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Original article

GENETIC DIVERSITY OF MITOCHONDRIAL DNA HAPLOGROUPS IN THE DON HORSE BREED

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The Don breed of horses, which became famous in Russia already in the middle of the 18th century, was obtained by long-term crossing of nomadic horses with cultured oriental breeds. Due to the reduction in the number of Don queens to 200 broodmares, the research of the genetic characteristics of this breed is of particular relevance. The purpose of the research was to study the matrilineal structure of the Don horse breed based on phylogenetic analysis of 530 nucleotide pairs [n.p.] of the hypervariable section of the D-loop of mitochondrial DNA [mtDNA] in 26 mares representing the main female families. Analysis of the mtDNA sequence was performed using the Neighbor-Joining [NJ] method in combination with bootstrap analysis in the MEGA7 software. In the mitochondrial genomes of the tested Don horses, 26 different haplotypes were identified, which are part of 10 mtDNA haplogroups, including A, B, D, G, L, M, N, O, P, and Q, according to the modern classification. Additionally, four new haplogroups were identified, leading the line from Black Sea mares and atypical for most European breeds. The sequenced fragment of the D-loop from 15471 to 16000 n.p. included 115 polymorphic sites, mainly represented by transversions. The mtDNA structure of the Don breed was dominated by haplogroups G (19.2%), B (15.4%), and L (11.5%). All the analyzed uterine families were clearly differentiated at the level of haplogroups and haplotypes with a high bootstrap support rate (58%–100%). This indicates a high genetic diversity of the genealogical matrilineal structure of the Don horse breed.

Keywords: *genetic; don horse; haplogroups mtDNA; female line; phylogenetic analysis*

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Научная статья

ГЕНЕТИЧЕСКОЕ РАЗНООБРАЗИЕ ГАПЛОГРУПП МИТОХОНДРИАЛЬНОЙ ДНК В ДОНСКОЙ ПОРОДЕ ЛОШАДЕЙ

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Донская порода лошадей, получившая известность в России уже в середине 18 века, была получена методом длительного скрещивания лошадей кочевников с культурными восточными породами. В связи с сокращением численности поголовья донских маток до 200 племенных кобыл изучение генетических особенностей этой породы приобретает особую актуальность. Целью проведенных исследований было изучение матрилинейной структуры донской породы лошадей на основании филогенетического анализа 530 пар нуклеотидов (п.н.) гипервариабельного участка D-петли митохондриальной ДНК (мтДНК) у 26 кобыл, представляющих основные женские семейства. Анализ последовательности мтДНК проводили с использованием Neighbor-Joining (NJ) метода в сочетании с бутстрэп-анализом в программе MEGA7. В митохондриальных геномах протестированных донских лошадей было выявлено 26 различных гаплотипов, входящих в состав 10 гаплогрупп мтДНК, включая A, B, D, G, L, M, N, O, P и Q, согласно современной классификации. Дополнительно было выявлено еще четыре новых гаплогруппы, ведущих линию от черноморских кобыл и нетипичных для большинства европейских пород. Секвенированный фрагмент D-петли с 15471 по 16000 п.н включал 115 полиморфных сайтов, в основном представленных трансверсиями. В структуре мтДНК донской породы преобладали гаплогруппы G (19.2%), B (15.4%) и L (11.5%). Все проанализированные маточные семейства были четко дифференцированы на уровне гаплогрупп и гаплотипов с высоким показателем бутстрэп-поддержки (58-100%), что свидетельствует о высоком генетическом разнообразии генеалогической матрилинейной структуры донской породы лошадей.

Ключевые слова: *генетика; донская лошадь; Гаплогруппы мтДНК; женская линия; филогенетический анализ*

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Introduction

The Don horse breed is one of the most valuable domestic breeds, the formation of which was started by the Cossacks in the 18th century. The basis of the breed was steppe horses of tribes that roamed in the southern Russian steppes and many breeds of Eastern origin. Subsequently, a thoroughbred riding breed was used to improve the Don horses. This contributed to obtaining a sufficiently tall, frisky, and hardy cavalry horse that fully meets the needs of the army. Systematic breeding work with the Don horse began in the 30s of the last century. It made it possible to obtain a large, hardy, versatile, and unpretentious herd horse [1; 2].

The intensification of agricultural production has led to a significant reduction in the number of horse livestock. The number of Don queens has practically decreased to 200 heads. According to modern criteria of the Food and Agriculture Organization of the United Nations [FAO] [17], the Don horse breed has a vulnerable risk status. Therefore it is important to monitor its genetic diversity. Previous research of polymorphism of 17 microsatellite loci in horses of domestic breeds has shown [4] that Don horses have a fairly high level of genetic diversity and are distinguished by the presence of rare alleles absent in other riding breeds of horses.

Recently, researchers have been greatly interested in studying the mitochondrial genome of horses of modern and ancient horse breeds [8; 9; 10; 12; 16;]. The high level of polymorphism of the mitochondrial DNA [mtDNA] D-loop sequence and maternal inheritance make the mitochondrial genome a unique object for studying the phylogeny of breeds and assessing the population diversity of populations. The modern classification of horse mtDNA variability includes 18 basic haplogroups [5] associated with the matrilineal origin of breeds [6; 13; 14; 15;]. An equally interesting practical aspect of studying mtDNA is the assessment of the genetic differentiation of female lines in horse breeds and the use of D-loop sequence analysis in identifying and controlling the origin of horses [3; 7; 11].

The research of the genetic characteristics of horses of the Don breed, created based on crossing many aboriginal and cultural breeds, is of undoubted interest for tracking its phylogenetic relationships. Evaluation of the genetic differentiation of established female families by mtDNA haplogroups and characterization of the matrilineal structure of the population are important for the preservation and further improvement of this unique small breed.

Materials and methods

The purpose of the research was to study the features of the hypervariable section of the D-loop of mtDNA in horses of the Don breed. In this regard, the following objectives were set:

- Study the genetic structure of mtDNA haplogroups in the Don breed of horses;
- Certify the royal families of the Don breed by mtDNA haplotypes;
- Analyze the phylogenetic relationships of Don horses considering their matrilineal structure.

The material for mtDNA analysis was hair samples from 26 horses of the Don breed belonging to 26 main uterine families. DNA was isolated from hair follicles using the ExtraGene DNA Prep 200 kit (Isogen LLC, Moscow). Amplification of the isolated DNA was carried out using specially selected primers to analyze the sequence of the studied section of the mtDNA D-loop, taking into account the reference sequence of the Swedish horse fossil X79547 [18]. The amplified DNA was sequenced using the BigDyeTM Terminator Cycle Sequencing Kit (PE Applied Biosystems, USA) on the ABI 3130xl genetic analyzer (PE Applied Biosystems, USA) in the laboratory of Genetics LLC, Moscow.

To align the obtained sequences, the authors used the BioEdit 7.2.1 program. They used the maximum composite likelihood [MCL] model to analyze the mtDNA D-loop section comprising 530 nucleotide pairs [n.p.] (from 15471 to 16000 bases). The authors constructed phylogenetic trees using the Neighbor-Joining [NJ] method. The statistical confidence of each node was estimated by 1000 random bootstrap runs. When analyzing mtDNA D-loop sequences, the authors used the GeneBank database for 18 basic haplogroups (JN398377-JN398457), according to the Achilli et al. classification [5]. Data analysis was carried out on the MEGA7 database and program.

Results

Sequencing of the hypervariable region of the mtDNA D-loop in 26 Don horses, belonging to the main families, showed the presence of 26 different haplotypes corresponding to 10 haplogroups (A, B, D, G, L, M, N, O, P, and Q), according to the classification of Achilli et al. [5]. In particular, the authors identified five original haplotypes representing four new haplogroups (Table 1). The ratio of the number of haplotypes to the number of haplogroups was 1, which indicates a high level of mtDNA variability in the Don breed of horses.

The results of the matrilineal analysis show that horses of 9 out of 26 uterine families are owners of the “family” mtDNA haplogroup, which can serve as a genetic marker of this female line (Fig. 1) [5]. Interestingly, the Benga family, belonging to haplogroup A, had a certain kinship with the sequence of the fossil Swedish horse (X79547).

Table 1.

Distribution of horses and Don breed families by mtDNA haplogroups

	Name of horse	Year of birth	Female line	Haplogroup mtDNA
1	Benga	2018	Alena 252	A
2	Trapeza 230	2019	Allegoria 91	G
3	Gulsara 23	2008	Vena 27	B
4	Tihonya 13	2017	Vena 46	L
5	Sidkiya	2017	Galka 107	B
6	Blagovest 4	2018	Daniya 282	New 1
7	Sviyaga 208	2018	Diva	M
8	Tropinka 223	2016	Jermelka 85	New 2
9	Tabiola 20	2016	Jestikulayciya 33	New 3
10	Baturka 205	2019	Zairka	New 4
11	Gabana 21	2012	Kolibri	N
12	Gordost 35	2000	Komanda 286	O
13	Gazeta 210	2019	Mamka 29	G
14	Slujebnaa	2019	Matematika 114	G
15	Tihaya Zavod 25	2010	Mirta 88	L
16	Trizna 221	2018	Molva 108	P
17	Gorelka 211	2019	Myshelovka	P
18	Zateya 209	2017	Nahodka 52	G
19	Trizna 8	2001	Nejnaya	D
20	Dorogaya 206	2015	Pepepelka 34	New 4
21	Glasnaya 15	2013	Pyshnaya 21	B
22	Garsa 22	2012	Remboka 242	G
23	Molotba 6	2015	Sudba 211	B
24	Sdoba 218	2016	Shamka 165	G
25	Dobabka 217	2019	Yupiterka 78	Q
26	Bombey 4	2013	Venera	L

The majority of mtDNA haplotypes of Don horses were represented by haplogroups G (19.2%), B (15.4%), and L (11.5%). The haplogroups A, D, M, N, and Q were relatively rare in the genomes of mares. The phylogenetic tree of the mitochondrial fragment of the D-loop of the tested Don breed horses, constructed using the NJ method, demonstrated a fairly high bootstrap value (59–100).

The analyzed sequence of the mtDNA D-loop fragment from 15471 to 16000 nucleotide positions contained 45 transitions and 70 transversions (Rs/v = 0.6). The number of nucleotide substitutions in relation to the length of the studied sequence was 0.217. This indicates a high level of nucleotide diversity of mtDNA in horses of the Don breed.

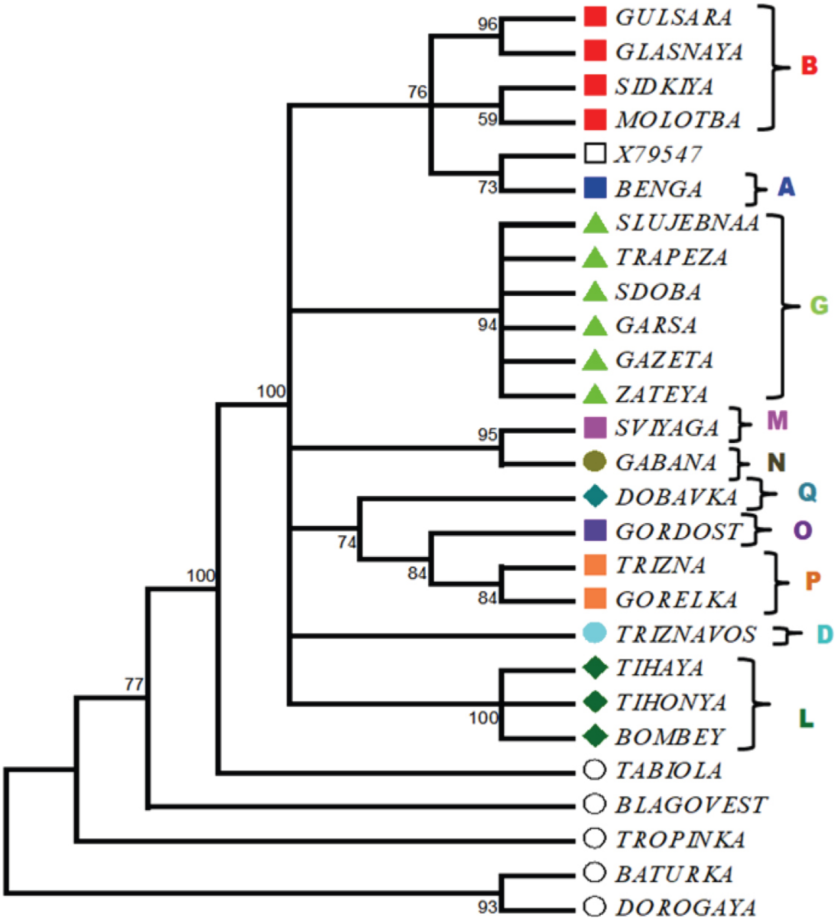

Fig. 1. Phylogenetic tree of mtDNA D-loop sequences from haplotypes of Don horses, constructed by the NJ method in combination with bootstrap analysis (bootstrap value > 50 are shown at nodes). For identification of haplogroups were used GenBank data (JN398377- JN398457).

Discussion

The results of the researches indicate a high level of variability of the hypervariable section of the mtDNA D-loop in horses of the Don breed. This confirms its heterogeneous origin with the participation of a number of aboriginal and cultural breeds. The presence in the genetic structure of this breed of haplogroup

A, characteristic of the fossil Swedish horse, as well as several new mtDNA haplogroups, may indicate a trace of unique ancient ancestors in its phylogeny. Another argument in favor of this hypothesis can be a relatively high level of nucleotide substitutions (0.217) at the analyzed site of 530 n.p., represented mainly by transversions (Rs/v = 0.6).

The genetic structure of the mitochondrial genome of Don horses has a certain similarity with that of Kabardian horses occupying a neighboring area in the foothills of the North Caucasus. In horses of these two riding breeds, nine common mtDNA haplogroups were identified, including rare variants A, D, P, and Q [14]. In general, haplogroups B and L were typical for horses of the Don, Vyatka [3], Kabardian, and other domestic breeds [14]. The frequency of occurrence of them exceeded 10%. Another feature of the mitochondrial genome of Russian horse breeds is the presence of unique additional haplogroups that are not found in horses of Western and Central Europe [3; 14; 16].

The assessment of the diversity of mtDNA haplogroups in the structure of female lines showed a high level of differentiation of haplotypes in 26 main brood families of the Don breed (bootstrap value 59–100). This testifies to the effective zootechnical work of specialists in the formation of the genealogical structure of the breed, which was started back in the 30^s of the last century. In conditions of reducing the number of breeding Don mares to 200 queens, it is very important to control the level of inbreed genetic diversity and maintain the established matrilineal structure of the breed.

Methodically, it is important that the research of the variability of mtDNA haplogroups is based on a sample of representatives of various female lines. This allows, even with a small amount of analysis, obtaining adequate information about the matrilineal structure of the breed. The research of the mitochondrial genome is an important part of the genetic certification of breeds, and can be used to identify and control the origin of animals [7; 11; 13; 15].

Conclusion

As a result of the researches of the sequence of the hypervariable section of the mtDNA D-loop of Don horses, the authors established high variability of their mitochondrial genome at the level of haplogroups, haplotypes, and nucleotides. Evidently, this is due to the participation in the creation of the female basis of the breed of a number of ancient horse breeds, traces of which can be traced in domestic horse breeds.

The authors found significant differences in the structure of mtDNA of horses of different breeding families. It confirms the success of zootechnical work on the formation of the genealogical structure of the breed. The carried out certification of brood families in the Don breed makes it possible to identify horses along the female line and use the data obtained in the control of the origin of horses.

The obtained data on the sequence of the mtDNA D-loop of Don horses are undoubtedly of interest for studying the genesis of this breed and the phylogenetic connections of domestic horse breeds, as well as for considering the mechanisms of breed formation themselves.

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Сергей И. Сорокин: conducting of the experiment, interpretation of the results, preparation of the text of the paper.

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Храброва Л.А.: осуществляла научное руководство, дизайн эксперимента, научное консультирование, оформление и редакция статьи.

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