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Genetic Diversity of Lori Goat Population Based on Microsatellite Marker

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Abstract: The current study aiming at Lori goat population in Iran based on microsatellite markers was undertaken. Genetic variation at 13 microsatellite loci was examined in this goat. All of 13 loci (LSCV36, TGLA122, MAF64, oarFCB304, oarJMP23, oarAE133, BM121, BM4621, ILSTS005, ILSTS022, ILSTS029, ILSTS033 and ILSTS34) were amplified successfully. The objectives of this study were to assess the genetic variability among Lori goat populations. The genetic characterizations of this genetic resource are essential to conservation and breeding programs. The average number of alleles observed across the studied microsatellite loci was 7.00 and that of effective alleles was 4.70. The average expected heterozygosity values were 0.778. The mean polymorphic information content value (0.725) further reflected high level of polymorphism across the loci. Hardy-Weinberg Equilibrium (HWE) had been tested based on likelihood ratio for different locus by POPGENE software.

Key words: Lori goat, microsatellite, marker, polymorphism

INTRODUCTION

During the last century, the selection for production traits of the main livestock species has led to a reduction is number of local populations with consequent loss of genetic variability (Sechi *et al*., 2005). Goats comprise one of the most important domestic livestock species in Iran and play an important role in the livelihood of a larg proportion of small and marginal farmers and landless labourers. Since, the goat provides a good source of meat, milk, fiber and skin, it is popularly known as the poor man's cow. The Lori goats were originally kept in the province of Lorestan. The Lori goats are medium-sized and mostly are black. Natural service is method of Breeding for this goat. The male and female have horns (Fig. 1).

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. The use of microsatellite regions, segments of the nuclear genom composed of tandem repeats of short sequence motifs, is well established and accepted as a method for the study of genetic information content of animal population (Goldestein and Schlotterer, 2000). A large number of highly polymorphic microsatellites have been characterized and mapped in domestic animals, including sheep, cattle and other ruminants (De-Gortari *et al*., 1997; Hayes *et al*., 1996; Jenkins *et al*., 1997), facilitating the use of these markers for detailed investigation of the genetic structure of a

Fig. 1: A Lori goat

population. In the case of genetic variability, the analysis of allelic distribution at highly polymorphic microsatellite loci can be used to monitor the genetic structure of populations and to detect changes in the frequency of alleles due to breeding. That also is proven, the microsatellite approach can be applied to the creation of mating schemes aimed to increase genetic variability, reduce consanguinity and enhance fitness within flocks (Tomasco *et al*., 2002). Microsatellite are highly polymorphic and randomly markers are the simple sequence motif not more than six bases long, that is randomly repeated e.g., (dC-dA)n. Microsatellite being polymorphic, they provide extremely useful markers for comparitive study of genetic variation, parentage contorol, linkage map analysis and could well be the marker of choice for analysis of population structure in domestic species. 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). The aim of this study is to investigate the genetic variability of Lori goat population through the analysis of 13 microsatellite markers.

MATERIALS AND METHODS

The blood samples were collected from the 46 Animals by puncturing the jugular vein in the vacutainer tubes having EDTA as blood anticoagulant was coll. Then bleeding were transferred them 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. This study was conducted in 2008.

In this study was used 13 microsatellite primer pairs 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 (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 rang and their location are shown Table 1.

All PCR reactions were continued the following component: 200 μM dNTPs, 3.5-6 mM $MgCl_2$, 0.25 μM each of primer, 0.5 unit Taq DNA polymerase, 150 ng DNA. The final volume

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

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

was 15 μL. Reactions were run on a thermal cycler (Biometra 96 block T-gradient ,Germany). in this study anealing temperature was modified as following: MAF64 (62.5°C), BM4621 (58°C), LSCV36 (55°C), oar FCB304 (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 programe (Crawford *et al*., 1995), for oarAE133 was used PCR programe (Hanrahan *et al*., 1994) and For ILSTS005, ILSTS022, ILSTS029, ILSTS033 and ILSTS34 primers, The touchdown PCR protocol was used. The alleles and genotypic frequencies directly were identified from the gel (Fig. 2).

Hardy-Weinberg equilibrium (HWE) had been tested based on likelihood ratio for different locus and the number of observed and effective alleles by POPGENE software (Yeh *et al*., 1999). Polymorphic Information Content (PIC) were estimated by HET software (Ott, 1989).

RESULTS

The PCR reactions were successfully done on all thirteen primers. seven allele in the TGLA122 locus observed in the prior studies on wild goats (capra ibex) but ten allele in Lori goats were observed. For the 13 microsatellites loci analyzed, expected heterozygosity estimates were calculated after Nei (1973), as implement in the POPGENE software to

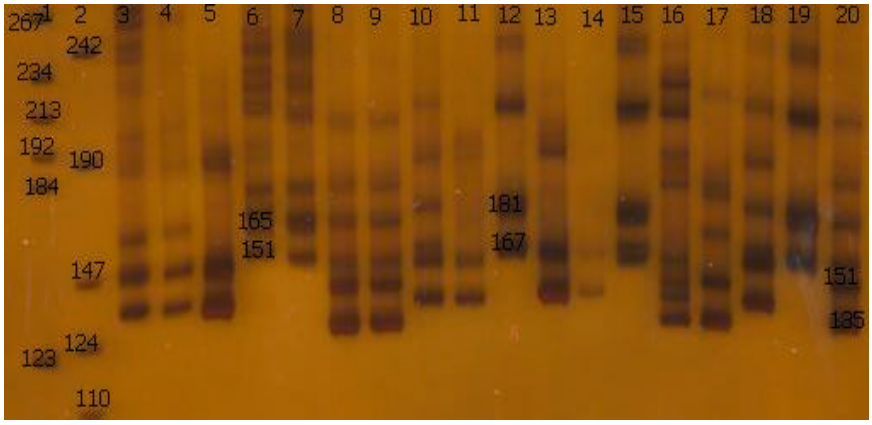

Fig. 2: Polyacrylamide nondenaturing gels (8%) showing alleles concerning oarJMP23 marker. DNA size markers are on wells 1, 2 . The alleles and sizes showed in bp

Table 3: n, n_e, H_e and PIC values at locus in Lori Goat population

Locus	n	n_e	H_e	PIC
BM121	7	5.3	0.836	0.793
BM4621	5	3.1	0.697	0.622
ILSTS005	8	5.1	0.805	0.801
ILSTS022	7	5.0	0.821	0.759
ILSTS029	6	4.3	0.799	0.742
ILSTS033	8	5.2	0.705	0.695
ILSTS34	9	6.6	0.785	0.708
LSCV36	8	4.5	0.798	0.751
MAF64	7	4.0	0.775	0.714
oarAE133	3	2.9	0.675	0.582
oarFCB304	6	3.2	0.708	0.618
oarJMP23	7	5.2	0.832	0.808
TGLA122	10	6.7	0.876	0.835
Mean	7	4.7	0.778	0.725
SD	1.78	1.21	0.060	0.080

determine genetic variation within the population. Heterozygosity is defined as the probability that a given individual randomly selected from a population will be heterozygous at a given locus. The observed and effective number of alleles was also calculated using POPGENE software (Kimura and Crow, 1964; Yeh *et al*., 1999). The tests for deviation from Hardy-Weinberg equilibrium were also derived using the exact test of POPGENE.

Number of allele (n), number of allele effective (n_e), expected the unbiased average of heterozygosity (H_e), and Polymorphic Information Content values (PIC) at locus showed in Table 3.

Yang *et al*. (1999) H_e value of oarFCB304 locus estimated 0.854 on Chinese goats but it was 0.708 in Lori goat.

Each 13 loci analysis was 100% polymorphic. Highest number of allele objective was 10 allele for TGLA122 locus and lowest number of allele objective was 3 allele for oarAE133 locus. Highest and lowest number of allele effective was 6.7 and 2.9 for TGLA122 and oarAE133 loci with, respectively.

All average the number of allele objective and effective was 7 and 4.70, respectively. Highest and lowest PIC value was 0.835 and 0.582 for TGLA122 and oarAE133, respectively.

The average of PIC value for this population was 0.725; it was between 0.746-0.8 in Chinese goats (Yang *et al*., 1999).

DISCUSSION

The Lori goats had substantial genetic variation based on their gene diversity and average number of alleles per locus. The average genetic variation (0.778) in Lori goats more than Indian indigenous goats breeds: Barbari, Jamnapari and Sirohi (Ganai and Yadav, 2001).

It also demonstrated that microsatellite genotyping is a useful tool for evaluating variation among important goat populations.

Test of genotype frequencies for deviation from HWE at each locus showed this goat population in several loci revealed significant departure from HWE. Deviation from HWE at microsatellite loci have, also been reported in various studies (Barker *et al*., 2001; 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. 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 (Callen *et al*., 1993; Pemberton *et al*., 1995)
- 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) (Kumar *et al*., 2006)

The result of this study suggests that there is substantial genetic variation and polymorphism across the studied loci in Lori goats. The study suggests scope for its further genetic improvement and to undertake appropriate breeding strategies to avoid inbreeding in the population. The information obtained in this study will aid their rational development, utilization and conservation.

This research presents an initial step in investigation of variability at the DNA level within elite breeding flock of Lori goat. The significance of this report is that it offers interesting perspectives for the incorporation of molecular genetic techniques to animal breeding in Iran. In addition the results of this study could provide basic molecular data for the research on germplasm characteristics of Lori goat.

Genetic markers are not only useful for measuring genetic distance between populations but they may also be used in measuring the similarity of individual genotypes with populations. Genetic similarity is a useful method of classifying individuals and populations based on marker genotype information. Further investigation is needed to study the exact properties of this new approach in populations of common origin and inbreed lines over generations.

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