Genetic diversity in maternal lines of cognate Mongolian horses of Mongolia, China and Russia

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Abstract. Analysis of D-loop of mitochondrial DNA of 4 populations of Mongolian horses from different origin was conducted. Nowadays Mongolian horses massive is divided to several countries. Among them there are Mongolia, Inner Mongolia part of China, and Kalmyk Republic of Russia. D-loop of mitochondrial DNA gives an inside into the maternal lines of population of horses. I was stated, that maternal lines of 4 populations from different territories have their similarities, as well as differences. More investigations are in need to understand, if similarities can be explained by the same origin of the populations, or due to other horse breeds, involved into the breed's genesis.

1 Introduction

Since 2006 Kalmyk horse population was investigated using classical techniques, such as phenotype description of animals. Even though the great work was done regarding selection breeding, nowadays, new genetic techniques are to be employed for genetic lines of horse breeds characterization.

Kalmyk horses population was divided from the main massive of Mongolian horses of Mongolia more than 400 years ago, together with the migration of people [1,2], since that Kalmyk horse obtained blood of Russian aborigine and European horse breeds, but still keeps main features of Mongolian horses. At the same time, Mongolian horses of Inner Mongolia in China were divided from the main population later.

Today, this is getting more and more interesting to investigate, what happened to the genotype of Kalmyk horses since genetic division, so, we decided to compare genome of contemporary Kalmyk horses with the genome of Mongolian horse from the territory of Mongolia and Inner Mongolia.

2 Materials and methods:

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2.1. Samples collection

Samples were collected from the private farms in Republic of Kalmykia (Russia), Inner Mongolia (China, region of Hukh-Hoto) and Mongolia (Tov region). There were collected: 44 animals from population №1, and 54 animals from population №2 from Yustinsky region of Republic of Kalmykia. 13 animals from Inner Mongolia, 11 animals from Tov region of Mongolia. There were collected hair samples from each animal (not less than 10 hairs with follicles).

2.2. Sanger sequencing processing:

Hair samples were used for DNA isolation (GenJet, Thermofisher Scientific). Isolated DNA was amplified using DT96 amplification machine (DNA Technologies) with the custom primers: (H-mtDNA F1) AGC TCC ACC ATC AAC ACC CAA A, (H-mtDNA R1) CCA TGG ACT GAA TAA CAC CTT ATG GTT G. Amplification was proceeded in the reaction master mix: 25 uL, 50 ng of genomic DNA, 0,25 M of each primer, 0,25 mM dNTPs, 2,5 mM MgCl₂, 1,5 U Taq DNA-polymerase in 10x buffer solution. Amplification protocol : 94°C (3 min), 34 cycles : 94°C (30 s), 72°C (30 s), 72°C (90 s). Amplicons, obtained as a result of the amplification were used for Sanger sequencing using ABI 3130 (BigDye 3.1.). Sanger sequencing of D-loop of mtDNA was proceeded as it was previously described [3].

2.3. uGene software analysis:

Results were gathered from Sanger sequencer as ABI files. After sequences were aligned using MUSCLE default setting, phylogenetic trees were built with use of PHYLIP Neighbor Joining algorithm. The results are presented here as a phylogenetic trees, in which all the animals are divided into clusters, which are presented in the table below each phylogenetic tree.

3 Results and discussion

Molecular markers are most powerful way to find out genetic variations and unique sites for animal populations. Such knowledge helping to save genetic diversity of agricultural and wild animals, to prevent inbreeding and improve selective breeding processes.

Meanwhile sequencing of D-loop of mitochondrial DNA brings inside into maternal lines of populations, the Y-chromosome analysis brings new information about paternal lines of genetic material transfer. Such data can be used for phylogenetic development analysis of the breed, as well as can help to find out the origin of the breed and presence of the genetic footprints from other breeds [4].

Y-chromosome, which almost doesn't undergo the recombination process, is inherited by the clonal type. At the same time investigation of the hypervariable region (D-loop) helps research on the diversification of inheritance on maternal lines inside the population, and also can help investigate the phylogenetic relationship between animals. Consequently, mitochondrial DNA is widely used as an important instrument of investigation of evolutionary and phylogenetic history of cattle [5], swine, chicken, as well as horses [6,7,8]. D-loop can be used for differentiation between the breeds and inside one breed [9]. Horses are characterized by great amount of mitochondrial haplotypes, and several authors are dividing such haplotypes into haplogroups [10].

Another investigation was done using population of horses of Iran. The population was described and structured using mitochondrial DNA data [11]. 11 haplotypes of the breed were identified, close relationships between different breeds was shown.

48 polymorphic mutations were defined, and all the population was divided into 52 haplotypes. Phylogenetic tree of Iranian aborigine breeds was created. The breed was divided into 11 haplogroups, including A, B, C, E, G, I, L, M, N, P, и Q. Numbers of genetic diversity and haplotype diversity (genetic diversity и haplotype diversity) are 0.0233 and 0.980 respectively. Nucleotide diversity (P_i) was varied in populations from 0.0172 to 0.0242. There were defined breeds of horses, inside the observed group, who was characterised by the most and less diversity in the genotype (F_{st} from 0.003 to 0.193). The results are reporting on genetic variability and great amount of maternal lines of population of horses of Iran.

Japanese investigators were using data on D-loop of mitochondrial DNA for the relict horse breed characterization [12]. This information, as well as information about 32 microsatellite loci helped to find out horse's pedigree, and genetic characteristics important for saving the breed were found out. Average number of alleles and was found out, heterozygosity and expected heterozygosity were found out.

It was shown, that the breed has overcome the moment, when it was cut out in the amount, but since this moment plenty of time is gone. Additionally to the microsatellite loci investigation, D-loop of mitochondrial DNA was investigated. Mitochondrial DNA is able for quick mutations appearance: up to 5-10 times higher mutation rate, than nuclear DNA.

It was shown, that Miyasaki horses have 3 mitochondrial haplotypes.

In one of the recent publications of Russian groups [13] in our field, we have seen combination of 3 techniques, used for the characterization of a horse breed : Y-chromosome, microsatellite loci and D-loop sequencing. Kabardinian horse breed from Northern Caucasus is known for its power, ability to go through long distances and endurance. At the same time this breed has a gap in the documentation of the breeding processes. It was found 64 haplogroups of D-loop. 4 haplogroups were found as most popular, and they are frequently met between racing horses of Asian region (Buryat, Kirgiz, Mongolian, Trans-baikal, and Tuva breeds). High diversity of mitochondrial genome, as well as diversity of paternal lines can be due to long history of the breed, as maternal lines were gathered from a wide spectrum of genetic material, with adding blood from Arabic and Thoroughbred stallions, to improve the breed.

If we talk about Mongolian horses and Kalmyk horses, this two breeds were divided between each other more than 400 hundred years ago. If Mongolian horses are investigated quite actively (which also can be explained by great number of animal on the territory of Mongolia and Inner Mongolia of China), than Kalmyk horse was recently restored and officially registered in Russia as a one of aborigine horse breeds. All the information of the breed was described in State Studbook of Kalmyk horse [14]. Since that, the genome of Kalmyk horses was investigated using genetic techniques [15]. It was shown, what kind genetic and phylogenetic variations are observed between main body of Kalmyk horse population and meat type "Tselinny", which was differentiated and registered using classic selection techniques.

Coming back to the results, which were obtained within the presented paper, below we present obtained phylogenetic trees of different combinations of populations investigated.

We made phylogenetic trees of population 1 of Kalmyk horses, population 2 of Kalmyk horses, population of Mongolian horses from the territory of Mongolia, population of Mongolian horses from the territory of Inner Mongolia.

Fig.1. Population’s №1 of Kalmyk horse phylogenetic tree (44 animals).

Table 1. 44 animals were divided into following populations due to their maternal lines:

№ cluster	of NN of horses
1	1p, 23p, 43p, 12p, 35p
2	7p, 9p
3	16p, 21p, 37p, 42p, 29p, 28p, 22p
4	11p, 41p, 38p, 31p, 25p, 34p, 17p, 39p, 10p
5	44p, 19p
6	4p male
7	14p
8	2p
9	13p, 15p, 8p
10	5p, 6p, 27p, 24p, 30p, 26p, 20p, 32p, 40p, 18p, 33p

Fig.2. Kalmyk horse population’s 2 phylogenetic tree (54 animals).

Table 2. There are following populations of horses from 54 animals:

№ of cluster	NN of horses
1	29k, 38k, 28k, 50k, 30k, 53k, 31k, 52k, 39k, 02k, 26k, 16k, 18k, 35k, 23k
2	27k, 20k, 19k
3	04k male
4	22k
5	11k
6	09k
7	37k
8	44k
9	03k, 05k, 48k, 06k, 10k, 25k, 14k, 24k, 17k, 01k, 40k, 15k, 08k, 41k, 13k, 49k, 36k, 45k, 51k, 33k, 43k, 07k, 12k, 32k male, 47k, 34k, 42k, 55k, 54k

Fig. 3. Mongolian horses (11 animals)

Table 3. There are following cluster groups of horses from 11 animals:

№ of cluster	NN of horses
1	19m, 2m
2	6m
3	20m, 21m
4	10m
5	9m
6	13m, 18m, 5m, 17m

Fig.4. Phylogenetic tree of a population of Mongolian horses of Inner Mongolia (13 animals)

Table 4. There are following populations of horses of 13 animals:

№ cluster	of NN of horses
1	10i
2	3i, 2i
3	8i, 7i, 10i
4	4i, 09i
5	6i
6	13m, 18m, 5m, 17m

Based on the phylogenetic trees obtained we can make following conclusions:

1. Population №1 of Kalmyk horses can be divided into 10 clusters. Population №1 of Kalmyk horses can be divided into 9 clusters. This means, that even though populations consist of quite close relatives, still they are characterized by high diversity in maternal lines. Also this could be due to adding of blood of other breeds, which were added to improve the breed.
2. Population of 11 animals from Mongolia can be divided into 6 clusters. This can be explained, by the fact, that the population of horses in Mongolia is much wider, than the population in Kalmykia, and there is great source of genetic material, including great number of maternal lines.
3. Population of 13 animals from Inner Mongolia can be divided into 6 clusters. The explanation is the same as for population from Mongolia. There is great genetic source on the territory of Inner Mongolia, which was divided from the main genetic source of Mongolia less than 100 year ago.

In addition to the phylogenetic trees of each population we are presenting in this paper, we have built trees for combinations of populations: Inner Mongolia population together with Mongolia population; all the investigated populations together in one phylogenetic tree.

Fig. 5. Phylogenetic tree of populations of Mongolian horses of Inner Mongolia and Mongolia as a solid population (24 animals).

Table 5. There are following populations of horses from 24 animals:

№ cluster	of NN of horses
1	2m
2	3i, 6m, 6m2, 2i
3	3m, 9i, 14m, 11m, 4i
4	12m, 10i
5	5m, 15m, 1m, 13m
6	7m
7	6i
8	8i, 7i, 10i2,

Based on the phylogenetic tree obtained, we can make following conclusion:
Populations of Inner Mongolia horses and Mongolian horses from Mongolia can be divided into 8 clusters, and 3 of them are the same for both populations. This fact is easily explained by the fact, that the population of horsed used to be the same genetic massive, and were divided less than 100 years ago from each other.

Fig. 6. Phylogenetic tree of two populations of Kalmyk horse (p and k), populations of Mongolian horse in Mongolia and Inner Mongolia (China), as a solid population.

Table 6. There are following populations of horses from all the investigated populations:

№ of cluster	NN of horses
1	49k, 25p, 34p, 17p, 10p, 38p, 11p, 39p, 40k, 35k, 42k, 20k, 13k, 39k, 36k, 1m
2	4p, 14p, 2p, 5p, 54k, 47k
3	6i, 14m, 10i, 12m, 9i, 18k, 11m, 12p, 4i, 43p, 23p, 28k, 53k
4	6m, 50k, 15m, 32k, 44km 34k
5	15k
6	26k, 43k, 29k, 51k, 1p
7	8i, 02k, 10k, 03k, 01k, 08k, 19k, 04k, 07k
8	7i, 12k, 2m, 55k, 17k, 5m, 27k, 7m, 45k, 25k
9	06k, 11k, 16k, 37k, 22k, 52k, 05k
10	13p, 40p, 32p, 32k, 14k, 24k, 20p, 3m, 27p, 6p, 23k, 38k, 33k, 10i2
11	33p, 19p, 41p, 31p, 35p, 18p, 28p, 22p, 9p, 30p, 26p, 24p, 15p, 8p, 44p, 30k
12	21p
13	41k
14	13m
15	48k
16	2i
17	4m
18	3i

19	37p
20	42p
21	29p
22	16p
23	7p
24	09k
25	4p, 14p, 2p, 5p, 54k, 47k

Based on the phylogenetic tree obtained, we can make following conclusions:

Population of all animals under investigation can be divided into 25 clusters. Even though there are clusters, which are typical for one population (clusters 9, 11), there are clusters, which have inside animals from different populations (clusters 1, 2, 3, 4, 6, 7, 8, 10, 25). This can be explained by several hypothesis, each of them need additional investigations. First possibility is that while breed's genesis maternal lines from the same other breeds were added, and the second possibility is that the Kalmyk horses and Mongolian horses, even after 400 years after they were genetically divided, keep similarities in the genetic material.

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