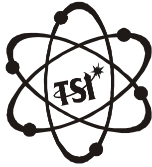
December 2008 Volume 2 Issue 3



Trade Science Inc.

BioTechnology

An Indian Journal

FULL PAPER

BTAIJ, 2(3), 2008 [142-145]

Analysis of the genetic structure of iranian indigenous raeni cashmere goat populations using microsatellite markers

Nahid Askari [1], **Amin Baghizadeh**[2]*, **Mohamadreza Mohamad Abadi**[3]

[1]Department of Animal Science, Ramin Agricultural and Natural Resources University of Ahvaz, (IRAN)

[2]International Center for Science, High Technology and Environmental Sciences, Kerman, (IRAN)

[3]Department of animal science, Faculty of Agriculture, Shaid Bahonar University of Kerman, (IRAN)

E-mail : baghizadeh@icst.ac.ir

Received: 24th September, 2008 ; Accepted: 29th September, 2008

ABSTRACT

In this paper, genetic diversity was evaluated in six Iranian indigenous Raeni Cashmere goat populations using eight microsatellite markers. Twenty individuals were randomly selected from each population. Total genomic DNA was extracted from the whole blood using optimized and modified salting-out method. The extracted DNA was amplified through polymerase chain reaction (PCR) of the eight microsatellite loci used in this study showed that all of the loci were polymorph within the six populations. Values of both polymorphic information content (PIC) and heterozygosity were high and the mean observed heterozigosity was 0.80±0.07. A Neighbor-joining (N-J) diagram based on Nei׳s genetic distances, yielded relationships of shared alleles between single individuals. The some specific alleles were shown to be important in the construction of the population structure and 19 specific alleles were shown to be important in the construction of the population structure. © 2008 Trade Science Inc. - INDIA

KEYWORDS

Raeini cashmere goat;
Microsatellite;
Polymorphism;
Heterozygosity.

1. INTRODUCTION

Raeni Cashmere goat is one of the most important breeds that spread in the southern area of Iran. Because the existence of a large gene pool is important for the potential future breeding preservation and for sustainable animal production system development, a concern about the conservation of genetic variability has arisen in the last years[7].

Microsatellite loci, because of their high degree of polymorphism, random distribution across the genome and possibility of automated scoring of genotypes, have been proven to be one of the most powerful tools for inferring genetic diversity[4].

Because of the genetic similarity among ungulate species, derived microsatellites developed in cattle and sheep also work in goat[13] and they can be used to analyse genetic diversity[14].

Biodiversity among domestic animals in developing countries is enormous. However, with the introduction of superior animals breeds with excellent performance, the native animal resources with good adaptability but lower productivity are in great danger (Mirhoseinie et al., 2005).

The purpose of this study was the genetic structure of the Raeni Cashmere goat group and it can be a model to be into account in controversies over breed recognitions in to the goat species, where the few levels of

TABLE 1 : Microsatellite markers used in this study of Raeni cashmere goats

Locus	Primer sequence	Ch. no*	Observed allele range (bp)	Ref.
MAF64	AAATACCCTATAAGGCACAGTACCAC	1	115-141	[3]
MAF64	CACCATGGCCACCTGGAATCAGG			
ILSTS034	AAGGGTCTAAGTCCACTTGGC	5	152-206	[8]
ILSTS034	GACCTGGTTTAGCAGAGAGC			
ILSTS059	AGTATGGTAAGGCCAAAGGG	13	160-197	[9]
ILSTS059	CGACTTGTGTTGTTCAAAGC			
BM1312	GGAATGTTACTGAACCTCTCCG	1	123-144	[5]
BM1312	CCATGTGCTGCAACTATGAC			
OraFCB20	AAATGTGTTTAAGATTCCATACATACATACAGTG	2	90-135	[3]
OraFCB20	GGAAAACCCCCATATATACCTATAC			
IL2RA	AGCAGAGGTACAGGTGGTAAGCA	13	132-146	[5]
IL2RA	GATATGCCTTGGAGAAGGTAGCGTAT			
LSCV11	CCTTCTGCTGAATATGCCAC	5	485-516	[14]
LSCV11	CACTATTTCATGCCCAAAATC			
LSCV24	CACAGAGAGGCAAACCCCTC	2	176-196	[14]
LSCV24	CTCAAGATAGTCCAGCCCAC			

*Ch. no: chromosome number

TABLE 2 : Measure of genetic variation at studied microsatellite loci: Raeni Cashmere goat

Locus	Sample size	na*	ne**	I***	PIC
MAF64	236	6	5.0929	1.7034	0.7757
BM1312	234	7	5.5805	1.8012	0.7962
LSCV24	238	6	4.4427	1.6114	0.7411
LSCV11	236	6	5.0140	1.6580	0.7697
OraFCB20	236	12	8.6350	2.3002	0.8737
ILSTS059	234	8	6.3170	1.9265	0.8216
IL2RA	238	6	4.1274	1.5912	0.7248
ILSTS034	232	9	6.9865	2.0641	0.8215
Mean	236	7.500	5.7745	1.8320	0.7905
St. Dev		2.138	1.4887	0.2497	

*na = Observed number of alleles; ** ne = Effective number of alleles; *** I = Shannon's Information index

intra-specific genetic diversity makes breed differentiation especially difficult.

2. MATERIALS AND METHODS

Blood sample were collected from 120 animals of mixed sex. They were obtained from jugular aseptic venipuncuture into vacutainers with EDTA as anticoagulant. Afterwards, they were frozen at -20^{0}C until required. DNA was extracted from the whole blood using optimized and modified salting-out method[10]. The selected microsatellites (TABLE 1) were amplified using Cinagen PCR Master Kit according to the instruction by the supplier. The reactions were carried out under the following conditions: 95^{0}C for 3 min followed by a hot- start and a touchdown of 1^{0}C for cycle from 60 to 53^{0}C; then 28 cycle of 95^{0}C for 45s, 53^{0}C for 45s and 72^{0}C for 1 min and a final extension step of min at 72^{0}C.

PCR products were run on 8% denaturing polyacrylamide gels using electrophoresis, 50bp ladder was used as a size standard for sizing PCR products. To visualize the PCR products, gels were stained using silver staining[2], the gels dried and scanned and then the genotypes were scored by UVIDOC software.

Allelic. Allelic frequencies, number of alleles for each locus and Shannon's Information index were calculated for each population using POPGENE ver.32[15]. Additionlly for eight microsatellite loci analyzed, observed and effective number of alleles was calculated in POPGENE software. Average expected theoretical heterozygosity from Hardy-Weinberg assumptions was calculated using the formula[6]:

$$H_e = 1 - \sum_{i=1}^{n} P_i^2$$

The test for deviation from Hardy-Weinberg equilibrium was derived using the exact test of POPGENE. Dendrogram resulting from a cluster analysis and graphic representation of all population were drawn by using NTSYS pc. ver 2.2. Polymorphic Information Content (PIC) was estimated by using HET software[12].

$$PIC = 1 - \left(\sum_{i=1}^{k} P_i^2\right) - \sum_{i=1}^{k-1} \sum_{j=i=1}^{k} 2P_i^2 P_j^2$$

3. RESULTS

The number of alleles observed across the

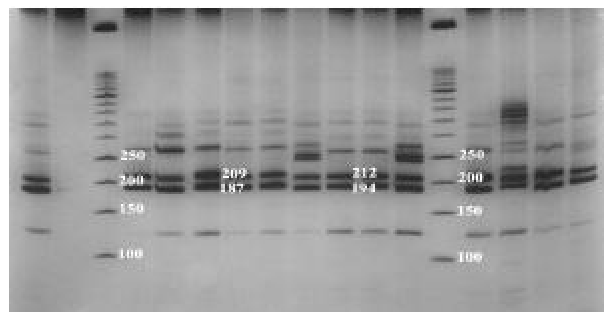


Figure 1: The PCR products obtained using ILSTS034 primer run on 8% denaturating page and stained by silver stain

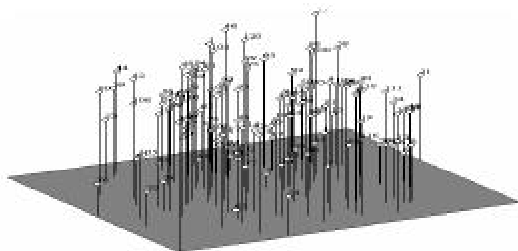


Figure 2: Correspondence analysis of allele frequencies from eight microsatellite loci genotyped in six Raeni cashmere goat population

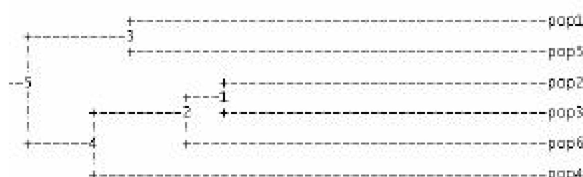


Figure 3: Dendogram depicting the relationship between six Raeni Cashmere goat populations

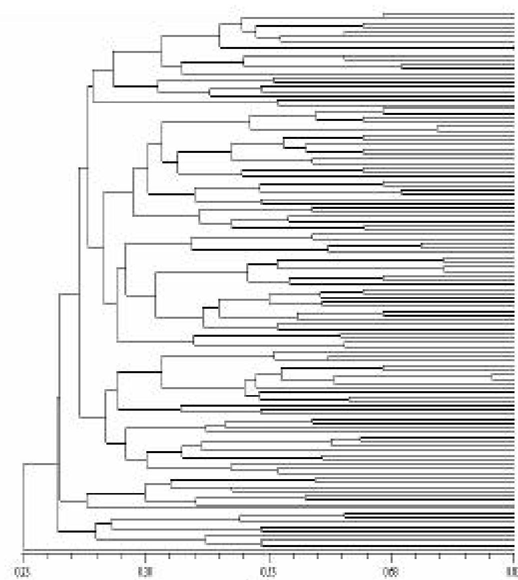


Figure 4: Neighbour-Joining tree of single individual

TABLE 3: Characterization of eight microsatellite markers in Raeni cashmere goat

Locus	H_o	H_e	H_{Nei}	H_{ave}
MAF64	0.7812	0.8071	0.8036	0.8036
BM1312	0.8162	0.8243	0.8208	0.8208
LSCV24	0.7715	0.7782	0.7749	0.7749
LSCV11	0.7922	0.8040	0.8004	0.8004
OraFCB20	0.8766	08880	0.8842	0.8842
ILSTS059	0.8205	0.8453	0.8417	0.8417
IL2RA	0.7607	0.7609	0.7577	0.7577
ILSTS034	0.8552	0.8606	0.8569	0.8569
Mean	0.8092	0.8210	0.8175	0.8175
St. Dev	0.0787	0.0424	0.0422	0.0422

microsatellite loci varied from 6 to 12 with an overall mean of 7.50±2.13 (TABLE 2). The Shannon's Information index (PIC) showed that all of the loci were highly informative indicating the polymorphism across the loci. The maximum and minimum of effective number of alleles were obtained at OraFCB20 (8.63) and IL2RA (4.12) respectively and the mean of effective number of allele was 5.77±1.48. An average of observed heterozygosity and PIC value were calculated from data 0.82±0.04 and 0.79 respectively.

Figure 1 showed the PCR products at ILSTS034 locus.

Five of the studied loci (LSCV11, OraFCB20, ILSTS059, IL2RA, ILSTS034) were at Hardy–Weinberg disequilibrium ($P<0.005$). Various measures of genetic variation are presented in TABLE 3.

The number of alleles observed totally was 60 that 19 of them can show 65% of diversity in these populations (Figure 2).

Population 4 showed the highest distance compared to the others, except for the population 6, probably due to the low number of sampled animals in both population (Figure 3). The N-J tree of individual distances, calculated as proportion of shared alleles (Figure 4), showed the variation among population. Not all the individuals of the same population clump together.

4. DISCUSSION

The precision of estimated genetic diversity is a function of the number of loci analyzed, the heterozygosity of these loci and the number of animals sampled in each population[1].

In total, 60 alleles were found at eight loci in this study. Barker[1] suggests that in diversities studies loci

with at least four different alleles should be used to reduce the standard error of the estimated distance.

Diversity studies on goat using microsatellite markers are rare in the literature. This selected microsatellites showed good performances in diversity studies in Raeni cashmere goats according to the number of alleles and heterozygosity. All eight microsatellite markers were found to be highly polymorphic, with the number of different per marker varying from six to twelve over all the populations studied. It is considered that, loci are highly polymorphic where PIC>0.7. In this population, heterozygosity varied from 0.7607 at IL2RA to 0.8766 at OraFCB20 locus. Within eight polymorphic loci, these two loci had the least and the most diversity, respectively. The study of Shannon index and PIC (Polymorphic Information Content) also indicated the least and the most diversity for IL2RA and OraFCB20 loci, respectively. An average of heterozygosity was estimated as 0.82 for this population. Yang et al.[15] studied diversity among autochthonous goat breeds from china using microsatellite markers and obtain results that the heterozygosity was high at 6 loci of the 13 analyzed loci, Amparo et al.[7] showed high polymorphism with an average of 9.95 allele per locus and gene diversity or unbiased expected heterozygosity (Nei, 1998) varied between 0.284 and 0.842.In general, it can be concluded that Raeini cashmere goat has approximately high genetic diversity. Therefore designing breeding project needs attention to conserving the genetic diversity, so the genetic resources will be conserved as a world's national investments.

These authors found a weak genetic structure among goat populations that probably resulted from recent intercontinental transportation of goat. This poor genetic differentiation causes the low bootstrap values in the phylogenetic tree. The tree obtain using distances among single individuals supported the genetic differences among populations and there weren't large differences among the populations in the percentage of individuals in the same cluster.

We conclude that microsatellites are a powerful tool to differentiate between goat populations. Further from the comparison of the microsatellite analyses with breeding histories, we conclude that again microsatellites are giving the correct answer, regardless of whether populations are closely related or not.

5. ACKNOWLEDGMENTS

This study has been supported by the International Center for Science & high Technology and Environmental Science.

REFERENCES

[1] J.S.F.Barker; Gelph: sd, 21, 501-508 **(1994)**.

[2] B.J.Bassam, G.Caetano-Anolles; Applied Biochemistry and Biotechnology, 42, 181-188 **(1991)**.

[3] M.D.Bishop, S.M.Kappes, J.W.Keele, R.T.Stone, S.L.F.Sunden; Genetics., **136**, 619-639 **(1994)**.

[4] M.W.Bruford, R.K.Wayne; Curr.Opin.Genet.Dev., 3, 939-943 **(1993)**.

[5] A.M.Crawford, K.G.Dodds, C.A.Pierson, A.J.Ede, G.W.Montgomery; Genetics, 140, 703-724 **(1995)**.

[6] P.W.Hedrick; 'Genetic of Populations', 2nd edition. Jones and Bartlett Publishers, Arizona State University, **(1999)**.

[7] D.Iamartino, A.Bruzzone, A.Lanza, M.Blasi, F.Pilla; Small Ruminant Research, **57**, 249-255 **(2004)**.

[8] S.J.Kemp, O.Hishida, J.WEambugu, A.Rink, M.L. Logeri, R.Z.Ma, J.Da, H.A.Lewin, W.Barendse, A.J.Teaale; Anim.Genet., **26**, 299-306 **(1995)**.

[9] R.Z.Ma, J.E.Beever, Y.Da, C.A.Green, I.Russ; J.Hered., (in press) **(1996)**.

[10] S.A.Miller, D.D.Dykes, H.F.Polesky; Nucleic Acid Research, **16**, 1215 **(1988)**.

[11] NTSYS pc; Numerical taxonomy system. version 2.2 for windows 98/NT/2000/xp.

[12] J.Ott, Program Het version 1.8. Utility programs for analysis of genetic linkage. Rockefeller University. New York, NY, USA. ftp://linkage. rockefeller. edu/software/utilities/, **(1988-2001)**

[13] L.Pepin, Y.Amigues, A.Lepingle, J.L.Berthier, Bensaid, D.Viman; Heredity, **74**, 53-61 **(1995)**.

[14] D.Vaiman, O.Koutita, A.Oustry, J.M.Eisen, E.Manfredi; Genome, **7**, 133-137 **(1996)**.

[15] F.C.Yeh, R.Yang, T.Boyle; POPGENE. Version 1.31. Microsoft Window-based Freeware for Population Genetic Analysis, University of Alberta. Edmonton, AB, Canada, **(1999)**.