Application of 11 STR markers for the evaluation of genetic variation in sheep

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Summary

It is important to study and monitor changes in genetic structure of small old sheep breeds. Microsatellite markers are widely used for estimating genetic diversity within and differentiation among populations. For the study we used the following set of 11 polymorphic STR markers: CSRD247, ETH152, INRA005, INRA006, INRA063, INRA172, MAF065, MAF214, McM042, McM527, OarFCB20 and AMEL *locus*.

The objective of the research was to study the genetic structure of 3 breeds included in the sheep genetic resources conservation programme in Poland (Wielkopolska – 100, Old Type Polish Merino – 93 and Olkuska – 88 animals) and to determine genetic differentiation between them based on polymorphism of 11 STR markers.

Genetic analyses were performed in an ABI 3130xl sequencer and the results were analysed using GeneMapper Software 4.0. The identified alleles were used to estimate observed (Ho) and expected heterozygosity (H_E), polymorphic information content (PIC) and Nei's genetic distance D_N (1987).

There were identified 101 alleles in 11 microsatellites loci with a mean of 6.87 alleles per *locus*. The range of H_O and H_E was from 0.2903 to 0.8710 and 0.3307 to 0.8370, respectively. All the markers were highly polymorphic. PIC values for each marker were high and exceeded 0.5 except for INRA172 locus in Merino (PIC=0.3171) and ETH152 locus in Olkuska sheep (PIC=0.4781). The highest polymorphism was observed in INRA63 of Merino sheep where PIC and Ho were 0.8710 and 0.8181, respectively. The estimated coefficient of genetic distance, calculated based on all markers, was low and ranged from D_N=0.0836 between Merino and Wielkopolska to D_N=0.2187 between Merino and Olkuska sheep. It can be concluded that each breed became genetically distinct.

Introduction

Farm animal genetic resources should be conserved due to their current and potential economic, scientific and cultural significance. Native Polish sheep breeds are very well adapted to local environmental conditions, have low feeding requirements, and are highly resistant to disease and poor living conditions. In order to save these breeds from extinction and perpetuate valuable characteristics in the population, they have been included in the genetic resources conservation programme (Kawecka and Krupinski, 2014).

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Sheep parentage verification based on blood groups, which was carried out in Poland since 1973, was replaced with analysis of DNA microsatellite polymorphism in 2016. The application of microsatellite markers has increased from their discovery in the 1980s. Today they are widely used for individual identification, parentage testing, biodiversity assessment, and in forensics (Rychlik *et al.* 2003; Radko *et al.* 2006).

In order to standardize sheep parentage testing based on DNA microsatellite sequences, the International Society of Animal Genetics (ISAG) has recommended a panel of 13 markers: AMEL, CSRD247, ETH152, INRA005, INRA006, INRA023, INRA063, INRA172, MAF065, MAF214, McM042, McM527, OarFCB20.

The objective of the study was to evaluate the polymorphism of selected loci and their usefulness for testing genetic structure, and to determine differences between the populations of three sheep breeds covered by the genetic resources conservation programme.

Material and methods

The material of the study comprised genomic DNA isolated from whole blood of 281 sheep: Old-Type Polish Merino (93 head), Olkuska (88 head) and Wielkopolska (100 head).

Extraction of genomic DNA from blood was carried out using the Sherlock AX kit (A&A Biotechnology) according to the manufacturer's directions.

For analysis of DNA polymorphism, 12 microsatellite markers recommended by ISAG were chosen and two multiplex sets were determined:

- Multiplex I: AME, INRA63, INRA006, MAF214, McM042, CSRD247, INRA172;
- Multiplex II: MAF065, McM527, OarFCB20, ETH152, INRA005.

For the analysis of microsatellite sequences, PCR reaction was performed in a Master Mix reaction mixture (Qiagen). The amplification conditions consisted of initial denaturation at 95°C for 5 min, 28 cycles: 95°C for 30 s, 61°C (multiplex I) / 58°C (multiplex II) for 3 min, 72°C for 30 s, and elongation at 72°C for 45 min. The primers used for the amplification were fluorescently labelled with four different dyes (FAM, VIC, NED, PET), which allowed for the simultaneous detection of twelve microsatellite markers in one gel lane. The PCR products obtained were electrophoresed on denaturing 7% polyacrylamide gel in the presence of 500 LIZ size standard, using the 3130xl Genetic Analyzer (Applied Biosystems). The size of separated DNA fragments and the genotypes were determined with GeneMapper® Software 4.0 (Applied Biosystems). Statistical calculations were performed using IMGBOVSTAT – IZOO PIB software.

Results and discussion

In the analysed material collected from 281 sheep, 101 alleles at 11 microsatellite loci were detected. The breeds differed in the size range and frequency of identified alleles (Table 1).

The mean number of alleles per locus varied from 5.6 in Olkuska sheep to 7.8 in Wielkopolska sheep (Table 1). The greatest number of alleles (12) was obtained at locus INRA005 in the Wielkopolska breed, and the smallest at loci ETH 152 – 4 alleles in each breed under study and CSRD247 – 4 alleles in Olkuska sheep (Table 2). For

Table 1. Allele size ranges (bp) and number of alleles per locus (for 11 markers) in the studied breeds.

Locus	Breed					
	Merino		Olkuska		Wielkopolska	
	Range	Number	Range	Number	Range	Number
CSRD247	211-239	8	213-229	4	211-239	9
ETH152	186-192	4	186-192	4	186-192	4
INRA005	125-149	10	125-145	6	125-151	12
INRA006	110-134	7	110-134	6	110-134	6
INRA172	126-164	6	126-162	5	126-162	7
INRA63	169-197	10	169-197	7	169-201	11
MAF065	125-137	7	123-135	5	125-139	7
MAF214	187-263	7	187-261	7	187-261	8
McM042	81-97	5	81-107	5	81-103	7
McM527	164-174	6	164-174	5	164-176	7
OarFCB20	87-113	9	87-113	8	87-107	8
Mean		7.2		5.6		7.8

Table 2. Degree of observed (Ho) and expected heterozygosity (He), inbreeding coefficient (Fis), and polymorphic information content (PIC).

Locus	Breed											
	Merino				Olkuska				Wielkopolska			
	HO	HE	FIS	PIC	HO	HE	FIS	PIC	HO	HE	FIS	PIC
CSRD247	0.5161	0.5365	0.0379	0.5020	0.5795	0.5799	0.0006	0.5167	0.6800	0.6173	-0.1016	0.5924
ETH152	0.5591	0.6719	0.1679	0.6109	0.6591	0.5387	-0.2235	0.4781	0.5400	0.6034	0.1051	0.5428
INRA005	0.7742	0.8184	0.0540	0.7938	0.8523	0.7480	-0.1394	0.7146	0.7400	0.8244	0.1024	0.8035
INRA006	0.7419	0.7113	-0.0431	0.6747	0.3750	0.5378	0.3028	0.5132	0.7400	0.7087	-0.0442	0.6725
INRA172	0.2903	0.3307	0.1220	0.3171	0.6136	0.5874	-0.0447	0.5174	0.6700	0.6655	-0.0068	0.6308
INRA63	0.8710	0.8370	-0.0406	0.8181	0.7273	0.6708	-0.0841	0.6264	0.7300	0.7892	0.0750	0.7585
MAF065	0.7957	0.7183	-0.1078	0.6687	0.8409	0.7510	-0.1197	0.7050	0.7900	0.7613	-0.0378	0.7196
MAF214	0.6344	0.6586	0.0367	0.5937	0.7045	0.7159	0.0159	0.6818	0.7400	0.7565	0.0217	0.7168
McM042	0.4731	0.5808	0.1853	0.5192	0.6705	0.5768	-0.1624	0.5265	0.7100	0.7504	0.0538	0.7123
McM527	0.6344	0.7211	0.1202	0.6762	0.7273	0.6642	-0.0950	0.6073	0.7300	0.7548	0.0329	0.7170
OarFCB20	0.6774	0.7090	0.0446	0.6804	0.8295	0.7829	-0.0596	0.7532	0.7400	0.7554	0.0204	0.7225

Table 3. Nei's genetic distance for the sheep groups under study.

Breed	Merino	Olkuska	Wielkopolska
Merino	0.0000	0.2187	0.0836
Olkuska	0.2187	0.0000	0.1990
Wielkopolska	0.0836	0.1990	0.0000

the ETH152 marker in Balochi sheep, Wajid et al. (2014) obtained 3 alleles in the 168-219 bp range. Greater differences in the number of alleles were reported by Yilmaz *et al.* (2015), Radha *et al.* (2011) and Rendo *et al.* (2011), who obtained from 7 to 23 alleles in the 209-261 bp range for locus CSRD247 in sheep.

To evaluate the polymorphism of the analysed markers, the observed (H_O) and expected heterozygosity (H_E) as well as the coefficient of inbreeding (Fis) were calculated (Table 2). The H_O and H_E values for all the studied breeds were high (above 0.5161) except for INRA172 in Merino sheep (H_O=0.2903, H_E=0.3307) and INRA006 in Olkuska sheep (H_O=0.3750). The highest values were observed for INRA63 in the Merino breed (H_O=0.8710, H_E=0.8370). Similar levels of heterozygosity for this marker were obtained by Kawecka and Piórkowska (2011) in Podhale Zackel (H_O=0.774, H_E=0.824) and by Radha (2011) in Kilakarsal sheep (H_O=0.740, H_E=0.790), and lower levels by Kawecka and Piórkowska (2011) in Swiniarka sheep (H_O=0.472, H_E=0.544).

The coefficients of inbreeding obtained for the studied breeds were similar. Average positive F_{IS} values were observed in Wielkopolska (F_{IS}=0.0201) and Merino sheep (F_{IS}=0.0525), and an average negative value was found in Olkuska sheep (F_{IS}=-0.0554), which may be due to selection. In the case of some *loci*, H_O differed from H_E. The greatest differences were observed in Olkuska sheep; they ranged from -0.2235 at ETH152 to 0.3028 at INRA006. F_{IS} estimates obtained by other authors showed varying levels of inbreeding: F_{IS}=0.09 in Colombian sheep (Ocampo *et al.*, 2016), F_{IS}=0.137 in Turkish breeds (Yilmaz *et al.*, 2015), and F_{IS}=0.2954 in Indian Vembur sheep (Pramod *et al.*, 2009).

An important parameter showing whether markers are suitable for population studies and for parentage verification is polymorphic information content (PIC). The most extreme values were observed in Merino. The lowest PIC (0.3171) was noted for INRA172, and the highest (0.8181) for INRA63 (Table 2). A comparably high PIC value of 0.891 for locus INRA63 was reported by Radha *et al.* (2011).

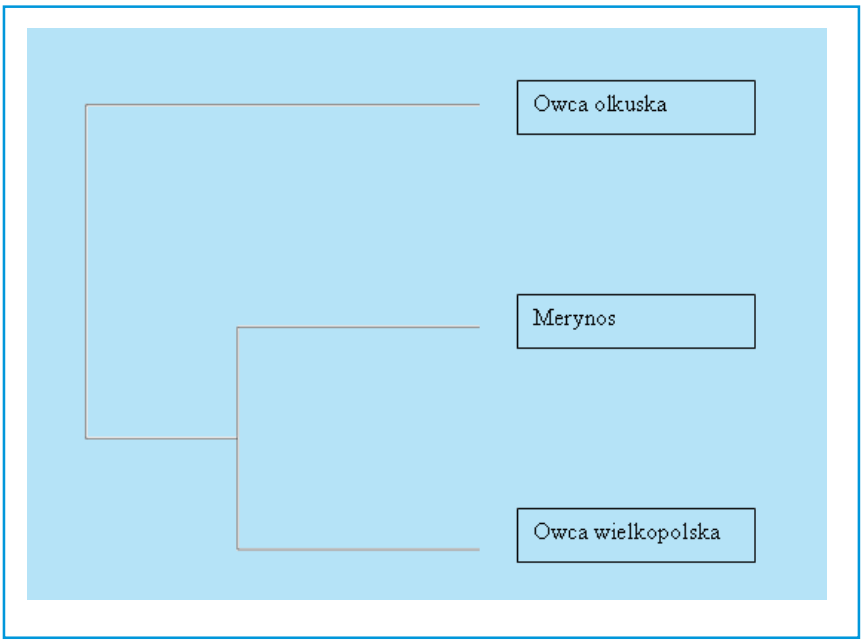

Figure 1. Dendrogram of genetic distance between the studied breeds.

Differences between the studied breeds were determined based on the values of Nei's genetic distance. They ranged from 0.0836 to 0.2187 (Table 3). Based on the genetic distances, unweighted pair group mean analysis (UPGMA) was performed to generate a dendrogram, which is a graphic representation of similarities between the studied breeds. Wielkopolska sheep was closely clustered with Merino and distantly clustered from Olkuska (Figure 1). This is supported by the historical origin of the Wielkopolska breed, because Merino sheep were used in composite crossing.

The results of the present study indicate that the tested set of 11 microsatellite markers can be used for evaluation of genetic structure and parentage testing in sheep.

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