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ANALYSIS OF GENETIC DIVERSITY AND STRUCTURE OF BALUCHI SHEEP BY MICROSATELLITE MARKERS

[ANÁLISIS DE DIVERSIDAD Y ESTRUCTURA GENÉTICA DEL BORREGO BALUCHI MEDIANTE MARCADORES MICROSATELITALES]

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SUMMARY

Allele diversity, genetic variability and population structure in two subpopulations of Baluchi sheep were estimated using seven microsatellite markers. A total of 503 individuals from two subpopulations were genotyped. Average number of alleles per locus for all loci was 5.57. The range of alleles per locus was from 4 in BM1853 and BMS1714 loci to 7 in MCM200 and RM0006 loci. The seven tested loci were all polymorphic in both subpopulations. The average observed heterozygosity over all the loci in each subpopulation was less than the expected heterozygosity. Test of genotype frequency deviation from Hardy-Weinberg equilibrium (HWE) at each locus, over all the population, revealed a significant departure from HWE. A slightly low rate of inbreeding within the two subpopulations was noticed (F_{is} = 0.003). Low genetic differentiation was detected based on the estimated F_{st} index between the two subpopulations. The genetic structure (AMOVA) analysis showed that about 2.4% of the total genetic variation was explained by population differences and 97.6 percent was corresponded to differences among individuals. The mean of polymorphism information content (PIC) value for all loci in Baluchi population was 0.65. In addition, the analysis of segregation in the populations showed that 85% of the individuals were informative, indicating the relatively high polymorphism in selected marker in Baluchi sheep.

Key words: DNA markers; Diversity; Inbreeding.

RESUMEN

Se estimó la diversidad de alelos, la variabilidad genética y la estructura poblacional de dos subpoblaciones del borrego Baluchi empleando siete marcadores microsatelitales en 503 individuos. El número promedio de alelos por locus para todos los locus fue de 5.57. El rango de alelos por locus fue de 4 en los locus BM1853 y BMS1714 a 7 en los locus MCM200 y RM0006. Los siete locus evaluados fueron todos polimórficos en ambas subpoblaciones. El promedio de heterozigocidad en todos los locus fue menor a la esperada. La prueba de la desviación de frecuencia genotípica en relación al equilibrio esperado de acuerdo a la ley de Hardy-Weinberg (HWE) en cada locus reveló una desviación significativa del valor esperado. Se observó un baja tasa de endogamia en dentro de cada población (F_{is} = 0.003). Se observa una baja diferenciación genética entre ambas poblaciones basado en el índice F_{st}. El análisis de estructura genética (AMOVA) mostró que 2.4% de la variación genética era explicada por diferencias poblacionales y 97.6% por diferencias individuales. La media del valor de información de polimorfismo (PIC) para todos los locus de la población Baluchi fue de 0.65. Además, el análisis de segregación de la población indicó que el 85% de los individuos proporcionaron información, indicando el relativamente alto nivel de polimorfismo en los marcadores seleccionados en el borrego Baluchi.

Palabras clave: Marcadores ADN; diversidad genética; endogamia.

INTRODUCTION

The total sheep population in Iran is 54 million heads, including 27 breeds and ecotypes (ASRI, 2004). Among them, Baluchi sheep is one of the most numerous breed, constitutes about 30% of total sheep population of Iran. It plays an important role in meat and wool production. Baluchi is an indigenous sheep breed, widely distributed in centre, east, north and southeast of Iran. This breed has been adapted to hard climate conditions in Iran, south of Afghanistan and southwest of Pakistan. Baluchi sheep have a white coat color and have lean medium fat-tail. Rams in this breed have usually long horns while ewes do not. The average body weight of the adult ewe and ram is 39 and 48 kg, respectively. The wool production in the adult ewe and ram is 1.3 and 1.8 kg, respectively (ASRI, 2004).

Similar to most of sheep breeds in Iran, Baluchi sheep is a multi-purpose breed producing meat, wool and milk. However, the breeding objective for this breed has been changed in recent years and the selection has been focused mainly on meat production.

Success in breeding programs depends on the amount of variation in population. Also, lack of diversity will limit success of any breeding program. In addition, the maintenance of genetic diversity is a key to the long term survival of most species (Zhang et al., 2009). The genetic polymorphism and diversity found in the animal breeds allow the farmers to develop new characteristics in response to changes in environment or market conditions (Zhang et al., 2009). Information about population of farm animals and their genetics is very important in animal breeding. The molecular markers such as microsatellites and STRs (short tandem repeats) are useful tools in estimating genetic diversity and genetic structure of the population (Esmail-Khanian et al., 2007).

Microsatellites occur regularly throughout the animal genome and are stable, polymorphic and easy to analyze. Also, microsatellites are co-dominant markers, so that all alleles can be scored. Nucleotide motifs are dispersed throughout the genome and have a high level of polymorphism compared with those of other molecular markers (Selkoe and Toonen, 2006).
Given the large number of the available microsatellite markers, study of genetic structure and other characteristics of sheep breeds using molecular techniques, is of interest. Over the past decade, numerous studies on genetic diversity in domestic livestock, based on the analysis of microsatellite loci, have been carried out worldwide (Dalvit et al., 2008; Mahmoudi and Babayev, 2009; Kusza et al., 2010; Arora et al., 2008). In Iran, a number of studies were done to evaluate the genetic diversity of Iranian sheep breeds (Osfory, 1999; Ghanbari, 2002; Daneshyar, 2003; Fendereski, 2004; Zahedy, 2004). Esmail-Khanian et al. (2007) used nineteen microsatellites to evaluate genetic variation in Baluchi sheep breed (using 45 animals per marker). 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. Nanekarani et al. (2010), using fifteen microsatellite, investigated the Iranian pelt sheep breed and found high level of genetic diversity and polymorphism in the markers they studied. Also, Sharifi-Sidani et al. (2009), Razban et al. (2009) and Molaei et al. (2011) investigated the genetic variation within and between different ecotypes of the Iranian sheep based on the analysis of microsatellite loci.

In this study, the allele diversity and genetic variability in two subpopulations of Baluchi sheep were investigated, by using microsatellite markers.

MATERIAL AND METHODS

Subpopulations history

The Research Centre of Baluchi breed in Mashhad was established in 1970. The population was divided in two subpopulations since the beginning of breeding plan. The two subpopulations were developed using 700 and 500 founder Baluchi ewes in subpopulations 1 and 2, respectively. In the recent years, the size of each subpopulation was increased to two thousand heads founder. The selection goal in subpopulation 1 (line 1) was based on increasing lamb production while in subpopulation 2 (line 2) the selection goal was for improving wool quality. Blood samples were taken from 503 animals including 13 sires and 490 progeny (289 animals from subpopulation 1 and 214 animals from subpopulation 2).

Microsatellite analysis

Seven microsatellite markers were selected based on their polymorphism and their location in chromosomes. The markers were taken from the available web-based sheep genetic map (http://www.thearkdb.org/arkdb/). The general characteristics of the markers and sequence of the primers are presented in Table 1.

DNA was extracted from frozen blood samples using DNA extraction kit (Diatom prep 100, Cinnagene Co., Iran). DNA concentration was determined using Nano drop machine and PCR amplifications were carried out in 25µl reactions using 20-50 ng genomic DNA as template. Reaction mixtures contained Taq DNA Polymerase, dNTP, Tris-HCL, KCL and MgCl2. The cycling protocol was conducted with an initial denaturation step at 95 ^{o}C for 10 min followed by 35

cycles of the following steps: 94 °C for 30s, 48-62 °C for 55s and 72 °C for 30s. The reactions were terminated by a final extension step at 72°C for 10 min. The primers and other information for markers are presented in Table 1. Amplification products were electrophoresed on 6 and 8% denaturing poly-acrylamide gels and the DNA bands were visualized following silver staining.

Statistical analysis

Different measurements of within breed genetic variation viz. the number of alleles per locus and population level, the effective number of alleles (Ne), Shannon's information index (I) as a measure of gene diversity, mean observed (Ho) and mean expected (He) heterozygosity value in each subpopulation and for the whole population were estimated. Test of departure from Hardy-Weinberg equilibrium was performed through Chi-square test and G-square test (G_{st}). The F-statistics parameters were calculated for three indices: F_{it} (inbreeding coefficient of an individual i relative to the total population), F_{is} (inbreeding coefficient of an individual i relative to the subpopulation) and F_{st} (the effect of subpopulation s compared with the total population). Then gene flow between two populations was calculated based on F_{st} estimates (Slatkin and Barton, 1989). Microsatellite allele frequency data was applied to calculate genetic distance (D_A) according to the method of Nei (1978) using the un-weighted pair group method with arithmetic mean for dendrogram construction. All the above analyses were carried out using the POPGENE program (Yeh et al. 1999).

Polymorphism information content (PIC) was calculated using the method of Botstein et al. (1980) applying HET software version 1.8 (Ott, 2001).

$$PIC = 1 - \sum_{i} p_i^2 - \sum_{i=j} 2p_i^2 p_j^2$$

In this formula i and j are the frequency of observed alleles in different populations. Analysis of molecular variance (AMOVA) was carried out using Arlequin software 3.1 (Excoffier et al. 2005) to statistically test the existence of differences among two subpopulations.

RESULTS

Allele diversity

The number of alleles (A), effective number of alleles (Ne) and Shannon's information index (I) for each of the seven microsatellites for individual subpopulations and overall population are presented in Table2. The total number of alleles was 39 for the seven loci studied. The number of identified alleles per locus ranged from 4 (BM7247, BM1853 and BMS1714 markers in subpopulation 1; BM1853 and BMS1714 in subpopulation 2) to 7 (BM6465 in subpopulation 1; MCM200 and RM0006 in subpopulation 2). However, the mean number of alleles per subpopulation and overall population for all loci were 4.86, 5.40 and 5.60 respectively. The effective number of alleles per locus ranged from 1.35 (BM1853) to 4.25 (MCM200) and Shannon's Information index varied from 0.53 (BM1853) to 1.61 (MCM200). The difference in frequency between two alleles was highest for RM0006 locus. Test for difference of allele frequencies in the two subpopulations was significant for a number of alleles (allele a in locus BM0741; alleles a and b in locus BM1853; alleles a and d in locus BM7247; alleles b and d in locus BMS1714; allele b in locus MCM200; alleles a, c and d in locus RM0006, $p<0.05$). Frequency of some alleles was zero in both subpopulations (allele g in BM6465 marker in subpopulation 1; allele e in BM7247 marker in subpopulation 2; allele f and g in MCM200 marker in subpopulation 1 and allele f and g in RM0006 marker in subpopulation 1). Frequency of alleles in BM6465 locus was similar in the two subpopulations. Twenty seven alleles (69 %) were found to be unique in both subpopulations. Highly informative markers like MCM200 and RM0006 showed specific alleles in subpopulation 2 (f and g alleles).

The polymorphism information content (PIC), for each of the loci is shown in Table 2. The PIC values for all loci ranged from 0.45 to 0.76 and the highest PIC was related to MCM200 locus while the lowest PIC was related to BM1853 locus. The PIC values between the two subpopulations were not significantly different (T-test, $p> 0.05$). The PIC values were relatively high, indicating that the selected loci are highly informative and are suitable for genetic studies of the Baluchi sheep breed.

Genetic variability

The observed and expected homozygosity and heterozygosity in each locus for both subpopulations and for overall population are shown in Table 3. The overall expected heterozygosity was 0.66 ranging from 0.47 in BM1853 locus to 0.76 in MCM200 locus. The mean expected heterozygosities in subpopulation 1 and 2 were 0.63 and 0.65, respectively. The mean observed heterozygosity values varied between 0.39 (BM1853) and 0.99 (MCM200) while the average expected heterozygosity values varied between 0.44(BM1853) and 0.75 (MCM200). Expected heterozygosity was higher than its corresponding observed values for loci BM1853, BM6465, BM7247 and BMS1714.

Table1. Information on markers studied, sequence of the PCR primers and PCR amplification conditions.

Marker name	Chr.	Po. (cM)	Primer F(forward) R(reverse)	PCR product (bp)	Annealing temperature (^{0}C)
RM0006	5	12.8	F: CTACAATATCTGGTCACTGGA R: GATCACCATATTTATGAGATGG	119-130	56
BM0741	5	36.2	F: GCCCCTGAAGGAATGGTG R: CCAAAAGGTCCTATCTCCAAA	156-186	55.5
MCM200	25	0.0	F: ACCAAACAGTGTCTCAACC R:GAACAGTCCTTAGATGCCA	130-150	57.3
BM1853	5	95.7	F:GCCTTTTGTAGGTGTTCATTG R:GGTTGCAAAGAGTCAGACATG	105-121	59
BMS1714	25	52.6	F:ATTTATCCCAAGAGGTTCCA R:TGAATCTGGTGAACAGGAAT	120-140	49
BM6465	1	80.8	F:TTTCCAAGGAGCAAGCATCT R:TTGCCAGGCTATAGAAGGACTT	119-133	62
BM7247	5	64.3	F:AAAGTAAGGCCTGCAGTAT R:CTTTCCCTAGAACTTACAAAG	105-121	58

Chr. = Chromosome ; Po. = Position of locus on chromosome

Table 2. Number of observed alleles, effective number of alleles, Shannon's index and polymorphism information content (PIC) in two subpopulations and overall population of Baluchi Sheep

LOCUS	Num. of Alleles	S-P 1			S-P 2			Overall		
		Ne	I	PIC	Ne	I	PIC	Ne	I	PIC
BM0741	5	1.8	0.8	0.44	2.3	1	0.55	2	0.9	0.51
BM1853	4	2.1	0.9	0.52	1.7	0.7	0.26	1.9	0.9	0.45
BM6465	7	3.7	1.5	0.73	3.5	1.4	0.71	3.6	1.4	0.72
BM7247	5	2.7	1.1	0.63	3.3	1.3	0.68	3.1	1.3	0.67
BMS1714	4	3.2	1.2	0.69	3.7	1.4	0.70	3.7	1.4	0.72
MCM200	7	3.8	1.5	0.74	4.3	1.6	0.76	4.2	1.6	0.76
RM0006	7	2	1.1	0.61	4	1.5	0.76	3.5	1.4	0.72

Ne = Effective number of alleles; I = Shannon's index; PIC= Polymorphism information content;
S-P1= Subpopulation 1; S-P2= Subpopulation 2

Test of genotype frequencies for deviation from Hardy-Weinberg equilibrium (HWE) was significant ($p<0.01$) for all of the loci in the whole population (G_{st} test, $p<0.05$). The G_{st} and F-statistics for each locus for overall population are given in Table 4. Three loci (BM0741, BM6465 and RM0006) in subpopulation 1 and two loci (BM1853 and RM0006) in subpopulation 2 were in Hardy-Weinberg equilibrium ($p>0.05$).

The values of fixation indexes (F_{is}, F_{st} and F_{it}) for the overall populations are given in Table 5. Most of the markers had positive values for F_{is} (BM1853, BM6465, BM7247 and BMS1714) showing a deficiency in heterozygosity. The F_{is} index was negative for MCM200, RM0006 and BM0741 markers indicating a high frequency of heterozygotes in these loci.

Table 3. Summary of heterozygosity statistics for all loci

Locus	S-P [a]	Ho	He*	Nei**	Ave-Ht
BM0741	S-P 1	0.47	0.444	0.442	
	S-P 2	0.529	0.558	0.556	
	All	0.502	0.509	0.509	0.49
BM1853	S-P 1	0.453	0.526	0.524	
	S-P 2	0.309	0.36	0.359	
	All	0.39	0.466	0.465	0.44
BM6465	S-P 1	0.615	0.731	0.728	
	S-P 2	0.627	0.715	0.713	
	All	0.623	0.723	0.722	0.72
BM7247	S-P 1	0.68	0.638	0.635	
	S-P 2	0.597	0.696	0.693	
	All	0.631	0.682	0.681	0.66
BMS1714	S-P 1	0.687	0.691	0.688	
	S-P 2	0.554	0.73	0.727	
	All	0.616	0.73	0.728	0.71
MCM200	S-P 1	0.991	0.742	0.74	
	S-P 2	0.984	0.77	0.768	
	All	0.99	0.764	0.763	0.75
RM0006	S-P 1	0.664	0.611	0.609	
	S-P 2	0.746	0.754	0.751	
	All	0.701	0.718	0.717	0.68
MEAN	-	0.64	0.66	0.66	0.64

* = Expected heterozygosity was computed using Levene (1949); **= Nei's (1973) expected heterozygosity; [a] subpopulations.

Population structure

Nei's (1978) genetic distance and genetic identity between the two subpopulations were 0.09 and 0.91, respectively. The genetic distance between the two subpopulations shows that they are very close to each other. The genetic distances between the two subpopulations of Baluchi breed calculated based on the markers are presented in Table 6. The smallest genetic distance was for BM6465 marker (0.024), and the largest genetic distance was for RM0006 marker (0.3518). The RM0006 marker had the largest number of alleles among other markers in this study and showed specific alleles in subpopulation 2.

The result of AMOVA is illustrated in Table 7. The AMOVA of the two subpopulations showed that 2.4 % of total variation was inter population and 97.6 % was related to intra population.

DISCUSSION

Knowledge on genetic diversity, population structure and genetic relationships among populations are essential for future breeding plan. In this respect, DNA markers such as microsatellites used in this study are useful tools to investigate within and between sheep breeds genetic characteristics.

The seven microsatellites used in this study were highly polymorphic. The mean number of alleles in whole population was 5.6. The total number of alleles per locus varied between 4 and 7. Molaei et al. (2011) in the study of six native Iranian native breeds detected 5.9 alleles per breed for microsatellites markers varying from 4 to 9 alleles in different breeds. Also, Nanekarani et al. (2010) reported that the total number of detected alleles in three sheep breeds varied from 6 to 12, and the mean numbers of alleles per locus were 8.1, 8.0 and 8.1 in Gray, Zandi and Karakul sheep breeds, respectively.

Table 4. Test for deviation from Hardy-Weinberg equilibrium by loci for Baluchi sheep subpopulations

Marker	Chi-square			G-square		
	S-P 1	S-P 2	Overall	S-P 1	S-P 2	Overall
BM0741	5.46NS	15.48NS	14.82NS	8.79NS	18.42*	18.85*
BM1853	21.35**	10.25NS	25.87**	22.05**	9.42NS	21.81**
BM6465	27.29NS	34.99**	52.99**	26.47NS	37.05**	56.97**
BM7247	18.83**	66.21**	97.68**	20.57**	74.89**	99.35**
BMS1714	21.28**	62.78**	85.51**	22.58**	57.9**	77.26**
MCM200	172.9**	120.1**	280.3**	217.7**	147.5**	335.9**
RM0006	13.83NS	20.40NS	47.45**	14.89NS	21.25NS	42.44**

* ,** Deviation from Hardy-Weinberg equilibrium is significant ($p<0.05$ and $p< 0.01$); NS= Deviation from Hardy-Weinberg equilibrium is not significant ($p>0.05$); S-P= subpopulations

Table 5. Information of genetic differentiation and gene flow

Locus	Genetic distance			Gene flow
	F_{it}	F_{is}	F_{st}	Nm*
BM0741	0.010	-0.001	0.011	22.86
BM1853	0.163	0.136	0.031	7.81
BM6465	0.142	0.138	0.005	54.02
BM7247	0.055	0.038	0.018	13.56
BMS1714	0.147	0.123	0.027	8.98
MCM200	- 0.292	- 0.31	0.014	17.47
Mean	0.027	0.003	0.024	10.14

F_{is}: fixation index (inter-individual); F_{st}: fixation index (subpopulations); F_{it}: fixation index (total population); * Nm = Gene flow estimated from $F_{st} = 0.25(1 - F_{st})/ F_{st}$.

Table 6. Nei's genetic distances between two subpopulations of Baluchi sheep

Pop. ID	MCM200	BMS1714	BM0741	RM0006	BM7247	BM6465	BM1853
1/2	0.0916	0.1356	0.0153	0.3518	0.0749	0.0240	0.0430

Table 7. AMOVA analysis of Baluchi sheep based on seven microsatellites markers

Source of variation	d.f.	SSD	MSD	% variance	EMS
Among populations	1	1.378	1.378	2.4	$\delta_w^2 + n\delta_b^2$
Within populations	501	673.84	1.345	97.6	δ_w^2
Total	502	692.76	1.380		

Nanekarani et al. (2010) observed negative values for F_{is} (-0.19) and F_{it} (-0.168) in the study of pelt sheep breed of Iran; while in the present study the positive F_{is} value for most of the markers in whole populations indicates a general potential risk for inbreeding. The F_{st} values of genetic differentiation ranged from 0.005 (BM6465) to 0.062 (RM0006). The value of F_{is} for most of the markers were higher than the F_{st} values, except for markers MCM200, RM0006 and BM0741 in which the values of F_{st} were higher than that of F_{is}. Multi-locus F_{st} values showed that about 2.4 % of the total genetic variation was explained by population differences and 97.6 % corresponded to differences among individuals. The values of F_{it} in the subpopulation for the most of markers were positive which shows the deficiency of heterozygosity. However, it was negative for MCM200 marker indicating that the heterozygosity was increased. This marker had specific alleles (g and f alleles) in the subpopulation and overall population.

The value of gene flow between the two subpopulations was positive and varied between 3.8 and 54.0 for different markers. This corroborated that animals were exchanged between the two subpopulations. The overall inbreeding coefficients F_{is}=0.003 and F_{it}=0.027 observed in the present study indicate a decrease of heterozygotes, thus the population is in the risk of inbreeding depression. The F_{st} value was 0.024 which shows the low differentiation between the two subpopulations. This result is similar to those reported for other breeds (Nanekarani et al., 2010; Molaei et al., 2011), where F_{st} estimates ranged between 0.03 and 0.08. The low genetic distance value (D_A=0.09) supported high genetic similarity between the two subpopulations. The genetic similarity observed between the two subpopulations is probably due to migration between them.

All of the investigated loci deviated from Hardy-Weinberg equilibrium ($p < 0.01$). This may be due to migration, high mutation rate in microsatellites and artificial selection in the two subpopulations. Deviation from HWE at microsatellites loci have also been reported in various native sheep breeds (Esmail-Khanian et al., 2007; Banabazi et al., 2007; Nanekarani et al., 2010; Razban et al., 2009 ; Molaei et al., 2011; Aminafshar et al., 2008 ; Sharifi-Sidani et al., 2009).

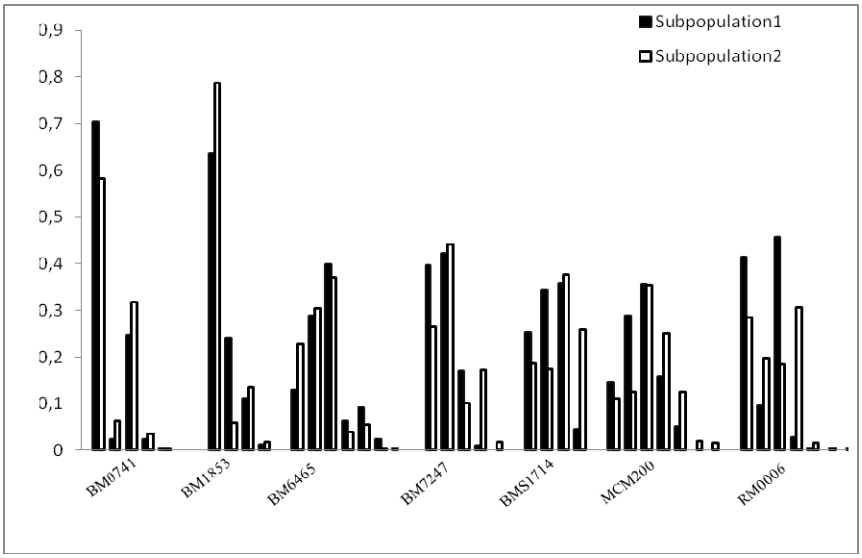

Figure 1.Distribution of allele frequency between two subpopulations for different markers

PIC is an index to measure genetic variation and describe the genetic variation of animal populations at the molecular level (Botstein et al., 1980) so that when PIC >0.5, the locus shows high polymorphism. Microsatellite markers with PIC over 0.70 are very useful in genetic linkage studies (Barker et al., 2001). In this study, all of the loci, except BM1853 locus, had the PIC values higher than 0.50 and the mean of PIC for all of the loci was 0.65 indicating that the selected loci are highly informative and suitable for molecular genetic studies of Baluchi sheep breed.

CONCLUSION

The seven tested microsatellite loci were all polymorphic in the two subpopulations of Baluchi sheep breed. The average observed heterozygosity over all the loci in each subpopulation was less than the expected heterozygosity. The results showed that about 2.4 % of the total genetic variation was explained by population differences and 97.6 % corresponded to differences among individuals indicating a very low genetic differentiation between the two subpopulations.

Then, this study show that more than 30 years trying to make separate lines could not be successful in research center of Baluchi sheep.

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