Bulgarian Journal of Agricultural Science, 32 (No 1) 2026, 201–209

Genetic characterization of Moldavian Karakul sheep populations: Microsatellite marker analysis and similarity assessment

Tatyana Lupolov[1*], Oleg Mashner[1], Petr Lyutskanov[1] and Tatiana Deniskova[2]

[1] *Public Institution National Institute for Applied Research in Agriculture and Veterinary Medicine, 2070, Chişinău, Moldova*

[2] *Federal Research Center for Animal Husbandry – All-Russian Institute of Animal Husbandry named after L. K. Ernst, Dubrovitsy, Russia*

[*] *Corresponding author:* talupolova@gmail.com

Abstract

Lupolov, T., Mashner, O., Lyutskanov, P. & Deniskova, T. (2026). Genetic characterization of Moldavian Karakul sheep populations: Microsatellite marker analysis and similarity assessment. *Bulg. J. Agric. Sci., 32*(1), 201–209

The genetic characterization of Karakul sheep populations was conducted affording the analysis of 10 microsatellite loci in order to evaluate genetic diversity and relatedness. Animals from following districts were studied: Anenii Noi, representing the New Moldovan type, Nisporeni, classic type, and Riscani, also classic type. Allelic diversity, heterozygosity levels, and genetic distances were determined to analyze populations' structure. This research revealed high allelic diversity (up to 15 alleles in the OarCP49 locus in the Nisporeni population), elevated heterozygosity in Riscani (H_o = 71.43%) and Nisporeni (H_o = 61.3%) populations, moderate heterozygosity in Anenii Noi population (H_o = 56.64%), and significant differentiation between populations. The smallest genetic distance was observed between Anenii Noi and Riscani (D = 0.132) and the largest between Anenii Noi and Nisporeni (D = 0.265) populations. Cluster analysis confirmed a close genetic relationship between Anenii Noi and Riscani populations, while Nisporeni one had formed a distinct group. These findings highlight the value of Karakul sheep as a genetic resource and provide insights for diversity conservation strategies and breeding program optimization.

Keywords: Karakul sheep; microsatellite markers; genetic diversity; population structure; allelic variation; genetic similarity

Introduction

Genetic diversity in domestic sheep is an important factor influencing their adaptation, productivity, and resilience to environmental challenges. Microsatellite markers are widely used to assess the genetic structure of sheep populations, enabling studies of their diversity, phylogenetic relationships, and the impact of selective breeding processes. For instance, Tapio et al. (2005) have investigated the genetic diversity and population structure of sheep in Northern Eurasia, revealing significant regional differences conditioned by geographic isolation and historical migrations. Similarly, Kijas et al. (2012) have used genome-wide analysis to demonstrate extensive historical mixing and strong selection pressures in global sheep breeds, highlighting the role of anthropogenic factors in shaping their genetics. The Karakul breed, renowned for its unique wool traits, has also been a focus of research. Deniskova et al. (2018) had characterized the genetic diversity of Russian sheep breeds, including Karakul, using microsatellite markers, while Hoda and Ajmone-Marsan (2012) have studied Albanian breeds, by identifying the local features of their genetic structure. Buzu (2012) had emphasized the influence of the indigenous Țușca breed on the resilience of Moldovan Karakul populations, and Buzu (2021)

had documented differences in pelt quality between classic types and the need for inbreeding monitoring. Further, Buzu (2023) is describing selective breeding efforts to develop the New Moldovan type in Anenii Noi, noting its adaptation to local conditions and improved wool characteristics. However, data on the genetic structure of Moldovan Karakul populations-particularly the New Moldovan type-remain limited, leaving gaps in understanding their genetic potential and relationship to classic types.

The novelty of this study consists in the comparative analysis of the genetic structure of three Karakul sheep populations: Anenii Noi (a new Moldovan type), Nisporeni, and Riscani (classical types). This analysis utilizes 10 microsatellite loci, including OarFCB11 and OarAE129, as described by Buchanan and Crawford (1993) and Penty et al. (1993). Unlike previous studies focusing on global or regional populations (Tapio et al., 2010; Kijas et al., 2012), this research concentrates on local Moldovan populations, enabling the identification of specific features of their genetic divergence and adaptation. The motivation for this study is driven by the need to preserve the genetic resources of the Karakul breed amid agricultural intensification and the risk of losing unique genetic lines, as highlighted in the guidelines for molecular genetic characterization of animals (Boettcher et al., 2011). Furthermore, understanding the genetic relationships among populations, is crucial for developing breeding strategies aimed at enhancing productivity and disease resistance.

Based on preliminary data, we hypothesize that the Anenii Noi population is more genetically similar to the Riscani population compared to the Nisporeni population. This similarity could be due to gene flow resulting from migration between these populations. The objective of this study is to assess the genetic diversity, heterozygosity, and genetic distances among the three Karakul sheep populations to determine their structure and phylogenetic relationships. Additionally, the study aims to provide recommendations for breeding programs.

Material and Methods

Objectives of study

The study was focused on the genetic analysis of Karakul sheep populations using 10 microsatellite loci (INRA005, SPS113, INRA23, MAF65, McM527, OarCP49, HSC, OarAE129, MAF214, and OarFCB11). These loci were selected as standard markers widely used in sheep genetic research due to their high polymorphism and ability to provide sufficient information for assessing genetic diversity, determining kinship, and studying populations' structure. These markers are frequently used in scientific research and breeding programs, aimed at improving breeds and preserving genetic diversity (Penty et al., 1993).

Karakul Sheep Populations Analyzed:

Three populations of Karakul sheep from different regions of Moldova (Fig. 1) were studied:

1. New Moldovan Type Population

– Composition: Hybrid ♀ "Tsushka" (indigenous breed) × ♂Karakul sheep of Uzbek selection.

– Sample Size: 24 individuals.

Fig. 1. Geographic distribution and composition of three Karakul sheep populations studied in Moldova
1 – Riscani District; 2 – Nisporeni District; 3 – Anenii Noi District
Source: Authors' own elaboration

– Location: Anenii Noi District (central Moldova, southeast of Chișinău; coordinates: 46.8794° N, 29.2283° E).
2. Classical Type Population
– Composition: Karakul sheep.
– Sample Size: 14 individuals.
– Location: Rîșcani District (northern Moldova; coordinates: 47.9061° N, 27.5914° E).
3. Classical Type Population
– Composition: Karakul sheep.
– Sample Size: 40 individuals.
– Location: Nisporeni District (central Moldova, west of Chișinău; coordinates: 47.1194° N, 28.0467° E) (Figure 1).

Experiment's organization

The genetic analysis was conducted at the Laboratory of Genetics and Genomics of Small Ruminants, at the All-Russian Research Institute of Animal Husbandry, named after Academician L.K. Ernst (VIZh). The experiments were organized in several stages:

1. DNA Extraction. DNA was extracted from tissue samples (ear notches) using a standard protocol with commercial DNA extraction kits, such as the QIAamp DNA Mini Kit (Qiagen), following the manufacturer's recommendations (Sambrook and Russell, 2001).

2. Polymerase Chain Reaction (PCR). Preparation of the Reaction Mixture: The PCR reaction mixture included PCR buffer, deoxynucleotides (dNTPs), specific primers for microsatellite loci, Taq polymerase, and template DNA. Primers were selected based on previously published data (Maddox et al., 2001).

PCR Conditions: Initial denaturation at 94°C for 2–5 minutes; denaturation at 94°C for 30 seconds per cycle; primer annealing at 55°C to 65°C (optimized for each primer pair) for 30 seconds. Elongation at 72°C for 30 seconds, with a final elongation at 72°C for 5–10 minutes. The number of cycles ranged from 30 to 40.

3. Separation of Amplified Fragments: Gel Electrophoresis: Used for preliminary verification of PCR products. Agarose gels (1–2%) were used for fragments ranging from 100 to 1000 base pairs, while polyacrylamide gels (6–8%) were used for more precise separation (Sambrook and Russell, 2001).

Capillary Electrophoresis: Performed using the Applied Biosystems 3130xl Genetic Analyzer. DNA fragments were separated by size in polymer-filled capillaries under the influence of an electric field. Detection and analysis of fragments were carried out by visualizing the fluorescence of marked primers.

Data analysis

For the analysis of capillary electrophoresis data, the GeneMapper software (Applied Biosystems, 2006) was used. This program automatically determines fragment sizes, genotypes alleles, and visualizes the results. Positive and negative controls were included to ensure the reliability of the results.

Statistical methods

Allele frequencies, genetic diversity indices, and populations' structure were analyzed using appropriate statistical tools (Ayala, 1988; Hartl and Clark, 1997). Each locus was tested for Hardy-Weinberg equilibrium.

The UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method was used to construct a dendrogram, that is visualizing the genetic similarity among the three sheep populations, using an algorithm implemented in the MEGA software package (Tamura et al., 2021).

To assess genetic similarity between Karakul sheep populations, Nei's genetic distance coefficient was calculated using the PowerMarker software (Liu and Muse, 2005). This method, based on alleles' frequency analysis, provides a quantitative measure of genetic differentiation between populations (Nei, 1972).

Results and Discussion

A genetic analysis of three populations of Karakul sheep (Anenii Noi – new Moldovan type, "Nisporeni" – classical type, and "Riscani" – classical type) was carried out using 10 microsatellite loci (*INRA005, SPS113, INRA23, MAF65, McM527, OarCP49, HSC, OarAE129, MAF214, OarFCB11*). Allele frequencies, heterozygosity, and genetic distances (based on Nei's coefficient) were analyzed to assess genetic diversity, population structure, and their phylogenetic relationships.

Allele frequencies and genetic diversity

Figure 2 presents the allele frequencies at 10 loci for the population of Karakul sheep (classical type) from the „Riscani" population. High-frequency alleles such as 129 at the *MAF65* locus (57.14%) and 153 at the *OarAE129* locus (71.43%) dominate the population, indicating their wide distribution.

A high level of observed heterozygosity (Ho) at the *INRA005* (0.9286) and *INRA23* (0.9286) loci indicates considerable genetic diversity (Table 1). However, the *OarFCB11* locus shows low heterozygosity (Ho = 0.2857), which may be due to inbreeding or selective pressure, which tell us about a high fixation index (F = 0.6445). The overall hetero-

Table 1. Genetic structure of the Karakul sheep population of the "Riscani" population

Locus	Number of alleles (Na)	Observed heterozygosity (Ho)	Expected heterozygosity (He)	Index's fixation (F)
INRA005	12	0.9286	0.8903	-0.0430
SPS113	7	0.6429	0.8087	0.2050
INRA23	10	0.9286	0.8673	-0.0706
MAF65	6	0.6429	0.6327	-0.0161
McM527	5	0.7857	0.7526	-0.0440
OaɔCP49	11	0.8571	0.8418	-0.0181
HSC	8	0.8571	0.8163	-0.0500
OaɔAE129	6	0.5000	0.4694	-0.0652
MAF214	8	0.7143	0.8061	0.1139
OaɔFCB11	8	0.2857	0.8036	0.6445
H_o = 71.43%				

Source: Authors' own elaboration

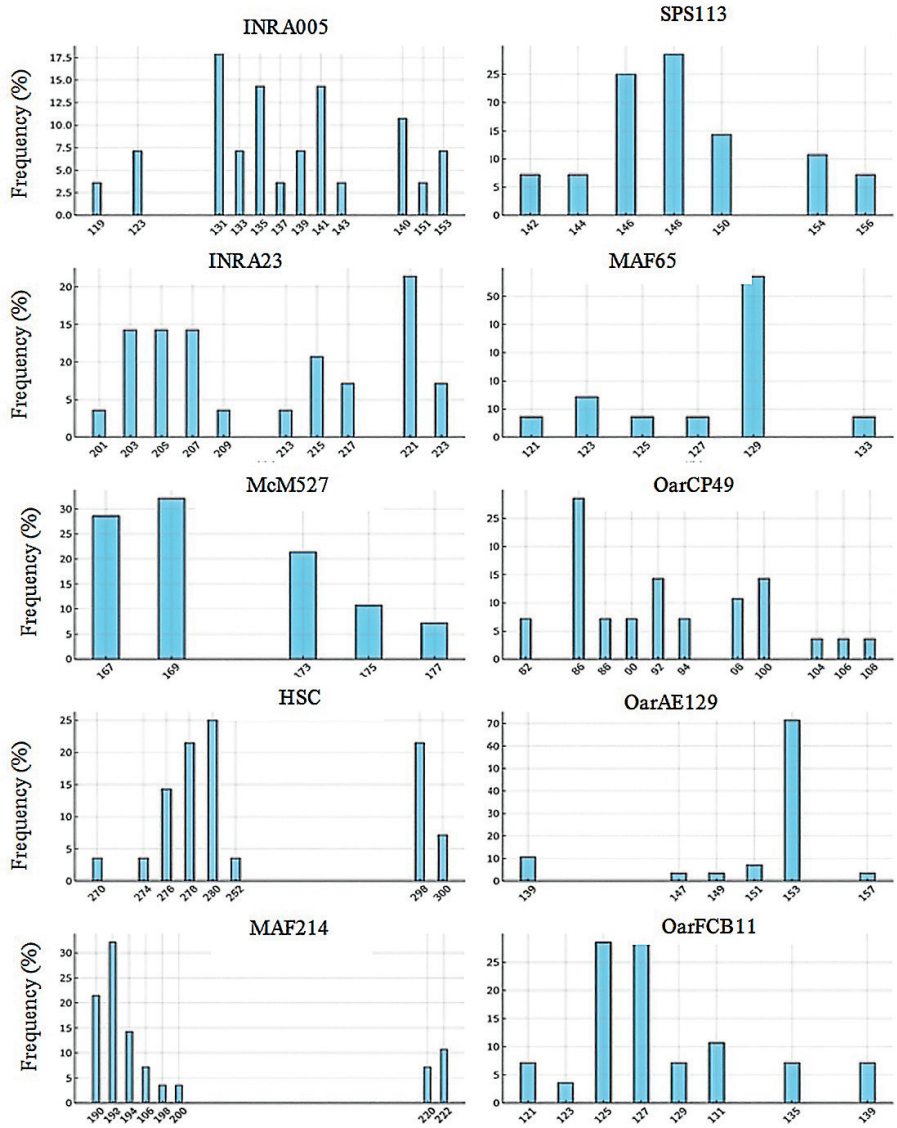

Fig. 2. Allele frequencies in the loci of Karakul sheep in the "Riscani" population
Source: Authors' own elaboration

Table 2. Genetic structure of the Karakul sheep in the "Anenii Noi" population

Locus	Number of alleles (Na)	Observed heterozygosity (Ho)	Expected heterozygosity (He)	Index's fixation (F)
INRA005	14	0.583	0.923	0.368
SPS113	10	0.458	0.859	0.467
INRA23	11	0.667	0.859	0.223
MAF65	6	0.583	0.704	0.172
McM527	10	0.583	0.817	0.286
OarCP49	11	0.875	0.817	-0.071
HSC	11	0.541	0.817	0.338
OarAE129	9	0.541	0.704	0.232
MAF214	6	0.25	0.704	0.645
OarFCB11	10	0.583	0.704	0.172
H_o= 0.5664				

Source: Authors' own elaboration

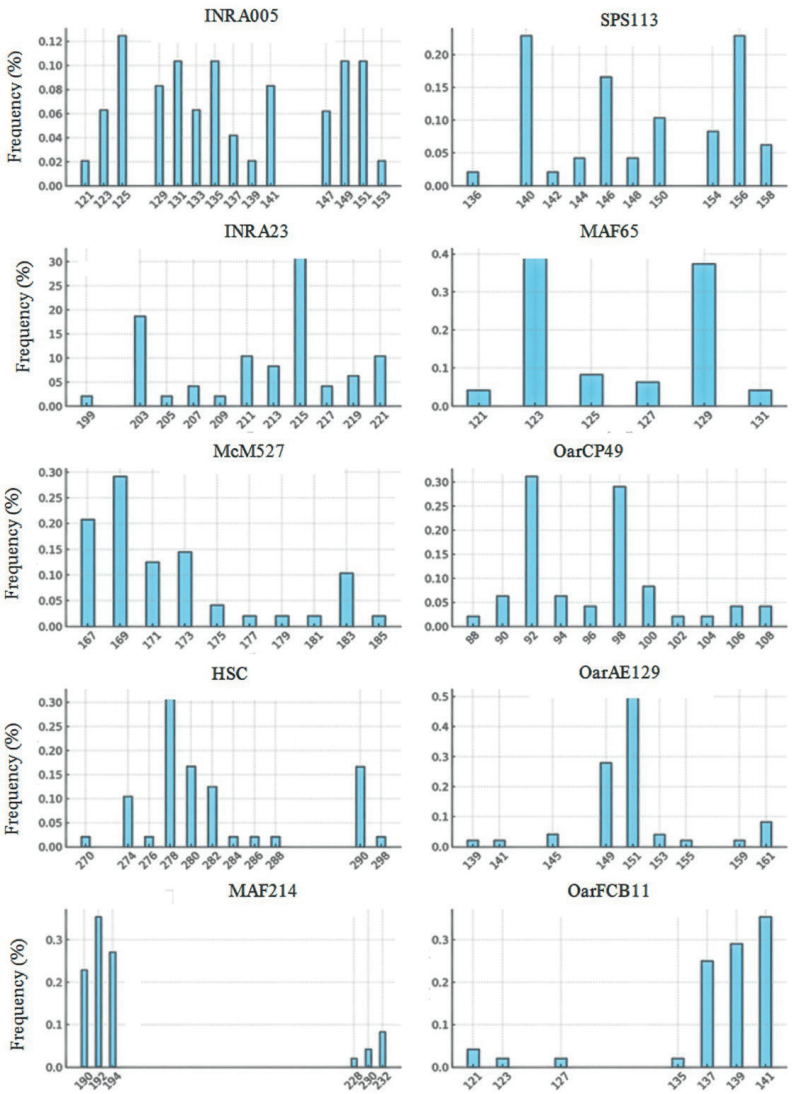

Fig. 3. Allele frequencies in the loci of Karakul sheep of the "Anenii Noi" population
Source: Authors' own elaboration

Table 3. Genetic structure of the Karakul sheep population of the "Nisporeni" population

Locus	Number of alleles (Na)	Observed heterozygosity (Ho)	Expected heterozygosity (He)	Index's fixation (F)
INRA005	11	0.37	0.763	0.377
SPS113	12	0.333	0.871	0.618
INRA23	9	0.8	0.858	0.068
MAF65	7	0.8	0.757	-0.057
McM527	10	0.6	0.728	0.176
OarCP49	15	0.85	0.861	0.013
HSC	13	0.9	0.814	-0.106
OarAE129	10	0.6	0.774	0.225
MAF214	9	0.55	0.753	0.270
OarFCB11	8	0.325	0.832	0.609
H_o = 0.613				

Source: Authors' own elaboration

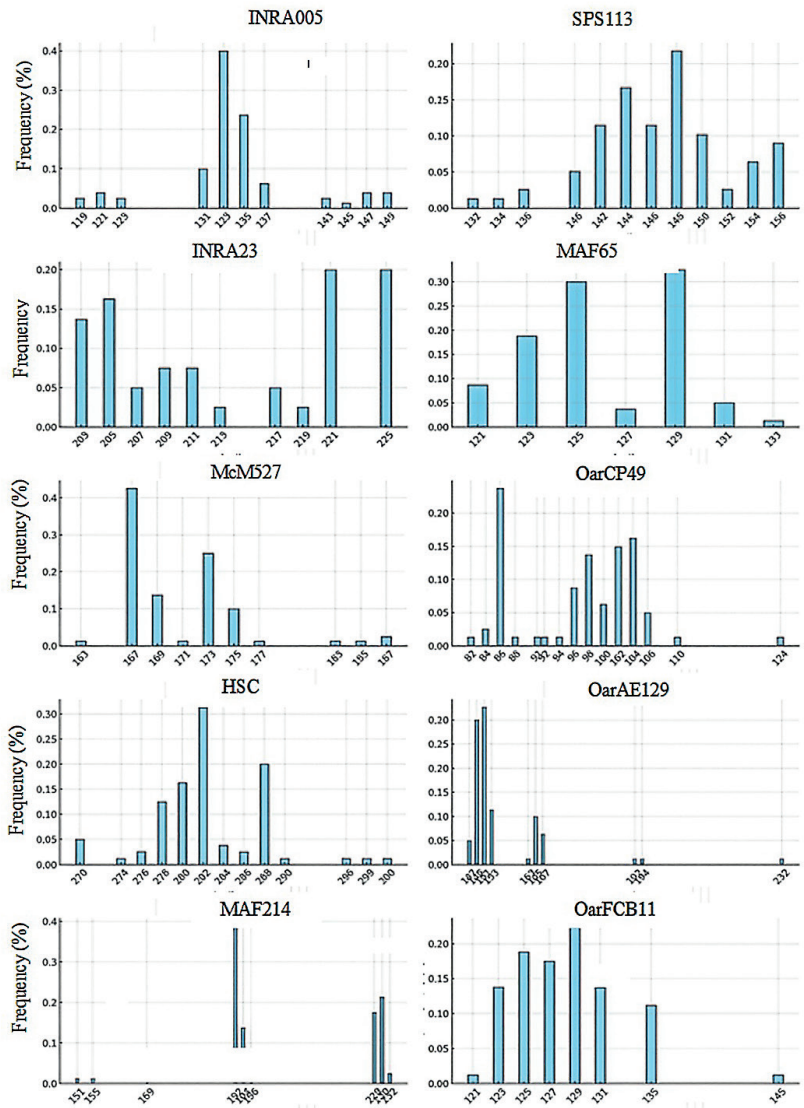

Fig. 4. Allele frequencies in the loci of Karakul sheep of the "Nisporeni" population
Source: Authors' own elaboration

zygosity of the population is 71.43%, which is a high indicator and reflects good genetic health and adaptive potential.

For the "Anenii Noi" population, the allele frequencies are shown in Figure 3. High-frequency alleles (frequency > 20%) include 125 (*INRA005*), 140, 156 (*SPS113*), 215 (*INRA23*), 129 and 123 (*MAF65*), 169 (*McM527*), 92 (*OarCP49*), 278 (*HSC*), 151 (*OarAE129*), 192 (*MAF214*) and 141 (*OarFCB11*) is not agree.

These alleles may indicate a selective advantage or genetic drift in a small population. The average observed heterozygosity (Ho = 0.5664) is lower than expected (He = 0.704–0.923), and positive values of the fixation index (F) for most loci (for example, 0.645 for *MAF214*) indicate a deficiency of heterozygotes, possibly due to inbreeding (Table 2).

In the "**Nisporeni**" population, allele frequencies (Figure 4) demonstrate high genetic diversity: 11 alleles at the *INRA005* locus and 15 at *OarCP49* **locus**. Dominant alleles, such as **133 (40%)** at *INRA005* and **167 (42.5%)** at *McM527*, contrast with rare alleles (frequency < 5%), indicating heterogeneity in the genetic structure.

The population is close to Hardy-Weinberg equilibrium at loci *INRA23* (F = 0.068) and *OarCP49* **(**F = 0.013**)**. The average observed heterozygosity (Ho = 0.613) is lower than the expected heterozygosity (He = 0.728–0.871), and the high fixation index for *SPS113* (F = 0.618) and *OarFCB11* (F = 0.609) confirms possible inbreeding (Table 3). However, negative F values at the MAF65 (-0.057) and *HSC* (-0.106) loci indicate an excess of heterozygotes, which may be due to negative assortative mating or migration.

Genetic distances and phylogenetic relationships

Genetic distances calculated using the Nei coefficient (Nei, 1972) show that the populations of "Anenii Noi" and "Riscani" have the greatest genetic proximity (D = 0.132, 86.8% similarity), and "Anenii Noi" and "Nisporeni" have the greatest genetic divergence (D = 0.265, 73.5% similarity). The distance between "Nisporeni" and "Riscani" was 0.160 (similarity 84.0%) (Table 4).

These data, visualized by the dendrogram (Figure 5), confirm the close relationship of "Anenii Noi" and "Riscani", which combine into a cluster, "Nisporeni" joins this cluster later, indicating its great genetic distance.

Table 4. Genetic distances (D) between populations of Karakul sheep

Population	Anenii Noi	Nisporeni	Riscani
Anenii Noi	0.000	0.265	0.132
Nisporeni	0.265	0.000	0.160
Riscani	0.132	0.160	0.000

Source: Authors' own elaboration

The results obtained indicate that "Anenii Noi" is genetically closer to "Riscani" than to "Nisporeni", which may be due to differences in breeding or geographical isolation.

The genetic structure of populations suggests the presence of two main groups: "Anenii Noi" and "Riscani." They form a closely related cluster, and Nisporen occupies an intermediate position.

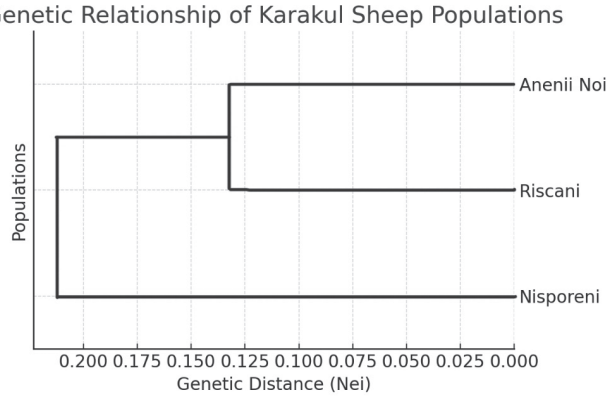

Fig. 5. Genetic relatioship of Karakul sheep populations
Source: Authors' own elaboration

Discussion

The high level of heterozygosity in the "Riscani" (71.43%) and "Nisporeni" (61.3%) populations indicates significant genetic diversity, which is a positive factor for resilience and breeding potential. This aligns with the findings of Mihailova et al. (2023) for Bulgarian autochthonous breeds (Ho = 0.67–0.78). Similarly, Gencheva et al. (2017) reported about Ho = 0.65–0.73 for Bulgarian breeds, highlighting the influence of local conditions in maintaining heterozygosity. In contrast, the "Anenii Noi" population (Ho = 0.5664) shows a heterozygote deficiency, which may be related to the small population size and intensive selection, as noted in Gaouar et al. (2016) for Moroccan breeds (Ho = 0.58–0.68). Low heterozygosity at the OarFCB11 locus across all three populations (especially in Riscani and Nisporeni), combined with a high fixation index, suggests the influence of inbreeding.

The genetic proximity between "Anenii Noi" and "Riscani" (D = 0.132) can be explained by the use of Botoșani Karakul rams (Romania) in the "Riscani" population. The Botoșani Karakul, developed by crossing Țurcana with Asian Karakul to improve pelt quality (Taftă and Ștefănescu, 1958; Drăgănescu et al., 1970), introduced genes responsible for silkiness and shine in wool, which likely enhanced its similarity to "Anenii Noi" – a new Moldovan type developed for similar purposes (Buzu, 2023). This also confirms

historical data on breeding practices in the region, where Botoșani rams were used to improve fleece quality (Buzu, 2023). These findings are consistent with those of Tapio et al. (2010), where genetic distances between closely related populations ranged from 0.10 to 0.15.

The significant divergence between "Anenii Noi" and "Nisporeni" (D = 0.265) is due to the influence of the indigenous "Țușca" (also known as Tsushca) breed on the "Nisporeni" population. "Țușca", known for its resilience to local climatic conditions and coarse wool (Buzu, 2012), likely contributed genetically, enhancing the genetic diversity in the "Nisporeni" population (11–15 alleles in key loci). This is confirmed by the studies of Taftă and Ștefănescu (1958, 1960), where crossing Țurcana with Karakul resulted in hybrids with unique morpho-productive traits, and by Drăgănescu et al. (1970), who emphasized the genetic heterogeneity of such populations. Genetic distances similar to those observed (0.20–0.30) have also been reported by Da Silva et al. (2014) for locally adapted breeds.

The high heterozygosity in the Riscani population likely correlates with pelt quality, which corresponds to the description of the Botoșani Karakul (Taftă and Ștefănescu, 1958), while Nisporeni retains the traits of Țușca resilience and robustness but with less valuable pelts (Buzu, 2021).

The low heterozygosity in the "Anenii Noi" population underscores the need for inbreeding monitoring, as suggested by Buzu (2012). Insights from Karakul breeding in Southwest Africa, as discussed in Fillingger's (1975) "60 Years of Karakul Sheep Breeding in Southwest Africa," reflect a global perspective on Karakul selection, where adaptation to extreme conditions parallels the influence of Țușca on the "Nisporeni" population.

The results obtained complement studies of local breeds (Mihailova et al., 2023) and historical work on Țurcana – Karakul crossbreeding (Taftă and Ștefănescu, 1960; Drăgănescu and Sandu, 1972). They underscore the unique genetic structure of Moldovan Karakul populations and their breeding potential. They also demonstrate the successful consolidation of the new Moldovan type "Anenii Noi", while preserving genetic diversity, as well as the potential of classical populations (Riscani and Nisporeni) for further breed improvement. The analysis of high-frequency alleles and heterozygosity provides valuable insights for breeders, enabling the optimization of breeding programs and the maintenance of genetic health in the populations.

Conclusions

A study of the genetic structure of three populations of Karakul sheep (Anenii Noi, Nisporeni, Riscani) revealed significant genetic diversity, especially in the "Riscani" (Ho = 71.43%) and "Nisporeni" (Ho = 61.3%) populations, which highlights their potential for breeding and sustainability. The smallest genetic distance between "Anenii Noi" and „Riscani" (D = 0.132) indicates their close relationship, confirming the hypothesis of a genetic flow, caused by migration of animals between these populations, while the large divergence of „Nisporeni" (D = 0.265) reflects differences in breeding processes and a lower impact of migration.

The high frequency of alleles, such as 129 (*MAF 65*) and 153 (*OarAE129*), can serve as a marker of adaptive traits, which is important for science and breeding. The practical significance lies in the recommendation of interbreeding between remote populations to preserve genetic diversity and improve the productivity of the breed.

Acknowledgments

This investigation was supported by the Ministry of Education and Research of Moldova for the project: SVR@ SZV/220101 "*Scientific Support for the Assessment of Resources in the Zoo-Veterinary Field: Selection and Adaptation of New Breeds and Hybrids Using Inoffensive Curative Technologies and Methods under Conditions of Climatic Resilience*"

References

Applied Biosystems. (2006). *GeneMapper software version 4.0: User guide.* Applied Biosystems.

Ayala, F. J. & Kiger, J. A. (1988). *Modern genetics*, *3*. Mir Publishing.

Boettcher, P., Ajmone-Marsan, P. & Lenstra, J. A. (2011). *Molecular genetic characterization of animal genetic resources.* Food and Agriculture Organization. FAO.

Buchanan, F. C. & Crawford, A. M. (1993). Ovine microsatellites at the OarFCB11, OarFCB128, OarFCB193, OarFCB266 and OarFCB304 loci. *Animal Genetics*, *24*(3), 145 – 146. https://doi.org/10.1111/j.1365-2052.1993.tb00278.x.

Buzu, I. (2012). *Type of sheep Karakul Moldovenesc Corpolent: theory and practice of creation and improvement* (monograph) (Ro).

Buzu, I. (2021). *Evaluation of the quality of Moldavian Karakul lambs: Scientific-methodical monograph.* Tipografia Centrală (Ro).

Buzu, I. (2023). *Theory and practice of creating new types (breeds) of Karakul and Tsigaie sheep.* Print-Caro (Ro).

Da Silva, E., McManus, C., Guimarães, M., Facó, O., Pimentel, D., Caetano, A. & Paiva, S. (2014). Validation of a microsatellite panel for parentage testing of locally adapted and commercial goats in Brazil. *Genetics and Molecular Biology*, *37*(1), 54 – 60. https://doi.org/10.1590/S1415-47572014000100010.

Deniskova, T. E., Dotsev, A. V., Selionova, M. I. & Zinovieva, N. A. (2018). Population structure and genetic diversity of 25

Russian sheep breeds based on whole-genome genotyping. *Genetics Selection Evolution*, *50*(1), Article 29. https://doi.org/10.1186/s12711-018-0399-5.

Drăgănescu, C., Petrescu, R. & Petrescu, G. (1970). Preliminary data on the quality of pelts in crosses between Black Karakul and Țurcană breed. *IANB Lucrări Științifice*, *13*(D Zootehnie), 245 – 253 (Ro).

Drăgănescu, C. & Sandu, G. (1972). Genetic analysis of Dulbanu Karakul]. *IANB Lucrări Științifice, 15*(D Zootehnie), 103 – 109 (Ro).

Fillingger, O. K. (1975). 60 years of Karakul sheep breeding in Southwest Africa. In *Karakul sheep breeding abroad,* 30 – 34. Kolos (Ru).

Gaouar, S. B. S., Kdidi, S. & Ouragh, L. (2016). Estimating population structure and genetic diversity of five Moroccan sheep breeds by microsatellite markers. *Small Ruminant Research, 144*, 299 – 306. https://doi.org/10.1016/j.smallrumres.2016.07.021.

Gencheva, D., Metodiev, S., Nikolov, V., Vassilev, D. & Todorovska, E. (2017). Genetic variation of Bulgarian autochthonous sheep breeds using microsatellite markers. *Genetika, 49*(1), 247 – 258. https://doi.org/10.2298/GENSR1701247G.

Hartl, D. L. & Clark, A. G. (1997). *Principles of population genetics* (3rd ed.). Sinauer Associates.

Hoda, A. & Ajmone-Marsan, P. (2012). Genetic characterization of Albanian sheep breeds by microsatellite markers. In R. Bouche, A. Derkimba, & F. Casabianca (Eds.), *New trends for innovation in the Mediterranean animal production,* 128 – 132. Wageningen Academic Publishers.

Kijas, J. W., Lenstra, J. A., Hayes, B. & Dalrymple, B. P. (2012). Genome-wide analysis of the world's sheep breeds reveals high levels of historic mixture and strong recent selection. *PLOS Biology, 10*(2), Article e1001258. https://doi.org/10.1371/journal.pbio.1001258.

Liu, K. & Muse, S. V. (2005). PowerMarker: An integrated analysis environment for genetic marker analysis. *Bioinformatics, 21*(9), 2128 – 2129. https://doi.org/10.1093/bioinformatics/bti282.

Maddox, J. F., Davies, K. P., Crawford, A. M., Hulme, D. J., Vaiman, D., Cribiu, E. P. & Eggen, A. (2001). An enhanced linkage map of the sheep genome comprising more than 1000 loci. *Genome Research, 11*(7), 1275 – 1289. https://doi.org/10.1101/gr.1350r.

Mihailova, Y., Rusanov, K., Rusanova, M., Vassileva, P., Atanassov, I., Nikolov, V. & Todorovska, E. (2023). Genetic diversity and population structure of Bulgarian autochthonous sheep breeds revealed by microsatellite analysis. *Animals, 13*(11), Article 1878. https://doi.org/10.3390/ani13111878.

Nei, M. (1972). Genetic distance between populations. *The American Naturalist, 106*(949), 283 – 292. https://doi.org/10.1086/282771.

Penty, J. M., Henry, H. M., Ede, A. J. & Crawford, A. M. (1993). Ovine microsatellites at the OarAE16, OarAE54, OarAE57, OarAE119 and OarAE129 loci. *Animal Genetics, 24*(4), 219 – 220. https://doi.org/10.1111/j.1365-2052.1993.tb00304.x.

Sambrook, J. & Russell, D. W. (2001). *Molecular cloning: A laboratory manual* (3rd ed.). Cold Spring Harbor Laboratory Press.

Taftă, V. & Ştefănescu, C. (1958). Results on crossing white Țurcană with Karakul and Karakul crossbred rams]. *Analele Institutului de Cercetări Zootehnice*, 16, 231 – 235 (Ro).

Taftă, V. & Ştefănescu, C. (1960). New data on crossing white Țurcană ewes with gray rams. *Analele Institutului de Cercetări Zootehnice*, *18*, 156 – 159 (Ro).

Tamura, K., Stecher, G. & Kumar, S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution, 38*(7), 3022 – 3027. https://doi.org/10.1093/molbev/msab120.

Tapio, M., Ozerov, M., Tapio, I. & Kantanen, J. (2005). Microsatellite-based genetic diversity and population structure of domestic sheep in northern Eurasia. *BMC Genetics, 6*, Article 27. https://doi.org/10.1186/1471-2156-6-27.

Tapio, M., Ozerov, M., Tapio, I. & Kantanen, J. (2010). Microsatellite-based genetic diversity and population structure of domestic sheep in northern Eurasia. *BMC Genetics, 11*, Article 76. https://doi.org/10.1186/1471-2156-11-76.

Received: **April, 28, 2025**; *Approved*: **May, 28, 2025**; *Published*: **February, 2026**