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Genetic Variation among Different Ecotypes of the Iranian Sanjabi Sheep

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Abstract: In order to investigate genetic variation of 3 ecotypes of Sanjabi sheep (Zardi, Kajal, Kolul), an experiment was planned, using 10 microsatellite markers (INRA023, SPS113, HSC, D5S2, McM527, MAF65, INRA005, INRA063, oarFCB304 and oarFCB11). Genomic DNA was extracted from 150 blood samples, using modified Salting out method. All loci were amplified by PCR except INRA023. Loci in all of the ecotypes were highly polymorphic. Significant departures from HWE were detected for all loci in all population, except for oarFCB11 in Kolul ecotype ($p<0.05$). INRA063 was the most polymorphic marker according to its effective number of alleles (equal to 6.58 in Zardi), expected heterozygosity (equal to 0.86 in Zardi), Shannon information index (equal to 2.01 in Zardi) and Polymorphism information content (equal to 0.83 in Zardi). However, MAF65 showed the lowest effective number of alleles (equal to 2.91 in Kajal), expected heterozygosity (equal to 0.66 in Kajal), Shannon information index (equal to 1.18 in Kajal) and Polymorphism information content (equal to 0.59 in Kajal). According to the observations, the Zardi ecotype was more polymorphic and showed highest genetic variation than the others as well as Kolul was lowest. The phylogenetic clusters presented that Zardi and Kolul ecotypes have minimum Genetic Distance to each other. Results showed that, high level of genetic diversity was observed in all ecotypes of Sanjabi breed and this breed was not at risk for conservation concept.

Key words: Sanjabi sheep, genetic variation, microsatellite markers, polymorphism, phylogenetic

INTRODUCTION

The history of breeding of native sheep's in Iran back to thousands of years ago. However some factors like, the mutational process, random drift, gene flow and selection may be affected genetic variation during the time but different breeds with high level of polymorphism observe in Iran, due to their extensive production system during the history (Saadatnoori and Siahmansur, 1993). The population size of the Sanjabi breed is more than other breeds of sheep (except *Baluchi breed*) in Iran and 3 ecotypes were detected for this breed (Tavakkolian, 2000).

Information about population genetics is one of the most important factors in animal breeding. In order to estimate genetic diversity in the populations, molecular markers like microsatellites are useful tools (Esmaeilkhanian and Banabazi, 2006). There were several researches about population genetic of sheep, used the molecular markers, in many countries (Gizaw *et al*., 2007; Esmaeilkhanian and Banabazi,, 2006). Therefore, the objective of this study is about genetic variation and phylogenetic relationship between all ecotypes of Sanjabi sheep, by microsatellite markers.

MATERIALS AND METHODS

One hundred and fifty blood sample of Sanjabi sheeps (47, 57 and 46 sample from Zardi, Kajal and Kolul ecotypes, respectively) were collected randomly. DNA was extracted from whole blood using modified Salting-out method (Miller *et al*., 1988). Genomic DNA was amplified in following condition of PCR; the PCR buffer in final concentration of 1X, 5 μM $MgCl_2$, 200 μM dNTPs, 1 unit per reaction Taq DNA polymerase, 150 ng genomic DNA and 0.25 μM of each primers in a total volume of 15 μL. The reactions were done with Gradient Master Cycler Eppendorf. The cycling protocol was as a follows; 2.5 min denaturing at 95°C followed by 32 cycles

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Table 1 Characterizations of microsatellites used in the analysis

Marker	Primer sequences (5'-3') forward and reverse	Accession number in gene bank or reference
oarFCB11	GCA AGC AGG TTC TTT ACC ACT AGC AC	L01531
	GGC CTG AAC TCA CAA GTT GAT ATA TCT ATC AC	
oarFCB304	CC TAG GAG CTT TCA ATA AAG AAT CGG	L01535
	CGC TGC TGT CAA CTG GGT CAG GG	
INRA063	GAC CAC AAA GGG ATT TGC ACA AGC	X71507
	AAA CCA CAG AAA TGC TTG GAA G	
INRA005	TTC AGG CAT ACC CTA CAC CAC ATG	X63793
	AAA TAT TAG CCA ACT GAA AAC TGG G	
MAF65	AAA GGC CAG AGT ATG CAA TTA GGA G	M67437
	CCA CTC CTC CTG AGA ATA TAA CAT G	
MCM527	GTC CAT TGC CTC AAA TCA ATT C	L34277
	AAA CCA CTT GAC TAC TCC CCA A	
D5S2	TAC TCG TAG GGC AGG CTG CCT G	Hoffmann *et al.* (2004)
	GAG ACC TCA GGG TTG GTG ATC AG	
HSC	CTG CCA ATG CAG AGA CAC AAG A	M33306
	GTC TGT CTC CTG TCT TGT CAT C	
SPS113	CCT CCA CAC AGG CTT CTC TGA CTT	Hoffmann *et al.* (2004)
	CCT AAC TTG CTT GAG TTA TTG CCC	

of denaturation at 95°C for 30 sec, Annealing at 50-63°C (depending on primers) for 30 sec, extension at 72°C for 30 sec and the final extension step at 72°C for 2.5 min.

PCR products were visualized by silver staining after electrophoresis on 8% Acryl amide gels and genotypes of animals were determined. Genotype and allele frequency were calculated by direct counting. Test of departure from Hardy-Weinberg equilibrium was performed using Chi-square (χ^2_t) and Likelihood Ratio or G-square (G^2_t) test (Hedrick, 2000). The observed number of alleles (Na), effective number of alleles (Ne), observed heterozygosity (Ho) and expected heterozygosity (He), were estimated (Hedrick, 2000; Aminafshar *et al*., 2008), using POPGENE software, version 1.31 (Yeh *et al*., 1999).

Since the maximum amount of heterozygosity is equal to 1, so for polymorphic markers like microsatellites, these values are not sufficiently sensitive to show increasing the variation. To prevalence on this deficiency, we used the Shannon information Index (I), as the following equation:

$$I = -\sum_i P_i \ln P_i$$

where:

P_i = The frequency of the ith allele (Aminafshar *et al*., 2008)

Polymorphic Information Index (PIC) was estimated using HET software, version 1.8 (Ott, 2001) as the following equation:

$$PIC = 1 - (\sum_{i=1}^{k} p_i^2) - \sum_{i=1}^{k-1} \sum_{j=i-1}^{k} 2p_i^2 p_j^2$$

where:

k = The number of alleles

P_i, P_j = The frequency of corresponding alleles (Mateescu *et al*., 2005; Nei *et al*., 1983; Nei, 1978)

Genetic Distance between ecotypes was estimated using Nei´s genetic Distance method as a follow equation:

$$D = -\ln\left(\frac{\sum_m \sum_i P_{1mi} P_{2mi}}{\left[\sum_m \sum_i P_{1mi}^2\right]^{1/2} \left[\sum_m \sum_i P_{2mi}^2\right]^{1/2}}\right)$$

where:

m = The number of loci

i = The number of alleles at the mth locus

P_{1mi} and P_{2mi} = The frequency of the ith allele at the mth locus in population 1 and 2, respectively

Barker (1994) suggests some standards to select microsatellites in diversity studies, such as selection loci with at least 4 different alleles, selection loci that was used in mapping studies previously and preferably to be unlinked, selection different forms of microsatellite that have the Mendelian inheritance and selection loci that was used in some relative species such as bovine and ovine. Microsatellite markers were chosen according to all above criteria and their characteristic was shown in Table 1 (Hoffmann *et al*., 2004).

RESULTS AND DISCUSSION

All microsatellite loci were amplified except INRA023. The range of allele size was between 81 bp (oarFCB11) to 319 bp (HSC). The size of amplified alleles (Table 2) was higher than reported allele size (Maddox *et al*., 2001). Difference in genetic structure and existence of the new allele may be the main reasons.

All loci were deviate from Hardy-Weinberg equilibrium ($p<0.05$) due to excess of heterozygote individuals than homozygote individuals, migration, high

Table 2: Annealing temperatures, reported and observed size of microsatellite Alleles

Locus	Annealing temp	Reported allele range	Observed allele range
Oar FCB 11	63	121-143	81-181
Oar FCB304	63	150-188	151-214
INRA063	54	165-199	133-236
INRA 005	50	120-180	126-204
MAF65	60	123-135	109-179
MCM 527	50	165-175	151-205
D5S2	55	190-210	176-230
HSC	55	269-301	251-319
SPS 113	55	138-150	133-172

Table 3: Population genetic parameter for each microsatellite marker in all ecotypes

Marker	Ecotype	Na	Ne	Ho	He	PIC	I
OarFCB11	Zardi	9.00	6.36	0.89	0.85	0.82	1.97
	Kajal	8.00	4.85	0.91	0.80	0.76	1.70
	Kolul	9.00	5.16	0.86	0.82	0.78	1.82
OarFCB304	Zardi	5.00	3.44	1.00	0.72	0.66	1.34
	Kajal	7.00	3.45	1.00	0.72	0.66	1.41
	Kolul	5.00	3.58	1.00	0.73	0.67	1.40
INRA063	Zardi	9.00	6.58	1.00	0.86	0.83	2.01
	Kajal	9.00	6.19	0.98	0.85	0.82	1.98
	Kolul	10.00	5.92	1.00	0.84	0.81	1.98
INRA005	Zardi	9.00	5.71	1.00	0.83	0.66	1.89
	Kajal	8.00	5.64	1.00	0.83	0.80	1.84
	Kolul	8.00	5.87	1.00	0.84	0.81	1.90
MAF65	Zardi	6.00	3.04	0.98	0.68	0.61	1.26
	Kajal	4.00	2.91	0.98	0.66	0.59	1.18
	Kolul	5.00	3.16	1.00	0.69	0.63	1.27
MCM527	Zardi	6.00	4.07	1.00	0.76	0.67	1.52
	Kajal	6.00	4.46	1.00	0.78	0.74	1.57
	Kolul	6.00	3.50	1.00	0.72	0.66	1.40
D5S2	Zardi	5.00	4.09	0.96	0.76	0.72	1.49
	Kajal	5.00	4.50	0.98	0.78	0.74	1.56
	Kolul	5.00	4.49	0.93	0.79	0.74	1.54
HSC	Zardi	5.00	3.35	0.98	0.71	0.64	1.30
	Kajal	5.00	3.37	1.00	0.71	0.65	1.33
	Kolul	4.00	3.24	1.00	0.70	0.63	1.24
SPS113	Zardi	6.00	4.40	0.99	0.78	0.73	1.55
	Kajal	6.00	4.51	1.00	0.78	0.74	1.57
	Kolul	5.00	4.39	1.00	0.78	0.73	1.53

Table 4: Average of population genetic parameter in all ecotypes

Ecotype	Na	Ne	Ho	He	PIC	I
Zardi	6.66	4.56	0.98	0.77	0.70	1.59
Kajal	6.44	4.43	0.98	0.76	0.72	1.57
Kolul	6.33	4.37	0.98	0.76	0.72	1.56
All (Sanjabi)	6.48	4.66	0.98	0.77	0.72	1.63

Table 5: Genetic distance (upper elements) and similarity (lower elements)

Ecotype	Zardi	Kajal	Kolul
Zardi	******	0.0300	0.0200
Kajal	0.9700	******	0.0300
Kolul	0.9800	0.9700	******

mutation rate in microsatellites and artificial selection in all ecotypes (Aminafshar *et al*., 2008). The population genetic parameters were showed in Table 3. According to the Table 3, maximum effective number of alleles (Ne), expected Heterozgosity (He), Polymorphism Information Content (PIC) and Shannon information index were equal to 6.58, 0.86, 0.83 and 2.01 at locus INRA063 in Zardi ecotype. However, minimum effective number of alleles (Ne), expected Heterozgosity (He), Polymorphism Information Content (PIC) and Shannon information index was equal to 2.91, 0.66, 0.59 and 1.18 at locus MAF65 in Kajal ecotype, respectively.

The average of genetic parameters was a little higher in Zardi ecotype than Kajal and Kolul, according to the Table 4. It seems, the differences are likely related to the sampling error not real differences among population.

According to the Table 5, the genetic distance between all ecotypes was very low and varies from 2-3%. By the other hand, all of the ecotypes are the same in this study. However, the minimum genetic distance was observed between Zardi and Kolul and it was equal to 0.02.

CONCLUSION

Finally, it was concluded, the Sanjabi breed possessed a considerable amount of genetic diversity due to the extensive production system, low pressure of artificial selection and possibility of random mating. The genetic diversity of that breed was higher in compared with genetic diversity in Indian sheep (Mukesh *et al*., 2006; Arora *et al*., 2008) and this breed was not at risk for conservation concept.

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