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Original scientific paper

GENETIC VARIATION OF BULGARIAN AUTOCHTHONOUS SHEEP BREEDS USING MICROSATELLITE MARKERS

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The aim of the study is focused on the design of a conservation sheep breeding programme for the studied autochthonous breeds based on SSR markers genetic variation analysis applied. Seven local Bulgarian autochthonous sheep breeds (Breznishka, Sofiiska, Copper-Red Shumenska, Karakachanska, Local Karnobatska, Blackhead Plevenska and Starozagorska) were differentiated using six microsatellite (SSR) markers, aiming to assess the genetic variation within and between breeds. Among the total of 96 identified alleles, eighteen population specific ones were detected across the all studied genotypes of 338 individuals, except in the Blackhead Plevenska sheep. All examined breeds indicated high level of genetic diversity, with an average of 0.792. The genetic differentiation between the examined sheep breeds was not significant and the values of genetic distances were relatively low. The analysis of molecular variances (AMOVA) showed low variation between the examined breeds (5.51%) in comparison to within population variation (94.49%). The greatest distance (0.643) was found between the populations Local Karnobatska and Starozagorska, while the smallest one (0.108), between the Copper-Red Shumenska and Karakachanska. The genetic distances calculated by Neighbour-Joining method, produced a phylogenetic tree which separates the investigated sheep breeds into two main clusters: one including Blackhead Plevenska Breznishka and Local Karnobatska, and the other one consisting of the four remaining breeds – Copper-Red Shumenska, Karakachanska, Sofiiska and Starozagorska sheep.

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The Factorial Correspondence Analysis (FCA) showed isolation of Local Karnobatska sheep and Starozagorska and an admixture of the other populations.

Key words: diversity, genetic distances, microsatellite (SSR) markers, *Ovis aries*

INTRODUCTION

The studies on the livestock genetic resources in Europe showed that despite the efforts for conservation of the local breeds their number continuously decreased and it is expected that some of them could disappear (SIGNORELLO *et al.*, 2004; TISDELL 2003). In Bulgaria, sheep breeding and the use of local sheep genetic resources have a longtime tradition. The country has a rich diversity of autochthonous domestic livestock breeds, of which nineteen are local sheep breeds (DIMITROV *at al.*, 1993; THE EXECUTIVE AGENCY OF ANIMAL BREEDING AND REPRODUCTION, 2011). Bulgarian local sheep breeds represent the natural basis of sheep industry in Bulgaria and play a considerable role in the breed formation of the contemporary sheep populations. In the last years despite the taken measures was observed a decrease in the number of animals of these breeds. This is a global tendency. Studies on livestock diversity by molecular genetic distances were in the focus of the researchers during the recent two decades. These studies were done mainly by microsatellite markers (SSRs) which are the most widely used molecular tool for assessment of genetic diversity (ALVAREZ *et al.*, 2004; TAPIO *et al.*, 2005; IVANKOVIC, 2005; HANDLEY *et al.*, 2007; LIGDA *et al.*, 2009; BOZZI *et al.*, 2009). There is only one report except our studies on molecular genetic diversity on Bulgarian sheep breeds (KUSZA *et al.*, 2008).

The main objective of this study is to determine the genetic differentiation in local Bulgarian sheep breeds using microsatellite markers. This is necessary for the development effective conservation breeding programs for the indigenous Bulgarian sheep populations.

MATERIALS AND METHODS

Samples and microsatellite analysis

A balanced sample of 338 unrelated ewes and rams of seven local Bulgarian sheep breeds were included in the study: Breznishka (BRSK, n=50), Sofiiska (Elin-Pelinska, SEPL, n=58), Copper-Red Shumenska (CRSH, n=37), Karakachanska (KKCH, n=38), Local Karnobatska (LKNB, n=48), Blackhead Plevenska (BHPL, n=59) and Starozagorska (LSTZ, n=48). DNA was extracted from blood samples with Illustra Blood GenomicPrep DNA Purification Kit (GE Healthcare, UK). The samples were screened for polymorphism by the use of a set of six microsatellite (SSR) markers: OarFCB20, MAF70, ILSTS11, MAF65, OarCP20 and OarJMP58. These markers were chosen preferably unlinked, on the base of the level of polymorphism and the location on the different autosomes. For the selection of the markers we also follow the recommendations of the Food and Agriculture Organization (FAO, 2011) and the International Society for Animal Genetics (ISAG). Primer sequences and allele size range of the markers were obtained from http://dad.fao.org/en/Home.htm.

PCR amplifications were carried out in a thermocycler GeneAmp 9700 (Applied Biosystems) in a total volume of 10µl, containing 50ng DNA, 1x AmpliTaq Gold PCR Master mix (Applied Biosystems, USA), 20pM of each fluorescently (Cy5) labelled forward and unlabelled reverse primers. PCR products were electrophoresed on 6% denaturing polyacrylamide gel (ReproGel High Resolution) using automated laser sequencer (ALF Express II, Amersham

Biosciences). The length of the fragments was determined with the software Allele Locator, v. 1.03 (Amersham Pharmacia Biotech).

Statistical analysis

POPGENE software, version 1.31 (YEH and YONG, 1999) was used to estimate the following parameters: allele diversity, number of alleles per locus and their richness, observed and expected heterozygosity (H_o and H_e), gene flow as well as the fixation indices for differentiation among the populations (WRIGHT, 1978) such as inbreeding coefficient within each population (F_{is}), coefficient of genetic differentiation between populations (F_{st}) and the inbreeding coefficient for all populations (F_{it}). The genetic distances (DA) between populations were estimated according to the method of NEI (1978) using the Neighbor-Joining algorithm for phylogenetic three reconstruction. For population data analysis were calculated: heterozygosity and gene diversity for each breed, Fst between all pairs of the tested breeds by the ARLEQUIN software, version 3.5.1.3 (EXCOFFIER and LISCHER, 2010). The same software was used to check deviation from Hardy-Weinberg equilibrium (HWE) and to perform the Analysis of Molecular Variance (AMOVA). Factorial Correspondence Analysis (FCA) was employed to establish further the differentiation of the breeds, taking into consideration a likely occurring of admixture between some of the populations. FCA was realized through the statistical package GENETIX 4.05 (BELKHIR *et al.*, 1996-2004).

RESULTS

A total of 96 alleles with an average of 16 alleles per locus were detected in the examined set of 7 sheep breeds using a panel of 6 highly polymorphic SSR markers (Table 1).

Table 1. Estimated allele parameters in the studied loci

Locus	Allele range (*bp*)	Na	Ne	Mn	Ho	He	Fis	Fst	Fit	Nm
OarFCB20	88-114	13	6.148	9.714	0.585	0.838	0.254	0.048	0.290	4.892
MAF70	121-185	31	13.333	19.000	0.834	0.926	0.078	0.031	0.107	7.684
ILSTS11	268-286	10	4.537	6.429	0.647	0.780	0.121	0.053	0.169	4.398
MAF65	122-140	11	5.070	7.000	0.378	0.804	0.501	0.060	0.531	3.900
OarCP20	67-91	11	4.728	7.571	0.547	0.789	0.256	0.073	0.311	3.152
OarJMP58	133-173	20	7.221	10.714	0.704	0.862	0.121	0.073	0.185	3.161
Mean		16	6.839	10.071	0.616	0.833	0.217	0.056	0.261	4.190

*Nm - gene flow; Fis - inbreeding within each population, Fst - coefficient of genetic differentiation between populations; Fit - the inbreeding coefficient for all populations

The mean number of alleles (Na) per locus varied between 6.42 (ILSTS11) and 19.00 (MAF70) while the effective number of alleles (Ne) from 4.53 (ILSTS11) to 13.33 (MAF70), thus showing that the marker MAF70 is the most polymorphic and informative. In addition,

together with the marker OarJMP58 it amplified the highest number of private alleles with frequencies < 1% (in total six alleles/locus).

The observed heterozygosity (H_o) is with the range from 0.378 to 0.834 with mean value of 0.616 (Table 1). The estimates of the expected heterozygosity are within 0.780 to 0.926 with a mean of 0.833. H_o was higher than 0.5 except for the marker MAF65 (0.378) and at each locus the observed one (H_o) was lower than the expected heterozygosity (H_e).

The estimated values of the parameters characterizing the inbreeding within each population (F_{is}), coefficient of genetic differentiation between populations (F_{st}) and the inbreeding coefficient for all populations (F_{it}) were 0.217, 0.056 and 0.261, respectively. The F_{is} varied from 0.078 (in MAF70 locus) to 0.501 (MAF65). The mean F_{is} value was higher than F_{st} (0.217>0.056) and heterozygosity deficiency at all tested loci was detected. The established gene flow levels in different loci range from 3.152 to 7.685.

In our study the total number of the population specific alleles (Table 2) is 18, which comprises 18.75% of the total number of the studied alleles.

Table 2. Population-specific alleles characteristics

Locus	Allele size (*bp*)	Frequency of specific alleles (*%*)	Breed
MAF70	121	4.167	LKNB
	171	15.789	KKCH
	175	10.417	LSTZ
	177	20.833	LSTZ
	183	4.167	LSTZ
	185	8.333	LSTZ
ILSTS11	268	8.621	SEPL
	274	1.724	SEPL
	286	5.263	KKCH
MAF65	122	2.000	BRSK
	126	3.448	SEPL
OarCP20	91	6.000	BRSK
OarJMP58	133	2.703	CRSH
	135	1.724	SEPL
	137	2.083	LKNB
	143	1.724	SEPL
	145	2.000	BRSK
	173	8.108	CRSH

The frequency of the population specific alleles varies from 1.724% (ILSTS11 and OarJMP58) to 20.833 % (MAF70). Population-specific alleles were detected in all breeds except in BHPL. Alleles with frequencies lower than 3% were detected in BRSK, SEPL, CRSH and LKNB. The SEPL breed showed expression of five population specific alleles, LSTZ – 4, BRSK – 3, while in the remaining three populations (CRSH, KKCH and LKBN) were found only 2 alleles. Several population-specific alleles of the microsatellite marker MAF70 (171bp, 175bp

and 177bp) were found with higher frequency of 15.789% in LKNB and of 10.417% and 20.833% in LSTZ respectively.

The mean Ho and He per studied populations were between 0.543-0.687 (KKCH–LSTZ) and 0.761-0.820 (LSTZ–SEPL), respectively (Table 3).

Table3. Estimated heterozygosity and related parameters for the studied breeds

Population	Heterozygosity		Fis	*xi*-square	P value
	Ho	He			
BRSK	0.596	0.810	0.264	27.87	0.0224
SEPL	0.574	0.820	0.300	17.18	0.3081
CRSH	0.621	0.812	0.235	11.80	0.6942
KKCH	0.543	0.774	0.298	9.02	0.8766
LKNB	0.607	0.768	0.210	9.48	0.8512
BHPL	0.666	0.802	0.170	24.26	0.0608
LSTZ	0.687	0.761	0.096	19.75	0.1817
Mean		0.792	0.225		

In all examined populations the Ho levels were lower than the expected heterozygosity (He). Generally, a heterozygote deficit could be detected in all examined loci and across the populations it was the highest in SEPL and the lowest in LSTZ. The estimated values of Fis were found positive in all examined breeds varying from 0.096 (LSTZ) to 0.300 (SEPL) with a mean 0.225, indicating a risk of inbreeding. In order to test the possible deviation from HWE, the P-values for the particular breeds were obtained. The investigated populations were in equilibrium, except BRSK and BHPL.

The AMOVA analysis (Table 4) revealed that percentage of variation among the examined breeds was 5.51% while the within populations was 94.49%.

Table 4. Genetic variation by the Analysis of Molecular Variance (AMOVA)

Source of variation	df	Sum of squares	Variance components	Percentage of variation	P value
Among population	6	94.405	0.138	5.51	0.000
Within population	669	1593.830	2.382	94.49	0.000
Total	675	1688.235	2.521		0.000
Fstat:	0.055				

The values of differences of the means within and between populations are significant. The genetic distances calculated by the method of NEI (1978) are given in Table 5.

Table 5. Nei's genetic distances between studied sheep breeds

Population	BRSK	SEPL	CRSH	KKCH	LKNB	BHPL
SEPL	0.261					
CRSH	0.179	0.231				
KKCH	0.219	0.209	0.108			
LKNB	0.229	0.492	0.361	0.449		
BHPL	0.185	0.189	0.189	0.118	0.303	
LSTZ	0.307	0.418	0.418	0.316	0.643	0.317

The greatest pairwise distance value (0.643) was found between the populations LKNB and LSTZ and the closest one (0.108) between CRSH and KKCH. Figure 1. depicts the phylogenetic tree calculated by Neighbour-Joining approach.

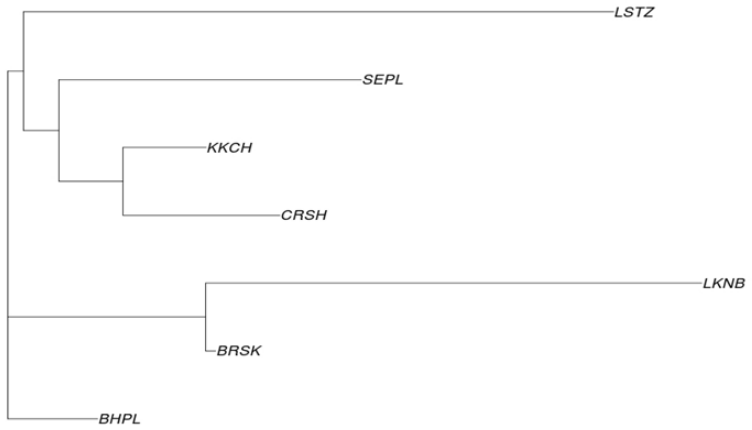

Figure 1. Rooted tree generated from Neighbour-Joining genetic distances of the studied breeds

The tree separated the investigated breeds into two clusters, one including LKNB, BRSK and BHPL and the second cluster includes LSTZ, SEPL, CRSH and KKCH.

The Factorial Correspondence Analysis (FCA) was used both to classify and visualize the individuals in 3-Dimensional scale within the particular breeds (Figure 2).

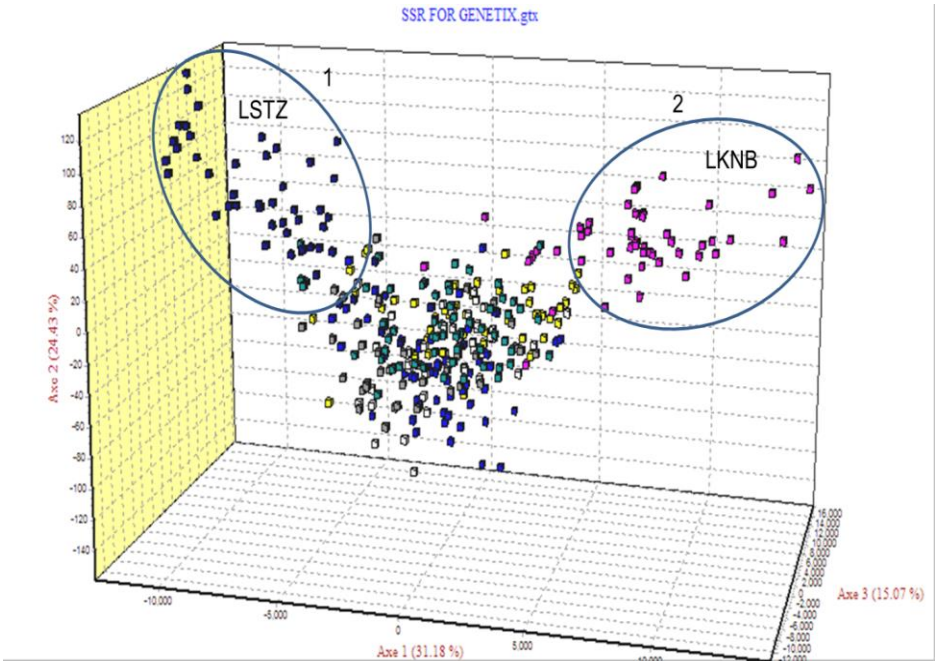

Figure 2. Factorial Correspondence Analysis for 3-D classification of the individuals within the studied sheep breeds

The FCA graph showed that with the exclusion of LSTZ (Circle 1) and LKNB (Circle 2). The remaining breeds cannot be clearly separated due to their low differentiation and admixture.

DISCUSSION

In the present study the allele diversity and genetic relationships of seven indigenous sheep breeds in Bulgaria were examined using a set of six polymorphic microsatellites. A positive correlation (r=0.439) was established between the sample size (i.e. the number of individuals from a given population included in the study, from 37 to 59) and the number of alleles identified across the breeds (in total 96).

The relatively high mean number of alleles per locus (10.07) indicates the potential usefulness of the selected markers for studying the genetic diversity in Bulgarian autochthonous sheep populations. The comparison of the effective number of alleles (Ne) with the number of observed alleles per locus (Na) provides an evidence for the predominance of certain alleles in each breed. In this sense, MAF70 locus could be considered as the most informative marker of our test panel. This observation is in accordance with other studies (ARRANZ *et al.*, 2001; KUSZA *et al.*, 2008, 2010) and confirms the suitability of this marker in studies of sheep genetic diversity. On the other hand, MAF70 showed the highest number of population specific alleles in the

breeds, especially in LSTZ and the lowest heterozygosity deficit (9.8% versus 52.8% in MAF65) (Table 1 and 2).

The values of the inbreeding within each population (F_{is}), coefficient of genetic differentiation between populations (F_{st}) and the inbreeding coefficient for all populations (F_{it}) (0.217, 0.056 and 0.261, respectively) indicated 26% (Table 1) heterozygosity deficit across the populations and around 22% (Table 3) within populations. The multilocus F_{st} showed that only 5.6% of the total genetic variation in Bulgarian sheep breeds is due to the population differences, while the remaining 94.4% corresponds to differences among individuals. The low value of F_{st} is an indication that the studied samples of the breeds are not differentiated enough. The lack of clear differentiation between Bulgarian local sheep breeds could be due to common origin, geographic proximity, similarity in environment and breeding practices but most likely to the past and present gene flow among them. These estimates for genetic differentiation were similar to those reported in other genetic diversity studies, e.g. 3.7% for local Greek sheep breeds (LIGDA *et al.*, 2009), 5.7% for Alpine and European and Middle-Eastern breeds (DALVIT *et al.*, 2008), 5.2% for West Balkan Pramenka sheep types (CINKULOV *et al.*, 2008), but lower than in Slovak Tsigai sheep (13.3%, KUSZA et al., 2008), Bardhoka breed in Albania and Kosovo (24%, HODA *et al.*, 2009).

The overall F_{is} was higher than that of F_{st} (0.217 versus 0.056). The F_{is} values were positive in all examined populations, indicating medium to high rate of heterozygosity loss, as for BHPL and LSTZ they were the least ones (0.170 and 0.096, respectively). Estimates for these heterozygosity deficit related parameters (F_{is}, F_{st}, F_{it}) were significant also for the local Greek breeds (LIGDA *et al.*, 2009), as well as for the Tsigai and Zakel type of sheep breeds from the Central-Eastern and Southern-European regions (KUSZA *et al.*, 2008) but lower in Sicilian sheep breeds. All examined populations in our study displayed heterozygosity deficiencies. The heterozygote deficit (He) was the highest in the SEPL and the lowest in LSTZ.

Regardless of significant heterozygosity deficit, the mean levels of Ho were relatively high in the investigated sheep breeds (0.543-0.687), Generally, all examined populations showed high level of genetic diversity with an average of 0.792 which is close to that published by OLIVEIRA *et al.* (2005) for Bordaleira de Entre Douro e Minho sheep (0.74), ALVAREZ *et al.* (2004) for Latxa sheep (0.77) and KUSZA *et al.* (2010) for five Bulgarian sheep breeds (0.736). A significant deviation from HWE in two of the examined breeds BRSK and BHPL was observed, even though a deficiency of heterozygosity was indicated in all studied sheep breeds. It could be explained with the possible natural processes of mutation, migration, non-random mating, genetic drift and both artificial and natural selection (DIEZ-TASCON *et al.*, 2000).

In all examined populations the Ho levels were lower than the expected heterozygosity (He). Generally, a heterozygote deficit could be detected in all examined loci and across the populations it was the highest in SEPL and the lowest in LSTZ. The estimated values of Fis were found positive in all examined breeds varying from 0.096 (LSTZ) to 0.300 (SEPL) with a mean 0.225, indicating a risk of inbreeding. In order to test the possible deviation from HWE, the P-values for the particular breeds were obtained. The investigated populations were in equilibrium, except BRSK and BHPL.

In this study the genetic distances among the examined populations were relatively low, but higher than 0.05 (Figure 3), which indicates certain differences in their genetic structure HARTL (1980).

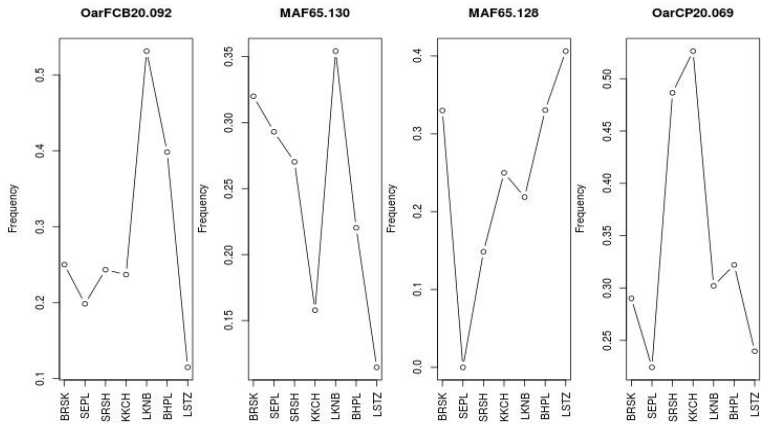

Figure 3. Most frequent alleles of the used markers by population

The closest genetic relatedness (0.108) was found between CRSH and KKCH which is in accordance with their similar exterior. Both breeds are short thin-tailed type with black-brown color of the wool, predominantly coarse. Among the examined sheep breeds, LKNB is most distinct from LSTZ (0.643). The two breeds originated from remoted regions and express different exterior type.

The genetic distances grouping of CRSH and LKNB at different clusters is surprising according their common breed origin and development. This indicates that there should be more detailed study with bigger number of loci and larger samples size.

The BHPL showed not clear genetic differences as it has been defined in the study of KUSZA *et al.* (2010). This could be explained with the absence of private alleles at the locus OarFCB20 which also has been included in our work. Because the number of defined population specific alleles depends not only on the markers used but also on the number of populations and individuals under the study and their genetic relatedness and even more specifically on the allele configuration at particular loci in each breed, more brought studies should be done. The most frequent alleles within the studied breeds shown on Figure 3 can be described as with values varying among the breeds.

Some alleles (as OarFCB20.092 and MAF65.130) are with higher frequencies for certain breeds as LKNB and BHLP which share the same cluster on the phylogenetic tree while the MAF65.128 and OarCO20.069 are with closer frequencies for the BRSK, SEPL, CRSH and KKCH populations.

The results of Factorial Correspondence Analysis are in agreement with the estimated genetic distances (Da), between the studied individuals of the included sheep breeds. It is depicted that the defined larger genetic distance between LSTZ and LKNB is confirmed by the phylogenetic analysis.

The major benefit of the study is that the applied microsatellite markers contribute to an obvious extent the defining of the genetic diversity within the studied local Bulgarian sheep breeds. The discussed marker alleles confirm the differences between the populations – giving a prevalence to certain breeds, which is confirmed also by the reconstruction of the phylogenetic

trees and the factorial component analysis. The outcomes of the study can be considered as a background for more extensive use of microsatellite markers for assessing the genetic diversity of all Bulgarian autochthonous sheep populations serving as a cornerstone for designing of effective conservation breeding programme.

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REFERENCES

ALVAREZ, I., L. ROYO, I. FERNANDEZ, J. GUTIERREZ, E. GOMEZ and F. GOYACHE (2004): Genetic relationships and admixture among sheep breeds from Northern Spain assessed using microsatellites. Journal of Animal Science, *82*: 2246-2252.

ARRANZ, J., Y. BAYOAN and F. SAN PRIMITIVO (2001): Genetic variation at microsatellite loci in Spanish sheep. Small Ruminant Research, *39*: 3-10.

BELKHIR, K., P. BORSA, L. CHIKHI, N. RAUFASTE and F. BONHOMME (1996–2004): GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, Montpellier (Fr) (http://www.genetix.uni-montp2.fr.)

BOZZI, R., P. DEGL'INNOCENTIA, P. RIVERA, L. NARDIA, A. CROVETTI, C. SARGENTINIA and A. GIORGETTI (2009): Genetic characterization and breed assignment in five Italian sheep breeds using microsatellite markers. Small Ruminant Research, *85*: 50-57.

ĆINKULOV M., Z. POPOVSKI, K. PORCU, B. TANASKOVSKA, A. HODŽIĆ, H. BYTYQI, H. MEHMETI, V. MARGETA, R. DJEDOVIĆ, A. HODA, R. TRAILOVIĆ, M. BRKA, B. MARKOVIĆ, B. VAŽIĆ, M. VEGARA, I. OLSAKE and J. KANTANEN (2008): Genetic diversity and structure of the West Balkan Pramenka sheep types as revealed by microsatellite and mitochondrial DNA analysis. Journal of Animal Breeding and Genetics, *125*: 417-426.

DALVIT, C., E. SACCA, M. CASSANDRO, M. GERVASO, E. PASTORE and E. PIASENTIER (2008): Genetic diversity and variability in Alpine sheep breeds. Small Ruminant Research, *80*: 45-51.

DIEZ-TASCÓN, C., R. LITTLEJOHN, P. ALMEIDA and A. CRAWFORD (2000): Genetic variation within the Merino sheep breed: analysis of closely related populations usingmicrosatellites. Animal Genetics, *31*: 243-251.

DIMITROV, TZ., I. DIMITROVA and D. VASSILEV (1993): Conservation of the Bulgarian Indigenous Livestock Resources. Proc. International Animal Conservation Strategy, FAO Workshop, 27-28.

EXECUTIVE AGENCY OF ANIMAL BREEDING AND REPRODUCTION, NICOLOV V. (ed.) (2011): Livestock breeds in the Republic of Bulgaria. Catalogue, 3rd Ed. Sofia, Bulgaria.

EXCOFFIER, L. and H. LISCHER (2010): Arlequin suite ver 3.5 (09.2011). A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources, *10:* 564-567. http://cmpg.unibe.ch/software/arlequin3.

FAO (2011): Molecular genetic characterization of animal genetic resources. FAO Animal Production and Health Guidelines 9, Rome.

HANDLEY, L., K. BYRNE, F. SANTUCCI, S. TOWNSEND, M. TAYLOR, M. BRUFORD and G. HEWITT (2007): Genetic structure of European sheep breeds. Heredity, *99*: 620-631.

HARTL, D. (1980): Principles of Population Genetics. Sinauer Press, Sunderland, 499.

HODA, A., H. BYTYQI, P. DOBI and H. MEHMETI (2009): Genetic diversity of Bardhoda breed in Albania and Cosova analyzes by microsatellite markers. Research Journal of Agricultural Science, *41*: 2.

IVANKOVIC, A., P. DOVC, T. KAVAR, P. CAPUT, B. MIOC, V. PAVIC, V. STUHEC and J. LETO (2005): Genetic characterisation of the Pag island sheep breed based on microsatellite and mtDNA data. Small Ruminant Research, *57*: 167-174.

KUSZA, S., I. NAGY, Z. SASVARI, A. STAGEL, T. NEMETH, A. MOLNAR, K. KUMED, Z. BOSZE, A. JAVOR and S. KUKOVICS (2008): Genetic diversity and population structure of Tsigai and Zackel type of sheep breeds in the Central-, Eastern- and Southern-European regions. Small Ruminant Research, *78*: 13-23.

KUSZA, S., D. DIMOV, I. NAGY, Z. BOSZE, A. JÁVOR and S. KUKOVICS (2010): Microsatellite analysis to estimate genetic relationships among five bulgarian sheep breeds. Genetics and Molecular Biology, *33(1)*: 51-56.

LIGDA, C., J. ALTARAYRAH, A. GEORGOUDIS and ECONOGENE CONSORTIUM (2009): Genetic analysis of Greek sheep breeds using microsatellite markers for setting conservation priorities. Small Ruminant Research, *83*: 42-48.

NEI, M. (1978): Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, *89*: 583-590.

OLIVEIRA, C., B. GUTIERREZ-GIL, S. PEDROSA, E. BARBOSA, R. DANTAS, J. LEITE, N. BRITO, J. ARRANZ and Y. BAYON (2005): Genetic characterization of Bordaleira de Entre Duoro e Minho and Serra da Estrela sheep breeds: Nuclear and mitochondrial DNA. Revista Portuguesa de Ciências Veterinárias, *100*: 175-180.

SIGNORELLO, G., G. CUCUZZA G. PAPPALARDO (2004): La tutela della biodiversit`a zootecnica italiana nei piani regionali di sviluppo rurale. Rivista di Economia Agraria, *59*: 1.

TAPIO, I., M. TAPIO, Z. GRISLIS, L. HOLM, S. JEPPSSON, J. KANTANEN, I. MICEIKIENE, I. OLSAKER H. VIINALASS and E. EYTHORSDOTTIR (2005): Unfolding of population structure in Baltic sheep breeds using microsatellite analysis. Heredity, *94*: 448-456.

TISDELL, C. (2003): Socioeconomic causes of loss of animal genetic diversity: analysis and assess. Ecological Economics, *45(3)*: 365-376.

WRIGHT, S. (1978): Evolution and the genetics of populations. Variability within and among natural populations. University of Chicago Press, Chicago, USA.

YEH, F. and R. YONG (1999): POPGENE version 1.31 (02.04.2011). Microsoft-based Freeware for Population Genetic Analysis. University of Alberta, Edmonton, Canada. http://www.ualberta.ca/~fyeh/fyeh

GENETIČKA VARIJABILNOST BUGARSKIH AUTOHTONIH SELEKCIJA OVCE PRIMENOM MIKROSATELITA

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[3] Izvršna agencija za selekciju životinja i reprodukciju, Sofija, Bugarska

Izvod

Cilj ispitivanja je fokusiran na dizajniranje programa konzervacije ovaca za ispitivanje autohtonih selekcija na osnovu SSR markera. Sedam lokalnih Bugarskih autohtonih selekcija ovce (Breznishka, Sofiiska, Copper-Red Shumenska, Karakachanska, Local Karnobatska, Blackhead Plevenska i Starozagorska) su diferencirane na osnovu šest mikrosatelita (SSR) markera, u cilju utvrđivanja genetičke varijabilnosti unutar i između rasa. Od ukupno 96 identifikovanih alela, 18 specifičnih za populaciju su identifikovani u svim ispitivanim geneotipovima 338 jedinki, osim u Blackhead Plevenska ovcama. Sve ispitane selekcije ukazuju na visoknivo genetičkog diverziteta, sa prosekom od 0.792. Genetička diferencijacija između ispitivanih selekcija ovaca nije bila značajna i vrednosti su bile relativno niske. Analiza molekularne varijanse (AMOVA) je pokazala nisko variranje između ispitivanih genotipova (5.51%) u poređenju sa varijabilnosti unutar populacija (94.49%). Najveća distance (0.643) je nađena između populacija Local Karnobatska i Starozagorska, dok je najmanja (0.108), između Copper-Red Shumenska i Karakachanska. Genetička distanca izračunata pomoću Neighbour-Joining metodom, daje filogenetsko drvo koje razdvaja ispitivane genotipove u dva glavna klastera: jedan koji uključuje Blackhead Plevenska Breznishka i Local Karnobatska, i drugi koji sadrži preostala četiri genotipa– Copper-Red Shumenska, Karakachanska, Sofiiska i Starozagorska. Factorialna Corespondeciona Analisa (FCA) pokazuje izolovanost Local Karnobatska i Starozagorska i nemešanje drugih populacija.

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