

## **Characteristic of genetic structure of goats in Poland**

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**The genetic structure of goats raised in Poland was assessed using 6 microsatellite loci. Samples of blood obtained from six breeds of goats: Saanen (SA), Anglo-Nubian (AN), Alpine (AL), Carpathian (CR), White Improved (WI) and Pygmy (PG), were investigated. The analyzed microsatellite sequences were chosen from a set of markers recommended by the Food and Agriculture Organization (FAO) for biodiversity studies. A total of 47 polymorphic variants at 6 loci were identified in the Polish goat populations studied. The highest mean number of alleles was identified for the Saanen breed (5.7) and the lowest for the Pygmy goat (4.33). The highest heterozygosity was characteristic of the White Improved breed (0.77). Saanen and White Improved breeds were the most similar in terms of the genetic structure. High genetic similarity was also found between the native Carpathian and Alpine goats. The Anglo-Nubian and Pygmy breeds were the most distant.**

**KEY WORDS: goats, microsatellite sequences, genetic structure**

Goat breeding in Poland has a long tradition but is still peripheral to livestock production. The population of goats in Poland is estimated to be around 119,000. The largest proportion is made up of White Improved goats, which originate from local, Saanen and White German Improved goats. A smaller group includes Fawn Improved goats, which originate from German Fawn, and Alpine goats, as well as imported breeds: Saanen, Alpine, Anglo-Nubian and Boer. There is also a large group of general-purpose scrub goats, which vary considerably in appearance and production traits, but are exceptionally resistant to adverse environmental conditions. The present goat population in Poland was influenced by the old local general-purpose breeds Carpathian, Sandomierska and Kazimierzowska. The native breeds Sandomierska and Kazimierzowska are extant, but efforts at restoring the Carpathian breed were successful and now a small population of these goats is raised in the Sub-Carpathian area.

Goats are most often kept in backyard systems, less frequently in large specialized herds and for hobby purposes. Pygmy goats, which are considered to represent feral goats, are raised in zoological gardens.

Studies on the genetic diversity of goats are not as extensive as in cattle or sheep. With advances in molecular techniques, this area of research attracts increasing attention also among goat researchers. Many research centres in the world use microsatellite markers to estimate genetic variation in farm animals. Such activities are also foreseen in the Global Plan of Action for Animal Genetic Resources, initiated by the *Food and Agriculture Organization (FAO)*. The FAO's project for Measurement of Domestic Animal Diversity (MoDAD) recommends a set of markers suitable for analyzing the genetic structure of farm animals, including goats [3]. Genetic variation is estimated based on frequency of alleles at microsatellite loci.

Microsatellite markers are used to estimate the inbreeding of goat populations, to verify parentages [7], to evaluate genetic distance between breeds [6, 10], as well as in phylogenetic studies [4].

The aim of the study was to characterize the genetic structure of goats raised in Poland using microsatellite markers recommended by FAO for biodiversity studies in goats.

### Material and methods

The material used in this study consisted of samples of blood, randomly collected from 215 goats of the following breeds: White Improved (n=44), Saanen (n=54), Alpine (n=46), Anglo-Nubian (n=26), Carpathian (n=20) and Pygmy feral goats (n=25) (Tab. 1).

**Table 1 – Tabela 1**

Breeds of goats used in the study

Rasy kóz w doświadczeniu

Breed Rasa	Symbol Oznaczenie	Size of group Liczebność grupy	Productive type Typ użytkowy
Saanen Saaneńska	SA	54	dairy mleczny
Alpine Alpejska	AL	46	dairy mleczny
Anglo-Nubian Anglo-nubijska	AN	26	general purpose ogólnoużytkowy
Carpathian Karpacka	KR	20	general purpose ogólnoużytkowy
Pygmy Kozka karłowata	KK	25	feral koza dzika
White Improved Biała uszlachetniona	KB	44	dairy mleczny

DNA was isolated using the DNA Wizard® Genomic DNA Purification Kit. The polymorphism of microsatellite sequences was analyzed using selected markers recommended for goat biodiversity studies by the Food and Agriculture Organization: CSR0247, ILSTS0087, INRA0023, McM0527, OarFCB0020, and SRCRSP0023 [3]. Primer sequences labelled with “Well Red” fluorescent markers were selected for each microsatellite marker. Simultaneous multiplex DNA amplification was possible because of similar annealing

temperature in all marker loci. The reaction was performed in 10 µl volume. The reaction mixture contained: 3mM MgCl<sub>2</sub>, 0.4 mM DTP, 1 ml buffer (10x), 0.15 mM CSR0247, 0.08 mM ILSTS0087, 1.2 mM INRA0023, 0.1 mM McM0527, 0.25 mM OarFCB0020, 0.25 mM SRCRSP0023, 100 ng DNA and 2U AmplitaqGold polymerase. The amplification conditions for selected loci were as follows: initial denaturation at 95°C for 10 min., 31 cycles: at 94°C for 30 s, at 55°C for 30 s and at 72°C for 1 min, elongation at 72°C for 5 min. To verify size, the fragments obtained were separated in denaturing polyacrylamide gel using capillary electrophoresis on a CEQ<sup>TM</sup>8000 Genetic Analysis System (Beckman Coulter). To visualize the genetic structure of the breeds studied, the results were analyzed using the POPGENE statistical package (ver. 3.2). Allele frequency was used to calculate genetic variation parameters: observed (Ho) and expected (He) heterozygosity, the observed (No) and effective number (Ne) of alleles, and the polymorphic information content (PIC).

## **Results and discussion**

As a result of separation of amplification products in the analyzed microsatellite sequences, 47 polymorphic variants were identified at all loci. The number of alleles for individual loci ranged from 6 (OarFCB0020, ILSTS0087 and McM0527) to 10 (SRCRSP0023 and CSR0247). The most frequent alleles were 156 bp at locus McM0527, 231 bp at CSR0247, 151 bp at ILSTS0087, 213 bp at INRA0023, 101 bp at OarFCB0020 and 105 bp at SRCRSP0023 (Tab. 2).

The microsatellite sequences used in the study were characterized by high polymorphism. The polymorphic information content (PIC) exceeded 0.5 for all loci (Tab. 3).

The highest mean number of alleles was observed in Saanen goats (5.7) and the lowest in Pygmy goats (4.33). In Carpathian and Anglo-Nubian goats, it was 4.7 and 4.8, respectively (Tab. 3). A Brazilian study [9] with a much greater pool of markers found a high number of alleles in dairy populations of the imported breeds Alpine, Toggenburg and Saanen, which were much lower than in Anglo-Nubian goats and native breeds of goats. Similar studies to characterize the structure of native goat populations were conducted in China [6] and India [4], where the mean number of alleles in these populations was 6.9 and 5.4, respectively.

The Polish Fawn goat was included among 45 European breeds of goats, whose genetic structure was analyzed by Canon et al. [2]. Their research is part of a large EU-funded ECONOGENE project (<http://www.econogene.eu>), which aims at increase of the existing knowledge of biodiversity of sheep and goats from Europe and the Central East, using molecular biology techniques with regard to socioeconomic conditions in the regions in which these animals are raised. In the project cited above [2], the mean number of alleles ranged from 5.2 to 8.3 according to the breed and was 6.9 in Polish Fawn goats.

To compare the degree of variation in microsatellite sequences of the analyzed breeds, we estimated the effective number of alleles for each locus (Ne). This parameter varied according to the group, with the highest value found for Saanen goats (3.83) and the lowest for Anglo-Nubian goats (2.5) (Table 2). The effective number of alleles was much lower

**Table 2 – Tabela 2**  
Allele frequency  
Frekwencja alleli

Breed Rasa	SA	AL	AN	KR	KB	Breed Rasa	SA	AL	AN	KR	KB
<b>McM0527</b>											
allele	0.500	0.435	0.673	0.76	0.036	allele	0.185	0.054	0.019	0.16	0.022
156	0.027	0.304			0.433	137	0.028	0.011	0.115	0.2	0.217
158	0.129		0.154	0.02	0.161	143	0.176	0.326	0.25	0.161	0.036
166		0.087			0.178	147	0.25	0.228	0.071	0.48	0.161
168	0.018	0.315	0.038	0.22	0.464	149	0.361	0.38	0.143	0.16	0.321
170	0.324		0.135		0.155	151					0.242
172											
<b>OarFCB0020</b>											
97	0.037	0.174	0.173	0.32	0.036	197	0.167		0.06	0.054	0.067
99	0.083	0.228	0.538	0.18	0.242	199	0.078	0.261	0.54	0.143	0.146
101	0.657	0.565	0.288	0.48	0.518	201	0.102	0.154	0.211	0.047	0.047
103	0.028	0.033			0.298	203			0.06	0.031	0.031
107	0.194			0.02	0.446	207	0.157	0.038	0.06	0.161	0.105
109					0.058	211	0.129	0.217	0.135	0.178	0.118
						213	0.333	0.163	0.442	0.2	0.34
						215	0.093	0.359	0.019	0.04	0.143
<b>CSRD0247</b>											
217	0.065		0.058		0.161	83			0.24	0.027	0.027
229	0.204	0.228	0.077		0.5	85	0.083	0.033	0.16	0.071	0.078
231	0.454	0.272	0.115	0.26		91		0.652		0.013	0.013
233		0.098		0.16		95	0.148	0.119	0.019	0.083	0.083
237			0.269		0.054	97	0.028	0.942	0.16	0.15	0.15
239	0.278	0.076	0.019		0.286	99	0.185	0.435	0.019	0.121	0.121
241		0.261	0.461	0.56		101	0.241	0.087	0.019	0.17	0.17
243		0.065			0.049	103	0.138	0.119	0.14	0.078	0.078
						105	0.120	0.446	0.14	0.202	0.202
						107			0.16	0.018	0.018
						111	0.055	0.054		0.214	0.214
					0.033	115		0.033		0.052	0.052
										0.007	0.007

than the observed number, which shows that a small number of alleles contributed to the genetic structure and the occurrence of many rare alleles in the population.

The highest heterozygosity was found for White Improved goats (0.77) and high values were obtained for Saanen and Alpine goats (0.71) (Table 3). Similar  $H_o$  values for these dairy breeds were found by Araujo et al. [1] and Iamartino et al. [5]. Oliveira et al. [9] reported that the observed heterozygosity in Saanen, Alpine and Anglo-Nubian goats was much lower (0.4). A Swiss study [10] found a 3-fold higher heterozygosity for domesticated breeds compared to Bezoar and Ibex feral breeds. A large-scale study [2] showed higher heterozygosity for the Middle Eastern populations of goats compared to European goats.

**Table 3 – Tabela 3**

Number of alleles (No), effective number of alleles (Ne), observed ( $H_o$ ) and expected heterozygosity (He), and polymorphic information content (PIC) for different breeds.

Liczba alleli (No), efektywna liczba alleli (Ne), heterozygotyczność obserwowana ( $H_o$ ) i oczekiwana (He), indeks stopnia polimorfizmu (PIC) dla poszczególnych ras.

Breed Rasa	No	Ne	$H_o$	He	PIC
SA	5.7	3.83	0.713	0.722	0.679
AL	5.5	3.56	0.710	0.729	0.678
AN	4.8	2.5	0.506	0.547	0.509
KR	4.7	3.3	0.667	0.657	0.599
KK	4.33	3.0	0.631	0.657	0.598
KB	5.16	3.74	0.776	0.729	0.679

The low value of the mean effective number of alleles and heterozygosity, observed in Anglo-Nubian goats, indicates a relatively low level of genetic variation in this breed compared to the other breeds.

Microsatellite markers have found wide application for analysis of evolutionary relationships between different breeds of goats. Genetic variability of a population can be determined from the frequency of alleles in microsatellite loci by estimating genetic distance. It reflects the degree of similarity between the analyzed breeds and serves to construct phylogenetic trees. Saitbekova et al. [10], who used microsatellite markers to illustrate the genetic distance between eight breeds of Swiss goats as well as Creole, Bezoar and Ibex goats, showed that Swiss goats are closely related but completely different from the other populations. Oliveira et al. [9], who studied the relationships between goat populations raised in Brazil, found three genetically distant groups of native Brazilian breeds (Moxoto, Caninde), imported breeds (Alpine, Saanen, Toggenburg and Anglo-Nubian) and local scrub goats. In the group of imported goats, the highest similarity was found between Saanen and Alpine breeds. A similar relationship between these two dairy breeds was reported by Araujo et al. [1], possibly suggesting that these two phenotypically different breeds share common ancestry.

Genetic variability between the breeds is shown in Table 4. The low genetic distance was noted for the Saanen and Alpine breeds, as did the authors cited above [1, 9], who observed these breeds to be similar. High genetic similarity was found for White Improved

**Table 4 – Tabela 4**

Genetic similarity (above diagonal) and genetic distance (below diagonal) [8]

Podobieństwo genetyczne (powyżej przekątnej) i dystans genetyczny (poniżej przekątnej) [8]

Breed Rasa	SA	AL	AN	KR	KK	KB
SA	****	0.7410	0.4949	0.5714	0.5512	0.7455
AL	0.2997	****	0.4975	0.7094	0.5166	0.5946
AN	0.7034	0.6981	****	0.6637	0.2376	0.5965
KR	0.5598	0.3433	0.4099	****	0.3994	0.5673
KK	0.5956	0.6604	1.4371	0.9177	****	0.4843
KB	0.2937	0.5198	0.5167	0.5668	0.7252	****

  genetic similarity – podobieństwo genetyczne  
  genetic distance – dystans genetyczny

and Saanen goats, which confirms the obviously close relationship between these breeds. The highest genetic distance was noted for the Anglo-Nubian and Dwarf breeds.

The results obtained reflect genetic variation of goats in Poland. However, it seems appropriate to extend this type of research with a greater number of microsatellite markers, which are a useful tool for analyzing genetic structure and comparing variation between different breeds of goats.

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## Charakterystyka struktury genetycznej kóz w Polsce

### Streszczenie

W przeprowadzonych badaniach podjęto próbę charakterystyki struktury genetycznej kóz występujących w Polsce z wykorzystaniem sekwencji mikrosatelitarnych. Materiał do badań stanowiły próbki krwi uzyskane od kóz sześciu ras: saaeńskiej, anglo-nubijskiej, alpejskiej, karpackiej, białej uszlachetnionej i kózki karłowatej. Sekwencje mikrosatelitarne wykorzystane w badaniach wybrano opierając się na markerach zalecanych przez FAO do oceny bioróżnorodności. U przebadanych populacji kóz występujących w Polsce zidentyfikowano 47 wariantów polimorficznych w 6 *loci*. Najwyższą średnią liczbę alleli zidentyfikowano w rasie saaeńskiej i białej uszlachetnionej – wynosiła ona 5,7, a najniższą u kózki karłowatej (4,1). Najwyższą heterozygotycznością charakteryzowała się rasa biała uszlachetniona (0,77). Pod względem struktury genetycznej określonej z wykorzystaniem 6 *loci* mikrosatelitarnych najbardziej zbliżone do siebie były rasy saaeńska i alpejska. Natomiast najwyższą wartość dystansu stwierdzono między rasą anglonubijską a kózką karłowatą.

**SŁOWA KLUCZOWE:** kozy / sekwencje mikrosatelitarne / struktura genetyczna