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Examination of Hungarian Tsigai variants based on control region of mitochondrial DNA

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Introduction

Domestic sheep (*Ovis aries*) are ruminant mammals kept for their productive value across the world. They were among the first animal species to be domesticated with domestication thought to have begun around 8-9000 B.C. within the area referred to as the Fertile Crescent (Meadows *et al*., 2006). These first ancient sheep breeds were fundamentally raised for their meat, milk and skin. Woolly sheep breeds began to develop 6000 B.C. with certain cultures namely Persian becoming dependent on this wool for trade. At present *Ovis aries* are wholly dependent on man to survive and are fully domesticated, with only a few feral breeds remaining, mostly in areas or places such as islands, and are free from predators (Zöldág, 2008).

Sheep are now kept for production of their meat, milk and wool. With regard to the Wool industry profits vary depending on the breed involved. The Merino sheep for example is highly valued and renowned for its wool, while with breeds such as the Texel, the cost of removing the fleece could exceed the value of the wool itself.

Sheep milk is used predominately in cheese and yogurt production. When compared to the bovine dairy industry, sheep produce a considerably lower amount of milk than cows but the milk that they do produce is significantly higher in total solids including fats, minerals and proteins.

The position of sheep meat in terms of global importance has diminished in recent years, once a stable source of protein at the transition period to agriculture, production has since decreased. A significant factor in this decrease being the slow reproduction times when compared to those of the chicken and pork industry.

The genetic preservation of the rare domestic animal species based on sequences analysis is a modern approach. The importance of the mitochondrial DNA (mtDNA) lies in the fact that it is inherited exclusively through the maternal side, therefore it can be a good opportunity for examining the maternal origin of specific breeds.

Our study is centred on the Hungarian native Tsigai breed of sheep. The Tsigai breed is an old, independent long-tailed sheep breed originating in Asia Minor. The breed was introduced into the country around 1700 and the 18th and 19th centuries witnessed the golden age of the breed. At this time it was unrivalled as a source of high quality wool, milk and

mutton. In **Figure 1.** we can see an image that we took of a Tsigai lamb that will be used for meat production. The breed possessed finer wool quality than the other contemporary home sheep breeds present. However the presence of the Hungarian Zackel and the appearance of the Merino ensured that the Tsigai never became dominant. At the end of World War II, a small number of native animals that had been maintained made it possible to establish a gathered flock of 22 ewes in Karcag in 1950. Due to the significance this breed has within the Hungarian Culture we feel the preservation of this breed, in spite of its lower production value, is of great importance. In this way the maintenance of a greater genetic diversity is essential on the course of preservation of our old, rare domestic animal breeds (Gaspardy, 2000)

The genetic preservation of the rare domestic animal species based on sequences analysis is a modern approach.

The importance of the mitochondrial DNA lies in the fact that it is inherited exclusively through the maternal side; therefore it can be a good opportunity for examining the maternal origin of specific breeds. In this study we investigated using mitochondrial DNA sequence data to provide us with greater insight into the maternal lineages of the Hungarian Tsigai breed with the aim of obtaining the best outcomes for Hungarian breeders.

Figure 1. *A Hungarian Tsigai lamb at one of our sampling locations*

Literary Survey

2.1 The decline of biodiversity and breeds such as the Tsigai as a result of the introduction of modern breeding and large scale production

It has been suggested that threats to biodiversity are increasing (Othman *et al*. 2014), whether it be in terms of extinction rate, loss of genetic diversity within the agricultural species or destruction of ecosystems. The formulation of the concept of modern breeding and large scale production occurred during the mid-1800s and resulted in significant changes in the livestock sector. The development of this type of large scale production saw farmers progressively substitute the less productive, locally adapted, native breeds with highly productive cosmopolitan breeds and progressively abandoned marginal areas. Therefore a significant number of cattle, sheep, and goat breeds already disappeared and many are presently endangered.

According to FAO (FAO, 2007), 20% of the breeds worldwide are classified as being critically endangered, critically maintained, endangered or endangered –maintained. Currently, more than 1300 different breeds of sheep are known for more than 1.1 billion animals (Othman *et al*., 2014). However, 181 breeds are now extinct more than 12% of identified breeds and many other breeds are threatened. In these conditions it is more strategically important than ever to preserve farm animal diversity to as great a degree as possible, to ensure a prompt and proper response to the needs of future generations.

With the increase of corporate agribusiness and the declined interest in small family holdings, many breeds of sheep are in danger of extinction, the native Hungarian Tsigai breed is one of such breeds. The reason being the preference for breeds of uniformity of characteristics and rapid growth rates have driven native heritage breeds, such as the Tsigai to the edge of farming and the sheep industry. Those that remain are maintained solely through the endeavours of conservation organisations and also farmers who believe in keeping the traditional breeds alive. Prior to this introduction of modern large scale production agriculture the breed was regarded as a highly developed multipurpose breed. This golden era was seen during the 18th and 19th century and saw the breed as an unrivalled source of fine wool, milk and mutton. During the last 200 years, the Tsigai were continuously present in Hungary but in varying proportion (1-10%) to other breeds. At the end of the 19th

century significant breeding programmes diminished and the Tsigai lost growing ascendency. They never became dominant because their expansion was by the presence of the Hungarian Zackel (Racka) and the appearance of the Merino.

At the end of the Second World War, a small number of native animals that had been maintained made it possible to establish a gathered flock of 200 ewes in Karcag. This limited stock can be considered as a base and starting point of the national gene conservation programme in the Tsigai breed (Gaspardy, 2004) As of the beginning of the 21st century, the registered seed-stock population of the Hungarian native Tsigai was approximately fifty rams and one thousand ewes. The majority of this seed stock population is managed by the National Parks of Hungary, however some of the population are also sustained individual private farmers and also by agricultural societies. In order to maintain the character of the breed the management of this Tsigai population is sustained as similarly as is feasible to the breed's original environmental conditions. In addition to these registered seed stock animals, it is thought that there are approximately another 3000 animals are dispersed across the country.

2.2 Mitochondrial DNA sequencing in ovine and bovine species

The bovine and the ovine mtDNAs are the only complete artiodactyl mtDNAs reported so far. The difference between the control region (cr) of Bos and Ovis, excluding the repeated region, was found as 27.6%. In a study (Hiendleder et al. 1998) the complete mitochondrial DNA molecule of the domestic sheep, *O. aries*, was sequenced together with part of the mtDNA of a specimen representing the other major *O. aries* haplotype group. It was determined that the length of the complete ovine mtDNA presented is 16,616 nucleotides, although that figure is not absolute due to heteroplasmy caused by the occurrence of different numbers of a 75-nt-long tandem repeat in the control region. This sequence data was then included in the analyses of intraspecific ovine molecular differences, molecular comparisons with bovine mtDNAs, and phylogenetic analyses based on complete mtDNAs (Hiendleder *et al.* 1998) The corresponding control region in the mtDNAs of both ovine and bovine species were compared and it was revealed that the difference between the bovid was 1.4 times greater than the intraspecific ovine difference. These findings (Hiendleder *et al.* 1998) suggest that the strains of a wild sheep from which the domestic sheep originate were more closely related than were the *B. primigenius* subspecies of cattle from which *B. indicus* and *B. taurus* arose. Further, dating based on the complete mtDNA sequences suggest that the

bovine and ovine lineages diverged about 30 million years before present which is earlier than previously suggested.

2.3 Mitochondrial DNA and its role in productive and reproductive trait inheritance

Studies have been carried out which have examined the role which mtDNA plays in productive and reproductive traits in cattle. A 1993 study by Schutz *et al*. examining the effect of mtDNA on production and health in dairy cattle suggested that maternal lineage effects, which are most likely indicative of mtDNA differences, may be important for milk production and reproductive success n dairy cattle, *B. taurus*. The same study looked at sequence variation of mtDNA in 36 maternal lineages of dairy cattle along with animal models to assess effects on milk productivity and reproductive traits.

Sequence polymorphisms of bovine mtDNA were shown to be associated with milk production, reproduction, and health costs incurred (Schutz *et al*. 1993). The results obtained showed that the effects of even a single base-pair substitution within the mtDNA had effects of economic importance and broad implications in genetic selection of dairy cattle. The specific examples documented in the study being one particular base-pair substitution which was associated with additional production of 842kg milk and 37kg milk fat per cow per lactation. While another single base-pair substitution was associated with a decrease of 36 days and one unsuccessful breeding between successive calvings (Schutz *et al*. 1993). New developments in reproductive technology and embryo manipulation seem poised to make this pathway of selection more viable.

Differences in mtDNA could be incorporated into embryo transfer breeding programs to better choose donor and recipient females to produce replacement heifers. Schutz *et al.* (1993) state that current cloning techniques require nuclear transplantation into an enucleated ovum without regard to cytoplasmic content. Potential exists for using mtDNA sequence polymorphism to identify ova of females with inferior nuclear genetics in superior mtDNA background as candidates for enucleation and subsequent introduction of nuclei with greater genetic potential.

In regard to mtDNA and selection differentials, studies have shown that the largest selection differentials are for the sire-bull pathway (Van Tassell *et al.*1991) However in this pathway the mtDNA polymorphism is not important as the mitochondrial genome is transmitted only from the female parents. It is suggested though (Schutz et al. 1993) that the dam-bull

pathway is equally as important as the sire-bull pathway, but that the accuracy of selection is less than at the sire-bull pathway. A bull's estimated transmitting ability based on pedigree may be biased if the contribution from his dam is not adjusted for mitochondrial influence on her records. This is most important in the first stage of bull selection. Although the bull would acquire mtDNA from the dam, it would not be transmitted to his offspring (Schutz *et al.* 1993).

Adjustment of bull-dam records for mtDNA influences would allow more accurate prediction of expected genetic contribution of a bull to his daughters. Such adjustment could be based upon genetic markers of the bull's mitochondria, because the entire genome is inherited only from the dam (Schutz *et al.* 1993).

Figure 2. *Native Hungarian Tsigai grazing*

2.4 Investigation of diversity amongst sheep breeds based on geographical location

This obtained sequence variation from mtDNA has been used to investigate genetic diversity within sheep breeds from Asia and Europe and in discovering different halogroups among sheep breeds based on geographical location. A study carried out (Meadows *et al*., 2005) showed comparison of 2027 base-pairs of sequence, from 121 animals, revealed 44 phylogenetically informative nucleotide positions and a single insertion/deletion. A total of 57 haplotypes were observed which formed two distinct clades, Haplotype A and Haplotype B. Type A haplotypes were found in breeds from Asia while Type B haplotypes were observed in greatest number within European breeds. The distribution of haplotypes indicates that sheep appear to have the weakest population structure and the highest rate of intercontinental dispersal of any domestic animal reported to date (Meadows *et al*., 2005).
Diagnostic restriction fragment length polymorphism polymerase chain reaction test to distinguish Type A and Type B haplotypes carried out on 223 animals from 17 breeds of European and Asian origin (Meadows *et al*., 2005). A mixture of the two lineages was found in every breed except Suffolk and the Indian Garole. These results indicate introgression has played a major part during breed development and subsequent selection. Further evidence of the dominance of the type B haplotype within European breeds can be seen in a 2006 study of the Iberian sheep breeds in Europe which showed that that the type B haplotype clearly predominates within the Iberian breeds (Pedrosa *et al*., 2006).
Similar experiments have also been carried out (Othman *et al.*, 2014) on Egyptian and Italian sheep breeds with similar results seen in that the type B haplotype was the most dominant haplogroup type. The study also showed that while haplogroups A and C were present (albeit in low numbers), haplogroups D and E were completely absent. Within all of the tested breeds within this study, the haplotype diversity and average number of pairwise differences were 0.97571 and 7.01484 respectively. Tapio *et al.* (2006) revealed geographical patterns in the distribution of haplotypes. First, Group C was present in the Caucasian and Central Asian areas but absent in the eastern fringe of Europe. A second recorded pattern was the absence of Group A in the 4 studied populations from south-eastern Europe.

2.5 Methods of haplogroup classification

In search of a quick and reliable haplogroup classification, a comparative study was carried out which looked at three simple molecular approaches in search of mtDNA haplogroup identification of domestic sheep (Yüncü *et al*., 2013). The three different molecular approaches tested were:

- The RFLP method applied to the mtDNA control region
- The single strand conformational polymorphism method applied to NADH dehydrogenase subunit 2
- And finally the SSCP method applied to NADH dehydrogenase subunit 4

The haplogroups of 622 sheep all from Turkey i.e. from the 'genetic diversity hotspot' of domestic sheep distribution were examined. The results obtained illustrated that the SSCP analysis of the MT-ND2 region exhibited higher discrimination power, sensitivity and specificity in haplogroup classification when compared with the SSCP analysis of MT-ND4 region and RFLP analysis of the control region.

Aims

The primary aim of our investigation is using obtained mitochondrial DNA sequence data to provide us with greater insight into the maternal lineages of the Hungarian Tsigai breed. To achieve this aim the initial step of the investigation is to determine the most ancient of maternal lines of the Hungarian Tsigai breed with the use of herd booking and obtain a blood sample which can be used in mitochondrial DNA sequencing. The objective of the mitochondrial DNA sequencing is to look at the control region of the sample and at the nucleotide deviation at this site in order to examine genetic diversity within the breed and determine the extent of unification of the maternal genetic background of the Tsigai breed.
Based on the haplotype variety in maternal families, we aim to insert our traditional breed into the other popular sheep breeds.
Ultimately our intention is to prove that the data obtained from the mitochondrial DNA sequencing supports the maintenance of preservation of the Hungarian Tsigai (see **Figure 3**.) and that this data can be provided to Hungarian breeders of the Tsigai with the goal of allowing them to obtain the best outcome within family selection.

Figure 3. *Native Hungarian Tsigai Sheep*

Materials and methods

The Hungarian Association of Sheep and Goat Breeders was established in 1991 and is composed of breeders and instructors. They are responsible for the organisation of the breeding work and herd booking of sheep and goat breeds in Hungary. They are also responsible for the ear tagging and selection of the breeding rams. Further, the testing of scrapie in breeding rams is obligatory in Hungary and it is the responsibility of the association to carry out the genotyping. Only rams possessing the resistant genotypes may be utilised for breeding.

We carried out our research in co-operation with the association and approached them for the herdbook of the Hungarian Tsigai breed. The current herdbook which we received was re-established in 1995 thus providing us with 20 years of data regarding the breed. There were approximately 28,000 individual sheep listed. The initial step that we took when analysing the data of these 28,000 sheep was to group them on the basis of their maternal lineages. Once we had completed this the next step was to select the most ancient lineages. Having determined the most ancient lineages we found 3 families 9 generations long, 19 lineages with 8 generations and 34 lineages which were 7 generations long. For our research, in addition to the lineage length, we also needed to consider the different variants of the Tsigai breeds.

In order to represent the different variants of the Tsigai breed in Hungary we chose the flock from Kardoskút (KM) and Csanádpalota (CS) to represent the lowland type. Individuals were selected from Debrecen /DB/ to represent the Csóka (Southland) type, from Cegléd /TC/ to represent the milking type and from Gödöllő (SF) to represent the yellow-faced type. The animals from the mountain type /BP/ were included in the investigation too.

Altogether 81 blood samples were taken (as can be seen in **Figure 4**.) from 2 members of each family, using EDTA-tubes. The samples were stored at -20 °C until further processing. DNA was purified using SIGMA GenElute Blood Genomic DNA Kit according to the manufacturer's recommendation. For the polymerase chain reaction (PCR) 25 µl reaction mixture was prepared containing 2.5 µl dNTP, 2.5 µl buffer, 1.5 µl MgCl, 2 µl primer, 1 µl BSA, 0.4 µl Taq-polimerase (Thermo Scientific) and ultra purified water.

According to Meadows et al. (2007) primers CR-F 5'-AACTGCTTGACCGTACATAGTA-3' and CR-R 5'-AGAAGGGTATAAAGCACCGCC-3' were used to amplify a 1059 bp

fragment part of the mtDNA control region (AF010406; Hiendleder et al. 1998, 15983-592 nucleotide). The PCR program was the following: 6 cycles 94 °C 30s - 54 °C 30s - 72 °C 45s, 6 cycles 94 °C 30s - 53 °C 30s - 72 °C 45s, and 18 cycles 94 °C 30s - 52 °C 30s - 72 °C 45s. The product was purified using SIGMA GenElute PCR Clean-Up Kit, and sequenced.

The results were aligned and analysed with MEGA6 (Tamura et al., 2013) software. Analyses were conducted using the Maximum Composite Likelihood model and nucleotide diversity was calculated within and between groups (breed variants). By the use of PopART (http://popart.otago.ac.nz) median-joining network (Bandelt et al., 1999) was designed to reveal the haplotypes and linkages among the individuals.

Figure 4. *Picture of me taking blood sample from Tsigai in the Bakony Mountain*

Results

After aligning and trimming the sequence we got a segment with 1059 nucleotides (AF010406; positions 15983-592). Differing nucleotides were found at 98 sites among all the animals (81 individuals), 47 of these were singletons. The number of differing nucleotides was various in the flocks: DB: 65, KM: 32, CS: 27, BP: 11, SF: 10, TC: 20.
The presence of the haplotypes in the breed variants are shown in **Table 1**. The relative frequency of the haplotypes is high, so most of the individuals represent an independent haplotype.

Table 1. *Prevalence of haplotypes in the Tsigai flocks*

flocks	n	number of haplotypes	frequency of haplotypes, %
KM	5	5	100
BP	6	6	100
CS	24	21	87.5
SF	5	4	80
TC	5	5	100
DB	36	30	83.3
overall	81	65	80.2

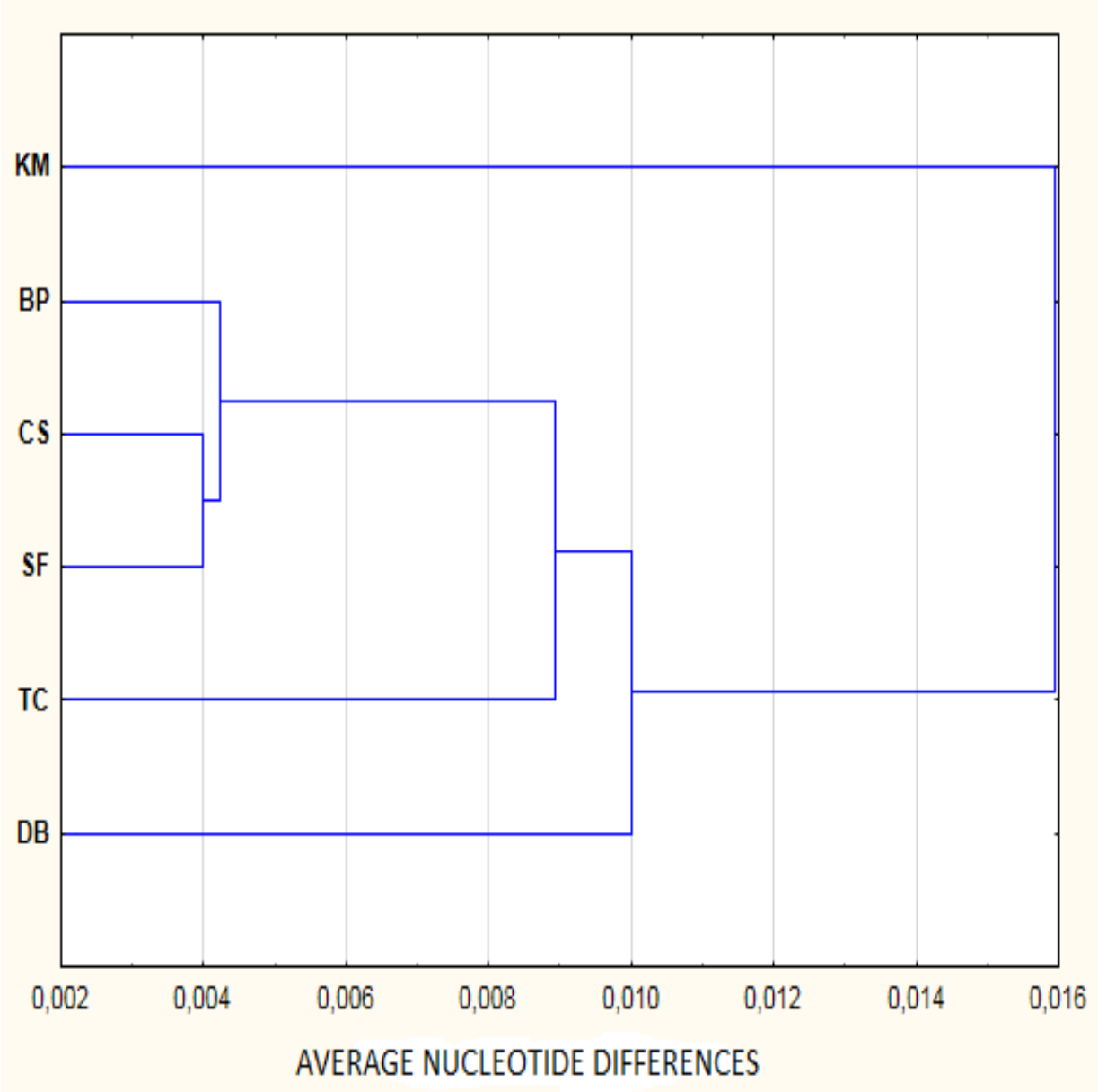
The within group distances from the average nucleotide substitution were the followings: DB: 0.007; KM: 0.014, CS: 0.004, BP: 0.005, SF: 0.004, TC: 0.010. According to these results it became clear, that the KM and TC flocks show the highest variability, these flocks have the most diverse genetic background. The number of base substitutions per site from the average of overall sequence pairs between groups is shown in **Table 2**. There was no great difference found between the populations CS, SF and BP, so we could assume that these flocks share common ancestors.

Table 2. *Estimates of evolutionary divergence over sequence pairs between groups*

	KM	BP	CS	SF	TC	DB
KM	0	0	0	0	0	0
BP	0.011	0	0	0	0	0
CS	0.010	0.004	0	0	0	0
SF	0.011	0.005	0.004	0	0	0
TC	0.014	0.009	0.008	0.008	0	0
DB	0.012	0.006	0.006	0.006	0.010	0

Using the Statistica program (StatSoft, Inc. (2013) a dendrogram was constructed based on the average nucleotide differences between the flocks. The dendrogram can be seen below in Figure 5. The figure illustrates the relationships between the flocks. The animals from the mountain type (BP) and one of the lowland type (CS) seem to be close to the yellow-faced variant (SF). We found resemblance between the milking type (TC) and the animals from the Southland (DB), this can be explained with the common geographical origin. The other lowland flock (KM) appeared to be far from the other variants. It could be a result of spontaneous mutation or due to the low number of samples taken from this variant.

Figure 5. *Average nucleotide differences between the flocks*



By the use of PopART (http://popart.otago.ac.nz) a median-joining network (Bandelt et al., 1999) was designed to reveal the haplotypes and linkages among the individuals. The median-joining network was constructed based on the sites which were informative for the haplogrouping. The comparison with the data from GenBank revealed that most of our samples (93.8 %) belong to haplogroup B, in 42 cases the animals showed total correspondence with the reference sequence B (DQ852175.1). Thus these sequences form the median result of our figure, visible as the largest circle. Each hatch mark on the figure represents one alteration in nucleotide sequence from the median.

Five animals (6.2 %; 2 DB, 2 TC, 1 SF) were found, which seemed to be closer to the haplotype A (DQ852101.1). Of these five animals which seemed to be closer to haplogroup A, two were of the milking type.

A single sequence (CS) differed at 4 positions from both the haplotypes B, and A of the GenBank database.

Of the 81 samples analysed, the median joining network demonstrated 12 main haplotypes. A dominant number of the investigated animals (51.9 %) belonged to haplogroup B. It is evident from the results that some animals originating from different flocks shared the same haplotype, e.g. DB and CS. While there were also some haplotypes possessed only by animals from the same flock.

It is also evident from the result that there were no individuals within our study that possessed haplotypes from the C, D and E haplogroups.

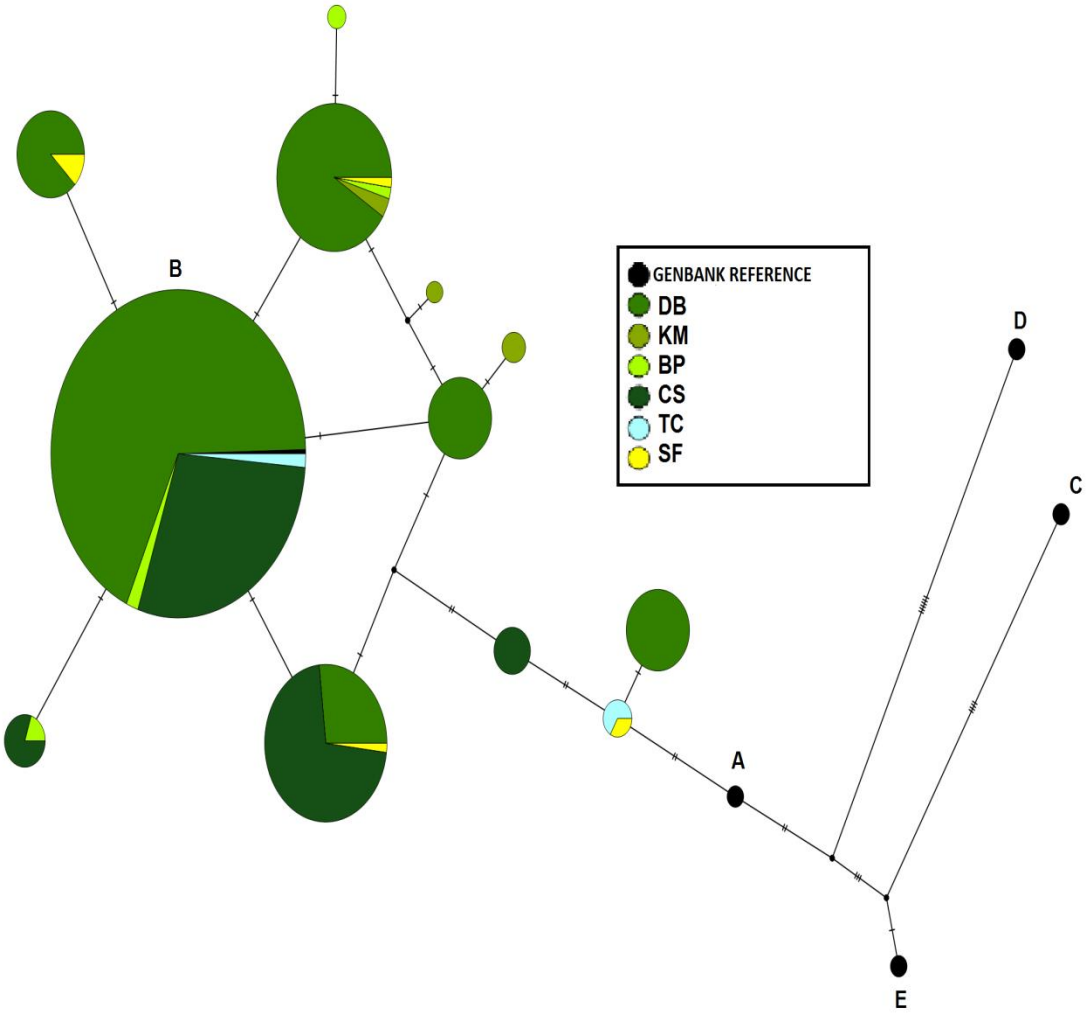


Figure 6. *Median-joining network showing the haplotypes of the Tsigai variants and the haplogroups to which they belong.*

Discussion

The results we obtained in **Table 1.**indicate that in 50% of the flock types of Tsigai breed there is 100% heterogeneity whilst overall heterogeneity within the individuals of the flock types tested was 80.2%. These figures indicate that the frequency of haplotypes within the individual flocks is high and as such variance within the flocks is high. This is important as it indicates that the inclusion of these individual flock members in the breeding programme will facilitate the maintenance of variation within the breed thus ensuring we preserve these breed variants of the Hungarian Tsigai.

Figure 7. shows some of the Tsigai from which we obtained blood samples. In the flocks where there were multiple individuals with identical haplotypes, this may indicate that the individual flock members that we obtained samples from were perhaps related, for example two of the individuals we tested were mother and daughter etc. The presence of more than one individual possessing a specific haplotype is redundant in the breeding program of the Tsigai with regard to preservation of the variation of haplotypes within the breed. As such, any surplus animal possessing identical haplotypes can be utilised for production and excluded from the breeding program.

Table 2. and Figure 5. illustrate the results we obtained when we looked at our nucleotide sequences in terms of evolutionary divergence over sequence pairs between groups. The results demonstrated the distance in terms of variability amongst the flocks tested. The results we obtained we would regard as somewhat unexpected. We can see from **Figure 5**. that the smallest variance between flocks appears to be between the Southland type flock and the yellow-faced flock. This is perhaps surprising to see that the yellow-faced flock possess one of the smallest variability when compared with the Southland flock as they are phenotypically the most different of the flocks. This would suggest that the phenotype of the flock is unrelated to the nucleotide sequence of maternal mtDNA which we examined and is possibly inherited from the paternal lineage. It may also suggest that the different characteristics of the breed variants came from the rams which were bred with the local ewes at that time.

From looking at **Figure 5.** it is also clear to see that the average nucleotide differences between individuals from the lowland type (CS) and the milking type (TC) are also small.

This is also somewhat unexpected as we have seen that the milking type flocks are closer to haplogroup A whilst the majority of the lowland types in our study have fallen into haplogroup B. The explanation for this diminished evolutionary divergence may be a geographical one. When we examine a map we can see that the regions where these flocks historically originate are quite close to each other so this may account for this apparent common ancestry.

Another unanticipated finding demonstrated by the results in **Figure 5.** is the apparent small average nucleotide difference between the mountain type (BP) and the lowland type (CS). Phenotypically these two flocks are quite different with the lowland type being larger than the mountain type. This would suggest that although they appear to possess a close common ancestry access to food for these flock types has resulted in differing appearance in the modern day individuals. The lowland type which are found on the Hungarian Great Plain would have had practically unlimited access to high quality feed whilst the grasslands in the mountains would not have been as abundant and of similar quality. This could be the reason the mountain type today is smaller than the lowland type.

Figure 7. *Tsigai Flock in the Bakony Mountain*

We can see from **Figure 6.** in the above results section that the majority of the samples (93.8%) corresponded to the reference sequence of haplogroup B which we obtained from Genbank, with 42 cases of the 81 analysed showing total correspondence to the reference sequence. For the constructing of this median joining network we only used the nucleotide differences which were informative for the haplogrouping. This result is of significant importance as it suggests that there is common ancestry of these samples. It supports our proposal that by examining the mitochondrial DNA of Tsigai sheep that are known to be from ancient families we can determine the individuals which have the truest lineage to the ancient breeds and thus knowing this information can provide it to breeders to ensure that these individuals are utilised in the breeding program.

With regard to the 5 cases which corresponded to haplogroup A; 2 of these were of the milking type, 2 were of the low-land type and 1 was of the yellow-faced type. An argument that may be made as to why some animals of the milking type were closer in sequence to the haplogroup A may be that in the past breeds of Near-East origin were introduced by Tsigai breeders and crossbred with their milking type Tsigai. The rationale behind this being that by cross breeding the Tsigai they could introduce traits to make the breed more productive within the dairy industry.

Although to an extent this has somewhat diluted the genetic homogeneity of some of these milking type Tsigai it is important that they also be preserved as they are variations within the ancient lineages and it is important that we aim to preserve these variants while preventing further dilution of the lineages.

Conclusion

The primary aim of our investigation was to use obtained mitochondrial DNA sequence data to provide us with greater insight into the maternal lineages of the ancient families of the Hungarian Tsigai breed. The significant number of nucleotide differences found in the mtDNA control region (98/1059) corroborates the variety of the investigated Tsigai flocks. We have shown that by utilising DNA sequencing on the control region of the mtDNA we can learn valuable information regarding the maternal lineage of the Tsigai sheep. Based on these results we can conclude that with regard to the maternal genetic background, the investigated populations are not as genetically different from each other as was previously suggested. We have shown that this information can be used to illustrate the haplotype of the individual animals tested and show variation within the flocks. This is information which we can now pass on to the Hungarian breeders of the Tsigai to help them maintain the existing variation within their flocks. Further these results validate our aim of proving that the data obtained from the mitochondrial DNA sequencing supports the maintenance of preservation of the Hungarian Tsigai as the information we obtained illustrates the common haplogroup of the flocks tested, haplogroup B, thereby indicating their common ancestry with the other European sheep breeds. Our investigation contributes to the global understanding of sheep mtDNA haplotypes and their importance in the breeding and preservation of the rare, native breed. Our recommendation, supported by these results, would be that these animals which we found to be of common haplogroup be included in a breeding programme to ensure the preservation of the traditional breed. In the future we want we continue using this method of investigation to examine other rare and native breeds and incorporate previous works and this investigation to use as a basis of comparison.

Summary

The Hungarian native Tsigai breed is an old, independent long-tailed sheep breed originating in Asia Minor. The breed was introduced into the country around 1700. Due to the significance this breed has within the Hungarian Culture we feel the preservation of this breed, in spite of its lower production value, is of great importance. In this way the maintenance of a greater genetic diversity is essential on the course of preservation of our old, rare domestic animal breeds. We are the first research group to analyse the genetic background by the use of mitochondrial DNA (mtDNA) sequence in the Hungarian native Tsigai breed, and compare it to the sequences of GenBank.

Our aim of this investigation is using obtained mitochondrial DNA sequence data to provide us with greater insight into the maternal lineages of the Hungarian Tsigai breed. With these results we aim to provide accurate information to the Hungarian breeders to obtain the best outcome within family selection.

At first we retrieved the necessary herd book from the Hungarian Association of Sheep and Goat Breeders and we analysed the pedigree data therein. From our pedigree analysis we chose the ancient maternal lineages to carry out sequence analysis. The blood samples were taken from a pair of descendants from each of the eldest families based on herd booking (and from two more breed variants, altogether from 81 individuals) in 2014.

From the blood samples taken, we purified the DNA and carried out PCR reaction and then sequencing. The control region of mtDNA showed nucleotide deviation at 98 sites. Among our 81 samples we could differentiate 65 haplotypes signifying a vast genetic diversity. However, the differences among the individuals were limited to few loci; so the maternal genetic background of the Tsigai breed seems to be unified. The genetic information confirmed the origin of the families/flocks known from the breed history. Ninety-four percent of the samples belonged to the ovine haplogroup B (in 42 cases with full matches with the reference of GenBank, DQ852175.1). This fact proves the common maternal origin of the Hungarian Tsigai with the other European sheep breeds.

A more intense focusing on the maternal side is motivated also by the fact that the females are present in greater number than the males. Further they remain in breeding for a longer period of time respectively, so they can be the depositaries of realization and maintenance

of genetic diversity to a larger extent. We believe the results of this investigation support the maintenance of preservation of the Hungarian Tsigai.

Figure 8. *Native Tsigai flock*

Összefoglaló

Az őshonos cigája Kis-Ázsiából származó, önálló régi hosszúfarkú juhfajta. Az 1700-as évek környékén került Magyarországra. A magyar hagyományok megőrzése szempontjából fontosnak tartjuk ennek a fajtának a fenntartását még alacsonyabb termelési mutatói mellett is. Ebből is látszik, hogy a nagyobb genetikai változatosság fenntartása létfontosságú a régi háziállatok fajtamegőrzésénél. Az őshonos cigája fajta genetikai hátterét elsőként vizsgáltuk és hasonlítottuk össze a génbankban található szekvenciákkal a mitokondriális DNS alapján.

Vizsgálatunk célja a mitokondriális DNS szekvencia adatok elemzése, hogy jobban megismerhessük az őshonos cigája anyai vonalait. A kapott eredményeinkkel szeretnénk elősegíteni a magyar juhtenyésztők munkáját, hogy megvalósíthassák a családon belüli szelekciót.

A Magyar Juh-és Kecsketenyésztő Szövetségtől kaptuk a cigája fajta törzskönyvét, ezeket az adatokat dolgoztuk fel először. A pedigré elemzése során kiválasztottuk az ősi anyai családokat, és ezeken végeztük el a szekvencia elemzéseket. A törzskönyv alapján választott ősi családok két-két képviselőjétől (emellett még 2 másik változattól, összesen 81 egyedtől) vettünk vérmintát 2014 nyarán.

A vérmintákból DNS-t tisztítottunk, PCR reakcióval felszaporítottuk a kívánt szakaszokat, majd szekvenálást végeztünk. A mitokondriális DNS kontroll régió szakasza 98 helyen mutatott nukleotid eltérést. A 81 mintaállat között 65 haplotípust tudtunk elkülöníteni, ez a fajta nagy genetikai változatosságát igazolja. Mivel az egyedek közötti eltérések csak pár nukleotidra korlátozódtak, a cigája fajta anyai háttere egységesnek tűnik. A genetikai vizsgálatok megerősítették a családok/nyájak törzskönyv alapján ismert származási helyét. A vizsgált egyedek 94 %-a a juhok B haplocsoportjába tartozott (42 esetben teljes egyezést mutatott a génbanki referencia szekvenciával DQ852175.1). Ez az eredmény a magyarországi cigájának az európai juhokkal számottevően közös anyai hátterét igazolja.

Az anyai oldal fokozottabb előtérbe állítását az is indokolja, hogy a nőivar nagyobb arányban van jelen, mint a hímivar, ill. hosszabb ideig marad tenyésztésben. Ezáltal nagyobb mértékben lehetnek a genetikai sokszínűség megvalósításának és fenntartásának letéteményesei. Úgy véljük, ezek az eredmények hozzájárulhatnak az őshonos magyar cigája fajtafenntartásához

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