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БИОРАЗНООБРАЗИЕ СВИНЕЙ РАЗЛИЧНЫХ ПОРОД НА ОСНОВЕ АНАЛИЗА D-ПЕТЛИ мтДНК

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Полиморфизм митохондриальной ДНК, представляющий собой один из наиболее распространенных генетических маркеров, активно применяется при изучении различных видов животных. Так как в пределах того или иного вида митохондриальный геном развивался на протяжении многих лет, это повлияло на фиксацию мутаций и образование митохондриальных линий, имеющих общее происхождение и формирующих митохондриальные геномы, называемые гаплогруппами мтДНК. Целями работы являлись оценка генетического разнообразия свиней двух материнских пород отечественной репродукции на основании анализа полиморфизма D-петли мтДНК, а также сравнение полученных результатов с идентичными последовательностями из базы данных NCBI в разрезе пород и их географического распространения. Были изучены 39 свиней породы ландрас и 49 – крупной белой породы. Митохондриальную ДНК выделяли из образцов тканей (ушной выщип). Для оценки генетического разнообразия определяли количество гаплотипов, гаплотипическое и нуклеотидное разнообразие, среднее число замен нуклеотидов на сайт. Обнаружено 23 полиморфных участка: 21 – у свиней породы ландрас, 17 – у представителей породы крупная белая. Всего в исследуемой популяции выявлено десять гаплотипов. В базе данных NCBI найдено 75 идентичных последовательностей, характерных для свиней различных пород, разводимых в разных странах мира. После определения нуклеотидных последовательностей фрагмента D-петли мтДНК свиней пород ландрас и крупная белая отечественной репродукции и их сравнения с последовательностями из базы данных NCBI было установлено наличие идентичных последовательностей у изучаемых нами животных и представителей европейских и азиатских пород, в том числе коммерческих и локальных. Полученные материалы показывают, что оценка полиморфизма мтДНК способствует идентификации филогенетических связей между популяциями, отслеживанию породообразовательных процессов и может быть рассмотрена как дополнительный критерий селекционно-племенной работы.

Ключевые слова: генетические маркеры, генетика, свиньи, мтДНК

BIODIVERSITY OF PIGS OF VARIOUS BREEDS BASED ON THE ANALYSIS OF mtDNA D-LOOP

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Mitochondrial DNA polymorphism, which is one of the most common genetic markers, is actively used in the study of various animal species. Since the mitochondrial genome has evolved over many years within a species, this has influenced the fixation of mutations and the formation of mitochondrial lineages that share a common origin and form mitochondrial genomes, called mtDNA haplogroups. The objectives of the work were to evaluate the genetic diversity of pigs of two maternal breeds of domestic reproduction based on the analysis of mtDNA D-loop polymorphism and to compare the results obtained with identical sequences from the NCBI database by breed and their geographic distribution. 39 Landrace pigs and 49 Large White pigs were used for the study. MtDNA was isolated from tissue samples (ear notch). The number of haplotypes, haplotypic and nucleotide diversity, and the average number of nucleotide substitutions per site were determined to assess the genetic diversity. 23 polymorphic sites were found: 21 in Landrace pigs, 17 in Large White pigs. A total of ten haplotypes were identified in the study population. The NCBI database found 75 identical sequences for pigs of different breeds bred around the world. After determining the nucleotide sequences of the D-loop fragment of the mtDNA of Landrace and Large White pigs of domestic reproduction and comparing them with the sequences from the NCBI database, the presence of identical sequences in the animals we studied and in the representatives of European and Asian breeds, including commercial and local was established. The materials obtained show that the assessment of mtDNA polymorphism contributes to the identification of phylogenetic relationships between populations, tracing of pedigree processes, and can be considered as an additional criterion of selection and breeding work.

Keywords: genetic markers, genetics, pigs, mtDNA

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Conflict of interest

The authors declare no conflict of interest.

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INTRODUCTION

Mitochondrial DNA (mtDNA) polymorphism, as one of the most common genetic markers, is widely used in the study of various animal species. This is due to its maternal inheritance and the high frequency of occurrence of mutational processes. Mitochondrial DNA is inherited through cytoplasmic inheritance and is not subject to recombination. Within a species, the mitochondrial genome has evolved over many years, leading to the fixation of mutations and the formation of different mitochondrial lineages that share a common origin, forming mitochondrial genomes known as mtDNA haplogroups. mtDNA haplotypes not only confer the advantages and disadvantages on organisms[1, 2] (e.g., influencing the growth and physical performance of animals, adaptation to warm and cold climates, sperm motility), but also determine economically important traits such as milk quality [1]. Furthermore, it has been shown that embryonic stem cell lines of different mtDNA haplotypes, with identical

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nuclear DNA, exhibit different levels of gene expression during cell differentiation [2], which may be influenced by the impact of mtDNA on phenotype realization.

Currently, both Asian (A, B, and C) and European (D and E) haplogroups have been identified in pigs. Pig breeds developed in China, Korea, and Japan are largely similar to each other but significantly differ from the European-type pigs. Commercial breeds of the European origin, such as Landrace, Large White, and Duroc, are bred worldwide, and their mtDNA may belong to either the Asian or non-Asian type depending on their origin and further improvement by individual breeding centers.

For domestic selection purposes, studies on mtDNA of pigs from breeding farms in the Russian Federation are of particular interest. In this regard, the objectives of our work were to assess the genetic diversity of two domestically reproduced maternal pig breeds based on the analysis of mtDNA D-loop polymorphism and to compare the obtained materials with identical sequences from the National Center for Biotechnology Information (NCBI) database in terms of the breeds and their geographical distribution.

MATERIALO AND METHODS

The study was conducted on the domestically reproduced Landrace pigs (*n* = 39) and Large White pigs (*n* = 49). DNA was extracted from tissue samples using the "K-Sorb-100" reagent kit (Syntol, Russia). The D-loop region (1046 bp) was amplified by PCR using the primers FW 5'-TGCAAACCAAAACGCCAAGT-3' and RW 3'-TTTTTGGGGTTTGGCAAGGC-5'. Gel-purification of the amplified fragments was performed using the Cleanup Mini kit (Eurogene, Russia). Sequencing was outsourced to Eurogene company. Editing and alignment of the sequences were performed using BioEdit 7.2.6 and MEGA 7 software. mtDNA haplotypes were denoted as Hap. The number of the haplotypes (H), haplotype diversity (HD), nucleotide diversity (π), and average number of nucleotide substitutions per site (k) were calculated using DnaSP 5.10. Calculations and construction of the phylogenetic tree were performed using the maximum likelihood method. The sequence number NC000845 (NCBI) was considered as the reference. Sequences were assigned to specific haplogroups based on comparison with known haplogroup sequences from the NCBI database (A-KT279758, B-KT261429, C-KT279759, D-KT279760, E-KT261430).

RESULTS AND DISCUSSION

During the study, nucleotide sequences of the variable D-loop region of mtDNA were determined for 88 tissue samples from Landrace and Large White pigs. For further analysis, a 638 bp fragment located within the range of 87-724 bp according to the reference sequence was used. Table 1 presents the parameters of genetic diversity in the studied pig groups.

The haplotype diversity for the Landrace breed was recorded at a level of 0.839, while for the Large White breed, it was 0.041 (see Table 1). Landrace pigs exhibited a higher number of nucleotide substitutions (k = 8.640) compared to Large White pigs (k = 0.735). The average nucleotide diversity was 0.013 (mostly due to the Landrace breed).

A total of 23 mutations were detected in the studied group, with 21 in Landrace pigs and 17 in Large White pigs. Ten haplotypes were identified in total (see Table 2).

Out of the 49 Large White pigs, 48 had the Hap-10 haplotype, while only one individual had the Hap-9 haplotype. Landrace pigs showed greater genetic diversity, with eight haplotypes identified. The most frequent haplotypes were Hap-1 and Hap-4 (see the figure).

Табл. 1. Генетическое разнообразие свиней пород ландрас и крупная белая

Table 1. Genetic diversity of Landrace and Large White pigs

Breed	N	S	H	HD	k	π
Landrace	39	21	9	0,839	8,640	0,0139
Large White	49	18	2	0,041	0,735	0,0012
On average	88	23	10	0,674	8,328	0,0134

The assignment of the identified haplotypes to haplogroups A, C, D, and E is shown on the phylogenetic tree (see figure). No individuals belonging to haplogroup B were found.

A search for identical sequences in the NCBI database revealed 75 sequences belonging to the representatives of different breeds (see Table 3). Out of these, 30 sequences were assigned to the Hap-10 haplotype, which we identified in Large White pigs. Previously, two sequences were found in wild Asian boars, 27 in Asian pig breeds[3, 4] [1–9], and one in a Spanish-origin Large White pig (see footnote 2). The individuals of the Asian origin with the Hap-10 haplotype were associated with local Chinese breeds [2, 5, 6, 8, 9]. This haplotype was also found in Mong Cai pigs [4, 8] and Kunekune pigs bred in New Zealand.

According to the NCBI data, identical Hap-1 haplotypes were identified in Landrace and Duroc pigs bred in Australia [1] and Japan[5], Iberian pigs (see footnote 2), and French wild boars (see footnote 1). Sequences identical to the Hap-2 haplotype were found in wild boars (see footnote 2) and Mangalica pigs [10]. Carriers of the Hap-5 haplotype are similar to the Landrace pigs of Chinese origin[6], Canadian pigs [11], and Japanese pigs[7]. The Hap-8 haplotype is fixed in Duroc pigs in Spain[8] and China (see footnote

Табл. 2. Гаплотипы свиней пород ландрас и крупная белая
Table 2. Haplotypes in Landrace and Large White pigs

n	Haplotype	Mutation																						
		109	124	131	145	153	158	180	185	241	248	279	294	306	323	390	405	452	560	575	640	692	704	706
		Landrace																						
9	1H	T	T	G	C	C	A	C	T	T	G	C	A	C	C	C	T	C	T	A	C	G	A	A
2	2H	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	A	–	–
5	3H	–	–	–	–	–	–	T	–	–	–	–	–	–	–	–	–	–	–	G	–	A	–	–
11	4H	C	A	A	T	T	G	–	–	C	–	T	G	T	T	T	–	–	C	G	–	A	G	G
2	5H	–	–	–	–	–	–	–	C	–	A	–	–	–	T	–	–	–	–	–	–	–	–	–
6	6H	–	A	–	–	–	–	T	–	–	–	–	–	–	T	–	–	–	–	–	T	–	–	–
2	7H	–	–	–	–	–	–	T	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2	8H	–	–	–	–	–	–	T	–	–	–	T	–	–	–	–	–	–	–	–	–	–	–	–
		Large White																						
1	9H	C	A	A	T	T	G	–	–	C	–	T	G	T	T	T	–	–	C	G	–	A	–	–
48	10H	C	A	A	T	T	G	–	–	C	–	–	G	T	T	T	–	–	–	G	–	–	G	G

Note. Positions are listed in accordance with Sequence No. NC000845.

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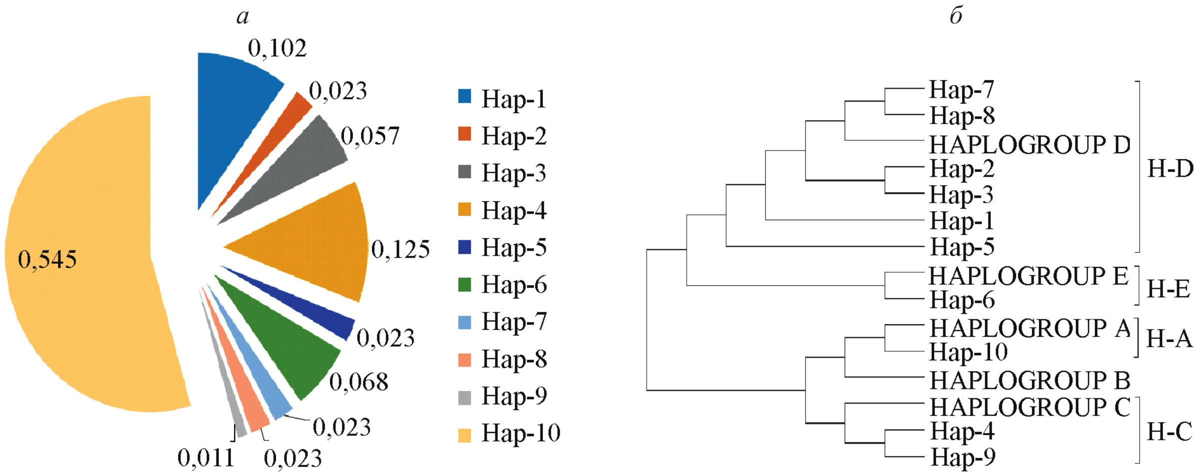
Табл. 3. Идентичные гаплотипы из базы данных NCBI
Table 3. Identical haplotypes from the NCBI database

Haplotype	Number in the NCBI	Breed	Country	Haplotype	Number in the NCBI	Breed	Country
Hap-1	KT279760	Commercial	Australia	Hap-8	KF569218	F2	Vietnam
Hap-1	FJ236997	Duroc	Spain	Hap-8	KC250275	Large White	China
Hap-1	AM040631	»	Taiwan	Hap-8	FJ236996	Duroc	Spain
Hap-1	AM040613	Landrace	»	Hap-8	EU660148	»	China
Hap-1	AM040615-AM040620	»	»	Hap-9	Hap-9 sequences are absent		
Hap-1	D42170	Commercial	Japan	Hap-10	MG250567	Yudong black	China
Hap-1	HM747198	Wild boar	Portugal	Hap-10	MG250564	Wuzhishan	»
Hap-1	AY232858	Iberian	Spain	Hap-10	MG250562	Wuzhishan	»
Hap-2	KJ746666	Mangalitsa	China	Hap-10	MG250556	Tunchang	»
Hap-2	HM747198	Wild boar	France	Hap-10	MG250532	Huizhong	»
Hap-2	JN601068	Mangalitsa	USA	Hap-10	MG250529	Huizhong	»
Hap-3	Hap-3 sequences are absent			Hap-10	MG250518	Bamaksyang	»
Hap-4	EU979134	Dapulian	China	Hap-10	KF913287	Kwangbaihai	»
Hap-5	EU660147	Landrace	»	Hap-10	KF913276	Cunjiangxiang	»
Hap-5	MH113791	Yorkshire and Landrace	»	Hap-10	KF913275	Cunjiangxiang	»
Hap-5	AB041499	Landrace	Japan	Hap-10	KF913271	Cunjiangxiang	»
Hap-6	Hap-6 sequences are absent			Hap-10	KF913253	Baixi	»
Hap-7	KC469587	Pietren	China	Hap-10	KF913252	Baixi	»
Hap-7	EU660181	Yorkshire	»	Hap-10	KX982643	Wild boar	Vietnam
Hap-7	EU660154	Duroc	»	Hap-10	KX982646	» »	»
Hap-7	EU660151	»	»	Hap-10	KX982647	» »	»
Hap-7	EU660150	»	»	Hap-10	KX982651	» »	»
Hap-7	EU660140	Landrace	»	Hap-10	KX982652	» »	»
Hap-7	AF034253	»	Taiwan	Hap-10	KX147100	Mong Cai	»
Hap-7	D16483	»	Japan	Hap-10	KU556691	Mong Cai	China
Hap-7	AM040625-AM040629	Duroc	Taiwan	Hap-10	KT279758	Large White	Australia
Hap-7	AM040612	Landrace	»	Hap-10	KP681243	Wuzhishan	China
Hap-7	AM040614	»	»	Hap-10	KM094194	Sandu black	»
Hap-7	AM040619	»	»	Hap-10	KF767443	Wuzhishan	»
Hap-7	AM040621	»	»	Hap-10	KC250274	Lantang	»
Hap-7	AM040622	»	»	Hap-10	FM244684	Wild boar	Thailand
Hap-7	HM747216	Bisaro	Portugal	Hap-10	FM244685	» »	»
Hap-7	AY232856	Iberian	Spain	Hap-10	EU979182	Chunghwai White	China
Hap-7	GQ169779	Duroc	Taiwan	Hap-10	AY884824	Kunekune	United Kingdom
Hap-7	AB041494	Large White	Japan	Hap-10	AY232882	Large White	Spain



Частота встречаемости гаплотипов мтДНК (*а*) и их распределение в соответствии с гаплогруппами A, B, C, D, E (*б*)

Frequencies of mtDNA haplotypes in the studied pigs (*a*). Distribution of haplotypes according to haplogroup A, B, C, D, E (*б*)

3) [4], as well as in Large White pigs in China [3]. It has been established that the divergence of European and Asian pig breeds based on mtDNA differences occurred long before their domestication [12]. Pigs and boars from China, Korea, and Japan demonstrate close relationships among themselves and are significantly different from European pigs in the maternal lineage [3].

CONCLUSION

As a result of the study, nucleotide sequences of the most variable fragment of the D-loop region of mtDNA in Landrace and Large White pigs have been determined. Comparison of these sequences with the variants deposited in the NCBI database revealed the presence of identical sequences among pigs of various Asian and European breeds. Thus, the assessment of mtDNA polymorphism contributes to the identification of phylogenetic relationships between populations, tracking breed formation processes, and can be considered as an additional criterion in breeding and breeding work.

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