POLYMORPHISM OF GENES ASSOCIATED WITH THE QUANTITATIVE TRAIT LOCI IN WILD BOAR (*Sus scrofa* L., 1758) IN RUSSIA

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Summary

The genetic studies of wild boar (*Sus scrofa* L., 1758) ($n = 89$) inhabited in Russia using ten DNA markers *RYR1*, *ESR*, *FSHB*, *NCOA1*, *BF*, *MUC4*, *IGF2*, *MC4R*, *POU1F1*, *ECRF18/FUT1* were carried out. The assignment of individuals to different territorial clusters was performed based on similarity coefficient (Q) calculation for $k = 2$. The average Q values in individuals inhabited on European part and in East Siberian was 0.984 ± 0.005 and in Irkutsk region and Khabarovsk Krai was 0.994 ± 0.001 . Five DNA markers (*RYR1*, *ESR*, *MUC4*, *IGF2*, *ECRF18/FUT1*) were monomorphic in both of territorial clusters. The non-significant differences in allele frequencies of *FSHB*, *BF*, *MC4R* and *POU1F1* genes between individuals assigned to the west and east clusters were observed: $p_A = 0.462$ and 0.250 , $p_A = 0.020$ and 0.143 , $p_A = 0.013$ and 0.000 , $p_C = 0.000$ and 0.111 , respectively. The studied territorial groups significantly differed in *NCOA1* allele frequencies: $p_{A1} = 0.938$ in wild boars assigned to the west cluster and $p_{A1} = 0.000$ to the east cluster.

Keywords: wild boar, DNA markers, polymorphism, *RYR1*, *ESR*, *FSHB*, *NCOA1*, *BF*, *MUC4*, *IGF2*, *MC4R*, *POU1F1*, *ECRF18/FUT1*.

Wild boar is a widespread representative of wild mammals. Phenotypic differences of *Sus scrofa* L. allow distinguishing four races of pigs (Western, Eastern, Indian and Indonesian), which, in turn, include 16 (1), or, to some view, 27 subspecies (2, 3).

In Russia, according to A.A. Danilkin (4), there are five native subspecies of wild boar: Central European (*S. s. Scrofa* L., 1758), Caucasian (*S. s. Attila Thorn*, 1912), Siberian (*S. s. Sibiricus Staffe*, 1922), Central Asian (*S. s. nigripes Blanf*, 1875), and Ussuri (*S. s. ussuricus Heude*, 1888). These subspecies inhabit the territories with no clear boundaries owing to natural dispersion of animals, seasonal migrations associated with feed resources, floods, dynamics in height of the snow cover, altitudinal migrations, etc. One of the most important factors that change genetic structure of subspecies is acclimatization. For example, Ussuri subspecies known as the largest wild boar was introduced in European part of Russia, for example, in the hunting reserve "Zavidovo" (Tver oblast) (5, 6). Introduction of wild pigs is plasticized in the present as well.

In recent years, genetic diversity of populations of wild pigs is being actively investigated in many countries including Russia (7-9), and these researches have both scientific and practical value.

The achievements of molecular genetics open up new possibilities for studying animal groups and their genetic differentiation. For wild boar, these methods may reveal the extent of genetic introgression of domestic pig resulting from accidental hybridization. This can be performed using different types of DNA markers: mtDNA (10), microsatellites (11), SNP-markers (12, 13), and DNA markers associated with quantitative trait loci (14). The fact that domestic pigs were among the maternal ancestors of wild boars is proved by the presence of Asian mtDNA haplotype (A) identified in domestic pig breeds with a frequency of 29% (10) owing to historical introgression in XVII-XIX centuries from Chinese pigs (15), while the animals of "pure" European populations have only E1 and E2 haplotypes. Along with it, the introgression of domestic pigs into populations of wild boar can be assessed by the frequency of genes associated with quantitative trait loci (QTL). The frequency of Q allele of *IGF2* gene (mutation G3072A) associated with enhanced growth of muscle tissue and meat quality in commercial pig breeds was found to be equal 86%, in local - 3%, and it was absent in 120 tested European wild boars (14). Y. Kuril et al. (16) characterized polymorphism of genes *RYR1*, *LEP*, *GH*, *MYOG*, *MYF5*, and *GDF8* in boars from the north-east of Poland. In this population, genes *RYR1*, *LEP*, and *MYF5* were monomorphic, while *MYOG*, *GH*, and *GDF8* were polymorphic with frequencies of alleles, respectively, 0,42 (allele *A*) and 0,58 (allele *B*); 0,56 (allele "+") and 0,44 (allele "-"); 0,63 (allele *C*) and 0,37 (allele *T*). In Asian and European populations, in was observed the segregation of different *MC1R* alleles responsible for skin color and difference in color between wild boars and domestic pigs (6), which was used in the study of introgression in wild boars of Greece (7).

The purpose of this research was identification of the polymorphism of genes associated with QTLs in different groups of wild boar native in the territory of Russia.

Technique. The studied samples of muscle tissue were derived from wild pigs in different populations on the territory of Russia ($n = 89$): Arkhangelsk oblast ($n = 3$), Bashkortostan ($n = 3$), Vladimir oblast ($n = 3$), Volgograd oblast ($n = 5$), Vologda oblast ($n = 2$), Ivanovo oblast ($n = 3$), Irkutsk oblast ($n = 2$), Kaliningrad oblast ($n = 3$), Kirov oblast ($n = 8$), Komi Republic ($n = 1$), Krasnodar krai ($n = 1$), Kurgan oblast ($n = 3$), Kursk oblast ($n = 3$), Leningrad oblast ($n = 8$), Mari-El Republic ($n = 3$), Nizhny Novgorod oblast ($n = 4$), Omsk oblast ($n = 3$), Orenburg oblast ($n = 3$), Penza oblast ($n = 3$), Saratov oblast ($n = 1$), Sverdlovsk oblast ($n = 3$), Smolensk oblast ($n = 3$), Tambov oblast ($n = 4$), the Republic of Tatarstan ($n = 1$), Tver oblast ($n = 3$), Udmurt Republic ($n = 2$), Khabarovsk krai ($n = 7$), and the Republic of Chuvashia ($n = 1$).

DNA was isolated using Nexttec columns and DNA extraction kit DIAtom™ DNA Prep100 ("Biokom", Russia) following the manufacturers' recommendations.

Genetic clustering of the animals was performed based on the analysis of 12 microsatellites (17) with subsequent cluster

analysis according to J.K. Pritchard et al. (18). The data were statistically processed in Structure software (v. 2.3.1) without the introduction of prior data about individual relations to particular groups for the number of populations (K-clusters), equal to 2.

The used DNA markers of QTL: ryanodine receptor gene (*RYR1*), estrogen receptor (*ESR*), β -subunit of follicle stimulating hormone (*FSHB*), coactivator of steroid hormones (*NCOA1*), properdin (*BF*), mucin 4 (*MUS4*), insulin-like growth factor 2 (*IGF2*), melanocortin receptor 4 (*MC4R*), pituitary transcriptional factor (*POUIF1*), receptor *Escherichia coli* (*ECRF18/FUT1*). Polymorphism of these genes was determined using the techniques developed in the Center for Biotechnology and Molecular Diagnostics (All-Russia Research and Development Institute for Livestock Husbandry).

The obtained information about alleles of each animal was summarized in Microsoft Excel spreadsheet. The resulting matrix of genotypes was statistically processed by standard methods (19, 20).

Results. The analysis of genetic structure of the studied total sample group of wild boars for $k = 2$ (Fig. 1) revealed a marked genetic differentiation of individuals in two clusters: conditionally western (hereinafter – the first cluster) and eastern (second one). The first includes the animals from the Central Russia, the Caucasus, Urals, and the Western Siberia, the second – from Khabarovsk Krai and Irkutsk region.

Fig. 1. Genetic structure of the studied groups of wild boar (*Sus scrofa* L., 1758) for 10 DNA-markers QTL: 1-3 — animals from Arkhangelsk oblast, 4-6 — from Bashkortostan, 7-9 — Vladimir oblast, 10-14 — Volgograd oblast, 15-16 — Vologda oblast, 17-19 — Ivanovo oblast, 20-22 — Kaliningrad oblast, 23-30 — Kirov oblast, 31 — Komi Republic, 32 — Krasnodar krai, 33-35 — Kurgan oblast, 36-38 — Kursk oblast, 39-46 — Leningrad oblast, 47-49 — Mari El Republic, 50-53 — Nizhny Novgorod oblast, 54-56 — Omsk oblast, 57-59 — Orenburg oblast, 60-62 — Penza oblast, 63 — Saratov oblast, 64-66 — Sverdlovsk oblast, 67-69 — Smolensk oblast, 70-73 — Tambov oblast, 74 — Republic Tatarstan, 75-77 — Tver oblast, 78-79 — Udmurt Republic, 80 — Republic Chuvashia, 81-82 — Irkutsk oblast, 83-89 — Khabarovsk krai; a — Q_1 , b — Q_2 . Calculations performed according to J.K. Pritchard et al. (18) for $k = 2$. Description of markers – see “Technique”.

Average value of similarity coefficient (Q) of wild boars from the European Russia and Western Siberia in the first group was equal to $Q_1 = 0,984 \pm 0,005$ with individual variations from 0,679 to 0,998. In 78 of the 80 tested animals Q_1 exceeded 0,900, and only two animals from Mari El Republic and Bashkortostan showed Q_1 of, respectively, 0,679 and 0,855, possibly, due to introgression of ancestors from the second cluster. In wild boars of Irkutsk oblast and Khabarovsk krai, average value of Q was equal to $Q_2 = 0,994 \pm 0,001$ with variations from 0,989 to 0,997. Thus, the obtained data allow the conclusion about reliable genetic differences between animals of these two clusters.

Frequency of genotypes in respect to DNA-markers QTL over the studied total sample of wild boar (*Sus scrofa* L., 1758)

Genotype, cluster		Frequency of genotype, %		
		Marker <i>RYR1</i>		
Genotype		<i>NN</i>	<i>Nn</i>	<i>nn</i>
in conventionally western cluster		100,0	0,0	0,0
in eastern cluster		100,0	0,0	0,0
		Marker <i>ESR</i>		
Genotype		<i>AA</i>	<i>AB</i>	<i>BB</i>
in conventionally western cluster		100,0	0,0	0,0
in eastern cluster		100,0	0,0	0,0
		Marker <i>FSHB</i>		
Genotype		<i>AA</i>	<i>AB</i>	<i>BB</i>
in conventionally western cluster		32,9	26,6	40,5
in eastern cluster		0,0	50,0	50,0
		Marker <i>NCOA1</i>		
Genotype		<i>A1A1</i>	<i>A1A2</i>	<i>A2A2</i>
in conventionally western cluster		87,5	12,5	0,0
in eastern cluster		0,0	0,0	100,0
		Marker <i>BF</i>		
Genotype		<i>AA</i>	<i>AB</i>	<i>BB</i>
in conventionally western cluster		1,4	1,4	97,3
in eastern cluster		0,0	28,6	71,4
		Marker <i>MUC4</i>		
Genotype		<i>CC</i>	<i>CG</i>	<i>GG</i>
in conventionally western cluster		100,0	0,0	0,0
in eastern cluster		100,0	0,0	0,0
		Marker <i>IGF2</i>		
Genotype		<i>QQ</i>	<i>Qq</i>	<i>qq</i>
in conventionally western cluster		100,0	0,0	0,0
in eastern cluster		100,0	0,0	0,0
		Marker <i>MC4R</i>		
Genotype		<i>AA</i>	<i>AG</i>	<i>GG</i>
in conventionally western cluster		0,0	2,5	97,5
in eastern cluster		0,0	0,0	100,0
		Marker <i>POUIF1</i>		
Genotype		<i>CC</i>	<i>CD</i>	<i>DD</i>

in conventionally western cluster	0,0	0,0	100,0
in eastern cluster	0,0	22,2	77,8
Marker <i>ECRF18/FUT1</i>			
Genotype	<i>AA</i>	<i>AG</i>	<i>GG</i>
in conventionally western cluster	0,0	0,0	100,0
in eastern cluster	0,0	0,0	100,0

The authors compared frequencies of genotypes and alleles for the mentioned DNA markers in tested animals (Table, Fig. 2). Five of the 10 studied DNA markers (*RYR1*, *ESR*, *MUC4*, *IGF2*, *ECRF18/FUT1*) were found to be monomorphic in both territorial clusters. Therefore, mutations that caused this genetic polymorphism have occurred after domestication of pigs. Conventionally western and eastern clusters showed insignificant intergroup differences in the frequency of alleles of DNA markers: *FSHB* (p_A – respectively, 0,462 and 0,250), *BF* (p_A – 0,020 and 0,143), *MC4R* (p_A – 0,013 and 0,000), and *POU1F1* (p_C – 0,000 and 0,111).

The studied territorial groups had reliable differences in the frequency of *NCOA1* allele: in the first cluster p_{A1} = 0,938, in the second cluster p_{A1} = 0,000. The presence of *A2 NCOA1* allele in the conventionally western cluster of wild boar can be seen as a result of introgression from Chinese pigs in XVII-XIX centuries (15). At the same time, the occurrence of *A2* allele in domestic pigs with a frequency of 0,722 to 1,000 (21) means the possibility of its introduction through hybridization with domestic pigs.

Fig. 2. Frequency distribution of alleles of DNA-markers QTL in wild boar (*Sus scrofa* L., 1758) in western (W) and eastern (E) territorial clusters: a, b — alleles 1 and 2, respectively. Allele 1 — *N* for *RYR1*, *A* for *ESR*, *A* for *FSHB*, *A1* for *NCOA1*, *A* for *BF*, *C* for *MUC4*, *Q* for *IGF2*, *A* for *MC4R*, *C* for *POU1F1*, *A* for *ECRF18/FUT1*; Allele 2 — *n* for *RYR1*, *B* for *ESR*, *B* for *FSHB*, *A2* for *NCOA1*, *B* for *BF*, *G* for *MUC4*, *q* for *IGF2*, *G* for *MC4R*, *D* for *POU1F1*, *G* for *ECRF18/FUT1*.

So, the examined populations of wild boar *Sus scrofa* differ in both anonymous DNA-markers and certain genes associated with quantitative trait loci in porcine genome.

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