Albanian j. agric. sci. 2014 (Special edition) *Agricultural University of Tirana*

REVIEW ARTICLE **(Open Access)**

Genetic diversity of albanian goat breeds estimated by molecular markers.

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Abstract:

Goats are one of the most important livestock species in Albania. The aim of this study is evaluation of genetic diversity, genetic structure and genetic distances between six Albanian local goat breeds, using three set of markers: 31 microsatellite markers, AFLP markers based on three primer combinations, and 26 SNP markers. A total of 185 individuals representing six different Albanian goat breeds (Capore, Muzhake, Dukati, Liqenasi, Hasi and Mati) were analyzed. All microsatellite markers were highly polymorphic. A total number of 331 alleles were observed at 30 microsatellite loci. The average observed heterozygosity was 0.673.The global heterozygosity deficit (F_{IT}) was estimated 0.11 and global breed differentiation evaluated by F_{ST}, was estimated 0.02. The AMOVA revealed that percentage of variation among populations was 2.04% and within populations was 97.96%. AFLP analysis using three primer combinations revealed 107 polymorphic markers. The F_{ST} value across all markers was 0.031, indicating that 3.1% of total genetic variation is due to breed differentiation. SNPs analysis indicated: Expected heterozygosity per locus ranged from 0.0059 to 0.526 with an average value for all loci, 0.316, while the values of observed heterozygosity (H_O) ranged from 0.0059 to 0.517, with an average value of 0.282. The results obtained here reflect gaot management in Albania. Based on the results of this study, we may conclude that Albanian goat breed are important reservoir of genetic diversity, have a low level of differentiation and high level of admixture.

Keywords: goat, Microsatellite, AFLP, SNP, heterozygosity, genetic diversity, genetic distance

1. 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 genetic characterization of a breed is very important for the evaluation of genetic variability, which is an important element in conservation of genetic resources and for breeding strategies. Genetic characterization can be done by different classes of molecular markers, such as Restriction Fragment Length Polymorphisms (RFLP), Single Stranded Conformation Polymorphisms (SSCP) , Random Amplified Polymorphic DNA (RAPD) markers, Amplified Fragment Length Polymorphisms (AFLP), Single Nucleotide Polymorphisms (SNP), and microsatellites.

Microsatellites are short tandem nucleotide repeats that are randomly distributed throughout eukaryotic genomes [2, 4]. They are inherited co dominantly in Mendelian fashion and are relatively easy to score directly. Microsatellite loci are often hypervariable with high mutation rates and therefore are highly polymorphic in most mammalian species. Microsatellites are used, in livestock species for estimating genetic variation within and among breeds and for assigning individuals to breeds [1, 5, 6, 7, 10, 11].

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. AFLP has been successfully applied to studies of genetic diversity and relationships in various domestic species, such as cattle, goat, poultry, dolphins and sheep [2, 8, 4].

Single nucleotide polymorphisms (SNPs) are biallelic markers and are considered as one of the most widely used genetic markers since they are abundant in the genome, easily reproduced in different laboratories, and simple to score. SNPs have been shown to be informative in ecological and conservation studies. SNPs are also suitable for the assignment of individuals to reference breeds [3, 9, 12]. There are many studies of genetic diversity based on SNP markers in several livestock species such as goats, sheep and/or cattle. In the present study, 26 SNP markers, have been used to estimate the genetic diversity and population structure of six local goat breeds.

The aim of this study is evaluation of genetic diversity, genetic structure and genetic distances between six Albanian local goat breeds, using three set of markers: 31 microsatellite markers, AFLP markers based on three primer combinations, and 26 SNP markers. The laboratory work was made possible in the frame of ECONOGENE project. The analysis at the level of genetic populations is carried out for the first time, in such wide and profound manner, considering a huge number of markers, and a vast set of parameters of population genetics.

2. Material and Methods

A total of 185 individuals representing six different Albanian goat breeds were analyzed. The breeds were: Capore, Muzhake, Dukati, Liqenasi, Hasi and Mati. For each breed 31, 31, 31, 31, 31, 30 respectively unrelated individuals were selected. Sampling was carried out in mountainous area, where still have pure breed individuals, from ten to eleven flocks.

Blood samples of 5–10 ml were collected in EDTA tubes and stored at –200C. DNA was isolated according to standard phenol-chloroform extraction method.

All samples were genotyped for 30 microsatellite markers. Allele frequencies, observed heterozygosity (H_O), expected heterozygosity (He) were estimated for 30 microsatellite markers using Genalex 6 program. Polymorphic information content (PIC) was estimated for all markers in all breeds using the Cervus software. Tests of genotype frequencies for deviation from Hardy-Weinberg equilibrium (HWE) as well as for linkage disequilibrium were carried out using Markov Chain Monte Carlo simulation (100 batches, 5000 iterations and a dememorization number of 10 000) implemented in the Genepop V.1.2 program. The program FSTAT, and estimation of Wright's fixation index. Estimates of genetic variability for each breed (He, Ho), mean number of alleles were computed using GENETIX program. Gene was calculated using the same program. Cavalli-Sforzas Chord Distance DC, Reynolds' DR distance, Nei's D_A distance and Nei's Standard Distance among breeds were computed using Populations program. Bootstrap (1000 replicates) resampling was performed to test the robustness of the dendrogram topology.

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). AFLP allele frequencies were estimated under the assumption of Hardy-Weinberg equilibrium. Values of Nei's (1973) expected heterozygosity (HE), Shannon information index of phenotypic diversity (I) and the number of effective alleles (Ne) were calculated using Genalex 6 program (Peakall and Smouse, 2006). Principal coordinate analysis of the Albanian goat breeds based on Nei's unbiased genetic distance was performed by Genalex 6 program. The NJ tree was created using the NEIGHBOR and CONSENSE modules in PHYLIP. STRUCTURE analysis is carried out as mentioned above on microsatellite markers. Exact tests of genotype frequencies for deviation from Hardy-Weinberg Equilibrium (HWE) were performed using the GENEPOP package as mentioned above. All the other analysis like structure, breed assignment ara carried out as it is described above for microsatellite markers.

3. Results and Discussion

3.1. Microsatellite markers

All microsatellite markers were highly polymorphic. A total number of 331 alleles were observed at 30 microsatellite loci. Except of MAF209, all the markers displayed 5 or more alleles. The total number of alleles varied from 4 (MAF209) to 30 (BM6444) with an overall mean of 11.03 alleles/locus.

The global heterozygosity deficit (F_{IT}) was estimated 0.11 and global breed differentiation evaluated by F_{ST}, was estimated 0.02. The contribution of the microsatellite markers for breed differentiation was estimated by the significance of the F_{ST} statistics. Sixteen markers had significant F_{ST} values and therefore contributed to breed differentiation. The overall estimates for F-statistics were significantly ($p < 0.05$) different from zero.

The values for observed heterozygosity (H_O) ranged from 0.407 to 0.879, with an overall mean value of H_O 0.673, while the values of expected heterozygosity (H_E) ranged from 0.379 to 0.901 with an overall mean value of H_E of 0,729. The mean value of Nei gene diversity, H_T was 0.754. The diversity within breeds (H_S) was 0.742 and diversity between breeds (D_{ST}) of 0,014. The diversity within breeds relative to the diversity of the whole population, G_{ST} value was 0.019. This value is similar with F_{ST} value.

Within breed mean expected heterozygosity varied from 0.700 in Mati to 0.745 in Muzhake having an overall mean value of 0.729. Mean estimate of observed heterozygosity overall breed and loci was 0.673. 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. All breeds displayed significant deviation from HWE in more than 6 loci.

The Factorial Correspondence Analysis (FCA) is performed to visualize the relationships between the individual using Genetix program, it is a multivariate method of analysis. Allele frequencies, of all populations and at all loci, are used as variables. Individuals of 4 breeds (Muzhake, Capore, Mati and Dukati) are grouped together indicating clear admixture among individuals.

3.2 AFLP markers.

Three different AFLP primer combinations (PCs) were used on a total of 183 individuals from 6 Albanian local goat breeds and a total of 107 of polymorphic markers were obtained. The number of polymorphic bands varied from 59 (Liqenasi) to 74 (Muzhake) (Table 5). 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.

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The heterozygosity at the level of local breeds (HS) was 0.186 and at the level of the whole goat population (HT) was 0.192. The Shannon's diversity index (I) had an average of 0.243 at the population level. Pearson correlation coefficient was rs=0.984 (p=0.0001) between Nei's expected heterozygosity and Shannon index, but was not significantly different among populations (Mann-Whitney U-test, $P > 0.05$). Mati displayed the lowest genetic diversity and the highest was displayed by Dukati.

The NJ tree, based on Reynolds' genetic distance displayed the relationship between six breeds. The principal component analysis (PCA) based on Nei's genetic distance showed that the first two factors explained 61.31% of the total variance. Nei's unbiased genetic distance and Reynolds' genetic distance values were very small between all pairs of breeds. The presence of individuals from different breeds in the same cluster is maybe due to the interchange of animals between different farms.

3.3 SNP markers

SNPs analysis indicated: Expected heterozygosity per locus ranged from 0.0059 to 0.526 with an average value for all loci, 0.316, while the values of observed heterozygosity (H_O) ranged from 0.0059 to 0.517, with an average value of 0.282. The frequencies of major alleles ranged from 0.524 to 0.997. All the other loci have frequencies of rare alleles higher than 5%. Significant deviation from HWE overall populations were observed in 5 loci.

Values of observed heterozygosity ranged from 0.239 (Capore) to 0.314 (Dukati). All breeds have close values of expected heterozygosity. Dukati and Liqenasi displayed negative F_{IS} values (-0.008 and -0.038) respectively.

Matrix of Nei's standard genetic distance is used to construct UPGMA phylogenetic tree. Bootstrap values at the nodes are lower than 50 %, displaying a low robustness of UPGMA tree.

Results of Factorial Component Analysis. It is clear that the breeds are not differentiated but show a high level of admixture.

Based on overall F_{ST} value, we may conclude that almost all of allelic variation were accounted within breeds and variation between breeds was very poor (1.8%). This was in accordance with the results obtained previously using microsatellite markers (2%) and AFLP markers (3%) .

4. Conclusions

The results obtained here reflect gaot management in Albania. Goat farming is an important activity for the farmers in Albania. Typically, the farms are small having 20–30 individuals with one reproducing male. Management system is extensive or semi-extensive. The animals graze on natural grasses from morning till evening, without any supplement feed. They provide an important source of milk, meat and wool, mainly for family consumption. Product marketing and processing is limited and difficult due to the low rural socio economic level, poor infrastructure and investments. There is no breeding program for these goat breeds. The mating is natural. In most of the cases there is only one reproducing male per flock that breed all the females in the flock. The reproducing males and females are housed and grazed together thereby no controlled mating is practiced at farmer's level. The reproducing males are selected by the farmer, trying to avoid the use of males from their own flock. Usually the farmer buys the reproducing males in the farm animal market, or from neighbor farms without any information or control of their origin, resulting in mating without parentage control. The lack of herd book, until nowadays, probably has facilitate the gene flow and the admixture of the breeds resulting to a low level of genetic differentiation. Based on the results of this study, we may conclude that Albanian goat breed are important reservoir of genetic diversity, have a low level of differentiation and high level of admixture. All this results may be used and help in starting a breeding strategy and policy involving the decision on crossbreeding or pure breeding.

5. References

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