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Genetic differentiation among autochthonous and cosmopolitan goat breeds based on information from microsatellite markers – a preliminary study

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Short communication

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The aim of the study was to evaluate the differentiation among three goat breeds – the autochthonous Carpathian goat and the cosmopolitan Alpine and Anglo-Nubian breeds – based on information from microsatellite markers. Blood samples of 64 goats were collected from three farms located in southern Poland. The population structure of the collected goats was investigated using the Bayesian approach, implemented in STRUCTURE 2.3.4. The Pairwise Population Fst analysis revealed that the largest genetic distinction was between the Anglo-Nubian and the Alpine breeds. In each of the analyses carried out, it was observed that the Carpathian breed is genetically more similar to the Alpine breed, while the Anglo-Nubian breed is a separate, much more distinct cluster. It therefore seems reasonable to carry out further research in the context of the genetic relationship between the two goat breeds (Carpathian and Alpine) adapted to the harsh conditions of mountainous areas.

Key words: diversity, population study, native breed, STR.

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Knowledge of population genetic diversity, intra-breed variation and the genetic linkages between populations allows for an estimate of the potential degree of endangerment and the identification of conservation strategies for a breed. Microsatellite markers (STR – short tandem repeats) are an extremely useful tool in selecting biodiversity conservation strategies for many livestock species (Cortes *et al.* 2022; Wenne 2023).

Although the goat population in Poland is estimated to be comprised of around 54,000 individuals, its active part, in which data is provided on the milk and breeding performance, is less than 1%. The assessed population is dominated by an indigenous goat breed called the Carpathian goat, which was rebuilt in the process of restitution. At one stage, the Carpathian goat, which used to be numerous in the Carpathian Mountains, was practically extinct. However, the process of restitution of the breed, which was initiated by the National Research Institute of Animal Production in Balice (Poland), has been successful – and at present the population numbers over 400 goats kept in 30 herds. The population is covered by the genetic resources protection programme. The Carpathian goat is a breed with great resistance and good health, longevity and fertility, with a perfect ability to adapt to difficult environmental conditions and an indiscriminate selection of feed. The goats have a characteristic long, white coat, good breeding characteristics and an average milk yield of

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approximately 300 kg per year (Kawęcka *et. al* 2021). The Carpathian goats included in the evaluation of the milk production performance account for as much as 70% of the assessed goat population in Poland (Kawęcka *et al.* 2022). The other breeds assessed are the Boer goat, Saanen goat, Alpine goat and the Anglo-Nubian goat. Alpine goats originate from the mountainous regions of France. The addition of blood from goats originating from Switzerland contributed greatly to the creation of the Alpine goats. Alpine goats have been imported to many countries, including Poland, not only for the purpose of having purebred animals, but also to improve the local colour goat breeds (Sikora 2003). The Anglo-Nubian is a dual-purpose goat that is reared both for goat's meat and for milk. The Anglo-Nubian breed originated in the British Isles, from cross-breeding between the traditional native British goats and a mixed population of goats imported from India, the Middle East and North Africa. The goats of this breed bred in Poland come mainly from this country, as well as from the Czech Republic, Slovakia and Belgium, and are mainly kept on agritourism farms (Kawęcka *et al.* 2021). The goat milk production in Poland is mainly from crossbreed goats. The dynamically developing Carpathian goat population is now not only an extremely valuable element of biodiversity, but also represents the potential for the development of a local market for goat milk products. A recent extensive study on Carpathian goats using microsatellite markers revealed the status of the genetic structure of the reconstituted population. The Carpathian goats maintained in Poland were characterised by relatively high genetic diversity (the average of the alleles per locus was 9.143), high values of heterozygosity and a low level for the inbreeding coefficient (Kawęcka *et al.* 2022). An attempt was also made to find out the genetic relationships between the native Carpathian breed and other dairy breeds in Poland.

The aim of this study was to evaluate the differentiation among three goat breeds: native Carpathian, Alpine and Anglo-Nubian, based on information obtained from microsatellite markers.

The experimental conditions in the present study were approved by the Local Ethics Committee (No. 267/219). Blood samples of 64 goats were collected from three farms located in southern Poland. Six samples of Alpine goats were obtained in Kluszkowce and 20 samples of Anglo-Nubian goats were obtained in Pisarzowice. In addition, 38 samples of Carpathian goat collected for previous studies (Kawęcka *et al.* 2022) were used. The herd in Odrzechowa was selected because the restitution of the Carpathian goats started in this locality.

DNA was extracted using QuickGene DNA whole blood kit (DB-S), following the suggested protocols of the manufacturer. The DNA concentrations were assessed using a NanoDrop™ 2000 Spectrophotometer (Thermo Fisher). Then, the genomic DNA was amplified using 14 microsatellite markers recommended by International Society for Animal Genetics (ISAG). A detailed description of the method can be found in the chapter 'Samples collection and microsatellite genotyping' and 'Table 1' in our previous article (Kawęcka *et al.* 2022).

The population structure of the collected goats was investigated using the Bayesian approach, implemented in STRUCTURE 2.3.4 (Pritchard *et al.* 2000). The analysis was performed with a burn-in period of 100,000 and 200,000 iterations, and the number of clusters (K) ranging from 1 to 10 with 10 runs for each K. STRUCTURE HARVESTER (Earl and VonHoldt 2012) using Evanno's method (Evanno *et al.* 2005) was used to select the best K. Principal Coordinate Analysis (PCoA), while the Pairwise Population Fst Analysis and Gene flow (Nm) was performed using GenAlEx (Peakall & Smouse 2012, 2006).

The Bayesian approach revealed that the most likely genetic structure for the collected goats was a structure composed of two genetic clusters (K=2) (Fig. 1). The first cluster contained the Anglo-Nubian individuals, while samples belonging to the Alpine goat breed were clustered together with the Carpathian goats (Fig. 2). Interestingly, in the case of K=3 in accordance with the phenotypic classification of breeds, the analysis showed separate clusters for the Anglo-Nubian, Alpine and Carpathian breeds, but the latter containing several individuals showing an admixture with the Alpine goat. According to STRUCTURE HARVESTER, K = 7 also had a relatively high indication. During the division of 64 individuals into seven subpopulations, it could be observed that individuals belonging to the Anglo-Nubian breed did not contain an admixture and formed a separate cluster, while the other two breeds showed high levels of admixture.

The PCoA performed on a complete dataset of 64 genotypes showed relationships between the individuals belonging to three breeds. Three different groups of genetic variation were observed. Interestingly, several individuals belonging to the Carpathian breed were included in the group consisting of the Alpine breed.

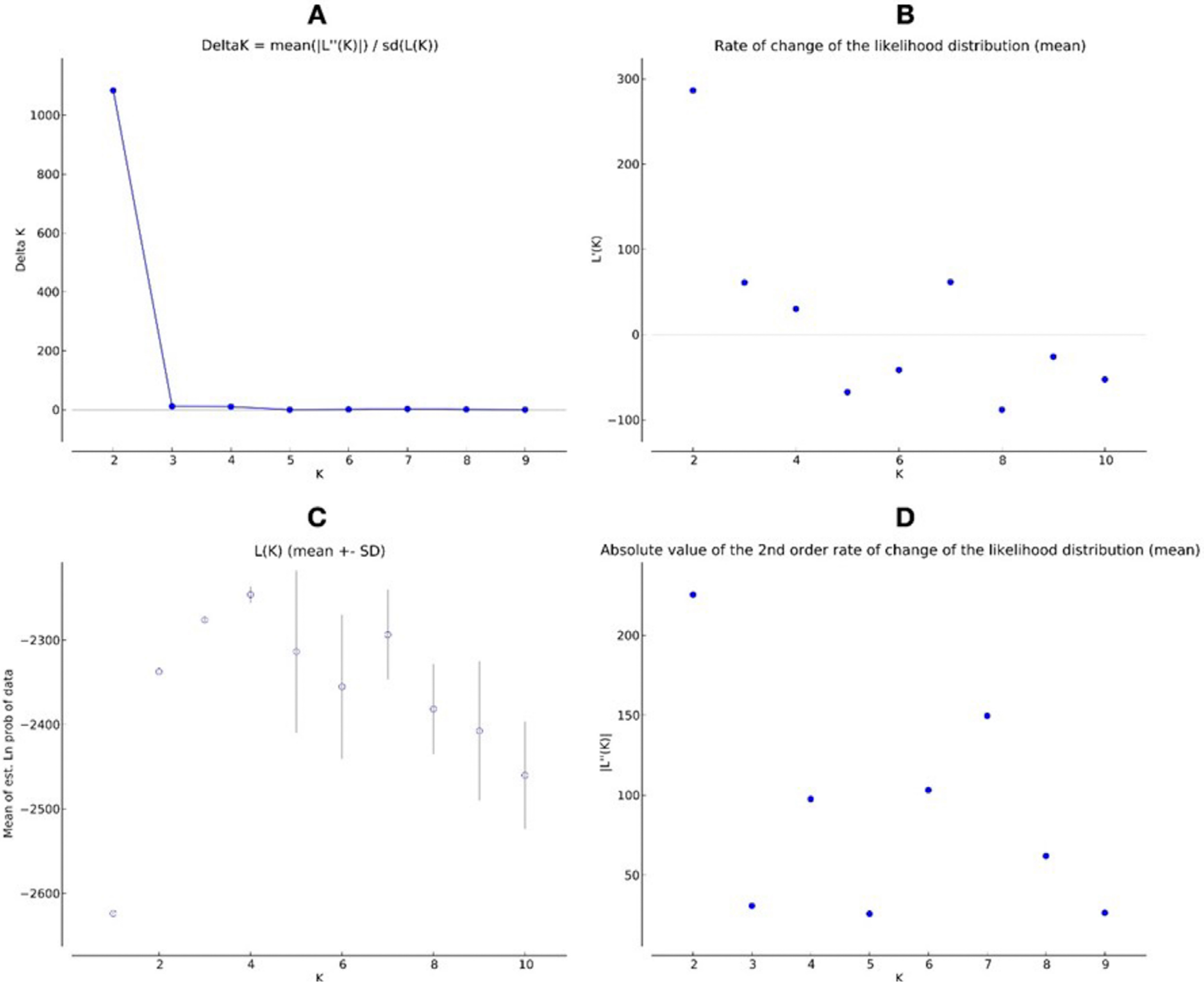

Fig. 1. Plots generated by the Evanno test using STRUCTURE HARVESTER. (A) deltaK; (B) lnPK; (C) meanLnProb; (D) lnPPK.

The Pairwise Population Fst (Table 1) revealed the highest genetic distance between the Anglo-Nubian and the Alpine breeds (0.114), and the lowest distance between the Alpine and Carpathian breeds (0.079). Accordingly, the highest gene flow was estimated to be between the Alpine and Carpathian breeds, while the lowest was between the Anglo-Nubian and Alpine breeds (Table 2). In each of the analyses carried out, it was observed that the Carpathian breed is genetically more similar to the Alpine breed, while the Anglo-Nubian breed is a separate, much more distinct cluster.

Table 1

Pairwise Population Fst Analysis for the three breeds

1	2	3	
0.000			1
0.114	0.000		2
0.093	0.079	0.000	3

1. Anglo-Nubian breed; 2. Alpine breed; 3. Carpathian breed.

Table 2

Gene flow (Nm) among the three breeds

1	2	3	
0.000			1
1.934	0.000		2
2.428	2.925	0.000	3

1. Anglo-Nubian breed; 2. Alpine breed; 3. Carpathian breed.

Estimating genetic diversity can be useful for selecting strategies for the sustainable conservation of endangered populations. An extensive study conducted on the entire population of Carpathians goat showed the state of the genetic structure of the reconstituted native breed (Kawęcka *et al*. 2022). The parameters obtained on the basis of information from microsatellite markers indicated the correctness of the breeding work carried out within the framework of the genetic resources protection programme, while providing hints for taking further

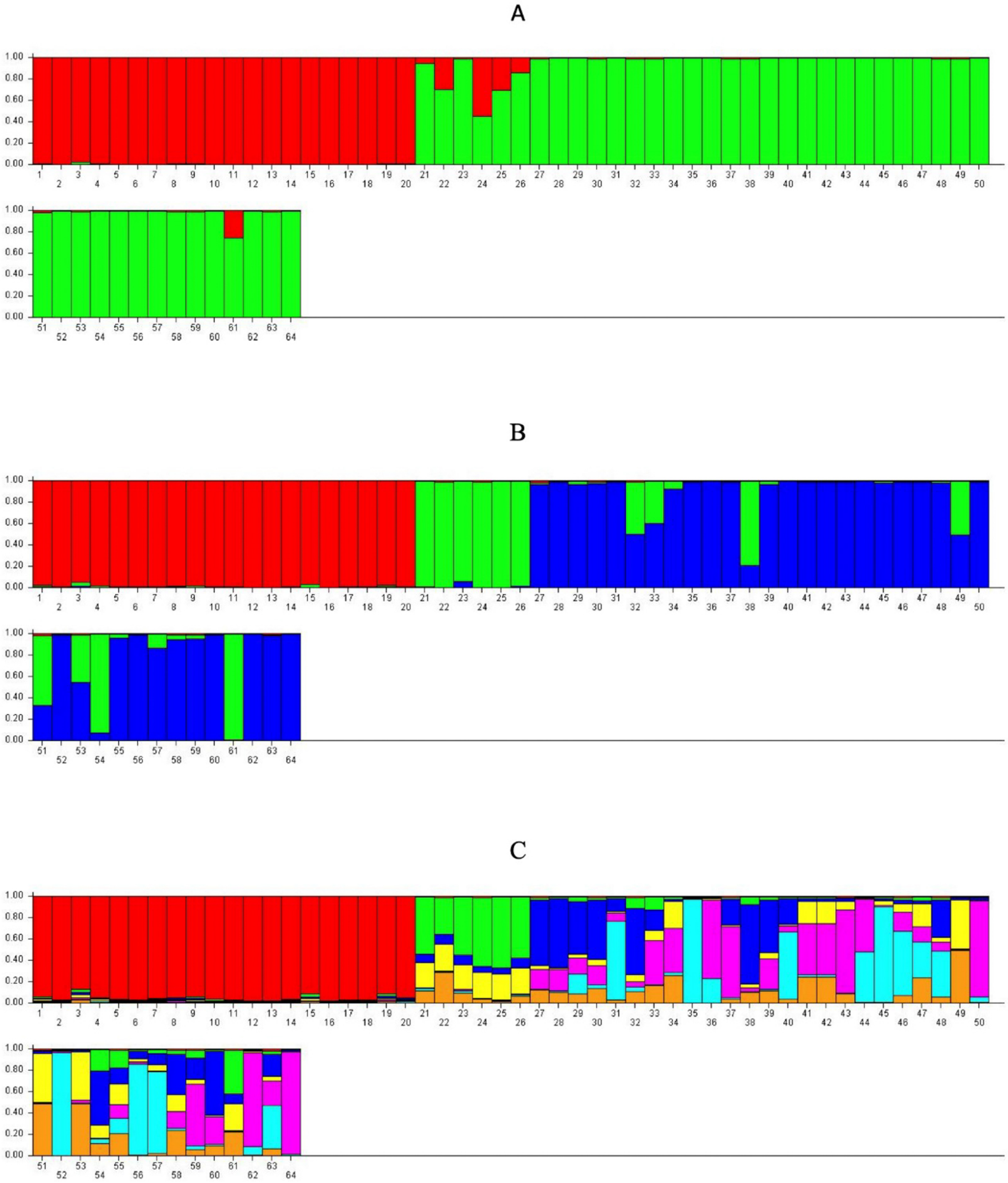

Fig. 2. Population structure for (A) K = 2; (B) K = 3; (C) K = 7. Individuals: 1-20, 21-26 and 27-64 included the Anglo-Nubian, Alpine and Carpathian breeds, respectively.

steps related to the breeding of this valuable native breed, which is becoming more and more popular among breeders. Since the Carpathian goat is a breed with an excellent ability to adapt to very different environmental conditions, it can be used throughout the country, just like the Alpine breed that adapts well to diverse, including mountainous, climatic conditions (Klir *et al*. 2015).

An attempt was made to study of the relationship between the currently rapidly-growing population of the Carpathian goat and other dairy breeds. The small number of samples included in the study was due to the lack of availability of breeding material for imported breeds in the country, but the results obtained give some idea of the relationship between these breeds.

The admixture analysis highlighted the clear differentiation of the Anglo-Nubian breed, which was separate from the rest of the sample. Similar results indicating a complete differentiation of native Italian breeds from a dairy Norwegian breed, which was the external group used for the phylogenetic analysis and for a study of the population structure, were obtained by Criscione *et al.* (2016).

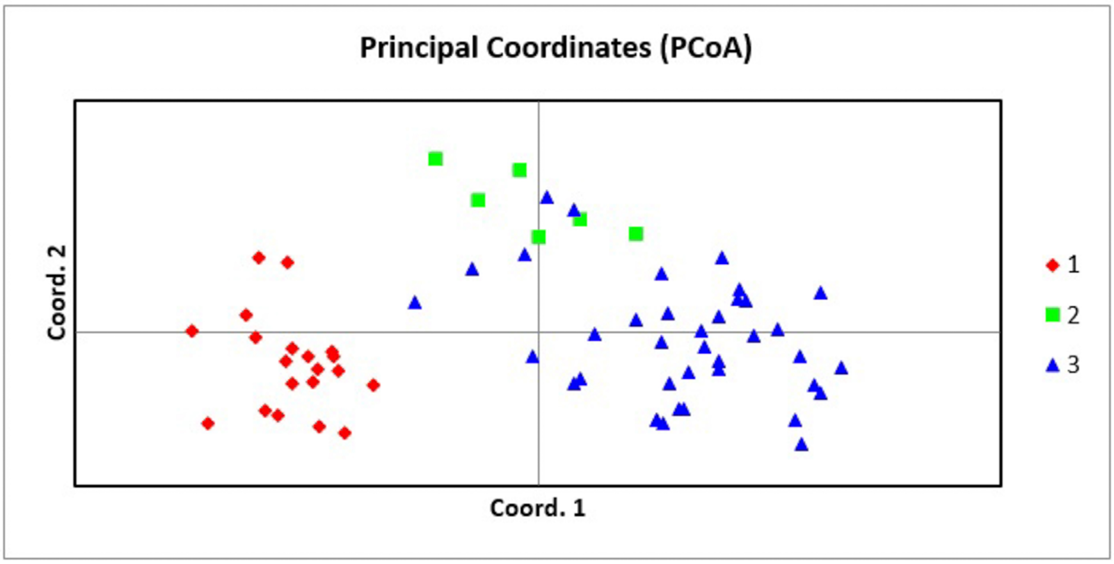

Fig. 3. Principal Coordinate Analysis (PCoA): 1. Anglo-Nubian breed; 2. Alpine breed; 3. Carpathian breed. Percentage of variation explained by the first 3 axes: 19.19, 9.67 and 7.87.

A very reliable parameter for assessing inter- or intra-population variation is the fixation coefficient Fst, which determines the decrease in heterozygosity of a subpopulation relative to the whole population due, for example, to selection or genetic drift. Its values indicate how intense the gene flow is between subpopulations, as well as the genetic distance between them. In our study, the highest genetic differentiation was found between the Anglo-Nubian breed (0.114), while for the native Carpathian and Alpine breeds this parameter was twice as low (0.079). In general, in all cases, the values of the distance between populations should be considered as moderate, i.e. between 0.05 and 0.25 (Mekuriaw *et al*. 2016). Cañon *et al*. (2006) described the average genetic variation between breeds for 45 local breeds in 15 countries in Europe and the Middle East as moderate (0.07). However, a genetic analysis of local goat breeds showed a high variation in this parameter, e.g. high (Fst = 0.17) for Swiss goat breeds (Saitbekova *et al*. 1999); while the low value for genetic variation (0.027) between Sicilian goat breeds (Mastrangelo *et al*. 2013) indicated that these populations were not very diverse, due to geographical proximity, similarities in their environment and breeding practices, and most likely past gene flow between them.

Conclusion

The results obtained in our study indicate some similarity between the native Carpathian and the Alpine breeds, despite breeding for breed purity and no exchanges of breeding animals between these populations. It therefore seems reasonable to carry out further research in the context of the genetic relationship between the two goat breeds adapted to the harsh conditions of mountainous areas.

Author Contributions

Research concept and design: A.K.; Collection and/or assembly of data: M.P., J.S.; Data analysis and interpretation: A.K., A.M.; Writing the article: A.K.; Critical revision of the article: A.M., M.P., J.S.; Final approval of article: A.K., M.P.

Conflicts of Interest

The authors declare no conflict of interest.

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