

## GENETIC DIVERSITY IN ALBANIAN SHEEP BREEDS ESTIMATED BY AFLP MARKERS

HODA ANILA<sup>1\*</sup> AJMONE-MARSAN PAOLO<sup>2</sup>, DOBI PETRIT<sup>1</sup>, BOZGO VILSON<sup>1</sup> AND ECONOGENE CONSORTIUM<sup>3</sup>

<sup>1</sup> Agricultural University of Tirana, Albania

<sup>2</sup> Istituto di Zootechnica, Università Cattolica del S. Cuore – Piacenza, Italy

<sup>3</sup> <http://www.econogene.eu>

\*corresponding author, email: [hodanila@yahoo.com](mailto:hodanila@yahoo.com)

### Abstract

In this investigation Amplified Fragment Length Polymorphism (AFLP) markers have been used to assess genetic diversity and relationship among three local Albanian sheep breeds. A total of 93 unrelated individuals were analysed by three *EcoRI/TaqI* primer combination that produced 92 AFLP markers. Nei's  $G_{ST}$  index was calculated to investigate the partition of diversity within and between breeds. The mean value of this index was 0.039, indicating that only 4% of the total genetic variation is due to between breeds differences, while 96% of the diversity is accounted by differences among individuals within breeds. The mean expected heterozygosity value for the whole population was 0.259, indicating that a high level of diversity is present in Albanian sheep compared to estimates in other regions. According to what indicated by the  $G_{ST}$  index, model-based clustering did not differentiate the breeds. The results obtained by AFLP data sets indicate high diversity in Albania but small genetic distances between breeds, confirming previous results obtained with microsatellites. These results reflect Albanian sheep management practices, which have facilitated a relevant gene flow between breeds. These results are useful to design proper breeding programs suited to conserve the genetic diversity presently existing in Albanian sheep.

**Key words:** local breed, genetic variability, genetic distance, cluster analysis, AMOVA

### 1. Introduction

In Albania, several local sheep breed are raised. The genetic characterization of these genetic resources is essential to design conservation and breeding programs. Amplified fragment length polymorphism (AFLP) [28] is PCR-based techniques and consist in the selective amplification of restriction fragments ligated to adapters of known sequence. It has been successfully applied to the identification and estimation of molecular genetic diversity in various

domestic animal species like cattle [5, 20, 21, 3, 4], goats [2], pigs [24, 16, 6], dogs [15, 11], poultry [7] and chicken [8]. Polymorphisms are indicated by the presence or absence of a band. AFLP markers can be generated relatively easily and do not require any prior knowledge about the genome sequence. They are dominant and biallelic [14]. The use of DNA markers for the analysis of Albanian local sheep breeds is quite important for the estimation of their genetic diversity. In the present study, carried

out in frame of Econogene project ([www.econogene.eu](http://www.econogene.eu)), AFLP markers have been used to estimate the genetic diversity and relationship among 3 local sheep breeds Bardhoka, Ruda and Shkodrane and to verify results obtained in recent studies assessing genetic diversity in Albanian sheep breeds by microsatellite markers [13].

## 2. Material and methods

Blood samples were collected from 93 unrelated animals belonging to 3 local Albanian sheep breeds (Bardhoka, Ruda and Shkodrane), based on the information provided by the farmer.

AFLP markers were generated using three *EcoRI/TaqI* primer combinations (E35/T32, E35/T38 and E45/T38). AFLP polymorphisms were binary scored as dominant markers: 1 for band presence and 0 for band absence, with the presence of the band dominant over the absence. AFLP allele frequencies were then calculated assuming populations in Hardy Weinberg proportions. The Genalex 6 program [25], was used to calculate the percentage of within population polymorphic loci; Nei's (1973) expected heterozygosity ( $H_E$ ) and Shannon information index of phenotypic diversity (I) [17]. Correlation coefficients among these indices were estimated using Pearson correlation coefficient by XLSTAT program [1, 27]. The Popgene program version 1.31 [29] was used to calculate indexes of total genetic diversity ( $H_T$ ), genetic diversity within population ( $H_s$ ) and proportion of total genetic diversity partitioned among

populations ( $G_{ST}$ ) [22]. Gene flow among populations was estimated as  $Nm = (1/GST - 1)/4$ .

Model-based clustering [26] was carried out using the STRUCTURE program (<http://pritch.bsd.uchicago.edu/structure.html>). The no-admixture ancestry model was used. A "burning period" of 300000 iterations and "period of data collection" of 300000 iterations were used. Samples were analyzed with a number of expected population  $K$  ranging from 2 to 4.

PhylTools [5] was used to generate Jaccard (Jaccard, 1901) distance matrices for datasets generated by bootstrapping original data, and to generate an input file for a consensus neighbour-joining (NJ) tree. The NJ tree was created using the NEIGHBOR and CONSENSE modules in PHYLIP [10].

$F$ -statistics was computed using Tools for Population Genetic Analysis (TFPGA) [19]. Estimates of 95% confidence intervals were obtained by bootstrapping 1000 replicates over loci. The TFPGA software was also used to calculate Nei's unbiased genetic distances and unbiased identity [23] between breeds. A dendrogram, using UPGMA method was constructed.

## 3. Results and discussion

### 3.1 AFLP polymorphism

In this study, AFLP marker were used to analyze genetic diversity within and between three Albanian sheep breeds. Three AFLP primer combinations (PCs) were used on 93 unrelated animals from small farms. The three PCs yielded a total of 92 polymorphic

bands with an average of  $30.67 \pm 2.52$  markers per primer pair (Table 1).

### 3.2 Genetic diversity

Genetic diversity indices are shown in

Table 2. Mean expected heterozygosity

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

Primer combination	Bardhoka	Ruda	Shkodrane	Across breeds
E35/T32	25	23	22	27
E35/T38	28	28	27	33
E45/T38	30	30	31	32
Total	83	81	80	92
Mean $\pm$ SD	$27.67 \pm 2.52$	$27.00 \pm 3.61$	$26.67 \pm 4.51$	$30.67 \pm 2.52$

**Table 2:** Genetic diversity indices within 3 local sheep breeds, averaged from three primer combinations.

Breeds	Nei's heterozygosity	St. error	Shannon index (I)	St. error
Bardhoka	0.248	0.022	0.367	0.030
Ruda	0.251	0.020	0.376	0.028
Shkodrane	0.278	0.021	0.411	0.029
Total	0.259	0.012	0.384	0.017

**Table 3:** Total genetic diversity ( $H_T$ ), genetic diversity within populations ( $H_S$ ) and coefficient of gene differentiation ( $G_{ST}$ ) across three sheep breeds

Primer combinations	$H_T$	$H_S$	$G_{ST}$
E35/T32	0.210	0.206	0.019
E35/T38	0.282	0.271	0.037
E45/T38	0.279	0.265	0.051
Across marker	0.255	0.245	0.039

The Shannon's diversity index ( $I$ ) had an average of 0.384 (Table 2) at the population level. Values of genetic diversity estimated by the three indexes (number of polymorphisms, Nei's heterozygosity and Shannon I index) were significantly correlated ( $P < 0.05$ ) and not significantly different among breeds (Mann-Whitney  $U$ -test,  $P > 0.05$ ). The values of pairwise Pearson rank correlation coefficient; were  $r = 0.996$  ( $P = 0.004$ ) between Nei's heterozygosity and the number of

values were similar (table 2) and indicate that breeds have a high level of within breed diversity when compared to other species pig [19], chicken [8], goats [2].

polymorphic loci,  $r = 0.981$  ( $P = 0.019$ ) between Shannon index and number of polymorphic loci,  $r = 0.994$  ( $P = 0.006$ ) between Nei's heterozygosity and Shannon index.

In table 3 are shown the indices of genetic diversity and genetic subdivision ( $G_{ST}$ ) for each primer combination across the three breeds. The mean  $G_{ST}$  differences value was 0.039, indicating that approximately 4% of total genetic variation was accounted by

breed differences and 96% by differences among individuals within breeds.

### 3.3 Population structure

Hierarchical AMOVA analysis confirmed  $G_{ST}$  estimates indicating that most of the variation is accounted for by

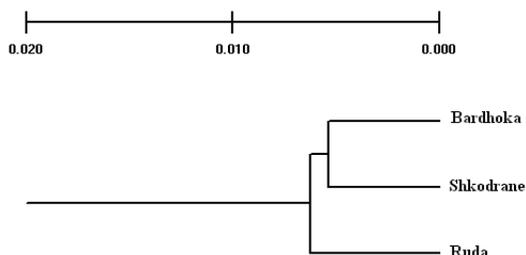
**Table 4:** Analysis of molecular variance AMOVA, for three Albanian sheep breeds

Source of variation	Sum of squares	Variance component	Percentage of total variation	P value
Among Pops	61.523	0.406	3.98	$p < 0.0001$
Within Pops	1511.086	9.808	96.02	$p < 0.0001$
Total	1572.609	10.214	100	

**Table 5:** Nei's (1978) unbiased genetic distance (below diagonal) and Nei's (1978) genetic identity (above diagonal), between three local sheep breeds

	Bardhoka	Ruda	Shkodrane
Bardhoka	****	0.992	0.993
Ruda	0.0077	****	0.992
Shkodrane	0.0068	0.0080	****

The number of migrants per generation ( $N_m$ ), based on  $G_{ST}$  values was estimated 11.176. Values for the fixation index ( $F_{ST}$ ) on the basis of dominant data [18] were estimated to be  $\theta = 0.0284 \pm 0.0073$  (95% CI). Genetic subdivision analyzed by model based clustering [26], at  $K= 2, 3, 4$  revealed the absence of genetic structure among the individuals analysed. Nei's unbiased genetic distance values were very small and similar between all pairs of breeds (Table 5).



**Figure 1:** Individual dendrogram constructed from Jaccard distance coefficient using UPGMA method.

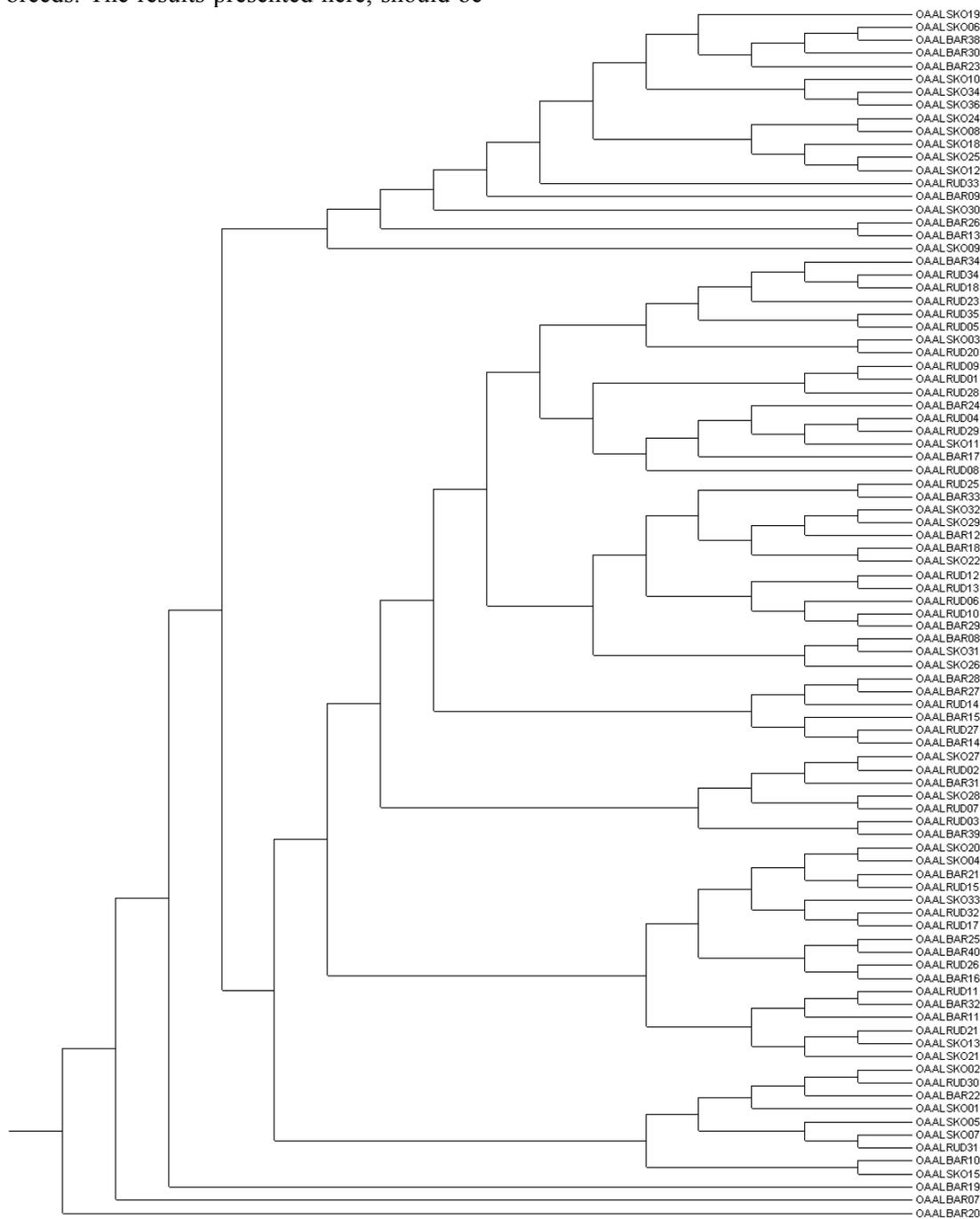
differentiation among individuals within populations (96%) and 4% among the populations (Table 4). The distinctiveness of breeds is low, since most of the variation is accounted by within breed diversity.

The UPGMA tree, based on Nei's (1978) unbiased genetic distance, is presented in figure 1, displaying the relationship between the three breeds. A dendrogram clustering individuals was constructed using the NJ clustering method, based on Jaccard's similarity coefficients matrix (figure 2).

A high genetic diversity was observed,  $P = 75\%$ ,  $I = 0.38$ . The high genetic homogeneity across populations is probably the result of a high level of gene flow. Very close genetic distance are observed between breeds, confirming results previously obtained [12] with a set of 31 microsatellite markers. Also model-based clustering of microsatellite genotypes [12] did not differentiated sheep breeds. The analysis displayed a high level of breed admixture. The results obtained in this paper reflect the

management of these local sheep breeds. Lack of herd books for the long period of 20 years have facilitated the exchange of reproducers and the high gene flow between breeds. The results presented here, should be

used to design breeding programs and policy, in order to prevent gene losses and conserve existing variation.



**Figure 2:** Neighbor-joining tree constructed from Jaccard distance matrix

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