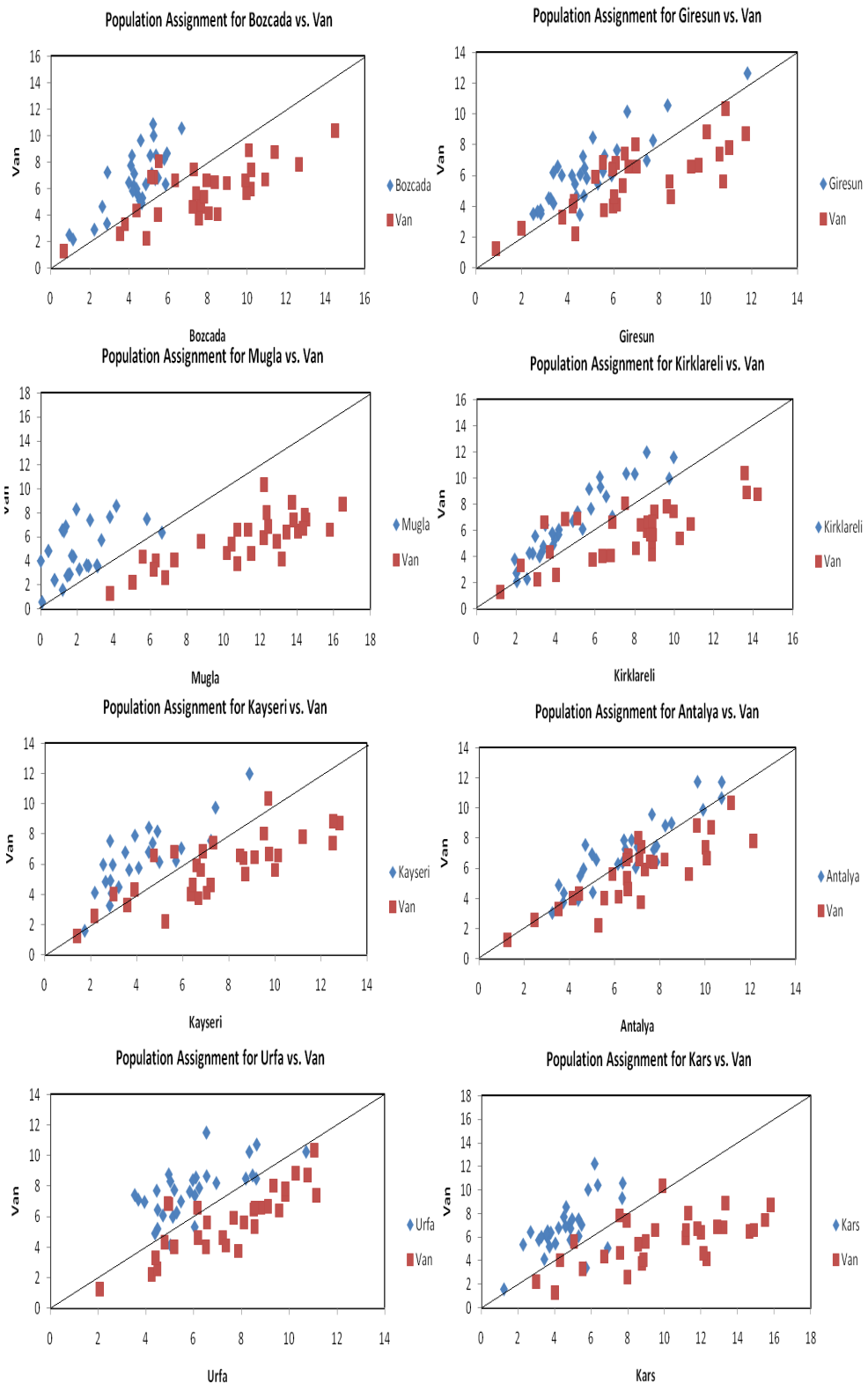

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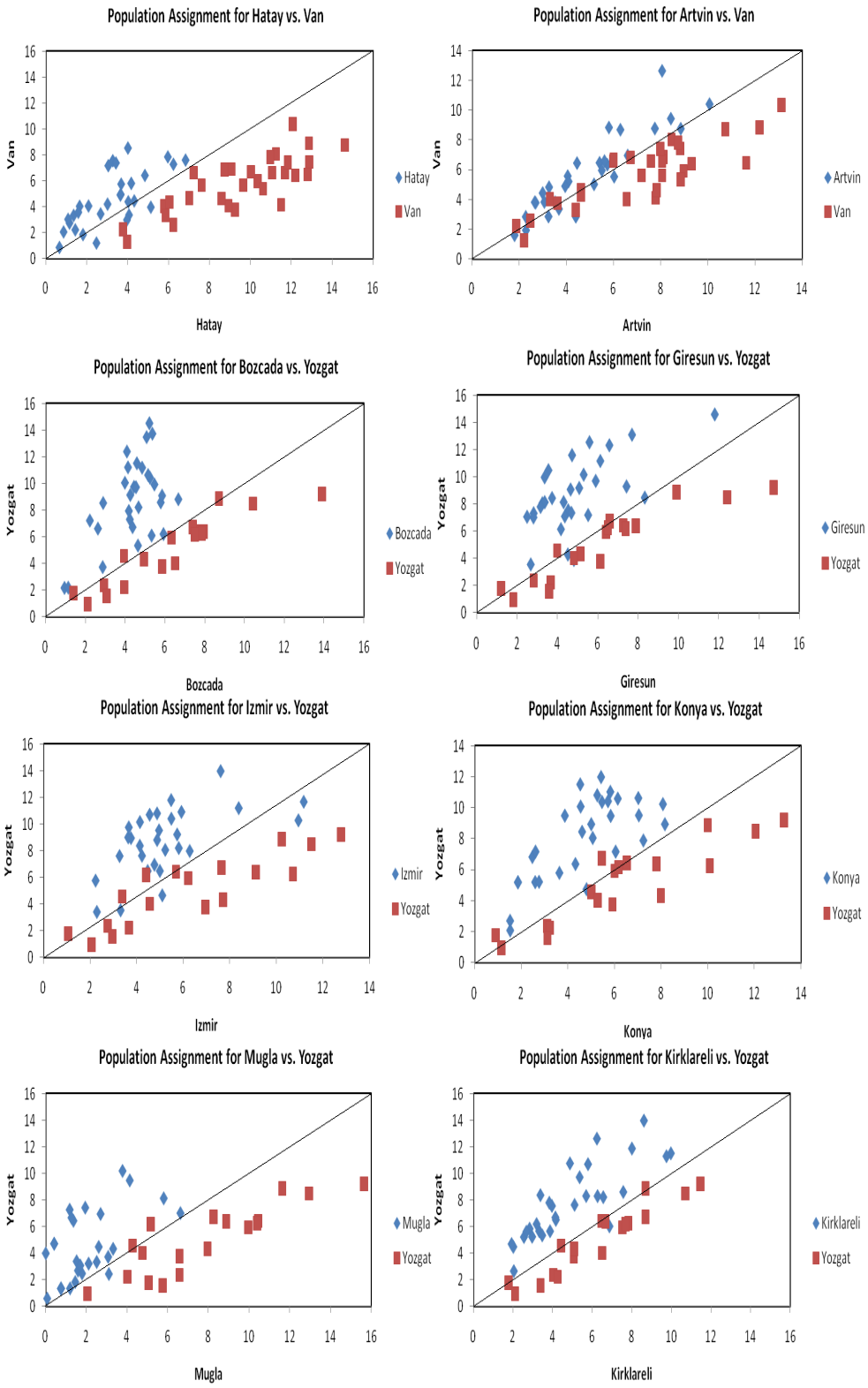

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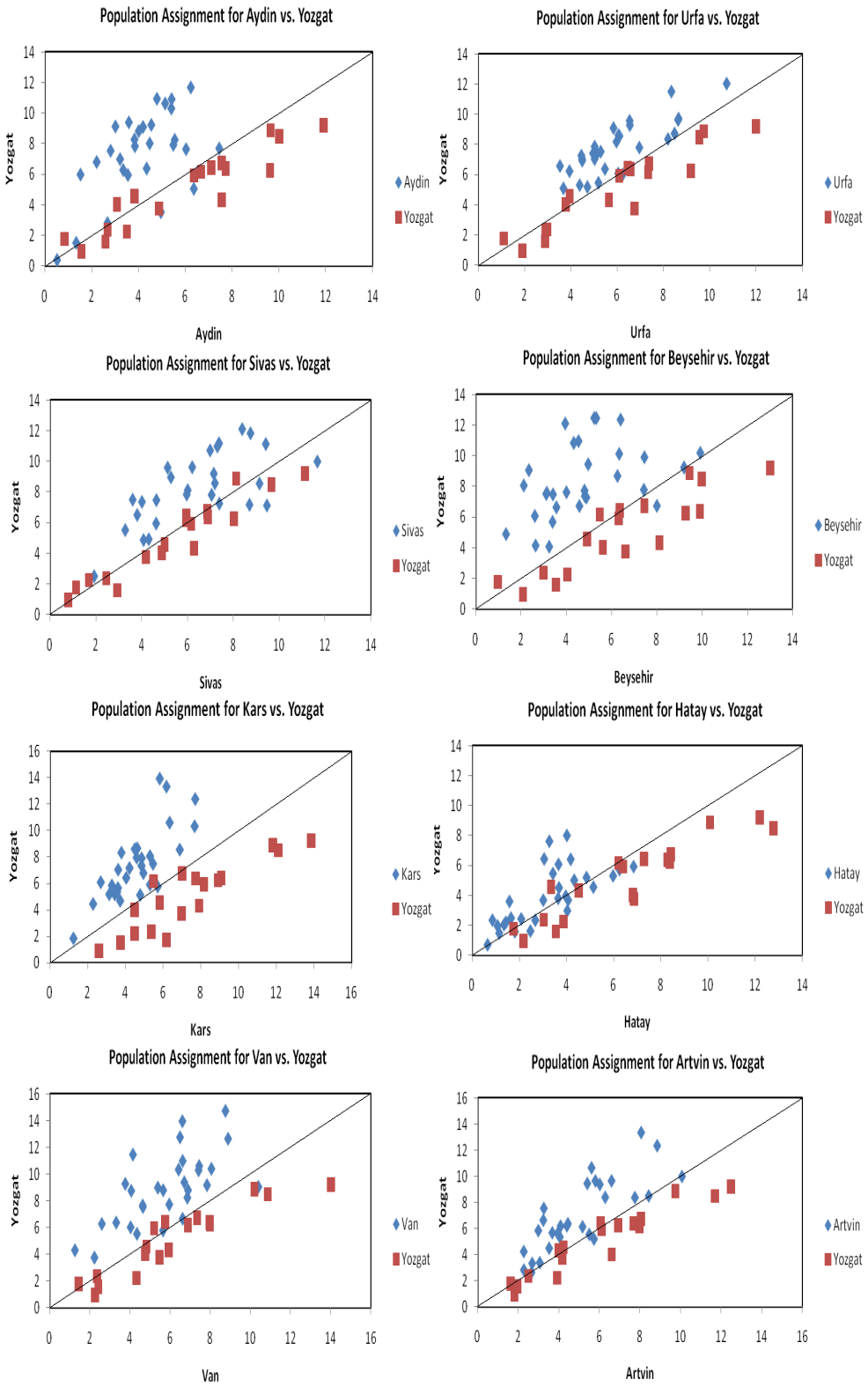

Figure E.1. Continued.

CIRRICULUM VITAE

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High School	Denizli High School, Denizli	1992

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2000-2002	Yuzuncu Yil University, Department of Animal Science, Agricultural Faculty,Van	Research Asistant
1997-2000	Primary School Teacher, Batman, İzmir	Teacher

PUBLICATIONS

Ivgin, R., G, Bilgen. 2002 Estimation of Genetic Distance in Meat and Layer Pure Lines Using Random Amplified Polymorphic DNA. *Turkish Journal of Veterinary and Animal Sciences*. 26(5), 1117-1120

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