Assessment of genetic diversity in the Tuvan downy goat breed based on microsatellite DNA loci analysis

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ABSTRACT

The study aims to assess the genetic diversity of the Tuvan cashmere goat breed, patented and registered in the State Register of Protected Breeds of the Russian Federation in 2024. The study was conducted using 14 microsatellite loci recommended by the International Society of Animal Genetics (ISAG). Microsatellite markers remain an important tool in population genetics due to their high polymorphism, reproducibility, and low cost. Their use in animal breeding covers tasks ranging from gene pool conservation to identifying links with economically valuable traits. The Tuvan cashmere goat breed is a unique genetic resource of Siberia, adapted to extreme climatic conditions. The wool of goats of this breed is distinguished by a high content (82–91%) of fine (16–18 μm) down fibers. Genetic studies of this new breed remain limited, making this research relevant. The study was conducted on a sample of 337 animals selected in the Republic of Tuva. A high genetic diversity of the population was established: the total number of alleles was 104, the average number of alleles per locus was 7.43. The most polymorphic loci were SRCRSP23 (11 alleles), MAF065 and SRCRSP5 (10 alleles each). The values of observed and expected heterozygosity were 0.722 and 0.715, respectively. The inbreeding coefficient (Fis = -

0.010) indicates the absence of inbreeding depression in the studied population. The obtained results characterize the Tuvan cashmere goat breed as a valuable genetic resource with a high level of genetic diversity and a stable population structure. The identified highly polymorphic markers are recommended for use in breed genetic monitoring programs and selection programs.

Keywords: Tuvan downy goat breed, Microsatellite markers, Genetic diversity, Heterozygosity, Inbreeding coefficient, Conservation of genetic resources

INTRODUCTION

Today, traditional methods alone are no longer sufficient to ensure rapid progress in goat breeding, making it necessary to integrate modern DNA technologies into selection programs. These technologies significantly enhance the efficiency of livestock breeding (Deniskova et al. 2020; Koshkina, Deniskova & Zinovieva 2020). Microsatellites (STRs, or short tandem repeats) are found in virtually all living organisms. They consist of tandemly repeated sequences and are located mainly in non-coding, and less frequently in coding, regions of the genome (Zinovieva, Popov & Ernst 1998; Kalashnikova, Dunin & Glazko 2000). Microsatellite loci are ideal genetic markers for a wide range of genetic studies due to their high polymorphism, codominant inheritance, and ease of genotyping via polymerase chain reaction (PCR). They are commonly used in livestock genetic testing for parentage verification, breed and line identification, genetic structure analysis, estimation of genetic distances between groups, and assessment of gene flow within and between populations (Groeneveld et al. 2010). Currently, microsatellites are widely applied in studies of genetic diversity, phylogenetic relationships, and population structure both within and among livestock breeds. They are also effective in detecting genetic differences between animals at the individual level. Genetic testing using microsatellites can verify parentage from DNA extracted from semen, blood, tissue, and other biological materials (Khabibrakhmanova et al. 2019). The relevance of microsatellite studies in animal husbandry lies in their universality, low cost, and broad applicability, from biodiversity conservation to livestock breeding, including goat improvement programs (Mekuriaw et al. 2016; Selionova et al. 2021). Population-genetic studies using microsatellite markers have been successfully conducted for various goat breeds worldwide (Seilsuth et al. 2016; Beketov et al. 2021), as well as for breeds raised in Russia, enabling evaluation of both genetic diversity and degrees of differentiation (Kharzinova et al. 2019; Selionova et al. 2020).

The Tuvan downy goat breed is a unique genetic resource developed through long-term within-breed selection of the indigenous goat population of the Republic of Tuva. These animals are distinguished by exceptional adaptation to the region's sharply continental climate (Figs. 1, 2). A multi-stage selection program, based on body weight, and the quantity and quality of down fiber, along with carefully planned mating, produced highly productive herds that combine improved performance traits with preserved adaptive potential. A key distinguishing feature of the breed (which received a patent as a breeding achievement in 2024) is its record-high proportion of down fibers in the fleece (82–91%) (Irgit et al. 2022). By this measure, Tuvan downy goats significantly outperform other Russian downy breeds: Dagestan White (65–80%), Gorno-Altai (65–75%), and Orenburg (45–50%) (Musalaev & Palaganova 2013; Nurzhanov 2007). The main breeding area of the Tuvan goats is the dry steppe zones of southern Tuva, characterized by extreme temperatures, down to , 58 °C in winter and up to +40 °C in summer. These climatic conditions are a key factor in the formation of fine down fibers of the cashemir type, which are highly valued in the global market (Fig. 3). The live weight of bucks averages 67.5 kg (up to 74.0 kg), and does average 45.9 kg (up to 53.5 kg), with low coefficients of variation (4.16–8.04%), indicating high herd uniformity (Irgit et al. 2023a). The animals have a compact build and strong constitution, with pronounced sexual dimorphism (Yuldashbaev et al. 2022). The breed also demonstrates good meat productivity: carcass weight of wethers reaches 11.04 kg at 8 months and 14.48 kg at 17.5 months (Amerkhanov et al. 2019; Irgit et al. 2021; Irgit et al. 2023). The down fibers of young animals meet the "downy" standard, while those of adult goats correspond to the "cashgora" type, with yields of 347–600 g and fiber lengths of 5.05–7.02 cm, meeting the requirements of the Russian processing industry (Irgit et al. 2018; Irgit et al. 2023a, 2023b; GOST 2260-2006).

Fig. 1. Buck – sire of the Tuvan downy breed.

Fig. 2. Doe of the Tuvan downy breed.

Fig. 3. Fleece of the Tuvan downy goat.

MATERIALS AND METHODS

Blood samples were collected from 337 Tuvan downy goats at APCC *Uurgay* in the Erzinsky District of the Republic of Tuva to study STR markers and evaluate genetic diversity. Blood was drawn into tubes containing the anticoagulant K_2-EDTA, in accordance with veterinary and ethical guidelines. In the DNA Technologies Laboratory of the Federal State Budgetary Scientific Institution All-Russian Research Institute of Animal Husbandry, leukocytes were isolated from whole blood by centrifugation and washed with EDTA and TBE buffer. DNA was then extracted from the leukocytes using the DNA-Extran-2 reagent kit (Scientific and Production Center *Syntol*, Moscow), following the manufacturer’s instructions. Microsatellite analysis was performed at 14 ISAG-recommended loci (INRA006, ILSTS87, ILSTS008, CSRD247, OarFCB20, ILSTS19, INRA063, SRCRSP8, MAF065, SRCRSP5, McM527, SRCRSP23, INRA005, INRA023) using the COrDIS Goat test system (LLC GORDIZ, Moscow), designed for multiplex PCR-based kinship testing and DNA identification in goats. PCR amplification followed the COrDIS Goat protocol. The PCR products were analyzed on an ABI 3130 capillary sequencer (Applied Biosystems, Japan) using POP-4 polymer (*Syntol*, Moscow), with the standard size marker GS500-ROX and a control sample included. Electrophoresis results were automatically processed with GeneMapper software, and the data were exported in Excel (XLS) format. Genetic diversity parameters for each microsatellite locus were calculated using GenAlEx 6.5 (Peakall & Smouse 2012).

RESULTS AND DISCUSSION

Table 1 summarizes the findings of the genetic analysis of 337 Tuvan downy goats**.** All 14 microsatellite loci examined were polymorphic, indicating genetic diversity in the studied population of Tuvan downy goats at the STR loci. The number of alleles detected varied by locus: the lowest number of alleles (3) was observed at INRA005, while the highest (11) occurred at SRCRSP23. Allele frequencies were unevenly distributed, most loci had one or several major alleles with high frequency, while the remaining alleles were minor and occurred less frequently. A clear example is ILSTS008**,** where allele 176 had a dominant frequency of 61.6%; similarly, at ILSTS19, allele 150 occurred at a frequency of 67.1%. A significant number of alleles were very rare (frequency <1%), such as OarFCB20-107 (0.1%), MAF065-123 and 135 (0.3%), and CSRD247-236 and ILSTS008-182 (0.4%). These rare alleles are important for assessing the uniqueness of the gene pool and serve as markers of genetic dynamics within the population.

To provide a comprehensive evaluation of the breed's genetic status, genetic diversity parameters were calculated (Table 2).

A high level of polymorphism was confirmed, with an average of 7.429 alleles per locus. The mean effective number of alleles (Ne = 3.758) indicates that the actual genetic diversity is somewhat lower due to the presence of dominant alleles. The loci contributing most to diversity were INRA006 **(**Ne = 4.917) and MAF065 **(**Ne = 4.675), while the lowest contribution was observed at ILSTS19 (Ne = 2.094). The average expected heterozygosity (He = 0.715) and observed heterozygosity (Ho = 0.722) values indicate a high level of genetic variation in the studied population. The close agreement between Ho and He, along with an average inbreeding coefficient near zero (F = –0.010), suggests that the population is generally in Hardy–Weinberg equilibrium. A statistically significant heterozygote deficit was observed at a few loci (SRCRSP5**,** F = +0.157; INRA005**,** F = +0.123), which may result from selection, population substructure, or the presence of null alleles. For most loci, negative Fis values were recorded, indicating an excess of heterozygosity in the population, likely due to balancing selection, which maintains genetic diversity by favoring heterozygous genotypes.

The average number of alleles with a frequency ≥5% was 4.571 per locus, reflecting the substantial contribution of common alleles to the genetic structure of the population. At the same time, the high number of private (unique) alleles (7.429) underscores the genetic distinctiveness and differentiation of this population compared to other groups (Fig. 4).

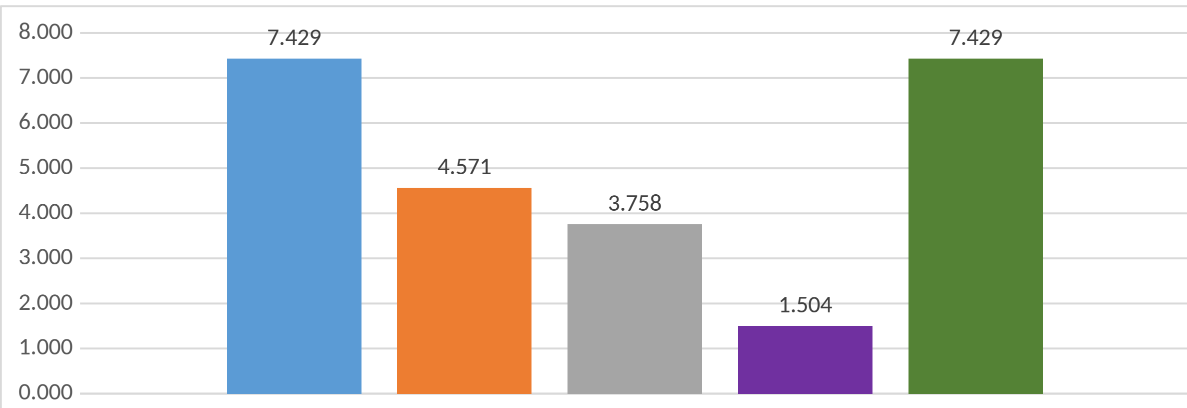

Fig. 4. Key genetic polymorphism parameters of the Tuvan downy goat population (n = 337) across 14 microsatellite loci. Na – mean number of alleles; Na Freq ≥5% – mean number of alleles with a frequency ≥5%; Ne – effective number of alleles; I – Shannon's index; No. Private Alleles – frequency of private (unique) alleles.

A comparative analysis with other goat breeds from Russia (Kharzinova et al. 2019) and Mongolia (Beketov et al. 2021) showed that the Tuvan downy goat exhibits a comparable or higher level of genetic diversity (Table 3). The comparative analysis of genetic diversity in the Tuvan downy goat breed versus Russian and Mongolian breeds provided valuable scientific insights. It was established that Tuvan downy goats are characterized by a high level of genetic diversity (Na = 7.43; He = 0.715; Ho = 0.722; see Table 3). Based on the set of studied parameters, the breed significantly surpasses Alpine goats (Na = 5.80; He = 0.671; Ho = 0.635) and is only slightly inferior to the Soviet Wool breed (Na = 8.20; He = 0.748; Ho = 0.744). The inbreeding coefficient (Fis = –0.010) is of particular importance, indicating the absence of inbreeding depression in the studied population. In comparison with Mongolian populations, Tuvan downy goats exhibit a similar level of observed heterozygosity (Ho = 0.722 vs. 0.713–0.740), but a slightly lower mean number of alleles (Na = 7.43 vs. 7.50–8.75).

CONCLUSION

The Tuvan downy goat population demonstrates high genetic diversity, is free from inbreeding, and can be regarded as both stable and genetically healthy. Loci showing consistent deviations from Hardy–Weinberg equilibrium, most notably SRCRSP5 and **I**NRA005, warrant closer attention in the design of breeding programs and may help identify markers linked to economically valuable traits; further research is recommended. These results align with earlier findings (Kharzinova et al. 2019; Beketov et al. 2021) and underscore the value of the Tuvan downy goat as an important genetic resource for future breeding efforts. Preserving this unique gene pool, characterized by broad allelic diversity and resilience against inbreeding, should be a top priority for the sustainable development of goat husbandry in Siberia.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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