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Maternal diversity of the Yellow-faced sheep of Kecskemét based on the mtDNA control region

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Abstract

Regarding the Yellow-faced sheep of Kecskemét or Sand-sheep, known as a variant of Yellow-faced Berke sheep, very little research has been undertaken to date and the small population, which is mainly found in the southern region of Hungary, is at risk of extinction. In this study blood samples from Yellow-faced sheep of Kecskemét were selected for Control Region (CR) sequencing and subsequent analysis. These were compared with CR data available from GenBank resources and other indigenous Hungarian breeds in order to compare and contrast the differences and similarities between these. A total of 40 individuals from 2 flocks in the South of Hungary were sampled in 2020. Investigations were performed based on the total number of sites (1174 bps) of the CR. It was revealed that the relative genetic diversity within the Yellow-faced sheep of Kecskemét (haplotype and nucleotide diversity 0.950 and 0.01635, respectively), in comparison to other indigenous breeds, albeit from a limited population, cannot be deemed a narrow genetic pool. The values of the Tajima D test, Fu's F_s statistic, Fu & Li's D^* - and F^* tests were found to be non-significant (in each case $P > 0.10$). Statistical evaluation does not indicate a lack of alleles. This study demonstrated three haplogroups within the Yellow-faced sheep of Kecskemét population – A, B and C. Haplogroup B was the most prevalent, which is typical for European sheep breeds, given an understanding about their arrival into Europe from the Near East. This is comparative to other Hungarian breeds, such as the Cikta and Polled Racka, and also similar to the native breeds of neighbouring countries. Regarding Haplogroup A, which was found to a minor frequency, being lower than that found in the Cikta or Polled Racka, but to a

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greater extent than Tsigai. To date, haplogroup C, which is typically demonstrated in sheep from Central Asia, had only been discovered in one native Hungarian breed: the Cikta, however its presence was also discovered in the Sand-sheep. The examination of the haplogroups seems to confirm the fact that the sheep came to Hungary not only from Asia Minor, but also from the interior of Asia in the past, and the mitochondrial genetic information of these latter animals maintained in some representatives of today's breeds, surviving the effects of crossbreeding and selection.

Keywords: Yellow-faced sheep of Kecskemét or Sand-sheep, mtDNA control region, maternal origin

Introduction

The yellow-faced sheep from Kecskemét is not an independently accepted breed, it is currently included in the herd book of the yellow-faced Berke (yellow-headed Tsigai) breed together with the Kovásznai (variant of Covasna) sheep in Hungary. Today, this joint official herd book collects the breeding and production data of a total of 80 dams, the same number of hoggets and 12 qualified rams of one state institute and four private farms.

The Tsigai sheep first came to the territory of Hungary at the end of the 18th century (1792) (RODICZKY, 1904). Its colour variants can be separated based on the colour of the fleece and the short hairs. Individuals with black, dark brown, light brown, yellowish-red, white and variegated (spotted) heads and legs were distinguished among the white-fleeced Tsigais. In the course of history, two basic colour variants of the white-wooly Tsigai, also belonging to the mountain type, spread in the Transylvanian parts. One of them is the brown-faced and small-legged, so-called Covasna Tsigai (ruĝine, rusty in Romanian). The first, most classic and almost yellow individuals of this version were the Tsigais from Hétfalu. Later, HAMMOND et al. (1961) also mention the reddish head but white or variegated fur and the completely black Romanian Tsigai. In the last decade, the yellow-faced (called also as red-faced) colour variants of the Tsigai native to Covasna (Kovászna) and Harghita (Hargita) counties have entered our country. Since 2016, they have been treated as an independent breed under the name yellow-headed Berke by the Hungarian Sheep and Goat Breeding Association. The other one, to SZENTKIRÁLYI's (1923) unifying proposal, is the variant with a completely black head and legs. The latter spread in other Hungarian historical areas. Further sub-variants were isolated in the Southern Region. The triple-purpose but more meaty csókai (variant of Čoka), the dairy zombori (variant of Sombor with a milk production of over 100 litres), as well as the ancient version, which is now extinct

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and most reminiscent of Transylvanian type, is the árpatarlóí (variant of Ruma) (ULMANSKI, 1922) and the large-bodied, also good-milking doroszlói (variant of Doroslovo) (KOVACS, 2000) was. All of them have chocolate brown or black head and legs, and white wool. In the territory of today's Hungary, it is worth distinguishing two sub-varieties according to their origin: one is the lowland one, which shows the greatest similarity with the csókai variant, and the other is the mountain one, which entered Jákotpuszta from the Highlands (Slovakia) three decades ago. Almost without exception, they have white fur and dark brown or black heads and legs.

According to the study of PÓCZOS (1934), there is no significant correlation between the colour of the head and the shearing weight and the amount of milk. Between the two world wars, based on the colour of the short hair on the head and legs, 46% of the mothers in the herd of Kisszállás Estate JSCo., originating from the southern region, were chocolate brown, 33% shiny black, 12% brown with snow and 9% reddish (yellow) found it. So, individuals with yellow heads and legs were regularly found in Tsigai flocks. This colour is still spreading today, and we should pay more attention to maintaining it.

The Yellow-faced sheep of Kecskemét, also known as the Sand-sheep, was a well-known and bred sheep throughout the Danube-Tisza region, but in recent decades it can only be found in the sandy wastelands around Kecskemét. Today, their total population in 8 farms can be estimated at around 850. Hornless individuals can be characterized by a uniform yellowish brown (rust brown) colored head and legs. Their skin is pigmented, their claws are slate gray, steely and hard. Their eyes are lively, their udders are well developed, and their ears are particularly long, which can also be linked to their selection for milk production. The neck is usually dewlap-free, although there is a small degree of longitudinal flap of skin, which may be the result of a former Merino effect. Their bones are strong, and their bellies are bulky (HEGEDŰS, 2021). Their fur is white consisting of larger staples, and black fibers may also occur in this. Their wool covers the neck and trunk, sometimes the wool also spreads to the forehead, where it appears as a white spot (rosy forehead). Most of their lambs are brown or dark brown (similar to calf or dog) at birth, but all of them turn white with time. The shepherds of Kecskemét considered only the one with a black spot on some part of the body to be a real Yellow-faced sheep (Figure 1). A lot of emphasis is placed on this even today.



Figure 1. Lamb of Yellow-faced sheep of Kecskemét with a spot on the shoulder

The aim of our study is to characterize the maternal background and maternal genetic diversity of the Yellow-faced sheep of Kecskemét. For this, we use the nucleotide sequence of the control region (CR) of the mitochondrial genome (mtDNA). We assume that as an inclusion variant, it can serve with a specific genetic pattern left over from the past. With our results, we would like to contribute to the genetic identification of the haplotypes (families) of that overlooked variety and its successful maintenance. The control region also carries important phylogenetic information, so our second goal is to place that variant in the kinship network formed by the gene bank control sequences and the sequences of the Hungarian indigenous breeds examined so far.

Material and methods

Presentation of the farms and sampling

On February 18, 2020, we collected biological samples from a total of 36 female and 4 male breeding animals in the herds of László HEGEDŰS in Kunbaracs (Bacsó farm) and then on March 10 in Mihály NÉMET's herd in Szentés (Bokros farm). This represents 50% sampling coverage of the officially registered stock. These two sampled farms purchased their individuals from the same seed stock. Although rams do not pass on the mitochondrial genome, their samples are suitable for assessing the maternal background and the diversity of the breed variant.

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DNA purification, amplification and sequencing of the mitochondrial control region
These steps are described in detail in TULLY's (2022) dissertation on the subject.

Evaluation of sequences

The mutations were evaluated with the test developed by FU and LI (1993), then the D-test proposed by TAJIMA (1989) was used as a population genetic evaluation method to analyze the detected mutations. The number of polymorphic base sites of the entire test sample was determined using the DNAsp 6.0 software, and then the average nucleotide difference within and between farms was calculated (ROZAS et al., 2017). We used the method of JUKES and CANTOR to determine the corrected base substitutions within sequences (JUKES, 1990). The distribution of haplotypes was plotted using Network 10.2.00 software (fluxus-engineering.com) (BANDELT et al., 1999). The samples were sorted into haplogroups based on the GenBank reference samples (HIENDLEDER et al., 2002; MEADOWS et al., 2005). Furthermore, we also selected a common representative haplotype sequence for each haplogroup from the samples of the Hungarian indigenous sheep breeds that we have typified so far (Cikta, Tsigai and Lowland Polled Racka) (KOVÁCS et al., 2020; GÁSPÁRDY et al., 2021 and 2022).

Results and discussion

The length of the aligned CR sequences was 1183 bp. During the alignment, data was lost at 9 base positions, so excluding these gaps, we evaluated the mtDNA control region based on a total of 1174 base pairs. In the CR region of the entire study sample, the number of monomorphic base sites was 1061, while the number of polymorphic base sites was 113 with 115 mutations. In the case of the latter, there was a singleton mutation in 25 places and a parsimony mutation in 88 places (with 2 triple mutant versions).

The mean of the nucleotide diversity (π) in the studied herd of Sand-sheep is $16.35 \cdot 10^{-3}$, and the standard deviation (SD) is $2.31 \cdot 10^{-3}$. The corrected value of nucleotide diversity according to JUKES and KANTOR is $\pi(JC)$ $16.63 \cdot 10^{-3}$. The value of the haplotype diversity (H_d) is 0.950, its standard deviation is $17 \cdot 10^{-3}$.

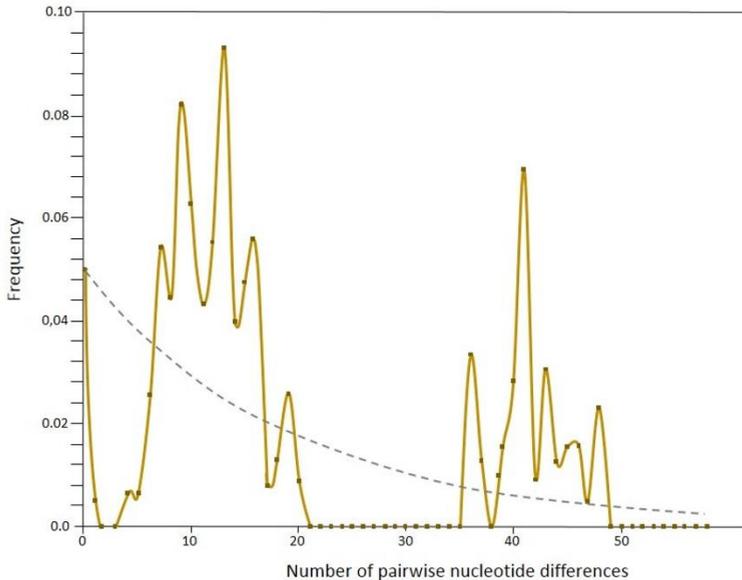


Figure 2. Frequency distribution of the number of sequence mismatches between pairwise combinations of Sand-sheep CR haplotypes

The average number of nucleotide differences between populations was 16.990. The average pairwise nucleotide difference (k) is 19.192. Nevertheless, the nucleotide difference is around 10 in the majority of the herd, and around 40 in the rest (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 Sand-sheep over time.

The average number of nucleotide exchanges per site between plants (D_{xy}) is $14.47 \cdot 10^{-3}$, and the number of net nucleotide exchanges per site between populations (D_a) is $0.80 \cdot 10^{-3}$. Table 1 refers to the DNA divergence between the two sampled populations. More mutations occurred in the Bokros farm, which has a larger number of individuals. Here, the average number of nucleotide differences (k) and nucleotide diversity (π) were higher than in individuals from the Bacsó farm (21.611 versus 10.489 and $18.41 \cdot 10^{-3}$ versus $8.93 \cdot 10^{-3}$). There were 26 shared mutations between the populations.

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Table 1: DNA divergence between the two farms

Parameter	Bacsó farm	Bokros farm
Number of sequences	10	30
Number of polymorphic sites	30	109
Total number of mutations	30	111
Average number of nucleotide differences (k)	10.489	21.611
Nucleotide diversity (π)	$8.93 \cdot 10^{-3}$	$18.41 \cdot 10^{-3}$
Shared mutations	26	

The D^* and F^* tests of FU and LI performed on the entire study sample did not give significant results, $53.62 \cdot 10^{-3}$ ($p > 0.10$) and -0.41194 ($p > 0.10$), respectively. Similarly, the value of the FU F_s statistic 0.806 was not significant ($p = 0.029$), just like the result of the TAJIMA D test was -1.06609 ($p > 0.10$).

The haplogroup and haplotype distribution is shown in Figure 3. The number of CR haplogroups identified in the Sand-sheep was three. The most populous of the haplogroups was B, followed by A, with 34, and 4 individuals, respectively. Furthermore, 2 Sand sheep individuals were included in haplogroup C.

Based on the CR region of the mtDNA of the samples, there are 3 haplogroups and 21 haplotypes. Haplogroups A, B and C were identified. Haplogroup A has 1 haplotype, haplogroup B has 19 haplotypes, while haplogroup C has 1 haplotype. Figure 3 reveals the taxonomic location of the individuals of the Sand-sheep in relation to each other and to the individuals used as controls. The 34 individuals of the Sand-sheep (on both farms) are closely related to the previously defined haplogroup B of the sheep. The mouflon also belongs to this haplogroup. It can be seen that the native breeds Cikta, Tsigai and Polled Racka also show individuals that are in this haplogroup. Four individuals (from both farms) belong to haplogroup A, which is genetically significantly distant from it. Haplogroup C was detected in the Sand-sheep with 2 individuals (only on the Bokros farm). So far, C has only been determined in the Cikta of our domestic sheep. However, it was not possible to detect haplogroups E and D in the Sand-sheep. Urial and Argali, included as out-groups, as distant relatives of the domestic sheep, are clearly separated from all of them.

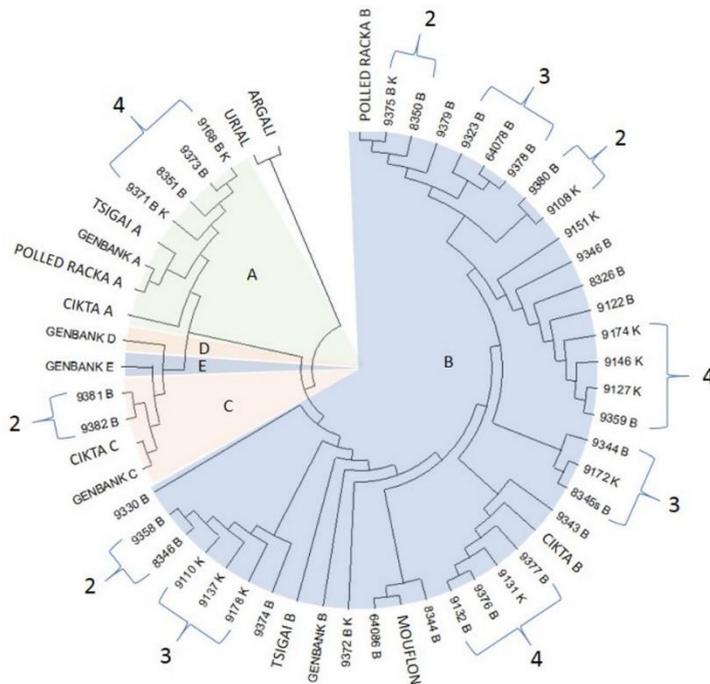


Figure 3. The composition of the Sand-sheep according to CR haplotype and haplogroup.

The figure also shows the location of Argali, Urial, Mouflon and the five GenBank haplogroups (A-HM236174, B-HM236176, C-HM236178, D-HM236180, E-HM236182 (MEADOWS et al., 2005); *O. musimon* Mouflon HM236184, *O. ammon* Argali HM236188, *O. vignei* Urial HM236186 (HIENDLEDER et al., 2002), as well as the most common haplotypes of Tsigai, Cikta (KOVÁCS et al., 2020; GÁSPÁRDY et al., 2021) and Polled Racka (GÁSPÁRDY et al., 2022). The capital letters indicate the haplogroups, while the colours associated with the letters indicate that which individual belongs to the given haplogroup. Additional numbers show individuals belonging to the same haplotype, where more than one individual represents a haplotype.

Conclusion and recommendation

Based on the above indicators, it can be concluded that the sand sheep show sufficient maternal mitochondrial (CR) genetic diversity and at the same time a relatively constant population size over time, taking into account the situation of other protected native sheep. If the pairwise nucleotide differences give a unimodal distribution curve, it would indicate that the herd has recently undergone a demographic expansion; a sudden expansion with foreign individuals.

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Despite the fact that the breed has bred in itself, it cannot be considered to have a narrow genetic stock in terms of molecular microevolution either. The TAJIMA D test, FU's Fs statistic, FU & LI's D* and F* test values are not significant (in each case $p > 0.10$). Our statistical evaluation does not indicate a lack of alleles, nor does it indicate a narrowing of the genetic diversity occurring in the history of the population.

Haplogroup B is the most common among Sand-sheep specimens (34.85%). This haplogroup is characteristic of European sheep domesticated in the Middle East, and correspondingly also of other native Hungarian sheep (97% in Tsigai, 81% in Cikta and 86% in Polled Racka). Investigations carried out in the neighbouring countries of the Balkan Peninsula pointed out that the origin of the Eastern Adriatic sheep breeds (FERENCAKOVIC et al., 2013) and the Romanian breeds (Turcana, Tsigai and Black-headed Ruda) (DUDU et al., 2016) is unanimously attributed to haplogroup B mothers. In our case, haplogroup A appeared with a lower frequency (4.10%). In the case of Cikta and Polled Racka sheep, the proportion of haplogroup A is 12% and 14%, respectively (KOVÁCS et al., 2020; GÁSPÁRDY et al., 2022), while this haplogroup is also present in Tsigai, but much less typically (3%) (GÁSPÁRDY et al., 2021). The haplogroup B is also dominant in the Western Balkan Pramenka sheep (ČINKULOV et al., 2008), Dubrovnik Ruda sheep and Istrian sheep (FERENCAKOVIC et al., 2013), but also few individuals are separated from it as belonging to haplogroup A. In our study, it can be considered a very special result that we observed the occurrence of haplogroup C in Sand-sheep (2.5%). This haplogroup is characteristic of Inner Asia (GANBOLD et al., 2019; CHEN et al., 2006; SULAIMAN et al., 2010) and the Indian subcontinent (77%) (LV et al., 2015) and has so far only been detected in Cikta (9%) of the Hungarian native breeds. In Europe, haplogroup C has only been found in the Iberian Peninsula (Portugal (PEREIRA et al., 2006) and Spain (PEDROSA et al., 2007)), Italy (MARIOTTI et al., 2013) and the southern countries of the Balkan Peninsula (Albania and Greece (PARISET et al., 2011)). The presence of haplogroup C supports the view that the Sand-sheep or Yellow-face sheep of Kecskemét has a complex maternal background. The examination of the haplogroups seems to confirm the fact that sheep came to Hungary not only from Asia Minor, but also from the interior of Asia in the past, and the mitochondrial genetic information of the latter survived the effects of crossing and selection and was preserved in the representatives of today's breeds.

However, the narrowing of genetic diversity resulting from inbreeding can generally be predicted for that breed variant. Therefore, as a future plan, we intend to set the further characterization of the Yellow-faced sheep of Kecskemét with the inclusion of nuclear markers and microsatellites, as well as its comparison with the Yellow-faced sheep of Covasna, in addition to joint pedigree registration, to what extent the mutual use of breeding rams can be recommended in terms of blood refreshment of the two variants.

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