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Genomic Characteristics and Diversity of Iranian Indigenous Goats

B. Mahmoudi^{1*}, M. Sh. Babayev², F. Hayeri Khiavi¹, A. Pourhosein¹ and M. Daliri³

¹Islamic Azad University, Meshkinshahr Branch, Meshkinshahr, Ardabil, Iran.

²Department of genetic, Faculty of Biology, Baku State University, Baku, Azerbaijan.

³Department of Animal Science, Genetic Engineering and Biotechnology Institute, Tehran, Iran.

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Investigation of genetic relationship among populations was traditionally based on the analysis of allele frequencies at different loci. The aim of this study was to analyze, the genetic diversity and variability of three native Iranian goat populations (Raeini, Korke Jonub Khorasan and Lori) through the use of 13 microsatellite markers. The 13 tested loci were all polymorphic in the three goat populations. Within the 13 polymorphic loci, allele frequencies, number of effective alleles (Ne), heterozygosity (He), polymorphism information content (PIC) and Nei's standard genetic distance (D) were calculated, and UPGMA phylogenetic tree was constructed based on allele frequencies. The average number of alleles was 7.57, ranging from 3 to 13 at the 13 assessed loci. The average values of Ne, He and PIC of all loci were 5.14, 0.797 and 0.757 respectively. Korke Jonub Khorasan showed the highest mean number of alleles (8.15), while the highest value for polymorphic information content was observed for Raeini population (0.78). Tests of genotype frequencies for deviation from the Hardy-Weinberg equilibrium (HWE), had been tested in the level of probability ($p < 0.005$). A UPGMA diagram based on Nei's standard genetic distances, yielded relationships between populations that agreed with what is known about their origin, history and geographical distribution.

Key words: Hardy-Weinberg equilibrium (HWE), goat, diversity, microsatellite.

INTRODUCTION

The maintenance of genetic diversity in livestock species requires the adequate implementation of conservation priorities and sustainable management programs, which should be based on comprehensive information regarding the structure of the populations, including sources of genetic variability among and within breeds. Species are the most recognized and protected units of biodiversity. Yet we tend to ignore the importance of biodiversity that is fundamental to new species (Crawford and Littlejohn, 1998). Genetic diversity is shaped by past population processes and affects the sustainability of species and populations in the future (Soule, 1987). The maintenance of genetic diversity is a key to the long-term survival of most species (Hall and Bradley, 1995). Farm animal genetic diversity is required to meet current production needs in various environments, to allow sustained genetic improvement, and to facilitate rapid adaptation to

changing breeding objective (Crawford and Littlejohn, 1998; Kumar et al., 2006). Genetic variation between and within breed is described as diversity. It is essential to characterize a breed for its conservation. Microsatellites are ideal molecular markers for characterization. This study attempted to analyze the diversity of three goat populations in the Iran by using thirteen microsatellites as molecular markers, so as to help breeders to implement rational decisions for conservation and improvement of valuable germplasm.

If genetic diversity is very low, none of the individuals in a population may have the characteristics needed to cope with the new environmental conditions or challenges. Such a population could be suddenly wiped out. Low amounts of genetic diversity increase the vulnerability of populations to catastrophic events such as disease outbreaks. Low genetic diversity may also indicate high levels of inbreeding with its associated problems of expression of deleterious alleles or loss of over-dominance. Change in the distribution of the pattern of genetic diversity can destroy local adaptations and break up co-adapted gene complexes. These problems combine to lead to a

*Corresponding author. E-mail: Bizhan.mahmoudi@gmail.com
Tel/Fax: +984525224293.

poorer 'match' of the population to its habitat increasing and eventually leading to the probability of population or species extinction. Microsatellite markers, also known as simple sequence repeats (SSRs) or short tandem repeats (STRs), are regions of DNA that exhibit short repetitive sequence motifs. Because of their high degree of polymorphism, random distribution across the genotypes, microsatellite markers have been proved to be one of the most powerful tools for evaluating genetic diversity and estimating genetic distances among closely populations of ruminant species (Moore et al., 1991; Buchanan et al., 1994; Ellegren et al., 1997). Microsatellite are highly polymorphic and randomly markers are the simple sequence motif not more than six bases long, that is tandemly repeated for example (dC-dA)_n. Microsatellite being polymorphic, they provide extremely useful markers for comparative study of genetic variation, parentage control, linkage map analysis and could well be the marker of choice for analysis of population structure in domestic species. There are close similarities between cattle, sheep and goat chromosomes (Crawford et al., 1994; Kemp et al., 1995; Vaiman et al., 1996).

Microsatellite markers present in all three species could be amplified with the same primer pair, so microsatellite markers developed in cattle and sheep also work in goats (Vaiman et al., 1996) and they can be used for the analysis of genetic diversity (Saitbekova et al., 1999). Indigenous livestock breeds are considered, for diverse reasons, as treasured genetic resources that tend to disappear as a result of new market demands, crossbreeding or breed replacement, and mechanized agricultural operations. There is a terrible risk that most breeds may perish before they have been exclusively recognized and exploited. The existence of a large gene pool is important for the potential future breeding preservation and for the development of a sustainable animal production system. Comprehensive knowledge of the existing genetic variability is the first step for the conservation and exploitation of domestic animal biodiversity (Li et al., 2002). The three distinct goat populations of Iran: Raeini, Korki Jonob Khorasan and Lori mainly distributed in Kerman, Khorasan and Lorestan province respectively. The breeds were phenotypically characterized but their genetic characterization was pending. Hence, this present study is aimed to characterize Lori, Korki Jonob Khorasan and Raeini goats (Figures 1A, B and C) at molecular level using microsatellite markers with the following objectives:

- 1) To estimate the alleles and allelic frequencies of different microsatellite gene loci and to establish a microsatellite profile for Lori, Korki Jonob Khorasan and Raeini goat using thirteen polymorphic markers.
- 2) To estimate the percent heterozygosity and polymorphic information content (PIC).
- 3) To assess genetic variability and relationships within and among the three goat populations.

4) To analyse the population for Hardy-Weinberg equilibrium.

5) To determine time of divergence for all three populations.

MATERIALS AND METHODS

Blood samples from unrelated animals of Raeini (49) (Figure 1C), Korki Jonob Khorasan (51) (Figure 1B) and Lori (53) (Figure 1A) populations of goat were collected at random from their respective home tract. Then bleeding was transferred to laboratory (in an ice-cooled box, where they were kept under -20°C in a deep freezer until DNA isolation) and DNA genomic was extracted by salting out method (Miller et al., 1988). We use both spectrophotometry and agarose gel (0.8%) for DNA quality definition. Hence, this study used 13 microsatellite primer pairs including MAF64, BM4621, BM121, LSCV36, TGLA122, oarJMP23, oarFCB304, oarAE133, ILSTS005, ILSTS022, ILSTS029, ILSTS033 and ILSTS34. Most of the primers used were independent and belonged to different chromosomes. These loci in prior studies had been amplified on the goat (Maudet et al., 2001; Yang et al., 1999; Hanrahan et al., 1994; Dixit et al., 2008). They showed polymorphism in the goat of world. Thirteen microsatellite markers, their sequences, type of repeat, size range and their location is shown in Table 1. All PCR reactions were continued the following component: 200 μM dNTPs, 3.5 to 6 mM MgCl₂, 0.25 μM each of primer, 0.5 unit *Taq* DNA polymerase, 150 ng DNA. The final volume was 15 μl. Reactions were run on a thermal cycler (Biometra 96 block T-gradient, Germany). In this study annealing temperature was modified as follows: MAF64 (62.5°C), BM4621 (58°C), LSCV36 (55°C), oarFCB304 (60.5°C) and BM121 (65.5°C). The rest of PCR process is in accordance with Table 2. For oarJMP23 and TGLA122 primers used PCR program (Crawford et al., 1995), for oarAE133 used PCR program (Hanrahan et al., 1994) and for ILSTS005, ILSTS022, ILSTS029, ILSTS033 and ILSTS34 primers, the 'touchdown' PCR protocol was used. PCR products were separated on a 10% polyacrylamid gel and detected by silver staining and visualized under white light on a BIO-RAD Gel Doc XR system. The alleles and genotypic frequencies directly were identified from the gel. Hardy-Weinberg equilibrium (HWE) had been tested based on likelihood ratio for different locus-population combinations and observed number of alleles (N), effective number of alleles (Ne) and expected heterozygosity (He) were computed by the software POPGENE (Version 3.2).

Polymorphism information content (PIC) was computed according to Botstein et al. (1980). Nei's standard genetic distance were calculated by POPGENE (Version 3.2), a phylogenetic tree was constructed by unweighted pair group method with arithmetic mean (UPGMA) method based on pair wise Nei's standard distances using the same software by a bootstrapping method.

Data analysis

Genotypes were assigned for each animal based on allele size data. On the basis of allele and genotypic frequencies, a likelihood ratio test (G^2_T) was conducted to test for deviations from Hardy-Weinberg equilibrium (Guo and Thompson, 1992). The most common measures of genetic diversity such as allelic diversity, heterozygosity and proportion of polymorphic loci were considered. The effective number of alleles (estimates the reciprocal of homozygosity) was calculated according to Hartl and Clark (1989). Nei unbiased expected heterozygosity ($H_e = 1 - \sum p_i^2$; where p_i is the frequency of allele (i) were estimated for all loci (Nei, 1978). These parameters were statistically analyzed using POPGENE software



Figure 1A. Lori goats.



Figure 1B. Korki Jonub Khorasan goat.

package version 1.31 (Yeh et al., 1999). Polymorphism information content (PIC) (Botstein et al., 1980) values were estimated in order to assess the relevance of each locus for linkage. The Hardy-Weinberg expected heterozygosity also defined as "gene diversity" (Weir, 1996) or polymorphism index content (PIC) (Botstein et al., 1980), was obtained from observed allele frequencies.

RESULTS

All the markers were successfully amplified in all the populations. Each 13 loci were found to be polymorphic in all populations. Korki Jonub Khorasan populations do not show the deviation of Hardy-Weinberg equilibrium (HWE). Lori and Raeini in some of loci showed the deviation of Hardy-Weinberg equilibrium (HWE). Most and least unbiased expected heterozygosity is for K.J.K. (0.809) and Lori (0.778) respectively. The population statistics generated by the thirteen microsatellite markers in three goat populations is presented in Table 3. Yang et al. (1999) He value of oarFCB304 locus estimated 0.854 on Chinese goats but it was 0.708, 0.702 and 0.635 in Lori, K.J.K. and Raeini goats populations respectively.



Figure 1C. Raeini goat.

The number of observed alleles for each locus ranged from 3 to 13. Highest number of allele's objective for oarJMP23 locus with the Raeini and for TGLA122 locus with the K.J.K. goats. Highest and lowest number of allele effective was 8.7 and 2.6 for oarJMP23 locus in Raeini and oarAE133 locus in K.J.K., respectively. All the average number of allele objective and effective was 7.67 and 5.14 respectively. Highest and lowest PIC value was 0.778 and 0.725 for Raeini and Lori respectively; it was between 0.746 to 0.8 in Chinese goats (Yang et al., 1999). The average expected heterozygosity overall loci in Raeini, Lori and K.J.K. are 0.805, 0.778 and 0.809, respectively. The mean effective number of alleles, polymorphism information content and expected heterozygosity over all populations were 5.14, 0.797 and 0.755, respectively. No significant difference in the number of alleles, Ne, He and PIC was found between these goat populations.

Among the three populations, K.J.K. populations displayed the highest values for mean He and Raeini PIC, while the Lori populations showed lower variability levels (Table 3). The standard genetic distances were calculated for these populations. The closest distance was observed between Raeini and K.J.K. ($D = 0.4891$) and the largest between Raeini and Lori ($D = 0.6298$), (Table 4; Figure 2).

DISCUSSION

The study of genetic variation plays an important role in developing rational breeding strategies for economical animal species (Maudet et al., 2002). The advantage of the use of microsatellites for estimating genetic variations among breed and among closely related populations has been investigated in farm animals such as, water buffalo (Barker et al., 1997; Moiola et al., 2001; Kumar et al., 2006), cattle (MacHugh et al., 1997), sheep (Gutierrez-Gil et al., 2006) and goat (Barker et al., 2001; Maudet et al., 2002). In the present study thirteen microsatellite loci were used to evaluate the genetic diversity within and

Table 1. Microsatellite markers, their sequences, type of repeat, size rang and location.

Locus	Primer sequence	Type of repeat	Size range	Chromosome No.
BM121	TGGCATTGTGAAAAGAAGTAAAA CTAGCACTATCTGGCAAGCA	(TC) ₁₈	165-185	16
BM4621	CAAATTGACTTATCCTTGGCTG TGTAACATATGGGCTGCATC	(CA) ₁₄	106-148	6
ILSTS005	GGAAGCAATGAAATCTATAGCC TGTTCTGTGAGTTTGTAAAGC	(nn) ₃₉	174-190	10
ILSTS022	AGTCTGAAGGCCTGAGAACC CTTACAGTCCTTGGGGTTGC	(GT) ₂₁	186-202	Ann
ILSTS029	TGTTTTGATGGAACACAGCC TGGATTTAGACCAGGGTTGG	(CA) ₁₉	148-191	3
ILSTS033	TATTAGAGTGGCTCAGTGCC ATGCAGACAGTTTTAGAGGG	(CA) ₁₂	151-187	12
ILSTS34	AAGGGTCTAAGTCCACTGGC GACCTGGTTTAGCAGAGAGC	(GT) ₂₉	153-185	5
LSCV36	GCACACACATACACAGAGATGCG AAAGAGGAAAGGGTTATGTCTGGA	(CA) ₁₆	524	19
MAF64	AATAGACCATTTCAGAGAAACGTTGAC CTCATCGAATCAGACAAAAGGTAGG	(TG) ₁₃	121-125	1
oarAE133	AGCCAGTAGGCCCTCACCAGG CCAACCATTGGCAGCGGGAGTGTGG	(TG) ₂₄	152	Ann
oarFCB304	CCCTAGGAGCTTTCAATAAAGAATCGG CGCTGCTGTCAACTGGGTCAGGG	(CT) ₁₁ (CA) ₁₅	119-169	Ann
oarJMP23	GSTATCTTGGGAGCCTGTGGTTTATC GTCCAGATGGGAATTGTCTCCAC	-	-	27
TGLA122	AATCACATGGCAAATAAGTACATAC CCCTCCTCCAGGTAAATCAGC	(CA) ₂₁	145	21

between Lori, K.J.K. and Raeini goat populations reared in Iran. The thirteen microsatellite are all polymorphic in the three goat populations. The use of microsatellites to evaluate the genetic diversity on the basis of allele frequency distribution has also been employed to differentiate between Italian, Greek and Egyptian buffalo populations (Moioli et al., 2001). The average expected heterozygosity overall loci in Lori, K.J.K. and Raeini are 0.778, 0.809 and 0.805 respectively. High value of average expects heterozygosity within the populations could be attributed to the large allele numbers detected in

the tested loci (Kalinowski, 2002). The average direct count of heterozygosity overall loci in each of the three goat populations is less than the expected heterozygosity. This finding is an evidence for the presence of overall loss in heterozygosity within the three tested goat populations (allele fixation) (De Araujo et al., 2006).

Test of genotype frequencies for deviation from HWE at each locus over all populations showed, Najdi goat populations except in two loci (LSCV41 and BM121), in other loci revealed significant departure from HWE. Deviatin from HWE at microsatellite loci have also been

Table 2. PCR reaction conditions for all loci exceptional TGLA122, oarJMP23 and oarAE133 loci.

Stage	PCR process	Temperature (°C)	Time
1	Denaturation	95	2.5 min
2	Denaturation	95	30 s
3	Annealing	-	30 s
4	Extension	72	30 s
5	Final extension	72	2.5 min
6	Maintenance	4	-

Table 3. Mean numbers of alleles per locus, Ne, He, PIC and related SD (Standard division) on three goat populations.

Population	Mean number of alleles	Ne	He	PIC
Raeini	7.85(2.67)	5.38(1.65)	0.80(0.07)	0.78(0.08)
Korki Jonub Khorasan	8.15(2.51)	5.34(1.51)	0.81(0.07)	0.76(0.09)
Lori	7.00(1.78)	4.7(1.21)	0.78(0.06)	0.73(0.08)

Table 4. Nei (1978) genetic distance (D) in three goat populations.

Goat populations	Raeini	Korki Jonub Khorasan
Raeini		
Korki Jonub Khorasan	0.5031	
Lori	0.6158	0.5831



Figure 2. UPGMA of 3 native goat populations by Nei (1978) genetic

reported in various studies (Braker et al., 2001; Hassan et al., 2003; Laval et al., 2000; Luikart et al., 1999). It is known that a population is considered to be within HWE only when it is able to maintain its relative allele frequencies. Heterozygosity deficiency is one of the parameters underlying departure from HWE. Heterozygosity deficiency may result from one or more of the following reasons:

- i) The presence of a null allele which is the allele that fails to multiply during PCR using a given microsatellite primer due to a mutation at the primer site (Callen et al., 1993; Pemberton et al., 1995);
- ii) Small sample size, where rare genotypes are likely to be included in the samples;
- iii) The Wahlund effect, that is presence of fewer heterozygotes in population than predicted on account of population subdivision;
- iv) The decrease in heterozygosity due to increased consanguinity (inbreeding) (Kumar, 2006).

The information obtained in this study will aid their rational development, utilization and conservation. The result of UPGMA was consistent with the background of the origin, history and geographical location of these populations. The UPGMA tree shows that two goat populations (K.J.K. and Raeini) are distinct from the other goat population (Lori). The close kinship between Najdi and Tali might suggest some past crossing between these two geographically close populations. However, only a small number of microsatellite loci populations were analyzed. Additional markers and samples are required to increase the accuracy of the results.

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