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DNA MICROSATELLITE INFORMATIVENESS, ALLELE FREQUENCIES AND THEIR DISTRIBUTION IN THE GENOME OF MACEDONIAN AUTOCHTHONOUS SHEEP POPULATIONS

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ABSTRACT

As a result of natural selection, management system, environment pressure, different populations of Pramenka sheep in South East Europe developed distinctive productive and phenotype characteristics which are sufficient to distinguish them. In this study, the informativeness of fifteen DNA microsatellite markers in the genome of 105 individuals from three indigenous sheep populations in the country (Sharplaninian, Karakachanian and Ovchepolian) was analysed. In overall population, 281 different alleles were observed. The average number of alleles per locus in total population was 18.73, the mean He in overall population was 0.824, so all loci were highly polymorphic with PIC value higher than 0.5. Mean number of alleles per locus, at population level, were 12.13, 11.53 and 13.27 alleles in the genome of Sharplaninian, Ovchepolian and Karakachanian sheep populations, respectively. Results from this research will improve knowledge of the sheep genetic resources in the country at DNA level and will provide appropriate information for further conservation.

KEYWORDS: pramenka, sheep, DNA, microsatellites.

INTRODUCTION

Genome characterization enables further knowledge of sheep genome, through determination of phylogeny and genetic differences among sheep populations. Further on, it can emphasize genome uniqueness and can develop breeding directions and specific breeding programs. The development and application of different molecular methods provide different levels of data that can be used further. DNA microsatellites are often used as genetic markers in genetic diversity studies, due to their distribution throughout the genome, high level of polymorphism,

co-dominant inheritance and possibility to analyse them easily (Cañon et al., 2006). Numerous sheep populations are characterized using DNA microsatellites and in those studies different number of them are applied to different sheep populations (Arora & Bhatia, 2004; Diez-Tascon et al., 2000; El-Nahas Soheir et al., 2008; Gutiérrez-Gil et al., 2006; Hassan et al., 2003). In the Western Balkan region, numerous phenotypes of Pramenka sheep population are present. Common characteristic of these phenotypes is that they evolved under different biogeographical and socio-cultural conditions (Porcu & Markovic, 2006). Major concern considering conservation of genetic diversity in autochthonous breeds is how to improve knowledge of the genetic resources in the specific breed (Zamani et al., 2015; Khodabakhshzadeh et al., 2016). Management of genetic diversity in livestock species requires recognition and development of breed conservation priorities and development of specific breeding programs. Data gained from different types of molecular studies can be used as foundation for further sustainable use of indigenous breeds. In our study, a set of fifteen FAO recommended microsatellite markers were used to evaluate the genetic diversity in the genome of three autochthonous sheep populations in the country.

MATERIAL AND METHODS

Genome of autochthonous Sharplaninian (Shar), Karakachanian (Kara) and Ovchepolian (Ovch) sheep populations was analysed with fifteen microsatellite loci. In total 105 samples were analysed, which means 35 adult animals per population. Blood samples were collected from different sheep flocks collecting no more than 8 samples per flock, exclusion from this rule was Karakachanian sheep where blood samples were collected from two *ex situ in vivo* conserved flocks. Blood samples were collected from the jugular vein into ethylenediamine tetra-acetic acid (EDTA)-containing vacutainer tubes. DNA was isolated from fresh blood using phenol-chloroform extraction according to established protocols (Efremov et al., 1998). DNA concentration was determined spectrophotometrically at 260 nm. Sheep genome was analysed with fifteen microsatellite markers. Forward primers for the following loci: BM 8125, ETH 225, ILSTS 11, OARJMP 58, OARFCB 193, SRCRSP 1, SRCRSP 7, SRCRSP 8, and McM 527 were labelled with IRDye^{TM}700, while forward primers for loci: ILSTS 28, MAF 214, OARFCB 11, OARFCB 48, SRCRSP 3, and SRCRSP 9 were labelled with IRDye^{TM}800. The PCR amplification was performed in Hybaid PCR Express Thermal Cycler, and afterward amplifications were run on two channel Licor4200 DNA Sequencing Analyser on 6% denaturing Polyacrylamide gel electrophoresis. Allele size was detected by Gen image IR4.05 Licor software program, using Licor IRD700 and 800 ladders. Allele frequency, the mean

number of alleles (MNA), polymorphic information content (PIC), observed heterozygosity (Ho) and heterozygosity expected (He) across the markers and the populations were calculated using the FORSTAT and Fstat software.

RESULTS AND DISCUSSION

As more microsatellite loci become available for use in genetic surveys of population structure, population geneticists can select appropriate loci to use them in such surveys. Analysis of selectively neutral molecular markers has become a common method for inferring the evolutionary history of populations and species. Technological advances have enabled population and conservation geneticists to describe increasingly complex and subtle genetic relationships (Kalinowski, 2002). In this study, 105 animals were genotyped with 15 microsatellites and 281 different alleles were observed. The average number of alleles per locus in the total population was 18.73. Highest number of different alleles, in total population, per locus were observed for loci MAF214 (32 alleles) and McM527 (32 alleles), while lowest number of different alleles (9) was observed for loci BM8125 and SRCRSP9. The best estimator of genetic diversity in the population is expected heterozygosity (He) (Kim et al., 2002). The mean He in the overall population in our study was 0.824 gained value that indicates high genetic diversity in analysed population (Tab. 1).

Overall, He was higher than the observed in the studies conducted by Ocampo et al. (2016) in Colombian sheep population (0.770), and Jevšinek et al. (2015) in Krajina Pramenka sheep (from Slovenia) and three Croatian breeds (Cres island sheep, Lika Pramenka sheep and Istrian Pramenka), where He was 0.756. At population level, locus McM527 have shown highest average expected heterozygosity (He = 0.919), while the locus SRCRSP9 had lowest average value (He = 0.737). Values for observed heterozygosity (Ho), at overall population, were in the range of 0.400 (for loci ETH22 and SRCRSP3) up to 0.905 (for loci OARJMP58 and SRCRSP8). Overall Ho at population level was 0.666. All loci were highly polymorphic and no marker from the selected microsatellite panel showed polymorphic information content (PIC) value less than 0.5 resulting with high average PIC value (0.824).

Table 1. Estimated diversity parameters for fifteen microsatellite loci in total population

Loci	n	A	He	Ho	*h*	PIC
BM8125	105	9	0.745	0.676	0.324	0.706
ILSTS11	105	14	0.817	0.762	0.238	0.793
ILSTS28	105	19	0.870	0.762	0.238	0.856
ETH225	105	13	0.830	0.400	0.600	0.809
SRCRSP1	105	13	0.789	0.676	0.324	0.779
SRCRSP3	105	16	0.825	0.400	0.600	0.806
SRCRSP7	105	20	0.866	0.562	0.438	0.854
SRCRSP8	105	24	0.877	0.905	0.095	0.874
SRCRSP9	105	9	0.737	0.676	0.324	0.700
OARFCB11	105	17	0.882	0.848	0.152	0.869
OARFCB48	105	17	0.868	0.590	0.410	0.856
OARFCB193	105	23	0.818	0.629	0.371	0.790
OARJMP58	105	23	0.902	0.905	0.095	0.898
MAF214	105	32	0.860	0.543	0.457	0.853
McM527	105	32	0.919	0.657	0.343	0.915
Mean	105	18.67	0.840	0.666	0.334	0.824

n- number of individuals, A - number of alleles per locus, Ho - average observed heterozygosity, He - average expected heterozygosity, *h*- average observed homozygosity, PIC - average polymorphic information content

Average PIC in total population ranged from 0.700 for SRCRSP9 up to 0.915 for McM527. High PIC values and the high average number of alleles per locus indicate that the selected panel of molecular markers used in this study are suitable and can be used for characterization of genetic diversity. A higher number of alleles than those detected in our study were noted by Hoda & Ajmone-Marsan (2012) in three different Albanian sheep breeds (348 observed alleles at 31 microsatellite loci in 93 individuals). Jevšinek et al. (2015) observed lower number of different alleles (106 different alleles, in 114 genotyped individuals, using 11 microsatellites) in the genome of Bela Krajina Pramenka sheep (n=29) from Slovenia and three Croatian breeds, Cres island sheep (n=25), Lika Pramenka sheep (n=25), and Istrian Pramenka (n=35). Same author reported 10.60 of alleles per locus in the genome of Bela Krajina Pramenka sheep and 3 Croatian breeds, Cres island sheep, Lika Pramenka sheep, and Istrian Pramenka. Salomon et al. (2012) and Salomon et al. (2015) studied the genome of Istrian sheep, Lika and Krk Pramenka sheep populations. They found that the great majority of markers were highly informative and polymorphic, 291 different alleles were found in 103 genotyped individuals. The average number of alleles per locus was 10.39. Ocampo et al. (2016) noted 157 alleles for the entire sheep population (549 animals genotyped for the 11 microsatellite loci), the most informative locus was ILSTS28 with 23 different alleles. Ocampo et al. (2016) reported highest number of alleles per locus in the creole hair sheep (11.91 alleles per locus) value that is higher than the 7.80 alleles per locus reported by Quiroz et al. (2007) in Mexican creole sheep, 7.25 alleles per locus reported by Ochipinti et al. (2012) in Paraguayan creole sheep, and 7.71 alleles

per locus reported by Blackburn et al. (2011) in North American creole sheep. Distribution of mean number of alleles is often connected to the sample size due to the presence of the unique alleles in populations. Those alleles often occur in low frequencies and because the number of observed alleles tends to increase depending on the population size (Ghazy et al., 2013). In addition, mean number of alleles is often connected to the presence of, or high level of heterozygous individuals in analysed population. Mean number of alleles per locus, at population level, were 12.13, 11.53 and 13.27 alleles in the genome of Sharplaninian, Karakachanian and Ovchepolian sheep population, respectively. The highest mean number of alleles per locus for Ovchepolian sheep population can be explained by presence of heterozygous genotypes in this population. Distribution of allele frequencies is presented in Tab. 2, 2a and 2b.

Table 2. Allele frequencies in analysed sheep populations

L	Na	Shar	Ovch	Kara	L	Na	Shar	Ovch	Kara	L	Na	Shar	Ovch	Kara
		35	35	35			35	35	35			35	35	35
OARJMP58	1	0.000	0.000	0.014	SRCRSP8	1	0.000	0.043	0.029	SRCRSP7	1	0.000	0.014	0.014
	2	0.000	0.000	0.029		2	0.214	0.100	0.229		2	0.071	0.100	0.000
	3	0.014	0.000	0.071		3	0.114	0.286	0.171		3	0.057	0.029	0.000
	4	0.057	0.000	0.186		4	0.100	0.014	0.029		4	0.014	0.014	0.043
	5	0.100	0.000	0.129		5	0.000	0.114	0.000		5	0.000	0.014	0.000
	6	0.171	0.029	0.057		6	0.014	0.057	0.029		6	0.000	0.000	0.029
	7	0.000	0.000	0.014		7	0.000	0.043	0.000		7	0.029	0.014	0.043
	8	0.071	0.057	0.014		8	0.000	0.029	0.000		8	0.100	0.186	0.071
	9	0.043	0.214	0.000		9	0.000	0.014	0.000		9	0.129	0.186	0.086
	10	0.086	0.057	0.000		10	0.014	0.000	0.000		10	0.114	0.143	0.029
	11	0.071	0.029	0.029		11	0.014	0.043	0.014		11	0.000	0.000	0.014
	12	0.043	0.043	0.086		12	0.086	0.000	0.014		12	0.100	0.086	0.029
	13	0.029	0.043	0.129		13	0.000	0.000	0.057		13	0.129	0.043	0.357
	14	0.114	0.043	0.043		14	0.129	0.071	0.143		14	0.043	0.057	0.200
	15	0.000	0.000	0.014		15	0.057	0.029	0.086		15	0.000	0.000	0.014
	16	0.014	0.100	0.000		16	0.043	0.000	0.014		16	0.100	0.071	0.057
	17	0.071	0.057	0.000		17	0.000	0.000	0.014		17	0.014	0.014	0.014
	18	0.071	0.057	0.071		18	0.000	0.071	0.000		18	0.057	0.000	0.000
	19	0.000	0.000	0.014		19	0.043	0.000	0.029		19	0.043	0.000	0.000
	20	0.043	0.014	0.100		20	0.071	0.014	0.014		20	0.000	0.029	0.000
	21	0.000	0.057	0.000		21	0.057	0.000	0.100	SRCRSP9	1	0.000	0.071	0.000
	22	0.000	0.086	0.000		22	0.000	0.071	0.014		2	0.529	0.329	0.343
	23	0.000	0.114	0.000		23	0.014	0.000	0.014		3	0.014	0.014	0.057
	/	/	/	/		24	0.029	0.000	0.000		4	0.286	0.214	0.200
	/	/	/	/		/	/	/	/		5	0.057	0.086	0.129
	/	/	/	/		/	/	/	/		6	0.114	0.129	0.171
	/	/	/	/		/	/	/	/		7	0.000	0.014	0.100
	/	/	/	/		/	/	/	/		8	0.000	0.100	0.000
	/	/	/	/		/	/	/	/		9	0.000	0.043	0.000

L= locus; Na= number of alleles; Shar = Sharplaninian; Ovch = Ovchepolian; Kara = Karakachanian.

Table 2a. Allele frequencies in analysed sheep populations

L	Na	Shar	Ovch	Kara	L	Na	Shar	Ovch	Kara	L	Na	Shar	Ovch	Kara
		35	35	35			35	35	35			35	35	35
BM8125	1	0.014	0.000	0.000	OARFCB193	1	0.014	0.000	0.000	OARFCB11	1	0.043	0.000	0.000
	2	0.043	0.000	0.000		2	0.000	0.000	0.043		2	0.100	0.000	0.014
	3	0.043	0.029	0.043		3	0.043	0.014	0.000		3	0.043	0.100	0.100
	4	0.400	0.200	0.329		4	0.057	0.043	0.171		4	0.257	0.100	0.186
	5	0.229	0.243	0.114		5	0.114	0.014	0.071		5	0.000	0.100	0.157
	6	0.186	0.329	0.371		6	0.000	0.014	0.043		6	0.029	0.029	0.043
	7	0.086	0.114	0.143		7	0.000	0.000	0.071		7	0.071	0.100	0.086
	8	0.000	0.043	0.000		8	0.000	0.014	0.014		8	0.000	0.000	0.043
	9	0.000	0.043	0.000		9	0.029	0.057	0.000		9	0.086	0.071	0.000
ILSTS11	1	0.029	0.000	0.029		10	0.257	0.086	0.000		10	0.243	0.057	0.071
	2	0.200	0.000	0.129		11	0.229	0.200	0.200		11	0.000	0.086	0.000
	3	0.057	0.000	0.300		12	0.086	0.229	0.129		12	0.057	0.057	0.114
	4	0.029	0.000	0.000		13	0.057	0.029	0.114		13	0.014	0.100	0.114
	5	0.271	0.000	0.000		14	0.057	0.071	0.071		14	0.029	0.057	0.043
	6	0.014	0.000	0.043		15	0.014	0.014	0.071		15	0.014	0.043	0.014
	7	0.029	0.000	0.129		16	0.014	0.071	0.000		16	0.014	0.014	0.014
	8	0.086	0.057	0.071		17	0.000	0.014	0.000		17	0.000	0.086	0.000
	9	0.114	0.243	0.086		18	0.029	0.014	0.000	OARFCB48	1	0.014	0.000	0.000
	10	0.171	0.014	0.000		19	0.000	0.029	0.000		2	0.000	0.071	0.000
	11	0.000	0.043	0.043		20	0.000	0.029	0.000		3	0.057	0.071	0.129
	12	0.000	0.100	0.057		21	0.000	0.014	0.000		4	0.043	0.171	0.171
	13	0.000	0.214	0.043		22	0.000	0.029	0.000		5	0.329	0.057	0.129
	14	0.000	0.329	0.071		23	0.000	0.014	0.000		6	0.286	0.186	0.029
SRCRSP1	1	0.000	0.043	0.000	SRCRSP3	1	0.000	0.029	0.000		7	0.029	0.100	0.029
	2	0.000	0.057	0.000		2	0.000	0.000	0.014		8	0.100	0.000	0.000
	3	0.000	0.029	0.014		3	0.000	0.014	0.000		9	0.071	0.143	0.057
	4	0.314	0.186	0.043		4	0.000	0.000	0.129		10	0.029	0.043	0.386
	5	0.414	0.200	0.029		5	0.000	0.000	0.029		11	0.014	0.071	0.000
	6	0.100	0.071	0.243		6	0.000	0.086	0.057		12	0.000	0.057	0.000
	7	0.071	0.071	0.014		7	0.029	0.043	0.014		13	0.014	0.014	0.043
	8	0.014	0.014	0.171		8	0.029	0.057	0.086		14	0.000	0.000	0.014
	9	0.043	0.129	0.000		9	0.143	0.000	0.100		15	0.000	0.014	0.014
	10	0.029	0.086	0.014		10	0.086	0.043	0.000		16	0.014	0.000	0.000
	11	0.014	0.086	0.129		11	0.043	0.043	0.157		/	/	/	/
	12	0.000	0.029	0.329		12	0.214	0.243	0.286		/	/	/	/
	13	0.000	0.000	0.014		13	0.314	0.286	0.100		/	/	/	/
	/	/	/	/		14	0.114	0.143	0.029		/	/	/	/
	/	/	/	/		15	0.000	0.014	0.000		/	/	/	/
	/	/	/	/		16	0.029	0.000	0.000		/	/	/	/

L= locus; Na= number of alleles; Shar = Sharplaninian; Ovch = Ovchepolian; Kara = Karakachanian.

Table 2b. Allele frequencies in analysed sheep populations

L	Na	Shar	Ovch	Kara	L	Na	Shar	Ovch	Kara	L	Na	Shar	Ovch	Kara
		35	35	35			35	35	35			35	35	35
ILSTS28	1	0.000	0.114	0.014	McM527	1	0.057	0.000	0.000	MAF214	1	0.000	0.014	0.000
	2	0.086	0.029	0.014		2	0.086	0.000	0.000		2	0.000	0.186	0.000
	3	0.014	0.000	0.000		3	0.086	0.000	0.000		3	0.000	0.014	0.000
	4	0.000	0.014	0.000		4	0.129	0.000	0.000		4	0.000	0.071	0.100
	5	0.014	0.000	0.000		5	0.043	0.043	0.000		5	0.000	0.129	0.171
	6	0.000	0.014	0.000		6	0.043	0.014	0.000		6	0.000	0.071	0.314
	7	0.000	0.157	0.014		7	0.029	0.029	0.000		7	0.000	0.114	0.000
	8	0.186	0.100	0.129		8	0.043	0.029	0.000		8	0.000	0.014	0.000
	9	0.029	0.000	0.000		9	0.086	0.000	0.000		9	0.014	0.043	0.000
	10	0.029	0.071	0.043		10	0.000	0.014	0.043		10	0.029	0.014	0.000
	11	0.000	0.071	0.029		11	0.129	0.000	0.000		11	0.257	0.043	0.000
	12	0.243	0.157	0.229		12	0.029	0.043	0.029		12	0.100	0.000	0.000
	13	0.029	0.071	0.029		13	0.071	0.000	0.000		13	0.057	0.000	0.000
	14	0.014	0.157	0.143		14	0.000	0.071	0.057		14	0.100	0.014	0.086
	15	0.100	0.043	0.143		15	0.014	0.029	0.000		15	0.129	0.043	0.143
	16	0.086	0.000	0.143		16	0.000	0.014	0.114		16	0.000	0.014	0.000
	17	0.114	0.000	0.071		17	0.029	0.071	0.043		17	0.014	0.100	0.000
	18	0.014	0.000	0.000		18	0.014	0.014	0.043		18	0.014	0.014	0.014
	19	0.043	0.000	0.000		19	0.043	0.043	0.014		19	0.000	0.000	0.171
ETH225	1	0.043	0.000	0.000		20	0.043	0.000	0.071		20	0.029	0.000	0.000
	2	0.100	0.000	0.014		21	0.000	0.000	0.171		21	0.057	0.000	0.000
	3	0.043	0.100	0.100		22	0.000	0.071	0.014		22	0.000	0.029	0.000
	4	0.257	0.100	0.186		23	0.014	0.014	0.071		23	0.029	0.029	0.000
	5	0.000	0.100	0.157		24	0.000	0.186	0.014		24	0.000	0.014	0.000
	6	0.200	0.143	0.329		25	0.014	0.000	0.071		25	0.000	0.029	0.000
	7	0.129	0.229	0.271		26	0.000	0.000	0.043		26	0.014	0.000	0.000
	8	0.186	0.129	0.100		27	0.000	0.057	0.029		27	0.014	0.000	0.000
	9	0.014	0.100	0.029		28	0.000	0.100	0.000		28	0.043	0.000	0.000
	10	0.000	0.000	0.014		29	0.000	0.000	0.071		29	0.029	0.000	0.000
	11	0.100	0.057	0.043		30	0.000	0.057	0.071		30	0.014	0.000	0.000
	12	0.000	0.043	0.014		31	0.000	0.100	0.014		31	0.014	0.000	0.000
	13	0.029	0.029	0.100		32	0.000	0.000	0.014		32	0.043	0.000	0.000

L= locus; Na= number of alleles; Shar = Sharplaninian; Ovch = Ovchepolian; Kara = Karakachanian.

Higher mean number of alleles than detected in this study are reported by Ghazy et al. (2013) in the genome of three Egyptian sheep breeds (the mean number of alleles per locus are 16.2, 15.3 and 15.8 in Sinai, Rahmani and Ossimi sheep breed, respectively). In addition, mean number of alleles is often related to presence of high level of heterozygoos individuals in analysed population. From the analysed loci, seven (ETH225, ILSTS11, MAF214, McM527, OARFCB193, OARFCB48 and OARJMP58) have shown differences in distribution of alleles with highest frequencies in analysed sheep population. Two out of three analysed sheep populations share the most frequent allele for the loci: BM8125 (Ovch and Kara), SRCRSP8 (Shar and Kara), SRCRSP 3 (Shar and Ovch), SRCRSP1 (Shar and Ovch) and ILSTS 28 (Shar and Kara). All three analysed sheep populations have at least one common allele with highest allele frequency for the OARFCB 11, SRCRSP7 and SRCRSP9 loci. The level of variation

depicted by the number of alleles at each locus serves as a measure of genetic variability having direct impact on differentiation of breeds within the populations (Arora & Bhatia, 2006).

CONCLUSIONS

High prevalence of traditional production system is present in sheep breeding in the country, pointing to free mating system that significantly affects the level of heterozygosity and contributes to loss of genetic diversity. This research is initial step in genetic characterization of autochthonous sheep populations in the country at DNA level. The gained results indicate that DNA microsatellite markers used in this study are highly informative and polymorphic in overall population and at individual population level. The data showed high values for observed alleles, high values for expected heterozygosity and PIC. Differentiation between analysed populations can be seen by differences between allele distribution and allele frequencies. Results from this research will contribute to better knowledge of the genome of studied sheep populations. This study also contributes to overall understanding of the genome of sheep populations that belong to Pramenka type and they enrich the database of original allele frequencies.

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