

Insertion of Hungarian Merino Sheep Breed into the European Merino Group according to Maternal Origin (mtDNA CR)

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Abstract

Merino sheep breeds have been widely distributed across the world. They represent one of the most important genetic resource of the sheep sector. But the crisis of the wool industry led to a drastic reduction in the number of European Merino breeds including the Hungarian Merino. The Hungarian Merino is a local sheep breed with a nearly 250-year old breeding history in Hungary. It has the largest number of individuals between both of the purebred (including nucleus and multiplier flocks) and commercial flocks. Most of the purebred flocks went through a liquidation after the year 1989. That also caused a drastic reduction in the number of purebred-ewes. The Hungarian Merino breeding stock consists of barely 3700 female individuals recently. This has led to declare the breed endangered in 2014. Our future plan is to analyse the genetic variability of the Hungarian Merino breed based on the approximately 1000 base pairs long mitochondrial DNA (mtDNA) D-loop sequences. So far, we collected DNA samples from 172 individuals from 10 genealogical lines. We would like to examine the haplotype diversity and compare the overall genetic distances within and among each genealogical lines. Our further plan is to compare our data to GenBank sequences of other Merino and founder breeds.

Keywords: Genetic Diversity, Hungarian Merino, Husbandry, Mitochondrial DNA, Sheep

1. Introduction

The World's sheep population is currently around 1-1.5 billion individuals, with more than six hundred sheep breeds worldwide. The Merino and Merino derived sheep breeds represent around 20 % of the World's sheep population [1] The first sheep reached Europe and the Mediterranean regions 6000 years B.C. approximately 5000 years after the first domestication event occurred in the Fertile Crescent [2-4]. During the first century B.C. fine-wool ewes from South-Italy were

introduced in the southern part of Spain and they were mated with coarse-wool rams from Africa. These breeds are believed to have been crossed with sheep imported by Arabs and are probably the main ancestor of the Merino breed [5]. The controlled breeding of this fine-wool sheep started during the 14th century in Spain. The Spanish Merino export was banned until the 18th century to sustain the monopoly of the fine-wool market in Spain. During the 18th century, Merino rams were imported to Germany and France, which have contributed to the development of the Merinolandschaf and Rambouillet Merino breeds. The first Merino breeding stock was established in Rambouillet, France in 1752 [6]. It has now only 7 genealogical lines. The Rambouillet breed was

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also exported to Central Italy where it gave rise to the Sopravissana and Mernizzata Italiana breeds [7]. The dispersal of Merino sheep in Eastern Europe (e.g. Hungary, Poland) and China also started in the 18th century. During the 19th century, the Merino breed was also exported to Australia, New Zealand, the North and South American continents and South-Africa. During the Soviet era, many composite fine-wool sheep breeds were developed in the vast URSS territory [8]. With the cheap wool being imported from Australia, and spreading across Europe starting 1950s. The prices of wool and added value have collapsed. That caused the increase in the number of mutton sheep breeds. European Merino sheep have experienced a dramatic numerical decrease to the point that they are considered as endangered breeds and no longer undergo active genetic improvement [9]. The Hungarian Merino also went through a huge reduction in number in the last decades. The first 300 Merino sheep were brought to Hungary from Spain for the request of the Queen Mária Terézia in 1765. The Hungarian Merino was developed by crossing with Rambouillet Merino and German Mutton Merino. After the World War II: the Hungarian Merino sheep were crossed with Russian Merino breeds.

After the year 1960 the new aim of the Hungarian Merino breeding programs was to develop the meat production. In this case the Hungarian Merino ewes were crossed with Merino Precoce rams from France and with German Mutton Merino rams. During the 1970s and 1980s the breed was crossed for finer wool with Kent, Corriedale and Australian Merino as well as with Booroola Merino to develop the prolificacy. The Hungarian Merino Herd Book was established in 1993. Since that time no other Merino breeds were used for crossing with the Hungarian Merino [10-11]. In the year 2000 the number of the pedigreed Hungarian Merino ewes was still 29.862 and the number of the breeders was 84. For the above mentioned reasons the number of the ewes decreased to 4.094 and the number of the breeders decreased to 36. According to a survey the number of the genealogy (paternal) lines was 39 in 2004 [12]. Now it reduced to 11 lines (Table 1). Recently the ram line 2, 5, 17 and 20 are in critical status. Now it is reasonable to conserve the genome of the breed. For these reasons the Hungarian Merino breed became endangered in 2014. The main aim of the conservation efforts is to conserve the genetic resource of the breed in situ in a sustainable manner.

Table 1. Current Hungarian Merino ram lines (Source: Hajduk, 2004)

Line	Establisher	Breeder	Nr. of rams 2004	Nr. of rams 2013	Nr. of rams 2014
2	20024 0030	New-Zeland import	21	2	1
3	15083 1586	Túrkevei (Vörös Csillag) Mgtsz, Túrkeve	21	18	33
8	15083 2616	Túrkevei (Vörös Csillag) Mgtsz, Túrkeve	15	62	61
9	15262 3166	Aranykalász Mgtsz, Jászboldogháza	11	4	5
10	20024 7234	New-Zeland import	10	17	12
11	15262 3051	Aranykalász Mgtsz, Jászboldogháza	9	5	5
12	15262 1058	Aranykalász Mgtsz, Jászboldogháza	9	6	9
16	20023 626	Australia import	6	3	7
17	20016 3107	German import	6	1	1
5	15382 62515	Aranykalász Mgtsz, Jászboldogháza	19	0	0
20	15010 6509	Közép Tiszai ÁG, Kunmadaras	4	0	0

A modern molecular genetic survey of the Hungarian Merino breed is now became necessary while it can be a base of the further breeding programs. The analysis of the genetic structure of the animal species can help us to determine the hereditary traits of the genotype groups that can be significant to conserve the genetic resources [13]. Among the Hungarian sheep breeds only the native breeds and the endangered Hungarian Merino have a closed population. The probability of migration is quite low. So that we have to pay

more attention to these breeds genetic variability [14]. Whereas the inbreeding – breeding without any cross-breeding – especially in the small populations can cause a reduction in the level of the genetic variability, and results a low value of heterozygosity [15].

Molecular genetics has proven highly informative to better understand the (recent) evolutionary history of livestock breeds, as well as to determine the level of their genetic variability, which is an essential aspect to consider when defining

conservation priorities and regional breed-specific programs [16].

Firstly, we intend to study the 1006 bp long sequence of the mtDNA D-loop region. The D-loop sequence is hypervariable. While the Hungarian Merino derived from many different breeds that genetically distant from each other, this method can be hopefully appropriate to determine the genetic diversity within the breed.

Mitochondrial DNA (mtDNA) is the genetic material that exists outside the nucleolus of eukaryotic cells. It has a simple molecular structure which is covalent and close. Including coding and non-coding regions, its length ranges from 15 to 20 kb in different species. The sheep mtDNA sequence is 16 kb long [17]. If a mutation occurs in the non-coding region, we can detect it in the next generation as a new mtDNA polymorphism. Most of the polymorphic mutations of the populations appeared a long time ago, and shows homoplasia. That means, that every cell's mitochondria have the same polymorphism [18]. The non-coding region is the control region. The rate of mtDNA evolution is about 5–10 times faster than that of nuclear DNA, and its genes do not recombine [26]. So mtDNA analysis has often been used to investigate haplotype diversity within species. The mtDNA D-loop region is known to be more variable than other regions of it, and it is often used to analyze the phylogeny of closely related breeds within species [19-21]. Examining the D-loop region is also used to determine the genetic diversity of animal breeds [16, 22-23].

A previous study on the relationships among Merino and non-Merino breeds was reported by Kijas et al. [24]. They analyzed 74 breeds from six continents using the Illumina Ovine SNP50 BeadChip and detected extensive haplotype sharing between Merino and other breeds, which likely results from the widespread use of Merino sires across Europe in the past centuries.

In modern European sheep breeds only two distinct mtDNA haplotype groups, termed A and B, have typically been found, suggesting the existence of two different maternal origins [25]. A recent study reported existence of a third mtDNA haplogroup (C) in European sheep [26]. However, studies including Middle Eastern, Caucasian and Turkish breeds revealed additional possible maternal origins for domestic sheep [3, 25, 27].

Most recently, a single Karachai animal sampled from the north Caucasus revealed control region sequence, which grouped separately from the three defined ovine mitochondrial DNA (mtDNA) clades [25]. This was taken as evidence for a fourth maternal lineage and termed group D.

Lancioni et al. [16] made a phylogenetic investigation with three different local sheep breeds (Gentile di Puglia, Sopravissana, Merinizzata Italiana). These local breeds went through a strong numerical reduction and eventually became endangered as well as the Hungarian Merino. The aim of their study was to assess the genetic diversity of these three Italian Merino-derived (IMd) breeds by examining the molecular information encoded in the maternally-inherited mitochondrial DNA (mtDNA). A parallel molecular investigation was performed on the putative paternal and maternal breeds, the Merino from Spain and the Appenninica from Italy, respectively, as well as on three unrelated dairy breeds (Sarda and Comisana from Italy, and Lacaune from France). When comparing the overall genetic distances, the three IMd breeds clustered together close to the Appenninica, thus confirming its parental role. Among the 90 IMd samples, 82 different haplotypes were observed, almost all belonging to haplogroup B, and only one to A. Thus, despite experiencing a drastic reduction in number the IMd breeds still represent a reservoir of distinctive mitochondrial variants, which could potentially contribute to the development of conservation and management programs of Italian sheep breeds.

According to the investigations of Ciani et al. [9], intensive gene flow, founder effects and geographic isolation are the main factors that determined the genetic makeup of current Merino and Merino-derived breeds. Model-based clustering, migration analysis and haplotype sharing indicated that a Merino-mediated gene flow to several Merino-derived populations around the world took place. The close relationship between Spanish Merino and other Spanish breeds was consistent with an Iberian origin for the Merino breed, with possible earlier contributions from other Mediterranean stocks. The Merino populations from Australia, New Zealand and China were clearly separated from their European ancestors. They observed a genetic sub structuring in the Spanish Merino population, which reflects recent herd management practices.

Macarthur and Andalusian Merino populations had low heterozygosity that reveals inbreeding. These breeds have closed nucleus. High levels of variability were found in the Spanish Merino population from Extremadura, in spite of a marked decrease in census size in the last 50 years. This variability may have been influenced by cross-breeding with Merino-derived breeds since the

1960s to direct the Merino breed towards meat production. However, the close relationship between the Merino breed and other Spanish modern breeds (Figure 1) and the admixture between the Merino breed and other Spanish breeds, both support an Iberian origin of the Merino breeds.

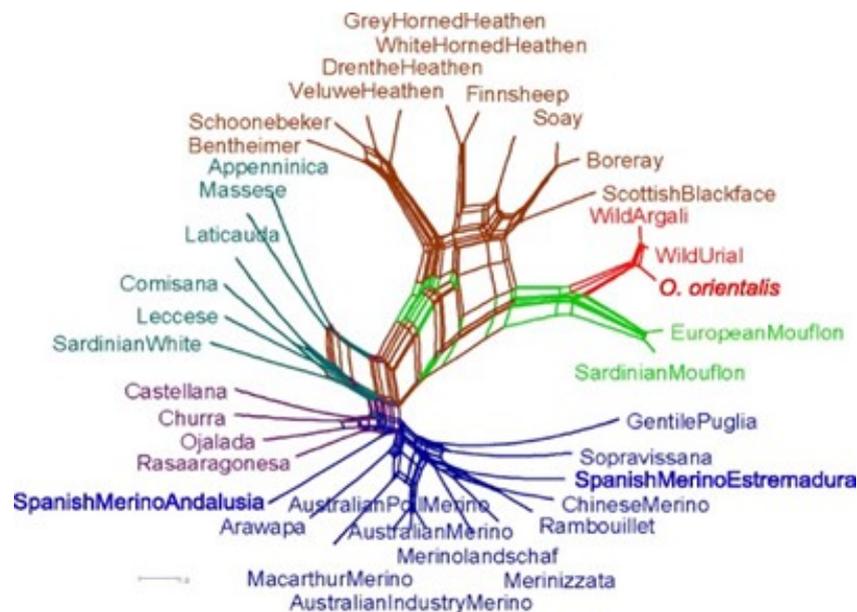


Figure 1. The phylogenetic tree of sheep breeds, From: Ciani et al., (2015) [2]

2. Planned investigations, partial results and expected results

We plan to study the genetic diversity within and among the recent genealogical lines of the Hungarian Merino breed by molecular genetic methods. Our further plan is to compare our data to other European, and former Merino breeds. According to the results we can develop the breeding directives.

We had collected hair-bulbs from Hungarian Merino lines and from 5 flocks. The samples were kept in paper pockets until the examinations. We collected samples from 10 lines and from 172 individuals. All the animals were registered in the Herd Book of the Hungarian Merino. The laboratory examinations take place in the Animal Genetic Laboratory of the University Debrecen.

We isolated genomic DNA from hair bulbs and optimize the PCR reaction.

We used the following primers for the PCR reaction:
 Forward: 5'-CCCACTATCAACACCCAAA-3';
 Reverse: 5'-

TGAGGATGCTCAAGATGCAG-3'. We amplified the 1006 base pairs long sequence of the mtDNA D-loop region. The amplified products were sequenced by the MacroGen Europe Company.

All sequences were trimmed to the shortest one by using the Mega7 software [28]. We would like to determine the haplotype diversity and haplotype sharing by every line and within the breed according to the mutations found in the sequences, and calculate the generally used diversity indices, respectively.

We would like to compare our sequences to the sequences of the founder Merino breeds that can be found in the GeneBank. We would like to use the mtDNA D-loop sequences of the following breeds primarily: German Merino, Spanish Merino, Polish merino, Transylvanian Merino.

First we analysed the haplotype diversity of 88 Hungarian Merino Sequences by using the DnaSP software [29]. The analysed sequences were 806 base pairs long. The number of haplotypes was 69.

The haplotype (gene) diversity (Hd) was 0.9937. 54 haplotypes were unique. That means that each haplotypes count only one individual. These data suggest that the Hungarian Merino breed probably has still a high genetic diversity.

3. Conclusion

Results However the pedigreed Hungarian Merino stock decreased by substitution or crossbreeding with commercial breeds. The breeding practices have changed driven by the economy. In the last few decades, the increased demand for meat and the advent of artificial fibers - that caused the crisis of the wool industry - triggered the necessity to conserve the Hungarian Merino breed.

The breeders Association must find a way to control the drastic reduction and also the dominance of some lines in case of maintaining the genetic variance of the breed.

The aim of the Hungarian Merino breeding system should be to increase the number of the pedigreed animals without increasing the inbreeding level and to maintain the genetic variance.

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