



Genetic diversity of modern lines of hybrid pigs based on variations in mitochondrial DNA sequence

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In the study, we evaluated the genetic specificity of haplotypes in the population of hybrid gilts (Large White × Landrace), (Landrace × Large White) from the SPE “Globinsky Pig Complex” LLC and breeding sows of the Large White breed from the SE “DG named after January 9th” using polymorphism of the lengths of the restricted fragments of mtDNA. The purpose of the study was to determine if the process of creating specialized parent lines (of modern cross-border breeds) reduces haplotype diversity. As a genetic material, bristles from sows of the Large White breed (n=7) were used and epithelial tissue of pigs (Large White × Landrace), (Landrace × Large White) — (n=37). DNA release from bristle samples was carried out using ion exchange resin *Chelex-100*. For the study of the D-loop of the mitochondrial genome of hybrid pigs (n=37) from the epithelial tissue of the auricle, a set of DNA-sorb-B nucleic acid extraction kit from “InterLabService-Ukraine” LLC was used. The samples of epithelial tissue of pigs’ ears were treated with fire from fuel tablet. For the analysis of the mitochondrial genome, the method of polymorphism of the lengths of restricted fragments was used, amplified with PCR. Genotyping of DNA samples of experimental pigs according to mitochondrial markers was carried out with the involvement of the polysite method in accordance with the methodological recommendations of K. F. Pochernyaev and M. D. Berezovsky (2014). The use of maternal inheritance type markers (mtDNA) allowed to identify 2 maternal lines with specific haplotypes, which participated in the creation of hybrid pigs and the formation of their haplogroup. The genetic diversity of mtDNA subspecies of wild and domesticated pigs is limited by the existing lines. Therefore, one haplotype of the mitochondrial genome does not indicate a specific breed, since, several breeds have the same haplotype mtDNA — A, G, C, N, and O. The concentration of haplotype A in tribal sows of Large White breed with a frequency (16%). In the hybrid gilts (Large White × Landrace), (Landrace × Large White) the concentration of detected haplotypes is: C (n=9) — Landrace, Hampshire, Wales, wild pig (20.5%); G — (n=5) Wales, wild pig (11.4%); O (n=5) — Landrace, wild pig (11.4%); N (n=11) — Large White, Berkshire, Asian wild pig (25%); D 9%, K 6.8% (n=7) — unknown among the breeds of domestic pig. We assume that pigs of a Large White breed with haplotype A and hybrid pigs (Large White × Landrace), (Landrace × Large White) with haplotype G, O, in particular D, K contain aboriginal genetic resources. However, in the middle of the XX century, subspecies of wild and domesticated pig breeds became less population-like due to decrease in the area of cultivation and increased pressure from foreign breeds with high growth rates and breeding grounds. Thus, there is a risk of extinction — existing haplotypes and those which have not yet been identified among domesticated pigs (D, K). This suggests that the study should focus on classifying and identifying the phylogenetic origin of pigs and the creation of a molecular genetic bank of producer boars for environmental activities. The domestication process puts strong selective pressure on *Sus scrofa* species through genetic processes such as inbreeding, genetic drift, natural and artificial selection according to the desired signs. Over the past 9–10,000 years, human intervention

has led to domesticated species that are morphologically, behaviorally, and genetically different from their ancestors' relatives. We believe that the "hybrid" subspecies of wild pigs with some morphological features of a domesticated pig had a higher proportion of the full-genomic ancestors of a domestic pig compared to the morphologically pure subspecies of wild pigs. Animals with haplotypes D, K are the result of hybridization with European boars. Representatives of haplotypes A (Large White, European-type Duroc, Mangalica); G (Wales, wild pig) — Italy; C (Landrace, Wales, Hampshire, wild pig) — Ukraine, Poland, France; O (landrace, wild pig) — Sweden, grouped into the European cluster of "mt-E" haplogroup. Pigs with the haplotype N — Large White (Asian type), Berkshire, a wild pig belongs to the Asian cluster of "mt-A" haplogroup. Over time, this led to almost complete disappearance of primary Middle Eastern ancestors in the nuclear genomes of European domesticated pigs. Phylogenetic reconstruction of mitochondrial genome data from hybrid pigs reflects a clear geographical division of mtDNA data — Eastern Europe and Asia. In particular, the subspecies of European and Asian wild pigs is the ancestral foundation on the maternal line, which preceded domestication and breeding pigs by hybridization. European and Asian haplotypes of wild pigs have shown that wild pigs from regions such as Italy, Poland, France, Scandinavia, and Ukraine were also either domesticated or at least initially included in domesticated pigs. The results of the study of the *S. s. domestica* mitochondrial genome showed an intra-breed genetic diversity of hybrid gilts. This is due to the selection strategy of international genetic centers, where, despite the consolidation of the genetic structure in the inside of the center, significant general genetic diversity of the breed is ensured. In addition, the above results indicate a connection between the frequency distribution of mtDNA haplotypes and adaptation to different climate conditions. As a whole, the presented results are an incentive to continue research on the study of the mitochondrial genome of modern lines of hybrid pigs. Carriers of haplotype C, O, G, and N are the basis of maternal breeding and improvement of the lines of hybrid pigs of the XXI century. It is necessary to take into account the fact that the cleanest mother nuclei (Wild pig, Great Yorkshire, Landrace) are really clean foundation for use in hybridization schemes, in the crossing over, in the formation and development of modern hybrid lines of pigs. Despite this, the diversity of the mitochondrial genome in the population of transboundary breeds persists.

Key words: origin, mtDNA, D-loop, haplotype, haplogroup, clade, cluster, breeding sows of the Large White, hybrid gilts (Large White × Landrace), (Landrace × Large White), PCR-RFLP analysis

Questions of the breed-creative process of domesticated pigs (*Sus scrofa domestica*) when creating modern hybrid lines of pigs, it is necessary to consider from the definition of the concept "Origin". Origin is the attribution of animals to certain breeds, lines, families, or subspecies of wild ancestors [18]. Eventually, the study of maternal lines using mitochondrial DNA markers. The microevolution process of breed formation has developed due to breeding conditions — a process of domestication, hybridization, selection, and selection of pairs. The requirements of modern breeding ensure the progressive development of the genome of domesticated pigs. To organize maternal lines, the most convenient way to study is mitochondrial DNA. The main source of the mechanism of breed-creative microevolution is hybridization, selection, and selection in the conditions of the intensive domestication process. The basis of the microevolution of *S. s. domestica* representatives as a morphogenetic transformation of the body of pigs are mutations and selection. It is microevolution that is the basis of labor formation in the conditions of domestication. Modern domestication is explained by the transformation of natural selection into artificial. It is worth considering the fact that the intraspecific hybridization

of the current distant descendants of subspecies of wild pigs in Eurasia, the selection process of populations that have undergone domestication is the result of artificial selection. Therefore, among modern representatives of high-performance hybrid pigs, there is a multiple cascading repetition and permanent use of Asian and European ancestral forms. This is effectively confirmed by molecular genetic analysis of PCR-RFLP using maternal inheritance markers. Studies of *S. s. domestica* microevolution at the molecular genetic level confirm the fact that the main source of the gene pool in the formation of the *S. s. domestica* genome was the mitochondrial genome of Asian and European subspecies of wild pigs (molecular-genetic Euro-Asian heterozygosity genome in the microevolution of *S. s. scrofa*). The study of *S. s. domestica* microevolution using molecular genetic methods made it possible to understand the specific direction of the mitochondrial genome of a large breed of pigs and hybrid pigs of foreign breeding. Subspecies of wild pigs of Asian origin took an active part in the microevolution of modern *S. s. domestica* representatives which come from the following subspecies: *S. s. orientalis*, *S. s. cristatus*, *S. s. vittatus* [4]. Commercial European domestic pigs are genetically more

diverse than European wild boars, although historically the latter represent the original population for domestication [3]. However, the genetic structure of pig breeds in Ukraine represents wide geographical regions and various climatic zones of Eurasia. Therefore, analysis of the genetic diversity and maternal structure of the population based on genomic data is useful for finding out the demographic history of pig breeds around the world.

Pigs (*Sus scrofa*) are among the first domesticated animals and remain one of the most important species in the agricultural communities of Eurasia [5, 19, 22]. *S. scrofa* have been domesticated 8,000 to 10,000 years ago in Eastern Anatolia and China [14, 21]. Most pigs were imported into Ukraine from the UK and China. Many breeds of pigs registered in the middle of the twentieth century were obtained by crossing several basic populations from different breeds, including Large White, Landrace, Hampshire, Berkshire, Mangalica, Asian pigs, and eventually the population of local subspecies of wild pigs. Although in Ukraine pigs come from imported breeds. This led to the emergence of unique characteristics that are specific to modern breeds and hybrid pigs, through intensive methods of selection work. The uniqueness is that modern pig populations differ from the “founding breed”, however, they are carriers of the haplotype of the maternal type of inheritance. The question is that today there is not enough literature on the breeds of pigs kept and bred in Ukraine. It is possible that these breeds represent unique genetic resources for local commercial pig lines. With this in mind, the purpose of our research was to determine, whether the process of creating specialized maternal lines in modern transboundary breeds leads to reduce haplotype diversity.

The Purpose of the Study

The purpose of the study was to determine whether the process of creating specialized maternal lines in modern transboundary breeds leads to a decrease in haplotype diversity among the studied sample of hybrid gilts (Large White × Landrace), (Landrace × Large White) from the “Globinsky Pig Complex” SPE LLC and breeding sows of the Large White breed from the “DG named after January 9th” SE using polymorphism of the lengths of the mtDNA restricted fragments.

Materials and Methods

The research was carried out in the conditions of the genetics laboratory of the Institute of Pig Breeding and Agricultural Production NAAS. To extract the D-loop of the mitochondrial genome of hybrid pigs (n=37) from the epithelial tissue of the auricle was used set *DNA-sorb-B nucleic acid extraction kit* from the “InterLabService-Ukraine” LLC. Samples of epithelial tissue from the ears of pigs were treated with fire from fuel tablet for 5–7 seconds.

After, the treated samples were crushed with a scalpel in the amount of 0.20 g per 1 sample. After the study, the samples were extracted by the sorbent method.

Samples of biological material — bristles from breeding pigs of the Large White breed (n=7) were investigated in SE “DG named after 9 January IPB and AIP NAAS”. DNA release from bristle samples was carried out using *Chelex-100* ion exchange resin according to the method [6]. If necessary, ready samples of matrix DNA from bristles and epithelial tissue were stored in the freezer at a temperature of –20°C. Genotyping of samples of epithelial tissue and bristles was carried out by PCR analysis with its own selection of thermodynamic characteristics of PCR taking into account the optimal concentration of amplified samples and the length of 2% of the agarose gel to confirm the results of 428 base pairs (table 1, 2).

Aliquot of PCR product (4 µL) was hydrolyzed with *TasI* endonuclease (*Thermo Scientific™*). DNA hydrolysis products were analyzed in 8% polyacrylamide gel in electrophoresis buffer 1×TBE. Enzymatic digestion was performed in a final volume of 10 µL, including 5 µL (~0.1–0.5 µg of DNA) of the PCR product, 0.1 µL of *Tas I* endonuclease (*Thermo Fisher Scientific™*) and 2 µL Buffer 10X, together with nuclease-free water 2.9 to reach final volume 9.9 µL. For electrophoresis in 8% PAAG for accurate separation of fragments of restricted samples, the optimal time and voltage of the electric field were selected. pBR322 DNA/*Msp I* and pUC19 DNA/*MspI* plasmid DNA was used as a molecular weight marker. Visualization of amplification and restraint products was carried out by staining with ethidium bromide and photographing on a transilluminator in ultraviolet light (*MicroDOC Gel Documentation Digital camera with UV Transilluminator, Cleaver Scientific*).

Table 1. The scheme of collection of components of PCR mixture with the expectation of 44 samples with a capacity of 0.25 cm³ test tubes

Date:	2021	25.11.2020 PCR	12.11– 20.12.2021 PCR
Gene	MTH	TH-01	TH-01
Program	T 63°C	7	37
H ₂ O	11.0/5.0 µl	77.0 µl	185.0 µl
10x(NH ₂) ₂ SO ₄	2.5/1.25 µl	17.5 µl	46.25 µl
2mmdNTP	2.0/1.25 µl	14.0 µl	46.25 µl
25mmMgCl	2.0/1.0 µl	14.0 µl	37.0 µl
Prim.MITPRO2F	1.0/0.25 µl	7.0 µl	9.25 µl
Prim.MITPROR	1.0/0.25 µl	7.0 µl	9.25 µl
Taq.Polymerases	0.5/0.5 µl	3.5 µl	18.5 µl
DNA	8–12 µl	at 20.0 µl	at 7.5 µl

Note. * MTH — abbreviated name of reagents for staging PCR mitochondrial DNA of a pig, *TH-01 — PCR program [Author's development]

Table 2. Structure of oligonucleotide primers for amplification of mitochondrial DNA of pigs in PCR

D-loop of mitochondrial DNA	Oligo-nucleotide	Structure	Size
NC_000845.1	MITPRO2F	CATACAAATATGTGACCCCAA	428 bp.
	MITPROR	GTGAGCATGGGCTGATTAGTC	

To determine the susceptibility of each region of the genome to the acquisition of variants normalized the total number of variants to the size (bp) of the D-loop area, as described in methodical recommendations [13].

Results and Discussion

The maternally inherited mitochondrial genome encodes key proteins of the electron transport chain, that produces to the vast majority of cellular ATP. The mitochondrial genome is necessary for the biochemical process of oxidative phosphorylation which generates most of the cellular energy [11]. OXPHOS is carried out in the electron transport chain and is the only cellular apparatus the subunits of which are encoded by the mitochondrial genome [2]. The pig mitochondrial genome has a size of 16,679 base pairs [17]. The total length of the pig's mitochondrial genome (KX094894.1) number is 16,731 base pairs [9]. The mitochondrial genome encodes 13 of the 90 subunits of the electron transport chain, 22 tRNAs and 2 rRNAs, and has one non-coding area of the D-loop which interacts with nuclear-encoded factors that transcribe and replicate mtDNA [16, 20]. The D-loop also has two hypervariable regions that identify maternal ancestry. Over billions of years, various maternal lineages have evolved, and based on their mtDNA sequences they are grouped together in groups known as mtDNA haplotypes. In order to determine the diversity of domesticated pigs in Ukraine was sequenced variable area of D-loop 44 pigs. Identified 5 haplotypes mtDNA (A, C, G, N, O) and 2 (D, K) — unknown among the breeds of domestic pig. The use of markers of mitochondrial DNA allows you to determine how many maternal lines with specific haplotypes participated in the creation of the breed and the formation of its haplogroup. It is also possible to identify wild subspecies of ancestors that were the basis of domestication and subsequently material for breeding work on improving economic qualities and fixing breed-specific features. For example, in this way, it was confirmed that the pigs of the Yorkshire breed of Canada and the United States are direct descendants of the English Large White breed [1]. The Great White Breed of Pigs (Yorkshire) was bred in the eighteenth century by crossing the local Great White Pig of Northern England with smaller ones, fat Chinese Pigs. The Great White Breed was recognized as a breed in 1868 [15]. The Large White breed of pigs is a popular breed in the commercial and breeding market around the world. The Large White breed is known as the “improved Greater Yorkshire” in Canada and the United States. The Great White Breed of Pigs is one of the first founding breeds of the National Pig Breeders Association. In the UK, Large White breed pigs are leaders in bacon production. In Canadian breeding, it is the Large White Breed that makes up the bulk and is imported to the United States. “improved Greater Yorkshire” and their descendants form the basis and are found in almost all breeding crossbreeding programs and rotation using

two or more breeds in the hybridization system. Sows of the Large White breed form the basis of female pigs F_1 . Modern breeding selection programs are designed taking into account the selection of individual queen lines to obtain purebred offspring, which differ in indicators: growth rate, % lean meat ratio and are included in terminal boar breeding programs (Maxgro). “Improved Greater Yorkshire” is widely used in intensive pig breeding due to its inherent maternal qualities, fertility, acclimatization, and high-performance signs [7]. In Ukraine, Large White pigs were introduced at the end of the XIX century. At the end of the XX — the beginning of the XXI century, the gene pool of pigs in Ukraine consists almost entirely of imported pigs from the advanced breeding centers of Denmark, France, England, the Netherlands, and Ireland.

It is believed that all descendants come from one high-performance ancestral and repeat its mitochondrial haplotype, this helps to establish the ancestor of all descendants in a number of generations [8]. To provide population evaluation, an effective approach is to study the polymorphism of the mitochondrial genome. Various mitochondrial DNA markers are found in the following breeds of pigs: A (fig. 1, 2).

Breed of pigs Mangalica, a representative of haplotype A, was bred during the Austro-Hungarian Empire, after Archduke Joseph Anton Johann received several Šumadian pigs from the Serbian prince and crossed them with the pigs Bakonyi and Szalontai — (Bakonia × Szalontai) × A Šumadija breed of Serbian origin (1833). Then the breeders made a rush of blood to female pigs of the “curly pig” breed Alföldi and the Croatian Šiška and Sirmien. “Curly pig” Mangalica belongs to the European unimproved breeds of pigs of the sebaceous direction of productivity (Iberian Black, Sicilian Black and Alentejan), which originated directly from populations of Wild pigs. Officially, in Europe, the Mangalica breed was recognized in 1927. In 1927, the National Society of Pig Breeders of Sebaceous Productivity was established to improve the “Mangalicatenyésztők Országos Egyesülete” breed. Mangalica “curly pig” was widespread in the Austro-Hungarian Empire in the early XX century. After the Great Patriotic War, their number decreased sharply. Hungary was on the side of the losers in both world wars, and reparations paid in kind also reduced the number of “curly pigs” in the country. After the 1970s, the introduction of new agricultural technologies led to the cessation of Mangalica production. Thus, it was gradually supplanted by other selected fast-growing imported breeds. And the mother nucleus of female pigs Mangalica began to be sharply squeezed out of the commercial market. However, due to valuable characteristics like resilience and excellent adaptability to extreme conditions of detention, maternal qualities, taste properties of meat have been recognized again since 2003.

One of the representatives of haplotype A — Duroc pigs were bred between 1822 and 1877 from pigs “Old Duroc” from New York and Red Jersey pigs “Duroc Jersey” from New Jersey. The Duroc breed has undergone wide exports, mainly to the regions of North and South

America. It is believed to be one of the sources of red or reddish-brown pigs found in the United States were those that came from the Guinean coast of Africa, pigs similar to those found on the coast of Guinea have been found in every country, where did the first ships engaged in the slave trade go. Reddish-brown “Red Berkshire” line pigs were introduced to the United States and are probably one of the founders of the Duroc breed. “Red Berkshire”, the modern Duroc is used in crossbreeding programs as a terminal boar-producer when crossing with sows (♀ Large White × ♂ Duroc), ($1/2$ ♀ Landrace/Large White) × ♂ Duroc. Since, the Large White breed of pigs is a plastic material for breeding development in the intensification of the pig industry, it is advisable to use breeding sows as a maternal base in 3 breed crossed, which ensures the use of two levels of heterosis (♀ Large White × ♂ Landrace) = hybrid sows F₁. (♀ F₁ × ♂ Duroc), (♀ F₁ × ♂ Piétrain) — sows F₁ mate with boars of specialized meat breeds or with boars of synthetic line — Maxter, OptiMus, MaxGrow.

C — Landrace, Hampshire, Wales, wild pig (Ukraine, Poland, France); G — Wales, wild pig (Italy); O — Landrace, wild pig (Sweden, France); N — Large White (Asian type), Berkshire, Asian wild pig (fig. 2, 3).

Representative of haplotype C — Hampshire, according to the American National Pig Registry — it is the fourth “most recorded breed” of pigs in the United States. The Hampshire breed originated from the local breeds of pigs in England, which by habitat were found in northern England and Scotland. Hampshire pigs are an “Aboriginal British” breed, as the original breeding stock in 1832 was imported from Wessex and Great Britain. Mainly the Hampshire breed is valued in the use of a crossover. Since 1890, in the United States, the Hampshire breed has also been called “The Thin Rind”, because, in terms of productivity, Hampshire pigs are characterized by lean meat, a high percentage of meat yield, and less salted. The Hampshire breed has a wide demand in the European market as breeding programs for the production of hybrid pigs in 3–4 generations (Large White × Large White) — (Large White × Landrace) — ($1/2$ Landrace/Large White × Hampshire). In 1975, the Hampshire breed became the breed champion at the English Royal Exhibition. In the world of commercial pig farming, the Hampshire breed is the best terminal breed as a used in breeding productivity programs on important economic indicators of hybrid pork production.

Inherited maternal haplotypes C and O — French Landrace, Danish Landrace, English Landrace, Polish Landrace, and Ukrainian Landrace belong to the breed of pigs from Scandinavia, Denmark, and Sweden. Landrace pigs have been introduced to France since the 1930s. Since then, the French Landrace has been effectively used in breeding. The Landrace is an important parent breed in the European and Ukrainian selection and commercial pig market. Widely used in reciprocal crossbreeding. Landrace pigs belong to any group of standardized breeds of domesticated pigs and hybrid pigs. The name of the breed is due to the fact that the basis of the Danish Landrace breed was made up of specimens of the local

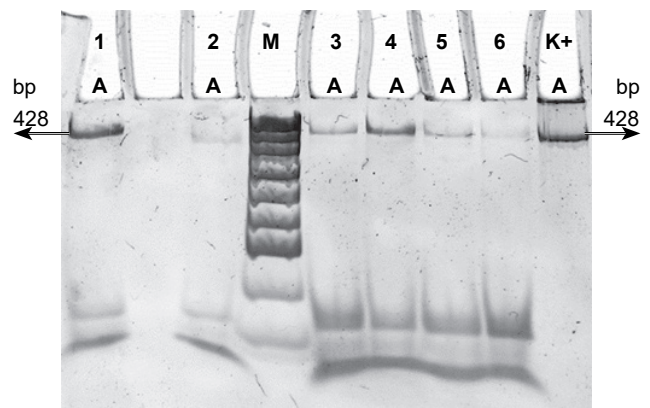


Fig. 1. Results of electrophoretic fractionation in 8% PAAG amplified in PCR and hydrolyzed using endonuclease Tas I mitochondrial DNA of pigs of the final hybrid (LW×L). M — molecular weight marker pUC19 DNA/MspI. [Author’s development]



Fig. 2. Map showing European continents, place of origin of European wild boar, Duroc (United Kingdom) no. 1 and Mangalica (Serbia, Hungary) no. 2 [Author’s development]

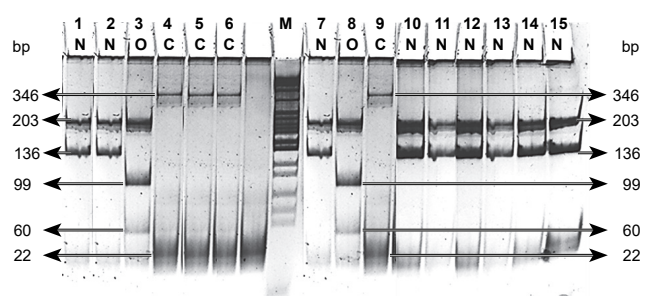


Fig. 3. Results of electrophoretic fractionation in 8% PAAG amplified in PCR and hydrolyzed using endonuclease Tas I mitochondrial DNA of pigs of the final hybrid (LW×L). M — molecular weight marker pBR322 DNA/Msp I. [Author’s development]

breed of pigs and a regional autochthonous breed originally from Denmark. The modern Landrace appeared as a result of crossing pigs of the Large White breed from England with a local pig. Thanks in large part to the use of Landrace, Denmark has become a major bacon exporting country and England became the main market.

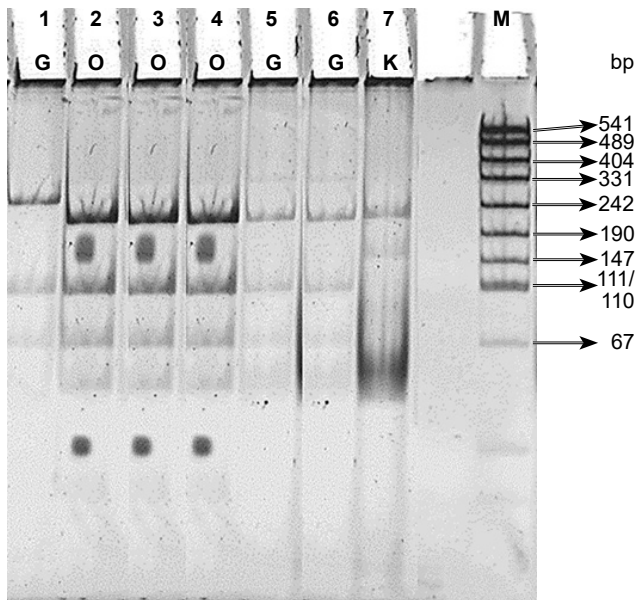


Fig. 4. Results of electrophoretic fractionation in 8% PAAG amplified in PCR and hydrolyzed using endonuclease Tas I mitochondrial DNA of pigs of the final hybrid (LW×L) × Maxgro. M — molecular weight marker pUC19 DNA/Mspl. [Author's development]



Fig. 5. Map showing European continents, place of origin of wild pigs and *S. s. domestica*, as well as the current geographical location of hybrid pigs (Large White × Landrace) × Maxgro. No. 1 C — Landrace, Hampshire, Wales, wild pig (Ukraine, Poland, France); no. 2 G — Wales, wild pig (Italy); no. 3 O — Landrace, wild pig (Sweden, France); no. 4 N — Large White (Asian type), Berkshire, Asian wild pig. [Author's development]

The basis of the American Landrace were those pigs that were bred in pure form or carried a small impurity (one sixteenth to one sixty-fourth) of polish-chinese blood. 38 boars and pigs carrying the blood of Norwegian, Danish and Swedish landraces were imported from Norway. Their blood mixes with U.S. Landraces and gives them a broader genetic base. Polish-Chinese breed of domesticated pigs, first bred in 1816 in the Miami Valley, state Ohio, USA. The basis of the Polish-Chinese pig is made up of Berkshire and Hampshire breeds. The Polish Landrace was based on an improved white German pig meat type productivity. The breed was modernized using the German and Swedish national breed. The Polish Landrace breed was finally identified and confirmed by a directive of the Ministry of Agriculture in 1962.

Since haplogroup — this is a set of related subclades, which are descended from even more ancient common ancestors. The mt-E and mt-A clades we have defined — it is a collection of carriers of the same SNPs, that is, all carriers, in this case, a population of hybrid gilts and breeding sows descended from one common maternal ancestor. All domesticated pigs are grouped into a single clade D with the inclusion of wild pigs from East Asia. Intra-clades A, mtDNA Asian domesticated pigs were grouped into a single clade D, and wild pigs from the East Asia region were interbred with European domesticated breeds of pigs. Therefore, wild pigs from East Asia and European domesticated breeds (Berkshire) belong to the clade E and A.

One of the representatives of the haplotype inherited from the maternal line — C and G, breed Wales, is the most commercially developed of all traditional breeds. Wales pigs are ideal for intensive pork production systems. Pigs wales have been found in the southern and western counties of the Principality. In the 1870s, Welsh and Shropshire pigs were in demand in the commercial trade in Cheshire. Thus, Wales and Shropshire have undergone migration through the commercial market in connection with the improvement of the productive qualities of local and domesticated pigs of Ukraiand and, Poland. In 1922, the Welsh Pig Society played an important role in increasing and spreading the number of brood stock of pigs, development of commercial characteristics of the Wales breed. In the 1950s, the production of commodity-type pigs was introduced into breeding practice — product lines for maternal and paternal. In this regard, the breed Wales was crossed with the breed Landrace (Wales × Landrace), (Landrace × Wales). As a result, in 1955 the improved Welsh pig was identified in “The Howitt Report” as one of the three main breeds (Yorkshire, British Landrace, Wales) on which modern pig breeding is based. However, the unrelenting desire to intensify and strengthen hybrids in commercial herds has led to a significant reduction in the number of breeds, and now it has become a threatened breed. This led to the decline of many other native breeds, which are known for their characteristics and performance in the counties of East Anglia, in midlands, and Yorkshire. These are good pig-breeding areas of England, where most of England's two dominant breeds are locat-

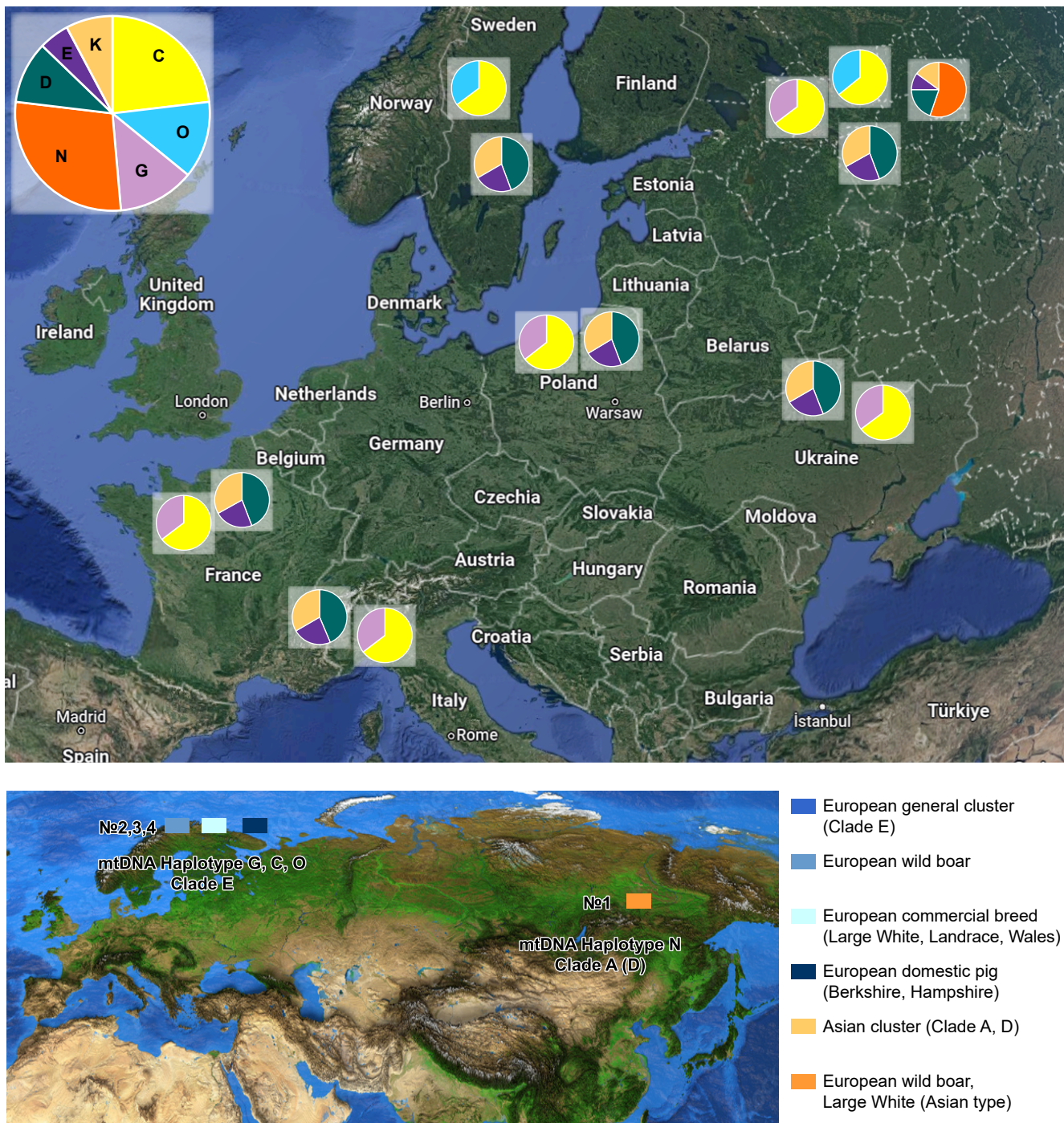


Fig. 6. Clustering of mtDNA haplotypes from the D-loop area of 37 commercially gilts and 7 breeding sows. Map showing Southeast Asia, place of origin of pig (*Sus scrofa*) species, as well as the current geographical distribution of wild and domesticated pigs. We also indicate (geolocation icon) pig domestication centers: no. 1 N — Large White (Asian type), Berkshire, Asian wild pig; no. 2 C — Landrace, Hampshire, Wales, wild pig (Ukraine, Poland, France); no. 3 O — Landrace, wild pig (Sweden, France); no. 4 G — Wales, wild pig (Italy) [Author's development]

ed — Large White and British Landrace. Commercial producers have used Welsh pigs as a White breed in cross-breeding programs (as nuclei on the maternal and paternal lines). Since the Breed Wales does not have a close relationship with the most famous breeds large White and British Landrace. Increased demand for pork and bacon during the First World War, when imports were restricted to Canada and the United States, led to the creation of the first hog breeding society in Wales. Old Glamorgan Pig Society was founded in 1918. The first volume of the stud

book was published in 1919. Pigs of this type were also bred in Cardigan, Pembroke, and Camarten.

The Berkshire Breed in 1790 in the Thames Valley, near Vantigieg. Characterized as a large black, red pig. Lord Barrington was thought to be largely responsible for improving the breed in 1825, when Chinese or East Asian blood was introduced into the breed. In the XIX century, the breed became very popular, enjoying the patronage of the aristocracy, including Queen Victoria. In 1823, the first Berkshire company was exported to the United States.

Table 3. The concentration of mitochondrial haplotypes identified among the sample of pigs studied

Reference sequence
(Accession: AJ002189.1) position

Haplo- types	Group	Population details	Base pairs	%
SE "DG named after January 9 th IPB and AIP NAAS"				
A	European commercial	wild pig, Mangalica, Duroc, Large White	406/22	16
SPE "Globinsky Pig Complex" LLC				
C	European wild / commercial	Wild boar, Landrace, Hampshire, Wales	346/60/22	20.5
G	European wild / commercial	Wild boar, Wales	247/99/60/22	11.4
O	European wild / commercial	Wild boar, Landrace	203/99/60/44/22	11.4
N	Asian wild / commercial and European wild / commercial	Wild boar, Large White, Hampshire	203/133/44/23/22	25
D	undetected among pigs of domestic breeds		346/37/23/22	9
K			203/159/44/22	6.8

[Author's development]

Over the past 17 years of the XIX century, the breed has produced 12 Smithfield champions, including pigs exhibited by members of the Royal Family. In the 1900s, the reduction in the Berkshire pig population led to the almost extinction of the breed in this country. However, with the introduction of new blood from Australia, New Zealand, and the United States, the breed has made great strides in recent years both in quantitative terms and as a carcass. As a hybrid breed, Berkshire is suitable for any breeding program, regardless of whether he is used as a father or mother. When crossing with a white breed, the resulting offspring will be white and inherit natural resources strong physique of Berkshires, and the ability to withstand extreme temperatures, as well as the easy level of feeding characteristic of this breed. Berkshire's herd is also in demand overseas, especially in Japan where the breed is very popular and sold as black pork at a higher price. Japanese shoppers still believe Berkshire from the UK has the

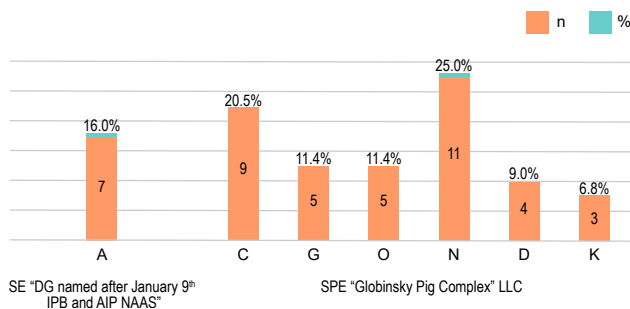


Fig. 7. Concentration of mitochondrial haplotypes in the study population of pigs SE "DG named after January 9th IPB and AIP NAAS" (n=7) and SPE "Globinsky Pig Complex" LLC (n=37) [Author's development]

best taste and aroma. Today, paternal lines are available to breeders (Orlando, Namatjira, Ambassador) and maternal lines (Suzanne, Mermaid, Farewell, Lady, Excelsa) [10].

Identification of alleles C, G, O, and N which have led subspecies of wild pigs to transformation, behavioral and physiological through artificial selection, represents the challenge we face from an interdisciplinary perspective in studying the process of domestication of wild and domesticated pigs. There is a general consensus that the Eurasian wild boar (*S. scrofa*) and other sister species, such as *S. celebensis* (Celebes warty pig), *S. verrucosus* (Java warty pig), *S. sebiferous* (Visayan warty pig), *S. philippensis* (Philippine warty pig) and *S. barbatus* (Bornean bearded pig), emerged in Southeast Asia in the early Pliocene, approximately 5.3–3.5 Myr ago [14]. We found that the Eurasian region of Eastern Europe was one of the key areas of pig domestication. Subspecies of wild pigs from Eurasia have a maternal line with modern domesticated pigs. Asian haplotype N demonstrates maternal genetic continuity dating back to the early Neolithic. Behind the emergence of agricultural societies in the Middle East for at least 12,500 years to date, the resettlement of farmers in the continents of Europe has followed. European domestic pigs dating from 7100 to 6000 years back, were of both Middle Eastern and European descent. Traditional methods of distinguishing between wild and domestic pigs are based on the study of the demographic profile. One possible mechanism explaining the apparent discrepancy between genotype and phenotype is the gene flow of indigenous European wild pigs into an introduced domesticated population. Domestic pigs have probably always interacted and interbred with wild populations, and this process is assumed wherever *S. s. domestica* appears [3].

Phylogenetic reconstruction of mitochondrial genome data from hybrid pigs reflects a distinct geographic division of mtDNA data — Eastern Europe and Asia. In particular, subspecies of European and Asian wild pigs are the pro-parent basis on the maternal line, which preceded the domestication and breeding of hybrid pigs by hybridization. European and Asian haplotypes of wild pigs showed, that wild pigs from regions such as Italy, Poland, France, Scandinavia, and Ukraine have also been or domesticated, or at least originally incorporated into domesticated livestock.

Carriers of the haplotype C, O, G, and N — represent the basis on the maternal line breeding and improving hybrid lines of pigs of the XXI century. It is necessary to take into account the fact that the purebred mother nucleus (Wild pig, Yorkshire, Landrace, Wales, Mangalica, Berkshire, and Hampshire) is a truly pure foundation for use in hybridization schemes, crossing over in the formation and development of modern hybrid lines of pigs.

Among the samples of breeding pigs of Large White breed from SE "DG named after January 9th IPB and AIP NAAS" mitochondrial DNA marker (haplotype) has been identified: A — wild pig of France, Mangalica, Duroc (n=7) 16%. The presence of haplotype A in the studied sample of pigs of the Large White breed indicates the presence

of crossbred pigs on the maternal line in a number of generations. This is due to the duration of breeding and selection from the beginning of the domestication process. The presence of haplotype A in the studied sample of pigs of the Large White breed indicates the presence of local pigs on the maternal line in a number of generations. This is due to the duration of breeding and selection from the beginning of the domestication process.

Among the samples of hybrid pigs (Large White × Landrace), (Landrace × Large White) the following haplotypes are installed: C (n=9) — Landrace, Hampshire, Wales, wild pig (20.5%); G — (n=5) Wales, wild pig (11.4%); O (n=5) — Landrace, wild pig (11.4%); N (n=11) — Large White, Berkshire, Asian wild pig (25%) (table 3, fig. 7).

In previous studies, among pigs of the large white breed of Ukraine, mitochondrial haplotypes were also found, with a frequency of N (5.91%); G (14.1%), C (9.2%), A (0.3%) [13]. Thus, studies have shown that mitochondrial haplotypes C, G, O, and N are defined in the hybrid sample SPE “Globinsky Pig Complex” LLC and breeding pigs of Large White breed SE “DG named after January 9th IPB and AIP NAAS” characteristic of pigs of large white breed of Ukraine. Haplotypes D 9%, K 6.8% (n=7) indicate that among the large white breed of pigs in Ukraine, haplotypes of local pigs have been preserved, which are extremely difficult to establish. These results indicate that different breeding schemes were used for different breeds even for breeds of close geographical origin. In general, the analysis shows that most breeds of pigs from Ukraine mainly have a European origin and contain different fractions of the ancestors of the Large White, Landrace, Duroc, and Hampshire. Nevertheless, it is possible that Asian pigs were directly involved in the creation of or subsequent crossing with local breeds from Ukraine.

Conclusions

1. PCR analysis of polymorphism of the D-loop area of mitochondrial DNA in a sample of breeding pigs of Large White breed in SE “DG named after January 9th IPB and AIP NAAS” 1 mitochondrial haplotype A — wild pig of France, Mangalica, Duroc (16%) was identified. The presence of haplotype A in the studied sample of pigs of the Large White breed indicates the presence of crossbred pigs on the maternal line in a number of generations.

2. Since, the Large White breed of pigs is a plastic material for breeding development in the intensification of the pig industry, it is advisable to use breeding sows from SE “DG named after January 9th IPB and AIP NAAS” as a maternal base in 3 breed crossed, which ensures the use of two levels of heterosis (♀ Large White × ♂ Landrace) = hybrid sows F₁ (♀ F₁ × ♂ Duroc), (♀ F₁ × ♂ Piétrain) — sows F₁ mate with boars of specialized meat breeds or with boars of synthetic line — Maxter, OptiMus, MaxGrow.

3. In a sample of hybrid pigs SPE “Globinsky Pig Complex” LLC identified 4 haplotypes characteristic of

(Large White × Landrace), (Landrace × Large White). Haplotype C — Landrace, Hampshire, Wales, wild pig (Ukraine, Poland) 20.5%; G — Wales, wild pig (Italy) 11.4%; O — Landrace, wild pig (Sweden) 11.4%; N — Large White (Asian type), Berkshire, Asian wild pig 25%.

4. The sample of hybrid pigs also has haplotypes: D 9%, K 6.8% — undetected among pigs of domestic breeds. This is due to the crossbreeding of pigs in a number of generations.

5. Since the mitochondrial genome is usually inherited only through the maternal line, the genetic diversity of mtDNA subspecies of wild and domesticated pigs, are likely to be limited to existing lines. Therefore, one haplotype of mitochondrial genomic indicates a specific breed, this indicates that several breeds have the same haplotype mtDNA — C and O. It is determined that wild pigs from East Asia and European domesticated pig breeds belong to the clade E and A.

6. Asia is the largest source of genetic variation of *S. scrofa* according to its geographical origin. The higher variability of European commercial pigs compared to European wild boars is largely due to the introgression of Asian haplotypes not a mixture of European origin.

7. It was found that in Europe the domestication of pigs represented a continuous process of domestication and hybridization, which led to the development of lines of hybrid pigs observed today.

8. These results confirm that Asian hybridization, which was used to improve the productive qualities of local breeds, left its mark on the genome of the commercial pigs we operate today.

9. Modern breeds themselves are not native breeds, because they are formal breeds, which are maintained by selective breeding rather than natural selection.

Prospects for Further Research

Due to the fact that the process of domestication leads to important modifications of phenotypic variability domesticated populations through artificial selection are compared to wild pig populations. Our task is to continue research to find out, whether domestication focused on the selection of new or permanent mutations, affecting several or many loci in hybrid pig lineage populations.

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Генетичне різноманіття сучасних ліній гібридних свиней на основі варіацій послідовності мітохондріальної ДНК

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Оцінено генетичну специфічність гаплотипів популяції гібридних свинок (велика біла × ландрас), (ландрас × велика біла) від ТОВ НВП «Глобінський свинокомплекс» та племінних свиноматок великої білої породи від ДП «ДГ імені 9 Січня» з використанням поліморфізму довжин рестриктивних фрагментів мтДНК. Метою дослідження було визначити, чи призводить процес створення спеціалізованих материнських ліній в сучасних транскордонних породах до зменшення гаплотипового різноманіття. Як генетичний матеріал використовували щетину від свиноматок породи велика біла (n=7) та епітеліальну тканину свинок (велика біла × ландрас), (ландрас × велика біла) — (n=37). Виділення ДНК зі зразків щетини проводили з використанням іонообмінної смоли «Челекс-100». Для дослідження D-петлі мітохондріального геному гібридних свиней (n=37) з епітеліальної тканини вушної раковини використовували набір ДНК-сорб-В для екстракції нуклеїнових кислот від ТОВ «ІнтерЛабСервіс-Україна». Зразки епітеліальної тканини вух свиней обробляли вогнем від «сухого спирту». Для аналізу мітохондріального геному використовували метод поліморфізму довжин рестриктивних фрагментів, ампліфікованих за допомогою ПЛР. Генотипування зразків ДНК дослідних свиней за мітохондріальними ДНК-маркерами було здійснено із залученням полісайтового способу згідно з методичними рекомендаціями К. Ф. Почерняєва, М. Д. Березовського (2014). Використання маркерів материнського типу усадкування (мтДНК) дозволило визначити дві материнські лінії з певними гаплотипами, котрі брали участь у створенні гібридних свиней та формуванні їхньої гаплогрупи. Генетична різно-

манітність мтДНК підвидів диких і одомашнених свиней обмежена наявними лініями. Тому один гаплотип мітохондріального геному вказує не на конкретну породу, оскільки кілька порід мають однаковий гаплотип мтДНК — А, G, C, N і O. Визначено концентрацію гаплотипів у племінних свиноматок великої білої породи — гаплотип А з частотою (16%). У гібридних свинок (велика біла × ландрас), (ландрас × велика біла) концентрація виявлених гаплотипів становить: С (n=9) — ландрас, гемпшир, вельс, дика свиня (20,5%); G — (n=5) вельс, дика свиня (11,4%); O (n=5) — ландрас, дика свиня (11,4%); N (n=11) — велика біла, беркширська, азійська дика свиня (25%). Визначені гаплотипи D 9%, K 6,8% (n=7) не були знайдені серед порід свині свійської. Ми припускаємо, що свині великої білої породи з гаплотипом А та гібридні свині (велика біла × ландрас), (ландрас × велика біла) з гаплотипом G, O, N, зокрема D, K містять аборигенні генетичні ресурси. Однак у середині ХХ ст. підвиди диких і одомашнених порід свиней стали малопопуляційними через зменшення площі вирощування і посилення тиску з боку іноземних порід з високою швидкістю росту та селекційними ознаками. Це свідчить про те, що дослідження варто зосередити на класифікації та ідентифікації філогенетичного походження свиней і створенні молекулярно-генетичного банку ДНК кнурів-виробників для природоохоронної діяльності. Процес одомашнення чинить сильний селективний тиск на види *Sus scrofa* через такі генетичні процеси, як інбридинг, генетичний дрейф, природний і штучний відбір за бажаними ознаками. За останні 9–10 тис. рр. втручання людини призвело до появи одомашнених видів, які морфологічно, поведінково та генетично відрізняються від своїх предків-співродичів. Ми вважаємо, що «гібридний» підвид диких свиней з деякими морфологічними особливостями одомашненої свині мав вищу частку повногеномних предків домашньої свині порівняно з морфологічно чистими підвидами диких свиней. Тварини з гаплотипами D, K є результатом гібридизації з європейськими свинями. З часом це призвело до майже повного зникнення первинних близькосхідних предків у ядерних геномах європейських одомашнених свиней. Представники гаплотипів А (велика біла, дюрорк європейського типу, мангалицька); G (вельс, дика свиня — Італія); С (ландрас, вельс, гемпшир, дика свиня — Україна, Польща, Франція); O (ландрас, дика свиня — Швеція) згруповані до Європейського кластеру гаплогрупи «mt-E». Свині з гаплотипом N — велика біла (азійський тип), беркшир, дика свиня — належать до Азійського кластеру гаплогрупи «mt-A». Філогенетична реконструкція даних мітохондріального геному від гібридних свиней відображає чіткий географічний поділ даних мтДНК — Східна Європа та Азія. Зокрема, підвиди європейських та азійських диких свиней є пребатьківською основою за материнською лінією, яка передувала одомашненню і виведенню гібридних свиней методом гібридизації. Європейські та азійські гаплотипи диких свиней продемонстрували, що дикі свині з таких регіонів, як Італія, Польща, Франція, Скандинавія та Україна, також були або одомашнені, або принаймні спочатку зараховані до одомашнених свиней. Результати вивчення мітохондріального геному *S. s. domestica* показали внутрішньопородне генетичне різноманіття гібридних свинок. Це пов'язане із селекційною стратегією міжнародних генетичних центрів, де, попри консолідацію генетичної структури всередині центру, забезпечують значне загальне генетичне різноманіття породи. Окрім вищесказаного, отримані результати свідчать про зв'язок частотного розподілення гаплотипів мтДНК з адаптацією до різних кліматичних умов. Загалом представлені результати є стимулом для продовження досліджень з вивчення мітохондріального геному сучасних ліній гібридних свиней. Носії гаплотипу С, O, G, і N є основою за материнською лінією розведення і поліпшення ліній гібридних свиней ХХІ ст. Необхідно враховувати той факт, що найчистіші материнські ядра (дика свиня, великий йоркшир, ландрас) є справді чистим фундаментом для використання в гібридизаційних схемах, кросинговері, в утворенні і розвитку сучасних гібридних ліній свиней. Незважаючи на це, різноманітність мітохондріального геному у популяції транскордонних порід зберігається.

Ключові слова: походження, мтДНК, гаплотип, гаплогрупа, клада, кластер, племінні свиноматки великої білої породи, гібридні свинки (велика біла × ландрас), (ландрас × велика біла), ПЛР-ПДРФ аналіз