REASSESSMENT OF GENETIC DIVERSITY IN NATIVE TURKISH SHEEP BREEDS WITH LARGE NUMBERS OF MICROSATELLITE MARKERS AND MITOCHONDRIAL DNA (MTDNA)

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Approval of the thesis:

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ABSTRACT

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In the present study, within and among breed genetic variability in seven native Turkish sheep breeds (Akkaraman, Dağlıç, Gökçeada, İvesi, Karayaka, Kıvırcık and Morkaraman) were analyzed based on 20 microsatellite loci. For the analysis, various statistical methods such as Neighbor-Net, Factorial Correspondence Analysis (FCA) and Structure were used.

High level of genetic variability within the Turkish breeds was observed. Gene pools of the breeds were visualized and found that they are highly overlapping with each other. As one of the reasons of this overlap, genetic exchange between the breeds was suggested. Dağlıç, claimed to be the ancestors of first domestic sheep in Anatolia, seemed to be the most admixed one. Yet Dağlıç, despite being the most introgressed one, still might be exhibiting its uniqueness. Observations implied that conservation practices concerning Dağlıç must be urgently revised.

Results of the present study do not support previous observations about the genetic differentiation patterns of the breeds within Anatolia. Possible reasons of the discrepancies between the observations were discussed.

Genetically extreme individuals can be identified by Structure, Assignment and FCA tests. These methods are found to be promising in establishing new relatively pure breeds or in saving the breeds from further genetic contamination. Genetically outlier individuals were shown not to exhibit any distinct morphological differences.

Unknown band patterns were found by RFLP and SSCP of mtDNA Control Region and the individuals harboring those were sequenced. They were shown to belong to the common haplogroups A, B or C. No novel haplogroup was found.

Keywords: Turkish native sheep breeds, microsatellites, morphology, genetic variation, sequencing

ÖZ

YERLİ TÜRK KOYUN IRKLARININ GENETİK ÇEŞİTLİLİKLERİNİN YÜKSEK SAYIDA MİKROSATELİT BELİRTEÇLERİ VE MİTOKONDRİYAL DNA (MTDNA) İLE YENİDEN ARAŞTIRILMASI

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Bu çalışmada yedi yerli Türk koyun ırkı (Akkaraman, Dağlıç, Gökçeada, İvesi, Karayaka, Kıvırcık and Morkaraman) arasındaki ve ırklar içerisindeki genetik çeşitlilik 20 mikrosatelit lokusu ile çalışılmıştır. Analiz için, Komşu-Ağ, Faktöriyel Birleştirici Analizi ve Yapı testleri gibi pek çok test uygulanmıştır.

Türk ırkları içerisinde, eskiden olduğu gibi, yüksek genetik çeşitlilik gözlenmiştir. Irkların gen havuzları gözlenmiş ve birbileriyle bir hayli kesiştiği görülmüştür. Bu kesişimin nedenlerinden biri olarak, ırklar arasındaki genetik alışveriş önerilmiştir. Anadolu'daki ilk yerli koyunların atası olduğu iddia edilen Dağlıç, en karışık birey olarak görülmektedir. En fazla koç katımına maruz kalmasına rağmen, Dağlıç hala eşsizliğini koruyor olabilir. Gözlemler, Dağlıç üzerindeki koruma pratiklerinin acilen gözden geçirilmesi gerektiğini gözler önüne sermektedir.

Bu çalışmanın sonuçları, Anadolu koyun ırklarının genetik farklılaşma örgüsüne dair önceki gözlemleri desteklememektedir. Bu gözlemlerdeki farklılıkların olası nedenleri üzerine tartışılmıştır.

Genetik olarak uç bireyler Yapı, Bireylerin Irklara Atanması Testi ve Faktöriyel Birleştirici Analizi ile tanımlanabilirler. Bu metodlar saf koyun ırklarının oluşturulmasında ya da ırkların daha fazla genetik kirliliğe maruz kalmamasında umut vaatetmektedir. Genetik olarak uç bireylerin görünümsel farklılık sergilemediği de görülmüştür.

RFLP ve SSCP metodları ile farklı bant örgüleri gösteren örneklerin mitokondriyal DNA kontrol bölgesi sekanslanmış ve bunların yaygın haplogruplar A, B ve C'ye ait oldukları görülmüştür. Yeni bir haplogrup bulunamamıştır.

Anahtar Kelimeler: Türk yerli koyun ırkları, mikrosatelit, morfoloji, genetik çeşitlilik, dizileme

To My Dear Family and Beloved Ones…

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I would like to begin with stating this period of my life was both inspiring and tiring. Throughout this journey, my supervisor Prof. Dr. İnci Togan was always there with her understanding, encouraging, engaging, smart and cheerful personality to discuss about everything; no matter what the subject is. She helped me to rediscover my scientific enthusiasm and my belief in my scientific capabilities. I cannot thank her enough.

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Finally, I would like to show my appreciation to my parents Ayla and Tayyar Doğan, my brother Arda Doğan, my aunt Zehra Kıran and my uncle Eyüp İlaslan for their reliance on me. Especially, my mother who never gave up believing in me… I could not succeed without you.

A last word: In the Netherlands, you should include a flyer in your PhD thesis in which you write your so-called proverbs or wise words you learned throughout your studies. It is a great tradition and I would like to do the same here: Working, especially in a very different sector, while doing masters / trying to perform science in Natural Sciences is an absolutely absurd and painful idea. Do not even think about it! 'Good' science demands and deserves your full time.

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INTRODUCTION

1.1. A Brief History of Domestication for Livestock Animals and Neolithic Demic-Diffusion Model

Human populations took a historic step when they abandoned foraging and hunting practices for farming and herding. This change in life style had a significant impact in the social and eventually intellectual lives of humans. However, the questions of where, when and how these momentous transitions occurred are still largely unknown. They still generate great interest and curiosity among researches and general public. Domestication provided independence from environmental fluctuations making it possible to manage food resources that later gave rise to permanent settlements. Via those, men developed new technologies, used those technologies for the well-being of himself and eventually human civilizations rose (Zeder, 2008).

The studies for domestication history were residing on archaeological findings, concerning the remains found at the excavation sites. Until the late 90's, archaeozoologists used morphological changes to identify the transformation of wild animals into herded livestock (Zeder *et al.*, 2006). It is thought that

domestication manifests itself via rapid reduction in body size (Uerpmann, 1979; Meadows, 1989). However, later new realizations suggested that domestication might have started at earlier times before the size reduction was detected. In herds, the females must be outnumbering the males because they are the ones that can give birth to new animals and produce milk. Male to female ratio change is now accepted as the earliest sign of domestication (Zeder, 2008).

Mainly the archeological results indicated that domestication could have occurred in three main areas: Southwest Asia, more specifically The Fertile Crescent (today's Israel, Jordan, Lebanon, west of Syria, southeast of Turkey, along the Tigris and Euphrates rivers, Iraq and the western of Iran) and Indus valley; East Asia: China; and Adnean chain of South America (Bruford *et al*., 2003). However, with the new realization, earliest domestication sites were mapped in the heart of Anatolia extending to Iran for sheep, goat, cattle and pig. The earliest domestication sites and which animals are thought to be domesticated in those sites can be seen in the map of Zeder (2008) in Figure 1.1.

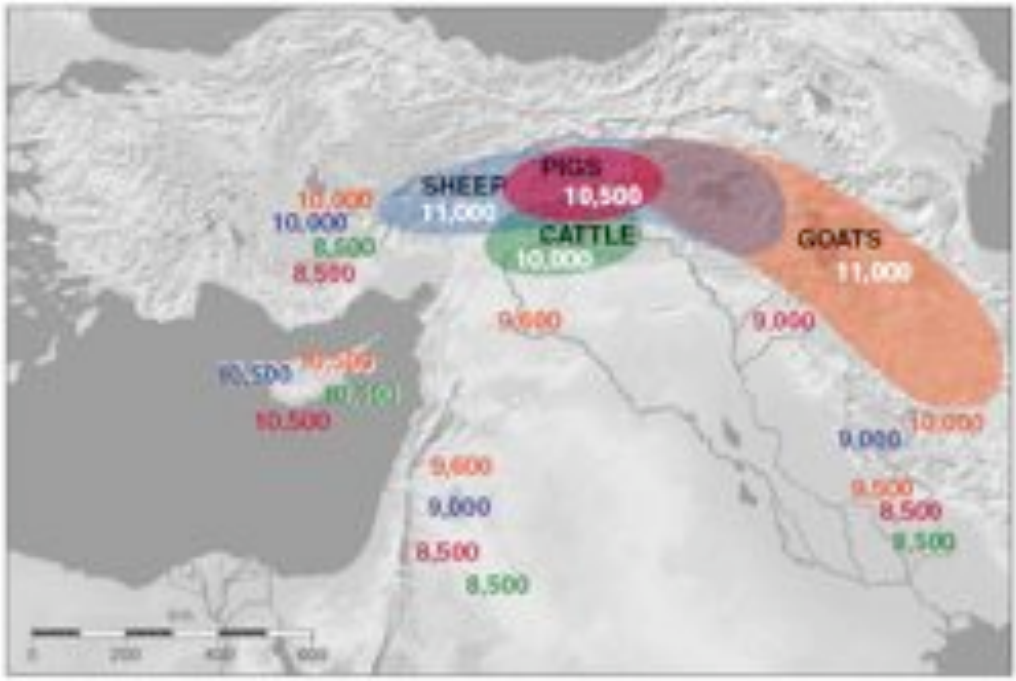


Figure 1.1 The domestication sites of important livestock animals and the approximate dates in before present (B.P.) when the domestication is thought to take place (Zeder, 2008). Dates outside of the shaded areas show the approximate date when the domesticate first appears in a region. (For this information, the color codes should be taken into consideration).

The map of Zeder (2008) reveals the importance of Anatolia as a center of domestication. According to Zeder (2008), it is highly probable for Anatolia to harbor the extents of the earliest domesticated animals as its native breeds. Furthermore, the complexity of domestication and the introgression of the products of different domestication sites should be kept in mind.

In the last two decades, genetics started to dominate the domestication studies and offered a variety of useful tools with maternal, paternal and autosomal origins, for such studies. It is assumed that the samples near the domestication centers had high genetic diversity and there was a decrease in genetic variation when one moves away from the domestication center (Bradley *et al*., 1996, Loftus *et al*., 1999).

For the human beings, peopling of Europe during the Neolithic ages (starting with the settlements of human beings about 11,000 years before present) it is generally assumed that people went from or through Anatolia to Europe. The Neolithic Demic-Diffusion model (NDD) is the well-known model proposed by Ammerman and Cavalli-Sforza (1973) postulating that extensive migrations of Near Eastern farmers during the Neolithic ages brought agricultural techniques to the European continent. The dispersal is thought to occur from Anatolia to Europe (Renfrew, 1991). Technological advances, especially in farming, increased the food supply and as a result population size increased. When the carrying capacity was reached, individuals felt an urge to move in to new areas. This was achieved by gradual dispersals of the small groups (demes) of Neolithic farmers. It is thought that the farmers brought new farming techniques as well as domesticated animals along with themselves (Barbujani *et al*., 1994), and the references therein). Human genetic studies (Barbujani *et al*., 1994; Chikhi *et al*., 2002) seem to support this model, as well as studies in goats (Troy *et al*., 2001) and sheep (Townsend, 2000; Meadows *et al*., 2005).

Apart from that, Renfrew (1991) suggested that a proto-indo-European language spoken within the East-central Anatolia propagated to Greece at 7000 BC then from Balkan Peninsula to Europe, which is also an advocating event for NDD.

1.2. Domestication of Sheep

The evolutionary history of domestic sheep is complex (Bruford *et al*., 2003; Bruford and Townsend, 2006) and still remains unclear and blurred. Sheep were one of the first animals that were domesticated and the possible place and time is considered to be the northern Zagros to central Anatolia approximately 11,000 before present (B.P.) (Bruford *et al*., 2003; Zeder, 2008).

Starting point in the evolutionary history of livestock animals can only give complete and conclusive results by the identification of wild ancestors. During the long and slow process of domestication, some gene variants were favored and selected. Yet, some variants are not explored at all or lost accidentally during the selection and / or colonization process of the domestic animals. Breeds, group of animals having specific morphology, yield and fitness characteristics have emerged. If we know the wild ancestors of the domestics we can guess the center of domestication and expect that the current day native breeds of this domestication center to harbor the highest genetic diversity with the variants of the traits having prime importance to be explored.

Even if the wild ancestor of modern sheep remains as an enigma, the molecular genetic studies using mitochondrial DNA (mtDNA) revealed that *Ovis gmelini* is the most likely domestic ancestor of modern sheep (Hiendleder *et al*., 1998b; Wu *et al*., 2003; Bruford and Townsend, 2006; Meadows *et al*., 2007; Koban *et al*., 2008). Other wild sheep populations, such as *Ovis vignei*, *Ovis ammon* found to be genetically distant to modern sheep (Hiendleder *et al*., 1998b), whereas *Ovis musimon* is considered to be a feral descendant of the first domestic sheep (Hiendleder *et al*., 2002; Pedrosa *et al*., 2005; Bruford and Townsend, 2006; Meadows *et al*., 2007).

Additionally, mtDNA control region sequence and RFLP analysis provided evidence for at least three possible domestication events. The first haplogroups (HPG) to be identified were HPG-A and HPG-B (Wood and Phua, 1996; Hiendleder *et al*., 1998a). Recent findings suggest that there are at least three more lineages, designated as HPG-C, HPG-D and HPG-E, were identified (Townsend, 2000; Guo *et al*., 2005; Pedrosa *et al*., 2005; Tapio *et al*., 2006; Bruford and Townsend, 2006; Meadows *et al*., 2007) indicating multiple domestication events. According to the results of the aforementioned studies, HPG A is mostly seen in Asia, HPG B is dominant across Europe, HPG C is in the region from east Europe to Asia, HPG D is solely confined in (eastern) Anatolia and HPG E, although low in frequency, observed from Sardinia to China. In one of the studies above, Guo (2005) a probable new haplogroup, which is named as HY030, is found. Koban *et al*. (2008) show that this haplogroup is not clustering with any of the known five haplogroups.

The importance of Anatolia came mainly from the fact that it harbors the archaeological sites exhibiting the earliest evidence of sheep domestication, Nevali Çöri and Çayönü (Peters *et al*., 1999) as well as it is very close to the other probable domestication areas, such as Zagros Mountains (Zeder, 1999).

1.3. Turkish Sheep Breeds

Sheep breeding in Turkey is carried out generally by small family enterprises. 97.3 % of the total sheep population in Turkey is comprised of native breeds. Akkaraman, mainly located in Central Anatolia, is the most common population among the native sheep breeds. Morkaraman, widespread in Eastern Anatolia, and Dağlıç, in Western Anatolia, are the 2nd and the 3rd. Kıvırcık is found in Aegean and Marmara regions, Karayaka in Black Sea region, Gökçeada in Gökçeada-Çanakkale region, Sakız (Chios) in coastal parts of Aegean-Marmara, Turkish

Merino in South Marmara-Central Anatolia regions, İvesi (Awassi) in South East Anatolia and Tuj (Tushin) breed in Kars province (FAO, 2004). There also exist crossbreeds that are not in the interest area of the study.

One of the most important problems that Food and Agricultural Organization of the United Nations (FAO) is drawing attention is the sharp decrease in the number of livestock animals. Turkey has been experiencing serious livestock number declining for the past 30 years. Between the years 1980 – 2000, the decline in sheep was 47.4 %. The same decline for the time period of 2000 – 2004 was 10.7 % (Oskam *et al*., 2005). This decline is thought to result from the taste shift from lamb to beef and poultry in urban areas, as well as political and social unrest in rural areas (Sarigedik, 2004).

1.4. Molecular Markers in Domestication Studies

1.4.1 Microsatellites as Marker

Microsatellites are stretches of DNA that consist of tandem repeats of a specific sequence of DNA, which contains mono, di, tri, or tetra tandem repeats. Microsatellites are found predominantly in the nuclear genome (usually non-coding parts of genome) in tens of thousands of localities in the genomes of most vertebrates and comprise short repetitive sequences. Microsatellites are inherited in a different way to mtDNA, with different evolutionary mechanisms and their variation can reach spectacular levels. They are inherited codominantly in a Mendelian fashion. Their variation is mostly neutral (Schlotterer, 2000).

Their distribution in the genomes, high levels of variability, ease and reliability of scoring, codominant inheritance, short lengths, and accumulation of major changes in allele frequencies very rapidly makes them ideal markers for measuring genetic variation, for genome mapping, for tracking the past

demographic events (for example changes in population size), for using them in parentage and relatedness analysis. Researchers are also enjoying the wealth of newly developed analytical and statistical approaches to the ample genetic information provided by microsatellite data (Luikart and England, 1999). To retrieve information from a marker with high allelic diversity, such as microsatellites, maximum likelihood, the coalescent and Bayesian statistical approaches gained great success and many statistical softwares were designed based on those approaches. By these methods, more accurate and detailed information about the evolutionary parameters, historical events, population parameters such as migration rates and effective population sizes has been dogged out of microsatellites (Luikart and England, 1999).

These markers are applied to tackle various problems. Their importance to livestock diversity was recognized in early 90's (Machugh *et al*., 1994) and the Food and Agriculture Organization of the United Nations (FAO), through Domestic Animal Diversity Information Service (DAD-IS) has attempted to standardize the use of these markers to characterize genetic variation within and among breeds. In livestock animals, microsatellites are used for measuring genetic variation within and among breeds (Diez-Tascon *et al*., 2000; Cronin *et al*., 2008; Dowling *et al*., 2008), for admixture studies (MacHugh *et al*., 1997; Freeman *et al*., 2006; Vicente *et al*., 2008) and for assigning individuals to breeds (Cornuet *et al*., 1999; Troy *et al*., 2001; Meadows *et al*., 2006).

1.4.2 Mitochondrial DNA (mtDNA) as Marker and Sheep mtDNA

The mitochondrion is a network of sub-cellular, double membrane organelles, constantly fusing and dividing. The outer membrane is separated from the inner membrane by the intermembrane space. The inner membrane is folded into cristae, maximizing its surface. The innermost compartment of the mitochondria is called the mitochondrial matrix, and contains the mitochondrial genome,

ribosomes, tRNAs and various proteins and enzymes required for mitochondrial function. The main function of the mitochondria is to use oxygen to generate the cell's major energy source, ATP. The origin of the mitochondrion is believed to be an ancient bacterium, which infected a primordial cell and became symbiotic. The benefit of this partnership is thought to have been the removal of the toxic compound oxygen, and the production of ATP a welcome side effect. The free-living organism with a genome most similar to the mitochondrion is the protobacterium *Rickettsia prowazekii* (Balaban *et al*., 2005).

Unlike most other cellular compartments, mitochondria have their own genomes, which encode a few mitochondrial proteins (most others being encoded by genes in the nucleus). Mitochondrial DNA (mtDNA) is a circular molecule of approximately 16,000 base pair. It became a key molecular marker for domestication studies, especially in the 80's not only to examine the evolutionary relationships among species but also to measure and partition genetic diversity within taxa. It is maternally inherited (usually clonally as a single copy) and does not undergo recombination. Mitochondrial DNA's evolving rate is way higher than its nuclear counterpart and certain regions evolve even faster. The control region (CR) is one of the examples for those regions. It is the most rapidly evolving region of mtDNA and this makes it an excellent candidate for livestock diversity studies. Nevertheless, mtDNA can scarcely give enough information for interpretation of the evolution of whole genomic diversity because of its acting as a one gene, not going under recombination and its having its own specific 'evolutionary dynamics' (Bruford *et al*., 2003).

One other region used for population studies in the ND4 region. Most of the energy needed in mammalian cells comes from the process of oxidative phosphorylation, OXPHOS. The respiratory chain carries out the process of OXPHOS, which uses oxygen and sugars to generate water and the cells' main energy source, adenosine-tri-phosphate, ATP. The respiratory is a set of five

multi-subunit enzyme complexes (complex I to complex V) that resides embedded in the inner mitochondrial membrane. The respiratory chain consists of over 100 different protein species, 13 of which are encoded by mtDNA. All complexes but complex II contain mtDNA-encoded subunits and are therefore dependent on mtDNA being present and decoded for their function. Complex I and II collect electrons from NADH and succinate, respectively, and pass them to ubiquinone. Complex I is also known as NADH dehydrogenase complex. It is a huge flavoprotein complex containing more than 25 polypeptide chains (subunits) (Balaban *et al*., 2005). ND4 is a 1378 base pair long gene that encodes NADH dehydrogenase subunit 4 (Hiendleder *et al*., 1998a). Guo *et al*. (2005) showed that Single Strand Conformational Polymorphism (SSCP) could be used to analyze partial ND4 region to determine the haplogroup of the sheep.

Complete sheep mitochondrial DNA is approximately 16,616 base pair. Length of the strands can vary because CR has a variable tandem repeat region of a ~75 bp repetitive unit occurring in 2-6 copies (Hiendleder *et al*., 1998a).

1.5. Statistical Analyses in Population Genomics Studies

In recent years, population genomic studies have benefited from both the use of new and stronger genetic markers as well as the increasing number of quality data obtained from those. Simultaneously, novel statistical methods have been proposed in order to keep up with the accumulating data, with the advances in computational power (Luikart and England, 1999). Bayesian methods, Markov chain Monte Carlo (MCMC) algorithms, maximum-likelihood and coalescent methods were among those unorthodox methods. The use of these methods, especially Bayesian methods, is revolutionizing the way genetic data analyses were handled (Beaumont and Rannala, 2004). Those markers and statistical analysis help to propose and compute more complex models, which reflects the reality for biological world. Nowadays, researchers are aiming to put non-genetic data (such as spatial and behavioral data) into the equation with the available

genetic data. The methods used, such as Bayesian methods, are fairly adequate but for those efforts will need further statistical approaches and new softwares for the computation of those (O'Hara *et al*., 2008).

1.6. Justification and Objectives of the Study

Today's world is facing with a sharp decline in biodiversity and biological richness. Conservation of those is one of the main concerns of most of the countries. Studies on resolving the phylogenetic relationships between breeds would be helpful in identifying the priorities in conservation of the animal genetic resources (Reist-Marti *et al*., 2003). With increasing knowledge about those, we can do more for the conservation of biodiversity.

Turkish native sheep breeds might be the closest living descendants of first domesticated sheep because of the fact that it harbors and/or is geographically very near to the putative earliest sheep domestication centers. Studying breeds located around putative domestication centers may provide invaluable insights into the evolutionary history of livestock animals.

Based on microsatellite markers, a number of molecular studies on native Turkish sheep breeds have been carried out. The studies are many but they did not have a holistic approach in asking the questions, as discussed by Koban *et al*. (2008). By some of them very few breeds and relatively few loci were used (Soysal *et al*., 2005), some used a higher number of markers but sampled relatively few individuals (Townsend, 2000) or breeds (Peter *et al*., 2007) or breeds from a restricted geographic region (Uzun *et al*., 2006). In this study, seven native Turkish sheep breeds (Akkaraman, Dağlıç, Gökçeada, İvesi, Karayaka, Kıvırcık and Morkaraman) were analyzed based on 20 microsatellite loci. The number of breeds is less but the microsatellite loci analyzed is twice as much when compared to microsatellite markers of Koban *et al*.'s (2008) study.

Objectives of the present study are as follows:

1) To analyze the diversity between the Turkish native sheep breeds including Kıvırcık, Gökçeada and İvesi which were not studied by extensive sampling.

2) To check the robustness of the previously observed genetic diversity patterns by the newly sampled individuals.

3) To see if 20 highly polymorphic microsatellites can define the gene pools of Turkish native sheep breeds.

4) To see if the outliers of the genetic distributions of the sheep breeds described by their individuals are associated with distinct / distorted morphology.

5) To see if genetic-statistical analyses can be of any use to select or purge individuals in the process of breed conservation.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

2.1.1 Samples, Sampled Breeds and Sampling

In this study, sampling was performed from individuals of seven breeds (Akkaraman, Dağlıç, Gökçeada, İvesi, Karayaka, Kıvırcık and Morkaraman). The total number of analyzed individuals was 280. Sampling was done by the experts under the supervision of Ministry of Agriculture and Rural Affairs (MARA) within the project TURKHAYGEN-I (Project No: 106G115). The sampling sites are as follows:

Akkaraman: Konya of Central Anatolia Region, sample size (n) = 39
Dağlıç: Afyon of Aegean Region, n = 42
Gökçeada: One of the two Aegean Islands of Turkey in Aegean Sea, near Çanakkale of Marmara Region, n = 31
İvesi: Şanlıurfa of Southeast Anatolia Region, n = 38
Karayaka: Ordu and Tokat of Black Sea Region, n = 44

Kıvırcık: Çukurpınar and Armutveren villages of Kırklareli of Thracian part of Turkey, n = 36
Morkaraman: Erzurum of Northeast Anatolia Region, n = 50

During sampling, extreme care is taken in order to select individuals that are not related. 2-3 individuals are sampled per herd. Figure 2.1 represents the location of the samples sites on a Turkey map.

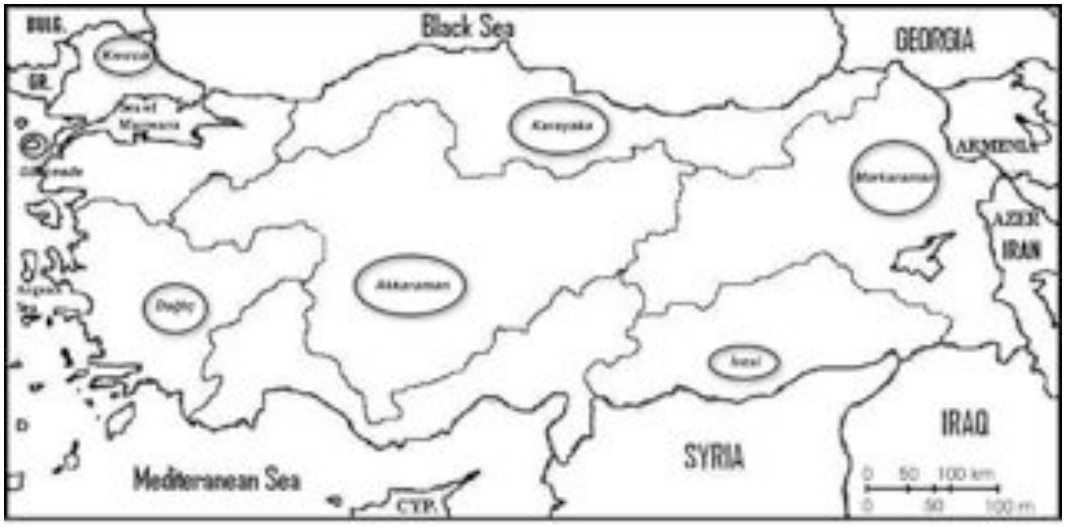


Figure 2.1 Location of the sampling sites.

The material sampled was blood. 10 ml of blood was collected in K_3EDTA containing tubes for the prevention of coagulation. Samples were stored at 4 °C until isolation of DNA. Ingredients of chemical solutions used in experiments can be found in Appendix A.

Turkish sheep breeds have adaptations for different climatic and topographic conditions of Turkey. One of the ways to distinguish the breeds is their tail feature: fat (and variation of fat tail) or thin (variation in thin) tail. Fat tail acts as an energy reservoir when the conditions are harsh and the temperature difference between the seasons is very high. The general features of Turkish native breeds used in this study are as follows (Acan, 2002; DAD-IS, 2009):

Akkaraman:

- Fat tailed
- White coat color, with black around the nose, and rarely around the eyes and on the legs.
- Rarely black specks are observed on the body.
- Males may have (about 10 %), whereas females do not have horns (very rarely small horns).
- Coarse and long outer coat and fine and short inner coat.
- Head, underside of neck and legs are usually devoid of wool.
- Adults' withers height is about 65 cm and live weight is 45 - 50 kg.

An example of Akkaraman sheep breed can be found in Figure 2.2.

Figure 2.2 An Akkaraman male. The photograph is taken for the TURKHAYGEN-I project and used by the permission of the project commission.

Dağlıç:

- Fat tailed
- White coat color with black spots around mouth, eyes and genital organs, and on the legs.
- Males have large spiral horns (95 %), whereas females do not have horns.
- Adults' withers height is about 58 cm and live weight is 35 - 40 kg.

An example of Dağlıç sheep breed can be found in Figure 2.3.

Figure 2.3 A Dağlıç male. The photograph is taken for the TURKHAYGEN-I project and used by the permission of the project commission.

Gökçeada:

- Thin tailed; the tail is thin and long, usually reaching below the hocks.
- White coat color with black spots around the mouth, eyes, nose and ears and rarely on the tip of the legs.
- The males are spiral-horned and the females are polled or have scurs.
- The head is narrow and its profile is straight.
- The wool is very coarse and long, and it covers the top of the head.
- Adults' withers height is about 58 cm and live weight is 35 - 40 kg.

An example of Gökçeada sheep breed can be found in Figure 2.4.

Figure 2.4 A Gökçeada female. The photograph is taken and kindly provided by Prof. Dr. M. İhsan Soysal and Ass. Prof. Emel Özkan of Namık Kemal University, Agriculture Faculty, Tekirdağ / Turkey.

İvesi:

- Fat tailed
- White coat color with brown marks on feet, ears and neck.
- Males have, whereas females do not have horns.
- Adults' withers height is about 65 cm and live weight is 45 - 50 kg.

An example of İvesi sheep breed can be found in Figure 2.5.

Figure 2.5 An İvesi female. The photograph is taken for the TURKHAYGEN-I project and used by the permission of the project commission.

Karayaka:

- Thin and long tailed, small fat deposit at the base of the tail.
- White coat color; eyes, head and legs are black in color. Occasionally black or brown animals are seen.
- Males have thick spiral, whereas females do not have horns.
- Tuft of wool on the forehead.
- Adults' withers height is about 60 - 62 cm and live weight is 35 - 40 kg.

An example of Karayaka sheep breed can be found in Figure 2.6.

Figure 2.6 A Karayaka female. The photograph is taken and kindly provided by Dr. Evren Koban of TÜBİTAK Kocaeli/ Turkey for the ECONOGENE project.

Kıvırcık:

- Thin and long tailed, usually reaching the hocks. In pure animals there is no fat deposit in the tail.
- White coat color with white or spotted faces.
- Males have horizontal spiral horns extending sideways, whereas females do not have horns.
- The ear is relatively short and extends horizontally.
- Adults' withers height is about 58 cm and live weight is 35 - 40 kg.

An example of Kıvırcık sheep breed can be found in Figure 2.7.

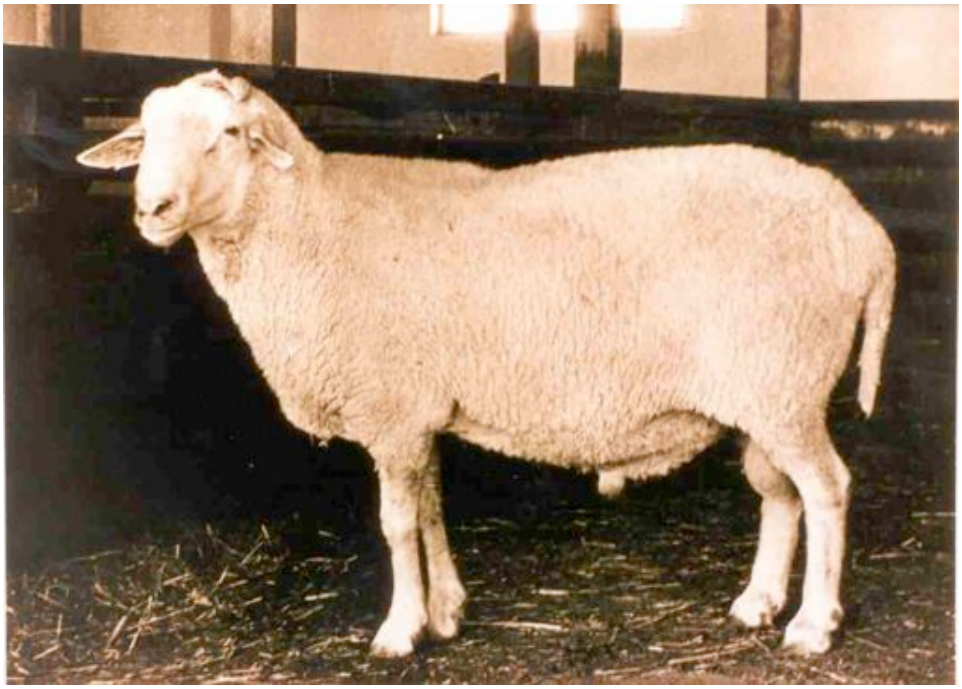


Figure 2.7 A Kıvırcık female. The photograph is taken and kindly provided by Prof. Dr. M. İhsan Soysal and Ass. Prof. Emel Özkan of Namık Kemal University, Agriculture Faculty, Tekirdağ / Turkey.

Morkaraman:

- Fat tailed
- Red or brownish coat color, the color is darker on head, neck and legs.
- In general, ewes and rams are polled; about 1 percent of ewes and 10 percent of rams may have small horns.
- The wool is of mixed, coarse carpet type, with low density and open head, neck, belly and legs.
- Adults' withers height is about 68 cm and live weight is 50 - 60 kg.

An example of Morkaraman sheep breed can be found in Figure 2.8.

Figure 2.8 A Morkaraman male. The photograph is taken for the TURKHAYGEN-I project and used by the permission of the project commission.

More photographs can be found in the website of the TURKHAYGEN-I (Project No: 106G115), http://www.turkhaygen.gov.tr.

2.2 Methods

2.2.1 DNA Isolation

Standard phenol: chloroform DNA extraction protocol is employed for DNA isolation. The samples (10 ml), collected into tubes containing K_3EDTA, were taken into a falcon tube containing 0.5 ml EDTA (0.5 M, pH 8.0). Volume is completed to 50 ml by adding 2 X lysis buffer (10 X Lysis solution: 770 mM NH_4Cl, 46 mM $KHCO_3$, 10 mM EDTA). Tubes were mixed by inversion for 10 minutes, and then put into ice for 30 minutes. Following, the tubes were centrifuged for 15 minutes at 3000 rpm at 4 °C. After the supernatants were disposed, 3 ml of salt-EDTA (pH: 7.5, 75 mM NaCl, 25 mM EDTA) solution, 0.3 ml SDS (10 %) and 0.15 ml proteinase K (10 mg/ml) were added to each tube. Incubation were performed overnight at 37 °C or for 3 hours at 55 °C. Afterwards, 3 ml phenol (pH 8.0) was added to each tube. The tubes were mixed vigorously for a minute by shaking and then by gentle inversion for 10 minutes. Another centrifugation was performed, again for 15 minutes at 3000 rpm at 4 °C. Then, the supernatants were transferred into new falcon tubes and phenol:chloroform:isoamylalcohol (in ratio of 25:24:1) solution was added to each tube. After vigorous shaking for a minute, 10 minute of gentle inversion step is employed. Tubes were again centrifuged for 15 minutes at 3000 rpm at 4°C. Later on, the supernatants were taken into glass tubes and 2 volume pure cold ethanol kept at -20 °C was added. The glass tubes were shaken abruptly, the condensed DNA was hooked out with a glass hook and transferred into 1.5 ml eppendorf tubes and 0.5 ml Tris-EDTA (10 mM Tris, 1 mM EDTA PH 7.5) buffer

was added to each one. DNA samples were stored in -20 °C (Sambrook *et al*., 1989)

2.2.1.1 Checking the Presence of DNA

After isolation, the concentration of DNA was checked by agarose gel electrophoresis. 0.8 % agarose gel was prepared by boiling agarose in 0.5 X TBE buffer. This solution was poured into an electrophoresis plate and left in room temperature for about 30 minutes for casting. Agarose plate was placed into an electrophoresis tank, which was filled with 0.5 X TBE buffer. DNA samples were prepared for loading by mixing 2 µl of newly isolated DNA with 3 µl 6 X loading buffer (bromophenol blue dye) and 3 µl dH2O and loaded into the wells of the gel. The gel was run at 100 V, for 20 minutes. After this period, the gel was put into ethidium bromide (EtBr) solution (0.5 µl/ml). 20 minutes later, the gel is visualized and photographed under UV light by a gel imaging system. The presence of DNA bands (intact DNA), smears (if any broken DNA) as well as the thickness of the bands (quantity) was checked. The DNA bands were compared according to their thickness and intensity with a DNA band of known concentration to decide about the concentrations of the samples, which are then used to adjust the concentrations of the sample DNAs prior to amplification. Furthermore, this information is used to decide how many µl of DNA sample should be put into the PCR mixture.

2.2.2 Microsatellites

Twenty microsatellite loci have been chosen for this study. The locus names and GenBank accession numbers (in parentheses) of this polymorphic loci are as follows: OarFCB226 (L20006), OarHH47 (L12557), OarVH72 (L12548), OarFCB128 (L01532), INRA063 (X71507), MAF33 (M77200), MAF214 (M88160), BM8125 (G18475), OarFCB304 (L01535), OarJMP29 (U30893),

OarJMP58 (U35058), ILSTS005 (L23481), OarFCB20 (L20004), OarFCB48 (M82875), MAF65 (M67437), MCM140 (L38979), ILSTS011 (L23485), OarCP34 (U15699), DYMS1 (), MAF209 (M80358)

They were chosen because there are extensive data available from the European Union (EU) 5th Framework project ECONOGENE (http://www.econogene.eu; (Peter *et al*., 2007) and the data obtained from this study will be comparable to the ones in ECONOGENE for later analysis. The names of these microsatellite loci, their allelic range, their origin and on which chromosome they are located were given in Table 2.1 below.

Table 2.1 Sheep microsatellite DNA markers, allelic ranges, origins and chromosome numbers.

	Microsatellite Loci	Allelic Range	Origin	Chromosome
1	OarFCB226	118 - 160	Ovine	2
2	OarHH47	121 - 163	Ovine	18
3	OarVH72	121 - 145	Ovine	25
4	OarFCB128	96 - 130	Ovine	2p
5	INRA063	156 - 212	Bovine	14
6	MAF33	116 - 147	Ovine	9
7	MAF214	134 - 264	Ovine	16
8	BM8125	106 - 128	Bovine	17
9	OarFCB304	145 - 191	Ovine	19
10	OarJMP29	113 - 167	Ovine	24
11	OarJMP58	137 - 177	Ovine	26
12	ILSTSOO5	174 - 218	Bovine	7
13	OarFCB20	86 - 130	Ovine	2q
14	OarFCB48	136 - 172	Ovine	17
15	MAF65	112 - 146	Ovine	15
16	MCM140	161 - 198	Ovine	6
17	ILSTS011	256 - 294	Bovine	9
18	OarCP34	110 - 136	Ovine	3p
19	DYMS1	157 - 211	Bovine	20
20	MAF209	109 - 142	Ovine	17

2.2.3 Polymerase Chain Reaction (PCR)

The microsatellite regions were amplified by using Polymerase Chain Reaction (PCR). This technique consists of three steps, which are called denaturation, annealing and extension. In denaturation DNA's single strands are separated from each other, in annealing oligonucleotide primers are bound to their complements on single stranded template DNA and in extension primers are extended in the 5'-

to- 3' direction. As a result, specific DNA regions were enzymatically amplified in microgram quantities (Klug *et al*., 2000).

All the DNA samples were amplified with the primers specific to these five twenty microsatellite loci by using Biometra T1 Thermocycler and BioRad MyCycler™ Thermal Cycler.

The forward (F) primers of the chosen microsatellite loci have been marked with different flourescent colors according to their expected allelic range. This flourescently labelled 20 microsatellite loci have been grouped into four according to their allelic ranges and colors. The groups and the flourescent markers, that the forward primers have, are as follows:

Group I: 5 loci	**Group II: 6 loci**
JMP58 + ILSTS005 ----- TET	BM8125 + FCB304 ----------HEX
FCB20 + FCB48----------HEX	FCB128 + INRA063----------FAM
JMP29 ----------FAM	MAF33 + MAF214 ----------TET

Group III: 6 loci	**Group IV: 3 loci**
MAF209 ----------HEX	FCB226 ----------FAM
CP34 + DYMS1----------TET	HH47 ----------TET
MAF65 + MCM140 + ILSTS0011--FAM	VH72 ----------HEX

From those flourescent markers, TET gives green, FAM blue and HEX black color signals. The forward and reverse primers used for the amplification of 20 microsatellite loci can be found in Appendix B.

The PCR reaction mix and PCR conditions for Group I: 5 loci are given in Tables 2.2 - 2.3.

Table 2.2 PCR reaction mix for Group I representing 5 loci.

	Concentrations	Quantities
dH_2O	N/A	5.9 µl
PCR Buffer	1 X	1.5 µl
$MgCl_2$	2 mM	1.2 µl
dNTP	100 µM	0.6 µl
Primers (F + R) 1) OarFCB48 2) OarFCB20 3) OarJMP29 4) OarJMP58 5) ILSTSOO5	 4 pmoles 3 pmoles 3 pmoles 2.5 pmoles 2 pmoles	 0.8 µl 0.7 µl 0.6 µl 0.5 µl 0.5 µl
DNA	100 - 150 ng	2.5 µl
Taq Polymerase	0.5 units	0.2 µl

TOTAL	**15 µl**

Table 2.3 PCR conditions for Group I representing 5 loci.

Steps	Temperature	Duration	
Denaturation	94 °C	2.5 minutes	
Denaturation	94 °C	20 seconds	35 cycles
Annealing	57 °C	20 seconds	
Extension	72 °C	40 seconds	
Final Extension	72 °C	20 minutes	

The PCR reaction mix and PCR conditions for Group II: 6 loci are are given in Tables 2.4 – 2.9:

- For OarFCB128, INRA063, MAF33, ve OarFCB304

Table 2.4 PCR reaction mix for Group II representing OarFCB128, INRA063, MAF33, ve OarFCB304.

	Concentrations	**Quantities**
dH_2O	N/A	5.1 µl
PCR Buffer	1 X	1.5 µl
$MgCl_2$	2 mM	1.2 µl
dNTP	200 µM	0.6 µl
Primers (F + R) 1) OarFCB128 2) INRA063 3) MAF33 4) OarFCB304	 3 pmoles 2 pmoles 2 pmoles 2 pmoles	 0.4 µl 0.4 µl 0.6 µl 2 µl
DNA	100 - 200 ng	3 µl
Taq Polymerase	1 unit	0.2 µl

TOTAL	**15 µl**

Table 2.5 PCR conditions for Group II representing OarFCB128, INRA063, MAF33, ve OarFCB304.

Temperature	Duration	
94 °C	3 minutes	
94 °C	20 seconds	
60 °C	35 seconds	
72 °C	45 seconds	3 cycles
94 °C	20 seconds	
57 °C	35 seconds	
72 °C	45 seconds	10 cycles
94 °C	20 seconds	
55 °C	35 seconds	
72 °C	45 seconds	10 cycles
94 °C	20 seconds	
53 °C	35 seconds	
72 °C	45 seconds	25 cycles
72 °C	20 minutes	

- For BM8125:

Table 2.6 PCR reaction mix for Group II representing BM8125.

	Concentrations	**Quantities**
dH_2O	N/A	8.55 µl
PCR Buffer	1 X	1.5 µl
$MgCl_2$	2 mM	1.2 µl
dNTP	200 µM	0.6 µl
Primers (F + R)	2.5 pmoles	1 µl
DNA	100 - 200 ng	2 u
Taq Polymerase	1 unit	0.15 µl

TOTAL	**15 µl**

Table 2.7 PCR conditions for Group II representing BM8125.

Steps	**Temperature**	**Duration**	
Denaturation	94 °C	3 minutes	
Denaturation	94 °C	20 seconds	35 cycles
Annealing	58.8 °C	25 seconds	
Extension	72°C	25 seconds	
Final Extension	72°C	20 minutes	

- For MAF214:

Table 2.8 PCR reaction mix for Group II representing MAF214.

	Concentrations	**Quantities**
dH_2O	N/A	9.25 µl
PCR Buffer	1 X	1.5 µl
$MgCl_2$	2 mM	1.2 µl
dNTP	200 µM	0.6 µl
Primers (F + R)	1.5 pmoles	0.3 µl
DNA	100 - 200 ng	2 µl
Taq Polymerase	1 unit	0.15 µl

TOTAL	**15 µl**

Table 2.9 PCR conditions for Group II representing MAF214.

Steps	**Temperature**	**Duration**	
Denaturation	94 °C	3 minutes	
Denaturation	94 °C	20 seconds	35 cycles
Annealing	62 °C	25 seconds	
Extension	72°C	35 seconds	
Final Extension	72°C	20 minutes	

The PCR reaction mix and PCR conditions for Group III: 6 loci are given in Table 2.10 – 2.13.

- For MAF65, ILSTS011, MAF209 ve MCM140

Table 2.10 PCR reaction mix for Group III representing MAF65, ILSTS011, MAF209 and MCM140.

	Concentrations	**Quantities**
dH_2O	N/A	6 µl
PCR Buffer	1 X	1.5 µl
$MgCl_2$	2 mM	1.2 µl
dNTP	200 µM	0.6 µl
Primers (F + R) 1) MAF65 2) ILSTS011 3) MAF209 4) MCM140	 3 pmoles 1.5 pmoles 3 pmoles 1.5 pmoles	 0.6 µl 0.8 µl 1.0 µl 0.6 µl
DNA	100 - 150 ng	2.5 µl
Taq Polymerase	0.5 units	0.2 µl

TOTAL	**15 µl**

Table 2.11 PCR conditions for Group III representing MAF65, ILSTS011, MAF209 and MCM140.

Steps	Temperature	Duration	
Denaturation	94 °C	3 minutes	
Denaturation	94 °C	20 seconds	35 cycles
Annealing	60 °C	35 seconds	
Extension	72 °C	45 seconds	
Final Extension	72 °C	20 minutes	

- For DYMS1 and OarCP34

Table 2.12 PCR reaction mix for Group III representing DYMS1 ve OarCP34.

	Concentrations	Quantities
dH_2O	N/A	6.7 µl
PCR Buffer	1 X	1.5 µl
$MgCl_2$	2 mM	1.2 µl
dNTP	200 µM	0.6 µl
Primers (F + R) 1) OarCP34 2) DYMS1	 2.5 pmoles 2.5 pmoles	 1 µl 1 µl
DNA	100 - 150 ng	2.5 µl
BSA	50 mg/ml	0.3 µl
Taq Polymerase	0.5 units	0.2 µl

TOTAL	15 µl

Table 2.13 PCR conditions for Group III representing DYMS1 ve OarCP34.

Temperature	Duration	
94 °C	3 minutes	
94 °C	20 seconds	15 cycles
60 °C	35 seconds	
72 °C	45 seconds	
94 °C	20 seconds	25 cycles
58 °C	35 seconds	
72 °C	45 seconds	
72 °C	20 minutes	

The PCR reaction mix and PCR conditions for Group IV: 3 loci are given in Tables 2.14 – 2.17.

- For OarFCB226 and OarHH47

Table 2.14 PCR reaction mix for Group IV representing OarFCB226 and OarHH47.

	Concentrations	**Quantities**
dH_2O	N/A	2.75 µl
PCR Buffer	1.5 X	2.75 µl
$MgCl_2$	4 mM	2.4 µl
dNTP	200 µM	0.6 u µl
Primers (F + R) 1) OarFCB226 2) OarHH47	 2 pmoles 3.75 pmoles	 0.8 µl 1.5 µl
DNA	100 - 200 ng	3 µl
BSA	0.01 µg/µl	1 µl
Taq Polymerase	1 unit	0.2 µl

TOTAL	**15 µl**

Table 2.15 PCR conditions for Group IV representing OarFCB226 and OarHH47.

Temperature	Duration	
95 °C	2 minutes	
94 °C	30 seconds	7 cycles
58 °C	60 seconds	
72 °C	60 seconds	
94 °C	30 seconds	10 cycles
52 °C	60 seconds	
72 °C	60 seconds	
94 °C	30 seconds	20 cycles
50 °C	60 seconds	
68 °C	60 seconds	
72 °C	10 minutes	

- For OarVH72:

Table 2.16 PCR reaction mix for Group IV representing OarVH72.

	Concentrations	**Quantities**
dH_2O	N/A	8.05 µl
PCR Buffer	1 X	1.5 µl
$MgCl_2$	2 mM	1.2 µl
dNTP	200 µM	0.6 µl
Primers (F + R)	3.75 pmoles	1.5 µl
DNA	100 - 200 ng	2 µl
Taq Polymerase	1 unit	0.15 µl

TOTAL	**15 µl**

Table 2.17 PCR conditions for Group II representing MAF214.

Temperature	**Duration**	
95 °C	2 minutes	
94 °C	30 seconds	10 cycles
60 °C	60 seconds	
72 °C	60 seconds	
94 °C	30 seconds	30 cycles
57 °C	60 seconds	
72 °C	60 seconds	
72 °C	10 minutes	

2.2.3.1 Checking the Presence of PCR Products

Visualization of the results of the PCR amplification was done by agarose gel electrophoresis. 2 % agarose gel was prepared by boiling agarose in 0.5 X TBE buffer. This solution was poured into an electrophoresis plate and left in room temperature for about 30 minutes for casting. Agarose plate was placed into the electrophoresis tank that was filled with 0.5 X TBE buffer. The load samples contained 5 μl PCR product with 1 μl, 6 X loading buffer. The gel was run at 90 V for 50 minutes. After that, EtBr staining was performed. The gel is visualized under UV light by a gel imaging system.

Microsatellite PCR products, co-loaded according to the groups mentioned, were analyzed on an automated ABI 310 DNA Analyzer using PE Tamra 350 internal size standard for sizing of the fragments. Genescan^{TM} v3.1 was used for data collection for producing electropherograms and analysis of the fragments.

2.2.4 Statistical Analyses

2.2.4.1 Genetic Variation Analysis

Allelic variation and heterozygosity analyses allow us to quantify the gathered information.

2.2.4.1.1 Allelic variation

The first step to statistical analysis of the data was calculation of allelic differences, which is an indication of genetic diversity. Thus, allele frequency is one of the measures of genetic variation that can be calculated as:

$$\hat{X}_i = \frac{1}{2_n}\left(2n_{ii} + \sum_{j \neq i} n_{ij}\right)$$

Where $\hat{X}_i$ is the gene frequency of the allele A_i, n represents the number of individuals in the sample, n_{ii} and n_{ij} represents the number of A_{ii} and A_{ij} genotypes, respectively (Nei, 1987).

Mean number of alleles per locus (n_a) or allelic richness is another component of genetic diversity, which is very sensitive to the sample size. It can be calculated as:

$$n_a = \frac{1}{r}\left(\sum_i n_{ai}\right)$$

Where n_{ai} is the number of alleles at the i^{th} locus and r is the total number of loci (Nei, 1987).

2.2.4.1.2 Heterozygosity estimations

Population heterozygosity, or gene diversity, is a useful measure of genetic variation in a population. The relative frequency of the heterozygote individuals in the sample in terms of the same locus gives the observed heterozygosity (H_o). Heterozygosity that helps to measure the genetic variation in a population was calculated with the formula:

H_0 = Number of heterozygotes / Total number of individuals

Nei (1987) formulated the unbiased estimate of the expected heterozygosity, or gene diversity, which eliminates the bias that may result from sample size. The expected heterozygosity (H_e) at a locus can be estimated by the formula:

$$\hat{X}_i = \frac{1}{2n-1} 2n \left(1 - \sum \hat{X}_i\right)$$

Where n is the number of individuals and $\hat{X}_i$ is the frequency of the allele A_i (Nei, 1987).

When multiple loci is the case, as in this study, the average of single locus heterozygosity values is considered to find observed (H_O) and expected (H_E) heterozygosities.

Calculation of the allele frequencies and the observed heterozygosities were performed with GENETIX 4.04 software (Belkhir *et al*., 1996).

2.2.4.2 *F*-statistics: Pairwise F_{st} Values and F_{is}

The genotype frequencies in each subpopulation do not necessarily follow Hardy-Weinberg equilibrium in natural populations. One of Wright's fixation indices, F_{ST}, measures the distance that computes the degree of genetic differentiation between pairs of populations of species (Nei, 1977). It can be calculated by the formula given below:

$$F_{ST} = 1 - \frac{H_S}{H_T}$$

where,

H_S = Mean expected heterozygosity within populations.

H_T = Expected heterozygosity when two populations are considered as one big population (Nei and Kumar, 2000).

F_{is} is defined as the correlation between homologous alleles within individuals in a given population. It measures the deviation from the Hardy-Weinberg equilibrium within the samples. It is estimated by the formula given below:

$$F_{IS} = 1 - \frac{H_O}{H_S}$$

where,

H_S = Mean expected heterozygosity within populations.

H_O = Mean observed heterozygosity within populations (Nei and Kumar, 2000).

The F indices proposed by Wright (1965) does not consider the unequal finite sample sizes and there is some disagreement on the interpretation of the quantities and on the method of evaluating them. Weir and Cockerham (1984) revised the F coefficients in order to unify various estimation formulas so that they are suited to small data sets. In this study, Weir and Cockerham's approach is used to examine the sample structure, permuted 1000 times over loci to test deviations from H-W equilibrium.

FST values by pairwise comparisons of seven breeds in this study, with their significance levels, and FIS values, within each population, were calculated by Arlequin package program (Excoffier *et al*., 2006) Significance of those were tested by applying 1000 random permutations.

2.2.4.3 Assignment Tests

Assignment tests were performed to test if the data on the twenty microsatellite loci provide enough genetic information to assign individuals to their original breeds. Genetic assignment methods are useful in addressing issues such as relationships, structure, and classification at the individual level. The test computes the probability of the multilocus genotype of each individual to be encountered in a given population. One of the resampling methods, Monte Carlo method, approximates the distribution of genotype likelihoods in a reference population sample and then compares the likelihood computed for the to-be-assigned individual to that distribution.

In this study, one of the Monte Carlo sampling methods, Rannala and Mountain's (1997) method is used to assign the individuals by simulating them 10000 times per samples. The probability criteria to reject the assignment of the individual was $P<0.05$. The software used for the assignment test was GeneClass2 (Piry *et al*., 2004).

2.2.4.4 Factorial Correspondence Analysis (FCA)

The Factorial Correspondence Analysis (FCA) (Lebart *et al*., 1984) is an analysis performed in order to visualize the individuals in multidimensional space and to explore the relationships between the individuals. The logic behind it suggests a linear transformation of the number of alleles at each locus for each individual. The allele copies could be 0, 1 or 2 at a particular locus. The first three axes are the most informative ones (Machugh *et al*., 1994). It is a good way to visualize how the individuals are related to each other on the independent axes chosen. Factorial Correspondence Analysis was performed to test the possible admixtures that occurred between the populations using GENETIX 4.04 software (Belkhir *et al*., 1996).

2.2.4.5 Structure

The software Structure implements a method to demonstrate the presence of population structure and identify distinct genetic populations (Pritchard *et al*., 2000). The underlying assumptions reside on a model in which there are K populations (where K may be unknown), each of which is characterized by a set of allele frequencies at each locus. Individuals in the sample are assigned jointly to two or more populations if their genotypes indicate that they are admixed. With the version 2.2 of Structure, microsatellites, Amplified Fragment Length Polymorphisms (AFLP), Restriction Fragment Length Polymorphisms (RFLP) and Single Nucleotide Polymorphisms (SNPs) are the data sets that could be used with Structure 2.2 (Falush *et al*., 2007).

The burn in length, the ancestry model and estimation of *K* (number of populations) are the most important factors to determine for *Structure* analysis.

Burn in length deals with how long to run the simulation before collecting data to minimize the starting configuration. Typically a burn in of 10,000 - 100,000 is more than adequate (Falush *et al*., 2007).

There are four main models for the ancestry of individuals. In this study "Admixture model" is used. This model assumes that individuals may have mixed ancestry. It is a reasonably flexible model for dealing with many of the complexities of real populations (Falush *et al*., 2003).

For the estimation of K (number of populations), there are 2 different methods proposed. The model choice criterion implemented in Structure to detect the true K is an estimate of the posterior probability of the data for a given K, Pr(X|K) (Pritchard *et al*., 2000). This value, called 'Ln P(D)' (Ln probability of data) in Structure output. For every K value, there exists an Ln P(D) value. Then, the

posterior probability of K is calculated by a formula. Here, a case with 5 populations is given. By the formula below, the probability for the K = 4 is calculated with Ln P(D) values.

$$\frac{e^{K=4}}{e^{K=1} + e^{K=2} + e^{K=3} + e^{K=4} + e^{K=5}}$$

The other probabilities for the K values for Ln P(D)s are also calculated and true number of populations is identified using the minimal value or the smallest value of K that captures the major structure in the data.

But, when there is real population structure, Pritchard *et al*.'s (2000) model could cause unrealistic K values. So, Evanno *et al*. (2005) proposed another method for calculation of K. The distribution of Ln P(D) (or L(K) as Evanno *et al*. (2005) call it) did not show a clear mode for the true K, but the second order rate of change of the likelihood function with respect to K (ΔK) did show a clear peak at the true value of K. A case with formulas and logic, taken from Evanno *et al*. (2005) can be seen in Figure 2.9.

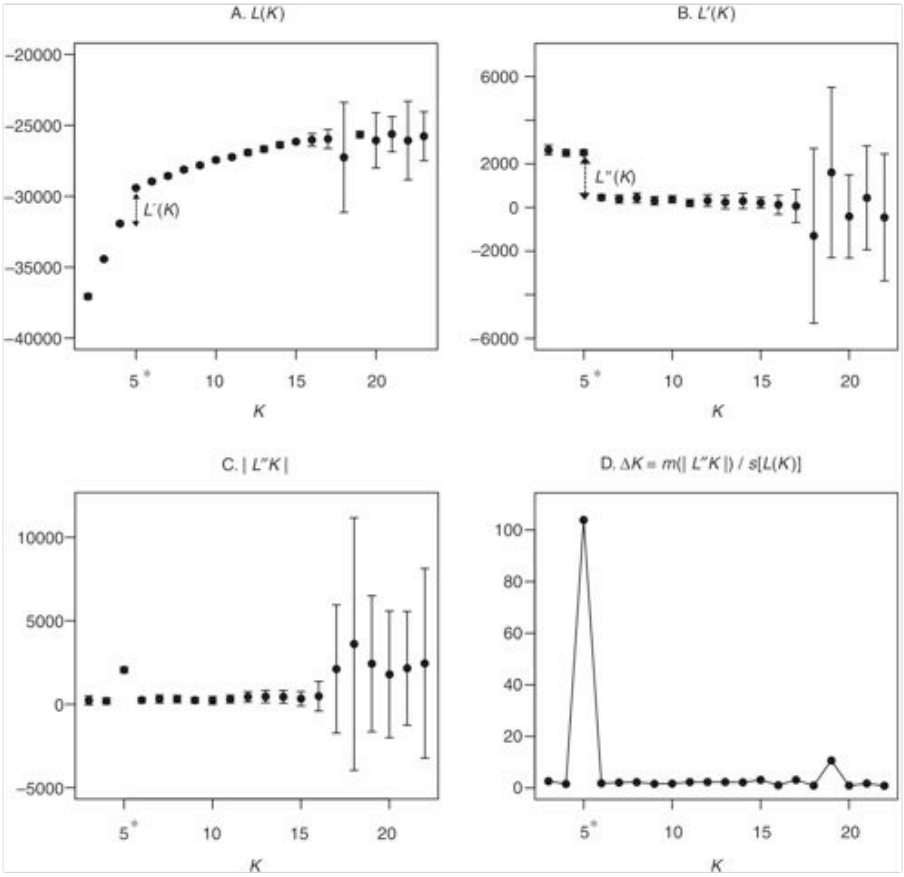


Figure 2.9 Description of the four steps for the graphical method allowing detection of the true number of groups *K*. (A) Mean L(K) (± SD) over 20 runs for each K value. (B) Rate of change of the likelihood distribution (mean ± SD) calculated as L′(K) = L(K) – L(K – 1). (C) Absolute values of the second order rate of change of the likelihood distribution (mean ± SD) calculated according to the formula: |L″(K)| = |L′(K + 1) – L′(K)|. (D) ΔK calculated as ΔK = m|L″(K)|/ s[L(K)]. The modal value of this distribution is the true K or the uppermost level of structure.

In this study, both approaches were employed.

The *Structure* 2.2 program can be obtained freely on http://pritch.bsd.uchicago.edu/software.

2.2.4.6 Genetic Distance Estimations and Phylogenetic Tree Construction

The distance matrix approaches used in this study are:

2.2.4.6.1 Nei's D_A Genetic Distance

The DA genetic distance is considered as the most appropriate method to obtain correct tree topology from microsatellite data (Takezaki and Nei, 1996). It is based on infinite allele model and calculated as:

$$D_A = 1 - \frac{1}{r}\sum_{j}^{r}\sum_{i}^{m_j}\sqrt{x_{ij}y_{ij}}$$

where,

x_{ij} = Frequencies of the i^{th} allele at the j^{th} locus in samples X.

y_{ij} = Frequencies of the i^{th} allele at the j^{th} locus in samples Y.

m_j =Number of alleles at the j^{th} locus.

r =Number of loci examined.

Nei's Genetic Distance (D_A) also change between 0 and 1 whereas value "0" is for identical populations and "± 1" is for populations that share no alleles.

Pairwise genetic distances of seven breeds in this study were calculated by GENDIST program in PHYLIP: PHYlogeny Inference Package software (Felsenstein, 1993).

2.2.4.6.2 Allele Sharing Distance

Allele Sharing Distance method uses the logic that alleles common in the samples of the same species are likely to have existed before the split of these samples. So, the proportion of shared alleles increases with increasing genetic similarity of the samples.

Shared allele distance between individuals (DSA_i) is calculated as:

$$DSA_i = 1 - \frac{\sum S}{2r}$$

where the number of shared alleles (S) is summed over all loci, r (Chakraborty *et al*., 1992; Bowcock *et al*., 1994).

2.2.4.6.3 Neighbor Joining (NJ) Tree

Neighbor Joining (NJ) tree construction method is a distance-based approach. It aims to visualize the genetic relationships of the populations and aims to minimize the total length of tree by sequentially finding the neighbors. NJ method performs better under non-uniform rates either among lineages or among sites because NJ tree does not assume equal rate of evolution of the breeds after the divergence.

In this study, NJ trees were constructed to observe the relationship of the seven populations. Neighbor-joining trees were produced by using the NEIGHBOR

program, 1000 bootstrap replicates were generated by the SEQBOOT program and a consensus tree was built with the CONSENSE program as implemented in PHYLIP 3.6 (Felsenstein, 1993).

2.2.4.7 Neighbor-Net Analysis

Phylogenetic networks are important for the reconstruction of evolutionary history. Implicit models such as split networks are very useful for exploring and visualizing the different signals in a data set. Neighbor-Net is a distance-based method for constructing phylogenetic networks based on the Neighbor-Joining (NJ) algorithm of Saitou and Nei (1987). Unlike NJ, Neighbor-Net can represent conflicting signals in the data, helping you to focus on those regions, with more detailed methods. Neighbor-Net is fast, consistent and efficient. However, the interpretation of the graphs is harder than the accustomed methods (Bryant and Moulton, 2004).

Neighbor-Net is available as part of the SplitsTree4 software package (Huson and Bryant, 2006).

2.2.4.8 Principal Component Analysis (PCA)

Principle components are a set of variables that define a projection that encloses the maximum amount of variation in a dataset. Principal Component (PC) analysis is used to determine the variance and relationships of the populations in terms of principle components. It is population-based; individuals cannot be represented. Relationship of the populations is explained by the visualization of the relative positions of populations on a space. The highest variation of the data is explained by the first compound axis, the next highest variation by the second compound axis, and so on. Weightings of the axes show the amount of contribution of the variable to the variation (Dytham, 2003).

2.2.4.9 Analysis of Molecular Variance (AMOVA)

When studying molecular variation, haplotypic data should be used so that there is no variation within individuals. Analysis of Variance (ANOVA) compares average gene frequencies among samples. Excoffier *et al.* (1992) modified ANOVA analysis to analyze of molecular variance to get the scent of differentiation between groups. That led AMOVA (Analysis of MOlecular VAriance) to become a player in the analysis scene for estimating the partitioning of total genetic variation among groups of populations, the populations within groups and/or the individuals within a population.

The design and formulas of the calculation for AMOVA for genotypic data, several groups of populations, within-individual level as can be seen in the Excoffier *et al*.'s (2006) Arlequin package program is in the table below:

Source of Variation:	Degrees of freedom	Sum of squares (SSD)	Expected mean squares
Among Groups	G - 1	SSD(*AG*)	$n''\sigma_a^2 + n'\sigma_b^2 + 2\sigma_c^2 + \sigma_d^2$
Among Populations / Within Groups	$P - G$	SSD(*AP/WG*)	$n\sigma_b^2 + 2\sigma_c^2 + \sigma_d^2$
Among Individuals / Within Populations	$N - P$	SSD(*AI/WP*)	$2\sigma_c^2 + \sigma_d^2$
Within Individuals	N	SSD(*WI*)	σ_d^2
Total:	$2N - 1$	SSD(*T*)	σ_T^2

Figure 2.10 General AMOVA table for genotypic data, several groups of populations, within-individual level taken from Arlequin package program (Excoffier *et al*., 2006).

where;

SSD (AG): Sum of squared deviations among groups of populations
SSD (AP/WG): Sum of squared deviations among populations, within groups
SSD (AI/WP): Sum of squared deviations among individuals, within populations
SSD (WI): Sum of squared deviations within individuals
SSD (T): Total sum of squared deviations
G: Number of groups in the structure
P: Total number of breeds

N: Total number of gene copies

In this study, Arlequin (http://lgb.unige.ch/arlequin) package program was used for AMOVA analysis (Excoffier *et al*., 2006).

2.2.5 Restriction Fragment Length Polymorphism (RFLP) Analysis of Sheep Mitochondrial DNA (mtDNA) Control Region (CR)

A restriction enzyme cuts double-stranded or single stranded DNA at specific recognition nucleotide sequences known as restriction sites (Roberts, 1976). In RFLP, a restriction enzyme is used for digesting the sequences to obtain different lengths of fragments according to the haplogroups (Lewis, 1999).

For the amplification of the CR region, the primers used are: forward primer 5'-CAT CGA AAA CAA CCT CCT CAA -3' and the reverse one 5'- GAT TCG AAG GGC GTT ACT CA -3'. The PCR mix and conditions are as follows:

Table 2.18 PCR reaction mix.

	Concentrations	Quantities
dH_2O	N/A	15.5 µl
PCR Buffer	1 X	2.5 µl
$MgCl_2$	2 mM	2 µl
dNTP	200 µM	0.5 µl
Primers (F + R)	5 pmoles	0.3 µl
BSA	1 mg / ml	1 µl
DNA	100 - 200 ng	3 µl
Taq Polymerase	1 unit	0.2 µl

TOTAL	**25 µl**

Table 2.19 PCR conditions for sequencing.

Steps	Temperature	Duration	
Denaturation	94 °C	3 minutes	
Denaturation	94 °C	30 seconds	35 cycles
Annealing	51 °C	45 seconds	
Extension	72°C	90 seconds	
Final Extension	72°C	15 minutes	

Afterwards, the samples were digested with *NsiI* enzyme (Bruford and Townsend, 2006; Koban *et al*., 2008) and incubated at 37 °C overnight. The enzyme digestion mixture for CR region comprises of 1X Enzyme Buffer, 20 µl PCR product, 2 units of restriction enzyme (*NsiI*).

After digestion, 4 µl 6X loading dye was put into each sample and samples were subjected to agarose gel electrophoresis in 3 % gels at 120 V for nearly 2 hours. Later, gel was stained by ethidium bromide solution (0.5µl / ml) and visualized and photographed under UV light by a gel imaging system.

2.2.6 Sequencing of Sheep Mitochondrial DNA (mtDNA) Control Region (CR)

Sanger's (1974) chain termination method was employed for sequencing the mtDNA CR of the samples. In sequencing PCR, SOAD (Wood and Phua, 1996) and HC2 (Townsend, 2000) internal primers in addition to the two PCR primers have been used. The original primers of Hiendleder (1998) did not work properly in our samples so after a series of optimization studies, new external primers are designed. The forward primer is 5'- CAT CGA AAA CAA CCT CCT CAA -3' and the reverse one is 5'- GAT TCG AAG GGC GTT ACT CA -3'. The PCR mix and conditions can be found in Table 2.18 and 2.19.

After cleaning the PCR products with the GeneClean Turbo for PCR kit (Q-BioGene), the samples were sequenced by a company called RefGen (http://refgen.com.tr/).

The raw sequence data obtained by RefGen were first analyzed using ChromasPro software (Technelysium Pty Ltd, available at http://www.technelysium.com.au/ChromasPro.html) The low quality sequences were trimmed from both ends to ease further alignment. Then, all the sequences in the contigs were aligned and compared with each other in terms of the base sequence. Following, a consensus sequence of the four was formed. Sheep mtDNA CR has a variable tandem repeat region of a ~75 bp repetitive unit occurring in 2-6 copies. After aligning the consensus sequences the repeat units within the CR were removed. Later, they are saved in FASTA format for further analysis. The sequences are visualized in the form of NJ tree with the help of the

software MEGA: Molecular Evolutionary Genetic Analysis v. 4.0 (Tamura *et al*., 2007).

CHAPTER 3

RESULTS

3.1 Results of the Laboratory Experiments

3.1.1 DNA Extraction and Results of Polymerase Chain Reaction (PCR)

Extracted DNA (by phenol:chloroform DNA extraction protocol as explained in Chapter 2), was checked in order to decide the suitability of the samples for further use in terms of quality and quantity. An example of the isolated DNA can be seen in Figure 3.1. According to the concentration of the samples that was decided comparing them against the standard size markers (here λ DNA HindIII digest), necessary dilutions were performed.

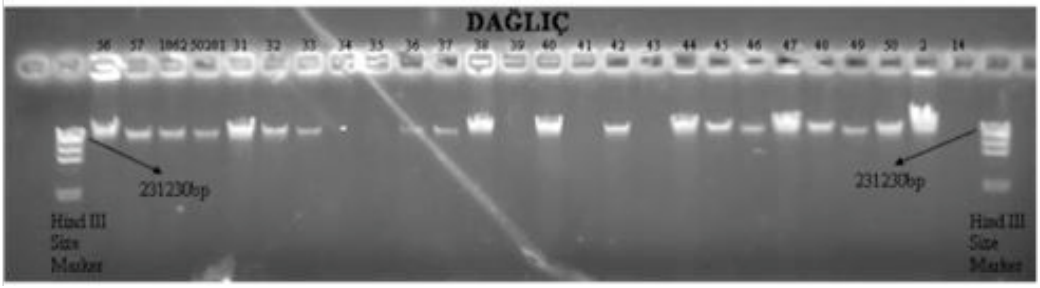


Figure 3.1 Agarose gel image of total DNA extracts after isolation. Numbers shown above the wells are the DNA sample numbers and name on the top of the image is the name of the breed (Dağlıç).

Amplifications of the 20 microsatellite loci were performed by Polymerase Chain Reaction (PCR) in total of 6 reactions and the results were run on ABI 310 DNA Analyzer in 4 groups. Figures 3.2, 3.3, 3.4 and 3.5 are the examples of the results from 3 Karayaka samples, namely 10, 17 and 18 for the 20 microsatellite loci grouped into four. Negative control is employed in order to check the presence of contamination.

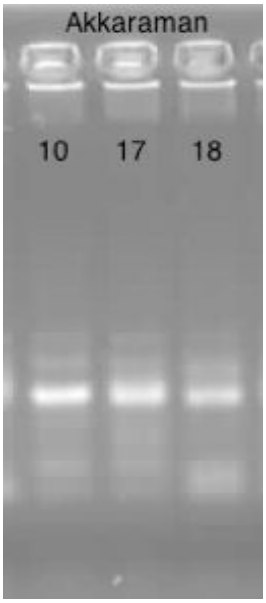


Figure 3.2 Agarose gel image of Group I representing 5 loci: JMP58 (137-177), ILSTS005 (174-218), FCB20 (86-130), FCB48 (136-172) and JMP29 (113-167). The allelic ranges of those loci were shown in brackets. Numbers shown under the wells are the individual numbers and name on the top of the image is the name of the breed (Akkaraman). The agarose gel is used only for checking the presence of PCR products.

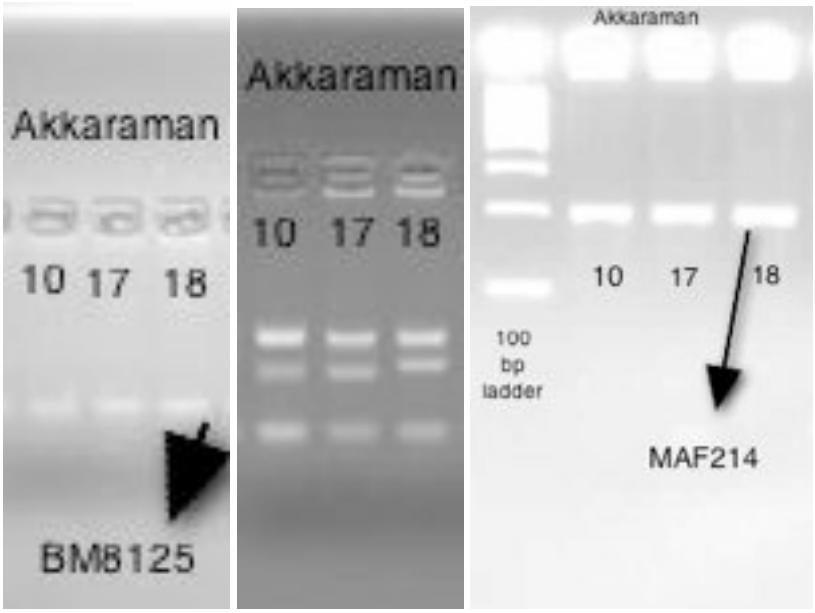


Figure 3.3 Agarose gel image of Group II representing 6 loci: BM8125 (106-128), FCB304 (145-191), FCB128 (96-130), INRA063 (156-212), MAF33 (116-147) and MAF214 (134-264). The allelic ranges of those loci were shown in brackets. Numbers shown under the wells are the individual numbers and name on the top of the image is the name of the breed (Akkaraman). The agarose gel image on the left belongs to microsatellite locus BM8125, the middle one represents four loci, namely FCB304, FCB128, INRA063 and MAF33, the right one belongs to locus MAF214. The bands representing the microsatellite loci were indicated where available. The agarose gel is used only for checking the presence of PCR products.

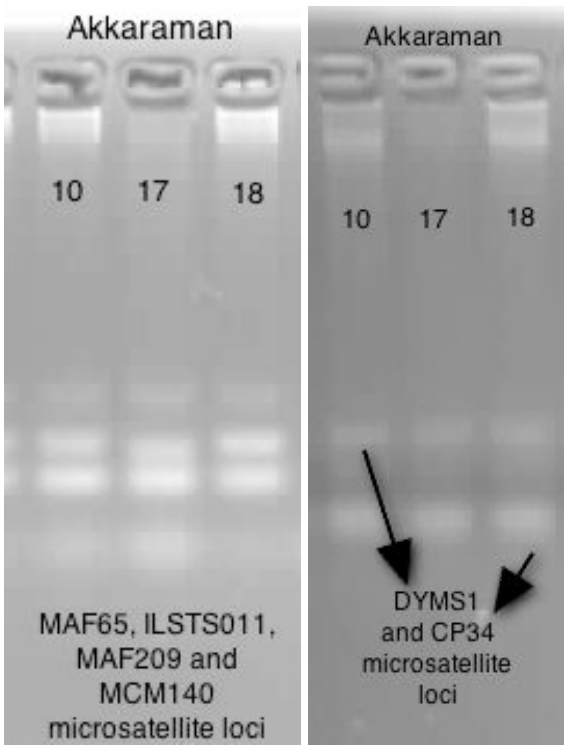


Figure 3.4 Agarose gel image of Group III representing 6 loci: MAF209 (109-142), CP34 (110-136), DYMS1 (157-211), MAF65 (112-146), MCM140 (161-198), ILSTS011 (256-294). The allelic ranges of those loci were shown in brackets. Numbers shown under the wells are the individual numbers and name on the top of the image is the name of the breed (Akkaraman). The agarose gel image on the left belongs to microsatellite loci MAF65, ILSTS011, MAF209 and MCM140, the right one belongs to loci DYMS1 and CP34. The bands representing the microsatellite loci were indicated where available. The agarose gel is used only for checking the presence of PCR products.

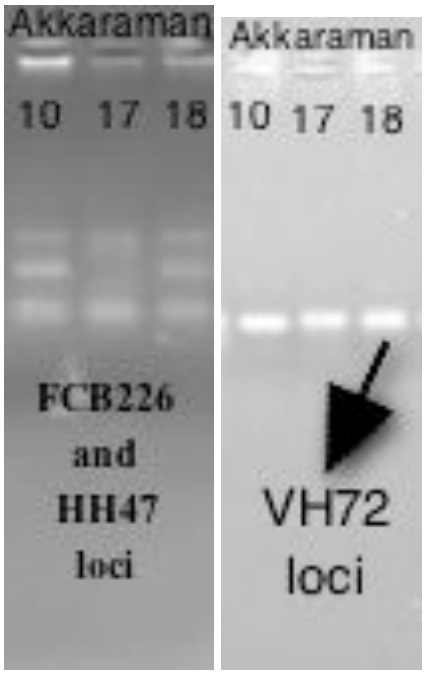


Figure 3.5 Agarose gel image of Group IV representing 3 loci: FCB226 (118-160), HH47 (121-163), VH72 (121-145). The allelic ranges of those loci were shown in brackets. Numbers shown under the wells are the individual numbers and name on the top of the image is the name of the breed (Akkaraman). The agarose gel image on the left belongs to microsatellite loci FCB226 and HH47, the right one belongs to locus VH72. The bands representing the microsatellite loci were indicated where available. The agarose gel is used only for checking the presence of PCR products.

3.1.2 Microsatellite Analysis

Microsatellite PCR products were analyzed on a automated ABI 310 DNA Analyzer using PE Tamra 350 internal size standard for sizing of the fragments. Genescan™ v3.1 was used for data collection and for producing

electropherograms and analysis of the fragments. The electropherogram results from one of the samples mentioned above (Karayaka 10) in terms of 20 microsatellite loci analyzed in four groups can be found in Figures 3.6, 3.7, 3.8 and 3.9.

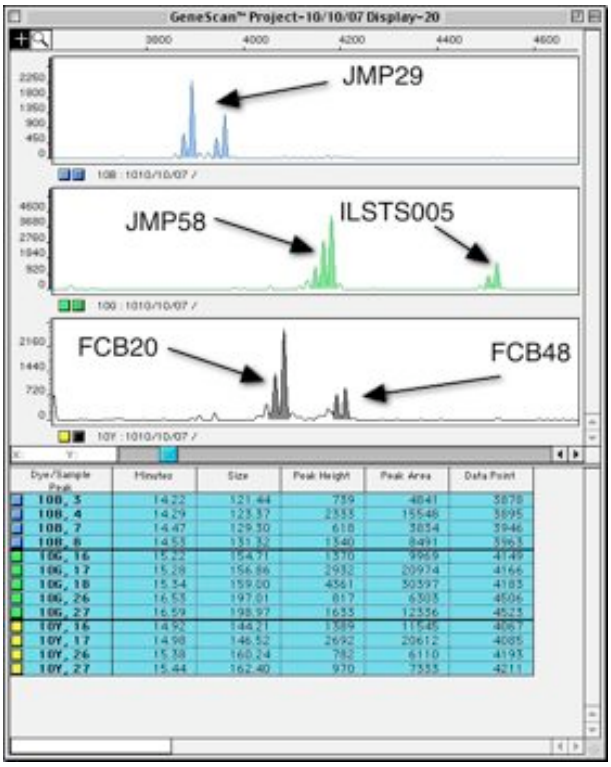


Figure 3.6 Microsatellite electropherogram of Group I representing 5 loci: JMP58, ILSTS005, FCB20, FCB48 and JMP29, obtained via Genescan™ v3.1. The loci names are indicated by arrows.

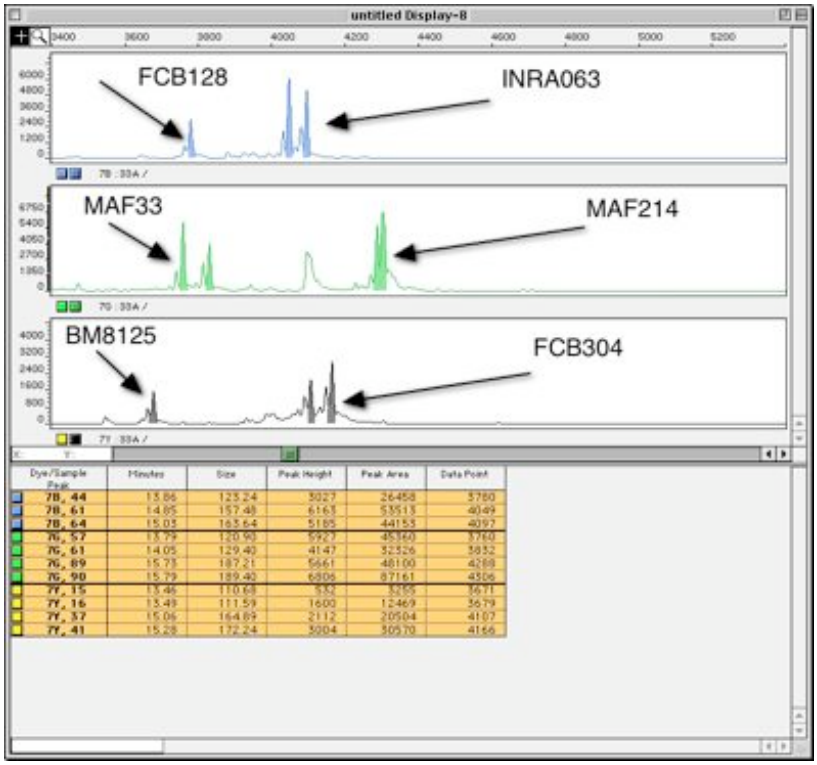


Figure 3.7 Microsatellite electropherogram of Group II representing 6 loci: BM8125, FCB304, FCB128, INRA063, MAF33 and MAF214, obtained via Genescan^{TM} v3.1. The loci names are indicated by arrows.

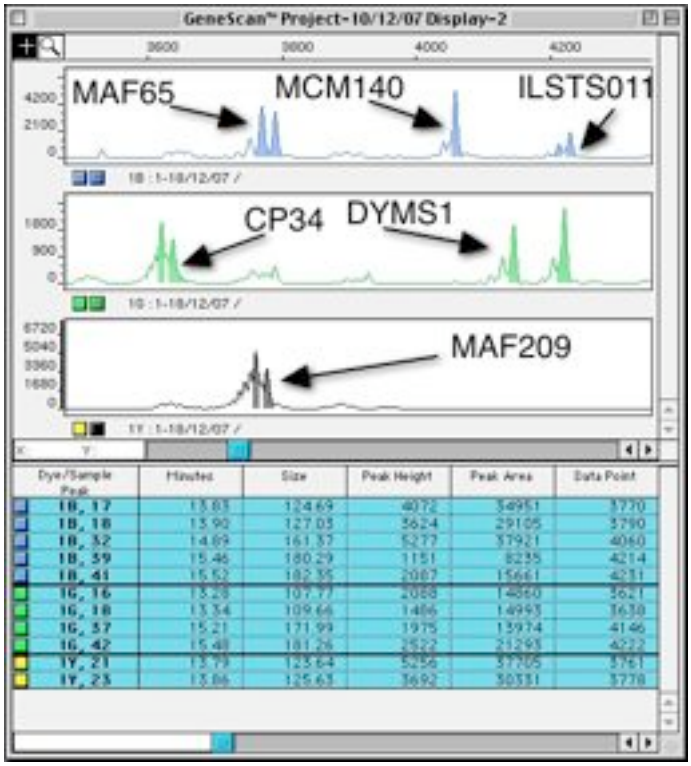


Figure 3.8 Microsatellite electropherogram of Group III representing 6 loci: MAF209, CP34, DYMS1, MAF65, MCM140, ILSTS011, obtained via Genescan^{TM} v3.1. The loci names are indicated by arrows.

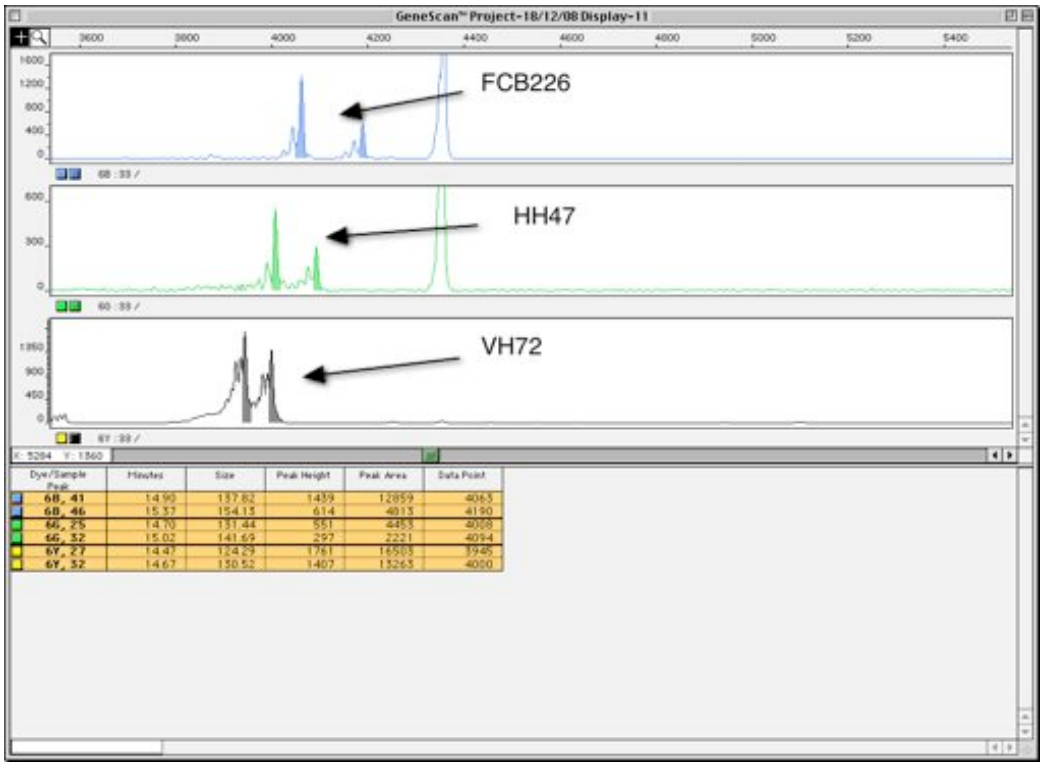


Figure 3.9 Microsatellite electropherogram of Group IV representing 3 loci: FCB226, HH47, VH72, obtained via Genescan^{TM} v3.1. The loci names are indicated by arrows.

3.2 Results of Statistical Analyses

The results of the allelic readings from 20 microsatellite loci analyzed in the present study can be found in Appendix C.

3.2.1 Breed Based Analyses

3.2.1.1 Genetic Variation Analysis

3.2.1.1.1 Allelic Variation

The total number of alleles observed for the loci used in the study is given in Table 3.1 below. The highest number of alleles observed for single locus is 23 for OarFCB304 and OarFCB48. The lowest one is 8 for MAF214 and BM8125. In the present study, the two of the loci analyzed, namely OarFCB128 and DYMS1, did not amplify properly for İvesi. These loci / breed are excluded for some statistical analysis (MNA / Pop and MNA / Locus).

Table 3.1 Total number of observed alleles in the seven breeds studied for the loci employed in the study.

Locus Name	Observed Number of Alleles
OarFCB226	18
OarHH47	16
OarVH72	11
OarFCB128	17
INRA063	21
MAF33	13
MAF214	8
BM8125	8
OarFCB304	23
OarJMP29	21
OarJMP58	20
ILSTSOO5	14
OarFCB20	22
OarFCB48	23
MAF65	16
MCM140	14
ILSTS011	10
OarCP34	10
DYMS1	18
MAF209	12

The number of alleles observed for each locus in each breed and the average numbers were given in Table 3.2.

Table 3.2 Total number of alleles observed for each locus in each breed, the mean number of alleles observed for each breed / sample (MNA/pop) and for each locus (MNA/locus)**.** The abbreviations are AKK (Akkaraman), DAĞ (Dağlıç), GÖK (Gökçeada), İVE (İvesi), KRY (Karayaka), KIV (Kıvırcık) and MOR (Morkaraman).

Locus Name	AKK	DAĞ	GÖK	İVE	KRY	KIV	MOR	**MNA / Locus**
OarFCB226	12	17	11	12	13	13	13	13.00
OarHH47	12	11	12	12	10	11	12	11.43
OarVH72	8	10	5	9	6	9	10	8.14
OarFCB128	10	12	10	0	10	8	11	10.17
INRA063	15	12	12	10	11	12	11	11.86
MAF33	10	10	6	9	12	8	8	9.00
MAF214	7	3	3	3	3	6	3	4.00
BM8125	7	6	7	6	6	7	5	6.29
OarFCB304	12	15	8	9	13	13	17	12.43
OarJMP29	12	9	9	14	13	11	12	11.43
OarJMP58	13	15	12	16	13	12	16	13.86
ILSTSOO5	7	10	4	9	8	8	10	8.00
OarFCB20	10	13	10	13	11	14	18	12.71
OarFCB48	12	14	10	17	10	13	17	13.29
MAF65	8	8	6	12	10	11	12	9.57
MCM140	11	12	10	10	11	11	14	11.29
ILSTS011	6	7	5	8	6	9	8	7.00
OarCP34	7	6	6	7	7	7	7	6.71
DYMS1	12	13	8	0	14	10	12	11.50
MAF209	9	10	9	9	8	8	10	9.00
MNA / Pop.	10.00	10.65	8.15	9.25	9.75	10.05	11.30	

The highest and lowest mean numbers of observed alleles per locus were 13.86 (OarJMP58) and 4.00 (MAF214), respectively. The highest and the lowest observed mean numbers of alleles per breeds were 11.30 (Morkaraman) and 8.15 (Gökçeada, an island population). Moreover, the mean number of alleles observed in the study per locus per sample (MNA / locus / pop) is 10.03.

Examining the frequencies of the observed alleles, it was seen that some of the alleles were present only in one of the breeds. These can be called as breed specific or private alleles. The names of the loci and the breeds that have private alleles, as well as the frequencies of those can be found in Table 3.3. The highest frequency is shown in bold.

Table 3.3 The distribution of private alleles and their frequencies.

Locus	Allele	Frequency	Breeds
OarFCB226	126	0.013	MORKARAMAN
OarHH47	123	0.019	GÖKÇEADA
	149	0.019	GÖKÇEADA
OarVH72	117	0.013	MORKARAMAN
INRA063	132	0.019	DAĞLIÇ
	134	0.019	DAĞLIÇ
	154	0.044	KARAYAKA
	182	0.015	KARAYAKA
	184	0.019	GÖKÇEADA
	192	0.019	AKKARAMAN
MAF33	116	0.015	DAĞLIÇ
	138	0.030	KARAYAKA
MAF214	164	0.016	KIVIRCIK
	194	0.054	AKKARAMAN
	200	0.014	AKKARAMAN
BM8125	120	0.013	İVESİ
OarFCB304	145	0.016	KIVIRCIK
	155	0.010	MORKARAMAN
	159	0.083	İVESİ

Table 3.3 (Cont'd)

OarJMP29	118	0.012	DAĞLIÇ
	146	0.041	İVESİ
	150	0.026	AKKARAMAN
	152	0.037	KARAYAKA
OarJMP58	133	0.014	İVESİ
	143	0.036	GÖKÇEADA
	169	0.014	KIVIRCIK
ILSTS005	180	0.022	AKKARAMAN
	206	0.014	İVESİ
	212	0.030	MORKARAMAN
OarFCB20	80	0.020	MORKARAMAN
	82	0.010	MORKARAMAN
	84	0.020	MORKARAMAN
	110	0.054	İVESİ
	118	0.014	KIVIRCIK
	126	**0.292**	AKKARAMAN
OarFCB48	125	0.013	AKKARAMAN
	127	0.030	İVESİ
	131	0.030	İVESİ
	133	0.010	MORKARAMAN
MAF65	107	0.029	İVESİ
	111	0.015	KIVIRCIK
	113	0.014	İVESİ
	115	0.030	KIVIRCIK
ILTSS011	182	0.014	İVESİ
	272	0.032	KIVIRCIK
OarCP34	106	0.014	İVESİ
	122	0.010	MORKARAMAN
MAF209	108	0.012	DAĞLIÇ

In total, 48 such alleles were observed. The highest number of private alleles, 6, was found in INRA063 and OarFCB20, both have high number of alleles and high mean number of alleles observed per locus – but not the highest. There are no private alleles found in MCM140 locus. These private alleles are mostly found at either end of the allelic range with a low frequency (between 0.01 and 0.08)

The ‘126’ allele of OarFCB20, also worth mentioning. It has a frequency of 0.292 in Akkaraman breed.

If the private alleles are analyzed breed-wise, it can be seen that Akkaraman has 7, Dağlıç has 5, Gökçeada has 4, İvesi has 12, Karayaka has 4, Kıvırcık has 7 and Morkaraman has 8 of those. Yet, except for “126” allele of OarFCB20 locus, all these alleles have very low frequencies.

3.2.1.1.2 Heterozygosity Analysis

The observed and expected heterozygosity values of each breed for each locus and the mean observed heterozygosity both per locus and per breed were analyzed with GENETIX software (Belkhir *et al*., 1996). Those values can be found in Table 3.4.

Table 3.4 The observed and expected heterozygosity values for each breed and locus; average observed and expected heterozygosity per breed and locus.

	Heterozygosity Values							
Locus Name	Mean / locus		Akkaraman		Dağlıç		Gökçeada	
	($H_{observed}$)	**($H_{expected}$)**	**($H_{observed}$)**	**($H_{expected}$)**	**($H_{observed}$)**	**($H_{expected}$)**	**($H_{observed}$)**	**($H_{expected}$)**
FCB226	0.6764	0.7533	0.6667	0.6785	0.4500	0.8994	0.8148	0.7250
OarHH47	0.7124	0.8267	0.7297	0.8689	0.7429	0.8388	0.8519	0.8416
OarVH72	0.7172	0.7701	0.6923	0.7304	0.7561	0.8287	0.5556	0.5857
OarFCB128	0.6015	0.8190	0.5938	0.7910	0.7027	0.8495	0.5000	0.7899
INRA063	0.6934	0.8336	0.7037	0.8669	0.7419	0.8710	0.7308	0.8151
MAF33	0.6285	0.8019	0.4800	0.7928	0.7059	0.8447	0.6923	0.7226
MAF214	0.2152	0.4890	0.3243	0.6870	0.2368	0.4900	0.1154	0.2692
BM8125	0.4178	0.5636	0.4615	0.6752	0.4000	0.5644	0.6552	0.6468
OarFCB304	0.6028	0.7274	0.7429	0.8029	0.6098	0.8275	0.6667	0.6008
OarJMP29	0.8100	0.8250	0.7436	0.8271	0.9286	0.8041	0.9310	0.8044
OarJMP58	0.8463	0.8300	0.7949	0.8228	0.9048	0.8526	0.9310	0.7776
ILSTS005	0.5828	0.6712	0.4783	0.6474	0.6190	0.6913	0.5862	0.6427
OarFCB20	0.7632	0.8772	0.5833	0.8507	0.9286	0.8727	0.7407	0.8573
OarFCB48	0.8082	0.7837	0.7632	0.7607	0.9286	0.8291	0.7586	0.7973
MAF65	0.7506	0.7592	0.7368	0.7663	0.8049	0.7454	0.6000	0.6080
MCM140	0.6865	0.8273	0.7105	0.8265	0.7561	0.8290	0.6400	0.7952
ILSTS011	0.6065	0.7626	0.5000	0.7164	0.7250	0.7234	0.6400	0.6496
OarCP34	0.6934	0.7544	0.7027	0.7776	0.6190	0.7446	0.6400	0.7336
DYMS1	0.7849	0.8008	0.8421	0.8331	0.8571	0.7436	0.7200	0.8000
MAF209	0.6474	0.7728	0.6842	0.8026	0.6341	0.7626	0.8400	0.8104
Mean / pop	**0.6622**	**0.7624**	**0.6467**	**0.7762**	**0.7026**	**0.7806**	**0.6805**	**0.7136**

Table 3.4 (Cont'd)

	Heterozygosity Values							
Locus Name	İvesi		Karayaka		Kıvırcık		Morkaraman	
	($H_{observed}$)	**($H_{expected}$)**	**($H_{observed}$)**	**($H_{expected}$)**	**($H_{observed}$)**	**($H_{expected}$)**	**($H_{observed}$)**	**($H_{expected}$)**
FCB226	0.6316	0.7645	0.7619	0.7911	0.7429	0.7110	0.6667	0.7038
OarHH47	0.6857	0.8498	0.8333	0.7920	0.4063	0.8018	0.7368	0.7940
OarVH72	0.7632	0.8099	0.7955	0.7960	0.7143	0.8180	0.7436	0.8222
OarFCB128	0.0000	0.0000	0.7037	0.8333	0.6087	0.8053	0.5000	0.8452
INRA063	0.5938	0.7866	0.7647	0.8651	0.6522	0.8270	0.6667	0.8033
MAF33	0.6667	0.7975	0.6061	0.8186	0.6000	0.8275	0.6486	0.8097
MAF214	0.1176	0.3326	0.2500	0.5228	0.2581	0.6233	0.2045	0.4982
BM8125	0.4737	0.4948	0.4419	0.6217	0.1250	0.5195	0.3673	0.4225
OarFCB304	0.2667	0.5556	0.7353	0.7829	0.7188	0.8076	0.4792	0.7146
OarJMP29	0.6757	0.8309	0.7805	0.8629	0.7941	0.7915	0.8163	0.8540
OarJMP58	0.6944	0.8191	0.7073	0.7965	0.9714	0.8657	0.9200	0.8756
ILSTS005	0.5946	0.7476	0.5641	0.5799	0.6571	0.7359	0.5800	0.6534
OarFCB20	0.8649	0.8919	0.7105	0.8726	0.7143	0.8910	0.8000	0.9040
OarFCB48	0.6970	0.7778	0.6585	0.6440	0.9118	0.8400	0.9400	0.8370
MAF65	0.7143	0.8155	0.8000	0.8019	0.8485	0.8209	0.7500	0.7565
MCM140	0.6053	0.8483	0.6750	0.8475	0.6061	0.7819	0.8125	0.8626
ILSTS011	0.5946	0.8269	0.5789	0.7607	0.4839	0.8585	0.7234	0.8029
OarCP34	0.5833	0.7072	0.6667	0.7442	0.7879	0.7929	0.8542	0.7804
DYMS1	0.0000	0.0000	0.8750	0.8278	0.7407	0.7908	0.6744	0.8096
MAF209	0.3235	0.7807	0.6750	0.7706	0.6250	0.7466	0.7500	0.7361
Mean / pop	**0.5859**	**0.7465**	**0.6792**	**0.7666**	**0.6484**	**0.7828**	**0.6817**	**0.7643**

Among the breeds, the mean number of observed heterozygosity per population is between 0.5859 (İvesi) and 0.7026 (Dağlıç); the mean number of expected heterozygosity per population is between 0.7136 (Gökçeada) and 0.7828 (Kıvırcık).

If the average heterozygosity per locus is considered, it is seen that MAF214 has the lowest values for both the observed and expected heterozygosity (0.2152 and 0.4890, respectively). MAF214 also had lowest mean number of observed alleles per locus. The highest average observed heterozygosity per locus belongs to OarFCB48 with the value, 0.8082. The highest value for average expected heterozygosity was 0.8772 for OarFCB20.

The mean expected heterozygosity values are all greater than the mean observed heterozygosity values. The case was the same with mean heterozygosities per locus, except for OarJMP58 and OarFCB48.

3.2.1.1.3 F-Statistics

3.2.1.1.4 Pairwise F_{st} Values

Pairwise F_{ST} values between the populations were estimated by Arlequin package program (Excoffier *et al*., 2006). The F_{ST} values, used for a determinant of genetic differentiation between populations, can be seen in Table 3.5.

Table 3.5 Pairwise F_{ST} values of the seven breeds studied. The abbreviations are AKK (Akkaraman), DAĞ (Dağlıç), GÖK (Gökçeada), İVE (İvesi), KRY (Karayaka), KIV (Kıvırcık) and MOR (Morkaraman).

	AKK	DAĞ	GÖK	İVE	KRY	KIV	MOR
AKK	-						
DAĞ	0.04103***	-					
GÖK	0.00713^{ns}	0.04202***	-				
İVE	0.02876***	0.02641***	0.02148***	-			
KRY	0.01286*	0.03361***	0.01864**	0.01722**	-		
KIV	0.02058**	0.01176*	0.01053*	0.01137*	0.01423*	-	
MOR	0.02701***	0.01601***	0.02489***	0.00634^{ns}	0.01664***	0.00743*	

ns: non-significant, *p<0.05, **p<0.01, ***P<0.001

According to the pairwise F_{ST} values, most of the breeds differentiated from each other with significance level smaller than 0.001. Dağlıç and Gökçeada seem to be the most genetically different breeds of all with their relatively high pairwise F_{ST} values as compared to others.

3.2.1.1.5 F_{IS} Values

The within breed variation observed in terms of the 20 loci analyzed was tested for Hardy-Weinberg equilibrium by using F_{IS} index of F statistics by Arlequin package program (Excoffier *et al*., 2006). The results and their significance levels were given in the Table 3.6.

Table 3.6 The estimated F_{IS} values of the breeds and samples and their significance results.

	F_{IS}
AKKARAMAN	0.11252 **
DAĞLIÇ	-0.03638 ^{ns}
GÖKÇEADA	-0.14114 ^{ns}
İVESİ	0.09367 **
KARAYAKA	0.07387 *
KIVIRCIK	0.02847 ^{ns}
MORKARAMAN	-0.01244 ^{ns}

ns: non-significant, *p<0.05, **p<0.01, ***P<0.001

The estimated F_{IS} values of Akkaraman and İvesi breeds were found to be significant with a probability of 0.01 and Karayaka breed with a probability of 0.05. A significant deviation from Hardy-Weinberg equilibrium can be seen in those breeds.

A positive F_{IS} value indicated the heterozygote deficiency. Among the seven breeds, Akkaraman, İvesi, Karayaka and Kıvırcık suffer from the aforementioned deficiency.

3.2.1.2 Genetic Distance Estimations and Phylogenetic Tree Construction

3.2.1.2.1 Nei's D_A Genetic Distance

Nei's D_A genetic distances for the seven populations were calculated with Populations 1.2.30 (Langella, 1999). Table 3.7 shows the Nei's D_A genetic distance matrix of the studied breeds.

Table 3.7 Pairwise Nei's D_A genetic distance values for the seven studied breeds. The abbreviations are AKK (Akkaraman), DAĞ (Dağlıç), GÖK (Gökçeada), İVE (İvesi), KRY (Karayaka), KIV (Kıvırcık) and MOR (Morkaraman).

	AKK	DAĞ	GÖK	İVE	KRY	KIV	MOR
AKK	0						
DAĞ	0.123851	0					
GÖK	0.163992	0.135474	0				
İVE	0.155998	0.126364	0.195792	0			
KRY	0.0986047	0.112444	0.144288	0.138213	0		
KIV	0.156512	0.114398	0.152664	0.133557	0.138271	0	
MOR	0.135086	0.0890602	0.179598	0.104914	0.124965	0.114837	0

According to these estimations, the highest D_A distance value is 0.195792 found between Gökçeada and İvesi; thus, they are the most diverged species.

3.2.1.2.2 Neighbor Joining (NJ) Tree Construction: Based on Nei's D_A Genetic Distance and Allele Sharing Distance (ASD)

3.2.1.2.2.1 Neighbor Joining (NJ) Tree based on Nei's D_A Genetic Distance

These pairwise D_A genetic distances were used in construction of NJ tree showing the genetic relationships between the breeds. For tree drawing, SplitTree4 (Huson and Bryant, 2006) and for a different visualization Populations 1.2.30 (Langella, 1999) were used. Figure 3.10 shows the NJ tree constructed by Nei's D_A values.

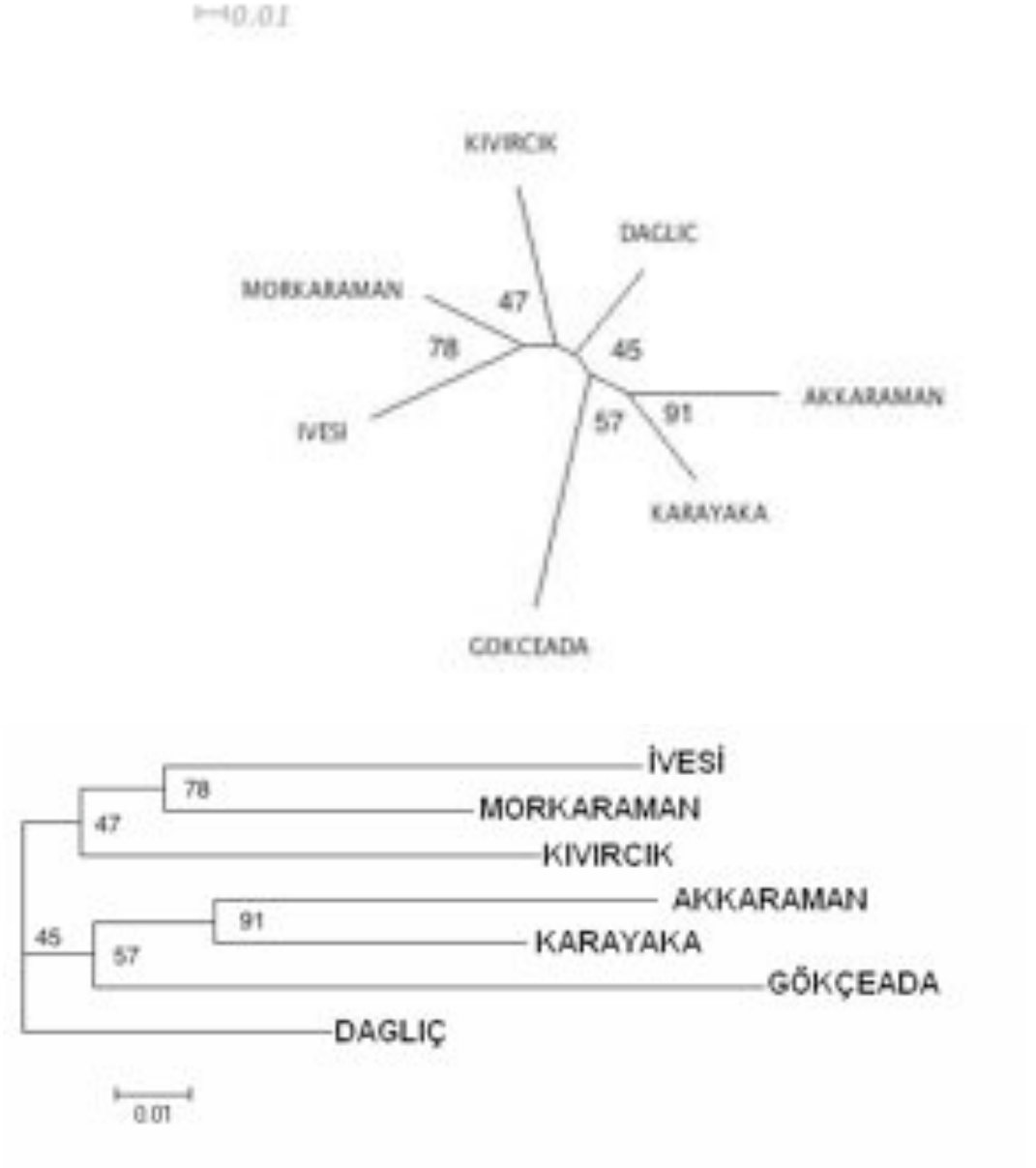


Figure 3.10 NJ tree constructed by Nei's D_A values with SplitTree4 and Populations 1.2.30 with bootstrap values on the branches.

Based on the NJ tree, Akkaraman and Karayaka breeds are tightly linked to each other with the highest bootstrap value (91) available in the tree. İvesi and Morkaraman are also forming the second group with a relatively high bootstrap value (78). Dağlıç seems to be the root in the second illustration and it seems to diverge more from the other breeds analyzed.

3.2.1.2.3 Neighbor Joining (NJ) Tree based on Allele Sharing Distance (ASD)

The NJ tree based on ASDs was drawn by Populations 1.2.30 (Langella, 1999). Figure 3.11 below shows the NJ tree constructed by ASD values.

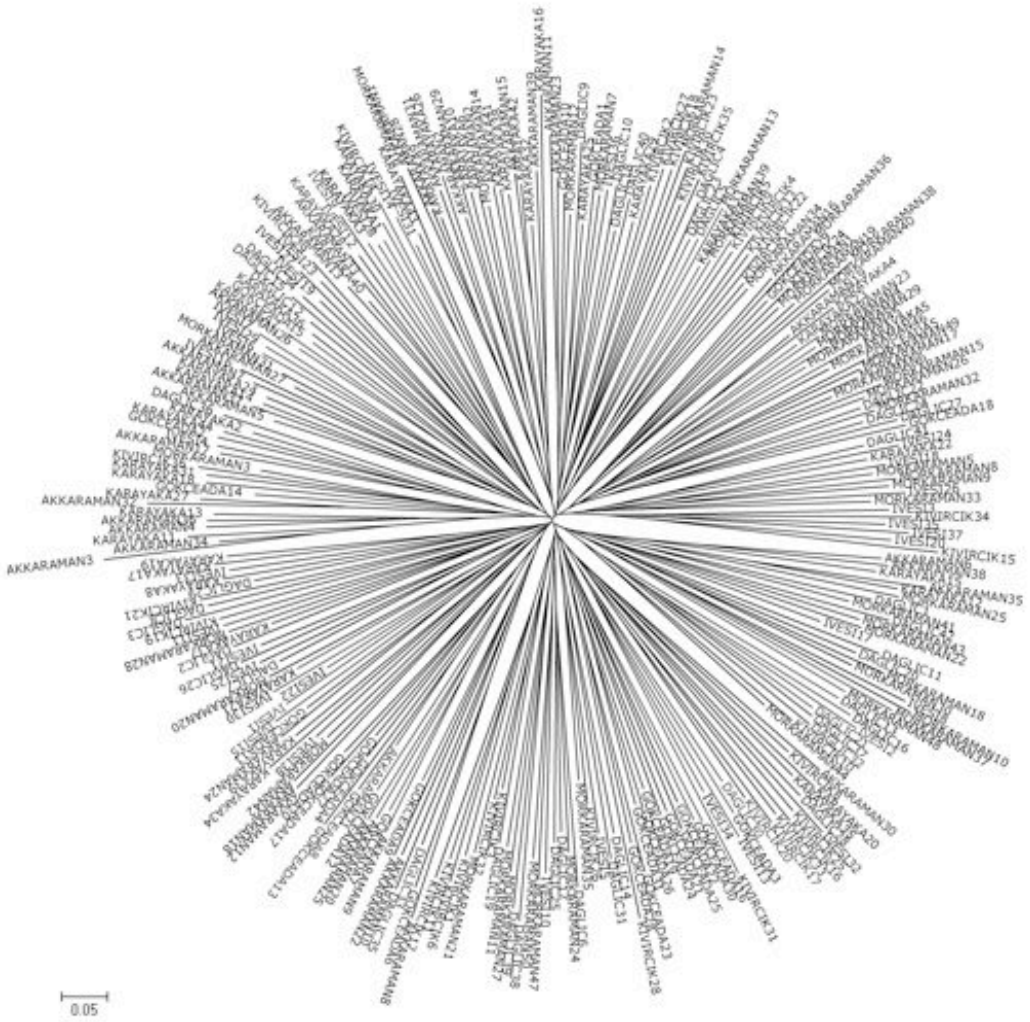


Figure 3.11 NJ tree based on Allele Sharing Distance (ASD).

The NJ tree is star-like with deep branches, which is a common future of ASD based trees of domestic animals. Branches representing the individuals of different breeds formed the external nodes of the tree. A single internal node holding individuals of the same breed solely was not seen. Likewise, any clear pattern for the node formation is not observed.

3.2.1.3 Neighbor-Net Analysis

Neighbor-Net (Bryant and Moulton, 2004) is a distance-based method for constructing phylogenetic networks based on the Neighbor-Joining (NJ) algorithm of Saitou and Nei (1987). It is available as part of the SplitsTree4 software package (Huson and Bryant, 2006). The Neighbor-Net Analysis result can be found in Figure 3.12.

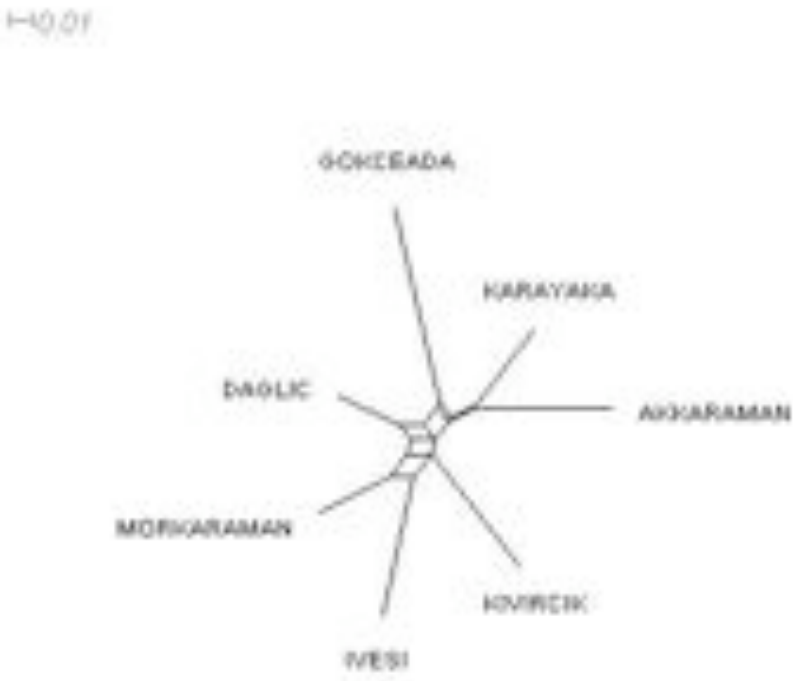


Figure 3.12 Neighbor-Net Analysis for the seven breeds studies.

The lengths of the networks are consistent with the differentiation of the breeds. For instance, Gökçeada is highly differentiated from other breeds. Karayaka and Akkaraman form a tight isolated group as was in NJ tree. Morkaraman and İvesi are again forming an isolated pair. The planar network surrounding Dağlıç represents genetic exchanges of Dağlıç with Morkaraman - İvesi, Kıvırcık, Gökçeada, and even with Karayaka - Akkaraman pair.

3.2.1.4 Principal Component Analysis (PCA)

Relative relatedness of populations was observed based on twenty microsatellite frequency data by performing Principle Component Analysis (PCA). The PCA was performed with PCA-GEN v. 1.2 (Goudet, 1999). Figure 3.13 represents two principal components of the PCA.

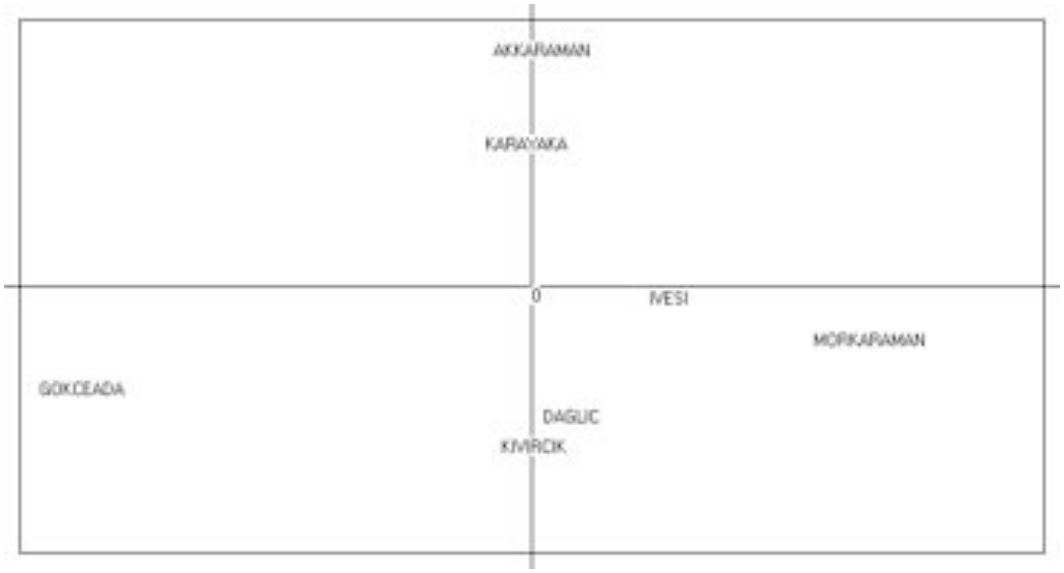


Figure 3.13 PCA of seven breeds based on twenty microsatellite frequencies.

The first axis has percentage inertia of 27.37 and the second one of 23.72. Together, they account for and explain more than 50 % of the total genetic variation. The x-axis defines three groups: Akkaraman, Karayaka and Kıvırcık; Morkaraman, İvesi, Dağlıç; and Gökçeada. The y –axis further discriminates among Akkaraman, Karayaka and Kıvırcık, the former two being closer to each other.

3.2.1.5 Analysis of Molecular Variance (AMOVA)

In order to see how the genetic variation was partitioned within and between the breeds, four different AMOVA analyses were performed by Arlequin package program (Excoffier *et al*., 2006).

In the first AMOVA analysis 3 groups were formed with respect to the results of the NJ trees. The first group was composed of Akkaraman, Karayaka and Gökçeada, the second one İvesi, Morkaraman, Kıvırcık and the third one, only Dağlıç. The AMOVA table can be seen in Table 3.8.

Table 3.8 AMOVA table for the 3 groups determined by NJ trees.

Source of Variation	*d.f.*	*Variance Components*	*Percentage of Variation*	*Fixation Indices*
Among Groups	4	0.02020	1.39	FCT: 0.01386*
Among Populations within Groups	2	0.01418	0.97	FSC: 0.00986***
Among Individuals within Populations	273	0.02887	1.98	FIS: 0.02028
Within Individuals	280	1.39464	95.66	FIT: 0.04338**
Total	559	1.45789		

*p<0.05 **p<0.01 ***p<0.001

Here, 95.66 % of the total variation was partitioned within individuals. The individuals within populations show no significant difference, but the differences among groups and among populations in those groups were significant. However, there were much more difference among population within groups than among groups.

Second, five groups were used: (1) İvesi, Morkaraman, (2) Kıvırcık, (3) Akkaraman, Karayaka, (4) Gökçeada and (5) Dağlıç. The results can be seen in the table below.

Table 3.9 AMOVA table for five groups.

Source of Variation	*d.f.*	*Variance Components*	*Percentage of Variation*	*Fixation Indices*
Among Groups	4	0.01727	1.32	FCT: 0.01188
Among Populations within Groups	2	0.01333	0.83	FSC: 0.00928*
Among Individuals within Populations	273	0.02887	1.98	FIS: 0.02028
Within Individuals	280	1.39464	95.86	FIT: 0.04090**
Total	559	1.45411		

*p<0.05 **p<0.01 ***p<0.001

In that grouping, no significance is found both in among individuals within populations and among groups. The significant differences were in among populations within groups and within individuals.

Third, four groups were used: (1) Gökçeada, Akkaraman and Karayaka, (2) Dağlıç, (3) Kıvırcık, (4) İvesi and Morkaraman. The results can be seen in the table below.

Table 3.10 AMOVA table for four groups.

Source of Variation	*d.f.*	*Variance Components*	*Percentage of Variation*	*Fixation Indices*
Among Groups	3	0.01757	1.32	FCT: 0.01207*
Among Populations within Groups	3	0.01441	0.83	FSC: 0.01002**
Among Individuals within Populations	273	0.02887	1.98	FIS: 0.02028
Within Individuals	280	1.39464	95.86	FIT: 0.04181**
Total	559	1.45550		

*p<0.05 **p<0.01 ***p<0.001

Here, the populations in the groups were significantly different from each other, not a uniform distribution in the groups can be seen. In addition, among groups there was difference with a significance value $p<0.05$.

In the fourth grouping, six groups were available: (1) İvesi and Morkaraman, (2) Dağlıç, (3) Akkaraman, (4) Karayaka, (5) Gökçeada and (6) Kıvırcık. The results can be seen in the Table 3.12.

Table 3.11 AMOVA table for six groups.

Source of Variation	*d.f.*	*Variance Components*	*Percentage of Variation*	*Fixation Indices*
Among Groups	5	0.02119	1.46	FCT: 0.01458*
Among Populations within Groups	1	0.00875	0.60	FSC: 0.00611
Among Individuals within Populations	273	0.02887	1.99	FIS: 0.02028
Within Individuals	280	1.39464	95.95	FIT: 0.04046***
Total	559	1.45345		

*p<0.05 **p<0.01 ***p<0.001

Here, the groups were significantly different from each other, whereas there were not any significant differences among populations within groups. Within individuals differences were significant as seen in all the four AMOVA tables.

Considering the results of AMOVA analysis, it can be concluded that grouping considered by the fourth AMOVA analysis revealed uniform population grouping. The partitioning of the total variation is found the most within the individuals.

3.2.2 'Individuals Within Population' Based Analyses

3.2.2.1 Assignment Tests

Assignment tests were performed to observe the inertia of the breeds based on the twenty microsatellite loci. GeneClass2 (Piry *et al*., 2004) is used for the assignment test. One of the Bayesian type likelihood methods (Rannala and Mountain, 1997) is used to assign the individuals by simulating them 10000 times. The probability criteria to reject the assignment of the individual was P<0.05. The results of the test could be found in Appendix D.

To interpret the results, the following criteria are used: An individual was accepted to assigned correctly if it was assigned to its original breed in the first place - with the highest probability. In addition, when the probability of the individual's likelihood to be assigned to breeds other than its original population estimated as greater than 90 %, then it was accepted to have a probability to be misleadingly assigned. According to those criteria:

97 % of Akkaraman breed, 71 % of Dağlıç breed, 87 % of Gökçeada breed, 89 % of İvesi breed, 93 % of Karayaka breed, 100 % of Kıvırcık breed and 92 % Morkaraman breed have been assigned correctly. More detailed results could be found in Table 3.12.

Table 3.12 The results of the Assignment tests. The second column is probabilities in respect to the proportion of the individuals assigned to their original breeds in the first place. The third column is for assignment of individuals to other breeds than their original population in the first place. The last column is the probability for the individuals assigned to their original breeds in the first place whereas their probability of assignment to other breeds was also higher than 90 %.

	To original breed	To other breed than original	To original breed but >90 % for other breeds
AKKARAMAN	97 %	3 %	13 %
DAĞLIÇ	71 %	29 %	18 %
GÖKÇEADA	87 %	13 %	32 %
İVESİ	89 %	11 %	8 %
KARAYAKA	93 %	7 %	14 %
KIVIRCIK	100 %	0 %	8 %
MORKARAMAN	92 %	8 %	20 %

Dağlıç, Gökçeada and İvesi seemed to have the least compact (the minimum inertia) and / or least isolated gene pools.

3.2.2.2 Factorial Correspondence Analysis (FCA)

The Factorial Correspondence Analysis (FCA) is performed in order to visualize the individuals in multidimensional space and to explore the relationships between the individuals. GENETIX v. 4.03 (Belkhir *et al*., 1996) is used for the analysis. The samples were examined on 3-Dimensional (3D) graphics with different triple combinations of the 10 factors (each represented by an axe) estimated by the software. The results of the analysis can be seen in Figures 3.14, 3.15 and 3.16.

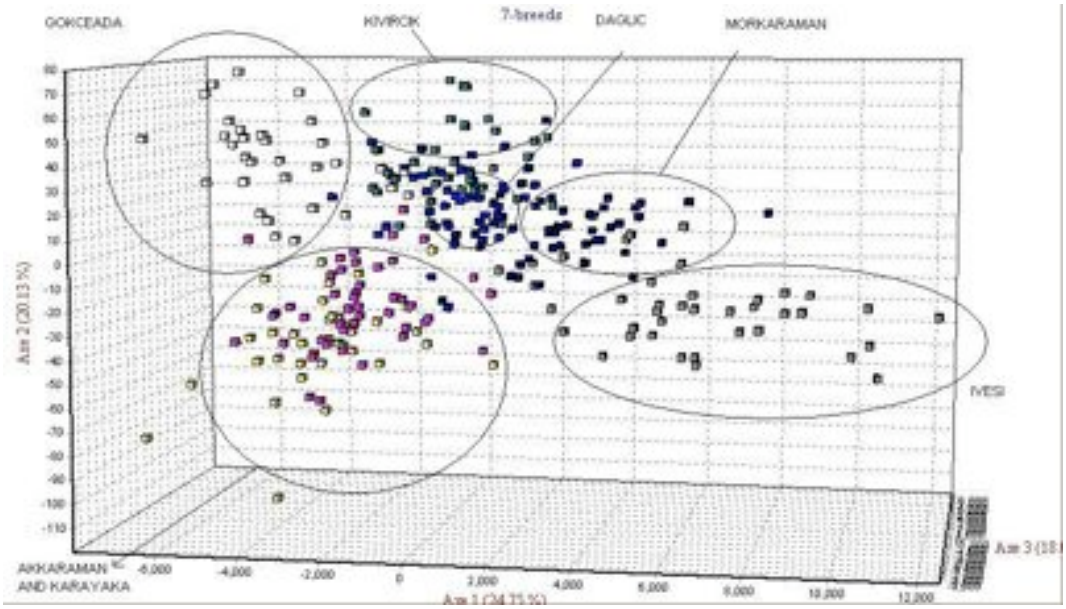


Figure 3.14 FCA result showing the relationship between all of the individuals analyzed in the study. The color labels and their corresponding breeds are as follows: Akkaraman (Yellow), Dağlıç (Blue), Gökçeada (White), İvesi (Gray), Karayaka (Pink), Kıvırcık (Turquoise) and Morkaraman (Purple).

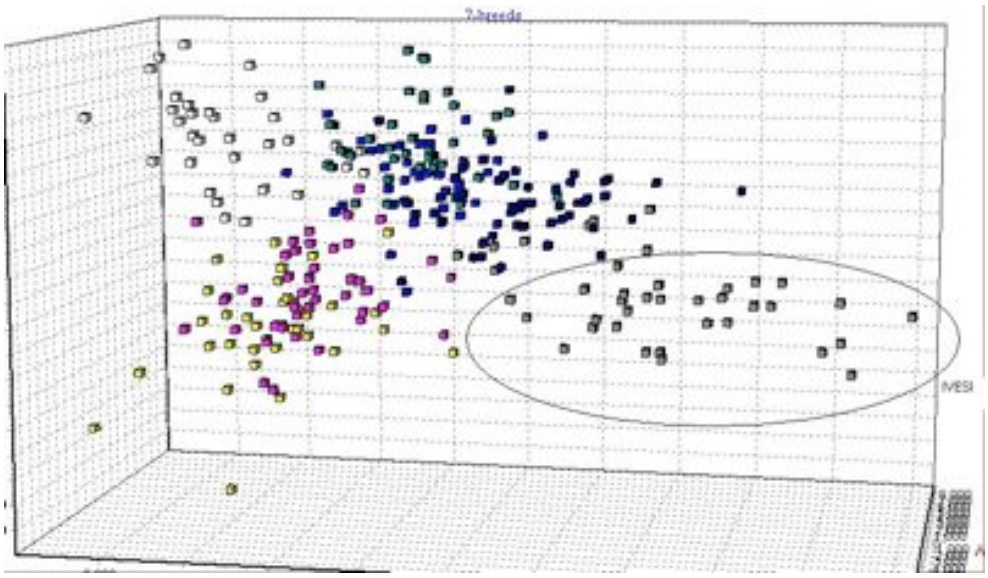


Figure 3.15 FCA results showing the relationship between all of the individuals analyzed in the study with a special emphasis on İvesi. The color labels and their corresponding breeds are as follows: Akkaraman (Yellow), Dağlıç (Blue), Gökçeada (White), İvesi (Gray), Karayaka (Pink), Kıvırcık (Turquoise) and Morkaraman (Purple).

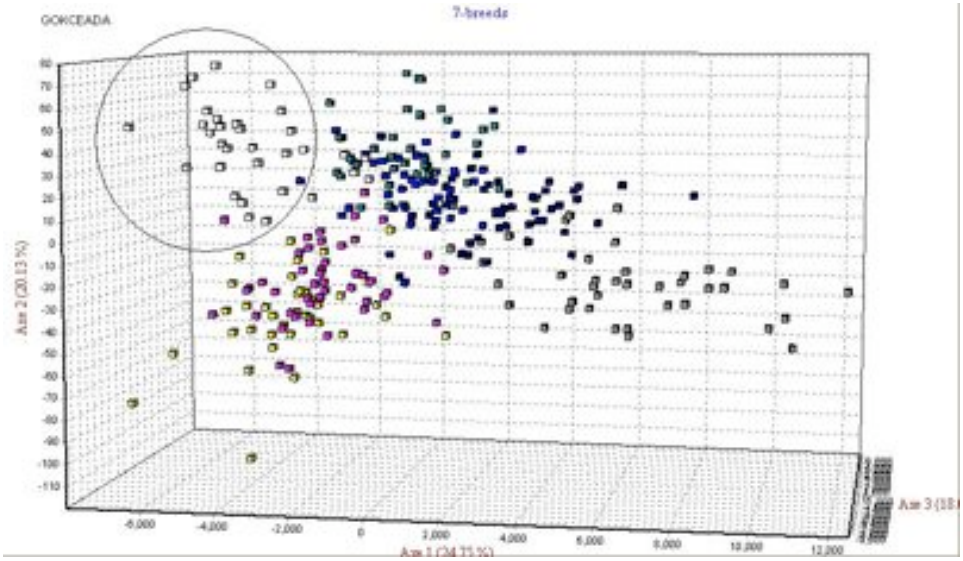


Figure 3.16 FCA results showing the relationship between all of the individuals analyzed in the study with a special emphasis on Gökçeada. The color labels and their corresponding breeds are as follows: Akkaraman (Yellow), Dağlıç (Blue), Gökçeada (White), İvesi (Gray), Karayaka (Pink), Kıvırcık (Turquoise) and Morkaraman (Purple).

Among all the breeds analyzed, samples of İvesi and Gökçeada, to some extent, fell apart from the rest of the samples. However, it is not easy to talk about a complete differentiation because as seen, some of the individuals of İvesi and Gökçeada were grouped with other breeds. Akkaraman and Karayaka (yellow and pink, respectively) clustered together. The same was true for Kıvırcık, Morkaraman and Dağlıç (turquoise, purple and blue colors, respectively). Dağlıç is the one that overlaps the most with the other breeds.

There were some individuals grouping with other breeds (such as some of the individuals of Gökçeada, Karayaka and İvesi). Those individuals' photographs were decided to be examined in order to determine if there were phenotypic differences existed which can differentiate them from their populations. To achieve that, a systematic approach was taken. Three breeds were chosen according to the extremity in their assignment values, the high number of their samples sizes and the number of microsatellite loci that are complete (for instance, İvesi has two missing loci). As a result, Dağlıç (has the minimum value in assignment test), Morkaraman and Akkaraman (high number of loci were examined) are decided to be further investigated by FCA in order to see the differentiated individuals in a better resolution and to compare those ones with other individuals of their population via photographs. A circle gathering 50 % and a rectangle gathering 85 % of the individuals of the breeds were drawn. Then, the photographs of the outliers in the 15 % were investigated. FCA with an emphasis to Dağlıç population and the pictures of the outliers can be seen in Figure 3.17.

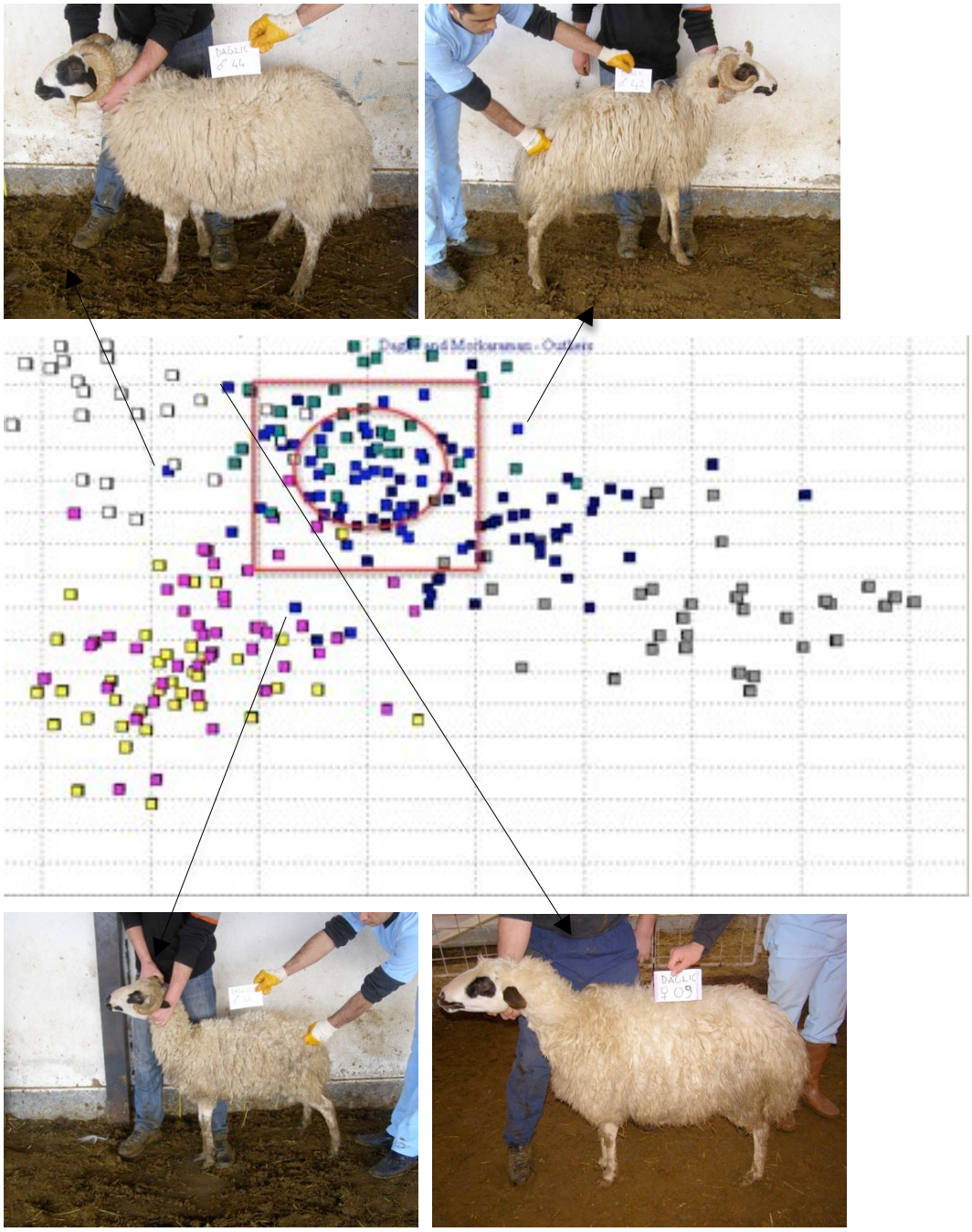


Figure 3.17 FCA result for Dağlıç (Blue). The circle represents the area that covers the 50 % of the individuals of the population. The rectangular represents the area that covers the 85 % of the individuals of the population. Outside those, the outliers exist. Photographs of 4 outliers from Dağlıç can be seen around the FCA figure.

Two individuals for Dağlıç from the 50 % circle of the FCA figure above (not the extreme ones but 'genetically typical' ones) can be found in Figure 3.18.

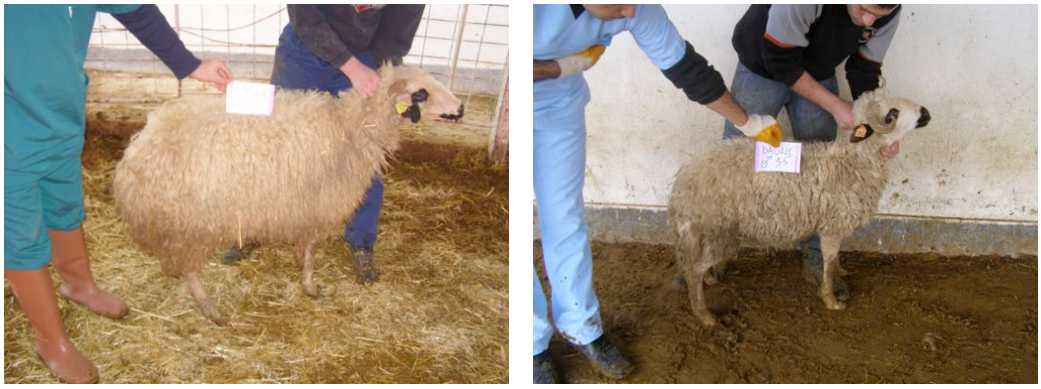
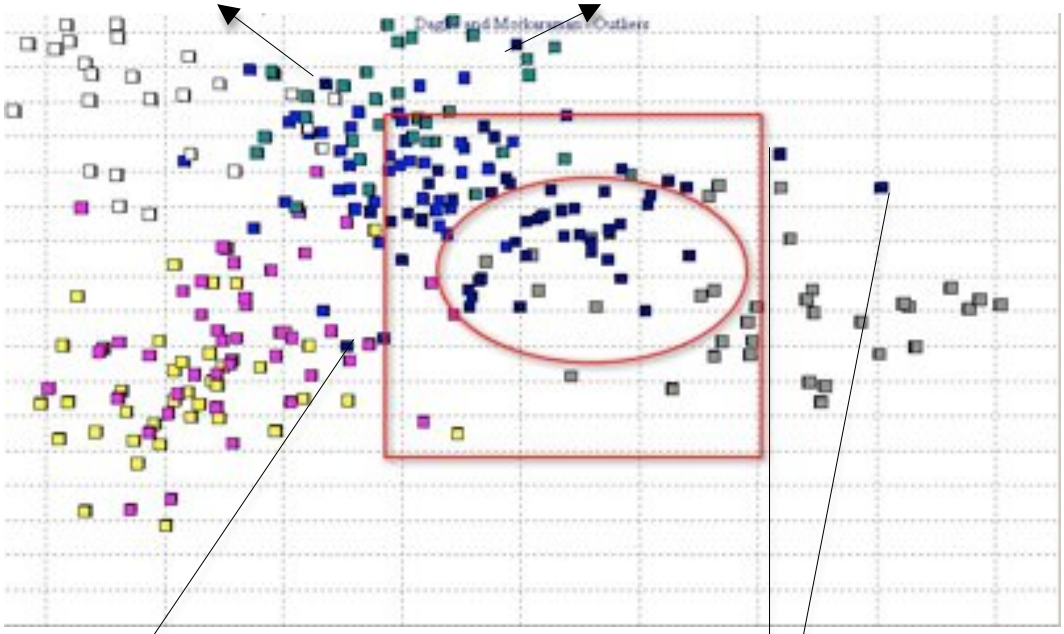


Figure 3.18 Two 'genetically typical' Dağlıç individuals; a female is on the left and a male on the right.

The same approach was held for Morkaraman. FCA of this population and the pictures of the outliers of Morkaraman can be seen in Figure 3.19.



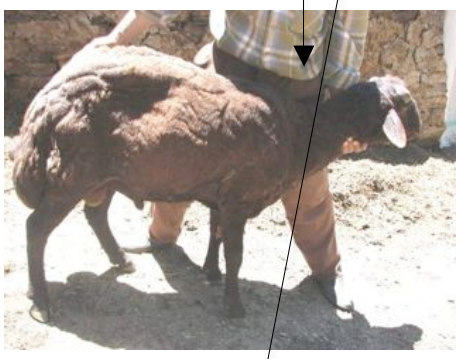


Figure 3.19 FCA result for Morkaraman (Purple). The circle represents the area that covers the 50 % of the individuals of the population. The rectangular represents the area that covers the 85 % of the individuals of the population. Outside those, the outliers exist. Photographs of 5 outliers from Morkaraman can be seen around the FCA figure.

Two individuals for Morkaraman from the 50 % circle of the FCA figure above (not the extreme ones but 'genetically typical' ones) can be found in Figure 3.20.

Figure 3.20 Two 'genetically typical' Morkaraman individuals; a male is on the left and a female on the right.

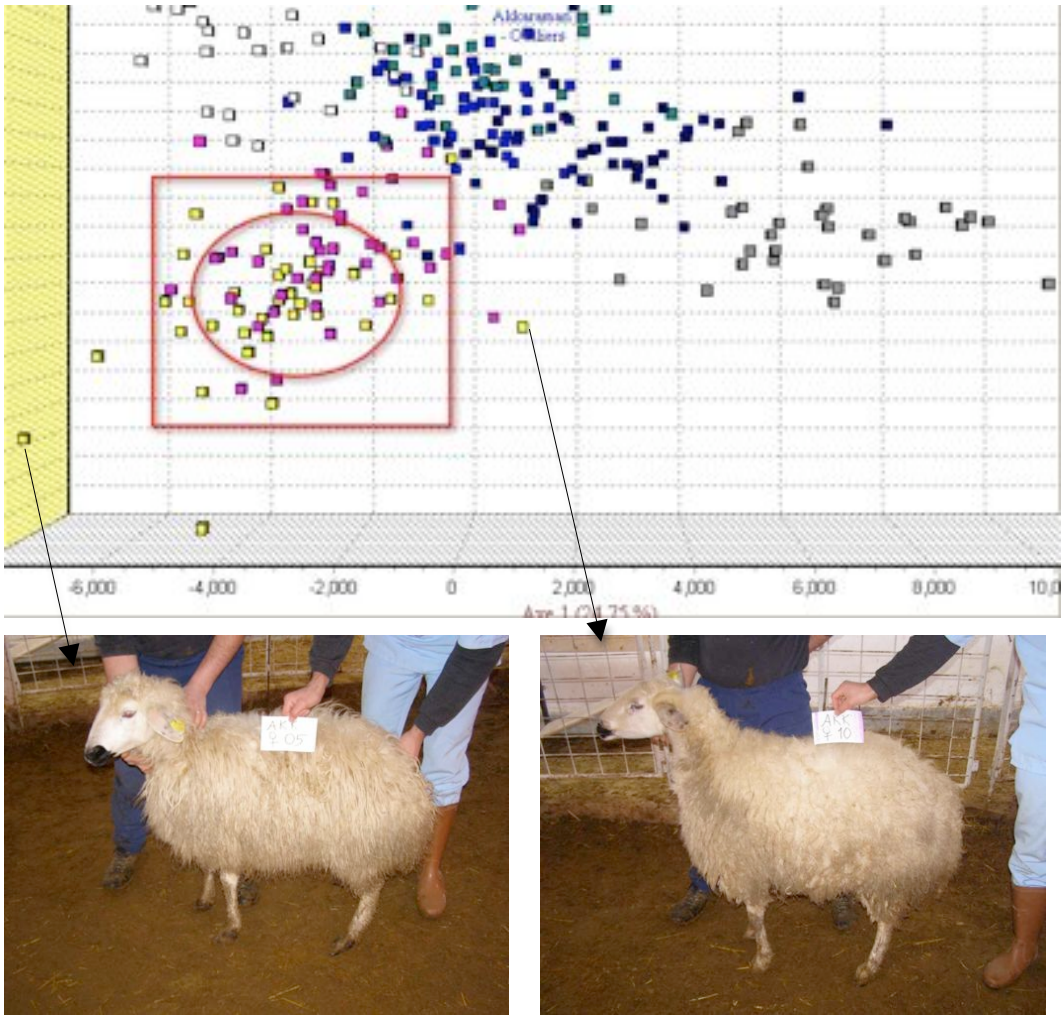


Figure 3.21 FCA result for Akkaraman (Yellow). The circle represents the area that covers the 50 % of the individuals of the population. The rectangular represents the area that covers the 85 % of the individuals of the population. Outside those, the outliers exist. Photographs of 2 of the 5 outliers from Akkaraman can be seen around the FCA figure.

Two individuals for Akkaraman from the 50 % circle of the FCA figure above (not the extreme ones but ‘genetically typical’ ones) can be found in Figure 3.22.

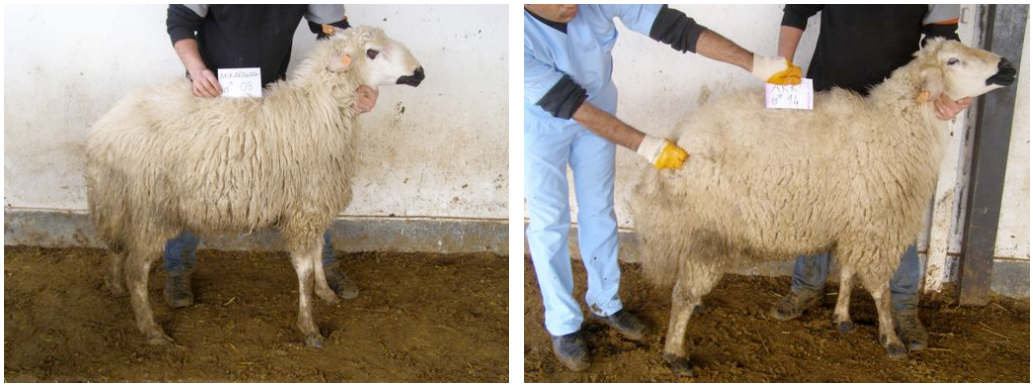


Figure 3.22 Two ‘genetically typical’ Akkaraman individuals; both are males.

After the examination for determining whether there are phenotypic differences (from the photographs not for geometric morphologies or meat/milk production capacities) exist for the breeds which can differentiate them from their populations that can provide us clues about the possible hybrids, it is decided that with the microsatellite information available in this study, such an observation is not very likely to make. The tail photographs of the ‘outlier’ and ‘genetically typical’ breeds of Dağlıç, Morkaraman and Akkaraman can be seen in Appendix E.

3.2.2.3 Structure

The Structure v2.2 software was used to analyze the presence of population sub-structuring and to identify genetically distinct populations among the seven sheep

breeds of the current study (Pritchard *et al*., 2000). The burn in length was set to 50.000, which is accepted as adequate (Falush *et al*., 2007). The "Admixture model" is used in this study. Possible number of distinct populations, shown by *K*, was tested from 1 to 15, and each *K* value was tested (iteration number) 20 times.

There are two methods used for the estimation of *K* using the simulation results of Structure: one was proposed by Pritchard *et al.* (2000) and the other was proposed by Evanno *et al.* (2005). Both methods were employed to estimate the best *K*; the best possible number of distinct groups based on genotypic data among the seven breeds analyzed.

In order to employ Prichard's method, first all a table was prepared using all the Ln P(D) estimates for each *K* value and for each iteration. Then average values for each K were estimated. The average Ln P(D) values estimated for each K from 1 to 15 of the current study can be found in Table 3.13.

Table 3.13 Average LN P(D) values for K= 1 to K=15.

K1	K2	K3	K4	K5	K6	K7	K8	K9	K10	K11	K12	K13	K14	K15
-20085.7	-20056.9	-19714.7	-19622.4	-19637.4	-19836.3	-19648.4	-19772.0	-19722.8	-19776.2	-19708.8	-19806.4	-19849.4	-20162.6	-20096.6

For *K*=4 the result was ≈ 0.999. For the other values of *K* the posterior probabilities were smaller than 0.999. So, according to Pritchard (2000) four distinct genetic populations were generating all 7 breeds.

It is claimed that, when there is real population structure, Pritchard *et al.*'s (2000) model could cause unrealistic *K* values. So, Evanno *et al.* (2005) proposed another method for calculation of *K*. According to this method, the second order rate of change of the likelihood function (ΔK = m|L″(K)|/ s[L(K)]) with respect to *K* shows a clear peak at the true value of *K*. After a series of calculations as explained in Chapter 2, the ΔK graph was drawn which can be seen in Figure 3.23.

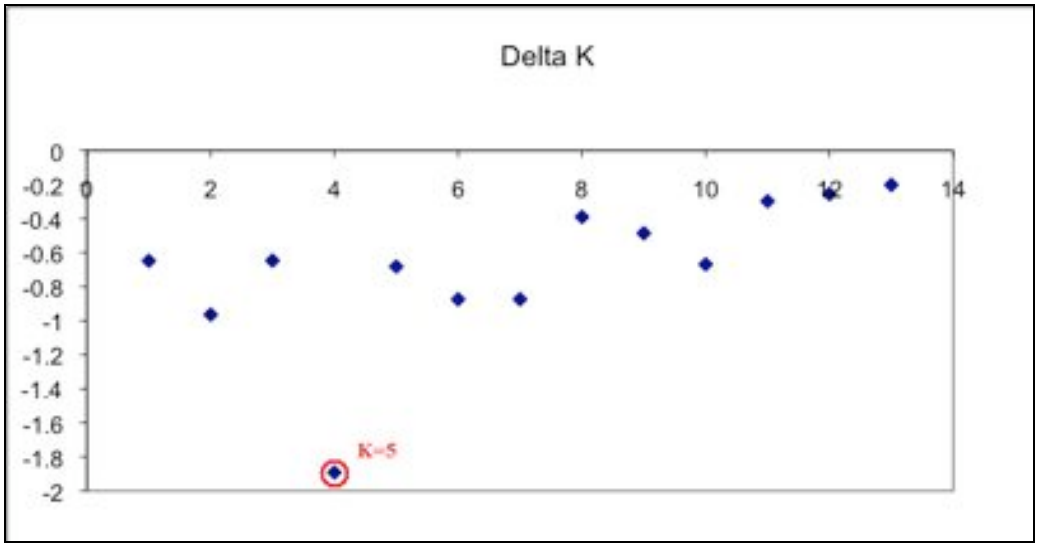


Figure 3.23 The second order rate of change of the likelihood function with respect to K graph.

So, according to Evanno *et al.* (2005) five distinct genetic populations were available.

There is difference in the estimated best possible number of populations among seven breeds, *K,* based on both Pritchard's and Evanno's methods. Pritchard's method suggested *K* as 4, whereas Evanno's as 5 among the phenotypically distinct 7 breeds analyzed. Both methods did not result in the estimation of the best *K* as 7 groups of populations parallel to the phenotypic grouping.

When *K* is 4 as suggested by Pritchard's method, the partitioning of the genetic variation among the breeds was shown in the Structure bar graph below.

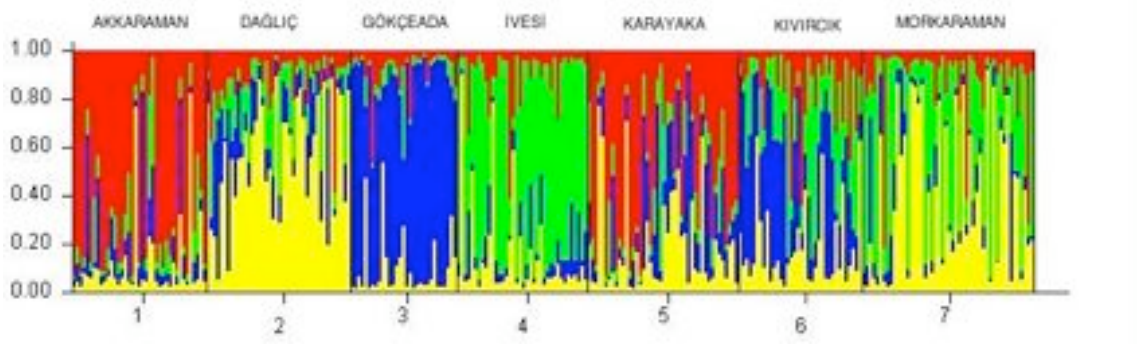


Figure 3.24 Structure Bar Plot based on LnP(D) when *K*=4, population number is given below the graph and the population names were given above the graph.

In Figure 3.24, it can be seen that none of the populations were represented by a single color, or by a color not seen in other populations, which would mean the distinctness of that population from the rest. Each color is observed in each

population mostly at different frequencies. Partitioning of the colors among the individuals within each breed is not the same, meaning that the diversity within each breed is high, rather than uniform. Yet, relatively high degree of uniform colors is associated with İvesi and Gökçeada, the two breeds having extreme distributions on the FCA plot. High degree of redness in Akkaraman and Karayaka, again greenness in Morkaraman and İvesi can be seen. Dağlıç has distinct yellow but also blue, red, green.

When K is 5 as suggested by Evanno *et al.*'s (2005) method, the partitioning of the genetic variation among the breeds was shown in the Structure bar graph below.

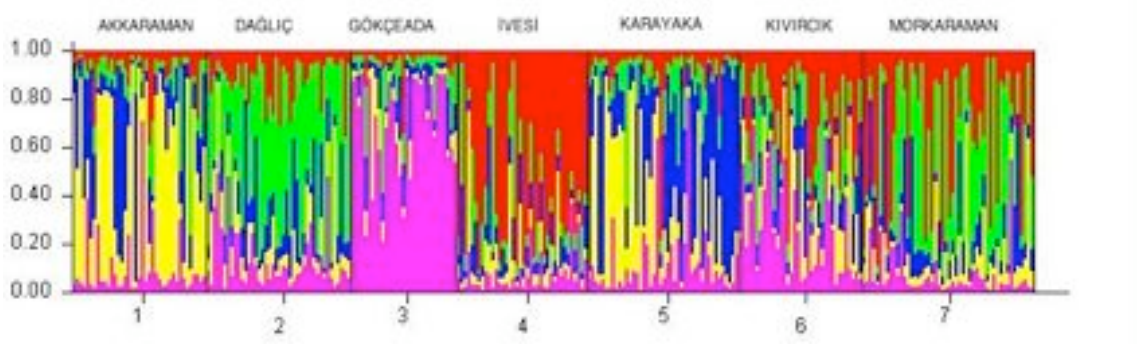


Figure 3.25 Structure Bar Plot based on LnP(D) when *K*=5, population number is given below the graph and the population names were given above the graph.

Results are more blurred but general nonuniformity in the breeds and similarity between them are quite the same when *K* is set to 4.

Another way of representation of the results is triangular plot. Each breed is assigned a color and the individuals of the breeds were placed within this triangular with respect to their genotypic data. The graph is given below.

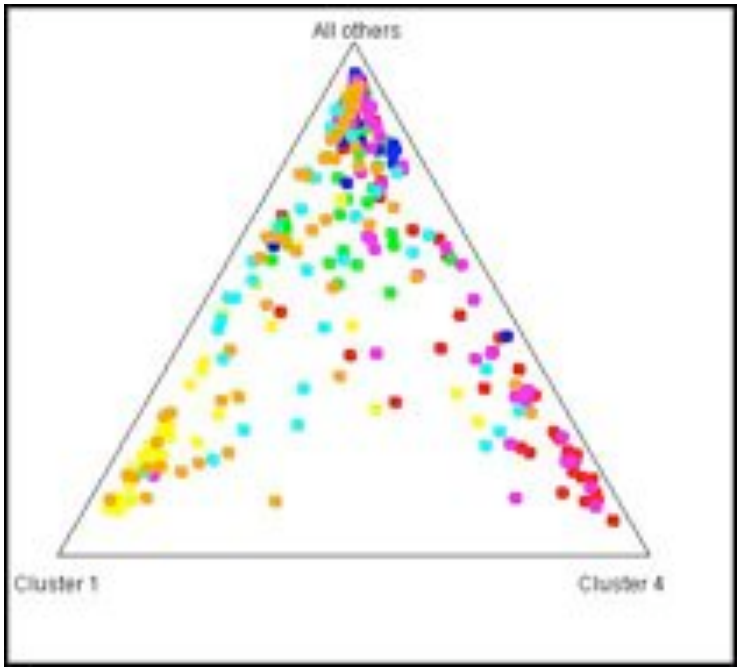


Figure 3.26 The triangle plot of individuals for seven breeds. The color codes are: Akkaraman (Red), Dağlıç (Green), Gökçeada (Blue), İvesi (Yellow), Karayaka (Pink), Kıvırcık (Turqouise) and Morkaraman (Orange).

Cluster 1 represents İvesi - Morkaraman individuals, Cluster 4 represents Akkaraman – Karayaka individuals as they were observed by NJ tree and FCA plot.

The results were also shown by plot in multiple lines. It can be seen in Figure 3.27.

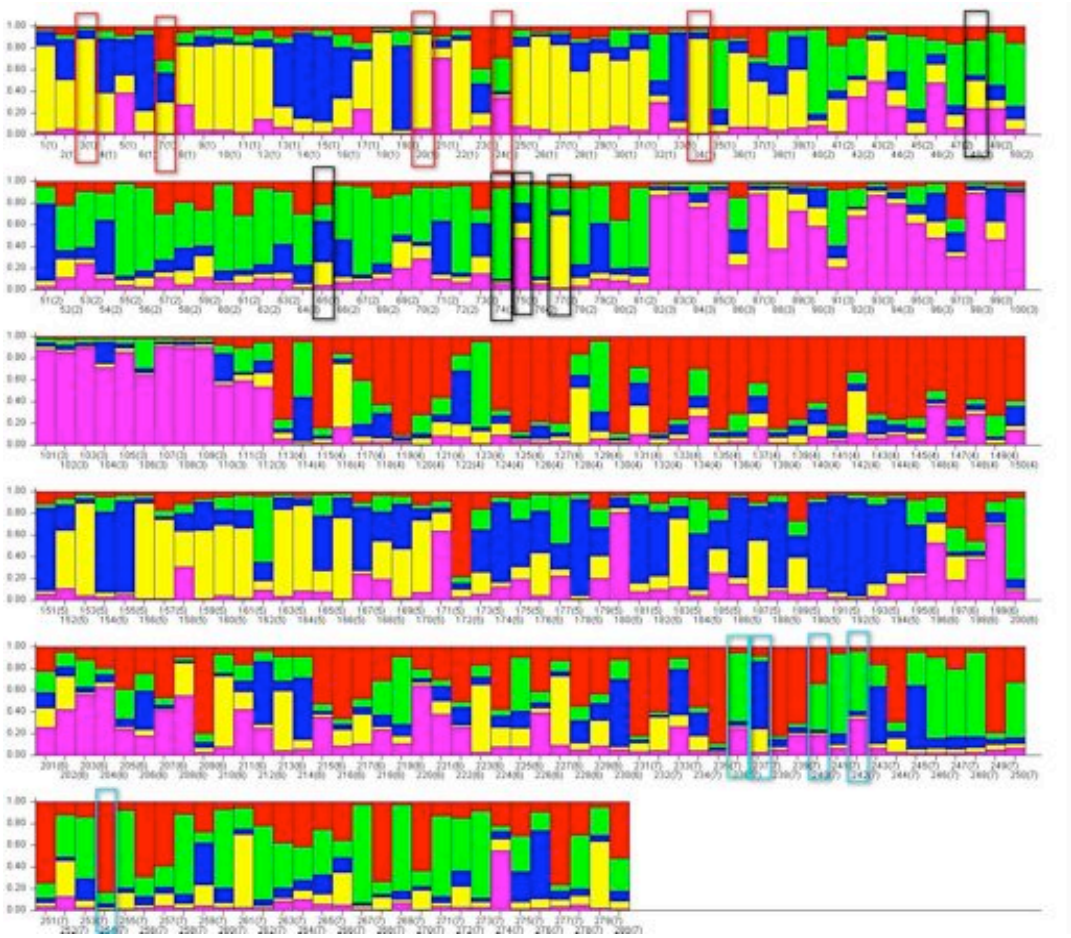


Figure 3.27 The plot in multiple lines. The red rectangles around the individuals denote Akkaraman individuals 5, 10, 26, 30 and 44, the black ones Dağlıç individuals 9, 28, 42, 44 and 46, and the turquoise ones Morkaraman individuals 6, 7, 10, 12 and 24, who are the outliers of FCA.

The outlier individuals can also be traced in *Structure*. The outliers were Dağlıç individuals 9, 28, 42, 44 and 46, Morkaraman individuals 6, 7, 10, 12 and 24, and Akkaraman individuals 5, 10, 26, 30 and 44. If the plot in multiple lines is observed, different colored rectangles, the color codes of which were given in Figure 3.27, can be seen.

The individuals are not grouped according to their breed of origins as it can be deduced by the K estimation results. There were mixing of İvesi and Morkaraman individuals in Cluster 1 and some mixing of Akkaraman and Karayaka in Cluster 4, as apparent from NJ and Neighbor-Net results. Rest of the individuals from these and the other breeds were included in "all others" cluster. No matter what the estimated K value is, either 4 or 5, the results show that there is high admixture in seven Turkish sheep breeds studied.

3.2.3 Sequencing of Sheep Mitochondrial DNA (mtDNA) Control Region (CR)

In the sequencing part, individuals harboring different patterns both in RFLP and SSCP according to Yüncü (2009) were sequenced in order to determine if they are the rare haplogroups D or E, or just some minor mutations causing them to drift apart from the commonly found haplogroups A, B or C. Those individuals were Akkaraman individual numbers 13, 38 and 46, Dağlıç individual numbers 9, 32, 55 and Morkaraman individual number 42. Sequencing is performed as previously told in Chapter 2. The consensus sequences are further clustered into NJ tree with the help of the software MEGA: Molecular Evolutionary Genetic Analysis v. 4.0 (Tamura *et al*., 2007). The other sequences employed in NJ tree formation were taken from Koban *et al*.'s study (Koban *et al*., 2008). The NJ tree can be seen in Figure 3.28.

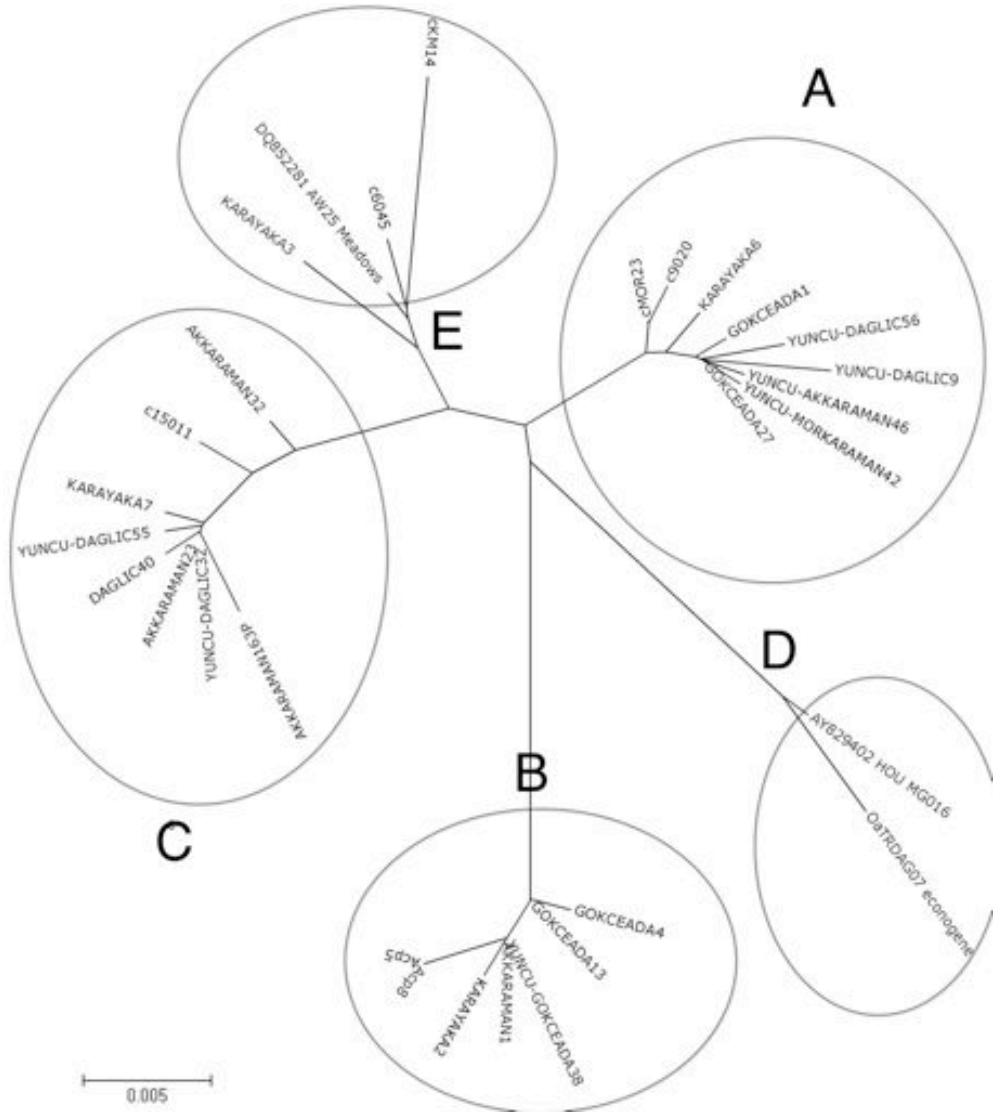


Figure 3.28 NJ Tree based on sequences of Yüncü's (2009) unknown band pattern revealing samples and previously known haplogroup containing individuals. A, B, C, D and E represents the haplogroups.

CHAPTER 4

DISCUSSION

In this study, seven native Turkish sheep breeds (Akkaraman, Dağlıç, Gökçeada, İvesi, Karayaka, Kıvırcık and Morkaraman) were investigated genetically using 20 microsatellite loci. One of the previous studies which was the most extensive in covered number of breeds (57) and loci (31), European Union (EU) Vth Framework Project ECONOGENE, indicated that Karayaka and Dağlıç are similar to European breeds based on microsatellite loci, whereas Akkaraman and Morkaraman are genetically more variable than those of the two Anatolian breeds and all of the European breeds (ECONOGENE, http://www.ECONOGENE.eu). Furthermore, these central and eastern Anatolian sheep breeds (Akkaraman and Morkaraman) are found to be genetically different than Karayaka and Dağlıç but very similar to Middle Eastern breeds from Saudi Arabia and Egypt (Lenstra and ECONOGENE Consortium, 2005; Peter *et al*., 2007). Koban *et al*.'s (2008) study confirmed these results. In addition, the same study (Koban *et al*., 2008) indicated that Hemşin and Norduz breeds are in the same group with those of Akkaraman and Morkaraman. Furthermore, based on mtDNA, hence indicating maternal composition of the breeds, it is observed that Dağlıç, Karayaka (Koban *et al*., 2008) and Kıvırcık (Yüncü, 2009) are possibly representing the earliest sheep of Anatolia; the rest of the breeds received migrant females from east and south east as was suggested by the presence of statistically different HPG clines. Again

Yüncü (2009) observed that Gökçeada breed represents an unexpectedly high Asian haplogroup, interpretable with the genetic drift experienced by Gökçeada sheep, an island breed.

The 20 microsatellite loci employed in this study were highly polymorphic, they did not have any problem in amplification, these loci were distributed evenly throughout the autosomes (Lenstra and ECONOGENE Consortium, 2005) and there are extensive data available from ECONOGENE (http://www.ECONOGENE.eu) (Lawson Handley *et al*., 2007; Peter *et al*., 2007). These loci were chosen by the national project TURKHAYGEN-I (106G005, In Vitro Conservation and Preliminary Molecular Identification of Some Turkish Domestic Animal Genetic Resources-I, http://www.turkhaygen.gov.tr/) so that once the individuals of ECONOGENE and the present study (which is part of the TUKHAYGEN-I) will be evaluated together with the same reference individuals, the results will be merged and data from a very wide geographic area (Europe-Turkey-Middle East) will be obtained.

By the present study, with an independent (7 years later than that of Koban *et al*.'s (2008) and ECONOGENE's (2005)) an extensive sampling microsatellite based variability patterns of the Turkish native sheep breeds will be revisited. Kıvırcık, Gökçeada and İvesi were not studied with the same standard of other breeds in the previous studies. Hence, these three breeds may provide some new insights or may enhance the observations made previously.

4.1. Genetic Variability based on Microsatellite Loci and Populations

The highest number of alleles observed for single locus is 23 for OarFCB304 and OarFCB48, the lowest one is 8 for MAF214 and BM8125 in this study. The highest and lowest mean numbers of observed alleles per locus were 13.86 (OarJMP58) and 4.00 (MAF214), respectively. To detect the possible reading

errors associated with some loci, Table 4.1 was constructed. Table 4.1 presents numbers of alleles observed in ECONOGENE (2005) 46 breeds sampled from all over Europe, 4 in Turkey and 7 from Near East. On the same table, the number of alleles observed for 4 Turkish breeds examined by ECONOGENE and in the present project is given. Observed number of alleles is quite compatible between the studies. Most of the time, 4 Turkish breeds exhibited a subset of the all alleles observed by the ECONOGENE and in the present study higher number of alleles for the 7 Turkish breeds were observed as expected. MAF214, which was represented with a very few alleles in the present study, was an exception.

Table 4.1 The number of alleles observed in ECONOGENE, 4 Turkish breeds as part of ECONOGENE and the present study.

	Econogene	*Econogene-TURK*	*Present*
Microsatellite locus	**Number of Alleles**		
OarFCB20	21	14	22
OarFCB48	NONE	NONE	23
OarFCB304	27	15	23
OarJMP29	23	18	22
OarJMP58	23	15	20
MAF65	13	12	16
MAF209	21	11	12
MAF214	33	12	8
ILSTS005	18	9	14
ILSTS011	19	6	10
INRA063	22	18	21
OarFCB226	18	14	18
OarHH47	20	14	16
OarVH72	10	8	11
OarFCB128	14	11	17
MAF33	14	11	13
BM8125	12	8	8
MCM140	17	13	14
OarCP34	14	8	10
DYMS1	24	16	18

One possible explanation for the discrepancy between the data could be missing observations in our data related to these loci. However, for MAF214, 88 % of the loci were completely read. Yet, such a big difference in the readings cannot be associated with reading errors. Nevertheless, readings will be repeated with the International Society for Animal Genetics' (ISAG) standards. Reference samples will be purchased soon. One last and the most likely explanation could be the

presence of ascertainment bias. All the loci were selected based on their high degree of polymorphism in tested breeds, which were mostly from Europe. This particular locus might be not so polymorphic in Eastern / Turkish breeds as it was observed in ECONOGENE data.

As one of the genetic variability measures, when mean numbers of alleles were taken into consideration, our study exhibited higher values. This supports the expectations of observing higher mean number of alleles / locus from the domestication centers and the number of mean number of alleles' being higher in Middle East region (Peter *et al*., 2007). Table 4.2 presents range of mean number of alleles per breed in comparable studies. Present study employed the most polymorphic 20 loci, therefore, mean number of alleles / breed was the highest among the comparative studies. Gökçeada exhibited the lowest MNA / population in Turkey as expected from an isolated, small and hence genetically drifting population.

Table 4.2 Comparison of Range of Mean Number of Alleles from Peter *et al.* (2007), Uzun *et al.* (2006), Koban *et al.* (2008) and the present study.

	Range of Mean Number of Alleles
Peter *et al.*	5.00 - 7.52
Uzun *et al.*	9.30 - 10.40
Koban *et al.*	6.70 - 10.20
Present Study	8.15 - 11.30

As a part of allelic variation, private alleles were investigated. In the present study, a total of 47 breed-specific alleles were detected at 20 loci. Yet, none of the alleles possess frequencies more than 3 %. The samples' (which contain private alleles) microsatellite results were revisited in order to be sure there is no misreading. There was no misreading and observation of similar proportion of private alleles by Peter *et al.* (2007) provided a further confidence for the observed private alleles, as they observed 65 breed-specific alleles at 24 loci, but none of them possessing frequencies over 5 %. Hence, it can be concluded that, private alleles found in these studies are not likely to be used for the breed assignment due to their low frequency. Moreover, Gökçeada has the lowest number of private alleles whereas İvesi and Morkaraman harbors the highest numbers of those alleles. Once more Gökçeada being an island population was expected to loose rare alleles along the genetic drift process (Allendorf and Luikart, 2007). It must also be emphasized that, all private allele data should be considered precociously. They are not informative unless far more number of breeds from a wider geographic area like Asia, Europe and Africa must be studied using the same microsatellite loci and the same automatic machine to decide the uniqueness of the allele. Most of the seemingly private alleles would lose their uniqueness when larger number of samples from wider areas employed in the study.

From the data in the literature, it was possible to compare the gene diversity, expected heterozygosity (second measure of genetic diversity), estimated for sheep breeds with the gene diversity estimations of the present study. Table 4.3 summarizes the expected heterozygosity values of different studies.

Table 4.3 The expected heterozygosity values of Peter *et al*. (2007), Uzun *et al*., 2006, Lawson Handley *et al*. (2007), Koban PhD Thesis (2004), Koban *et al*. (2008), Diez-Tascón *et al*. (2000) and the present study.

	H_e (ranged between)
Peter *et al.*	0.63 - 0.77
Uzun *et al.*	0.69 - 0.74
Lawson Handley *et al.*	0.54 - 0.81
Koban *et al.*	0.72 - 0.79
Koban (PhD Thesis)	0.68 - 0.81
Diez-Tascón *et al.*	0.69 - 0.77
Present Study	0.71 - 0.78

Expected heterozygosity values of the present study were in the range found in the literature. Among the breeds, the mean number of expected heterozygosity per population is between 0.7136 (Gökçeada) and 0.7828 (Kıvırcık). With its second highest observed heterozygosity value (0.7626), Dağlıç should also be taken into account.

Results in relation to genetic diversity are consistent with those of the previous results (Uzun *et al*., 2006; Peter *et al.*, 2007; Lawson Handley *et al.*, 2007; Koban *et al.*, 2008). Turkish sheep breeds are generally more variable than those of European breeds. It is generally accepted that a high degree of genetic variability is expected in the breeds close to domestication centers (Loftus *et al*., 1999; Bruford *et al*., 2003). These results are considered as an evidence for Turkish sheep breeds being descendants of the first domestic sheep, and they are the descendents of first sheep domesticated in Anatolia as was archeologically shown (Zeder, 1999). However, in Koban *et al*.'s (2008) and Yüncü's (2009) studies, presence of admixture was indicated. Admixture must also be contributing to high genetic diversity. Indeed, the highest admixture was observed in Morkaraman

(Yüncü, 2009) and the highest MNA / population was in Morkaraman in the present study.

Yet, in the present study, highly different than those of the previous two studies (Koban *et al*., 2008; Lenstra and ECONOGENE Consortium, 2005) the most genetically variable breed based on HE was Kıvırcık (from the western Anatolia), Dağlıç (from the western), Karayaka (from the northern Anatolia). Two of these were studied before and they were the least variable among the Turkish breeds (Peter *et al*., 2007). Hence, there is an important difference between the previous studies and the present study, regarding the HE distribution within the native breeds.

4.2. Genetic Differentiation Between the Breeds

Pairwise F_{ST} values revealed that most of the breeds differentiated from each other with significance level smaller than 0.001. Dağlıç and Gökçeada seem to be the most genetically different breeds of all with their relatively high pairwise F_{ST} values as compared to others. The range of F_{ST} values in the present study is 0.00634 - 0.04202 and this range is lower than that of the lowest of another study (Lawson Handley *et al*., 2007) where the range is 0.100 - 0.288. In that study, sheep samples were obtained from different European countries, one (Soay) is feral living island population, and differentiation between them reaches to 7 times more than those of Turkish breeds. These results are consistent with the general observation of less degree of differentiation in eastern European and Middle Eastern ruminant breeds (Canon *et al*., 2006; Lawson Handley *et al*., 2007).

One of the other F statistics, F_{IS}, showed positive results for Akkaraman, İvesi, Karayaka and Kıvırcık, indicating overall heterozygote deficiencies. These can be contributed to Wahlund effect. That is there could be substructuring within those breeds.

Pairwise F_{ST} values were not employed for tree construction because they can be biased when there are high levels of within group variation (Hendrick, 1999). So, instead of F_{ST}, Nei's D_A is used for construction of NJ tree. Nei's D_A is one of the many genetic distances used for constructing phylogenetic trees (Allendorf and Luikart, 2007). With Nei's D_A, based on information obtained from 20 microsatellites, a single value is derived to see the among population differentiation. The trees constructed has the constraint of showing the genetic differentiation of breeds only in one dimension, yet, it has high power to detect the breeds with high similarities. NJ tree is the one which is used most widely, it is less time consuming, allows to have different rate of evolution for the taxa from the node of divergence and simulation studies indicate that it catches the topology quite efficiently (Graur and Li, 1994).

Based on the NJ tree, Akkaraman and Karayaka breeds are tightly linked to each other, as İvesi and Morkaraman. Dağlıç seems to be the root (or highly admixed breed) seemingly different from other breeds as was exhibited by high pairwise F_{st} values. No evident geographical differentiation, which is consistent relatedness within neighbors in the direction of east to west, as was observed with respect to mtDNA haplogroups by Koban *et al.* (2008) and Yüncü (2009), was observed. For example, Morkaraman was not genetically close to Akkaraman but was close to Dağlıç and even to Kıvırcık. NJ tree revealed that Gökçeada is highly differentiated as it posses long branch, from other breeds. As previously explained, Gökçeada's, being an island population, effective population size is small and isolated, therefore it must be genetically drifted.

Karayaka – Akkaraman (with a high bootstrap value: 91) and Morkaraman – İvesi (with relatively high bootstrap value: 78) formed two groups in NJ tree. Members of these pairs are geographically neighboring breeds. It is highly likely that there is a gene flow between them. Gene flows, presence of genetic exchanges / introgression can be seen by the Neighbor Net analysis. The planar network

surrounding Dağlıç possibly represents genetic exchanges of Dağlıç with Morkaraman - İvesi (contribution from Middle Eastern breeds: Akkaraman / Morkaraman, to Dağlıç was also observed by Lenstra and ECONOGENE Consortium (2005)), Kıvırcık, Gökçeada, and even with Karayaka - Akkaraman pair. Those genetic events were not possible to be observed by NJ trees. It must be pointed out Akkaraman- Karayaka pair seemed not to have genetic exchanges. In the light of previous observations (Lenstra and ECONOGENE Consortium, 2005; Koban *et al*., 2008), Akkaraman - Karayaka similarity is another unexpected result of the present study. Karayaka is a well-known thin tailed breed, which is found to be highly related with the European breeds, whereas Akkaraman is fat tailed, Middle Eastern breed (Peter *et al*., 2007).

When genetic relatedness (or differentiation) of the breeds on two dimensions are observed by means of PCA similar groups Akkaraman- Karayaka, and Morkaraman- İvesi are seen. The first two axes represent the 50 % of the total variation that is between the breeds. Hence, the whole picture was not depicted. PCA did not revealed any new level of differentiation between the breeds.

Results of the present study can be examined comparatively with those of the previous ones and some interpretations can be suggested. First, in Koban's PhD Thesis study (2004), the NJ tree shown in Figure 4.1 was obtained from 5 microsatellite markers.

Figure 4.1 NJ tree of purebred Turkish samples based on Nei's D_A from Koban (2004).

In this tree, Morkaraman, İvesi and Kıvırcık are forming a group. However, Dağlıç's position is different than the one in present study. It is in the same group with Karayaka. Furthermore, Akkaraman is not forming a bond with Karayaka. At first glance, one can talk about three reasons for this inconsistency. Dağlıç samples of Koban (2004) were collected nearly 5 years ago, by two independent sampling (therefore, NJ tree shows samples called Dağlıç1 and Dağlıç2), from two slightly different geographic areas. During these years, there could be increasing introgression in Dağlıç breed. It is known that 10 years ago, Dağlıç owners were using Akkaraman males to obtain larger animals. Recently, this practice was abandoned and Kıvırcık males were used for hybridization with Dağlıç in order to meet the demands of market economy (Dr. Bekir Ankaralı, Prof. Dr. Muhittin Özder, personal communication). Furthermore, in the region, Acıpayam hybrid breed existed until very recently. Acıpayam was a hybrid

between İvesi (50 %), Dağlıç (25 %), and Eastern Freeze (25 %). Some of these might be introgressed into Dağlıç, adding İvesi to Dağlıç gene pool. All these must be causing increasing introgression and hence genetic pollution in Dağlıç breed, leading to different results in two studies conducted 5 years apart.

One may raise the question of why, based on the same individuals of the present study, no discrepancy was seen regarding the mtDNA haplogroups (Yüncü, 2009) and between the one conducted by Koban *et al.* (2008). It should be noted here that, mtDNA results tell the history of a species from the maternal side, whereas microsatellites and other autosomal markers are affected by paternal as well as maternal evolutionary history. In African cattle, it is well known that Eastern African cattle are maternally *Bos taurus* yet they have *Zebu* morphology and microsatellite characteristics are again *Zebu* related, because of the repeated introgression of *Zebu* cattle males in East Africa (Hanotte *et al*., 2000). Introgression through rams could have changed the gene pool of Dağlıç in a relatively short time but not changing results based on mtDNA.

NJ consensus-tree of the 57 sheep breeds (Peter *et al*., 2007) indicated that Dağlıç (and Karayaka) are at the root of all European breeds. Moreover, it has very unique mitochondrial DNA haplogroups, such as D and E (Koban *et al*., 2008). Furthermore, as was confirmed by Yüncü (2009) Haplogroup B is the most common among native breeds. This haplogroup is also the most commonly found one in Europe, even being called as European haplogroup. This observation and absence of wild sheep in Europe during domestication (Clutton-Brock, 1981) strongly suggests that the first domestic sheep in Anatolia was carrying haplogroup B (Koban *et al*., 2008). Since Dağlıç and Karayaka showed the highest frequency of haplogroup B (Koban *et al*., 2008; Yüncü, 2009) they might be the closest relatives of the first domesticated sheep, later giving rise to most of the European sheep. In the present study, Dağlıç seems to be the root of NJ tree and despite the fact that it has genetic exchanges with all breeds according to

Neighbor-Net, its F_{ST} value is significantly different from other breeds suggesting that it still proposes itself as the root of many breeds.

If proposed market economy related hybridization scenario is true and if the predictions of the previous studies that Dağlıç is one of the closest descendents of the oldest sheep of Anatolia are valid, in that case, this breed of prime importance in conservation studies is almost lost. It is under the severe threat of heavy hybridization. Dağlıç is under the *in situ* conservation by Ministry of Rural Affairs and Agriculture (MARA). They are conserved as 300 sheep in three farms. Yet, sufficiency of breeding practices, possibility of enlarging the size of populations must be urgently discussed.

Similarly, Karayaka is under *in situ* conservation. Again, an extreme care must be taken for its conservation, because it may have changed in the last 5 years time.

Another possible reason for the differences between the preset and the previous results (Dağlıç is related with many breeds but not Karayaka; Karayaka is higly resembling to Akkaraman; Central - Eastern breeds are not genetically more variable than those of the western Turkish breeds) could be the sampling error (mistakenly obtaining samples that are not representing gene pools of the breeds) in both of the studies. In Turkey there are no herd books kept for sheep breeds, there is no isolation between the breeds because rams are easily exchanged between the breeds. Hence, gene pools of the breeds are not well defined and isolated. There are some guidelines for the morphological characteristics of the breeds. Yet, it is well known that morphology is controlled by the small subset of the genes, a very typical looking individual of the breed may exhibit untypical genetic makeup of the breed as will be discussed below. Unfortunately, there is no other way of choosing the individuals of the breeds. Extreme care should be taken to sample unrelated individuals and from a wide variety of relatively well isolated

area that could represent the distribution of the breed. Otherwise, a complete genetic characterization cannot be achieved.

Another possibility for the observed differences between the studies is that, the results and interpretations will differ when all of the Turkish native sheep breeds and all of the 20 microsatellite loci (after calibration with the standards) will be characterized and analyzed accordingly. Especially in population genetics studies, inconsistencies will arise from not employing enough individuals, breeds or genetic markers. Such a phenomenon was seen previously. Uzun *et al.* (2006). employed Akkaraman, Morkaraman, Karayaka, Hemşin, Tuj and Churra breeds in their study and according to their NJ tree results, they concluded that fat tailed breeds are separated based on microsatellite loci. But, if they had employed Dağlıç in their study, they could easily see that Dağlıç is also fat tailed, yet it does not join to the group, which is composed by other fat-tailed breeds (Peter *et al*., 2007; Koban *et al*., 2008).

As the last part of whole population based analysis, AMOVA, it became evident that the highest proportion of the total variation is found within the individuals. The fourth combination of the AMOVA analysis, compromising İvesi and Morkaraman as one group and the other breeds as separate groups revealed non-significant within population grouping.

As a summary there is a high level of genetic variability within the Turkish breeds (AMOVA), there might be substructuring within the breeds (positive F_{IS}); yet, breeds are significantly different from each other (significant F_{ST} values and when groups of the breeds are assumed within groups became statistically significant). However, genetic differentiation is relatively low between the Turkish breeds (F_{ST} values are relatively low compared to those of European breeds). Genetic exchange between the breeds was suggested (Neighbor Net analysis). Dağlıç, despite being the most introgressed one, still might be exhibiting its uniqueness

(generally high F_{ST} value). Generally, results of the present study are not fully confirming the results of the previous studies possibly due to the evolution of breeds in the last decade and / or wrong sampling and / or error in reading the alleles of microsatellites and / or presence of missing data.

4.3. Individual based analyses

The assignment tests, Factorial Correspondence Analysis (FCA) and Structure analysis provide an opportunity to discover meaningful variance components at the individual level.

According to assignment tests Dağlıç, Gökçeada and İvesi (but especially Dağlıç) seemed to have the minimum inertia and / or least isolated gene pools. Dağlıç is at the center of the breeds as can be seen from FCA. As was discussed above, Neighbor Net analysis revealed that it had genetic exchanges but as suggested by the practices of the farmers these exchanges can be assumed as mostly from other breeds to Dağlıç. Yet, as can also be visualized by Structure analysis with K=4 and K=5 cases, nearly 50 % of Dağlıç individuals were assigned to Dağlıç but not to any other breed. On the other hand, genetic outliers (as defined by the outer 15 % of the individuals based on the genetic distribution of the breed) did not exhibit any morphological feature, which would lead us to be suspicious about the breed identity of the genetically extreme individual. If an introduction from another breed (lets remember that a breed is exhibiting high degree of variability and breeds are not highly differentiated) was made more than two generations ago chromosomes of the foreign individual will be segregated in different individuals. There will be individuals with mixed genetic material in different proportions. We cannot expect an individual that is uniformly exhibiting hybrid chromosomes. Recombination may add to increase the degree of mixture. Assuming that there are nearly 30,000 genes a genetic outlier based on 20 microsatellite loci need not catch an individual with an “atypical” breed look. However, genetic analysis may help to detect and remove highly mixed ones from the gene pool if the gene pool

can be defined by, for instance ‘colors’ in structure and / or a central 50 % distribution by FCA. Outliers determined based on the distributions of breed in FCA were examined with the help of Structure analysis Figure 3.27, Akkaraman outliers (5,10, 26, 30, 44) 10 and 30 seemed to be highly mixed ones. These might be outliers because they might be representing highly introgressed genomes. Can we identify individuals which represent highly mixed ones with the help of Structure (and perhaps with the help of FCA)? What about the other outliers (Akkaraman 5, 26, 44)? These outliers present a more uniform structure, mostly yellow, which seems to be the ‘color’ of Akkaraman. That raises the question: So, why are they outliers? Is it because they are highly homozygote and this fact makes them outliers or do they have private alleles and therefore they become outliers? These questions could not be fully answered yet but Table 4.4 presents a preliminary attempt to understand the effects of these various factors to generate outliers. Private alleles also seemed to contribute to define the outliers. If one can understand the effects of these factors then one may use them to select and purge the highly mixed ones.

Table 4.4 The outlier and 'normal' individuals and their in-depth investigation for assignment test results, private allele existence, homozygote alleles percentage and number of subgroups they manifest in Structure analysis.

Outlier Individual Breed / Number	***Assignment Test Results***	***Private Allele***	***Homozygote %***	***Structure Results***
AKKARAMAN / 5	51 % AKKARAMAN	NONE	58 %	5
AKKARAMAN / 10	87 % AKKARAMAN	NONE	26 %	5
AKKARAMAN / 26	86 % AKKARAMAN	**MAF214:** 200	29 %	4
AKKARAMAN / 30	78 % AKKARAMAN	NONE	42 %	5
AKKARAMAN / 44	70 % AKKARAMAN	NONE	36 %	5
DAĞLIÇ / 9	52 % KIVIRCIK, 41 % DAĞLIÇ	**MAF33:** 116	25 %	5
DAĞLIÇ / 28	43 % DAĞLIÇ	NONE	43 %	5
DAĞLIÇ / 42	20 % DAĞLIÇ	**JMP29:**118	21 %	3
DAĞLIÇ / 44	94 % DAĞLIÇ	NONE	50 %	4
DAĞLIÇ / 46	48 % DAĞLIÇ, 46 % MORKARAMAN	NONE	36 %	5
MORKARAMAN / 6	97 % MORKARAMAN	NONE	23 %	5
MORKARAMAN / 7	86 % MORKARAMAN	NONE	29 %	5
MORKARAMAN / 10	12 % MORKARAMAN	**FCB20**:84	33 %	5
MORKARAMAN / 12	90 % DAĞLIÇ, 85 % MORKARAMAN	NONE	42 %	5
MORKARAMAN / 24	95 % İVESİ, 89 % KIVIRCIK, 85 % MORKARAMAN	NONE	29 %	4

Table 4.4 (Cont'd)

Normal Individual Breed / Number	***Assignment Test Results***	***Private Allele***	***Homozygote %***	***Structure Results***
AKKARAMAN / 18	99 % AKKARAMAN, 98 % MORKARAMAN	NONE	50 %	5
AKKARAMAN / 28	91 % AKKARAMAN	**MAF214:** 194	27 %	5
AKKARAMAN / 34	86 % AKKARAMAN	NONE	38 %	5
AKKARAMAN / 45	86 % KIVIRCIK, 78 % AKKARAMAN	NONE	17 %	5
AKKARAMAN / 49	86 % AKKARAMAN	NONE	28 %	5
DAĞLIÇ / 16	85 % DAĞLIÇ	NONE	20 %	5
DAĞLIÇ / 31	99 % DAĞLIÇ, 97 % DAĞLIÇ, 96 % KIVIRCIK, 94 % AKKARAMAN	NONE	37 %	5
DAĞLIÇ / 33	97 % DAĞLIÇ	NONE	25 %	5
DAĞLIÇ / 45	99 % DAĞLIÇ	NONE	30 %	5
DAĞLIÇ / 47	52 % DAĞLIÇ	NONE	20 %	5
MORKARAMAN / 26	99 % MORKARAMAN	NONE	45 %	5
MORKARAMAN / 28	92 % MORKARAMAN	NONE	25 %	5
MORKARAMAN / 33	96 % DAĞLIÇ, 96 % MORKARAMAN	NONE	30 %	5
MORKARAMAN / 37	74 % MORKARAMAN	NONE	45 %	4
MORKARAMAN / 50	87 % MORKARAMAN	NONE	24 %	4

If we go back to examine breeds individually, Kıvırcık has the highest assignment value (100 %) and all of its members assigned to itself. But few Kıvırcık, as well as the individuals of other breeds, were assigned not only to their original breeds but also to other breeds as well. The results can be found in Table 3.12. There was no significant pattern in assignment of the individuals of the same breed to other breeds. Taking the 20 microsatellite loci into consideration, individuals of most of the breeds can not be discriminated from the others as most of the alleles were shared by all populations with similar probabilities. Private alleles, with their very low frequencies, that did not help in discrimination.

Individuals, analyzed by FCA, did not form independent, isolated groups according to their breed origins. This was true even for Gökçeada, an island population. Overlaps in the genetic distributions, in FCA plots, of some European breeds were observed even for those having complete isolations (Bryne *et al*., in press). Yet, the degree of overlap between the gene pools of the breeds in Turkey was higher. This is a visual implication of the facts that Turkish sheep breeds were never under systematic selection, for example towards higher milk yield and were never fully isolated. They seemed to be isolated by distance with respect to mtDNA (Koban *et al*., 2008; Yüncü, 2009) but not in relation to microsatellites.

Furthermore, Dağlıç, Morkaraman and Akkaraman are decided to be further investigated by FCA in order to see if the highly differentiated individuals are morphologically distinct. Again no such differences could be seen.

Another, study on sheep (Quiroz *et al*., 2008), when analyzed by Structure, revealed that breeds are quite distinctly and uniformly defined in Europe as can be seen by the Figure 4.2. In that study different *K* values were tried sequentially. It must also be tried for Turkish breeds with the hope that another *K* value may provide better distinction between the Turkish breeds. However, this approach requires very long computing time. Therefore, faster computer service will be searched for this data analysis.

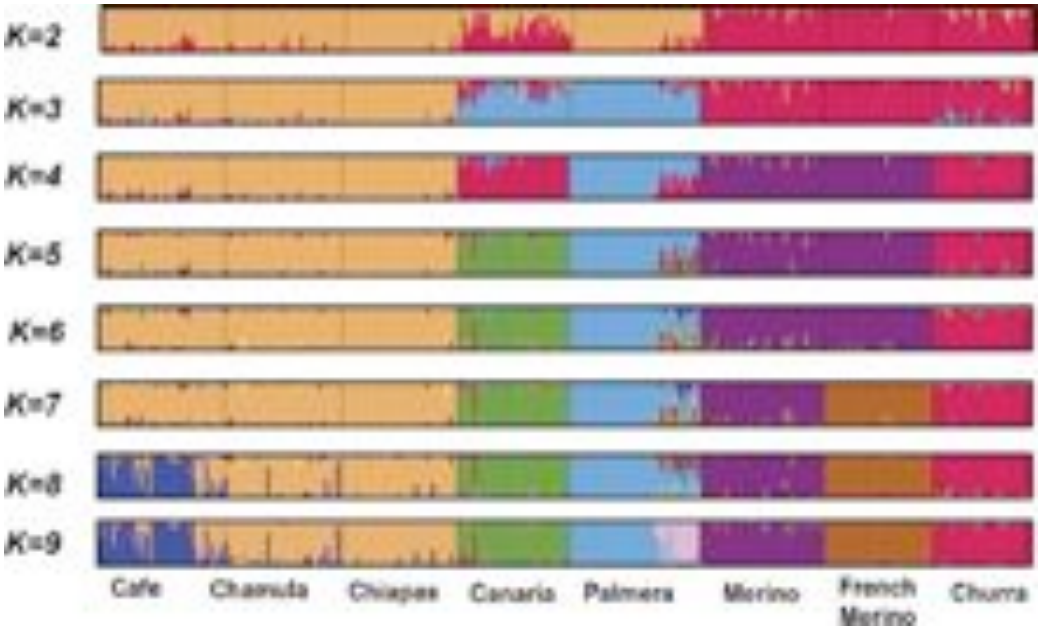


Figure 4.2 Estimation of the population structure with different *K* values from Quiroz *et al.* (2008) study.

As a summary, generally high degree of genetic similarity between the individuals of breeds was observed by the Assignment tests, FCA analysis and Structure analysis. Hence, one cannot talk about the distinct gene pools of the breeds, at least based on the loci employed. Yet, genetically extreme individuals can be identified by the help of these tests. It is observed that these individuals are not exhibiting any morphological signals. Why they are being displayed as extremes must be studied. Highly mixed ones among the extremes can be taken of the gene pool of the breed. Thereby, genetic analysis may directly contribute to the establishment and conservation studies of pure breeds.

4.4. Sequencing of Sheep Mitochondrial DNA (mtDNA) Control Region (CR)

As a part of her master studies, Eren Yüncü (2009) worked with sheep mtDNA. Haplogroup analysis of mtDNA CR region was carried out by Restriction Fragment Length Polymorphism (RFLP), whereas the haplogroup analysis of mtDNA ND4 region was performed by Single Strand Conformation Polymorphism (SSCP) method (Yüncü, 2009).

During RFLP, Akkaraman individual number 46 and Dağlıç individual number 56 showed different patterns than expected. Furthermore, SSCP analysis revealed that Akkaraman individual number 13, 38, Dağlıç individual number 9, 32, 55, and Morkaraman individual 42 were showing different patterns than expected. Figure 4.3 shows the RFLP results of the mtDNA CR after digestion with *NsiI* restriction enzyme. Figure 4.4 shows the conformational polymorphism profiles of unidentified band patterns in Yüncü's study (2009).

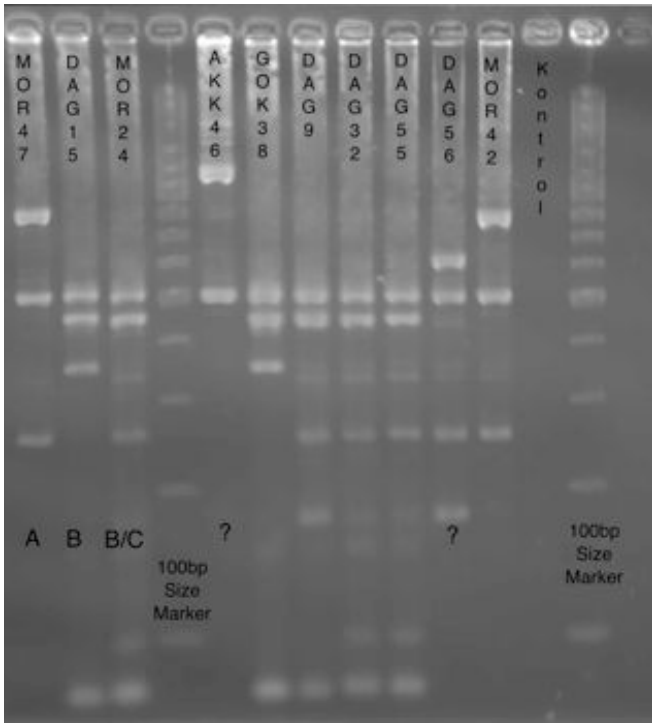


Figure 4.3 Restriction enzyme profiles of mtDNA CR analysis of the unknown band harboring samples both from RFLP and SSCP. Numbers shown above the wells are the DNA sample numbers, abbreviations are breed names and letters at the bottom of the patterns are haplogroups that are represented by digestion profiles above them. Unidentified band patterns were symbolized by "?". The abbreviations are: MOR: Morkaraman, DAG: Dağlıç, AKK: Akkaraman, GOK: Gökçeada.

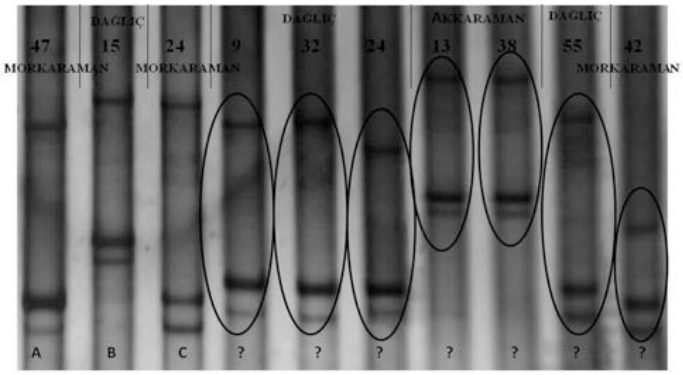


Figure 4.4 Conformational polymorphism profiles of unidentified band patterns taken from Yüncü (2009). Numbers shown above the patterns are the DNA sample numbers, names on the top of the image are the name of the breeds and letters at the bottom of the patterns are haplogroups that are represented by conformational polymorphism profiles above them. A vertical line separates samples that belong to two different breeds. Unidentified band patterns were symbolized by "?" and shown in an ellipse.

The last column of Table 4.5, which was missing in Yüncü's (2009) study, is completed with those sequencing experiments. No new haplogroup or rare haplogroup was observed. There was just one mismatch between the RFLP/or SSCP result and sequencing result, which was observed in relation to Dağlıç 9.

Table 4.5 The RFLP, SSCP and sequence results of the unknown band pattern giving individuals of Yüncü (2009). Unidentified band patterns were symbolized by "?".

Breeds	Individual	SSCP Result	RFLP Result	Sequence Result
Akkaraman	46	HPG-A	?	HPG-A
Gökçeada	38	HPG-C	HPG-B	HPG-B
Dağlıç	9	?	HPG-B/HPG-C	HPG-A
Dağlıç	32	?	HPG-B/HPG-C	HPG-C
Dağlıç	55	?	HPG-B/HPG-C	HPG-C
Dağlıç	56	HPG-A	?	HPG-A
Morkaraman	42	?	HPG-A	HPG-A

The unknown band formations can be due to minor mutations in the recognition sequence of the restriction enzyme *NsiI* or tandem repeats which can exist in various numbers in CR region. The unknown bands in RFLP (Akkaraman 46 and Dağlıç 56) were not identical, yet they gave the same sequence result. It can be concluded that these unknown band patterns are not informative for the haplogroup determination without sequencing according to those preliminary results. More future studies should be conducted. It must also be emphasized that, to be able to catch the rare haplogroups sequencing is required and, unfortunately, large number of sequencing must be done in order to be sure not to miss these rare haplogroups.

CHAPTER 5

CONCLUSION

- Extensive sampling of Kıvırcık, Gökçeada and İvesi breeds revealed that those are the most variable native Turkish breeds. Previous studies (Lenstra and ECONOGENE Consortium, 2005; Koban *et al*., 2008) proposed the direct contrary.

- Karayaka – Akkaraman and Morkaraman – İvesi pairs are genetically close. Dağlıç is related with many breeds, but not with Karayaka (again on the contrary of the previous two studies). Central – Eastern Anatolian breeds are not genetically more variable than those of the western Turkish breeds.

- Dağlıç could be experiencing severe admixture through introgression leading to change in its gene pool. Assuming that Dağlıç is one of the closest descendants of first domesticated sheep in Anatolia, better conservation practices should be implemented for this breed. Otherwise, Dağlıç's precious genetic architecture would be lost.

- Despite the fact that individuals of the breeds present high degree of genetic similarity, nonoverlapping distinct gene pools of the breeds cannot be achieved at least by the 20 microsatellite loci employed.

- Genetically outlier individuals were shown not to exhibit any distinct morphological differences. Yet, genetically extreme individuals can be identified by the help of Structure, Assignment and Factorial Correspondence Analysis tests.

- The combination of morphologic and genetic analyses may directly contribute to the establishment and conservation studies of pure breeds by eliminating the genetically polluted individuals from the gene pool of the breeds.

- Unknown band patterns found as a result of RFLP and SSCP of control region mtDNA by Yüncü's (2009) study were sequenced, but they were shown to belong to the previously defined and the most common haplogroups A, B or C.

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APPENDIX A

CHEMICAL SOLUTIONS USED IN THIS STUDY

1) 10 X Lysis Buffer:
   770 mM NH_4Cl
   46 mM $KHCO_3$
   10 mM EDTA

2) Salt-EDTA Buffer
   75 mM NaCl
   25 mM EDTA

3) Sodium Dodecyl Sulfate
   10 % (w/v) SDS

4) Proteinase-K
   10 mg/ml (w/v) Proteinase K

5) Sodium Acetate (NaAc)
   3 M NaAc

6) Tris-EDTA (TE) Buffer (pH: 7.5)
   10 mM Tris
   1 mM EDTA

7) Sodium Hyroxide (NaOH) Solution

50 mM NaOH

8) Tris-HCl Solution (pH:8)

1M Tris-HCl

9) 5X Tris Borate EDTA (TBE) Buffer (pH: 8.0)

0.45 M Tris (Base)

0.45 M Boric Acid

APPENDIX B

The sequences of forward and reverse primers used for the amplification of 20 microsatellite loci.

Microsattelite Loci	F/R	Sequence (5'-3')
OarHH47	Forward	TTTATTGACAAACTCTCTTCCTAACTCCACC
OarHH47	Reverse	GTAGTTATTTAAAAAAATATCATACCTCTTAAGG
OarCP34	Forward	GCTGAACAATGTGATATGTTCAGG
OarCP34	Reverse	GGGACAATACTGTCTTAGATGCTGC
DYMS1	Forward	AACAACATCAAACAGTAAGAG
DYMS1	Reverse	CATAGTAACAGATCTTCCTACA
JMP58	Forward	GAAGTCATTGAGGGGTCGCTAACC
JMP58	Reverse	CTTCATGTTCACAGGACTTTCTCTG
ILSTS005	Forward	GGAAGCAATGAAATCTATAGCC
ILSTS005	Reverse	TGTTCTGTGAGTTTGTAAGC
MAF33	Forward	GATCTTTGTTTCAATCTATTCCAATTTC
MAF33	Reverse	GATCATCTGAGTGTGAGTATATACAG
MAF214	Forward	AATGCAGGAGATCTGAGGCAGGGACG
MAF214	Reverse	GGGTGATCTTAGGGAGGTTTTGGAGG
ILSTS011	Forward	GCTTGCTACATGGAAAGTGC
ILSTS011	Reverse	CTAAAATGCAGAGCCCTACC
MCM140	Forward	GTTCGTACTTCTGGGTACTGGTCTC
MCM140	Reverse	GTCCATGGATTTGCAGAGTCAG
OarFCB226	Forward	CTATATGTTGCCTTTCCCTTCCTGC
OarFCB226	Reverse	GTGAGTCCCATAGAGCATAAGCTC
OarVH72	Forward	GGCCTCTCAAGGGGCAAGAGCAGG
OarVH72	Reverse	CTCTAGAGGATCTGGAATGCAAAGCTC
OarFCB128	Forward	ATTAAAGCATCTTCTCTTTATTTCCTCGC
OarFCB128	Reverse	CAGCTGAGCAACTAAGACATACATGCG
INRA063	Forward	ATTTGCACAAGCTAAATCTAACC
INRA063	Reverse	AAACCACAGAAATGCTTGGAAG
BM8125	Forward	CTCTATCTGTGGAAAAGGTGGG
BM8125	Reverse	GGGGGTTAGACTTCAACATACG
OarFCB304	Forward	CCCTAGGAGCTTTCAATAAAGAATCGG

OarFCB304	Reverse	CGCTGCTGTCAACTGGGTCAGGG
OarJMP29	Forward	GTATACACGTGGACACCGCTTTGTAC
OarJMP29	Reverse	GAAGTGGCAAGATTCAGAGGGGAAG
OarFCB20	Forward	GGAAAACCCCCATATATACCTATAC
OarFCB20	Reverse	AAATGTGTTTAAGATTCCATACATGTG
OarFCB48	Forward	GACTCTAGAGGATCGCAAAGAACCAG
OarFCB48	Reverse	GAGTTAGTACAAGGATGACAAGAGGCAC
MAF209	Forward	GATCACAAAAAGTTGGATACAACCGTGG
MAF209	Reverse	TCATGCACTTAAGTATGTAGGATGCTG
MAF65	Forward	AAAGGCCAGAGTATGCAATTAGGAG
MAF65	Reverse	CCACTCCTCCTGAGAATATAACATG

APPENDIX C

The data collected in the present study. The first column is the abbreviation of the breed (AKK: Akkaraman, DAĞ: Dağlıç, GÖK: Gökçeada, İVE: İvesi, KRY: Karayaka, KIV: Kıvırcık and MOR: Morkaraman), the second column is the sample number, the remaining 20 columns are for the alleles (in bp) of each sample for each loci used in the study.

	FCB226	116116	116128	152152	116116	116112	094108	126126	000000
	HH47	133137	127141	137145	133141	127133	123133	121121	123133
	VH72	125129	123133	121131	121121	123133	121123	096110	110112
	FCB128	108110	110110	000000	108120	110112	094108	126126	000000
	INRA063	160166	160164	160160	164164	160164	126126	156172	164172
	MAF33	120132	122122	130130	120122	122122	122122	122122	135141
	MAF214	186190	192192	000000	192192	190190	192192	192192	190190
	BM8125	110110	112118	110110	112116	112112	112118	112118	112112
	FCB304	163163	163165	173173	173185	165173	169171	169171	165165
	JMP29	132136	124134	136136	122124	124138	136142	136142	136144
	JMP58	141145	151159	147147	163165	141145	141157	141157	141165
	ILSTS005	192192	192192	200200	184194	198200	000000	000000	192200
	FCB20	000000	000000	000000	126126	094108	126126	126126	000000
	FCB48	000000	147169	147169	147147	125145	147147	147147	141163
	MAF65	121125	125127	129133	125131	125127	127129	127129	121131
	MCM140	178178	174182	172180	180188	184184	174184	174184	184190
	ILSTS011	268268	268268	282282	268282	268282	274282	274282	274280
	OarCP34	112116	112116	116128	108116	116116	110112	110112	112116
	DYMS1	195195	181193	171181	181201	181197	181183	181183	179195
	MAF209	124124	112118	126126	124126	124126	114114	114114	114122

AKK	AKK	AKK	AKK	AKK	AKK	AKK	AKK	AKK	AKK	AKK	AKK	AKK	AKK	AKK	AKK
30	29	28	27	26	24	23	22	21	20	19	18	17	16	15	13
116150	118152	128128	116128	116130	116116	116128	128130	116128	128146	116128	116116	116128	116116	116116	116116
125141	133133	129129	131133	000000	133133	129129	131141	127141	133141	131135	135141	129139	137137	121133	127145
123127	121133	121131	121123	121121	121123	121121	121121	121127	123131	127133	123123	121121	121127	123123	123125
112122	112122	120122	000000	110118	000000	096110	122122	120120	108108	110110	110110	110120	096110	110120	120120
164172	000000	000000	000000	186190	000000	162164	164178	164166	000000	172172	160164	164170	170170	162162	172172
122122	130132	000000	000000	120120	000000	128128	122132	122124	000000	122122	122122	128128	120132	118120	122132
166166	192192	192194	190190	194200	192192	188190	190190	192192	190192	190192	192192	190190	188188	188188	190192
108112	112112	114114	110112	112112	112112	112114	110116	110112	112112	112112	112116	110110	108110	110110	112114
165165	165183	171179	000000	165171	161165	163165	165165	165173	165181	165173	163185	163175	167183	163185	165179
134134	124144	122134	124134	124132	122132	132136	124124	136142	124124	132136	124134	134148	124136	124134	142150
141141	141155	147157	141163	157165	141155	137159	157157	141149	141141	141165	141165	147151	141147	151165	141161
200200	000000	192192	196200	000000	000000	000000	192200	192192	000000	194200	000000	000000	192192	192200	180200
000000	000000	102104	000000	000000	000000	000000	000000	000000	000000	126126	000000	000000	092094	108126	102116
151153	147147	147149	147147	143147	147147	145147	147149	149163	145149	147147	147147	147149	145147	147147	145149
127127	125129	127127	125127	127133	117125	127133	127127	125135	131131	125133	125127	127127	125129	127127	127133
180184	180182	180184	184192	180180	174180	172172	184184	184188	174174	174182	180184	180184	180182	180184	180180
282282	268268	268278	268282	268282	274274	268268	282282	268274	274280	280280	268276	282282	268268	268278	268282
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183187	181195	181181	195197	181185	181195	179201	173197	181195	181185	185195	181185	183187	181195	181181	181185
110124	112122	114122	120124	122124	114116	124124	122124	114124	114122	114126	114114	110124	112122	114122	122124

DAĞ	AKK	AKK	AKK	AKK	AKK	AKK	AKK	AKK	AKK	AKK	AKK	AKK	AKK	AKK	AKK
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000000	127127	121139	131141	133141	133133	133147	129133	131131	000000	127127	129135	131135	129133	129129	129141
000000	121123	121125	123133	121127	123127	121125	121121	123123	123127	125131	123137	121123	121121	121121	121123
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172188	158162	000000	000000	000000	170192	000000	000000	166176	164172	188190	166172	000000	156162	000000	164164
118128	000000	000000	000000	000000	118126	000000	000000	130130	132132	120132	122122	000000	000000	000000	120136
190192	190190	192192	192192	000000	190190	190192	190190	192192	190192	190192	188188	192192	186190	190190	194194
110112	110116	110110	112112	104108	108108	112112	112112	114118	110112	112112	112114	112112	112112	112112	112116
165181	163163	165171	165171	000000	161163	000000	000000	167167	165175	163173	165173	165165	165165	171173	165171
124128	124134	132134	120124	122136	124134	136136	124124	134134	124150	112142	134144	134144	132132	124124	124124
139157	151159	137139	141141	141141	147165	141141	147157	147165	161165	147157	151157	141155	141141	141157	141165
192192	192192	200200	000000	192192	000000	000000	194200	000000	200200	192192	000000	000000	000000	192200	000000
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143149	147169	147171	149163	147149	147155	147169	145147	147147	143149	145171	145147	143163	143145	145169	143145
127129	121131	127129	125127	125131	127129	129133	125127	129129	125125	000000	125127	121125	125127	125129	125125
180184	184190	174184	184184	180188	176188	172180	174180	184184	174180	000000	174182	178178	180186	172184	180190
274274	274280	274282	268282	268282	274274	282282	280280	282282	282282	000000	268268	268268	268282	276282	274282
110110	112116	110112	116116	108116	110112	116128	110110	110110	112116	000000	112116	112116	110116	116118	108108
183197	179195	181183	181197	181201	175181	171181	173185	181185	195201	000000	181193	195195	195201	193193	183185
114114	114122	114114	124126	124126	120126	126126	114114	122122	124124	000000	112118	124124	114126	114124	122124

DAĞ	DAĞ	DAĞ	DAĞ	DAĞ	DAĞ	DAĞ	DAĞ	DAĞ	DAĞ	DAĞ	DAĞ	DAĞ	DAĞ	DAĞ	DAĞ
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121133	127127	133143	000000	131135	131141	127133	000000	131135	000000	129139	121141	000000	129133	129133	119133
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108120	120124	110110	124124	096108	096108	110126	110110	104106	120122	110122	108110	108124	124124	122122	108120
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165165	165167	173173	173181	165173	153165	173191	173173	171171	173185	165165	165165	153165	153189	183185	171171
132134	134142	130132	130142	132134	132134	122134	124134	124134	122132	124136	122132	122124	124134	130134	134134
139165	139159	139165	159165	139161	143147	165165	137139	137139	139159	151159	139155	151157	141151	141147	141147
192194	192200	192196	192192	200200	192194	192192	194198	192192	192192	192200	186192	192200	192200	200200	200200
090094	090098	092108	090108	102102	092094	092094	090100	096108	100102	096102	096106	092092	092104	092102	090102
145147	143147	145147	147163	159165	147149	141145	143147	143147	157165	147161	145147	147165	149163	157163	147165
125127	127135	125127	125127	127131	127129	125127	121125	127135	129129	129129	125131	125127	125125	125131	121129
180182	172180	180190	182182	180192	180182	180180	180182	182186	180184	180184	170170	188188	186190	184184	170180
274274	274282	268274	274278	274282	268282	276282	274282	268268	274278	274282	274274	268278	278282	274278	274276
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179183	187195	189199	183199	181183	185195	183191	183197	187197	185197	181197	183185	183183	181183	183197	181183
114114	114124	114114	114126	120124	108114	112124	112114	114116	112116	114122	114114	114124	116124	124124	114114

DAĞ	DAĞ	DAĞ	DAĞ	DAĞ	DAĞ	DAĞ	DAĞ	DAĞ	DAĞ	DAĞ	DAĞ	DAĞ	DAĞ	DAĞ	DAĞ
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158172	164174	158164	156156	166172	164166	000000	000000	164172	164172	168172	000000	000000	000000	000000	158164
120122	122130	120130	118132	132132	120120	122134	000000	118130	134134	134134	000000	000000	000000	118118	126134
190190	192192	190190	190190	190192	192192	192192	190190	190190	192192	190192	192192	000000	000000	190190	190190
112112	112112	112112	112114	000000	112112	112116	112118	108116	112112	112112	112114	112112	112116	112112	112112
167185	165165	165173	167173	165165	171173	167173	157161	165165	165165	167185	171171	161161	165165	163165	171173
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137139	139147	139165	141151	139139	139159	143143	139145	139155	157159	157159	139165	139157	139147	139155	139157
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127133	127135	127135	125127	125127	125127	127127	000000	125127	127129	125127	127127	127127	125129	127129	125127
182186	178192	182186	186188	180182	176186	174180	000000	182190	180184	182186	184184	182186	180184	182186	180182
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108110	108116	110114	108116	110116	112112	116116	116116	116116	116116	116118	112114	110112	108114	108116	116116
183187	181195	179183	183195	183183	183195	179183	183195	183195	183185	185191	183185	183187	183195	177183	159183
114122	114124	112126	112114	114114	112114	126130	000000	114114	114126	110112	112114	114124	112124	124124	112112

GÖK	GÖK	GÖK	GÖK	GÖK	GÖK	GÖK	DAĞ	DAĞ	DAĞ	DAĞ	DAĞ	DAĞ	DAĞ	DAĞ	DAĞ
20	19	17	12	11	10	2	50	49	48	47	46	45	44	42	38
116148	142152	152156	116152	116136	116148	116148	148156	130136	116116	118128	128128	144144	128136	128142	154154
127135	127129	127133	123133	127129	131133	133139	133141	131133	133133	127131	129133	121127	127127	000000	129129
123129	121123	123123	123129	123129	127129	127133	123135	123123	123131	133137	121127	121129	121133	119131	121135
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188190	000000	000000	190192	190190	000000	190190	190190	190192	190190	190190	000000	190192	188190	192192	190190
112112	112112	000000	110112	112116	000000	114114	110114	112112	110112	108108	000000	112112	112112	108112	112112
000000	000000	000000	173173	173175	000000	173173	167173	171185	173177	163169	000000	165165	173173	181185	165163
126134	124132	120132	124134	124134	124130	124134	130142	134134	134134	134142	136142	124134	124134	118128	130132
141159	149151	141157	137141	141155	141173	141155	139155	147167	139163	139165	163165	139159	151151	135165	157165
200200	188200	192192	192200	188194	188188	200200	192198	192198	184192	192198	192192	192198	198198	192198	192198
102104	088096	108108	104104	000000	000000	094108	090092	094102	088106	094112	090102	096102	092104	088102	092104
143143	143143	145161	145147	141153	143147	147147	147155	147155	147147	145163	145163	147163	145147	137159	147149
127133	127127	127129	125127	125127	127127	000000	127135	129131	125129	125127	133133	127127	119127	125135	127135
180180	192192	180180	182184	188192	184184	000000	180180	182184	176180	180180	172186	180180	184184	182186	190192
268282	268268	268282	268268	268278	282282	000000	274282	000000	268274	282282	282282	274282	282282	268274	274282
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181185	183183	189197	183197	183185	181181	000000	183183	183183	183183	183189	183183	183195	181195	183189	183201
122124	122122	114114	112122	114126	112114	000000	124124	124124	116116	116122	116126	120124	114114	114122	114114

GÖK	GÖK	GÖK	GÖK	GÖK	GÖK	GÖK	GÖK	GÖK	GÖK	GÖK	GÖK	GÖK	GÖK	GÖK	GÖK
42	41	40	39	36	35	34	33	30	27	26	25	24	23	22	21
116148	116128	116116	000000	116116	128152	148152	116116	000000	116116	130146	000000	000000	138146	116128	116154
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İVE	İVE	İVE	İVE	İVE	İVE	İVE	İVE	GÖK	GÖK	GÖK	GÖK	GÖK	GÖK	GÖK	GÖK
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İVE	İVE	İVE	İVE	İVE	İVE	İVE	İVE	İVE	İVE	İVE	İVE	İVE	İVE	İVE	İVE
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KRY	KRY	İVE	İVE	İVE	İVE	İVE	İVE	İVE	İVE	İVE	İVE	İVE	İVE	İVE	İVE
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KRY	KRY	KRY	KRY	KRY	KRY	KRY	KRY	KRY	KRY	KRY	KRY	KRY	KRY	KRY	KRY
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KRY	KRY	KRY	KRY	KRY	KRY	KRY	KRY	KRY	KRY	KRY	KRY	KRY	KRY	KRY	KRY
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KIV	KIV	KIV	KIV	KIV	KIV	KIV	KIV	KIV	KIV	KIV	KIV	KIV	KIV	KIV	KIV
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141141	139141	153157	141151	139141	141157	151165	147157	151159	157159	153157	141153	139151	141157	147165	141169
192192	192192	194202	192192	194194	194202	192194	192194	188194	188192	192200	192200	192200	192192	192192	192198
102102	096104	090104	102102	090102	104108	094104	106118	094094	088090	096096	088090	096096	094112	104104	092098
143149	151153?	145147	149165	147153	145145	155171	147155	147171	143147	153165	143147	153153	147163	145149	143147
115129	131135	125127	119123	127127	125125	125125	000000	121127	127129	123129	123127	125127	127135	121125	115127
178182	190190	174182	172180	176178	174186	186186	000000	186186	190192	174186	186186	186186	182182	192192	182184
270270	276284	000000	268268	280280	268278	268268	000000	268268	270270	280282	276276	276282	272276	268274	282282
112116	110116	108116	108110	108112	108118	110110	000000	116120	114116	112112	112116	108110	108110	108110	110116
181187	183191	000000	183197	000000	183183	183183	000000	000000	187197	187197	187197	000000	187187	183191	000000
114124	116122	114114	114114	114128	112114	114114	000000	114124	114114	114124	112112	112124	114114	114126	114124

MOR	MOR	KIV	KIV	KIV	KIV	KIV	KIV	KIV	KIV	KIV	KIV	KIV	KIV	KIV	KIV
2	1	48	47	44	43	42	41	40	39	38	36	34	33	31	30
116130	116116	146154	116154	116154	116138	116142	116116	116150	128140	116116	116148	116134	116116	000000	116138
000000	131133	127127	135135	131131	127145	135143	127127	133133	129135	000000	133133	131131	137145	000000	133133
123133	123127	121123	123133	121121	121121	123133	123133	123135	121121	135135	123127	127127	121125	000000	121125
000000	000000	110124	000000	110124	000000	110124	128128	000000	000000	112124	000000	122124	122122	110122	110110
000000	000000	158168	000000	172178	000000	158164	168172	000000	000000	160176	000000	160160	166172	158164	156172
000000	000000	134134	000000	120132	000000	122134	132132	000000	000000	128128	000000	120134	118134	118122	126126
188190	192192	192192	000000	192192	188188	190190	190190	000000	190190	166190	000000	190190	190192	192192	164190
112114	104112	112112	114114	112116	112112	112112	114114	000000	112112	112112	000000	112112	112112	112114	110110
165165	165165	165179	171171	165173	171171	165165	163181	000000	171171	165173	000000	165173	167173	173187	171189
136136	132136	122130	132132	132134	132144	132134	134142	112136	112136	130132	126136	000000	124132	134136	124132
157161	161165	141159	141147	139153	151157	155157	137139	141153	141157	157167	157167	000000	137139	157159	141165
194200	192192	194196	192200	190194	194194	192194	194202	192194	192192	196196	194196	000000	194194	192192	188200
092096	096096	094112	094108	096104	104108	090102	096116	108108	102102	092094	096096	000000	088090	088090	096108
147149	147149	147147	161163	155165	147149	147149	147149	147163	147153	149163	143147	000000	147153	147163	147153
127131	121127	000000	127129	000000	123127	127129	119125	125127	111127	131137	123125	123127	121121	121127	125127
186186	186190	000000	174182	000000	186186	186192	172174	182186	180180	180192	180186	182186	186186	186186	182188
276276	276284	000000	270280	000000	268268	278282	274278	270270	276276	280280	268280	000000	274274	270276	270284
110116	112116	000000	112116	000000	110110	112114	108112	108116	116116	108108	112114	108116	108108	108110	110110
000000	183193	000000	183187	000000	183197	183197	187203	183201	199199	000000	183185	183183	173187	183183	197199
112124	112114	000000	124124	000000	114116	124124	000000	126126	114124	112116	122124	112114	112114	112114	122124

MOR 18	MOR 17	MOR 16	MOR 15	MOR 14	MOR 13	MOR 12	MOR 11	MOR 10	MOR 9	MOR 8	MOR 7	MOR 6	MOR 5	MOR 4	MOR 3
116128	000000	116130	116152	128142	126142	136146	116140	116116	116116	116150	116116	116144	116128	000000	000000
143147	000000	133133	129141	133133	133133	133133	127133	133139	129133	133133	119133	131133	119133	000000	000000
123123	000000	123123	127127	123127	135135	123129	123135	129129	129135	127127	121125	123129	123123	000000	000000
108108	110122	096108	096096	110110	130130	120120	114122	000000	112120	112112	000000	000000	112112	000000	110110
172172	166176	156170	164158	164172	164168	160164	164164	000000	158164	172172	000000	000000	166176	164164	164164
118132	118132	118118	000000	120132	000000	000000	118134	000000	122134	120134	000000	000000	122122	000000	122134
192192	192192	190190	192192	192192	192192	190190	190190	000000	000000	192192	000000	190192	192192	192192	192192
112112	112112	112112	112112	114114	112112	112112	112116	108116	112112	112112	000000	112112	112112	112112	112112
165165	165165	165173	163163	165183	177177	171185	153165	165171	165165	163163	000000	149149	165165	165165	165175
128130	128132	128128	130142	114124	122124	134134	122136	114116	122134	122134	124134	132136	132134	132136	130134
137139	141159	147163	137139	147153	141151	141165	139165	135137	147155	147155	141155	151159	141155	141141	141159
192194	192192	198198	192212	186194	192200	192192	192194	194194	188194	188194	192192	188192	192192	192192	192200
108116	092104	100112	092092	080080	092108	088106	090102	082094	094096	094096	094120	096108	096102	096096	104104
141153	145147	159161	143147	133135	147161	147153	143143	151153	149149	147149	145147	145147	147149	147153	147147
000000	125125	117125	125127	125129	129133	125127	127127	127127	125129	127127	121125	125127	119119	127127	125127
000000	190194	182186	182182	182190	186188	182182	176182	184190	182184	182186	174184	182182	182184	172188	180192
000000	274282	276282	274274	270282	268274	268274	282282	274282	276276	270284	276280	274274	284284	276284	282282
000000	112114	110110	112116	108116	110116	110114	108112	110112	108116	108116	116116	110112	112114	110110	116116
000000	189189	185185	189199	193197	181197	183197	181183	000000	183197	181181	173181	183191	183183	181181	197197
000000	114116	112124	114114	114124	114120	114114	114116	110114	114116	114124	114114	114114	114114	114124	114126

MOR	MOR	MOR	MOR	MOR	MOR	MOR	MOR	MOR	MOR	MOR	MOR	MOR	MOR	MOR	MOR
34	33	32	31	30	29	28	27	26	25	24	23	22	21	20	19
134150	116128	128128	116148	116128	128150	128150	116148	116116	116142	000000	116116	130148	116128	116116	128150
133133	133141	131133	127135	127133	133133	119141	133141	127135	133145	000000	119127	131135	127131	119131	131141
123127	127133	131133	117121	123127	125131	123133	123133	123123	121123	000000	121135	123123	125133	123131	121137
108110	122122	122122	108120	108120	110110	108124	120120	118118	108108	108110	108110	110120	122122	96096	96096
172176	158164	164164	156178	164164	164178	174174	164164	156164	166166	172176	164172	166172	172172	168168	158164
120132	122122	134134	120132	118118	120130	118134	120134	118118	120120	118118	120134	120132	122132	118132	118128
190192	190190	192192	000000	192192	192192	192192	190190	192192	192192	190190	192192	190190	190190	190190	190190
112112	112112	108112	112112	112112	112112	112112	112116	112112	108112	108112	112112	112112	112112	112116	112116
173181	165165	183183	165165	165165	157165	165181	163163	165173	149165	165165	161173	167175	165167	181191	147147
132136	134134	132136	130132	130134	132132	134142	136144	132142	134134	132132	132132	132154	132134	132142	132136
141167	157165	139165	147165	137145	141151	139145	147165	141157	155165	139141	157165	139155	145149	141157	141155
192192	198202	192204	192200	192194	192192	200200	192192	192212	192192	192196	192204	192192	192196	198200	192194
106108	102102	092100	084104	084092	096096	094106	100104	096108	102106	094096	094106	102104	096104	088102	096108
145147	145163	147163	145171	147161	143147	147149	145149	147163	143147	145147	143147	149163	145147	149153	143147
121127	119129	127127	125127	129129	127127	129135	129135	135137	127129	119123	000000	127133	131139	127129	125127
182184	182184	184188	180184	180180	180184	180186	180192	182182	184188	182182	000000	176180	186188	184190	184186
274282	274282	274282	268274	280282	278282	268282	276276	270274	274274	278284	000000	268282	270276	274282	276284
114116	116118	110116	110116	108114	110116	110116	112116	112116	112114	112116	000000	108114	112114	110116	110116
179181	185197	000000	181197	183197	173181	197197	183183	197197	193197	159183	000000	183193	183183	197197	197197
112114	112114	112114	114124	116124	114116	114122	116116	114114	126126	112114	000000	114124	118124	112114	114122

MOR	MOR	MOR	MOR	MOR	MOR	MOR	MOR	MOR	MOR	MOR	MOR	MOR	MOR	MOR	MOR
50	49	48	47	46	45	44	43	42	41	40	39	38	37	36	35
000000	000000	000000	000000	000000	000000	116116	116146	116130	116116	128128	116128	128128	116116	000000	116128
000000	000000	000000	000000	000000	000000	127127	119133	131131	127141	129135	127133	141141	125141	000000	127133
000000	000000	000000	000000	000000	000000	121133	123133	127131	123133	121133	127127	121131	121133	000000	123127
108122	110122	120122	108122	096110	096126	000000	096122	110110	120120	96096	122122	000000	122124	120120	000000
164172	170172	172176	168172	164170	164164	000000	160164	156164	168172	164170	164168	000000	172172	158170	000000
118000	118118	120120	130134	134134	120132	000000	132136	120128	118132	118118	118118	000000	120132	130134	000000
190192	188188	192192	000000	190192	192192	190192	000000	190192	190192	192192	192192	190192	192192	192192	190190
112112	112112	116116	112112	112112	112112	112112	112116	108112	112114	112116	108112	112116	112116	114114	112116
165165	149163	165181	155165	163165	165165	173000	167173	163165	165165	165183	167167	163183	165165	165165	165165
122122	126142	128134	124132	124132	122132	124132	130132	122134	124134	136142	124134	136142	128130	132142	132136
141147	141141	141141	141147	139141	139141	141147	139159	139147	137139	161165	151157	141159	137137	141159	141155
200200	192192	192200	194194	186192	192212	192200	192200	192200	192200	192192	192192	192194	192192	192192	192200
102104	090108	104108	102108	090092	094096	096096	090100	100102	090100	106108	088092	102102	086086	092108	098116
145149	145147	147149	143145	149163	145147	149163	151163	141147	157163	147149	139147	149165	157159	145149	147167
127135	127129	127127	127127	125127	125127	127135	119127	125127	127129	125127	125127	129135	127127	125129	125133
170178	172186	182186	178190	178188	180188	184190	180180	180186	184188	170188	180182	162180	180180	186188	182186
270282	274282	274282	270270	274274	274282	268282	274282	268274	000000	270284	274282	276276	274282	276276	274282
110118	108112	108110	114116	112122	116118	108110	110116	110112	116116	116116	110116	112114	112118	110110	110116
173183	000000	181197	181197	173183	173183	183197	183193	181187	000000	197197	181183	197197	191193	183193	183199
114116	112114	118124	114114	114122	114122	124124	114124	116124	112114	114124	114124	124124	126126	122128	112114

APPENDIX D

The results of the assignment tests. The first column is the breed name and sample number. The next 7 columns are the probability of assignment of the individual in the first column to the breeds specified. The abbreviations are AKK: Akkaraman, DAĞ: Dağlıç, GÖK: Gökçeada, İVE: İvesi, KRY: Karayaka, KIV: Kıvırcık and MOR: Morkaraman. The last column is the number of loci used for the assignment tests.

	AKK	DAĞ	GÖK	İVE	KRY	KIV	MOR	Nb. of loci
Assigned sample	probability							
AKKARAMAN/3	0.817	0.031	0.002	0.007	0.260	0.437	0.041	16
AKKARAMAN/4	0.991	0.408	0.222	0.419	0.785	0.808	0.502	17
AKKARAMAN/5	0.509	0.013	0.001	0.000	0.029	0.003	0.000	16
AKKARAMAN/7	0.999	0.332	0.002	0.083	0.195	0.140	0.269	18
AKKARAMAN/8	0.999	0.556	0.685	0.570	0.932	0.640	0.593	18
AKKARAMAN/9	0.992	0.165	0.043	0.054	0.816	0.285	0.101	17
AKKARAMAN/10	0.872	0.415	0.004	0.728	0.427	0.543	0.679	17
AKKARAMAN/12	0.401	0.003	0.001	0.000	0.003	0.213	0.020	17
AKKARAMAN/13	0.970	0.062	0.036	0.008	0.232	0.158	0.175	18
AKKARAMAN/15	0.993	0.469	0.036	0.008	0.294	0.182	0.044	18
AKKARAMAN/16	0.944	0.053	0.047	0.017	0.236	0.350	0.185	18
AKKARAMAN/17	0.796	0.073	0.053	0.007	0.405	0.196	0.084	16
AKKARAMAN/18	0.998	0.843	0.311	0.823	0.972	0.938	0.986	16
AKKARAMAN/19	0.988	0.071	0.014	0.005	0.478	0.356	0.138	18
AKKARAMAN/20	0.754	0.216	0.179	0.027	0.538	0.528	0.573	14
AKKARAMAN/21	0.958	0.436	0.300	0.105	0.971	0.522	0.737	17
AKKARAMAN/22	0.998	0.787	0.530	0.396	0.852	0.721	0.670	17
AKKARAMAN/23	0.898	0.550	0.091	0.003	0.358	0.517	0.238	15
AKKARAMAN/24	0.783	0.643	0.234	0.288	0.702	0.570	0.976	14
AKKARAMAN/26	0.861	0.130	0.226	0.018	0.167	0.223	0.040	15
AKKARAMAN/27	0.990	0.705	0.906	0.704	0.556	0.628	0.518	14
AKKARAMAN/28	0.905	0.285	0.044	0.023	0.225	0.406	0.154	16
AKKARAMAN/29	0.760	0.312	0.143	0.161	0.214	0.513	0.589	15

AKKARAMAN/30	0.784	0.108	0.244	0.424	0.183	0.171	0.461	17
AKKARAMAN/31	0.904	0.216	0.137	0.166	0.402	0.362	0.394	16
AKKARAMAN/32	0.971	0.394	0.523	0.533	0.578	0.601	0.378	15
AKKARAMAN/33	0.967	0.817	0.550	0.792	0.964	0.923	0.827	15
AKKARAMAN/34	0.767	0.150	0.176	0.469	0.510	0.864	0.712	14
AKKARAMAN/35	0.779	0.167	0.201	0.077	0.406	0.757	0.155	16
AKKARAMAN/37	0.920	0.215	0.017	0.000	0.236	0.483	0.118	13
AKKARAMAN/38	0.997	0.225	0.382	0.110	0.785	0.529	0.522	17
AKKARAMAN/39	0.662	0.271	0.222	0.069	0.319	0.318	0.439	16
AKKARAMAN/40	0.975	0.638	0.804	0.437	0.899	0.966	0.440	14
AKKARAMAN/44	0.703	0.024	0.023	0.027	0.308	0.050	0.225	13
AKKARAMAN/45	0.199	0.131	0.002	0.004	0.111	0.043	0.074	16
AKKARAMAN/46	0.948	0.206	0.275	0.123	0.478	0.410	0.787	14
AKKARAMAN/47	0.825	0.372	0.438	0.734	0.764	0.449	0.704	14
AKKARAMAN/48	0.645	0.255	0.072	0.019	0.461	0.360	0.068	15
AKKARAMAN/49	0.856	0.413	0.164	0.052	0.504	0.404	0.100	16
DAĞLIÇ/1	0.846	0.852	0.174	0.018	0.671	0.732	0.552	15
DAĞLIÇ/2	0.300	0.762	0.046	0.228	0.447	0.817	0.709	16
DAĞLIÇ/3	0.096	0.352	0.031	0.007	0.064	0.042	0.040	17
DAĞLIÇ/4	0.344	0.775	0.305	0.135	0.544	0.801	0.399	18
DAĞLIÇ/5	0.246	0.774	0.089	0.100	0.282	0.472	0.259	17
DAĞLIÇ/6	0.070	0.442	0.000	0.017	0.019	0.017	0.211	18
DAĞLIÇ/7	0.940	0.911	0.928	0.384	0.795	0.718	0.717	18
DAĞLIÇ/8	0.063	0.701	0.003	0.027	0.057	0.279	0.274	16
DAĞLIÇ/9	0.127	0.409	0.004	0.000	0.105	0.524	0.080	18
DAĞLIÇ/10	0.355	0.870	0.176	0.012	0.422	0.714	0.592	17
DAĞLIÇ/11	0.838	0.788	0.040	0.465	0.624	0.677	0.828	18
DAĞLIÇ/12	0.325	0.971	0.207	0.319	0.690	0.228	0.530	18
DAĞLIÇ/13	0.124	0.655	0.057	0.185	0.153	0.161	0.138	17
DAĞLIÇ/14	0.520	0.894	0.326	0.341	0.482	0.880	0.870	16
DAĞLIÇ/15	0.085	0.968	0.200	0.231	0.561	0.725	0.710	18
DAĞLIÇ/16	0.148	0.853	0.028	0.001	0.101	0.085	0.256	18
DAĞLIÇ/17	0.850	0.990	0.113	0.720	0.725	0.822	0.794	17
DAĞLIÇ/18	0.872	1.000	0.559	0.807	0.960	0.998	0.917	18
DAĞLIÇ/19	0.852	0.870	0.095	0.420	0.730	0.944	0.784	17
DAĞLIÇ/20	0.600	0.768	0.390	0.696	0.617	0.736	0.898	15
DAĞLIÇ/21	0.274	0.938	0.064	0.227	0.312	0.244	0.490	15
DAĞLIÇ/23	0.426	0.759	0.186	0.684	0.287	0.833	0.927	15
DAĞLIÇ/24	0.586	0.983	0.266	0.110	0.747	0.894	0.965	18
DAĞLIÇ/25	0.549	0.986	0.616	0.695	0.903	0.963	0.961	18
DAĞLIÇ/27	0.610	0.987	0.092	0.534	0.626	0.535	0.806	18
DAĞLIÇ/28	0.091	0.426	0.008	0.054	0.044	0.018	0.096	12
DAĞLIÇ/29	0.108	0.639	0.094	0.008	0.179	0.260	0.254	17
DAĞLIÇ/30	0.043	0.552	0.002	0.066	0.104	0.239	0.449	18
DAĞLIÇ/31	0.940	0.997	0.563	0.840	0.920	0.957	0.973	17
DAĞLIÇ/32	0.761	0.845	0.133	0.303	0.465	0.897	0.945	18
DAĞLIÇ/33	0.119	0.968	0.097	0.121	0.418	0.757	0.318	18
DAĞLIÇ/36	0.469	0.780	0.367	0.231	0.542	0.519	0.787	18
DAĞLIÇ/37	0.022	0.733	0.012	0.014	0.168	0.268	0.312	18

DAĞLIÇ/38	0.242	0.861	0.040	0.445	0.385	0.901	0.433	18
DAĞLIÇ/42	0.000	0.197	0.000	0.000	0.001	0.000	0.023	17
DAĞLIÇ/44	0.607	0.940	0.700	0.024	0.652	0.581	0.545	18
DAĞLIÇ/45	0.740	0.991	0.170	0.351	0.259	0.381	0.647	18
DAĞLIÇ/46	0.437	0.483	0.000	0.017	0.207	0.060	0.457	13
DAĞLIÇ/47	0.250	0.522	0.000	0.000	0.030	0.213	0.074	18
DAĞLIÇ/48	0.230	0.835	0.269	0.007	0.690	0.193	0.242	18
DAĞLIÇ/49	0.633	0.782	0.058	0.214	0.562	0.367	0.373	15
DAĞLIÇ/50	0.097	0.863	0.002	0.004	0.548	0.467	0.171	18
GÖKÇEADA/2	0.949	0.850	0.900	0.135	0.939	0.719	0.347	13
GÖKÇEADA/10	0.041	0.140	0.981	0.426	0.216	0.363	0.538	12
GÖKÇEADA/11	0.262	0.238	0.848	0.004	0.237	0.437	0.131	17
GÖKÇEADA/12	0.983	0.691	0.999	0.247	0.559	0.811	0.537	18
GÖKÇEADA/17	0.468	0.294	0.939	0.241	0.353	0.219	0.484	13
GÖKÇEADA/19	0.177	0.054	0.891	0.061	0.141	0.230	0.112	14
GÖKÇEADA/20	0.604	0.324	0.944	0.103	0.431	0.522	0.470	15
GÖKÇEADA/21	0.897	0.485	0.971	0.236	0.763	0.830	0.180	18
GÖKÇEADA/22	0.987	0.961	1.000	0.690	0.990	0.998	0.724	17
GÖKÇEADA/23	0.048	0.338	0.887	0.217	0.489	0.914	0.343	16
GÖKÇEADA/24	0.827	0.548	0.973	0.446	0.890	0.828	0.692	15
GÖKÇEADA/25	0.561	0.138	0.966	0.045	0.753	0.873	0.262	15
GÖKÇEADA/26	0.295	0.205	0.946	0.083	0.480	0.489	0.161	17
GÖKÇEADA/27	0.780	0.404	0.960	0.059	0.225	0.708	0.696	18
GÖKÇEADA/30	0.725	0.568	0.954	0.000	0.890	0.950	0.232	15
GÖKÇEADA/33	0.632	0.376	0.935	0.394	0.679	0.672	0.556	18
GÖKÇEADA/34	0.530	0.330	0.997	0.052	0.449	0.288	0.059	18
GÖKÇEADA/35	0.576	0.278	0.789	0.026	0.445	0.118	0.140	18
GÖKÇEADA/36	0.874	0.702	0.997	0.428	0.780	0.992	0.834	13
GÖKÇEADA/39	0.750	0.641	0.996	0.065	0.889	0.661	0.252	15
GÖKÇEADA/40	0.902	0.556	0.998	0.694	0.930	0.863	0.602	18
GÖKÇEADA/41	0.536	0.813	0.998	0.113	0.832	0.976	0.884	18
GÖKÇEADA/42	0.227	0.224	0.945	0.004	0.533	0.450	0.108	18
GÖKÇEADA/43	0.961	0.929	0.999	0.627	0.942	0.952	0.881	18
GÖKÇEADA/44	0.042	0.161	0.928	0.000	0.084	0.269	0.113	18
GÖKÇEADA/45	0.694	0.749	1.000	0.084	0.375	0.903	0.555	13
GÖKÇEADA/46	0.902	0.977	0.997	0.680	1.000	0.995	0.865	13
GÖKÇEADA/47	0.109	0.777	0.939	0.085	0.808	0.795	0.522	13
GÖKÇEADA/48	0.305	0.641	0.855	0.229	0.931	0.818	0.725	13
GÖKÇEADA/49	0.403	0.577	0.972	0.050	0.443	0.610	0.354	13
GÖKÇEADA/50	0.685	0.955	0.978	0.523	0.867	0.999	0.772	13
İVESİ/1	0.238	0.170	0.025	0.990	0.223	0.524	0.579	18
İVESİ/2	0.015	0.117	0.002	0.472	0.158	0.023	0.073	17
İVESİ/4	0.349	0.293	0.027	0.981	0.291	0.612	0.703	18
İVESİ/5	0.537	0.123	0.294	0.786	0.283	0.284	0.057	17
İVESİ/7	0.670	0.647	0.072	0.957	0.547	0.660	0.816	17
İVESİ/9	0.737	0.577	0.144	0.977	0.596	0.606	0.664	15
İVESİ/10	0.008	0.024	0.002	0.924	0.001	0.316	0.096	17
İVESİ/12	0.022	0.015	0.000	0.443	0.020	0.046	0.122	18
İVESİ/14	0.035	0.033	0.025	0.541	0.100	0.324	0.179	18

İVESİ/15	0.169	0.083	0.036	0.631	0.397	0.384	0.404	18
İVESİ/16	0.370	0.383	0.002	0.570	0.141	0.113	0.575	15
İVESİ/17	0.656	0.076	0.039	0.795	0.340	0.455	0.278	17
İVESİ/18	0.104	0.168	0.002	0.924	0.054	0.426	0.186	17
İVESİ/19	0.325	0.150	0.091	0.968	0.450	0.192	0.298	18
İVESİ/20	0.045	0.047	0.000	0.665	0.005	0.234	0.462	18
İVESİ/23	0.695	0.199	0.002	0.522	0.346	0.414	0.282	17
İVESİ/24	0.982	0.921	0.866	0.956	0.861	0.964	0.969	17
İVESİ/26	0.064	0.093	0.001	0.903	0.067	0.405	0.832	16
İVESİ/27	0.787	0.662	0.464	0.979	0.745	0.593	0.620	18
İVESİ/28	0.812	0.188	0.035	0.988	0.327	0.576	0.622	18
İVESİ/30	0.153	0.231	0.013	0.906	0.314	0.508	0.436	18
İVESİ/31	0.916	0.885	0.514	0.997	0.753	0.579	0.910	16
İVESİ/32	0.123	0.201	0.002	0.948	0.149	0.405	0.426	16
İVESİ/33	0.069	0.183	0.009	0.833	0.202	0.358	0.206	16
İVESİ/34	0.890	0.784	0.533	0.878	0.656	0.876	0.623	17
İVESİ/35	0.229	0.078	0.001	0.887	0.178	0.337	0.334	17
İVESİ/36	0.005	0.041	0.000	0.816	0.020	0.024	0.183	17
İVESİ/37	0.678	0.434	0.151	0.928	0.703	0.295	0.455	14
İVESİ/38	0.191	0.644	0.330	0.992	0.355	0.973	0.957	15
İVESİ/39	0.498	0.276	0.011	0.786	0.387	0.485	0.200	17
İVESİ/40	0.364	0.415	0.391	0.994	0.486	0.775	0.714	15
İVESİ/41	0.011	0.033	0.000	0.315	0.002	0.167	0.163	18
İVESİ/42	0.009	0.031	0.000	0.744	0.018	0.122	0.083	17
İVESİ/43	0.603	0.636	0.767	0.981	0.624	0.963	0.825	17
İVESİ/44	0.731	0.150	0.115	0.966	0.389	0.771	0.555	16
İVESİ/46	0.249	0.240	0.264	0.885	0.375	0.833	0.630	16
İVESİ/48	0.022	0.100	0.000	0.876	0.180	0.381	0.550	18
İVESİ/50	0.114	0.144	0.115	0.908	0.511	0.338	0.595	17
KARAYAKA/2	0.364	0.197	0.194	0.589	0.974	0.468	0.507	16
KARAYAKA/4	0.926	0.458	0.316	0.343	0.998	0.748	0.709	17
KARAYAKA/5	0.866	0.394	0.274	0.139	0.952	0.562	0.413	17
KARAYAKA/6	0.168	0.164	0.014	0.032	0.826	0.394	0.164	18
KARAYAKA/7	0.141	0.291	0.073	0.028	0.860	0.312	0.137	13
KARAYAKA/8	0.978	0.428	0.044	0.130	0.958	0.715	0.580	13
KARAYAKA/9	0.666	0.134	0.014	0.156	0.932	0.628	0.734	18
KARAYAKA/10	0.223	0.692	0.043	0.538	0.891	0.557	0.239	17
KARAYAKA/11	0.751	0.236	0.063	0.415	0.979	0.532	0.609	13
KARAYAKA/12	0.628	0.594	0.335	0.044	0.967	0.471	0.164	18
KARAYAKA/13	0.256	0.100	0.000	0.013	0.867	0.037	0.138	17
KARAYAKA/14	0.275	0.288	0.135	0.021	0.660	0.091	0.155	15
KARAYAKA/15	0.269	0.024	0.067	0.089	0.912	0.367	0.043	16
KARAYAKA/16	0.990	0.147	0.505	0.288	0.998	0.707	0.812	17
KARAYAKA/17	0.665	0.221	0.318	0.007	0.981	0.422	0.117	18
KARAYAKA/18	0.192	0.052	0.000	0.000	0.548	0.079	0.041	18
KARAYAKA/19	0.688	0.624	0.822	0.260	0.982	0.722	0.784	18
KARAYAKA/20	0.720	0.760	0.701	0.518	0.926	0.695	0.674	17
KARAYAKA/21	0.454	0.242	0.083	0.019	0.982	0.140	0.247	18
KARAYAKA/22	0.073	0.022	0.002	0.001	0.584	0.450	0.117	18

KARAYAKA/23	0.988	0.776	0.867	0.851	0.996	0.993	0.975	18
KARAYAKA/24	0.544	0.651	0.060	0.652	0.973	0.751	0.536	18
KARAYAKA/25	0.853	0.416	0.447	0.777	0.982	0.887	0.855	17
KARAYAKA/27	0.408	0.540	0.569	0.373	0.993	0.887	0.505	18
KARAYAKA/28	0.812	0.869	0.626	0.618	0.970	0.791	0.712	15
KARAYAKA/29	0.211	0.345	0.183	0.033	0.862	0.400	0.113	17
KARAYAKA/30	0.092	0.520	0.476	0.279	0.819	0.334	0.387	14
KARAYAKA/31	0.801	0.529	0.409	0.117	0.973	0.615	0.651	14
KARAYAKA/32	0.418	0.785	0.162	0.368	0.982	0.908	0.880	18
KARAYAKA/33	0.876	0.591	0.562	0.061	0.868	0.788	0.091	13
KARAYAKA/34	0.030	0.022	0.062	0.001	0.705	0.165	0.173	14
KARAYAKA/36	0.990	0.809	0.787	0.553	0.994	0.834	0.978	15
KARAYAKA/37	0.893	0.821	0.662	0.678	0.890	0.484	0.882	13
KARAYAKA/39	0.074	0.077	0.052	0.025	0.705	0.211	0.463	13
KARAYAKA/40	0.122	0.075	0.009	0.050	0.462	0.790	0.150	15
KARAYAKA/41	0.722	0.504	0.711	0.509	0.999	0.951	0.764	17
KARAYAKA/42	0.891	0.731	0.318	0.633	0.986	0.655	0.575	15
KARAYAKA/43	0.769	0.288	0.415	0.023	0.978	0.707	0.160	18
KARAYAKA/44	0.753	0.431	0.206	0.607	0.931	0.622	0.406	13
KARAYAKA/45	0.988	0.805	0.899	0.975	1.000	0.997	0.989	18
KARAYAKA/46	0.799	0.575	0.515	0.078	0.997	0.743	0.275	17
KARAYAKA/47	0.404	0.593	0.139	0.025	0.839	0.649	0.455	13
KARAYAKA/48	0.229	0.150	0.194	0.242	0.986	0.582	0.298	17
KARAYAKA/49	0.540	0.317	0.585	0.181	0.973	0.391	0.184	13
KIVIRCIK/1	0.200	0.173	0.048	0.044	0.632	0.972	0.309	17
KIVIRCIK/3	0.633	0.323	0.322	0.140	0.478	0.949	0.296	16
KIVIRCIK/4	0.948	0.853	0.387	0.928	0.820	0.961	0.538	16
KIVIRCIK/5	0.144	0.447	0.062	0.399	0.172	0.995	0.487	17
KIVIRCIK/6	0.468	0.443	0.684	0.046	0.747	0.992	0.707	18
KIVIRCIK/7	0.003	0.026	0.003	0.019	0.030	0.564	0.123	13
KIVIRCIK/9	0.042	0.041	0.036	0.006	0.081	0.698	0.235	16
KIVIRCIK/10	0.929	0.508	0.285	0.123	0.362	0.932	0.593	18
KIVIRCIK/11	0.523	0.541	0.327	0.134	0.541	0.991	0.875	16
KIVIRCIK/12	0.213	0.343	0.385	0.319	0.681	0.998	0.458	18
KIVIRCIK/13	0.013	0.116	0.075	0.556	0.434	0.868	0.620	16
KIVIRCIK/14	0.036	0.035	0.065	0.072	0.292	0.979	0.420	13
KIVIRCIK/15	0.043	0.332	0.196	0.086	0.054	0.998	0.505	18
KIVIRCIK/16	0.359	0.208	0.063	0.074	0.320	0.965	0.174	18
KIVIRCIK/17	0.100	0.111	0.006	0.056	0.032	0.645	0.160	13
KIVIRCIK/19	0.150	0.078	0.001	0.019	0.183	0.814	0.153	16
KIVIRCIK/20	0.552	0.375	0.267	0.257	0.196	0.960	0.271	17
KIVIRCIK/21	0.030	0.231	0.354	0.010	0.690	0.965	0.368	18
KIVIRCIK/22	0.039	0.052	0.001	0.003	0.305	0.563	0.049	16
KIVIRCIK/24	0.290	0.265	0.058	0.085	0.257	0.994	0.538	17
KIVIRCIK/28	0.092	0.165	0.162	0.117	0.280	0.896	0.414	16
KIVIRCIK/29	0.568	0.096	0.025	0.363	0.495	0.992	0.780	18
KIVIRCIK/30	0.029	0.049	0.002	0.058	0.162	0.581	0.017	18
KIVIRCIK/31	0.148	0.386	0.073	0.121	0.677	0.999	0.505	15
KIVIRCIK/33	0.151	0.332	0.004	0.059	0.186	0.944	0.445	18

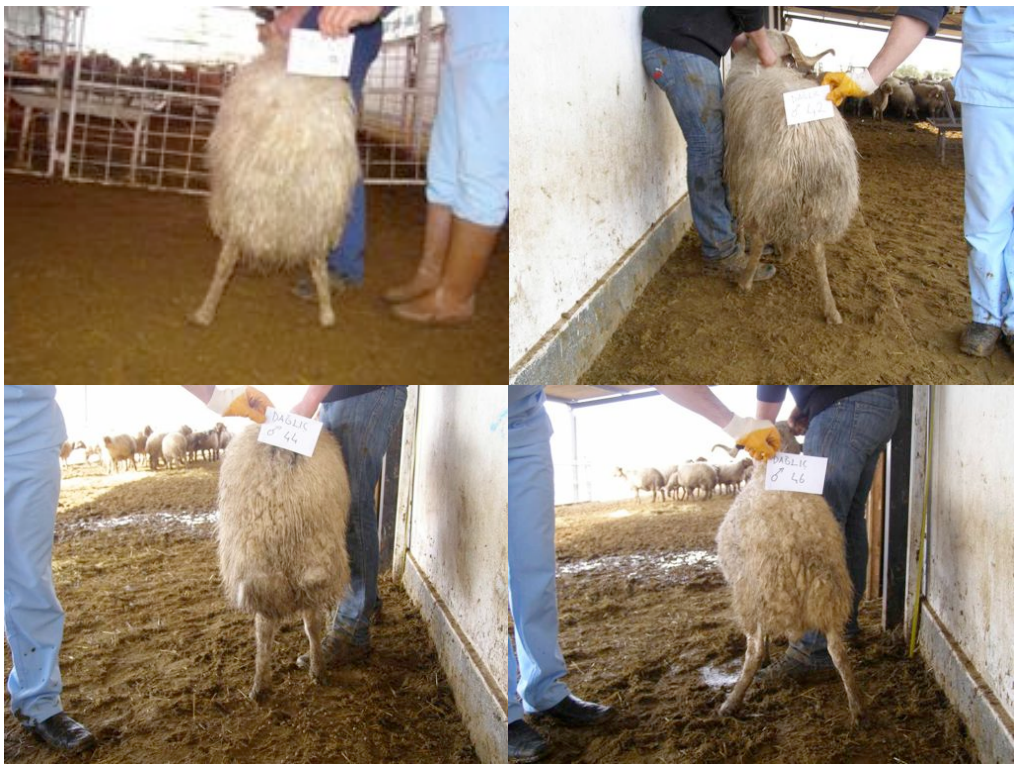
KIVIRCIK/34	0.183	0.739	0.472	0.440	0.698	1.000	0.801	12
KIVIRCIK/36	0.051	0.037	0.073	0.054	0.287	0.828	0.426	13
KIVIRCIK/38	0.002	0.006	0.000	0.002	0.003	0.675	0.026	17
KIVIRCIK/39	0.128	0.099	0.028	0.024	0.207	0.680	0.094	16
KIVIRCIK/40	0.040	0.038	0.047	0.169	0.243	0.928	0.593	13
KIVIRCIK/41	0.204	0.188	0.001	0.001	0.081	0.721	0.465	17
KIVIRCIK/42	0.619	0.953	0.731	0.890	0.871	1.000	0.950	18
KIVIRCIK/43	0.076	0.053	0.019	0.145	0.158	0.927	0.097	16
KIVIRCIK/44	0.109	0.216	0.001	0.256	0.200	0.904	0.113	13
KIVIRCIK/47	0.210	0.188	0.152	0.044	0.429	0.790	0.227	15
KIVIRCIK/48	0.176	0.342	0.012	0.182	0.204	0.872	0.269	13
MORKARAMAN/1	0.566	0.173	0.014	0.663	0.504	0.892	0.997	16
MORKARAMAN/2	0.504	0.287	0.060	0.624	0.668	0.739	0.830	15
MORKARAMAN/3	0.992	0.868	0.935	0.982	0.955	0.982	0.985	15
MORKARAMAN/4	0.954	0.270	0.543	0.814	0.940	0.981	0.998	14
MORKARAMAN/5	0.371	0.227	0.162	0.744	0.481	0.899	0.999	18
MORKARAMAN/6	0.223	0.510	0.686	0.310	0.189	0.679	0.968	16
MORKARAMAN/7	0.737	0.162	0.104	0.082	0.689	0.741	0.860	13
MORKARAMAN/8	0.273	0.314	0.002	0.695	0.397	0.992	0.993	18
MORKARAMAN/9	0.323	0.599	0.196	0.597	0.523	0.956	0.985	17
MORKARAMAN/10	0.001	0.002	0.002	0.005	0.000	0.004	0.115	15
MORKARAMAN/11	0.141	0.937	0.043	0.133	0.174	0.754	0.956	18
MORKARAMAN/12	0.472	0.898	0.820	0.224	0.527	0.775	0.845	17
MORKARAMAN/13	0.022	0.177	0.002	0.007	0.102	0.084	0.588	17
MORKARAMAN/14	0.011	0.005	0.000	0.005	0.003	0.075	0.479	18
MORKARAMAN/15	0.304	0.659	0.066	0.142	0.606	0.377	0.951	17
MORKARAMAN/16	0.010	0.104	0.000	0.000	0.005	0.017	0.523	18
MORKARAMAN/17	0.498	0.380	0.092	0.381	0.281	0.532	0.957	15
MORKARAMAN/18	0.063	0.408	0.048	0.007	0.164	0.300	0.902	13
MORKARAMAN/19	0.239	0.171	0.000	0.227	0.308	0.850	0.902	18
MORKARAMAN/20	0.196	0.573	0.011	0.130	0.604	0.576	0.895	18
MORKARAMAN/21	0.183	0.067	0.000	0.224	0.090	0.289	0.710	18
MORKARAMAN/22	0.775	0.628	0.581	0.058	0.580	0.519	0.831	17
MORKARAMAN/23	0.316	0.828	0.065	0.086	0.238	0.800	0.943	13
MORKARAMAN/24	0.104	0.097	0.002	0.952	0.209	0.888	0.846	15
MORKARAMAN/25	0.563	0.410	0.077	0.260	0.432	0.542	0.907	18
MORKARAMAN/26	0.392	0.440	0.022	0.428	0.468	0.875	0.996	18
MORKARAMAN/27	0.376	0.324	0.001	0.370	0.114	0.491	0.902	18
MORKARAMAN/28	0.115	0.606	0.000	0.074	0.320	0.302	0.921	18
MORKARAMAN/29	0.813	0.850	0.133	0.398	0.686	0.785	0.990	18
MORKARAMAN/30	0.347	0.618	0.137	0.056	0.308	0.584	0.988	18
MORKARAMAN/31	0.649	0.482	0.033	0.192	0.312	0.552	0.938	17
MORKARAMAN/32	0.386	0.892	0.025	0.277	0.266	0.233	0.990	18
MORKARAMAN/33	0.698	0.962	0.063	0.834	0.784	0.842	0.956	18
MORKARAMAN/34	0.441	0.556	0.163	0.247	0.281	0.965	0.989	18
MORKARAMAN/35	0.673	0.677	0.409	0.684	0.805	0.680	0.979	16
MORKARAMAN/36	0.327	0.081	0.018	0.023	0.603	0.646	0.674	15
MORKARAMAN/37	0.063	0.148	0.002	0.001	0.039	0.029	0.741	18
MORKARAMAN/38	0.070	0.093	0.000	0.055	0.062	0.088	0.581	16

MORKARAMAN/39	0.808	0.881	0.413	0.334	0.783	0.928	0.990	18
MORKARAMAN/40	0.239	0.232	0.000	0.043	0.086	0.151	0.964	18
MORKARAMAN/41	0.791	0.935	0.090	0.661	0.497	0.763	0.993	17
MORKARAMAN/42	0.790	0.850	0.002	0.691	0.554	0.678	0.961	18
MORKARAMAN/43	0.112	0.724	0.190	0.227	0.331	0.412	0.876	17
MORKARAMAN/44	0.992	0.832	0.933	0.866	0.952	0.983	0.991	15
MORKARAMAN/45	0.994	0.778	0.332	0.883	0.736	0.804	0.999	15
MORKARAMAN/46	0.618	0.289	0.093	0.402	0.897	0.606	0.846	15
MORKARAMAN/47	0.215	0.183	0.053	0.353	0.125	0.777	0.756	14
MORKARAMAN/48	0.786	0.252	0.082	0.266	0.606	0.633	0.946	15
MORKARAMAN/49	0.467	0.047	0.004	0.012	0.559	0.714	0.694	15
MORKARAMAN/50	0.459	0.381	0.041	0.430	0.124	0.424	0.870	14

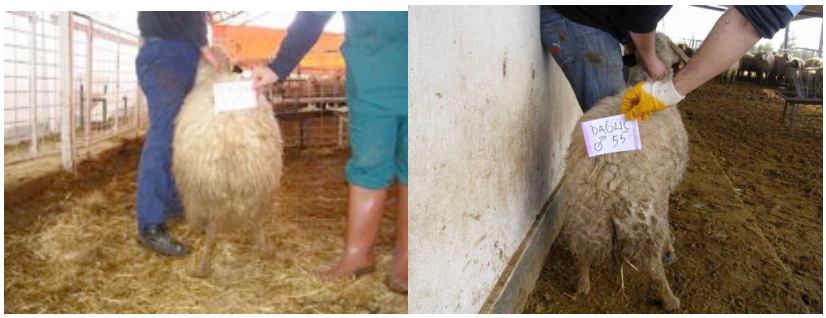
APPENDIX E

The tail photographs of the ‘outlier’ and normal individuals as stated in FCA.

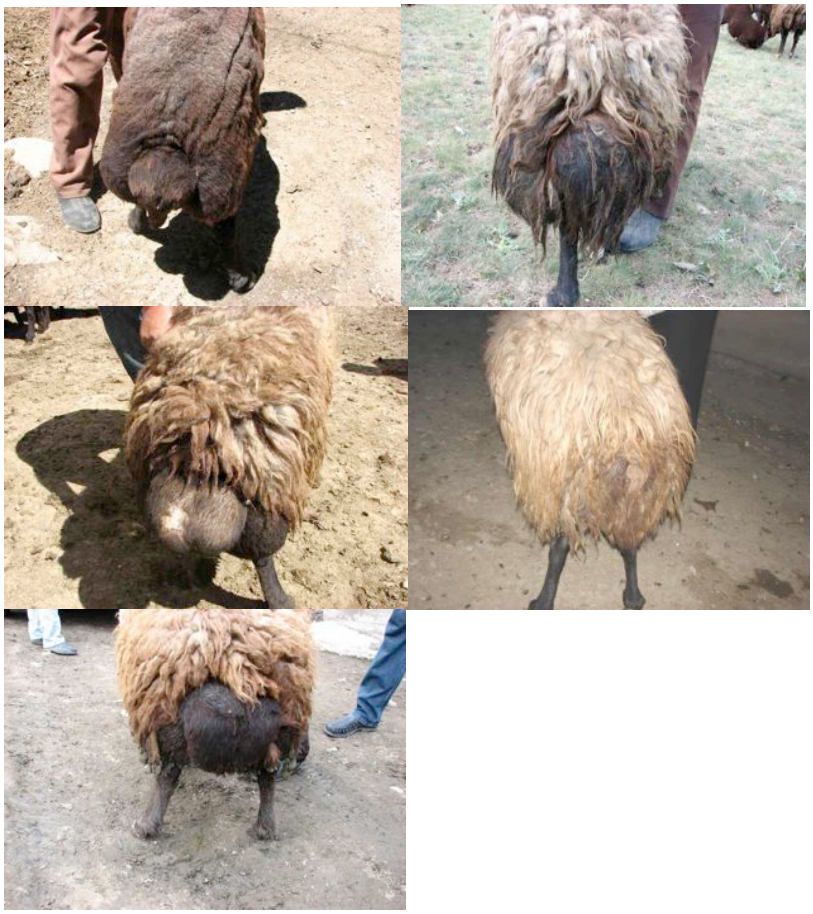
Dağlıç ‘outlier’ individuals; numbers 9, 42, 44 and 46, respectively:



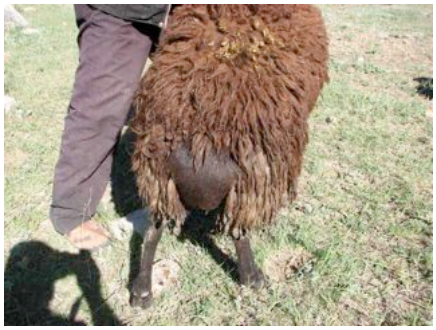
Dağlıç ‘normal’ individuals; numbers 24 and 55, respectively:



Morkaraman ‘outlier’ individuals; numbers 6, 10, 17, 20 and 24, respectively:



Morkaraman ‘normal’ individuals; numbers 2 and 26, respectively:




Akkaraman ‘outlier’ individuals; numbers 5 and 10, respectively:


Morkaraman ‘normal’ individuals; numbers 8 and 14, respectively: