

GENETIC DIVERSITY REVEALED BY AFLP MARKERS IN ALBANIAN GOAT BREEDS

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Abstract – The amplified fragment length polymorphism (AFLP) technique with three EcoRI/TaqI primer combinations was used in 185 unrelated individuals, representative of 6 local goat breeds of Albania, and 107 markers were generated. The mean Nei's expected heterozygosity value for the whole population was 0.199 and the mean Shannon index was 0.249, indicating a high level of within-breed diversity. Wright's F_{ST} index, Nei's unbiased genetic distance and Reynolds' genetic distance were calculated. Pairwise F_{ST} values among the populations ranged from 0.019 to 0.047. A highly significant average F_{ST} of 0.031 was estimated, showing a low level of breed subdivision. Most of the variation is accounted for by differences among individuals. Cluster analysis based on Reynolds' genetic distance between breeds and PCA were performed. An individual UPGMA tree based on Jaccard's similarity index showed clusters with individuals from all goat breeds. Analysis of population structure points to a high level of admixture among breeds.

Key words: local breed, goat, genetic variability, genetic diversity, population structure, Albania

INTRODUCTION

Albania is a west-Balkan country. Goats are one of the most important livestock species in Albania, providing 6% of total milk production and 11% of total meat production. Sixty percent of goat flocks are managed in hilly and mountainous areas of Albania and the predominant production system is extensive. There are several local goat breeds. In Albania 99% of the goat population consist of the local ecotypes. The main characteristics included in the objective of goat breeding programs are the reproduction and milk production performances.

The Hasi goat lives in northeastern Albania, in the Albanian Alps. It is the larger mountain version of the Albanian breed. The breed is kept for both milk and meat production; it is reddish in coloration with lop ears. Population size is estimated at 25,000.

Dukati is a small animal, with a small head, straight horns and black mantel. These goats are capable of grazing in difficult terrains and pastures. The area of distribution is in the southern part of Albania. Population size is estimated at 3,000 heads.

Mati is a medium-sized animal, with a robust, deep body, and arched-to-twisted horns, strong legs, and a reddish-to-brown mantel. The area of distribution is in northern part of Albania, the Mati district. Population size is estimated at 18,000 heads.

Muzhake is a small-sized animal, with a mainly grey mantel (other colors occurring are black and white), curled/arched horns, developed skeleton, short neck, straight profile line, small ears. The area of distribution is in the southern part of Albania. Population size is estimated at 100,000 heads.

Capore is a small-to-medium sized animal, with a white-to-reddish mantel, short neck, arched nasal profile of male and straight nasal profile of female. Males have arched horns, while females are without horns and have well-developed round udders. The length of hair is 40-45 cm. The area of distribution is in the southeastern part of Albania, in the Pogradeci district. Population size is estimated at 450 heads. Capore is bred for milk and meat. However, as the casein content in the milk is high, cheese production is common.

Liqenas is a medium-sized animal, with a black mantel, developed skeleton and short neck. Males have arched and twisted horns, while females have arched horns. The area of distribution is in southeastern part of Albania (Prespa Lake, bordering Greece and Macedonia). Population size is estimated at 2,000-2,500 heads.

The genetic characterization of these resources is essential to conservation and breeding programs. A very limited number of studies have been carried out for the characterization of local goat breeds based on microsatellite markers (Canon et al., 2006). Analysis based on 30 microsatellite markers (Canon, et al., 2006) indicates that the Albanian local goat breeds have a low level of genetic differentiation. In the present study, AFLP markers were used in order to estimate the genetic diversity and relationship among 6 local goat breeds: The study, carried out in the framework of the Econogene Project (www.econogene.eu), presents the genetic variability within and between local Albanian goat breeds.

AFLP is a PCR-based technique that uses primers complementary to the synthetic adapters that are ligated to the 'sticky ends' of restriction fragments generated by restriction enzymes. It does not require any prior knowledge about the genome, it is dominant, biallelic and highly reproducible (Ajmone-Marsan et al., 1997; Bagley et al., 2001). The amplified fragment length polymorphism (AFLP) technique (Vos et al., 1995) easily generates a large numbers of markers spanning the whole genome without any prior knowledge about it. Polymor-

phisms are indicated by the presence or absence of the band. AFLP has been successfully applied to studies of genetic diversity and relationships in various domestic species, such as cattle (Ajmone-Marsan et al., 1997; Ajmone-Marsan et al., 2002; Buntjer et al., 2002; Negrini et al., 2006; Negrini et al., 2007), goat (Ajmone-Marsan et al., 2001; Ovilo et al., 2000; Kim et al., 2002; Cameron et al., 2003; Foulley et al., 2006), poultry (Cassandro et al., 2004), chicken (De Marchi et al., 2006), dolphins (Kingston and Rosel, 2004), and sheep (Hoda et al., 2010).

MATERIALS AND METHODS

Populations and AFLP markers

Blood samples were collected from 31 unrelated individuals, randomly chosen from each of 6 local goat breeds of Albania (Muzhake, Capore, Liqenasi, Mati, Hasi and Dukati). The selection of individuals was based on information provided by the farmer. DNA was isolated by phenol-chloroform extraction, proteinase K digestion and ethanol precipitation of DNA (Sambrook et al., 1989). The AFLP marker data were generated using three EcoRI/TaqI primer combinations, E32/T38, E43/T33 and E45/T32, according to the procedure explained in details by Negrini et al. (2010).

Statistical analysis

AFLPs were scored as 1 for band presence and 0 for band absence. AFLP allele frequencies were estimated under the assumption of Hardy-Weinberg equilibrium. Values of Nei's (1973) expected heterozygosity (H_E), Shannon information index of phenotypic diversity (I) (Lewontin, 1972) and the number of effective alleles (N_e) were calculated using Genalex 6 program (Peakall and Smouse, 2006). The correlation coefficient among these indices was estimated using the Pearson rank correlation coefficient by statistiXL software version 1.8. (Vekemans, 2002). The gene flow among these populations was estimated using the Genetix program (Belkhir et al., 2001).

The pairwise F_{ST} values (Lynch and Milligan, 1994), Nei's and Reynolds' genetic distance between the populations were calculated on the basis of non-uniform prior distribution of allele frequencies, generating matrices for each of 1000 bootstrapped data sets using the program AFLP-SURV (Vekemans, 2002). Principal coordinate analysis of the Albanian goat breeds based on Nei's unbiased genetic distance was performed by Genalex 6 program (Peakall and Smouse, 2006). Analysis of molecular variance (ANOVA) Excoffier et al., (2005) was used to separate variance between and within the Albanian goat populations.

The program PhylTools (Buntjer et al., 2002) was used to generate Jaccard distance matrices for the bootstrapped datasets, and to generate an input file for a consensus neighbor-joining (NJ) tree. The NJ tree was created using the NEIGHBOR and CONSENSE modules in PHYLIP (Felsenstein, 1993).

The Bayesian approach implemented in the program STRUCTURE (Pritchard et al., 2000; Falush, et al., 2003) was used to analyze the genetic structure of the Albanian goat breeds. The program infers the number of populations into which the analyzed genotypes can be divided. The admixture ancestry model, assuming that the allele frequencies with an inferred α having an initial value of 1.0 for all populations, was used. The samples were analyzed with K from 1 to 7 applying 20 independent runs for each of the different values of K , with "burning period" of 50 000 iterations and "period of data collection" of 100 000 iterations. Evanno's method (Evanno et al., 2005) was used to identify the appropriate number of clusters using the *ad hoc* statistic Δk , which is based on the second order rate of change of the likelihood function with respect to successive values of K .

RESULTS

AFLPs

Three different AFLP primer combinations (PCs) were used on a total of 155 individuals from 5 Albanian local goat breeds and a total of 107 of poly-

morphic markers were obtained. The number of polymorphic bands varied from 59 (Liqenasi) to 74 (Muzhake) (Table 1).

Genetic diversity

Genetic diversity indices, the number of effective alleles and the total number of polymorphic bands from three primer combinations are shown in Table 2. The gene diversity index varied from 0.145 (Mati) to 0.176 (Dukati) with an average of 0.156, showing a low level of heterozygosity. The heterozygosity at the level of local breeds (H_S) was 0.186 and at the level of the whole goat population (H_T) was 0.192. The Shannon's diversity index (I) had an average of 0.243 (Table 2) at the population level. Pearson correlation coefficient was $r_s = 0.984$ ($p = 0.0001$) between Nei's expected heterozygosity and Shannon index, but was not significantly different among populations

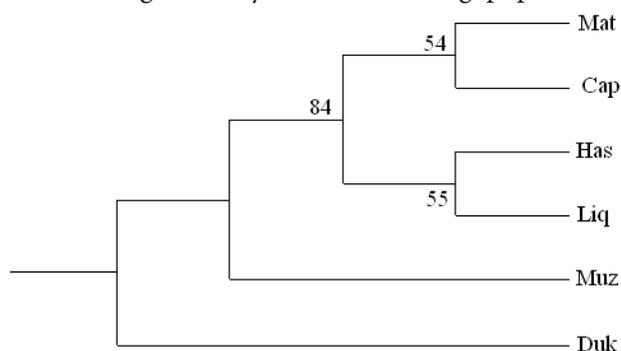


Fig. 1. Dendrogram constructed from Reynolds' genetic distance using the NJ method.

(Mann-Whitney U -test, $P > 0.05$). Mati displayed the lowest genetic diversity and the highest was displayed by Dukati.

Population structure

The NJ tree, based on Reynolds' genetic distance (Table 3) is presented in Fig. 1, displaying the relationship between five breeds. The principal component analysis (PCA) based on Nei's genetic distance (Table 3) is presented in Fig. 2. The first two factors explained 61.31% of the total variance. Nei's unbiased

Table 1. Number of polymorphic bands per primer combination within and across breeds.

Primer combination	Muzhake	Capore	Liçenasi	Hasi	Dukati	Mati	Across breeds
E32/T38	16	16	14	16	14	17	24
E45/T33	26	31	23	23	23	28	34
E45/T32	32	23	22	21	28	22	37
Total	74	70	59	60	65	67	95

Table 2. Genetic diversity indices within 5 local goat breeds, averaged from three primer combinations.

Breeds	Nei's heterozygosity	Shannon index (I)	Number of effective alleles per locus (Ne)
Muzhake	0.167	0.261	1.279
Capore	0.148	0.235	1.240
Liçenasi	0.151	0.232	1.253
Hasi	0.149	0.232	1.240
Dukati	0.176	0.268	1.296
Mati	0.145	0.229	1.234
Mean	0.156	0.243	1.257

Table 3. Nei's genetic distance (below diagonal) after Lynch & Milligan, and Reynolds' genetic distance (above diagonal), between five local goat breeds.

	Muzhake	Capore	Liçenasi	Hasi	Dukati	Mati
Muzhake	****	0.0191	0.0256	0.0289	0.0175	0.0334
Capore	0.0043	****	0.0286	0.0280	0.0450	0.0226
Liçenasi	0.0059	0.0063	****	0.0224	0.0479	0.0367
Hasi	0.0060	0.0047	0.0056	****	0.0470	0.0337
Dukati	0.0037	0.0093	0.0123	0.0115	****	0.0520
Mati	0.0076	0.0049	0.0081	0.0063	0.0113	****

Table 4. Pairwise F_{ST} values among Albanian goat populations (above diagonal) and gene flow (below the diagonal).

	Cap	Muz	Liq	Has	Duk	Mat
Cap	****	0.019	0.025	0.029	0.017	0.0329
Muz	12.87	****	0.028	0.028	0.044	0.0223
Cap	6.71	4.61	****	0.022	0.047	0.0360
Liq	4.99	4.58	5.09	****	0.046	0.0331
Has	7.85	5.93	4.02	5.65	****	0.0506
Mat	8.01	6.72	3.54	3.38	4.53	****

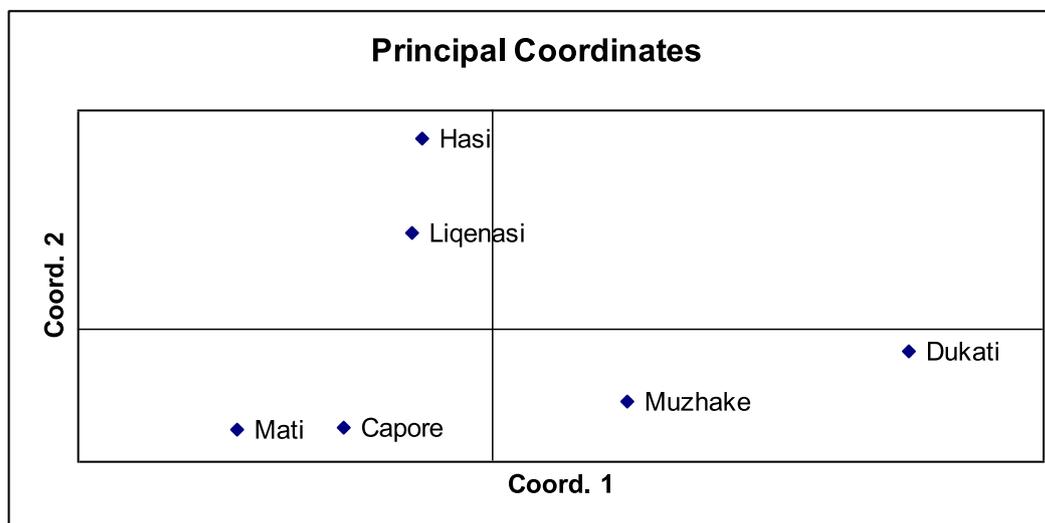


Fig. 2. Principal component analysis based on Nei's genetic distance.

genetic distance and Reynolds' genetic distance values were very small between all pairs of breeds. An individual dendrogram was constructed using the NJ clustering method, based on Jaccard's similarity coefficients matrix (Fig. 3). It displays clusters with individuals from all the goat breeds. The presence of individuals from different breeds in the same cluster is maybe due to the interchange of animals between different farms.

The number of migrants per generation (N_m), based on F_{ST} values, was estimated to be 7.01. The highest gene flow was observed between Capore and Muzhake (Table 4). Values for the fixation index (F_{ST}) on the basis of dominant data (Lynch and Milligan, 1994) were estimated to be 0.032, $P < 0.0001$. Therefore, approximately 3% of total gene diversity was observed among the breeds and 97% can be explained by differences among individuals. F_{ST} values for each of the pairwise population comparisons ranged from 0.019 to 0.029 (Table 4). Hierarchical ANOVA analysis indicated that only 3% of the total genetic variability was due to differences among populations.

The program STRUCTURE (Pritchard et al., 2000; Falush et al., 2003) was run independently 20 times, with K ranging from 1 to 7, in order to choose the appropriate value of K . Our data displayed the

highest value of $\ln P(D)$ at $K = 2$ (Fig. 4). The Evanno method (Evanno, 2005) was applied and ΔK was calculated, an *ad hoc* statistic based on the second order rate of change of the likelihood function with respect to K . This statistic peaked at $K = 2$ (Fig. 4) indicating strong support for 2 groups. Graphic representation of the estimated membership coefficients to the clusters for each individual, ($K = 2$) is shown in Fig. 5.

DISCUSSION

AFLP analysis using three primer combinations revealed 107 polymorphic markers. There was no significance between Nei's expected heterozygosity and the Shannon index, but the rank correlation of these estimates was highly significant for each breed. Dukati exhibited the highest level of genetic diversity and Hasi the lowest. These values are lower than the values obtained in Italian goat breeds (range 0.21–0.24) (Ajmone-Marsan et al., 2001), or Italian goats in the Alps of the Lombardy region (range 0.260 – 0.290) (Gorni et al., 1999). This result is probably due to the small size of the populations and to the limited areas of diffusion.

The F_{ST} value across all markers was 0.031, indicating that 3.1% of total genetic variation is due

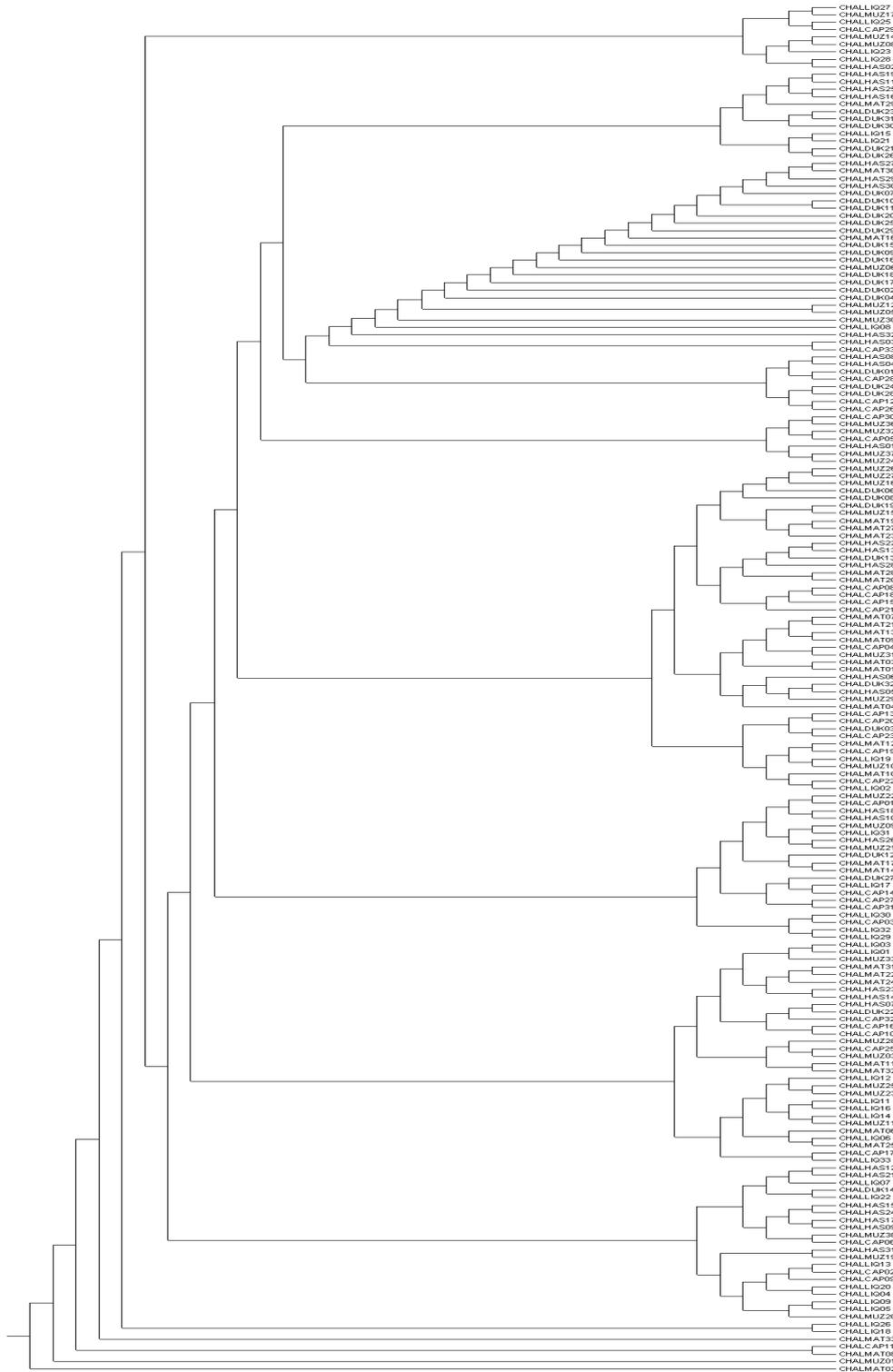


Fig. 3. Neighbor-joining tree constructed from the Jaccard distance matrix.

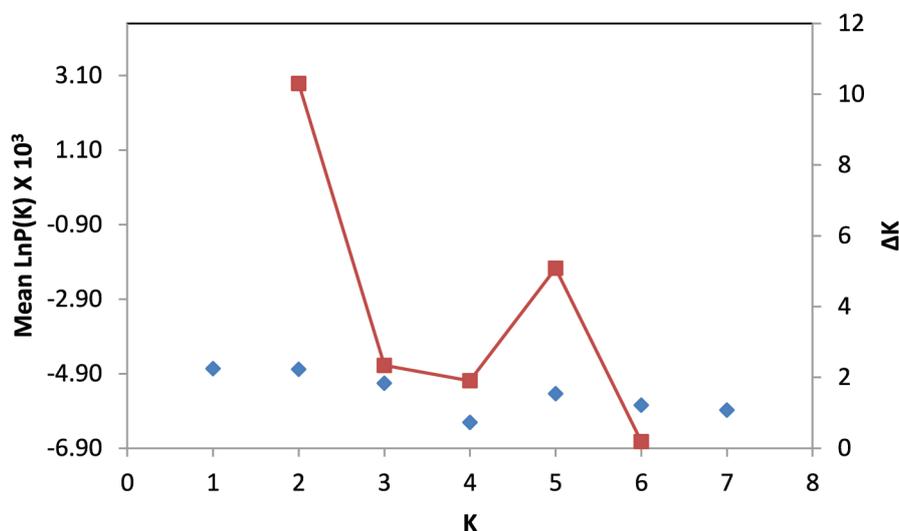


Fig. 4. Results of the STRUCTURE analysis showing mean Ln P(D) and ΔK values.

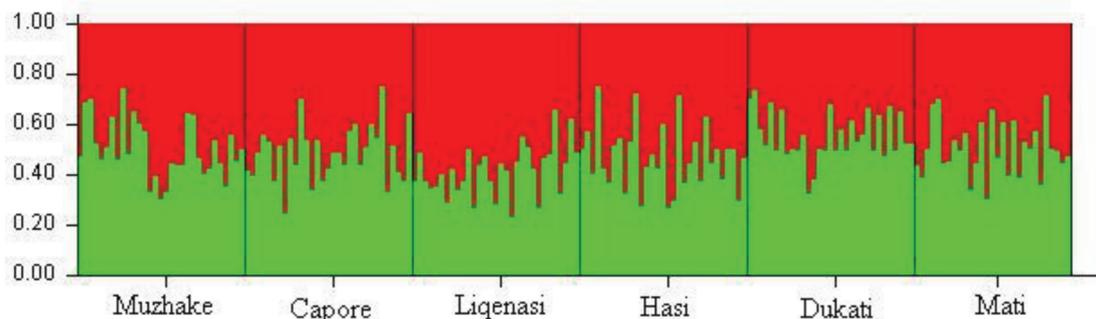


Fig. 5. Graphical representation of the estimated membership coefficients (Q) for each individual in each cluster for K= 2 in six goat breeds.

to breed differentiation. The value is lower than the value ($F_{ST} = 0.11$) for the Italian goat population. This value is also lower than those found in other livestock species from AFLP analysis: 0.15 found in cattle (Negrini et al., 2007), 0.39 and 0.11 in pigs (Kim et al., 2002 and Foulley et al., 2006, respectively), and 0.40 in chickens (De Marchi et al., 2006).

The presence of individuals from different breeds in the same cluster is probably due to the interchange of animals between farms in the same area and from region to region. The presence of Hasi and Liqenas in the same cluster as well as Mati and Capore is explained by the interchange of males from the Korce district (Liqenas breed) to the Hasi district

(Hasi breed) and from the Pogradeci district (Capore breed) to the Mati district (Mati breed) during the period 1970-1990 when a system of centralized economy was implemented in Albania.

Also, hierarchical ANOVA analysis indicated that most of the variation (97%) is accounted for by within-breed diversity. The values for the variation within the populations were higher than those observed in Italian native goats by Ajmone-Marsan et al. (2001) and Crepaldi et al. (2001), who found 75% and 91.2%, respectively, for the variation within the populations by AFLP markers, or the value (71.55%) found in the Moxoto goat breed (Oliveira et al., 2005), by RAPDs markers.

The results presented in this paper reflect the management of local goat breeds. There were no herd books for a long period, not only in the last 20 years of market economy, but also during the period of centralized economy. Goat breeding was ignored in Albania and the very extensive management system explains it. In addition, there is no Animal Genetic Resources Conservation program or a policy development framework, which are more than necessary, especially for some of the endangered breeds (Capore breed) and those at risk (Dukati and Liqenas). There is a lack of parentage control, since the breeding is realized by the farmers themselves without any breeding program. This kind of management has probably facilitated breed admixture. The results presented here should be used to design breeding programs and policy frameworks and actions in order to prevent complete gene losses and to conserve the existing variations.

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