Chapter

Reality of Mitogenome Investigation in Preservation of Native Domestic Sheep Breeds

András Gáspárdy

Abstract

This chapter deals with the study of extranuclear hereditary material and the possibilities of using it to maintain endangered animal breeds. The chapter characterizes mtDNA, presents its genes and their functions, while also emphasizing the hypervariable control region. It reports on the results of previous researches, referring to international publications. It sheds light on promising areas of mitogenomic research. It shows the maternal genetic background of local native varieties according to the results of the study of available country/geographical region. It deals with reasons for endangerment and the arguments for preservation of autochthonous breeds. In addition, it gives place to discuss some exciting professional concepts in rare breed preservation.

Keywords: mitogenome, sheep, genetic diversity, haplogroup, haplotype, breed preservation, maternal lineages, within-family selection

1. Introduction

For our domesticated animals, their domestication history has long preoccupied professionals. Substantially earlier evolution of domesticated species is also an area of research.

In the case of the horse, from the *Phenacodus* onwards, the last 60 million years have been exceptionally well known through a chain of transitional species, sometimes separations.

The phylogeny of family *Bovidae* (e.g., cattle, sheep, and goats) is less resolved. Here, the *Hypertragulidae* appeared as the first identifiable primitive ancestor around 50 million years ago (Mya) in Southeast Asia [1]. The complex, functional stomach developed about 40 million years ago. The molecular dating applied to cytochrome b gene, which is located in mitogenome showed that the separation of the sub-family *Caprinae* occurred 6.2 ± 0.4 million years ago, but there are proposed earlier radiation from about 14 Mya [2]. The *Myotragus*, which is basal to the *Ovis* clade within sub-family *Caprinea* stood out 5.35 million years ago.

In regard of evolutionary questions, besides nuclear microsatellites [3], SNPs [4], retrovirus integrations [5], and Y chromosomal mutations [6] mitochondrial DNA (mtDNA) represents a very informative genomic element. At the same time, this part of the hereditary material can also be efficiently used to better understand domestication. Nowadays, the study of mtDNA plays a role in the genetic

characterization and differentiation of our animal species living with us. Looking to the future, we can believe that this will be essential for the conservation of genetic resources and preservation of endangered autochthonous animal breeds all over the world.

2. Mitochondrial hereditary material

2.1 Small circular genome

Majority of animal DNA as genetic information (about $3.3x10^9$ base pairs) is stored in chromosomes within the cell nucleus. However, a minor part of DNA is located in chromosomes of mitochondria, outside the nucleus, in the cytosol. The circular mitochondrial genome is also built up of double-stranded DNA like nuclear genome, and consists of about 16,500 base-pairs. It is a semi-autonomous asexually reproducing genome in eukaryotic organisms [7]. Mitochondria are late descendants of free-living bacteria capable of metabolizing oxygen maintained by endosymbiosis in eukaryotic cells.

The mitochondrial DNA (mtDNA) which is not enveloped like nuclear DNA in chromosomes, is located in the mitochondrial matrix which can be found inside the inner mitochondrial membrane. The outer compartment of a mitochondrion is surrounded by the outer and inner membrane. The outer membrane contains porins through which smaller or larger proteins can enter the mitochondria. While, the inner mitochondrial membrane has all the elements of the electron transport system and the ATP synthase complex [8].

Considering that a cell has multiple mitochondria, and that a mitochondrion carries multiple copies of its own genome as opposed to the nuclear genome, the difference between the two remains significant.

2.2 High mutation rate

The mitochondrial genome or mitogenome mutates more frequently (approx. 100 times more often) than nuclear ones causing divergence in mtDNA at within-mitochondrion and between-mitochondrion level. Therefore, the mitogenome can be considered heterogeneous and heteroplasmic homoplasmic instead.

Little is known about the movement and segregation of mitochondrial DNA during mitotic growth or meiotic divisions. When a cell divides, mitochondria enter the progeny cells at random. If the DNA of the mitochondria of the dividing cell differs for several mitochondria, it is possible that the two daughter cells will receive the same genetic information, but it is also conceivable that they will not. Thus, it is hard to estimate the outcome of the transfer of genetic information, including defects. The random mutations that occurred further complicate that situation.

Because of the constantly frequent mutation rate of mitogenome, it has been widely used as a phylogenetic marker for both cladogram building and molecular dating. Brown et al. [9] first implemented the mitochondrial molecular clock in primates using fossil data. According to that work, scientists considered the 2% substitution rate per one million year as a reasonable reference in case of missing of relevant fossil data in vertebrates [10]. Since then, studies (e.g. [11]) have reported significant differences between species, it was found that because of the not fully clock-like evolution of the species the mtDNA mutation rate is of limited use in a comparison. The median (from 3 to 14.3 million years) of divergence dates

between species are not related to body mass or generation interval [12]. However, Galtier et al. [13] found a proven correlation between mutation and longevity, which is closely related to the generation interval, and suggested a low (somatic) mutation rate could be responsible to achieve long life, in concordance with the mitochondrial theory of ageing. According to Song et al. [14] mitochondria may have a nucleotide imbalance that leads to higher mitochondrial DNA mutation rates. Their research suggests increased dGTP (deoxyguanosine triphosphate) level in free deoxynucleotide triphosphate (dNTP) pool which increases the rate of T to C substitutions.

2.3 Maternal inheritance

The offspring receive mostly mtDNA from the maternal ooplasm (sometimes this material from the sperm can also be included), but in the adult embryo only the maternal mtDNA remains functional. So, mitochondrial inheritance is considered as clonal or maternal, as one of the cases of uniparental inheritance. Paternal mtDNA, even if it enters the ovum, loses its function before (in crayfish [15]), during (in Ascidia [16]) or after (in mouse [17]) fertilization. That condition prevents an effective recombination. However, the paternal inheritance of mtDNA was displayed by Zhao et al. [18], what is to show the mtDNA patterns of progeny were identical to that of its male parent. Systematic surveys of within-species mtDNA data revealed departure from the clonality assumption in several species [19]. These prove that mitochondrial recombination is possible, and caution when in constructing and interpreting within-species mtDNA genealogies [20].

Thus, mtDNA testing can reveal the maternal background of individuals; which maternal lineage of mitochondria they belong to. But, of course, it can also be used to prove maternal kinship. Mitochondrial DNA sequencing has gained significance also in human rights cases [21].

It was observed that some characteristics (e.g. behavioral) do not follow Mendelian segregation. If a trait is such, it is either polygenic or extranuclear.

2.4 Perspectives of mtDNA research

Mitochondrial gene content is strongly conserved across animals, with very few duplications, no intron, and very short intergenic regions [22]. At the same time, mitogenome also contains a very limited presence of non-coding regions, approximately 3%, as opposed to nuclear DNA, where its proportion is 93%. These highly variable non-coding regions (e.g. the control region) are typically flanked by highly conserved ones (e.g. ribosomal DNA). The elevated mutation rate of highly variable regions creates the condition for monitoring the population history over relatively short time frames.

One of the perspectives of mitogenome research is therefore the discovery of mitochondrial genetic disorders (next to accelerated ageing, neurodegenerative disease, cancer, diabetes), and the study of their mechanism of action (e.g. [23]).

Another promising area is the mapping of evolutionary branches and the determination of the more precise taxonomic location and movement of different species (including humans [24]). Mutations will be passed over into all maternal progenies homoplasmic making individuals of a maternal lineages the same in mitogenome, especially, when they share entirely homoplasmic mitochondrial pool.

For the third time, it is worth mentioning the unfolding of the microevolutionary web of our domesticated animals and the knowledge of the origin (at the same time geographical) of the breeds that have developed today (e.g. [25]).

3. Non-coding and coding region on mtDNA

3.1 Control region

On the circle of the mtDNA, there is a specialized sequence, called control region (CR), and also called D (displacement)-loop because of its peculiar protrusion. The CR is made up of a triplex DNA structure at the site of origin of the heavy strand. This region is critical for the initiation of transcription and translation [7].

Zardoya et al. [26] determined the nucleotide sequence of the sheep mitochondrial DNA CR and its flanking tRNA genes. They found that several conserved motifs characteristic mammals have been identified along the 1189-bp sequence of the sheep control region: ten termination-associated sequences (TASs) and one conserved sequence block (CSB-1). CSB-2 and CSB-3, which are frequently determined in most species, are not present in the sheep CR, which shows instead a short direct repeat at their usual localization.

The CR contains hypervariable sites (mutational hotspots). This unstable segment gives the basis for dating estimation in many mammalian species. However, according to some authors (e.g. [27]) the high occurrence of recurrent mutations may bias dating estimates. Also within the sheep species, according to Pedrosa et al. [28], the time of separation of haplogroups is significantly earlier if hypervariable CR is taken into account. Researches on human mtDNA raise the concerns that focusing exclusively on CR can be inadequate [29].

3.2 Genes

A total of 37 genes of mtDNA are coding 2 ribosomal RNAs, 22 transfer RNAs, and 13 mitochondrial proteins as well in mammals. The latter, without exception, direct cells to produce protein subunits for enzyme complexes of the oxidative phosphorylation system. Mitogenome has a very similar conservative nucleotide sequence in all organisms.

A meta-analysis study [30] of over 1500 animal species revealed that the average within-species level of mtDNA diversity *per se* is remarkably similar across animal phyla. Reason for that is a recurrent selective sweeps which would affect mtDNA evolution in species causing frequent drops in diversity at the whole genome level. Based on that hypothesis and being high conservative gene dense, according to the report of Galtier et al. [31] mtDNA is by and large not the satisfactory marker of molecular diversity and representation of population history. After that, it may come as a surprise the most popular marker of molecular diversity in animals, a mitochondrial fragment, COX1, was recently elected as the standardized tool for molecular taxonomy and identification [32].

Some genes even overlap. In the mitochondrial genome, some triplet codons may be the final stage of one gene but also (in a functional overlapping) the initial stage of the next gene. Another feature is that the mtDNA is transcribed from several structural genes to the messenger RNA at the same time. Thus, large mitochondrial mRNAs contain instructions for the synthesis of different proteins. The inheritance of the extranuclear genes are independent of nuclear genes, but, they interact with each other in function. The mitochondrial genome is not able to produce all the proteins required for phosphorylation on its own, so mitochondria are highly demanding of gene products produced by the nuclear genome.

Investigation of Rocky Mountain bighorn sheep (*O. canadenesis,* [33]) revealed 16,466 bps, with about 40% GC content. Further on, it confirmed also the bighorn sheep mitochondrial genome has 22 tRNA genes, 2 rRNA genes (12S and 16S), and

13 respiratory genes (ATP6, ATP8, CYTB, COX1, COX2, COX3, ND1, ND2, ND3, ND4, ND4L, ND5, and ND6). Comparison of the genome of bighorn sheep with the genome sequence of other sheep showed 99.6% identity, indicating the separation of the bighorn 3 million years ago from the sheep living at that time.

Higher mutation rate of mtDNA will cause increased rate of genetic diseases of mitochondrial origin [34]. Therefore, such diseases are also all of maternal origin. Males can be carriers of a given genetic defect and can be affected by its manifestation, however, they are not responsible for transmitting that disease into their progenies.

Since mitochondria acts as the powerhouses of cells, tissues that have high energy demands (brain, retina, skeletal muscle, and cardiac muscle) are particularly vulnerable to the harmful consequences of mutation. As an inherent part of energy production, mitochondria create reactive oxygen species (ROS) as well which is seen to cause further mitochondrial mutations. Elevated levels of ROS negatively affect cellular metabolism, thereby accelerating the cell ageing process and increasing the likelihood of cell death. Symptoms of mitochondrial diseases in humans can usually include: poor growth; muscle- weakness, pain, low tone, exercise intolerance, and movement disorders; vision and/or hearing problems; learning disabilities and mental retardation; autism and autism-like features; heart-, liver- or kidney diseases; gastrointestinal disorders, swallowing difficulties, diarrhea or constipation, unexplained vomiting, and cramping, reflux; diabetes; increased risk of infection; neurological problems, seizures, migraines; strokes; thyroid problems; respiratory (breathing) problems; lactic acidosis; dementia [35]. Fortunately, next-generation sequencing techniques have substantially improved genetic diagnosis.

Individuals resulting from cloning procedures (*nuclear transplantation* or *somatic cell fusion,* [36]) are heteroplasmic. The initial heteroplasmic stage of chimeric offspring cell turns usually into homoplasmy with prevailing mitochondria of the host oocyte [37]. It is likely that heteroplasmy of mitochondrial genomes will be terminated by selective elimination of donor or recipient mitochondria by chemical or other means. Establishing biotechnical approaches allows women with mitochondrial diseases to have reproductive options. Recent advances in these including *in vitro* fertilization techniques with mitochondrial donation, will serve as a solution in the future [38].

Mutation in mtDNA-coded ribosomal RNA, called RNR1 indicates the presence of an environmental effect. That mutation causes deafness in children, but the clinical symptoms of the deafness are related to the administration of certain antibiotic type [39].

The percentual manifestation (penetrance) of a mitochondrial disease in males and females differing from the expectations points to the likely involvement of other (nuclear) genes and environmental factors. *Leber hereditary optic neuropathy* (LHON) is an example for mitochondria-associated disorders which is manifested in loss of vision [40].

First reported Pal et al. [41] the association of cytochrome b (Cyt b, CYTB) gene with disease traits in sheep. Mutations of Cyt b gene (non-synonymous substitutions: F33L and D171N) interferes with the site of heme binding domain and calcium binding essential for electron transport chain causing anemia, malfunctioning of most of the vital organs. This discovery raises the possibility that the sheep may come into play as a model of man.

Results of Reicher et al. [42] revealed ovine mitogenome genetic variation in protein- and tRNA coding genes (26 and 8 mutant sites, respectively) and emphasize that sequence variation is associated with ewe prolificacy.

Yüncü et al. [43] tested the restriction fragment length polymorphism (RFLP) method (applied to CR) and the single strand conformational polymorphism

(SSCP) method (applied to NADH dehydrogenase subunit 2 and 4) for reliability in haplogroup classification. Among these the SSCP analysis of NADH dehydrogenase subunit 2 exhibited the highest discrimination power among these. Starting with that, authors advice a stepwise screening, when whole sequencing is not easy available.

4. Mitochondrial investigation in sheep

4.1 Whole mitogenome

Over the half (58%) of the mitogenome was completely (included CR and CYTB, ND2, ND3, ND4L, COX3, and 12 tRNA genes, and the origin of L strand replication), and partially (12S and 16S rRNA and an additional six protein coding and six tRNA genes) analyzed by Hiendleder [44]. In that research, the CRs and the coding regions shown 4.34% and 0.44% divergence in the comparison of sheep haplogroup A and B, respectively.

Table 1 represents the complete mtDNA molecular sequencing of the domestic sheep (*Ovis aries*) achieved by Hiendleder et al. [45]. The length of the complete ovine mtDNA presented is 16,616 nucleotides (nt), which length is variable, due to heteroplasmy caused by the occurrence of different numbers of a 75-nt-long tandem repeat in the CR. The majority of domestic sheep contained four copies of that 75 bp repeat unit in work of Meadows et al. [46] resulting in a mitogenome of 16,616 or 16,620 bps.

Using 14 restriction enzymes Hiendleder et al. [47] evaluated haplotypes of restriction fragment length polymorphisms (RFLP) based on pairwise nucleotide sequence divergence between haplotypes, and proved that the domestic sheep come exclusively from *Ovis orientalis*.

Sanna et al. [48] reported the first complete mitogenome of the (*Ovis gmelini ophion*), and compared to the known five mitochondrial haplogroups. They suggest that the Cyprus Mouflon, a feral variant of domestic sheep diverged from urial (*O. vignei*) and argali (*O. ammon*) about 0.89 and 1.11 million years ago.

Lv et al. [49] performed a meta-analysis using complete and partial mitogenomic sequences. They suggest sheep individuals migrating east from Fertile Crescent on the Mongolian Plateau region may have formed another centre for the further spread of domestic sheep 3–5 thousand year B.C., at the same time extending the haplogroup C.

The complete mitochondrial genome provides complex information for knowledge of phylogeography and population genetics in sheep. The mitochondrial genomes of several breeds of domestic sheep (*Ovis aries*) have now been mapped by Davenport et al. [33].

4.2 Examination of fossils

The sheep (*Ovies aries*), together with the dog are the earliest domesticated animal species, and had remarkable role in the life of ancient societies. Sheep were domesticated around 7–9 thousand years B.C. in the area of the Fertile Crescent. Demirci et al. [50] found a time-dependent change in the incidence and proportions of haplotypes in Anatolia. Ancient samples showed the presence of haplogroup E (3%) in the Bronze Age and the presence of haplogroup C (6%) in the Hellenistic age, while haplogroups A and B were continuously present (with nearly 50–50 percent).

Feature	From	To	Size	Start codon	Stop codon[b]	3′ spacer
tRNA-Phe	1	68	68			
12S rRNA	69	1,026	958			
tRNA-Val	1,027	1,093	67			
16S rRNA	1,094	2,667	1,574			
tRNA-Leu (UUR)	2,668	2,742	75			AA
NADH1	2,745	3,700	956	ATG	TAa	
tRNA-Ile	3,701	3,769	69			
tRNA-Gln (L)	3,767	3,838	72			AT
tRNA-Met	3,841	3,909	69			
NADH2	3,910	4,951	1,042	ATA	Taa	
tRNA-Trp	4,952	5,018	67			A
tRNA-Ala (L)	5,020	5,088	69			A
tRNA-Asn (L)	5,090	5,162	73			
Origin of L-strand repl.	5,163	5,194	32			
tRNA-Cys (L)	5,195	5,262	68			
tRNA-Tyr (L)	5,263	5,330	68			C
COI	5,332	6,876	1,545	ATG	TAA	
tRNA-Ser (UCN) (L)	6,874	6,944	71			TAAAC
tRNA-Asp	6,950	7,017	68			T
COII	7,019	7,702	684	ATG	TAA	AAT
tRNA-Lys	7,706	7,773	68			T
ATPase8	7,775	7,975	201	ATG	TAA	
ATPase6	7,936	8,615	680	ATG	TAa	
COIII	8,616	9,399	784	ATG	Taa	
tRNA-Gly	9,400	9,468	69			
NADH3	9,469	9,815	347	ATA	TAa	
tRNA-Arg	9,816	9,884	69			
NADH4L	9,885	10,181	297	ATG	TAA	
NADH4	10,175	11,552	1,378	ATG	Taa	
tRNA-His	11,553	11,621	69			
tRNA-Ser (AGY)	11,622	11,681	60			A
tRNA-Leu (CUN)	11,683	11,753	71			
NADH5	11,754	13,574	1,821	ATA	TAA	
NADH6 (L)	13,558	14,085	528	ATG	TAA	
tRNA-Glu (L)	14,086	14,154	69			ACTA
Cyt b	14,159	15,298	1,140	ATG	AGA	CAA
tRNA-Thr	15,302	15,371	70			

Feature	From	To	Size	Start codon	Stop codon[b]	3′ spacer
tRNA-Pro (L)	15,371	15,436	66			
Control region	15,437	16,616	1,180			

[a]Nucleotide number 1 is the 5′ end of the tRNA-Phe-specifying gene. Anticodons for the two tRNA-Leu and the two tRNA-Ser are given in parentheses. (L) denotes light-strand sense. Positions include the 58 and 38 nt of each feature. ATPase6 and ATPase8, genes encoding subunits 6 and 8 of ATPase; COI-III, genes encoding subunits I–III of cytochrome c oxidase; Cyt b, gene encoding cytochrome b; NADH1–6, genes encoding subunits 1–6 of nicotinamide adenine dinucleotide dehydrogenase.
[b]Incomplete stop signals are denoted by lowercase letters.

Table 1.
Features of the Ovis aries *mitochondrial genome[a] [45].*

Dymova et al. [51] carried out archeological mitochondrial DNA D-loop fragment analysis based on about 4,000–1,000 years old sheep bone remains in Altai. They found all the previously determined haplogroups (A, B, C, D and E lineages). That richness of diversity led them to conclude that the Altai region had been a migratory area for many sheep and peoples in the past.

Study of Horsburgh and Rhines [52] evaluating sheep finds excavated in a South African Neolithic Age cave shown their assignation to haplogroup B.

In comparative study of samples dated primarily by archeological context and ranged from Late Bronze Age, through Iron Age to post-medieval period Rannamäe et al. [53] identified four novel ancient haplotypes specific to Estonia (H3, H4, H5 and H9), and haplogroups A and B in a ratio of one to two.

4.3 Displacement-loop

Mitochondrial displacement-loop (D-loop), called also as control region (CD) is a frequently investigated sequence in researches, also in sheep.

Divergence times estimated for types B and A (which was about 1.5–0.45 Mya) can be overestimated when it is based solely on hypervariable sequence of CR [54]. In regard of calibrating a molecular clock, the consideration of the codifying cytochrome b gene seems to be more accurate. To eliminate the distortive effect (known heteroplasmic behavior) of CR the repeat unit located within the CR region was removed before phylogenetic inference made by Meadows et al. [46].

The purpose of sequencing is, in addition to the genetic characterization of a given breed (population), to compare its genetic material to genetic material of other already sequenced breeds (GenBank sequences). Thus, we try to get an answer to the origin of the given breed and its genetic relatives. This is important when studied in the former natural range of the sheep species (primarily Asia, then Europe and Africa), but it is also important on the continents (America and Australia) where sheep individuals later entered with migrants.

For example, based on the CR, Annus et al. [55] confirmed the common origin of the Hungarian Tsigai with European sheep after finding that they are belonging to the haplogroup B (with the exception of 6% to the haplogroup A). An example of the latter case is the evaluation of the Mexican Creole sheep carried out by Alonso et al. [56], which revealed a narrow Iberian maternal origin.

Lancioni et al. [57] discovered relationships of the three Italian Merino-derived sheep breeds, and obtained that these are representatives of the predominant haplogroup B (99%). Since almost all the animals are carrying an own individual haplotype these are characterized with a diverse genetic background. On the other hand, this processing gives an example of how, despite upgrading (with Merino), the maternal background (Appeninica) is clearly discernible.

4.4 Cytochrome b gene

It was observed cytochrome b (Cyt b) gene is also quite mutable than other mtDNA coding regions. For this reason, phylogenetic studies have used that marker too to investigate the genetic relationships among breeds. By use of cytochrome b gene and displacement-loop together or individually, like before, five haplogroups of sheep can be distinguished from each other [46].

In the phylogenetic sequence analysis based on cytochrome b gene Bunch et al. [58] revealed an about 3.12 million yearlong evolution of true sheep (*Ovis*). On the course of its evolutionary history there have been three major genetic groups developed. Foremost, the Argaliforms (*Ovis ammon*, 2n = 56) and Moufloniforms (*Ovis musimon* or *O. orientalis*, 2n = 54, and Urial/*Ovis vignei* 2n = 58) diverged from the initial ancestral stock 2.3 million years ago and spread on Eurasian continent. The domestic sheep (*Ovis aries*, 2n = 54) descend solely from *O. orientalis*. Second, the snow sheep group (*Ovis nivicola*, 2n = 52) as a variant of Pachyceriforms took their own shape in Eastern Asia from about 1.96 million years ago, then the other variants of Pachyceriforms (*O. canadiensis*, 2n = 54; *O. dalli*, 2n = 54) separated from them at about 1.41 million years ago, and evolved further in North America. Argaliforms are represented by only one species, *O. ammon*. The ancestral karyotype had 2n = 60 chromosomes. During the evolutionary development of variants of the species, also acrocentric fusions of chromosomes are observed.

According to the initial studies (e.g. [59, 60]) haplogroup A predominates in Asian sheep, while haplogroup B predominates in European sheep. Nevertheless, haplogroup C seems to have a wide geographical distribution [61]. Pedrosa et al. [28] and Chen et al. [61] suggested the divergence time of haplogroup C from haplogroup A and B to be approximately 0.42–0.76 million year ago and approximately 0.45–0.75 Mya from the analysis of control region and Cyt b gene sequences, respectively. However, a study of Meadows et al. [46] using 12 protein-coding genes of mtDNA puts the separation between the haplogroups less early (0.59–1.17 Mya between A and B, and 0.26–0.09 Mya between C and E.

4.5 ND5 gene

Tserenbataa et al. [62] collected 71 argali sheep (*O. ammon*) samples from three main geographical regions of Mongolia, and additionally from Kazakhstan and Kyrgyzstan. Based on the sequenced 556 bp of the mitochondrial ND5 gene. They differentiated 17 haplotypes which were differed far from each other by transitional substitutions in most of the cases. Nucleotide diversity was low within the three regions from Mongolia ($\pi = 0.0029$) compared to Kazakhstan and Kyrgyzstan. While the variance occurred within populations was as much as 85.76%. Finally, the use of mitochondrial ND5 gene provided an opportunity to detect divergence between the Altai and Gobi, and Altai and Khangai populations at a significant level.

4.6 Haplogroup dispersion

For phylogenetic relationship between mitochondrial haplogroups of domestic sheep Meadows et al. [46] observed the greatest distance between B and C (nucleotide difference, D = 163.5), closely followed by B–E and C–D (D = 162.0, identically). The lowest number of nucleotide differences was 93.0 and 58.5 between A and B, and C and E, respectively. **Table 2** reveals the genetic distance between domestic sheep haplogroups in addition to Urial, Argali to us.

	HA	HB	HC	HD	HE	Mouflon	Urial	Argali
HA	—	0.57	0.93	0.75	0.90	0.58	2.19	2.53
HB	93	—	1.01	0.81	1.00	0.07	2.31	2.59
HC	150.5	163.5	—	1.00	0.36	1.00	2.33	2.65
HD	122.5	131.5	162	—	0.98	0.81	2.27	2.61
HE	147	162	58.5	159.5	—	0.98	2.30	2.63
Mouflon	94	11	162.5	131.5	160	—	2.31	2.60
Urial	357.7	377	380.3	370.5	375.7	377.3	—	2.32
Argali	413	423	433	425.5	429	424	379	—

The average number of nucleotide differences (D) is given below the diagonal and nucleotide substitutions per site (K, given as a percentage) are given above the diagonal for the full mitochondrial sequence after removal of both indels and the repetitive component of the control region.

Table 2.
Genetic diversity observed between domestic and wild sheep mitogenomes [46].

The distinct haplogroup diversity of sheep mtDNA is comparable with what is observed in goats and cattle, although the divergence of sheep haplogroups is less pronounced than the *taurine–zebu* divergence [63]. Also, sheep haplogroups show little association with the geographical origin, in contrast to bovine haplotypes. A given sheep haplogroup can assume several regions of origin, or the coexistence of several different maternal lineages in a domestication centre can be suspected.

4.6.1 Haplogroups A, B, C, D, and E in Asia

In a today phylogenetic study of Ganbold et al. [64] revealed three haplogroups (A, B, and C) in Mongolian native sheep. The Mongolian Plateau, as mentioned above played a determining role in the arrival of sheep in eastern Asia. And, as a consequence of it, they observed a small genetic differentiation between breeds from Mongolia and China.

The Moghani sheep of Iranian plateau was identified in haplogroup A [65].

Haplogroups D and E are the least frequent and have only been identified in samples from Turkey and the Caucasus [66, 67]. Slowly, haplogroup E was detected also in Iran [68]. In a paper of Liu et al. [69], the proportion of haplotypes of lineage D was 0.157% in Tibetan sheep, further demonstrating that lineage D is the rarest of the mtDNA lineages.

4.6.2 Haplogroups A, B, C, and D in Europe

Haplogroup B is scattered in numerous countries of Europe (e.g. [57, 70]. The haplogroup B seems to be expanded around 6,400 years ago and reached Western Europe before the haplogroup A [48].

Within Europe haplogroup C has been found, so far, only on the Iberian Peninsula (in Portugal [71] and in Spain [72]) and in the southern countries of the Balkan Peninsula (in Albania and Greece [73]. Haplogroup D is present in Italy in breeds Bergamasca and Laticauda [74].

4.6.3 Haplogroups B and C in Africa

Haplogroup B is also dominant in Africa as it was revealed in some today publications: in Benin in breed Djalonke [75], in Mauritania in breeds Peul and

Touareg [76], in Somalia and Kenya in Red Massai and Blackhead, respectively [77], and in Egypt in Barki and Ossimi breeds [78].

Ghernouti et al. [79] found thin-tailed Arabic breeds in Algeria belong to haplogroup B (87%) and C (13%). Authors believe the presence of haplogroup C in breed Ouled Djellal is a proof of the Middle Eastern origin of that breed. The haplotype C, also identified in Egyptian breeds is in agreement with the assumption of early spread in sheep [78]. Studying the Siroua sheep in Morocco two haplogroups (haplogroups B and C) were also identified by Kandoussi et al. [80] with a predominance of haplogroup B.

4.6.4 Haplogroup B in America

Analyzing mtDNA control region of 40 unrelated domestic sheep in Mexico, Campos et al. [81] revealed 31 different haplotypes with 74 polymorphic sites. The phylogenetic analysis identified all Mexican sheep as belonging to haplogroup B. Sheep from other American regions (Brasilia and Cuba) in that analysis made sure the high frequency of an ancestral haplotype (h15) in Ibero-American countries as well.

Revelo et al. [82] identified the Creole sheep as exclusive haplogroup B, and justified that the two seriously different types of Creole sheep (wooly and hairy) of Colombia descent from an Iberian and an African ancestor.

4.6.5 Haplogroup a and B in Australia

Hiendleder et al. [54] suggest that the high frequency of haplogroup A (beside B) in New Zealand resulted from early imports of fat-tailed Indian sheep (beside mouflon specimens) into Australia in accordance with the sheep stream hypothesis.

4.7 Haplotype diversity

With the third of the aforementioned goals of mitochondrial research, we can relate that subchapter the most. In characterizing the haplotypes of the breeds and comparing them with each other, CR may fulfill the expectations placed on it. Going further, CR can also gain ground in research into the genetic structure of the breed (sub-breed, variety, family).

D-loop of two Tibetan sheep breeds was analyzed by Wang et al. [83]. The length of the D-loop sequences varied considerably (between 1,107 bp and 1,259 bp) according to the copy numbers of a 75 bp tandem repeat located from 640 bp to 1,140 bp. That variability was most characteristic for haplogroup C, less so for A and B. Fu's test showed that the populations had not been expanded historically ($0.10 > p > 0.05$). Results are useful for the conservation and utilization of Chinese sheep genetic resources.

In randomly collected samples of four Nigerian breeds 96 haplotypes were observed with a high mean haplotype diversity of 0.899 ± 0.148. The high percent of variation (99.77) found by Agaviezor et al. [84] within populations indicates common origin of these breeds. However, the evolutionary divergence of the breeds (Yankasa, West African Dwarf, Balami, and Uda) based on mitochondrial DNA D-loop sequence may be coincident with their geographical distribution in Nigeria.

Arora et al. [85] compared 19 Iranian sheep breeds in their extended CR study. They confirmed the majority of the breeds belong to haplogroup A solely, and five breeds appear with of haplogroup B as well. Both haplogroups show unimodal patterns of mismatch distribution curves, and the significant minus F_S statistics values indicate population expansion in Indian sheep population.

The control region of mtDNA showed polymorphisms at 32 sites in the Hungarian Cikta evaluated by Kovács et al. [86]. However, herds shared 24 polymorphic sites, so the maternal background of the Cikta appears to be genetically uniform. The total number of haplotypes were 13, furthermore, most of the samples belonged to the haplogroup B of sheep. The average number of pairwise differences (k) and the average nucleotide diversity (π) were 6.863 and 5.95 × 10–3, respectively. The values of the Cikta population were not significant ($p < 0.10$) neither by the Tajima D-test (0.107) nor by Fu's Fs statistics (2.533), meaning that the greatly reduced population size of the breed known from the breed history did not cause genetic drift, it is in genetic equilibrium regarding its ancient families. The Cikta shown some degree of genetic narrowing based on Cyt b gene [87]. However, the average number of pairwise nucleotide differences is relatively high, which indicates different genetic characteristics of the families occurring in the farms.

Kusza et al. [88] investigated the two variants of Wallachian sheep by country sequencing 599 bps of the D-loop region. They isolated altogether, 42 haplotypes, of which 23 were common in both eco-types. Since they estimated a very low level of genetic differentiation between the Gyimesi Racka (in Hungary) and Turcana (in Rumania) breeds, therefore these are really two variants of one transboundary breed.

According to the haplotype diversity results Kirikci et al. [89] stated the Karayaka breed from Northern Anatolia cannot be categorized as a genetically homogeneous population. That breed not only has not suffered from a genetic bottle neck effect, but even has four different haplogroups (A, B, C, and E).

5. Animal genetic resources

Term animal genetic resources is defined shortly as a potential of domestic animals that is used for production of food and fiber [90]. Animal genetic resource management is necessary on a global scale and its improvement requires careful thinking. While the contribution of livestock sector to 43 percent of world's agricultural Gross Domestic Product, which in some developing countries accounts for about 30 percent of national agricultural GDP. Actual economic modeling estimates that for those rural populations, poverty is limiting, economic growth suggested to be critically low. The fate of poor people and their livestock is interlinked, so none should be overlooked in future food security efforts [91]. The World Bank forecasts that contribution of livestock sector to agricultural GDP in undeveloped regions will be necessary by about 80 percent between 2000 and 2030.

Sheep are very important in the socio-economic lives of the people. However, their potential is not realized under poor conditions because of low productivity resulting from high mortality and weak performance among others. That fact calls the attention to the environment of production. But a given loss of animal genetic resources concerns the loss of genetic diversity within improved, cosmopolitan breeds and not only the extinction of traditional breeds [92]. The first reason for loss, the uniformity with increasing homozygosity as consequence of enormous development of highly improved breeds has led to growing concerns about the erosion of genetic resources [93]. Lenstra et al. [94] give a detailed review about molecular tools and analytical approaches for the characterization of farm animal genetic diversity.

Integration of local breeds threatened by extinction but carrying appropriate alleles into the further refinement of breeds for mass production result in effective management of erosion of farm animal genetic resources (FAnGR, [95]). Therefore, the maintenance of old, local breeds is in any case justified by this requirement.

However, autochthonous breeds are the national treasure of a given country and, as such, their maintenance is the duty of that state. In addition, in my personal opinion, access to the benefits of this treasure needs to be regulated for one's own country, but especially for other countries.

6. Maintenance of endangered breeds

6.1 Reasons for endangerment/extinction

FAO [96] listed the broad categories of threats in three major groups: trends in livestock sector, disasters and emergencies, and animal disease epidemics and lack of control measures.

Considering the first one, the global reliance on a very limited number of international, specialized (single purpose) breeds suited to the needs of high input high output industrial agriculture can be mentioned. This expansion was accompanied by the grading-up of local breeds, by changes feeding-, housing, and reproduction technologies.

Under the second group of treats the lack of development interventions, appreciation, sustained breeding programmes, and loss of labour force (migration to urban areas in search of employment), traditional knowledge associated with livestock herding, further on changes in land use (destruction of native habitats), inappropriate management of climate change and natural disasters (floods, drought, famine). In many places, to this is added the local conflict (socio-political), and a range of political instability (civil strife, war).

The third means: inadequate control of disease epidemics, lack of disease control, preventive treatments, genetic control of inheritable defects, as well as lack of identification, transport, traceability, food chain controlled.

6.2 Arguments for preservation

The experts collect the following economic, scientific, human cultural, socio-economic, and environmental rationales for preservation beside the needs for development and sustainability mentioned formerly.

Genetic variation is the raw material for animal improvement. Prudent economy demands conservation. Lost flexibility will limit the ability of future generations to respond to changed markets and opportunities. Old breeds are of unique physiological or other traits. They can show specific adaptation ability, resistance to diseases Biotechnology will need to reveal unique sequences of DNA. Based on microsatellites, Agaviezor et al. [97] concluded that these associated with unique ancestral alleles of certain functional genes may reflect a better adaptability in more agro-ecological zones. Firestone et al. [98] shown through simulations that with samples of at least 30–40 individuals found the correct ratio of private alleles in most cases can be. Due to the low frequencies of the private alleles in a study, the results should be interpreted cautiously and viewed more as a trend.

Animal husbandry is a special characteristic of human culture. It is comparable to other great reminders of man's past. Rare breeds are results of human creation (worth preserving and conserving as any other work of art, like monuments or buildings). They are kept for demonstration and showing of historical development of animal husbandry, and are of great advantage and value for physiological and genetic comparative studies. Some domestic animal breeds are historically closely linked to different farming cultures, environment and regions, traditional and regional. Livestock are part of life style in all the countries.

Impact of changed animal genetic diversity affects their whole life style. In integrated crossing programs with high yielding breeds it may be economical (crossed progenies for market and pure bred progenies for herd replacement!). Under unfavorable (harsh) conditions it can be bred with minimum input.

Our old, native breeds are multipurpose animals. However, we have become accustomed to using several benefits. Therefore, in addition to primary production (which is already known to be low), it is necessary to emphasize the use of animals in several ways during their lives (e.g. grazing/landscape care, traction, tourist attraction) and efficient processing of slaughtered animal parts through traditional handicrafts (e.g. fur and horned skull/trophy). What is very important, and what is realized exclusively in *in situ* gene conservation, is the regular use of animals. In the original, traditional environment, the genetic ability of the breed can be manifested. Raw materials of animal origin obtained directly or indirectly must be processed regularly and the product sold (although this is often seasonal).

Animal, plant, forest, fish, wildlife genetic resources are equal major components of global biological diversity and must be viewed together. The environmental implications of livestock are huge. Billions of livestock in human care moving over large areas of land where they affect the soil, water and vegetation, and interacting with other life. Therefore, the environment must be considered as a whole.

6.3 Breed history for preservation

In my opinion, maintaining a variety is more than finding and currently using a beneficial gene. The rationale for the preservation of old breeds is given by the history and former significance of the breed. Regarding the breed history, I would like to call the attention to the *in libro* concept of conservation worded by us formerly [99]. A longer version of the technical term is *in libro conservation in causa emoriendi*, namely the conscious preservation of an already extinct domestic animal breed or a lost characteristic entered. The meaning of the entered (in a book; booked) conservation in broader sense is the preservation of all the remaining knowledge, keepsake, documents and material heritage of a still living rare breed. As a reason for the *in libro* conservation the same arguments can be presented as for the *in vivo* and *in vitr*o approaches of conservation. The keeping in life of an extinct breed does not crop up but the "keeping alive" its one-time presence in the common knowledge is an important role. The cradle of the breed should not be forgotten.

In contrast to mtDNA, Peter et al. [100] among others, reports the disadvantage of microsatellites in search for breed history, especially in open flocks. Although microsatellites on nuclear chromosomes describe the current genetic nature and genetic relatedness of the herds, the genetic character depends heavily on the genetic makeup of the rams being actually used. The breeds can no longer be clearly assigned to one of the breed groups, but can be found in the mixed populations.

6.4 Herd booking and pedigree analysis

The official, mandatory individual animal numbering is essential for everyday breeding work. The parentage control is also essential for reliable pedigree registration. Of course, among the pedigree data we also store the regularly recorded (in many cases seemingly redundant) production data. Most multi-purpose indigenous breeds are utilized solely through their meat production today, however, in order to preserve the valuable traits, not only the fattening and slaughter values, but also, for example, the wool production traits must be recorded and selected for.

Pedigree data are fundamental to the assessment of the demographic structure and risk status of livestock breeds. Careful investigation of herd book data will

serve knowledge on pedigree completeness [101], effective population size [102], factual number and effective number of founders and ancestors, respectively (these two later explain the complete genetic diversity of a population [103]), and founder genome equivalents [104].

The length of the generation interval (defined as the average age of parents at the birth of their progeny kept for reproduction) is one of the keys to demanding breed maintenance. The four pathways (sire-son, sire-daughter, dam-daughter, and dam-son) of generation interval should be as long and similar as possible. The higher this is, the lower the possibility of an annual gain even in the case of a preserving section that may not be perfectly implemented.

Based on pedigree data the average relatedness coefficient of each individual is evaluated together with the inbreeding coefficient. Inbreeding coefficient (autozygosity) is influenced by the length and completeness of pedigree, the longer and more complete the available pedigree is the more reliable the estimated inbreeding coefficient.

6.5 Preserving selection

When maintaining endangered animal breeds, care must be taken not to preserve the name of the breed in the new, upgraded population, or to ensure that the critical herd size is maintained for generations. The purpose of breed maintenance is to preserve the original level of phenotypic (and underlying genetic) characters of the breed. The constancy and possible, unexpected change of the production level can be verified in historical comparative study (e.g. [105–107]. That is, in order to maintain within-breed diversity, it is necessary to leave individuals from all phenotype groups (production levels, ideally based on their breeding value) for further breeding. This is the aim of preserving selection. The principle of diversity-preserving selection is very far from conventional truncation selection. The selection limit is not determined by the performing-ability, but by the measure of the remount rate proportional to the Gaussian curve.

6.6 Founder sampling

The genetic composition of a given sheep breed is also likely to be mixed in terms of mtDNA haplotypes. As a breed has a remarkable higher number of different mutant sites overall it can be assumed that more founder animals were present in this population have to. The long-known history of this breed (sheep herd of the area) and the current genetic mapping can provide certainty, confirming the colorful background, possibly the contribution of several breeds or sheep subspecies to the gene pool.

From the current point of view, the essence of the pedigree study is the identification of female founder individuals and the families derived from them. In order to capture full diversity the DNA samples should be taken from the living descendants of the eldest families based on herd booking. In the study of mtDNA of two Hungarian native sheep breeds (Tsigai and Racka), sample collection and analysis [55, 87] was preceded by the processing of pedigree data and the identification of ancient families [108, 109].

6.7 Maternal lineages

As previously described, reliable characterization of the breed for mtDNA should be performed on samples taken from representatives of maternal lineages. The genetic background and the current diversity thereby reliably discovering in

any breeds. The number of haplotypes representing individuals in the sampled families or providing the sample is likely to be less than the number of samples. Unfortunately, it can also be predicted that the number of surviving founder families will decrease from generation to generation. Thus, it is recommended to leave breeding offspring from all families, from all haplotypes, to replenish the female herd. Since the number of individuals is higher than the number of haplotypes a rigid selection focussing on haplotype maintenance can lead to loss of many other genetic information (like in selection against scrapy genotypes).

Of course, it is a big question whether, in the case of outlier haplotypes, we choose to save or discard it in the endangered population of small size. Each cell contains many copies of mtDNA which, except in very rare cases of heteroplasmy, are identical and shared by all members of the maternal lineage. Another problem may then be the treatment of the heteroplasmy.

To my mind, the searching for ancient families is very crucial aspect of breed conservation. I consider it important to inform farmers about the family and haplotype of their sheep. In this respect, close long-term cooperation is needed between breeders and breeders' associations, and even with the breeding authority in obtaining state subsidies. In other words, the individual sheep genetic knowledge gained from the researches should be communicated to the animal owners.

6.8 Within-family selection

If differentiated families with their specific haplotype are already available, it is reasonable to select offspring for further breeding within these. Using a within-family selection, potential offspring are identified by sophisticated breeding software, while the breeder remains free to choose which one to actually stay for breeding.

At this point, I would like to draw attention to the differences between the professional work of the association and the ideas of the private breeder, and the necessary cooperation, or the antagonism in many places that the breeder is the owner of the animal but the breed must be preserved by the state.

7. Conclusions

During the phylogenetic investigation of sheep species, and characterization for improvement and conservation of sheep breeds, mtDNA diversity plays an important role. This is indeed noticeable as it is being applied more and more widely. However, opinions differ on the degree of success of the study in terms of its purpose. Here, too, it is true that any doubt encourages further appropriate work.

For comparison between domestic and wild sheep the less mutagen gene (Cyt b) sequences are advisable. But, for the exploration of haplogroup relationships among domestic sheep processing of a Cyt b gene dataset combined with CR is recommended. Then, for a precise differentiation of haplotypes a hypervariable sequence set of CR seems to be the most reliable. High levels of mutations observed in the control region (emphatically hypervariable sequence) may skew dating estimates for many mammalian species. Before drawing phylogenetic conclusions the removal or reduction of repetitive sequence elements located within the CR is to advice because of its known heteroplasmic behavior.

Coding regions are conservative, for function with sameness making them less useful for isolating species, breeds, and individuals. Investigation of these is important in screening for deleterious alleles.

At the same time, there is a need to consider more segments of the mitogenome which are used to outline the phylogeography of the species (since a single segregating locus does not adequately represent the origin of the entire genome), and the more of these, the more reliable they are. However, consideration of one or a few sequences in the context of species domestication may be advantageous in itself. In addition to the number of sequences investigated, their length (number of mutant sites) and the number of individuals sampled should not be overlooked in terms of processing reliability and comparability.

The fluent determination genetic diversity and its maintenance at a high level is indispensable on the course of preservation of our old, rare landraces. Indigenous breeds are national treasures and an aim of the saving of such breeds is not only to be prepared to compensate for the genetic erosion of another breed, but manufacture local products, preserve ancient knowledge and conserve natural landscape, furthermore transfer historical and cultural values continuously.

In order to map the broad genetic background biological samples should be taken from the descendants of the founders. During the future preservation work special attention should be paid to maternal families and representatives of ancient families should be preserved. A more intense focusing on the maternal side is motivated also by the fact that the females exceed in number the males, respectively they remain in breeding for a longer period of time, so they can at larger extent be the depositaries of realization and maintenance of genetic diversity.

Acknowledgements

This project is supported by the European Union and co-financed by the European Regional Development Fund (ERDF) (Grant Contract No: VEKOP-2.3.2.-16-2016-00012).

Conflict of interest

The Author declares no conflict of interest.

Author details

András Gáspárdy
University of Veterinary Medicine, Budapest, Hungary

*Address all correspondence to: gaspardy.andras@univet.hu

References

[1] Hackmann TJ, Spain JN. Mint: Invited review: Ruminant ecology and evolution: Perspectives useful to ruminant livestock research and production. Journal of Dairy Science. 2010;93(4):1320-1334. DOI: 10.3168/jds.2009-2071

[2] Lalueza-Fox C, Castresana J, SampietroL,Marquès-BonetT,AlcoverJA, Bertranpetit J. Mint: Molecular dating of caprines using ancient DNA sequences of Myotragus balearicus, an extinct endemic Balearic mammal. BMC Evolutionary Biology. 2005;5:70. DOI: 10.1186/1471-2148-5-70

[3] Joost S, Bonin A, Bruford MW, Després L, Conord C, Erhardt G, Taberlet P. Mint: A spatial analysis method (SAM) to detect candidate loci for selection: towards a landscape genomics approach to adaptation. Molecular Ecology. 2007;16(18):3955-3969. DOI: 10.1111/j.1365-294X.2007.03442.x

[4] Kijas JW, Townley D, D alrymple BP, Heaton MP, Maddox JF, McGrath A, Wilson P, Ingersoll RG, McCulloch R, McWilliam S, Tang D, McEwan J, Cockett N, Oddy VH, Nicholas FW, Raadsma H for the InternationalSheepGenomicsConsortium. Mint: A genome wide survey of SNP variation reveals the genetic structure of sheep breeds. PLoS ONE. 2009;4(3):e4668. DOI: 10.1371/journal.pone.0004668

[5] Chessa B, Pereira F, Arnaud F, Amorim A, Goyache F, Mainland I, Kao RR, Pemberton JM, Beraldi D, Stear M, Alberti A, Pittau M, Iannuzzi L, Banabazi MH, Kazwala R, Zhang Y-P, Arranz JJ, Ali BA, Wang Z, Uzun M, Dione M, Olsaker I, Holm L-E, Saarma U, Ahmad S, Marzanov N, Eythorsdottir E, Holland MJ, Ajmone-Marsan P, Bruford MW, Kantanen J, Spencer TE, Palmarini M. Mint: Revealing the history of sheep domestication using retrovirus integrations. Science. 2009;324(5926):532-536. DOI: 10.1126/science.1170587.

[6] Meadows JRS, Hawken RJ, Kijas JW. Mint: Nucleotide diversity on the ovine Y chromosome. Animal Genetics. 2004;35(5):379-385. DOI: 10.1111/j.1365-2052.2004.01180.x

[7] Cummins J. Mint: Mitochondrial DNA in mammalian reproduction. Reviews of reproduction. 1998;3(3):172-182. DOI: 10.1530/ror.0.0030172.

[8] Chial H, Craig J. Mint: mtDNA and mitochondrial diseases. Nature Education. 2008;1(1):217

[9] Brown WM, George M, Wilson AC. Mint: Rapid evolution of animal mitochondrial DNA. Proceedings of the National Academy of Sciences of the United States of America. 1979;76(4):1967-1971. DOI: 10.1073/pnas.76.4.1967

[10] Moritz C, Dowlin TE, Brown WM. Mint: Evolution of animal mitochondrial DNA: relevance for population biology and systematics. Annual Review of Ecology and Systematics, 1987;18(1):269-292. DOI: 10.1146/annurev.es.18.110187.001413

[11] Xu W, Jameson D, Tang B, Higgs PG. Mint: The relationship between the rate of molecular evolution and the rate of genome rearrangement in animal mitochondrial genomes. Journal of Molecular Evolution. 2006;63(3):375-392. DOI: 10.1007/s00239-005-0246-5

[12] Nabholz B, Glémin S, Galtier N. Mint: Strong variations of mitochondrial mutation rate across mammals – the longevity hypothesis. Molecular Biology and Evolution. 2008;25(1):120-130. DOI: 10.1093/molbev/msm248

[13] Galtier N, Jobson RW, Nabholz B, Glémin S, Blier PU. Mint: Mitochondrial whims: metabolic rate, longevity, and the rate of molecular evolution. Biology Letters. 2009;5(3):413-416. DOI: 10.1098/rsbl.2008.0662

[14] Song S, Pursell ZF, Copeland WC, Longley MJ, Kunkel TA, Mathews CK. Mint: DNA precursor asymmetries in mammalian tissue mitochondria and possible contribution to mutagenesis through reduced replication fidelity. Proceedings of the National Academy of Sciences of the United States of America. 2005;102(14):4990-4995. DOI: 10.1073/pnas.0500253102

[15] Moses MJ. Mint: Spermiogenesis in the crayfish (*Procambarus clarkii*) II. description of stages. The Journal of Biophysical and Biochemical Cytology. 1961;10:301-333. DOI: 10.1083/jcb.10.3.301

[16] Ursprung H, Schabtach E. Mint: Fertilization in tunicates: Loss of the paternal mitochondrion prior to sperm entry. Journal of Experimental Zoology. 1965;159(3):379-383. DOI: 10.1002/jez.1401590310

[17] Sutovsky P, Moreno RD, Ramalho-Santos J, Dominko T, Simerly C, Schatten G. Mint: Ubiquitin tag for sperm mitochondria. Nature. 1999;402(6760):371-372. DOI: 10.1038/46466

[18] Zhao X, Chu M, Li N, Wu C. Mint: Paternal inheritance of mitochondrial DNA in the sheep (Ovine aries). Science in China Series C: Life Sciences. 2001;44(3):321-326. DOI: 10.1007/BF02879339

[19] Ujvari B, Dowton M, Madsen T. Mint: Mitochondrial DNA recombination in a free-ranging Australian lizard. Biology Letters. 2007;3(2):189-192. DOI:10.1098/rsbl.2006.0587

[20] Hey J. Mint: Human mitochondrial DNA recombination: can it be true? Trends in Ecology and Evolution. 2000; 15(5):181-182. DOI: 10.1016/s0169-5347(00)01856-5

[21] Owens KN, Harvey-Blankenship M, King M-C. Mint: Genomic sequencing in the service of human rights. International Journal of Epidemiology. 2002;31(1):53-58. DOI: 10.1093/ije/31.1.53

[22] Gissi C, Iannelli F, Pesole G. Mint: Evolution of the mitochondrial genome of Metazoa as exemplified by comparison of congeneric species. Heredity. 2008;101(4):301-320. DOI: 10.1038/hdy.2008.62

[23] Polyak K, Li Y, Zhu H, Lengauer C, Willson JKV, Markowitz SD, Trush MA, Kinzler KW, Vogelstein B. Mint: Somatic mutations of the mitochondrial genome in human colorectal tumours. Nature Genetics. 1998;20:291-293. DOI: 10.1038/3108

[24] Ingman M, Kaessmann H, Pääbo S, Gyllensten U. Mint: Mitochondrial genome variation and the origin of modern humans. Nature. 2000;408(6813):708-713. DOI:10.1038/35047064

[25] Kim YS, Tseveen K, Batsukh B, Seong J, Kong HS. Mint: Origin-related study of genetic diversity and heteroplasmy of Mongolian sheep (*Ovis aries*) using mitochondrial DNA. Journal of Animal Reproduction and Biotechnology. 2020;35(2):198-206. DOI: 10.12750/JARB.35.2.198

[26] Zardoya R, Villalta M, López-Pérez MJ, Garrido-Pertierra A, Montoya J, Bautista JM. Mint: Nucleotide sequence of the sheep mitochondrial DNA D-loop and its flanking tRNA genes. Current Genetics. 1995;28(1):94-96. DOI: 10.1007/BF00311887.

[27] Achilli A, Bonfiglio S, Olivieri A, Malusà A, Pala M, Kashani BH, Perego UA, Ajmone-Marsan P, Liotta L, Semino O, Bandelt H-J, Ferretti L, Torroni A. Mint: The Multifaceted Origin of Taurine Cattle Reflected by the Mitochondrial Genome. PLoS ONE. 2009;4(6):e5753. DOI: 10.1371/journal.pone.0005753

[28] Pedrosa S, Uzun M, Arranz J-J, Gutiérrez-Gil B, Primitivo FS, Bayón Y. Mint: Evidence of three maternal lineages in near eastern sheep supporting multiple domestication events. Proceedings of the Royal Society of London, Series B: Biological Sciences. 2005;272(1577):2211-2217. DOI: 10.1098/rspb.2005.3204

[29] Torroni A, Achilli A, Macaulay V, Richards M, Bandelt H-J. Mint: Harvesting the fruit of the human mtDNA tree. Trends in Genetics. 2006;22(6):339-345. DOI: 10.1016/j.tig.2006.04.001

[30] Bazin E, Glémin S, Galtier N. Mint: Population size does not influence mitochondrial genetic diversity in animals. Science, 2006;312(5773):570-572. DOI: 10.1126/science.1122033

[31] Galtier N, Nabholz B, Glémin S, Hurst GDD. Mint: Mitochondrial DNA as a marker of molecular diversity: a reappraisal. Molecular Ecology. 2009;18:4541-4550. DOI: 10.1111/j.1365-294X.2009.04380.x

[32] Ratnasingham S, Hebert PDN. Mint: BOLD: The Barcode of Life Data System (http://www.barcodinglife.org). Molecular Ecology Notes. 2007;7(3):355-364. DOI: 10.1111/j.1471-8286.2006.01678.x

[33] Davenport KM, Duan M, Hunter SS, New DD, Fagnan MW, Highland MA, Murdoch BM. Mint: Complete mitochondrial genome sequence of bighorn sheep. Genome Announcements. 2018;6(23): e00464-18. DOI: 10.1128/genomeA.00464-18

[34] Taylor RW, Turnbull DM. Mint: Mitochondrial DNA mutations in human disease. Nature Reviews Genetics. 2005;6(5):389-402. DOI:10.1038/nrg1606

[35] United Mitochondrial Disease Foundation (UMDF) https://www.umdf.org/

[36] Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KHS. Mint: Viable offspring derived from fetal and adult mammalian cells. Nature. 1997;385:810-813. DOI: 10.1038/385810a0

[37] Evans MJ, Gurer C, Loike JD, Wilmut I, Schnieke AE, Schon EA. Mint: Mitochondrial DNA genotypes in nuclear transfer-derived cloned sheep. Nature Genetics. 1999;23(1):90-93. DOI: 10.1038/12696

[38] Gorman GS, Chinnery PF, DiMauro S, Hirano M, Koga Y, McFarland R, Suomalainen A, Thorburn DR, Zeviani M, Turnbull DM. Mint: Mitochondrial diseases. Nature reviews. Disease primers. 2016;2:16080. DOI: 10.1038/nrdp.2016.80

[39] Prezant TR, Agapian JV, Bohlman MC, Bu X, Öztas S, Qiu W-Q, Arnos KS, Cortopassi GA, Jaber L, Rotter JI, Shohat M, Fischel-Ghodsian N. Mint: Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness. Nature Genetics. 1993;4:289-294. DOI: 10.1038/ng0793-289

[40] Yu-Wai-Man P, Griffiths PG, Brown DT, Howell N, Turnbull DM, Chinnery PF. Mint: The epidemiology of Leber hereditary optic neuropathy in the North East of England. American Journal of Human Genetics. 2003;72(2):333-339. DOI: 10.1086/346066

[41] Pal A, Pal A, Banerjee S, Batabyala S, Chatterjee PN. Mint: Mutation in Cytochrome B gene causes debility and adverse effects on health of sheep. Mitochondrion. 2019;46:393-404 DOI: 10.1016/j.mito.2018.10.003

[42] Reicher S, Seroussi E, Weller JI, Rosov A, Gootwine E. Mint: Ovine mitochondrial DNA sequence variation and its association with production and reproduction traits within an Afec-Assaf flock. Journal of Animal Science. 2012;90(7):2084-2091. DOI: 10.2527/jas.2011-4673

[43] Yüncü E, Demirci S, Baştanlar EK, Doğan ŞA, Taşdemir U, Togan I. Mint: Comparative study of three simple molecular approaches in search of mtDNA haplogroup identification of domestic sheep. Small Ruminant Research. 2013;114(1):64-71. DOI: 10.1016/j.smallrumres.2013.05.014

[44] Hiendleder S. Mint: A low rate of replacement substitutions in two major *Ovis aries* mitochondrial genomes. Animal Genetics. 1998;29(2):116-122. DOI: 10.1046/j.1365-2052.1998.00295.x

[45] Hiendleder S, Lewalski H, Wassmuth R, Janke A. Mint: The Complete Mitochondrial DNA Sequence of the Domestic Sheep (*Ovis aries*) and Comparison with the Other Major Ovine Haplotype. Journal of Molecular Evolution. 1998;47:441-448. DOI: 10.1007/PL00006401

[46] Meadows JRS, Hiendleder S, Kijas JW. Mint: Haplogroup relationships between domestic and wild sheep resolved using a mitogenome panel. Heredity. 2011;106(4):700-706. DOI: 10.1038/hdy.2010.122

[47] Hiendleder S, Mainz K, Plante Y, Lewalski H. Mint: Analysis of mitochondrial DNA indicates that domestic sheep are derived from two different ancestral maternal sources: no evidence for contributions from urial and argali sheep. Journal of Heredity. 1998;89(2):113-120. DOI: 10.1093/jhered/89.2.113

[48] Sanna D, Barbato M, Hadjisterkotis E, Cossu P, Decandia L, Trova S, Pirastru M, Leoni GG, Naitana S, Francalacci P, Masala B, Mereu P. Mint: The first mitogenome of the Cyprus Mouflon (Ovis gmelini ophion): New insights into the phylogeny of the genus Ovis. PloS ONE. 2015;10(12): e0144257. DOI: 10.1371/journal.pone.0144257

[49] Lv F-H, Peng W-F, Yang J, Zhao Y-X, Li W-R, Liu M-J, Ma Y-H, Zhao Q-J, Yang G-L, Wang F, Li J-Q, Liu Y-G, Shen Z-Q, Zhao S-G, Hehua E, Gorkhali NA, Vahidi SMF, Muladno M, Naqvi AN, Tabell J, Iso-Touru T, Bruford MW, Kantanen J, Han J-L, Li M-H. Mint: Mitogenomic meta-analysis identifies two phases of migration in the history of Eastern Eurasian sheep. Molecular Biology and Evolution. 2015;32(10):2515-2533. DOI: 10.1093/molbev/msv139

[50] Demirci S, Baştanlar EK, Dağtaş ND, Pişkin E, Engin A, Özer F, Yüncü E, Doğan ŞA, Togan I. Mint: Mitochondrial DNA diversity of modern, ancient and wild sheep (Ovis gmelinii anatolica) from Turkey: new insights on the evolutionary history of sheep. PLoS One. 2013;8(12):e81952. DOI: 10.1371/journal.pone.0081952

[51] Dymova MA, Zadorozhny AV, Mishukova OV, Khrapov EA, Druzhkova AS, Trifonov VA, Kichigin IG, Tishkin AA, Grushin SP, Filipenko ML. Mint: Mitochondrial DNA analysis of ancient sheep from Altai. Animal Genetics. 2017;48(5):615-618. DOI: 10.1111/age.12569

[52] Horsburgh KA, Rhines A. Mint: Genetic characterization of an archaeological sheep assemblage from South Africa's Western Cape. Journal of Archaeological Science.

2010;37(11):2906-2910. DOI: 10.1016/j.jas.2010.06.035

[53] Rannamäe E, Lougas L, Niemi M, Kantanen J, Maldre L, Kadorova N, Saarma U. Mint: Maternal and paternal genetic diversity of ancient sheep in Estonia from the Late Bronze Age to the post-medieval period and comparison with other regions in Eurasia. Animal Genetics. 2016;47:208-218. DOI: 10.1111/age.12407

[54] Hiendleder S, Kaupe B, Wassmuth R, Janke A. Mint: Molecular analysis of wild and domestic sheep questions current nomenclature and provides evidence for domestication from two different subspecies. Proceedings of the Royal Society of London, Series B: Biological Sciences. 2002;269:893-904. DOI: 0.1098/rspb.2002.1975.

[55] Annus K, Maróti-Agóts Á, Pásztor K, Vada E, Sáfár L, Gáspárdy A. Mint: Characterisation of Hungarian Tsigai variants based on control region of mtDNA (with English summary). Magyar Állatorvosok Lapja. 2015;137(10):625-631.

[56] Alonso R, Ulloa-Arvizu R, Gayosso-Vázquez A. Mint: Mitochondrial DNA sequence analysis of the Mexican Creole sheep (*Ovis aries*) reveals a narrow Iberian maternal origin. Mitochondrial DNA Part A. 2017;28(6):793-800. DOI: 10.1080/24701394.2016.1192613

[57] Lancioni H, Di Lorenzo P, Ceccobelli S, Perego UA, Miglio A, Landi V, Antognoni MT, Sarti FM, Lasagna E, Achilli A. Mint: Phylogenetic relationships of three Italian Merino-derived sheep breeds evaluated through a complete mitogenome analysis. PLoS ONE. 2013;8(9): e73712. DOI: 10.1371/journal.pone.0073712

[58] Bunch TD, Wu C, Zhang Y-P, Wang S. Mint: Phylogenetic Analysis of Snow Sheep (*Ovis nivicola*) and Closely Related Taxa. Journal of Heredity. 2006;97(1):21-30. DOI: 10.1093/jhered/esi127

[59] Guo J, Du LX, Ma YH, Guan WJ, Li HB, Zhao QJ, Li X, Rao SQ. Mint:, 2005. A novel maternal lineage revealed in sheep (*Ovis aries*). Animal Genetics. 2005;36(4):331-336. DOI: 10.1111/j.1365-2052.2005.01310.x

[60] Meadows JRS, Li K, Kantanen J, Tapio M, Sipos W, Pardeshi V, Gupta V, Calvo JH, Whan V, Norris B, Kijas JW. Mint: Mitochondrial sequence reveals high levels of gene flow between breeds of domestic sheep from Asia and Europe. Journal of Heredity. 2005;96(5):494-501. DOI: 10.1093/jhered/esi100

[61] Chen, S.Y., Duan, Z.Y., Sha, T., Xiangyu, J., Wu, S.F., Zhang, Y.P., 2006. Mint: Origin, genetic diversity, and population structure of Chinese domestic sheep. Gene. 2006;376(2):216-223. DOI: 10.1016/j.gene.2006.03.009

[62] Tserenbataa T, Ramey IIRR, Ryder OA, Quinn TW, Reading RP. Mint: A population genetic comparison of argali sheep (*Ovis ammon*) in Mongolia using the ND5 gene of mitochondrial DNA; implications for conservation. Molecular ecology. 2004;13(5):1333-1339. DOI: 10.1111/j.1365-294X.2004.02123.x

[63] Bruford MW, Bradley DG, Luikart G. Mint: DNA markers reveal the complexity of livestock domestication. Nature Reviews Genetics. 2003;4(11):900-910. DOI: 10.1038/nrg1203.

[64] Ganbold O, Lee S-H, Seo D, Paek WK, Manjula P, Munkhbayarc M, Lee JH. Mint: Genetic diversity and the origin of Mongolian native sheep. Livestock Science. 2019;220:17-25. DOI:10.1016/j.livsci.2018.12.007

[65] Mohammadhashemi A, Pirany N, Nassiri MR, Daloii TA, Kohnegroz BB. Mint: Studying the partially sequenced mtDNA hypervariable region1 (HVR1) of Iranian Moghani sheep. Annals of Biological Research. 2012;3(6): 2906-2910

[66] Tapio M, Marzanov N, Ozerov M, Ćinkulov M, Gonzarenko G, Kiselyova T, Murawski M, Viinalass H, Kantanen J. Mint: Sheep mitochondrial DNA variation in European, Caucasian, and Central Asian areas. Molecular Biology and Evolution. 2006;23(9):1776-1783. DOI: 10.1093/molbev/msl043

[67] Meadows JRS, Cemal I, Karaca O, Gootwine E, Kijas JW. Mint: Five Ovine Mitochondrial Lineages Identified From Sheep Breeds of the Near East. Genetics. 2007;175(3):1371-1379. DOI: 10.1534/genetics.106.068353

[68] Rafia P, Tarang A. Mint: Sequence Variations of Mitochondrial DNA Displacement-Loop in Iranian Indigenous Sheep Breeds. Iranian Journal of Applied Animal Science. 2016;6(2):363-368

[69] Liu J, Ding X, Zeng Y, Yue Y, Guo X, Guo T, Chu M, Wang F, Han J, Feng R, Sun X, Niu C, Yang B, Guo J, Yuan C. Mint: Genetic Diversity and Phylogenetic Evolution of Tibetan Sheep Based on mtDNA D-Loop Sequences. PLoS ONE. 2016;11(7): e0159308. DOI: 10.1371/journal.pone.0159308

[70] Ćinkulov M, Popovski Z, Porcu K, Tanaskovska B, Hodžić A, Bytyqi H, Mehmeti H, Margeta V, Djedović R, Hoda A, Trailović R, Brka M, Marković B, Važić B, Vegara M, Olsaker I, Kantanen J. Mint: Genetic diversity and structure of the West Balkan Pramenka sheep types as revealed by microsatellite and mitochondrial DNA analysis. Journal of Animal Breeding and Genetics. 2008;125(6):417-426. DOI: 10.1111/j.1439-0388.2008.00742.x

[71] Pereira F, Davis SJM, Pereira L, McEvoy B, Bradley DG, Amorim A. Mint: Genetic Signatures of a Mediterranean Influence in Iberian Peninsula Sheep Husbandry. Molecular Biology and Evolution. 2006;23(7):1420-1426. DOI: 10.1093/molbev/msl007

[72] Pedrosa S, Arranz J-J, Brito N, Molina A, Primitivo FS, Bayón Y. Mint: Mitochondrial diversity and the origin of Iberian sheep. Genetics Selection Evolution. 2007;39:91-103. DOI: 10.1051/gse:2006034

[73] Pariset L, Mariotti M, Gargani M, Joost S, Negrini R, Perez T, Bruford M, Marsan PA, Valentini A. Mint: Genetic Diversity of Sheep Breeds from Albania, Greece, and Italy Assessed by Mitochondrial DNA and Nuclear Polymorphisms (SNPs). The Scientific World Journal. 2011;11:1641-1659. DOI: 10.1100/2011/186342

[74] Mariotti M, Valentini A, Marsan PA, Pariset L. Mint: Mitochondrial DNA of seven Italian sheep breeds shows faint signatures of domestication and suggests recent breed formation. Mitochondrial DNA (The Journal of DNA Mapping, Sequencing, and Analysis). 2013;24(5):577-583. DOI: 10.3109/19401736.2013.770493

[75] Brahi OHD, Xiang H, Chen X, Farougou S, Zhao X. Mint: Mitogenome revealed multiple postdomestication genetic mixtures of West African sheep. Journal of Animal Breeding and Genetics. 2015;132(5):399-405. DOI: 10.1111/jbg.12144

[76] Álvarez I, Capote J, Traoré A, Fonseca N, Pérez K, Cuervo M, Fernández I, Goyache F. Mint: Mitochondrial analysis sheds light on the origin of hair sheep. Animal Genetics. 2013;44(3):344-347. DOI: 10.1111/j.1365-2052.2012.02398.x

[77] Resende A, Gonçalves J, Muigai AWT, Pereira F. Mint:

Mitochondrial DNA variation of domestic sheep (Ovisaries) in Kenya. Animal Genetics. 2016;47(3):337-381. DOI: 10.1111/age.12412

[78] Othman OE, Pariset L, Balabel EA, Marioti M. Mint: Genetic characterization of Egyptian and Italian sheep breeds using mitochondrial DNA. Journal of Genetic Engineering and Biotechnology. 2015;13(1):79-86. DOI: 10.1016/j.jgeb.2014.12.005

[79] Ghernouti N, Bodinier N, Ranebi M, Maftah D, Petit D, Gaouar SBS. Mint: Control Region of mtDNA identifies three migration events of sheep breeds in Algeria. Small Ruminant Research. 2017;155:66-71. DOI: 10.1016/j.smallrumres.2017.09.003

[80] Kandoussi A, Boujenane I, Piro M, Petit D. Mint: mtDNA genetic characterization of an isolated sheep breed in South of Moroccan Atlas. Small Ruminant Research. 2020;193:106250. DOI: 10.1016/j.smallrumres.2020.106250

[81] Campos E, Cuéllara J, Salvador O, García-Trejo EA, Pereira F. Mint: The genetic diversity and phylogeography of Mexican domestic sheep. Small Ruminant Research. 2020;187:106109. DOI: 10.1016/j.smallrumres.2020.106109

[82] Revelo HA, López-Alvarez D, Landi V, Rizzo L, Alvarez LA. Mint: Mitochondrial DNA Variations in Colombian Creole Sheep Confirm an Iberian Origin and Shed Light on the Dynamics of Introduction Events of African Genotypes. Animals. 2020;10:1594. DOI: 10.3390/ani10091594

[83] Wang X, Chen H, Lei CZ. Mint: Genetic diversity and phylogenetic analysis of the mtDNA D-loop region in Tibetan sheep. Asian-Australasian Journal of Animal Sciences. 2007;20(3)313-315. DOI: 10.5713/ajas.2007.313

[84] Agaviezor BO, Adefenwa MA, Peters SO, Yakubu A, Adebambo AO, Ozoje MO, Ikeobi CON, Ilori BM, Wheto M, Okpeku M, De Donato M, Imumorin IG. Mint: Mitochondrial D-loop genetic diversity of Nigerian indigenous sheep. Animal Genetic Resources. 2012;50:13-20. DOI: 10.1017/S2078633612000070

[85] Arora R, Yadav HS, Mishra BP. Mint: Mitochondrial DNA diversity in Indian sheep. Livestock Science. 2013;153(1-3):50-55. DOI: 10.1016/j.livsci.2013.02.006

[86] Kovács E, Maróti-Agóts Á, Harmat L, Annus K, Zenke P, Tempfli K, Sáfár L, Gáspárdy A. Mint: Characterisation of Hungarian Cikta sheep based on the control region of mtDNA (with English summary). Magyar Állatorvosok Lapja. 2020;142(7):421-428.

[87] Kovács E, Harmat L, Tempfli K, Sáfár L, Becskei Zs, Maróti-Agóts Á, Gáspárdy A. Mint: Ergebnisse der Sequenzanalyse des mitochondrialen Gens Cyt-b von Cikta Schafen (with English summary). Danubian Animal Genetic Resources. 2020;5(1):19-25.

[88] Kusza Sz, Zakar E, Budai Cs, Cziszter L, Padeanu I, Gavojdian D. Mint: Mitochondrial DNA variability in Gyimesi Racka and Turcana sheep breeds. Acta Biochimia Polonica. 2015,62(1):273-280. DOI: 10.18388/abp.2015_978

[89] Kirikci K, Noce A, Cam MA, Mercan L, Amills M. Mint: The analysis of mitochondrial data indicates the existence of population substructure in Karayaka sheep. Small Ruminant Research. 2018;162:25-29. DOI: 10.1016/j.smallrumres.2018.02.007

[90] FAO. The state of food and agriculture 2007. Rome. Agriculture Series No. 38

[91] Salmon GR, MacLeod M, Claxton JR,Pica Ciamarra U, Robinson T, Duncan A, Peters AR. Mint: Exploring the landscape of livestock 'Facts'. Global Food Security. 2020;25:100329. DOI: 10.1016/j.gfs.2019.100329

[92] Taberlet P, Coissac E, Pansu J, Pompanon F. Mint: Conservation genetics of cattle, sheep, and goats. Comptes Rendus Biologies. 2011;334(3):247-254. DOI: 10.1016/j.crvi.2010.12.007

[93] FAO. The State of the World's Animal Genetic Resources for Food and Agriculture. FAO, Rome. 2007

[94] Lenstra JA, Groeneveld LF, Eding H, Kantanen J, Williams JL, Taberlet P, Nicolazzi EL, Sölkner J, Simianer H, Ciani E, Garcia JF, BrufordMW,Ajmone-MarsanP,WeigendS. Mint: Molecular tools and analytical approaches for the characterization of farm animal genetic diversity. Animal Genetics. 2012;43(5):483-502. DOI: 10.1111/j.1365-2052.2011.02309.x

[95] Groeneveld LF, Lenstra JA, Eding H, Toro MA, Scherf B, Pilling D, Negrini R, Finlay EK, Jianlin H, Groeneveld E, Weigend S, GlobalDiv Consortium. Mint: Genetic diversity in farm animals – a review. Animal genetics. 2010;41(Suppl 1):6-31. DOI: 10.1111/j.1365-2052.2010.02038.x

[96] FAO. Molecular genetic characterization of animal genetic resources. FAO Animal Production and Health Guidelines. No. 9. Rome 2011 (available at http://www.fao.org/docrep/014/i2413e/i2413e00.htm). Google Scholar

[97] Agaviezor BO, Peters SO, Adefenwa MA, Yakubu A, Adebambo AO, Ozoje MO, Ikeobi CON, Wheto M, Ajayi OO, Amusan SA, Ekundayo OJ, Sanni TM, Okpeku M, Onasanya GO, De Donato M, Ilori BM, Kizilkaya K, Imumorin IG. Mint: Morphological and microsatellite DNA diversity of Nigerian indigenous sheep. Journal of Animal Science and Biotechnology. 2012b;3(1):38.

[98] Firestone KB, Houlden BA, Sherwin WB, Geffen E. Mint: Variability and differentiation of microsatellites in the genus Dasyurus and conservation implications for the large Australian carnivorous marsupials. Conservation Genetics. 2000;1(2):115-133. DOI: 10.1023/A:1026578821339

[99] Gáspárdy A, Csóri Zs, Daróczi-Szabó M. Legend of the four horned Racka or whether in the sight of what did Eugene of Savoy delight? Paper on Joint Annual Meeting of ÖNGENE and of DAGENE; 30-31 August 2012; Wels, Austria [Internet]. Available from: http://www.dagene.eu/docs/ongene_dagene_2012/legendo4_gaspardy_2012.pdf [Accessed: 2020-12-10]

[100] Peter C, Bruford M, Perez T, Dalamitra S, Hewitt G, Erhardt G, ECONOGENE Consortium. Mint: Genetic diversity and subdivision of 57 European and Middle-Eastern sheep breeds. Animal Genetics. 2007;38(1):37-44. DOI: 10.1111/j.1365-2052.2007.01561.x

[101] Boichard D, Maignel L, Verrier É. Mint: The value of using probabilities of gene origin to measure genetic variability in a population. Genetic Selection Evolution. 1997;29:5-23. DOI: 10.1186/1297-9686-29-1-5

[102] Gutiérrez JP, Cervantes I, Goyache F. Mint: Improving the estimation of realized effective population size in farm animals. Journal of Animal Breeding and Genetics. 2009;126(4):327-332. DOI: 10.1111/j.1439-0388.2009.00810.x.

[103] Huby M, Griffon L, Moureaux S, De Rochambeau H, Danchin-Burge C,

Verrier É. Mint: Genetic variability of six French meat sheep breeds in relation to their genetic management. Genetic Selection Evolution. 2003;35(7):637-655. DOI:10.1051/gse:2003044

[104] Caballero A, Toro MA. Mint: 2000 Interrelations between effective population size and other pedigree tools for the management of conserved populations. Genetics Research. 2000;75(3):331-343. DOI: 10.1017/s0016672399004449

[105] Gáspárdy A, Simon Cs, Andrásofszky E, Sáfár L, Kósa E. Mint: Historical evaluation of milk producing ability of the Hungarian native Tsigai sheep (with English summary). Állattenyésztés és Takarmányozás. 2016;65(1):24-36

[106] Mederle A, Maróti-Agóts Á, Matiuti M, Gáspárdy A. Mint: Challenges in conservation of Tyrolean Grey Cattle. Danubian Animal Genetic Resources. 2020;5(1):11-17

[107] Ledwith L, Kőrösi A, Daróczi-Szabó M, Gáspárdy A. Mint: Comparative skull geometry of recently lived Hungarian Rackas. Danubian Animal Genetic Resources. 2020;5(2):55-63

[108] Annus K, Arkenberg H, Prikoszovich M, Oláh J, Maróti-Agóts Á, Gáspárdy A. Mint: Characterisation of the female Tsigai population by use of Hungarian herd-book data. In: Hajas P, Gáspárdy A, editors. 25 years with DAGENE. Printed by Palatia Nyomda és Kiadó Kft. Győr, ISBN 978-963-12-3101-4, 2015;108-113

[109] Posta J, Kovács E, Tempfli K, Sáfár L, Gáspárdy A. Mint: Pedigree analysis of a population bottlenecked, the Cikta with special regard to its maternal lineages (with English summary). Magyar Állatorvosok Lapja. 2019;141(3):171-180.