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Maternal genetic diversity of the Red-faced sheep of Covasna based on the mtDNA control region

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Abstract

One of the variants of the Tsigai sheep is the reddish-headed and -legged, small-sized, so-called Redface Covasna. In 2014, the Institute for Farm Animal Gene Preservation brought in a small herd of these from Transylvania with the aim of national conservation of the breed. The aim of the study is to characterize the maternal background and maternal genetic diversity of the Red-faced sheep of Covasna. For this, the nucleotide sequence of the control region (CR) of the mitochondrial genome (mtDNA) was used. The sampling was achieved from 34 individuals 2021 and 2022. The length of the aligned CR sequences is 621 bp with the number of polymorphic base sites 88. The number of haplotypes is 30 (from haplogroups A and B, which proves its origin in Asia Minor). The value of haplotype diversity (H_d) is 0.991, and its variance is 0.010. The mean of the nucleotide diversity (π) and the average nucleotide difference per pair (k) in the investigated flock are $21.27*10^{-3}$ and 13.21, respectively. The value of the Tajima D test (-1.4427, $p > 0.10$) does not indicate a lack of alleles, nor does it indicate a narrowing of the genetic diversity occurring in the history of the population.

Keywords: bimodal mismatch distribution, maternal origin, breed variant

Introduction

After RODICZKY (1904), the Tsigai (cigája or berke) sheep first came to the historical territory of our country at the end of the 18th century (1792). The demand

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of the domestic post factories and Brassó's (Brașov, Kronstadt) flourishing wool trade encouraged the farmers of the Transylvanian counties to replace their herds of coarse woolly Țurcanăs (gyimesi racka) with finer (albeit 31-42 micron, "crossbred") wool-producing Tsigai (ESZES and GÁSPÁRDY, 1997). It was SZENTKIRÁLYI (1885) who was the first and corrected to state that the fleece of the Tsigai sheep consists entirely of wool fibres; therefore, it is not a mixed wool sheep, as many people mistakenly claimed.

According to SCHANDL (1941), "sharply defined sub-breeds and ecotypes are not usually distinguished in the Tsigai. Nevertheless, it can be established that the nutritive power of the nugget makes its impact felt". The existence and different origin of the Tsigai became aware of those who dealt with the breed later (VERESS, 1996; DUNKA, 1997; KESZTHELYI, 1997; TÓTH, 1997; KÓSA, 1998).

The colour variants of the Tsigai breed can be separated based on the colour of the fleece and the short hairs. According to the colour of the head and legs, individuals with black, dark brown, light brown, yellowish-red, white and variegated (spotted) heads and legs were distinguished among the white fleecy Tsigais. Spots were more common on the legs than on the head. In the course of history, two basic colour variants of the white-wool Tsigai, also belonging to the mountain type, spread in the Transylvanian parts.

One of them, on SZENTKIRÁLYI's (1923) unifying proposal, is the variant with a completely black head and legs. This spread in other Hungarian historical areas: the Uplands, the South, the Great Plains and, to an extent, the Transdanubia. Several more sub-variants were separated in the Southern Region: the triple-purpose but meatier variant of Čoka (csókai) and the dairy variant (with a milk production of over 100 litres) of Sombor (zombori). Further on, there were the ancient variants, the Tsigai Rumska (árpatarlói cigája; ULMANSKI, 1922) which is now extinct and most reminiscent of Transylvanian Tsigai, and the large-bodied, also with a high yield of milk, the Doroslovo Tsigai (doroszlói cigája; KOVACS, 2000). All of them have chocolate brown or black head and legs, and white fleece. In the territory of today's Hungary, it is worth distinguishing two sub-varieties according to origin: one is the lowland one, which shows the greatest similarity with the variant of Čoka, and the other is the mountain one, which entered Jákotpuszta from the Highlands three decades ago. Almost without exception, they have white fleece and dark brown or black heads and legs.

The other one, is the brown-faced and short-legged, so-called Covasna Tsigai (kovásznai vörösképű cigája, in Hungarian; ruğine, rusty or red-faced, in Romanian; KUKOVICS, 2006). The first, most classic and almost yellow individuals of this variant were the Tsigais from Hétfalus. Later, HAMMOND et al. (1961) also mention the reddish head but white or variegated wool and the completely black Romanian Tsigais.

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The variants of the Tsigai breed group in other countries have very different names, for example, Karbanat in Bulgaria, Ruda in Albania, and Kivircik in Turkey. These are now not detailed, just like the single purpose dairy-type Tsigais.
In the last decades, the yellow-headed colour variants of the Tsigai native to Covasna and Hargitha counties (BODNÁR et al., 2021) have entered our country. Since 2016, the Hungarian Sheep and Goat Breeding Association has treated them as a standalone breed (thus separated from the black-headed Hungarian native Tsigai) under the name Yellow-headed Berke.
The aim of the study is to characterize the maternal background and maternal genetic diversity of the Red-faced sheep of Covasna. For this, the nucleotide sequence of the control region (CR) of the mitochondrial genome (mtDNA) is used. It is assumed that as an enclosure culture, it can serve with a specific genetic pattern left over from the past. With these results, the genetic identification of the haplotypes (families) of this ecotype is revealed and their successful maintenance is established.

Material and methods

In 2014, the Institute for Farm Animal Gene Preservation (HGI) brought in a small herd of Red-faced sheep of Covasna from Transylvania with the aim of national conservation of the breed. The sampling was achieved from 34 individuals (20 ewes and 14 rams) 2021 and 2022. Of these, 21 come from the HGI's "ex situ" herd (Gödöllő), which were imported from Transylvania. The other 13 samples were collected in Transylvania from 4 different "in situ" herds.
The blood samples were taken from the jugular vein in EDTA blood collection tubes, then the blood inhibited in coagulation was transferred into Eppendorf tubes and frozen at -20 °C the same day. The blood samples were taken from the selected animals as part of routine veterinary procedures for the detection of infectious diseases (e.g. Ovine brucellosis). The further use of these samples obtained during general clinical veterinary procedures for research purposes according to Act XXVIII of 1998 and Government Decree no 40/2013 does not qualify as animal testing, so ethical permission was not necessary.
DNA was extracted and purified using the GenElute Blood Genomic DNA kit (Sigma-Aldrich, St. Louis, MO, USA). A previously described primer pair was used to amplify the segment to be examined (HIENDLEDER et al., 1998). The programmable Thermal Cycler 2720-type PCR equipment (Applied Biosystem, Waltham, MA, USA) was used to amplify the DNA sequence. The PCR products were cleaned with the SIGMA GenElute™ PCR Clean Up Kit (Sigma-Aldrich, St. Louis, MO, USA) following the protocol. For the sequencing reaction, a BigDye® Terminator version 3.1 Cycle Sequencing Kit (ThermoFisher Scientific, Waltham, MA, USA) was used as recommended by the manufacturer. The ABI Prism 3130XL Genetic Analyzer (Applied Biosystems, ThermoFisher Scientific, Waltham, MA,

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USA) was used to analyse and detect the sequence data, according to the manufacturer's instructions. Sequence data were analysed using Sequencing Analysis Software 5.1 (Applied Biosystems, ThermoFisher Scientific, Waltham, MA, USA) and subsequently aligned using SequencherTM 4.1.2 software (Gene Codes Corp, Ann Arbor, MI, USA).
The sequences were evaluated using the DnaSP 6.0 software (ROZAS et al., 2017). The samples were sorted into haplogroups based on the GenBank reference samples (A-HM236174, B-HM236176, C-HM236178, D-HM236180, E-HM236182 (MEADOWS et al., 2005).

Results and discussion

The length of the aligned CR sequences is 621 bps. In the entire study sample, the number of monomorphic base sites was 533, while the number of polymorphic base sites was 88 (with singleton variable sites and parsimony informative sites 35 and 53, respectively).
The number of haplotypes is 30. This means that the 34 individuals almost without exception represented their own haplotype. Eight individuals, which are identical shared a pairwise haplotypes (that is, belonging to the same maternal line, not necessarily siblings). Individuals can be classified into two haplogroups. The presence of haplogroups A and B proves the origin of the Red-faced sheep of Covasna in Asia Minor.
The haplotypes of the individuals transported to Hungary match well with the haplotypes of the Transylvanian animals, in some cases falling into the same haplotype. The haplotype distribution of the Hungarian individuals therefore represents the breed well and is a basis for preserving genetic diversity.

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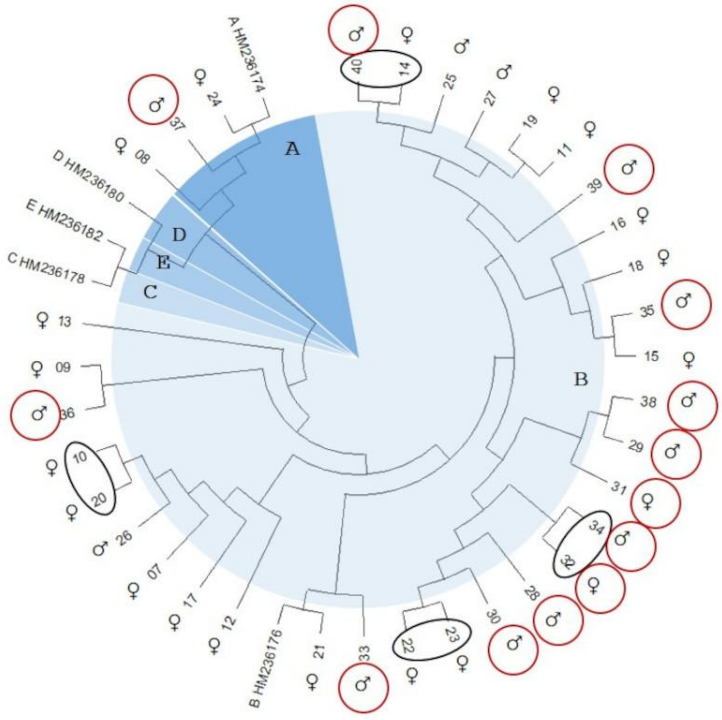

Figure 1. Composition of Red-faced sheep of Covasna according to CR haplotype and haplogroup, with GenBank control haplogroups ((A-HM236174, B-HM236176, C-HM236178, D-HM236180, E-HM236182; MEADOWS et al., 2005). The solid line connects individuals belonging to the same haplotype and are genetically identical. The individuals circled in red live in Transylvania.

The value of haplotype diversity (Hd) is 0.991, and its variance is 0.010. The mean of the nucleotide diversity (π) and the average nucleotide difference per pair (k) in the investigated flock are 21.27*10-3 and 13.21, respectively. Nevertheless, the nucleotide difference is around 10 in most of the herd, and around 35 in the remainder (Figure 2). The dashed line represents the expected distribution for a constant stock size; its course is moderate. Points connected by a solid line reflect a bimodal observed distribution. The distinct peaks indicate that there are two dominant groups of haplotypes associated with a relatively constant population size of the Red-faced sheep of Covasna over time.

The D* and F* tests of FU and LI performed on the entire test sample did not give significant results, -1.1246 ($p > 0.10$) and -1.4632 ($p > 0.10$), respectively. Unlike these, the value of the FU's Fs statistic of -13.813 was significant ($p < 0.001$). The value of the Tajima D test (-1.4427, $p > 0.10$) does not indicate a lack of alleles, nor

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does it indicate a narrowing of the genetic diversity occurring in the history of the population.

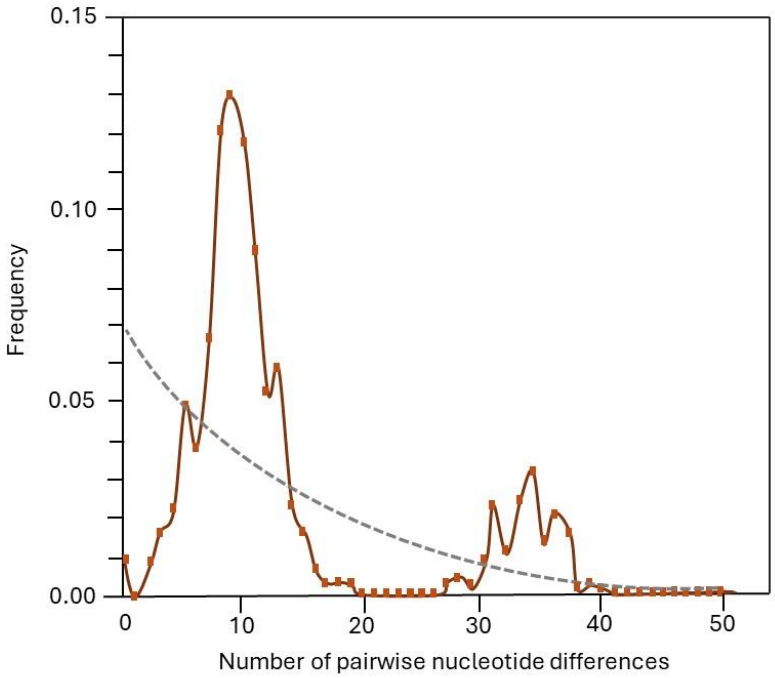

Figure 2. Frequency distribution of the number of sequence mismatches between pairwise combinations of CR haplotypes of Red-faced sheep of Covasna

Conclusion and recommendation

The first such processing of the Red-faced sheep of Covasna was successfully carried out. The haplogroups A and B determined prove the historically known origin of the Tsigai in Asia Minor, including the Red-faced sheep of Covasna. The breed came to Hungary from Turkey via the Balkans. Haplogroup C has been identified in other Hungarian native sheep breeds. Thus, the breeds like Cikta (GÁSPÁRDY et al., 2022) and Yellow-faced sheep of Kecskemét (TULLY et al., 2023) have a more complex maternal background.

The number of haplotypes in the studied population is high. This means that almost every individual in the flock belongs to a different maternal lineage, i.e. the maternal genetic background is advantageously diverse. The pedigree of the imported stock is incomplete; therefore, knowledge of the maternal background is essential for

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pairings. Haplotype diversity (Hd) is also high. These observations can also be paralleled by the strongly negative value of Fu's Fs statistic, which indicates a remarkable excess number of alleles. Together, these facts create the possibility of maintaining the genetic diversity of the breed.

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