GENETIC DIVERSITY OF NATIVE AND CROSSBREED SHEEP BREEDS IN ANATOLIA

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EVREN KOBAN

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Prof. Dr. Canan ÖZGEN

Director

I certify that this thesis satisfies all the requirements as a thesis for the degree of Doctor of Philosophy.

_____

Prof. Dr. Semra KOCABIYIK

Head of the Department

This is to certify that we have read this thesis and that in our opinion it is fully adequate, in scope and quality, as a thesis for the degree of Doctor of Philosophy.

_____

Prof. Dr. İnci TOGAN

Supervisor

Examining Committee Members

Prof. Dr. Zeki KAYA	(METU, BIO)	_____
Prof. Dr. İnci TOGAN	(METU, BIO)	_____
Prof. Dr. M. İhsan SOYSAL	(Trakya Ünv., TZF)	_____
Prof. Dr. Mehmet NİZAMLIOĞLU	(Selçuk Ünv., Vet. Fak.)	_____
Prof. Dr. Okan ERTUĞRUL	(Ankara Ünv., Vet. Fak.)	_____

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Evren KOBAN

ABSTRACT

GENETIC DIVERSITY OF NATIVE AND CROSSBREED SHEEP BREEDS IN ANATOLIA

Koban, Evren
Ph.D., Department of Biology
Supervisor: Prof. Dr. İnci Togan

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In this study the genetic diversity in Turkish native sheep breeds was investigated based on microsatellite DNA loci. In total, 423 samples from 11 native and crossbreed Turkish sheep breeds (Akkaraman, Morkaraman, Kıvırcık, İvesi, Dağlıç, Karayaka, Hemşin, Norduz, Kangal, Konya Merinosu, Türkgeldi) and one Iraqi breed (Hamdani) were analyzed by sampling from breeding farms and local breeders.

After excluding close relatives by Kinship analysis, the genetic variation within breeds was estimated as gene diversities (H_E), which ranged between 0.686 and 0.793. The mean number of observed alleles (MNA) ranged between 5.8 and 11.8. The allele frequency distribution across Turkey showed no gradient from east to west expected in accordance with the Neolithic Demic Diffusion model. The differentiation between different samples of Akkaraman, Dağlıç and Karayaka breeds was tested by F_{ST} index. Akkaraman1 sample from the breeding farm was

significantly (P<0.001) different from the other two Akkaraman samples. Deviation from HW expectations observed for Akkaraman1, İvesi, Morkaraman and Hemşin breeds. AMOVA analysis revealed that most of the total genetic variation (~90%) was partitioned within the individuals. In parallel to this observation, when factorial correspondence analysis and shared alleles distances were used to analyze the relationship between the individuals of the breeds, there was no clear discrimination between breeds. Moreover, NJ tree constructed based on D_A genetic distance, and PC analyses were used to analyze among breed differentiation. Delaunay Network drew 4 genetic boundaries (two of them being parallel to geographic boundaries) between breeds. All the results indicated that Kıvırcık was the most differentiated breed. Finally, Mantel Test and Bottleneck analysis did not reveal a significant result.

Kıvırcık breed, among all native Turkish breeds, was found to be the genetically closest to the European breeds based on the loci analyzed. The genetic variation in Turkish breeds was not much higher than that of European breeds, which might be a consequence of the recent sharp decrease in sheep number.

Keywords: Genetic variation, microsatellite, sheep, Turkish breeds.

ÖZ

ANADOLU YERLİ VE MELEZ KOYUN IRKLARININ GENETİK ÇEŞİTLİLİĞİ

Koban, Evren
Doktora, Biyoloji Bölümü
Tez Yöneticisi: Prof. Dr. İnci Togan

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Bu çalışmada, Türk koyun ırklarında mevcut genetic çeşitlilik Mikrosatelit DNA lokusları kullanılarak incelenmiştir. Devlet üretim çiftlikleri, üniversite üretim çiftlikleri ve yerel yetiştiricilerin elinde bulunan sürülerden yerli ve melez onbir Türk ırkı (Akkaraman, Morkaraman, Kıvırcık, İvesi, Dağlıç, Karayaka, Hemşin, Narduz, Kangal, Konya Merinosu, Türkgeldi) ile bireyleri Irak'tan getirilmiş yabancı bir ırkı (Hamdani) temsil eden toplam 423 örnek bu çalışmada kullanılmıştır.

Öncelikle üretim çiftliklerinden toplanan populasyonlardan akrabalık derecesi yüksek bireylere ait veriler yakınlık (kinship) analizinin sonuçları doğrultusunda çıkartılmıştır. Genetik varyasyonun ölçütlerinden beklenen heterozigotluk (H_E) 0.686 ile 0.793 arasında, ortalama gözlenen alel sayıları (OAS) ise 5.8 ile 11.8 arasında değişmiştir. Türkiye üzerinde alel frekans dağılımları Neolitik Gruplarca Yayılma Modeli'nin beklediği gibi bir değişim göstermemiştir. F_{ST} indeksi Akkaraman, Karayaka ve Dağlıç'ta aynı ırkın farklı populasyonlarındaki farklılaşmayı ölçmek için kullanılmıştır ve yetiştirme çiftliğinden alınan Akkarman1'in diğer iki

Akkaraman populasyonundan istatistiki önemle ($P<0.001$) farklı olduğu bulunmuştur. F_{IS} indeksi ile ırklar Hardy-Weinberg dengesi açısından test edilmiş, Akkaraman1, İvesi, Morkaraman ve Hemşin'de H-W'den sapma tespit edilmiştir. AMOVA analizi toplam genetik varyasyonun büyük bir kısmının (~%90) bireyler içinde ayrışarak paylaşıldığını göstermiştir. Paralel sonuçlar bireyler arası genetik ilişkinin incelendiği faktöryel benzerlik analizi ve alel paylaşım uzaklığı ile de elde edilmiş ve ırklar arası belirgin bir fark görülmemiştir. D_A genetik uzaklığı ile çizilen NJ ağacı ve temel ögeler analizi ise populasyonlar arası farklılaşmayı incelemek için kullanılmıştır. Delaunay örgüsü ırklar arasında 4 adet (ikisi coğrafi bariyer ile paralel) genetik sınır belirlemiştir. Sonuçların hepsi Kıvırcık'ın diğerlerinden çok farklı olduğu yönündedir. Mantel testi ve Bottleneck testi istatistiksel olarak anlamlı bir sonuç ortaya koymamıştır.

Avrupa ırklarının çoğuna genetik olarak en yakın bulunan Kıvırcık'tır. Türk ırklarında Avrupa ırklarından farklı ve yüksek bir genetik çeşitlilik belirlenmemiştir. Bunda son yıllarda koyun sayısında yaşanan hızlı düşüş etkili olmuş olabilir.

Anahtar Kelimeler: Genetik varyasyon, mikrosatelit, koyun, Türk ırkları.

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To My Grandmothers..

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CHAPTER I

INTRODUCTION

The onset of the permanent settlements built by Neolithic hunter-gatherers is when the climatic conditions became favorable to grow plants. The first settlements are seen in the region called Fertile Crescent in the Near East. It extends from the southern Levant through Syria till southeastern Turkey in the north and Iran in the east including northern Iraq.

The domestication of food items started with plants. The archaeological evidences from several excavation sites in the Old World reveal that the cereals were already domesticated about 10.000 years ago (Zohary and Hopf, 2000). Thus, wheat cultivation in this area should have started even earlier.

Animal domestication started with dogs. Hunters first used the dog to track down and kill other wild animals. They also provided companionship. Sheep and goat were the first domesticated animals as the food items among the other livestock animals. Domestication ensured a steady food supply. Mainly mammals and birds were domesticated to provide meat, milk, and eggs as well as to provide wool and hides. Larger mammals were used to carry or pull heavy loads. Land management became easier and tasks became quicker. So, the animals provided assistance with farm work, clothing, protection, as well as food and in return, they received protection and a constant food supply.

In this chapter, important findings, based on molecular markers, in the literature about the domestication history of livestock animals will be summarized. In these studies, one of the most commonly used markers was microsatellites. Along with their basic properties the reasons of their preference will be given. Finally, the aims of the study will be presented.

I.1. Domestication History of Livestock Animals and Neolithic Demic Diffusion Model

After the recent advances in molecular genetic techniques and the development of computational methods it became possible to study the domestication history of plants and animals based on genetic data. Before that, there were only archaeological findings, concerning the remains found at the excavation sites, telling about the possible location and timing of the domestication events. However, it must be noted that for the identification of wild ancestors of the domestics is equally important, which is possible by genetic studies. Only then conclusive decisions can be made about the evolutionary history of animals. Since the beginning of 90's, the geneticists are working on the genetics of livestock animals using different kinds of molecular markers. Together with the archaeological findings, the results of the genetic studies are providing a more detailed view of the domestication history of the livestock. This in turn, may help to develop proper conservation strategies for modern day sheep breeds. Furthermore, genetic analyses on domestics provide new realizations about the genetic compositions of the breeds.

The archaeological and genetic studies suggest that the two of the three main areas for livestock domestication are Southwest Asia and East Asia, where domestication of cattle, sheep, goat, pig, camel and buffalo took place (Bradley *et al*., 1996; Tanaka *et al.*, 1996; Lau *et al.*, 1998; Hanotte *et al*., 2000; Luikart *et al*., 2001; Troy *et al*. 2001; Bruford *et al*., 2003).

With every single genetic study completed, it is better seen that the domestication process was very complex. First domestication took place in different

places, and then the products of different domestication centers might have been introgressed. This introgression was sometimes asymmetric with respect to sexes. Also, there were differences in domestication of males and females of a species, may be because the herders usually slaughtered the males but kept the females to produce offspring or they kept few males for breeding in every generation. Although each species has its own domestication history, a common finding is the East-West duality in the domestication events suggesting that cattle (MacHugh *et al.*, 1997), pig (Guiffra *et al.*, 2000), sheep (Hiendleder *et al.*, 2002), and water buffalo (Tanaka *et al.*, 1996; Lau *et al.*, 1998) were domesticated at least twice, independently at different sites. The phylogenetic trees constructed from the results of these studies are given in Figure I.1.

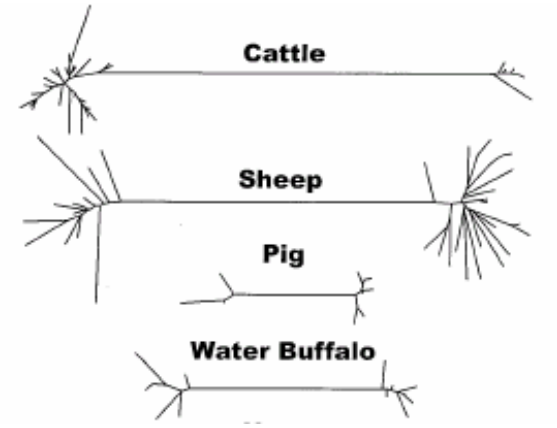

Figure I.1. Unrooted neighbor joining trees constructed by using uncorrected mtDNA sequence divergences (taken from MacHugh and Bradley, 2001).

The common features of these neighbor-joining trees are;

* The sequences cluster in two distinct groups separated by a long internal branch. It is estimated that the two groups were diverged hundreds of thousands

years ago, well before the domestication. Hence, there are at least two distinct domestication centers.

* These two distinct groups represent the samples from different geographic origin.

* These two distinct groups in cattle also follow the taxonomic categorization based on morphology and represent zebu (humped, eastern cattle) and taurine (western) cattle.

* Similarly, in water buffalo, the two distinct groups represent river and swamp buffalo, the two taxonomic classification based on morphology.

* The two distinct groups in pigs suggest two domestication events; one from an Asian and one from a Near Eastern or European wild boar species.

However, the "East-West Duality" found in mtDNA and visually presented in Figure I.1 is prone to modifications. A more recent study (Bruford and Townsend, 2004) suggested the possible presence of third domestication center and all three of them are possibly in the southwestern Asia.

In addition, the above studies revealed 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).

In accordance with the neolithic demic diffusion model (NDD) as first stated by Ammermann and Cavalli-Sforza in 1973, farming culture was developed in or near Fertile crescent nearly 10.000 years ago in Neolithic period. Technological advances (eg. farming techniques) resulted in increased food supply and, in turn, in increased population size. When the carrying capacity was reached, individuals needed to move in to new areas. This was done by gradual dispersals of the small groups (demes) of Neolithic farmers. The farming techniques may have been carried to new areas by these local movements of peasants from the farming regions to the regions where farming was not practiced, yet (Barbujani *et al.*, 1994 and the

references there in). Human genetic studies (see for example Barbujani *et al.*, 1994; Chikhi *et al.*, 2002) seem to support this model.

There are 4 nuclear zones (centers of domestications for both plants and animals) and slow migrations from these zones, as shown in Figure I.2, took place in the expended form of the NDD model (Renfrew *et al.*, 1991).

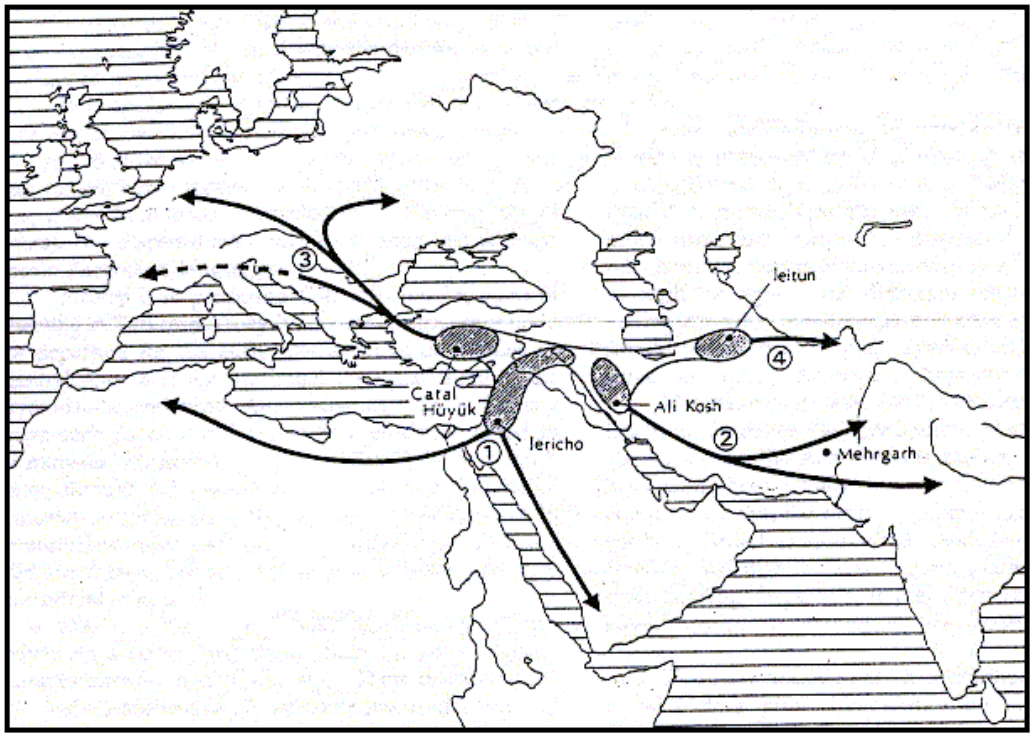

Figure I.2. The migration routes of the Neolithic farmers of related proto-languages originally located within the 4 nuclear zones of domestication as predicted by NDD model (taken from Renfrew, 1991); (1) Afro-Asiatic, (2) Elamo-Dravidian, (3) Indo-European and (4) Altaic language families.

Although the three ancestral mtDNA lineages found in goats are not associated well with the geographic structure (Luikart *et al*., 2001), the two ancestral mtDNA lineages of cattle show a gradual decline in mtDNA diversity from southwestern Asia to northeastern Europe (Troy *et al*., 2001). This finding was in accordance with the predictions of the NDD model. In addition, the decrease of diversity proportional to distance from the domestication center was detected in European sheep of mtDNA lineage A (Townsend, 2000; Bruford and Townsend, 2004) providing that Germany, Netherlands and Hungary samples are excluded. The geographic pattern of domestic sheep mtDNA lineages still needs further investigations.

I.1.1. Cattle

Cattle are the one of the livestock species studied in detail. While the probable wild ancestors of sheep, goat and pigs still survive the ancestor of domestic cattle is extinct. The common ancestor of domestic cattle was *Bos primigenius*, the auroch. Genetic studies conducted by using different molecular markers reveal different parts of the story of the domestication and spread of cattle breeds. Today, there are two different types of cattle, taurine (*Bos taurus*) and humped back zebu (*Bos indicus*). Taurine cattle are found in Europe, Middle East, North and West Africa and zebu cattle is found in Eastern Eurasia and Eastern Africa.

A mtDNA control region sequence study (Troy *et al.,* 2001) among the samples of taurine and zebu cattle together with the remains of 6 British aurochs (*Bos primigenius*) and a study on the whole mtDNA RFLP analysis (Loftus *et al*., 1994) revealed that there were two domestication centers in Near East Asia; one close to southeastern Anatolia (for taurine cattle) and one close to Baluchistan (for zebu cattle) (see also Bradley *et al*., 1996). British breeds are the descendants of Near Eastern taurine cattle and not the wild populations (aurochs) of Britain. The two lineages were separated hundreds of thousands of years ago. The last finding suggested, having known that its domestication event took place about 10.000 years ago, domestication of cattle occurred in two different regions from different

subspecies of the ancestral wild cattle. MtDNA diversity decreased from Near East to Europe indicating the migration along that direction in parallel to the expectation of NDD. These mtDNA studies also revealed that African zebu cattle were different from Asian zebu cattle. In fact, the mtDNA of African zebu cattle were similar to that of African and European taurine cattle. Moreover, the genetic studies conducted by using microsatellites (MacHugh *et al*, 1997; Loftus *et al.*, 1999) and Y chromosome markers (Bradley *et al*., 1994) showed that African zebu cattle was in fact most similar to Asian zebu cattle. These findings revealed that the origin of African zebu cattle was as follows: African taurine cattle females were interbred with Asian zebu bulls, and in the next generations the crossbreed females were again mated with zebu males. Hence, there was an introgression from East to West (from zebu to taurine).

Diversity decrease in microsatellite variability from neareast to Europe was also observed by Loftus and collaborators (1999). Furthermore, they have detected 13 alleles in 6 microsatellite loci among 20 they used, which they classified as diagnostic of zebu cattle. These diagnostic alleles revealed the introgression from zebu cattle into Near Eastern taurine cattle. There was a gradual decrease in the allele frequencies from East Indian to West Anatolia and alleles were not detected in European breeds. In a similar study by MacHugh and colleagues (1997), some alleles of the 10 loci among 20 that they analyzed, were classified as diagnostic of zebu.

The presence of zebu specific microsatellite alleles is parallel to the dualism found in mtDNA studies of cattle. These alleles help to analyze the admixture between the two cattle types (MacHugh *et al.*, 1997; Loftus *et al.*, 1999) and to track the migration routes (Hanotte, 2002).

I.1.2. Goat

A detailed study by Luikart and colleagues (2001) on goat mtDNA Hyper Variable Region-I revealed that there were three matrilineal roots for goats. They analyzed 406 goat samples of different origin together with 14 samples from wild

capra species. None of the samples of the wild goat species was grouped with 3 domestic lineages; A, B and C. They estimated the coalescence time for domestic goat using sequences of complete cyt-b region of samples from all these three lineages, which was found to be 20-280 thousand years ago. The mismatch distribution suggested sample expansion in all three lineages. The lineage A was the oldest and the most diverse haplogroup. Then lineage C was the second and lineage B was the youngest.

Based on the distribution of the lineages and the archaeological evidences, it is concluded that the oldest goat domestication was in Near East close to Anatolia (Luikart *et al*., 2001). An important finding of this study is the distribution of these lineages across the old world. Only lineage A is found everywhere. Lineage B is found exclusively in breeds of Southern Asia. Moreover lineage C is confined to a small number of European breeds, and also a single sample from Mongolia was grouped in this lineage. The absence of lineage C in Near East is a question that was not answered, yet. Increasing the sample size and addition of different kinds of molecular markers can provide a better picture on the origin and distribution of these lineages.

I.1.3. Sheep

Several archaeological and genetics studies have shed light on sheep domestication (e.g. Clutton-Brock, 1981; Uerpmann, 1996; Hiendleder *et al.*, 2002; Bruford and Townsend, 2004). However, the information on the evolutionary history of domestic sheep and particularly their relationship to wild species remained limited. According to the archeological finds, the domestication of sheep is believed to have occurred approximately 10.000 BP in the region of the Zagros Mountains on the border of Turkey and Iran (Legge, 1996; Uerpmann, 1996).

The studies on sheep domestication in the literature contain samples mainly from Europe and New Zealand (Hiendleder *et al.*, 1998; Hiendleder *et al.*, 1999; Townsend, 2000; Hiendleder *et al.*, 2002). The total number of analyzed samples of

wild *Ovis* species is quite low in these studies and does not include all the subspecies of *Ovis gmelini*. Therefore, it is still unclear which wild species or subspecies was/were the ancestor(s) of the modern day domestic sheep, and where and how many times the domestication of sheep took place.

In the last decade, the molecular genetic studies using mtDNA revealed that *Ovis gmelini* is the most likely domestic ancestor (Hiendleder *et al*., 1998; Hiendleder *et al*., 1999; Townsend, 2000; Hiendleder *et al*., 2002). Moreover, MtDNA control region sequence and RFLP analysis provided evidence for two domestication events (Hiendleder *et al*., 1998), where the two distinct lineages were named as A and B. In addition, Townsend (2000) has found evidence for a possible third domestication event as a part of her PhD thesis and the third lineage found was named as C. However, it is important to mention here that the lineages, named as "A" and "B" by Hiendleder and collaborators (1998), were named as "B" and "A" by Townsend (2000), respectively (see also Bruford and Townsend, 2004). She included many samples across Europe, few samples from Turkey and Near East into her study including an *Ovis gmelini anatolica* sample grouped in lineage B. She also had few samples of wild species. Unfortunately, none of the wild species samples is grouped within domestic sheep clusters in these studies. Only *Ovis musimon*, the European mouflon samples are grouped within clusters A and B, but not in cluster C. Today, *Ovis musimon* is not accepted as the members of the wild species but as the feral remnants of the first domestic populations (Bruford and Townsend, 2004).

The increasing data on genetics of sheep breeds using different genetic markers will help to understand the evolutionary history of sheep better. In addition, it will help to refine the definition of breed (see Soysal and Özkan, 2002).

I.2. The Significance of the Native Turkish Sheep Breeds and the Justification of the Present Study

The archaeological and genetic evidences point that the sheep domestication took place either in a region close to eastern/southeastern Turkey or within Turkey.

Thus, it is highly likely that the Turkish native sheep breeds of today are the oldest/one of the oldest living descendants of their first domesticated ancestors. Given the fact that no wild sheep species naturally existed in Europe, there is a high probability that Turkish native sheep breeds gave rise to most/all of the European sheep breeds of today.

Today's European so called "economically important" breeds lost their ability to survive on the extremes of climatic conditions and on poor food on the way of migration and breed improvement. The adaptation of Turkish sheep breeds to the harsh environmental and poor feeding conditions, and to some diseases is much greater than European breeds. For example the Turkish breeds can survive on extremes of heat. There is about 20-30°C difference between summer and winter temperatures in the distribution region of some Turkish native breeds, like Morkaraman, which can also survive on a range of altitude from 1500 to 3500 meters. However, the survival rate of Welsh Mountain decreases sharply with an altitude difference of 500 meters (personnel communication).

With every single extinction event, if there is any genetic information confined only in that breed is also lost. Therefore, the characterization of the native genetics resources before being lost and developing proper conservation strategies are important.

Bruford and colleagues (2003) pointed that ancestral populations and closely related species might be a source of alleles of economic value that have been lost by chance during domestication, and the eastern-most Asian breeds or those nearest the putative centers of domestication contain greater genetic diversity and therefore, these higher diversity breeds should receive a concomitant higher priority for conservation. Since the Turkish native sheep breeds are close to one of the domestication centers, their genetic diversity must be studied and those, which have the highest diversities, must be identified for conservation with highest priority.

One of the most important problems that Food and Agricultural Organization (FAO) of the United Nations draws attention to is the sharp decrease in the number of livestock animals (FAO, 1996). The 22.5% of the total livestock of the European local breeds was also extinct and replaced by economically important breeds. Turkey is one of the countries affected by the decrease in the number of livestock animals with about 47% decrease in sheep number in the last two decades. “Genetic erosion” and “genetic pollution” are the two important factors causing an important decrease in the number and size of the livestock breeds of Europe. Probably, both factors are operating in Turkey. Furthermore, social unrest is also a very important reason for the decline and extinction in eastern and southeastern Turkey. The heaviest genetic toll among the Turkish native sheep breeds was ‘perhaps’ on the most precious ones, that is, on the ones nearest to the putative centers of origins. Therefore, genetic diversity of the native sheep breeds must be studied both to understand the evolutionary history of the sheep and to develop proper conservation strategies for the sheep breeds.

I.3. Microsatellite DNA Markers

In evolutionary history studies of domestic animals one of the most preferred DNA markers is microsatellites. They are stretches of DNA that consist of tandem repeats of a specific sequence of DNA bases or nucleotides, which contains mono, di, tri, or tetra tandem repeats (for example, AAT repeated 15 times in succession). In the literature they can also be called simple sequence repeats (SSR), short tandem repeats (STR), or variable number tandem repeats (VNTR). Alleles at a specific location (locus) can differ in the number of repeats. Microsastellites are inherited in a Mendelian fashion.

Microsatellites are "junk" DNA, and the variation is mostly neutral. They usually don't have any measurable effect on phenotype. In humans, 90% of known microsatellites are found in noncoding regions of the genome. When found in human coding regions, microsatellites are known to cause disease. Interestingly, when found in coding regions, microsatellites are usually trinucleotide repeats. Any other type of

nucleotide repeat would be too detrimental to the coding region, as it would cause a frameshift mutation.

Microsatellite loci are highly abundant and almost uniformly distributed over the entire genome (Ortí *et al*., 1997; Schlötterer, 1998). They exhibit exceptionally high mutation rate, high polymorphism and they are relatively easy to survey. It is estimated that microsatellites mutate 100 to 10,000 as fast as base pair substitutions. This makes microsatellites useful for studying evolution over short time spans (hundreds or thousands of years), whereas base pair substitutions are more useful for studying evolution over long time spans (millions of years).

The highly polymorphic nature of microsatellites provide an important source of molecular markers (Goldstein and Shlötterer, 2000) for many areas of genetic research such as studying relationships among closely related species or samples of a single species (Bowcock *et al*., 1994), determination of paternity and kinship analyses, forensic studies (Edwards *et al.*, 1992), linkage analysis (Francisco *et al*., 1996; Mellersh *et al.*, 1997) and the reconstruction of phylogenies (Bowcock *et al*., 1994).

The microsatellite loci have been increasingly used for evolutionary purposes. Yet, a concensus has not been achieved on a particular mutational model generating the allelic variation at these loci. There are several mutation models considered for microsatellites. The infinite allele model (IAM, Kimura and Crow, 1964) and the stepwise mutation model (SMM, Kimura and Ohta, 1978), are the two extreme models. The SMM states that mutation of microsatellite alleles occurs by the loss or gain of a single tandem repeat. So, alleles mutate towards allele states already present in the sample. However, in the IAM, mutation involves any number of tandem repeats and always results in an allele state not previously encountered in the sample. Two-phase model (TPM, DiRienzo *et al*., 1994) is intermediate to the SMM and IAM. It describes mutation at microsatellite loci by loss or gain of X repeats where P is the probability of X equals 1 (like SMM) and 1-P is the probability of X following a geometric distribution (like IAM).

The statistical analysis methods used for classical genetic markers do not account for microsatellites. That's why new methods are developed to retrieve information from microsatellite data. Luikart and England (1999) have summarized the most recent and innovative statistical methods and computer programs to analyze microsatellite data.

The use of microsatellites in livestock animal studies started in the beginning of 90s (MacHugh *et al.*, 1994) and FAO conducted studies to standardize the microsatellite loci to be used in analyzing the genetic variation within and among breeds. The list of the microastellite loci suggested by FAO can be found from its webpage: http://dad.fao.org/en/refer/library/guidelin/marker_without_link.pdf. However, not much data based on these have accumulated, yet.

There are mainly three uses of microsatellites in livestock animals:

i) To measure the genetic variation within and among breeds (e.g., Diez-Tascón *et al.*, 2000),
ii) To determine the "genetic admixture" experienced by the samples (e.g., MacHugh *et al.*, 1997),
iii) To assign the individuals to the breeds according to their genetic resemblence (Cornuet *et al.*, 1999).

I.4. The Objectives of the Study

The aims of the study are listed below:

1. The samples collcted from Turkish native sheep breeds were analyzed based on 5 microsatellite loci;

- To determine the genetic diversity among the breeds analyzed.
- To assess their degree of variability compared to that of European breeds.
- To assess the genetic distinctiveness of the Turkish breeds and to compare it with that of found among the breeds from Europe.

2. To compare the results with literature;
    - To find out if some of the breeds are associated with different domestication events.
    - To assess if Kıvırcık breed is the closest relative of the European breeds.
    - To find out if the traces of NDD can be identified in the form of spatial genetic diversity distribution of the sheep.
    - To examine the presence of the admixture in Anatolian sheep breeds as it was in the case for cattle breeds.
3. The genetic data obtained will be assessed;
    - To determine the genetic variation between different samples of the breeds. Hence, to obtain a better understanding for the term “gene pool of a breed”.
    - To use the results of the study to develop conservation strategies for Turkish native sheep breeds.

CHAPTER II

MATERIALS AND METHODS

II.1. Samples

In this project, samples were collected from individuals of 12 breeds; Kıvırcık, Türkgeldi, Dağlıç, Karayaka, Hemşin, Akkaraman, Konya Merinosu, Kangal, İvesi, Morkaraman, Norduz and Hamdani. The total number of individuals that were analyzed was 423. The sampled material was 10 ml. of blood collected in tubes containing K_3EDTA.

All the breeds in this study were represented by one sample except for Akkaraman, Karayaka and Dağlıç breeds. Regarding the time and place of the sampling, Akkaraman sampling was repeated three times while Karayaka and Dağlıç were sampled twice.

The distribution of the native sheep breeds of Turkey is given in the Figure II.1 below. The most widely distributed breed is Akkaraman.

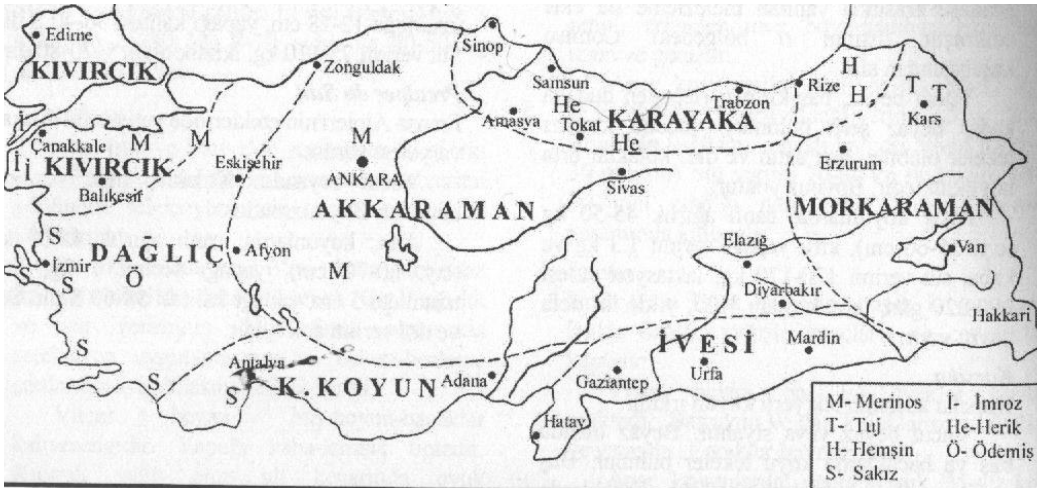

Figure II.1: The distribution of the breeds across Turkey (taken from Akçapınar, 2000).

Different parts of Turkey have different topographic and climatic conditions. The Turkish native breeds are naturally adapted to these conditions. These breeds can be classified in to two main groups according to their tail features: fat tail and thin tail. The fat tail provides advantage in places where the environmental conditions are harsh and the temperature change between the seasons is big. Genral features of Turkish native breeds are as follows:

Akkaraman: It is a fat tailed breed. It has a white coat and black nose. Occasionally it can have black around the eyes. The females do not have horns, but males may have horns. Its wool type is classified as carpet wool. Adult animals' withers height is about 65 cm and live weight is 45-50 kg. Its main use is for meat, then wool and milk productions come.

Kangal: It is believed to be a variety of Akkaraman. It is a fat tailed breed. It has a white coat and black nose. Occasionally it can have black colored zones around the eyes. The females do not have horns, but males may have horns. Its wool type is

classified as carpet wool. Adult animals' withers height is about 85 cm and live weight is 80 kg. Its main use is for meat, then wool and milk productions come.

Norduz: It is a fat tailed breed. It has a white coat color with some brown or grey colored regions on it. There are black spots on head, neck and legs. Usually the ears and eyes are in black, too. The females do not have horns, but males may have horns. Its wool type is classified as carpet wool. Adult animals' withers height is about 65-70 cm and live weight is 45-55 kg. Its main use is for meat, then milk and wool productions come.

Morkaraman: It is a fat tailed breed. It has a red or brownish coat color. The females do not have horns, but males may have horns. Its wool type is classified as carpet wool. Adult animals' withers height is about 68 cm and live weight is 50-60 kg. Its main use is for wool, then meat and milk productions come.

İvesi: It is a fat tailed breed. It has a white coat color with brown marks on feet, ears and neck. The females do not have horns, but males do have horns. Its wool type is classified as carpet wool. Adult animals' withers height is about 65 cm and live weight is 45-50 kg. Its main use is for milk, then meat and wool productions come.

Dağlıç: It is a fat tailed breed. It has a white coat color with occasional black marks around mouth, nose and eyes. The females do not have horns, but males may have horns. Its wool type is classified as carpet wool. Adult animals' withers height is about 58 cm and live weight is 35-40 kg. Its main use is for wool, then meat and milk productions come.

Kıvırcık: It is a thin tailed breeds. It has a white coat color and may have black spots on legs and face. The females may have horns, but males always have horns. Its wool type is classified as carpet wool. Adult animals' withers height is about 58 cm and live weight is 35-40 kg. Its main use is for wool, then meat and milk productions come.

Karayaka: It is a thin tailed breeds. It has a white coat color and black eyes, head and legs. The females do not have horns, but males do have horns. Its wool type is classified as carpet wool. Adult animals' withers height is about 60-62 cm and live weight is 35-40 kg. Its main use is for wool, then meat and milk productions come.

Hemşin: It is a thin tailed breeds with a fat deposition at the tail base. It has a mixed coat color of white, brown and black. The females may have horns, but males always have horns. Its wool type is classified as carpet wool. Adult animals' withers height is about 65 cm and live weight is 45-50 kg. Its main use is for meat, then wool and milk productions come.

Konya Merinosu: It is a thin tailed crossbreed of German meat Merino (80%) and Akkataman (20%). It has a white coat color. Adult animals' withers height is about 66 cm and live weight is 54-56 kg. Its main use is for meat, then wool and milk productions come.

Türkgeldi: It is a thin tailed crossbreed of East Friesian (9/16) and Kıvırcık (7/16), It has a white coat color. It is found in Thrace, Turkey. They are a dairy breed also used for meat and wool productions.

Hamdani: It is a fat tailed breed found in Iraq and Iran. It has a white coat color with brown/black color on the face, ears and legs. Unfortunately, there is not much information found in the literature about the breed characteristics of Hamdani.

There were two sampling strategies used during sample collection;

1. Some of the samples of the breeds were taken from governmental enterprises or university farms. The names of the breeds and where they were taken from are as follows: Akkaraman1 and Konya Merinosu breeds were sampled from Konya Stud of Selçuk University, Konya; İvesi breed was sampled from Gözlü Agricultural Enterprise, Konya; Türkgeldi breed was sampled from Research and Application Unit of Trakya University in Tekirdağ and Kıvırcık breed was sampled from İnanlı Agricultural Enterprise, Tekirdağ.

2. All the rest of the samples of the breeds, which are Dağlıç, Akkaraman, Kangal, Karayaka, Hemşin, Morkaraman, Norduz and Hamdani, were collected from local breeders and flocks. The flocks and the individuals to be sampled were chosen with the help of veterinarians, veterinary technicians and/or agricultural engineers so that morphologically the best representatives of each breed were tried to be sampled. From each flock visited, 2-4 samples were collected, considering the total size of the flock. By this way, the maximum genetic variation within a breed was tried to be captured.

In table II.1 the names of the breeds and their repeated samples were given together with the some details about these samples.

Table II.1. Description of the study material and sampling. FB: fat and big tail; FS: fat and short tail; T: thin and long tail; FTB: thin tail which is fat at the base.

Breeds	**Abbrev.**	**Sample size**	**Breeding Farm (B)/Flock (F)**	**Native (N)/ Foreign (F)**	**Pure (P)/ Crossbreed (C)**	**Tail type**
Akkaraman	***Akk***	***52***	***B and F***	***N***	***P***	***FB***
Akkaraman1	Akk1	28	B	N	P	FB
Akkaraman2	Akk2	10	F	N	P	FB
Akkaraman3	Akk3	14	B and F	N	P	FB
İvesi	İve	35	B	N	P	FB
Kıvırcık	Kıv	23	B	N	P	T
Morkaraman	Mork	35	F	N	P	FB
Hemşin	Hem	34	F	N	P	FTB
Karayaka	***Kry***	***57***	***F***	***N***	***P***	***T***
Karayaka1	Kry1	28	F	N	P	T
Karayaka2	Kry2	29	F	N	P	T
Dağlıç	***Dağ***	***64***	***F***	***N***	***P***	***FS***
Dağlıç1	Dağ1	32	F	N	P	FS
Dağlıç2	Dağ2	32	F	N	P	FS
Norduz	Nor	26	F	N	P	FB
Kangal	Kngl	22	F	N	P	FB
Hamdani	Ham	22	F	F	P	FB
Konya Merinosu	KM	29	B	N	C	FS
Türkgeldi	TG	24	B	N	C	T

During sample collection as many places as possible visited for each breed. The area covered during sampling is shown in Figure II.2. Details about the addresses of the sampling places were given in Appendix A where possible.

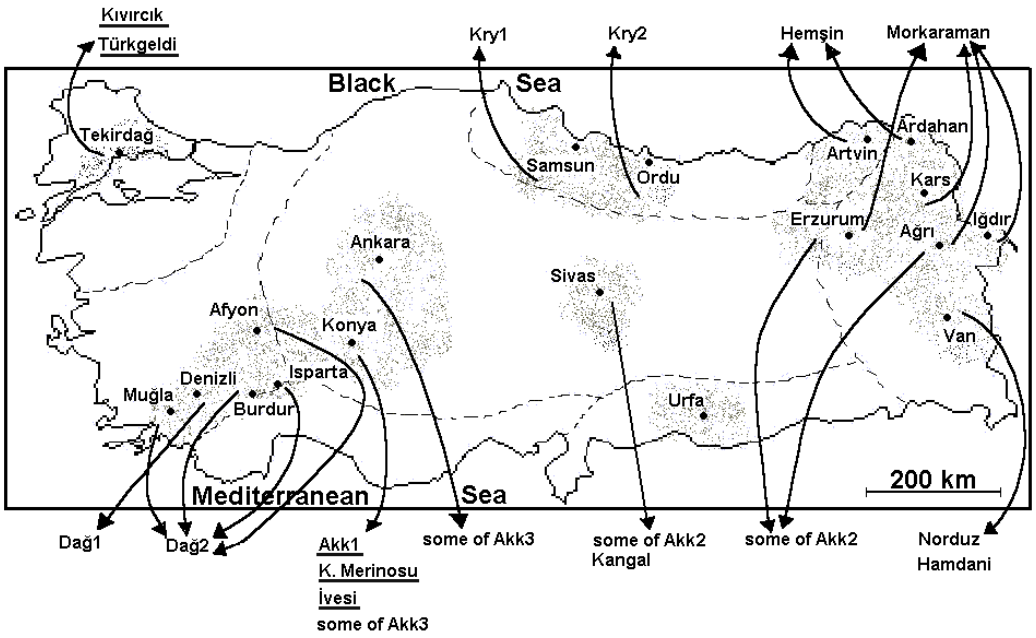

Figure II.2. Sampling locations. The dashed lines discriminate between the distribution areas of the breeds and the area covered during sampling was shadowed. The names of the sampled breeds and the names of the cities within the sampling area are given. Underlined breeds are from governmental and university farms.

II.2. DNA Isolation

Standard phenol: chloroform DNA extraction protocol (Sambrook *et al.*, 1989) was used for extracting DNA from the blood samples collected. All the DNAs were extracted at Middle East Technical University, Department of Biology.

10 ml of blood sample was put in 0.5 ml EDTA (0.5 M; pH 8.0) containing falcon tube and 2X lysis buffer (10X Lysis solusyonu: 770 mM NH_4Cl, 46 mM $KHCO_3$, 10mM EDTA) was added onto it until the volume was 50 ml. After mixing the content of the tube well by inversions for 10 min. the tubes were centrifuged at 3000 rpm at +4°C for 10 min. The supernatant was poured off and 3 ml of salt/EDTA (75mM NaCl, 25 mM EDTA) was added onto the pellet and mixed by vortex. After the addition of 0.3 ml of %10 SDS solution and 150 µl of proteinase K (10 mg/ml) solution, the samples were incubated at 55°C for 1-3 hr. When the time is over, 3 ml of phenol (pH 8.0) was added on to the samples, the tubes were shaken vigorously for 20 s and then by gentle inversions for 5 min. Afterwards, the tubes were centrifuged at 3000 rpm at +4°C for 10 min. The supernatant was transferred into new sterile tubes labeled properly and 3 ml of phenol:cloroform:isoamyl alcohol (25:24:1) was added on to the supernatant, which was then shaken vigorously for 20 s and then by gentle inversions for 5 min. Moreover, the tubes were centrifuged at 3000 rpm at +4°C for 10 min for the last time and the supernatant was transferred into a sterile glass tube, to which 2 volumes of ice cold. (kept at –20°C) EtOH was added. The glass tubes were shaken abruptly, the condensed DNA was hooked out with a glass hook and transferred in to 1.5 ml eppendorf tubes containing 0.5 ml of TE buffer (10mM Tris, 1mM EDTA PH 7.5). The DNA solution can be either stored at +4°C (if it is going to be used immediately) or at -20°C (for long term storage).

II.3. Microsatellite Used

Five microsatellite loci have been chosen for this study after consulting with Prof. Mike Bruford from Cardiff University, Dr. Kate Byrne from London Institute of Zoology and FAO webpage (http://dad.fao.org/en/refer/library/guidelin/marker_without_link.pdf). They were chosen because they were all polymorphic, a data from European samples were available, and data are accumulating based on these loci. The names of these microsatellite loci, their origin, on which chromosome they are located and their allelic range were given in Table II.2 below.

Table II.2. Microsatellite markers used in the study; their names, origins, chromosome numbers and allelic ranges.

Locus	Origin	Chromosome #	Allelic Range
MAF33	Ovine	9	122-154
MAF65	Ovine	15	117-139
MAF209	Ovine	17	104-136
JMP29	Ovine	24	115-155
JMP58	Ovine	26	140-176

II.4. Polimerase Chain Reaction (PCR) Conditions

All the DNA samples were amplified with the primers specific to these five microsatellite loci by using Biometra, Stratagene and Perkin Elmer 3700 Polymerase Chain Reaction (PCR) machines.

The amplified products were either visualized by radioactive labeling using ^{33}P dATP (for the samples analyze at Middle East Technical University, Department of Biology) or by fluorescent labeling using FAM, TET and HEX flourophores (for the samples analyzed at Cardiff University, School of Biosciences).

- For radioactive labeling, 1X PCR mixture contained 1X PCR buffer, 2.5 mM $MgCl_2$; 200mM of dTTP, dCTP and dGTP; 20 mM of dATP; 0.1 µl of 1000mCi ^{33}P dATP; primers of the locus to be amplified and sterile distilled water to adjust the volume. The primer concentrations used in the 1X PCR master mix are as follows; 6 pmol from each of forward and reverse MAF 33 primers, 4 pmol from each of forward and reverse MAF 65 primers, 5 pmol from each of forward and reverse MAF 209 primers, 7 pmol from each of forward and reverse JMP29 and JMP58 primers. This mixture was distributed to PCR tubes containing 50-100 ng DNA samples and then 1 unit of Taq DNA polymerase was added into each tube and the tubes were placed in PCR machine for amplification.

- For fluorescent labeling, 1X PCR mixture contained 1X PCR buffer, 2.5 mM $MgCl_2$; 200mM of each of dNTP; primers of the locus to be amplified and sterile distilled water to adjust the volume. The primer concentration used was the same, but the primers were fluorescently labeled. Similar to radioactive labeling the mixture was distributed to PCR tubes containing 50-100 ng DNA samples and then 1 unit of Taq DNA polymerase was added into each tube and the tubes were placed in PCR machine for amplification.

The PCR amplification conditions are as follows: 1 cycle of denaturation at 94°C for 2 min; 30 cycles of amplification process where the samples are incubated at 94°C for 20 s, then at the annealing temperature specific for the primers for 20 s, and then at 72°C for 40 s; 1 cycle of final extension at 72°C for 10 min. The annealing temperatures of the loci used in the study are 57°C for MAF33, MAF65, JMP29 and JMP58 loci, and 60°C for MAF209 locus.

The PCR products were checked on 1.5% agarose gels for amplification.

II.5. Polyacrylamide Gel Electrophoresis and Data Collection

There were two methods used for polyacrylamide gel electrophoresis as there were two labeling methods used:

- For radioactive labeling (used for the samples analyzed at Middle East Technical University, Department of Biology); 6X loading dye (with formamide) was added to the PCR products, and then they were kept at 95°C for 3 min for denaturation and kept on ice for loading. Then 3 µl of samples were loaded onto 6% denaturing polyacrylamide gel. After running the gel for 2-4 hr at 1600V, the glass plates were separated. As Sigmacote was applied to the back plate, the gel was only sticked to the front plate. Then the gel is transferred carefully on to 3M whatman paper and stretch film is placed on the gel. Afterwards, the gel was dried in vacuum gel dryer at 60°C for about an hour. Finally the gel was placed in exposure cassette and radioactive sensitive film was placed on it properly. According to the radioactive labeled

dNTP's delivery date the film was exposed to the gel for 3 days to 15 days before it was washed.

- For fluorescent labeling (used for the samples analyzed at Cardiff University, School of Biosciences); 1.5 µl of each PCR product were mixed with 1.2 µl of Tamra350 internal size standard (labelled with red colour). After incubating the mixture at 95°C for 3 min. samples were loaded onto 4.2% non-denaturing polyacrylamide gel in an ABI 377 semi-automated DNA analyzer. The raw data were collected by GeneScan software of Perkin Elmer. After the data is collected, electrophenograms of the amplified alleles were checked and the allelic sizes were determined in comparison with the internal size standard by using Genotyper software of Perkin Elmer.

II.6. Data Analysis

The first collected samples were from the governmental and university farms (Akkaraman1, Kıvırcık, İvesi, Konya Merinosu, Türkgeldi). Right after completion of the data from these breeds the results were tested for the presence of close relatives within the sample and some of the individuals from each of the 5 samples were excluded using the software Kinship (Goodnight and Queller, 1999).

After completion of the data collection, data matrix file was constructed so that it could be analyzed using GENETIX 4.02 software (Belkhir et al. 1996-2004) which is available at the website: http://www.univ-montp2.fr/~genetix/genetix.htm. This program computes several basic parameters of sample genetics such as Nei's D and H, Wright's F-statistics (using the Weir-Cockerham's and Robertson-Hill's estimators). For each of them, the distribution of the parameter values under the null hypothesis is generated by the appropriate resampling scheme of the relevant objects (e.g. alleles between individuals in the case of F_{IS}) using permutations. The permutation-based statistical inference procedures implemented in GENETIX estimates the probability value of departure from the null hypothesis.

In addition, GENETIX file format was used in further analyses performed by using the softwares Geneclass (Cornuet *et al.*, 1999) for the assignment tests, Bottleneck (Cornuet and Luikart, 1996) for the analysis of the probability of a recent reduction in the sample size. Furthermore GENETIX program converts the data file into the GENEPOP file format, which was used in Populations 1.0 software (http://www.cnrs-gif.fr/pge/bioinfo/samples) to construct the neighbour-joining trees based on the D_A genetic distance between samples and the proportion of shared alleles between the individuals, and into the Arlequin (Excoffier *et .al.*, 1992) file format, which was used for Mantel Test, AMOVA and F_{ST} estimations. Furthermore, the allele frequencies used for Principle Component analysis performed by NTSYSpc (www.exetersoftware.com/cat/ntsyspc/ntsyspc).

II.6.1 Kinship Analysis

In Turkey, pedigree records are not taken in sheep breeding. Therefore, the relationship between the individuals of each of the five breeds (Akkaraman1, Kıvırcık, İvesi, Türkgeldi and Konya Merinosu), which are taken from the governmental enterprises and the university breeding farms, is not known. There may be close relatives within the same sample, which in turn will affect the results of the statistical analyses. Therefore, after completing the data collection from these five breeds, the results were first tested for relatedness and parentage probabilities by using the software Kinship (Goodnight and Queller, 1999; see the webpage at http://www.gsoftnet.us/GSoft.html). It performs maximum likelihood tests of pedigree relationships between pairs of individuals in a population based on the genotype information for single-locus, codominant genetic markers (e.g. microsatelites). The user enters two hypothetical pedigree relationships, a primary hypothesis and a null hypothesis. Then, the program estimates likelihood ratios comparing the two hypotheses for all possible pairs in the data set. In addition, it generates simulated data sets from the given genetic data to test the significance of results. Moreover, it estimates pairwise relatedness statistics. The resulting file is a matrix showing the likelihood ratio between primary and null hypotheses.

II.6.2 Genetic Variation Analysis

Allelic variation and heterozygosity analyses allow us to quantify this information.

a) Allelic variation

Allelic differences in a sample is an indication of genetic diversity. Thus, allele frequency is one of the measures of genetic variation which can be calculated as:

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

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:

$$n_a = \frac{\sum_i n_{ai}}{r}$$

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

b) Heterozygosity estimations

Population heterozygosity, or gene diversity, is a useful and widespread measure of genetic diversity. The relative frequency of the heterozygote individuals in the sample in terms of the same locus gives the observed heterozygosity (h_o).

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 ($\hat{h}_e$) at a locus can be estimated by the formula:

$$\hat{h}_e = \frac{2n\left(1 - \sum \hat{x}_i^2\right)}{(2n-1)}$$

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

In case of multi loci, the average of single locus heterozygosity values is taken to find sample's observed (H_O) and expected (H_E) heterozygosities.

II.6.3. F-statistics

In actual sample, the genotype frequencies in each subpopulation do not necessarily follow Hardy-Weinberg equilibrium. Wright's fixation indices F_{IS}, F_{IT} and F_{ST} measure the deviations from Hardy-Weinberg expectations in terms of genotype frequencies in a subdivided sample. These coefficients are used to allocate the genetic variability to the total sample level (T), samples (S) and individuals (I), and they are useful to understand the breeding structure of the sample.

These three F-coefficients are interrelated so that;

$$1 - F_{IT} = (1 - F_{ST})(1 - F_{IS}) \qquad \text{or} \qquad F_{ST} = \frac{F_{IT} - F_{IS}}{1 - F_{IS}}$$

Nei (1987) showed that F_{IS}, F_{IT} and F_{ST} can be defined in terms of expected and observed heterozygosities and still satisfies the above equation.

F_{IS} is defined as the correlation between homologous alleles within individuals relative to the samples. It deals with the inbreeding in induviduals at the subpopulation level so that it measures the deviation from the Hardy-Weinberg equilibrium within the samples. It is estimated by the following formula:

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

F_{IT} is defined as the correlation of the corresponding alleles within individuals relative to the total sample and it accounts for both the effects of inbreeding within samples and the effects of sample subdivision. F_{IT} quantity measures the deviation from Hardy-Weinberg equilibrium over the total sample. The following formula estimates F_{IT}:

$$F_{IT} = \frac{H_T - H_O}{H_T}$$

F_{ST} is a measure of genetic differentiation of samples. It deals with inbreeding in samples relative to the total sample and it can be estimated by the following formula:

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

Where;

H_O = average observed heterozygosity of the samples

H_S = average expected heterozygosity in the samples

H_T = average heterozygosity of the total sample

(Hedrick, 1983; Nei 1987, Nei and Kumar, 2000)

The F indices proposed by Wright (1951) 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 offer some unity to various estimation formulae suggested by different authors. They used the parameters F, θ and f for F_{IT}, F_{ST} and F_{IS} respectively. These estimators do not make assumptions concerning numbers of samples, sample sizes or heterozygote frequencies and they are suited to small data sets. F, θ and f parameters are estimated as follows:

$$F = 1 - C/(B + C)$$
$$\theta = A/(A + B + C)$$
$$f = 1 - C/(A + B + C)$$

Where;

A=inter-sample component of allelic frequency variance

B=component of allelic frequencies variance between individuals in each sample

C=component of allelic frequencies variance between gametes in each individual

In this study, Weir and Cockerham's approach is used to examine the sample structure, but the parameters are denoted by F_{IT}, F_{ST} and F_{IS} instead of F, θ and f.

In order to test the significance of estimated F-coefficients, the data were permuted for 1000 times and the distribution of the calculated values (F_{IS} and F_{ST}) from the permuted data was generated under the null hypothesis (no sample differentiation for F_{ST}, and HW equilibrium for F_{IS} and F_{IT}). The probability of obtaining original estimated F-coefficients under the null hypothesis was calculated as the proportion of the distribution having values larger than the original value. For F_{IS}, alleles were permuted within each sample whereas for F_{ST}, genotypes were permuted among the samples.

II.6.4. Anaysis of Molecular Variance (AMOVA)

With the new advances in molecular genetic techniques and the new devices developed, it is easier to collect information on allele frequencies, as well as on the amount of differences (mutations) between alleles. 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. That is why, Excoffier and his collaborators (1992) modified ANOVA analysis to incorporate the molecular information in it and named it as AMOVA (Analysis of Molecular Variance). A variety of molecular data – molecular marker data (for example, RFLP or AFLP), direct sequence data, or phylogenetic trees based on such molecular data – may be analyzed using this method (Excoffier, et al. 1992).

Analysis of Molecular Variance (AMOVA) is a method for studying molecular variation within a species and the results are tested. It estimates the partitioning of total genetic variation;

- Among groups of populations,
- Among the populations within groups,
- Among the individuals within a population.

The raw molecular data is treated as a Boolean vector $\mathbf{p}_i$ in AMOVA. The data are converted in to a 1xn matrix of 1s and 0s, 1 indicating the presence of a marker (1) and its absence (0). Then AMOVA is performed using Euclidean distances derived from vectors of 1s and 0s, which is unlikely to follow a normal distribution. A null distribution is therefore computed by resampling of the data (Excoffier, et al. 1992). In each permutation, each individual is assigned to a randomly chosen population while holding the sample sizes constant. These permutations are repeated many times, eventually building a null distribution. Hypothesis testing is carried out relative to these resampling distributions.

There are some assumptions included in AMOVA (Excoffier, et al. 1992): The individuals from which haplotypes are sampled should be chosen independently and at random, or coarse. The mating in the sampled population is entirely random and non-assortative and no inbreeding occurs. Thus, if non-random mating or inbreeding is occurring, it will result in lower heterozygosity, and if the rates of non-random mating or inbreeding differ between populations, fixation estimates will be confounded.

The AMOVA design and the formulae are given below;

Source of Variation	d.f.	sum of squares	mean squares	Expected mean squares
Among groups	G-1	SS(AG)	$\frac{SS(AG)}{G-1}$	$\sigma_w^2 + n^n\sigma_b^2 + n^m\sigma_a^2$
Within groups among samples	d-G	SS(AD)	$\frac{SS(AD)}{d-G}$	$\sigma_w^2 + n^{'}\sigma_b^2$
Within samples	n-d	SS(WD)	$\frac{SS(WD)}{n-d}$	σ_w^2
Total	n-1	SS(T)		

$$SS(T) = \frac{1}{2n}\sum_i^n \sum_j^n \delta_{ij}{}^2$$

$$SS(AG) = SS(T) - \sum_g^G \frac{1}{2n_g}\sum_k^d \sum_{k'}^d \times \sum_i^{n_{gk}} \sum_j^{n_{gk}} \delta_{ij}{}^2$$

$$SS(AD) = \sum_g^G \frac{1}{2n_g}\sum_k^{d_g} \sum_{k'}^{d_g} \times \sum_i^{n_{gk}} \sum_j^{n_{gk}} \delta_{ij}{}^2 - SS(WD)$$

$$SS(WD) = \sum_g^G \sum_k^{d_g} \frac{1}{2n_{gk}} \times \sum_i^{n_{gk}} \sum_j^{n_{gk}} \delta_{ij}{}^2$$

$$d = \sum_g^G d_g$$

$$n = \sum_g^G \sum_k^{d_g} n_{gk}$$

$$n_g = \sum_k^{d_g} n_{gk}$$

$$n' = \frac{1}{d}\sum_{g=1}^{G}\sum_{k=1}^{d_g} n_{gk} - \sum_{g=1}^{G}\frac{1}{n_g}\sum_{k=1}^{d_g} n_{gk}^2$$

$$n'' = \frac{1}{G-1}\sum_{g=1}^{G}\sum_{k=1}^{d_g} n_{gk}^2 - \sum_{g=1}^{G}\sum_{k=1}^{d_g} n_{gk}^2$$

$$n''' = \frac{1}{G-1}n - \frac{1}{n}\sum_{g=1}^{G}\left(\sum_{k=1}^{d_g} n_{gk}\right)^2$$

In this study AMOVA analysis was employed to analyze how the total genetic variation was partitioned within and among breeds (for similar application, see Tserenbataa *et al.*, 2004).

II.6.5. Genetic Distance Estimations and Tree Construction

The distance matrix approaches used in this study are as follows:

(i) *Nei's D_A Genetic Distance*

The D_A 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} and y_{ij} are the frequencies of the *i*th allele at the *j*th locus in samples X and Y, respectively and m_j is the number of alleles at the *j*th locus, and r is the number of loci examined.

(ii) *Allele Sharing Distance*

This method is based on the idea that alleles which are common in all samples of the same species are likely to have existed before the split of these

samples; so that they might also be more frequent than the newly formed ones that might not shared by all. As a result, the proportion of shared alleles increases with increasing genetic similarity of the samples.

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

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

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

Phylogenetic tree construction method used:

Neigbor Joining (NJ) tree construction method is a distance based approach which aims to minimize the total length of tree by sequentially finding the neighbors. This method is used rather than UPGMA, which is another distance based approach, because NJ does cluster analysis allowing for unequal rates of molecular change among branches (Avise, 1994). Also, the comparative studies among different tree construction methods have suggested that NJ method performs better than the others under nonuniform rates either among lineages or among sites (Saitou and Nei, 1987; Li, 1997).

II.6.6. Factorial Correspondance Analysis (FCA)

The Factorial Correspondence Analysis (FCA) is performed to visualize the individuals in multidimensional space and to explore the relationships between the individuals. It involves a linear transformation of the number of alleles at each locus for each individual, which can have 0, 1 or 2 copies of an allele at a particular locus. The coefficients are chosen to maximize the variation of the transformed data measured along each coordinate axis. The first three axes are the most informative ones (MacHugh *et al*., 1997; Byrne *et al*., in press). As a result, it is possible to visualise how the individuals are related to each other on the independent axis chosen.

II.6.7. Assignment Test

Assignment tests were performed to test if the data on the five microsatelite loci chosen for the study provide enough genetic information to assign individual samples only to their original breeds. There are two types of methods for assigning individuals to samples: (i) likelihood-based methods in which individuals are assigned to the sample where the likelihood of their genotype is highest and (ii) genetic distance-based methods in which individuals are assigned to the (genetically) closest sample.

In addition, every assignment method can be used in two different ways. In the first way, noted "direct" in the interface, the chosen criterion (likelihood or genetic distance) is used directly to assign the individual. In the second way, noted "simulation", a "probability" that the individual belongs to each sample is computed. It can be used to exclude samples as origins of individuals.

In this study, Bayesian type likelihood method is used to assign the individuals by simulating them 10000 times per samples. Also 5 different probability criteria to reject the assignment of the individual to the sample of interest were used, which are $P<0.001$, $P<0.01$, $P<0.05$, $P<0.2$, and $P<0.5$.

II.6.8. Principal Component (PC) Anaysis

By definition, principle components are a set of variables that define a projection that encapsulates the maximum amount of variation in a dataset and is orthogonal (and therefore uncorrelated) to the previous principle component of the same dataset. Principle Component (PC) analysis is designed to capture the variance in a dataset in terms of principle components. In this analysis, individuals cannot be shown. A sample is represented as a single point in space constructed by PCs

In the vector space, PC analysis identifies the major directions and the corresponding strengths of the variation in the data. PC analysis achieves this by computing the eigenvectors and eigenvalues of the covariance matrix of the data.

Keeping only a few eigenvectors corresponding to the largest eigenvalues, PC analysis can also reduce the dimensions of the data while retaining the major variation of the data.

For the PC analysis analysis, NTSYSpc software was used (Exeter software; www.exetersoftware.com/cat/ntsyspc/ntsyspc).

II.6.9. Delaunay Network Analysis

Delaunay network is performed, by applying Thiessian polygons on the study area (Monmonier, 1973). First the map of the study area is taken and divided into regions. In this case the regions are the distribution areas of the studied breeds. Then points are placed in the center of the regions as representatives of the regions or breeds. Afterwards, the outer boundary of the study area is drawn with joining the points at the outer regions. The next step is to join all the interior points based on "shortest distance" criterion to form triangles. The resulting figure is called "Delaunay Network".

In order to find about the possible genetic barriers pairwise genetic differences between the breeds are written on the corresponding edges of the triangles joining the points (Brassel and Reif, 1979). A perpendicular is drawn to the one of the triangle edges in the outer boundary, which has the highest pairwise genetic distance. Then the perpendicular line is directed towards the one of the remaining two edges of that triangle, which has the highest pairwise genetic distance and you are either in the neighboring triangle or outside of the outer polygon. Drawing of the perpendicular lines is continued until you are outside of the polygon, which is considered as a barrier. The drawing of the second barrier is started from one of the edges of the outer polygon, which is untouched by the first barrier and has the highest pairwise genetic distance than the rest of the untouched edges of the polygon. Drawing of the second barrier continues till it meets the first barrier or it goes out of the polygon. The drawing of the barriers continues until all the edges of the outer polygon are tested. If the barrier does not go out of the polygon, but drive

circles inside it, then it means there is no barrier. The order with which the barriers are drawn also means as the order of their priority.

II.6.10. Mantel Test

The principle of mantel test is to test the presence of correlation between two distance matrices (Mantel, 1967; Rousset and Raymond, 1997). For example, the relation between genetic distance matrix and geographic distance matrix can be tested for the presence of isolation by distance. Using proper statistics, it is not only possible to investigate the relationship between the elements of two matrices but also it is possible to test the statistical significance of the results by permutation test.

II.6.11. Bottleneck Analysis

This analyis is used to investigate the probability for the occurence of a recent bottleneck in the sample of interest. The software, named as Bottleneck, used for this test is based on the hypothesis that the allelic diversity (H_E) is reduced faster than the heterozygosity (H_O) if samples experience a recent reduction of their effective sample size.

In order to detect recent effective sample size reductions, the program Bottleneck uses the allelic frequencies and it computes for each sample sample and for each locus the distribution of the heterozygosity expected from the observed number of alleles (k), given the sample size (n) under the assumption of mutation-drift equilibrium. This distribution is obtained through simulating the coalescent process of n genes under three possible mutation models, the IAM (Infinite allele model), TPM (Two-phased model, both IAM and SMM in different percentages used in simulation) and the SMM (Stepwise mutation model) using either genetic distance or bayesian approaches. This enables the computation of the average (H_{exp}), which is compared to the observed heterozygosity to establish whether there is a heterozygosity excess or deficit at this locus. In addition, the standard deviation (SD) of the mutation-drift equilibrium distribution of the heterozygosity is used to

compute the standardized difference for each locus ((H_O-H_E)/SD). The distribution obtained through simulation enables also the computation of a P-value for the observed heterozygosity.

Once all loci available in a sample sample have been processed, the three statistical tests (sign test, Wilcoxon test and standardized differences test) are performed for each mutation model and the allele frequency distribution is established in order to see whether it is approximately L-shaped (as expected under mutation-drift equilibrium) or not (recent bottlenecks provoke a mode shift).

In this study, TPM was employed with bayesian approach and Wilcoxon test (as recommended by the authors for microsatellite data) was used to test the significance of the results after permuting the data 1000 times (see Cornuet and Luikart, 1996 for details).

II.6.12. List of Statistical Analysis Methods Applied and the Softwares Used

The summary list of the statistical methods used in data analysis of these study and the names of the softwares used to perform these statistical test are given (in paranthesis) below:

- Exclusion of one of the relatives (Kinship 1.1)
- Allelic diversity and gene diversity estimations (Genetix 4.02)
- F statistics (Genetix 4.02, Arlequin 2.001)
- Analysis of Molecular Variance (Arlequin 2.001)
- Genetic distance estimations and NJ tree construction (Populations)
- Factorial Correspondence Analysis (Genetix 4.02)
- Assignment Test (GeneClass)
- Principle Component Analysis (NTSYSpc)
- Delaunay Network for construction of genetic barriers
- Mantel Test (Arlequin 2.001)
- Bottleneck Test (Bottleneck)

The webpage addresses for these softwares are:

- Kinship 1.3.1. : http://www.gsoftnet.us/GSoft.html
- Genetix 4.02 : http://www.univ-montp2.fr/~genetix/genetix.htm
- Arlequin 2.001 : http://anthropologie.unige.ch/arlequin/
- Populations : http://www.pge.cnrs-gif.fr/bioinfo/wini386/samples.exe
- GeneClass : http://www.ensam.inra.fr/URLB/
- NTSYSpc : http://www.exetersoftware.com/cat/ ntsyspc/ntsyspc
- Bottleneck : http://www.ensam.inra.fr/URLB/

CHAPTER III

RESULTS

In this chapter first the results of the analyzed data will be presented. The allelic data collected for this study were given in the Appendix B. This data were analyzed by all the statistical methods explained in the previous chapter.

III.1. Kinship

Before analyzing all the data, first, the samples collected from breeding farms were tested for relatedness and some individuals were discarded as they were closely related to some of the individuals in the same sample collected from governmental and university farms (which are Akkaraman1, Kıvırcık, İvesi, Konya Merinosu and Türkgeldi). The initial population sizes of each of the 5 samples were 35. After excluding the relatives and some samples that did not amplify for all the five loci, the sample sizes (n) for Akkaraman1, Kıvırcık, İvesi, Konya Merinosu and Türkgeldi became 28, 23, 35, 29 and 24, respectively. For this exclusion process, the results of the kinship analysis were used and the criteria there in (Goodnight and Queller, 1999).

III.2. Allelic Variation

The total number of alleles observed for the loci used in the study is given in Table III.1 below. The highest and the lowest number of alleles observed for single locus is 21 for JMP29 and 11 for MAF 33, respectively. The number of alleles observed for each locus in each breed and the average numbers were given in Table III.2.

Table III.1. Total number of observed alleles for the loci employed in the study.

Locus Name	Observed Number of Alleles
MAF33	11
MAF65	13
MAF209	15
JMP29	21
JMP58	18

Table III.2. Total number of alleles observed for each locus in each breed and sample, the mean number of alleles observed for each breed/sample (MNA/pop) and for each locus (MNA/locus)

	MAF33	MAF65	MAF209	JMP29	JMP58	MNA/pop
Akkaraman	***6***	***7***	***10***	***14***	***13***	***10***
Akkaraman1	4	5	8	10	10	7.4
Akkaraman2	4	5	3	9	8	5.8
Akkaraman3	6	6	5	10	9	7.2
İvesi	7	8	9	11	11	9.2
Kıvırcık	8	6	7	9	10	8
Morkaraman	9	9	10	12	9	9.8
Hemşin	8	8	9	8	10	8.6
Karayaka	***10***	***9***	***8***	***14***	***16***	***11.4***
Karayaka1	10	8	8	12	14	10.4
Karayaka2	7	8	6	11	14	9.2
Dağlıç	***9***	***9***	***10***	***12***	***13***	***10.6***
Dağlıç1	6	7	8	10	11	8.4
Dağlıç2	8	9	9	12	11	9.8
Norduz	8	8	7	12	9	8.8
Kangal	7	7	8	11	9	8.4
Hamdani	5	7	9	7	6	6.8
Konya Merinosu	4	4	7	8	11	6.8
Türkgeldi	5	5	7	7	5	5.8
MNA/locus	6.6	6.9	7.5	9.9	9.8	*8.2*

The highest and lowest mean numbers of observed alleles per locus were 9.9 (JMP29) and 6.6 (MAF 33), respectively. The highest and the lowest observed mean numbers of alleles per sample were 10.4 (Karayaka1) and 5.8 (Türkgeldi and Akkaraman2), respectively. The observed mean number of alleles per sample is greater than 8 for all the samples of the study except for Akkaraman1, Hamdani, Konya Merinosu and Türkgeldi. Moreover, the mean number of alleles observed in the study per locus per sample is 8.4.

When the frequencies of the observed alleles were examined, given in Appendix C, it was determined that some of the alleles were present only in one of

the breeds. These alleles are called as breed specific or private alleles. The names of both the loci and the breeds for which the private alleles were observed, and the number of private alleles are given in the Table III.3.

Table III.3. The distribution of private alleles observed in the study and their frequencies. Two alleles with highest frequencies were shown in bold.

Locus	Allele	Frequency	Sample found
JMP29	113	0.010	Akkaraman1
	119	0.009	Karayaka1
	153	0.026	Karyaka1
	159	0.023	Kıvırcık
JMP58	134	0.018	Karayaka1
	138	**0.456**	**Kıvırcık**
	146	0.009	Karayaka2
MAF65	109	0.023	Kangal
	119	0.038	Norduz
MAF209	108	0.023	Kangal
	110	**0.273**	**Kıvırcık**
	136	0.015	Morkaraman

In total, 12 such alleles were observed. The highest number of private alleles (4) was found in JMP29, which also has the highest number of alleles and the highest mean number of alleles observed per locus. There are no private alleles found in MAF33, which has the lowest number of alleles observed. There are 2 private alleles observed for MAF65 locus and 3 private alleles in both MAF209 and JMP58 loci. Kıvırcık and Karayaka1 samples have 3 private alleles each; Kangal has 2; Karayaka2, Akkaraman1, Morkaraman and Norduz have 1 private allele. In general, these private alleles are found at either end of the allelic range with a low frequency (between 0.01 and 0.05). As their frequencies are quite low, they cannot be used as in identification of the breeds. However, two private alleles observed in Kıvırcık breed are significant due to their high frequencies, which are allele-110 of MAF209 locus (frequency: 0.27) and allele-138 of JMP58 locus (frequency: 0.46).

In order to visualize the alleles observed for each breed and their frequencies frequency distribution histograms were constructed and given in the Appendix D. Alleles 124 and 136 are the most common alleles observed in MAF33 locus in the samples analyzed. Alleles 127, 129 and 131 are the most common ones observed in MAF65 locus in general. In MAF 209 locus, allele-118 is the most common allele observed with high frequency (0.2-0.5) in all of the breeds except for Kıvırcık in which allele-110 (confined to Kıvırcık) and allele-112 have the highest frequencies and the frequency of allele-118 is lower than 0.1. The most common alleles of JMP29 locus are 135, 137 and 139. Finally, alleles 142 and/or 144 are the two most common alleles observed in all breeds except for Kıvırcık in which allele 138 (Confined to Kıvırcık) has the highest frequency and both 142 and 144 have frequencies lower than 0.1.

The frequency distribution graphs of alleles (Appendix D) observed for each breed were placed on a map with respect to the distribution of the breeds across Turkey in order to see if there is a particular pattern (e.g. West-East, North-South; Northeast-Southwest or Southeast-Northwest) in the distribution of the alleles. This was repeated for each locus analyzed and the graphs are given in Figure III.1 – III.5.

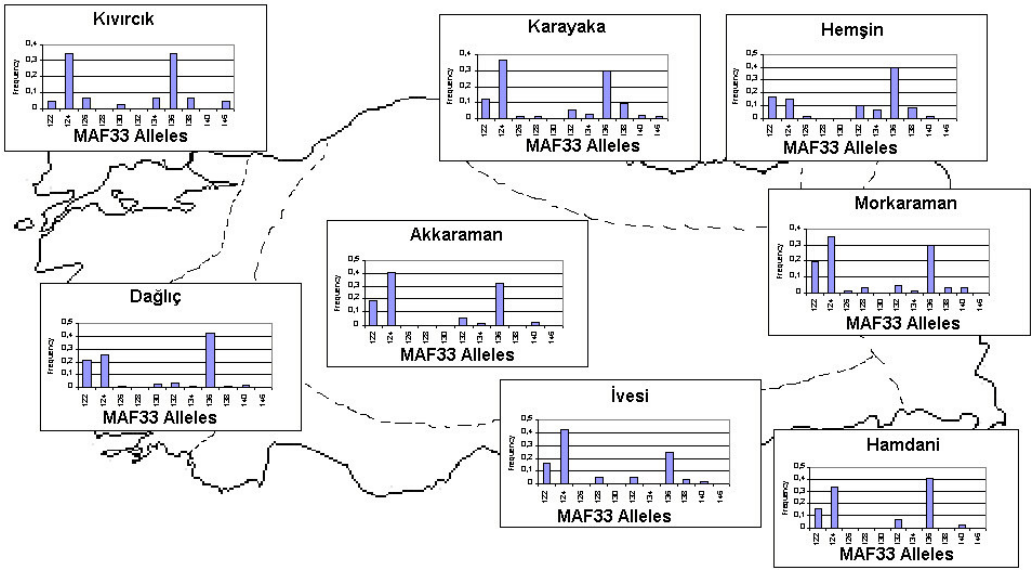

Figure III.1. The allele frequency distribution charts of MAF33 alleles for Turkish native breeds and Hamdani placed on map of Turkey.

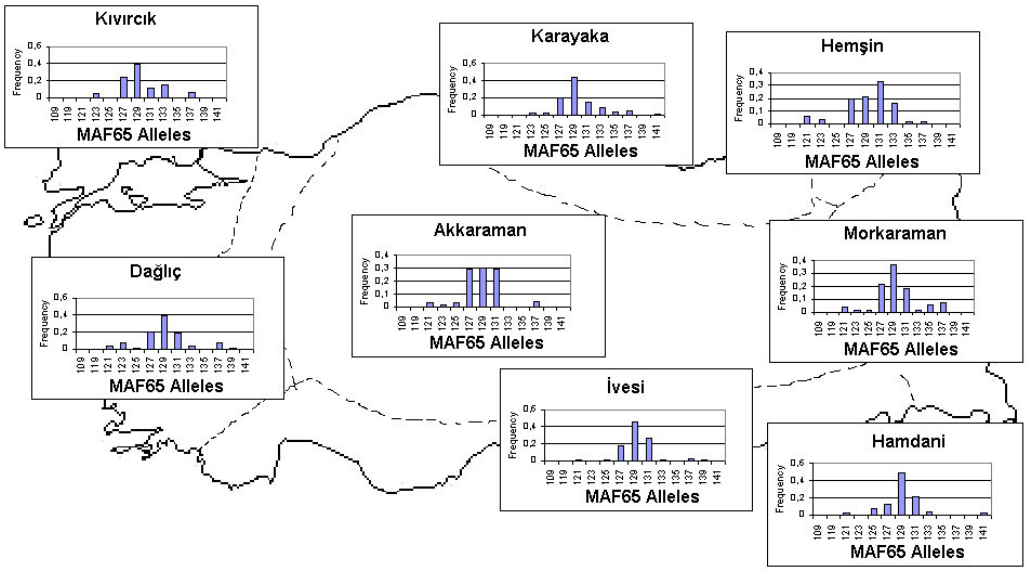

Figure III.2. The allele frequency distribution charts of MAF65 alleles for Turkish native breeds and Hamdani placed on map of Turkey.

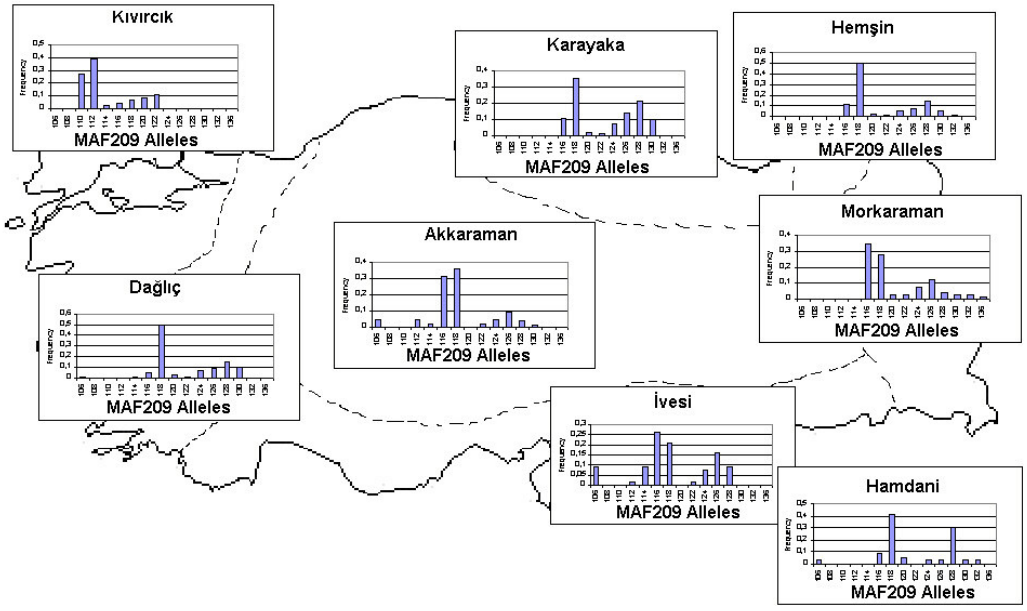

Figure III.3. The allele frequency distribution charts of MAF209 alleles for Turkish native breeds and Hamdani placed on map of Turkey.

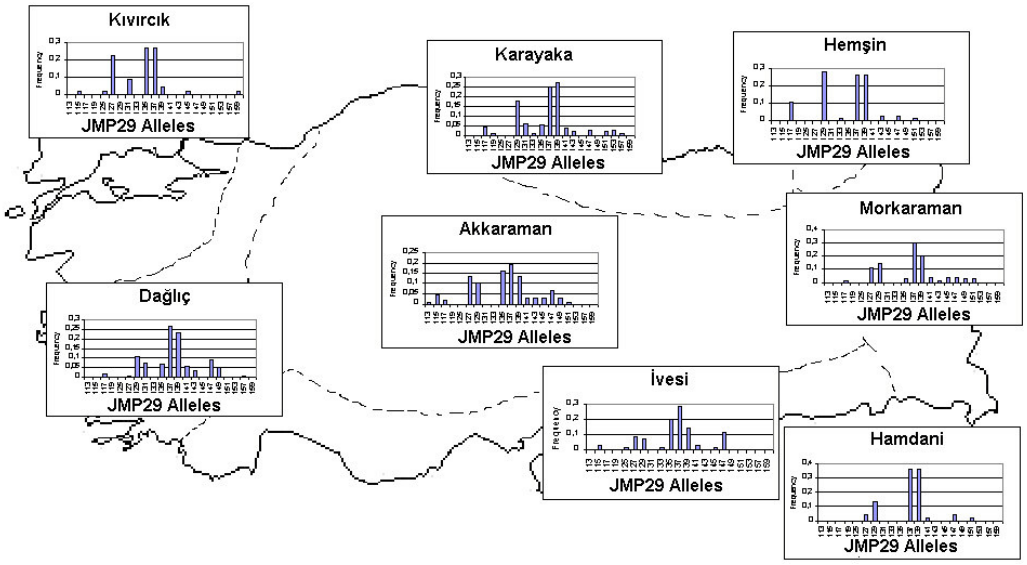

Figure III.4. The allele frequency distribution charts of JMP29 alleles for Turkish native breeds and Hamdani placed on map of Turkey.

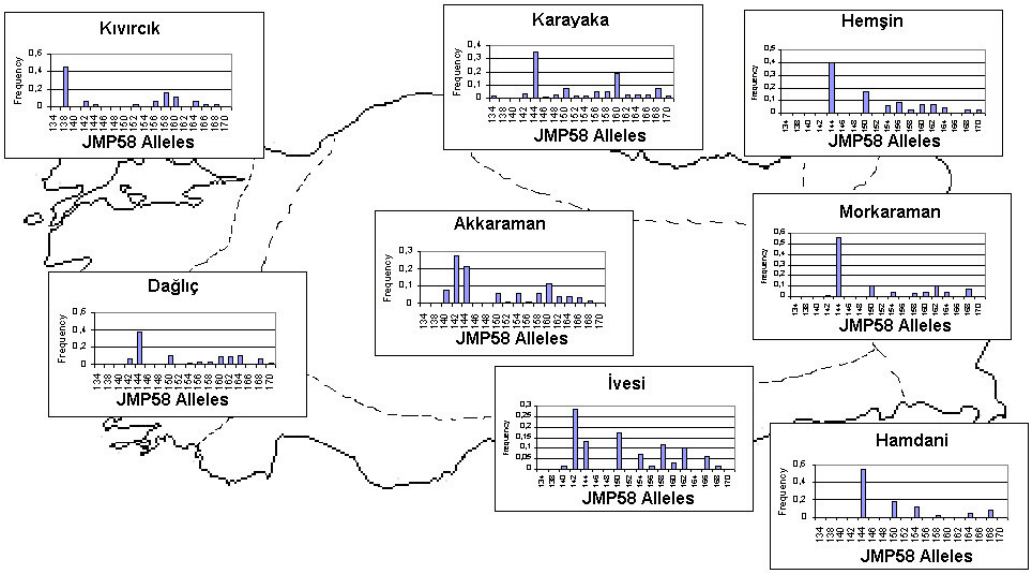

Figure III.5. The allele frequency distribution charts of JMP58 alleles for Turkish native breeds and Hamdani placed on map of Turkey.

As can be seen from the figures, there is no significant pattern in the distribution of alleles among breeds across Turkey. Also, there is no particular difference between eastern breeds and western breeds or between thin tail breeds and fat tail breeds in terms of alleles or allele frequencies they posses. Similarly, there is no significant difference between northern breeds and southern breeds. Moreover, there is no gradual change observed in the breeds from northeast to southwest and from southeast to northwest with respect to their alleles observed in the loci analyzed.

III.3. Heterozygosity Analysis

The observed heterozygosity (H_O) values of each breed/sample for each locus and the mean observed heterozygosity both per locus and per breed/sample were given in Table III.4. Among the breeds, Dağlıç, which has the 2nd highest mean allele number, has the highest average H_O and Norduz has the 2nd highest average H_O. The

lowest average H_O values were found in Akkaraman1 (0.633) and Türkgeldi (0.679). If we consider the average H_O per locus values, the highest two numbers belong to JMP29 (0.735) and MAF33 (0.726) loci, which has the highest and the lowest total number of observed alleles, respectively. The average H_O per locus that belong to the other loci are as follows; 0.720 (MAF65), 0.679 (MAF 209) and 0.703 (JMP58).

Table III.4. The observed heterozygosity (H_O) values for each breed/subpopulation in terms of each locus analyzed and the average H_O values per sample and per locus with standard deviations. The italic raws are not included in average estimations.

	MAF33	**MAF65**	**MAF209**	**JMP29**	**JMP58**	**Mean/pop. .± St.Dev.**
Akkaraman	***0.638***	***0.745***	***0.471***	***0.654***	***0.827***	***0.667* ± 0.133**
Akkaraman1	0.607	0.679	0.593	0.536	0.750	0.633 ± 0.083
Akkaraman2	0,800	0,778	0,200	0,800	0,900	0.696 ± 0.281
Akkaraman3	0,643	0,857	0,429	0,786	0,929	0.729 ± 0.198
İvesi	0.679	0.743	0.706	0.743	0.686	0.711 ± 0.031
Kıvırcık	0.773	0.826	0.727	0.773	0.478	0.715 ± 0.137
Morkaraman	0.647	0.815	0.485	0.857	0.686	0.698 ± 0.148
Hemşin	0.767	0.735	0.548	0.677	0.735	0.692 ± 0.087
Karayaka	***0.764***	***0.717***	***0.737***	***0.842***	***0.673***	***0.746* ± 0.063**
Karayaka1	0.885	0.708	0.750	0.821	0.577	0.748 ± 0.117
Karayaka2	0.655	0.724	0.724	0.862	0.759	0.745 ± 0.076
Dağlıç	***0.813***	***0.738***	***0.661***	***0.836***	***0.734***	***0.756* ± 0.060**
Dağlıç1	0.844	0.724	0.667	0.828	0.719	0.756 ± 0.076
Dağlıç2	0.781	0.750	0.656	0.844	0.750	0.756 ± 0.068
Norduz	0.769	0.615	0.808	0.808	0.760	0.752 ± 0.080
Kangal	0.636	0.727	0.682	0.773	0.818	0.727 ± 0.072
Hamdani	0.727	0.619	0.778	0.727	0.682	0.707 ± 0.060
Konya Merinosu	0.621	0.542	0.857	0.593	0.880	0.698 ± 0.158
Türkgeldi	0.875	0.762	0.870	0.542	0.348	0.679 ± 0.229
Mean/locus	0.732	0.725	0.655	0.748	0.716	0.715
St. Dev.	±0.093	±0.082	±0.173	±0.107	±0.150	±0.033

The average H_O values among the breeds and among the loci are quite similar to each other, differing at most by 0.123. The estimated H_E values from the allelic data for each locus and breed analyzed, and their average numbers were given in Table III.5. The highest and the lowest average estimated H_E values are 0.793

(Kıvırcık) and 0.686 (Türkgeldi), respectively. Hamdani, which has the 2nd lowest MNA/sample value, has the 2nd lowest average H_E with 0.706, as well. When we consider the average H_E per locus values, JMP29 has the highest average H_E with 0.824, and JMP58 has the second highest average H_E with 0.757. The rest of the average H_E locus values are 0.744, 0.730 and 0.718 estimated for MAF209, MAF65 and MAF33 loci, respectively.

Table III.5. The estimated expected heterozygosity (H_E) values each breed has for each locus, and the average H_E values both per locus and per sample with standard deviations. The italic raws are not included in average estimations.

	MAF33	**MAF65**	**MAF209**	**JMP29**	**JMP58**	**Mean/pop.±St.Dev.**
Akkaraman	***0.702***	***0.739***	***0.760***	***0.887***	***0.852***	***0.788*.± 0.078**
Akkaraman1	0.653	0.707	0.731	0.848	0.746	0.737.± 0.071
Akkaraman2	0,822	0,771	0,468	0,895	0,784	0.748 ± 0.164
Akkaraman3	0,751	0,735	0,429	0,849	0,770	0.707 ± 0.161
Kıvırcık	0.766	0.765	0.765	0.806	0.759	0.772 ± 0.191
İvesi	0.734	0.691	0.845	0.842	0.852	0.793 ± 0.075
Morkaraman	0.759	0.783	0.790	0.840	0.668	0.768 ± 0.063
Hemşin	0.781	0.797	0.715	0.781	0.796	0.774 ± 0.034
Karayaka	***0.758***	***0.745***	***0.794***	***0.830***	***0.831***	***0.792*.± 0.040**
Karayaka1	0.800	0.695	0.836	0.827	0.753	0.782 ± 0.058
Karayaka2	0.693	0.787	0.756	0.833	0.862	0.786 ± 0.066
Dağlıç	***0.707***	***0.765***	***0.709***	***0.849***	***0.812***	***0.768*.± 0.063**
Dağlıç1	0.707	0.730	0.713	0.844	0.813	0.761 ± 0.063
Dağlıç2	0.716	0.795	0.713	0.860	0.822	0.781 ± 0.065
Norduz	0.753	0.695	0.759	0.860	0.722	0.758 ± 0.063
Kangal	0.677	0.744	0.697	0.846	0.777	0.748 ± 0.067
Hamdani	0.702	0.698	0.740	0.728	0.661	0.706 ± 0.031
Konya Merinosu	0.555	0.659	0.849	0.804	0.862	0.746 ± 0.134
Türkgeldi	0.702	0.656	0.800	0.774	0.500	0.686 ± 0.119
Mean/locus	0.723	0.732	0.725	0.827	0.759	0.753
St.Dev.	±0.064	±0.048	±0.119	±0.041	±0.092	±0.032

In this table, the average H_E values of the breeds and the loci differed by at most 1.07, and the estimated average H_E/locus/breed was 0.753. When we considered the average observed and expected heterozygosities, JMP29 has the highest average

value for both H_O and H_E. The mean H_E values are all greater than the mean H_O values, except for Hamdani in which both values are equal. Dağlıç, Norduz and Karyaka breeds have high values for both H_O and H_E. The crossbreed Türkgeldi has one of the lowest values for both average observed and expected heterozygosity values (Table III.6).

Table III.6. The average observed (H_O) and expected (H_E) values for the breeds analyzed. The italic raws are not included in average estimations.

	Average Ho	**Average He**
Akkaraman	***0.667***	***0.788***
Akkaraman1	0.633	0.737
Akkaraman2	0.696	0.748
Akkaraman3	0.729	0.707
İvesi	0.711	0.793
Kıvırcık	0.715	0.772
Morkaraman	0.698	0.768
Hemşin	0.692	0.774
Karayaka	***0.746***	***0.792***
Karayaka1	0.748	0.782
Karayaka2	0.745	0.786
Dağlıç	***0.756***	***0.768***
Dağlıç1	0.756	0.761
Dağlıç2	0.756	0.781
Norduz	0.752	0.758
Kangal	0.727	0.748
Hamdani	0.707	0.706
Konya Merinosu	0.698	0.746
Türkgeldi	0.679	0.686
Ort./lokus	0.715	0.753

III.4. Pairwise F_{ST} comparisons of the samples of Akkaraman, Karayaka and Dağlıç breeds

Before going further in the data analysis, the pairwise F_{ST} values between the samples of Akkaraman, Karayaka and Dağlıç breeds were estimated and permutation

tests were performed to test the significance of these pairwise F_{ST} values. The results are given in the Table III.7.

Table III.7. Pairwise F_{ST} values of Akkaraman, Karayaka and Dağlıç samples and their significance test results.

	AKK1	**KRY1**	**DAĞ1**	**AKK2**	**DAĞ2**	**KRY2**	**AKK3**
AKK1	-	***	***	***	***	***	***
KRY1	0.103	-	ns	*	ns	*	*
DAĞ1	0.117	0.002	-	ns	ns	**	ns
AKK2	0.146	0.031	0.010	-	ns	***	ns
DAĞ2	0.106	0.005	0.008	0.010	-	*	ns
KRY2	0.098	0.016	0.020	0.044	0.013	-	**
AKK3	0.146	0.029	0.013	0.002	0.005	0.029	-

(ns: not significant,*P<0.05, **P<0.01, ***P<0.001)

This analysis was performed to see if there were significant differences between the samples of the same breeds. There was a significant difference between Akkaraman1 and Akkaraman2, and Akkaraman1 and Akkaraman3, but not between Akkaraman2 and Akkaraman3. For this reason, Akkaraman2 and Akkaraman3 samples were considered as one sample (n=24), so that its size became comparable to the sample sizes of the other breeds. Akkaraman1 sample was kept as it is.

The pairwise F_{ST} value between Dağlıç samples is not significant. However, the pairwise F_{ST} value between Karayaka samples was significant (P<0.001). Hence, they are kept as separate samples. Although the two Dağlıç samples were not significantly different from each other, they are kept separate to minimize the bias that may result from sample size differences in further analysis. Because; if Dağlıç samples are considered as one sample, the size of the sample becomes twice as much the size of the other breeds. In addition, in some analysis only one subpopulation of the Dağlıç and Karayaka breeds were considered. In this case, Dağlıç2 and Karayaka2 samples were chosen as during their sampling the flocks visited were more apart from each other and the number of flocks visited were more than that of visited for the other subpopulation of the breeds.

III.5. Within Breed Variation and Hardy-Weinberg Equilibrium

The within breed variation observed in terms of the 5 loci analyzed can be tested for Hardy-Weinberg equilibrium by using F_{IS} index of F statistics. For this reason breed and subpopulation F_{IS} values were estimated and permutation tests were performed to test their significance. The results were given in the Table III.8. The estimated F_{IS} values of İvesi, Morkaraman and Hemşin breeds were found to be significant with a probability of 0.05 and of Akkaraman1 sample with a probability of 0.001. In other words, there is a significant deviation from Hardy-Weinberg expectations in these breeds/sample.

Table III.8. The estimated F_{IS} values of the breeds and samples and their significance test results.

Population	N	F_{IS}	Statistical significance
Akkaraman	***52***	***0.16***	***
Akkaraman1	28	0.14	**
Akkaraman2	24	0.02	NS
Kıvırcık	23	0.08	NS
İvesi	35	0.10	*
Morkaraman	35	0.09	*
Hemşin	34	0.11	*
Karayaka	***57***	***0.06***	***NS***
Karayaka1	28	0.04	NS
Karayaka2	29	0.05	NS
Dağlıç	***64***	***0.02***	***NS***
Dağlıç1	32	0.01	NS
Dağlıç2	32	0.03	NS
Norduz	26	0.01	NS
Kangal	22	0.03	NS
Hamdani	22	0.00	NS
Konya Merinosu	29	0.06	NS
Türkgeldi	24	0.01	NS

(NS: not significant,*P: 0.05, **P: 0.01, ***P: 0.001)

There is no other significant deviation observed from Hardy-Weinberg expectations in the rest of the breeds and their repetitive samples.

III.6. Analysis of Molecular Variance (AMOVA)

In order to see how the genetic variation was partitioned within and between the breeds, 5 different AMOVA analyses were performed using the data only from the native breeds (Kıvırcık, Dağlıç, Karayaka, Hemşin, Akkaraman, Morkaraman, Norduz and İvesi).

In the first AMOVA analysis, each breed was treated as one group. In total 8 groups were formed. Each of Akkaraman, Karayaka, and Dağlıç groups was composed of their repetative samples and each of Morkaraman, İvesi, Kıvırcık; Dağlıç and Norduz was composed of their only one sample. The result revealed that 94.22% of the total variation was attributed to within the samples and only 5.03% of the total variation was partitioned between the samples of the breeds. Moreover, only 0.75% of the total variation was between the breeds, which is not significant.

In the second AMOVA analysis two groups were formed, namely fat tail (Akkaraman2, Morkaraman, İvesi) and thin tail (Karayaka2, Kıvırcık) in order to see the partitioning of the genetic variation within and among fat tailed and thin tailed breeds. In this approach, 90.30% of the total variation was partitioned within the samples and 9.36% was partitioned among the breeds within groups formed based on their tails. Also there was not a significant difference between the groups of thin tailed and fat tailed breeds ($P<0.34$). In addition, the differentiation among samples within groups was increased by 4.33% with respect to the first analysis. There were much more difference within the groups than among the groups.

In the third AMOVA analysis, three groups were formed, in order to analyze the pattern in the partitioning of the total genetic variation among the Northwestern, Central and Southeastern Turkish breeds. The name of the groups and the breeds included in the groups are; Northwestern breeds (Kıvırcık), Central Anatolian breeds (Akkaraman2 and Kangal) and Southeastern breeds (Morkaraman, İvesi, Hamdani and Norduz). This analysis also revealed similar results. 91.32% of the total variation was partitioned within the breeds, 2.51% of the total variation was between the breeds of geographic groups and 6.17% of the total variation was among the groups

(P<0.003). The difference among groups was higher than that of among samples within the groups. Thus, there seems to be a significant difference between the groups of breeds in Northwest-Southeast direction.

In the fourth AMOVA analysis, three groups were formed as well to analyze any pattern in genetic variation partitioning in north-south direction. The groups were; Northern breeds (Kıvırcık, Karayaka2 and Hemşin), Central Anatolian breeds (Akkaraman2, Dağlıç2, Norduz and Morkaraman) and Southern breeds (İvesi and Hamdani). The purpose was to analyze the pattern in the partitioning of the total genetic variation in North-South direction. The results were not different from the previous AMOVA analysis. 94.86% of the total variation was within the samples, 5.09% was among samples within groups and 0.04% was among groups. There was not a significant pattern (P<0.367) in the partitioning of the total genetic variation among the groups of breeds from North to South.

In the final AMOVA analysis, all the native breeds were put in one group (Akkaraman2, Kıvırcık, Dağlıç2, Karayaka2, Hemşin, Morkaraman, İvesi and Norduz) in order to analyze the partitioning of the genetic variation among the breeds. The results showed that 94.58% of the total variation was within the samples and 5.42% was among the breeds (P<0.001). This significant result suggested that there was at least one sample differentiated from the rest.

III.7. Allele Sharing Distance (ASD) and Factorial Correspodance Analysis (FCA)

There are two analyses performed in order to visualize the genetic relationship between all the individuals analyzed in the study: Neighbor-Joining (NJ) tree based on Allele Sharing Distances (ASD) between the individuals and Factorial Correspondence Analysis (FCA).

The NJ tree constructed based on ASDs has deep branches and has a star like shape, which is expected for domestic animals, because domestication starts with bottlenecks or founder effects followed by a rapid demographic expansion. Branches

representing the individuals of different breeds formed the nodes of the tree. There was not a single node holding individuals of the same breed solely. Moreover, there was not any clear pattern in the formation of the nodes. As the tree containing all the individuals of 9 native breeds (Kıvırcık, Dağlıç, Karayaka, Hemşin, Akkaraman, Kangal, Morkaraman, Norduz and İvesi) had a very crowded, compact and complex structure (see Figure III.6 below), 5 individuals from each breed were randomly chosen and the tree was reconstructed using these randomly chosen individuals, in order to see if there was a pattern in the distribution of the individuals. The tree is given in Figure III.7 in the next page.

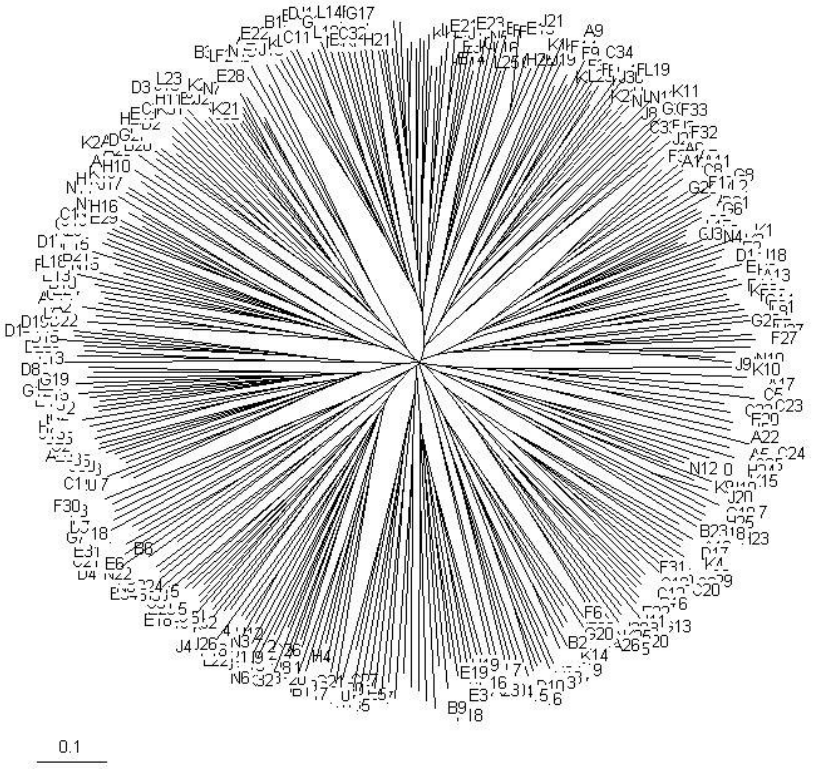

Figure III.6. The allele sharing distance tree of all native Turkish sheep breeds.

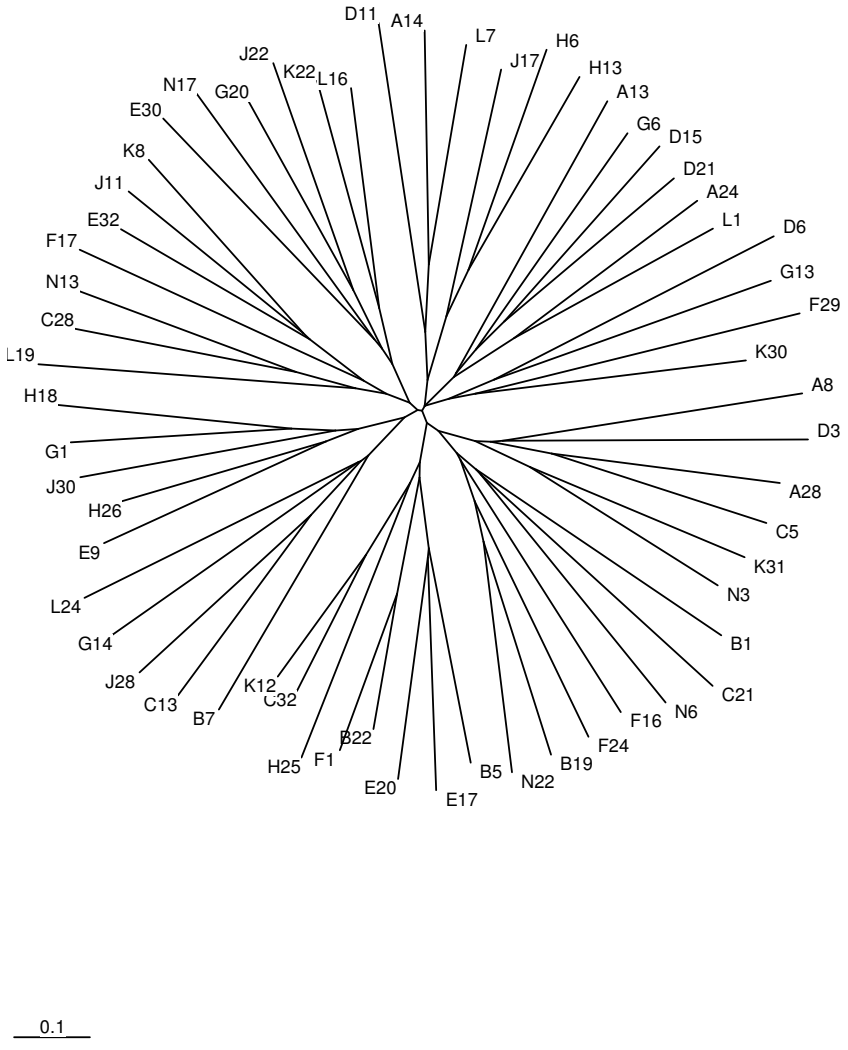

Figure III.7. The NJ tree constructed based on ASD between the randomly chosen 5 individuals of each breed (Kıvırcık, Dağlıç, Karayaka, Hemşin, Akkaraman, Kangal, Morkaraman, Norduz and İvesi). The legends for the breeds are; A: Akkaraman 1, B: Akkaraman 2, C: İvesi, D: Kıvırcık, E: Morkaraman, F: Hemşin, G: Karayaka 1, H: Karayaka 2, J: Dağlıç 1, K: Dağlıç 2, L: Norduz, N: Kangal.

The FCA was performed first by using only the native breeds and second, by using all the breeds analyzed in the study. In both analysis, the samples were examined on 3D graphics with different triple combinations of the 10 factors (each

represented by an axe) estimated by the software. Among all the breeds analyzed, only the samples of Kıvırcık fell apart from the rest, clearly (Figure III.8). Although there was not a nice and clear grouping, Akkaraman1 and Konya Merinosu samples were together and detectable. Moreover, İvesi and Türkgeldi individuals grouped together. All the rest of the samples tended to cluster together.

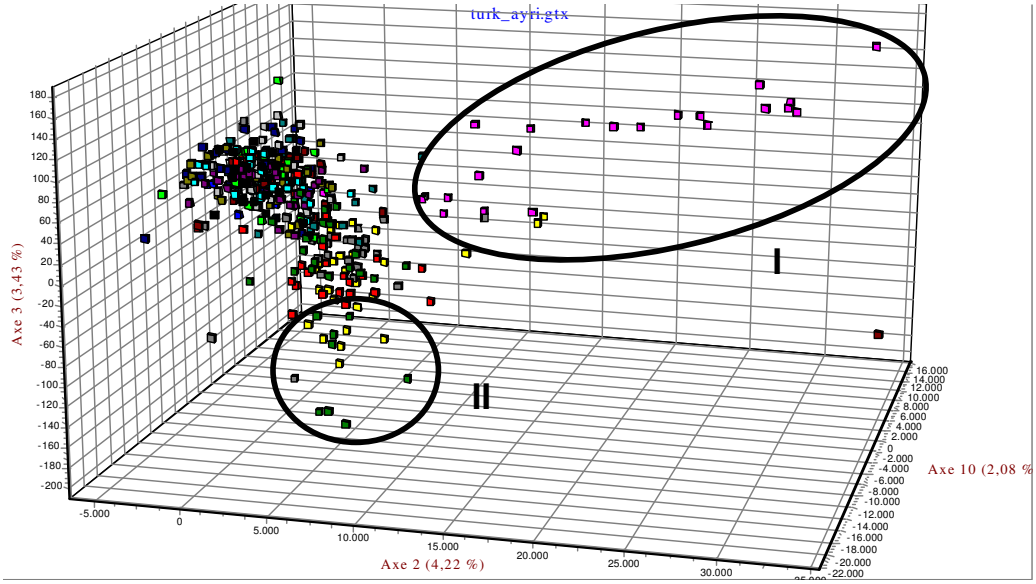

Figure III.8. The FCA results showing the relationship between all the individuals analyzed in the study.

Although there is no clear separation for the majority of the samples, this analysis grouped samples from governmental and university breeding farms together in general. In circle I, there are Kıvırcık individuals, except for the two Akkaraman1 individuals (yellow). In circle II, there are Akkaraman1 (yellow) and Konya Merinosu samples (green).

III.8. Assignment Test Results

In the assignment test analysis, different combinations of the data set were considered with different α-criteria for the rejection of the assignment. First only native breeds included in the test. Second, both native and crossbreed breeds were included. Then repeated samples of the breeds were treated first as separate samples and then as single pooled sample.

As a result, except for the Kıvırcık samples, all the rest of the samples were assigned to some/all of the breeds analyzed without any significant pattern. Of the 23 Kıvırcık samples, 20 samples were assigned only to Kıvırcık breed, 1 was not assigned to any breed, 1 was assigned to one more breed and 1 was assigned to two more breeds except for Kıvırcık.

III.9. Genetic Distance Estimates and Genetic Relationships Between the Breeds

The pairwise D_A distances between the breeds, which are estimated from the allelic data, were given in the Table III.9. According to these estimations the lowest D_A distance value is 0.041 found between Dağlıç1 and Dağlıç2, and the highest D_A distance value is 0.448 found between Kıvırcık and Hamdani.

These pairwise D_A genetic distances were used in construction of 3 NJ trees showing the genetic relationships between the breeds and their samples. Bootstrapping tested the robustness of the tree. In the first NJ tree (Figure III.9), all the breeds and their repeated samples were included. In the second NJ tree (Figure III.10) only purebred samples were included omitting the Akkaraman1, Karayaka1 and Dağlıç1 samples. In the third NJ tree (Figure III.11), all the native and crossbreed breeds were included, but the same samples mentioned above were omitted. The NJ trees are given below.

Table III.9. Pairwise D_A genetic distances between the breeds and samples.

	Akk1	**Akk2**	**İve**	**Kıv**	**TG**	**KM**	**Mork**	**Hem**	**Kry1**	**Krya2**	**Dağ1**	**Dağ2**	**Nor**	**Ham**	**Kngl**
Akkaraman1	*														
Akkaraman2	0,297	*													
İvesi	0,112	0,146	*												
Kıvırcık	0,304	0,404	0,330	*											
Türkgeldi	0,296	0,343	0,248	0,415	*										
Konya Merinosu	0,158	0,262	0,174	0,368	0,366	*									
Morkaraman	0,255	0,075	0,126	0,366	0,323	0,231	*								
Hemşin	0,376	0,115	0,209	0,419	0,375	0,328	0,098	*							
Karayaka1	0,311	0,130	0,165	0,344	0,314	0,250	0,094	0,093	*						
Karayaka2	0,310	0,143	0,177	0,384	0,318	0,287	0,128	0,098	0,088	*					
Dağlıç1	0,309	0,081	0,171	0,365	0,342	0,263	0,097	0,107	0,085	0,099	*				
Dağlıç2	0,287	0,079	0,142	0,354	0,282	0,234	0,091	0,090	0,081	0,090	**0,041**	*			
Norduz	0,340	0,098	0,169	0,411	0,352	0,269	0,100	0,122	0,116	0,108	0,118	0,106	*		
Hamdani	0,368	0,108	0,184	**0,448**	0,374	0,262	0,103	0,115	0,129	0,160	0,132	0,114	0,098	*	
Kangal	0,318	0,065	0,160	0,371	0,329	0,271	0,095	0,113	0,116	0,110	0,113	0,089	0,090	0,106	*

Note: Minimum and maximum D_A values are in bold numbers.

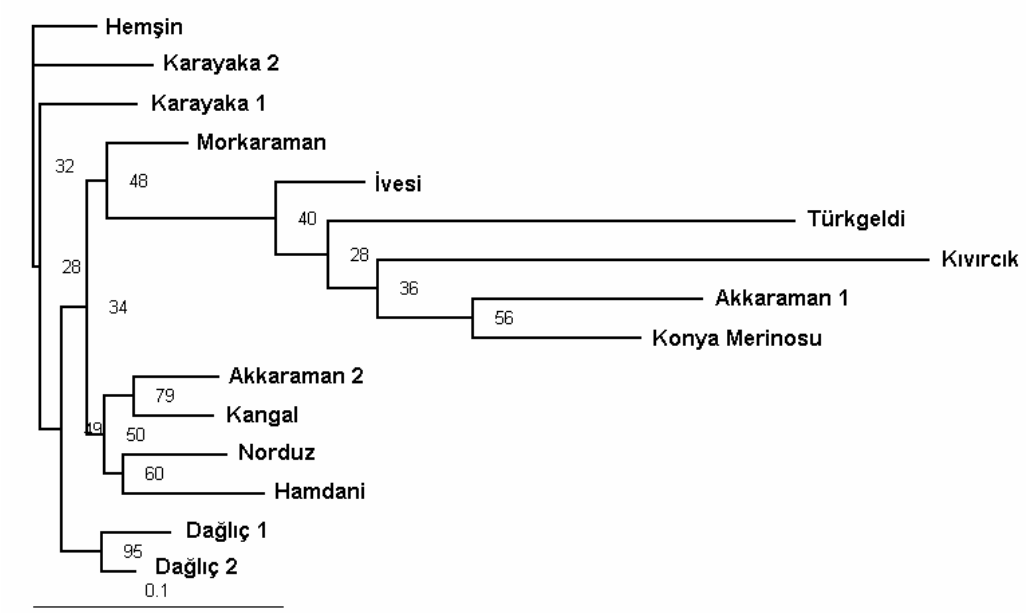

Figure III.9. NJ tree of all samples analyzed based on pairwise D_A genetic distances.

In this tree (Figure III.9) above, statistically indifferent samples of Dağlıç, which are Dağlıç1 and Dağlıç2, grouped together with a 95% bootstrap value. Then Central Anatolian breeds Akkaraman2 and Kangal (which is considered to be a variety of Akkaraman by Akçapınar, 2000) grouped together with a 79% bootstrap value. Then the Southeastern breeds Narduz and Hamdani formed a group with a 60% bootstrap value and further back joined with the group of Akkaraman2 and Kangal with a 49% bootstrap value. In this tree, the farm samples (Akkaraman1, Kıvırcık, İvesi, Türkgeldi and Konya Merinosu) formed a group where Kıvırcık and Türkgeldi had the deepest branches indicating their high degree of differentiation. Lastly, northern breeds, Hemşin, Karayaka1 and Karayaka2, formed a group with a low bootstrap value (32%).

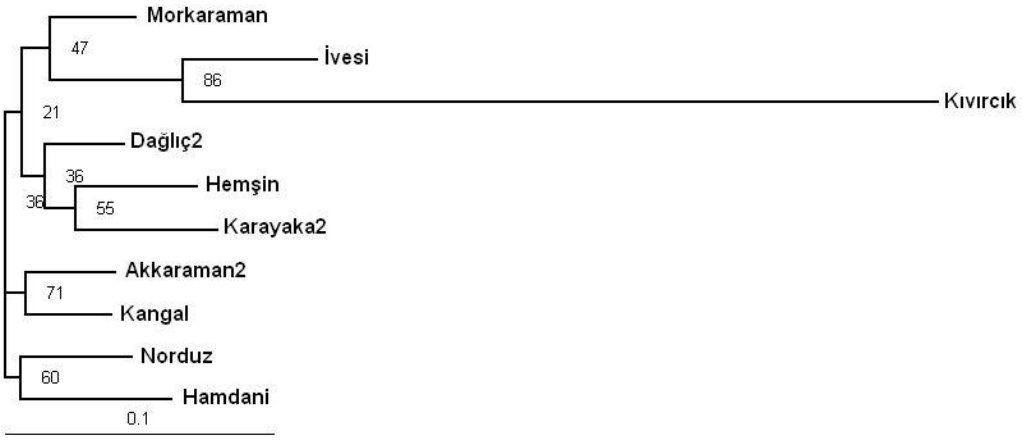

Figure III.10. NJ tree of purebred samples based on pairwise D_A genetic distances

In the above tree (Figure III.10) the crossbeeds (Konya Merinosu and Türkgeldi) were omitted together with Akkaraman1, Karayaka1 and Dağlıç1 samples. Thus, a single population represented each breed. After the omission of these samples, the groupings of Akkaraman2 with Kangal and Norduz with Hamdani didnot change. Apart from the previous tree, the Northern breeds Hemşin and Karayaka-2 grouped together and further back joined with Dağlıç-2. The last group included Morkaraman, İvesi and Kıvırcık, which have the deepest branch of all.

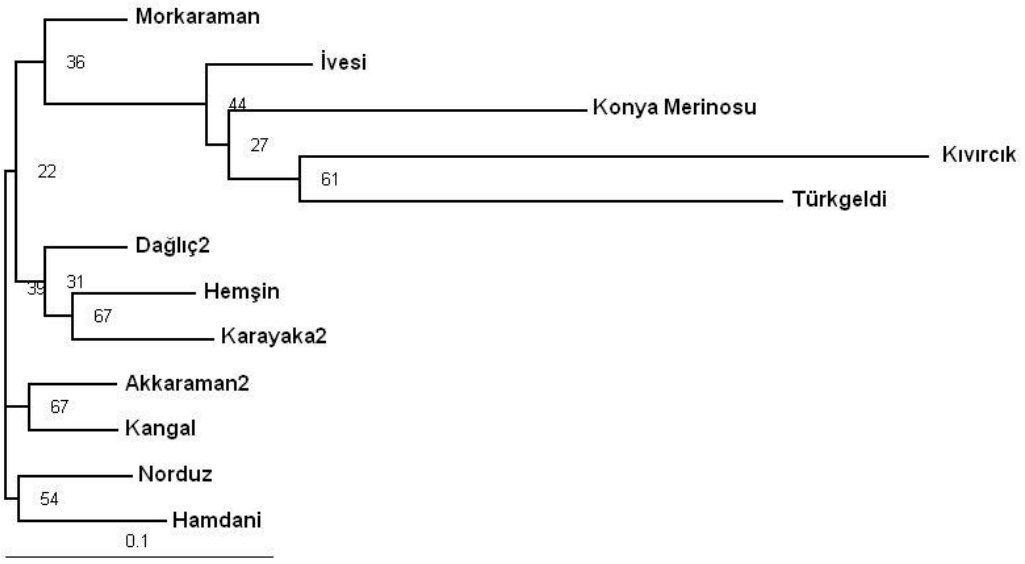

Figure III.11. NJ tree, based on D_A distances, of all samples analyzed except for samples Akkaraman1, Karayaka1 and Dağlıç1.

In this last NJ tree (Figure III.11), all the samples including the crossbreeds were incuded, but Akkaraman1, Karayaka1 and Dağlıç1. Similar to the previous tree (Figure III.10), Norduz grouped with Hamdani (both from Southeastern Turkey), Akkaraman-2 grouped with Kangal (both from Central Anatolia) and Karayaka-2 grouped with Hemşin (both from Northern Turkey), which further grouped with Dağlıç-2. The four native and crossbreed samples (İvesi, Kıvırcık, Türkgeldi and Konya Merinosu) raised in governmental or university farms grouped together and then further grouped with Morkaraman. Kıvırcık, Türkgeldi and Konya Merinosu had the deepest branches.

III.10. Principal Component (PC) Analysis

The 62.31% of the total genetic variation present between the breeds was explained by the first three axes of the PC analysis. The presentation of the breeds on 3D plot of PC analysis is given in Figure III.12. At first sight Konya Merinosu,

Kıvırcık, Akkaraman1 and Türkgeldi fell apart from the rest. However, with a lesser degree it can be seen that the first three axes can separate most of the breeds.

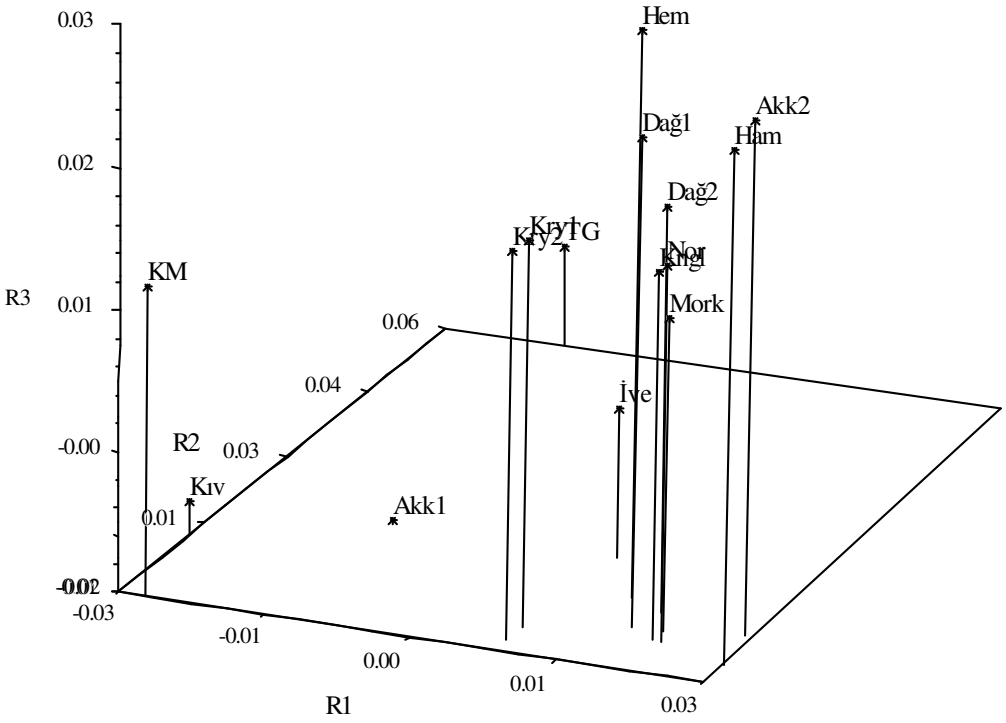

Figure III.12. The 3D PC analysis plot of all the breed analyzed. The legends for the sample names are in accordance with the Table II.1.

From left to right, the name of the breeds that are separated by the first axis are as follows; Kıvırcık, Konya Merinosu, Türkgeldi, Akkaraman1, two samples of Karayaka, İvesi, Hemşin, Dağlıç1, Dağlıç2, Kangal, Morkaraman, Norduz, Akkaraman2 and Hamdani. In this axis, the discrimination between Karayaka1 and Karayaka2 is not clear, but they are separated by the second axis.

III.11. Delaunay Anaysis Based on D_A Genetic Distance

Delaunay network analysis was performed using all the data except for the crossbreeds (Konya Merinosu and Türkgeldi) and the samples Akkaraman1, Karayaka1 and Dağlıç1. In this analysis, the pairwise D_A genetic distances were used to construct the genetic barriers on the map. First, the dots representing the breeds were placed on the map considering their distributions and sampling places. Then the thiessian triangles were formed based on the "shortest distance" criteria and starting from the edge having the highest D_A value the genetic barriers were drawn. As the significance of the barriers is correlated with the order by which they are drawn, the barriers were numbered accordingly (Figure III.13).

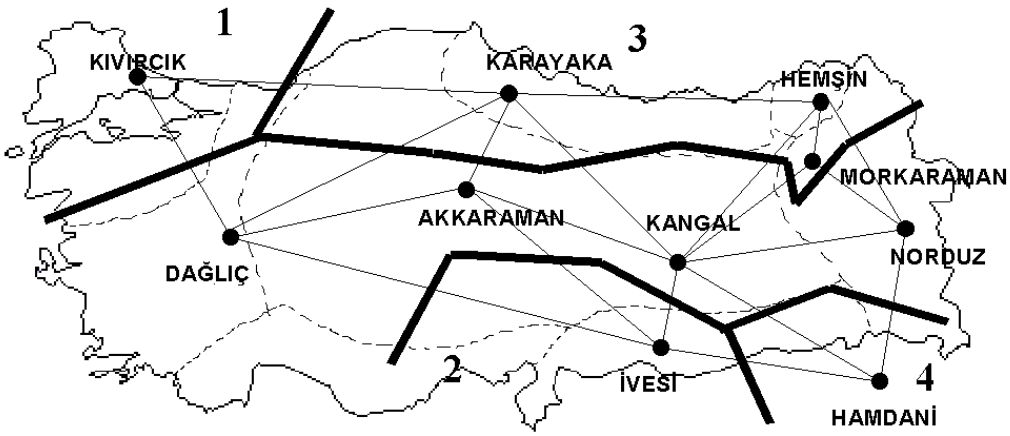

Figure III.13. Delaunay network analysis for the genetic barriers based on D_A genetic distances.

The first barrier separates Kıvırcık from the rest. The second barrier separates İvesi from the rest and the third barrier separates the Northern breeds Karayaka and Hemşin together with Morkaraman from the rest. Finally, the fourth barrier separates Hamdani from all of the Turkish breeds.

III.12. Mantel Test

This analysis was performed to test whether there is a significant correlation between the geographical distance and genetic distance between the native breeds. For this reason, on a map of Turkey marks were put showing the centers of the distribution areas of each breed. Then, the geographical distance matrix formed by measuring the pairwise distances between these marks. Afterwards, this physical distance matrix and genetic distance matrix of D_A distances were subjected to mantel test. The permutation test results revealed no significant correlations (P=0.094) between these two matrices.

III.13. Bottleneck Test

The bottleneck analysis did not reveal a statistically significant result indicating a recent bottleneck for any of the Turkish native breeds analyzed. The null hypothesis "the population is at migration-drift equilibrium" could not be rejected as all the probabilities estimated were higher than 0.4.

CHAPTER IV

DISCUSSION

In the first half of the discussion, the results of the present study was evaluated (1) to see if there is a gradient in the allele frequencies in accordance with a) the admixture from the east/south east of Anatolia b) NDD model (ii) to explain the deviations from H-W equilibrium in some samples of breeds. (iii) to see how distinct the native, Turkish sheep breeds are ancestors of some other native breeds in Turkey, (v) to emphasize the distinctness of the farm samples of the breeds. (vi) to help for the development of conservation strategies of the Turkish native sheep breeds. (vii) To have a better understanding for the "gene pool of a breed" concept.

In the second half of discussion results from same European and Awassi breeds (Bryne *et al*., in press.) will be considered together with the results of present study (i) to compare the genetic variants levels of the eastern and western sheep breeds in Europe and Western Asia (ii) to reconsider the distinctness of Turkish sheep breeds compared to these European breeds. (iii) to assess the possible genetic relatedness of Kıvırcık breed to the European breeds. (iv) to identify the possible precedents of 3 domestications among the Turkish native breeds.

IV.1. Discussion of the Results of the Present Study

The results of the different statistical analyses used in the present study were discussed below.

VI.1.1. Inferences from the Allelic Data

The presence of zebu specific microsatellite alleles in cattle (MacHugh *et al*., 1997; Loftus *et al*., 1999) made it possible to study the admixture between the two cattle types originated from different domestication events. To detect the presence of such phenomena in sheep, first, the histograms of the allelic frequencies (Appendix D) of the samples were examined to see if there were any particular alleles only seen in particular breeds in accordance with their geographic distribution. However, there were no such alleles specific only to some of the breeds in the present study. In order to see the distribution of the allele frequencies across Turkey, the histograms were placed on a map. The allele frequencies were checked for a pattern present among the breeds analyzed from east to west, north to south, northwest to southeast. None of the alleles of the five loci exhibited any gradient in these directions. However, 6 of the 20 loci studied were diagnostic loci for zebu admixture in cattle (Loftus *et al*., 1999). Perhaps, if there is a similar genetic admixture (if there is any) it can be detected with the employement of higher number of loci. Another possible explanation for seeing the marks of admixture is given below.

The mtDNA studies are suggesting two, possibly three domestication events in sheep (Hiendleder *et al.*, 1998; Townsend, 2000; Bruford and Townsend, 2004). The distribution of mtDNA lineages corresponding to these domestications do form a gradient such that mainly “A” being in the west of Turkey, “A”, “B” and “C” lineages on the east of Turkey, and “C” frequently being high on the southeast of Turkey. May be the early descendants of the first domesticated sheep were replaced with the migrations from the east and this is done mainly through the sires and hence microsatellites can not detect the admixture anymore as it was in African zebu (Loftus *et al.,* 1999).

Even if there is no admixture in the sheeps of Turkey, still the gradients for some of the alleles were expected in accordance with the Neolithic Demic Diffusion. The absence of gradients might be another support for the male mediated invasion of Anatolia after NDD. Other possible explanations for the lack of gradients can be;

(i) At least in modern times, exchange between the breeds is very high. Therefore, all the traces of admixture and/or NDD have been erased. The relatively high overlap between the members of the breeds observed in FCA, and assignment test. Furthermore very low genetic variation observed between the breeds (5%), when the genetic variation is partitioned into within samples, between the samples of the breeds and between the breeds, supports this argument. Except for samples of some breeds (Kıvırcık, Akkaraman1 and Ivesi) as shown in FCA, NJ tree based on D_A, Delaunay analysis, one can not talk about the genetic distinctness of the native Turkish sheep breeds. This last observation is going to be discussed further in this section. Lastly, the absence of significant correlation between the genetic and geographic distances in Mantel test, and the absence of correlation between the location of the breeds on 3D space in PC analysis and their geographic distribution across Turkey, are the other evidences for high degree of interchange between the breeds and/or relatively recent high degree of gene flow by the migrations from the east.

(ii) Such gradients can be detected in larger geographic scales. These last arguments can be checked with higher number of loci and with higher number of breeds from all over Eurasia.

IV.1.2. Inferences from the Heterozygosity Estimations and Inbreeding Test

Akkaraman1, Akkaraman2, Morkaraman and Hemşin samples have average H_O values smaller than 0.7; but their average H_E values are much higher. The F_{IS} statistics revealed that there was a deviation from H-W expectations in Akkaraman1, Morkaraman and Hemşin. In addition, a deviation from H-W expectations was also found in İvesi breed. Both Akkaraman1 and İvesi are farm samples of the breeds. The current size and the size of the founders in these breeding farm samples of the breeds are important factors. Small effective population sizes in the farms and

inbreeding cause deviations from H-W expectations. Hemşin breed is confined to a small mountainous area in Artvin, Turkey. It has relatively small total population size with respect to the other Turkish native breeds. The small population size and the topology of the area by causing a highly structed destribution of the Hemsin individuals may have resulted in the observation of the significant F_{IS} value. Morkaraman breed has a larger distribution area and a higher total number than Hemşin. Still, there was a deviation from H-W expectations. The Morkaraman individuals were sampled over a wide range, where there may be groupings within the breed. Populations close to each other by physical distance may be exchanging individuals but not populations distant from each other. For example, the populations in Erzurum and eastern Sivas may be exchanging individuals with each other but not with the ones in Iğdir or Kars. This structure, named as Wahlund effect, may be the cause of the positive F_{IS} value found in Morkaraman indicating heterozygote deficiency. Also, the effects of social unrest in eastern Turkey, which is the distribution region of Morkaraman, should not be ignored. In the last two decades, it resulted in a sharp decrease in agriculture in the region as the villagers migrated from eastern to western Turkey and left their farming practices. Yet, the bottleneck test revealed that, there was no sign for a recent bottleneck for this breed.

IV.1.3. Inferences from AMOVA Results

In AMOVA analysis, the most of the variation (>90%) was partitioned within samples in all of the five analyses. There was no significant difference among the groups when each native breed was treated as one separate group (Akkaraman, Ivesi, Kıvırcık, Karayaka, Dağlıç, Hemsin, Morkaraman, Norduz). When the partitioning of the variation between thin tailed and fat tailed sheep groups was analyzed, the two groups were not significantly different ($P<0.34$), either. But, the 9.36% of the total variation was found among the breeds within the groups of the two tail types. In other AMOVA analyses of Turkish native breeds, this value was usually found about 5-6%. This result revealed that on the contrary to expectations the breeds having the same tail type are actually different from each other more than they are from the one having the other tail type. There is no special differentiation between the Turkish native breeds associated with the tail types.

The AMOVA analysis of the three groups of breeds in the northwest-southeast direction revealed a significant difference among groups (P<0.003). That means there is significant difference at least between the two of the groups. The grouping shows similarity with the results of the Delaunay network analysis result. The northwest group contains Kıvırcık, which is separated by the first genetic barrier of the Delaunay network. Then the central Anatolian group contains Akkaraman2 and Kangal samples that there were no barrier between these two found by Delaunay. Finally, the southwest group contains Morkaraman, İvesi, Hamdani and Norduz, and this group has no similarity with the Delaunay network results. As a result, although it is not clear from the alleles and allele frequency analysis, there seems to be a difference among the breeds in the northwest-southeast direction. However, this can also be associated with the Kıvırcık breed being the most distinctive one among the others as revealed by different statistical analyses used in this study.

When all the Turkish native samples were included in the same single group in the fifth AMOVA analysis, the 5.42% of the variation is partitioned among samples and it is found to be statistically significant (P<0.001). Thus, at least one of the samples is significantly different from the others.

IV.1.4. Inferences from genetic distance estimations, NJ trees constructed, and FCA results

The NJ tree based on the allele sharing distances between the individuals did not group the individuals of the same breed together around the same node. The result was in consistence with the results of the assignment test and the FCA analysis. In assignment test, Kıvırcık was the only breed that 20 out of 23 individuals of it were only assigned to Kıvırcık. In FCA, the group formed by the Kıvırcık samples, which are all sampled from the same breeding farm, was the only one separate from the rest. The remaining of the individuals of the breeding farm samples (Akkaraman1, İvesi, Kıvırcık, Konya Merinosu, Türkgeldi) was together in one end of the FCA graph. Also they were grouped together in the NJ tree constructed from D_A genetic distances between the breeds. Moreover, in the 3-D PC analysis result,

these same breeds were placed separate from each other and from the rest. Results suggest that farm samples are not the typical representatives of the breeds. Possible explanation is that they are in low effective population sizes in the farms and thus, they are under the effect of random genetic drift. Therefore, in conserving the breeds in the governmentally managed farms, high effective population sizes must be maintained. Perhaps, many such farms should be established for each breed. Furthermore, exchange of samples between the farms would slow down the genetic erosion that will take in the breed.

IV.1.5. Infrences from Delaunay Network analysis results

Although the distinctness of farm samples Kıvırcık, Ivesi, Konya Merinosu, Turkgeldi, for example in NJ tree, based on D_A distances, difference of Akkaraman1 and Akkaraman2 (basically composed of samples collected from the local breeders) suggest that the farm samples are much more different than the rest of the samples of the breeds. The degree of their differentiation may at least partially reflect the divergence of the breeds that they represent. For example, Kıvırcık sample in this study is very distinct partly because it is founded by the samples of geographically distinct breed and also it was kept in farm with a low effective population size. Hence it diverged due to random genetic drift. Moreover, the first barrier resembles the barrier associated with both of İstanbul Bosporus and Çanakkale Bosporus (a geographic barrier) found by Ergüven (1997) in her PhD study on protein polymorphism and blood groups in Turkish human populations. She also found a similar barrier to the 3rd barrier of this present study, which is parallel to the north Anatolian mountain chains. This barrier was also found in a different independent study conducted by Önde and Kence (1995), based on ABO blood groups of humans. Finally, the central Anatolian corridor found in the present study is also parallel with the results of these two studies (Önde and Kence, 1995; Ergüven, 1997). Hamdani, separated by the 4th barrier is not a native breed to Turkey.

Presence of parallel genetic barriers associated with geographic barriers in two different organisms; sheep and humans, indicates the presence of genetic differentiation between the geographically isolated groups. However, the

differentiation is weak as seen from the NJ tree based on allele sharing distances or from the FCA results.

IV.1.6. Evaluation of the statistical analysis results of Turkish samples together

Before the results of this study was obtained, it was believed that morphologically different, geographically distinct and distant native sheep breeds would have relatively compact gene pools to be observed in the FCA or in NJ tree constructed by the allele sharing distances. This was not the case a very high degree of overlap between the gene pools of the breeds was observed. Whether this is a special case for Turkish native sheep breeds or whether it is a general problem will be discussed in the second half of the discussion. Yet it must be pointed out that with the employment of higher number of microsatellite loci the resolution between the different members of the breeds in expected to improve. When the independent repetitive samples of the same breeds are considered provided that they are collected from the local breeders, they were quite similar as it was the case for Dağlıç 1 and 2, Akkaraman 2 and 3. However, when one of the samples in from the farm the genetic difference was very high as measured by F_{ST}, D_A, as detected by AMOVA, FCA and PC plots. As a conclusion it can be said that individuals of the breeds exhibit a great genetic overlap and this blurs the genetic differentiation which is present between the breeds for example as detected by the Deleunay analysis.

Are there clusters of breeds indicating that they are the descendants of the different domestication events? When D_A distances and NJ tree constructed based on these distances were considered:

(i) Besides the distinctness of farm samples, relatively high genetic similarity of Morkaraman to farm samples: among these to Ivesi and Kıvırcık,

(ii) Central position of Dağlıç to all breeds, especially high degree of similarity to Karayaka, Hemsin and Akkaraman

(iii) a group formed by Norduz and Hamdani were observed.

It must be emphasized that bootstrap values are low in this tree and hence observations given above and interpretations given below must be considered cautiously. The first observation may suggest that Morkaraman is the ancestor of Kıvırcık and Ivesi, or rather Ivesi. Kıvırcık is in this cluster just because it is highly diverged from the rest of the breeds in the farm. Alternatively, this cluster represents the distinct breeds (long branches) and Morkaraman has a positive significant F_{IS} exhibiting deviation from Hardy-Weinberg equilibrium. The second result may indicate that Dağlıç is one of the breeds from which Karayaka, Hemsin and Akkaraman have evolved. Perhaps it contributes to all other breeds with a varying degree. There may be some other explanations to this second observation. One possible explanation is that Dağlıç is the breed having the highest input from all other breeds and therefore it occupies the central position.

There is another grouping represented by Norduz and Hamdani. There is a possibilitt that these 3 groups represent the three domestications events and their descendants. However, high degree of admixture low number of microsatellite loci employed and asymmetric evolutionary history of the dams and sires might be blurring the picture. For more conclusive results more detailed studies based on higher number of microsatellite loci, mtDNA and Y chromosome dependent markers are needed.

One of the reasons for the close resemblance observed between Karayaka and Dağlıç could be as follows: as well as Karayaka, Herik, which is crossbreed of Dağlıç has overlapping distribution with that of Karayaka. Sample collections of Karayaka were done twice in each case with the accompaniment of the local veterinarians. Hence, Karayaka looking samples were collected. However, it is likely that certain degree of admixture between Karayaka and Herik caused a convergence between Karayaka and Dağlıç gene pools.

Detection of relatively high degree of genetic similarities between Akkaraman 2 and Kangal (as Kangal is accepted to be the variant of Akkaraman),

between Kıvırcık and its crossbreed Türkgeldi, between Akkaraman1 and its crossbreed Konya Merinosu proves the reliability of genetic results.

IV.2. Discussion Including The New Analyses From The Literature

In this part, the results will be compared and discussed with those given in the literature.

IV.2.1. Allelic Diversity and Gene Diversity Comparisons

The mean number of alleles observed in different breeds of cattle, goat, sheep, pig and horse as reported in the literature were summarized in Table IV.1, which includes the results of the present study.

Table IV.1. The mean number of alleles observed in different livestock breeds and their references.

Name of the species	Mean # of alleles (Ranged between)	References
Cattle	5.2 and 6	Edwards *et al.* (2000)
Cattle	4.4 and 8.8	Loftus *et al.* (1999)
Cattle	4.9 and 6.7	Martin-Burriel *et al.* (1999)
Goat	5.2 and 6.8	Luikart *et al.* (1999)
Pig	3.4 and 5.8	Martínez *et al.* (2000)
Horse	3.6 and 4.5	Vila *et al.* (2001)
Sheep	4 and 11.5	Byrne *et al.* (in press)
Sheep	**5.8 and 11.8**	**Present study**

According to this summary table, Turkish sheep breeds have higher mean number of alleles. Although the MNA/breed estimated in Turkish sheep breeds ranged between 5.8 and 11.8; the average MNA/breed was 8.4, which is higher from all the upper limits of the MNAs observed for the livestock breeds except for two studies (Loftus *et al*., 1999; Bryne *et al.*, in press).

During the data collection, some of the samples of this study were PCR amplified and analyzed together with some of the samples from Byrne *et.al.*'s (in press) study. That is why; it was possible to include the data of 26 European sheep breeds from Byrne *et.al.* (in pres) in the analysis. There are 4 microsatellite loci in common with this study.

First of all, the mean numbers of alleles (MNA) of the Turkish and European breeds were compared with respect to the 4 loci in common. The MNA of Turkish breeds ranged between 6.0 (Türkgeldi) and 11.8 (Karayaka) with an average MNA/population of 9.0. The MNA found in European breeds in terms of the 4 loci in common ranged between 5.25 (Soay, which is a small feral sheep breed) and 11.5 (Turcana, sheep breed related with Turkish breeds) with an average MNA/population of 7.78.

The allelic results were, then, compared in terms of the private alleles observed in the present study (see Table IV.2 below). The breed specific alleles observed in Kangal sample for MAF65, Akkaraman1 sample for JMP29 and Karayaka1 sample for JMP58 are still specific to these breeds. However, they are at the edge of their allelic ranges and their frequencies are quite low. They donot lend themselves to be used as breed identification marker. Except for these alleles, the other breed specific alleles detected in Turkish native breeds are also present in some of the European breeds (Bryne *et al.*, in press). On the other hand, the private allele observed in Kıvırcık breed for JMP58 is in the middle of the allelic range with a relative frequency of 0.46 and was only observed in Sarda (Italy) with a relative frequency of 0.048. Thus, this allele can still be used as a marker for breed identification.

Table IV.2. Private alleles after comparing the allelic data with the European allelic data (Bryne *et al*., in press) as indicated by red arrows. The green arrow indicates the allele-138 of JMP58 locus observed in Kıvırcık, which can be used as marker in later studies.

Locus	Allele	Frequency	Sample found
JMP29	113	0.010	Akkaraman1
	119	0.009	Karayaka1
	153	0.026	Karyaka1
	159	0.023	Kıvırcık
JMP58	134	0.018	Karayaka1
	138	**0.456**	**Kıvırcık**
	146	0.009	Karayaka2
MAF65	109	0.023	Kangal
	119	0.038	Norduz
MAF209	108	0.023	Kangal
	110	**0.273**	**Kıvırcık**
	136	0.015	Morkaraman

From the data in the literature, it was possible to compare the gene diversity estimated for different livestock breeds with the gene diversity estimations of the present study. In Table IV.3 the summary of the H_E values reported in the literature are given.

Table IV.3. Average H_E values estimated for different livestock breeds and their references.

Name of the species	H_E (Ranged between)	References
Cattle	0.43 and 0.53	Hanslik *et al.* (2000)
Cattle	0.58 and 0.71	Edwards *et al.* (2000)
Cattle	0.54 and 0.79	Loftus *et al.* (1999)
Cattle	0.56and 0.68	Matrin Burriel *et al.* (1999)
Goat	0.56 and 0.67	Luikart *et al.* (1999)
Goat	0.51 and 0.58	Saitbekova *et al.* (1999)
Pig	0.46 and 0.64	Martínez *et al.* (2000)
Horse	0.49 and 0.63	Vilà *et al.* (2001)
Sheep	0.55 and 0.86	Byrne *et al.* (in press)
Sheep	0.69 and 0.77	Diez-Tascón *et al.* (2000)
Sheep	**0.68 and 0.81**	**Present study**

The expected heterozygosities estimated for each breed analyzed in this study are within the range of sheep studies in the literature. In general, goat, pig and horse have lower H_E values than cattle and sheep.

The average H_E values estimated for the Turkish (present study) and European breeds (Byrne *et al*., in press) were compared with respect to the 4 loci in common. The average H_E values of Turkish breeds ranged between 0.683 (Türkgeldi) and 0.810 (Karayaka2 and Akkaraman) with an average H_E/population value of 0.767. The average H_E values estimated for the European breeds ranged between 0.591 (Comisana of Spain) and and 0.865 (Sumavka) with an average H_E/population value of 0.738. If we rank the microsatellite loci in terms of descending average H_E/locus, the order for Turkish breeds is MAF65, MAF209, JMP29 and JMP58; but the order for the European breeds is JMP29, JMP58, MAF209 and MAF65. The average H_E/locus values ranged between 0.730 for the Turkish breeds and between 0.711 and 0.781 for the European breeds.

The comparison of the Turkish and European breeds in terms of the 4 common loci results in slightly higher values for Turkish breeds. In the literature, it has been shown that the breeds/populations near the domestication centers have higher genetic diversity in terms of microsatellite (Loftus *et al.* 1999) and based on

mtDNA sequences (Luikart *et al.*, 2001). So, these allelic and gene diversity estimates of the present study do not support well the findings in cattle (Loftus *et al.*, 1999) and goat (Luikart *et al.*, 2001). But it also does not conflict with the assumptions about Turkish populations being one of the closest descendants of their ancestors, and the possible domestication place being in Anatolia or at a close site.

The crossbreeds exhibited the lowest diversity values among the Turkish breeds. Because recently it went through a bottleneck during its formation, this result was expected. High values displayed by Turcana and Sumavka could be due to their admixal status from Turkey and north of Europe (Bruford and Towsend, 2004). Furthermore the loci chosen were suggested by M. Bruford and K. Byrne (Byrne *et al.*, in press), hence, there might be a bias in these loci such that they are exhibiting higher diversity in European breeds.

Furthermore, after the recent dramatic size decrease in sheep populations (39%) could have reduced the genetic variability in native sheep breeds. In this case many potentially important information might be lost forever. Not to cause another such information lost urgent conservation strategies must be formed and conservation must start.

IV.2.2. AMOVA Results

In order to analyze the partitioning of the total genetic variation among the sheep breeds in different European countries AMOVA analysis was performed for each of the 3 groups formed containing breeds of the same country, separately. The groups are**:** one group of 3 Greek breeds, one group of 3 German breeds and one group of 3 Italian breeds. The data on Greek, German and Italian breeds were taken from Byrne and colleagues (in press)'s study. These AMOVA analyses revealed that 83-87% of the total genetic variation was within samples and 13-17% was among the samples.

Afterwards, the AMOVA analysis was repeated for each of the three groups of Turkish breeds, separately. These three groups were formed from different triple

combinations of the Turkish breeds analyzed, which are phenotypically and geographically distant. In the first analysis the group was composed of Akkaraman1, Morkaraman and Dağlıç2 samples. In the second analysis, the group was composed of Kıvırcık, Morkaraman and Karayaka2 samples. In the final analysis, the group was composed of Kıvırcık, Akkaraman1 and İvesi samples. In these analyses, the great portion (about 90%) of the total genetic variation was within the samples. Unlike European breeds, which had 13-17% of the total variation present among breeds, at most 12.4% of the total variation was partitioned among the samples. The results for Turkish breeds are biased towards the maximum possible among breed variations. Because in each trial one or more of the differentiated farm samples were included. When breed samples other than farm samples were considered, the contribution of between breed variations to the total variation is only 5%.

Finally one last AMOVA analysis was performed for the group composed of all Turkish and European sheep breeds. The results revealed that 88% of the total variation was within the samples and 12% was among the samples, which was 5% when only Turkish native samples were used.

Among group differentiation is higher in European sheep breeds, than in Turkish sheep breeds. When the intense artificial selection and inbreeding practices during the formation of these breeds is considered, this result is expected. Furthermore, unrecorded sporiadic mixture of members of different breeds and may be the admixture of breeds by the migration from the east/south east of Anatolia (could not be detected in the present study) also contributed to lower the diversity between the breeds. Establishment of farms is important to stop further homogenization of the breeds. By the help these farms, uncontrolled hybridizations of members of different breeds can be avoided. Yet, the results points that there is still considerable differentiation among Turkish breeds.

IV.2.3. FCA Results

All the data from the Turkish (present study) and European (Byrne *et al.*, in press) sheep breeds were analyzed together by using Factorial Correspondence Analysis in order to visualize the genetic relationship between all the individuals. Among the 10 factors; 2, 5 and 6 are the three axes that can separate the samples on 3D plot with a better resolution. The result is given in Figure IV.1. By this way, some clusters of individuals were detected. So, the individuals of the same breed that seem to fell apart from the big clustering in the middle are taken into circles.

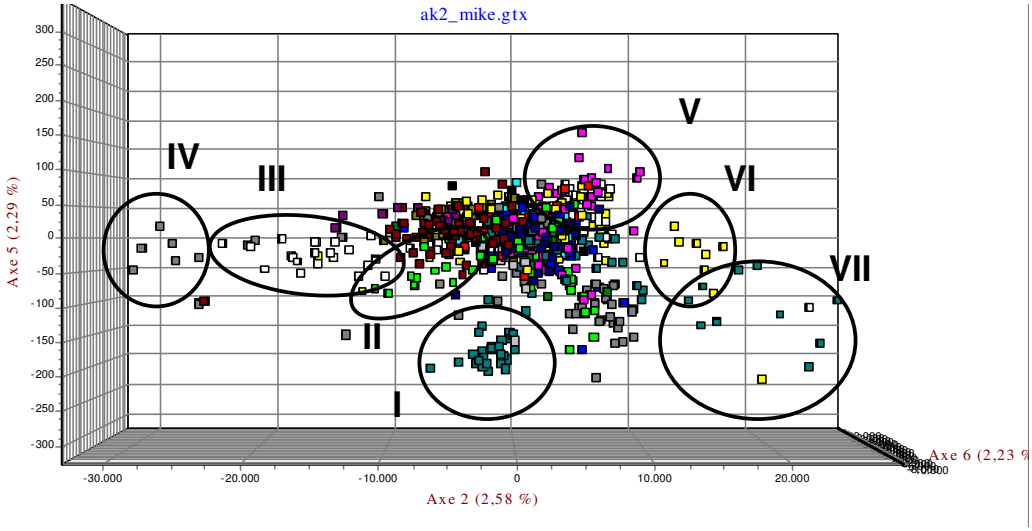

Figure IV.1. The FCA plot of Turkish and European sheep samples.

There are 7 groupings in this figure above. Group I was mainly composed of Comisana (Spanish) samples. Group II mainly included Merino sheep samples (green), and group III Skudde individuals (German). Group IV was composed of Kıvırcık (Turkish) sheep and Group V includes Türkgeldi individuals (purple). Group VI and VII contain individuals from Akkaraman1 (yellow) and NRonalds (green) (British) individuals.

In NJ tree constructed using all the Turkish and European breeds (Figure III.19), Kıvırcık, Skudde, NRonalds, Comisana and Merino grouped together. These individuals of these breeds are also identifiable by the FCA analysis.

Above results indicate that (i) there is a great overlap between the members of the sheep breeds in Europe as well as in Turkey, (ii) the distinct among the European breeds are present (Comisana, Skudde. NRonalds, Merino), but even more distinct ones are the Turkish breed samples from the farms (Kıvırcık, Turkgeldi and Akkaraman). This last result, especially the one for Akkaraman confirms once more that farm samples in Turkey are genetically differentiating from the main gene pool of the breed.

IV.2.4. Assignment Test Results

Results of the assignment test was not different than those observed for Turkish native samples. Kıvırcık breed samples again assigned only to Kıvırcık breed except for the three: one was not assigned to any breed; two assigned to 1-2 more breeds except for Kıvırcık. The next breed having most of its samples assigned only to itself was Skudde (Germany). Thirdly, Comisana (Spain) samples were usually assigned only Comisana to breed. All the rest of the samples, both Turkish and European, were assigned not only to their original breeds but also to other breeds as well. There was no significant pattern in assignment of the individuals of the same breed to other breeds. Using different α criteria for the rejection of the assignment did not increase the percentage of the individuals being assigned only to their breeds of origin for both Turkish and European sheep samples.

Both FCA and assignments test result indicated that, at least based on the 5 microsatellite loci studied, individuals of most of the European breeds can not be discriminated from the others just as it was the case for Turkish sheep individuals.

IV.2.5. Genetic Relationship Between Turkish and European Sheep Breeds

In a study by Diez-Tascon and collaborators (2000) genetic variation within and among the 6 populations of Merino sheep breed was analyzed, all from different countries: Spain, Portugal, France, Germany and New Zealand. All were derived from Spanish Merino within the last 400 years. The results revealed high genetic variation in Iberian populations, but the genetic variations were also high in other populations. The first Merino sheep were imported to France, Germany and New Zealand in the 18^{th} century. Portuguese imported Merino sheep from Spain for centuries, but since 1930, French mutton was introduced to direct the production towards early maturation.

The study revealed that although Spanish Merino was the origin of these populations, the rest of the 5 populations also have high allelic diversity and heterozygosity values. So, the effects of founder events were not detectable from these measures. But, the pairwise genetic distances revealed the differentiation between the populations. The lowest genetic distance (0.086) was between Spanish Merino and Portuguese Black and the highest genetic distance (0.356) was between New Zealand and French populations. Similarly, the D_A genetic distance found between two Akkaraman samples of the present study, which are Akkaraman1 and Akkaraman2, found to be 0.297. The results of both studies suggested that it was possible to detect the impact of founder effect and or random genetic drift by genetic distance estimates and F_{ST} estimates.

In order to investigate the genetic relationships between Turkish and European breeds, first pairwise D_A genetic distances were estimated, then the NJ tree of all breeds, except for the two Turkish crossbreed populations, was constructed as given in Figure IV.2. In this tree, only one of the repetitive samples of the breeds Karayaka, Akkaraman and Dağlıç were included, which are Karayaka2, Akkaraman2 and Dağlıç2.

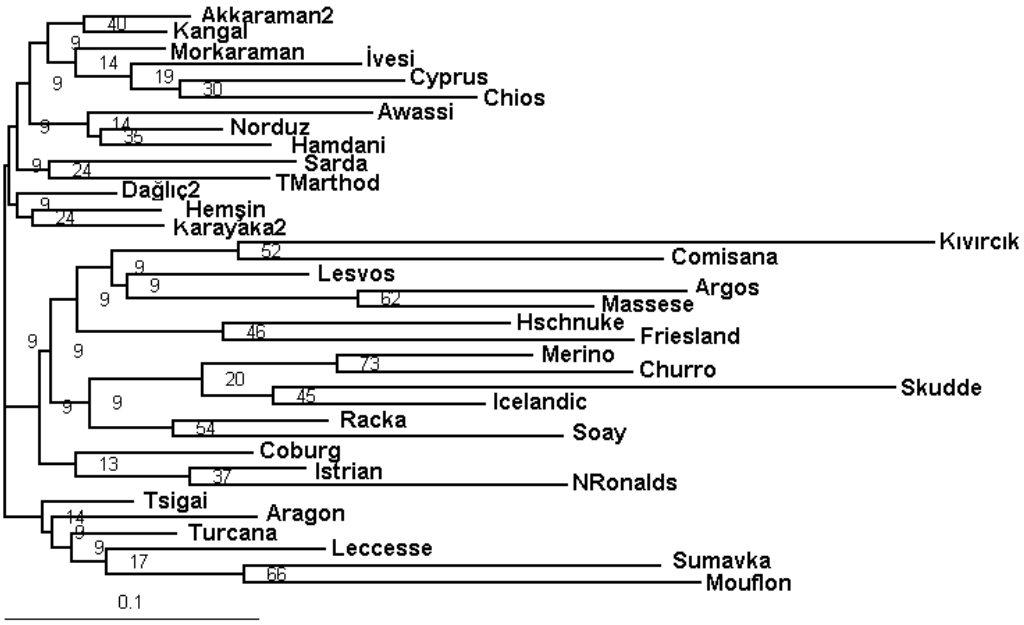

Figure IV.2. The NJ tree of all Turkish (except for the crossbreeds; Türkgeldi and Konya Merinosu) and European breeds (Byrne *et al.,* in press) based on D_A genetic distances.

In this tree, among all the bootstrap values only 5 of them were above 50%. The highest bootstrap value belongs to Merino and Churro node with 73%. The second one belongs to Sumavka (Czech Republic) and Mouflon (Italy, France..) node with 66%. The third one belongs to Argos (Greece) and Massese (Italy) node with 62%. The fourth one belongs to Racka and Soay (British) node with 54%. The last one belongs Kıvırcık and Comisana (Spain) node with 52%.

There are mainly three groupings in the three:

1. All the Turkish breeds except for Kıvırcık were grouped around the same node. This group also included Cyprus fat tail and Chios of Greece (which grouped with İvesi), Awassi of Near East (which grouped with Norduz and Hamdani breeds that are geographically close to Awassi), as well as Sarda (Italy) and TMarthod (France) breeds (grouped together). Among the Turkish breeds,

Akkaraman and its variety, Kangal, were in the same group, which were then grouped with the branch of Morkaraman, İvesi, Cyprus fat tail and Chios breeds. Moreover, Dağlıç was grouped with Hemşin and Karayaka. In this group previous grouping in Turkish breeds were modified.

Dağlıç-Hemşin-Karayaka remained together. Chios, Cyprus grouped with Morkaraman; Akkaraman2 and Kangal joined to this group Norduz, Hamdani, and Awassi (not a farm sample) formed the last group. These three groups may represent the descendents of 3 domestication events.

2. This group included more than half of the European breeds and Kıvırcık, which first grouped with Comisana (Spain) and then with Lesvos (Greece), Argos (Greece) and Massese (Italy). Hschnuke (Germany) and Friesland (Netherlands) formed a branch whilst Skudde (Germany) grouped with Icelandic sheep (Iceland) and Coburg (Germany) grouped with Istrian (Hungary) and NRonalds (British Isles). Furthermore, from the remaining breeds Merino grouped with Churro (both Spanish), and Racka grouped with Soay.

3. This group included Mouflon, Sumavka, Leccesse (Italy), Turcana (Romania), Aragon (Spain) and Tsigai (Romania).

The branches of the first group, which has all the Turkish samples except for Kıvırcık are not long compared to the branches of the other two. These deep branches are associated with the impacts of bottleneck, founder effects or random genetic drift. The breeds having the longest branches are the most differentiated ones. The individuals of these breeds (Kıvırcık, Comisana, Skudde and NRonalds) also formed groups separate from the rest in FCA analysis. Furthermore, the assignment test results for Kıvırcık, Skudde and Comisana are in accordance with this tree. Similar results for Kıvırcık was also found in studies by Soysal and collaborators (2001) and Bulut (2004), where Kıvırcık breed was also differentiated from the rest.

This NJ tree (in Figure IV.2) indicates that among the Turkish breeds, Kıvırcık breed is the closest one to the most of the European breeds in terms of the genetic analysis. Having known that the European breeds were formed from the sheep populations migrated along with the farmers from Fertile Crescent to Europe. It is a possibility for Kıvırcık to be the ancestor of these breeds. The morphology of Kıvırcık, which is a thin tailed breed, also supports this result.

IV.2.6. The PC analysis Plot of the Turkish and European Sheep Breeds

This 3D plot (Figure IV.3) separates some of the European breeds from the rest, which are Mouflon Sumavka, Churro, Merino, Iceland, Skudde, Argos, Hschnuke, Friesland, Chios and Cyprus fat tail. In addition, some Turkish breeds are also separated from the others, namely Akkaraman2, İvesi, Kıvırcık, and Morkaraman. All the rest of the breeds form a cluster.

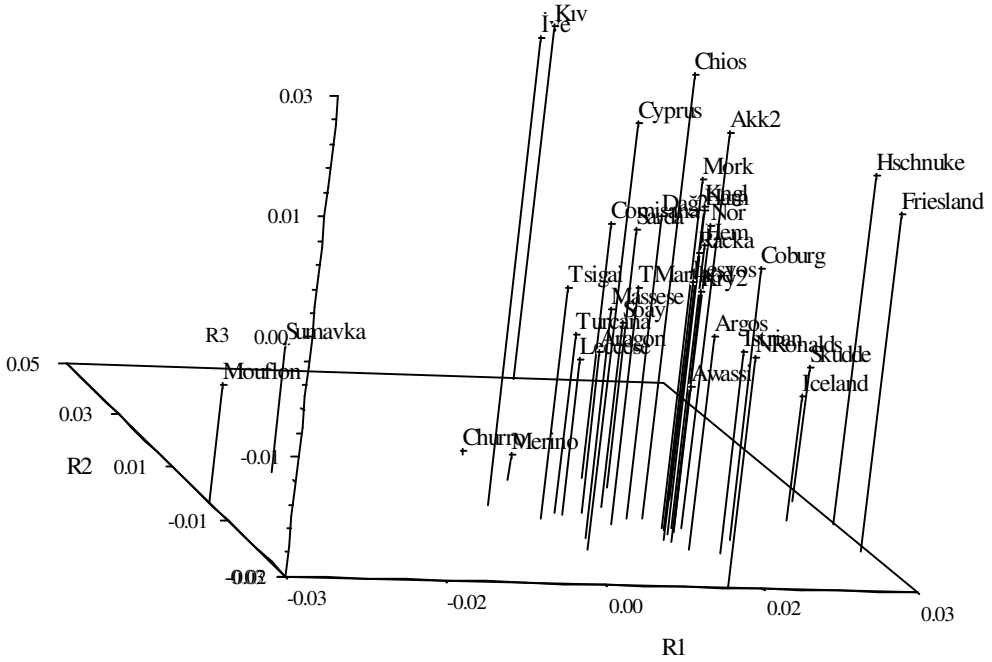

Figure IV.3. The PC analysis plot of the Turkish (except for the crossbreeds, Akkaraman1, Karayaka1 and Dağlıç1) and European sheep breeds.

When two-dimensional plot is analyzed, the names of the breeds that can be discriminated by the first axis of the PC plot are as follows (from left to right): Mouflon, Sumavka, İvesi, Tsigai, Cyprus fat tail, Turcana, Massesse, Leccesse, Aragon, TMarthod, Soay, Dağlıç2, Hemşin, Akkaraman2, Istrian, Racka, Coburg, Iceland, Skudde, Friesland and Hschnuke.

Unlike the FCA analysis, most of the breeds could be separated from each other by the PC analysis. In the FCA analysis, the individuals placed in the plot as single entries. Hence, the wide distributions of the breeds were visualized. However in the PCA, the breeds are represented by single points. Only approximately 12 % of the total genetic variation observed in AMOVA (Turkish x European breeds) was investigated. The first axis of the PC analysis could distinguish 20 out of 36 breeds. Yet, there is also little separation between the remaining breeds in the first axis as can be seen from the figure IV.4 below. Furthermore, central position of Turkish breeds (perhaps because they are the ancestors) on the axis, which describes the highest genetic differentiations that is present between the breeds.

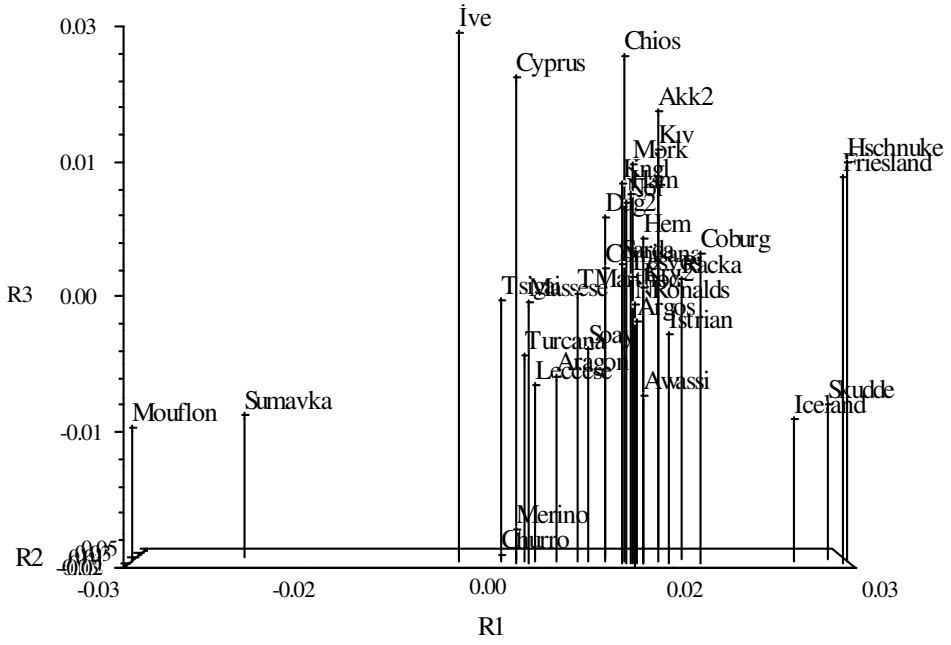

Figure IV.4. The 2-D PCA plot of the Turkish (excluding the crossbreeds, Akkaraman1, Karayaka1 and Dağlıç1) and European sheep breeds (Bryne *et al.*, in press).

IV.3. Summary of the Results

As a summary, it can be said that results are very much affected by the sampling procedure. A sample from the farm does not represent the breed. Despite the drawback introduced by the presence of farm samples, it is observed that Kıvırcık is genetically the most related sample to the European samples and Turkish breeds do not reveal clear cut high levels of genetic diversity as was expected from the descendents of the domestication centers. However, possible 3 groups as the candidates of descendents from three domestication events were suggested.

Furthermore, sheep breeds are generally not very differentiated in Europe, but more so in Turkey, at least based on the results of 5 microsatellite loci. Except for İvesi and Kıvırcık (both are the farm samples), all of the Anatolian breeds in PCA

occupy a central position. This is compatible with the hypothesis that they form a domestic sheep pool from which different breeds were developed, some of them, such as Sumavka, Mouflon, Friesland and Hschnuke, diverged more under various evolutionary forces.

CHAPTER V

CONCLUSIONS

The results of this study and comparisons of the results with the literature can be concluded as follows:

- No gradient in allele frequencies indicating admixture from east to west in parallel to the expectations predicted by NDD model was detected.

- When all the Turkish native samples were included in the same single group in the AMOVA analysis, the 5.42% of the variation is partitioned among samples and it is found to be statistically significant ($P<0.001$).
- Genetic differentiation between the breeds in parallel to geographic barriers was detected by Delaunay Network analysis. However, individuals of the breeds exhibit a great genetic overlap as seen in FCA and NJ tree based on ASD, which blurs the genetic differentiation between the breeds detected by Delaunay Network analysis.
- The results suggest that farm samples are not the typical representatives of the breeds.
- Preliminary results suggest that Morkaraman, Dağlıç-Karayaka-Hemşin, Norduz-Hamdani groups are the descendents of the three domestication events in sheep that must be verified by further more detailed studies.

- Observing the high similarity between Akkaraman and Kangal (variety of Akkaraman), Kıvırcık and Türkgeldi (crossbreed of Kıvırcık), Akkaraman1 and Konya Merinosu (crossbreed of Akkaraman1) enhanced confidence in the results.

- Establishment of farms is important to stop further homogenization of the breeds. The farms can help to avoid uncontrolled hybridization of different breeds.

- Both FCA and assignments test result indicated that, at least based on the 5 microsatellite loci studied, individuals of most of the European breeds can not be discriminated from the others just as it was the case for Turkish sheep individuals.

- Groupings of Turkish native breeds in general suggested by the NJ trees were as follows: Dağlıç-Hemşin-Karayaka remained together. Chios, Cyprus grouped with Morkaraman; Akkaraman2 and Kangal joined to this group Norduz, Hamdani, and Awassi (not a farm sample) formed the last group. These three groups may represent the descendents of 3 domestication events.

- The analysis results of the present study and the morphologic features indicates that there is a high possibility for Kıvırcık, thin tailed sheep, to be the ancestor of some of the European breeds.

- Genetic variability of the native Turkish breeds was not much higher than those observed in Europe. One of the reasons could be the recent reduction in their population sizes. Hence, urgent conservation strategies must be made and must be operated.

- The results of the present and forthcoming studies based on different types of molecular markers should be evaluated comparatively and carefully in developing conservation strategies.

Important things to consider in future studies can be listed as below:

- More sampling from Turkish native breeds is necessary.
- The sampling should include few samples from distant flocks in the distribution area of the breed.
- Turkish native breeds which were not analyzed in this study, like Kara Koyun from southern Turkey, should also be analyzed.
- All the samples should be analyzed by using more and different types of molecular markers; microsatellites, mtDNA and Y-chromosome.
- The samples from the wild sheep Ovis gmelini and its subspecies must be included in the future studies as it is the most likely ancestor of domestic sheep.
- Collaborative studies, for example with Greece, may enhance the results.
- Better sampling in Balkans to have further in sight of Kıvırcık differentiation.

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APPENDICES

APPENDIX A: Sampling Places

The addresses of the sampling places are given below:

Akkaraman1 sample

All the individuals were sampled from Konya Stud of Selçuk University, Konya in Spring 2000.

İvesi sample

All the individuals were sampled from Gözlü Agricultural Enterprise, Konya in Spring 2000.

Kıvırcık sample

All the individuals were sampled from İnanlı Agricultural Enterprise, Tekirdağ in April 2000.

Konya Merinosu sample

All the individuals were sampled from Konya Stud of Selçuk University, Konya in Spring 2000.

Türkgeldi sample

All the individuals are from Trakya University, Faculty of Agriculture Research and Application farm, Tekirdağ in April 2000.

Akkaraman2 sample

Individuals of Akkaraman2 sample were sampled in June 2002 from the following places;

Sample ID	*Sampled from..*
1,2,3	Mağara Önü village in Hafik district of Sivas province.
4,5,6	Çukurca ağıl village in Hafik district of Sivas province.
7,8,9,10,11	Taşkesen village in Diyadin district of Ağrı province.
12	Başgedikler village in Merkez district of Kars province.
13,14	Başgedikler village in Merkez district of Kars province.
15	Kulveren village, Arazoğlu in Kars province.

Akkaraman3 sample

Individuals of Akkaraman3 sample were sampled in May 2003 from the following places;

Sample ID	*Sampled from..*
1,2,3	From a flock in a village in the west of Ankara province.
4,5,6	From another flock in the same village of Ankara province.
7,8,9	From a flock in another village in the west of Ankara province.
10,11,12	Agricultural Enterprise in Ereğli district of Konya province.
13,14,15,16,17,18	Agricultural Enterprise in Gözlü district of Konya province.

Kangal sample

All the individuals of Kangal breed were sampled from Merkez, Kocakurt villages of Kangal district of Sivas province, in July 2003.

Morkaraman sample

Individuals of Morkaraman breed were sampled in June 2002 from the following places;

Sample ID	*Sampled from..*
1,2,3	Dumlu city of Erzurum province.
4,5	Köşk village of Erzurum province.
6,7	Çayırtepe village of Erzurum province.
8,9	Murat Mahallesi of Diyadin district of Ağrı province.
10,11,12,13,14	Sağdıç village of Doğu Beyazıt district of Ağrı province.
15,16	Suveren village of Diyadin district of Ağrı province.
17,18	Korhan plateau of Ağrı Mountain in Iğdır province.
19,20	Karakuyu vilaage of Iğdır province.
21,22,23	Aşağı Alican village of Iğdır province.
24,25,26	Aşağı Alican village of Iğdır province.
27	Saklıca village of Kars province.
29,30,32	Saklıca village of Kars province.
33,34	Karakaş village in Merkez district of Kars province.

Hemşin sample

All individuals of Hemşin breed were sampled from the villages of Merkez, Şavşat, Borçka and Ardanuç districts of Artvin province, in August 2002.

Karayaka1 sample

Individuals of Karayaka1 sample were sampled in July 2002 from the following places;

Sample ID	*Sampled from..*
1,2,3	Küçükkızoğlu village in Ladik district of Samsun province.
4,5,6	Aşağı Ladik plateau in Ladik district of Samsun province.
7,8,9	Ladik district of Samsun province.

Sample ID	*Sampled from..*
10,11,12	Ladik district of Samsun province.
13,14,15	Ladik district of Samsun province.
16,17,18,19	Küçükkızoğlu village in Ladik district of Samsun province.
20,21,22	Çırakman village in Tekeköy district of Samsun province.
23,24,25	Ladik district of Samsun province.
26,27,28	Ladik district of Samsun province.
29,30,31,32	19 Mayıs University breeding farm in Samsun province.

Karayaka2 sample

All individuals of Karayaka2 sample of Karayaka breed were sampled from 10 different flocks grazing in plateaus of Ordu province, in July 2003.

Dağlıç1 sample

All individuals of Dağlıç1 sample of Dağlıç breed were sampled from village in Acıpayam district of Denizli province, in March 2002.

Dağlıç2 sample

Individuals of Dağlıç2 sample were sampled in April 2003 from the following places;

Sample ID	*Sampled from..*
1,2,3	Yatağan district of Muğla province.
4,5,6	Muratlar village in Merkez district of Muğla province.
7,8	Fethiye district of Muğla province.
9	Fethiye district of Muğla province.
10,11	Serinhisar district of Denizli province.
12,13,14	Yeşilova plateau in Burdur province.
15,16	Yeşilova plateau in Burdur province.
17,18,19	Gelendost district of Isparta province.
20,21,22,23	Gelendost district of Isparta province.
24,25	Yalvaç district of Isparta province.

26,27,28	Eğirdir district of Isparta province.

Sample ID	***Sampled from..***
29,36,37	Konarı village in İşçehisar district of Afyon province.
30,31,32,33	Konarı village in İşçehisar district of Afyon province.
34,35	Seydiler village in İşçehisar district of Afyon province.
38,39	Seydiler village in İşçehisar district of Afyon province.

Norduz sample

Individual samples of Norduz breed were collected from the villages of Merkez, Muradiye and Gürpınar districts of Van province, in June 2003.

Hamdani sample

Individual samples of Hamdani breed were collected from the villages of Merkez, Muradiye and Gürpınar districts of Van province, in June 2003. We have been informed that the individuals of this breed were brought to Van from Iraq.

APPENDIX B: Table of Allelic Data

Table B: The data collected in the present study. The first two columns are for the name of the breed and the sample number, the remaining 5 columns are for the alleles (in bp) of each sample for each loci used in the study.

Breed	Sample no	JMP29	JMP58	MAF65	MAF33	MAF209
AKKARAMAN1	1	127127	142156	129131	124136	106126
AKKARAMAN1	2	127137	142142	127129	124124	112112
AKKARAMAN1	3	137137	142158	131131	124136	000000
AKKARAMAN1	4	135135	142142	127129	122132	112112
AKKARAMAN1	5	135135	150160	125127	122124	116116
AKKARAMAN1	7	139139	142142	127129	122124	114116
AKKARAMAN1	8	127135	142142	127129	122124	112116
AKKARAMAN1	11	137143	140160	127127	136136	122126
AKKARAMAN1	12	115135	140158	131131	136136	124126
AKKARAMAN1	13	147147	140142	127127	124124	116116
AKKARAMAN1	15	127135	142154	127129	122124	116124
AKKARAMAN1	16	137137	142142	127129	122124	116116
AKKARAMAN1	17	139139	153166	127137	124136	106122
AKKARAMAN1	18	127139	140140	137131	124124	116116
AKKARAMAN1	19	113115	158160	127127	122136	106126
AKKARAMAN1	21	127145	158160	127131	124136	116124
AKKARAMAN1	22	127127	140142	129131	136136	106116
AKKARAMAN1	23	115127	142160	127131	136136	126126
AKKARAMAN1	24	127145	142160	127131	122136	118124
AKKARAMAN1	25	135147	142142	129129	122124	116116
AKKARAMAN1	26	135135	142166	127129	124136	106116
AKKARAMAN1	27	135139	142160	129131	124124	116124
AKKARAMAN1	29	135139	140142	125127	124124	116116
AKKARAMAN1	30	135135	142162	131131	124136	116126
AKKARAMAN1	31	115115	160168	129129	124124	116118
AKKARAMAN1	32	135147	142166	127127	122136	116116
AKKARAMAN1	34	117137	142150	127131	124136	116116
AKKARAMAN1	35	137137	140142	127131	136136	116126

Table B cont'd…

Breed	Sample no	JMP29	JMP58	MAF65	MAF33	MAF209
İVESİ	1	139147	142142	129129	124128	116116
İVESİ	2	137137	142150	129129	136138	126126
İVESİ	4	127135	142158	129129	136136	106116
İVESİ	5	115115	140150	127131	122136	116126
İVESİ	6	135135	154162	129131	136136	116126
İVESİ	7	133147	166166	127131	124132	106116
İVESİ	9	135137	142166	127131	124136	114116
İVESİ	10	000000	142148	127127	000000	102104
İVESİ	11	137137	142154	127129	122124	106116
İVESİ	12	137147	142162	127131	124132	124128
İVESİ	13	135137	142162	129131	124136	116116
İVESİ	14	127137	142158	129129	124136	126126
İVESİ	17	135139	150158	127131	124136	116116
İVESİ	18	127145	160166	129129	122124	116126
İVESİ	19	139147	142158	127129	124124	106126
İVESİ	20	125137	142162	129129	122136	126128
İVESİ	21	127137	154154	129131	124124	114126
İVESİ	22	135147	142158	129131	124124	114116
İVESİ	24	137137	154162	129129	124124	106114
İVESİ	26	127137	142162	129131	124124	114116
İVESİ	28	127135	142162	127131	124132	112122
İVESİ	29	137147	142142	129137	124140	116124
İVESİ	30	137137	158158	129131	124136	106114
İVESİ	32	135137	142156	129131	128128	116126
İVESİ	34	135135	142160	133137	122138	116124
İVESİ	35	135135	142142	121129	122124	000000
KIVIRCIK	1	132132	142156	127129	124136	000000
KIVIRCIK	2	127137	158164	127129	136136	110120
KIVIRCIK	3	135139	142158	127133	136136	112120
KIVIRCIK	4	127139	152160	129137	124146	110116
KIVIRCIK	5	137137	160160	123127	124126	110112
KIVIRCIK	8	135135	164164	129133	134138	112112
KIVIRCIK	9	135159	138168	127129	124124	112112
KIVIRCIK	11	127137	138138	133137	134136	112120
KIVIRCIK	12	127137	138158	131133	000000	112118
KIVIRCIK	16	135135	138158	129133	122136	110110
KIVIRCIK	18	127135	138138	123129	124124	118120
KIVIRCIK	20	137137	142160	127131	136136	110122
KIVIRCIK	21	125137	138138	129137	122126	112122
KIVIRCIK	22	127135	138138	127129	130136	112122
KIVIRCIK	23	131135	138138	127127	124136	112122
KIVIRCIK	24	131137	156166	129133	124138	110110
KIVIRCIK	25	115137	158160	127131	124136	110110
KIVIRCIK	27	000000	144156	131133	124136	114116
KIVIRCIK	29	127135	158158	129129	124134	112122
KIVIRCIK	30	127137	138138	129131	126146	110112
KIVIRCIK	33	135145	138138	127129	124136	112118

Table B cont'd…

Breed	Sample no	JMP29	JMP58	MAF65	MAF33	MAF209
KIVIRCIK	34	127135	138138	129129	124136	112112
KIVIRCIK	35	127137	138138	129129	124138	110112
TÜRKGELDİ	1	137147	000000	000000	124136	114116
TÜRKGELDİ	3	127127	142142	127137	136138	118130
TÜRKGELDİ	5	137147	142142	000000	124136	114126
TÜRKGELDİ	6	137137	144144	127133	124136	116128
TÜRKGELDİ	8	137139	144144	000000	124132	000000
TÜRKGELDİ	11	137137	154158	129137	134136	114130
TÜRKGELDİ	12	125137	142142	127127	132136	114116
TÜRKGELDİ	17	125137	142142	133137	136138	114114
TÜRKGELDİ	18	137137	154158	129129	134136	116128
TÜRKGELDİ	19	147157	142158	127137	124136	114116
TÜRKGELDİ	20	139139	140142	127129	124134	116118
TÜRKGELDİ	21	137137	142142	127139	134136	116130
TÜRKGELDİ	22	125137	142142	127127	132136	114116
TÜRKGELDİ	23	137157	142142	127129	124136	118130
TÜRKGELDİ	25	131147	142142	127129	136136	114114
TÜRKGELDİ	26	157157	142154	127137	134136	116130
TÜRKGELDİ	27	137139	142142	127129	136136	114116
TÜRKGELDİ	28	139139	142154	127129	132134	128130
TÜRKGELDİ	29	125125	142142	127137	134136	116126
TÜRKGELDİ	30	137137	142154	127127	136136	116124
TÜRKGELDİ	31	147147	142142	127137	134136	114130
TÜRKGELDİ	32	137147	142158	127137	134136	114126
TÜRKGELDİ	33	131137	142142	127127	124136	114114
TÜRKGELDİ	34	147157	142142	127129	124132	118126
KONYA MERİNOSU	1	000000	000000	127131	136136	116124
KONYA MERİNOSU	2	135137	000000	000000	124124	118126
KONYA MERİNOSU	3	127127	142166	129129	124136	106118
KONYA MERİNOSU	4	143143	142160	129129	124134	106118
KONYA MERİNOSU	5	115115	142142	129129	122136	118126
KONYA MERİNOSU	6	135135	154158	127129	122136	116126
KONYA MERİNOSU	7	127127	000000	000000	136136	116124
KONYA MERİNOSU	8	127127	140142	000000	136136	106116
KONYA MERİNOSU	9	127139	142160	125129	122136	118126
KONYA MERİNOSU	10	115115	160168	000000	124136	116126
KONYA MERİNOSU	11	115135	150162	125129	124136	106126
KONYA MERİNOSU	12	135137	144160	125129	124136	106106
KONYA MERİNOSU	13	127137	160168	127127	136136	118128
KONYA MERİNOSU	14	115127	144144	129129	124136	118126
KONYA MERİNOSU	15	115115	142158	129129	136136	120128
KONYA MERİNOSU	17	137137	142144	129129	122136	126126
KONYA MERİNOSU	18	127143	160162	131131	124136	106124
KONYA MERİNOSU	19	139145	142160	127129	124136	116118
KONYA MERİNOSU	21	127127	142144	125129	136136	118126
KONYA MERİNOSU	24	000000	150160	125125	136136	118126
KONYA MERİNOSU	25	137143	144158	000000	124136	118120

Table B cont'd…

Breed	Sample no	JMP29	JMP58	MAF65	MAF33	MAF209
KONYA MERİNOSU	26	115127	000000	125125	136136	000000
KONYA MERİNOSU	27	115127	140140	125129	124136	116126
KONYA MERİNOSU	28	115115	140168	125129	136136	106124
KONYA MERİNOSU	30	125137	160168	125129	122124	120128
KONYA MERİNOSU	31	115127	144160	125129	136136	128128
KONYA MERİNOSU	32	127143	144170	127129	122136	126128
KONYA MERİNOSU	33	115127	158160	127127	122136	120120
KONYA MERİNOSU	35	137143	142160	125129	122136	106118
MORKARAMAN	1	137139	150160	129129	122122	000000
MORKARAMAN	3	129137	144154	127129	122136	000000
MORKARAMAN	4	137145	144144	127137	124128	116116
MORKARAMAN	5	127137	144160	127137	122124	116126
MORKARAMAN	6	135139	144144	127127	136140	116122
MORKARAMAN	7	127137	144144	129137	126128	120124
MORKARAMAN	8	139139	144168	121129	124124	116128
MORKARAMAN	9	135151	144154	127129	124136	124132
MORKARAMAN	10	137139	150162	125129	124136	126130
MORKARAMAN	11	137139	144144	127129	122124	126126
MORKARAMAN	12	127139	144168	127135	124136	126126
MORKARAMAN	13	127137	142162	000000	136136	118126
MORKARAMAN	14	129137	144150	121129	124124	120124
MORKARAMAN	15	117139	144144	000000	124136	118118
MORKARAMAN	16	137139	150154	131135	122132	118118
MORKARAMAN	17	137139	160168	000000	136136	116116
MORKARAMAN	18	127139	144162	129131	124132	116116
MORKARAMAN	19	137141	144162	129135	122122	118118
MORKARAMAN	20	127145	144144	127129	124138	116116
MORKARAMAN	21	137137	144162	129131	122124	118124
MORKARAMAN	22	137137	144150	123131	136136	118118
MORKARAMAN	23	127129	164168	131131	124136	118124
MORKARAMAN	24	129139	144144	129129	124132	116130
MORKARAMAN	25	139147	144144	127131	124124	122126
MORKARAMAN	26	147151	144158	000000	134136	118118
MORKARAMAN	27	137141	144162	000000	000000	118118
MORKARAMAN	28	137141	144168	000000	136136	118118
MORKARAMAN	29	129129	144150	129129	136136	116132
MORKARAMAN	30	137139	144144	129131	124124	116136
MORKARAMAN	31	145149	144144	000000	124138	116128
MORKARAMAN	32	137147	144158	129137	124140	116116
MORKARAMAN	33	143149	162164	127131	122124	118128
MORKARAMAN	35	129129	144144	000000	124136	116116
MORKARAMAN	36	127129	144164	127133	122136	116116
MORKARAMAN	Mork	129137	144150	129131	122122	116116
HEMŞİN	1	129129	144164	129131	122136	118128
HEMŞİN	2	137147	144150	121129	132138	116118
HEMŞİN	3	137139	000000	129131	000000	118126
HEMŞİN	4	129137	144144	131131	136136	122124

Table B cont'd…

Breed	Sample no	JMP29	JMP58	MAF65	MAF33	MAF209
HEMŞİN	5	129129	156160	131133	000000	118130
HEMŞİN	6	129129	000000	131131	000000	120128
HEMŞİN	7	117137	156160	127133	000000	118118
HEMŞİN	8	129137	144144	127127	122136	118118
HEMŞİN	10	137137	150150	131133	136136	118126
HEMŞİN	11	137137	144156	129135	000000	118130
HEMŞİN	12	137139	144154	131131	136136	126126
HEMŞİN	13	137139	150162	131133	124134	118118
HEMŞİN	14	139143	162162	127129	136136	116116
HEMŞİN	15	129143	158162	129129	136136	118118
HEMŞİN	16	117129	144156	129133	122136	118120
HEMŞİN	17	137139	144158	131131	136138	118128
HEMŞİN	18	137137	144154	131137	124124	118128
HEMŞİN	19	117117	150150	131131	122136	118118
HEMŞİN	20	117129	144150	127129	122124	128128
HEMŞİN	21	117129	150164	123133	132134	116116
HEMŞİN	22	129139	160170	121131	122124	118118
HEMŞİN	23	129139	160170	121131	122124	118118
HEMŞİN	24	139139	144150	127127	122138	118124
HEMŞİN	25	129137	144144	127131	122136	124132
HEMŞİN	27	129139	154164	129131	124136	116128
HEMŞİN	28	137139	144168	129133	132132	118118
HEMŞİN	29	129129	144150	121127	122136	118126
HEMŞİN	30	139139	144160	129129	136140	118126
HEMŞİN	31	139139	144168	129131	126136	128128
HEMŞİN	32	129137	156162	123131	134136	118128
HEMŞİN	33	129133	150154	127131	124132	120130
HEMŞİN	34	139151	144156	127133	000000	116118
HEMŞİN	35	139139	144144	127131	124132	118118
HEMŞİN	36	129147	144144	133133	136138	000000
HEMŞİN	37	117137	144150	131133	134136	000000
HEMŞİN	38	137139	144144	127129	136138	000000
KARAYAKA1	1	137139	144160	129137	122124	118120
KARAYAKA1	2	139153	144160	129129	122124	126126
KARAYAKA1	3	117129	144144	127129	124136	124128
KARAYAKA1	4	119139	134134	131135	000000	118128
KARAYAKA1	6	135139	158158	000000	124132	118124
KARAYAKA1	7	129135	152158	127129	124136	116128
KARAYAKA1	8	129135	144170	125129	000000	130130
KARAYAKA1	9	133137	148164	127129	126132	128128
KARAYAKA1	10	137139	150154	129129	136136	118118
KARAYAKA1	11	129141	144144	000000	132134	126130
KARAYAKA1	12	129139	000000	127129	122138	118118
KARAYAKA1	13	137151	000000	129129	128136	128130
KARAYAKA1	14	135137	164164	127131	136138	116116
KARAYAKA1	15	139139	142144	129129	122140	118126
KARAYAKA1	16	137137	142158	129133	134136	122130

Table B cont'd…

Breed	Sample no	JMP29	JMP58	MAF65	MAF33	MAF209
KARAYAKA1	17	129137	144144	131131	124136	116128
KARAYAKA1	18	129139	144160	129131	124136	126128
KARAYAKA1	19	129139	156160	129133	122124	118126
KARAYAKA1	20	129135	144156	129129	122124	118124
KARAYAKA1	21	139139	144144	127131	122136	126130
KARAYAKA1	22	129131	144144	000000	136136	118120
KARAYAKA1	23	137153	144160	000000	124136	116118
KARAYAKA1	24	137137	144144	127129	122124	118124
KARAYAKA1	25	137153	144144	127129	124136	118128
KARAYAKA1	26	137139	160162	129129	136138	126126
KARAYAKA1	27	131147	144144	123125	138146	118128
KARAYAKA1	29	137139	000000	127131	134136	000000
KARAYAKA1	30	129137	144168	127131	136136	118124
KARAYAKA1	31	137137	144168	127129	122136	116128
KARAYAKA1	32	000000	000000	127135	124136	128130
DAĞLIÇ1	1	137137	144162	000000	132136	118120
DAĞLIÇ1	2	000000	160160	127129	122136	118118
DAĞLIÇ1	3	139141	144150	129129	130140	000000
DAĞLIÇ1	4	000000	144160	000000	122136	118128
DAĞLIÇ1	5	000000	152160	121126	136140	118118
DAĞLIÇ1	6	137137	148158	129131	122124	116126
DAĞLIÇ1	7	137141	158160	129131	122136	118128
DAĞLIÇ1	8	129139	162164	129129	124136	118118
DAĞLIÇ1	9	135137	162168	129129	124136	118128
DAĞLIÇ1	10	139139	144168	127129	122124	118128
DAĞLIÇ1	11	129149	144144	131133	122124	118126
DAĞLIÇ1	12	139149	164168	000000	122124	118128
DAĞLIÇ1	14	137141	150162	000000	136136	118118
DAĞLIÇ1	15	137149	144168	129129	136136	116130
DAĞLIÇ1	16	000000	142144	123127	124136	126126
DAĞLIÇ1	17	137147	142144	131131	124136	118128
DAĞLIÇ1	18	129129	144144	123129	124136	116130
DAĞLIÇ1	19	131139	144150	121131	124136	118130
DAĞLIÇ1	20	129143	142156	127129	122132	118126
DAĞLIÇ1	21	137139	144144	123127	124136	124130
DAĞLIÇ1	22	139143	164164	129131	122124	118126
DAĞLIÇ1	23	137139	142160	123137	122136	116130
DAĞLIÇ1	25	131139	144144	121129	122136	124124
DAĞLIÇ1	26	137137	144150	129129	124136	118118
DAĞLIÇ1	27	131149	144150	129133	122136	118130
DAĞLIÇ1	28	131149	144164	127131	122136	118130
DAĞLIÇ1	29	135137	162162	129137	122136	118118
DAĞLIÇ1	30	137139	144150	129129	124130	000000
DAĞLIÇ1	31	129147	142156	129129	122124	128122
DAĞLIÇ1	32	117147	144164	129131	136136	118118
DAĞLIÇ1	33	137139	144160	123127	122136	118128
DAĞLIÇ1	34	135137	144144	127129	136136	118128

Table B cont'd…

Breed	Sample no	JMP29	JMP58	MAF65	MAF33	MAF209
DAĞLIÇ1	35	135139	144144	127129	136136	118118
AKKARAMAN2	1	129137	000000	000000	136140	118118
AKKARAMAN2	2	137147	150160	121131	122132	118118
AKKARAMAN2	3	143149	144154	127131	122136	118118
AKKARAMAN2	4	129129	142152	000000	122132	114118
AKKARAMAN2	5	127129	144150	000000	000000	118118
AKKARAMAN2	6	137139	144154	129131	132136	118118
AKKARAMAN2	7	139147	144162	129131	000000	118118
AKKARAMAN2	8	137139	144158	000000	000000	118128
AKKARAMAN2	9	137143	144144	129137	000000	118118
AKKARAMAN2	10	137139	144164	129129	000000	116116
AKKARAMAN2	11	137137	144158	127133	000000	000000
AKKARAMAN2	12	127127	144154	129129	000000	118118
AKKARAMAN2	13	127139	144144	000000	000000	114118
AKKARAMAN2	14	141145	144162	121126	000000	116116
AKKARAMAN2	15	137139	144150	121131	124124	116118
DAĞLIÇ2	7002	137141	144168	127129	130134	114124
DAĞLIÇ2	7004	137137	158160	127131	132136	118128
DAĞLIÇ2	7005	137141	144162	127129	122124	106124
DAĞLIÇ2	7006	129129	144164	127131	124132	118126
DAĞLIÇ2	7008	137147	142144	131137	124136	118128
DAĞLIÇ2	7009	137141	160162	129137	136136	118118
DAĞLIÇ2	7010	141147	144164	129129	124136	118118
DAĞLIÇ2	7011	139147	164168	127131	122124	119128
DAĞLIÇ2	7012	129147	144144	129131	122124	118126
DAĞLIÇ2	7013	139139	144168	127127	122124	118128
DAĞLIÇ2	7014	135137	162168	129137	124132	118128
DAĞLIÇ2	7017	129139	162164	127131	124136	118118
DAĞLIÇ2	7018	131149	144150	123137	122136	118130
DAĞLIÇ2	7019	131139	144144	122127	122136	124124
DAĞLIÇ2	7021	131137	150150	129133	122124	120130
DAĞLIÇ2	7022	137139	142160	127129	122136	116130
DAĞLIÇ2	7023	139143	164164	121131	122124	118126
DAĞLIÇ2	7024	137139	142144	123129	124136	124130
DAĞLIÇ2	7025	131139	144150	123127	136136	118130
DAĞLIÇ2	7027	129139	144144	129129	124136	116130
DAĞLIÇ2	7028	135139	144144	129131	136136	118118
DAĞLIÇ2	7029	137137	144144	131131	136136	118128
DAĞLIÇ2	7030	137139	144160	129129	122136	118128
DAĞLIÇ2	7031	117147	144164	129137	136136	118118
DAĞLIÇ2	7032	129147	142156	127131	122124	128128
DAĞLIÇ2	7033	147147	144150	131131	124136	118118
DAĞLIÇ2	7034	135137	162162	129129	122136	118118
DAĞLIÇ2	7035	137143	150154	129139	126136	118126
DAĞLIÇ2	7036	135139	144170	127137	124124	118128
DAĞLIÇ2	7037	127131	144160	127131	124136	118126
DAĞLIÇ2	7038	139157	154158	129129	136136	118118

Table B cont'd…

Breed	Sample no	JMP29	JMP58	MAF65	MAF33	MAF209
DAĞLIÇ2	7039	129139	150170	125133	136138	118118
KARAYAKA2	6033	117141	158160	129129	124124	126126
KARAYAKA2	6034	139141	144154	129137	124136	118126
KARAYAKA2	6035	117129	146166	133141	124124	118128
KARAYAKA2	6036	139147	144144	127129	132136	118128
KARAYAKA2	6037	137139	156160	129129	124124	124128
KARAYAKA2	6038	131139	150150	127135	124124	118124
KARAYAKA2	6039	129141	156160	129129	136138	126128
KARAYAKA2	6040	129139	160160	131137	136138	118118
KARAYAKA2	6041	129137	162168	127129	136138	118128
KARAYAKA2	6042	137143	160160	127127	124136	118126
KARAYAKA2	6043	137139	148168	129129	136136	116128
KARAYAKA2	6044	139139	160168	127129	124136	118118
KARAYAKA2	6045	131139	144160	122133	124124	118130
KARAYAKA2	6046	139139	152166	129129	122136	118118
KARAYAKA2	6047	117131	150162	127127	124124	118118
KARAYAKA2	6048	129129	144160	129131	124124	118128
KARAYAKA2	6049	129137	150160	129133	124138	116128
KARAYAKA2	6050	137139	144170	129137	124124	126130
KARAYAKA2	6051	137139	160168	127129	122124	116116
KARAYAKA2	6052	117137	144144	127131	124132	118128
KARAYAKA2	6053	139143	144168	129131	124136	118118
KARAYAKA2	6054	137137	142166	131133	136136	126130
KARAYAKA2	6055	131151	148150	131131	134140	118130
KARAYAKA2	6056	129137	150150	133137	122124	124128
KARAYAKA2	6057	129139	156160	129131	124138	118130
KARAYAKA2	6058	137139	142160	129131	124136	118128
KARAYAKA2	6059	135139	144168	133135	122124	116128
KARAYAKA2	6060	131157	144160	129135	124138	118118
KARAYAKA2	6061	139147	144144	127133	132136	116128
AKKARAMAN3	16	139139	144160	131131	124136	118126
AKKARAMAN3	19	127129	150164	127129	124124	118118
AKKARAMAN3	20	135149	144150	129131	122122	118118
AKKARAMAN3	21	129147	144168	129131	122136	118128
AKKARAMAN3	22	129137	144154	129131	124140	118118
AKKARAMAN3	23	135137	144160	127131	122140	118118
AKKARAMAN3	24	117139	144164	129129	122124	116128
AKKARAMAN3	25	137151	144162	125127	124136	118130
AKKARAMAN3	27	129137	142144	129137	132136	118118
AKKARAMAN3	28	129137	142144	127131	136136	118128
AKKARAMAN3	29	141149	144144	123129	124124	118128
AKKARAMAN3	31	129129	144164	129131	124136	118118
AKKARAMAN3	32	137141	158160	127131	124124	118118
AKKARAMAN3	33	129129	144158	129131	134136	118118
NORDUZ	13001	129139	144162	131131	124136	106118
NORDUZ	13003	127137	142168	127129	122132	128128
NORDUZ	13005	129147	144144	127129	132140	118128

Table B cont'd…

Breed	Sample no	JMP29	JMP58	MAF65	MAF33	MAF209
NORDUZ	13006	129129	144150	127127	122132	120128
NORDUZ	13007	137151	144162	129129	124136	120128
NORDUZ	13008	137151	142144	129129	124136	118120
NORDUZ	13009	137141	160162	127129	124124	116116
NORDUZ	13010	139139	000000	127129	122122	120128
NORDUZ	13011	137147	150154	127129	124132	118128
NORDUZ	13012	139147	144162	129131	124136	116118
NORDUZ	13013	139143	144144	129131	124136	106118
NORDUZ	13014	129129	142144	127129	124124	118118
NORDUZ	13015	129129	144156	129129	124138	116118
NORDUZ	13016	117137	144162	127135	124136	116118
NORDUZ	13017	139139	150152	129129	122124	128130
NORDUZ	13018	129137	144144	119135	124136	118128
NORDUZ	13019	135139	144144	127129	124136	128130
NORDUZ	13020	127137	144150	119133	124124	128132
NORDUZ	13021	131141	144154	123131	124134	118128
NORDUZ	13023	137151	144154	129129	122136	118128
NORDUZ	13024	137141	160168	127129	128136	118118
NORDUZ	13025	129141	144160	131135	132136	118118
NORDUZ	13026	139145	150162	129129	136136	106118
NORDUZ	13027	135141	144150	129129	122132	128130
NORDUZ	13029	129137	144144	129129	124136	118130
NORDUZ	13028	129137	144144	131141	136136	118128
HAMDANI	14001	137139	144150	125129	124136	118128
HAMDANI	14002	137139	144154	121125	136140	118120
HAMDANI	14004	129129	144144	127131	124136	118128
HAMDANI	14005	137147	144150	129129	122136	116118
HAMDANI	14006	139151	144154	131131	124124	000000
HAMDANI	14007	137139	144168	129131	132136	128130
HAMDANI	14008	129139	164168	129129	122136	118118
HAMDANI	14009	139139	144144	000000	124136	116118
HAMDANI	14010	137139	144154	127133	124136	116128
HAMDANI	14011	139141	144144	129129	122136	000000
HAMDANI	14012	137139	144150	125129	122124	124128
HAMDANI	14013	137139	154164	127129	136136	000000
HAMDANI	14014	137139	144144	129129	124132	000000
HAMDANI	14015	139147	150154	129129	124136	120128
HAMDANI	14016	137137	144144	127129	122136	118118
HAMDANI	14017	137137	144144	131141	136136	126128
HAMDANI	14018	127129	144150	129131	136136	118118
HAMDANI	14019	137137	158168	131131	122124	118128
HAMDANI	14020	127137	150168	129131	122136	106128
HAMDANI	14021	139139	144144	127129	124124	118128
HAMDANI	14022	129139	144150	129129	124132	128132
HAMDANI	14023	129137	144150	129133	124124	118118
KANGAL	15001	135147	144160	129131	124124	118120
KANGAL	15002	141147	144162	129129	122124	116120

Table B cont'd…

Breed	Sample no	JMP29	JMP58	MAF65	MAF33	MAF209
KANGAL	15003	125145	144160	127129	136136	118118
KANGAL	15004	137141	144150	127129	124136	116120
KANGAL	15005	129137	144168	127131	124136	116128
KANGAL	15006	127129	160168	131137	124140	118118
KANGAL	15007	139147	144144	129131	136136	126128
KANGAL	15008	129137	144164	127133	124124	128128
KANGAL	15009	129129	160160	127127	124124	118118
KANGAL	15010	129137	144168	127133	000000	000000
KANGAL	15011	139139	144168	129131	122124	114118
KANGAL	15012	129129	144160	123131	124124	116118
KANGAL	15013	137139	144144	129131	122124	118118
KANGAL	15014	137143	154164	127129	124140	118128
KANGAL	15015	129147	158160	129129	124136	118118
KANGAL	15016	151151	144150	129129	124124	118128
KANGAL	15017	137137	160168	131131	124136	114118
KANGAL	15018	139151	144144	109131	132136	118126
KANGAL	15019	129139	150154	131131	122124	118130
KANGAL	15020	137139	142168	129131	124124	114118
KANGAL	15022	129147	144164	127131	124138	108118
KANGAL	15023	129137	144160	127129	122132	118128
KANGAL	15024	129137	144168	127133	122134	118118

Table C: The allele frequency distribution of each locus across the samples. The name of the locus and the alleles are given in the first column, N is the sample size for the corrsponding locus.

	Akk1	Akk2	Ive	Kıv	TG	KM	Mork	Hem	Kry1	Kry2	Dağ1	Dağ2	Nor	Ham	Kngl
MAF33															
(N)	28	19	28	22	24	29	34	30	26	29	32	32	26	22	22
122	0,179	0,211	0,161	0,046	0,000	0,138	0,191	0,167	0,173	0,069	0,234	0,188	0,135	0,159	0,136
124	0,446	0,342	0,429	0,341	0,188	0,224	0,353	0,150	0,231	0,483	0,234	0,281	0,365	0,341	0,523
126	0,000	0,000	0,000	0,068	0,000	0,000	0,015	0,017	0,019	0,000	0,000	0,016	0,000	0,000	0,000
128	0,000	0,000	0,054	0,000	0,000	0,000	0,029	0,000	0,019	0,000	0,000	0,000	0,019	0,000	0,000
130	0,000	0,000	0,000	0,023	0,000	0,000	0,000	0,000	0,000	0,000	0,031	0,016	0,000	0,000	0,000
132	0,018	0,105	0,054	0,000	0,104	0,000	0,044	0,100	0,058	0,052	0,031	0,047	0,115	0,068	0,046
134	0,000	0,026	0,000	0,068	0,188	0,017	0,015	0,067	0,039	0,017	0,000	0,016	0,019	0,000	0,023
136	0,357	0,263	0,250	0,341	0,479	0,621	0,294	0,400	0,346	0,259	0,438	0,422	0,308	0,409	0,205
138	0,000	0,000	0,036	0,068	0,042	0,000	0,029	0,083	0,077	0,103	0,000	0,016	0,019	0,000	0,023
140	0,000	0,053	0,018	0,000	0,000	0,000	0,029	0,017	0,019	0,017	0,031	0,000	0,019	0,023	0,046
146	0,000	0,000	0,000	0,046	0,000	0,000	0,000	0,000	0,019	0,000	0,000	0,000	0,000	0,000	0,000
MAF65															
	Akk1	Akk2	Ive	Kıv	TG	KM	Mork	Hem	Kry1	Kry2	Dağ1	Dağ2	Nor	Ham	Kngl
(N)	28	23	35	23	21	24	27	34	24	29	29	32	26	21	22
109	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,023
119	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,039	0,000	0,000
121	0,000	0,065	0,014	0,000	0,000	0,000	0,037	0,059	0,000	0,000	0,052	0,016	0,000	0,024	0,000
123	0,000	0,022	0,000	0,044	0,000	0,000	0,019	0,029	0,021	0,017	0,086	0,063	0,019	0,000	0,023

Table C cont'd…

MAF65															
	Akk1	Akk2	Ive	Kıv	TG	KM	Mork	Hem	Kry1	Kry2	Dağ1	Dağ2	Nor	Ham	Kngl
(N)	28	23	35	23	21	24	27	34	24	29	29	32	26	21	22
125	0,036	0,022	0,014	0,000	0,000	0,271	0,019	0,000	0,042	0,000	0,000	0,016	0,000	0,071	0,000
127	0,411	0,152	0,186	0,239	0,524	0,167	0,222	0,191	0,208	0,190	0,172	0,219	0,212	0,119	0,227
129	0,250	0,370	0,457	0,391	0,214	0,500	0,370	0,206	0,500	0,379	0,466	0,328	0,500	0,500	0,341
131	0,268	0,326	0,271	0,109	0,000	0,063	0,185	0,324	0,146	0,155	0,155	0,219	0,135	0,214	0,318
133	0,000	0,000	0,014	0,152	0,048	0,000	0,019	0,162	0,042	0,121	0,035	0,031	0,019	0,048	0,046
135	0,000	0,000	0,000	0,000	0,000	0,000	0,056	0,015	0,021	0,052	0,000	0,000	0,058	0,000	0,000
137	0,036	0,044	0,029	0,065	0,191	0,000	0,074	0,015	0,021	0,069	0,035	0,094	0,000	0,000	0,023
139	0,000	0,000	0,014	0,000	0,024	0,000	0,000	0,000	0,000	0,000	0,000	0,016	0,000	0,000	0,000
141	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,017	0,000	0,000	0,019	0,024	0,000
MAF209															
	Akk1	Akk2	Ive	Kıv	TG	KM	Mork	Hem	Kry1	Kry2	Dağ1	Dağ2	Nor	Ham	Kngl
(N)	27	24	34	22	23	28	33	31	28	29	30	32	26	18	22
106	0,093	0,000	0,088	0,000	0,000	0,161	0,000	0,000	0,000	0,000	0,000	0,016	0,058	0,028	0,000
108	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,023
110	0,000	0,000	0,000	0,273	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
112	0,093	0,000	0,015	0,386	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
114	0,019	0,021	0,088	0,023	0,326	0,000	0,000	0,000	0,000	0,000	0,000	0,016	0,000	0,000	0,068
MAF209															
	Akk1	Akk2	Ive	Kıv	TG	KM	Mork	Hem	Kry1	Kry2	Dağ1	Dağ2	Nor	Ham	Kngl
(N)	27	24	34	22	23	28	33	31	28	29	30	32	26	18	22
116	0,482	0,125	0,265	0,046	0,261	0,125	0,349	0,113	0,107	0,103	0,067	0,031	0,096	0,083	0,091

Table C cont'd

MAF209															
	Akk1	Akk2	Ive	Kıv	TG	KM	Mork	Hem	Kry1	Kry2	Dağ1	Dağ2	Nor	Ham	Kngl
(N)	27	24	34	22	23	28	33	31	28	29	30	32	26	18	22
118	0,037	0,729	0,206	0,068	0,087	0,214	0,273	0,500	0,286	0,414	0,500	0,500	0,385	0,417	0,523
120	0,000	0,000	0,000	0,091	0,000	0,089	0,030	0,032	0,036	0,000	0,017	0,031	0,077	0,056	0,068
122	0,037	0,000	0,015	0,114	0,000	0,000	0,030	0,016	0,018	0,000	0,017	0,000	0,000	0,000	0,000
124	0,093	0,000	0,074	0,000	0,022	0,071	0,076	0,048	0,089	0,052	0,050	0,078	0,000	0,028	0,000
126	0,148	0,021	0,162	0,000	0,087	0,232	0,121	0,081	0,161	0,121	0,100	0,078	0,000	0,028	0,046
128	0,000	0,083	0,088	0,000	0,065	0,107	0,046	0,145	0,196	0,224	0,133	0,156	0,289	0,306	0,159
130	0,000	0,021	0,000	0,000	0,152	0,000	0,030	0,048	0,107	0,086	0,117	0,094	0,077	0,028	0,023
132	0,000	0,000	0,000	0,000	0,000	0,000	0,030	0,016	0,000	0,000	0,000	0,000	0,019	0,028	0,000
136	0,000	0,000	0,000	0,000	0,000	0,000	0,015	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
JMP29															
	Akk1	Akk2	Ive	Kıv	TG	KM	Mork	Hem	Kry1	Kry2	Dağ1	Dağ2	Nor	Ham	Kngl
(N)	28	24	35	22	24	27	35	34	28	29	29	32	26	22	22
113	0,018	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
115	0,089	0,000	0,029	0,023	0,000	0,259	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
117	0,018	0,021	0,000	0,000	0,000	0,000	0,014	0,103	0,018	0,069	0,017	0,016	0,019	0,000	0,000
119	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,018	0,000	0,000	0,000	0,000	0,000	0,000
125	0,000	0,000	0,014	0,023	0,104	0,019	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,023
127	0,196	0,063	0,086	0,227	0,042	0,315	0,114	0,000	0,000	0,000	0,000	0,016	0,039	0,046	0,023
129	0,000	0,229	0,071	0,000	0,000	0,000	0,143	0,279	0,196	0,155	0,103	0,109	0,231	0,136	0,273
131	0,000	0,000	0,000	0,091	0,042	0,000	0,000	0,000	0,036	0,086	0,069	0,078	0,019	0,000	0,000

Table C cont'd…

JMP29															
	Akk1	Akk2	Ive	Kıv	TG	KM	Mork	Hem	Kry1	Kry2	Dağ1	Dağ2	Nor	Ham	Kngl
(N)	28	24	35	22	24	27	35	34	28	29	29	32	26	22	22
133	0,000	0,000	0,014	0,000	0,000	0,000	0,000	0,015	0,018	0,000	0,000	0,000	0,000	0,000	0,000
135	0,268	0,042	0,200	0,273	0,000	0,093	0,029	0,000	0,089	0,017	0,069	0,063	0,039	0,000	0,023
137	0,161	0,229	0,286	0,273	0,417	0,148	0,300	0,265	0,286	0,207	0,293	0,234	0,231	0,364	0,227
139	0,125	0,146	0,143	0,046	0,125	0,037	0,200	0,265	0,232	0,310	0,224	0,234	0,173	0,364	0,159
141	0,000	0,063	0,029	0,000	0,000	0,000	0,043	0,000	0,018	0,052	0,052	0,063	0,096	0,023	0,046
143	0,018	0,042	0,000	0,000	0,000	0,111	0,014	0,029	0,000	0,035	0,035	0,031	0,019	0,000	0,023
145	0,036	0,021	0,014	0,023	0,000	0,019	0,043	0,000	0,000	0,000	0,000	0,000	0,019	0,000	0,023
147	0,071	0,063	0,114	0,000	0,167	0,000	0,043	0,029	0,018	0,035	0,052	0,125	0,058	0,046	0,114
149	0,000	0,063	0,000	0,000	0,000	0,000	0,029	0,000	0,000	0,000	0,086	0,016	0,000	0,000	0,000
151	0,000	0,021	0,000	0,000	0,000	0,000	0,029	0,015	0,018	0,017	0,000	0,000	0,058	0,023	0,068
153	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,054	0,000	0,000	0,000	0,000	0,000	0,000
157	0,000	0,000	0,000	0,000	0,104	0,000	0,000	0,000	0,000	0,017	0,000	0,016	0,000	0,000	0,000
159	0,000	0,000	0,000	0,023	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
JMP58															
	Akk1	Akk2	Ive	Kıv	TG	KM	Mork	Hem	Kry1	Kry2	Dağ1	Dağ2	Nor	Ham	Kngl
134	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,039	0,000	0,000	0,000	0,000	0,000	0,000
138	0,000	0,000	0,000	0,457	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
140	0,143	0,000	0,014	0,000	0,022	0,080	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
142	0,464	0,063	0,286	0,065	0,696	0,220	0,014	0,000	0,039	0,035	0,078	0,063	0,060	0,000	0,023
144	0,000	0,458	0,129	0,022	0,087	0,160	0,557	0,397	0,481	0,224	0,391	0,375	0,500	0,546	0,409
146	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,017	0,000	0,000	0,000	0,000	0,000

Table C cont'd…

JMP58															
	Akk1	Akk2	Ive	Kıv	TG	KM	Mork	Hem	Kry1	Kry2	Dağ1	Dağ2	Nor	Ham	Kngl
148	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,019	0,035	0,016	0,000	0,000	0,000	0,000
150	0,036	0,083	0,171	0,000	0,000	0,040	0,100	0,177	0,019	0,121	0,094	0,109	0,120	0,182	0,068
152	0,000	0,021	0,000	0,022	0,000	0,000	0,000	0,000	0,019	0,017	0,016	0,000	0,020	0,000	0,000
154	0,036	0,083	0,071	0,000	0,109	0,020	0,043	0,059	0,019	0,017	0,000	0,031	0,060	0,114	0,046
156	0,018	0,000	0,014	0,065	0,000	0,000	0,000	0,088	0,039	0,052	0,031	0,016	0,020	0,000	0,000
158	0,071	0,042	0,114	0,152	0,087	0,080	0,029	0,029	0,077	0,017	0,031	0,031	0,000	0,023	0,023
160	0,143	0,083	0,029	0,109	0,000	0,240	0,043	0,074	0,115	0,259	0,094	0,078	0,060	0,000	0,205
162	0,018	0,063	0,100	0,000	0,000	0,040	0,100	0,074	0,019	0,035	0,094	0,094	0,120	0,000	0,023
164	0,000	0,083	0,000	0,065	0,000	0,000	0,043	0,044	0,058	0,000	0,094	0,109	0,000	0,046	0,068
166	0,054	0,000	0,057	0,022	0,000	0,020	0,000	0,000	0,000	0,052	0,000	0,000	0,000	0,000	0,000
168	0,018	0,021	0,014	0,022	0,000	0,080	0,071	0,029	0,039	0,103	0,063	0,063	0,040	0,091	0,136
170	0,000	0,000	0,000	0,000	0,000	0,020	0,000	0,029	0,019	0,017	0,000	0,031	0,000	0,000	0,000

APPENDIX D: Allele Frequency Distribution Graphs

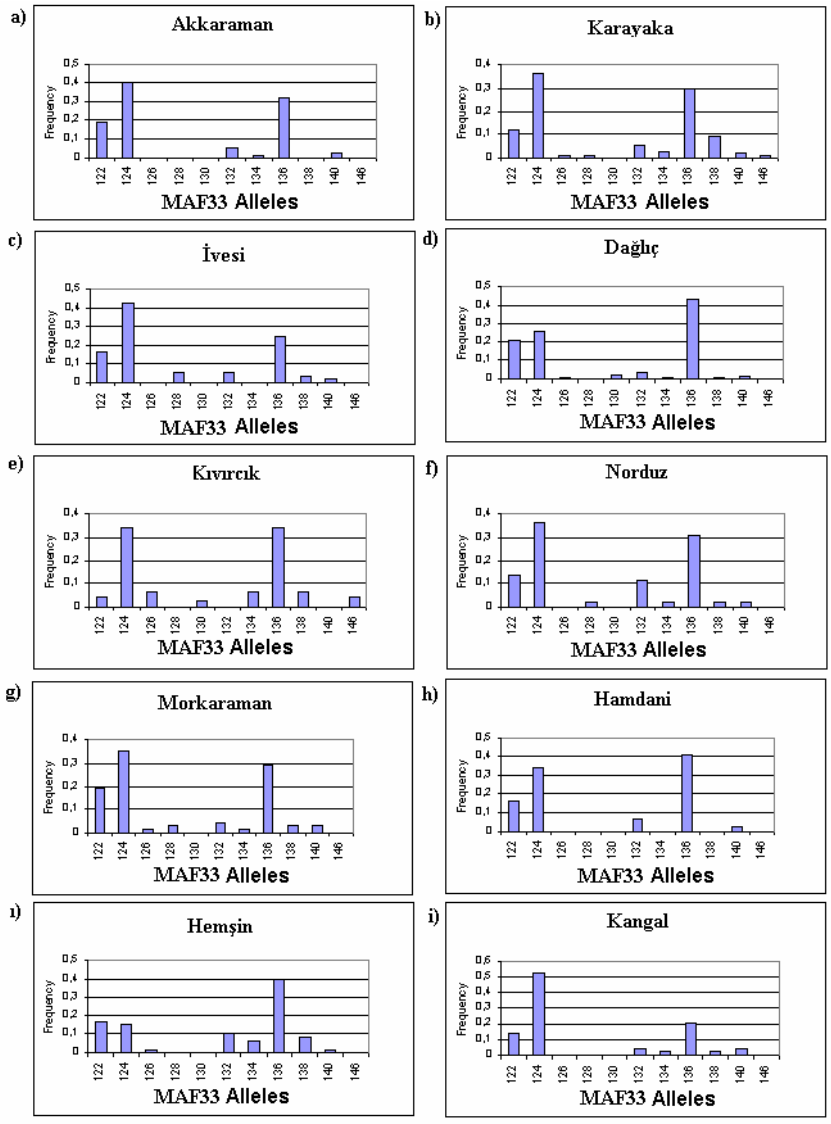

Figure D.1. The frequency distribution of the alleles of MAF33 locus for native breeds

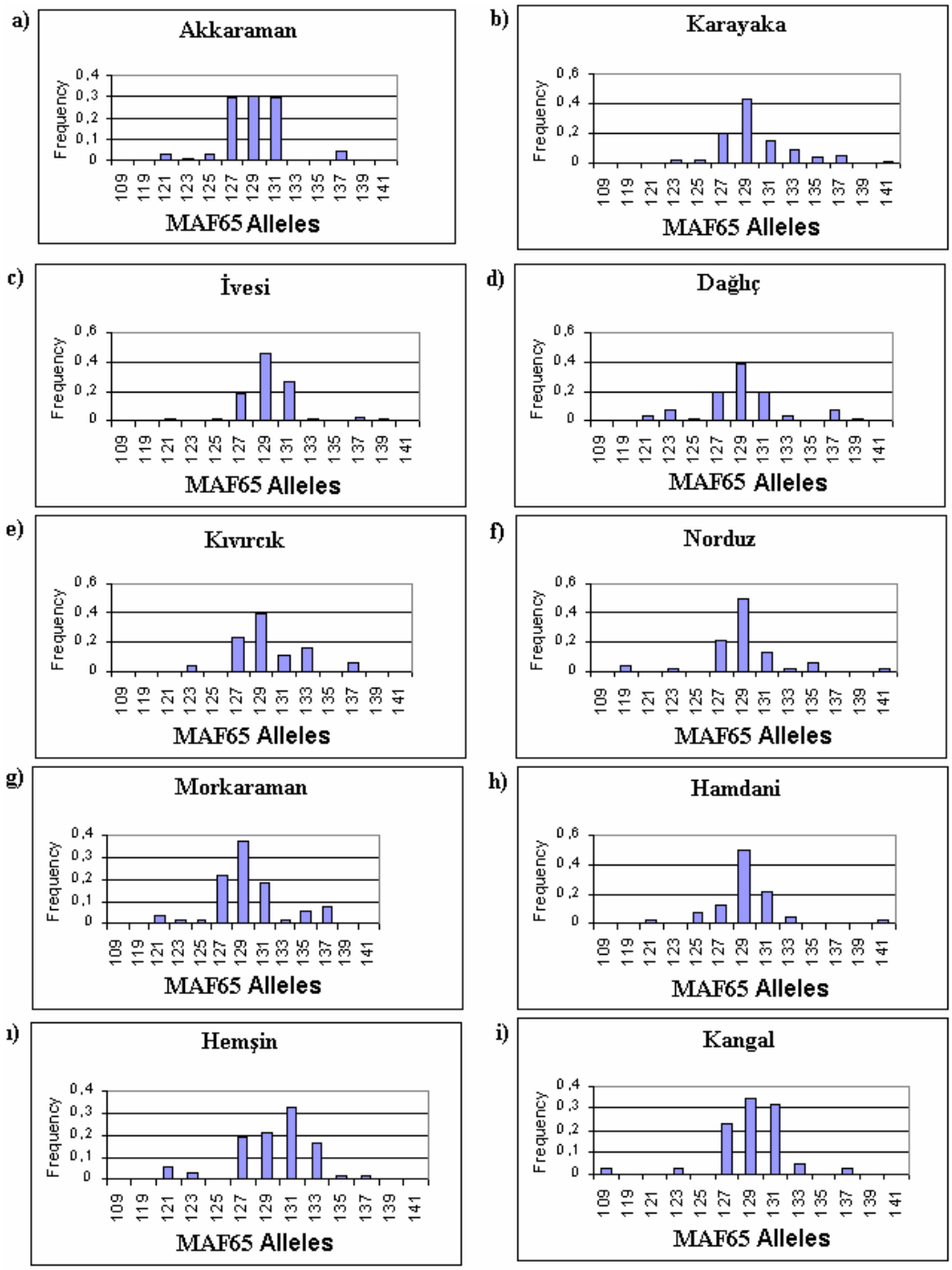

Figure D.2. The frequency distribution of the alleles of MAF65 locus for native breeds and Hamdani.

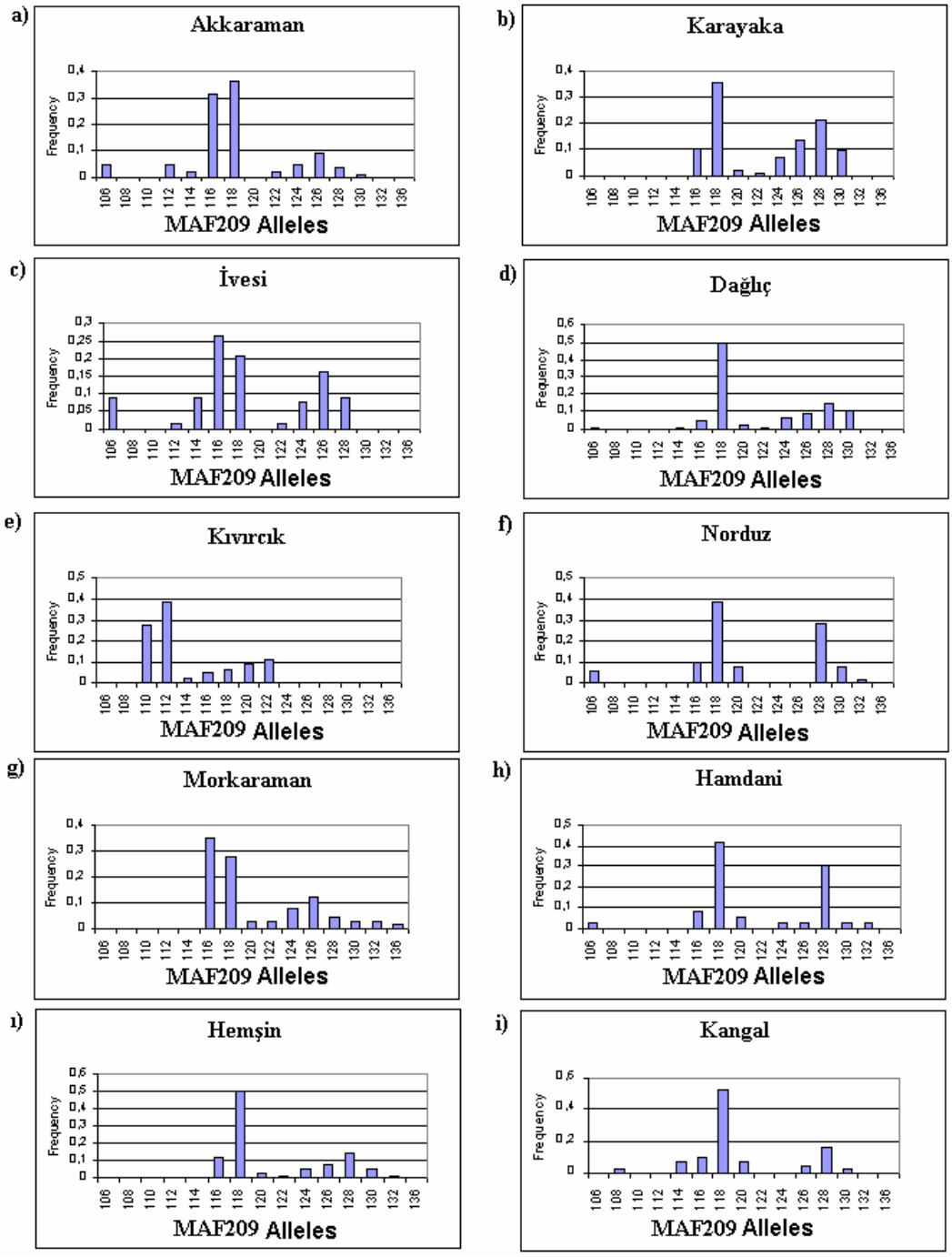

Figure D.3. The frequency distribution of the alleles of MAF209 locus for native breeds and Hamdani.

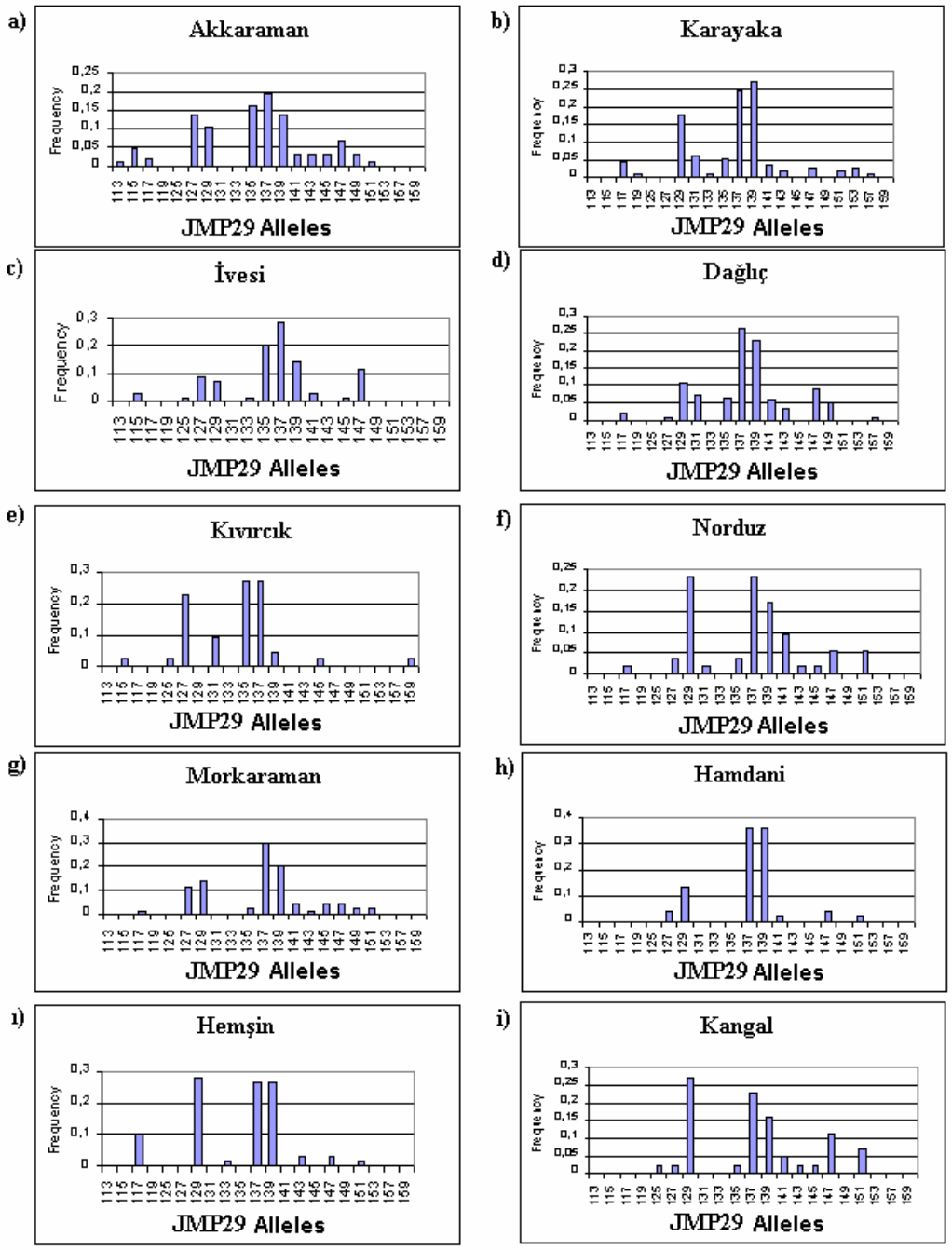

Figure D.4. The frequency distribution of the alleles of JMP29 locus for native breeds and Hamdani.

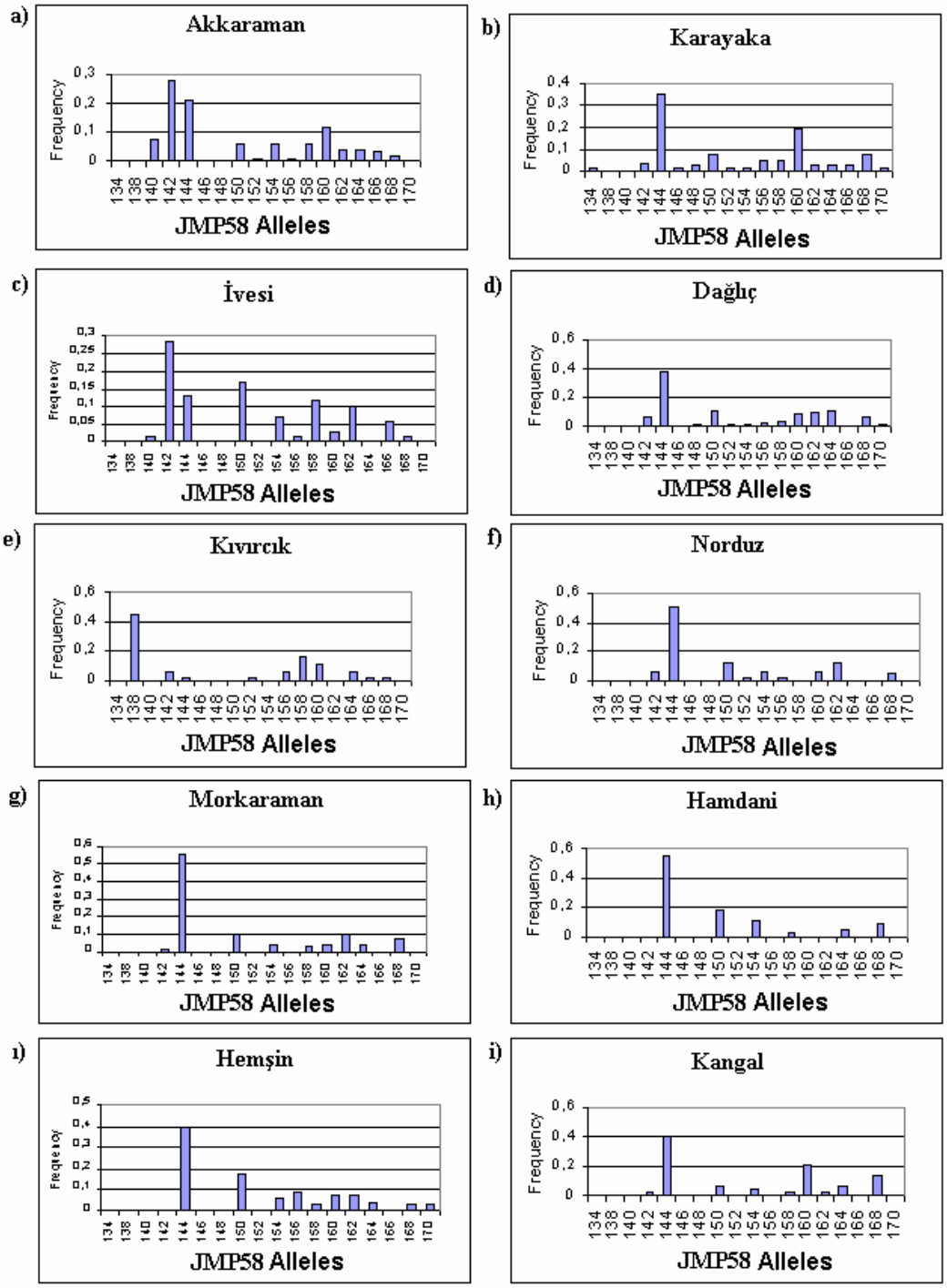

Figure D.5. The frequency distribution of the alleles of JMP58 locus for native breeds and Hamdani.

CURRICULUM VITAE

PERSONAL INFORMATION

Surname, Name: Koban, Evren
Nationality: Turkish (TC)
Date and Place of Birth: 11 June 1976, İstanbul
Marital Status: Single
Phone: +90 312 210 5179
Fax: +90 312 210 1289
E-mail: evrenkoban@yahoo.com

EDUCATION

Degree	Institution	Year of Graduation
MS	METU, Biology	2001
BS	METU, Biology Education	1998
High School	Ayrancı Lisesi, Ankara	1993

WORK EXPERIENCE

Year	Place	Enrollment
2004-present	METU Central Lab, Molecular Biology and Biotechnology Research and Development Unit	Research Assistant

FOREIGN LANGUAGES

Advanced English

PUBLICATIONS

Soysal M. İ., Koban E., Özkan E., Altunok V., Bulut Z., Nizamlıoğlu M. and Togan İ. Evolutionary relationship among three native and two crossbreed sheep breeds of Turkey: preliminary results. *Revue de Medecine Veterinaire,* in press.

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PROJECTS WORKED AS RESEARCHER

TBAG-2127 (2001-2004): "Genetik diversity among native sheep breeds". Directed by Prof. Dr. İnci Togan. Supported by Turkish Scientific and Technical Research Council (TUBITAK).

2000-2003: "The study on the native domestic sheep breeds in Anatolia; genetic diversity and distinctiveness". Directed by Prof. Dr. İnci Togan and Prof. Dr. Mike Bruford. Supported by the British Council Turkey.

TOGTAG/TARP-1811 (1998-2001): "Conservation of rainbow trout (*Oncorhynchus mykiss*) stocks in South Aegean and Western Mediterranean regions in Turkey based on molecular methods*"*. Directed by Prof. Dr. İnci Togan. Supported by TUBITAK.

*VHAG-1553 (1999-2001): "*A study on the genetic structure of native and crossbred sheep breeds of Turkey based on microsatellite markers*"*. Directed by Prof. Dr. M. İhsan Soysal. Supported by TUBITAK.

*VHAG-1396 (1998-1999): "*Conservation of brown trout (*Salmo trutta*) living in Lake Abant and Southeastern Anatolia*"*. Directed by Prof. Dr. İnci Togan. Supported by TUBITAK.

WORKSHOPS ATTTENDED

Population Genetics for Animal Conservation. 4-7 Eylül 2003, Trento, Italy.

International Workshop on Shallow Lakes and Wetlands: Ecology, Eutrophication and Restoration. 28-30 Kasım 2001, Orta Doğu Teknik Üniversitesi, Ankara, Turkey.

Population Viability Analysis (PVA) Workshop: Applications of RAMAS. 12-14 Mayıs 1999, Orta Doğu Teknik Üniversitesi, Ankara, Turkey.