

Determination of the genetic structure of pro-maternal pig breeds of Irish selection using mitochondrial DNA markers

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Traditionally, the mitochondrial genome is characterized as a "molecular clock" for tracking the history of phylogeny along the maternal line. Particular attention is paid to the distribution of mitochondrial DNA haplotypes among commercial pigs (Large White × Landrace) × Maxgro from RPE "Globinsky Pig Farm", Globyno town, Poltava region, Ukraine. For the study of the genetic structure of the pigs' hybrid markers of mitochondrial DNA — a maternal type of inheritance was used. DNA markers are a convenient tool for investigating the origin of pro-maternal pig breeds. Application of multiplex analysis PCR-RFLP (Polymerase chain reaction-restriction fragment length polymorphism) when examining the variable area of the D-loop between sites 15558–15917 mitochondrial genome of hybrid pigs made it possible to determine the pro-maternal haplotypes of the experimental sample (n=20). Thus, according to the multisite system developed by Pochernyaev K. F., determination of mitochondrial haplotypes of pigs, which are denoted by Latin letters from A to P allowed to determine the true pro-maternal haplotypes of the experimental sample of pigs (n=20), as evidenced by the presence of the Tas I website in the above-mentioned provisions what actually determine the haplotypes of mitochondrial DNA. According to the results of the study defined haplotypes characterize different breeds, namely 4 animals with haplotype C — Landrace (Ukraine, Poland). 6 pigs have mitochondrial haplotype N — Large White (Asian type) and 7 pigs with mitochondrial haplotype O — Landrace. 1 animal with haplotype G — wild pig and crossborder breed Wales (Italy). 2 representatives of haplotype D — not found among the breeds of domestic pigs. According to the established pro-maternal haplotypes of hybrid pigs, animalscarriers of haplotype O are representatives of Scandinavian female pigs F₁ as used in uterine herds in Sweden and Ireland with the participation of the Maxgro terminal parent line in the hybridization system. Identified mitochondrial haplotypes were found to be breed-specific to hybrid pigs of Irish breeding, this is confirmed by the established polymorphism of the mitochondrial genome which is an objective marker even in complex hybridization schemes. The work was done with the support of the National Academy of Agrarian Sciences of Ukraine 31.01.00.07.F. "Investigate the pleiotropic effect gens that the SNP use in marker-associated pig breeding". DR no. 0121U109838. Following the example of the developed systematization of the combination of restricted fragments by Pochernyaev K. F. in the future, I propose to create a database of reference haplotypes of mitochondrial DNA of pigs' final hybrid. In the future, it will be used in further research to reconstruct the demographic history of commercial pigs of cross-border breeds.

Key words: pigs, final Irish hybrid, (Large White × Landrace) × Maxgro, mitochondrial DNA, haplotype, PCR-RFLP

The maternally inherited mitochondrial genome (mtDNA) is necessary for the biochemical process of oxidative phosphorylation (OXPHOS), which generates most of the cellular energy (ATP) [17]. The pig (Sus scrofa domesticus) mitochondrial genome consists of 16,679 bp in size. The mitochondrial genome looks like

an annular double-stranded molecule. The mitochondrial genome encodes 13 of the 90+ subunits of the electron transfer chain, 22 tRNAs, and 2 rRNA,s and has one non-coding variable region — the D-loop. The variable region of the D-loop of the mitochondrial genome of the pig interacts with nuclear factors that transcribe

and replicate mtDNA [17-19]. Characteristic of the mitochondrial genome is the presence of a hypervariable region — D-loop, which is used in molecular genetics to identify maternal hereditary patterns, the transmission of mitochondrial DNA (mtDNA) in a number of generations, and most importantly the evolutionary history of migration worldwide [9, 12]. Over billions of years, various maternal lines of representatives (Sus scrofa) have experienced evolutionary development based on their mtDNA sequences. Thus, the polymorphism of mtDNA historically determines the specific sequence of the pig genome, it characterizes the mitochondrial genome of an individual organism — haplotype. It is known that the mitochondrial sequences of animals evolve rapidly, and the location of their genes often remains unchanged over long periods of evolutionary time [1]. There are at least six species of the Genus Sus, of which Sus scrofa shows the largest geographic distribution. Scientists it is estimated that around 3.0-3.5 million years ago Sus scrofa emerged from South East Asia and colonized Asia, Europe, and North Africa [4, 11]. Eurasian domestic pigs were subsequently transported to Oceania, Africa, and America explaining the current worldwide distribution of Sus scrofa [10]. Pigs were independently domesticated in Asia and Europe and subsequently selected for traits valuable to humans for thousands of years. The resulting European (English) breeds, thanks to their improved production characteristics, subsequently became the founders of several currently recognized international commercial pig breeds [3]. Pig breeds around the world have well-defined origins, in some cases cross breedings from populations of different origins. In addition, it is likely that human migrants transported domesticated pigs to different geographic locations. As a result of migration, the share of subspecies of wild pigs was the result of taming (domestication process), which led to between-breeding hybridization the result of which are modern cross-border breeds of pigs, who are representatives of the promaternal genetic structure of the subspecies of wild pigs.

Assessing the breeding and genetic structure of modern lines of hybrid pigs is an important necessity in order to preserve the diversity of pigs of local and foreign breeds. As an example, to determine the phylogenetic relationship between Croatian autochthonous breeds of pigs, some Asian and European pigs became possible only in the study of the mitochondrial genome. This indicates that polymorphism of the variable region D-loops sequence is characterized by a high degree of genetic variability in ethno-historical aspects [5].

In a scientist-led study of wild boar phylogeny and phylogeography in six countries of Central and Eastern Europe based on 101 complete mitogenome sequences, 29 new haplotypes were identified. Among the 548 mtDNA control region sequences (D-loop) analyzed, the scientists identified 19 different haplotypes. Mitochondrial genomic analysis has allowed scientists to identify seven phylogenetic clades of the wild boar — in the geographical area from North Africa to the main-

land of Europe, to Dagestan in the North Caucasus. Thus, the clades from Italy and Dagestan are representatives of the old evolutionary branch. The Italian clade is a sister haplogroup to the boar clade from Europe and North Africa [13]. Since the representatives of subspecies (*Sus scrofa*) are wild ancestors (*Sus scrofa domesticus*). Under the influence of the domestication process of wild pigs, modern commercial lines are the result of hybridization [2, 14]. This piqued the interest of our study. Since the wild boar is the coexisting wild ancestor of the domesticated pig, the genetic structure of domesticated pigs of Irish selection is of growing interest in the academic context.

The purpose of the study was to determine the genetic structure experimental sample (n=20) of hybrid pigs (Large White × Landrace) × Maxgro using polymorphism of the lengths of restriction fragments of mitochondrial DNA.

Materials and Methods

For the study were used pigs of the final hybrid (Large White × Landrace) × Maxgro (n=20) from RPE "Globinsky Pig Farm", Globyno town, Poltava region, Ukraine. DNA was isolated from bristle samples using Chelex-100 ion exchange resin [7]. PCR amplification of fragment D-loop located between positions 15558-15758 of the mitochondrial genome, conducted on the amplifier Tertsyk-2 (DNA-Technologies) using oligonucleotide primers: forward — MITPRO2F CATACAAATATGTGACCCCAAA and reverse -MITPROR GTGAGCATGGGCTGATTAGTC, concentrations of 258.2 µmol and 233.6 µmol. Plasmid 1 kb Ladder DNA was used as a molecular weight marker to read electrophoregrams of amplified samples in 2% agarose gel. Alikvot of PCR product (4 µL) was hydrolyzed with *Tas* I endonuclease (*Thermo Scientific*™). DNA amplification and hydrolysis products were analyzed in 8% polyacrylamide gel in an electrophoretic device in the 1xTBE buffer. As a marker of molecular weight, pBR322 DNA/MSPI plasmids was used. Visualization of amplification and restriction products was carried out by painting with ethidium bromide and photographing on a transilluminator in ultraviolet light (MicroDOC Gel Documentation digital camera with UV Transilluminator, Cleaver Scientific).

Results and Discussion

To determine the degree of mtDNA genetic diversity among an experimental sample of hybrid pigs (n=20), the variable region of the mitochondrial genome D-loop was investigated by PCR. Five different mitochondrial haplotypes have been identified. Identified haplotypes of the studied sample of pigs (Large White × Landrace) (n=20) indicate that each sow (mother) is a descendant

of one of the five common ancestors. Based on the results obtained experimental samples of the studied pigs, five mtDNA haplotypes are representative of the commercial lines and cover different breeds of pigs. European pig breeds consist of pigs with Asian and non-Asian mitochondria, some of which are descended from closely related maternal ancestors [6, 17].

Thus, analyzed the site of the D-loop of the mitochondrial genome of a pig measuring 428 bp (with *Tas* I recognition sites in positions 15558, 15580, 15616, 15714, 15758 bp). Thanks to the multi-site system for determining the mitochondrial haplotypes of pigs developed by K. F. Pochernyaev (table) [8, 15, 17], 5 mitochondrial haplotypes were identified.

Table. The scheme of characteristics of mitochondrial haplotypes of a pig, defined by single-nucleotide polymorphisms for the use of endonuclease *Tas* I

Nº no.	Haplo- type	Polymorphic positions of the fragment D-loops of the pigs' mitochondrial genome (endonuclease <i>Tas</i> I (AATT)					The size of DNA restriction fragments, bp					
		15558	15580	15616	15714	15758	The Gize of Bry treeshed in lagine he, pp					
1	Α	Т	С	С	С	С	406	22				
2	B1	Т	Т	С	С	С	383	23	22			
3	B2	Α	Т	С	С	С	383	45				
4	С	Т	С	Т	С	С	346	60	22			
5	D	Т					346	37	23	22		
6	Е	Т	С	С	T	С	247	159	22			
7	F						247	136	23	22		
8	G	Т	С	Т	T	С	247	99	60	22		
9	Н	Т					247	99	37	23	22	
10	1	Т	С	С	С	Т	203	203	22			
11	J1	Α	Т	С	С	Т	203	180	23	22		
12	J2	Т	Т	С	С	Т	203	180	45			
13	K						203	159	44	22		
14	L	Т	С	Т	С	Т	203	143	60	22		
15	М	Т	Т	Т	С	Т	203	143	37	23	22	
16	N	Т	Т	С	Т	Т	203	136	44	23	22	
17	0	Т	С	Т	Т	Т	203	99	60	44	22	
18	Р	Т	Т	Т	Т	Т	203	99	44	37	23	22

Representatives of the haplotype (N) of a large white breed were grouped with Asian pigs which indicates that those pro-maternal Asian pigs were involved in the development of the hybrid young pigs we studied. European breeds of pigs consist of pigs with mitochondria of Asian and non-Asian type, some of which were formed from closely related maternal ancestors, which are descended from the wild descendants of pigs inhabiting Asia and Europa, grouped with Asian pigs, demonstrating the Asian origin of their mitochondria. Landrace, Hampshire and Wales grouped with subspecies of wild pig, which indicates that subspecies of wild pigs participated in the development of these breeds, according to the established haplotype (C) inhabiting Ukraine and Poland.

Representatives of haplotype G-inhabiting Italy are wild pigs and pigs of the transboundary breed Wales.

As you know, the ancestor of the pig breed Wales is the Large White Breed. Wales pigs or also called "Italian Landrace" were bred in England and in Denmark at the end of the nineteenth century as a result of crossing Landrace boars with Wales pigs, the livestock of which was brought to Italy after World War II, this is confirmed by representatives of haplotype (G) — (n=1) (fig.).

This is the second-largest breed of pigs in Italy after the Large white. Pigs Wales are the result of a cross-border commercial breed, because it is widely used in complex interbreeding crossbreeding programs. "Italian Landraces" effectively improved pig productivity in Italy, as they interbred with breeds of Scandinavian roots and native (local) pigs. Representatives of haplotype (C) and (G) are demanding commercial lines in modern breeding — bacon direction of productivity.

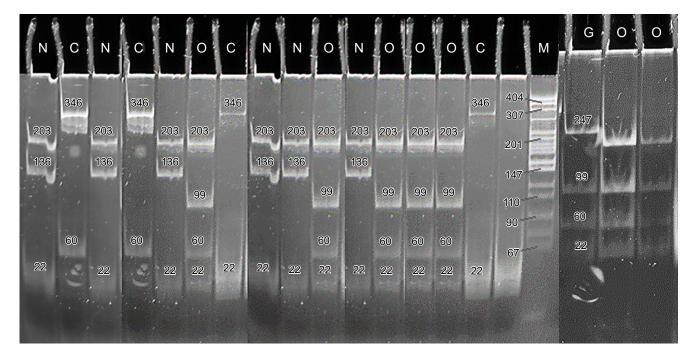


Fig. Results of electrophoretic fractionation in 8% PAAG amplified in PCR and hydrolyzed using endonuclease *Tas* I mitochondrial DNA of pigs of the final hybrid (LW× L) × Maxgro: M — molecular weight marker pBR322 DNA/Msp I.

Landrace, a subspecies of Wild pig with haplotype (O) inhabiting Scandinavian. The Sweden Landrace is popular as a breeding and commercial herd core. Landrace is one of the transboundary breeds worldwide, in demand for export. The export of genetic material is accordingly explained by the migration of this breed, especially to England, Ireland and Northern Ireland. The popularity of the genetic bank of Irish selection is explained by the fact that most of the pigs F_1 (Scandinavian Landrace × Yorkshire) produced in nucleus herds in Sweden, and (Scandinavian Landrace × Maxgro) in the nucleus herds of Ireland.

Because the mitochondrial genome is usually inherited only through the maternal line, genetic diversity at the mtDNA representatives of subspecies of wild and domesticated pigs' level is likely to be limited by existing lines. Therefore, one haplotype of the mitochondrial genome is unlikely to indicate a specific breed, it is likely that several breeds have the same mtDNA haplotype.

The studied pig population allows us to observe the influence of modern breeding features of agriculture on the diversity of the specific mitochondrial genome, and what is the evolutionary result of breed formation. This is important for understanding how modern human society was shaped by agricultural practice. Based on mtDNA sequences, it follows that European wild pigs were hybridized with domesticated Middle Eastern pigs. Moreover, in the early stages of domestication, offspring with the same mtDNA haplotype may have possessed both wild and more domesticated traits, depending on their chromosomal genes [17]. Two animals with mitochondrial haplotype (D) (not found among the breeds of domestic pigs) are also an example of this.

As a hypothesis, it can be assumed that many haplotypes of the mitochondrial genome of subspecies of wild pigs were inadvertently eliminated in the process of domestication. Modern hybrid pigs are the result of hybridization in generations and the evolutionary development of the domestication process. Thus, mtDNA haplotypes are an invaluable source for studying the history of ethnic origin and development along the maternal line, monitoring of selection and genetic selection of descendants of pigs that are carriers of a valuable branch of the pro-maternal basis.

Conclusion

- 1. Through the use of mitochondrial DNA markers in the study of the variable site of the D-loop of hybrid pigs, five different pro-motherly haplotypes were identified: 4 animals with haplotype C Landrace (Ukraine, Poland). 6 animals with haplotype N Large White (Asian type) and 7 with haplotype O Landrace. 1 animal with haplotype G wild pig and cross-border breed Wales (Italy). 2 representatives of haplotype D not found among the breeds of domestic pigs.
- 2. It was found that haplotype O is also a haplotype of Scandinavian pigs F_1 , because it is used in the nucleus herds of Sweden and Ireland with the participation of the terminal paternal line of Maxgro in the hybridization system.
- 3. This study highlights the potential contribution of genetic variation in pro-maternal basis pigs to the genetic diversity of modern domesticated commercial pigs. A modern commercial line that resulted from (Large White × Landrace) × Maxgro as a hybrid young.

Prospects for Further Research

Maxgro Pigs (\circlearrowleft) × [Landrace (\circlearrowleft) × Large White (\hookrightarrow)], Maxgro (\circlearrowleft) × [Large White (\circlearrowleft) × Landrace (\hookrightarrow)] is a popular hybrid pig that makes the most of heterosis in the world. Continue research on the determination of mitochondrial haplotypes of X-maternal and Y-paternal basis in order to determine the associations of haplotypes with signs of fattening productivity of hybrid pigs.

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Визначення генетичної структури проматеринських порід свиней ірландської селекції з використанням мітохондріальних ДНК-маркерів

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Традиційно мітохондріальний геном характеризується як «молекулярний годинник» для відслідковування історії філогенії за материнською лінією. Особливу увагу приділяють розподіленню гаплотипів мітохондріальної ДНК серед комерційних свиней (велика біла × ландрас) × Maxgro від ТОВ НВП «Глобинський свинокомлекс», Полтавська обл., Україна. Для вивчення генетичної

структури фінального гібрида свиней використовують маркери мітохондріальної ДНК — материнського типу успадкування. ДНК-маркери є зручним інструментом для дослідження походження проматеринських порід свиней. Застосування мультиплексного аналізу ПЛР-ПДРФ (Polymerase chain reaction-restriction fragment length polymorphism — PCR-RFLP) у дослідженні варіабельної ділянки D-петлі між сайтами 15558–15917 мітохондріального геному гібридних свиней дозволило визначити проматеринські гаплотипи експериментальної вибірки (n=20). Згідно з розробленою Почерняєвим К. Ф. багатосайтовою системою, визначення мітохондріальних гаплотипів свиней, які позначаються латинськими літерами від А до Р, дозволило визначити істинні проматеринські гаплотипи експериментальної вибірки свиней (n=20), про що свідчить наявність сайту *Tas* I у вищезазначених положеннях. Власне, це і визначає гаплотипи мітохондріальної ДНК. Згідно з результатами проведеного дослідження, визначені гаплотипи характеризують різні породи, а саме 4 тварини з гаплотипом С — ландрас (Україна, Польща). 6 свиней мають мітохондріальний гаплотип N — велика біла (азійський тип), 7 свиней з мітохондріальним гаплотипом О — ландрас. 1 тварина з гаплотипом G — дика свиня і транскордонна порода Уельс (Італія). 2 представники гаплотипа D не знайдені серед тварин свійських порід. Згідно зі встановленими гаплотипами гібридних свиней, успадкованих за материнською лінією, тварини носії гаплотипу О є представниками скандинавських самок свиней F₁, оскільки використовуються у маточних стадах Швеції та Ірландії за участі термінальної батьківської лінії Махого в системі гібридизації. Визначені мітохондріальні гаплотипи виявились породоспецифічними для гібридних свиней ірландської селекції. Підтвердженням цього є встановлений поліморфізм мітохондріального геному, котрий є об'єктивним маркером навіть за складних схем гібридизації. Роботу виконано за підтримки Національної академії аграрних наук України 31.01.00.07.Ф «Дослідити плейотропний ефект генів, SNP яких використовують в маркер-асоційованій селекції свиней». ДР №0121U109838. За прикладом розробленої систематизації комбінування рестриктних фрагментів Почерняєвим К. Ф. у майбутньому пропонуємо створити базу референтних гаплотипів мітохондріального геному фінальних гібридів свиней. У перспективі це буде використано у подальших дослідженнях для реконструкції демографічної історії комерційних свиней транскордонних порід.

Ключові слова: свині, фінальний ірландський гібрид, (велика біла × ландрас) × Махдго, мітохондріальна ДНК, гаплотип, ПЛР-ПДРФ