GENETIC DIVERSITY OF GAZELLES (*GAZELLA MARICA* AND *GAZELLA GAZELLA*) IN SOUTHEAST TURKEY: WITH A SPECIAL EMPHASIS ON ONGOING CONSERVATION STUDIES OF *GAZELLA MARICA* IN TURKEY

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ABSTRACT

GENETIC DIVERSITY OF GAZELLES (*GAZELLA MARICA* AND *GAZELLA GAZELLA*) IN SOUTHEAST TURKEY: WITH A SPECIAL EMPHASIS ON ONGOING CONSERVATION STUDIES OF *GAZELLA MARICA* IN TURKEY

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The present study was conducted to confirm the recently suggested taxonomic status of gazelles in Turkey and also to investigate genetic diversity that exists between and within populations of *Gazella marica* (One from Kızılkuyu State Farm, Şanlıurfa, n=48; one from Erikçe State Farm, Gaziantep, n=25) and *Gazella gazella* (population from Kırıkhan County, Hatay, n=4). In the frame of the study, partial mtDNA *cyt-b* sequence (amplified by the primers: L14724, H15149), 17 microsatellite loci (RT1, ETH10, OARFCB304, MM12, BM143, BM757, IDVGA29, BM848, BM4505, BMC1009, INRA40, ETH152, INRABERN172, TGLA122, ILSTS005, CSSM39, CSSM43), two Y-chromosome microsatellite loci (INRA126, UMN0103) and restriction profiles of mtDNA *cyt-b* region for two restriction enzymes (*HinfI, HaeIII*), were employed.

First, the taxonomic status of the gazelles species in Southeastern Anatolia (*Gazelle marica* and *Gazelle gazella)* on the basis of mtDNA *cyt-b* sequences was confirmed with the newly collected samples (n=27 and n=4, respectively), independently from the previous studies. Additionally, there were no variations detected within populations (n=3).

The informativenesses of the microsatellite loci were investigated and 12 microsatellite loci out of 17 (RT1, ETH10, OARFCB304, BM848, BMC1009, INRA40, BM4505, INRABERN172, TGLA122, ILSTS005, BM757 and CSSM43) were found to be promising for the future studies to be carried out in gazelle species. Then, we analyzed the within population diversities by means of effective population sizes and the differentiation of three different gazelle populations by estimating the F_{ST} based on microsatellite data. Captive populations (*Gazella marica* population from Kızılkuyu, n=48; *Gazella marica* population from Erikçe, n=25) not only showed low effective population sizes (for Kızılkuyu N_e=9.7, for Erikçe N_e= 8.9) but also gave the signals of inbreeding depression due to low birth ratios. Moreover, they diverged from each other: F_{ST}=0.04 for Kızılkuyu/Erikçe and they diverged from each other almost significantly; F_{ST}= 0.44 and F_{ST}= 0.46 for the Kızılkuyu/Hatay and Erikçe/Hatay, respectively and both of these F_{ST} values were highly significant. While investigating the degree of admixture levels of the populations, interestingly, possible wild individuals in the sampling groups were detected by the help of Structure analysis.

Furthermore, it was shown that the sequence of Y chromosome based microsatellite locus (INRA126, approximately 240 bp long) differentiated *Gazella marica* and *Gazella gazella* males. This preliminary Y-chromosome data may serve as a reference point for further studies covering Y-chromosome diversity within and among gazelle species.

Finally, using Restriction Fragment Length Polymorphism (RFLP) method based on mtDNA *cyt-b* fragment, observations on the basis of two restriction enzymes (*HinfI, HaeIII*) suggested that there is an easy and less time consuming method to differentiate the three gazelle species (*Gazella marica, Gazella gazella, Gazella subgutturosa*).

The project was carried out on behalf of Turkish Ministry of Forestry and Water Affairs and was supported by the Scientific and Technological Research Council of Turkey (TUBITAK, project no: KAMAG 109G016).

Keywords: Gazelle, population genetics, conservation, captive breeding, microsatellite

ÖZ

TÜRKİYE'DE SÜRMEKTE OLAN *GAZELLA MARICA* KORUMA ÇALIŞMALARI ODAKLI, GÜNEYDOĞU ANADOLU'DAKİ CEYLANLARIN GENETİK ÇEŞİTLİLİĞİ (*GAZELLA MARICA* VE *GAZELLA GAZELLA*)

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Mevcut çalışma, Türkiye'deki ceylan türlerinin yakın zamanda önerilmiş taksonomik konumlarının doğrulanması ve ceylan popülasyonları içerisindeki ve popülasyonlar arasındaki genetik çeşitliliğin saptanması amacıyla yürütülmüştür. Çalışma için üç farklı popülasyon örneklenmiştir: Kızılkuyu Üretim Çiftliği'nden *Gazella marica* (Şanlıurfa, n=48), Erikçe Üretim Çiftliği'nden *Gazella marica* (Gaziantep, n=25) ve Kırıkhan Beldesi'nden *Gazella gazella* (Hatay, n=4). Sunulan çalışmada, 17 mikrosatelit lokusu (RT1, ETH10, OARFCB304, MM12, BM143, BM757, IDVGA29, BM848, BM4505, BMC1009, INRA40, ETH152, INRABERN172, TGLA122, ILSTS005, CSSM39, CSSM43),

iki Y kromozomu mikrosatelit lokusu (INRA126, UMN0103), mtDNA sitokrom-*b* bölgesinin (L14724 ve H15149 primerleri ile yükseltgenen) bir parçası ve iki enzim için (*HinfI, HaeIII*) restriksiyon profilleri kullanılmıştır.

İlk olarak, lokusların bilgi vericiliği test edilmiş ve 17 lokus içerisinden 12'sinin (RT1, ETH10, OARFCB304, BM848, BMC1009, INRA40, BM4505, INRABERN172, TGLA122, ILSTS005, BM757 and CSSM43) ceylan türleri üzerine yapılacak çalışmalarda umut verici olduğu saptanmıştır. Daha sonra, efektif popülasyon büyüklükleri yoluyla popülasyon içi çeşitlilikler ve mikrosatelit verilerine dayanarak hesaplanan F_{ST} ile de popülasyonlar arası farklılaşma saptanmıştır. Çiftlik popülasyonları (Kızılkuyu Üretim Çiftliği'nden *Gazella marica* popülasyonu, n=48; Erikçe Üretim Çiftliği'nden *Gazella marica* popülasyonu, n=25) düşük efektif popülasyon büyüklükleri göstermenin yanı sıra (Kızılkuyu için N_e=9.7, Erikçe için N_e= 8.9), düşük doğum oranları sebebiyle kendileşme baskısı sinyallerini de sergilemiştir. Dahası, popülasyonlar arasındaki genetik farklılaşma istatistiki olarak anlamlı çıkmıştır (sırasıyla, Kızılkuyu/Erikçe, Kızılkuyu/Hatay ve Erikçe/Hatay için F_{ST} = 0.04, 0.44 ve 0.46). Structure analiziyle popülasyonların genetik olarak karışım oranları incelenirken, örneklem gruplarındaki olası yaban kökenli bireyler açığa çıkarılmıştır.

Bu bulgulara ek olarak, Y kromozomu üzerindeki mikrosatelit lokus dizisinin (INRA126, yaklaşık 240 bç uzunluğunda) *Gazella gazella* ve *Gazella marica* türlerinin erkeklerini ayırmada başarılı olduğu gösterilmiştir. Başlangıç niteliğindeki bu Y kromozomu verisi, ceylan türlerinde genetik çeşitlilik üzerine ileride yapılacak çalışmalara referans noktası olarak kullanılabilir.

Güneydoğu Anadolu'daki ceylan türlerinin (*Gazelle marica* ve *Gazelle gazella)* taksonomik konumları, toplanan örneklerin (sırasıyla n=27 ve n=4) mtDNA sitokrom-*b* bölgesinin dizilerine dayanarak, daha önce yürütülen çalışmalardan bağımsız olarak doğrulanmıştır. Ayrıca, popülasyonlar içerisinde farklı haplotiplere rastlanmamıştır (n=3).

Son olarak mtDNA'nın sitokrom-*b* bölgesinde Restriksiyon Parça Uzunluk Polimorfizmi (RPUP) metodu denenmiş, çalışmada kullanılan kesim enzimlerine (*HinfI, HaeIII*) dayanarak üç ceylan türünü ayırmada (*Gazella marica, Gazella gazella, Gazella subgutturosa*) daha kolay ve az zaman gerektiren bir metodun varlığı gösterilmiştir.

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Anahtar Kelimeler: Ceylan, popülasyon genetiği, koruma, üretim istasyonu, mikrosatelit.

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This study was the part of a large-scale national project, named "Analysis, Conservation and Management of Large Mammals in Context of National Strategies on Conservation of Biodiversity and Genetic Resources" and it was conducted by TÜBİTAK Marmara Research Center with the help of the Ministry's GDNPNP. The project was supported by the Turkish Scientific and Technological Research Council (project no: KAMAG 109G016).

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% : Per Cent

°C : Degrees Celsius

aDNA: Ancient DNA

Arlequin: An Integrated Software Package for Population Genetics Data Analysis

bp: Base Pair

BSA: Bovine Serum Albumine

cyt-b: Cytochrome *b*

dH2O: Distilled Water

DNA: Deoxyribonucleic Acid

dNTP: Deoxyribonucleotide Triphosphates

EDTA : Ethylene Diamine Tetra Acetic Acid

e.g: For example

EtBr: Ethidium Bromide

K_3EDTA: Potassium EDTA

M: Molar

MCMC : Markov Chain Monte Carlo

ME: Minimum Evolution

MEGA : Molecular Evolutionary Genetics Analysis

mg: Miligram

$MgCl_2$: Magnesium Chloride

min: Minute

µl: Microliter

ml: Milliliter

mM: Millimolar

mtDNA: Mitochondrial DNA

Ne: Effective Population Size

ng: Nanogram

NJ: Neighbor Joining

PCR: Polymerase Chain Reaction

rpm: Revolutions per minute

RFLP: Restriction Fragment Length Polymorphism

sec: Second

Taq: Thermus aquaticus

TBE: Tris Borate EDTA

UV: Ultra Violet

CHAPTER 1

INTRODUCTION

1.1 Anatolia: A Home for Some of the World's Large Mammals

Grey wolf (*Canis lupus*), brown bear (*Ursus arctos*), Eurasian lynx (*Lynx lynx*), caracal (*Caracal caracal*) and striped hyena (*Hyaena hyaena*) are the large carnivores found in Anatolia. Three deer species (*Dama dama*, *Cervus elaphus*, *Capreolus capreolus*), two gazelle species (*Gazella gazella*, *Gazella marica*), wild goat (*Capra aegagrus*), chamois (*Rupicapra rupicapra*), mouflon (*Ovis orientalis*) and wild boar (*Sus scrofa*) are the large herbivores that inhabit Anatolia.

The Ministry of Forestry and Water Affairs, General Directorate of Nature Protection and National Parks (GDNPNP) is responsible for the conservation and monitoring of wildlife in Turkey. They have been carrying out monitoring studies and re-introduction studies as well as establishing and managing wild animal breeding and protection centers. To develop conservation strategies, they are using tools to maximize the number of individuals in the captivity without using the genetics as a tool. However, molecular genetics has been a tool for conservation studies for the last few decades and there has been a need to include genetic data in conservation studies conducted by the Ministry in Turkey. Taking this need into consideration; a large scale national project, named "Analysis, Conservation and Management of Large Mammals in Context of National Strategies on Conservation of Biodiversity and Genetic Resources", was conducted by TÜBİTAK Marmara Research Center with the help of

the Ministry's GDNPNP. The project was supported by the Turkish Scientific and Technological Research Council (project no: KAMAG 109G016). It included all of the above mentioned large mammal species of Anatolia. Samples were collected by the project team conducting field work and by the Ministry's provincial organization. The samples were genotyped using autosomal DNA, mtDNA and Y chromosome markers. The samples are being stored in DNA and tissue banks established in the context of the project. The present study is a part of this national project, which presents and evaluates the molecular genetic data obtained by the analysis of the samples from *Gazella* spp. found in Southeast Anatolia, collected between the years 2010 and 2014.

1.2 *Gazella* Genus: From Africa to Asia

Gazelles are ungulate mammals. They are classified as a member of *Gazella* genus in Bovidae family which is the largest and most diverse family of ungulates (Groves and Grubb, 2011) in the order, *Artiodactyla*. This genus shows a distribution from Africa to Northern Asia including Arabian Peninsula and South-eastern Anatolia (Lerp et al., 2013). According to Groves and Grubb's taxonomic study based on the morphological evaluations and comparisons in 2011 (Table 1.1), 23 different species with their subspecies were identified under the genus of *Gazella*.

Many gazelle species are in the IUCN Red List of Threatened Species, even if they were classified as "vulnerable", due to rapid loss of population sizes in the wild (IUCN/SSC Antelope Specialist Group, 2008). As a result of this situation, conservation actions such as captive breeding programs have been initiated for specific gazelle species (Mallon & Kingswood, 2001). However, false taxonomical classifications based on the morphological evaluations can hamper the conservation efforts by mixing the gene pools of different subspecies in the re-introduction or captive-breeding programs (Wronski et al., 2010; Lerp et al., 2013).

Table 1.1 Taxonomy of genus, *Gazella* according to Groves and Grubb's (2011) study. The species/ subspecies marked with an asterik are found in Southeast Anatolia and will be analyzed in this study.

Common Name	**Scientific Name**	**Subspecies**
Farasan Gazelle	*Gazella arabica*	
Deccan Chinkara	*Gazella bennettii*	*Gazella bennettii*
Gujarat Chinkara		*Gazella b. christyi*
Eastern Jebeer		*Gazella b. fuscifrons*
Western Jebeer		*Gazella b. shikarii*
Salt Range Gazelle		*Gazella b. salinarum*
Bilkis Gazelle	*Gazella bilkis*	
Cuvier's Gazelle	*Gazella cuvieri*	
Dorcas Gazelle	*Gazella dorcas*	*Gazella dorcas*
Pelzeln's Gazelle		*Gazella d. pelzelni*
Mountain Gazelle	*Gazella gazella*	*Gazella gazella* *
Acacia Gazelle		*Gazella g. acaciae*
Arabian Desert Gazelle		*Gazella g. cora*
Farrur Gazelle		*Gazella g. dareshurii*
Arabian Coastal Gazelle		*Gazella g. erlangeri*
Bushehr Gazelle		*Gazella g. karamii*
Slender-horned Gazelle	*Gazella leptoceros*	
Saudi Gazelle	*Gazella saudiya*	
Speke's Gazelle	*Gazella spekei*	
Persian/Goitered Gazelle	*Gazella subgutturosa*	*Gazella subgutturosa*
Turkmen Gazelle		*Gazella s. gracilicornis*
Sand Gazelle		*Gazella s. marica* *
Yarkand Gazelle		*Gazella s. yarkandensis*

All gazelle species have adapted to live in arid, semi-desert habitats, mostly away from the water sources and many of them have camel-like adaptions to survive in the conditions of extreme seasonal temperatures. As observed in the metabolism of many herbivores adopted to the desert conditions, gazelles are also homeotherms which means they can set their body temperature between specific range of temperatures according to environment (Babor et al., 2014). Many of gazelle species are known to be diurnal, however both nocturnal and diurnal species of *Gazella* genus exist, such as *Gazella thomsonii* (Grzimek's Animal Life Encyclopedia, 2004).

The semi-desert habitats preferred by gazelles are grassy plains and surrounded by little hills. They feed on grass, roots and on the other supplements in the vegetation. Although the number of individuals in a herd shows seasonal changes which varies between different species, all species live in herds including females, calves and mostly territorial one male.

All gazelle species under *Gazella* genus are known as fast animals with their slim legs, pale-colored pelage (which is even paler in their calves) and graceful morphological appearance. The black stripes as a fur ornamentation can be observed around the body and face, in some species. Additionally, in some species, the horns are observed only on male individuals whereas there are many species where the horns appear not only on males but also on females. The sexual dimorphism can be observed depending on the species of gazelles (Groves and Grubb, 2011).

Mating season of gazelles varies in accordance with populations' locations. It takes place between November to January, gestation lasts around 5-6 months and ends generally between March and July when the abundance of food is highest. Generally, only mothers play a role in nursing the fawns and their life span varies around 12-18 years (Durmuş, 2010).

1.3 Gazelles in Anatolia

1.3.1 Observations from Anatolia

The distribution areas of gazelles in Anatolia seemed to bridge the different gazelle species of old continents. However, not only the south-eastern part of Anatolia contains vegetation and climate conditions convenient for gazelles, but also central Anatolia has areas covered with many dry, desert like plains with hilly geographical structures and similar climate conditions. In accordance with today's observation, as explained by Kasparek (1986), William Francis Ainsworth, an English surgeon and also traveler, recorded "herds of gazelles" around Bolvadin (Afyon) in 1839 during his travel to Mesopotamia and published this observations in the book called, "Travels and Researches in Asia Minor, Mesopotamia, Chaldea and Armenia" (1842). Also, Ainsworth noted about the great abundance of gazelles in Çukurova plains (Adana) in the same study. Another observation from Adana province by Russegger and Kotschy (1843), again mentioned by Kasparek (1986), was: "full of numerous gazelles and deer, stretched out in front of us in an incredible uniformity". Finally, Danford and Alston (1877; 1880) observed the gazelles and described as "common" in the valley of Ceyhan (Adana Province), (Kasparek, 1986).

One of the more recent studies, which belongs to Kumerloeve (1969; 1975), drew the distribution lines of gazelles from the Turkey-Syria border to Northern plains of Şanlıurfa, making observations especially around Ceylanpınar. Another recent study published by Turan (1984), gave the distribution of the gazelles along the south-eastern border of Turkey; from Northern Hatay (Kırıkhan) to Şırnak (Cizre). He also mentioned that once gazelles were seen over the plains of Iğdır and Aralık (Eastern Anatolia).

As explained by Kasparek (1986), Ainsworth (1842) did not give information about the species of the gazelles observed. Kotschy visited the same area twice (Adana province). At his first report, he specified one species which was very similar to *Gazella dorcas* (considered as mainly the species of Africa) but had bigger features

(1856) and at his second report, he defined two different species in the plains of Adana (1858). Danford and Alston also visited the area, twice; at first they identified all gazelles as *Gazella dorcas* (1877). After their second visit, they stated that there was another species located further eastern of area, possibly *Gazella subgutturosa* (considered as mainly the species of northern Arabic Peninsula and Western Asia).

However, according to Kumerloeve (1975), Dorcas Gazelle (*Gazelle dorcas*) never reached farther than Lebanon, therefore he emphasized that the herds mentioned in older records should have belonged to *Gazella gazella* (considered as the species of Levantin and Arabic Peninsula). Whereas, Turan (1984) identified the gazelles in South-eastern Turkey as *Gazella subgutturosa* and also noted the possible existence of another gazelle species living in South-eastern Anatolia as *Gazella dorcas*. Yet, he also drew attention to the possibility of Kumerloeve's point.

Genetic and ecological studies were carried out on the gazelles around Şanlıurfa; specifically, their karyotype (Tez et al., 2009) and habitat preferences (Durmuş, 2010) were analysed. However, the existence of two different gazelle species (*Gazella gazella*, *Gazella marica*) in the borders of Turkey were showed by Kankılıç et al. (2012). That study also emphasized that the gazelle species live in Kızılkuyu State Farm are closer to *Gazella marica* than *Gazella subgutturosa* based on the mtDNA cytochrome *b* gene.

1.3.2 Current Taxonomic Status of Gazelles in Anatolia

The genus *Gazella*, is one of the most complex and least examined unit among mammals (Groves and Harrison 1967; Groves, 1969). Even with great progress achieved by the employment of genetics in taxonomical studies, the genus still harbors conflicts regarding to its classification based on skull morphometry, phenotypic features and genetic information (Lerp et al., 2013).

Table 1.2 Summarizes both common and scientific names of the gazelle species existing today and gazelle species thought to exist in the past together with their geographic distributions in the old continents.

Table 1.2 Distribution and common names of Anatolian gazelles: those existed in the past or exist currently.

Common Name(s)	**Scientific Name**	**Distribution Area**
Dorcas Gazelle	*Gazella dorcas*	Sahelo-Saharan Region, Southern Israel, Syria, Jordan
Mountain Gazelle **Idmi** **Arabian Gazelle**	*Gazella gazella*	Mountains near the Coastal Area of South-eastern Turkey, Lebanon, Palestine, Golan, Western Jordan
Persian Gazelle **Goitered Gazelle** **Black-tailed Gazelle**	*Gazella subgutturosa*	Tigris/Euphrates Basin, Caucasus, Iran, Turkmenistan, China, Mongolia
Sand Gazelle **Reem/Rheem** **Arabian Sand Gazelle**	*Gazella marica/* *Gazella s. marica*	Iraq, Jordan, Turkey, Syria Oman, Southern Arabia, United Arab Emirates

- **Dorcas Gazelles (*Gazella dorcas;* Linnaeus, 1758):** Range of this species begins from the northern Africa extends to Israel through Sinai Peninsula (Yom-Tov et al., 1995). Although, there were observations reporting on the existence of this species in Anatolia (Danford and Alston, 1877; Kotschy, 1856), there is no genetic data supporting these observations. Even if Dorcas gazelle lived in Anatolia in the past, this species does not exist in this area at the present time.

- **Mountain Gazelles (*Gazella gazella*; Pallas, 1766):** A recent molecular study based on mtDNA *cyt-b* (Kankılıç et al., 2012) proved that gazelles living around northern Hatay in Turkey belonged to species *Gazella gazella*. In addition to southern Anatolia, this species is widely distributed in Arabian Peninsula including Israel, Oman, Yemen, Saudi Arabia and United Arab Emirates (Mallon and Kingswood, 2001).

- **Goitered Gazelles (*Gazella subgutturosa*; Güldenstaedt, 1780) and Sand Gazelles (*Gazella subgutturosa marica/Gazella marica*; Thomas, 1897):** Sand gazelles were first described by Thomas (1897), as a separate, full species. In 1951, this species was considered as African slender horned gazelle (*Gazelle leptoceros*) due to habitat choice and similar behaviors on dune systems (Ellerman & Morrison-Scott, 1951). Afterwards, the morphometric investigations on the basis of skull shape and horn together with the karyological similarities between *Gazella marica* and *Gazella subgutturosa*, sand gazelles was assigned as subspecies of goitered gazelles and named as *Gazella subgutturosa marica* (Groves and Harrison, 1967). However, recently published phylogenetic studies based on mtDNA cytochrome *b* gene were emphasized that *Gazella marica* is clearly more closely related to the North-African species, *Gazella leptoceros* and *Gazella cuvieri* and they are considered as a species of their own (Hammond et al., 2001; Wacher et al., 2010). In the present study this species will be referred as *Gazella marica* but not as *Gazella subgutturosa marica*.

Studies on the gazelles of Anatolia grouped them into *Gazella subgutturosa* species until 2010 (Kumerloeve, 1967, 1969, 1975; Turan, 1977, 1984; Kasparek, 1986; Olcer, 2001; Çobanoğlu, 2010; Durmuş, 2010). However those gazelles, previously accepted as *Gazella subgutturosa* in Anatolia, were found to be similar to *Gazella marica* based on mtDNA cytochrome *b* (*cyt-b)* gene (Kankılıç et al., 2012). By the help of this

genetic information, the border between the distributions of these two gazelle species, *Gazella subgutturosa* and *Gazella marica*, was determined. While goitered gazelles are distributed from eastern of Arabian Peninsula, through Iran to Turkmenistan (Mallon and Kingswood, 2001; Zachos et al., 2010); the sand gazelles are observed from open habitats through Arabian Peninsula, through Syria to South-eastern Turkey (Wacher et al., 2010; Kankılıç et al., 2012).

In Figure 1.1, two different species of gazelles (*Gazella gazella*, *Gazella marica*) encountered in Turkey were photographed. Photos labelled with A and C represent *Gazella gazella* species and photos labelled B and D belong to *Gazella marica* species. As it can be seen in Figure 1.1, male *Gazella gazella* individual shows more slender morphology with thinner neck, whereas male *Gazella marica* individual has bigger body shape with remarkable thick neck.

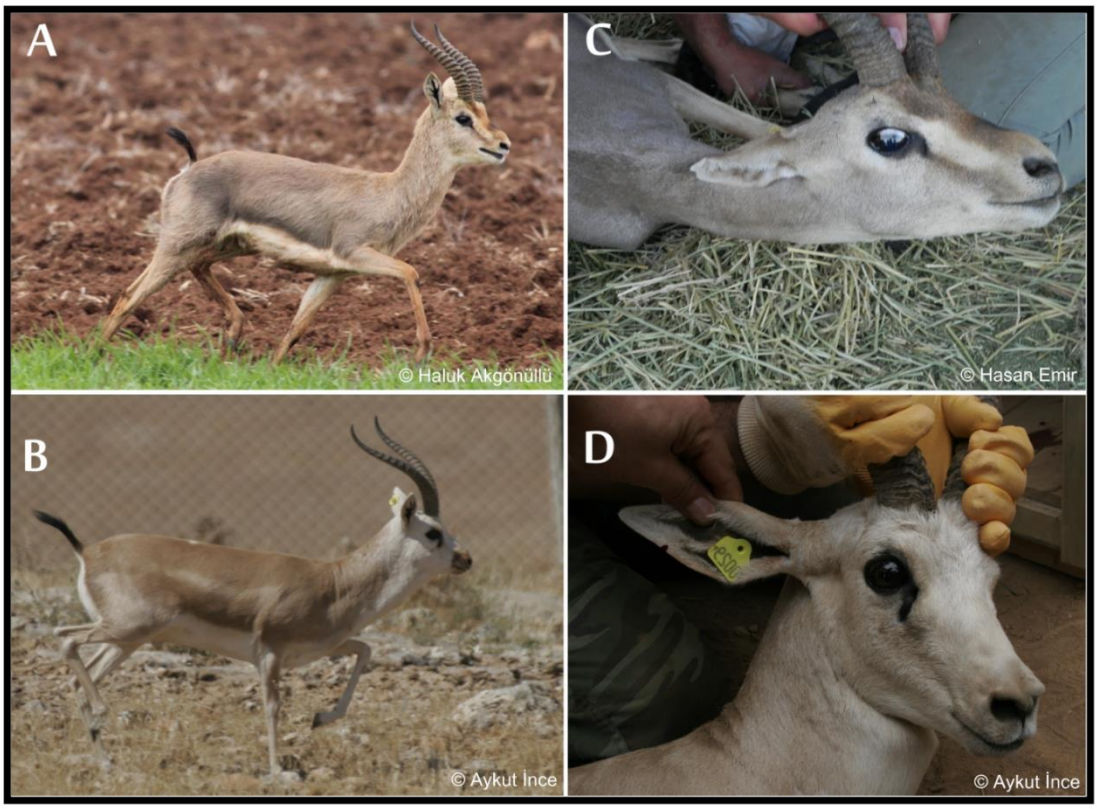

Figure 1.1 Photos of two *Gazella* spp. in Turkey. Photos labeled A and C represent the male *Gazella gazella* individual whereas photos labelled B and D represent the male *Gazella marica* individual.

1.4 Conservation Studies for *Gazella marica* in Turkey

Gazelles in Anatolia cannot be considered as "common" as they were in the reports from 19th century (Ainsworth, 1842; Russegger and Kotschy, 1843; Danford and Alston, 1877). Although there is a law enforced against illegal hunting to prevent the decrease of population size since 1957, the population size of gazelles in Southeastern Turkey decreased from 3000 individuals to 300 individuals (Ceylanpınar, Şanlıurfa) between the years 1968 and 1978 (Oğurlu, 1992; IUCN/SSC Antelope Specialist Group, 2001). After this rapid decline in the population size, the following conservation actions were taken:

- Ceylanpınar State Farm was founded by 5 gazelle individuals collected from wild (4 females, 1 male) by General Directorate of National Parks, Game and Wildlife in the Ministry of Forestry in Şanlıurfa, 1978.
- In 1982, when the number of individuals reached to 23 in Ceylanpınar State Farm, the management of this farm was left to the General Directorate of Agricultural Studies (TIGEM).
- The second state farm (Gölpınar State Farm), founded by Ministry of Forestry, was established with 24 individuals taken from Ceylanpınar State Farm in 1998. After that, in 2007, the state farm was moved to Kızılkuyu region (Şanlıurfa). Because the region is closer to the reintroduction site and the region already had its own wild gazelle population, it was considered to be more suitable for gazelles.
- The third state farm also known as Erikçe State Farm was established by 29 individuals. They were transported from Ceylanpınar State Farm, to Erikçe, in 1999.
- The last state farm for captive-breeding was founded by 8 individuals, transported from Kızılkuyu State Farm, to Hekimhan (Malatya), in 2005.
- The first gazelle reintroduction to wild was carried out in Şanlıurfa, 2005. Eighty six individuals from Gölpınar State Farm (later it will be called as Kızılkuyu State Farm) were marked and released to Payamlı region near the Kızılkuyu State Farm. The number of gazelles living in the wild increased from 50 to 500 between the years 2005 to 2013 in Kızılkuyu Wildlife Conservation and Improvement Area.

According to the reports obtained from the Ministry of Forestry and Water Affairs, six *Gazella marica* individuals from Ceylanpınar were added into the Kızılkuyu State Farm. Also, one *Gazella marica* individual taken from Kızılkuyu wild area, was transported to the Erikçe State Farm in Gaziantep. The locations of the state farms and Kırıkhan, Hatay from which samples were taken in the present study were given in Figure 1.2, above.

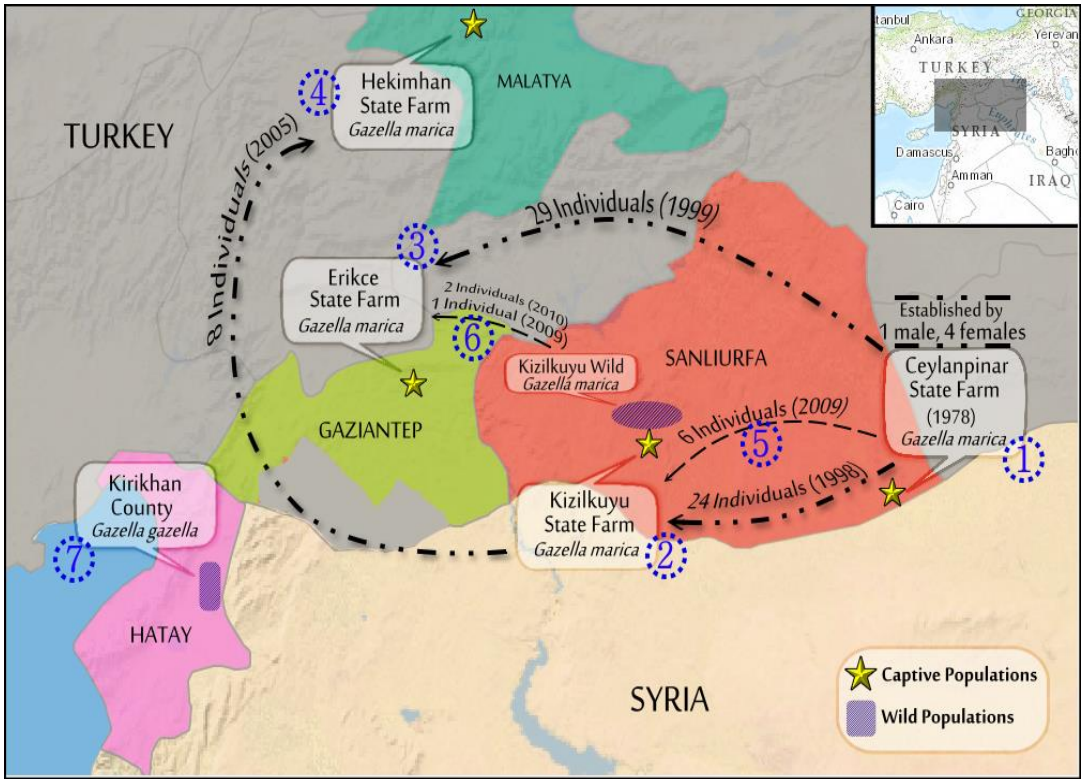

Figure 1.2 The origins of the gazelle individuals studied in the present study (Erikçe and Kızılkuyu State Farms) and Kırıkhan, Hatay are underlined. Also, the years in which the state farms were founded, the founding number of individuals and their relationship to the source population were given.

The introduction/reintroduction studies done in the frame of conservation actions were summarized in Table 1.3. All data were collected from General Directorate of Nature Protection and National Parks at the end of the December of 2014.

Table 1.3 Summary table on introduction/re-introduction studies carried out by General Directorate of Nature Protection and National Parks. The name of the introduction area, the name of the state farm, the year of introduction were given in columns.

Year	State Farm	Introduction/Reintroduction Area	Number of Individuals
2005	Kızılkuyu	Şanlıurfa – Payamlı	86
2008	Kızılkuyu	Şanlıurfa – Payamlı	25
2011	Kızılkuyu	Ankara – Polatlı	44
2012	Kızılkuyu	Şanlıurfa – Payamlı	44
2013	Kızılkuyu	Şanlıurfa – Payamlı	6
2014	Kızılkuyu	Şanlıurfa – Payamlı	79
2014	Erikçe	Iğdır – Aralık	51
			Total: 335

As can be seen from the Table 1.3, mainly Kızılkuyu State Farm population served as the source of the introduced *Gazella marica* individuals. Yet, this state farm was originated from Ceylanpınar as was Erikçe state farm. The exact birth and death rates observed in these state farms are not known. However, with the available data these were estimated and given in the discussion part of the manuscript. Another State Farm located in Hekimhan, Malatya. However, this captive-breeding station has the lowest breeding number from 2005 to 2014 due to low natality. Therefore, there is no introduction/reintroduction study in relation to this state farm.

Apart from these, a number of *Gazella marica* individuals were sent from Ceylanpınar State Farm to eastern Georgia as a part of the re-introduction of *Gazella subgutturosa* studies in this country in 2008. By that time, the gazelle species in Ceylanpınar was known as *Gazella subgutturosa.* Later, gazelles sent to Georgia were transferred to Azerbaijan after the rest of the population disappeared in the wild areas of Georgia. These two species (*Gazella marica* and *Gazella subgutturosa*) are living together under captivity in Azerbaijan, Shirvan National Park (Durmuş et al., 2010; Murtskhvaladze et al., 2012).

1.4.1 Conservation Status of Sand Gazelles (*Gazella marica*)

In Anatolia, the dramatic decrease of the population size between the years 1968-1978 caused almost local extinction of the main (or only) gazelle of Anatolia: the sand gazelles. In addition to illegal hunting, capturing the calves in breeding season, heavily used pesticides by farmers and locals, overgrazing of domestic livestock and also disturbances by shepherd and feral dogs are other reasons for this rapid decrease in the number of sand gazelles in Anatolia (Turan, 1977; Durmuş, 2010).

Sand gazelles are threatened not only in the borders of Turkey but also in other countries where the species is present because of hunting and overgrazing by domestic livestock (Mallon and Kingswood, 2001). According to an IUCN report (by Antelope Specialist Group in 2008), the state of *Gazella marica/Gazella subgutturosa marica* populations were declared as “Vulnerable” with the criteria, C2a(i). A key for this criteria is given in Figure 1.3, below.

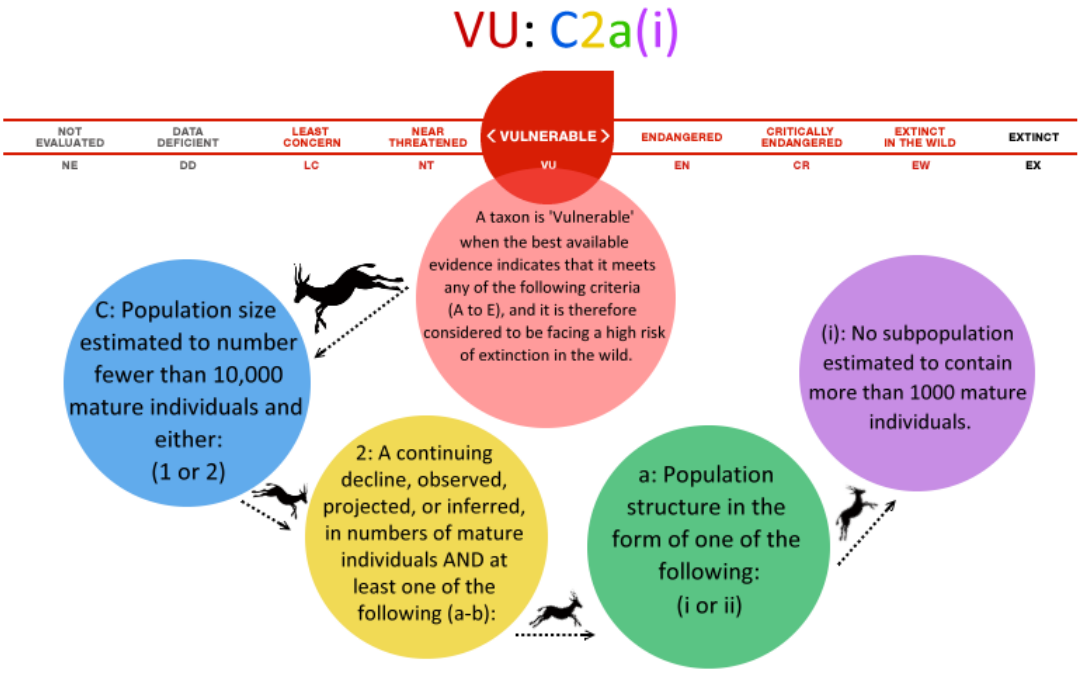

Figure 1.3 The key for the IUCN's "Vulnerable" C2a(i) status criteria as given in the version 3.1 of IUCN Red List Categories and Criteria (SSC/IUCN Antelope Specialist Group, 2008).

Briefly; "VU:C2a(i)" criteria of IUCN emphasizes that the interested organism bears high risk of extinction, since the estimated population size is under the 10,000 individuals. Moreover, there is no subpopulations more than 1000 individuals in the wild and the number is in continuous decline.

1.5 Genetics and Conservation Biology in Brief

Many scientist indicated that the sixth mass extinction has already begun based on the human induced reasons such as accelerated climate change, growth of the human population (Frankham et al., 2002; Novacek, 2007; Wake and Vredenburg, 2008) and some studies estimate that around 40% of the current species will be lost by 2050 (e.g.

Butchart et al., 2010). The loss of genetic variation plays a major role in the extinction of the populations and species (Frankham, 2005), because they suffer from inbreeding depression (for instance their birth rate decrease and or death rate increase) (Allendorf and Luikart, 2007) and they do not have the variation to cope with the newly developing environmental conditions. In other words, they lose their potential to evolve (Spielman et al., 2004). Since genetics is a tool to measure, monitor and thus help to manage the genetic variations in gene pools of the species, it is widely used to plan long-term survival strategies for the endangered species by using various molecular markers (Frankham, 2010; Avise 2012).

Microsatellites, PCR based markers, consist of short DNA segments with 1- 6 bp long repeats and they are repeated up to about 100 times. Microsatellites have high mutation rate around 10^2-10^6 per generation which makes them a useful tool for measuring genetic diversity within populations and between closely related populations. By the help of microsatellite markers, many genetic diversity monitoring and managing studies were held within the frame of conservation studies. For example, in a recent study on individuals of Arabian oryx (n=24), one of the relatives of genus *Gazella*, by estimating the molecular diversity based on seven microsatellite loci present both in the captive breeding and re-introduced populations, they showed the importance of the microsatellites for the management of captive breeding programs (Arif et al., 2010).

Current population sizes and the trend in the change of population sizes are the two parameters of prime interest in conservation studies. For populations under threat of extinction if there is no census data to trace these parameters, microsatellites can be used to describe the genetic diversity of the population. Then, this data serves to estimate both the current effective population size (N_e), and the rate of the population size change. Incidentally, effective population size is the size of the ideal population that will result in the same amount of genetic drift as in the actual population being considered (Allendorf et al., 2013). One remarkable study, which was done to conserve

the orangutans in the Borneo, shows how current effective population size decreased because of the human induced affects such as deforestation in the last thousand years (Goossens et al., 2006). In that study, N_e estimates of the current and past populations were done using the data from microsatellite loci analysis.

Microsatellites can also be used to measure the genetic differentiation between subpopulations, for instance by using the F_{ST} measure (Wright, 1978). Moreover, using microsatellites admixtures can be detected between subpopulations. For this purpose, Structure software (Pritchard et al., 2000) can be employed. Thereby, this marker type and the methods are widely used in conservation studies of subpopulations or species. However, the limitations of the microsatellites (such as existence of null alleles) should be considered when employing these markers.

There is another marker used in population and conservation studies; maternally inherited mtDNA. This DNA does not recombine, and protein coding mtDNA genes, such as *cyt-b,* have moderate mutation rates. This marker is used for revealing maternal evolutionary history in lower taxonomic levels, such as genera and species. Because it is not recombined and it has relatively high mutation rate, mtDNA is one of the widely used genetic markers to resolve taxonomic uncertainties in conservation genetics studies (Arif et al., 2010). Taxonomic studies solely based on morphology may cause erroneous phylogeny (Avise, 1989). To prevent the hybridizations based on wrong taxonomical status, many phylogenetic studies have been done on gazelles recently. For instance, sequences of mtDNA *cyt-b* and control region showed the existence of possible reciprocally monophyletic lineages of two *Gazella gazella* populations, one of which is restricted to a small area on the Golan Heights and may be considered as a separate species (Wronski et al., 2010; Lerp et al., 2011).

1.6 Justifications and Aims of the Study

Here, in the present study, samples from two gazelle (*Gazella marica*) populations, which are Kızılkuyu State Farm (n=48) and Erikçe State Farm (n=25), will be analysed using 17 autosomal microsatellite loci, two Y chromosome loci and partial mtDNA *cyt-b* region (by sequencing and by RFLP analysis). Additionally, 4 individuals from *Gazella gazella* population in Hatay will also be tested to distinguish the differences between these two species (*Gazella marica* and *Gazella gazella*) based on the genetic markers employed in the study.

Within the limits of our data, following questions will be addressed:

(i) Genetic diversities within and among populations will be estimated not only to observe the effects of captive-breeding on gene pools but also to estimate their effective population sizes.

(ii) An attempt to determine the Y chromosome based diversity of the two gazelle species (three gazelle populations) will be made. It will provide the first attempt of distinguishing Y chromosomes of different gazelle species.

(iii) Existence of two different gazelle species in Southeastern Anatolia (*Gazelle marica* and *Gazella gazella)* will be confirmed based on the mtDNA *cyt-b* sequences of the samples collected independently from the previous studies.

(iv) Restriction Fragment Length Polymorphism (RFLP) method will be tested on mtDNA *cyt-b* fragments to suggest an easy and quick way to distinguish gazelles across the two species (*Gazella marica* and *Gazella gazella*).

It is hoped that results of the present study will contribute to the conservation studies of *Gazella marica* currently existing in the Southeastern border of Turkey. All the samples introduced to Aralık (Iğdır) from Erikçe were genotyped within the context of this study. This data will serve as a start point for long term monitoring study for this population.

CHAPTER 2

MATERIALS AND METHODS

2.1 Sampling

In the present study, blood and/or tissue samples of 77 individuals from 3 different gazelle populations from 2 different gazelle species (*Gazella gazella*, *Gazella marica*) were collected in context of the project "Analysis, Conservation and Management of Large Mammals in Context of National Strategies on Conservation of Biodiversity and Genetic Resources" conducted by TÜBİTAK Marmara Research Center and owned by the Ministry of Forestry and Water Affairs' General Directorate of Nature Protection and National Parks. The blood samples were collected in 10 ml K_3EDTA (anticoagulant) containing vacuum tubes and the tissue samples were collected in tubes containing ethanol.

***Gazella marica*, Kızılkuyu samples (n=48):** These were largely representing individuals which were kept in Kızılkuyu State Farm. This State Farm was established by transporting 24 individuals from Ceylanpınar State Farm in 1998. Later another 6 individuals were transported from Ceylanpınar to Kızılkuyu State Farm in 2009. However, some of the Kızılkuyu samples of the present study were the samples hunted from the wild Kızılkuyu individuals. It is also known that some captive individuals were introduced to the Kızılkuyu wild population from time to time just before hunting seasons. Since the area of the farm is quite large, it is likely that during the illegal

hunting attempts, some wild individuals could have entered to the farm without being recorded. Similarly individuals from the captive population could have entered to the wild population. The location of the Kızılkuyu State farm is illustrated in Figure 1.2.

***Gazella marica*, Erikçe samples (n=25):** These samples were all from the Erikçe State Farm (Figure 1.2) which was established in 1999. Individuals of this state farm (n=29) were transferred from Ceylanpınar State farm. To our knowledge, only one wild male individual is introduced to Erikçe from Kızılkuyu wild samples in 2010.

***Gazella gazella*, Kırıkhan County, Hatay samples (n=4):** These samples are from Kırıkhan County (Figure 1.2) either captured alive (new born, sick or injured) by the locals (and taken to the university) or found dead between the years 2013-2014.

2.2 Laboratory Experiments

2.2.1 DNA Extraction

DNA was isolated by performing standard: phenol-chloroform isoamyl alcohol method (25:24:1) (Sambrook et al., 1989). All DNA isolations from blood were done at TUBITAK GMBE laboratories and the procedure followed is given below:

- At first, 10 ml blood samples of each individual were taken put into a tube containing 0.5 ml EDTA (0.5 M, pH 8.0), and then, 40 ml of 2X lysis buffer (10X Lysis Buffer: 770 mM NH_4Cl, 46 mM $KHCO_3$, 10 mM EDTA) was added.
- The tubes were inverted for 10 minutes to mix the content and after keeping in ice for 30 minutes, the tubes were subsequently centrifuged at 3000 rpm at 4°C for 10 minutes.
- After centrifuging, the supernatant was discarded, 3 ml salt/EDTA (75 mM NaCl, 25 mM EDTA) was added in to the tubes and mixed by the help of vortex.

- Then 0.3 ml of 10 % SDS and 150 µl of proteinase K (10 mg/ml) solution were added, samples were incubated at 55°C for 3 hours.
- After the incubation, 3 ml phenol (pH 8.0) was added to each tube and tubes were shaken vigorously for 20 seconds and then by gentle inversions for 5 minutes.
- Then, tubes were centrifuged at 3000 rpm at 4°C for 10 minutes. Seemed pure supernatants were transferred to the new tubes labelled properly, 3 ml phenol: chloroform: isoamyl alcohol (25:24:1) was added into the each tube.
- After switching the tubes, the tubes were shaken vigorously for 20 seconds, by gentle inversions for 5 minutes and centrifuged at 3000 rpm at 4°C for 10 minutes.
- The supernatants were transferred into new sterile 15 ml falcon tubes labelled properly, ice cold 96% EtOH (kept at -20°C) was added at a volume of twice the supernatant, and the tubes were shaken rapidly to precipitate the extracted DNA well.
- Finally, the precipitated DNAs were transferred into 1.5 ml eppendorf tubes, washed once with 70% alcohol, by a micropipette, air dried and dissolved in approximately 1 mL of Tris-HCl/EDTA buffer (10mM Tris-HCl, 1mM EDTA, pH 8).

The extracted DNA samples were either stored in dilutions at +4°C for short-term use or at -20°C as stocks for long term storage.

2.2.2 Checking the Quality and Estimating the Quantity of the DNAs Extracted

The quality and quantity of the isolated DNAs were checked on 1 % agarose gel prepared with 1X TAE (Tris-acetate-EDTA) buffer and EtBr (0.5 ug/ml). To be able to visualize migration of the DNAs during electrophoresis, 3 µL of DNA samples are mixed with 3 µL of 2X loading dye (bromophenol blue, xylene cyanol FF, glycerol) and then, dyed samples were run on agarose gels applying 100 volts for 30 minutes

in a horizontal tank containing 1X TAE buffer. The gels were viewed under the UV light using the SYNGENE Ingenius LHR visualizing device. Invitrogen 50 bp DNA ladder was used as DNA ladder and as a reference for sample DNAs' concentration estimation. Furthermore, the quality and quantity of each DNA sample were also checked by NanoDrop (Thermo Scientific) device. Then dilutions (~50 ng/ul) for each DNA sample were prepared to be used in setting up the PCR reactions.

2.2.3 Microsatellites

In the present study, 17 microsatellite loci were employed. The loci were chosen from the literature as they were used in studies on either gazelles or other species from Bovidae family. The names of the microsatellite loci, type of them and related references were given in Table 2.1, below.

Table 2.1 The names of the microsatellite loci used in the study, their types and related references.

Loci	*Primer 5'-3'*	*Type of Loci*	*Reference*
RT1	TGCCTTCTTTCATCCAACAA CATCTTCCCATCCTCTTTAC	Polymorphic	*Wilson et al., 1997*
ETH10	GTTCAGGACTGGCCCTGCTAACA CCTCCAGCCCACTTTCTCTTCTC	Polymorphic	*Toldo et al., 1993*
OarFCB304	CCCTAGGAGCTTTCAATAAAGAATCGG CGCTGCTGTCAACTGGGTCAGGG	Polymorphic	*Buchanan & Crawford, 1993*
MM12	CAAGACAGGTGTTTCAATCT ATCGACTCTGGGGATGATGT	Polymorphic	*Mommens et al., 1994*
BM848	TGGTTGGAAGGAAAACTTGG CCCTCTGCTCCTCAAGACAC	Polymorphic	*Bishop et al., 1994*
BMC1009	GCACCAGCAGAGAGGACATT ACCGGCTATTGTCCATCTTG	Polymorphic	*Kappes et al., 1997*
INRA40	TCAGTCTCCAGGAGAGAAAAC CTCTGCCCTGGGGATGATTG	Polymorphic	*Vaiman et al., 1994*
IDVGA29	CCCACAAGGTTATCTATCTCCAG CCAAGAAGGTCCAAAGCATCCAC	Polymorphic	*Mezzelani et al., 1995*
BM4505	TTATCTTGGCTTCTGGGTGC ATCTTCACTTGGGATGCAGG	Polymorphic	*Bishop et al., 1994*
ETH152	TACTCGTAGGGCAGGCTGCCTG GAGACCTCAGGGTTGGTGATCAG	Polymorphic	*Steffen et al., 1993*
INRABERN172	CCACTTCCCTGTATCCTCCT GGTGCTCCCATTGTGTAGAC	Polymorphic	*Saitbekova et al., 1999*

Table 2.1 (continued)

TGLA122	CCCTCCTCCAGGTAAATCAGC AATCACATGGCAAATAAGTACATAC	Polymorphic	*Georges & Massey, 1992*
ILSTS005	GGAAGCAATGAAATCTATAGCC TGTTCTGTGAGTTTGTAAGC	Polymorphic	*Kemp et al., 1995*
BM757	TGGAAACAATGTAAACCTGGG TTGAGCCACCAAGGAACC	Polymorphic	*Bishop et al., 1994*
BM143	ACCTGGGAAGCCTCCATATC CTGCAGGCAGATTCTTTATCG	Polymorphic	*Bishop et al., 1994*
CSSM39	AATCGGAACCTAGAATATTTTGAG AGATAAAATGTGAGTGTGGTCTCC	Polymorphic	*Moore et al., 1994*
CSSM43	AAAACTCTGGGAACTTGAAAACTA GTTACAAATTTAAGAGACAGAGTT	Polymorphic	*Moore et al., 1994*

Two additional microsatellite loci (Table 2.2) located in Y- chromosome were also employed as they were reported as highly polymorphic in the literature. The names of the microsatellite loci, their types and the related references were given in Table 2.2, below.

Table 2.2 The microsatellite loci of Y chromosome used in the study: their primer sequences, types and related references.

Loci	**Primer 5'-3'**	**Type of Loci**	**Reference**
INRA126	GTTGTTGCCTCTGCAGAGTAGG GACACTCTTTCTATTTTCAAGG	Polymorphic	Vaiman et al., 1994
UMN0103	ACACAGAGTATTCACCTGAG ATTTACCTGGGTCAAAGCAC	Polymorphic	Liu et al., 2002

2.2.4 Cytochrome *b* (*cyt-b*) Region of mtDNA

In this study, a 381-bp-long region on mtDNA cytochrome *b* gene of gazelle samples were sequenced and analyzed. The primers amplifying this region are L14724 and H15149 (Kocher et al., 1989; Irwin et al., 1991). The sequences of the primers are:

L14724: 5'-CGAAGCTTGATATGAAAAACCATCGTTG-3'

H15149 : 5'-AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA-3'

2.2.5 Setting up Polymerase Chain Reactions (PCRs)

2.2.5.1 PCR Conditions for Microsatellite Loci

In the present study, 17 microsatellite loci were analyzed by PCR amplification. The reactions were set by grouping these loci (multiplexing) according to their expected size ranges and the fluorescent dye they were labelled with. PCRs were performed on Biometra T-1 thermoblock. All the 17 loci were analyzed in 5 different multiplex sets. The reaction mixes and the reaction conditions are given below:

Table 2.3 The multiplex sets of microsatellite loci, PCR mixes and conditions of them.

PCR mixes			
	S1 /S2 (ul)	**S3/S4 (ul)**	**S5 (ul)**
dH2O	8,42 / 8,7	7,8 / 7,6	7,55
10X Buffer	1,5	2	2,25
25 mM MgCl2	1,2	1,5	1,5
10 mM dNTP	0,3	0,3	0,3
Primer	S1 ETH10 0,3 ul RT1 0,18 ul OARFCB304 0,5 ul S2 MM12 0,4 ul BMC1009 0,15 ul BM848 0,15 ul INRA40 0,5	S3 IDVGA29 0,3 ul BM4505 0,3 ul ETH152 0,4 ul S4 ILSTS05 0,2 ul INRABERN 0,2 ul 172 TGLA122 0,3 ul BM757 0,5	BM143 0,6 ul CSSM39 0,2 ul CSSM43 0,3 ul
DNA (50 ng)	2	2	2
Taq	0,1	0,1	0,1
BSA	-	0,3	0,2

PCR Condition (S1-2-3-4)		
95°C	3 min	
94°C	20 sec	
57°C	35 sec	30 cycles
72°C	1 min	
72°C	10 min	

PCR Condition (S5)		
95°C	3 min	
94°C	20 sec	
52°C	40 sec	30 cycles
72°C	50 sec	
72°C	10 min	

The PCR products were checked on 1% agarose gels containing EtBr (0.5 ug/ml) under UV light after electrophoresis. For the multiplex sets; S3, S4 and S5: 3 µl of each PCR products were loaded to each well of the agarose gel. For the multiplex sets; S1 and S2: The PCR products were mixed in 1:2 ratio (respectively), and they were co-loaded

to each well of the agarose gel. After checking the PCR products on agarose gels and making sure that the reactions worked and produced amplified DNA bands, 2 µl from each PCR product were mixed with 0.2 µl of Beckman Coulter's DNA Size Standard Kit - 400 and 30 µl of sample loading buffer. Then the samples were loaded on to Beckman Coulter CEQ8800 Genetic Analysis System and analyzed by capillary electrophoresis. After electrophoresis, sizing of the alleles observed were performed by the fragment analysis tool implemented in the Beckman Coulter CEQ8800 Genetic Analysis System.

For the Y chromosome microsatellite loci, the primers were not fluorescently labelled. Therefore the reactions were set separately. The PCR mixes for both of the Y chromosome loci were given in Table 2.4, below.

Table 2.4 The content of the PCR mixes for the Y chromosome microsatellite loci analyzed in the study.

PCR Mix of INRA126 (15 ul)	
dH2O	8,4
10X Buffer	1,5
25 mM MgCl2	1,4
10 mM dNTP	0,3
Primer	0,5
DNA (50 ng)	2
Taq	0,1
BSA	0,8

PCR Mix of UMN0103 (15 ul)	
dH2O	7,9
10X Buffer	1,5
25 mM MgCl2	1,4
10 mM dNTP	0,3
Primer	0,5
DNA (50 ng)	2,5
Taq	0,1
BSA	0,8

The PCR reaction conditions were as follows: **INRA 126 locus**; first denaturation at 95°C for 3 minutes, then 30 cycles of amplification (30 sec at 94°C for denaturation, 35 sec at 54°C for annealing, 40 sec at 72°C for extension), then one cycle for final

extension at 72°C for 5 minutes. **UMN0103 locus**; first denaturation at 95°C for 3 minutes, then 30 cycles of amplification (20 sec at 94°C for denaturation, 40 sec at 54°C for annealing, 50 sec at 72°C for extension), then one cycle for final extension at 72°C for 5 minutes.

After PCR amplifications, the PCR products (of the loci INRA 126 and UMN0103) were checked on 1% agarose gels containing EtBr (0.5 ug/ml) under UV light after electrophoresis. Afterwards, the PCR products were purified with GeneJET PCR Purification Kit to remove the PCR left overs following the procedure below:

1- For every 15 µl of PCR product after amplification, 15 µL of binding buffer were added and mixed until the yellow colored solution was obtained.
2- Then 15 µl of 100% isopropanol was added into the tubes containing binding buffer – PCR product and mixed again.
3- The obtained solutions for each sample were transferred to the purification columns. After centrifuging at 15000 rpm for 1 minute, the flow-through was extracted from the columns.
4- For an each sample, 700 µL of washing buffer were added into columns and centrifuged at 15000 rpm for a minute. After the flow-through was extracted, the last products were placed back into the collection tubes with their purification columns and the columns were centrifuged at 15000 rpm for a minute again to remove the left overs of washing buffer.
5- As a final step, the solutions in the purification columns were replaced into the UV-sterilized 1.5 mL centrifuge tubes. After adding 20 µl of elution buffer at the center of the columns membranes, at first tubes were left around ten minutes to elute the Y chromosome fragments and then, they were centrifuged at 15000 rpm for a minute.
6- The purified Y chromosome fragments were stored at +4^{o}C until the sequencing.

Then the purified PCR products of the two Y chromosome loci were sent to RefGen Company (http://www.refgen.com/) for sequencing. The company uses ABI PRISM® 3100 Genetic Analyzer system for sequencing.

2.2.5.2 PCR Conditions for Cytochrome *b* (*cyt-b*) Region of mtDNA

The PCR primers for mtDNA *cyt-b* region are given above in the section "2.2.4" within this chapter. For the reactions, Biometra T-1 Thermoblock PCR device was used. The reaction mixture was prepared as given in Table 2.5, below:

Table 2.5 the PCR mixture of the amplified mtDNA *cyt-b* fragments

	PCR mix (25 ul)
dH2O	16,6
10X Buffer	2,5
25 mM MgCl2	2
10 mM dNTP	0,4
Primer	0,4
DNA (50 ng)	3
Taq DNA polymerase	0,1

The PCR reaction conditions were as follows: first denaturation at 95°C for 3 minutes, then 30 cycles of amplification (30 sec at 94°C for denaturation, 35 sec at 54°C for annealing, 40 sec at 72°C for extension), then one cycle for final extension at 72°C for 5 minutes.

After PCR amplification, the amplified fragments were checked by electrophoresis (1% agarose gel containing EtBr (0.5 ug/ml), 1XTAE) and visualized under the UV light using the SYNGENE Ingenius LHR visualizing device.

2.2.6 Sequencing of Cytochrome *b* (*cyt-b*) Region of mtDNA

After checking the PCR products by electrophoresis, first the concentration of the amplification products were measured by using Thermo NanoDrop device, and then the samples were diluted to a concentration of 50 – 60 ng/µl. After making the dilutions, the sequencing reactions were prepared for each of the diluted PCR product. In these sequencing reactions, the previously prepared PCR products were used as DNA templates. These DNA templates are sequenced by Sanger et al.'s (1973) chain termination method using the Beckman Coulter's GenomeLab Dye Terminator Cycle Sequencing with Quick Start Kit which contains fluorescently labelled dideoxynucleotides along with the unlabeled deoxynucleotides. It is this labelled dideoxynucleotides terminating the DNA chain when incorporated to the growing end of the DNA chain by the polymerase enzyme. The reactions were prepared separately for forward and reverse primers. In other words, each sequencing reaction contained only one primer, providing one way sequence of the PCR template analyzed. The sequencing reaction mixture is prepared as given in Table 2.6, below.

Table 2.6 Sequencing PCR mixture.

	Sequence PCR mix (ul)
dH_2O	4.5
dITP mix (Beckman's kit)	2
PCR Product (DNA template)	2
4 µM Primer	1.5

For sequencing reactions, Biometra T-1 Thermoblock PCR device was used. The sequencing conditions were as follows: first denaturation at 96°C for 1 minutes, then 30 cycles of amplification (20 sec at 96°C for denaturation, 20 sec at 50°C for

annealing, 4 min at 60°C for extension). After sequencing reactions were completed, the Sequencing PCR products were purified by ethanol precipitation method as suggested by the Beckman Coulter's GenomeLab Dye Terminator Cycle Sequencing with Quick Start Kit's manual. The procedure is as follows:

- At first, 5 µl Stop solution/Glycogen mixture for each sample was prepared with 2 µl of 3M of Sodium Acetate (pH 5.2), 2 µl of 100 mM Na2-EDTA (pH 8.0) and 1 of 20 mg/mL of glycogen.

- Each Products of Sequence PCR were transferred to their own the labeled, sterile 0.5 mL microfuge tubes and 5 µl Stop solution/Glycogen mixtures were added in tubes with the help of micropipette.

- Then 60 µl ice cold 95% (v/v) ethanol/dH_2O were added and the samples centrifuged at 14,000 rpm at 4°C for 15 minutes. After the process, supernatants were removed by not touching the visible pellets.

- The pellets were washed 2 times with ice cold 200 µl 70% (v/v) ethanol/dH_2O after centrifuging at 14,000 rpm at 4°C for 2 minutes and removing supernatants carefully.

- At last, pellets were dried by the help of vacuum and the samples were resuspended by adding 40 µl of the Sample Loading Solution (SLS) provided with the kit.

These resuspended samples were loaded on to Beckman Coulter CEQ8800 Genetic Analysis System and analyzed by capillary electrophoresis. After capillary electrophoresis was finished, the forward and the reverse sequences of each sample was obtained separately by using the Sequencing Analysis program implemented in the Beckman Coulter CEQ8800 Genetic Analysis System. The sequences were then analyzed visually by using ChromasPro software (http://www.technelysium.com.au/ChromasPro.html), the forward and reverse sequences were aligned for each

individual and contig sequences were obtained. Then, the contig sequences were exported in FASTA format. This FASTA file of contig sequences was edited (aligned by using ClustalW Multiple Alignment Tool and trimmed) and data file was prepared for further statistical analysis by using BioEdit software version 7.2.5 (Hall, 1997-2013).

2.3 Data Analysis

2.3.1 Statistical Analyses for the Microsatellite Data

2.3.1.1 Reliable Genotyping by the Microsatellites

-Detection of the Null Alleles

Null alleles are alleles that exist at the microsatellite loci, yet they are not amplified after polymerase chain reaction (PCR mutations at the primer sites and low quality and/or low quantity DNA templates are the causes for the observation of loci with null alleles). If there are null alleles, heterozygotes will be erroneously read as homozygotes. If the null alleles are ignored then within-population genetic diversity will be underestimated (Paetkau and Strobeck, 1995).

MICRO-CHECKER 2.2.3 software (Oosterhout *et al.*, 2004) detects the null alleles and by capturing the significant excess of homozygotes. According to previously published references (Dakin and Avise, 2004; Chapuis and Estoup, 2007) the loci with null allele frequencies bigger than 0.2 were considered as loci revealing an unreliable data.

- Linkage Disequilibrium (LD)

Linkage disequilibrium is an approach to determine the non-random associations between alleles at two loci (Lewontin and Kojima, 1960), in other word, it occurs when the genotypes at the two loci are not independent. Gene flows, random

gene drifts and even mutations in non-recombining regions of the genome can be caused linkage disequilibrium. Also, in the small and isolated populations, linkages between loci are expected to be seen due to high inbreeding ratio (Hill and Robertson, 1968).

D is known as the most common coefficient of LD and calculated as:

$$D = (x_{11}x_{22}) - (x_{11}x_{22})$$

where x_{11}, x_{22}, x_{11} and x_{22} are the frequency of the four gametes or haplotypes like AB, Ab, aB, and ab respectively. So, if a population is in the linkage equilibrium, D value will be observed as "0" in order to random association of alleles in the population. If not so, whereas a population is in LD, D value will not be equal the "0" which means the alleles in two loci are not associated randomly. Since the population size worked on is small and isolated, some of the loci pairs are expected to be in LD due to expected high inbreeding ratio.

In the present study, LD estimation was performed for the pairwise comparison of 17 loci by using software, Arlequin v.3.5.1.3. (Excoffier *et al*., 2006). Markov chain steps were set as 10000 and dememorization steps were set as 1000 with 5000 batch. The results were evaluated after Bonferroni correction.

2.3.1.2 Analyses of Genetic Variation

2.3.1.2.1 Allelic Richness

The number of alleles in a locus is known as allelic richness which is one of the commonly used estimator of genetic variation in populations. Allelic richness is known to be more sensitive than the heterozygosity to the loss of genetic variation while working on small sample sizes (Allendorf, 1986). The allelic richness of 16 loci was calculated by using FSTAT V.2.9.3 package program (Goudet, 2001).

2.3.1.2.2 Polymorphism Information Content (PIC)

The Polymorphism Information Content (PIC) is a measure which ranks the markers used in terms of their informativeness. PIC is estimated with the total number of alleles and allele frequencies in a population. PIC for a locus is calculated by the following equation:

$$PIC = 1 - \sum_{i=1}^{n} p_i^2 - 2\left[\sum_{i=1}^{n-1} \sum_{j=i+1}^{n} p_i^2 p_j^2\right]$$

where p_i is the frequency of the i^{th} allele of the j^{th} locus, and n is the number of alleles (Botstein et al., 1980). PIC values were determined by the help of CERVUS 3.0 (Kalinowski et al., 2007).

2.3.1.2.3 Private Alleles

"Private allele" is a term used for the alleles found in only one of several populations (Slatkin, 1985). The presence of private alleles was linearly related to the mean number of migrants exchanged per generation between sub-populations (Nm) (Barton and Slatkin, 1986). In the present study, frequencies of private alleles for each locus of each population was determined by using GenAlEx version 6.501 (Genetic Analysis in Excel) software (Peakall and Smouse, 2012).

2.3.1.2.4 Heterozygosity

Hardy-Weinberg equilibrium (HWE) is a null model that estimates the expected heterozygosity in a locus and to measure the genetic variation within populations (Hardy, 1908; Weinberg, 1908). HWE predicts that allelic frequencies of the populations will remain the same from the generation to generations in a large and

randomly mating populations, provided that there is no evolutionary force (mutation, gene migration, selection or genetic drift) acting on the population. Expected Heterozygosity at the Hardy-Weinberg Equilibrium is calculated as:

$$H_e = 1 - \sum_{i=1}^{n} p_i^2$$

where p_i is the frequency of the i^{th} of n alleles.

Since the expected heterozygosity is not affected significantly by sample size (Gorman and Renzi, 1979), all populations in the present study were tested for HWE. Expected heterozygosities (He) for each locus of each population and deviations from HWE were calculated by using the software, Arlequin v.3.5.1.3. (Excoffier *et al*., 2006), Markov chain steps were set as 10000 and dememorization steps were set as 1000. For the significance tests Bonferroni correction was calculated as the threshold probability for the significance divided by the number of tests for each population.

2.3.1.3 Measures of Genetic Differentiation: F-statistics

F-statistics is the oldest and the most commonly used metric of the genetic differentiation which is defined by the Wright (1965) for loci with two alleles and extended to more alleles by Nei in 1977 (Hartl and Clark, 1997). In a structured population F-statistics measure the deviations from HWE in different levels: within subpopulations (F_{IS}), genetic divergence among subpopulations (F_{ST}), overall deviations from HWE in the entire base population and allele frequency divergence among subpopulations (F_{IT}) (Allendorf and Luikart, 2007).

In the present study, the F_{IS} and F_{ST} measures were used to observe the genetic differentiation within and among populations.

F_{IS} is used to estimate the deviations from HWE within subpopulations by the help of the following formula:

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

where H_0 is the average observed heterozygosity over all subpopulations, and H_S is the average expected heterozygosity over all subpopulations. If the F_{IS} exhibits a positive value, it indicates heterozygote deficiency, otherwise, it indicates the excess of the heterozygotes in the population.

F_{ST} is used to determine the genetic divergence among subpopulations by using the formula below:

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

where H_T is the expected heterozygosity for the entire base population and H_S is the average expected heterozygosity over all subpopulations. F_{st} values range between zero (where all subpopulations have equal allele frequencies) to one (where all the subpopulations are fixed for different alleles).

While inbreeding coefficients were being designed by Wright (1965), all estimators were set on the idea of infinite population size which means the equal sample size for each subpopulation at the same time. FSTAT V.2.9.3 (Goudet, 2001) was used to calculate F_{IS} values and pairwise F_{ST} values. In the tests, 1000 random permutations were done to test the significance of the results after Bonferroni correction was calculated.

2.3.1.4 Differentiation of the Populations Based on Individuals

2.3.1.4.1 Factorial Correspondence Analysis (FCA)

Factorial correspondence analysis (FCA) (Lebart, et al,. 1984) is an analysis performed on genotypes described by multi-allelic loci and it enables us to visualize on a three dimensional space, not only the genetic differentiation between populations but also the genetic differentiation between individuals within a population. For this purpose, GENETIX Software version 4.05 (Belkhir et al. 1996–2004; http://univ-montp2.fr/~genetix) was used by choosing the "populations + individuals" option.

2.3.1.4.2 Structure Analysis

STRUCTURE v2.3.4 (Pritchard et al., 2000) is a software which uses the Bayesian clustering approach to observe the genetic admixture of the populations based on the assumed number of ancestors generating the populations. For each assumed number of ancestors (K), genotypes of the individuals are evaluated. Irrespective of their populations/species, their genotypes are illustrated in colors determined by the assumed K. Then they are assigned to their populations. When the population is characterized by one color, it indicates the absence of admixture.

The two parameters were defined for the software model which used admixture option; burn-in length to minimize the effect of the starting configuration before collecting data, and to determine K value by using designed methods which are also counted as the most significant parameters of the analysis. Since the 10,000 – 100,000 burn-in length is more than adequate (Falush et al., 2003), burn-in length was set as 10,000. Additionally, K values were given from 1 to 10 and 10 iterations were performed for each K value.

In the present study, two common methods were performed to estimate the most likely K value: (1) Evanno et al.'s method (2005) is based on calculating second order rate of change of the likelihood function (Delta K= mean [|L"(K)|] /sd[L(K)]) and taking the K value as a true K where the result gave a clear peak on the graph and (2) Tapio et al.'s method (2010) gives the possible true K value where the similarities of the independent runs reaches the highest value. CLUMPP v1.1.2 (Jakobsson and Rosenberg, 2007) was used to detect the similarity coefficients and Distruct v1.1 (Rosenberg et al., 2004) was performed to obtain the illustrations for Tapio's method.

2.3.1.5 Estimating Effective Population Size (N_e)

Definition of the effective population size was made by the Wright (1938) as the number of breeding individuals in an idealized population that would show the same amount of dispersion of allele frequencies under random genetic drift or the same amount of inbreeding as the population under consideration. Since a population in the wild can barely provide the circumstances of an ideal population (e.g. equal number of sexes, random mating etc.), effective population size is almost always considerably less than the census population size (Frankel and Soulé, 1981). Effective Population Size (N_e) is estimated by the following equation:

$$N_e = \frac{4N_m N_f}{N_m + N_F}$$

where, N_M is the number of breeding males and N_F is the number of breeding females.

In the present study, NeEstimator V.2.01 (Do et al., 2014) was used to estimate the effective population size of a population. The software can handle four different methods to estimate the effective population size: (1) Linkage disequilibrium method (Hill, 1981), (2) Heterozygosity-excess method (Pudovkin et al., 1996), (3) Temporal

method (Polak, 1983) and (4) Molecular co-ancestry method (Nomura, 2008). Since the type of data was only suitable for Nomura's molecular co-ancestry method (2008), the effective population size of breeders (N_{eb}) was calculated with this method in the present study.

2.3.2 Statistical Analyses for the mtDNA *cyt-b* Region

2.3.2.1 Constructing a Phylogenetic Tree

Phylogenetic trees are the diagrams which, presumably, represent the evolutionary relationships between species. In the present study, the phylogenetic tree was constructed by neighbor joining method after detecting the nucleotide substitution model.

2.3.2.1.1 Nucleotide Substitution Model

Before constructing the phylogenetic tree between the samples used in this study and the other related samples worked on similar studies, the evolutionary model has to be examined to detect the best-fitting model to our data set. The basic purpose of this process is to find proportional number of mutations over the nucleotides.

In this study, substitution model for mtDNA *cyt-b* region was determined by using the software MEGA version 6.06 (Tamura et al., 2013). The method, "RelTime" which was implemented by the software, is the fastest method to analyze large numbers of sequences comprising contemporary data sets (Tamura et al., 2012). Moreover, the software tests the models by estimating the likelihoods according to number of criteria with/without invariable site (+I) and gamma distribution of substitution rate (+G).

2.3.2.1.2 Neighbor Joining (NJ) Tree

Neighbor joining method (Saitou and Nei, 1987) aims to find topology by using minimum evolutionary method (ME)(Rzhetsky and Nei, 1992) with the evolutionary distance correlated branch lengths at the final tree. Since NJ method is suitable for data harboring any kind of evolutionary distance model and the algorithm tests the many topologies faster than other methods, this method was performed in the present study.

NJ tree was constructed with 1000 bootstrap value by the same software used for detecting nucleotide substitution model that is MEGA version 6.06 (Tamura et al., 2013).

2.3.2.2 Restriction Fragment Length Polymorphism (RFLP)

The Restriction Fragment Length Polymorphism (RFLP) analysis is a technique which investigates the variations between the homologous DNA sequences. In this method, restriction endonucleases are used to cut the DNA into pieces. Each restriction endonuclease recognizes a certain sequence on the DNA and cuts the DNA into pieces at a site specific to that endonuclease. Then, the resulting restriction fragments are separated according to their lengths by gel electrophoresis.

In the present study, two different RFLP enzymes (*HaeIII*, *HinfI*; Wacher et al., 2010), were used to cut the amplified partial mtDNA *cyt-b* gene of the samples (see section 2.2.5.2. for the PCR amplification of this region) in order to separate the two gazelle species (Wacher et al., 2010). The sequences recognized by these two enzymes and their restriction sites were indicated in Figure 2.1.

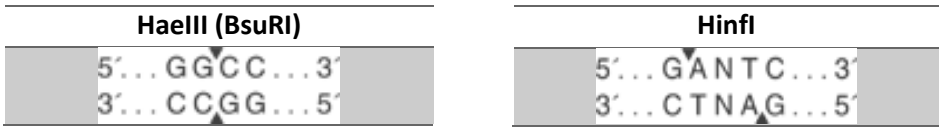

Figure 2.1 The restrictions site sequence of the restriction enzymes, HaeIII (BsuRI) and HinfI. The black arrows indicate the restriction sites.

First the partial mtDNA *cyt-b* region of the samples were PCR amplified as explained in the section "2.2.5.2" above in this chapter. Then, the PCR products were checked by electrophoresis (1% agarose gel, 1X TAE). Afterwards, 4 µl from each of the amplification products were transferred to sterile 0.5 mL microfuge tubes. Finally, 5 µl of a mixture containing dH2O, enzyme buffer and the enzyme is added to these tubes. The mixture for one reaction is composed of 4.7 µl dH2O, 1 µl restriction enzyme buffer, and 0,3 µl restriction enzyme containing 3 units of enzyme. A master mix of this mixture is prepared when more than one PCR template is analysed by RFLP.

The prepared tubes were incubated at 37°C for at least 6 hours or overnight. Then, the restriction fragments were checked by agarose gel electrophoresis (2,5% agarose gel containing EtBr (0.5ug/ml), 1X TAE). All the reaction mixture for each sample was loaded in the wells contained on the agarose gels. After electrophoresis, the gels were viewed under the UV light using the SYNGENE Ingenius LHR visualizing device. Invitrogen 50 bp DNA ladder was used as DNA ladder and as a reference for sizing of the fragments.

CHAPTER 3

RESULTS

3.1 Results of the Laboratory Experiments

3.1.1 DNA Extraction

DNA was isolated by performing standard: phenol-chloroform isoamyl alcohol method (25:24:1) (Sambrook et al., 1989). The quality and quantity of the isolated DNAs were checked on 1% agarose gel prepared with 1X TAE (Tris-acetate-EDTA) buffer and Et-Br (0.5 ug/ml). The gels were viewed under the UV light using the SYNGENE Ingenius LHR visualizing device. Invitrogen 50 bp DNA ladder was used as DNA ladder and as a reference for sample DNAs' concentration estimation. The quality and quantity of the DNA samples were also checked by NanoDrop (Thermo Scientific) device. Then dilutions (~50 ng/ul) for each DNA sample were prepared. In Figure 3.1, DNA bands can be observed before preparing the dilutions. As it can be seen some of the bands represent highly degregaded DNAs as "Gm10" and "Gm12" in Figure 3.1. DNAs of these individuals were isolated once more.

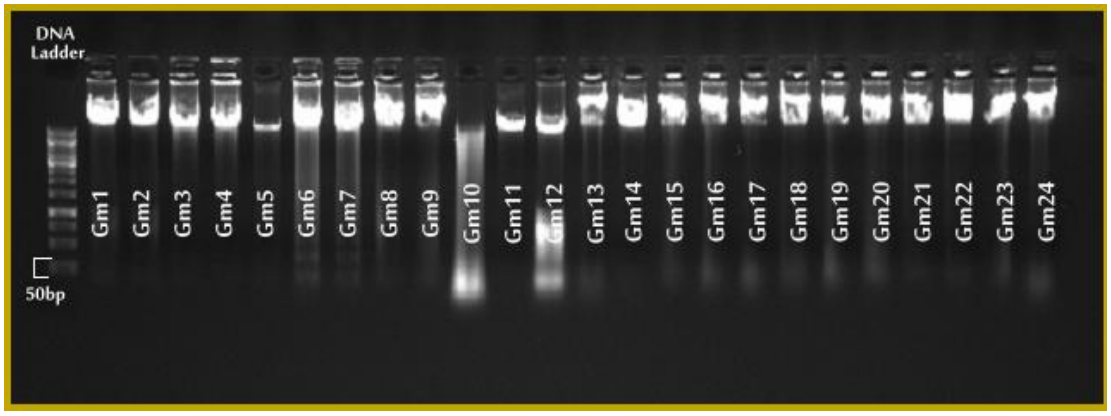

Figure 3.1 After DNA isolation, DNA bands were checked on 1% agarose gel under the UV light using the SYNGENE Ingenius LHR visualizing device. The first 24 individuals of *Gazella marica* (Gm) were represented in this figure as an example. DNA ladder was Invitrogen 50 bp DNA ladder.

An example of sample DNAs checked on agarose gel (1X TAE) after preparing the dilutions at about 50 ng/ul concentrations is given in Figure 3.2.

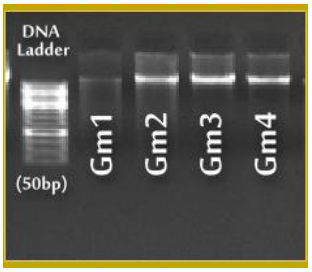

Figure 3.2 A sample of diluted DNAs checked on agarose gel (1X TAE). Invitrogen 50 bp DNA ladder was used as reference.

3.1.2 Microsatellites

3.1.2.1 Amplifying the Microsatellite Loci by PCR

In total, 17 loci (RT1, MM12, OARFCB304, ILSTS30, ETH10, BM848, BMC1009, INRABERN172, TGLA122, IDVGA29, ILSTS005, BM4505, ETH152, INRA40, BM757) were amplified by PCR in this study (Table 2.1). To save time and consumables 5 multiplex sets were arranged by grouping the primers according to their different fluorescent labels and allelic ranges. PCR were performed as described in Section 2.2.5.1 in Chapter 2 under the title of "PCR Conditions for Microsatellite Loci".

After PCR amplifications, the DNA band were first checked on the 1.5% agarose gel (1X TAE) under the UV light using the SYNGENE Ingenius LHR visualizing device. Also, Invitrogen 50 bp DNA ladder was used to determine the size of fragments and to distinguish the non-specific fragments in the case of contamination.

3.1.2.2 Collecting Microsatellite Data

Fluorescently labeled PCR products were analyzed by using a capillary electrophoresis device, Beckman Coulter CEQ8800 Genetic Analysis System, in order to size the fragments. Beckman fragment analysis tool was used to process the raw data and to determine the allele sizes. Beckman Coulter's DNA Size Standard Kit – 400 was used as an internal size standard within every sample of PCR product loaded on the device. Both fluorescent labels and allelic ranges helped to differentiate and identify the products of each microsatellite loci, employed in the study, on the electropherograms obtained by Beckman Coulter CEQ8800 Genetic Analysis System. The information on the multiplex sets, the amount of each primer put in the reaction and the contents of the PCR mixtures were presented in Table 2.3.

The Figure 3.3, below, is a representative graphic, or electropherogram, showing the observed alleles of three different microsatellite loci analyzed in a multiplex set.

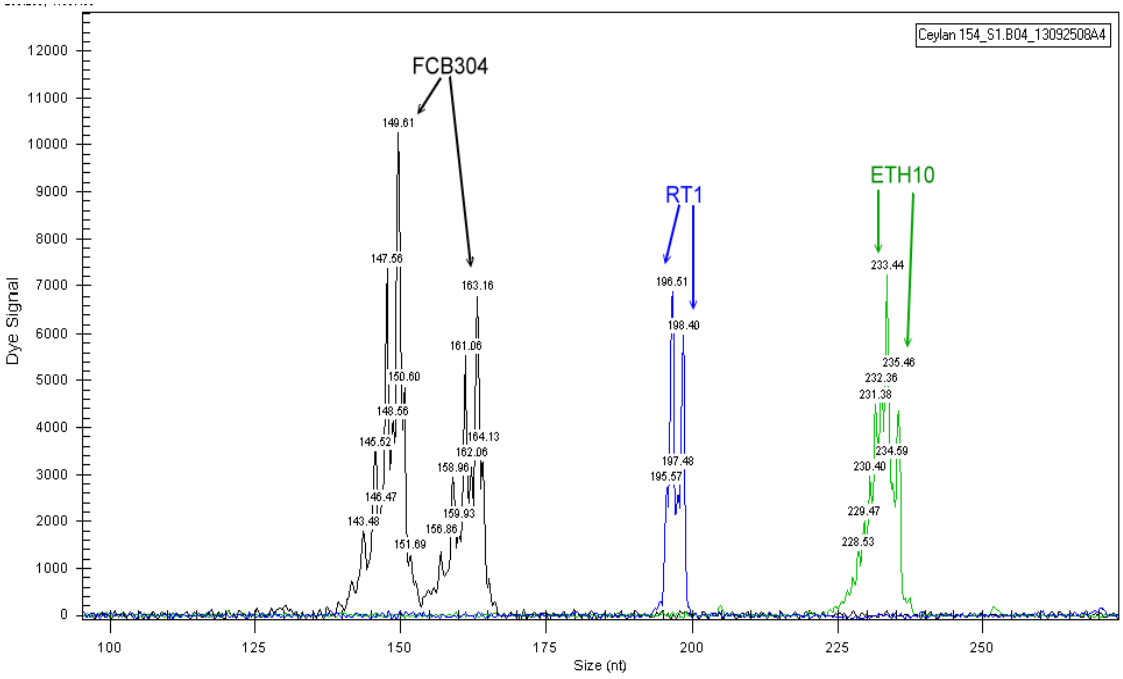

Figure 3.3 An electropherogram of the microsatellite loci included in the multiplex set 1. Three loci, OARFCB304, RT1 and ETH10 each labelled with a different fluorescent dye. Alleles of each loci were indicated on the graph with arrows. The graphic was obtained by Beckman Coulter CEQ8800 Genetic Analysis System.

With respect to the three loci of multiplex set-1 (Figure 3.3), the genotype of the individual was detected as 149-163 (OARFCB304), 196-198 (RT1) and 233-235 (ETH10). The genotypes of the individuals were given in the Appendix A.

One locus, IDVGA29, is excluded from the analysis as will be explained in the section "3.2.1.1 Reliability of Microsatellite Data".

3.1.3 Y Chromosome Analysis Based on Microsatellite Loci

The microsatellite primers for chromosome Y (UMN0103, INRA126) were used to amplify the two fragments of Y chromosomes. These loci did not amplify any fragment in female gazelles. A sample from the gel views of amplified Y chromosome loci are displayed in Figure 3.4, below.

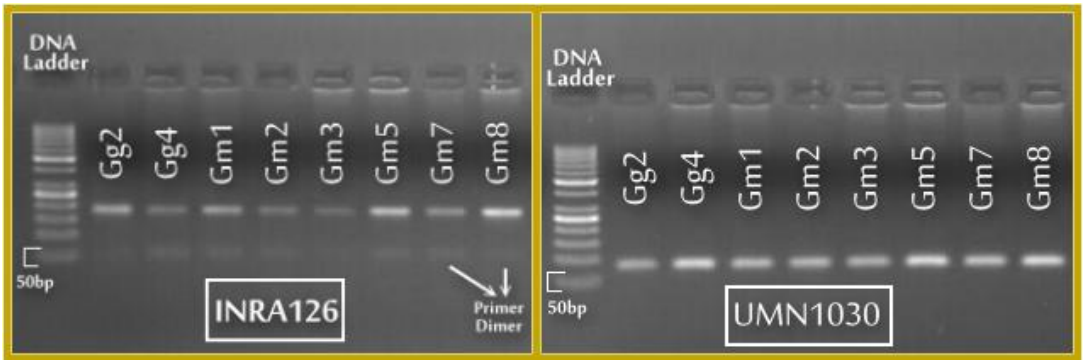

Figure 3.4 Gel view of amplified Y chromosome loci for the two sets of primers (INRA126, UMN0103), for *Gazella gazella* (Gg2 and Gg4) and *Gazella marica* individuals (Gm1 to Gm8). Invitrogen 50 bp DNA ladder was used as reference for the size of the PCR amplified DNA bands.

After the purification procedure, amplified Y chromosome DNA bands were sent to RefGen Company (http://www.refgen.com/) for sequencing and the sequences were analyzed using ABI PRISM® 3100 Genetic Analyzer. However, only INRA126 alleles could be sequenced properly. The resulting sequences distinguished males of *Gazella gazella* species (Hatay population) from males of *Gazella marica* species with a single nucleotide difference at the 216th base (Figure 3.5).

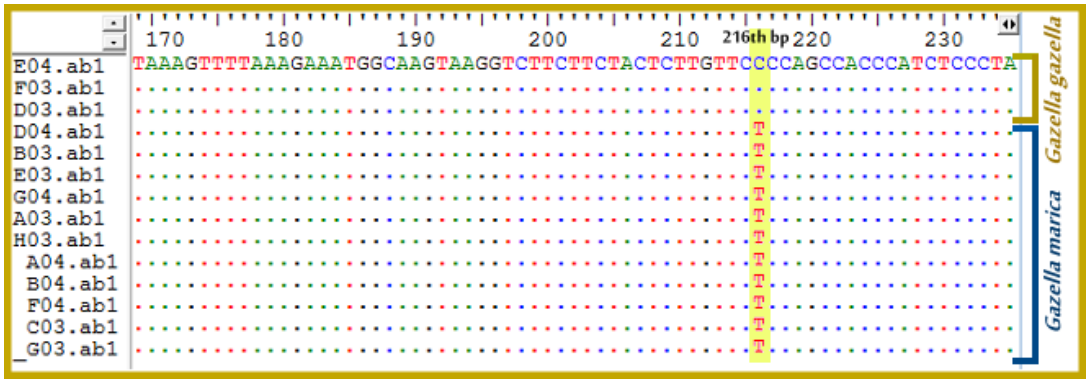

Figure 3.5 The alignment view of Y chromosome INRA126 locus sequences obtained in the study. Male individuals of *Gazella marica* and *Gazella gazella* showed a single base difference at the 216th bp as highlighted in yellow.

3.1.4 Cytochrome *b* gene of mtDNA

3.1.4.1 Amplifying the Fragment of Cytochrome *b* (*cyt-b*) Region and Sequencing

mtDNA *cyt-b* fragments of 77 individuals were successfully amplified from the extracted DNAs. A gel image of amplified mtDNA *cyt-b* fragments (381 bp long) including the negative control is displayed in Figure 3.6.

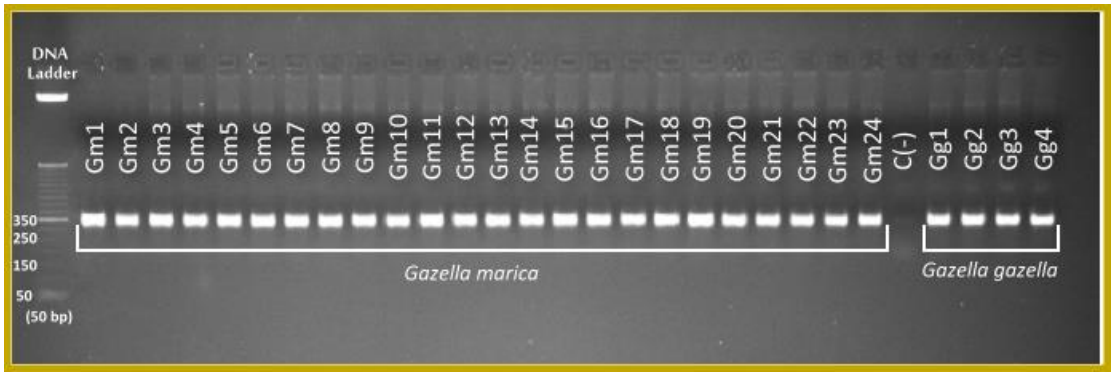

Figure 3.6 Amplified mtDNA *cyt-b* fragment of the samples were run on the 1% agarose gel containing EtBr and viewed under UV light by using the the SYNGENE Ingenius LHR visualizing device. The image presents the PCR bands from 24 *Gazella marica* individuals, all four *Gazella gazella* individuals and the negative control reaction (C(-)). Invitrogen 50 bp DNA ladder was as the reference for sizing the DNA bands.

After checking the PCR amplification results on agarose gel, randomly chosen 15 individuals from Kızılkuyu (Şanlıurfa) and 8 individuals from Erikçe (Gaziantep) were sequenced. Sequences were edited and aligned using the softwares, Chromas Pro version 2.4.3 (Technelysium Pty Ltd, Tewantin QLD) and BioEdit version 7.2.5 (Hall, 1997-2013), respectively. As a result, 381 bp long sequences were obtained. In Figure 3.7, a part of the aligned sequences can be observed.

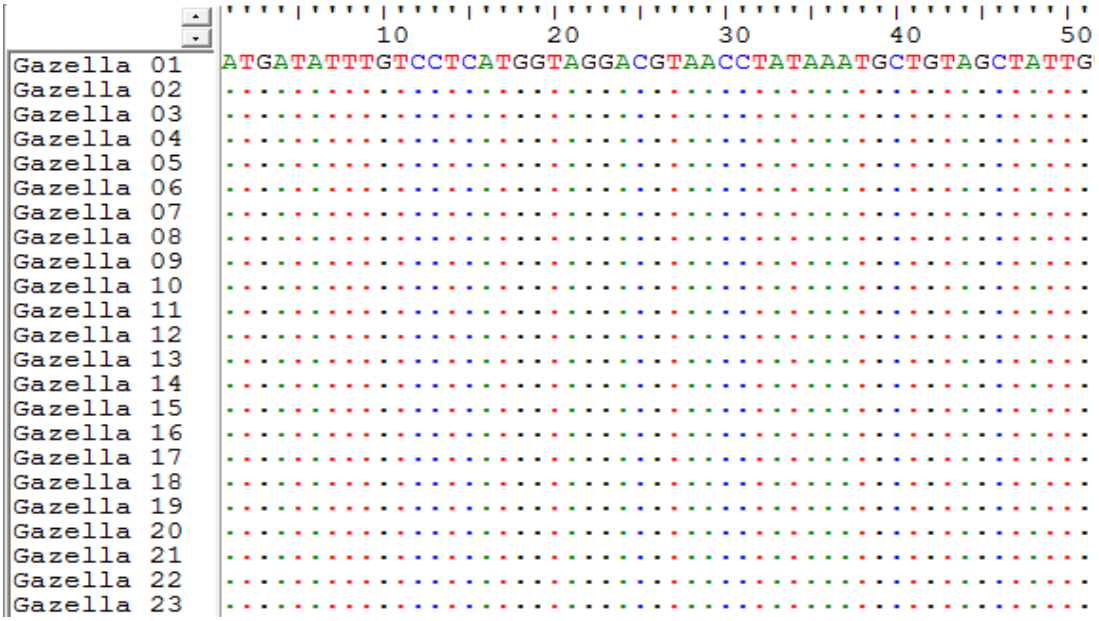

Figure 3.7 View of 51 bp long part of the aligned sequences. Snapshot was taken from the BioEdit version 7.2.5 (Hall, 1997-2013). Sequences from "Gazella 01" to "Gazella 15" belong to the gazelles from Kızılkuyu (Şanlıurfa), the rest of the sequences belong to the gazelles from the Erikçe (Gaziantep).

For this part of the sequences there was no polymorphism neither within nor between the *Gazella marica* populations. In fact, there were no polymorphism found within this 381 bp region neither within nor between the populations of the *Gazella marica* populations.

Additionally, there was no polymorphism detected between the sequences of 4 *Gazella gazella* individuals. However, these two species (*Gazella marica* and *Gazella gazella*) were found to be different at 23 site out of 381 (23/381).

Identity of the individuals for which the sequences were obtained, were given in Appendix B.

3.2 Statistical Analysis

3.2.1 Microsatellites

In the present study, 17 microsatellite loci were examined over three populations: *Gazella marica*, Kızılkuyu population from Şanlıurfa (n=48), *Gazella marica*, Erikçe population from Gaziantep (n=25) and *Gazella gazella* population from Hatay (n=4).

3.2.1.1 Preliminary Evaluation of Microsatellite Data

- Null Alleles

Before analyzing the microsatellite data, first MICRO-CHECKER 2.2.3 software (Oosterhout *et al.*, 2004) was used to detect the presence null alleles and thereby to prevent the bias that can be caused from the excess of false homozygotes in microsatellite data.

Null allele frequencies of 3 populations detected for the 17 microsatellite loci used in the study were given in Table 3.1. Also, in Table 3.1, those frequencies bigger than 0.2 were indicated with asterik. The data of a locus with a frequency value >0.2 was considered as the indication of data with low reliability (Chapuis and Estoup, 2007). The locus IDVGA29 gave the signal for the presence of null allele in one of the populations (Kızılkuyu). Therefore, this locus was not used in further analysis.

Table 3.1 Null allele frequencies of 3 populations for 17 microsatellite loci calculated by the software, MICRO-CHECKER 2.2.3 software (Oosterhout *et al.,* 2004). Cells with an asteriks indicate the presence of null alleles in the given population (Kızılkuyu).

Locus	Kızılkuyu (n:48)	Erikçe (n:25)	Hatay (n:4)
RT1	0,0056	-0,0731	0
ETH10	0,0123	-0,0505	-0,1948
OARFCB304	0,0598	0,0016	0
MM12	0	0,1211	0
BM848	0,0168	0,0187	0,0731
BMC1009	-0,0344	-0,0857	-0,5
INRA40	0,0293	0,0471	0
IDVGA29	0,3265*	0	0
BM4505	0,0611	0,0818	0
ETH152	0	0.00100	0,4142
INRABERN172	-0,0777	-0,1066	-0,2929
TGLA122	-0,0362	-0,1986	0
ILSTS005	0,0573	-0,0353	0
BM757	-0,0502	0,0556	0
BM143	0	0	-1
CSSM39	0	-0,0619	0
CSSM43	0,0234	0,0069	0

- Linkage Disequilibrium (LD)

After evaluating Bonferroni Correction ([0.05/120] = 0.0004) for the pairwise comparison of 16 loci, there is no significant value obtained which increases the confidence of the reliability on the data.

In the table Appendix D, p values of the genotypic disequilibrium were given for the pairwise comparison of 16 loci over three populations.

3.2.1.2 Analyses of Genetic Diversity within and between the Populations

In this part of the study, the genetic diversity that is present among and within the populations are examined.

3.2.1.2.1 Allelic Richness

There are 115 alleles were counted for the 16 employed microsatellite loci over 2 populations of *Gazella marica* (Kızılkuyu, n=48; Erikçe, n=25) as presented in Table 3.2. The loci having the maximum number of alleles were found as OARFCB304 and INRA40 with 10 and 12 alleles, respectively. The two loci ETH152 and BM143 were monomorphic in both of the populations in the present study.

Table 3.2 The allelic ranges, number of observed alleles for each locus in each population which was calculated by FSTAT V.2.9.3 package program (Goudet, 2001).

Locus	Allelic Ranges	Kızılkuyu (n:48)	Erikçe (n:25)	Total
RT1	196-200	3	3	3
ETH10	213-245	10	8	10
OARFCB304	144-174	10	9	12
MM12	79-81	2	2	2
BM848	207-229	5	5	6
BMC1009	274-300	8	5	8
INRA40	201-297	12	7	12
BM4505	196-254	10	5	10
ETH152	192-210	1	1	1
INRABERN172	229-251	8	6	9
TGLA122	122-126	3	3	3
ILSTS005	179-195	5	3	6
BM757	159-201	4	2	4
BM143	84-114	1	1	1
CSSM39	177-183	1	2	2
CSSM43	246-264	9	7	9

After analyzing the observed alleles, the allelic richness of the populations was estimated. Since the size of the *Gazella gazella* (Hatay) population was low (n=4) allelic richness for this population was not estimated.

The results for the allelic richness values found per locus and population (Kızılkuyu, n=48; Erikçe, n=25) were presented in Table 3.3. The maximum and minimum mean allelic richnesses of loci were 9.338 (OARFCB304) and 1.000 (ETH152, BM143) for Kızılkuyu and Erikçe populations, based on minimum sample size of 25.

As can be seen in Table 3.3 the allelic richness estimates of the two populations of *Gazella marica* species are quite similar. Yet, in 13 (out of 16) loci allelic richnesses are either equal or slightly more in Kızılkuyu population compared to Erikçe population. In fact, Kızılkuyu and Erikçe populations were observed to be significantly different ($p<0.05$) with respect to their allelic richnesses by Wilcoxon-Signed rank test (Sokal and Rohlf, 1995).

Table 3.3 Calculated allelic richnesses per locus and per population based on minimum sample size of 25 individuals.

Locus	Kızılkuyu (n:48)	Erikçe (n:25)	Mean/Locus
RT1	2.894	3.000	2.980
ETH10	8.880	8.000	9.115
OARFCB304	8.565	9.000	9.338
MM12	2.000	2.000	2.000
BM848	4.515	5.000	5.280
BMC1009	7.213	5.000	6.647
INRA40	10.054	7.000	9.152
BM4505	8.014	5.000	7.235
ETH152	1.000	1.000	1.000
INRABERN172	6.686	6.000	6.798
TGLA122	3.000	3.000	3.000
ILSTS005	4.188	3.000	4.599
BM757	3.667	2.000	3.288
BM143	1.000	1.000	1.000
CSSM39	1.000	2.000	1.719
CSSM43	8.177	7.000	8.069

3.2.1.2.2 Polymorphic Information Content (PIC)

The PIC values for the microsatellite loci analyzed in the study were calculated by using software CERVUS 3.0 (Kalinowski et al., 2007). The least informative loci were determined as ETH152 and BM143 (0.000), whereas the most informative locus was determined as ETH10 (0.801) as given in Table 3.4 below. Due to its low sample size, *Gazella gazella* population (Hatay, n=4) was excluded from this part of the analysis, too.

Table 3.4 Polymorphism information content table of the 16 loci based on the allelic frequencies of the 73 individuals analyzed. Informative loci were indicated with asterisk (PIC > 0.7).

Locus	Kızılkuyu (n:48)	Erikçe (n:25)	Overall PIC
RT1	0.408	0.493	0.451
ETH10	0.817	0.784	0.801*
OARFCB304	0.810	0.739	0.775*
MM12	0.305	0.341	0.323
BM848	0.580	0.620	0.600
BMC1009	0.765	0.719	0.742*
INRA40	0.823	0.766	0.795*
BM4505	0.720	0.519	0.620
ETH152	0.000	0.000	0.000
INRABERN172	0.665	0.769	0.717*
TGLA122	0.569	0.410	0.490
ILSTS005	0.436	0.521	0.479
BM757	0.445	0.373	0.409
BM143	0.000	0.000	0.000
CSSM39	0.000	0.106	0.053
CSSM43	0.814	0.757	0.786*

3.2.1.2.3 Private Alleles

In the small sized populations, if the gene flow between the populations is low, the number of private alleles tends to increase through mainly the drift process on microsatellites, since presumably, they represent neutral loci. The frequencies of the private alleles observed in the study were given in Table 3.5 below, indicating the name of the loci and the population it belongs to. There seemed to be 28 private alleles in Kızılkuyu population (*Gazella marica*, n=48) and 7 in Erikçe population (*Gazella marica*, n=25). From the table it can be seen that, the most of these allele frequencies are quite low. Arbitrarily, 0.05 was considered as threshold value and those with the frequencies above 0.05 were considered as alleles with high frequencies. There were 3 such private alleles in each of the populations (Kızılkuyu and Erikçe) as indicated with asteriks in Table 3.5. Hatay Population (*Gazella gazella*, n=4) was excluded from the analysis since there is no other population of *Gazella gazella* to compare with.

Table 3.5 Frequencies of the private alleles observed as calculated by GenAlEx version 6.501 (Genetic Analysis in Excel) software (Peakall and Smouse, 2012). The name of the loci and the population in which they are observed are indicated in the table. The frequencies above 0.05 were indicated with asterisk.

Locus	Allele	Frequency	Population
ETH10	225	0,010	Kızılkuyu
	233	0,073*	
OARFCB304	160	0,021	Kızılkuyu
	168	0,052	
	170	0,010	Erikçe
	144	0,020	
	174	0,040	
INRA40	269	0,010	Kızılkuyu
	279	0,010	
	281	0,021	
	283	0,010	

Table 3.5 (continued)

	287	0,021	
BM4505	196	0,010	Kızılkuyu
	234	0,063*	
	244	0,010	
	254	0,010	
	256	0,010	
CSSM39	181	0,060*	Erikçe
INRABERN172	237	0,021	Kızılkuyu
	249	0,010	
	251	0,010	
	231	0,040	Erikçe
ILSTS005	177	0,021	Kızılkuyu
	183	0,010	
	185	0,031	
	195	0,160*	Erikçe
BM757	163	0,021	Kızılkuyu
	201	0,031	
CSSM43	244	0,010	Kızılkuyu
	256	0,063*	
BM848	217	0,010	Kızılkuyu
	209	0,160*	Erikçe
BMC1009	284	0,021	Kızılkuyu
	290	0,011	
	296	0,032	

3.2.1.2.4 Heterozygosity

Expected heterozygosities (He) for each locus and population were calculated by using the Software, Arlequin v.3.5.1.3. Moreover, the probabilities (p values) for each of the He estimate were calculated and the significances were checked by applying Bonferroni correction (P= [0.05/13]=0.003 for Kızılkuyu, [0.05/14]=0.004 for the Erikçe and [0.05/7]=0.007 for Hatay). Consequently, there was no locus in any of the populations which exhibited a deviation from Hardy-Weinberg equilibrium.

The average number of expected heterozygosity per locus per population was calculated as 0.69 (Kızılkuyu), 0.63 (Erikçe) and 0.602 (Hatay). The Expected Heterozygosity estimations (He) with p-values for each locus in each population and the average estimates per locus per population were given in Table 3.6, below.

Table 3.6 The expected heterozygosity (He) estimates and their p-values.

	Kızılkuyu (n:48)	**Erikçe (n:25)**	**Hatay (n:4)**
Locus	**He**	**He**	**He**
RT1	0.51557(0.4444)	0.58122(0.2675)	Monomorphic
ETH10	0.84561(0.4440)	0.82449(0.9026)	0.85714(0.6638)
OARFCB304	0.84013(0.0319)	0.78612(0.2993)	Monomorphic
MM12	0.37895(1.0000)	0.44408(0.1899)	Monomorphic
BM848	0.65351(0.6866)	0.69224(0.2352)	0.67857(1.0000)
BMC1009	0.80302(0.0107)	0.77551(0.5126)	0.53571(1.0000)
INRA40	0.84978(0.1188)	0.81224(0.5280)	Monomorphic
BM4505	0.76272(0.0573)	0.57306(0.7225)	Monomorphic
ETH152	Monomorphic	Monomorphic	0.57143(0.0863)
INRABERN172	0.71908(0.0342)	0.81551(0.6756)	0.42857(1.0000)
TGLA122	0.65088(0.5562)	0.49224(0.2204)	Monomorphic
ILSTS005	0.52193(0.0082)	0.60490(1.0000)	0.57143(1.0000)
BM757	0.55066(0.0134)	0.50694(0.6878)	Monomorphic
BM143	Monomorphic	Monomorphic	0.57143(0.3139)
CSSM39	Monomorphic	0.11510(1.0000)	Monomorphic
CSSM43	0.84430(0.3523)	0.80163(0.0806)	Monomorphic
Population Average	**0.69**	**0.6304**	**0.602**

3.2.1.3 F-Statistics

3.2.1.3.1 Estimating F_{IS} Values

F_{IS} is a measure to determine the deviation from Hardy-Weinberg Equilibrium within a population. As given in Table 3.7, F_{IS} values for 3 populations based on 16 loci were calculated by using FSTAT V.2.9.3 (Goudet, 2001). After applying the Bonferroni correction as [0.05/34]=0.0015, there was no significant F_{IS} value observed which means that each of the populations as a whole are in Hardy-Weinberg equilibrium. Estimated P values after Bonferroni correction as [0.05/34]=0.0015 were given in Appendix C.

Table 3.7 F_{IS} values estimated among 16 loci for each of the 3 populations.

Locus	Kızılkuyu (n:48)	Erikçe (n:25)	Hatay (n:4)
RT1	0.031	-0.103	Monomorphic
ETH10	0.040	-0.069	-0.200
OARFCB304	0.133	0.034	Monomorphic
MM12	0.011	0.284	Monomorphic
BM848	0.044	0.077	0.294
BMC1009	-0.061	-0.138	-0.500
INRA40	0.069	0.116	Monomorphic
BM4505	0.127	-0.048	Monomorphic
ETH152	Monomorphic	Monomorphic	1.000
INRABERN172	-0.131	-0.182	-0.200
TGLA122	-0.047	-0.308	Monomorphic
ILSTS005	0.123	-0.059	0.143
BM757	-0.060	0.134	Monomorphic
BM143	Monomorphic	Monomorphic	-1.000
CSSM39	Monomorphic	-0.043	Monomorphic
CSSM43	0.063	0.053	Monomorphic
Average	**0.029**	**-0.020**	**-0.010**

In Kızılkuyu population, 4 loci out of 13 loci analyzed revealed some heterozygosity excess (but not significant) whereas it was 8 out of 14 loci in Erikçe population due to negative Fıs values (Table 3.7).

3.2.1.3.2 Estimating Pairwise F_{ST} Values

F_{ST} is a measure that might be used to determine the genetic divergence among subpopulations. The pairwise F_{ST} values were estimated by using FSTAT V.2.9.3 package program (Goudet, 2001). To test their significances, p values were determined, also. The pairwise F_{ST} values were given in the lower triangle in Table 3.8. Level of significances (p values) were indicated in the upper triangle. Although the pairwise F_{ST} value for the populations of Kızılkuyu and Erikçe was estimated as significant, they showed a little genetic differentiation according to Wrigth's interpretation (1978) (F_{ST}=0.0444, $p< 0.05$), whereas the pairwise F_{ST} values between Hatay and each of the other two populations showed a significant genetic differentiation (Table 3.8).

Table 3.8 Pairwise F_{ST} estimates with p values based on 3000 permutations and Bonferroni corrections.

Pairwise F_{ST}	**Kızılkuyu (n:48)**	**Erikçe (n:25)**	**Hatay (n:4)**
Kızılkuyu (n:48)		**	**
Erikçe (n:25)	0.0444		**
Hatay (n:4)	0.4378	0.4588	

(p<0.001**)

3.2.1.4 Differentiation of the Populations Based on Individuals

3.2.1.4.1 Factorial Correspondence Analysis (FCA)

In this study, factorial correspondence analysis (FCA) (Lebart, et al,. 1984) was performed to visualize genetic differentiation between individuals within a population in a multidimensional platform. The software, GENETIX version 4.05 (Belkhir et al. 1996–2004; http://univ- montp2.fr/~genetix) was used to obtain the 3-dimensional FCA graphic.

The analysis was performed over 16 loci and 3 populations. Two axes of the graph represents 85.17% and 14.83% and thus 100 percent of the total genetic variation. Each of the individuals were placed in a 3-D graphic based on their allelic compositions as shown in Figure 3.8. Since Hatay population (*Gazella gazella*, n=4) belongs to a different gazelle species, it is observed as the most distinct population in Figure 3.8.

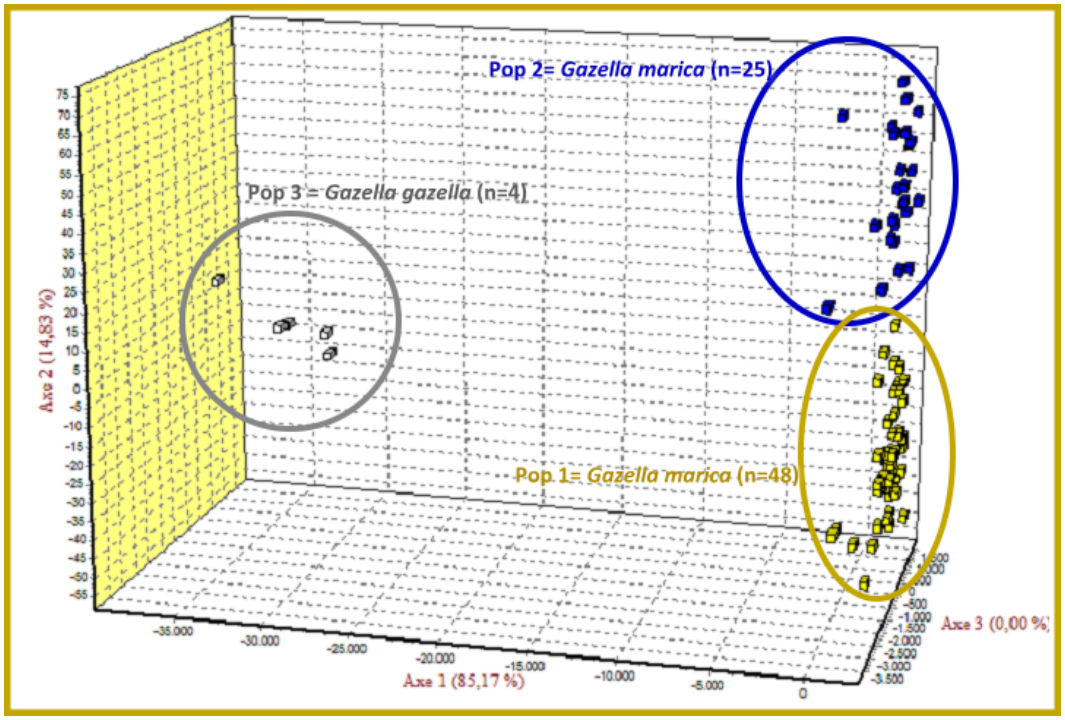

Figure 3.8 FCA analysis indicating the genetic variation between the three populations. (Pop 1=*Gazella marica* population from Kızılkuyu, Şanlıurfa, n=48; Pop 2=*Gazella marica* population from Erikçe, Gaziantep, n=25 and Pop 3=*Gazella gazella* species from Hatay, n=4).

Individuals of the two *Gazella marica* populations shows some differentiation from each other in one dimension with almost no overlapping as seen in the FCA graphic (Figure 3.8).

3.2.1.4.2 Structure Analysis

Firstly, Evanno et al.'s method (2005) was employed to obtain the most probable number for K. It was determined by finding the maximum second order rate of change of the likelihood function (Delta K= mean [|L"(K)|] /sd[L(K)]). In Figure 3.9, Delta K variation for different K values according to Evanno's method can be observed. Thus, K=3 was suggested as the number of ancestral populations for the given data (obtained by the microsatellites) set by the Evanno et al.'s method (2005).

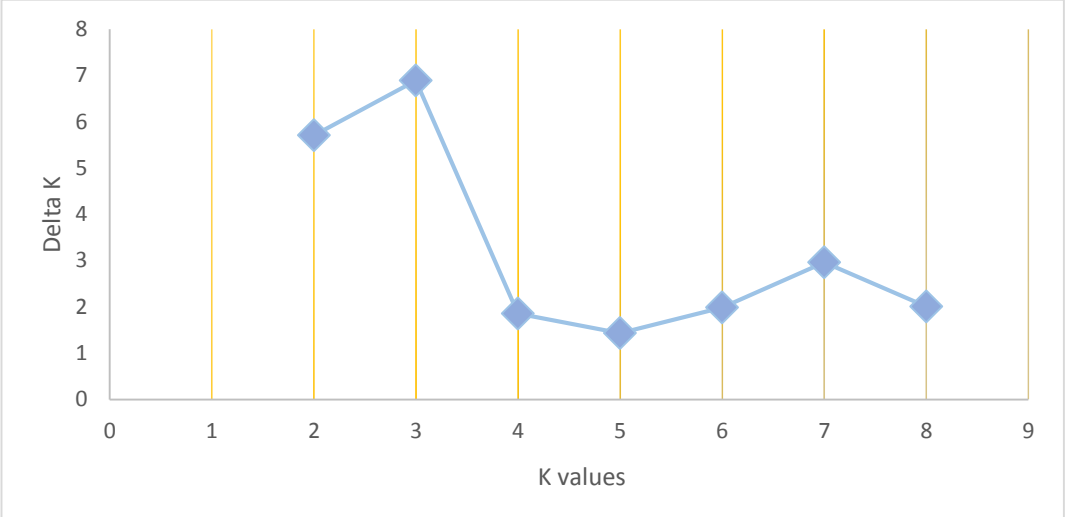

Figure 3.9 Estimation of the most probable K value with the help of Evanno's method (2005). The estimated delta K was maximum for K=3. Therefore K=3 was chosen for the analysis.

Another method used to determine the most probable value of K is Tapio's similarity test done by calculating the maximum value of similarity coefficients, H', between the independent runs of the analysis (Tapio, et al. 2010). The algorithm was run by the help of CLUMPP software (Jakobsson et al., 2007) and the most probable K values were observed as: K=2 and K=4, where the two highest peaks for H' were observed (Figure 3.10).

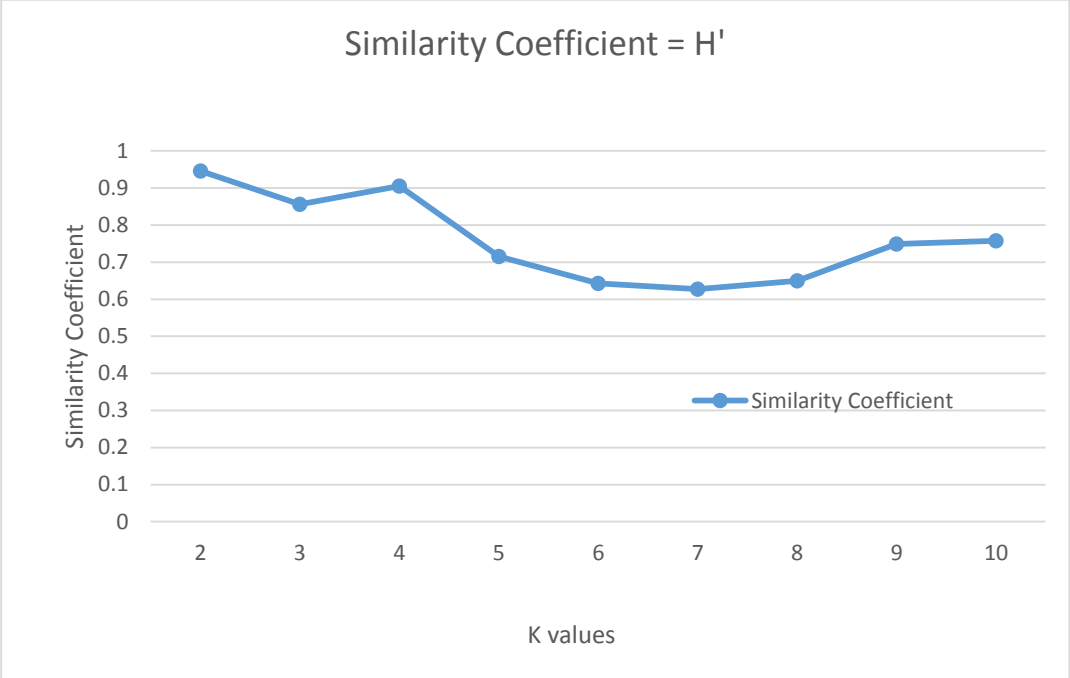

Figure 3.10 Similarity coefficients (H') for each of the different K values by using the Tapio's method.

After estimating the probable K values (K=2, K=3 and K=4), the microsatellite data were analyzed using Structure software and by setting K values from 2 to 4 (Figure 3.11, below).

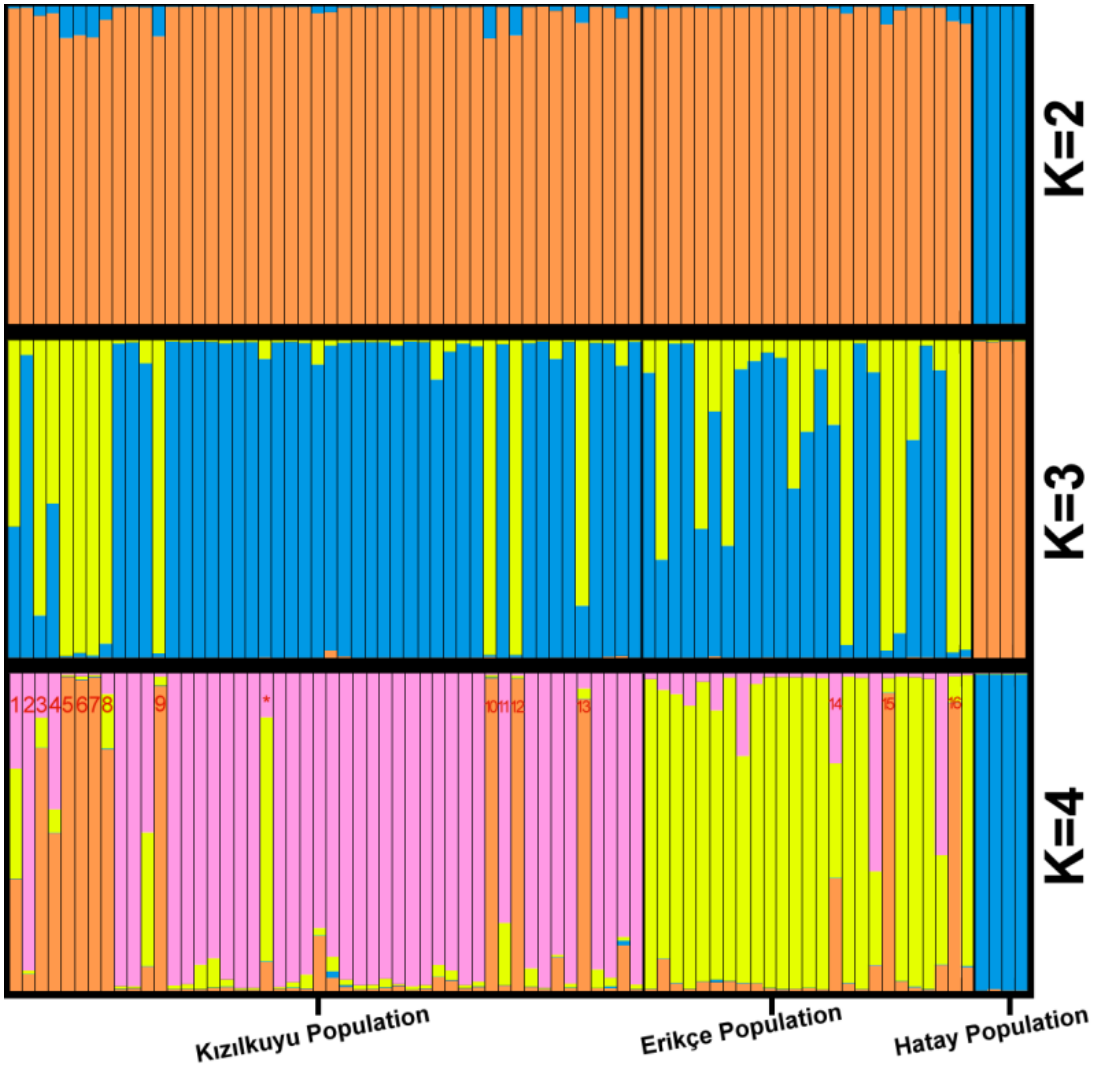

Figure 3.11 Bar plots of genetic admixture analysis of the three populations obtained by using the software STRUCTURE v2.3.4 (Pritchard et al., 2000). Each individual is represented by a bar plot. For K=4 analysis, the numbered individuals from Kızılkuyu population are hunted individuals from the wild Kızılkuyu population based on the information provided by the Ministry of Forestry and Water Affairs. The individuals indicated with an asterisk from Kızılkuyu population showed the characteristics of Erikçe State Farm. Finally, the numbered individuals from Erikçe population were the individuals closer to the wild Kızılkuyu individuals.

It is clearly seen from the Structure analysis (K=2-4) that *Gazella gazella* individuals are very different from the individuals of the *Gazella marica* populations. K=3 shows that there are heterogeneity within the *Gazella marica* populations between the individuals, whereas K=4 also differentiates between the two *Gazella marica* populations. Although within population heterogeneity is present both in Erikçe and Kızılkuyu, it seems that they are differing from each other. The Kızılkuyu gene pool is mainly represented by pink color but Erikçe gene pool is represented mainly by yellow color in the K=4 analysis' result plot. The fourth color (indicated by the light brown) was present in both of the *Gazella marica* populations, but more heavily in Kızılkuyu population. When the samples which were taken from the hunted animals (wild) around Kızılkuyu state farm were considered it was seen the brownish color is highly associated with them. There were 2 individuals in Erikçe population mainly exhibiting this very same color as can be seen in Figure 3.11, K=4 bar plot.

Although there was no known wild individual in sampled population of Erikçe State Farm, the contribution of a wild individual (a male), which was released into Erikçe State Farm in 2010, might have been observed with the same, light brownish color. There were 2 individuals in Erikçe population whose bar plots, almost mainly, exhibited this very same brown color in Figure 3.11 when K=4.

In the light of this discovery, since these individuals were observed in two *Gazella marica* populations, it can be suggested that the degree of genetic differentiation among populations might have been underestimated. Therefore, the pairwise F_{ST} values were calculated one more time, after removing the wild individuals from *Gazella marica* populations and results are given in Table 3.9. As a result, the pairwise F_{ST} value for Kızılkuyu population (*Gazella marica*, n=48) and Erikçe population (*Gazella marica*, n=25) increased. It is now a statistically significant divergence between the two populations with a p value of 0.0545 and it is in the "moderately differentiated" range in accordance to Wright's scale (Wright, 1978). Wright's scale can be summarized as: If pairwise F_{ST} is <0.05 it indicates a non -significant

differentiation; If it is in the range of 0.05< F_{ST} <0.15 the differentiation is in the range of moderate differentiation; If F_{ST} is 0.15< F_{ST} <0.25 it indicates the presence of significant differentiation; finally if F_{ST}> 0.25, the differentiation between the populations is very significant. Hatay, population seems to be very significantly different than the two populations of *Gazella marica*.

It should be noted that the only individuals, which were reported in seasonal hunting reports, were excluded from Kızılkuyu population, since the other wild type detected individuals maintain the genetic contribution to the next generations.

Table 3.9 Pairwise F_{ST} values re-estimated based on 3000 permutations after removing the "wild" individuals from *Gazella marica* populations.

Pairwise F_{ST}	**Kızılkuyu (n:48)**	**Erikçe (n:25)**	**Hatay (n:4)**
Kızılkuyu (n:48)		**	**
Erikçe (n:25)	0.0545		**
Hatay (n:4)	0.4561	0.4723	

($p \leq 0.001$**)

3.2.1.5 Estimating Effective Population Size (N_e)

In the present study, the effective population sizes of the three populations were estimated by using the molecular co-ancestry method of Nomura (2008) which is implemented in recently developed software, NeEstimator V.2.01 (Do et al., 2014).

Basically, the aim is to choose among eligible pairs, the one that yields the smallest average value of co-ancestry indices taken across loci (Do et al., 2014).

The effective population sizes were estimated as 9.7, 8.9 and 6.4 for Kızılkuyu population (*Gazella marica*, n=48), Erikçe population (*Gazella marica*, n=25) and Hatay population (*Gazella gazella*, n=4), respectively. Furthermore, the sibling populations, Kızılkuyu and Erikçe were pooled together and N_e was estimated for this pooled population. N_e for the pooled population was estimated as 24.5. At last, when the wild individuals were excluded from the Kızılkuyu population, the final effective population size of the captive population was estimated as, 8.9.

3.2.2 Cytochrome *b* gene of mtDNA

3.2.2.1 Constructing a Neighbor Joining (NJ) Tree

Since the clustering algorithm of NJ method allows us to observe big data sets from different taxa in the same phylogenetic tree (Saitou and Nei, 1987), sequences from different gazelle species found in the literature were included in the analysis and the sequences from related bovid species were used as out-group samples. Totally, 28 samples were used from GenBank; 3 *Gazella arabica* (Lichtenstein, 1827) (GenBank accesion numbers: KC188740, KC188741, KC188744), 3 *Gazella bennettii* (Sykes, 1831) (GenBank accession numbers: JN410340, JN410341, JN410357), 2 *Gazella cuvieri* (Ogilby, 1841) (GenBank accession numbers: JN410342, JN410343), 3 *Gazella dorcas* (Linnaeus, 1758) (GenBank accession numbers: JN410332, JN41036, JN410337), 4 *Gazella gazella* (Pallas, 1766) (GenBank accession numbers: KC188773, KC188774, KC188775, KC188776), 3 *Gazella leptoceros* (F. Cuvier, 1842) (GenBank accession numbers: JN410344, JN410345, JN410346), 3 *Gazella subgutturosa* (Güldenstädt, 1780) (GenBank accession numbers: AF036282, HQ316159, AF187716), 3 *Gazella subgutturosa marica* (Thomas, 1897) (GenBank accession numbers: HQ316160, HQ316161, HQ316162). As out-group; an *Antidorcas marsupialis* (Zimmermann, 1780), a *Nanger granti* (Brooke, 1872), an *Antilope cervicapra* (Linnaeus, 1758), an *Aepyceros melampus* (Lichtenstein, 1812) were also

included whose GenBank accession numbers were AF036281, AF034723, AF036283, AF036289, respectively. The origin of the species, whether they are captive or wild and their references harboring the relevant information can be seen in Table 3.10.

Table 3.10 The summary information about the samples taken from the literature. The geographic origins of the samples are given if they were available. Moreover, the status of the samples; i.e. whether they were kept captive or wild, the accession numbers and related references were given in the table. Abbreviations: EEZA – Estación Experimental de Zonas Áridas, Spain; KKWRC – King Khalid Wildlife Research Centre, Riyadh, Saudi Arabia; WA-SWC – Wadi Al-Safa Wildlife Center, Dubai; MNHN: Muséum National d'Histoire Naturelle, Paris.

Species	Origin	Captive/Wild	Accession Number	Reference
G. Arabica	Southern Arava Valley, Israel	Wild	KC188740	Lerp et al., 2012
G. Arabica	Southern Arava Valley, Israel	Wild	KC188741	Lerp et al., 2012
G. Arabica	Southern Arava Valley, Israel	Wild	KC188744	Lerp et al., 2012
G. bennettii	KKWRC, Thumamah	Captive	JN410340	Lerp et al., 2011
G. bennettii	KKWRC, Thumamah	Captive	JN410341	Lerp et al., 2011
G. bennettii	KKWRC, Thumamah	Captive	JN410357	Lerp et al., 2011
G. cuvieri	EEZA, Almeria	Captive	JN410342	Lerp et al., 2011
G. cuvieri	EEZA, Almeria	Captive	JN410343	Lerp et al., 2011
G. dorcas	KKWRC, Thumamah	Captive	JN410332	Lerp et al., 2011
G. dorcas	KKWRC, Thumamah	Captive	JN410336	Lerp et al., 2011
G. dorcas	Tunisia	Wild	JN410337	Lerp et al., 2011
G. gazelle	Central Israel	Wild	KC188773	Lerp et al., 2013
G. gazelle	Central Israel	Wild	KC188774	Lerp et al., 2013
G. gazelle	Central Israel	Wild	KC188775	Lerp et al., 2013
G. gazelle	Central Israel	Wild	KC188776	Lerp et al., 2013
G. leptoceros	Tunisia	Wild	JN410344	Lerp et al., 2011
G. leptoceros	Tunisia	Wild	JN410345	Lerp et al., 2011
G. leptoceros	Western Desert, Egypt	Wild	JN410346	Lerp et al., 2011
G. subgutturosa	MNHN, Paris	Unspecified	AF036282	Hassanin et al., 1999

Table 3.10 (continued)

G. subgutturosa	Aksu, Chinese Turkistan	Wild	HQ316159	Wacher et al., 2010
G. subgutturosa	Samarra, Iraq	Wild	AF187716	Hammond et al., 2001
G. s. marica	Ramlat Fasad, Oman	Wild	HQ316160	Wacher et al., 2010
G. s. marica	WA-SWC, United Arab Emirates	Captive	HQ316161	Wacher et al., 2010
G. s. marica	Wadi Abu Al Jir, Iraq	Wild	HQ316162	Wacher et al., 2010
Outgroup				
Antidorcas marsupialis	MNHN, Paris	Unspecified	AF036281	Hassanin et al., 1999
Nanger granti	MNHN, Paris	Unspecified	AF034723	Hassanin et al., 1998
Antilope cervicapra	MNHN, Paris	Unspecified	AF036283	Hassanin et al., 1999
Aepyceros melampus	MNHN, Paris	Unspecified	AF036289	Hassanin et al., 1999

All the *cyt-b* sequences (present study and the ones taken from the literature) were aligned by using ClustalW Multiple Alignment Tool in the BioEdit version 7.2.5 (Hall, 1997-2013). As there was no differentiation observed among the *Gazella marica cyt-b* sequences in the study, only five of them included together with the four sequences of *Gazella gazella* when constructing the phylogenetic tree. The best nucleotide substitution model was determined as Kimura 2-parameter nucleotide substitution model (K2) with the gamma distributed rate heterogeneity (G) value as 0.23. The bootstrap value was taken as 1000. The Neighbor Joining tree was constructed with the help of the software MEGA version 6.06 (Tamura et al., 2013) and shown in Figure 3.12.

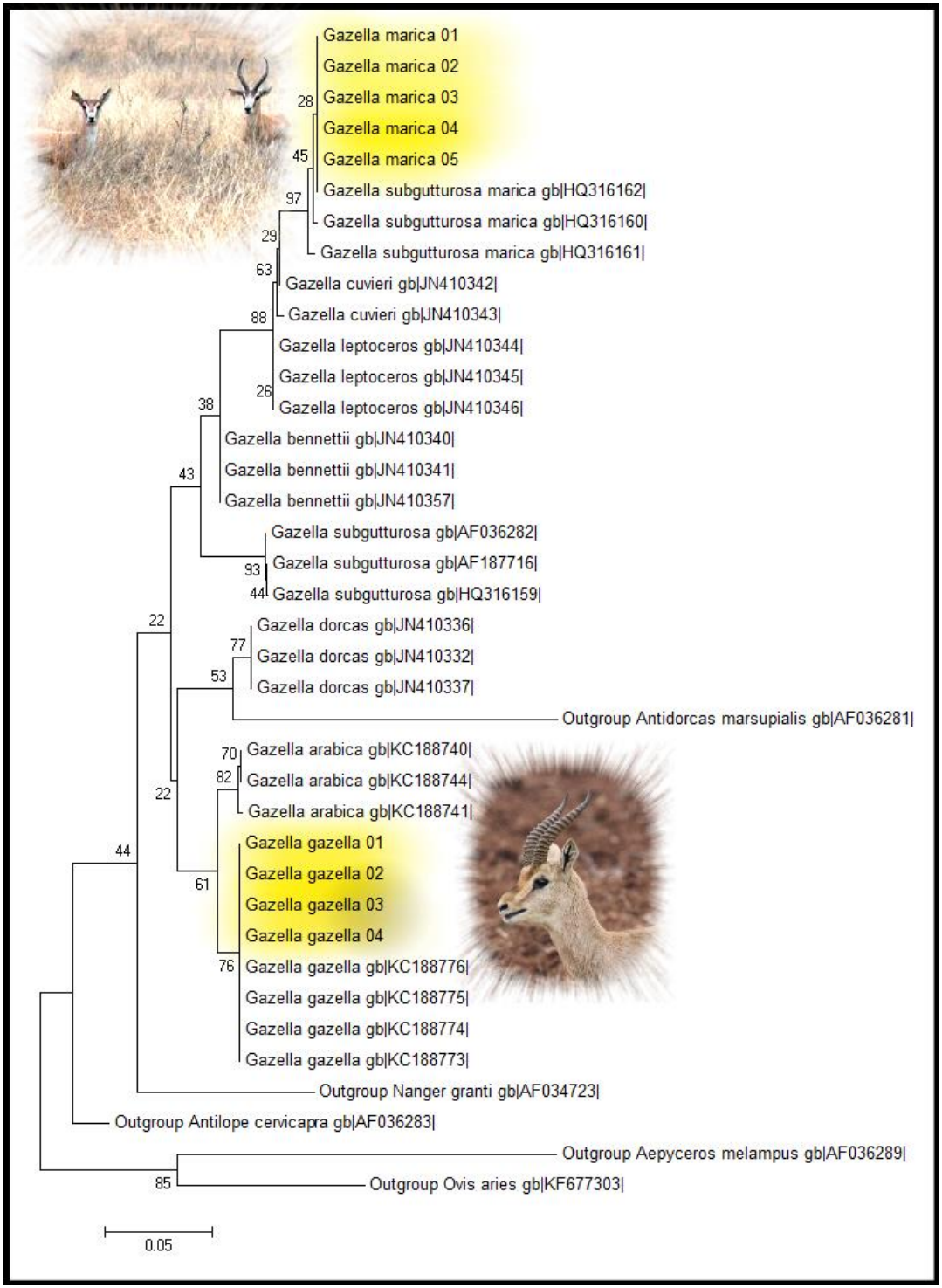

Figure 3.12 Phylogenetic tree constructed using NJ algorithm with a 1000 Bootstrap value and employing K2 nucleotide substitution model with gamma distribution (G=0.23). The GenBank Accession numbers were given at the end of the sample names. The highlighted samples are the samples analyzed in the present study. The software MEGA v6.06 (Tamura et al., 2013) was used for the analysis.

According to Figure 3.12:

1) mtDNA *cyt-b* of recently detected Gazelle individuals from northern Hatay were clustered with the *Gazella gazella* individuals as already observed by Kankılıç et. al, (2012).

2) mtDNA *cyt-b* of individuals who were originated from Şanlıurfa, Ceylanpınar State Farm individuals grouped with the individuals which were originally called as *Gazella subgutrosa marica*, but now called as *Gazella marica* (Wacher et. al, 2010). The individuals of this species were sampled from Arabian Peninsula; from Oman and Iraq in the present days. There seems to be some genetic variation between the mtDNA *cyt-b* of these individuals (Figure 3.12).

Thus, based on the mtDNA *cyt-b* sequences analyzed in this study, presence of two different species: *Gazella marica* and *Gazella gazella* in the borders of Turkey were confirmed.

3.2.2.2 Restriction Fragment Length Polymorphism (RFLP)

In the obtained sequences of these two populations of the same gazelle species (*Gazella marica*), there were no polymorphic sites or nucleotide diversities detected in the analyses done by the Polymorphism tool which is included within the software, DnaSP version 5.1. (Librado and Rozas, 2009).

There are restriction enzymes used in a recent article (Wacher et. al, 2010), *HaeIII* (also known as *BsuRI*) and *HinfI*, suggested for discriminating between the gazelle species. As the sequences from both gazelle species (*Gazella marica* and *Gazella gazella*) obtained in the study also confirmed this information; all 77 individuals included in the present study were analyzed by RFLP method employing these two restriction enzymes: *HaeIII* and *HinfI*. After incubation of the PCR amplified DNAs

with the restriction enzymes, the samples were run on 2,5% agarose gel containing EtBr and visualized under UV light. Invitrogen 50 bp DNA ladder was used as reference for size determination of the restriction fragments. A gel picture is provided below (Figure 3.13) showing the banding patterns obtained for the two enzymes in each of the two gazelle species.

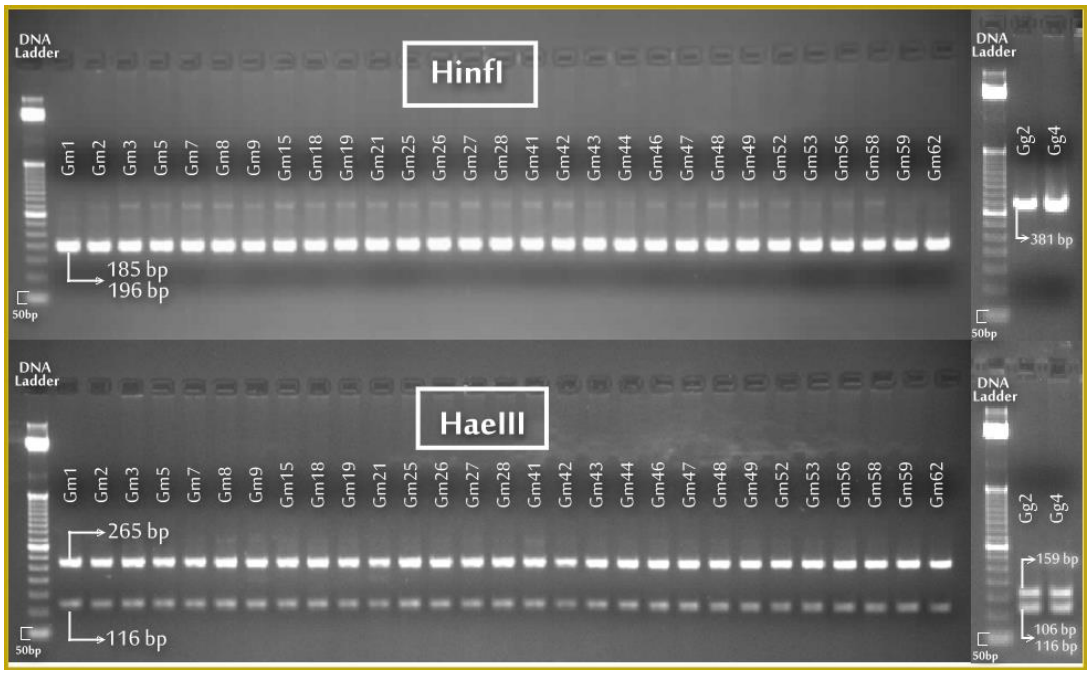

Figure 3.13 mtDNA partial *cyt-b* gene RFLP analysis result of the samples belonging to two different gazelle species. Each sample is labelled. 'Gm..' labeled samples are the gazelles from Kızılkuyu (Şanlıurfa) and Erikçe (Gaziantep). On the right end of the gel, 'Gg..' labelled samples belong to *Gazella gazella* (Hatay Mountain Gazelle) from Hatay.

As the sequencing analysis suggested, there was no within species differentiation among the samples in terms of their RFLP genotypes, but there was among species differentiation observed between the two gazelle species of the study. All the individuals from Kızılkuyu and Erikçe (*Gazella marica* samples) exhibited one single haplotype, and all the 4 individuals from Hatay belonging to *Gazella gazella* had another single haplotype based on both restriction endonucleases; *HinfI* and *HaeIII*.

CHAPTER 4

DISCUSSION

In the present study, to investigate the variation between and within gazelle populations, and to clarify the taxonomic states of gazelles in Anatolia; 17 microsatellite loci, part of the mtDNA *cyt-b* sequence, 2 microsatellite loci (and the sequences of one locus) on Y chromosome and two RFLP enzymes on part of the *cyt-b* region of mtDNA were employed. For microsatellite analysis and mtDNA RFLP analysis 77 samples were used. For *cyt-b* gene partial sequencing 27 individuals and for the Y chromosome analysis 15 gazelle individuals belonging to the three populations of the two gazelle species were used.

4.1 Choosing the Markers and Notes about the Experiments

It was reported by the Ministry of Forestry and Water Affairs that one of the State Farm populations of *Gazella marica* namely Erikçe population was suffering from very low fertility (in 2013 no birth was observed) and the death rate was increasing (from the reports obtained from the Ministry of Forestry and Water Affairs). This observation required the use of markers which can evaluate the genetic diversity within gazelle populations. Microsatellites are the markers with a relatively high mutation rate and mostly used to investigate differentiation within closely related species, populations or subpopulations and individuals. They are highly preferred in criminology studies as they can differentiate between individuals. Therefore they are chosen for the present study.

There are a few microsatellite loci used in gazelle species and the most of them were published after the experimental stage of this study was completed (Zachos et al., 2009; Lerp et al., 2014; Hadas et al., 2015; Duo et al., 2015; Okada et al., 2015). Therefore, in the present study, 20 microsatellite primers were chosen among the bovine primers found in the literature. They were tested in the framework of the national project "Analysis, Conservation and Management of Large Mammals in Context of National Strategies on Conservation of Biodiversity and Genetic Resources". After the optimization experiments, 17 promising loci were used in the present study.

Another observation in relation to gazelle related studies was that there was not a single study employing the Y chromosome based markers. However, species specific Y chromosome related markers were urgently needed to understand if there was introgression of one sex from one species into another. For instance, maternal introgression from *Gazella marica* females into *Gazella subgutturosa* and formation of a hybrid was suggested, previously (Murtskhvaladze et al., 2012; Lerp et al., 2013).

To start the establishment of Y chromosome related markers, two pairs of primers for Y chromosome were chosen from the published literature by considering the high degree of polymorphism they exhibited between the bovid species. INRA126 (Vaiman et al., 1994) is one the Y chromosome locus showing high polymorphism in different bovid species (Hanotte et al., 1997; Edwards et al., 2000). The other locus chosen for this study, UMN0103 (Liu et al., 2002), is commonly used to detect the polymorphism as well as to trace the demographic history of the cattle breeds (Perez-Pardal et al., 2010; Xin et al., 2014).

Finally, mtDNA *cyt-b* region is the most commonly used marker in recently published literature (Wronski et al., 2010; Wacher et al., 2010; Lerp et al., 2011; Kankılıç et al., 2012; Murtskhvaladze et al., 2012; Lerp et al., 2013) for *Antilopinae* subfamily which includes gazelles and its close relatives. The high preference for the usage of this

primer pair is because the amplified mtDNA region functions as a good discriminator at the species level. Already accumulated data (sequences of *cyt-b* fragment) in the Genbank provided means for comparative studies within and between *gazella spp* which was lacking for microsatellites and Y chromosome related markers.

Wacher et al., (2010) demonstrated that by means of RFLP analysis in mtDNA *cyt-b* region, one can also separate the gazelle subspecies and can also reveal the variations within the species. In the present study, the same RFLP enzymes (*HaeIII* also known as *BsuRI* and *HinfI*) from Wacher et al.'s study (2010) were utilized.

All blood samples were collected by veterinarians who were authorized by the Ministry of Forestry and Water Affairs within the context of the project "Analysis, Conservation and Management of Large Mammals in Context of National Strategies on Conservation of Biodiversity and Genetic Resources". The author of this thesis have accompanied the field works involving sample collection for several times between the years 2013, 2014. The laboratory analysis using the microsatellite markers, sequencing of the mtDNA *cyt-b* region and mtDNA *cyt-b* region RFLP analysis were performed at Genetic Engineering and Biotechnology Institute of TUBITAK Marmara Research Center under the supervision of Dr. Evren Koban. Only the Y chromosome related studies was carried out at Middle East Technical University supervised by Prof. Dr. Inci Togan and the sequence data was obtained through a private company: RefGen (http://www.refgen.com).

4.2 Evaluation of the Microsatellite Data and Recommended Microsatellite Loci

Microsatellite data may suffer from null alleles. If there is a mutation on the primer site of some of the chromosomes, it will prevent the amplification of that region on the chromosome. If an individual is heterozygous for that mutation, then only the non-mutant primer site will be amplified and the individual will be recorded as a homozygote. Therefore, the presence of null allele will cause an excess of homozygotes in all of the alleles (Oostherhout et al., 2004). A general agreement is

that if the frequency of the estimated null allele for a locus is above 0.2, then that locus must be excluded from the further analysis (Chapuis and Estoup, 2007) with the argument that the individuals could not be genotyped correctly for it. In parallel to these arguments, in the present study, out of 17 microsatellite loci analyzed, 1 locus (IDVGA29) was excluded from the statistical analyses.

Regarding the assumptions of the 'STRUCTURE' method (Pritchard et al., 2000), non-significant deviations from Hardy-Weinberg equilibrium and presence of linkage equilibrium observed in all loci in all the three populations.

Out of 16 microsatellite loci, two of them (ETH152 and BM143) were monomorphic in all the 3 (Kızılkuyu, Erikçe, Hatay) populations. In this study, the loci, OARFCB304 and INRA40, with 12 alleles each, were found to be the richest loci in terms of the allelic diversity, which is a measure that is sensitive the sample sizes of the populations. Results can be erroneous where the sample sizes of different populations are not similar. Therefore, the allelic richness, a measure of the allelic diversity, which takes into account the sample sizes by using the rarefaction algorithm (El Mousadik and Petit, 1996), was used (with the smallest population size, n=25) for each of the loci analyzed in Kızılkuyu and Erikçe populations. According to the results, the locus OARFCB304 showed the highest allelic richness.

In relation to diversity measures for a locus, allelic richness is calculated based on the number of alleles at a locus, whereas the Polymorphic Information Content (PIC) determines the informativeness of the locus by using not only the allele numbers observed at a locus but also its allelic frequencies. The loci with PIC values higher than 0.4 can be interpreted as moderately informative, whereas the loci with PIC values higher than 0.7 can be considered as highly informative (Hildebrand et al., 1994). In the light of this information, 6 out of 16 loci were found as highly informative whereas the most informative locus was ETH10 (0.801). Also there were 6 loci observed as

moderately informative. Thus, twelve loci: RT1, ETH10, OARFCB304, BM848, BMC1009, INRA40, BM4505, INRABERN172, TGLA122, ILSTS005, BM757 and CSSM43 were observed as being informative. These loci can be used in the future studies of gazelles to determine genetic diversity of populations or their pedigree relations.

4.3 Effective Population Sizes (N_e) of the Studied Populations, Variability in mtDNA and Y Chromosome

For both of Kızılkuyu and Erikçe populations the source population was Ceylanpınar Sate Farm population. Ceylanpınar State Farm population is known to be started by 1 male and 4 females. Since in a highly distorted sex ratio case N_e is nearly 4 times the number of the rare sex (Allendorf and Luikart, 2007), it can be assumed that at the start of Ceylanpınar State Farm Population in 1978, N_e was nearly 4 (i.e. assuming that they were not related). Due to low number of individuals in the years after 1978, unequal contribution of the individuals in the gene pool and overlapping generations, the N_e must have been quite low. Populations with low Ne will show wild fluctuations in their allele frequencies and during the genetic drift, they are expected to lose variability especially in mtDNA and Y chromosome since they are haploid in number. It is known that Ceylanpınar State farm started with one male, thus one haplotype of Y chromosome. In fact, during the dramatic bottleneck experienced by the wild Ceylanpınar population, in 1970s, variability in mtDNA and Y chromosome must have already been reduced. Initially, even if there were more than one mtDNA haplotype in Ceylanpınar State Farm population, with a low Ne under random drift, it is very likely to see a single haplotype for mtDNA. Twenty years later when populations of Kızılkuyu and Erikçe State Farms were started by individuals transported from Ceylanpınar, they must have started with very low values of Ne with the same single haplotypes of Y chromosome and mtDNA that were present in Ceylanpınar. Therefore, no variation observed in both mtDNA and Y chromosome is fitting the expectations for these populations.

Microsatellite based N_e estimations for both Kızılkuyu and Erikçe populations indicated that they are low (9.7 for Kızılkuyu and 8.4 for Erikçe) but not less than 4 (an estimate for the starting population of Ceylanpınar State Farm population). This calculation suggests that these populations might be receiving individuals from some other sources, possibly from wild populations. In fact the documented transportation of 1 male from Kızılkuyu wild population to Erikçe, and again transportation of 6 individuals from Ceylanpınar to Kızılkuyu support this suggestion. If these estimations are approximately correct still the N_e~ 10 is indicating a small population size.

For Hatay population, the estimated N_e was 6.4 which might be related with its low sample size or alternatively, it has a small N_e. This estimation should be repeated when more samples from this population are analyzed.

4.4 Small Populations:

- <u>Diversity</u>

For small populations, one expects to see low genetic variability. Here it was assessed by two measures: Expected heterozygosity (H_e) and allelic richness, both of which are not effected much from low sample sizes.

In both of these measures Erikçe population exhibited lower values (Average H_e: 0.63 and allelic richness: 4.31) than those of Kızılkuyu population (0.69, 5.05). For Hatay population, allelic richness was not considered and the heterozygosity estimated was 0.6. Additionaly, Kızılkuyu and Erikçe populations were observed to be significantly different ($p<0.05$) with respect to their allelic richnesses by Wilcoxon-Signed rank test (Sokal and Rohlf, 1995).When a randomly sampled population with the same size of Hatay population (n=4) from Kızılkuyu population was selected, the mean He was estimated as 0.68 which is higher than the one observed for Hatay population. This possibly indicates that Hatay population has even lower genetic diversity than both of the two captive *Gazella marica* populations.

Unfortunately, there are no data regarding within population diversity measures in gazelle presented in the literature. However, for instance, heterozygosities in sheep are all >0.75, and allelic richness estimates ranged between 7.8 and 9.8 in sheep breeds of Turkey (Açan, 2012). Therefore, one can say that diversity is low in the studied populations but more so in Erikçe compared to Kızılkuyu. Furthermore, although overall F_{IS} values of the populations are not significant, number of negative F_{IS} values per locus is high in Erikçe (8/13) compared to Kızılkuyu (4/12). Excess of heterozygotes for many loci in these populations might be indicating that the populations are really small.

- <u>Divergence between the Populations:</u>

For the small and isolated populations one expects to see divergence in their gene pools. Kızılkuyu and Erikçe were founded from the same source approximately 16 years ago. Although they may not be strictly isolated, they must be subject to random drift, thus divergence, for about 10 generations (generation time of gazelle was assumed as 1 or 2). They exhibited almost a significant pairwise F_{ST} value (0.044) and their individuals are almost exclusively separated by the FCA analysis (Figure 3.8). Since Hatay population is representing wild individuals of *Gazella gazella*, it was not considered here in this section.

- <u>Private Alleles:</u>

Regarding the private alleles, it is likely that the same alleles were not introduced in the founding stages of the populations. In addition, they were isolated from each other (at least partially). Moreover, since each of these state farm populations were low in their effective population sizes, many alleles were expected to be lost randomly in both of the populations, and thus revealing a high number of private alleles in them. Observed number of private alleles In Kızılkuyu (28) and Erikçe (7) indicates that they are isolated from each other and they are under the effect of random drift. These

numbers might be overestimates because we might be missing the same allele in the other population just by sampling effect; especially if it has a low frequency. However, it is also possible that these alleles are erroneously recorded alleles, especially if their frequencies are low. Arbitrarily, 0.05 was considered as threshold value for a private allele frequency and those with the frequencies above 0.05 were considered as alleles with high frequencies. There were 3 such private alleles in each of the populations (Kızılkuyu and Erikçe) as presented in Table 3.5.

These are true private alleles observed more than once in each of the populations, without any doubt. However, it must be pointed out that, genotyping was done by an experienced researcher and both of the populations were screened by the same person. If these alleles are true private alleles, they might be drifting in the population having relatively higher population size (Kızılkuyu ranges from 38 to 215 whereas Erikçe ranges from 32 to 112 between the years 2005-2014). However, differences between the population sizes are not too high to explain the high number of private alleles observed in Kızılkuyu. The high number of private alleles in Kızılkuyu suggests that it might have received alleles from another relatively less related gazelle population such as from wild gazelles.

- <u>Inbreeding Depression:</u>

Along with the loss of diversity, homozygosity of deleterious alleles increase and perhaps selective advantage of the heterozygotes cannot be attained in small populations (Allendorf and Luikart, 2007). As a consequence of loss of diversity 'inbreeding depression' (for instance decrease in growth rate in mosquitofish, increase in juvenile mortality rate of mammals in captivity, reduced survival rate of mammals in captivity compared to wild populations) is observed (Allendorf and Luikart, 2007).

It can be argued that Erikçe population having lower mean H_e and allelic richness than Kızılkuyu is, already, suffering from inbreeding depression by exhibiting no births recorded in 2013 and having high death rate. It seems that Kızılkuyu population is not at that stage yet. Birth and death rates of the populations will be discussed in the section "4.8".

4.5 Admixture Estimation by STRUCTURE Analysis

By employing the Structure Analysis (Pritchard et al., 2000) populations' gene pools can be visualized at individual level. Furthermore the admixture between them or from an unknown source population can be identified. As a very first step of the analysis, the most likely K (number of ancestral populations presumably generating the populations under consideration) were determined by using two different methods, Evanno et. al's (2005) and Tapio et. al.'s method (2010). The most likely K values were found as 2, 3 and 4.

In Figure 3.11, STRUCTURE analysis results were given in bar plots (each bar corresponds to an individual) for different K values (K=2-4). Each bar can have different number of colors, between 1 to K, represents the genotype of an individual. If it is presumably representing just one ancestral population it will be in a single color. When individuals (bars) are examined in their population, one may see mainly a single color representing the gene pool of that population.

Under three different K values, the Population 3 (*Gazella gazella* from Hatay, n=4) did not exhibit any common pattern with the other two populations and had completely different coloring in the result graphics. This result emphasizes that Hatay population has a completely different gene pool than those of Erikçe and Kızılkuyu, which was expected since Hatay and the other two population are from different gazelle species.

For K=4, Population 1 (Kızılkuyu) and Population 2 (Erikçe) were distinguished from each other: Kızılkuyu gene pool was mainly represented by pink color but Erikçe mainly by yellow color. The fourth color (indicated by the light brown) was present in both of the *Gazella marica* populations, but more heavily in Kızılkuyu population. Before the analyses were done, all of the individuals sent from Kızılkuyu were considered as the members of captive population. When the background story of those individuals which seemed to be different than common Kızılkuyu gene pool were searched, it was understood that they could be the ones which were hunted from the wild population of Kızılkuyu. Because 9 out of 11 hunted ones exhibited this light brown color.

However, among the hunted individuals, numbered as "2" and "11" in Figure 3.11, were largely pink (presenting the gene pool of captive Kızılkuyu population). This observation could suggest that these were among the ones which were released from Kızılkuyu into wild previously. Furthermore among the individuals of Kızılkuyu there were two more individuals exhibiting presumably Kızılkuyu wild type. These might be still hunted ones or introduced ones from wild to Kızılkuyu captive population.

4.6 Loci of Chromosome Y

The Y chromosome locus, INRA126 was amplified and sequenced. Now, the sequences for the amplified part of Y chromosome are available for *Gazella gazella* and *Gazella marica* species. This sequence seems to be differentiating these two gazelle species. To our knowledge, this is the first sequence that is reported for the Y chromosomes of gazelles. However, high number of wild samples from both of the species must be tested to confirm the discrimination power of this sequence. Then these sequences may be useful in Y chromosome based comparative studies for gazelle species such as:

(i) If there is a hybrid zone in Turkey for *Gazella marica* and *Gazella subgutturosa* as suspected in the previous studies (Wacher et al., 2010; Murtskhvaladze et al., 2012), male individuals of *Gazella subgutturosa* and "truly" *Gazella marica* can be tested by the help of the locus, INRA126. If INRA126 is discriminating males of *Gazella subgutturosa* and *Gazella marica* then it can be applied to the populations from Anatolia to see if they were hybrids generated by maternal introgression of *Gazella marica* into *Gazella subgutturosa*.

(ii) Since the amplification product of INRA126 is short (approximately 240 bp) as required by ancient DNA (aDNA) studies, by examining the ancient bone samples of the gazelles in Anatolia, the early distribution maps of the gazelle species (whether they belong to *Gazella marica* or *Gazella gazella*) or the densities of them in Central, Southern, Eastern and Southeastern Anatolia can be revealed by INRA126 Locus.

4.7 Evaluation of mtDNA *cyt-b* Sequence and RFLP

When 381 bp long fragment of mtDNA *cyt-b* was sequenced for 27 (n=15 from Kızılkuyu, n=8 from Erikçe and n=4 from Hatay) samples out of 73, no haplotype diversity was observed between the two populations of *Gazella marica* species, as expected (as was discussed above). Then, the rest of the individuals sampled from *Gazella marica* populations and also 4 individuals of *Gazella gazella* species were examined using the RFLP enzymes (*HaeIII* and *HinfI*): (i) to detect if there is a variation between *Gazella marica* individuals (ii) to separate the populations at the species level.

Since the sequenced *Gazella marica* samples from both populations (Kızılkuyu and Erikçe) gave the same haplotype, only 5 of them were included within the Neighbor Joining (NJ) tree reconstruction. Also, the sequences of all the 4 *Gazella gazella*

samples were used in the construction of the phylogenetic tree. The tree was reconstructed after addition of the sequences from 28 samples found in GenBank (Table 3.10). The NJ tree (given in Figure 3.12) allowed us to evaluate the gazelle species comparatively based on the partial mtDNA *cyt-b* sequences.

Recent publications based on the mtDNA *cyt-b* sequences proved that, maternally, *Gazella marica* (also *G.s. marica* in the tree) is more closely related to the North-African species, *Gazella leptoceros* and *Gazella cuvieri* than *Gazella subgutturosa*. Thus, it was suggested to separate *Gazella marica* as a species (Hammond et al., 2001; Wacher et al., 2010). Consecutively, few individuals from Kızılkuyu State Farm were analyzed and suggested to be classified as *Gazella marica* (Kankılıç et al., 2012). As it can be observed in Figure 3.12, findings of the present study supported these previous studies based on data from 23 individuals; sampled from two populations in two different locations. Additionally, the presence of another species of gazelles, *Gazella gazella*, was confirmed for the second time (Kankılıç et. al, 2012).

RFLP enzymes, *HaeIII* and *HinfI*, separated the amplified DNA fragments of *Gazella marica* and *Gazella gazella* in this study (Figure 3.13). In addition, the reference sequences of *Gazella marica* and *Gazella gazella*, taken from GenBank and used in phylogenetic tree re-construction, were also checked for the restriction sites in terms of these two enzymes. All *Gazella marica* and *Gazella gazella* sequences, including the samples of the present study, exhibited the same restriction sites (there were no restriction site for *HinfI* in *Gazella gazella* sequences). The same two restriction sites of the two enzymes (*HaeIII* and *HinfI*) were also searched on the mtDNA *cyt-b* sequences (retrieved from the GenBank) of *Gazella subgutturosa*. The sum of the observations was given in Table 4.1.

Table 4.1 RFLP enzymes and their restriction site according to three gazelle spp.

RFLP Enzyme	Species	Restriction Site
HaeIII	*Gazella marica*	116th bp
	Gazella subgutturosa	116th and 275th bp
	Gazella gazella	116th and 275th bp
HinfI	*Gazella marica*	185th bp
	Gazella subgutturosa	185th and 302nd bp
	Gazella gazella	None

HinfI separated these three species from each other; whereas, the RFLP enzyme, *HaeIII*, could only separate *Gazella marica* and *Gazella subgutturosa* based on the amplified mtDNA *cyt-b* region. Thus the previous observation that these two enzymes can discriminate *Gazella marica* and *Gazella subgutturosa* species (Wacher et al., 2010) was confirmed and in this study it was further shown that *HaeIII* can also discriminate *Gazella gazella* from the other two.

If maternal type of a gazelle is the question of interest in and around Anatolia, first *Gazella gazella* can be identified visually by looking at the smaller body-shape and the preference of the home range (hills whereas *Gazella marica* prefers the plains). Since it is reported that some of the perfectly *Gazella subgutturosa* looking individuals exhibited *Gazella marica* type of mtDNA (Murtskhvaladze et al., 2012), perhaps instead of sequencing the *cyt-b* fragment, it can be amplified with the L14724, H15149 primers and then one can try any (or both) of the *HaeIII* and *HinfI* to differentiate *Gazella marica* and *Gazella subgutturosa* as was used a way to confirm the speciation previously (Wacher et al., 2010). However, these observations must be confirmed with higher number of observations.

If the sample DNA is fragmented and there is difficulty in PCR amplification (as in the case of old bones), then *HaeIII* restriction sites can be amplified and sequenced to associate the sample to one of the three (*Gazella gazella*, *Gazella marica* and *Gazella subgutturosa*) possible species.

4.8 In relation to Conservation Efforts of *Gazella marica* in Anatolia

In the age of extinction risk for many organisms, well applied captive breeding programs are not only the useful wildlife sources but they may be considered as the best immediate action to prevent the extinction of the organisms in the wild. After focusing on the importance of genetic diversity on the viability of the species (Avise, 1989), many conservation studies were done on maintaining the genetic variation rather than increasing the number of individuals in a population.

The key for the success of captive breeding programs is maximizing the effective population size and minimizing the effects of genetic drift. Since the genetic variation is the basis of the adaptation to rapidly changing environmental conditions, it should be desirable, especially in the captive breeding programs to increase the survival skills of individuals which will be reintroduced into the wild (Ballou et al., 2010). Before discussing the observations and inferences made for the captive populations sampled for the present study, the goals of captive breeding programs can be summarized as: Captive breeding is carried out (i) to obtain demographic and genetic source for further studies, (ii) to prevent the extinction of species from wild and to use the captive populations as the source of reintroduction, (iii) to prevent the extinction of species which have no chance to survive in the wild.

There is a concern that many captive populations exhibit behavioral change because of adaptation to captivity (Allendorf and Luikart, 2007). As a function of time, loss of genetic diversity reduces the survival of the populations in the wild. Additionally, lack of interchanging genetic materials between wild and captive populations cause genetic

differentiation and released individuals from captivity may change the unique characteristics of original wild populations perhaps adversely affecting the fitnesses of the wild populations. Soule (1987) stated that long-term persistence and adaptation of a species or a population under captivity was one of the most difficult and challenging intellectual problems in conservation biology. Additionally, it is suggested that retaining 90% of the heterozygosity in a population for 200 years should be the main goal of the captive breeding programs to bring the populations into wild and ensure their long-term persistence in there (Soule, 1985).

Another concern is that, according the widely known "50/500" rule (Franklin,1980), only the populations with a higher Ne than 500, may have the chance of viability in the wild, whereas populations with Ne between 50 and 500 are under extinction risk in the long-term and populations with Ne under 50 can go to extinct in a short time interval. Although, this rule is still controversial due to feasibility problems for various organisms, it is a widely accepted rule by conservation biologists.

The present study proves the usefulness of population genetics approaches based on molecular markers in conservation studies. First of all, the estimations of the effective population sizes of the captive populations could be done and seen that they were very low (<10). Again with the molecular markers (microsatellites) divergence between Kızılkuyu and Erikçe populations can be quantified (F_{ST}= 0.044 and F_{ST}= 0.0545; significant F_{ST} according to Wright's scale (1978) when individuals having the light brown bars are removed). Statistical analysis (with the STRUCTURE analysis) suggested that some of the heterogeneity within the populations could possibly be attributed to the wild Kızılkuyu individuals. As an implication of this observation, if they were representing the gene pool of wild individuals, it can be suggested that captive populations are different than the Kızılkuyu wild population. Again, if the two hunted individuals exhibiting the characteristic of Kızılkuyu captive gene pool is true, it can be suggested that introduced ones did survive until they were hunted and they might have survived to introduce their genetic makeup into wild.

Captive populations, having low effective population sizes and being relatively closed populations, were expected to suffer from inbreeding depression. There were documents kept by the Ministry of Forestry and Water Affairs for Kızılkuyu and Erikçe populations. However, year to year census values did not seem to be consistent. Still, an approximate calculations were carried out in an attempt to estimate the birth and death rates for Kızılkuyu and Erikçe populations and they were given in Table 4.2 and presented in Figure 4.1, below.

Constructing the table, the birth ratio was determined by dividing the absolute difference between the census sizes of the populations before and after birth period (from April to end of the May) to the number of females before birth. In addition, the death rate was calculated by dividing the number of deaths to census size of the populations including newborns of the year.

Table 4.2 Birth and Death rates of the populations studied per year. "NA" is used for the missing information.

	Kızılkuyu (n:48)		**Erikçe (n:25)**	
Year	Birth Rate	Death Rate	Birth Rate	Death Rate
2005	1.71	0.028	0.44	0.07
2006	2.43	0.29	0.46	0.038
2007	1.06	0.046	0.2	NA
2008	1.538	0.05	0.48	0.046
2009	1.29	0.025	0.39	NA
2010	1.25	0.023	0.454	0.011
2011	0.93	0.093	0.605	NA
2012	0.755	0.125	0.135	0.1
2013	1.16	1.16	0	0.028
2014	1.08	0.076	0.044	0.24

Using this table above, the birth and death rates for both of the captive populations were presented in graphics, below (Figure 4.1 both Kızılkuyu and Erikçe populations give the signals of inbreeding depression; in general, birth rates are decreasing and death rates are increasing). In Erikçe population, birth rates seemed to be lower than those of Kızılkuyu and death rates are higher than those of Kızılkuyu for which lower genetic diversity (allelic richness and heterozygosity) was observed through the analyses of the present study.

In Figure 1.4, the reintroduction/introduction studies and the individuals added into the state farms were also indicated with arrows. As it can be seen from the graph, for Kızılkuyu, birth and death ratios gave peaks at the same time after reintroduction/introduction studies done with high number of individuals (also see Table 1.3). Raising birth ratio for Kızılkuyu State Farm may indicate the competitive mating. Comparatively, Erikçe State Farm showed lower birth and death rates than Kızılkuyu State Farm. The wild type Kızılkuyu individuals which were added into Erikçe State Farm in 2010 and 2011 may prevent the possible deaths and keep the birth rates stable for a while. However, the dramatic decrease and sudden increase can be observed in Erikçe after 2011.

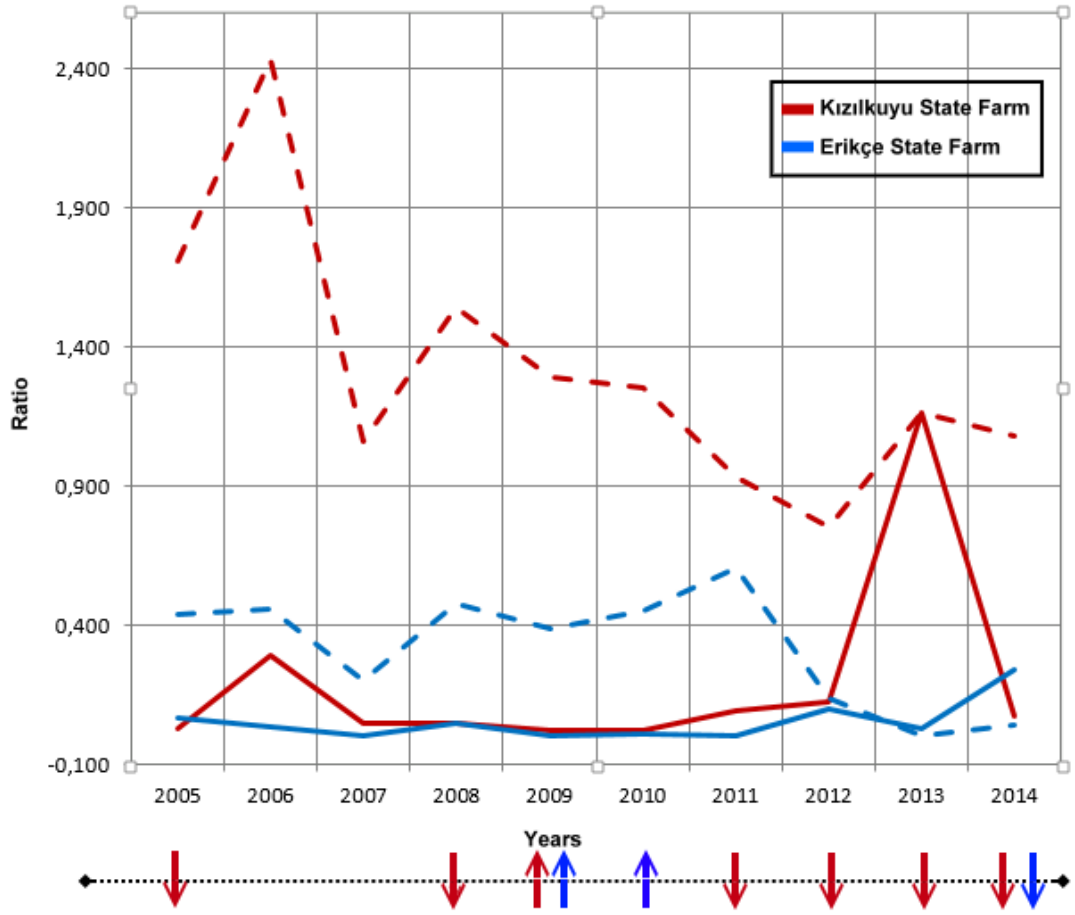

Figure 4.1 Approximate birth (dashed lines) and death rates (continued lines) of Kızılkuyu and Erikçe State Farm populations are given. The outer arrows from graph indicate the reintroduction/introduction studies where as inner ones indicate the years that new individuals were added into state farms.

Incidentally, when the two captive populations were pooled, calculated Ne increased to 24.5 and 29.5 (when DNA samples from "hunted" individuals are excluded). It can be assumed that when all the wild (Kızılkuyu) and state farm populations (Ceylanpınar and Hekimhan as well as Kızılkuyu and Erikçe) were pooled, then the Ne can be estimated as even higher. However, it is quite unlikely that it will reach to more than 50.

In context of conservation studies, Ceylanpınar and Hekimhan (Malatya) captive populations and the wild populations of Kızılkuyu (Şanlıurfa) must also be studied with at least the same molecular markers to estimate the total effective population size. Since the signals of inbreeding depression for the populations and divergence between the populations are apparent, may be with a corridor (real or by translocation of the individuals) the drift and high genetic loss in every generation can be slowed down and inbreeding depression can be overcome. Molecular markers can also be employed to manage the translocations between the populations and to slow down the inbreeding by controlling the introduction of new borns such that genetic loss will be minimized in each generation. Furthermore after checking the mtDNA cytochrome *b* gene and Y chromosome based diversity, translocation of *Gazella marica* individuals between Oman, Iraq or United Arab Emirates can be considered for the benefit of conserving *Gazella marica* species in Turkey and in the world.

4.9 Conclusion

Remembering the aims, in the present study:

(i) Genetic diversities within and among captive populations of *Gazella marica* species were examined on the basis of 16 microsatellite loci of which 12 (RT1, ETH10, OARFCB304, BM848, BMC1009, INRA40, BM4505, INRABERN172, TGLA122, ILSTS005, BM757 and CSSM43) were found to be promising for the future studies to be carried out in gazelle species. The divergence between the populations (F_{ST}=0.04, 0.44 and 0.46 for the Kızılkuyu/Erikçe, Kızılkuyu/Hatay and Erikçe/Hatay, respectively) and their effective population sizes (for Kızılkuyu N_e=9.7, for Erikçe N_e= 8.9 and for Hatay N_e= 6.4) were estimated,

(ii) It was shown that Y chromosome based microsatellite locus (INRA126) sequence (approximately 240 bp) seemed to differentiate between *Gazella marica* and *Gazella gazella* males. This is the first result reporting the differentiation based on Y chromosomes of different gazelle species.

(iii) Existence of two different gazelle species in Southeastern Anatolia (*Gazelle marica* and *Gazelle gazella)* on the basis of mtDNA *cyt-b* sequences was confirmed with collected samples (n=27 and n=4, respectively), independently from the previous studies.

(iv) With the restriction enzymes *HinfIII* and *HaeIII*, RFLP method was tested on mtDNA *cyt-b* fragment (amplified by the primer pair, L14724 and H15149). It was observed that *Gazella marica* and *Gazella gazella* individuals can be differentiated. Thus, RFLP method with these enzymes can be utilized for quick identification of the mtDNA of an individual to find out if it belonged to *Gazella marica* or *Gazella gazella* species.

Finally, regarding the conservation studies of *Gazella marica* species in Anatolia, it is observed that: (i) captive Kızılkuyu and Erikçe populations have low effective population sizes, (ii) they diverged from each other almost significantly (may be they are also from Kızılkuyu wild population) and (iii) they are both giving the signals of inbreeding depression. It might be useful to establish a corridor between the populations of *Gazella marica* species which can be slow down the diversity loss from the populations and their rate of differentiation by genetic drift. Furthermore, by using the markers employed in this study, marker assisted management for reducing the genetic erosion in the species can be implemented in the conservation studies for this species.

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APPENDICES

APPENDIX A

THE GENOTYPES OF THE INDIVIDUALS

DNA Bank ID	RT1	ETH10	OARFCB304	MM12	BM848	BMC1009
G.mar-001	198-198	219-219	164-164	081-081	215-215	284-298
G.mar-002	196-200	219-227	156-164	079-081	215-219	286-298
G.mar-003	196-196	227-231	146-170	081-081	213-219	286-300
G.mar-004	196-196	219-227	158-164	081-081	215-219	286-294
G.mar-005	196-196	233-233	158-168	079-079	213-215	294-298
G.mar-006	196-198	233-235	150-164	079-081	215-215	286-298
G.mar-007	196-198	233-235	149-163	079-081	215-215	286-298
G.mar-008	196-198	219-219	162-164	081-081	213-215	284-290
G.mar-009	196-198	219-235	146-156	081-081	207-219	286-298
G.mar-011	196-196	227-229	150-156	081-081	215-215	294-298
G.mar-012	196-196	227-229	146-156	081-081	213-219	294-300
G.mar-015	196-196	229-229	146-172	079-079	213-215	296-296
G.mar-018	196-198	227-235	146-164	081-081	215-219	286-294
G.mar-019	196-196	235-245	156-162	079-081	215-219	286-300
G.mar-020	198-198	219-229	146-164	081-081	219-219	294-294
G.mar-021	196-198	227-227	156-164	081-081	215-215	286-300
G.mar-022	196-198	219-229	146-146	081-081	219-219	294-298
G.mar-023	198-198	219-235	146-164	081-081	207-219	294-298
G.mar-024	198-198	219-235	146-162	081-081	207-219	294-298
G.mar-025	196-198	223-227	150-150	081-081	213-215	286-300
G.mar-026	196-198	219-227	146-162	081-081	219-219	286-298
G.mar-027	196-198	227-245	150-156	079-081	207-219	286-300
G.mar-028	196-198	227-245	146-164	081-081	215-219	286-300
G.mar-029	198-198	225-229	156-160	081-081	215-215	294-300
G.mar-030	196-196	227-235	162-162	079-081	213-219	292-300
G.mar-031	198-198	219-245	150-156	079-081	215-215	294-294

APPENDIX A (continued)

G.mar-032	198-198	219-235	146-156	081-081	215-215	294-294
G.mar-033	196-196	229-235	164-164	079-081	207-215	292-298
G.mar-034	196-198	227-235	156-162	079-081	207-215	292-300
G.mar-035	196-198	229-245	150-164	081-081	207-219	298-300
G.mar-036	198-198	229-245	156-162	079-081	215-219	294-294
G.mar-037	196-198	219-229	150-156	079-081	215-219	294-294
G.mar-038	196-198	219-235	150-162	079-081	217-219	292-298
G.mar-039	196-198	219-223	156-156	081-081	215-219	294-300
G.mar-040	196-196	219-239	164-164	081-081	219-219	294-298
G.mar-041	196-198	219-235	162-162	079-081	215-219	286-298
G.mar-042	196-198	223-233	168-168	079-081	215-215	294-296
G.mar-043	196-198	227-245	164-164	081-081	215-219	286-300
G.mar-044	196-196	233-233	168-168	079-081	213-213	000-000
G.mar-045	196-198	239-245	150-164	081-081	215-219	294-300
G.mar-046	196-196	227-235	156-156	081-081	219-219	286-294
G.mar-047	196-196	227-227	146-156	079-081	215-215	286-294
G.mar-048	196-196	223-235	156-162	079-081	215-219	286-300
G.mar-049	196-200	227-227	146-150	081-081	215-219	286-292
G.mar-050	196-200	219-227	150-164	081-081	215-219	292-294
G.mar-051	196-196	235-239	164-164	081-081	219-219	294-294
G.mar-052	196-196	219-235	150-160	079-081	219-219	294-300
G.mar-053	196-198	235-235	156-162	079-079	215-219	286-292
G.mar-054	196-198	229-239	146-164	081-081	209-213	292-294
G.mar-055	196-198	219-227	146-156	081-081	213-213	292-294
G.mar-056	196-198	227-227	164-174	079-079	207-209	286-294
G.mar-057	198-198	223-227	146-162	081-081	215-215	292-294
G.mar-058	198-198	231-245	146-164	079-081	213-215	286-300
G.mar-059	196-198	219-235	164-164	079-081	213-213	286-294
G.mar-061	198-198	229-231	164-164	079-081	213-215	286-294
G.mar-062	198-200	229-239	164-164	079-079	213-219	292-300
G.mar-063	196-200	227-229	146-164	079-081	213-215	292-294
G.mar-064	196-198	227-229	150-164	081-081	209-213	294-294
G.mar-065	196-198	229-229	156-164	081-081	209-215	292-294
G.mar-066	198-198	229-231	146-156	081-081	213-215	292-294
G.mar-067	198-200	223-235	146-146	079-081	213-215	286-292
G.mar-068	198-198	223-229	146-162	081-081	215-215	292-294
G.mar-069	200-200	229-245	146-164	079-079	213-215	300-300
G.mar-070	196-198	223-239	146-172	081-081	213-219	292-292

APPENDIX A (continued)

G.mar-072	198-198	227-229	146-164	079-081	209-209	286-294
G.mar-073	196-200	229-235	146-146	079-081	215-215	286-292
G.mar-074	196-196	227-235	146-158	081-081	213-213	286-298
G.mar-075	196-198	223-231	144-162	081-081	213-215	286-292
G.mar-076	198-198	223-229	146-162	081-081	213-215	292-298
G.mar-077	196-198	227-227	156-174	081-081	209-209	286-292
G.mar-078	196-198	229-235	146-164	079-079	215-219	294-300
G.mar-079	196-198	227-229	158-162	079-081	213-213	298-300
G.mar-080	196-198	229-231	172-172	081-081	213-215	286-300
G.gaz-001	196-196	215-219	152-152	079-079	223-223	274-298
G.gaz-002	196-196	213-217	152-152	079-079	223-231	274-274
G.gaz-004	196-196	215-219	152-152	079-079	223-229	274-298
G.gaz-006	196-196	219-223	152-152	079-079	229-229	274-298

DNA Bank ID	INRA40	IDVGA29	BM4505	ETH152	INRABERN172
G.mar-001	285-297	099-099	242-242	192-192	229-233
G.mar-002	273-291	099-099	234-234	192-192	247-247
G.mar-003	273-291	099-099	232-256	192-192	229-247
G.mar-004	201-289	099-099	196-232	192-192	229-235
G.mar-005	281-289	099-099	250-250	192-192	229-249
G.mar-006	291-297	099-099	232-236	192-192	233-237
G.mar-007	291-297	099-099	232-236	192-192	233-237
G.mar-008	297-297	099-099	242-242	192-192	229-233
G.mar-009	289-297	099-099	232-240	192-192	235-247
G.mar-011	273-273	099-099	234-242	192-192	229-229
G.mar-012	287-289	099-099	242-242	192-192	229-235
G.mar-015	273-281	099-099	236-242	192-192	229-247
G.mar-018	201-273	099-099	232-240	192-192	229-229
G.mar-019	201-289	099-099	242-242	192-192	235-247
G.mar-020	201-289	099-099	240-242	192-192	229-235
G.mar-021	201-289	099-099	242-242	192-192	229-247
G.mar-022	201-289	099-099	232-240	192-192	229-247
G.mar-023	297-297	099-099	232-232	192-192	229-247
G.mar-024	297-297	099-099	232-232	192-192	229-247
G.mar-025	291-295	099-099	242-242	192-192	239-247
G.mar-026	297-297	099-099	232-232	192-192	229-247
G.mar-027	201-291	099-099	232-240	192-192	229-235
G.mar-028	289-297	099-099	232-240	192-192	235-247

APPENDIX A (continued)

G.mar-029	201-201	099-099	232-250	192-192	229-229
G.mar-030	201-297	099-099	242-244	192-192	229-235
G.mar-031	201-291	099-099	232-242	192-192	247-247
G.mar-032	201-297	099-099	234-242	192-192	235-247
G.mar-033	201-273	099-099	240-242	192-192	235-247
G.mar-034	201-291	099-099	240-242	192-192	235-247
G.mar-035	273-273	099-099	240-240	192-192	235-235
G.mar-036	201-291	099-099	232-242	192-192	229-247
G.mar-037	201-285	099-099	232-242	192-192	229-247
G.mar-038	285-285	099-099	242-242	192-192	229-229
G.mar-039	291-297	099-099	232-250	192-192	229-247
G.mar-040	201-285	099-099	242-250	192-192	229-247
G.mar-041	201-285	099-099	242-242	192-192	229-247
G.mar-042	269-283	099-099	234-242	192-192	229-239
G.mar-043	287-295	099-099	232-240	192-192	235-247
G.mar-044	273-279	099-099	236-242	192-192	229-229
G.mar-045	295-297	099-099	242-242	192-192	229-247
G.mar-046	289-297	099-099	232-240	192-192	229-235
G.mar-047	273-285	099-099	240-254	192-192	229-235
G.mar-048	201-201	099-099	232-242	192-192	229-229
G.mar-049	201-297	099-099	232-240	192-192	233-251
G.mar-050	201-297	099-099	232-242	192-192	229-247
G.mar-051	201-295	099-099	232-250	192-192	229-247
G.mar-052	273-289	099-099	234-242	192-192	239-247
G.mar-053	201-201	099-099	242-242	192-192	229-247
G.mar-054	201-285	099-099	240-242	192-192	229-235
G.mar-055	201-297	099-099	236-242	192-192	229-233
G.mar-056	295-295	099-099	232-232	192-192	235-247
G.mar-057	201-295	099-099	240-242	192-192	235-239
G.mar-058	289-289	099-099	242-242	192-192	233-247
G.mar-059	289-295	099-099	242-242	192-192	229-247
G.mar-061	289-297	099-099	232-242	192-192	233-247
G.mar-062	201-289	099-099	232-242	192-192	247-247
G.mar-063	201-201	099-099	242-242	192-192	229-233
G.mar-064	201-295	099-099	242-242	192-192	229-239
G.mar-065	201-289	099-099	242-242	192-192	233-235
G.mar-066	295-297	099-099	232-242	192-192	233-247
G.mar-067	295-295	099-099	232-242	192-192	235-247
G.mar-068	289-291	099-099	232-242	192-192	229-235
G.mar-069	289-295	099-099	242-250	192-192	235-247
G.mar-070	289-289	099-099	236-242	192-192	233-239

APPENDIX A (continued)

G.mar-072	201-201	099-099	232-242	192-192	229-235
G.mar-073	273-297	099-099	242-242	192-192	229-247
G.mar-074	273-285	099-099	236-242	192-192	229-231
G.mar-075	289-291	099-099	236-242	192-192	229-235
G.mar-076	201-289	099-099	242-242	192-192	229-239
G.mar-077	289-295	099-099	232-242	192-192	235-239
G.mar-078	201-289	099-099	242-242	192-192	229-235
G.mar-079	285-285	099-099	236-236	192-192	231-235
G.mar-080	285-295	099-099	236-242	192-192	235-239
G.gaz-001	217-217	099-099	244-244	192-192	239-239
G.gaz-002	217-217	099-099	244-244	210-210	239-239
G.gaz-004	217-217	099-099	244-244	192-192	239-241
G.gaz-006	217-217	099-099	244-244	210-210	239-241

DNA Bank ID	TGLA122	ILSTS005	BM757	BM143	CSSM39	CSSM43
G.mar-001	122-124	179-179	167-167	084-084	183-183	250-264
G.mar-002	122-122	179-179	167-167	084-084	183-183	252-260
G.mar-003	122-122	179-179	167-201	084-084	183-183	250-260
G.mar-004	122-124	179-179	167-201	084-084	183-183	250-264
G.mar-005	122-122	181-185	165-167	084-084	183-183	250-256
G.mar-006	124-124	181-185	165-167	084-084	183-183	252-252
G.mar-007	124-124	179-183	165-167	084-084	183-183	252-252
G.mar-008	124-124	179-179	167-201	084-084	183-183	252-252
G.mar-009	000-000	179-181	165-165	084-084	183-183	256-260
G.mar-011	126-126	179-179	165-167	084-084	183-183	264-264
G.mar-012	122-124	179-181	165-167	084-084	183-183	252-264
G.mar-015	122-124	181-181	165-167	084-084	183-183	250-256
G.mar-018	124-126	179-179	165-167	084-084	183-183	248-260
G.mar-019	122-126	179-179	165-167	084-084	183-183	248-250
G.mar-020	122-122	179-181	167-167	084-084	183-183	250-264
G.mar-021	124-126	179-181	165-167	084-084	183-183	248-260
G.mar-022	122-124	181-181	167-167	084-084	183-183	250-264
G.mar-023	122-126	179-181	165-167	084-084	183-183	260-264
G.mar-024	122-126	179-181	165-167	084-084	183-183	260-264
G.mar-025	124-126	179-179	165-167	084-084	183-183	250-264
G.mar-026	122-126	179-181	167-167	084-084	183-183	260-260
G.mar-027	122-124	179-181	165-167	084-084	183-183	260-260
G.mar-028	122-126	179-181	165-167	084-084	183-183	252-260
G.mar-029	124-126	181-181	165-165	084-084	183-183	248-252
G.mar-030	126-126	179-181	165-167	084-084	183-183	250-260

APPENDIX A (continued)

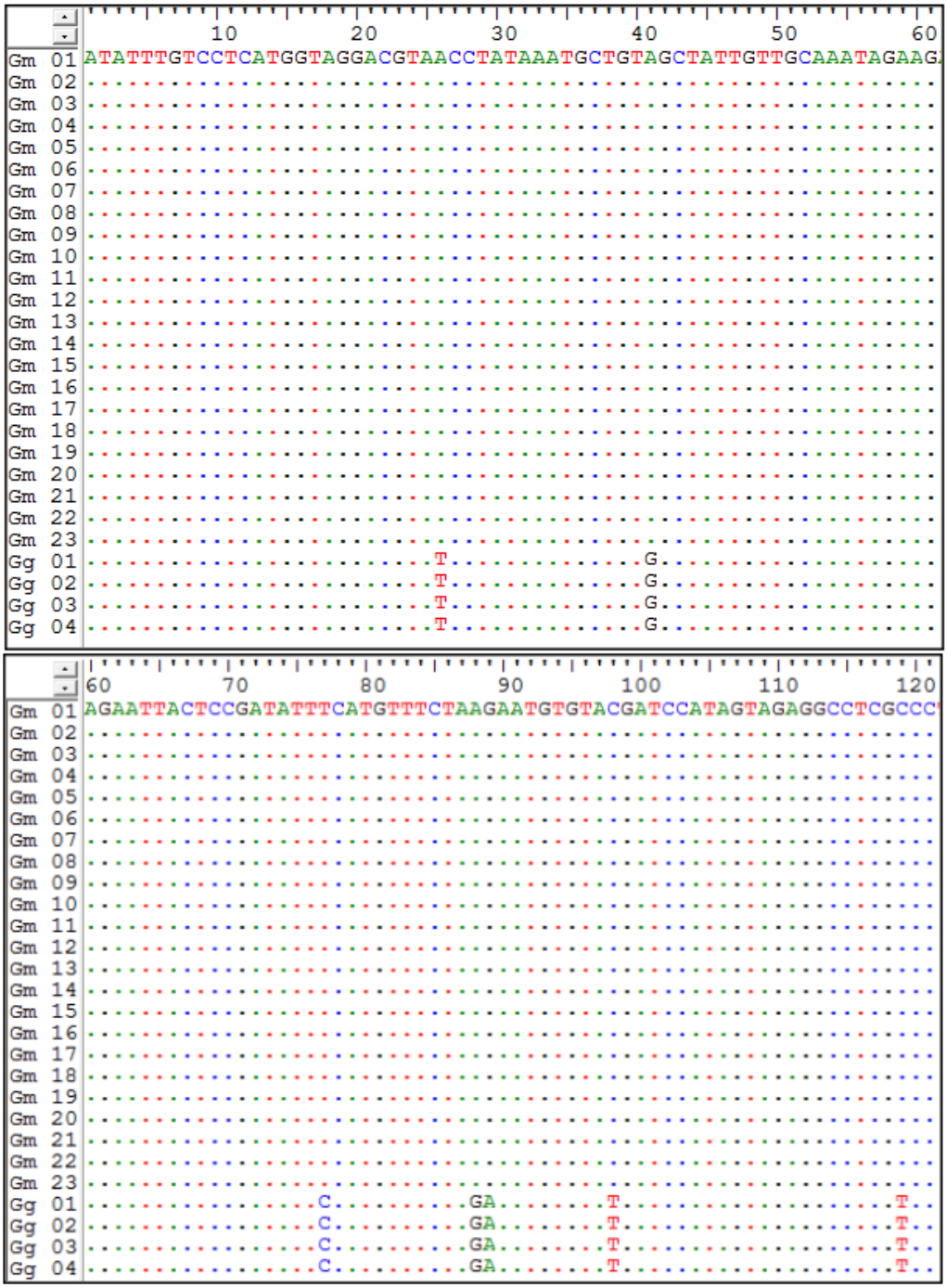
G.mar-031	122-126	179-179	165-167	084-084	183-183	250-262
G.mar-032	122-126	179-181	165-167	084-084	183-183	252-264
G.mar-033	122-126	179-179	165-167	084-084	183-183	248-264
G.mar-034	124-126	179-181	165-167	084-084	183-183	250-260
G.mar-035	122-122	179-181	167-167	084-084	183-183	248-248
G.mar-036	122-126	179-181	165-167	084-084	183-183	264-264
G.mar-037	122-126	179-181	165-167	084-084	183-183	256-260
G.mar-038	122-126	179-179	167-167	084-084	183-183	246-260
G.mar-039	122-124	179-179	165-167	084-084	183-183	250-250
G.mar-040	122-126	179-179	165-167	084-084	183-183	246-264
G.mar-041	122-126	179-179	165-167	084-084	183-183	246-250
G.mar-042	122-124	181-185	165-165	084-084	183-183	244-248
G.mar-043	122-126	179-181	165-167	084-084	183-183	252-260
G.mar-044	122-122	179-179	165-165	084-084	183-183	248-250
G.mar-045	122-126	179-181	167-167	084-084	183-183	250-256
G.mar-046	122-126	179-179	167-167	084-084	183-183	260-264
G.mar-047	126-126	181-181	165-165	084-084	183-183	248-256
G.mar-048	126-126	179-179	165-165	084-084	183-183	248-250
G.mar-049	122-124	177-177	163-163	084-084	183-183	248-250
G.mar-050	122-122	179-179	167-167	084-084	183-183	250-264
G.mar-051	122-126	179-179	165-165	084-084	183-183	262-264
G.mar-052	122-124	181-181	167-167	084-084	183-183	248-248
G.mar-053	126-126	179-181	165-165	084-084	183-183	250-264
G.mar-054	122-124	179-195	167-167	084-084	183-183	250-260
G.mar-055	122-122	181-195	165-167	084-084	181-183	264-264
G.mar-056	122-124	179-181	165-167	084-084	183-183	250-260
G.mar-057	122-126	179-179	165-167	084-084	183-183	260-260
G.mar-058	122-124	181-181	165-165	084-084	183-183	246-250
G.mar-059	122-122	179-181	167-167	084-084	183-183	246-262
G.mar-061	122-124	179-181	165-165	084-084	183-183	250-252
G.mar-062	122-124	179-181	165-165	084-084	183-183	246-264
G.mar-063	122-126	179-179	165-167	084-084	183-183	246-250
G.mar-064	122-122	179-179	167-167	084-084	183-183	250-250
G.mar-065	122-124	179-181	165-167	084-084	183-183	250-260
G.mar-066	122-122	181-195	165-165	084-084	183-183	250-260
G.mar-067	122-124	181-195	165-167	084-084	183-183	246-260
G.mar-068	122-122	179-195	167-167	084-084	183-183	260-260
G.mar-069	122-124	181-181	165-165	084-084	183-183	248-248
G.mar-070	122-124	179-179	167-167	084-084	183-183	250-252
G.mar-072	122-124	179-179	165-167	084-084	183-183	250-252
G.mar-073	122-122	179-181	165-167	084-084	183-183	246-264
G.mar-074	122-124	179-181	167-167	084-084	183-183	246-250
G.mar-075	122-122	179-195	165-167	084-084	183-183	260-264

APPENDIX A (continued)

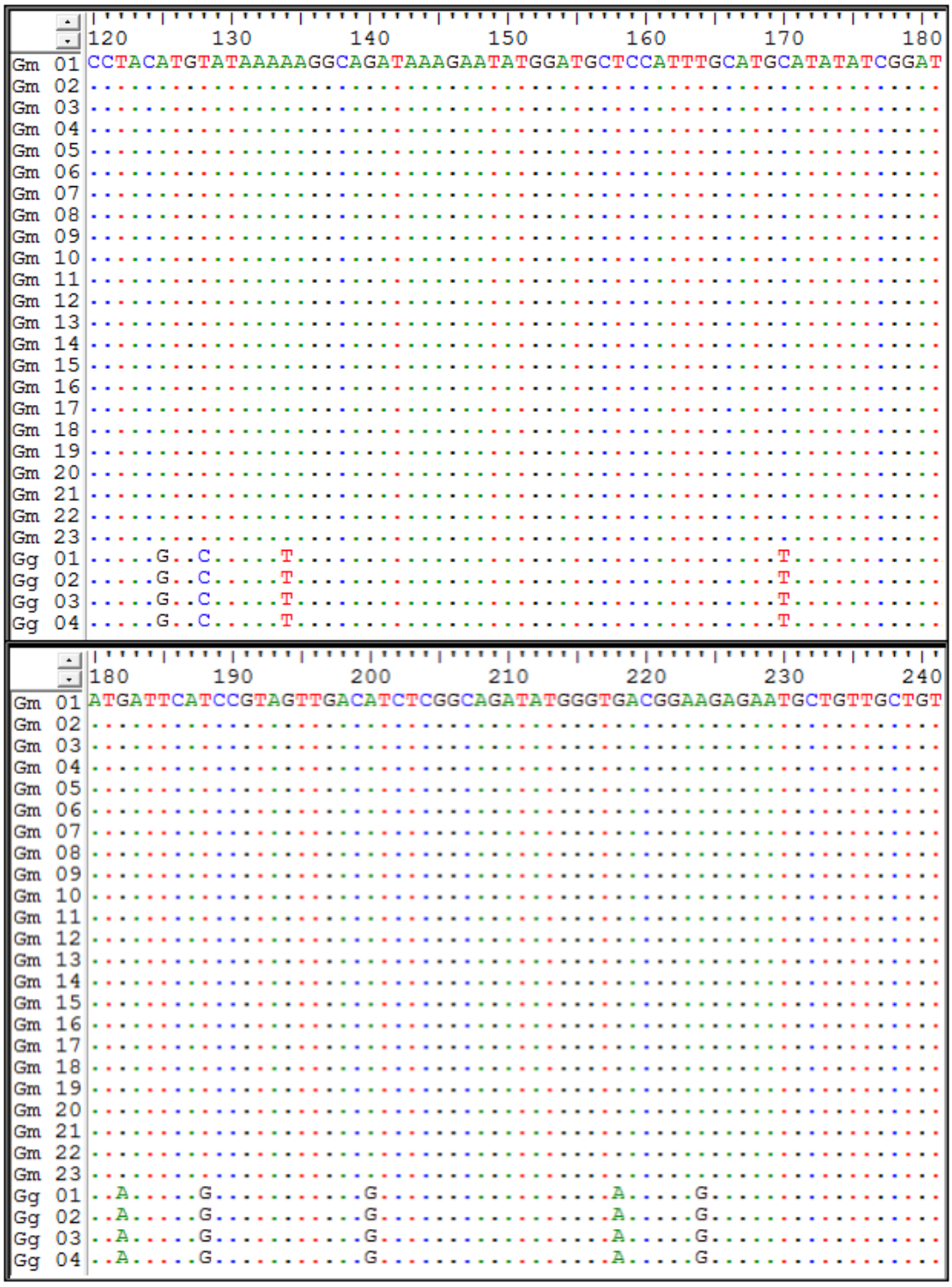
G.mar-076	122-124	179-195	167-167	084-084	183-183	250-260
G.mar-077	124-126	179-181	167-167	084-084	183-183	250-260
G.mar-078	122-124	179-179	165-165	084-084	181-183	248-250
G.mar-079	122-122	179-179	165-167	084-084	181-183	250-250
G.mar-080	122-122	179-195	165-167	084-084	183-183	252-262
G.gaz-001	124-124	181-189	159-159	084-144	177-177	262-262
G.gaz-002	124-124	189-189	159-159	084-144	177-177	262-262
G.gaz-004	124-124	181-181	159-159	084-144	177-177	262-262
G.gaz-006	124-124	181-189	159-159	084-144	177-177	262-262

APPENDIX B

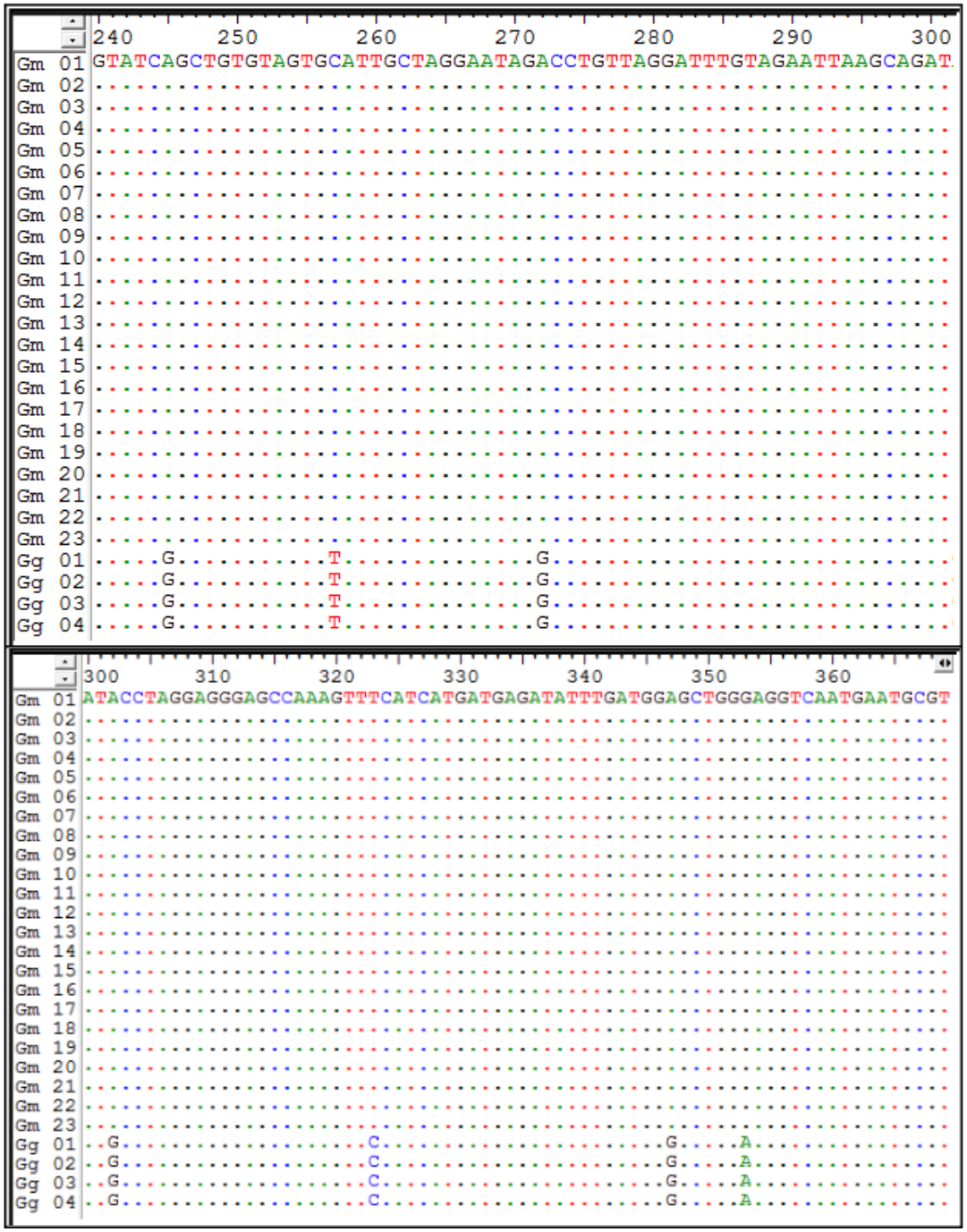
THE SEQUENCES OF SAMPLES OBTAINED



APPENDIX B (continued)



APPENDIX B (continued)



APPENDIX C

ESTIMATED PROBABILITY VALUES FOR F_{IS} BASED ON 960 RANDOMIZATIONS

Locus	Kızılkuyu (n:48)	Erikçe (n:25)	Hatay (n:4)
RT1	0.4927	0.8167	NA
ETH10	0.3188	0.8521	1.0000
OARFCB304	0.0354	0.4646	NA
MM12	0.6021	0.1677	NA
BM848	0.4073	0.3323	0.4271
BMC1009	0.8510	0.9542	1.0000
INRA40	0.1760	0.1688	NA
BM4505	0.0698	0.7354	NA
ETH152	NA	NA	0.0938
INRABERN172	0.9646	0.9927	1.0000
TGLA122	0.7427	0.9802	NA
ILSTS005	0.1938	0.7552	0.7625
BM757	0.7229	0.4198	NA
BM143	NA	NA	1.0000
CSSM39	NA	1.0000	NA
CSSM43	0.1896	0.3813	NA

APPENDIX D

THE P-VALUES OF LINKAGE DISEQUILIBRIUM

Kızılkuyu Population			Erikçe Population			Hatay Population		
Locus X Locus		P-values	Locus X Locus		P-values	Locus X Locus		P-values
RT1	ETH10	0,018	RT1	ETH10	0,1245	RT1	ETH10	1
RT1	FCB304	0,9649	RT1	FCB304	0,4581	RT1	FCB304	1
ETH10	FCB304	0,0016	ETH10	FCB304	0,5241	ETH10	FCB304	1
RT1	MM12	0,1955	RT1	MM12	0,014	RT1	MM12	1
ETH10	MM12	0,0115	ETH10	MM12	0,0825	ETH10	MM12	1
FCB304	MM12	0,0711	FCB304	MM12	0,5632	FCB304	MM12	1
RT1	BM848	0,0284	RT1	BM848	0,0927	RT1	BM848	1
ETH10	BM848	0,0651	ETH10	BM848	0,4133	ETH10	BM848	0,5411
FCB304	BM848	0,1713	FCB304	BM848	0,4828	FCB304	BM848	1
MM12	BM848	0,1205	MM12	BM848	0,4707	MM12	BM848	1
RT1	BMC1009	0,0394	RT1	BMC1009	0,0039	RT1	BMC1009	1
ETH10	BMC1009	0,0000	ETH10	BMC1009	0,0705	ETH10	BMC1009	0,2611
FCB304	BMC1009	0,0918	FCB304	BMC1009	0,0383	FCB304	BMC1009	1
MM12	BMC1009	0,0374	MM12	BMC1009	0,7055	MM12	BMC1009	1
BM848	BMC1009	0,0169	BM848	BMC1009	0,9278	BM848	BMC1009	0,8444
RT1	INRABERN172	0,2893	RT1	INRABERN172	0,3687	RT1	INRABERN172	1
ETH10	INRABERN172	0,0027	ETH10	INRABERN172	0,3207	ETH10	INRABERN172	1
FCB304	INRABERN172	0,0024	FCB304	INRABERN172	0,9171	FCB304	INRABERN172	1
MM12	INRABERN172	0,0079	MM12	INRABERN172	0,6143	MM12	INRABERN172	1
BM848	INRABERN172	0,1439	BM848	INRABERN172	0,2214	BM848	INRABERN172	1
BMC1009	INRABERN172	0,0799	BMC1009	INRABERN172	0,1609	BMC1009	INRABERN172	1
RT1	BM4505	0,3019	RT1	BM4505	0,6375	RT1	BM4505	1
ETH10	BM4505	0,0139	ETH10	BM4505	0,1699	ETH10	BM4505	1
FCB304	BM4505	0,0038	FCB304	BM4505	0,1537	FCB304	BM4505	1
MM12	BM4505	0,0048	MM12	BM4505	0,2146	MM12	BM4505	1
BM848	BM4505	0,0104	BM848	BM4505	0,3744	BM848	BM4505	1
BMC1009	BM4505	0,3336	BMC1009	BM4505	0,0152	BMC1009	BM4505	1
INRABERN172	BM4505	0,0039	INRABERN172	BM4505	1	INRABERN172	BM4505	1
RT1	ETH152	1	RT1	ETH152	1	RT1	ETH152	1
ETH10	ETH152	1	ETH10	ETH152	1	ETH10	ETH152	1
FCB304	ETH152	1	FCB304	ETH152	1	FCB304	ETH152	1

APPENDIX D (continued)

MM12	ETH152	1	MM12	ETH152	1	MM12	ETH152	1
BM848	ETH152	1	BM848	ETH152	1	BM848	ETH152	1
BMC1009	ETH152	1	BMC1009	ETH152	1	BMC1009	ETH152	1
INRABERN172	ETH152	1	INRABERN172	ETH152	1	INRABERN172	ETH152	1
BM4505	ETH152	1	BM4505	ETH152	1	BM4505	ETH152	1
RT1	INRABERN172	0,2853	RT1	INRABERN172	0,253666	RT1	INRABERN172	1
ETH10	INRABERN172	0,0818	ETH10	INRABERN172	0,61991	ETH10	INRABERN172	0,7465
FCB304	INRABERN172	0,4031	FCB304	INRABERN172	0,204252	FCB304	INRABERN172	1
MM12	INRABERN172	0,1689	MM12	INRABERN172	0,169854	MM12	INRABERN172	1
BM848	INRABERN172	0,1084	BM848	INRABERN172	0,436102	BM848	INRABERN172	0,0755
BMC1009	INRABERN172	0,2098	BMC1009	INRABERN172	0,733658	BMC1009	INRABERN172	0,1990
INRABERN172	INRABERN172	0,0082	INRABERN172	INRABERN172	0,301512	INRABERN172	INRABERN172	1
BM4505	INRABERN172	0,0001	BM4505	INRABERN172	0,58995	BM4505	INRABERN172	1
ETH152	INRABERN172	1	ETH152	INRABERN172	-	ETH152	INRABERN172	1
RT1	TGLA122	0,7035	RT1	TGLA122	0,1357	RT1	TGLA122	1
ETH10	TGLA122	0,0038	ETH10	TGLA122	0,5807	ETH10	TGLA122	1
FCB304	TGLA122	0,2345	FCB304	TGLA122	0,0078	FCB304	TGLA122	1
MM12	TGLA122	0,7932	MM12	TGLA122	0,0051	MM12	TGLA122	1
BM848	TGLA122	0,0985	BM848	TGLA122	0,4936	BM848	TGLA122	1
BMC1009	TGLA122	0,068	BMC1009	TGLA122	0,6843	BMC1009	TGLA122	1
INRABERN172	TGLA122	0,1957	INRABERN172	TGLA122	0,3525	INRABERN172	TGLA122	1
BM4505	TGLA122	0,5919	BM4505	TGLA122	0,0328	BM4505	TGLA122	1
ETH152	TGLA122	1	ETH152	TGLA122	0,0169	ETH152	TGLA122	1
INRABERN172	TGLA122	0,0005	INRABERN172	TGLA122	1	INRABERN172	TGLA122	1
RT1	ILSTS005	0,0881	RT1	ILSTS005	0,9921	RT1	ILSTS005	1
ETH10	ILSTS005	0,0374	ETH10	ILSTS005	0,208	ETH10	ILSTS005	1
FCB304	ILSTS005	0,0107	FCB304	ILSTS005	0,8054	FCB304	ILSTS005	0,5462
MM12	ILSTS005	0,0382	MM12	ILSTS005	0,4713	MM12	ILSTS005	1
BM848	ILSTS005	0,8039	BM848	ILSTS005	0,1429	BM848	ILSTS005	1
BMC1009	ILSTS005	0,1892	BMC1009	ILSTS005	0,4989	BMC1009	ILSTS005	0,6985
INRABERN172	ILSTS005	0,2893	INRABERN172	ILSTS005	0,7553	INRABERN172	ILSTS005	1
BM4505	ILSTS005	0,132456	BM4505	ILSTS005	0,3417	BM4505	ILSTS005	1
ETH152	ILSTS005	1	ETH152	ILSTS005	1	ETH152	ILSTS005	0,2970
INRABERN172	ILSTS005	0,0002	INRABERN172	ILSTS005	0,6254	INRABERN172	ILSTS005	1
TGLA122	ILSTS005	0,5631	TGLA122	ILSTS005	0,1372	TGLA122	ILSTS005	1
RT1	BM757	0,0699	RT1	BM757	0,0782	RT1	BM757	1
ETH10	BM757	0,0099	ETH10	BM757	0,1024	ETH10	BM757	0,1434
FCB304	BM757	0,0225	FCB304	BM757	0,0482	FCB304	BM757	1
MM12	BM757	0,0829	MM12	BM757	0,6242	MM12	BM757	1
BM848	BM757	0,0847	BM848	BM757	0,0679	BM848	BM757	1
BMC1009	BM757	0,0485	BMC1009	BM757	0,0449	BMC1009	BM757	1
INRABERN172	BM757	0,3982	INRABERN172	BM757	0,5053	INRABERN172	BM757	1

APPENDIX D (continued)

BM4505	BM757	0,1544	BM4505	BM757	1	BM4505	BM757	1
ETH152	BM757	1	ETH152	BM757	1	ETH152	BM757	1
INRABERN172	BM757	0,0709	INRABERN172	BM757	0,0559	INRABERN172	BM757	1
TGLA122	BM757	0,0046	TGLA122	BM757	0,2580	TGLA122	BM757	1
ILSTS005	BM757	0,0014	ILSTS005	BM757	0,0042	ILSTS005	BM757	1
RT1	BM143	1	RT1	BM143	0,1005	RT1	BM143	1
ETH10	BM143	1	ETH10	BM143	0,6605	ETH10	BM143	1
FCB304	BM143	1	FCB304	BM143	0,0012	FCB304	BM143	1
MM12	BM143	1	MM12	BM143	0,3082	MM12	BM143	1
BM848	BM143	1	BM848	BM143	0,0219	BM848	BM143	0,4734
BMC1009	BM143	1	BMC1009	BM143	0,3994	BMC1009	BM143	1
INRABERN172	BM143	1	INRABERN172	BM143	0,5802	INRABERN172	BM143	1
BM4505	BM143	1	BM4505	BM143	1	BM4505	BM143	0,8797
ETH152	BM143	1	ETH152	BM143	0,0054	ETH152	BM143	0,5724
INRABERN172	BM143	1	INRABERN172	BM143	0,7155	INRABERN172	BM143	1
TGLA122	BM143	1	TGLA122	BM143	0,1165	TGLA122	BM143	1
ILSTS005	BM143	1	ILSTS005	BM143	1	ILSTS005	BM143	1
BM757	BM143	1	BM757	BM143	1	BM757	BM143	0,8095
RT1	CSSM39	1	RT1	CSSM39	1	RT1	CSSM39	1
ETH10	CSSM39	1	ETH10	CSSM39	1	ETH10	CSSM39	0,8827
FCB304	CSSM39	1	FCB304	CSSM39	1	FCB304	CSSM39	1
MM12	CSSM39	1	MM12	CSSM39	1	MM12	CSSM39	1
BM848	CSSM39	1	BM848	CSSM39	1	BM848	CSSM39	1
BMC1009	CSSM39	1	BMC1009	CSSM39	1	BMC1009	CSSM39	1
INRABERN172	CSSM39	1	INRABERN172	CSSM39	1	INRABERN172	CSSM39	1
BM4505	CSSM39	1	BM4505	CSSM39	1	BM4505	CSSM39	1
ETH152	CSSM39	1	ETH152	CSSM39	1	ETH152	CSSM39	1
INRABERN172	CSSM39	1	INRABERN172	CSSM39	1	INRABERN172	CSSM39	1
TGLA122	CSSM39	1	TGLA122	CSSM39	1	TGLA122	CSSM39	1
ILSTS005	CSSM39	1	ILSTS005	CSSM39	1	ILSTS005	CSSM39	1
BM757	CSSM39	1	BM757	CSSM39	1	BM757	CSSM39	1
BM143	CSSM39	1	BM143	CSSM39	1	BM143	CSSM39	1
RT1	CSSM43	0,8245	RT1	CSSM43	0,2617	RT1	CSSM43	1
ETH10	CSSM43	0,2908	ETH10	CSSM43	0,7354	ETH10	CSSM43	1
FCB304	CSSM43	0,7707	FCB304	CSSM43	0,7139	FCB304	CSSM43	1
MM12	CSSM43	0,4995	MM12	CSSM43	0,6462	MM12	CSSM43	1
BM848	CSSM43	0,0039	BM848	CSSM43	0,2354	BM848	CSSM43	1
BMC1009	CSSM43	0,1042	BMC1009	CSSM43	0,5579	BMC1009	CSSM43	1
INRABERN172	CSSM43	0,0075	INRABERN172	CSSM43	0,629	INRABERN172	CSSM43	1
BM4505	CSSM43	0,1956	BM4505	CSSM43	0,2244	BM4505	CSSM43	1
ETH152	CSSM43	1	ETH152	CSSM43	1	ETH152	CSSM43	1
INRABERN172	CSSM43	0,3988	INRABERN172	CSSM43	0,3336	INRABERN172	CSSM43	1

APPENDIX D (continued)

TGLA122	CSSM43	0,1199	TGLA122	CSSM43	0,6497	TGLA122	CSSM43	1
ILSTS005	CSSM43	0,0239	ILSTS005	CSSM43	0,7996	ILSTS005	CSSM43	1
BM757	CSSM43	0,7947	BM757	CSSM43	0,2909	BM757	CSSM43	1
BM143	CSSM43	1	BM143	CSSM43	1	BM143	CSSM43	1
CSSM39	CSSM43	1	CSSM39	CSSM43	0,0024	CSSM39	CSSM43	1