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Genetic Variation Within and Between Five Iranian Sheep Populations Using Microsatellites Markers

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Abstract: Genetic variation within and between five Iranian sheep populations; Sanjabi (SAN), Kordi Kordistan (KKO), Kordi Khorasan (KKH), Mehraban (MEH) and Moghani (MOG) was assessed using 6 microsatellite markers (McMA2, McMA26, MAF64, OarAE64, OarCP26 and OarFCB304). There are significant deviation from Hardy-Weinberg equilibrium in all population-locus combinations except the locus McMA2 in the MOG population ($p<0.005$). The lowest D_A genetic distance (0.234) was obtained between KKH and KKO and the highest (0.388) between SAN and MOG. D based on D_A distances, neighbor-joining revealed one cluster containing KKO, KKH and SAN and another cluster with MEH and MOG. The average expected heterozygosity within populations ranged from 0.744 to 0.847 for KKH and MEH, respectively. The estimated time of divergence for two Kordi populations (KKO and KKH) was 445 years that has accordance with historical evidence. This study showed that microsatellite loci can be an useful tool for evaluating variation evolutionary relationships among local sheep populations.

Key words: Iranian sheep, microsatellite genetic variation, heterozygosity, polymorphism

INTRODUCTION

Iranian sheep are about 60 million and are considered as the most important domestic animal which mainly used to produce meat. There are different indigenous sheep in different locations of Iran without well definition as distinct breeds. However, they are considered as geographically defined populations. Study on genetic variation among Iranian sheep populations is a critical necessity.

Microsatellites are valuable genetic markers due to their dense distribution in the genome, great variation, co-dominant inheritance and easy genotyping. In recent years, they have been extensively used in parentage testing, linkage analyses, population genetics and genetic studies (Goldstein and Pollock, 1997). They have greatly used to investigate on genetic structure in local sheep populations. For example, Arranz *et al.* (2001) were genotyped nineteen microsatellite loci in five indigenouse Spanish breeds of sheep to determine genetic relationships among them. Stahlberger-Saitbekova *et al.* (2001) estimated genetic relationships between Swiss sheep breeds on basis of microsatellite analysis. Buchanan *et al.* (1994) determined evolutionary relationships among six British sheep breeds and both Australian and New Zealand Merino using eight ovine microsatellite loci. They have also calculated the time of divergence between the Australian and the New Zealand Merino. Farid *et al.* (2000) were assessed genetic variability of ten sheep breeds using ten microsatellite loci.

The present study is the first research on genetic variation within and between five Iranian sheep populations using microsatellite loci. Another objective of the study was to investigate accordance variation resulted from microsatellites with historical and geographical evidences.

MATERIALS AND METHODS

Genetic variation at 6 microsatellite loci including McMA2, McMA26, MAF64, OarAE64, OarCP26 and OarFCB304 were analyzed for five Iranian sheep consisting of sanjabi (SAN), kordi kordistan (KKO), kordi khorasan (KKH), mehraban (MEH) and moghani (MOG). Whole blood samples were collected from spreading areas of these sheep (Fig. 1). The number of DNA samples were 35, 32, 25, 25 and 24 for SAN, MOG, KKO, KKH and MEH, respectively. The properties of 6 loci are given at Table 1.

Genomic DNA was extracted by the salting-out method (Miller *et al.*, 1988) with some modifications. All PCR reactions contained the following components: 200 μM dNTPs, 3.5 mM $MgCl_2$, 0.25 μM each of primer, 1U *Taq* polymerase, 100-200 ng DNA. The final volume

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Table 1: Characteristics of the 6 sheep microsatellites used in the study

Cession numbers	Annealing temperature (°C)**	Chromosome	Primers (5′-3′)*	Name
AF098773	52	13	TCACCCAACAATCATGAAAC TTAAATCGAGTGTGAATGGG	McMA2
AF098961	52	18	TCTCTGCTTTCCAGCCTTATTC AGAGCTTTTAGGACAGCCACC	McMA26
M62993	64	1	AATAGACCATTCAGAGAAACGTTGAC CTCATGGAATCAGACAAAAGGTAGC	MAF64
U15698	62	4	GGCCTAACAGAATTCAGATGATGTTGC GTCACCATACTGACGGCTGGTTCC	OarCP26
L01535	61	7	CCCTAGGAGCTTTCAATAAAGAATCGG CGCTGCTGTCAACTGGGTCAGGG	OarFCB304
L13869	-	19	TGCAAGAAGGGCAGACCTTGGAG CAGACCACTCTCTTCCCTCCACG	OarAE64

* Primer sequences were cited from Ede *et al.* (1995). ** These temperatures were set up for this study

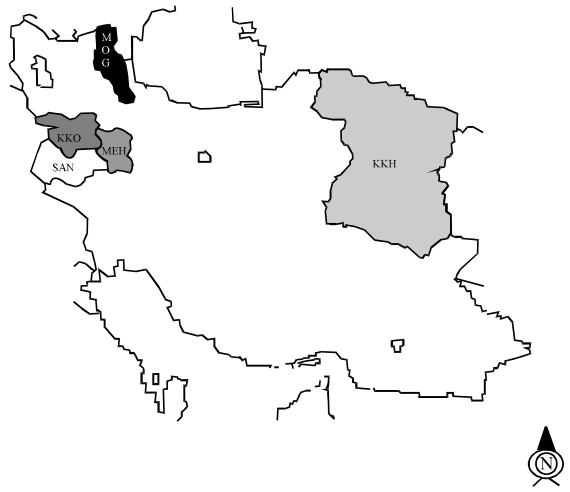

Fig. 1: The spreading regions of five Iranian sheep populations

was 15 μL. Reactions were run on a thermal cycler(Biometra) using two thermal cycling programs: (a) For McMA2 and McMa26 loci, one cycle of denaturation at 95°C (2.5 min), 29 cycles of denaturation at 95°C (30 sec), annealing 52°C (30 sec), extension 72°C (30 sec) and one cycle of extension 72°C (2.5 min) (Maddox *et al.*, 2000) and (b) For other loci, seven cycles of denaturation at 95°C (30 sec) and annealing as in Table 1 (1min) followed by 20 cycles of denaturation at 90°C (30 sec) and annealing as in Table 1 (1 min). No extension step was used (Ede *et al.*, 1995). The products were electrophoresed on 8% nondenaturing polyacrylamide gels and bands visualized by rapid silver staining (Sanguinetti *et al.*, 1994).

The allele and genotypic frequencies were directly estimated from the gel. Hardy-Weinberg equilibrium (HWE) based on likelihood ratio (Weir, 1996) were tested for different locus-population combinations by POPGENE software (Yeh *et al.*, 1999). Nei (1972) standard genetic distance and D_A genetic distance (Nei *et al.*, 1983) matrices were calculated by MICROSAT software (Minch *et al.*, 1995) and dendrograms were constructed using neighbor-joining (NJ) (Saitou and Nei, 1987) by POPTREE software (Takezaki, 2000) with 1000 bootstrap replications. The unbiased average expected heterozygosity (H_e) (Nei, 1978) was calculated by POPTREE software. Polymorphism criteria such as polymorphic information content (PIC) (Buchanan and Thue, 1998) and the number of observed and effective alleles (Hedrick, 1999) were also estimated by HET (Ott, 1989) and POPGENE software, respectively. The time of divergence between two kordi populations (KKH and KKO) was estimated using $D = 2\alpha t$ equation (Nei, 1976) where t and D are the divergence time and Nei (1978) unbiased genetic distance, respectively. In order to estimate t, we assumed $\alpha = 4.5*10^{-4}$ (observed spontaneous mutations per locus per gamete in humans) as the rate of change in repeat number. The estimated time was compared with historical evidence for divergence about 400 years ago. Mutation rates were then calculated for each locus and per all loci using the estimated time of divergence as Buchanan *et al.* (1994) have described.

RESULTS AND DISCUSSION

PCR reactions were successfully done with all primers excepted to OarAE64. The possible explanations for this failure are mutation at the primer site or a mistake during primer synthesis. A few alleles were found in Iranian sheep that haven't been previously reported in the same loci for other sheep (Farid *et al.*, 2000; Buchanan *et al.*,1994; Buchanan and Crawford, 1993; Arranz *et al.*, 2001; Stahlberger-Saitbekova *et al.*, 2001; Diez-Tascon *et al.*, 2000; Maddox *et al.*, 2000). There are significant deviation from Hardy-Weinberg equilibrium in all population-locus combinations except the locus McMA2 in the MOG population ($p<0.005$).

Table 2 shows genetic distance matrices based on D_A (upper diagonal matrix) and D_S (lower diagonal matrix) using 1000 bootstrap replications. Due to long geographic

Table 2: Genetic distance matrices based on D_A (upper diagonal matrix) and D_S (lower diagonal matrix) using 1000 bootstrap replications

	SAN	KKO	KKH	MEH	MOG
SAN		0.180	0.450	0.180	0.559
KKO	0.264		0.271	0.347	0.467
KKH	0.307	0.234		0.395	0.498
MEH	0.263	0.304	0.353		0.293
MOG	0.388	0.371	0.371	0.260	

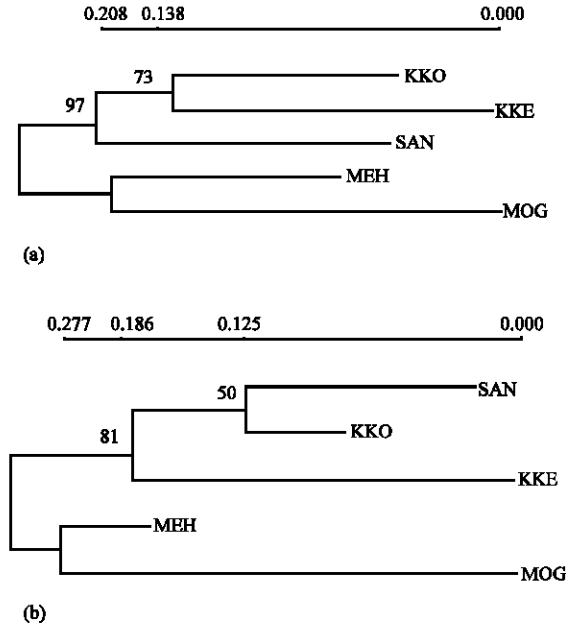

Fig. 2: Dendrograms showing evolutionary relationships among five Iranian sheep based on D_A (a) and D_S (b) using NJ algorithms with 1000 bootstrap replications The scale shows arbitrary genetic distance. The numbers at nodes represent robustness of nodes

distance and natural barriers (mountains and sea) between MOG and other populations, we considered MOG as an outgroup. So, it is expected to find high genetic distance between MOG and others. Longtime usage of MEH rams for crossing with other sheep may be occurred low distance between MEH and MOG and intermediate distance MEH with three other populations.

Both dendrograms (Fig. 2) have the same topology that reflects the geographical locations of these five Iranian sheep. Support of nodes is are relatively weak (except KKO and KKH branch), although only 5 microsatellites were used. There are two separate clusters on both dendrograms. One includes KKO, KKH in a branch and then SAN. Another consists MEH and MOG. A possible explanation for this topology was expressed above.

PIC values and interpopulation diversities (Unbiased average expected heterozygosity) are given in Table 3. The values of H_e are relatively high and have wide range (0.506 to 0.915). However values per population (average of all loci) had narrow range from 0.743 (KKH) to 0.847 (MEH). PIC and heterozygosity values were very close to other studies (Farid *et al*., 2000; Buchanan *et al*.,1994; Buchanan and Crawford, 1993; Arranz *et al*., 2001; Stahlberger-Saitbekova *et al*., 2001; Maddox *et al*., 2000). Small population size and short time for variability may be a possible reason for the low H_e in KKH. Large population size and gene flow could be explain the relatively high H_e for MEH and KKO. PIC per population (average of all loci) indicating variability in each population had narrow range (from 0.692 to 0.796 for KKH and KKO, respectively). PIC for each locus had also narrow range (from 0.761 to 0.901 for OarFCB304 and OarCP26, respectively). However, these values for different locus-population combinations had wide range (0.4679-0.8595). The number of observed alleles per locus varied from 7 at OarFCB304 to 13 at McMA2 and OarCP26. The highest and lowest number of effective alleles were 10.9 and 4.7 for OarCP16 and OarFCB304 loci, respectively. Although sample sizes were small, polymorphism criteria such as PIC values and number of alleles indicate high polymorphism at studied loci and sheep. These loci will be useful for further studies of population relationships, demographic history and the traceability of animals and food products.

The divergence time of two kordi populations (KKH and KKO) was estimated 445 years old (with generation interval equal 3.5 years old). This time is very similar to historical evidences (about 400 years old). Mutation rates per locus were calculated using the estimated time and then compared with proposed mutation rate. Table 4 shows Nei`s unbiased genetic distances (D) and mutation rates (α).

The range of mutation rate is very wide (from $0.97*10^{-4}$ to $12.52*10^{-4}$). The mutation rates of McMA2 and for all loci were very near to the assumed rate. The correlation between two distance criteria was very high (0.903). Since D_A has been found more useful to obtaining of correct topology (Takezaki and Nei, 1996), we present only the results of D_A. The lowest D_A was between KKH and KKO (0.234) and between SAN and KKO (0.246). These distances are rational due to co-descendant of two kordi sheep, short time passed from their separation (about 400 years old) and neighboring geographic distributions. Their phenotypic similarity also agree with these distances. The highest D_A and D_S were between SAN and MOG (D_A = 0.388, D_S= 0.559).

Table 3: H_e and PIC values at locus-population combinations, per population (average of loci) and per locus

	SAN		KKO		KKH		MEH		MOG			
	H_e	PIC	H_e	PIC	H_e	PIC	H_e	PIC	H_e	PIC	H_e	PIC
McMA2	0.889	0.841	0.846	0.795	0.881	0.833	0.862	0.813	0.860	0.807	0.904	0.889
McMA26	0.839	0.782	0.869	0.820	0.765	0.689	0.835	0.783	0.860	0.807	0.877	0.859
MAF64	0.648	0.548	0.770	0.716	0.710	0.653	0.825	0.768	0.852	0.799	0.814	0.786
OarCP26	0.905	0.860	0.876	0.828	0.862	0.819	0.890	0.845	0.894	0.749	0.915	0.901
OarFCB304	0.786	0.718	0.869	0.820	0.506	0.468	0.821	0.765	0.648	0.548	0.792	0.762
Average	0.813	0.750	0.846	0.796	0.743	0.692	0.847	0.795	0.823	0.762		

Table 4: Mutation rates (α) and Nei`s unbiased genetic distances (D)

Locus	D	α
McMA2	0.293	11.36
McMA26	0.123	4.76
MAF64	0.073	2.82
OarCP26	0.025	0.97
OarFCB304	0.323	12.52
All Loci	0.114	4.42

CONCLUSIONS

This research showed high variation within and between studied Iranian sheep populations for five microsatellite loci. This study showed that microsatellite loci can be an useful tool for evaluating variation evolutionary relationships among local sheep populations. Microsatellite-based estimates of population relationships were consistent with known demographic history and geographic distances.

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