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Genetic Diversity among Three Goat Populations Assessed by Microsatellite DNA Markers in Iran

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Abstract: The genomes of three native goat populations were screened by using microsatellite as molecular markers; the populations were Markhoz, Najdi and Tali goats. A total of 13 microsatellite markers were used and genetic diversities and genetic distances were also determined. The thirteen tested loci were all polymorphic in the three goat populations. Within the thirteen 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 7.3, ranging from two to eleven at the thirteen assessed loci. The average values of Ne, He and PIC of all loci were 4.764, 0.759 and 0.720 respectively. The Markhoz population showed the highest variability (PIC=0.767, He=0.799). Tests of genotype frequencies for deviation from the Hardy-Weinberg equilibrium (HWE), had been tested in the level of probability ($p<0.005$). An unweighted pair group method with arithmetic means (UPGMA) diagram based on Nei's standard genetic distances, yielded relationships between populations that agreed with what known about their origin, history and geographical distribution. It was concluded that microsatellite technique is a useful tool for evaluation of genetic variation among of domesticated animals.

Key words: Microsatellite · HWE · Biodiversity · Goat · Genetic

INTRODUCTION

Species are the most recognized and protected units of biodiversity, yet if the importance of biodiversity that is fundamental to new species is ignored [1]. Genetic diversity is shaped by past population processes and affects the sustainability of species and populations in the future [2]. The maintenance of genetic diversity is a key to the long-term survival of most species [3]. 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 [1,4]. 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 [5]. 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. Also, low genetic diversity may 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

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complexes. These problems combine to lead to a 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 snd estimating genetic distances among closely populations of ruminant species [6-8]. There are close similarities between cattle, sheep and goat chromosomes [9-11]. Microsatellite markers present in all three specice could be amplified with the same primer paire, so microsatellite markers developed in cattle and sheep also work in goats [11] and they can be used for the analysis of genetic diversity [12].

Indigenous livestock breeds are considered, for diverse reasons, as treasured genetic resorces that tend to disappear as a result of new market demands, crossbreeding or breed replacement and mechanized agricultural operations. There is terrible risk that most breed 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 knowlege of the existing genetic variability is the first step for the conservation and exploition of domestic animal bidaiversity [5].

This study attempted to analyze the diversity of three goat populations in the Iran by using thirteen microsatellite as molecular markers, so as to help breeders to implement rational decisions for conservation and improvement of valuable germ plasm.

MATERIAL AND METHODS

Characterizations of Three Native Goat Populations: The Markhoz is mainly used for wool, which is sold as mohair. Markhoz was originally kept in the province of Kordestan. The Markhoz goats are medium-sized and mostly are black, white and chocolate brown colored. Natural service is method of breeding for this goat. The male and female have horns. Height at shoulder and body weight is 60cm and 45kg in adult male and 50cm and 35kg in adult female goat, respectively (Figure 1.A). The Najdi goats are medium-sized and adapted to extremely high temperatures, grey, dark brown, brown with a black back line colored. This goat was originally kept in the province of Khouzestan. Height at shoulder is 70 and 60cm in adult male and female goat, respectively. This goat is polled (Figure 1.B). The Tali goats are medium-sized and mostly are brown or light brown colored. Most animals is polled. Natural service is method of breeding for this goat. Its main distribution areas are the coastal region of Hormozgan province along the gulf and in some parts of Boushehre province especially near the towns of Minab, Bandarabbas, Khamir, Bandarlengeh and on Qeshm Island in the Strait of Hormuz. The male and most of female have horns. Height at shoulder is 76cm in adult male and 68cm in adult female goat (Figure 1.C).

Blood samples were collected from (146) goats the by puncturing the jugular vein in the vacationer's tubes having EDTA as blood anticoagulants were cooled, transferred to laboratory (in an ice-cooled bo and they were kept under-20°C in a deep freezer until DNA isolation) and DNA genomic was extracted by salting out method [13]. both spectrophotometery and agarose gel (0.8%) were used for DNA quality definition.

In this study, 13 microsatellite primer pairs were used, including MAF64, BM4621, BM121, LSCV36, TGLA122, oarJMP23, oarFCB304, oarAE133, ILSTS005, ILSTS022, ILSTS029, ILSTS033 and ILSTS34. Most of primers used were independent and belonged to different chromosomes. These loci in prior studies had been amplified on the goat [14-17]. They showed polymorphism in the goat of world. Thirteen microsatellite markers, their sequences, type of repeat, size rang and their locations are showed in Table 1.

All PCR reactions were continued the following componenet: 200µM dNTPs, 3.5-6mM $MgCl_2$, 0.25µM each of primer, 0.5 units *Taq* DNA polymerase, 150ng DNA. The final volume was 15µl. Reactions were run on a thermal cycler (Biometra 96 block T-gradient,Germany). In this study anealing temperature was modified as folowing: 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 the Table 2.

For oarJMP23 and TGLA122 primers were used PCR program was used [18], for oarAE133 PCR program was used [16] and For ILSTS005, ILSTS022, ILSTS029, ILSTS033 and ILSTS34 primers, The 'touchdown' PCR protocol was used.

(A) (B)

(C)

Fig. 1A-C: A. Markhoz Goats, B. Najdi Goat, C. Tali Goat

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 TGTTCTGTGAGTTTGTAAGC	$(nn)_{39}$	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	AATAGACCATTCAGAGAAACGTTGAC CTCATCGAATCAGACAAAAGGTAGG	$(TG)_{13}$	121-125	1
oarAE133	AGCCAGTAGGCCCTCACCAGG CCAACCATTGGCAGCGGGAGTGTGG	$(TG)_{24}$	152	Ann
oarFCB304	CCCTAGGAGCTTTCAATAAAGAATCGG CGCTGCTGTCAACTGGGTCAGGG	$(CT)_{11}(CA)_{15}$	119-169	Ann
oarJMP23	GTATCTTGGGAGCCTGTGGTTTATC GTCCCAGATGGGAATTGTCTCCAC	-	-	27
TGLA122	AATCACATGGCAAATAAGTACATAC CCCTCCTCCAGGTAAATCAGC	$(CA)_{21}$	145	21

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	30s
3	anealing	-	30s
4	extension	72	30s
5	Final extension	72	2.5 min
6	maintenance	4	-

The alleles and genotypic frequencies were identified directly 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 hetrozygosity (He) were computed by the software POPGENE (Version 3.2). Polymorphism information content (PIC) was computed according to Botstein *et al*. [19]. Nei's standard genetic distance were calculated by POPGENE (Version 3.2), a phylogenetic tree was constructed by 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 [20]. 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 [21]. Nei unbiased expected heterozygosity ($He=1-\Sigma p^2_i$ where p_i is the frequency of allele i) were estimated for all loci [22]. These parameters were statistically analyzed using POPGENE software package version 1.31 [23]. Polymorphism information content (PIC) [19]. values were estimated in order to assess the relevance of each locus for linkage.

RESULTS

All the markers were successfully amplified in all the populations. Each 13 loci were found to be polymorphic in all populations.

Morkhoz and Tali populations present no deviation of HWE in locus-population component at the level of probability ($p<0.005$). Najdi in two loci (LSCV41, BM121) showed the deviation of Hardy-Weinberg equilibrium (HWE).

Most and least unbiased expected hetrozygosity is for Markhoz (0.799) and Tali (0.736). The population statistics generated by the thirteen microsatellite markers in three goat populations is presented in Table 3.

The number of observed alleles for each locus ranged from 2 to 11. Highest number of allele's objective for oarJMP23 locus with the Tali goats and lowest number of allele's objective for oarAE133 locus with the Najdi goats.

Highest and lowest number of allele effective was 8.8 and 2 for oarJMP23 locus in Tali and oarAE133 locus in Najdi, respectively.

All average number of objective and effective alleles was 7.3 and 4.8, respectively. Highest and lowest PIC value was 0.767 and 0.688 for Markhoz and Najdi, respectively; it was between 0.746-0.8 in Chinese goats.

The average expected hetrozygosity overall loci in Tali, Markhoz and Najdi were 0.736, 0.799 and 0.741, respectively.

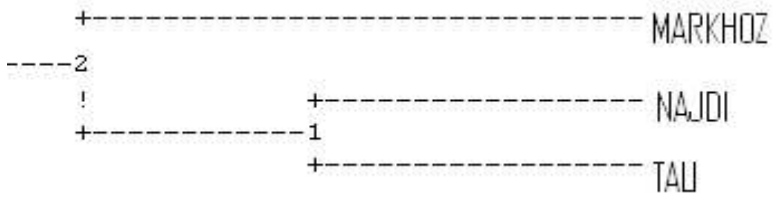

Fig. 2: UPGMA of 3 native goat populations by Nei's (1978) genetic distance

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

Population	Mean number of alleles	Ne	He	PIC
Markhoz	8.08(1.59)	5.262(1.84)	0.799((0.09)	0.767(0.08)
Najdi	6.46(2.47)	4.177(1.78)	0.741(0.12)	0.688(0.14)
Tali	7.38(2.26)	4.854(2.22)	0.736(0.11)	0.704(0.11)

He value of oarFCB304 locus estimated 0.854 on Chinese goats but it was 0.884, 0.541 and 0.543 in Markhoz, Najdi and Tali goats populations respectively.

Table 4: Nei's (1978) genetic distance (D) in three goat populations

goat populations	Markhoz	Najdi
Markhoz		
Najdi	0.4395	
Tali	0.4261	0.2708

The mean effective number of alleles, polymorphism information content and expected hetrozygosity over all populations were 4.764, 0.720 and 0.759, respectively. No significant difference in the number of alleles, Ne, He and PIC was found among these goat populations. Among the three populations, Tali populations displayed the highest values for both mean He and PIC, while the Najdi populations showed lower variability levels (Table 3).

The standard genetic distances were calculated for these populations. The closest distance was observed between Tali and Najdi (D=0.2708) and the largest between Markhoz and Najdi (D=0.4395), (Table 4; Figure 2).

DISCUSSION

The study of genetic variation plays an important role in developing rational breeding strategies for economical animal species [24]. The advantage of the use of microsatellites for estimation genetic variations among breed and among closely related populations has been investigated in farm animals such as, water buffalo [4,25,26], cattle [27], sheep [28] and goat [24,29].

In the present study, thirteen microsatellite loci were used to evaluate the genetic diversity within and between Markhoz, Najdi and Tali goat populations reared in Iran. The thirteen microsatellite are all polymorphic in the three goat populations. Major differences between the three goat populations were observed. The use of microsatellites to evaluate the genetic diversity on the basis of allel frequency distribution has also been employed to differentiate between Italian, Greek and Egyptain buffalo populations [26]. The average expected heterozygosity overall loci in Markhoz, Najdi and Tali were 0.799, 0.741 and 0.736, respectively. High value of average expects heterozygosity within the populations could be attributed to the large allele numbers detected in the tested loci [30]. 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) [31]. Test of genotype frequencies for deviation from HWE at each locus over all populations showed only Najdi goat population in some loci, revealed significant departure from HWE. Deviation from HWE at microsatellite loci have, also been reported in various studies [29,32,33]. It was known that a population is considered to be within HWE only when it is able to maintain its relative allele frequencies. Heterozygosis deficiency is one of the parameters underlying departure from HWE. Heterozygosis deficiency may results from one or more of the following reasons:

- 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 [34,35].
- Small sample size, where rare genotypes are likely to be included in the samples;
- The Wahlund effect, i.e. presence of fewer heterozygotes in population than predicted on account of population subdivision;
- The decrease in heterozygosity due to increased consanguinity (inbreeding) [4].

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 breeds. The UPGMA tree shows that two goat populations (Najdi and Tali) are distinct from the other goat population (Markhoz). 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. In conclusion this research shoed high variation within and between studied Iranian goat's populations for 13 microsatellite loci. It also demonstrated that microsatellite genotyping is a useful tool for evaluating variation evolutionary relationships among important goat populations. Microsatellite-based estimates of population relationships were consistent with know demographic history and geographic distances.

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