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GENETIC STRUCTURE OF RECONSTITUTED NATIVE CARPATHIAN GOAT BREED BASED ON INFORMATION FROM MICROSATELLITE MARKERS*

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Abstract

The aim of the study was to evaluate the genetic structure of the reconstituted native Carpathian goat breed based on information from microsatellite markers. The study analysed 14 microsatellite markers recommended by the International Society for Animal Genetics (ISAG) for goats individual identification and parentage testing. Blood samples were taken from 249 Carpathian goats from 14 farms. All microsatellite markers employed in this analysis showed sufficient polymorphism to assess genetic variation in Carpathian goats and the ISAG-recommended panel for goat individual identification and parentage testing is a highly useful one. The present study showed the status of the genetic structure of the reconstituted population of Carpathian goats. Carpathian goats maintained in Poland were characterized by relatively high genetic diversity (the average of alleles per locus was 9.143), high values of heterozygosity and a low level of inbreeding coefficient. The obtained parameters indicate the correctness of the breeding activities carried out within the framework of the programme for the protection of genetic resources and give guidelines for taking further steps related to the breeding of this valuable native breed.

Key words: Carpathian goat, native breed, genetic structure, microsatellite markers

Native breeds of livestock, despite their unique features and adaptability to harsh environmental conditions, are at risk of declining in numbers and subsequent extinction, due to the low profitability of breeding. Such a fate has been suffered by many global livestock breeds, some becoming irreversibly extinct; according to FAO (2019), cattle and sheep are the species with the highest number of breeds considered extinct. In the case of goats, the aforementioned report found that out of 687 identified goat breeds worldwide, the endangered status of more than 60% of them was set as unknown; only 73 breeds were considered non-endangered, and more than 130 breeds had a critical or endangered status, and 21 breeds were recorded as extinct.

Goats have been kept in Poland for centuries, particularly on small farms; their main advantages included low nutritional requirements, early breeding maturity, good prolificacy and, above all, the possibility of obtaining a range of products such as milk and meat or leather. In the 20th century, the population of goats in Poland numbered around 300 000 livestock population, and there were local breeds such as Pokucka, Carpathian, Sandomierska, Kazimierzowska and Silesian goats. In the post-war period, the emerging population was based on imported breeds, and the growing interest in dairy products meant that, for a long period, the majority of the herd consisted of animals that were kept for dairy purposes only; in the 1990s these included imported breeds such as the Saanen goat, Alpine goat, as well as improved domestic stock: improved white goat and improved coloured goat. The native goat breeds previously present in Poland were gradually displaced.

Actions taken in many countries aimed at the broadly understood protection of biodiversity led to attempts to restore old, forgotten Polish goat breeds in the first decade of the 21st century. The restoration initiative was successful concerning three goat breeds, of which the Carpathian breed, typical for mountain and foothill regions, appears to be the most promising for goat breeding in Poland (Sikora et al., 2020). Currently, it accounts for 50% of the goat population (Kawęcka et al., 2021) of the active goat population.

Carpathian goats have a long white coat, are horned, and often have a fringe over their eyes; bucks have large

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spreading horns with a spiral twist, as well as a very abundant beard and mane (Figure 1). The Carpathian breed of goats is distinctive for its autochthonous populations; their features include good health, longevity, excellent adaptation to difficult environmental conditions, and indiscriminate choice of fodder. These goats show good breeding characteristics, and their annual milk yield is about 500 kg per year (Sikora and Kawęcka, 2017). At the beginning of 2005, the Carpathian goat-type specimens were found in Poland. The stud bucks and goats with their offspring were placed at the Odrzechowa breeding farm belonging to the National Research Institute of Animal Production. The herd was systematically enlarged and at the same time, other new herds were raised. In subsequent years, activities were taken to open herd registers for the breed and to include the breed under the genetic resources protection programme. Interest in breeding and financial support under the Rural Development Programme resulted in the current population of over 300 does and 30 bucks kept in 21 herds. The dynamically growing population of Carpathian goats is an extremely valuable element of biodiversity and a potential for developing the local market for dairy goat products.

The aim of the study was to evaluate the genetic structure of the reconstituted Carpathian goat breed based on information from microsatellite markers and to assess the usefulness of applying this tool for the individual identification and parentage testing of goats.

Material and methods

Samples collection and microsatellite genotyping

The experimental conditions in the present study were approved by the Local Ethics Committee (No. 267/2019). Blood samples were taken from 249 Carpathian goats from 14 farms located in the foothills of southern Poland (Figure 2) with the distribution of the individuals (in brackets): 1. Bolechowice (6), 2. Bóbrka (7), 3. Odrzechowa (38), 4. Szlembark (14), 5. Łąki Kozielskie (19), 6. Żernica (18), 7. Kluszkowce (32), 8. Nowa Biała (19), 9. Pisarzowice (12), 10. Mogilno (7), 11. Kobylec (13), 12. Poronin-1 (8), 13. Poronin-2 (9) and 14. Bańska Niżna (47). DNA was extracted using QuickGene DNA whole blood kit S (DB-S) following the suggested protocols of the manufacturer. Genomic DNA was used as the template for multiplex PCR. DNA concentrations were assessed using a NanoDrop™ 2000 Spectrophotometer (Thermo Fisher).

This study analysed 14 microsatellite markers recommended by the International Society for Animal Genetics (ISAG) for goats individual identification and parentage testing: CSRD247, ILSTS008, ILSTS19, ILSTS87, INRA005, INRA006, INRA023, INRA063, MAF65, McM527, OarFCB20, SRCRSP23, SRCRSP5, SRCRSP8 (Table 1). The multiplex PCR reaction was prepared and optimized for amplification and was performed using SimplyAmp Thermo Cycler (Applied Biosystems, Foster City, CA, USA).

The volume of each component, primers concentration and temperature conditions were selected experimentally. The master mix mixture contained the following components: 11.2 µl Type-it Microsatellite PCR Kit (QIAGEN, GmbH, Hilden, Germany) and 1.2 µl primer mix. 1 µl DNA about the concentration of 40 ng/µl was added to each of the samples. The primer mix was prepared by the 100 uM primers synthesized by Thermo Fischer, which was diluted by nuclease-free water diethylpyrocarbonate (DEPC-treated water) (Applied Biosystems, Foster City, CA, USA) to the appropriate concentrations, determined experimentally. The forward primer from each pair was labelled with fluorescent dye, respectively 6-FAM, VIC, NED, PET (Table 1). PCR conditions for all reactions entailed an initial denaturation of 95°C for 5 min, followed by 30 cycles of 95°C 30 s, 60°C 90 s and 72°C 30 s, extension step was followed at 60°C for 30 min. Capillary electrophoresis was performed using 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). For each sample was measured 11 µl formamide, 0.4 µl GeneScan™ 500 LIZ™ dye Size Standard (Applied Biosystems, Foster City, CA, USA) and 1 ul of PCR product and then was denatured for 5 min at 95°C. The results of capillary electrophoresis were analysed using GeneMapper v. 4.0 Software (Applied Biosystems, Foster City, CA, USA).



Figure 1. Goat and buck of Carpathian breed (photo M. Pasternak, M. Brejta)

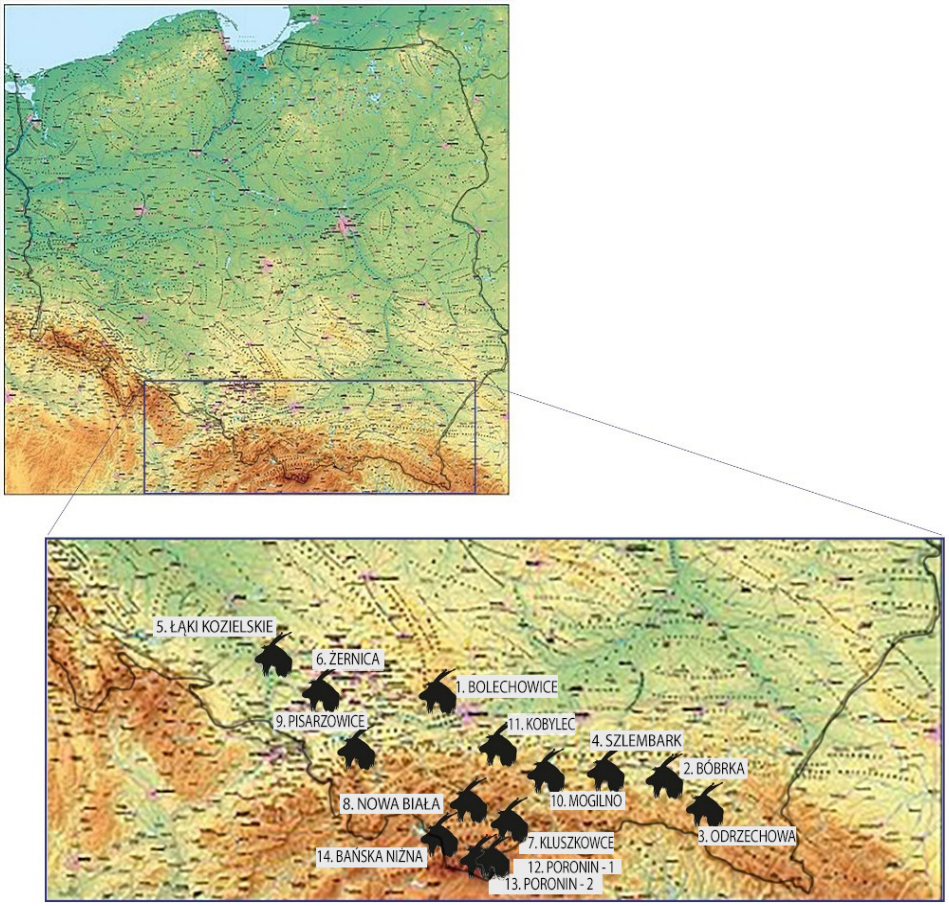


Figure 2. Location of herds of Carpathian goats

Population structure analysis

The genetic structure of the studied population was investigated using the STRUCTURE software (Pritchard et al., 2000). The group of 249 Carpathian goat individuals was divided into 14 subpopulations according to their place of occurrence. The analysis was performed with a burn-in period of 100,000 and 200,000 iterations fitting K from 1 to 20 with 10 runs for each K. To select the best K, the STRUCTURE HARVESTER was used (Earl and Von Holdt, 2012) and to graphically visualize the best K the CLUMPAK program was used (Kopelman et al., 2015).

Statistical analysis

The obtained results for each marker were analysed statistically. The number of different alleles (Na), number of effective alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He) and inbreeding coefficient (F) and genetic distance for each subpopulation were calculated using GenAlEx 6.5 (Peakall and Smouse, 2006, 2012). The genetic distance dendrogram was constructed using the unweighted pair group method with arithmetic mean (UPGMA) (Garcia-Vallvé et al., 1999). The tree was manipulated by iTOL v5 (Letunic and Bork, 2021).

Then, the values of genetic diversity of all individuals of Carpathian goat maintained in Poland were estimated. The number of alleles per microsatellite (Na), number of effective alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), a deficit of heterozygotes (P), Hardy–Weinberg equilibrium (HWE), the inbreeding coefficient within individuals relative to the subpopulation (Fis), the inbreeding coefficient within individuals relative to the total (Fit), the inbreeding coefficient within subpopulations relative to the total (Fst) were calculated using GenAlEx 6.5 (Peakall and Smouse, 2006, 2012). The parentage testing parameters: polymorphism information content (PIC), the null allele frequencies (Fnull), non-exclusion first parent (NE-1P), non-exclusion second parent (NE-2P), non-exclusion parent pair (NE-PP), non-exclusion identity (NE-I), non-exclusion sibling (NE-SI), combined exclusion probability – first parent (CE-1P), combined exclusion probability – second parent (CE-2P), combined exclusion probability – parent pair (CE-PP), combined exclusion probability – identity (CE-I), combined exclusion probability – sib identity (CE-SI) were calculated by CERVUS 3.0.7 (Kalinowski et al., 2007).

Table 1. Characteristic of 14 microsatellite loci included in the multiplex PCR reactions

Locus	Primer (5' -3') forward	Primer (5' -3') forward	Size range (bp)	Dye
CSRD247	GGACTTGCCAGAACTCTGCAAT	CACTGTGGTTTGTATTAGTCAGG	215–250	VIC
ILSTS008	GAATCATGGATTTTCTGGGG	TAGCAGTGAGTGAGGTTGGC	170–195	NED
ILSTS19	AGGGACCTCATGTAGAAGC	ACTTTTGGACCCTGTAGTGC	141–160	NED
ILSTS87	AGCAGACATGATGACTCAGC	CTGCCTCTTTTCTTGAGAGC	130–160	6-FAM
INRA005	TTCAGGCATACCCTACACCACATG	AAATATTAGCCAACTGAAAACTGGG	110–130	PET
INRA006	AGGAATATCTGTATCAACCGCAGTC	CTGAGCTGGGGTGGGAGCTATAAATA	95–140	NED
INRA023	GAGTAGAGCTACAAGATAAACTTC	TAACTACAGGGTGTTAGATGAACTC	195–220	PET
INRA063	GACCACAAAGGGATTTGCACAAGC	AAACCACAGAAATGCTTGGAAG	170–185	6-FAM
MAF65	AAAGGCCAGAGTATGCAATTAGGAG	CCACTCCTCCTGAGAATATAACATG	116–150	VIC
McM527	GTCCATTGCCTCAAATCAATTC	AAACCACTTGACTACTCCCCAA	155–175	VIC
OarFCB20	GGAAAACCCCCATATATACCTATAC	AAATGTGTTTAAGATTCCATACATGTG	90–115	VIC
SRCRSP23	TGAACGGGTAAAGATGTG	TGTTTTTAATGGCTGAGTAG	75–110	6-FAM
SRCRSP5	GGACTCTACCAACTGAGCTACAAG	TGAAATGAAGCTAAAGCAATGC	160–190	PET
SRCRSP8	TGCGGTCTGGTTCTGATTTCAC	CCTGCATGAGAAAGTCGATGCTTAG	200–295	6-FAM

Results

Genetic diversity across the studied microsatellite loci

Table 2 shows the genetic diversity estimates across 14 polymorphic loci. A total of 128 alleles were found for the microsatellites analysed. The overall mean of the observed number of alleles was 9.143, while the mean effective number 3.798 of alleles (Ne) was lower. The most polymorphic marker turned out to be MAF65 with 13 alleles per locus, in contrast to the INRA005 with 5 alleles per locus. The mean value of observed heterozygosity was 0.671 with the highest value obtained for SRCRSP23 (0.847) and the lowest for INRA063 (0.382) marker, for which also the lowest value Ne was found (1.636). Similar results were obtained for the expected heterozygosity, the average of which was 0.701. Ten out of 14 loci showed significant deviations from HWE. The indicators included in the F-statistics parameters showed the mean value of Fis with a negative value of -0.047, Fit at 0.045. A relatively high level of genetic differentiation was found between the subpopulations under study (mean Fst=0.088). Among the loci analysed, MAF65 locus was the most differentiating (Fst=0.122). The least differentiation was found at ILSTS008 locus (Fst=0.060) (Table 2).

Table 2. Genetic diversity and F-statistics parameters across 14 microsatellite markers

Locus	Na	Ne	Ho	He	P	HWE	Fis	Fit	Fst
SRCRSP23	12	7.027	0.847	0.858	0.010	**	–0.089	–0.006	0.076
ILSTS87	11	4.071	0.751	0.754	0.977	ns	–0.093	–0.001	0.084
INRA063	6	1.636	0.382	0.389	0.000	***	–0.050	0.017	0.065
SRCRSP8	10	3.117	0.614	0.679	0.000	***	–0.018	0.090	0.106
OarFCB20	7	3.194	0.679	0.687	0.479	ns	–0.004	0.057	0.061
MAF65	13	3.762	0.667	0.734	0.000	***	–0.032	0.094	0.122
MCM527	7	2.894	0.610	0.654	0.076	ns	0.028	0.131	0.106
CSRD247	10	4.015	0.747	0.751	0.003	**	–0.111	–0.007	0.094
INRA006	12	6.179	0.779	0.838	0.000	***	–0.061	0.065	0.118
ILSTS19	8	4.111	0.719	0.757	0.001	***	–0.036	0.062	0.095
ILSTS008	7	2.297	0.590	0.565	0.013	*	–0.172	–0.101	0.060
INRA005	5	2.992	0.622	0.666	0.142	ns	0.016	0.094	0.079
SRCRSP5	9	4.236	0.695	0.764	0.000	***	0.003	0.093	0.090
INRA023	11	3.640	0.691	0.725	0.000	***	–0.036	0.048	0.081
Mean	9.143	3.798	0.671	0.701			–0.047	0.045	0.088
Total	128								

Key: ns=not significant, *P<0.05, **P<0.01, ***P<0.001; Na, number of different alleles; Ne, number of effective alleles; Ho, observed heterozygosity; He, expected heterozygosity; P, a deficit of heterozygotes; HWE, Hardy–Weinberg equilibrium; Fis, the inbreeding coefficient within individuals relative to the subpopulation; Fit, the inbreeding coefficient within individuals relative to the total; Fst, the inbreeding coefficient within subpopulations relative to the total.

Table 3. Parentage testing of the markers across 249 individual Carpathian goat samples

Locus	PIC	NE-1P	NE-2P	NE-PP	NE-I	NE-SI	F(Null)
SRCRSP23	0.842	0.448	0.286	0.121	0.036	0.330	0.0050
ILSTS87	0.713	0.648	0.472	0.289	0.101	0.398	0.0039
INRA063	0.365	0.921	0.782	0.635	0.397	0.655	0.0337
SRCRSP8	0.630	0.725	0.557	0.368	0.152	0.449	0.0481
OarFCB20	0.633	0.732	0.566	0.388	0.152	0.445	0.0011
MAF65	0.709	0.642	0.457	0.251	0.096	0.407	0.0580
MCM527	0.615	0.748	0.572	0.378	0.159	0.463	0.0412
CSRD247	0.713	0.645	0.468	0.280	0.100	0.400	–0.0008
INRA006	0.818	0.490	0.321	0.148	0.046	0.342	0.0380
ILSTS19	0.722	0.634	0.455	0.265	0.094	0.395	0.0285
ILSTS008	0.505	0.828	0.683	0.518	0.250	0.530	–0.0361
INRA005	0.606	0.758	0.598	0.428	0.172	0.460	0.0300
SRCRSP5	0.727	0.632	0.454	0.270	0.093	0.391	0.0490
INRA023	0.692	0.661	0.480	0.280	0.108	0.414	0.0253
Mean	0.664	0.679	0.511	0.330	0.140	0.434	0.023
CE-1P	0.9964271						
CE-2P	0.99994721						
CE-PP	0.99999994						
CE-I	0.99999999						
CE-SI	0.99999309						

PIC, polymorphic information content; NE-1P, non-exclusion first parent; NE-2P, non-exclusion second parent; NE-PP, non-exclusion parent pair; NE-I, non-exclusion identity; NE-SI, non-exclusion sibling; F(null), estimated frequency of null allele; CE-1P, combined exclusion probability – first parent; CE-2P, combined exclusion probability – second parent; CE-PP, combined exclusion probability – parent pair; CE-I, combined exclusion probability – identity; CE-SI, combined exclusion probability – sib identity.

The average value of PIC was 0.664 (Table 3), where the most useful marker turned out to be SRCRSP23 (0.842), and the least of INRA063 (0.365). This result is so low that it would be necessary to consider replacing this marker with another marker in the tests concerning parentage testing of Carpathian goats. The null allele frequency was not high and was in the range of –0.0008 for the CSRD247 marker and 0.0580 for the MAF65 marker. The probability of excluding an individual after one and the other parent, as well as the parent pair, was estimated at 99.64%, 99.99% and 99.99999%, respectively.

Genetic diversity within population

Genetic diversity parameters for each subpopulation are summarized in Table 4. The largest number of different and effective alleles was estimated for the subpopulation in Bańska Niżna, and the smallest for Bóbrka. The highest value of observed and expected heterozygosity was obtained in the Poronin-2, while the lowest in the subpopulation Bóbrka. In the analysed herds, the studied parameters were differentiated. The mean number of alleles in the locus ranged from 3 in the subpopulations from Bolechowice to 8.214 in the subpopulation in Bańska Niżna. The values of observed and expected heterozygosity ranged from the lowest (Ho = 0.439 and He = 0.467, respectively) in the Bóbrka herd, to the highest in the Poronin-2 herd (Ho = 0.802 and He = 0.743). The coefficient of inbreeding (Fis) ranged from −0.166 in Szlembark to 0.046 in Bóbrka, remaining relatively low and not indicative of inbreeding in subpopulations.

Table 4. Characteristic of 14 Carpathian goat subpopulations based on 14 microsatellite loci

Subpopulation	N	Na	Ne	Ho	He	Fis
Bolechowice	6	3.000	2.401	0.560	0.538	–0.042
Bóbrka	7	2.714	2.034	0.439	0.467	0.046
Odrzechowa	38	5.071	3.062	0.656	0.632	–0.031
Szlembark	14	3.214	2.450	0.622	0.526	–0.166
Łąki Kozielskie	19	5.857	3.175	0.647	0.667	0.022
Żernica	18	5.214	3.468	0.690	0.658	–0.050
Kluszkowce	32	5.857	3.254	0.670	0.655	–0.027
Nowa Biała	19	5.643	3.405	0.662	0.659	0.001
Pisarzowice	12	4.786	3.224	0.708	0.661	–0.065
Mogilno	7	3.286	2.664	0.653	0.551	–0.193
Kobylec	13	3.571	2.754	0.687	0.607	–0.127
Poronin-1	8	5.286	3.715	0.688	0.685	–0.006
Poronin-2	9	6.429	4.269	0.802	0.743	–0.080
Bańska Niżna	47	8.214	4.468	0.714	0.733	0.025

N, number of individuals; Na, number of different alleles; Ne, number of effective alleles; Ho, observed heterozygosity; He, expected heterozygosity; Fis, inbreeding coefficient.

Population structure and genetic relationship

The Delta K method (Evanno et al., 2005) indicated that the best K was K = 2 (Figure 3 a). This data suggests the existence of two major clusters in the Polish Carpathian goat population (Figure 3 b). It can also be seen that subpopulation 14 (Bańska Niżna) consists of a different cluster than subpopulations 4 (Szlembark) and 11 (Kobylec) and they have a little mutual admixture of each other. The remaining 11 subpopulations are mixtures of individuals from two genetic clusters.

The genetic relationship between the fourteen subpopulations is shown in Figure 4. UPGMA generated dendrogram based on genetic distance indicated that the most distant subpopulations were Szlembark (4) and Bańska Niżna (14). The analysis of breeding records confirmed the correlations that were presented. The Bóbrka (2), Poronin-2 (13) and Poronin-1 (12) herds were created mostly of material derived from the Bańska Niżna herd. The herds that follow on the dendrogram include animals descending in large part from the oldest herd in Odrzechowa. The herds from Nowa Biała (8) onwards had no contact with the Bańska Niżna (14) herd.

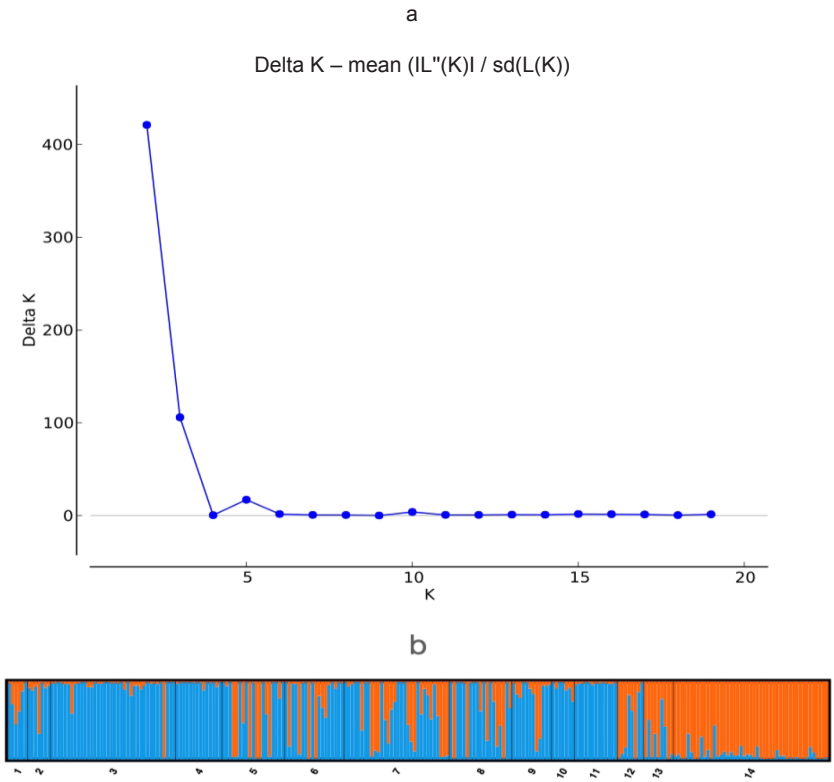


Figure 3. (a) Delta K values obtained with STRUCTURE HARVESTER (b) structural analysis for K = 2 obtained for the 14 subpopulations with values from 1 to 14: Bolechowice, Bóbrka, Odrzechowa, Szlembark, Łąki Kozielskie, Żernica, Kluszkowce, Nowa Biała, Pisarzowice, Mogilno, Kobylec, Poronin-1, Poronin-2 and Bańska Niżna, respectively

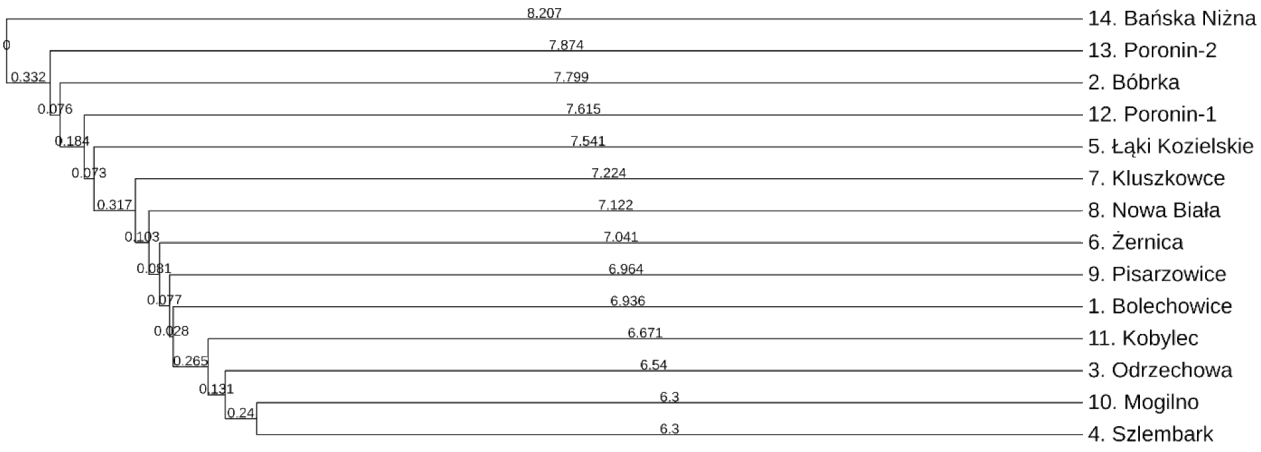


Figure 4. Dendrogram of genetic distance between all individuals by UPGMA algorithm

Discussion

In recent years, there has been a great deal of interest both in preserving local livestock breeds and populations that are disappearing, but also in restoring those that once existed, which is extremely important in terms of the existence of a large gene pool. It is also crucial for the preservation of future breeding and the development of sustainable livestock production systems. Local breeds, especially small ruminants, are able to adapt to marginal and difficult production environments, particularly in mountain regions, and their role is now being increasingly recognised, not only as a source of raw materials for products with high nutritional and dietary value. Sheep and goats play an important role as a key element in maintaining unique rural landscapes and agro ecosystems, preserving cultural heritage, or providing ecosystem services. Breeding efforts in such small, often endangered populations require special care and knowledge of the population structure is essential for the management of their genetic resources.

Microsatellite markers suitability for assessment of genetic variation in goats

Contemporary researches provide numerous molecular tools that can be helpful in assessing the diversity of livestock populations. Microsatellite sequences, also known as short tandem repeat (STR) sequences, have been popular in this type of analysis for a long time, and despite the increasingly widespread use of genome-wide single nucleotide polymorphism (SNP), are still an appropriate solution for assessing genetic structure (Laoun et al., 2020).

An appropriate selection of markers for the purpose of the study was shown by Mekuriaw et al. (2016) in a paper in which they collected the results of genetic diversity studies conducted on more than 120 goat populations in different parts of the world. The authors found the poor performance of the markers used in many analyses, resulting from the low number of alleles and low heterozygosity value per marker. As part of our study, the number of alleles ranged from 5 to 13 and the average heterozygosity for all markers was 0.671. Hoda et al. (2011) analysing the diversity of local Albanian goat breeds using 30 microsatellite markers, found a high number of alleles for each locus, confirming their suitability for this type of analysis. According to the standard given by FAO (2004) in the Secondary Guidelines for Development of the National Farm Animal Resources Management Plans, at least four alleles should occur at microsatellite loci. The microsatellite markers used in our analyses showed sufficient polymorphism to assess intra-population genetic variation in Carpathian goats.

One more parameter indicating the suitability of a given marker for molecular analyses is PIC value, which can vary from 0 to 1. According to Asroush et al. (2018), low polymorphism of a marker is referred to if PIC value<0.25, medium if 0.25<PIC value<0.50 and high when PIC value>0.50, and a population with high PIC is distinguished by high genetic diversity. According to this classification, there was only one marker (INRA063) in our study with medium polymorphism (0.365), and the others with high polymorphism (PIC value ranged from 0.505 to 0.842). Large variations in PIC values per locus are reported by Mekuriaw et al. (2016) for Egyptian goats (0.22–0.87), Ceccobelli et al. (2020) for Italian goats (0.18–0.80) and Seilsuth et al. (2016) for Indian goats (0.08–0.90) and those kept in Thailand (0.205 to 0.657). The high PIC values obtained for the markers used in our study demonstrate that they are highly polymorphic and can be successfully used for the molecular characterization of goats. According to Asroush et al. (2018) the variability in PIC values found in the literature may be due to the different mating methods used in the study populations.

Significant deviations from HWE were recorded for most markers, but very similar observations were reported for Ardi, Holandi and Shami (Hossam Ahmoud et al., 2020), as well as Katjang (Erni Muneerah et al., 2021) goats. This may be due to the magnitude of homoplasy of the microsatellites tested, as no significant value was observed to indicate the presence of null alleles in the studied population, and also most of the markers (except one – ILSTS008) indicated the absence of heterozygote excess phenomenon in the population (lower Ho than He values). The Fnull allele parameter for most markers was estimated at a highly satisfactory level. It can therefore be concluded that the panel proposed by ISAG is better selected than the other goat panels (Serrano et al., 2009).

Microsatellite markers suitability in parentage testing

Correct pedigree information is essential for genetic evaluation, avoiding incorrect estimates and low accuracy of estimated breeding values. In Poland, genetic evaluation of goats is not undertaken and the active population of goats accounts for 0.1%. The Carpathian goat population restored as part of the restitution process is the most numerous breed represented, so there is a need to implement pedigree information from this source in practice. Sequences selected for parentage testing should be characterised by high polymorphism, balanced allele frequency at loci and high values of indicators such as number of alleles per locus, PIC Ho as already mentioned when discussing microsatellite markers suitability in assessing genetic variation in goats.

The parameters used strictly for origin analysis (NE-1P, NE-2P, NE-PP, NE-I, NE-SI) indicated values that once again confirm that the markers chosen by ISAG are properly selected. INRA063 marker, mentioned earlier, is also questionable. This is another evidence to consider replacing this marker with another more useful one in the future. Nevertheless, it should be noted that in case of the study on the Girgentana breed (Siwek and Knol, 2010), in which the authors obtained slightly better val-

ues in this marker, among the 21 markers MCM527 had worse parameters than INRA063. A poor value for the PIC parameter (0.360) was also observed at this locus. These data confirm that such values are variable according to the breed studied and should be tested for each individual. This study demonstrates that the MCM527 marker is highly useful for analysing Carpathian goats.

Da Silva et al. (2014) performed a validation of a panel of microsatellites for the purpose of parentage testing in goats of local and commercial breeds raised in Brazil. The number of alleles and expected heterozygosity (He) per marker ranged from 4 to 18 and from 0.051 to 0.831, respectively. The panel with 14 microsatellites showed adequate exclusion power (99.99%) when both parents were tested. However, in case of unilateral testing, the value obtained (99.64%) also confirms that the employed markers are well selected for individual identification and origin control of goats and can be used for routine pedigree verification of restituted Carpathian goats. In the future, Polish researchers should consider checking the usefulness of this panel for other goats maintained in Poland.

Genetic diversity within populations

The genetic description requires knowledge of the genetic variation that can be effectively measured within and between subpopulations, which provides an important tool for sustainable management as well as for the conservation of a specific population. Indeed, limited genetic diversity may hamper the long-term adaptation of a population to its local environment. Heterozygosity is a good measure of genetic variation in a population. The obtained mean expected heterozygosity for Carpathian goats was 0.701 and was higher than the observed (0.671) value. Similar mean values of He and Ho for local Albanian goat populations were obtained by Hoda et al. (2011) – 0.74 and 0.67 respectively. The aforementioned Mekuriaw et al. (2016) presented a broad overview of the knowledge of genetic diversity in domestic goats using STR in the context of supporting breeding programmes. The authors showed the global status of genetic variability of different goat breeds. Estimates of genetic diversity (He and Ho) observed in goats from Sri Lanka, Australia, Korea, Botswana and in some Indian and Brazilian goat populations were below 0.5. Heterozygosity of nine domestic Swiss goat breeds was higher (He ranged from 0.51 to 0.58) than wild goat and Bezoar goats (0.17 to 0.19) (Saitbekova et al., 1999). The low estimates suggest that there may have been high selection pressure, small population size, minimal or no immigration of new genetic material into the population. The importance of genetic structure studies was shown by Ernie Muneerah et al. (2021) following the example of the endangered, only native Malaysian Katjang goat. This breed is the only native domesticated goat in Malaysia and is perfectly adapted to the local environment, but such low genetic diversity (very low Ho=0.29, high FIS=0.46) may result in a risk to its future existence if not properly maintained and protected to prevent further genetic erosion of this breed.

The inbreeding coefficient in the Carpathian goat population (Fis) ranged from –0.166 to 0.046, remaining at a relatively low level, which indicates that there is no risk of backcrossing in the studied population. According to Hoda et al. (2011) positive values of this parameter for Albanian goats indicated a shortage of heterozygotes in the breeds and explained it by inbreeding and the Wahlund effect. Cañon et al. (2006) for 45 goat breeds from Europe and the Middle East reported a mean Fis value of 0.1, which, according to the authors, could be due to small population size, small number of breeding males and their limited geographical area of breed dispersal. In the above-mentioned project, the Polish Fawn Coloured goat breed had a high inbreed coefficient (0.11), while the Romanian Carpathian goat had one of the lowest coefficients.

A study using STR on the genetic characterisation of the autochthonous Sicilian Mascaruna and Girgentana goat breeds and hybrids of different breeds was presented by the team of Mastrangelo et al. (2013). The Mascaruna breed, with only 100 animals, which does not have a herd register or any other form of breed certification, is not subject to breeding programmes or a milk yield control system. The genetic diversity results showed the existence of a specific genetic structure, despite the lack of systematic breeding work, the possibility of influx from other breeds and the limited availability of breeding goats. The high level of inbreeding and the relatively high expected heterozygosity in the significantly more numerous Girgentana breed is probably due to the presence of local bottlenecks. The hybrid group showed the greatest genetic diversity as it derived from a mixture of other populations.

Population genetic relationship and structure

Genetic diversity or fixation indices form the parameters for assessing inter- or intra-population variation. The most commonly used one is FST, which measures the degree of genetic differentiation between subpopulations by calculating the standardised variance of allele frequencies between subpopulations. The FST-fixation coefficient measures the decrease of heterozygosity in a subpopulation relative to the entire population, due to e.g., selection or genetic drift. Its values indicate how intense the gene flow is between subpopulations. It indicates the genetic distance between subpopulations or populations. High genetic distance (Fst) is the one that takes values exceeding 0.25, moderate genetic distance is between 0.05 and 0.25, and low genetic distance is below 0.05 (Mekuriaw et al., 2016). The mean genetic variation between all analysed stocks was 0.088, which means that about 8.8% of the total genetic variation was explained by subpopulation differences, and 91.2% corresponds to differences between individuals within each stock. Cañon et al. (2006) described average genetic variation between breeds for 45 local breeds in 15 countries in Eu-

rope and the Middle East at 7% as moderate, although it ranged from a very low 0.004 (0.4%) for the Greek breed to 0.12 (12%) for the Swiss Valais Black Neck. The genetic analysis of local FST Albanian goat breeds was 2%, which was very low according to the authors. High values were reported for Swiss goats Fst=17% (Saitbekova et al., 1999), 14% for Asian goats and 10.5% for Chinese goats (Di et al., 2010). The low value of genetic diversity (0.027), as was the case between Sicilian goat breeds (Mastrangelo et al., 2013), indicates that these populations had little diversity, due to geographical proximity, similarities in environment and breeding practices, but most likely due to past gene flow between them.

Bayesian clustering revealed two main genetic clusters from which the currently breeding Carpathian goats originate, confirming the restoration process of these animals. The Carpathian goat was originally restored from two herds (Sikora and Kawęcka, 2014). At the time of its formation, the herd consisted of 6 adult goats, 4 female goats, 2 stud goats and 2 little goats. The herd, which has steadily increased, currently contains 30 mother goats. In the course of the development, further herds were created from individuals in this farm, and at the same time a large herd was established in Bańska Niżna in the Lesser Poland Province, in the foothills of the Tatra Mountains, using non-origin animals from neighbouring farms. The new herds used breeding material from these two initial herds, hence their positioning between them.

Microsatellite data are commonly used to assess genetic relationships between populations and individuals by estimating genetic distances. The most commonly used measure of genetic distances is Nei's standard genetic distance or modified Cavalli-Sforza distance for closely related populations where genetic drift is a major factor in genetic variation, as is often the case in livestock populations (Mekuriaw et al., 2016). In phylogenetic tree analysis, the use of both the neighbour joining (N-J) method and UPGMA can be useful. Applied in this study, UPGMA generated dendrogram based on genetic distance indicated that the most distant populations were Szlembark and Bańska Niżna. The analysis of breeding records for herds including Carpathian goats confirmed the relationships drawn on the dendrogram.

In the course of the restitution, further herds were created from individuals of the original farm, and, at the same time, a large herd was formed in Bańska Niżna in the Lesser Poland Province, in the foothills region of the Tatra Mountains, from individuals with no pedigree, coming from neighbouring farms. The resulting new herds used breeding material from these two original ones, hence these herds were placed between them on the dendrogram.

When breeding, especially in small, endangered populations where the most important aim is to maintain or increase the size of the breed and to maintain the stability and preservation of the breed pattern, factors that can contribute to a decrease in gene diversity, such as selection pressure, the use of artificial insemination and uncontrolled mating of related individuals, must be particularly controlled. The breeding procedures used to date have resulted in the reproduction of a breed with relatively high genetic diversity, despite the two flocks initially employed.

Conclusion

All microsatellite markers employed in this analysis showed sufficient polymorphism to assess genetic variation in Carpathian goats and the number of loci (14) was sufficiently useful for the planned purposes. The parameters calculated for the parental analysis showed that the ISAG-recommended panel for goat individual identification and parentage testing is a highly useful one. Carpathian goats maintained in Poland were characterized by relatively high genetic diversity (the average of alleles per locus was 9.143), high values of heterozygosity and a low level of inbreeding coefficient, which indicates the absence of a risk of inbreeding in the studied population. The values of the fixation coefficient were at a medium level which indicates a moderate differentiation between the subpopulations of this breed.

The present study showed the status of the genetic structure of the reconstituted population of Carpathian goats. The obtained parameters indicate the correctness of the breeding activities carried out within the framework of the programme for the protection of genetic resources and give guidelines for taking further steps related to the breeding of this valuable native breed.

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