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Full Length Research Paper

Genetic variation among pelt sheep population using microsatellite markers

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Genetic variation in three Iranian pelt sheep breeds namely: Gray Shiraz, Zandi and Karakul were investigated using fifteen microsatellite loci. Genomic DNA was extracted from 360 blood samples by extraction kits and salting-out procedure with some modifications. The total number of alleles ranged from 6 to12 in loci. The fifteen tested loci were all polymorphic in the three breeds. The average direct count of heterozygosity overall loci in each tested breed was more than the expected heterozygosity. Tests of genotype frequencies for deviation from the Hardy-Weinberg equilibrium (HWE) were performed at each locus of overall breeds and revealed significant departure from HWE ($P < 0.001$) due to heterozygote excess. Polymorphism information content value in Gray Shiraz, Zandi and Karakul were 0.815, 0.808 and 0.808, respectively. Rate of inbreeding within the three breeds was not noticeable (global $F_{is} = - 0.19$). Low genetic differentiation was detected by estimation of F_{st} index between all pairs of breeds. Results showed that high level of genetic diversity was observed in pelt sheep. The phylogenetic tree based on Nei distances were drown using the neighbor-joining (NJ) and unweighted pair-group method using an arithmetic average (UPGMA). With both methods, Gray Shiraz and Karakul sheep populations were located together at one cluster and Zandi sheep population at another. The results can be useful in the development of breeding strategy for genetic improvement of pelt sheep in Iran.

Key words: Microsatellites, sheep, genetic, diversity.

INTRODUCTION

Awareness of the value of animal genetic resource is currently prompting deliberate efforts at optimum utilization and conservation of the species. The maintenance of genetic diversity is a key to the long-term survival of most species (Hall and Bardley, 1995). The genetic polymorphism and diversity found in the domestic breeds allows farmers to develop new characteristics in response to changes in environment or market conditions. So, the importance of increasing, maintaining and conserving the genetic diversity in these animals has been recognized for the future (Zhang et al., 2009). Studies of genetic diversity in domestic animals are based on an evaluation of the genetic variation within breeds and genetic relationship among them.

The total sheep population in Iran is 54 million heads. At present, a total of 1.6 million people are directly working in sheep and goat sector (ASRI, 2004). Most of sheep breeds are multipurpose producing lamb, wool and milk. Gray Shiraz, Zandi and Karakul are three well-known breeds of sheep for pelt production in Iran with a

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Abbreviations: PIC, Polymorphism information content; **PCR,** polymerase chain reaction; **dNTPs,** deoxynucleoside triphosphates; **HWE,** Hardy-Weinberg equilibrium.

population of 500,000, 500,000 and 300,000, respectively (ASRI, 2004). In the past, the Gray Shiraz, Karakul and Zandi were selected for production of good quality pelts mainly for exports. Now, due to the increased demand of the meat in the country, these breeds are mainly oriented towards the meat and lamb production (Moradpour, 1993). The Gray Shiraz and Karakul sheep breeds are from the same origin. Gray Shiraz has a slightly smaller size than Karakul (Tavakolian, 1999). Gray Shiraz is found in South of Iran (Fars province). Karakul is very resistant to harsh condition and is raised mostly under the semi nomadic system of management. Karakul breed is mainly found in the plain of Sarakhs in the province of Khorasan, neighboring Turkmenistan. Zandi breed is similar to Gray Shiraz and Karakul (Moradpour, 1993). It has a slightly smaller size than both two mentioned breeds. This breed is mainly found in parts of Tehran, Semnan, Ghom provinces and some parts of the Central province. These breeds are medium size, meat and pelt type, with coarse wool breed (Tavakolian, 1999). 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). Microsatellites are stable, polymorphic and easy to analyze; this occurs regularly throughout an animal genome as well.

Microsatellites are co-dominant markers, so that all alleles can be scored. Several studies had investigated the genetic diversity in sheep using microsatellites (El Nahas, 2008; Dalvit et al., 2008; Mahmoudi and Babayev, 2009; Sharifi sidani et al., 2009; Kusza et al., 2010). The aim of this study is to use the molecular data to evaluate genetic variability, gene flow and inbreeding in sheep flocks, and also for designing a breeding strategy aimed at incrementing genetic diversity within and between pelt sheep breeds.

MATERIALS AND METHODS

Blood sampling and DNA extraction

Blood samples of 360 sheep of both sexes, were randomly collected from three pelt breeds: Gray Shiraz, Zandi and Karakul from distant located experimental stations belonging to the Animal Science research Institute. Genomic DNA was extracted from fresh or frozen blood using modified salting-out (Miller et al., 1988) and DNA Extraction kit (Diatom DNA Prep 100). DNA concentration was determined using a UV spectrophotometer.

Microsatellite polymorphism detection

Fifteen microsatellite markers were selected in respect of polymorphisms, a non-linkage criterion for syntenic loci and criterion of location on different chromosomes (Table 1). Microsatellites were amplified with polymerase chain reaction (PCR) using genomic DNA extracted from individual animals. The PCR was performed for each locus in 15 µl reaction mixture consisting: 1X buffer, 200 µM dNTPs, 1.5 - 4.5 mM $MgCl_2$, 0.25 µM of each Primers , 1 units Taq polymerase and 100 - 200 ng DNA. The reactions were done with Gradient Master Cycler Ependorf. The cycling protocol was as follows: 5 min denaturing at 95°C followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 52 - 63°C (depending on primers) for 30 s, extension at 72°C for 45 s and the final extension at 72°C for 5 min. Amplification products were electrophoresed on 8% denaturing polyacrylamide gels and stained according to rapid silver staining procedure. The gel was photographed using Gel-Doc XR (BioRad). Patterns of the different genotypes for each microsatellite locus were analyzed using Gel-Pro analyzer, version 3.1 for windowsTM, which determines the allele's sizes in each animal.

Statistical analysis

Allele frequencies at each locus for each breed were calculated using a computer program GenAlEx 6 (Peakall and Smouse, 2006). GenAlEx 6 software program was also used for calculating the observed and expected heterozygosity overall loci in each of the three breeds (Levene, 1949; Nei, 1978). Test of departure from Hardy-Weinberg equilibrium at each locus overall breeds were performed using Chi-square and Likelihood ratio or G-square test (Hedrick, 2000) using GenAlEx 6 program and POPGENE software version1.31 (Yeh et al., 1999), respectively. GenAlEx 6 program was also used to calculate F-statistics that include three indices: Fit, Fst (genetic differentiation) and Fis (within breed heterozygosity deficit). Polymorphism information content (PIC) was calculated using HET software version 1.8 (Ott, 2001). The genetic distance (DS) Nei (1972) was calculated using the GenAlEx 6 program. Nei genetic distances was used to construct phylogenetic tree using the neighbor-joining method (NJ) and the unweighted pair group method with arithmetic mean (UPGMA) using MEGA software version 3 (Kumar, 2004).

RESULTS

The number of alleles for each of the fifteen microsatellite loci in each of the three breeds is presented in Table 2. The total number of detected alleles varied from 6 (BM1815, BM1815, OARCP26, OARFCB20 and MAF64) to 12 (MCMA2 and BMS678). The mean numbers of alleles per locus are 8.1, 8 and 8.1 in Gray, Zandi and Karakul, respectively. The mean number of alleles shared between Gray and Zandi, Gray and Karakul and also Zandi and Karakul are 7.7, 7.9 and 7.8, respectively, whereas the mean number of the alleles shared by the three breeds is 7.6. It is worth mentioning that all alleles representing ten loci are present in the three breeds and the only difference found is at BMS460, OARCP26, OARAE129, MAF64 and BM6444 loci where some alleles at loci are not present in Gray, Zandi or Karakul.

Table 3 presents the alleles frequency distribution at the analyzed loci in the three breeds. At loci MCMA26, the same alleles are at the highest frequency in Gray, Zandi and Karakul breeds. Gray and Karakul breeds have similar alleles at the highest allele frequency at six loci. Whereas Gray and Zandi; Zandi and Karakul breeds show similar alleles at the highest allele frequency at three loci. Gray and Zandi breeds have the highest allele frequency at OARCP26 locus.

The average direct count of heterozygosity (observed

Table 1. Characteristics of the microsatellites under investigation.

Name	Primer sequence	Chr. no.	Accession no.	Allelic range	Annealing Temperature (°C)	References
MCMA2	TCACCCAACAATCATGAAAC / TTAAATCGAGTGTGAATGGG	13	AF098773	157-201	52	Maddox et al., 2000.
MCM63	CCCAATTTGGCAACAGCTACG / ATTGGCCTCTCTCTGATGCAC	9	L37889	120-168	55	Smith et al., 1995.
BMS460	TGCCCCATAGTGTAGTGCTC / GCCAGCAGAGAATTGTAGCA	3	G18836	120-148	58	Maddox et al., 2000.
BM1815	AGAGGATGATGGCCTCCTG / CAAGGAGACAAGTCAAGTTCCC	20	G18389	Not available	55	Bishop et al., 1994.
OARCP26	GGCCTAACAGAATTCAGATGATGTTGC / GTCACCATACTGACGGCTGGTTCC	4	U15698	120-170	55	Ede et al., 1995.
OARFCB20	AAATGTGTTTAAGATTCCATACAGTG / GGAAAACCCCCATATATACCTATAC	2	L20004	83-123	55	Buchanan et al., 1993.
OARAE129	AATCCAGTGTGTGAAAGACTAATCCAG / GTAGATCAAGATATAGAATATTTTTCAACACC	5	L11051	133-159	52	Penty et al., 1993.
MAF64	AATAGACCATTCAGAGAAACGTTGAC / CTCATGGAATCAGACAAAAGGTAGG	1	M62993	109-141	63	Swarbrick et al., 1991.
BMS332	GACAAAACCCTTTTAGCACAGG / AATTGCATGGAAAGTTCTCAGC	22	G18841	127-157	57	Maddox et al., 2000.
LSCV38	GTTGCAAAGAGCTGGACGTG / CTGGATGGCAAAGTGATTCAG	12	G40990	102-122	54	Maddox et al., 2000.
BM6444	CTCTGGGTACAACACTGAGTCC / TAGAGAGTTTCCCTGTCCATCC	2	G18444	128-165	55	Bishop et al., 1994.
BMS995	AATTCTTCCAACCTCCAGTGC / ACTTTTCAAGCAGGGCTCAC	13	G18766	121-147	58	Maddox et al., 2000.
MCMA26	TCTCTGCTTTCCAGCCTTATTC / AGAGCTTTTAGGACAGCCACC	18	AF098961	188-212	52	Maddox et al., 2000.
BMS678	ACCATCTACTGTGCTATGGCTT / GCAGAAACACAATACTCAGTGC	2	G18734	100-130	54	Gortari et al., 1997.
OARCP49	CAGACACGGCTTAGCAACTAAACGC / GTGGGGATGAATATTCCTTCATAAGG	17	U15702	85-107	63	Ede et al., 1995.

Chr. = Chromosome; no. = number.

Table 2. Number of alleles at each microsatellite locus in the three breeds and the number of alleles shared between breeds.

Locus	Number of alleles							
	Total	Gray	Zandi	Karakul	Shared by Gray-Zandi	Shared by Gray-Karakul	Shared by Zandi-Karakul	Shared by all
MCMA2	12	12	12	12	12	12	12	12
MCM63	8	8	8	7	8	7	7	7
BMS460	7	6	7	6	5	5	6	5
BM1815	6	6	6	6	6	6	6	6
OARCP26	6	6	4	6	4	6	4	4
OARFCB20	6	6	6	6	6	6	6	6
OARAE129	7	7	6	7	6	7	6	6
MAF64	6	6	5	4	5	4	4	4
BMS332	7	7	7	7	7	7	7	7
LSCV38	8	6	8	8	6	6	8	6
BM6444	9	9	8	9	8	9	8	8
BMS995	11	11	11	11	11	11	11	11
MCMA26	11	11	11	11	11	11	11	11
BMS678	12	12	12	12	12	12	12	12
OARCP49	9	9	9	9	9	9	9	9
Mean	8.3	8.1	8.0	8.1	7.7	7.9	7.8	7.6

Table 3. Allele frequencies at each microsatellite locus in the three sheep breeds.

Allele no.	MCMA2			Allele no.	BMS678		
	Gray	Zandi	Karakul		Gray	Zandi	Karakul
1	0.114	0.062	0.059	1	0.063	0.090	0.174
2	0.082	0.075	0.090	2	0.059	0.085	0.136
3	0.091	0.071	0.069	3	0.072	0.056	0.065
4	0.055	0.049	0.053	4	0.095	0.098	0.114
5	0.082	0.058	0.048	5	0.050	0.090	0.054
6	0.077	0.058	0.085	6	0.050	0.060	0.060
7	0.068	0.049	0.074	7	0.126	0.124	0.054
8	0.082	0.124	0.085	8	0.081	0.051	0.049
9	0.141	0.080	0.122	9	0.108	0.115	0.054
10	0.059	0.137	0.090	10	0.113	0.081	0.065
11	0.077	0.142	0.112	11	0.108	0.060	0.076
12	0.073	0.097	0.112	12	0.077	0.090	0.098
Allele no.	**MCM63**			**Allele no.**	**LSCV38**		
	Gray	Zandi	Karakul		Gray	Zandi	Karakul
1	0.045	0.081	0.128	1	0.000	0.144	0.128
2	0.067	0.242	0.267	2	0.000	0.051	0.059
3	0.067	0.119	0.133	3	0.117	0.051	0.064
4	0.147	0.085	0.067	4	0.230	0.292	0.239
5	0.179	0.199	0.211	5	0.157	0.157	0.096
6	0.183	0.161	0.133	6	0.052	0.051	0.053
7	0.121	0.064	0.000	7	0.261	0.203	0.250
8	0.192	0.051	0.061	8	0.183	0.051	0.112
Allele no.	**OARAE129**			**Allele no.**	**BMS332**		
	Gray	Zandi	Karakul		Gray	Zandi	Karakul
1	0.050	0.284	0.066	1	0.118	0.173	0.121
2	0.073	0.208	0.055	2	0.236	0.261	0.253

Table 3. Contd.

3	0.055	0.127	0.093	3	0.264	0.235	0.275
4	0.161	0.161	0.181	4	0.086	0.111	0.082
5	0.275	0.169	0.181	5	0.145	0.084	0.104
6	0.206	0.051	0.159	6	0.082	0.084	0.115
7	0.179	0.000	0.264	7	0.068	0.053	0.049
Allele no.	**BM1815**			**Allele no.**	**OARFCB20**		
	Gray	**Zandi**	**Karakul**		**Gray**	**Zandi**	**Karakul**
1	0.197	0.118	0.304	1	0.140	0.291	0.134
2	0.272	0.193	0.380	2	0.331	0.261	0.274
3	0.145	0.218	0.065	3	0.182	0.145	0.226
4	0.154	0.160	0.065	4	0.144	0.107	0.075
5	0.127	0.261	0.082	5	0.093	0.090	0.118
6	0.105	0.050	0.103	6	0.110	0.107	0.172
Allele no.	**OARCP26**			**Allele no.**	**MAF64**		
	Gray	**Zandi**	**Karakul**		**Gray**	**Zandi**	**Karakul**
1	0.049	0.114	0.137	1	0.134	0.325	0.397
2	0.433	0.452	0.170	2	0.196	0.213	0.196
3	0.357	0.333	0.335	3	0.165	0.254	0.326
4	0.063	0.101	0.181	4	0.335	0.163	0.082
5	0.049	0.000	0.099	5	0.085	0.046	0.000
6	0.049	0.000	0.077	6	0.085	0.000	0.000
Allele no.	**BMS995**			**Allele no.**	**MCMA26**		
	Gray	**Zandi**	**Karakul**		**Gray**	**Zandi**	**Karakul**
1	0.077	0.141	0.130	1	0.047	0.057	0.104
2	0.128	0.098	0.089	2	0.060	0.070	0.066
3	0.068	0.103	0.120	3	0.085	0.110	0.115
4	0.098	0.051	0.052	4	0.175	0.162	0.159
5	0.081	0.090	0.073	5	0.145	0.149	0.066
6	0.120	0.077	0.130	6	0.098	0.079	0.088
7	0.077	0.073	0.052	7	0.051	0.070	0.099
8	0.068	0.090	0.068	8	0.073	0.057	0.066
9	0.068	0.098	0.125	9	0.094	0.061	0.055
10	0.077	0.051	0.052	10	0.120	0.101	0.066
11	0.137	0.128	0.109	11	0.051	0.083	0.115
Allele no.	**BM6444**			**Allele no.**	**OARCP49**		
	Gray	**Zandi**	**Karakul**		**Gray**	**Zandi**	**Karakul**
1	0.174	0.058	0.050	1	0.133	0.098	0.059
2	0.071	0.096	0.050	2	0.150	0.107	0.128
3	0.192	0.233	0.111	3	0.168	0.171	0.117
4	0.089	0.175	0.150	4	0.164	0.197	0.122
5	0.121	0.254	0.150	5	0.053	0.051	0.170
6	0.089	0.067	0.083	6	0.093	0.051	0.096
7	0.063	0.075	0.122	7	0.093	0.150	0.053
8	0.116	0.042	0.161	8	0.053	0.090	0.112
9	0.085	0.000	0.122	9	0.093	0.085	0.144
		Allele no.	**BMS460**				
			Gray	**Zandi**	**Karakul**		
		1	0.000	0.143	0.000		
		2	0.000	0.237	0.043		

Table 3. Contd.

		3	0.127	0.147	0.085		
		4	0.145	0.116	0.197		
		5	0.219	0.112	0.234		
		6	0.219	0.174	0.319		
		7	0.149	0.071	0.122		
		8	0.140	0.000	0.000		

The highest allele frequency/breed is in bold typeface. no. = Number.

Table 4. Mean heterozygosity and polymorphism information content (PIC) in the three sheep breeds.

Heterozygosity and PIC	Gray	Zandi	Karakul
Mean observed heterozygosity ± SD	0.9841 ± 0.0297	0.9857 ± 0.0152	0.9885 ± 0.0117
Mean expected heterozygosity ± SD	0.8366 ± 0.0619	0.8297 ± 0.0654	0.8306 ± 0.0643
Mean PIC ± SD	0.8155 ± 0.0743	0.8077 ± 0.0803	0.8081 ± 0.0780

SD, Standard deviation.

Table 5. PIC, F_{IT}, F_{ST}, G_{ST} and F_{IS} values, and chi-square and G-square test for HWE for each locus over all breeds.

LOCUS	PIC	F_{IT}	F_{ST}	F_{IS}	G_{ST}	Chi-square	G-square	Df
MCMA2	0.906	-0.075	0.005	-0.080	0.005	653.2***	618.3***	66
MCM63	0.842	-0.158	0.021	-0.183	0.021	402.6***	394.2***	28
BMS460	0.828	-0.172	0.034	-0.213	0.034	558.6***	467.6***	28
BM1815	0.786	-0.216	0.030	-0.253	0.030	302.9***	300.3***	15
OARCP26	0.680	-0.361	0.032	-0.406	0.032	726.2***	444.7***	15
OARFCB20	0.781	-0.219	0.011	-0.233	0.011	222.2***	266.5***	15
OARAE129	0.830	-0.167	0.042	-0.218	0.042	483.2***	477.3***	21
MAF64	0.743	-0.224	0.039	-0.274	0.039	359.9***	333.4***	15
BMS332	0.796	-0.190	0.003	-0.193	0.003	247.5***	278.3***	21
LSCV38	0.807	-0.196	0.013	-0.212	0.013	600.6***	513.5***	15
BM6444	0.859	-0.137	0.019	-0.159	0.019	424.7***	407.1***	36
BMS995	0.896	-0.082	0.004	-0.086	0.004	275.1***	268.5***	55
MCMA26	0.891	-0.111	0.005	-0.117	0.005	676***	619***	55
BMS678	0.906	-0.082	0.007	-0.090	0.007	634.9***	588.4***	78
OARCP49	0.869	-0.127	0.010	-0.139	0.010	617.7***	585.7***	36
Mean	0.828	-0.168	0.018	-0.19	0.018			

*** $p < 0.001$.

heterozygosity) overall loci in Gray, Zandi and Karakul breeds are 0.984, 0.986 and 0.988, respectively. Whereas the average expected heterozygosity overall loci in the three breeds are 0.837, 0.830 and 0.831, respectively (Table 4). These results show more heterozygosity than expected in each breed. Mean PIC overall loci in Gray, Zandi and Karakul breeds are 0.8155, 0.8077 and 0.8081, respectively (Table 4). As expected, fairly high level of genetic heterogeneity was further reflected within three breeds by a mean PIC value. These high estimates of PIC substantiated the suitability of used set of markers to applications such as parentage control, linkage-mapping programs in addition to genetic polymorphism studies in Iranian sheep too.

The PIC values for all loci ranged from 0.680 (OARCP26) to 0.906 (MCMA2). The PIC values are very high, indicating that these loci are highly informative and suitable for genetic studies of sheep breeds (Table 5).

Tests of genotype frequencies for deviation from Hardy-Weinberg equilibrium (HWE), at each locus overall breeds, reveal significant departure from HWE ($P > 0.001$) (Table 5). G_{ST} and F-statistics for each locus overall

Table 6. Per pair Fst values between all pairs of the tested breeds.

Breed	Gray	Zandi
Gray		
Zandi	0.013	
Karakul	0.013	0.015

Table 7. Nei's genetic distances between the studied breeds.

Breed	Gray	Zandi
Gray		
Zandi	0.141	
Karakul	0.139	0.155

breeds are given in Table 5. The global F_{IS}, F_{ST} and F_{IT} are -0.19, 0.018 and -0.168, respectively. All markers had negative values of F_{IS}, showing an excess of heterozygotes. F_{ST} values of genetic differentiation and GST values of breed differentiation were similar and ranged from 0.003 (BMS332) to 0.042 (OARAE129). Per pair estimator of FST, which is the measure of differentiation among population, is 0.013 between Gray and Zandi, 0.013 between Gray and Karakul and 0.015 between Zandi and Karakul (Table 6). The calculated genetic distance matrix is shown in Table 7. The distance between Gray and Zandi, and Gray and Karakul was smaller than the distance between Zandi and Karakul. Nei' (1978) genetic distances among breeds are presented in Table 7, with the corresponding UPGMA dendrogram and neighbour-joining tree in Figures 1 and 2, respectively. The smallest genetic distance (0·139) was estimated between Gray and Karakul. There is little differentiation between the pelt sheep breeds with Nei distances ranging from 0·139 to 0·155. The cluster analysis shows that Gray and Karakul breeds cluster independently from Zandi breed.

DISCUSSION

Recently, the preservation of unique, genetically distinct breeds of domesticated animals, especially indigenous, has received much attention. Knowledge and information on genetic diversity, population structure and genetic relationships between populations are absolute prerequisites for defining and accomplishing effective preservation strategies (Kusza et al., 2010). This study aimed to characterize the genetic diversity and structure of pelt sheep populations by using fifteen microsatellites. Over the past decade, numerous studies on genetic diversity in domestic livestock (mainly in small ruminants), based on the analysis of microsatellite loci, have been carried out worldwide. Investigation of genetic variation in Taleshi goat using microsatellite loci indicated substantial genetic variation based on their gene diversity and average number of alleles per locus (Mahmoudi and Babayev, 2009). Genetic characterization of Alpine sheep breeds was established on the basis of individual genotypes at microsatellite loci (Dalvit et al., 2008). Study of genetic relationships among bulgarian sheep breeds using microsatellite loci indicated a high level of variation in the tested breeds (Kusza et al., 2010). Arora et al. (2008) examines the genetic variability in Jalauni, an important sheep of northwestern arid and semi arid region of India, at 25 microsatellite loci. Also, genetic diversity and relationships within and among Magra, Marwari and Sonadi sheep breeds of India, were distinguished based on microsatellite markers (Arora et al., 2008).

In Iran, many studies were performed to evaluate the genetic diversity of Iranian sheep breeds. Esmail et al. (2007) used nineteen microsatellites to evaluate genetic variation within Baluchi sheep breed. Banabazi et al. (2007) studied the genetic variation within and between five Iranian sheep populations including Sanjabi, Kordi Kordistan, Kordi Khorasan, Mehraban and Moghani using six microsatellite Markers. Also, genetic variation among different ecotypes of the Iranian sanjabi sheep was investigated based on the analysis of microsatellite loci (Sharifi et al., 2009). In the present study, fifteen microsatellite loci were used to evaluate the genetic diversity within and between pelt sheep breeds reared in Iran. The fifteen microsatellites are all polymorphic in the three breeds. The total number of alleles was 122 at the 15 studied loci. Major differences between the three breeds were not observed. The average expected heterozygosity overall loci in Gray, Zandi and Karakul are 0.837, 0.830 and 0.831, respectively. High value of average expected heterozygosity within the breed could be attributed to the large allele numbers detected in the tested loci (Kalinwski, 2002). The average direct count of heterozygosity overall loci in each of the three sheep breeds is more than the expected heterozygosity.

All loci were derived from Hardy-Weinberg equilibrium ($p < 0.001$) due to excess of heterozygote individuals than homozygote individuals, migration, high mutation rate in microsatellite and artificial selection in all breeds (Aminafshar et al., 2008). Deviation from HWE at microsatellites loci have, also been reported in various studies (Barker et al., 2001; Laval et al., 2000; El Nahas, 2008; Aminafshar et al., 2008; Sharifi sidani et al., 2009). It is known that a population is considered to be within HWE only when it is able to maintain its relative allele frequencies.

The global inbreeding coefficients F_{IS} (-0.19) and F_{IT} (-0.168) observed in the present study indicate an excess of heterozygotes and so it does not probably encounter problems that results from inbreeding depression. This result may explain the observed high value of direct count of heterozygosity in each breed and the deviation from HWE which were detected in all loci overall breeds.

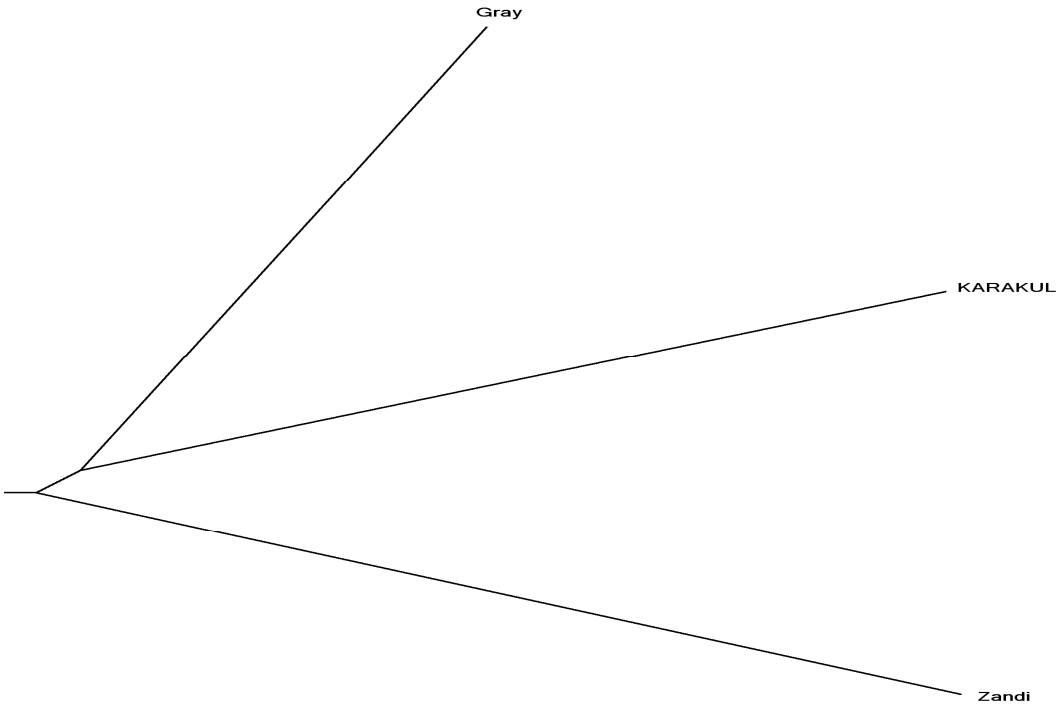

Figure 1. UPGMA phylogenetic radial tree based on Nei's standard genetic distances of the three sheep breeds.

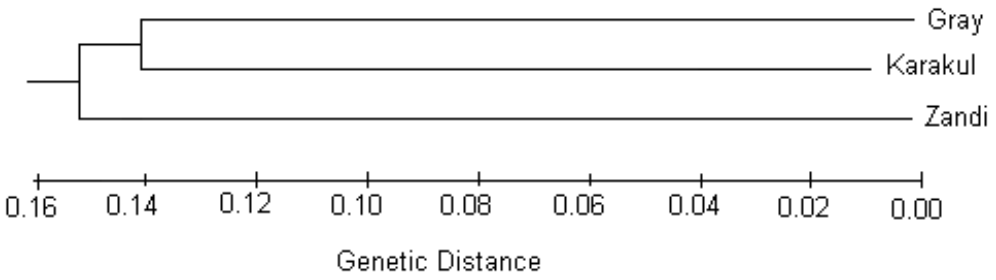

Figure 2. Neighbour-joining phylogenetic tree based on Nei's standard genetic distances.

According to Hartl (1980), per pair F_{ST} value equals 0.05 is indicative for moderate differentiation between populations. The per pair FST values reported in the present investigation between all pairs of the tested breeds are less than 0.05 which may indicate a low differentiation between populations under investigation. The estimated F_{ST}, which corresponds to the proportion of genetic variability accounted for by differences among breeds, was 0.018. These results indicate that genetic diversity quantified by microsatellite markers shows very little differentiation among pelt sheep breeds. Our results are similar to those reported for other sheep breeds, where F_{ST} estimates range between 0.03 and 0.08 (Arranz et al., 1998; Alvarez et al., 2004; Rendo et al., 2004; Sodhi et al., 2006; Peter et al., 2007; El Nahas et al., 2008). The genetic similarity observed among three breeds is probably a result of migration among populations that may have a common origin and which have been selected mostly for morphological traits associated with the breed standard. Migration has a great effect on the reduction of genetic differentiation between popula-tions (Laval et al., 2000). Additionally, low genetic distance values (Ds = 0.139 - 0.155) supported high genetic similarity between these three breeds and were in similar range with those cited by Arranz et al. (1998) and Sodhi et al. (2006) for closely related Spanish sheep breeds (Ds = 0.21 – 0.36) and Nali and Chokla sheep (Ds = 0.229), respectively. In summary, the three breeds were found to be genetically and closely related to each other, although there was a significant geographical distance between the three populations.

In conclusion, the tested microsatellites, being all polymorphic in the three breeds, could be fruitfully used for the differentiation between breeds. The evaluation of

genetic variations within and between pelt sheep breeds may be used as basis for the development of common breeding strategy for genetic improvement of these breeds.

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