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Population genetic structure and performing assignment test on six Iranian native goats using simple sequence repeat markers

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Abstract

The native goat breeds could be better managed and their genetic diversity to be conserved through identification of population genetic structure. Total of 299 animals from six goat breeds, which are major native breeds of Iran, were used to study their genetic structure and understand relationship among the breeds using SSR markers on 13 microsatellite loci. The breeds were selected from different geographic regions of Iran. The results indicated that there is high genetic diversity at the population level (H_S of 0.78) and at the species level (H_T of 0.86). The level of inbreeding was low across the breeds and even genetic diversion was observed among them, indicating a low level of gene flow at the regional scale. Some level of admixtures was observed among breeds, which supported by clustering of the breeds based on their geographic origin. Analysis of the population genetic structure indicated that all breeds are grouped into four clusters. The assignment accuracy per locus ranged from 40.1% (BM4621) to 66.9% (oarJMP23). The assignment power of microsatellites based on the Bayesian method had positive correlation with the number of alleles and gene differentiation coefficient (Gst) per locus. In conclusion, this study provided a genetic profile for the conservation and improvement and origin of the studied breeds.

Keywords: conservation, genetic diversity, goat, microsatellite

Introduction

Goats constitute important source of proteins, having crucial effects on the life of human population especially in less favored regions around the world (Dubeuf et al., 2004). They are one of the well adapted livestock species to various climate regions

(Luikart et al., 2001; MacHugh and Bradley, 2001). In addition to the nutrient importance and the high adaptability of goats, they could strongly contribute in controlling weeds, ecosystem preservation, improving wild-life habitants and decreasing the incidences of wildfire (El Aich and Waterhouse, 1999).

Developing countries are home to 60% of total of 921 million worldwide. Iran as a country with diverse climates and as a rapidly developing country is home to 22 million goats (Food and Agriculture Organization, 1997). They could be found in various climate regions including cold mountain area (Lorestan province) and very warm prairie (Khozestan province) in Iran (Bizhan, 2011). Goats are considered as multi-purposes animals which are raised for milk, meat, and fiber, and revenue of many nomadic tribes solely depends on this livestock husbandry (Mahmoudi and Babayev, 2009). They are raised nomadically and mainly named by their demographic position. Iranian breeds are identifiable by their physical appearance and production performance (Vahidi et al., 2014).

Studying genetic diversity and population structure of goats are very essential from prospective of conversation their invaluable genetic resources (Hedrick, 2011). Native breeds are invaluable resources because of their genetic diversity and adaptation to the environment over the years. In the recent years, however, the diversity of native breeds is concerned since there are non-systematic crossbreeding practices among farmers and there is not any association or organized record track. In the other hand, another factors, such as climate changes, environmental and ecological factors, natural barriers, human activities, migration, gene flow, or combination of these factors, impose these invaluable genetic resources in the risk of elimination (Wright, 1949; Noss, 1990; Frankham et al., 2002). To address the issue of preservation genetic variation of goat breeds, many researches have been conducted in many countries such as Portugal (Bruno-de-Sousa et al., 2011), China (Chen et al., 2005; Di et al., 2011), Europe and the Middle East (Canon et al., 2006), Italia (Sacchi et al., 2005), and Pakistan (Sultana et al., 2003). There is, however, limit reports about the genetic variation and population structure of Iranian native breeds. Vahidi et al. (2014) investigated genetic diversity among seven Iranian native goat breeds, namely Markhoz, Najdi, Taleshi, Khalkhali, Naini, native Abadeh and Turki-Ghashghaei. They reported that Iranian breeds possess a remarkably genetic diversity within-breed component and also gene flow within and between regions. In previous studies, findings showed that Iranian native breeds, Markhoz (MAR), Najdi (NAJ), Korki Jonub Khorasan (KJK), Taleshi (TAL), Raeini (RAE), and Lori (LOR), are phylogenetically grouped into two cluster. It has also been indicated that there is a signature of bottleneck in Tali and Markhoz populations (Mahmoudi et al., 2012; Mahmoudi et al., 2013; Mahmoudi et al., 2014).

The available information about Iranian native goats is not enough to manage and watch genetic resources of goats in Iran. In order to provide knowledge about Iranian native goats, population genetic structure of some dominant breeds of Iran was investigated. This study tests genetic relationships between Iranian native breed populations to discover possible in danger population.

Material and methods

Breeds and sample collection

Six dominant breeds were selected to include in this study. The breeds were Markhoz (MAR), Najdi (NAJ), Korki Jonub Khorasan (KJK), Taleshi (TAL), Raeini (RAE), and Lori (LOR). Markhos breed (MAR) is found in Kurdistan province of Iran and is well known for fine fiber and adaption to the harsh winter of Zagros Mountains. Najdi (NAJ) breed is predominated in Khuzestan province and are reared for milk and fleece. This breed is well adapted to high temperature of over 40 ºC for more than 6 months in a year. The Korki Jonub Khorasan (KJK) breed is adapted to southern of Khorasan province, which has cold and hot climate of desert and semi-arid conditions. The Taleshi (TAL) breed is reared in Hormozgan and Kerman provinces and well known for their high milk yield. Whereas in the same region, Raeini breed (RAE) is reared for their fine fiber. Hormozgan and Kerman provinces have high temperature and humidity over 6 months of the year. Finally, the Lori (LOR) breed goats are raised in Lorestan province where is mountainy area with cold winter and mild summer. This breed is medium size with good chevron performance.

Blood samples were collected from total of 299 individuals which 51, 53, 45, 56, 49, and 45 samples were KJK, LOR, MAR, NAJ, RAE, and TAL breeds, respectably. These animals were located in six different environments and geographical areas. The blood samples were collected into vacationer containing EDTA and stored at temperature -20 ºC for further analysis.

DNA extraction

The DNA was extracted from whole blood sample based on the method described by Sambrook (Sambrook et al., 1989). The DNA isolation procedure was as following: lysis of red blood cells, digestion of protein by using proteinase K, and precipitation of protein by using phenol, chloroform, and isoamyl alcohol with the ratio of 25:24:1.

Microsatellite loci and the polymerase chain reaction (PCR) procedure

Microsatellite markers or simple single repeats (SSR), 20 number, were tested from which 5 SSR failed to amplify and 2 SSR showed monomorphic pattern. Finally, 13 polymorphic SSRs were selected to use in this study. The selected SSRs were MAF64, BM4621, BM121, LSCV36, TGLA122, oarJMP23, oarFCB304, oarAE133, ILSTS005, ILSTS022, ILSTS029, ILSTS033 and ILSTS034. None of these loci was linked to each other. The DNAs were amplified by implementing polymerase chain reaction (PCR) followed with 60 ng of target DNA within 25 mL PCR reaction-containing PCR buffer, 50 ng primer, 200 mM of dNTPs, 0.5 unit Taq DNA polymerase, and 1.5 mM $MgCl_2$.

The thermo cycle setting was set to:

(1) 3 cycles with 45 sec at 95 °C and 1 min at 60 °C

(2) 3 cycles with 45 sec at 95 °C and 1 min at 57 °C

(3) 3 cycles with 45 sec at 95 °C and 1 min at 54 °C

(4) 3 cycles of 45 sec at 95 °C and 1 min at 51 °C

(5) 20 cycles with 45 sec at 92 °C and 1 min at 48 °C

In all cycles, elongation temperature and time were 72 °C and 1 min. The SSR markers were resolved in 6% polyacrylamide and stained with silver staining. Then, alleles were scored using unlabeled primers (Bassam et al., 1991). The genotypes of individuals were recorded by direct counting at 13 microsatellite loci.

Diversity analysis

Mean of alleles, number of observed heterozygosity (H_O), expected heterozygosity (H_E), average genetic diversity (H_S), total gen diversity (H_T), and Wright's F-statistics were calculated to estimate measures of genetic diversity using software packages of POPGENE (Yeh et al., 1997) and GenAlex v.6.0 (Peakall and Smouse, 2006).

Wright's F-statistics and F_{ST} are the most widely used descriptive statistics in population and evolutionary genetic studies to provide great insight in population structure and variation within and among populations. The regions of genome that was targeted by selection can be determined by F_{ST}, and comparing estimates of F_{ST} from different parts of genome can shed light on demographic history of population. F_{ST} directly related to allele frequency and conversely related to the degree of resemblance of allele among individuals. The lower value of F_{ST} means individuals are similar and the higher value means they have different allele frequency.

Wright defined three interrelated parameters F_{IT}, F_{IS}, and F_{ST}. The estimates of F_{IT} show the correlation between gametes within an individual relative to the entire population. F_{IS} shows correlation between an individual relative to a subpopulation from which that individual belongs to. F_{ST} shows between gametes chosen randomly with same sub-population relative to entire population.

Molecular variance (AMOVA) was calculated using Arlequin v.3.5.1.2 software package (Excoffier and Lischer, 2010). AMOVA is a method similar to ANOVA in a way that detects a population differentiation using DNA markers. MVSP-A multivariate statistical Package v.3.22 was used to calculate Principal Component Analysis (PCA) and pattern of genetic variation among populations. PCA shows variation and patterns of a dataset in the simple way. This technique was used to visualize and explore easily the six breed differentiations based on allele frequencies.

Population structure analysis

Population genetic structure was assessed by Structure software package (Pritchard et al., 2000). This software is based on Bayesian clustering approach and groups in clusters of similar genetic structure. The most probable number of population/breed (K value) was used to determine the best clustering value (K values) using Structure

Harvester software package (Earl, 2012). The K was also calculate using the observed genotypic data by performing 10 independent runs for each K (K=1-8) with both burn-in length and MCMC iterations at 100,000. Degree of admixture (alpha parameter) was extracted from the data using the default settings of Structure Harvester software package and an admixture model with correlated allele frequencies (Falush et al., 2003). Degree of admixture is used to detect levels of genetic admixture across populations.

Assignment test was performed using Geneclass2 (Piry et al., 2004), which calculates various genetic assignment criteria to assign or exclude reference populations. This software includes several Monte Carlo resampling algorithms that compute probability for each individual belonging to each reference population or probability to be a resident in the population where it was sampled. The percentage of individuals correctly assigned to source breed was calculated for distinct threshold values of breed. Total of nine models were used for this purpose, including Bayesian method, Nei's standard distance, DA of Nei et al. (1983), Cavilli-Sforza, Gene frequency, Nei's minimum distance, Shared allele distance, $(\mu\delta)^2$ of Goldstein, and Paetkau et al. (1995) model.

Results

Genetic diversity of markers

Total of 13 SSRs markers were used in this study and the results showed no evidence of linkage disequilibrium within each locus (P>0.001 after Bonferroni corrections). A total of 134 different alleles were identified among 290 animals. The mean effective number of alleles (Ne) was 7.5 across all loci, which was lower than observed mean number of each allele, 10.3. The loci oarAE133 showed the highest total number of alleles (T_A), 15, and the loci oarJMP023 indicated the lowest value, 7, among 13 tested SSRs. Most of the loci were highly polymorphic with mean of 0.75. Based on polymorphism information content (PIC) values, the locus oarAE133 with 0.58 had the lowest value and both the loci BM4621 and ILSTS0050.81 were 0.81. Other loci showed PIC values between 0.58 and 0.81 (Table 1).

Deviation from Hardy-Weinberg Equilibrium (HWE) was observed in at least one locus in all population (P<0.05). The expected (H_e) and observed (H_o) heterozygosity across breeds were 0.782 and 0.804, respectively. Within all breeds, except TAL, H_o was higher than H_e (Table 2).

Genetic differentiation across breeds

The population differentiation varied significantly (P<0.05) between 0.052 for the locus BM4621 and 0.145 for the locus LSCV36. The mean of F_{ST} value was 0.1 for all markers**.** The population-inbreeding coefficient relative to subpopulation (F_{IS}) ranged from -0.12 to 0.03 and population-inbreeding coefficient relative to total population (F_{IT}) ranged from 0.017 to 0.13. Means for total diversity (H_T), variability within breeds (H_S), and intra-variety genetic diversity ($D_{ST} = H_T - H_S$) were 0.86, 0.78, and 0.08, respectively (Table 1). Analyzing the genetic differentiation of the breeds showed that The F_{IS} values for breeds were in the range of -0.06 for the breed KJK to 0.02 for the

breed TAL (Table 2). Although gene differentiation coefficient (G_{ST}) was low among breeds, 9.5% variation, it was high, 90.5%, within breeds. The results of the pairwise F_{ST} distance analysis for pairs of six breeds showed that the F_{ST} value was in the range of 0.05 for the pair TAL–NAJ to 0.15 for the pair of LOR–NAJ. The F_{ST} value of LOR was between 0.11 and 0.15 meaning that this population have possessed the highest genetic differentiation compared to other populations (Table 3).

Analysis of molecular variance (AMOVA), index for population differentiation, confirmed existence of 89% variation within breeds. There was, however, low amount of genetic differentiation (11%) across breeds. Of 134 alleles, 28% were common among breeds, and only 9% were breed specific alleles.

Individual breed assignment to determine accuracy of methods

In order to implement assignment test on 13 studied SSR markers, nine models were tested. The model $(\mu\delta)^2$ of Goldstein showed the lowest accuracy (16.39% correctly assignment) while other studied models showed correct accuracy of more than 94%. Bayesian method indicated 100% correctly assignment (Table 4). Then, the Bayesian method was chosen and used to analyze the performance of assignment for the 13 microsatellites. Results showed that the correct assignment based on the single locus was in the range of 40.1% for BM121 locus to 66.9% for oarJMP23 locus. Based on the results of assignment test, two distinct groups of loci were established. The evaluation of these groups through Bayesian method showed that six loci (MAF64, BM121, LSCV36, oarJMP23, oarAE133, ILSTS034) had the high individual score, correct assignment of 99.7%. The remaining loci (BM4621, TGLA122, oarFCB304, ILSTS005, ILSTS022, ILSTS029, ILSTS033) had the low individual score with correct assignment value of 84.3% (Table 5).

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Table 1. Genetic diversity of 13 microsatellite loci in 290 individuals across six breeds

Locus	Location on chromosome	T_A	Ne	H_o	H_S	H_T	F_{IT}	F_{ST}	F_{IS}	N_m	PIC	D_{ST}	G_{ST}
BM121	16	11	4.44	0.805	0.813	0.866	0.069	0.069	0	3.37	0.78	0.053	0.061
BM4621	6	10	8.02	0.829	0.838	0.877	0.053	0.052	0.001	4.549	0.81	0.038	0.044
ILSTS005	10	12	10.37	0.854	0.834	0.905	0.055	0.087	-0.034	2.64	0.81	0.071	0.079
ILSTS022	3	8	7.42	0.756	0.786	0.867	0.126	0.102	0.027	2.204	0.75	0.081	0.094
ILSTS029	3	9	8.17	0.84	0.823	0.878	0.042	0.071	-0.032	3.257	0.79	0.056	0.063
ILSTS033	12	10	7.57	0.815	0.798	0.873	0.065	0.094	-0.031	2.423	0.76	0.075	0.086
ILSTS034	5	9	6.86	0.793	0.768	0.856	0.072	0.11	-0.043	2.026	0.72	0.088	0.102
LSCV36	19	9	7.26	0.769	0.744	0.863	0.108	0.145	-0.044	1.472	0.7	0.119	0.138
MAF64	1	9	5.93	0.802	0.739	0.833	0.035	0.12	-0.096	1.834	0.69	0.094	0.113
oarAE133	Not known	7	5.01	0.728	0.654	0.802	0.091	0.19	-0.123	1.063	0.58	0.148	0.184
FCB304	19	11	7.66	0.822	0.794	0.871	0.055	0.096	-0.045	2.357	0.76	0.077	0.088
JMP23	27	15	9.01	0.8	0.791	0.891	0.101	0.119	-0.021	1.847	0.77	0.099	0.112
TGLA122	21	14	6.66	0.84	0.786	0.851	0.011	0.084	-0.08	2.726	0.75	0.065	0.077
Mean	Not applicable	134	7.5	0.804	0.782	0.864	0.068	0.103	-0.04	2.177	0.75	0.082	0.095

T_A - Total Number of Alleles, Ne - Effective Number of Alleles, H_o - Observed Heterozygosity, H_s - Gene Diversity, H_T - Overall Gene Diversity, F_{IT} - Population Inbreeding Coefficient Relative to Total Population, F_{ST} - The Effect of Subpopulations Compared to The Total Population, F_{IS} - Population Inbreeding Coefficient Relative to Subpopulation, N_m - The Number of Effective Migrants, PIC - Polymorphism Information Content, D_{ST} - Intra-variety Genetic Diversity, G_{ST} - Gene Differential Coefficient.

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Table 2. Genetic differentiation of six Iranian native goat breeds based on 13 microsatellite loci

	Breeds	n	TNA	MNA	SD	AR	SD	Ho	SD	He	SD	F_{IS}	PIC
1	RAE	49	92	7.077	1.977	4.91	0.28	0.808	0.06	0.796	0.046	-0.016	0.76
2	KJK	51	98	7.538	2.106	5.217	0.385	0.845	0.093	0.798	0.08	-0.059	0.76
3	LOR	53	90	6.923	2.216	4.987	0.437	0.817	0.094	0.786	0.077	-0.04	0.75
4	MAR	45	87	6.692	2.057	5.21	0.454	0.824	0.114	0.799	0.064	-0.031	0.76
5	NAJ	56	81	6.231	2.006	4.309	0.413	0.782	0.057	0.751	0.078	-0.042	0.71
6	TAL	45	85	6.538	1.898	4.604	0.404	0.749	0.104	0.765	0.096	0.021	0.72

TNA - Total number of Alleles, MNA - Mean Number of Alleles, AR - Allelic Richness, SD - Standard Deviation, H_O - Observed Heterozygosity, H_E - Expected Heterozygosity, F_{IS} - Population Inbreeding Coefficient, PIC - Polymorphism Information Content. RAE - Raeini, KJK - Korki Jonub Khorasan, LOR - Lori, MAR - Markhoz, NAJ - Najdi, TAL - Tali.

Table 3. Nei's DA genetic distance matrix and Pairwise FST distance between six Iranian goat breeds

	RAE	KJK	LOR	MAR	NAJ	TAL
RAE		0.082	0.12	0.123	0.145	0.134
KJK	0.464		0.112	0.104	0.123	0.113
LOR	0.756	0.681		0.112	0.15	0.131
MAR	0.845	0.655	0.693		0.077	0.084
NAJ	0.89	0.679	0.898	0.362		0.053
TAL	0.833	0.644	0.769	0.437	0.224	

The zero values indicate diagonal, FST are represented above and DA are represented below of diagonal. RAE - Raeini, KJK - Korki Jonub Khorasan, LOR - Lori, MAR - Markhoz, NAJ - Najdi, TAL - Tali.

Table 4. Assignment test of 13 microsatellite loci

Methods	Correctly assignment (%)
Bayesian method	100
Nei's standard distance	94.31
DA of Nei et al. (1983)	99.33
Cavilli-Sforza	98.66
Gene frequency	99.33
Nei's minimum distance	95.65
Shared allele distance	95.65
$(\mu\delta)^2$ of Goldstein	16.39
Paetkau et al. (1995)	99.3

Table 5. The correct assignment of 13 microsatellite loci based on the single locus

Locus	Assignment (%)	Quantitative indexes
BM121	53.5	40.41
BM4621	40.1	30.45
ILSTS005	51.2	43.99
ILSTS022	46.8	39.14
ILSTS029	46.5	34.37
ILSTS033	40.8	34.61
ILSTS034	55.2	42.82
LSCV36	58.5	46.24
MAF64	59.5	51.44
oarAE133	61.5	56.44
oarFCB304	51.2	41.99
oarJMP23	66.9	53.02
TGLA122	47.5	39.89

Individual breed assignment to compare breeds

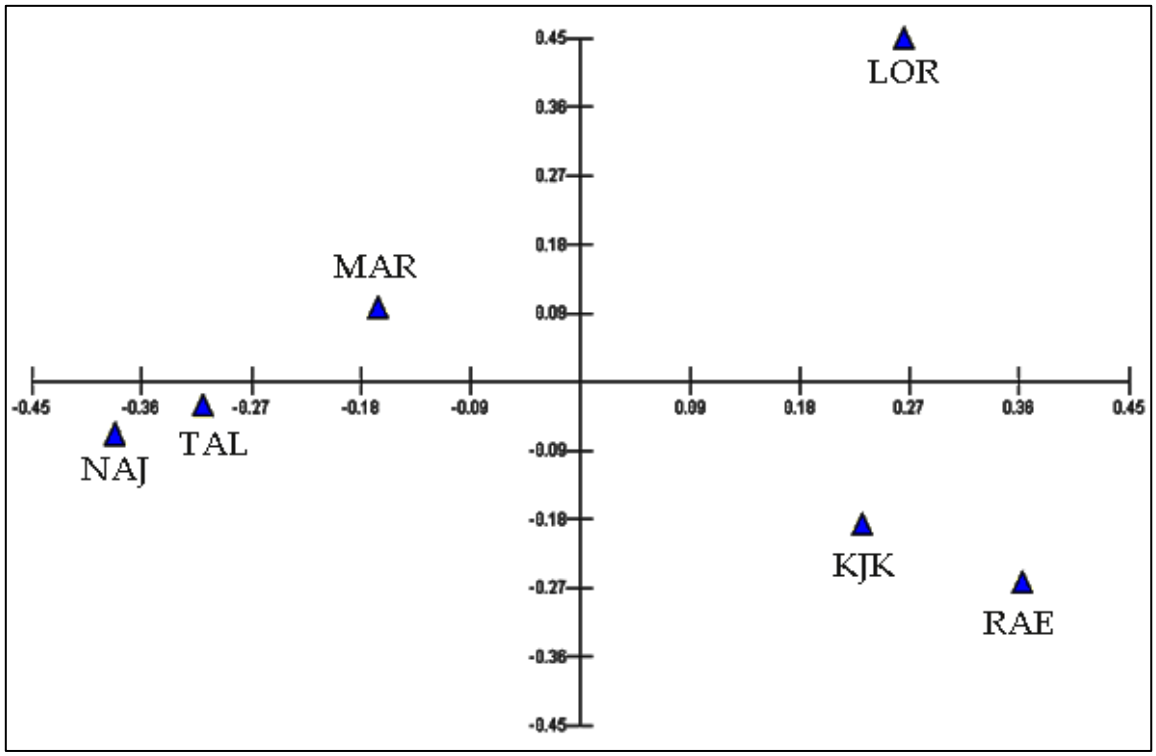
All individuals from different breeds were mixed and reassigned to breeds using assignment approaches based on multi-locus genotypes. The results from Bayesian method based on 13 microsatellites were analyzed using approach presented by Cornuet and Luikart (1996). The results indicated that the NAJ and KJK breeds had extreme pattern while other breeds had intermediate patterns. The log-likelihood average could be indicator of uniformity or heterogeneity among the genotypes, and it showed that NAJ breed had low uniformity (14.79±0.23) compared to other breeds. The degree of divergence among NAJ breed and other breeds was also confirmed by distributions of assignment criteria, which were 14.79±0.23 vs. 35.72±0.65 for NAJ and non-NAJ animals. The log-likelihood results also showed that KJK breed had the highest heterogeneity (16.61±0.24) among breeds. However, overlapping was observed among of the population noticeably in KJK goats with log-likelihood averages of 16.61±0.24 for KJK vs. 32.33±0.27 for other animals (Table 6).

Table 6. Log-likelihood average of six Iranian native goat breeds

	log	Standard error		log	Standard error
Raeini (RAE)	16.29	0.19	Non-RAE	35.92	0.34
Korki Jonub Khorasan (KJK)	16.61	0.24	Non-KJK	32.33	0.27
Lori (LOR)	16.01	0.22	Non-LOR	36.56	0.26
Markhoz (MAR)	16.18	0.21	Non-MAR	33.4	0.46
Najdi (NAJ)	14.79	0.23	Non-NAJ	35.72	0.65
Tali (TAL)	15.63	0.25	Non-TAL	33.01	0.58

Population structure analysis and genetic relationship

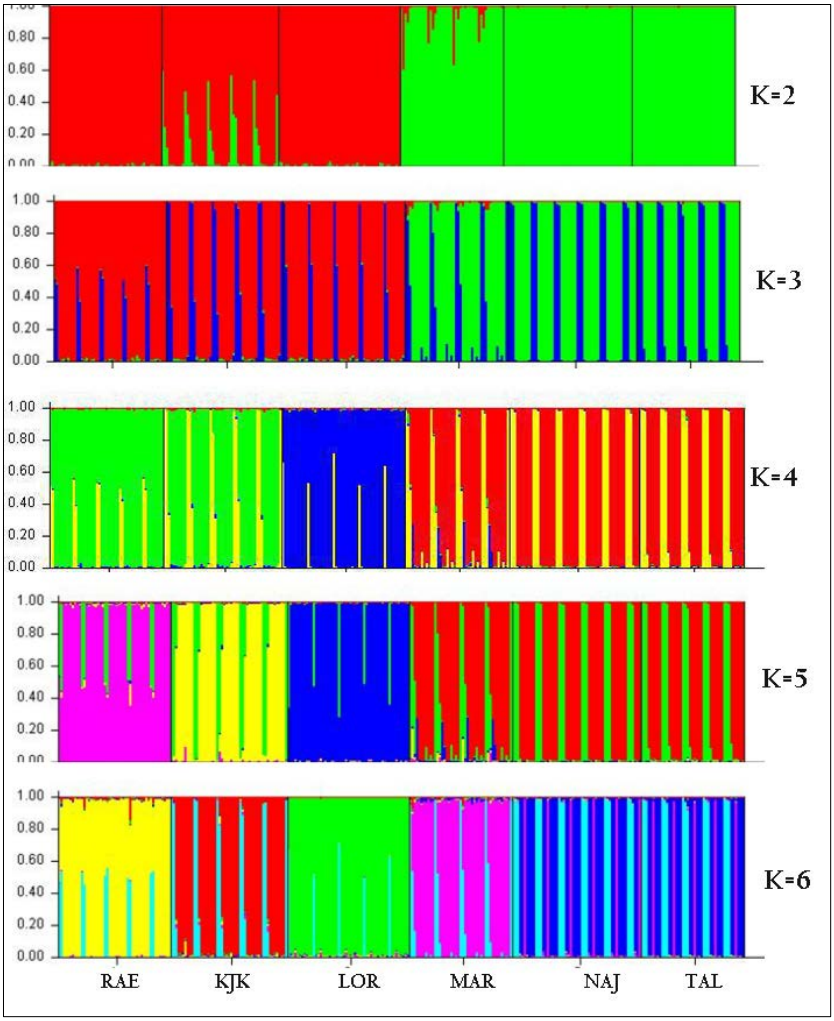
The population-based principal component analysis (PCA) was performed by using allele frequency of the markers. The PCA results showed that PC1 and PC2 (the first two components) accounted for 38.37% and 23.48% of the total variance, respectively. The first component of the PCA (PCA1) separated the breeds into two distinct groups: the breeds MAR, NAJ, TAL and RAE as group 1 and the breeds KJK and LOR in group 2. This pattern of grouping was not consistent with breeds demographic distribution. The second component of PCA (PCA2) grouped the breeds into four groups in which MAR was from west, TAL and NAJ were from south, LOR was from south-west, RAE and KJK were from east. This grouping was more in agreement with demographic distribution of the breeds (Figure 1).



RAE - Raeini, KJK - Korki Jonub Khorasan, LOR - Lori, MAR - Markhoz, NAJ - Najdi, TAL - Tali.
X-axis = 38.7% and Y-axis = 23.48%.

Figure 1. Principal component analysis (PCA) of six Iranian native goat breeds based on allele frequencies of 13 microsatellite loci

Results from Structure software and Structure Harvester software indicated that breeds were descended from three ancestral populations. The K equal to 4 yielded optimum number of clusters in Structure Harvest software package (Figure 2). At the K value of 4, MAR, NAJ and TAL were grouped into cluster 1, LOR alone into cluster 2, RAE, KJK into cluster 3. None of the six breeds were grouped in cluster 4. Cluster 3 and cluster 4 had the least and highest F_{ST} values of 0.13 and 0.22, respectively (Table 7). NAJ-TAL pair remained together in same cluster even at K=6 (Table 8).



Different color represents a different breed; Blue - Tali (TAL) and Najdi (NAJ), Violet - Markhoz (MAR), Green - Lori (LOR), Red - Korki Jonub Khorasan (KJK), Yellow - Raeini (RAE).

Figure 2. Population structure of six Iranian native goat breeds implementing 13 microsatellite loci using Structure software package

Discussion

Genetic diversity is the main factor on adaptability of organisms to the change of environmental conditions (Amos and Harwood, 1998). Diversity studies are very critical to conserve, utilize, and improve the existing germplasms (Zeb et al., 2009; Groeneveld et al., 2010). The results of this study showed that, Iranian goat breeds have high genetic diversity at both of the population and species level (Tables 1, 2). These diversities provide them with ability to be acclimated in various climate regions including cold mountain area and very warm prairie. However, the amount of genetic diversity for the six breeds in this study were lower than European breeds with allele number of 5.2 to 9.1, an average of 7.1, and allelic richness values of 6.1 to 7.9. However, it was high compared to East Asian indigenous populations with 2.5 to 7.6 mean number of alleles, an average of 5.8, and 2.3–6.89 allelic richness values (Canon et al., 2006; Nomura et al., 2012).

The KJK and MAR had more diversity among the six breeds (Table 2), which could be due to gene flow by mainly migration of male animals (Álvarez et al., 2004) and sampling variation within the breeds. High level of variation also could be due to selection of special management system or breeding practices (Barton and Slatkin, 1986; Woolaston and Piper, 1996; Hedrick, 2011).

The six Iranian goat breeds had significant diversity among the population with F_{ST} values of 0.11 (Table 2). These six breed diversity were higher than European indigenous goat breeds with F_{ST} value of 0.07 and lower than East Asia and Chines goat population with F_{ST} values of 0.13, 0.129, respectively (Canon et al., 2006; Nomura et al., 2012). This could be due to extent environment variation for Iranian breeds.

The assignment methods are very important to study the genomics of breeds for the different objectives such as to determine their migration, descendants, wildlife management, animal tracking, and hybridization detection (Paetkau et al., 1995; Cornuet and Luikart, 1996; Manel et al., 2002; Maudet et al., 2002). Animals in this study were clearly classified into their origin (Tables 4, 5) using nine assignment tests. Among the nine methods Bayesian method (Cooper and Herskovits, 1992), DA distance method (Saitou and Nei, 1987), and gene frequency method (Weir, 1990) had high accuracy; while, the $(\delta\mu)^2$ of Goldstein method (Goldstein, 1995) had the lowest accuracy. The lower performance for the $(\delta\mu)^2$ of Goldstein method was expected since this method performs better for the large and divergent breeds. Cornuet et al. (1996) reported the same order of accuracy for the methods.

Many factors could affect the efficiency of assignment methods, noticeably, the loci and allele numbers, the polymorphisms information content, genetic heterozygosity, and gene differential coefficient (Paetkau et al., 2004; Manel et al., 2005). The results of the study suggested that accuracy of the Bayesian method is related to the allele numbers and gene differential coefficient. However, it was not related to genetic heterozygosity and the polymorphism information content, which are based on allele numbers and frequencies in each locus. Furthermore, the alleles with extreme values should be treated carefully and rare unique alleles should be ignored in the breed assignment because the alleles with extreme values can mislead the assignment.

The percentage of correct assignment was variable in range of 40.1% (BM4621) to 66.9% (oarJMP023) based on single locus among the applied microsatellites. The assignment efficiency increases corresponding with increasing of microsatellite numbers and the population genetic differentiation. The breed assignment results could be useful to detect admixture, to identify exotic animals, and to select animals to be used in the breeding programs while to maintain the integrity of each breed.

The structure analysis of Iranian native goats revealed that some degrees of admixture and genetic relationship exist across the breeds. Interestingly, none of the six breeds were assigned in cluster 4 (Figure 3, Table 7), which it could show the influence of other existing breeds/populations of Iranian goats. For example, Adani, Mamasani and Nadoshan breeds in south of Iran could influence NAJ and TAL breeds since they are raised in the same region. This leads to a high level of admixture and a low genetic purification, and thereby creating of cluster 4.

The MAR breed was grouped with NAJ and TAL breeds within one cluster and remaining of the three breeds were grouped into three distinct clusters at K equal to

five. The animals were grouped in five when K set to six, five clear clusters in which RAE, KJK, LOR, MAR clustered as separate cluster and NAJ, TAL clustered yet in the same group together. Inclusion of NAJ and TAL in one cluster was further confirmed by the lower F_{ST} values for both of the breeds. Both of the NAJ and TAL counted for 55% of cluster 3 and over 31% of cluster 6 (Table 8). These results showed clear form of admixture in a way that both breeds could structurally be considered as one breed. These two breeds also have more similarity in their morphology. These two breeds also have the same origin, which is from Saudi Arabia and introduced to the southern provinces of Iran (Khuzestan and Hormozgan).

The LOR, NAJ, and TAL breeds were more heterozygosity among other breeds. However, the LOR breed had the highest breed specific alleles, which could be indicator of isolation of LOR breed from other breeds, although it is located geographically between MAR and NAJ breeds. The results of Bayesian clustering confirmed that the Iranian native goat breeds are highly conserved breeds with a small amount of admixture. The gene flow between LOR and MAR breeds was low, although they are from a relatively small and same geographical area. However, the lower gen flow among them could be due to mountain as a geographical boundary. The NAJ and MAR breed had a high amount of gene flow though they are located far from each other. It could be due to raising these breeds by the similarly named tribe of Iran and Iraq and possible interaction between them. The efficiency of Iranian native goat breeds clustering confirmed further by results of the PCA analysis. The clustering pattern and the genetic relationships for studied breeds were based on their natural geographical locations, but not based on their artificial selection for producing meat, milk, and cashmere. The results also indicated that the genetic differentiation is low for the breeds with close geographical distance, and the differentiation was increased as geographic distance was increased.

Table 7. Inferred clusters of Iranian native goat breeds based on Pritchard et al. (2000) at K=4

Breeds	Inferred clusters			
	1	2	3	4
RAE	0.006	0.884	0.006	0.104
KJK	0.008	0.736	0.009	0.248
LOR	0.004	0.005	0.93	0.061
MAR	0.716	0.008	0.034	0.243
NAJ	0.674	0.003	0.003	0.321
TAL	0.652	0.004	0.004	0.341
F_{ST}	0.168	0.128	0.187	0.22

RAE - Raeini, KJK - Korki Jonub Khorasan, LOR - Lori, MAR - Markhoz, NAJ - Najdi, TAL - Tali, F_{ST} - Fixation Index-Statistics.

Table 8. Inferred clusters of Iranian native goat breeds based on Pritchard et al. (2000) at K=6

Breeds	Inferred clusters					
	1	2	3	4	5	6
RAE	0.013	0.004	0.004	0.869	0.004	0.106
KJK	0.75	0.005	0.004	0.009	0.006	0.226
LOR	0.004	0.923	0.003	0.005	0.006	0.06
MAR	0.007	0.005	0.01	0.004	0.8	0.174
NAJ	0.003	0.002	0.563	0.002	0.112	0.318
TAL	0.003	0.003	0.552	0.003	0.111	0.329
F_{ST}	0.184	0.189	0.251	0.186	0.165	0.247

RAE - Raeini, KJK - Korki Jonub Khorasan, LOR - Lori, MAR - Markhoz, NAJ - Najdi, TAL - Tali, F_{ST} - Fixation Index-Statistics.

Conclusion

The objective of this study was to investigate population genetic structure of six Iranian native goats to provide information on their genetic relationships and differentiation. The results showed that Iranian native goat breeds have maintained their genetic diversity. However, the low amount of gene flow was estimated among the breeds. Structure analysis indicated that these six breeds were highly structured. Considering K value of 4, RAE and KJK breeds were grouped in one cluster, and the LOR breed grouped in a separate cluster. The MAR, NAJ, TAL clustered together, even though some degree of admixture was also observed among them. The NAJ and TAL showed a high similarity, while the LOR breed indicated the most homogeneity among other breeds. The breeds with close geographic distance showed lower genetic differentiation, while this index increased corresponding with increasing geographic distance of the breeds from each other. Therefore, Iranian native goats, essentially NAJ and TAL breeds, must be managed in order to manage their genetic diversity. There is, however, need for complementary studies about Iranian native goats to put in account for taking management decisions. Although microsatellite markers provide sufficient genetic information, using other novel approaches such as SNPs and high-throughput sequencing approaches could help understanding the population genetic structure of Iranian native goats. Furthermore, studying more samples and also samples from neighbor countries could further provide integrative information.

References

Álvarez, I., Royo, L., Fernandez, I., Gutiérrez, J., Gómez, E., Goyache, F. (2004) Genetic relationships and admixture among sheep breeds from Northern Spain assessed using microsatellites. Journal of Animal Science, 82 (8), 2246-2252. DOI: https://dx.doi.org/10.2527/2004.8282246x

Amos, W., Harwood, J. (1998) Factors affecting levels of genetic diversity in natural populations. Philosophical transactions of the Royal Society of London, Series B: Biological sciences, 353, 177-186. DOI: https://dx.doi.org/10.1098/rstb.1998.0200

Barton, N., Slatkin, M. (1986) A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. Heredity, 56(3), 409-415.

Bassam, B.J., Caetano-Anollés, G., Gresshoff, P.M. (1991) Fast and sensitive silver staining of DNA in polyacrylamide gels. Analytical Biochemistry, 196 (1), 80-83. DOI: https://dx.doi.org/10.1016/0003-2697(91)90120-I

Bizhan, M. (2011) Breed characteristics in Iranian native goat populations. Journal of Cell and Animal Biology, 5 (7), 129-134.

Bruno-de-Sousa, C., Martinez, A., Ginja, C., Santos-Silva, F., Carolino, M., Delgado, J., Gama, L. (2011) Genetic diversity and population structure in Portuguese goat breeds. Livestock Science, 135 (2), 131-139. DOI: https://dx.doi.org/10.1016/j.livsci.2010.06.159

Canon, J., García, D., García-Atance, M., Obexer-Ruff, G., Lenstra, J., Ajmone-Marsan, P., Dunner, S. (2006) Geographical partitioning of goat diversity in Europe and the Middle East. Animal genetics, 37 (4), 327-334. DOI: https://dx.doi.org/10.1111/j.1365-2052.2006.01461.x

Chen, S.-Y., Su, Y.-H., Wu, S.-F., Sha, T., Zhang, Y.-P. (2005) Mitochondrial diversity and phylogeographic structure of Chinese domestic goats. Molecular Phylogenetics and Evolution, 37 (3), 804-814. DOI: https://dx.doi.org/10.1016/j.ympev.2005.06.014

Cooper, G.F., Herskovits, E. (1992) A Bayesian method for the induction of probabilistic networks from data. Machine Learning, 9 (4), 309-347. doi.org/10.1007/BF00994110

Cornuet, J.M., Luikart, G. (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics, 144 (4), 2001-2014.

Di, R., Farhad Vahidi, S., Ma, Y., He, X., Zhao, Q., Han, J., Guan, W.-J., Chu, M., Sun, W., Pu, Y. (2011) Microsatellite analysis revealed genetic diversity and population structure among Chinese cashmere goats. Animal Genetics, 42 (4), 428-431. DOI: https://dx.doi.org/10.1111/j.1365-2052.2010.02072.x

Dubeuf, J.-P., Morand-Fehr, P., Rubino, R. (2004) Situation, changes and future of goat industry around the world. Small Ruminant Research, 51 (2), 165-173. DOI: https://dx.doi.org/10.1016/j.smallrumres.2003.08.007

Earl, D.A. (2012) Structure harvester: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources, 4 (2), 359-361.

El Aich, A., Waterhouse, A. (1999) Small ruminants in environmental conservation. Small Ruminant Research, 34 (3), 271-287. DOI: https://dx.doi.org/10.1016/S0921-4488(99)00079-6

Excoffier, L., Lischer, H.E. (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources, 10 (3), 564-567. DOI: https://dx.doi.org/10.1111/j.1755-0998.2010.02847.x

Falush, D., Stephens, M., Pritchard, J.K. (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics, 164 (4), 1567-1587.

Frankham, R., Briscoe, D.A., Ballou, J.D. (2002) Introduction to conservation genetics. Cambridge: Cambridge University Press.

Food and Agriculture Organization (1997) Statistics. Rome: Food and Agriculture Organization.

Groeneveld, L., Lenstra, J., Eding, H., Toro, M., Scherf, B., Pilling, D., Negrini, R., Finlay, E., Jianlin, H., Groeneveld, E. (2010) Genetic diversity in farm animals – a review. Animal Genetics, 41 (S1), 6-31. DOI: https://dx.doi.org/10.1111/j.1365-2052.2010.02038.x

Hedrick, P.W. (2011) Genetics of populations. Jones & Bartlett Learning.

Luikart, G., Gielly, L., Excoffier, L., Vigne, J.-D., Bouvet, J., Taberlet, P. (2001) Multiple maternal origins and weak phylogeographic structure in domestic goats. Proceedings of the National Academy of Sciences, 98 (10), 5927-5932. DOI: https://dx.doi.org/10.1073/pnas.091591198

MacHugh, D.E., Bradley, D.G. (2001) Livestock genetic origins: goats buck the trend. Proceedings of the National Academy of Sciences, 98 (10), 5382-5384. DOI: https://dx.doi.org/10.1073/pnas.111163198

Mahmoudi, B., Babayev, M.S. (2009) The investigation of genetic variation in Taleshi goat using microsatellite markers. Research Journal of Biological Sciences, 4 (6), 644-646.

Mahmoudi, B., Esteghamat, O., Shahriyar, A., Babayev, M.S. (2012) Genetic characterization and bottleneck analysis of Korki Jonub Khorasan goats by microsatellite markers. Journal of Cell and Molecular Biology, 10 (2), 61-69.

Mahmoudi, B., Habibi, R., Montazeri, S., Babayev Sh, M. (2013) Bottleneck analysis and microsatellite based genetic diversity in Najdi goat population. Journal of Animal Science Advances, 1 (3), 544-550.

Mahmoudi, B., Panahi, B., Mohammadi, S.A., Daliri, M., Babayev, M.S. (2014) Microsatellite based phylogeny and bottleneck studies of Iranian indigenous goat populations. Animal Biotechnology, 25 (3), 210-222. DOI: https://dx.doi.org/10.1080/10495398.2013.850431

Manel, S., Berthier, P., Luikart, G. (2002) Detecting wildlife poaching: identifying the origin of individuals with Bayesian assignment tests and multilocus genotypes. Conservation Biology, 16 (3), 650-659. DOI: https://dx.doi.org/10.1046/j.1523-1739.2002.00576.x

Manel, S., Gaggiotti, O.E., Waples, R.S. (2005) Assignment methods: matching biological questions with appropriate techniques. Trends in Ecology & Evolution, 20 (3), 136-142. DOI: https://dx.doi.org/10.1016/j.tree.2004.12.004

Maudet, C., Miller, C., Bassano, B., Breitenmoser-Würsten, C., Gauthier, D., Obexer-Ruff, G., Michallet, J., Taberlet, P., Luikart, G. (2002) Microsatellite DNA and recent statistical methods in wildlife conservation management: applications in Alpine ibex [*Capra ibex* (ibex)]. Molecular Ecology, 11 (3), 421-436. DOI: https://dx.doi.org/10.1046/j.0962-1083.2001.01451.x

Nomura, K., Ishii, K., Dadi, H., Takahashi, Y., Minezawa, M., Cho, C., Faruque, M., Nyamsamba, D., Amano, T. (2012) Microsatellite DNA markers indicate three genetic lineages in East Asian indigenous goat populations. Animal Genetics, 43 (6), 760-767. DOI: https://dx.doi.org/10.1111/j.1365-2052.2012.02334.x

Noss, R.F. (1990) Indicators for monitoring biodiversity: a hierarchical approach. Conservation Biology, 4 (4), 355-364. DOI: https://dx.doi.org/10.1111/j.1523-1739.1990.tb00309.x

Paetkau, D., Calvert, W., Stirling, I., Strobeck, C. (1995) Microsatellite analysis of population structure in Canadian polar bears. Molecular Ecology, 4 (3), 347-354. DOI: https://dx.doi.org/10.1111/j.1365-294X.1995.tb00227.x

Paetkau, D., Slade, R., Burden, M., Estoup, A. (2004) Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. Molecular Ecology, 13 (1), 55-65. DOI: https://dx.doi.org/10.1046/j.1365-294X.2004.02008.x

Peakall, R., Smouse, P.E. (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes, 6 (1), 288-295. DOI: https://dx.doi.org/10.1111/j.1471-8286.2005.01155.x

Piry, S., Alapetite, A., Cornuet, J.-M., Paetkau, D., Baudouin, L., Estoup, A. (2004) GENECLASS2: a software for genetic assignment and first-generation migrant detection. Journal of Heredity, 95 (6), 536-539. DOI: https://dx.doi.org/10.1093/jhered/esh074

Pritchard, J.K., Stephens, M., Rosenberg, N.A., Donnelly, P. (2000) Association mapping in structured populations. The American Journal of Human Genetics, 67 (1), 170-181. DOI: https://dx.doi.org/10.1086/302959

Sacchi, P., Chessa, S., Budelli, E., Bolla, P., Ceriotti, G., Soglia, D., Rasero, R., Cauvin, E., Caroli, A. (2005) Casein haplotype structure in five Italian goat breeds. Journal of Dairy Science, 88 (4), 1561-1568. DOI: https://dx.doi.org/10.3168/jds.S0022-0302(05)72825-3

Saitou, N., Nei, M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution, 4 (4), 406-425. DOI: https://dx.doi.org/10.1093/oxfordjournals.molbev.a040454

Sambrook, J., Fritsch, E.F., Maniatis, T. (1989) Molecular cloning. New York: Cold Spring Harbor Laboratory Press.

Sultana, S., Mannen, H., Tsuji, S. (2003) Mitochondrial DNA diversity of Pakistani goats. Animal Genetics, 34 (6), 417-421. DOI: https://dx.doi.org/10.1046/j.0268-9146.2003.01040.x

Vahidi, S.M.F., Tarang, A.R., Anbaran, M.F., Boettcher, P., Joost, S., Colli, L., Garcia, J.F., Ajmone-Marsan, P. (2014) Investigation of the genetic diversity of domestic Capra hircus breeds reared within an early goat domestication area in Iran. Genetics Selection Evolution, 46 (1), 27. DOI: https://dx.doi.org/10.1186/1297-9686-46-27

Weir, B.S. (1990) Genetic data analysis. Methods for discrete population genetic data. Sinauer Associates Inc. Publishers.

Woolaston, R., Piper, L. (1996) Selection of Merino sheep for resistance to Haemonchus contortus: genetic variation. Animal Science, 62 (3), 451-460. DOI: https://dx.doi.org/10.1017/S1357729800014995

Wright, S. (1949) The genetical structure of populations. Annals of Eugenics, 15 (1), 323-354. DOI: https://dx.doi.org/10.1111/j.1469-1809.1949.tb02451.x

Yeh, F.C., Yang, R.C., Boyle, T.B., Ye, Z., Mao, J.X. (1997) POPGENE, the user-friendly shareware for population genetic analysis. Edmonton: Molecular biology and biotechnology centre, University of Alberta.

Zeb, B., Khan, I., Ali, S., Bacha, S., Mumtaz, S., Swati, Z. (2009) Study on genetic diversity in Pakistani wheat varieties using simple sequence repeat (SSR) markers. African Journal of Biotechnology, 8 (17).