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Short communication

Genetic relationships among six Iranian indigenous sheep populations based on microsatellite analysis

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ABSTRACT

Six Iranian indigenous sheep populations: Ghashghaee (GSH), Bakhtiyari (BKH), Kabude Shiraz (KSH), Sanjabi (SAN), Lori (LRI) and Arabi Khuzestan (ARB) were genotyped for 10 microsatellite markers recommended by the MoDAD program [FAO, 1998. Food and Agriculture Organisation of the United Nations Secondary Guidelines for Development of National Farm Animal Genetic Resources Management Plans. Measurement of Domestic Animal Diversity (MoDAD). Recommended Microsatellite Markers]. All population per locus combinations were at Hardy–Weinberg Equilibrium except MAF214 for KSH and BM1824, MAF214 and MCM527 for SAN ($P < 0.05$). All loci were polymorphic in all populations. There was substantial genetic variability within sheep populations, with average heterozygosity range of 0.747–0.792 based on expected hetrozygosity. The three sheep populations showing the highest level of inbreeding on the basis of heterozygote deficiency. The lowest genetic distance (0.166) was obtained between LRI and BKH and the highest genetic distance (0.378) between SAN and GSH. The phylogenetic tree based on unbiased distances was drawn using UPGMA. To study the genetic relationships among sheep populations, a principal coordinate analysis (PCA) based on Nei standard distances was performed.

1. Introduction

Sheep (*Ovis aries*) was domesticated from at least three ancestral subspecies of the wild Mouflon (*O. gmelini*, Gmelin 1774) approximately 9000 years before present (YBP) in Southwest Asia (Lawson Handley et al., 2007). Today, over 850 sheep breeds are recognized worldwide (United Nations Food and Agriculture Organisation, FAO, 2000). Iran has 27 sheep populations, which vary in their genetic potential for production of milk, meat and wool; disease resistance; fecundity, etc. (Tavakolian, 2000). The first step for the sustainable use of domestic animal genetic resources is gathering knowledge about the genetic variability in the populations (Grigalinait et al., 2003). Very little information is currently available to compare different sheep populations from Iran. Thus, although we have used only six representative sheep populations, the present study may be regarded as a beginning to try and understand the genetic diversity of indigenous sheep populations in Iran. Further investigations including more native Iranian sheep populations would be useful to clarify their recent origin and the relationships between them. This study used microsatellite markers because they are a powerful tool for tracking alleles through a population and to estimate genetic variability and inbreeding (Zajc et al., 1997). The aim of this study is the use of molecular data for evaluating genetic variability and genetic relationships of the Iranian indigenous sheep populations.

2. Materials and methods

Genetic relationships among six Iranian sheep populations: Ghashghaee (GSH), Bakhtiyari (BKH), Kabude Shiraz (KSH), Sanjabi

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Table 1
Information on six indigenous sheep populations, such as phenotypic traits (colour and body weight), number of DNA studied and number of flocks.

Population	Colour	Weight ♂	Weight ♀	DNA studied	Flock
SAN	White, dark head and legs	65	58	45	5
KSH	Grey, black and brown	60	55	45	6
GSH	Brown (from light to black)	63	57	45	7
LRI	White	74	67	45	7
BKH	White	90	68	45	9
ARB	White, black and brown	65	60	45	8

(SAN), Lori (LRI) and Arabi Khuzestan (ARB) were obtained using 10 microsatellite markers isolated from domestic sheep (MAF33, OarCP34, BM8125, MAF214, MAF70, DYMS1, MCM527, OarJMP29, OarJMP58 and BM1824). These loci were selected because of their high polymorphism and high number of alleles previously detected in sheep (FAO, 2000). Three populations (LRI, BKH and GSH) are composed of native sheep for the Zagros Mountains and three populations (ARB, SAN and KSH) are composed of native sheep for the desert (Tavakolian, 2000). Iranian sheep populations that are studied here can be classified according to their colour and body weight (Saadat Noori and Siah Mansoor, 1982). These breeds, such as other Iranian native sheep breeds except Zel breed, are fat-tailed (Saadat Noori and Siah Mansoor, 1982). Information on six indigenous sheep populations is shown in Table 1. Fig. 1 is a map showing the sampled areas. Not more than one-tenth of each herd or village population was sampled to ensure that the animals sampled were as unrelated as possible. Genomic DNA was isolated from 1 ml blood aliquots by a standard procedure (Miller et al., 1988). The quantity and quality of the isolated DNA was determined by spectrophotometry based on absorbance at 260 and 280 nm, respectively. Polymerase chain reaction (PCR) was performed according to Touch down method, as described by Crawford et al. (1995). Amplification products were run on 6% denaturing polyacrylamide gels by BIO-RAD sequencing. Bands visualized by rapid silver staining (Sanguinetti et al., 1994). The loci per locus frequencies and tests of genotype frequencies for deviation from Hardy–Weinberg Equilibrium (HWE) were carried out using the exact tests of the GenAlEx version 6 (Peaka and Smouse, 2006). The POPGENE program (Yeh et al., 1999) was employed for the calculation of mean observed heterozygosity (H_o) and mean expected heterozygosity (H_e) for populations. Nei (1972) standard genetic distances were estimated using the GenAlEx and POPGENE software packages. The genetic relationships among the six analysed populations were evaluated by the UPGMA method. To individualize the animals, an approach based on the computation of the log-likelihood of each individual genotype to belong to each population (Paetkau et al., 1995) was performed using GenAlEx version 6. The principal coordinate analysis (PCA) was performed on Nei standard distances by the Jacobi method (Press, 1992). In order to assess the presence of inbreeding within population, the Wright (1978) fixation index (F_{IS}) as a measure of heterozygote deficiency was calculated for each population according to original formulas, a bootstrap analysis on 1000 replicates was performed to estimate the standard deviation of F_{IS}.

3. Results

All loci were found to be polymorphic in the six sheep populations, and generated a total of 73 loci across the 10 locus from the 270 individuals analysed. The number of loci detected for each microsatellites marker ranged from 4 to 9. Of the 60 population per locus combinations, 56 (93.3%) were at Hardy–Weinberg Equilibrium except MAF214 for KSH and BM1824, MAF214 and MCM527 for SAN ($P < 0.05$).

Genetic variability parameters are presented in Table 2. Although varying among populations, mean observed heterozygosity was lower than the mean expected heterozygosity for all the populations (see Table 2). The average gene diversity was 0.778 for the three desert study sites, 0.769 for the three Zagros Mountains sites, and 0.773 overall. KSH had the highest gene diversity for all the loci ($H_e = 0.792$) with average number of loci per population (6.50), while GSH had the lowest gene diversity ($H_e = 0.747$) with average number of loci per population (6.00) (Table 2). The mean number of loci per locus ranged from 5.9 in LRI to

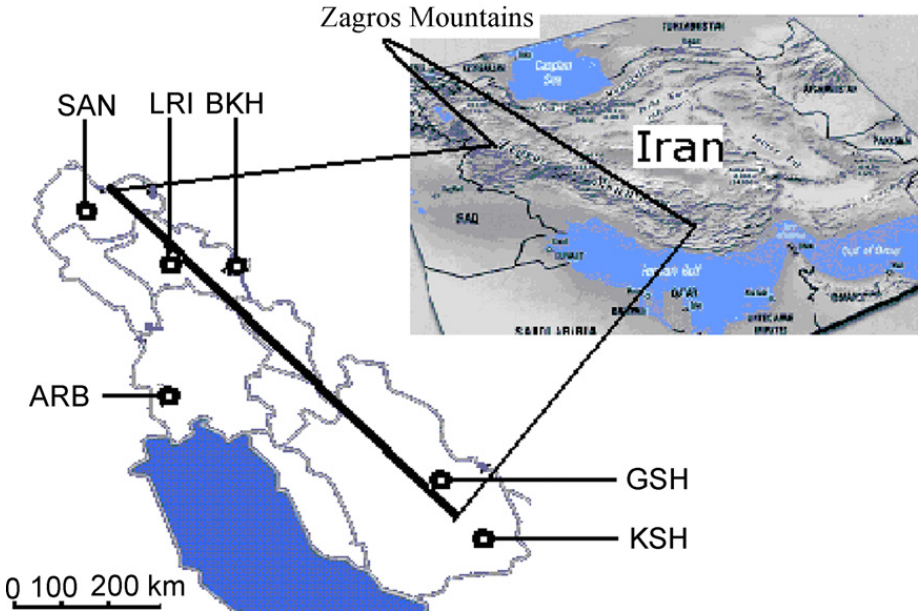

Fig. 1. Location of the six study sites in Iran. The putative subspecies are indicated as Ghashghaee (GSH), Bakhtiyari (BKH), Kabude Shiraz (KSH), Sanjabi (SAN), Lori (LRI) and Arabi Khuzestan (ARB).

Table 2
Genetic variability parameters in six Iranian sheep populations.

Population	MNL	SE	MNEL	SE	Mean heterozygosity				F_{IS}	m.l.c.a.
					H_o	SE	H_e	SE		
GSH	6	1.15	4.28	1.01	0.654	0.19	0.747	0.08	0.124	0.777
BKH	6.6	1.075	5.134	1.209	0.629	0.147	0.789	0.043	0.202	0.666
KSH	6.5	0.849	4.942	0.592	0.7	0.15	0.792	0.026	0.116	0.755
SAN	6.6	1.349	4.708	0.01	0.685	0.193	0.776	0.059	0.117	0.8
LRI	5.9	0.99	4.499	0.907	0.599	0.102	0.771	0.043	0.223	0.777
ARB	6.3	0.948	4.415	0.765	0.584	0.101	0.768	0.039	0.239	0.711

Notes: MNL = mean number of loci per locus; MNEL = mean number of effective loci per locus; SE = standard error; H_o = observed heterozygosity; H_e = expected heterozygosity (Nei, 1978); Wright's (1978) fixation index (F_{IS}) as a measure of heterozygote deficiency. Percentage of correct assignment of individual genotypes to the flock of origin by maximum likelihood (m.l.c.a.).

Table 3
Matrix of Nei's original measures of genetic identity and genetic distance among six Iranian sheep populations: the Nei (1972) standard genetic distance (below diagonal) and genetic identity (above diagonal).

Population	GSH	BKH	KSH	SAN	LRI	ARB
GSH	***	0.81	0.763	0.684	0.786	0.754
BKH	0.21	***	0.815	0.753	0.846	0.795
KSH	0.269	0.203	***	0.802	0.787	0.803
SAN	0.378	0.282	0.22	***	0.694	0.828
LRI	0.24	0.166	0.238	0.365	***	0.815
ARB	0.281	0.229	0.219	0.188	0.203	***

6.6 in SAN and BKH, with a mean of 6.3 loci per locus overall. BM1824 had the most loci overall with the 9 loci, while MAF214 had just 5 loci overall. All populations had substantial levels of genetic variation as shown by the observed hetrozygosity (H_o) and mean unbiased estimates of gene diversity (H_e) (Nei, 1978).

The log-likelihood-based assignment of genotypes correctly attributed a great proportion of individuals to their population of origin (Table 2). The assignment of individual genotypes to flocks performed by the log-likelihood analysis represents a quick and easy method for identifying rams more likely to increase genetic diversity (Pariset et al., 2003).

The lowest genetic distance (0.166) was obtained between LRI and BKH and the highest genetic distance (0.378) between SAN and GSH (see Table 3).

We used the pairwise *D* values to build the phylogenetic tree in Fig. 2, which provides one method of summarizing the genetic relationships between study sites. As can be seen, only two population clusters received strong support from the data: the three study populations in the Zagros Mountains (BKH, LRI and GSH) in one cluster and the three study sites with sheep from desert (ARB, SAN and KSH) in another cluster.

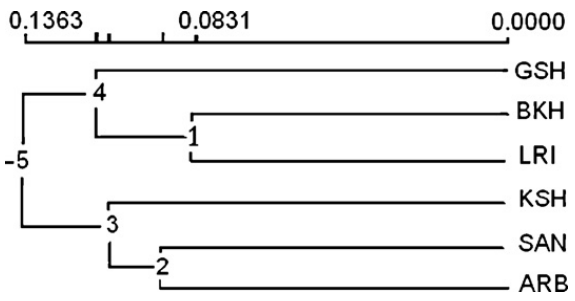

Fig. 2. UPGMA phylogenetic tree based on Nei (1978) genetic distance.

4. Discussion

In the global test of deviation from Hardy–Weinberg Equilibrium, the deviations from the expected value may be due to a variety of causes: population subdivision owing to genetic drift (Lawson et al., 1989). The mean number of loci per locus and heterozygosity values were not significantly different among the six sheep populations. All six indigenous sheep populations had a substantial amount of genetic variation, and none of them can be considered genetically impoverished. However, observed heterozygosity was always lower than expected. This finding could be due to population subdivision in each region, local inbreeding or the presence of null alleles (Lawson Handley et al., 2007; Peter et al., 2007; Pariset et al., 2003). The different F_{IS} values of the populations reflect different levels of inbreeding (Pariset et al., 2003). The three sheep populations (BKH, LRI and ARB) showing the highest level of inbreeding (See Table 2) should be considered to be at risk as this condition can lead to reduced fitness. Since the set of microsatellites markers we used showed a little higher variability than that of the microsatellites markers used in the genetic diversity analysis of Sarda sheep breeds of central Italy (Pariset et al., 2003), European sheep breeds (Peter et al., 2007), desert bighorn sheep of Arizona, California, New Mexico, Alberta and Canada (Gustavo et al., 2000), we interpreted our higher gene diversity as reflections of both the choice of the microsatellite markers and the choice of breeds.

The rugged topography and Zagros Mountains barriers (see Fig. 1) limit movement of sheep and impose reproductive isolation between SAN and GSH population distribution areas. So, is a high genetic distance between SAN and GSH populations. The LRI sheep population had a common geographical location (see Fig. 1) and a similar morphological appearance to that of the BKH sheep population (Saadat Noori and Siah Mansoor, 1982; Tavakolian, 2000), therefore the highest similarity is expected between LRI and BKH sheep population.

Taking into account the spatial distribution of the populations explained by PCA (Fig. 2) is partially different with the geographical locations of the farms in the study area (see Fig. 1), indicating that the gene flow among the populations is not limited (Fig. 3).

The log-likelihood of individual genotypes belonging to a population can be a method to select rams suitable for exchanging between two or more populations. In fact, if individuals can be attributed to more populations, then

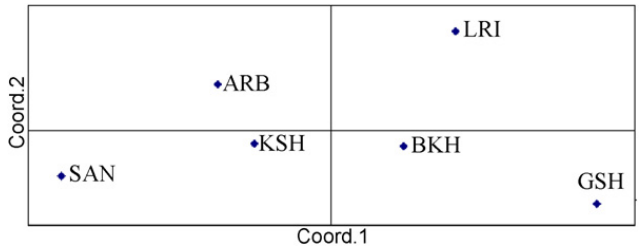

Fig. 3. Principal coordinate analysis (PCA) of loci per locus frequencies from 10 microsatellite loci typed in six sheep populations from Iran, defined by the first dimensions.

any exchange between them is not likely to improve the genetic variability, as they are likely to carry the same alleles (Pariset et al., 2003).

It is interesting to note that the spatial distribution of the flocks explained by UPGMA phylogenetic tree based on Nei (1978) genetic distance partially fits with the geographical locations (mountain sheep populations or desert sheep populations) of the farms in the study area indicating that the gene flow among the flocks is rather limited between mountain sheep populations and desert sheep populations too.

According to the selective standard of microsatellite loci, microsatellite loci ought to have at least 4 loci per locus to be considered useful for the evaluation of genetic diversity. Based on this criterion, the 10 microsatellite loci used in the present study can be considered useful for the evaluation of genetic diversity within and among populations and for the selection of breeding animals from divergent groups maximizing genetic variation and consequently fitness.

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