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Genetic Diversity of Raeini Goat Population Based on Microsatellite

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Abstract: The current study aiming at Raeini 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 Raeini 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 7.84 and that of effective alleles was 5.38. The average expected heterozygosity values were 0.805. The mean polymorphic information content value (0.778) 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: Raeini goat, microsatellite markers, genetic variation, polymorphism, variability

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 speciese in Iran and play an important role in the livelihood of a large 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'. Raeini group of goats is represented in Africa by the Angora breed, which is native to the province of Ankara in Turkey from which it gets its name. Historical evidence shows that these goats were long used for their mohair. Not until 1838 was an attempt made outside Turkey to exploit their unique characteristics; in this year they were first imported into South Africa and developed since then for Mohair production. As one of the two types of fleece-bearing goats the Angora or mohair goat is of special interest. It is a lop-eared spiral-horned goat whose fleece has been selected in a manner parallel to that of the Merino sheep, i.e., by an increase in the number of secondary fibers and a reduction in the diameter and degree of medullation of the primary fibers (Fig. 1). The

Fig. 1: A Raeini goat

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predominant distribution area is the region of northern Kerman. However, because the breed is managed mainly under the nomadic system, flocks migrate to winter pastures, in the hot areas of southern Kerman. They also spend 4-5 months of the year in winter pastures in northern hormozgan province and western Sistan and Baluchistan Province.

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.

Microsatellite are highly polymorphic and randomly markers are the simple sequence motif not more than six bases long, that is tandomly 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 snd estimating genetic distances among closely populations of ruminant species (Moor *et al*., 1991; Buchanan *et al*., 1994; Ellegren *et al*., 1997).

The aim of this study is to study the genetic variability of Raeini goat population through the analysis of 13 microsatellite markers.

MATERIALS AND METHODS

The blood samples were collected from the 30 animals by puncturing the jugular vein in the vacutainer tubes having EDTA as blood anticoagulant were col. 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 metod (Miller *et al*., 1988). We use both spectrophotometry and agarose gel (0.8%) for DNA quality definition.

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 choromosomes. 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 showed Table 1.

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	-

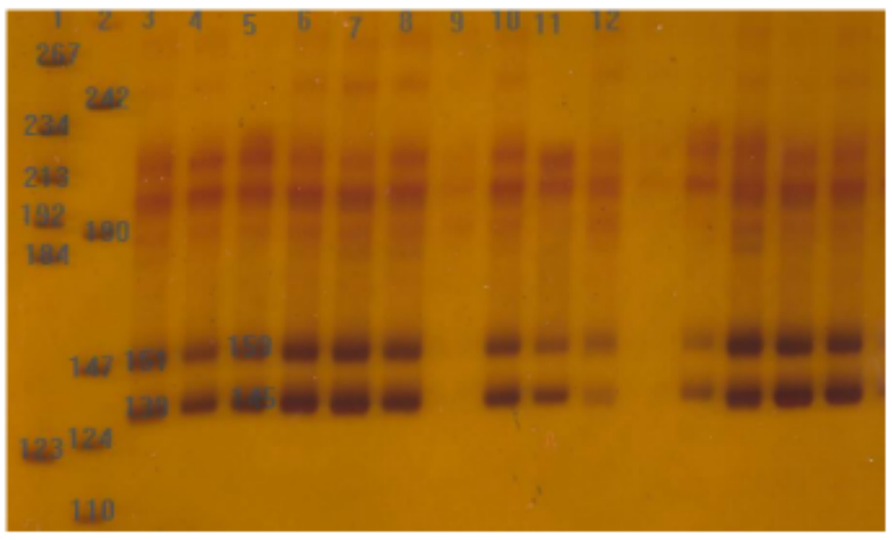

Fig. 2: Polyacrylamide nondenaturing gels (8%) showing alleles concerning oarAE133 marker. DNA size markers are on wells 1, 2. The alleles and sizes showed in bp

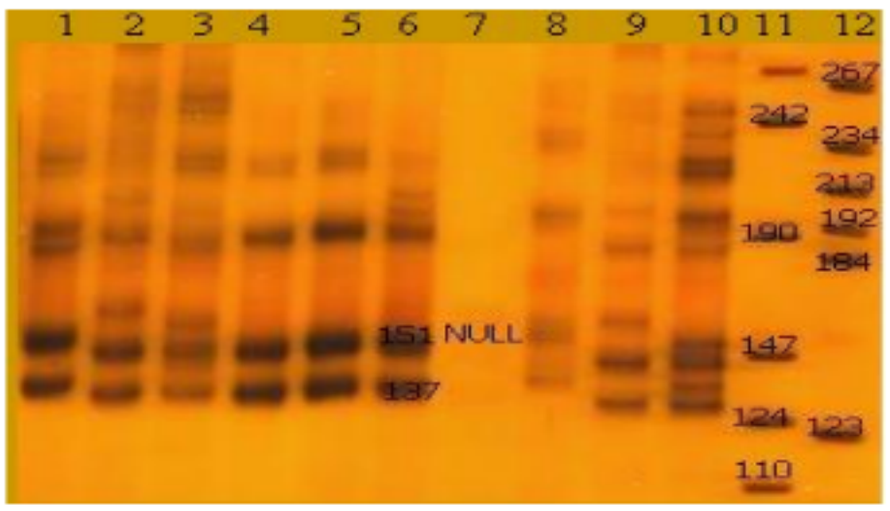

Fig. 3: Polyacrylamide nondenaturing gels (8%) showing alleles concerning MAF64 marker. DNA size markers are on wells 11, 12. The alleles and sizes showed in bp

All PCR reactions were continued the following componenet: 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 was 15 μL. 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 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 and 3).

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 softwar (Ott, 1989).

RESULTS AND DISCUSSION

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 eight allele in Raeini goats were observed. For the 13 microsatellites loci analyzed, expected heterozygosity estimates were calculated after Nei (1973), as implement in the POPGENE software to 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 (ne), expected the unbiased average of Heterozygosity (He) and Polymorphic Information Content values (PIC) at locus showed in Table 3.

Yang *et al*. (1999) He value of oarFCB304 locus estimated 0.854 on Chinese goats but it was 0.635 in Raeini goat.

Each 13 loci analysis was 100% polymorphic. Highest number of allele objective was 13 allele for oarJMP23 locus and lowest number of allele objective was 3 allele for oarFCB304 locus. Highest and lowest number of allele effective was 8.7 and 2.7 for oarJMP23 and oarFCB304 loci, with respectively.

All average the number of allele objective and effective was 7.84 and 5.38, respectively. Highest and lowest PIC value was 0.913 and 0.624 for oarJMP23 and oarAE133, respectively. The average of PIC value for this population was 0.778; it was between 0.746-0.8 in Chinese goats (Yang *et al*., 1999).

The Raeini goats had substantial genetic variation based on their gene diversity and average number of alleles per locus. The average genetic variation (0.805) in Raeini goats more than Indian indigenous goats breeds: Barbari, Jamnapari and Sirohi (Ganai and Yadav, 2001).

Table 3: n, n_e, H_e and PIC values at locus in Raeini goat population

Locus	n	n_e	H_e	PIC
BM121	12	7.9	0.888	0.866
BM4621	6	5.1	0.817	0.775
ILSTS005	9	6.1	0.782	0.725
ILSTS022	9	5.7	0.801	0.751
ILSTS029	8	6.0	0.831	0.782
ILSTS033	7	3.9	0.791	0.701
ILSTS34	8	5.2	0.841	0.752
LSCV36	8	5.2	0.821	0.785
MAF64	6	3.6	0.734	0.850
oarAE133	5	4.2	0.777	0.624
oarFCB304	3	2.7	0.635	0.702
oarJMP23	13	8.7	0.903	0.913
TGLA122	8	5.7	0.839	0.886
Mean±SD	7.84±2.67	5.38±1.65	0.805±0.07	0.778±0.08

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; 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. 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)

CONCLUSION

The result of this study suggests that there is substantial genetic variation and polymorphism across the studied loci in Raeini 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.

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