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Genetic Diversity of Tendürek Mouflon Population

Tendürek Müflon Populasyonunun Genetik Çeşitliliği

Balal SADEGHI

Food Hygiene and Public Health Department, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran

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ORCID IDs of the authors: B.S. 0000-0002-8284-3022.

Abstract

Genetic diversity is an important factor influencing a population's capability of long term survival. Studying biodiversity is a basic approach to make suitable decisions to protect the endangered species. For this purpose, 75 biological samples were collected from Tendürek National Park mouflon population. High quality genomic DNA was extracted from blood, meat, hair and bone samples. A set of ten microsatellite loci were selected for analysis of genetic variability. Based on PCR and electrophoresis results, all studied markers were found polymorphic. Across the 10 microsatellite loci, total 61 alleles with an average number of 6.1 allele per locus were detected. The highest number of observed alleles belonged to MAF36 locus (8 allele) and the lowest was for OarFCB304 and OarHH47 loci (5 allele). Mean observed heterozygosity and mean expected heterozygosity were 0.5842 and 0.7849 respectively. Mean Fixation index (FST) in this study was 0.2508. Results revealed that inbreeding in Tendürek mouflon population has increased to dangerously high levels. These findings indicate the reduction of genetic diversity in this population that is so necessary for survival of this species.

Keywords: Biodiversity, Tendürek, mouflon, microsatellite

Öz

Genetik çeşitlilik, bir populasyonun uzun süreli hayatta kalma kabiliyetini etkileyen önemli bir faktördür. Biyoçeşitliliğin çalışılması, nesli tükenmekte olan türlerin korunması için uygun kararların alınmasındaki temel yaklaşımdır. Bu amaçla, Tendürek Milli Parkı müflon populasyonundan 75 adet biyolojik örnek toplandı. Kan, et, yapağı ve kemik örneklerinden yüksek nitelikli genomik DNA ekstrakte edildi. Genetik çeşitlilik analizi için 10 mikrosatelit lokusluk bir dizin seçildi. PCR ve elektroforez sonuçlarına göre çalışılan tüm belirteçlerin polimorfik olduğu bulundu. On mikrosatelit lokusu boyunca her bir lokus için ortalama 6,1 alel içeren toplam 61 alel saptandı. Gözlenen en yüksek allel sayısı (8 alel) MAF36 lokusuna; en düşüğü (5 alel) ise OarFCB304 ve OarHH47 lokusuna ait idi. Gözlenen ve beklenen ortalama heterozigosite değerleri sırasıyla 0,5842 ve 0,7849 olarak belirlendi. Çalışmada, ortalama fiksasyon indeksi (FST) 0,2508 idi. Sonuçlara göre Tendürek müflon populasyonunda soy içi eşleşmenin tehlikeli derecede yüksek oranlara ulaştığı gözlendi. Bulgular, türün hayatta kalması için oldukça büyük önem taşıyan genetik çeşitliliğin bu populasyonda azaldığını işaret etmektedir.

Anahtar kelimeler: Biyoçeşitlilik, Tendürek, müflon, mikrosatelit

Address for Correspondence: Balal SADEGHI • **E-mail:** sadeghi.balal@uk.ac.ir

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Introduction

The increasing rate of habitat destruction and loss of animal species have prompted a new global vision for wildlife and habitat conservation (Bulte and Rondeau, 2007). Iran as a large country in the Middle East with varied ecosystems is one of the most important area for conservation of biological diversity (NBSAP, 2000). As a result of uncontrolled hunting and habitat destruction, Iran has already lost two of its most worthy carnivores, the Persian Lion and the Caspian Tiger (NBSAP, 2000). Some other valuable species are threatened which demonstrate the urgent need for conservation measures (NBSAP, 2000).

However, one way of conserving biological diversity is inclusion of environmental concerns in all national and regional development policies (Mace and Baillie, 2007). For this goal, the national parks are unique areas of keeping biodiversity in natural ecosystems. Relatively vast natural areas having specific characteristics and national significance from the geological, ecological, biogeographical, and scenic areas points of view are selected as national parks with purpose of maintaining the biological and natural conditions, improvement of the population of animal species and vegetation sites and also recreational utilization. National parks are suitable places for educational and research activities as well as ecotourism. In order to fundamentally protect the biodiversity, genetic reserves, ecological integrity and scenic areas, consumer and residential utilizations are prohibited in these areas. Stronger legislative supports are provisioned for national parks as compared with other protected areas for the same reason. Such areas are certain reservations for protecting many wild species and help researchers to study the process of changing ecosystems (NBSAP, 2000). National Parks serve dual functions of conservation and ecotourism, and are typically selected as outstanding examples of ecological biodiversity, and geological scenic resources that are of national and global importance (Leopold et al., 1963).

Tendürek National Park which is located at North-East of Iran has an area level of 37080 hectare. This park is the habitat of many wild species including animals and plants. Iranian wild sheep or mouflon *(Ovis orientalis)* is one of these attractive species (NBSAP, 2000).

Sheep breeds have evolved over thousands of years probably from mouflons. Natural selection has made considerable diversity in appearance, size, shape and color, adaptability, disease resistance, reproductive performance and weight gain of sheep breeds (Pardeshi et al., 2007).

Little is known about the actual numbers of Iranian mouflons. It has been estimated that about 5000–6000 mouflons are living in Tendürek national park. Mouflon's height is between 85 to 100 cm at the shoulder and it weighs around 35 to 80 kg. Females are smaller than males (Valdez, 1976; Bon et al., 1993). The gestation period is between 150–180 days and single or twin lambs are born in mid-April to early May in Tendürek. Their life span is about 10–11 years. Their powerful sight, hearing and sense of smell are all actually developed. They are excessively wary, depending upon early detection of approaching danger and fight for their survival (Ptak et al., 2002).

The microsatellites have become a standard method for estimating genetic diversity (Diez-Tascón et al., 2000; Arranz et al., 2001; Peter et al., 2007). Allele number is a measure of genetic diversity that has a direct impact on breed development within a species (Buchanan et al., 1994). High levels of polymorphism coupled with the ease of analysis of the PCR makes microsatellites as one of the most widely used method for genetic analysis. This marker also has been widely used in construction of linkage maps in many species (Crawford et al., 1995; Kappes et al., 1997; de Gortari et al., 1998; Maddox et al., 2001). Microsatellites have been recommended by the Food and Agriculture Organization (FAO) of the United Nations for characterization of animal biodiversity (Baumung et al. 2004). Our study is based on the genotypes of 10 autosomal microsatellites in Tendürek National Park's Mouflons for studying of biodiversity in order to make suitable decisions to protect this species.

Materials and Methods

Study area

The studied population was distributed over 308 km^2 of Tendürek National Park which is located at North-East highlands of Iran (37°29′E to 37°33′E, 58°33′N to 58°54′N) in Khorasan Razavi province near to Turkmenistan border (Figure 1).

Sample collection

Biological samples (blood, meat, hair and bone) were collected from 75 individuals of legally harvested Iranian mouflon in three geographic regions of Tendürek National Park during 2 years from same herd (2013-2014). Hair samples were placed into individual envelopes. Small pieces of tissue samples were placed into cryovials containing desiccant beads. Samples were stored at -20°C as soon as possible.

DNA extraction

Two commercial kits; DNA IQ™ System kit (Promerga, USA) and DNA SV Genomics Purification Kit (Promerga, USA); were used for DNA extraction according to the manufacturer's instruction. The quantity and quality of extracted DNA was assessed using NanoDrop ND1000 spectrophotometer (USA) and electrophoresis in 0.8% agarose gels. Then DNA samples were diluted to 10 nanograms per mL concentration using TE buffer (10mM Tris-HCl, pH 8.0, 0.1mM EDTA).

Microsatellite genotyping

Genotypes of 10 microsatellite markers were determined for all samples. The used microsatellite markers are listed in Table 1. The microsatellite markers were recommended by International Society of Animal Genetics (ISAG) under FAO's MoDAD program for sheep diversity studies (http://dad.fao.org/).

Table 1. Microsatellite markers used in this study

Locus	Accession no.	Chromosome	Ta	Primer
OarFCB128	L01532	2	64	F: CAGCTGAGCAACTAAGACATACATGCG
				R: ATTAAAGCATCTTCTCTTTATTCCTCGC
McM527	McM527	5	63	F: GTCCATTGCCTCAAATCAATTC
				R: AAACCACTEACTACTCCCCAA
OarFCB304	L01535	19	67	F: CCCTAGGAGCTTTCAATAAAGAATCGG
				R: CGCTGCTGTCAACTGGGTCAGGG
MAF36	M80519	22	65.5	F: TTGCGAAAGTTGGACACAATTGAGC
				R: CATATACCTGGGAGGAATGCATTACG
MAF65	M67437	15	64	F: AAAGGCCAGAGTATGCAATTAGGAG
				R: CCACTCCTCCTGAGAATATAACATG
MAF214	M88160	16	69.5	F: GGGTGATCAGGGAGGTTTTGGAGG
				R: AATGCAGGAGATCTGAGGCGGACG
OarHH47	L12557	18	63	F: TITATTGACAAACTCTCTTCCTAACTCCACC
				R: GTAGTTATITAMAAAATATCATACCTCTTAAGG
CSSM031	U03838	23	56.5	F: CCAAGTITAGTACTTGTAAGTAGA
				R: GACTCTCTAGCACTITATCTGTGT
BM8125	G18475	17	63	F: CTCTATCTGTGGAAAAGGTGGG
				R: GGGGGTTAGACTTCAACATACG
OarCP34	U15699	3	66	F: GCTGAACAATGTGATATGTTCAGG
				R: GGGACAATACTGTCTTAGATGCTGC

Table 2. True and effective number of alleles

Locus	Size Range	*na	**ne	Shannon index (H')
OarFCB128	100-128	6	4.9901	1.6733
McM527	160-184	6	4.8254	1.6796
OarFCB304	154-191	5	4.4193	1.5370
MaF36	84-116	8	5.0314	1.7231
MaF65	160-184	6	4.4897	1.6297
MaF214	175-267	6	4.1961	1.5744
OarHH47	126-146	5	4.1977	1.5121
CSSM031	136-171	7	4.8033	1.7056
BM8125	108-120	6	4.1614	1.5490
OarCP34	105-133	6	4.5072	1.5779
Mean		6.0	4.5622	1.6162
SD		0.8756	0.3311	0.0758

*na = Observed number of alleles
**ne = Effective number of alleles

Table 3. The observed (Ho), Expected (He) and average heterozygosity for different loci

Locus	H_o	H_e	Average Het.	Fis
OarFCB128	0.7763	0.8049	0.7996	-0.2367
McM527	0.6579	0.7980	0.7928	-0.2568
OarFCB304	0.4605	0.7788	0.7737	0.0932
MaF36	0.5658	0.8066	0.8012	-0.0263
MaF65	0.6184	0.7824	0.7773	-0.2359
MaF214	0.2237	0.7667	0.7617	0.5312
OarHH47	0.6316	0.7668	0.7618	-0.3232
CSSM031	0.6711	0.7971	0.7918	-0.2854
BM8125	0.6053	0.7647	0.7597	-0.2764
OarCP34	0.6316	0.7833	0.7881	-0.2623
Mean	0.5842	0.7849	0.7798	-0.1312
SD	0.1497	0.0160	0.0159	

Individual microsatellites were genotyped by polymerase chain reaction (PCR). All primer sets were amplified in separated reactions. Primer sequences, size ranges, multiplexing information and PCR protocols of the markers are available from the FAO website (http://dad.fao.org/en/home.htm) which ranks markers by typing efficiency (e.g. PCR-amplification, scoring reliability and lack of ambiguity) and shown in Table 1. PCR amplification was accomplish in a final volume of 25 mL containing 75 mM Tris–HCl (pH 8.8), 1 unit of Platinum Taq DNA Polymerase (Invitrogen, USA), 0.2 mM each of dATP, dCTP, dGTP, dTTP (Pharmacia, Uppsala, Sweden), 1.5 mM MgCl2, 10 pmol of primers and 10 ng of DNA template. According to MoDAD project,

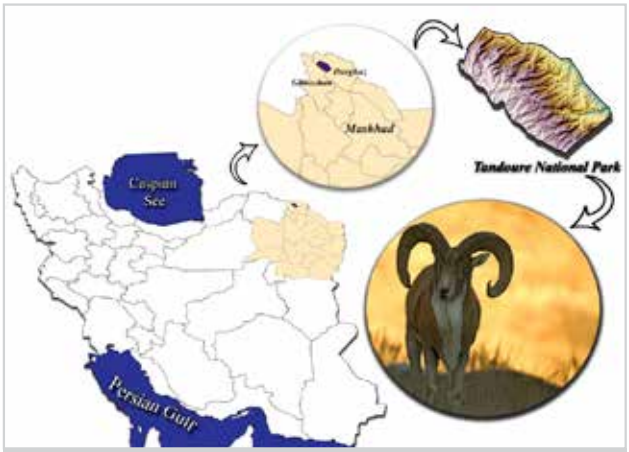

Figure 1. Geographic position of Tendürek National Park in Iran

thermocycler conditions were: initial denaturation at 94°C for 3 min, 30 cycles of 30 s at 94°C, 75 s at the annealing temperature (Table 1) and followed by final extension for 5 min at 72°C. PCR was performed with no extension step based on to MoDAD project data. Genotyping was performed by electrophoresis on denaturing 8% polyacrylamide gels at 75 W (Bio-Rad, USA) and visualized by silver staining. Allele sizes were estimated using 10-bp ladder (Invitrogen, USA).

Statistical analysis

Once genotypes were determined that allelic frequencies calculated. Allelic frequencies for each primer in this population were computed simply by dividing the counts for each allele by the total number of alleles found in that population (for that certain primer). Different measurements of within breed genetic variations like observed and expected heterozygosity, Shannon index and polymorphic parameters (the number of actual alleles and the number of effective alleles) were calculated using POPGENE software package (Yeh et al., 1999).

Using POPGENE intrabreed genetic variation was estimated on the basis of observed heterozygosity (Ho) and mean unbiased estimates of gene diversity (He) (Nei 1978). To assess the population's genetic structure, all F-statistics parameters (Weir and Cockerham, 1984) were estimated using POPGENE computer program.

Results

Microsatellite loci

All 10 microsatellites were amplified in the designed PCR reactions. All studied microsatellite loci were polymorphic. Size ranges of amplified alleles were completely in agreement with FAO report (2004). Allele number is a measure of genetic diversity that has a direct impact on breed development within a species (Buchanan et al., 1994). At the 10 microsatellites, a total of 61 alleles were detected. The mean allele number per locus was 6.1, ranging from 5 (OarFCB304, OarHH47) to 8 (MaF36) (Table 2). Also the highest mean effective number of alleles was for MaF36 (5.0314) (Table 2). Two of independent tests for Hardy-Weinberg equilibrium were rejected at $p<0.05$. Excess of homozygotes in MaF214 caused its deviation from Hardy–Weinberg equilibrium (HWE) ($p<0.05$). On the other hand, slight excess of homozygotes in OarFCB304 did not affect HW proportions and this locus was still in HWE ($p<0.05$). In other studied loci there was not any excess of homozygotes therefore no significant departure ($p<0.05$) from HWE proportions was revealed in these loci. In general, observed HWE deviations were not consistent.

Genetic variation

Observed heterozygosity (H_o) varied from 0.224 (MaF214) to 0.776 (OarFCB128) with an average about 0.584 while the average expected heterozygosity (H_e, gene diversity) for all loci was 0.785 with variation between 0.765 (BM8125) and 0.807 (MaF36). MaF36 displayed the highest level of intrapopulation variation in terms of expected heterozygosity while BM8125 was slightly less variable than the other studied loci. The average mean values for various genetic diversity measures, suggested that all the studied loci contained high level of genetic variability. All loci were in relatively same level of within-breed diversity in terms of H_e ($p<0.05$).

High levels of expected heterozygosity can be attributed to some factors such level of inbreeding, low selection pressure and high allele number. Since wild sheep breed naturally and no intentional control made on their reproduction process, so no artificial selection pressure or inbreeding can be imagined.

Within-population inbreeding estimates (Fis) were positive in 2 loci (MaF214 and OarFCB304) and significantly different from zero ($p<0.05$) but were negative in other 8 studied loci. Although the present samples do not allow for examination of Mendelian inheritance of the microsatellite alleles, the results indicate that the deficiency of heterozygotes at MaF214 and OarFCB304 microsatellites could be due to the presence of non-amplifying null alleles. Positive f estimates for 2 studied microsatellites could be assumed as an indication for inbreeding.

Although it is difficult to envisage the exact basis of this departure, however, the presence of low frequency null alleles segregating at these loci may be the possible reason as described by Peter et al. (2007). This deviation could also be linked to fairly high positive Fis (within-population inbreeding estimate) values (Mukesh et al. 2004; Mukesh et al., 2006) observed in the investigated population ($p<0.05$, Table 3). The shortage of heterozygotes and excess of homozygotes (Fis >0) exhibited by the investigated population might be attributed to a number of factors such sample relatedness, population heterogeneity or null alleles (Nei 1987; Peter et al. 2005). However, the foremost reason for significant Fis values in this population seems to be relatedness of few samples under range conditions.

Heterozygote deficiency analysis revealed that one of the studied loci exhibited significant deviations from HWE ($p<0.05$). It may be due to the presence of low frequency null alleles segregating at these loci as described by Peter et al. (2005). This lack of deviation could also be linked to fairly high negative Fis (within-population inbreeding estimate) values (Mukesh et al. 2004) observed in this study ($p<0.05$).

Discussion

From the demographic structure of these breed, it is apparent that rams breed with some of the ewes in the flock, as the rams and ewes grazed together thereby no controlled mating can be imagined. On the other hand, in industrial husbandry systems generally few rams breed with all the ewes in the flock and using related individuals for reproduction may cause high heterozygote deficiency observed in those systems. Generally, small sample size in this study is a cause of individual relatedness in the sample and it might be responsible for heterozygote deficiency observed in this study.

Major efforts caused by unsuitable management practices to recover individuals and to assure intentional breeding mating can induce an undesirable loss of genetic diversity, (Goyache et al. 2003).

Since the studied samples were collected from one region and the size of samples was small, hidden genetic structure could not be ruled out.

The present data also display high allelic variation in Iranian mouflon as represented by mean expected heterozygosity (ranging from 0.765 to 0.807). Direct comparison of diversity estimates presented in different studies is complicated by differences in marker sets and number of analyzed individuals.

However, findings of the present study of high variability of the Iranian mouflons found in this study is in agreement with previous sheep diversity studies based on analysis of autosomal microsatellites and show the existence of a genetic diversity 'hot spot' for wild mouflon in the northeast of Iran. Domestic sheep breeds originating from the Near East, and surrounding areas such as the Caucasian and southeast European regions, typically display elevated levels of genetic variation because they have retained more variation from the ancestral wild species, mouflon (*Ovis orientalis*) (Tapio et al. 2006; Peter et al. 2007).

Several distinct phenotypes of the Iranian mouflon have been developed in distinct geographical regions and their classification was based on their phenotypic traits. Here we provide new information on the genetic diversity of the Iranian mouflon by analyzing autosomal microsatellite loci.

In conclusion, values of genetic variation indicated that Tendürek mouflon population harbor distinct and estimable reservoirs of diversity. It can be inferred from our analysis that breed descriptions are often due to geographical borders and management history, in addition to effective phenotypic and particularly genetic distinctiveness.

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