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GENETIC DIVERSITY OF ALBANIAN GOAT BREEDS BASED ON MICROSATELLITE MARKERS

DIVERSIDAD GENÉTICA DE RAZAS CAPRINAS DE ALBANIA, BASADA EN MARCADORES MICROSATÉLITES

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ADDITIONAL KEYWORDS

Albanian local breeds.

PALABRAS CLAVE ADICIONALES

Razas locales de Albania.

SUMMARY

The domestic goat is one of the most important livestock species in mountainous area of Albania. In this study thirty microsatellite markers in 183 unrelated individuals from 6 local goat breeds are analyzed. Twenty nine markers had five or more alleles. All loci were polymorphic and a total of 331 alleles were detected. The average number of alleles per locus was 11.03. Within breeds, the mean number of alleles ranged from 7.8 to 8. Mean expected heterozygosity (He) ranged from 0.712 to 0.758. Allelic richness varied from 7.61 to 8.19. Inbreeding for all population is rather high F_{IS}= 0.093, ranging from 0.075 to 0.103. The mean F_{ST} (≈ 0.02) demonstrated that 98% of total genetic variation is due to genetic differentiation within each population.

RESUMEN

La cabra doméstica es una de las especies ganaderas más importantes en la zonas montañosas de Albania. En este trabajo se analizaron 30 marcadores microsatélites procedentes de 183 individuos no relacionados, pertenecientes a 6 razas locales de cabras. Veintinueve marcadores mostraron 5 o más alelos. Todos los *loci* fueron polimórficos habiéndose detectado un total de 331 alelos. El número medio de alelos por *locus* fue

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11,03. Dentro de las razas, el número medio de alelos osciló entre 7,8 y 8. La heterocigosidad media esperada (He) osciló entre 0,712 y 0,758. La riqueza alélica varió de 7,61 a 8,19. La consaguinidad para toda la poblacón es más bien alta F_{IS}= 0,093 con un rango de 0,075 a 0,103, la F_{ST} media (≈ 0,02) demostró que el 98% de la variación genética total se debe a la diferenciación genética dentro de cada población.

INTRODUCTION

The domestic goat is one of the most important livestock species in mountainous area of Albania. Goat breeds are defined mainly by the geographic location, morphological characteristics and production performance.

Polymorphic DNA markers are very useful in assessment of genetic diversity within and between breeds. Microsatellites are widely used as genetic markers for the analysis of genetic variability within and between breeds due to their high number, distribution throughout the genome and the efficacy of genotyping.

There are several studies on genetic diversity of goats, based on microsatellite markers, such as Swiss breeds (Saitbekova *et al.*, 1999), Chinese indigenous populations (Li *et al.*, 2002, Li *et al.*, 2004), Indian domestic goats (Rout *et al.*, 2008), goats from Europe (including also the breeds represented here) and middle east (Cañon *et al.*, 2006). In this study, carried out in the frame of econogene project, we intend to determine the levels of genetic variation and relationships among six local goat breeds, representing diverse morphological characteristics. This is the first effort to characterize the Albanian local goat breeds through DNA markers.

MATERIAL AND METHODS

SAMPLE COLLECTION AND MICROSATELLITE MARKERS

A total of 183 randomly sampled animals representing 6 different Albanian goat breeds were analyzed. For each breed a maximum of three unrelated individuals (two females and one male) per flock were sampled, based on the information provided by the farmer. Sampling was carried out from an average of 11 flocks per breed. The breeds are marginally farmed and locally distributed. Thirty microsatellite markers according to MoDAD (ISAG/FAO standing committee) were used to genotype the sampled animals using procedures detailed elsewhere (Cañon *et al.*, 2006).

STATISTICAL ANALYSES

Allele frequencies and tests of genotype frequencies for deviation from Hardy-Weinberg Equilibrium (HWE) were carried out using exact tests of the Genepop V.1.2 program (Rousset *et al.*, 2008). Genetix version 4.05.2 (Belkhir *et al.*, 2001) (http://www.univ-montp2.fr//~genetix/genetix/genetix/htm) was used for the calculation of observed heterozygosity (H_o), mean unbiased estimates of gene diversity (H_e) and the F-statistics (Wright, 1978, Wright, 1965), F_{IS}, F_{IT} and F_{ST} including their significance level by a permutation test. The program Fstat (Goudet *et al.*, 1995) (http://jhered.oxfordjournals.org/cgi/content/citation/86/6/485) was used for the calculation of corrected allele diversity (allelic richness).

Nei genetic distance (Nei, 1972) was calculated and used for the construction of UPGMA consensus tree (Saitou and Nei, 1987) with phylip package (http//evolution.genetics.washington.edu/phylip.html). Bootstrap (1000 replicates) resampling was performed to test the robustness of the dendrogram topology. Nei genetic distance calculated from the allele data was plotted as PCA using GenAlEx program (Peakall and Smouse, 2006).

Assignment of individual to their reference population was evaluated using GeneClass v.1.0.02 (http://www.ensam.inra.fr/URLB/geneclass/geneclass.html) (Cournet

Table I. *Number of microsatellite alleles observed (NA), allele size, observed heterozygosity (H_O), expected heterozygosity (H_E), fixation indices (F_{IS}, F_{IT}, F_{ST}), deviations from HWE for each marker in 6 Albanian goat breeds.* (Número de alelos microsatélites observados (NA), tamaño de alelos, heterocigosidad observada (Ho), heterocigosidad esperada (He), índices de fijación (F_{IS}, F_{IT}, F_{ST}), desviación para HWE para cada marcador en 6 razas albanas de cabras).

Marker	NA	Allele range (bp)	H_O	H_E	F_{IS}	F_{IT}	F_{ST}	HWE deviations
CSRD247	11	220-248	0.69	0.76	0.067	0.095	0.03	1
DRBP1	14	195-225	0.43	0.76	0.427	0.433	0.011	6
ILSTS011	9	264-280	0.75	0.77	0.029	0.037	0.008	0
ILSTS087	11	135-155	0.74	0.82	0.097	0.109	0.014	2
INRA023	10	195-213	0.75	0.81	0.074	0.071	-0.003	1
INRA063	6	172-182	0.62	0.66	0.057	0.051	-0.006	0
InraBern172	9	232-252	0.80	0.82	0.023	0.027	0.004	0
MAF65	16	119-159	0.88	0.89	-0.004	0.018	0.022	0
McM527	9	158-178	0.76	0.80	0.008	0.051	0.043	1
OarAE54	15	111-139	0.81	0.84	0.018	0.045	0.028	0
OarFCB20	8	94-110	0.73	0.74	-0.001	0.025	0.026	0
OarFCB48	11	151-171	0.82	0.85	0.012	0.031	0.019	0
SPS113	16	131-163	0.78	0.83	0.052	0.058	0.006	0
SRCRSP09	13	115-143	0.77	0.79	0	0.019	0.019	1
SRCRSP23	16	81-111	0.88	0.89	0.004	0.016	0.012	0
SRCRSP3	7	110-124	0.53	0.63	0.119	0.157	0.044	3
MAF70	13	138-166	0.69	0.80	0.12	0.131	0.012	1
SRCRSP5	12	160-182	0.71	0.78	0.083	0.087	0.005	3
ILSTS005	6	186-198	0.55	0.64	0.125	0.15	0.029	2
ETH10	5	204-212	0.69	0.68	-0.058	-0.021	0.035	0
TGLA53	13	137-161	0.73	0.75	0.019	0.026	0.008	0
SRCRSP8	14	212-242	0.77	0.81	0.048	0.05	0.002	2
BM6444	30	122-198	0.68	0.93	0.259	0.267	0.01	5
P19	11	172-194	0.48	0.86	0.434	0.446	0.021	6
MAF209	4	100-106	0.43	0.46	-0.022	0.074	0.094	0
SRCRSP7	6	119-131	0.46	0.68	0.303	0.326	0.033	3
ILSTS029	6	150-166	0.48	0.66	0.256	0.279	0.03	4
SRCRSP15	7	181-195	0.63	0.66	0.033	0.039	0.007	0
TCRVB6	14	223-251	0.74	0.86	0.127	0.148	0.024	1
INRABERN185	9	264-286	0.41	0.41	-0.006	0.017	0.024	1
mean	11.03		0.67	0.75	0.090	0.109	0.020	1

*$p<0.001$.

et al., 1999). For all breeds was carried out a direct assignment of individuals and a exclusion analysis based on 10 000 simulated individuals (Cournet *et al.*, 1999). The methods based on allele frequencies (Paetkau *et al.*, 1995) as well as bayesian approach (Rannala and Mountain, 1997) were used. The calculations were carried out using always a *leave one out* procedure.

The program structure (Pritchard *et al.*, 2000) is used for the analysis of population structure by a clustering analysis based in bayesian model. The program uses markov chain monte carlo method the program was

***Table II.** Measures of genetic variability in Albanian goats.* (Medidas de variabilidad genética en cabras albanas).

Breed	n	TNA	AR	He	Ho	MNA	ENA	F_{IS}	HWE deviations
Capore	31	233	7.57	0.74	0.67	7.77	3.9	0.105	8
Muzhake	30	240	7.81	0.76	0.69	8.00	4.1	0.088	7
Dukati	30	233	7.61	0.74	0.69	7.77	3.9	0.076	6
Liqenasi	31	236	7.66	0.75	0.68	7.87	4	0.093	8
Hasi	31	240	7.72	0.74	0.67	8.00	3.8	0.097	6
Mati	30	236	7.64	0.71	0.64	7.87	3.5	0.101	8
Total	183	331	7.67	0.74	0.67	11.03	3.87	0.093	

n: sample size; TNA: total number of alleles; AR: allelic richness; H_E: expected heterozygosity; H_O: observed heterozygosity; MNA: mean number of alleles; ENA: effective number of alleles.

run under the *admixture model*, with *burning period* of 100 000 iterations and *period of data collection* of 100 000 iterations. The samples were analyzed with *K* from 2 to 7, applying 3 running.

RESULTS AND DISCUSSION

MICROSATELLITE LOCI

All the markers were polymorphic. The total number of alleles and allele size for each locus are presented in **table I**. In total 331 alleles were observed over all loci across the 183 individuals. The mean number of alleles per locus was 11 and varied from 4 (MAF209) to 30 (BM6444). Out of 30 markers, 29 showed 5 or more alleles. The total number of alleles per locus in the present study ranged from 5 to 30, except of MAF 209 which had 4 alleles. Observed H_o per locus ranged from 0.41 (InraBern185) to 0.8 (SRCRSP23), with an average of 0.67. A total of 43 breed specific alleles were detected at 25 loci, but only 5 of these alleles had frequencies ≥5%. Departures from Hardy-Weinberg proportions were revealed in 43 of the 180 locus-population comparisons ($p<0.05$). The loci DRBP1, P19 deviated in all populations and BM6444 deviated in five populations. These markers were excluded from the genetic differentiation analyses.

GENETIC VARIATION WITHIN AND AMONG BREEDS

In **table II** the measures of genetic variability of 6 Albanian goat breeds are presented. The most diverse breeds were

***Table III.** Matrix of Nei (D_S) genetic distance (above) among 6 goat populations and number of migrants (below).* (Matriz de distancia genética de Nei (D_S), arriba, entre 6 poblaciones de cabras, y, abajo, número de migrantes).

	Capore	Muzhake	Dukati	Liqenasi	Hasi	Mati
Capore	-	0.072	0.080	0.137	0.101	0.071
Muzhake	31.8	-	0.075	0.122	0.091	0.075
Dukati	21	28.5	-	0.153	0.117	0.085
Liqenasi	8.5	10.7	7.5	-	0.129	0.147
Hasi	12.8	16.8	10.4	8.9	-	0.075
Mati	21.9	21.1	15.6	6.8	19.2	-

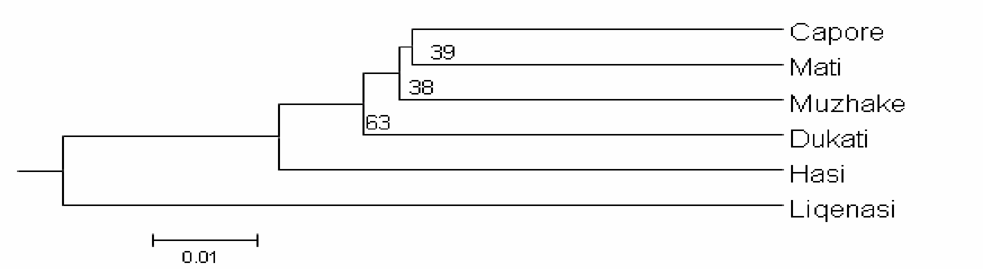

Figure 1. *NJ tree constructed from Ds, among six goat breeds.* (Árbol NJ construido a partir de Ds entre 6 razas de cabras).

Muzhake and Hasi with the highest total number of alleles (TNA) of 240 and the highest mean number of alleles (MNA) of 8. Capore and Dukati have the lowest TNA of 233 and the lowest MNA of 7.8. Expected heterozygosity was lowest in Mati of 0.71 and the highest in Muzhake of 0.76. Allelic richness (AR) ranged from 7.6 in Capore to 7.8 in Muzhake with an average of 7.7 alleles per breed. The mean expected He ranged from 0.71 in Mati to 0.76 in Muzhake. Observed H_o varied from 0.64 in Mati to 0.69 in Muzhake. Mean values of H_e and H_o, overall loci and breeds were 0.74 and 0.67 respectively. The mean number of alleles per locus (MNA) varied from 7. 8 in Capore and Dukati to 8 in Hasi and Muzhake. F_{IS} values ranged from 0.076 in Dukati to 0.105 in Capore. **Table II** shows the number of loci deviating significantly (p<0.05) from HWE, per each breed.

All breeds displayed significant deviation from HWE in more than 6 loci. The positive F_{IS} values showed heterozygotes deficiency within breeds. This deficit might be because of inbreeding and wahlund effect. Inbreeding values obtained for all the breeds varied from 0.076 in Dukati to 0.105 in Capore with a mean value of 0.093. Cañon *et al.*, 2006 reported an average F_{IS} value of 0.10 for the 45 goat breeds from Europe (including also 6 Albanian local breeds) and Middle East analyzed with the same set of microsatellite markers. These high inbreeding values could be due to their small population size, small number of

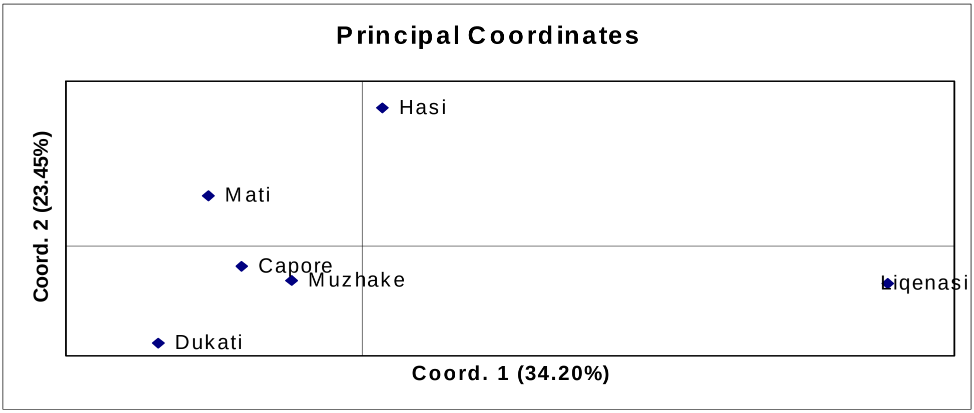

Figure 2. *Principal component analysis based on allele frequencies.* (Análisis de componentes principales basados en la frecuencia de los alelos).

breeding males and their limited geographical area of dispersion (Dobi *et al.*, 2006). Another possible reason for the high values of inbreeding may be the wahlund effect, because the sampling is carried out in 11 flocks per breed with a probable genetic differences among them.

GENETIC DIFFERENTIATION

The average genetic differentiation between all breeds (F_{ST} value) was 0.02, significantly different from zero ($p<0.001$), which means that about 2% of the total genetic variation was explained by population differences and 98% correspond to the differences among individuals within each breed.

Genetic distance are small and ranged from 0.071 to 0.153. Small genetic distances have also been found among Subsaharian Africa goat breeds (Muema, 2009), or among Indian goat breeds (Shadma 2006). Corresponding to these small genetic distances high gene flow between breeds is expected under an island model and values found ranged from 7.5 (Liqenasi-Dukati) to 31.8 (Capore-Muzhake).

Nei's distance (Nei, 1972) (D_S) between each pair of populations **(table III)** was generated based on allele frequencies and used to build a dendrogram with the NJ algorithm **(figure 1)**. The number at the nodes are values for 1000 bootstrap resampling of the typed loci. The NJ tree revealed a cluster of Capore and Mati, although bootstrap value was low.

The principal component analysis (PCA) based on allele frequencies is presented in **figure II**. It is clear that Capore, Muzhake and Dukati form one group. Again, Liqenasi seems quite separate in a single quadrate. The first component clearly discriminate Liqenasi from the rest, while the second component discriminate Hasi, Mati, Dukati and the rest three breeds.

The genetic analysis of 6 Albanian local goat breeds with 30 microsatellite markers showed high gene diversity. The high number of alleles for each locus and the high gene diversity for each breed showed the appropriateness of the markers to analyze diversity in Albanian local goat breeds.

Both NJ tree and PCA analysis showed that Liqenasi breed is distinct from the other breeds. This breed is located in an isolated area in South east of Albania where population has the Macedonian nationality, therefore they prefer to have limited relationship with other areas of Albania (Dobi *et al*, 2006). F_{ST} among the local goat breeds in this study is 2%, that is very low compared to the values of 6.9% reported

Table IV. *Percentage of individuals from each goat breed correctly assigned to their population of origin by direct frequency and Bayesian assignment methods.* (Porcentaje de individuos de cada raza, correctamente asignados a su población de origen, mediante los métodos de asignación directa de frecuencia y bayesiano).

Breed	No	Frequency Direct	Frequency Simulation	Bayesian Direct	Bayesian Simulation
Capore	31	45.16	6.45	54.84	3.23
Muzhake	30	40.00	3.33	50.00	16.67
Dukati	30	63.33	3.33	63.33	6.67
Liqenasi	31	77.42	22.58	74.19	25.81
Hasi	31	58.06	9.68	61.29	6.45
Mati	30	63.33	3.33	63.33	3.33
Total	183	57.92	8.20	61.20	10.38

***Table V**. Proportion of membership of Albanian goat breeds when inferred clusters are 2 and 3.* (Proporción de miembros de razas albanas de cabras, cuando los cluster inferidos son 2 y 3).

Breed	Inferred clusters K= 2		Inferred clusters K= 3		
	1	2	1	2	3
Capore	0.53	0.47	0.44	0.25	0.31
Muzhake	0.50	0.50	0.36	0.38	0.26
Dukati	0.53	0.47	0.51	0.21	0.28
Liqenasi	0.40	0.60	0.15	0.73	0.13
Hasi	0.51	0.49	0.19	0.24	0.57
Mati	0.55	0.45	0.40	0.20	0.40

by Cañon *et al.*, 2006, the 14% for Asian goats (Barker *et al.*, 2001), the 17% for Swiss goat breeds (Saitbekova *et al*, 1999), the 10.5% for Chinese goat breeds (Li *et al*, 2002), 5.4% West African Dwarf goat (Mujibi, 2005), or even the 5% found for indigenous goats of Sub-Saharan Africa (Muema, 2009). All pairwise F_{ST} values were significant ($p<0.01$). The lack of herd book, until nowadays, probably has facilitate the admixture of the breeds contributing to a high level of gene flow between the breeds reducing, as a consequence, the level of genetic differentiation. In practice, the reproducing males are selected by the farmers, who try to avoid the use of males from their own flock, but usually buy them in the farm animal market, or from neighbor farms without any information or control of their origin, resulting in mating without parentage control.

The assignment (direct) and exclusion (simulation) of individuals to their reference population **(table IV)** is carried out by frequency, and bayessian methods. The confidence level was 99%. The Bayes approach performed better for assigning animals to their breed. Muzhake was the breed with a lower rate of animals correctly assigned, and Liqenasi, the breed with the higher rate of correctly assigned animals, being also this breed which showed the higher rate of excluded animals (25.8). The relatively low percentage of correctly assigned individuals is in concordance with the small genetic distance between breeds and with the low genetic differentiation ($F_{ST\approx}2$).

After running the program structure, with the *K* ranging from 2 to 7 we found that the highest value ln Pr(X|K) was obtained for K= 2-3, but the variance is higher at K= 3. In **table V** the proportions of membership of each breed in inferred clusters for K= 2 and K= 3 are presented. In both cases Liqenasi breed is more differentiated, 60% (K= 2) and 73% (K= 3) of individuals are assigned in a single cluster. The other breeds show a high level of admixture.

The small ruminant farming is one of the main production activities in hill and mountainous area of Albania and consists in small farms with limited number of effectives managed on extensive or semi-extensive system with natural mating, which provide an important source of meat and milk mainly for family consumption. Product marketing and processing is limited and difficult due to the low rural socio economic level, poor infrastructure and investments. Goat well adapted to the harsh mountainous environment, poor pasture, and resistant to diseases, use natural pastures wit spontaneous flora resources (concentrated feed is used only before and after weaning). The number of goats is decreasing rapidly in the last years the main reason being possibly the movement of rural population towards urban areas, youth emigration abroad, due to the low economic development of these regions. There is no breeding program for these goat breeds, and the genetic characterization of these breeds carried in the present study, may be used to start a breeding strategy and a policy in order to conserve valuable breeds.

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