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GENETIC STRUCTURE OF UKRAINIAN LARGE WHITE PIGS, ESTIMATED USING MITOCHONDRIAL DNA-MARKERS

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Aim. To study the genetic structure of Ukrainian Large White pigs using mitochondrial DNA-markers. **Methods.** Multiplex PCR-AFLP analysis, remarkable for the investigation of D-loop fragment between positions 15531 and 15959 of the pig mitochondrial genome with one monomorphic 15558W and five polymorphic sites *TasI* 15580T > C, 15616T > C, 15714T > C, 15758T > C, 15916A > T, was used. The presence or absence of site *TasI* in the abovementioned positions determines mitochondrial DNA haplotypes, indicated with Latin letters from *A* to *P*. **Results.** Seven mitochondrial DNA haplotypes of different frequency were revealed among Ukrainian Large White pigs. The highest frequency was noted for haplotypes *G* (0.141), *JI* (0.133) and *N* (0.591), previously described for wild boars of Belgium, France, Italy (*G*); wild boars of Japan and China, Meishan and Large White breeds (*JI*) and wild boars of Japan and China, Berkshire and Large White breeds (*N*). The frequency for haplotype *L* (Belgian wild boar and Large White) is 0.022; haplotype *A* (French wild boar, Mangalica, Duroc) – 0.003; haplotype *C* (wild boar of France, Belgium, Poland, Israel and Landrace, Welsh breeds) – 0.092. Haplotype *BI* (0.019) was revealed in Myrgorod breed, Poltava Meat and Red White Belted breeds, bred on its basis. **Conclusions.** The haplogroup of mitochondrial DNA of Ukrainian Large White pigs includes seven haplotypes of mitochondrial DNA. Pigs with haplotypes *G*, *JI*, *N* and *L* may be referred to representatives of Ukrainian Large White pigs, which did not have introgression of maternal line of other breeds.

Keywords: Large White pigs, haplotype, haplogroup, PCR, AFLP, mitochondrial DNA.

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INTRODUCTION

At present the condition of animal genetic resources in the sphere of food production and agriculture is the subject of monitoring of relevant institutions, including the Food and Agriculture Organization (FAO) of the United Nations Organization. Since 2000 the breeds with specified molecular and genetic characteristics have been added into the Global Databank of Farm Animal Genetic Resources. It may be explained by the fact that sometimes it is rather hard to have a clear distinction between breeds, as some of them have similar features, especially useful for economy [1]. Due to this the determination of specific characteristics of the breed requires the study of its genetic structure. At present this study involves a wide application of DNA markers known for different types of inheritance. First

of all, these are microsatellite and single nucleotide polymorphisms (SNPs), localized on autosomes and inherited from both parents. Secondly, these are markers, localized on the part of Y-chromosome, which do not recombine with X-chromosome and therefore are inherited only in paternal line. Finally, these are mitochondrial DNA markers, inherited in maternal line. Comprehensive information, obtained using all types of DNA-markers, will allow obtaining the integral picture of breed genetic structure.

The prevailing majority of transboundary breeds of pigs, including Large White, have already been analyzed for their molecular and genetic characteristics including the application of amplified fragment length polymorphism (AFLP) [2], microsatellites [3] and mitochondrial DNA polymorphism [4].

There are no complete molecular and genetic characteristics for Ukrainian Large White pigs. Therefore, the aim of the work was to estimate the genetic structure of this breed using mitochondrial DNA markers.

MATERIALS AND METHODS

The samples of blood, sperm or hair of 369 Ukrainian Large White boars and breeding sows, bred at breeding farms of Ukraine, were used in the study.

DNA was isolated using ion-exchange resin, Chelex 100 [5]. The D-loop fragment between positions 15531 and 15959 of the pig mitochondrial genome with one monomorphic 15558W and five polymorphic sites *TasI* 15580T > C, 15616T > C, 15714T > C, 15758T > C, 15916A > T, was analyzed, and the presence or absence of site *TasI* in the abovementioned positions was studied to determine mitochondrial DNA haplotypes, indicated with Latin letters from *A* to *P*. The oligonucleotide primers MITPRO2F: catacaaatattgtgaccccaa and MITPROR: gtgagcatgggctgattagtc were designed from the sequence AJ002189 [6] and synthesized in Metabion GmbH (Germany). The set of reagents (TAPOTILI, RF), was used to amplify D-loop fragment of the pig mitochondrial genome. The amplification was conducted using the Biometra TRIO Thermocycler (Biometra GmbH, Germany) following the program: initial denaturation at 94 °C (5 min), 30 amplification cycles, each including denaturation (95 °C, 20 s), annealing (63 °C, 30 s) and elongation (72 °C, 30 s). The final synthesis was performed at 72 °C for 3 min. The PCR results were found to be positive, if the amplicon of 426 b.p. was obtained.

The aliquot of the PCR product (10 µl) was hydrolyzed using endonuclease *TasI* (Thermo Scientific™, Lithuania). When the restriction of amplicons using endonuclease *TasI* was completed, the fragments obtained were separated by electrophoresis in 8 % polyacrylamide gel in 1 × TBE buffer. *pUC19/MspI* (*HpaII*) (Thermo Scientific™) was used as a molecular weight marker. The products of amplification and restriction were visualized by staining with ethidium bromide and photographing at the transilluminator in ultraviolet light.

RESULTS AND DISCUSSION

Seven mitochondrial DNA haplotypes of different frequency were revealed among Ukrainian Large White pigs. Haplotypes *G* (0.141), *JI* (0.133) and *N* (0.591) were the most frequent; considerably less frequent – haplotypes *A* (0.003), *BI* (0.019), *C* (0.092) and *L* (0.022) (Figure).

Mitochondrial DNA haplotypes have their specificities of inheritance, as the introgression of mitochondrial genomes in the population of domestic animals occurs only due to inclusion into female population (maternal type of inheritance). Taking into consideration the fact that mitochondrial DNA haplotypes are known for many subspecies of wild boars and breeds of domestic pigs, it is possible to determine their impact on the formation of haplogroup of breeding sows of a specific breed.

The method of multiplex PCR-AFLP, used in this work, allows determining only six SNP (Table) which is less than the number of nucleotide substitutes, found on the D-loop site. For instance, this was approximately the site, where Fang *et al.* found 28 SNP [7], Alves *et al.* – 34 [8] and Giuffra *et al.* – 27 [9].

The nomenclature of haplotypes, suggested in this work, is commensurate with the one, suggested by Fernández *et al.** [8], Fang *et al.*** [7] and Marinics *et al.**** [10]. Haplotype *A* matches haplotypes (EH12, EH31)**, (H01–H07, H25)***, haplotype *C* – (H2–H10, H13, H14, H21, H25, H26)*, (EH7, EH25, EH28)**, (H08, H11–H15, H24, H26)***, *G* – (H1, H15, H16, H18, H27, H29)*, (EH29, H09)***, *JI* – H24*, (AH7, AH19, AH31, AH33, AH34)**, (H18, H20)***, *L* – (H11, H12, H28)*, (EH15, EH30)**, H174P***, *N* – (AH4, AH30)**, (H21, H22, H23)***. Haplotype *BI* was not found in subspecies of wild boar and breeds of domestic pigs.

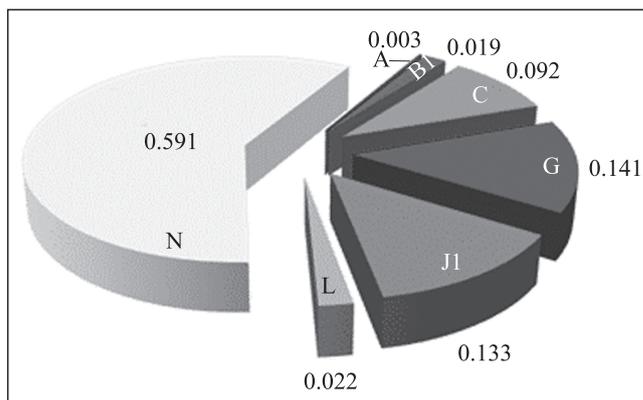
Therefore, haplotypes, revealed among the Ukrainian Large White pigs, and the previously described ones are in good agreement. For instance, haplotype *G* was previously found in wild boars of Belgium, France, Italy, haplotype *JI* – in wild boars of Japan, China, Meishan and Large White, *N* – in wild boars of Japan, China, Berkshire and Large White, haplotype *L* was previously found in wild boars of Belgium and Large White. The haplotypes *A* (0.003), previously described for French wild boar, Mandalica, Duroc, and *C* (0.092) – for wild boars of France, Belgium, Poland, Israel and Landrace, Welsh breeds, had insignificant frequency. Haplotype *BI* was revealed in Myrgorod breed and Poltava Meat and Red White Belted breeds, bred on its basis [11].

There may be several explanations for the presence of haplotypes, not remarkable for purebred Large White, in the haplogroup of Ukrainian Large White pigs. First of all, it is a long period of breeding Ukrainian Large White pigs.

GENETIC STRUCTURE OF UKRAINIAN LARGE WHITE PIGS, ESTIMATED USING MITOCHONDRIAL

The export of pigs of this breed started in the territory of modern Ukraine at the end of the XIX century. At the beginning of the XX century their number increased due to agricultural exhibitions. At that time the breeding of Large White pigs, imported from Shchepkin's farm, was conducted in Maynivka agricultural school (Chernihiv region). In 1910–1912 the pig breeding departments of Poltava and Nosovska agricultural research stations started their work in creating pedigree herds of Large White. During the World War I, followed by the civil war, the Large White pig population was destroyed almost completely. All the pedigree animals were gathered in Poltava and Nosovska agricultural research stations and amounted to 11 boars and 66 breeding sows. In 1927 the specialists of the Poltava agricultural research station created the first State Herd-book of Large White pigs. During 1928–1930 194 boars and 871 breeding sows were registered therein. Since 1930 the network of breeding state-owned farms and breeding collective farms was established in Ukraine. The breeding state-owned farms were mainly supplied the animals from breeding farms of the Moscow region, while the breeding collective farms received their pigs from the Poltava agricultural research station. In 1933 the Poltava and Starokonstantyniv (Khmelnysk region) state breeding centers of Large White were organized.

During World War II almost all the animals were eliminated, except for the best animals of the Poltava agricultural research station and the breeding farm of Maynivka state-owned farm-Dedovo technical school. After the war these animals were brought back to Ukraine. In 1945–1955 the breeding farm of the Maynivka state-owned farm-technical school became the leading breeding center.



The mitochondrial DNA haplogroup of Ukrainian Large White pigs and haplotype frequencies, comprising it

By 1970 Large White pigs were bred in Ukraine in 11 breeding centers, 9 breeding farms and 48 breeding collective farms. The number of pedigree Large White boars was 98.3 % of the total number of boars of this breed, and that of breeding sows was 38.9 % [12]. Therefore, it is possible that haplotypes of meat pigs *A* and *B1* were preserved among Ukrainian Large White pigs.

Large White breed had a considerable impact on the process of breed formation in Ukraine. These pigs were used to breed Ukrainian White Steppe (1926–1934), Myrgorod (1921–1940), Ukrainian Spotted Steppe (1938–1961), Poltava Meat (1963–1993), Ukrainian Meat (1981–1993) and Red White Belted breeds of meat pigs (1976–2006).

At present Large White breed has a dominating position in Ukraine, amounting to almost 70 % in comparison to other breeds. At this stage of industry de-

The polymorphism positions of D-loop fragment of the pig mitochondrial genome and sizes of restrictive fragments of endonuclease *TasI*

Haplotype	Polymorphism positions of <i>TasI</i>							Size of restrictive fragments, b.p.
	15553 15556	15558 W	15580 T > C	15616 T > C	15714 T > C	15758 T > C	15916 A > T	
A	AATT	W	C	C	C	C	A	404/24
B1	AATT	W	T	C	C	C	A	381/23/24
C	AATT	W	C	T	C	C	A	344/60/24
G	AATT	W	C	T	T	C	A	245/99/60/24
J1	AATT	W	T	C	C	T	A	201/180/47
L	AATT	W	C	T	C	T	A	201/143/60/24
N	AATT	W	T	C	T	T	A	201/136/44/23/24

velopment the pedigree foundation is presented by 74 breeding centers and 60 pedigree sow farms. The genealogical structure of the breed includes intrabreed types, farm lines and families (maternal lines). Three main selection directions have been established for the breed: 1) aimed at the creation of ULW-1 intermediate maternal type Ukrainian Large White 1 with high maternal and growing-fattening features; 2) aimed at the creation of intermediate paternal type ULW-2 with improved meat qualities, and 3) complex selection ULW-3.

Due to extensive import of Large White from abroad for the last 30 years, the number of lines of Ukrainian selection reduced to 10 % compared to the newly brought ones. The main countries, from which Large White pigs are imported, are Great Britain, Ireland, France and Hungary.

Some countries have developed their domestic Large White breeds, for instance, Hungary has Hungarian Large White with the haplotype C (according to the nomenclature of Marincs *et al.*, haplotypes H08, H11–H15, H24, H26) [10]. In Ukraine these breeds are registered as pedigree animals of Large White. This may be another reason of the presence of the haplotype, not remarkable for Large White breed.

CONCLUSIONS

The mitochondrial DNA haplogroup of Ukrainian Large White pigs includes seven haplotypes. Three mitochondrial DNA haplotypes – *A*, *BI*, *C* – in the haplogroup of Ukrainian Large White pigs are not remarkable for pedigree British Large White. Pigs with haplotypes *G*, *JI*, *N* and *L* may be referred to representatives of maternal lines, which did not have introgression of mitochondrial genomes of other breeds.

SUPPORT

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Генетична структура свиней великої білої породи України, оцінена за допомогою мітохондріальних ДНК-маркерів

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Meta. Дослідити генетичну структуру свиней великої білої породи України на основі поліморфізму довжини

рестриктних фрагментів (ПДРФ) мітохондріальної ДНК. **Методи.** Використано метод багатосайтового ПЛР-ПДРФ-аналізу, особливість якого полягала у вивченні фрагмента D-петлі між позиціями 15531 і 15959 мітохондріального геному свині, де розташовані один мономорфний 15558W і п'ять поліморфних сайтів *TasI* 15580T > C, 15616T > C, 15714T > C, 15758T > C, 15916A > T. Присутність або відсутність сайту *TasI* у зазначених вище позиціях визначає мітохондріальні гаплотипи, позначені латинськими літерами від *A* до *P*. **Результати.** З-поміж свиней великої білої породи України виявлено сім мітохондріальних гаплотипів, які зустрічаються з різною частотою. З найбільшою частотою зустрічались гаплотипи *G* (0,141), *JI* (0,133) і *N* (0,591), раніше описані у дикої свині Бельгії, Франції, Італії (*G*); дикої свині Японії, Китаю, порід мейшан і великої білої (*JI*) і дикої свині Японії, Китаю, порід беркшир і великої білої (*N*). Частота зустрічальності гаплотипу *L* (дика свиня Бельгії і велика біла порода свиней) становить 0,022; гаплотипу *A* (дика свиня Франції, породи мангалиця, дюрорк) – 0,003; *C* (дика свиня Франції, Бельгії, Польщі, Ізраїлю і породи ландрас, уельс) – 0,092. Гаплотип *BI* (0,019) знайдено у миргородської породи і створених на її основі полтавської м'ясної і червоної білопоясої порід м'ясних свиней. **Висновки.** Гаплогрупа мітохондріальної ДНК свиней великої білої породи України включає сім гаплотипів мітохондріальної ДНК. До тварин великої білої породи України, у яких не відбулося інтрогресії по материнській лінії інших порід, можна віднести свиней з гаплотипами *G*, *JI*, *N* і *L*.

Ключові слова: велика біла порода свиней, гаплотип, гаплогрупа, ПЛР, ПДРФ, мітохондріальна ДНК.

Генетическая структура свиней крупной белой породы Украины, оцененная с помощью митохондриальных ДНК-маркеров

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Цель. Изучить генетическую структуру крупной белой породы свиней Украины на основе полиморфизма длинных рестриктных фрагментов (ПДРФ) митохондриальной ДНК. **Методы.** Использован метод многосайтового ПЦР-ПДРФ-анализа, особенность которого состояла в изучении фрагмента D-петли между позициями 15531 и 15959 митохондриального генома свиньи, где расположены один мономорфный 15558W и пять полиморфных сайтов *TasI* 15580T > C, 15616T > C, 15714T > C, 15758T > C, 15916A > T. Наличие или отсутствие сайта *TasI* в указанных выше позициях определяет митохондриальные гаплотипы, обозначенные латинскими

буквами от *A* до *P*. **Результаты.** Среди свиней крупной белой породы Украины обнаружены семь митохондриальных гаплотипов, встречающихся с различной частотой. С наибольшей частотой встречались гаплотипы *G* (0,141), *JI* (0,133) и *N* (0,591), ранее описанные у дикого кабана Бельгии, Франции, Италии (*G*); дикая свинья Японии, Китая, пород мейшан и крупной белой (*JI*) и дикого кабана Японии, Китая, пород беркшир и крупной белой (*N*). Частота встречаемости гаплотипа *L* (дикий кабан Бельгии и крупная белая порода свиней) составляет 0,022; гаплотипа *A* (дикий кабан Франции, породы мангалица, дюрок) – 0,003; *C* (дикий кабан Франции, Бельгии, Польша, Израиль и породы ландрас, уэльс) – 0,092. Гаплотип *B1* (0,019) обнаружен у миргородской породы и созданных на ее основе полтавской мясной и красной белопопоясой пород мясных свиней. **Выводы.** Гаплогруппа митохондриальной ДНК крупной белой породы свиней Украины включает семь гаплотипов митохондриальной ДНК. К животным крупной белой породы Украины, в которых не произошла интрогрессия по материнской линии других пород, можно отнести свиней с гаплотипами *G*, *JI*, *N* и *L*.

Ключевые слова: крупная белая порода свиней, гаплотип, гаплогруппа, ПЦР, ПДРФ, митохондриальная ДНК.

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