GENETIC DIVERSITY OF BARDHOKA BREED IN ALBANIA AND KOSOVA ANALYZED BY MICROSATELLITE MARKERS

DIVERSITETI GJENETIK I DELES SË RACES BARDHOKË, QË MBARËSHTOHET NË SHQIPËRI DHE KOSOVË, ME ANË TË MARKERËVE MIKROSATELITË.

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Abstract: *Sheep are considered as an important livestock species in Albania and Kosova. Bardhoka is an autochthonous breed and is the most milk productive sheep among the long tail breeds that lives in both countries. Our study aimed at comparative analysis of microsatellite polymorphism in 6 loci in Bardhoka sheep breed in Albania and Kosova. It is the first time that these local populations of Bardhoka breed from two countries are compared based on DNA markers. It is analyzed genetic diversity of these populations. Allele diversity, observed heterozigosities, expected heterozigosities, F-statistics, G_{ST} estimates is calculated, using different software package. A total of 72 alleles were found. The average number of alleles per locus was 8.15. Within breeds, the mean number of alleles ranged from 5.8 in population from Kosova to 7.66 in population from Albania. The Albanian population has higher values of expected and observed heterozigosity, higher allele number and higher F_{IS} value. Mean expected heterozygosity (He) ranged from 0.68 in Bardhoka from Kosova (Kobar) to 0.76 in Bardhoka from Albania (Albar). Both populations showed a significant heterozigote deficit. Several factors that could cause this deficit are discussed. Gene flow (0.81) is rather low. The mean F_{ST} (0.238) demonstrated that 76.2% of total genetic variation is due to genetic differentiation within each population. Genetic differentiation between populations was much higher than reported by other authors. Genetic differentiation might be caused by long term isolation of these populations and application of different breeding strategies in each country. The research will go further in the future, increasing the number of the markers.*

Përmbledhje: *Delet konsiderohen si një specie e rëndësishme e kafshëve bujqësore në Shqipëri dhe Kosovë. Bardhoka është një racë autoktone dhe është një nga racat kryesore bishtgjata, prodhuese të qumshtit që mbarështohet në dy vendet. Studimi ynë ka për qëllim analizën krahasuese të polimorfizmit të 6 lokuseve mikrosatelitë në delen Bardhoka në Shqipëri dhe Kosovë. Është hera e parë që këto popullata lokale të racës Bardhoka të dy vendeve krahasohen mbi bazën e markerëve të ADN-së. Është analizuar diversiteti gjenetik i këtyre popullatave. Diversiteti alelik, heterozigotia e vëzhguar, e pritur, statistika F dhe G_{ST} janë llogaritur duke përdorur programe të ndryshme kompjuterike. Në total u hasën 72 alele. Numri mesatar i aleleve për lokus është 8.15. Brenda racës, numri mesatar i aleleve varionte nga 5.8 për popullatën nga Kosova deri në 7.66 për popullatën nga Shqipëria. Popullata shqiptare ka vlera më të larta të heterozigotisë së vëzhguar dhe të pritur. Heterozigotia mesatare e pritur (He) varion nga 0.68 në Bardhokën nga Kosova deri në 0.76 në Bardhokën nga Shqipëria. Të dy popullatat shfaqën një deficit sinjifikant të heterozigotëve. Janë analizuar faktorë të ndryshëm që mund të kenë shkaktuar këtë deficit. Fluksi i gjeneve (0.81) është relativisht i ulët. F_{ST} mesatare (0.238) tregon se 76.2% e variacionit gjenetik total është për shkak të ndryshimeve gjenetike brenda çdo popullate. Ndryshimet gjenetike midis popullatave janë më të larta se ato të treguara nga autorë të tjerë. Diferencimi gjenetik mund të jetë shkaktuar nga izolimi afatgjatë i këtyre popullatave dhe nga aplikimi i strategjive të ndryshme në secilin vend. Ky studim do të vijojë më tej në të ardhmen, duke rritur numrin e markerëve.*

Key words*: microsatellite, genetic diversity, gene flow, sheep, local populations*

Fjalë kyçe: *mikrosatelitë, diversitet gejnetik, fluks gjenesh, dele, popullata lokale*

INTRODUCTION

Bardhoka is an autochthonous breed and is the most milk productive sheep among the long tail breeds that lives in both countries, Albania and Kosovo. Bardhoka breed is classified in long tail group. Its origin is from Tropoja area in north/northeast of Albania as well as in western part of Kosovo. This is a sheep with triple productive profile, milk, lamb and wool.

Earlier, phenotypic traits, or biochemical markers were used to characterize the populations of Bardhoka in both countries, separately. ZORAQI, 1991 has studied Bardhoka breed in Albania, based on visible traits, as well as milk and blood polymorphism. The visible genetic and phenotypic profile, correlations of quantitative and qualitative traits of Bardhoka sheep in Kosovo were studied by MEHMETI, 2000. It is the first time that these local populations are compared, using DNA markers

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.

Recently they are used for the study of diversity in European sheep breeds (ÁLVAREZ et al. 2004, ARRANZ et al. 1998, ARRANZ et al. 2001, DIEZ-TASCÓN et al. 2000, FARID et al. 2000, PARISET et al. 2003, RENDO et al. 2004, STAHLBERGER-SAITBEKOVA et al. 2001, TAPIO et al. 2005, TAPIO et al. 2003, PETER et al., 2007). This study regards 6 microsatellite markers in 2 local sheep populations of Bardhoka breed, located in Albania and Kosova in order to evaluate genetic diversity and genetic distances between them.

MATERIAL AND METHODS

Sample collection and microsatellite markers

A total of 52 randomly sampled animals representing Bardhoka breed, in Albania (Albar) and Kosova (Kobar) were analyzed. For each population were sampled maximum three unrelated individuals (two females and one male) per flock. Sampling was carried out from an average of 11 flocks per breed. The populations are marginally farmed and indigenous. There are used 6 microsatellite markers: BM8125, MAF65, OarCP34, OarFCB304, OarHH47, and OarVH72.

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 package (Raymond and Rousset, 2001), performing a probability test using Markov chain Monte Carlo simulation (dememorization 10,000, batches 1000, iterations per batch 5,000). Significant levels were calculated per locus, per population, and over all loci and populations combined. GENETIX version 4.05.2 (http://www.univ-montp2.fr) was used for measuring genetic diversity within population, by the calculation of observed heterozygosity (H_O) and mean unbiased estimates of gene diversity (H_e), (Nei 1978), mean number of alleles (MNA) per locus, the number of private alleles (PA, alleles found in only one breed). The program FSTAT (http://www/2.until.ch.popgen.softwares/fstat.htm) was used for the calculation of corrected allele diversity (allelic richness). Also, FSTAT was used to compute F-statistic parameters, according to Weir and COCKERHAM (1984) (F_{IT} (W&C), F_{IS} (W&C and F_{ST} (W&C). The significance was tested by 1000 permutations (GOUDET 1995).

There were calculated gene flow (Nm) and two genetic distances from allele frequencies using GENETIX software, Nei genetic distance (D_A) that is useful in case of

closely related populations, where the genetic drift is the primary factor of genetic differentiation. It was calculated also standard genetic distance of Nei (1972) (D_S).

RESULTS AND DISCUSSIONS

The allele and genotype frequencies of six microsatellite loci were determined in 2 populations of Bardhoka breed, located in Albania and Kosova. In table 3 are summarized the estimates of various measures of diversity at the breed level. All the markers were polymorphic. The total number of alleles and allele size for each locus are presented in Table 1. A total of 72 alleles were detected over all loci in 52 individuals. The number of alleles for each of six microsatellite loci in both populations is presented in table 3. The total number of detected alleles varied from 8 (BM8125) to 16 (Oarfcb3). PIC (*Polymorphism Information Content*) range from 0.796 (OAFCB3) to 0.887 (OARCP3). The loci used in the analysis were located in different chromosomes, therefore were independent. The microsatellite markers were used to evaluate the genetic diversity within and between both populations of Bardhoka breed, located in Albania and Kosova. All the markers were polymorphic, showing more than 5 alleles and having PIC values higher than 0.5. All the markers appeared to be highly informative.

Table 1

Number of alleles (NA), allelic richness, PIC values for each of the microsatellite markers, in each population, PIC values

Loci	*Chromos.*	*PIC*	*Number of alleles (NA)*			*Allelic richness (AR)*		
			AlBar	KoBar	Total	AlBar	KoBar	Total
BM8125	17	0.772	6	7	8	5.808	7.000	7.400
MAF65	15	0.852	8	6	14	7.606	6.000	11.764
OARCP3	3	0.887	6	5	11	5.833	5.000	10.420
OAFCB3	19	0.796	10	6	16	9.428	6.000	11.802
OARHH4	18	0.822	8	4	12	7.828	4.000	10.241
OARVH7	25	0.819	8	7	9	7.966	7.000	8.582

Observed heterozygosis (H_O) per locus ranged from 0.481 (OARFCB3) to 0.837 (OARVH7). Observed and expected heterozygosis in Albanian population are higher. In total 12 locus-population comparisons revealed Hardy-Weinberg proportions. Nei genetic diversity H_T for all 6 loci, was 0.848, including the diversity within populations H_S, of 0.733 and diversity between populations of 0.115. G_{ST} value is 0.238.

Table 2

Nei heterozygosis and F_{IS}, F_{ST} and F_{IT} values estimates

Loci	*Ho*	*Hs*	*Ht*	F_{IT}	F_{ST}	F_{IS}	*Gst*
BM8125	0.727	0.704	0.809	0.201	0.231	-0.039	0.230
MAF65	0.755	0.737	0.869	0.236	0.261	-0.034	0.263
OARCP3	0.800	0.809	0.904	0.200	0.191	0.011	0.191
OAFCB3	0.481	0.642	0.821	0.521	0.356	0.256	0.358
OARHH4	0.588	0.675	0.837	0.413	0.322	0.135	0.325
OARVH7	0.837	0.831	0.845	0.031	0.033	-0.002	0.033
Total	0.698	0.733	0.848	0.274	0.238	0.048	0.238

There is a deficit of heterozygotes of 0.048 for both populations and loci. It contributed to F_{IT} (W&C) 0.274. F_{IS} (W&C) values varied from -0.002 (OARVH7) to 0.256 (OAFCB3). Markers BM8125, MAF65 and OARVH7 had negative values of F_{IS} (W&C),

showing an excess of heterozygotes. Genetic differentiation between populations, measured by F_{ST}(W&C) was 0.238. F_{ST} values ranged from 0.033 (OARVH7) to 0.356 (OAFCB3).

F_{ST} values of genetic differentiation and G_{ST} values of breed differentiation were similar. The genetic differentiation (F_{ST}) among local populations is high, 23.8%. F_{ST} values indicated that about 24% of the total genetic variation was explained by population difference and 76% correspond to the differences among individuals. The values of genetic differentiation are higher than those reported by other authors. The genetic differentiation were 5.7% between 57 European and Middle Eastern sheep breeds (PETER et al., 2007), 5.2% between West Balkan pramenka sheep types (CINKULOV et al 2008), 8% between finish sheep breed, (TAPIO et al., 2003), 8.8% between Baltic sheep breeds (TAPIO et al., 2005), or 8.5% (FORBES et al, 1995). In all these cases the number of markers was much higher than in our case.

Table 3

Gene flow, pair wise F_{ST}, genetic distances.

	Gene flow (*Nm*)	Pairwise F_{ST}	Nei D_S	Nei D_A
Albar-Kobar	0.8	0.237	2.001	0.289

Gene flow *Nm*, between populations, pair wise F_{ST} and Nei genetic distances D_S and D_A are computed with GENETIX and the data are presented in table 3. *Nm* is rather low, showing a value of 0.8. High genetic differentiation between two Bardhoka populations may be explained with isolation of both populations for about 100 years due to state borders established after year 1913'. This has brought to a high reduction of gene flow between Albar and Kosbar populations. Even there were not genuine breeding programs for this breed in both countries, development of the breed went in different ways.

Table 4

Details of 6 microsatellite loci, typed in 2 local populations

	H_E	H. n. b.	AR	H_O	MNA	F_{IS}
Albar	0.7557	0.7686	7.41	5.83	7.6667	0.061
Kobar	0.6820	0.6959	5.83	0.6733	5.8333	0.033

Inbreeding values for both populations were positive. The values are given in table 4. F_{IS} values are 0.061 and 0.033 for Albar and Kobar respectively. There were found some breed specific alleles. The values for Albanian population were statistically different from zero. The consanguinity, produced by mating between relatives, can be one of the causes for the loss of heterozygosity. Since this deficit does not affect all or, most of the loci in a similar way, we might exclude consanguinity as the principal cause for the loss of heterozygotes. A more feasible reason might be the presence of population substructure within the breed, which may lead to Wahlund's effect. Sampling was carried out in 11 flocks per populations.

The mean number of alleles (MNA) and expected heterozygosity (H_E) are useful in estimating the genetic diversity of a breed. Both populations indicated a high level of genetic diversity. Allelic richness showed high values for each locus, in each population and in total as well. Allelic richness (AR) ranged from 5.83 in Kobar to 7.41 in Albar. The mean expected heterozygosity (H_E) ranged from 0.682 in Kobar to 0.756 in Albar. Therefore these markers were appropriate for measuring genetic variation. The breeds live in extensive condition, grazing in the pasture. This may be the explanation for the high degree of genetic variability. Observed heterozygosity (H_O) varied from 0.67 in Kobar to 0.72 in Albar. The mean number of alleles per locus (MNA) varied from 5.8 in Kobar to 7.7 in Albar. F_{IS} values ranged from 0.033 in Kobar to 0.061 in Albar. All markers showed more than 5 alleles, in each population. Allelic richness (5.83-7.41), pair wise F_{ST} estimates and gene flow are shown in table. The populations in this study are widespread geographically. They have a small population size. There is no artificial insemination and breeding improvement programs are absent. This might be the

reason for the limited gene flow between both populations and the high F_{ST} values (23.8%). The high degree of genetic differentiation found between both populations was supported by low level of gene flow between them. The populations under study have conserved the allelic variation. These populations represented a reservoir of allelic and genetic diversity, that have to be in consideration in breeding programs and in husbandry.

CONCLUSION

The genetic diversity of Bardhoka breed located in Albania and Kosova was analysed for the first time using 6 microsatellite markers.

All markers were polymorphic, showing more than five alleles per locus, high PIC values, high level of heterozygosity, therefore were very suitable for the genetic diversity study.

The diversity of Albanian population is higher than Kosova population.

The mean F_{ST} (0.238) demonstrated that 76.2% of total genetic variation is due to genetic differentiation within each population and was much higher than reported by other authors.

Gene flow (0.8) is rather low.

Genetic differentiation might be caused by long term isolation of these populations and application of different breeding strategies in each country.

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