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Genetic Diversity Analysis of Four Sheep Breeds of Iran: Towards Genetic Maintenance and Conservation Decision

Research Article

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ABSTRACT

The present study was conducted to measure the genetic diversity and population genetic structure of four Iranian sheep breeds, namely; Ghezel, Makui, Kurdi and Baluchi (utilizing 20 animals per breed from both sexes) using 12 short tandem repeats markers. Our results demonstrated that the observed number of alleles and the median number of effective alleles for the Ghezel Makui, Kurdi, and Baluchi breeds were (50, 48, 44 and 43) and (2.49, 2.46, 2.45 and 2.47), respectively. Also, the mean Shannon Index was 0.982, 0.939, 0.965, and 0.959 values for Ghezel, Makui, Kurdi, and Baluchi breed, respectively. Moreover, the polymorphic information content (PIC) values were ranked from 0.09 (ILSTS011) to 0.75 (TGLA13). Additionally, the average inbreeding coefficient (F_{IS}) was from 0.144 (Kurdi) to 0.306 (Makui). Furthermore, the lowest genetic distance was seen between Makui and Baluchi breeds (0.120), but the highest was between Ghezel and Kurdi breeds (0.884). The outcomes of this research showed the normal 'L'-shaped distribution of the mode-shift analysis test and the lack of bottleneck in the studied populations. On this basis, we conclude that the investigated Iranian sheep breeds carry reasonable within and between genetic diversity.

KEY WORDS genetic diversity, Iranian sheep breeds, short tandem repeats markers.

INTRODUCTION

Sheep are among the most important and oldest ruminants that were domesticated by humans in the Fertile Crescent, 9000 years ago (Peter *et al*. 2007). There are more than 850 breeds of sheep in the world (Rege and Gibson, 2003). Sheep are ancient small ruminants with large production for human society in the world. These species have seasonal mating which was affected through photoperiods (Rosa and Bryant, 2002). In aspect of climatology, Iran is divided into different regions, classified as dry, semi-arid, temperate, and cold, with majority of arid and semi-arid regions. Iran is known as one of the world's largest national sheep populations with a population of approximately 39 million in 2018 (FAOSTAT, 2020). There are at least 27 native breeds in Iran which are classified according to size, shape, type, and wool colour (Tavakolian, 2000). Most Iranian sheep are mainly used for meat production, but Ghezel, Makui, Kurdi, and Baluchi breeds are also good sources for wool production (Valdez *et al*. 1978). Due to the danger of existence of this invaluable genetic resources, maintaining their genetic variation is critical for food safety and rural development. Currently, several major factors threat survivability of diversity in Iranian sheep, including climate change, occurrence of natural disasters flooring, new resurgent disease, immigration of farmers from village to cities, new

research on human nutritional needs and changing market conditions or changing social needs (FAO, 2011).

Genetic diversity is an essential requirement for animal breeding, whereas a high genetic variation is necessary for the genetic improvement of domestic animals (Askari *et al.* 2011). Moreover, genetic diversity studies have a significant role in constructing the breeding strategies for economically important animal species (Gaouar *et al.* 2011). Therefore, evaluation of genetic variation is considered the first step to conservation genetic resources and is considered one of the most important stages for the maintenance of breeds (Kevorkian *et al.* 2010). Short tandem repeats (STRs) have been widely used as a choice of the marker to analyze the genetic variation in dromedary (Cherifi *et al.* 2017), cattle (Rahal *et al.* 2020), sheep (Abdelkader *et al.* 2017; Abdelkader *et al.* 2020), and goats (Tefiel *et al.* 2018;) due to the similarities between their chromosomes. The SSRs markers used to measure the genetic distance between close breeds of different geographies. The STRs markers are widely used to measure the genetic distance between close breeds of different geographies. These markers have several advantages such as relative ease of acquisition, high polymorphism, neutrality, and easy to sample preparation. It also detects changes in simple nucleotide repetitions, random and abundant distribution in the genome and their alignment which used for several types of studies (Abdul-Muneer, 2014).

In sheep linkage map, there are 504 SSRs markers assigned for 54 chromosomes (De-Gortari *et al.* 1998), and there is a list of suitable STRs markers for genetic study recommend by FAO and genetic society for international comparison of obtained outputs (FAO, 2011).

A summary of previous genetic diversity of the same breeds was reported using fewer loci and in the past (Esmaeilkhanian and Banabazi, 2006; Saberivand *et al.* 2010; Ebrahimi *et al.* 2017). Therefore, the main focus of this research was to unravel the genetic diversity of Iran sheep breeds using 12 STRs markers in an extended manner.

MATERIALS AND METHODS

Animals and sampling

To perform this research, in overall, 80 individuals (n=20 per breed from both sexes) was randomly selected from different geographical regions, particularly from villages. Figure 1 and Table 1 describe the characteristics of the studied breeds. The location of sampling, owner and ID of animals recorded carefully and 10 mL of blood was taken from the jugular-vein from each animal by using tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant and instantly stored in 4 ˚C and then stored at -20 ˚C. According to the FAO and the International Association of Animal Genetics (ISAG) (FAO, 2004), we selected 12 STRs markers with different chromosomal positions (Table 2).

Molecular analysis

The genomic DNA was purified from blood according to the Samadi Shams conventional protocol (Samadi Shams *et al.* 2011). Gel monitoring and NanoDrop 2000/2000c spectrophotometer methods (Thermo Fisher Scientific, USA) were used for quality and quantity measurement test. PCR amplification was performed in a total volume of 15 µL containing master mix kit (Ampliqon, Denmark) 7.5 Ml master mix 2X, 1 pmol of each primer (Forward and Reverse), and 4.9 µL ddH_2O, and 1.5 µL of genomic DNA (all these steps were done on ice). Model of PCR Machin for amplification of fragment was Biometra Company and the PCR Program was used in the PCR machine to replicate the STR loci of the Touch-down PCR specific program was designed to simultaneously amplify the locus and minimize nonspecific and starter bands. The "touchdown" PCR protocol used with initial denaturation of 95 ˚C for 8 min, followed by the first stage of amplification of 12 cycles involving a denaturation step at 94 ˚C for 1min, annealing based on reducing the temperature from 68 ˚C to 52 ˚C for 40 sec, and extension at 72 ˚C for 30 sec, and finally, the final reproduction temperature was set at 72 ˚C for 8 min. The PCR products were determined on 6% agarose gels stained with metaphor in 1X TAE buffer. Allele sizes were estimated using the 11 lines (25-755-bp) ladder (Life Science Company).

Statistical analysis

Individual genotypes are measured at each location or allele size using UVdoc 99.02 analysis software (UVI Tech, Cambridge, UK), then for preparation of input files for each specific package, CONVERT version 1.31 (Glaubitz, 2004), and CREAT version 1.1 packages (Coombs *et al.* 2008) were employed. The molecular measurements indexes such as genotype and allele frequencies, the observed number of alleles (Na), effective number of alleles (Ne), observed (Ho), expected (He) heterozygosity's, Shannon index (I), Polymorphic Information Content (PIC), Inbreeding coefficient (F_{IS}), F-statistics (F_{IS}, F_{IT} and F_{ST}), genetic distance, allele admixture and population structure, clustering, analysis of molecular variance (AMOVA), and bottleneck index were calculated. From used different software and the computational package include POPGENE version 1.31 (Yeh *et al.* 1997), ARLEQUIN version 3.5.2.2 (Excoffier and Lischer, 2010), GenAlEx version 6.5 (Peakall and Smouse, 2012), STRUCTURE version 2.3.4 (Pritchard *et al.* 2000), BOTTLENECK version 1.2.02 (Piry *et al.* 1999).

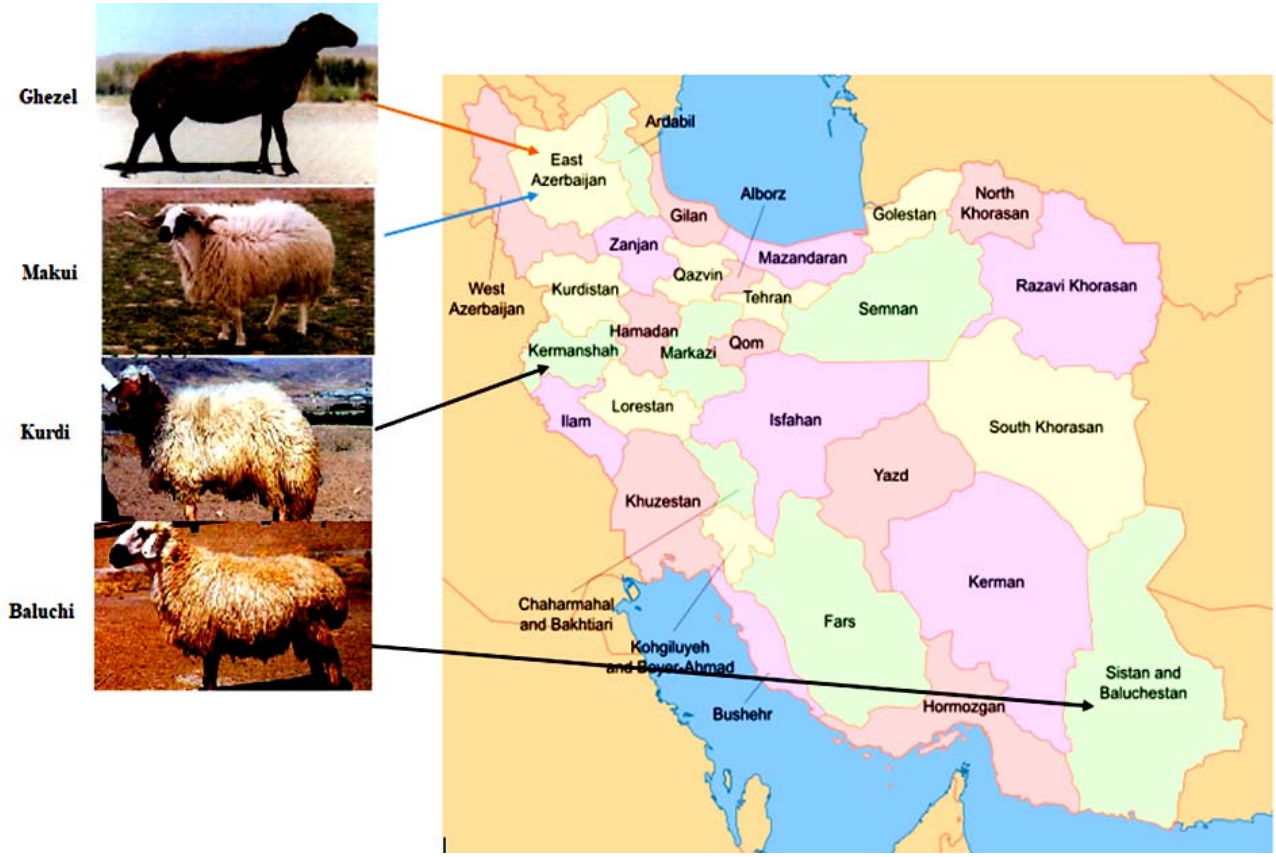

Figure 1 Geographical distribution of investigated sheep breeds in Iran map

Table 1 Characteristics of the studied Iran sheep breeds

Breeds	Number of sample	Present location	Geographical origin	Uses	Mature weight Yearling (kg)	BW (kg)	Twining rate %
Ghezel	20	Iran	East Azerbaijan	Milk and meat	41.7-38.2	3.8-4.47	8
Makui	20	Iran	Eastern and Western Azerbaijan	Meat, wool and milk	48-51	3.5-3.7	5
Kurdi	20	Iran	Kermanshah	Meat	45-64	5.5-6.4	10
Baluchi	20	Iran	Sistan and Baluchistan	Wool and meat	35-40	2.5-2.8	6

RESULTS AND DISCUSSION

They describe the results of the genotype pattern, which showed successful shape amplification of PCR products almost in all investigated loci. To the best of our knowledge, as the existence of stutter band accounted as natural barriers and disadvantages of a STR marker, some di motif loci have a tendency to show an artificial band which may interrupt estimation of the actual band and imposes genotyping error (reminder STR marker have to indicate two or one bands in each individual) and we try to minimize stutter using touch-down strategy. Figure 2 shows the successful implication gel photograph for some loci investigated population.

The first part of statistical analyses of assessment of STR across the population was showed that allele frequency ranged from 0.0063 to 0.9241. Table 3 summarizes various allele frequency measures estimated for each locus in Iranian sheep breeds. Some markers were a few good shapes. The values of the total Na, Ne, and I are shown in Table 4. The observed number of alleles in Ghezel, Makui, Kurdi, and Baluchi breeds was 50, 48, 44, and 43, respectively. The mean numbers of observed alleles were as 4.16, 4.00, 3.66, and 3.58, respectively, and the numbers of effective alleles averaged 2.49, 2.46, 2.45, and 2.47 in Ghezel, Makui, Kurdi, and Baluchi breeds, respectively. Some of the STR markers studied were sufficiently polymorphic and are suitable for analysis of genetic variation.

Table 2 Characteristics of selected STRs in present study

Marker (chromosome)	Primers (5´-3´)	Annealing temperature (˚C)	References
OARAE129 (5)	F:AATCCAGTGTGTGAAAGACTAATCCAG R:GTAGATCAAGATATAGAATATTTTTCAACACC	56	(Kappes *et al*. 1997)
ILSTS011 (9)	F:GCTTGCTACATGGAAAGTGC R:CTAAAATGCAGAGCCCTACC	50	(Brezinskly *et al*. 1993)
INRA063 (14)	F:ATTTGCACAAGCTAAATCTAACC R:AAACCACAGAAATGCTTGGAAG	56	(Vaimand *et al*. 1992)
McM527 (5)	F:GTCCATTGCCTCAAATCAATTC R:AAACCACTTGACTACTCCCCAA	58	(Maddox *et al*. 2001)
OarFCB20 (2)	F:AAATGTGTTTAAGATTCCATACAGTG R:GGAAAACCCCCATATATACCTATAC	63	(Buchanaen *et al*. 1994)
OARFCB226 (2)	F:CTATATGTTGCCTTTCCCTTCCTGC R:GTGAGTCCCATAGAGCATAAGCTC	56	(Buchanaen *et al*. 1994)
MCM064 (2)	F:TACAGTCCATGGGGTCACAAGA R:TCTGAATCTACTCCCTCCTCAGAGC	50	(Maddox *et al*. 2001)
TGLA137 (5)	F:GTT GAC TTG TTA ATC ACT GAC AGC C R:CCT TAG ACA CAC GTG AAG TCC AC	55	(Georges and Massey, 1992)
INRA011 (1)	F:CGAGTTTCTTTCCTCGTGGTAGGC R:GCTCGGCACATCTTCCTTAGCAAC	55	(Bishop *et al*. 1994)
RM32 (2)	F:AGTCTACGTGGTGTACACGTGG R:TGCGGCCTGCCGTTTGTGAG	55	(Bishop *et al*. 1994)
TGLA13 (2)	F:CATTTAATATACATATATGACTATGTGCC R:GAGCCTCTTTTTTACTTGTGCTCC	59	(Georges and Massey, 1992)
BMI853 (5)	F:AGCCTTTTGTAGGTGTTCATTG R:ATGGGGTTGCAAAGAGTCAG	61	(Bishop *et al*. 1994)

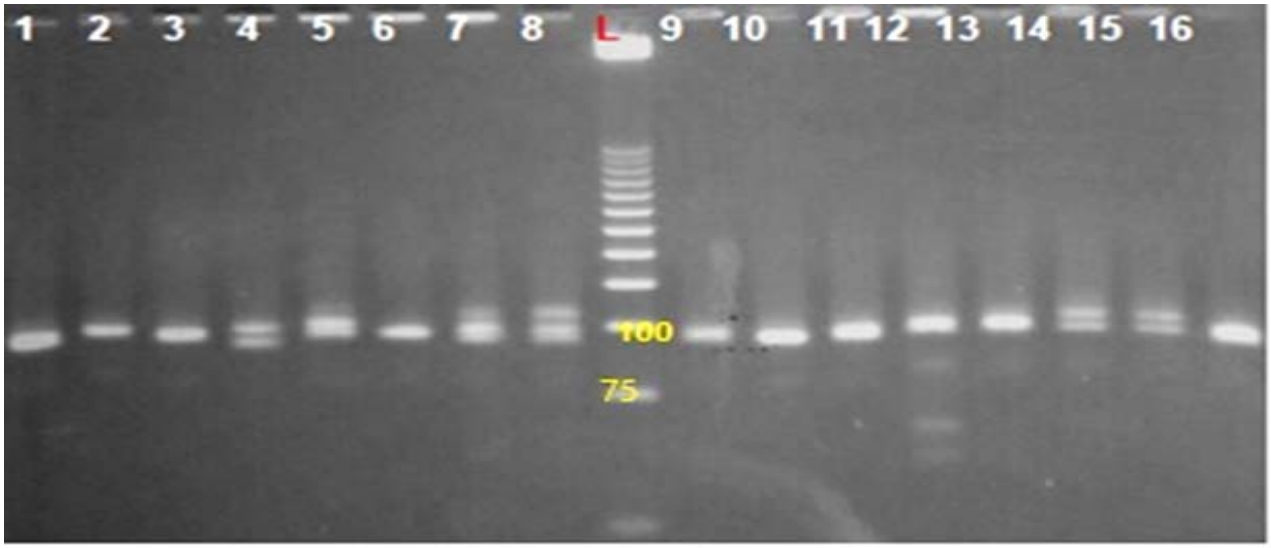

Figure 2 Electrophoresis pattern for some PCR products of OarFCB226 in a Ghezel sheep

The results observed in this research was lowest than for four Tunisian sheep breeds with 30 STR markers (Kdidi *et al*. 2015), in three local sheep breeds in Saudi Arabia, using 17 STR markers (Mahmoud *et al*. 2017) and in four Romania native sheep breeds with 18 STR markers (Dudu *et al*. 2020).

The Shannon index values (I) ranged from 0.19 (ILSTS011) in the Ghezel breed to 1.68 (TGLA13) in the Makui breed. Mean (I) were 0.98, 0.93, 0.96, and 0.95 for Ghezel, Makui, Kurdi, and Baluchi breeds, respectively. Shannon's information index, a measure of biodiversity, was low for four sheep breed of Iran showed that most of

the loci in this study were lowly polymorphic (Table 4). These results are highest then (Greguła-Kania *et al*. 2015) in Poland, while lowest than (Jyotsana *et al*. 2010) in India, (Musthafa *et al*. 2012) in Saudi Arabia and (Hussain *et al*. 2019) in Pakistan. The Ho, He, PIC, and F_{IS} are shown in Table 5. Means of observed Ho were 0.42, 0.40, 0.44, and 0.44 in Ghezel, Makui, Kurdi, and Baluchi sheep populations, respectively. While, means of expected He were 0.54, 0.52, 0.54, and 0.55 in Ghezel, Makui, Kurdi, and Baluchi sheep populations, respectively. The Ho index ranged from 0.00 (OarFCB20 and TGLA137) to 1.00 (TGLA13) and the He index ranged from 0.09 (ILSTS011) to 0.81 (TGLA13) in Iranian sheep breeds. The degree of He was higher than that Ho in all the studied breeds. The range in values for He and Ho observed in this study compare with previously published data (Kusza *et al*. 2010) in Bulgaria, (Ocampo *et al*. 2016) in Colombia and (Dossybayev *et al*. 2019) in Kazakhstan. The polymorphic information content (PIC) is a parameter indicant of the scale of the formativeness of a marker. The PIC determines the amount of polymorphism in each locus, the PIC value less than 0.25 indicates a low polymorphism, a value between 0.25 and 0.5 average polymorphism and a value higher than 0.5 a highly polymorphic locus (Botstein *et al*. 1980). Hence, the degree of formativeness of a marker reveals its utility in diversity analysis of a breed.

A higher value of PIC means rather alleles and greater polymorphism at that locus. The studies on Ghezel, Makui, Kurdi, and Baluchi breeds of sheep revealed that the mean PIC values were found to be 0.47, 0.44, 0.48, and 0.46, respectively (Table 5). The PIC values for all loci ranged from 0.09 (ILSTS011) in the Ghezel breed to 0.75 (TGLA13) in the Makui breed. These results are similar to those observed in several China local sheep breeds (Zenga *et al*. 2010), sheep breeds in South Africa (Qwabe, 2011), and (Greguła-Kania *et al*. 2015) in Poland, and lower from Jordanian (Jawasreh *et al*. 2018) and Iranian (Ebrahimi *et al*. 2017) sheep breeds. Some factors, such as mutations and selection, affect the amount of polymorphism in STR loci. Most of the studied markers are shown to be at high PIC values, and these markers are important for genetic diversity.

Table 3 Allele frequency profile of studied Iranian sheep population

Locus	Allele A	Allele B	Allele C	Allele D	Allele E	Allele F	Allele G	Allele H
OarFCB20	0.2532	0.4051	0.3418					
OarAE129	0.8938	0.1062						
ILSTS011	0.9241	0.0759						
TGLA137	0.1437	0.6813	0.1750					
RM32	0.2375	0.3625	0.1875	0.2125				
MCMC527	0.5886	0.4114						
INRA011	0.3354	0.0696	0.2405	0.3544				
INRA063	0.0127	0.0063	0.0253	0.0506	0.3987	0.2658	0.2405	
BM1853	0.4873	0.0696	0.0633	0.3734	0.0063			
OarFCB226	0.1812	0.0375	0.5000	0.0813	0.0688	0.0063	0.1125	0.0125
MCMC64	0.0063	0.0127	0.0570	0.2975	0.1139	0.0063	0.4430	0.0633
TGLA13	0.1250	0.0250	0.2500	0.0875	0.1562	0.1875	0.1688	

Table 4 Genetic diversity measures at the 12 STRs loci analyzed between the four Iranian sheep breeds

Locus	Breeds											
	Ghezel			Makui			Kurdi			Baluchi		
	Na	Ne	I	Na	Ne	I	Na	Ne	I	Na	Ne	I
OarFCB20	3	2.06	0.78	3	2.06	0.78	3	1.66	0.66	3	2.15	0.84
OarAE129	2	1.21	0.32	2	1.28	0.37	2	1.21	0.32	2	1.21	0.32
ILSTS011	2	1.10	0.19	2	1.16	0.26	2	1.17	0.27	2	1.21	0.32
TGLA137	3	2.06	0.88	2	1.21	0.32	3	2.60	1.01	2	1.60	0.56
RM32	4	3.36	1.27	4	2.42	1.00	4	2.58	1.11	4	3.18	1.22
MCMC527	2	1.83	0.64	2	1.91	0.67	2	1.99	0.69	2	1.88	0.66
INRA011	2	1.72	0.61	4	1.89	0.88	4	3.19	1.25	3	2.77	1.05
INRA063	6	3.53	1.40	5	3.29	1.27	5	3.29	1.35	5	2.97	1.27
BM1853	5	2.86	1.22	4	2.43	1.01	4	2.26	0.98	4	2.78	1.15
OarFCB226	8	3.61	1.63	7	3.73	1.59	5	2.60	1.17	6	2.86	1.31
MCMC64	6	2.97	1.28	6	3.41	1.39	5	2.86	1.28	5	2.96	1.32
TGLA13	7	3.53	1.51	7	4.76	1.68	5	3.98	1.44	5	4.04	1.44
Mean	4.16	2.49	0.98	4	2.46	0.93	3.66	2.45	0.96	3.58	2.47	0.95

Na: number of alleles observed; Ne: effective number of alleles and I: Shannon index.

Table 5 Some parameters characterizing genetic diversity

Locus	Breeds															
	Ghezel				Makui				Kurdi				Baluchi			
	Ho[1]	He[1]	PIC[1]	F_{IS}[1]	Ho	He	PIC	F_{IS}	Ho	He	PIC	F_{IS}	Ho	He	PIC	F_{IS}
OarFCB20	0.05	0.52	0.40	0.903	0.05	0.52	0.40	0.903	0.31	0.41	0.33	0.211	0.00	0.54	0.43	1.000
OarAE129	0.20	0.18	0.16	-0.111	0.05	0.22	0.19	0.771	0.20	0.18	0.16	-0.111	0.20	0.18	0.16	-0.111
ILSTS011	0.10	0.09	0.09	-0.052	0.15	0.14	0.12	-0.081	0.15	0.14	0.13	-0.085	0.20	0.18	0.16	-0.111
TGLA137	0.11	0.52	0.46	0.805	0.00	0.18	0.16	1.000	0.15	0.63	0.53	0.756	0.00	0.38	0.30	1.000
RM32	0.60	0.72	0.64	0.145	0.15	0.60	0.50	0.744	0.45	0.62	0.55	0.266	0.55	0.70	0.62	0.198
MCMC527	0.50	0.46	0.35	-0.098	0.57	0.49	0.36	-0.211	0.35	0.51	0.37	0.298	0.45	0.48	0.35	0.040
INRA011	0.00	0.43	0.33	1.000	0.05	0.48	0.43	0.893	0.10	0.70	0.63	0.846	0.00	0.65	0.56	1.000
INRA063	0.73	0.73	0.66	-0.027	0.70	0.71	0.63	-0.005	0.60	0.71	0.64	0.138	0.60	0.68	0.61	0.096
BM1853	0.57	0.66	0.58	0.110	0.50	0.60	0.50	0.150	0.40	0.57	0.48	0.284	0.60	0.65	0.57	0.064
OarFCB226	0.80	0.74	0.69	-0.105	0.95	0.75	0.70	-0.296	0.85	0.63	0.56	-0.379	0.90	0.66	0.60	-0.382
MCMC64	0.73	0.68	0.60	-0.110	0.70	0.72	0.65	0.010	0.75	0.66	0.60	-0.151	0.80	0.67	0.62	-0.207
TGLA13	0.75	0.73	0.68	-0.045	0.95	0.81	0.75	-0.202	1.00	0.76	0.70	-0.335	1.00	0.77	0.70	-0.328
Mean	0.42	0.54	0.47	0.201	0.40	0.52	0.44	0.306	0.44	0.54	0.48	0.144	0.44	0.55	0.46	0.188

Ho: observed heterozygosity; He: expected heterozygosity; PIC: polymorphic information content and F_{IS}: coefficient of inbreeding.

Table 6 Summary of F-statistics and gene flow for all short tandem repeats loci in Iranian sheep breeds

Locus	F_{IS}	F_{IT}	F_{ST}
OarFCB20	0.788	0.841	0.247
OarAE129	0.143	0.144	0.001
ILSTS011	-0.087	-0.082	0.004
TGLA137	0.851	0.871	0.130
RM32	0.324	0.402	0.115
MCMC527	0.011	0.029	0.018
INRA011	0.930	0.944	0.207
INRA063	0.049	0.070	0.022
BM1853	0.147	0.154	0.007
OarFCB226	-0.285	-0.265	0.015
MCMC64	-0.112	-0.074	0.034
TGLA13	-0.229	-0.120	0.088
Mean	0.185	0.255	0.085

F_{IS}: within-population inbreeding coefficient; F_{IT}: total population inbreeding coefficient and F_{ST}: among-population genetic differentiation coefficient.

The mean inbreeding coefficient (F_{IS}) values for Ghezel, Makui, Kurdi, and Baluchi sheep breeds was 0.201, 0.306, 0.144, and 0.188 respectively, with a range from -0.005 (INRA063) to 1.000 (TGLA137 and INRA011) as were shown in Table 5. Positive values of F_{IS} indicate loss of heterozygosity in some STR markers, similar to the results reported by (Hristova *et al*. 2014), (Vahidi *et al*. 2016), and (Karsli *et al*. 2020). The positive F_{IS} value suggested inbreeding to be one of the main causes of lack of heterozygotes in Iranian sheep. Low heterozygotes and excess of homozygotes within the studied populations may be related to several factors such as the mating system of animals, number of samples, selection (Genetic hitchhiking), and null alleles (Nei, 1987).

The estimated values of the parameters characterizing the within-population inbreeding coefficient (F_{IS}), total population inbreeding coefficient (F_{IT}), and among-population genetic differentiation coefficient (F_{ST}), for each of the 12 loci studied across four Iranian sheep population are given in Table 6.

The within-breed deficit in heterozygote as evaluated by the F_{IS} parameter ranged between -0.087 (ILSTS011) to 0.930 (INRA011) having a total mean of 0.185 for all loci. The F_{IT} value expressing general heterozygosity loss was higher in INRA011 (0.944) locus than the other and having a total mean of 0.255 for all loci. Global breed differentiation evaluated by F_{ST}, was estimated at 0.085 with a range of 0.001 (OarAE129) to 0.247 (OarFCB20). According to (Hartl, 1980), pairwise F_{ST} values up to 0.05 is represented moderate differentiation between populations. The estimated F_{ST} value, which corresponds to the proportion of genetic variation calculated by the differences between breeds, was 0.085.

This result illustrates that genetic diversity quantified by STR markers indicates moderate differentiation between breeds. The reason for the moderate distinction between breeds of Iranian sheep can be due to environmental similarities, geographical proximity, and breeding programs, but they are likely to lead to past and present gene flow among them.

Table 7 Matrix of Nei's standard genetic distances between the studied breeds[1]

Population	Ghezel	Makui	Kurdi	Baluchi
Ghezel		0.829	0.886	0.863
Makui	0.187		0.849	0.884
Kurdi	0.120	0.163		0.852
Baluchi	0.147	0.122	0.159	

[1] Genetic identity (above diagonal) and genetic distance (below diagonal).

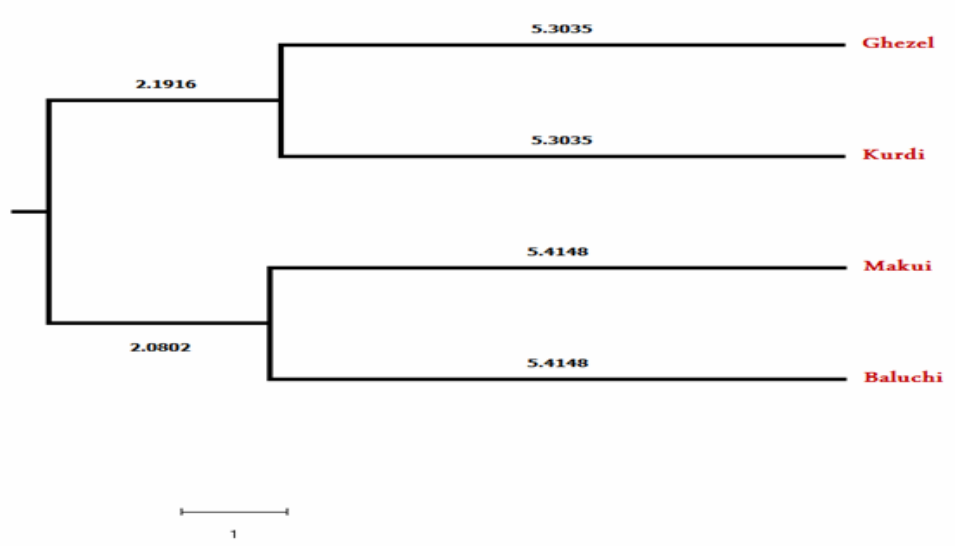

Figure 3 Dendrogram showing the genetic distance between the sheep breeds

Table 8 Analysis of molecular variance (AMOVA) for 80 animals in four Iranian sheep breeds

Source of variation	D.F.	Sum of squares	Variance components	Percentage variation
Among populations	3	46.763	0.29284 Va	8.35
Among individuals (within populations)	76	294.425	0.65888 Vb	18.78
Within individuals	80	204.500	2.55625 Vc	72.87
Total	159	545.688	3.50797	100

The mean values of F_{ST}, F_{IS}, and F_{IT} were highest than the values reported by (Kevorkian *et al.* 2010) in Romania, and (Ebrahimi *et al.* 2017) in Iran, and lowest than the values reported by (Agaviezor *et al.* 2012) in Nigeria and (Amareswari *et al.* 2017) in India.

The highest genetic distance in the four selected sheep breeds was observed between Ghezel and Makui with (0.187) while the lowest distance (0.235) between the Iran sheep populations was realized between Ghezel and Kurdi (Table 7). The highest genetic identity was observed between Ghezel and Kurdi breeds (0.886). The lowest genetic identity (0.829) was between Ghezel sheep and Makui sheep. These results are higher than those detected in Tunisian (Kdidi *et al.* 2015), Indian (Ramachandran *et al.* 2015), and Turkish (Karsli *et al.* 2020) sheep breeds.

The phylogenetic relationship between breeds made by the neighbor-joining tree is shown by the genetic distance matrix.

These Iranian sheep breeds were divided into two groups: the first group of Ghezel and Kurdi group, and the second was Makui and Baluchi group (Figure 3).

The close relationship between these breeds might suggest some related among these geographically close breeds which suggest the possibility of genetic admixture between these breeds or migration. The tree phylogeny received agreed with the results of (Yilmaz *et al.* 2015) for the four Turkish sheep breeds as shown by moderate genetic differentiation.

Analysis of molecular variance (AMOVA) provided an estimate of the measure of population genetic differentiation within and among populations. The AMOVA showed that 8.35 percent of the genetic variation among Iranian sheep breeds is attributed to among populations compared with 18.78 percent due to variation between individuals within populations and 72.87 percent due to variation within individuals (Table 8).

Table 9 Test of the null hypothesis under three short tandem repeats evolution models

Test/model	I.A.M	T.P.M	S.M.M
Sign test			
Heterozygosity excess expected (Hee)	6.21	6.55	6.59
Heterozygosity deficiency (Hd)	1	4	6
Heterozygosity excess (He)	11	8	6
Probability	0.00410	0.29200	0.47498
Standard difference test			
(T_2 value)	3.573	2.374	0.427
Probability	0.00018	0.00879	0.33451
Wilcoxon rank test			
P (one tail for **H** deficiency)	0.99976	0.99329	0.60449
P (one tail for **H** excess)	0.00037	0.01709	0.42505
P (two tails for **H** excess or deficiency)	0.00073	0.03418	0.85010

I.A.M: infinite allele model; T.P.M: two-phase model and S.M.M: stepwise mutation model.

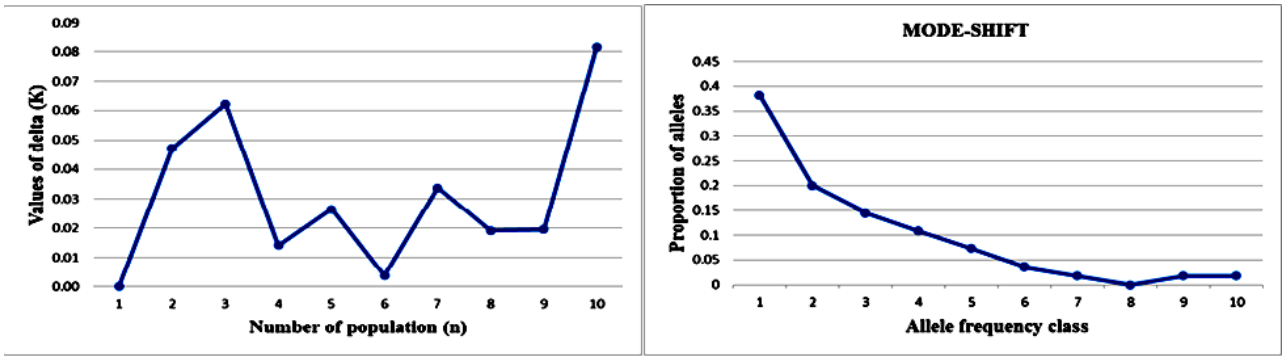

Figure 4 Mode shift analysis and the plot of delta "K" values from the structure analyses of Iranian sheep breeds

Table 10 The relationship of the membership of every of the four sheep breeds in the three clusters (K=3) inferred using structure software

Predefined populations	Inferred cluster			Number of animals
	1	2	3	
Ghezel	0.517	0.430	0.053	20
Makui	0.072	0.427	0.502	20
Kurdi	0.168	0.791	0.041	20
Baluch	0.543	0.065	0.392	20

The hierarchical analysis of variance showed 0.085 for F_{ST} (population among populations). The value obtained in this study was moderate but significant (P<0.001), indicating breeds difference. The significant and high within individuals' source of diversity suggests that breeds exhibited different genetic variations useful in utilizing these genetic resources as breeders strive to addition productivity and the breed's competitive advantage.

The diversity between allelic changes and heterozygosity has been used as a basis for statistical tests to uncover the curtain of recent genetic bottlenecks in the population (Piry *et al*. 1999). Four sheep populations included Ghezel, Makuii, Kurdi, and Baluchi were included in the study using 12 STR loci. The sign test, standardized difference test, and Wilcoxon rank test. The results take from the bottleneck analysis are given in Table 9.

The expected numbers of loci with heterozygosity excess were 6.21, 6.55, and 6.59 out of 12 loci under IAM, SMM, and TPM, respectively. The results obtained for the standardized difference test in which the T_2 values were computed. The T_2 values for the 3 mutation models were 3.573, 2.374, and 0.427 thus null hypotheses of mutation-drift equilibrium are accepted for IAM and TPM model but rejected for the SMM mutation model. The Wilcoxon rank tests as well as revealed similar results (the probability value for heterozygosity excess existence more than 0.05) under the TPM and SMM mutation models accepting the null hypothesis, i.e. no bottleneck in sheep breeds. In addition, the quantitative measure of the genetic bottleneck was tested using the mode shift indicator method and it displayed a normal 'L' shaped curve, confirming there is no bottleneck in Iran sheep breeds (Figure 4).

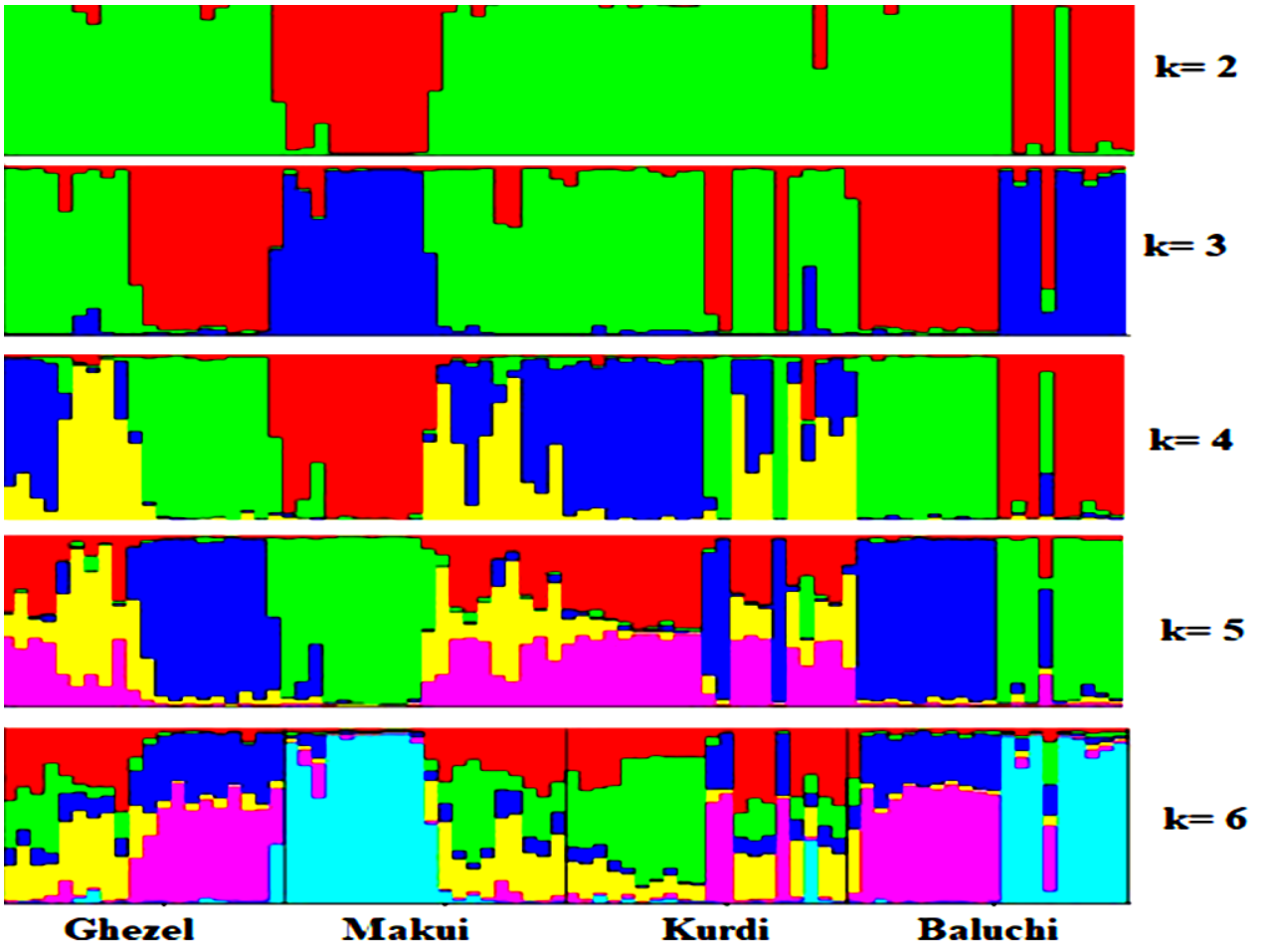

Figure 5 Genetic structures of four sheep populations for "K" ranging from 2 to 6

Similar detections were also reported by earlier research workers (Manjari *et al*. 2018; Mohankishore *et al*. 2019).

The population structure was computed using Structure (Pritchard *et al*. 2000). In structure analysis, the Ln Pr (X|K) increased clearly from K= 2 to K= 6 and reached a plateau at K= 3, and it did not show significant oscillation from K= 4 to 6 (Figure 4). So K= 3 was taken as the most likely number of inferred populations. In Table 10 the ratio of individuals of each of the populations in the three most probably clusters inferred by the Structure (Evanno *et al*. 2005) are reported, and this corresponded to the four different Iranian sheep breeds included in the research. Also, the cluster 1 includes the Ghezel and Baluchi individuals; cluster 2 includes most of the Kurdi genotypes, and Ghezel and Makui individuals; and, finally, cluster 3 includes Makui and Baluchi individuals. The proportions of each breed that contributed to each of the three clusters are shown in (Figure 5). This is also highlighted that Ghezel, Makui, and Kurdi were well-differentiated in comparison with Baluchi. The result of the clusters revealed the presence of admixtures which are indicative of gene flow between these breeds. Baluchi is the only breed with the least admixture. This probably due to the geographical scheme of the breeds and the management practice of the sheep owners. Also, the level of genetic variation could be attributed to a loss of artificial selection pressure between breeds typical of traditional breeding systems.

These results will help future researchers as a key guide to better understanding the genetic relationships and breed differences in Iranian sheep breeds for making future breed policies and programs to protect any breed of sheep in the country.

CONCLUSION

This present study demonstrated genetic similarity with and between individuals in four Iranian sheep breeds and in summary statistical output indicated the ability of STR for estimation of genetic diversity in sheep. Finally, further investigation for genetic variability of the genome in sheep is needed.

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