

Study of population-genomic structure of Vyatka horses in interline aspect

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Abstract. The population-genomic structure of the Vyatka horses was studied in the context of 10 male genealogical lines bred in the breed. **Purpose.** Evaluation of the genetic and breeding potential of the Vyatka breed, as well as the influence of factory breeds. **Methods.** Isolation of DNA from the hair follicles of Vyatka horses ($n = 38$) was performed using “ExtraGene DNA Prep 2000” by allele-specific PCR. Statistical calculations of allele frequencies and the types of studied loci were performed using MS Excel 10 software. **Results.** The polymorphism of MSTN, GYS1, DMTR3, CAST, and PRLR genes in Vyatka breed lines was studied for the first time. According to the frequency of occurrence CAST G/A (0.472) and PRLR G/C (0.417), as well as the genotype MSTN T/T (0.579) is somewhat dominated by the heterozygous genotypes characteristic of local breeds. They revealed polysaccharide accumulation mutation PSSM1 (0.189), characteristic of draft horses, and the DMRT3 mutation (0.087), which determines the ability for a non-standard gait-amble, noted among Oryol trotters, which indicates the presence of draft and trotting blood in the lines carrying these alleles. MSTN/C and DMRT3/A alleles, which are not typical for aborigines, are noted in Znatok line, whose representatives are distinguished by a lightweight body type and productive movements. The carriers of the mutant defective GYS1 allele (PSSM1) were identified in five lines: Bob, Gabizon, Buran, Dobrik, and Malakhit. Genotyping of all used stallions for genes associated with economically useful traits will allow more efficient selection in the Vyatka breed and prevent the spread of unwanted alleles, which is especially important for small breeds. **Scientific novelty.** For the first time, an intrapopulation genomic analysis of the domestic horse breed was studied in the interline aspect, and the influence of factory breeds on certain genealogical lines was shown. The polymorphism of the calpastatin (CAST) and prolactin receptor (PRLR) genes has not previously been studied in horse breeding.

Keywords: horses, Vyatka breed, genetics, breeding, DNA markers, genotype assessment, genealogical lines.

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Introduction

To study and protect animal genetic resources, it is necessary to pay attention to breeding work aimed at preserving the linear structural diversity of breeds. 10 lines have been bred in the Vyatka breed (Bob, Gabizon, Bubenchik, Radius, Dobrik, Buran-Sobor, Znatok, Vorobey-Bagulnik, Kabur, Malakhit), the ways of selection and breeding work have been outlined in order to preserve genetic diversity. The Vyatka breed is extremely small, there are only 85 stallions in it [1, pp. 22, 24]. Applied breeding should be inextricably linked

with genetic methods, which is especially important in small breeds. The use of these technologies will make it possible to multiply the intensity of breeding, avoid the accumulation of negative genetic mutations, obtain up-to-date information and transfer of genetic complexes responsible for the severity of the main breeding traits among the horses of different lines and types [2, p. 13].

In recent years, Russian scientists have begun to introduce genomic analysis technologies in fundamental research to improve horse breeding [2, pp. 13–16; 3, pp. 14–15; 4, pp. 66–68; 5, pp. 57–62; 6, pp. 26–27;

7, pp. 258–266; 8, pp. 4–6; 9, p. 6]. Based on generalization of the study analysis of structural genes and DNA sections (STR, SNP) polymorphism among horses, they presented the trends of marker breeding use in the industry [2, pp. 13–16]. Among them, the selection was noted according to the markers associated with economically useful traits, as well as the diagnosis of hereditary diseases [2, pp. 13–16]. Studies have begun in the Vyatka breed on the genotypic features of the paint, as a valuable breeding marker of the breed [9, p. 6]. Scientists have determined the structure of the Vyatka breed of horses by mtDNA haplogroups [8, pp. 4–7].

The myostatin gene (MSTN) has been studied sufficiently in horse breeding. Myostatin is a regulator of skeletal muscle growth in many mammalian species. Mutations g.66493737 T > C and g.66493737 C > T were found in horses [4, p. 66; 5, p. 57; 6, p. 26; 10, p. 91]. The results of the study of myostatin polymorphism for the nucleotide substitution g.66493737 T > C indicate that this mutant allele is typical for many native horse breeds [4, p. 66; 5, p. 58; 6, p. 26; 7, p. 262–263]. The presence of the nucleotide substitution g.66493737 T > C in the MSTN locus of local breed horses suggests that it existed in the genome of their ancient ancestors during the period of domestication [7, p. 263]. The g.66493737 C > T was not found in the donkey and zebra, which allows us to conclude that the T-substitution is more ancient (wild), which is consistent with the herbivorous lifestyle of wild horses, when they needed to travel long distances in search of better pastures [4, p. 66]. The relationship between the polymorphism g.66493737 C > T and the distance qualities of racehorses was revealed. The horses with the C/C genotype are adapted for short distances, those with the C/T genotype for medium distances, and the horses with the T/T genotype are the best stayers [3, p. 15; 11, p. 29–33; 12, pp. 56–63]. Histological analysis confirmed that the horses with the MSTN C/C genotype had the musculature with well-developed short muscle fibers, which ensured a powerful start and productive muscle work over short distances, while the horses with the MSTN T/T genotype demonstrated mainly stayer abilities [3, pp. 14–15]. Scientists have found that the motivation of racehorses to win is conditioned by one of the genes of the peptidase family, encoded by PRCP with the function of cellular metabolism activation [12, pp. 56–63]. The success of horses in classical equestrian sports is also fixed at the genetic level [5, pp. 59–61; 9, pp. 92–63; 13, pp. 965–974]. The animals with the C/C and C/T genotypes are superior to the horses with the T/T genotype in terms of athletic qualities and measurements in equestrian disciplines [9, pp. 92–63]. The horses with the MSTN C/T genotype have some advantages in equestrian competitions in terms of the number of starts and the number of prize-winning places [5, pp. 59–61; 13, pp. 965–974].

The mutation in DMRT3 gene also became the history of microevolution of horse breeds due to the physiological effect on the coordination and variability of movements among horses and led to the appearance of amble and other "non-standard" gaits [7, p. 260]. This gene has replenished the gene pool of many breeds of horses and was named "Gait kee" [14]. A recessive mutation of DMRT3 gene, which determines the ability of a horse to move with an amble, was found among the horses of all breeds where pacers are found, also among some trotter breeds, including the Orlov trotter [15, p. 278]. During the study of Russian native breeds, a nonsense mutation in DMRT3 gene g.22999655 C > A was found among Altai, Bashkir, Vyatka, Mezen and Tuva horses [7, p. 264; 14, pp. 276–278]. Only two genotypes, A/C and C/C, were identified when studying the polymorphism of DMRT3 gene (g.22999655 C > A) among the horses of local Russian breeds. The majority of horses tested (90.0 %) had homozygous DMRT3C/C wild-type genotype. The frequency of the DMRT3A mutant allele occurrence in these breeds ranged from 0.043 (Vyatka) to 0.167 (Tuva). At the same time, the variability of the DMRT3 gene was found both among the horses of the northern forest breeds of the European part of the Russian Federation (Vyatka and Mezen), and among Tuva horses living in Southwestern Siberia and with a high level of the mutant allele fixation (0.167). It is assumed that the improvement of the local stock of horses by Oryol trotters led to the introduction of the DMRT3/A allele into some breeds [7, pp. 264–265].

A point mutation in GYS1 glycogen synthase locus (g.18940324 G > A) leading to polysaccharide accumulation myopathy (PSSM1) may be the reason for the horse performance reduction. Increased activity of mutant glycogen synthase leads to excessive accumulation of polysaccharides, mainly glycogen, in skeletal muscles and is the cause of rhabdomyolysis during exercise of horses. Clinical signs include muscle rigidity, sweating, weakness, reluctance to move, and often lameness [16, pp. 163–164]. This undesirable mutation in GYS1 gene is prevalent mainly among draft horses [7, pp. 265–266; 16, p. 164]. Among all the studied horses of domestic breeds, the homozygous defective A/A genotype in this locus was noted only in the Russian draft breed. The mutant allele GYS1A was found with a low frequency (0.016–0.050) among the horses of three local breeds - Bashkir, Buryat and Vyatka, which indicates a low probability of this mutation fixation in aboriginal populations. It is assumed that the presence of this defective allele among the horses of local breeds is conditioned by their long-term crossing with heavy draft breeds [7, pp. 265–266].

Thanks to modern methods of studying the horse genome, more than two dozen genes responsible for working capacity have been identified. Genotyping of horses based on DNA chips of different density, which can be used for early prediction of their working quali-

ties, drawing up an optimal training plan and maximizing the potential of animals is the most effective one. The task of assessing the genetic potential of horse performance is inextricably linked with the control problem of latent defects that reduce working qualities. DNA genotyping techniques for six such anomalies caused by single-locus mutations are available in our country.

In general, the polymorphism of GYS1, DMTR3 and MSTN genes associated with the working qualities of horses was studied in 10 Russian local breeds: Vyatka Altay, Bashkir, Mezen, Tuva, Tavdinskaya, Polesskaya, Priobskaya and Yakut breeds [7, pp. 262–267]. The C/C genotype of the MSTN gene was found, and the DMRT3/A and PSSM-1/A alleles, rare for native breeds, were also identified in the Vyatka breed, the only one of all native breeds. The genetic variability of horses at GYS1 and MSTN loci can affect the breeding efficiency, so FAO (2015) recommends using these markers when assessing the status of horse breeds and populations [7, p. 258].

When identifying marker genes, economically useful traits, other studies that affect the genetic conditionality of various physiological processes may be of particular interest. These genetic factors include the calpastatin (CAST) and prolactin receptor (PRLR) genes, which have not yet been studied in horses. The protein encoded by the CAST gene is an endogenous inhibitor of calpain (calcium-dependent cysteine protease). It is involved in the proteolysis of the amyloid precursor protein. The calpain/calpastatin system is involved in numerous membrane fusion events such as exocytosis of nerve vesicles and aggregation of platelets and erythrocytes [17, pp. 70–75]. The studies of the CAST gene conducted by Russian scientists in sheep breeding have shown that it encodes growth factors, their receptors, transport and regulatory proteins, i.e. participates in the formation of muscle mass and, therefore, affects the growth rate [18; 19, pp. 2–3]. The prolactin receptor (PRLR) refers to membrane receptors associated

with cytoplasmic protein kinases. It is a hormonal signal conductor for the gonadotropic hormone prolactin and pituitary growth hormone. The hormones of this family to some extent affect anabolic, hyperglycemic, lipotitic, lactogenic, and growth activity [20, pp. 1–2]. The influence of prolactin receptor (PRLR) genotypes on the milk productivity of cows and the reproductive qualities of sows was revealed [20, p. 8; 21, pp. 37–38].

Intrapopulation genomic analysis of domestic horse breeds has not been previously studied in the interline aspect. The scientists assessed the genealogical structure of the Vyatka breed using the analysis of microsatellite DNA polymorphism [22, pp. 462–466]. However, microsatellites do not carry key information about the genetic (phenotypic) characteristics of the horses in which they are detected. Microsatellite analysis is mainly focused on the main applied problem solution – control and certification of the horse origin reliability, implicitly the results of these studies will make it possible to judge intrabreed genetic diversity and genealogical relationships. Only a bioinformatic analysis of genomes and the selection of suitable snips, also encoding phenotypic traits, can provide valuable information both about horse breeds and their populations in general, and about individual animals. Genomic analysis can also be used, among other things, to identify the origin of animals [23, pp. 284–285].

The aim of the research is to study the population-genomic structure of the Vyatka horses in the interline aspect to assess the genetic and breeding potential of the Vyatka breed and the influence of factory breeds. To do this, the task was set in the context of the genealogical lines of the Vyatka breed to study the polymorphism of genes that affect the health and working qualities of horses, in particular, the loci that cause the accumulation mutation of polysaccharides GYS1 (PSSM type 1), non-standard gait (DMTR3), myostatin genes (MSTN), as well as calpastatin (CAST) and prolactin receptors (PRLR).

Table 1
Genotype polymorphism in the genes of calpastatin (CAST), prolactin receptor (PRLR) and myostatin (MSTN) among Vyatka breed lines (P)

Line	CAST			PRLR			MSTN					
	n	AA	GA	GG	n	CC	GC	GG	n	CC	TC	TT
Bob	9	0.444	0.111	0.444	9	0.444	0.444	0.111	8	0.250	0.000	0.750
Bubenchik	1	0.000	1.000	0.000	1	0.000	1.000	0.000	1	0.000	0.000	1.000
Gabizon	4	0.750	0.250	0.000	4	0.250	0.750	0.000	5	0.000	0.400	0.600
Buran	7	0.286	0.714	0.000	7	0.286	0.143	0.571	2	0.000	0.500	0.500
Znatok	–	–	–	–	–	–	–	–	7	0.143	0.429	0.429
Radius	1	0.000	1.000	0.000	1	0.000	0.000	1.000	2	0.000	0.500	0.500
Dobrik	13	0.231	0.538	0.231	13	0.308	0.308	0.385	5	0.000	0.600	0.400
Malakhit	–	–	–	–	–	–	–	–	4	0.000	0.250	0.750
Vorobey	–	–	–	–	–	–	–	–	2	0.000	0.000	1.000
Kabur	1	0.000	1.000	0.000	1	0.000	1.000	0.000	2	0.000	1.000	0.000
Total	36	0.333	0.472	0.194	36	0.306	0.417	0.278	38	0.079	0.342	0.579

Methods

The object of the study is the horses of the Vyatka breed (stallions, mares) in the amount of 38 heads. Sampling was carried out during the period of 2019–2022 among the horses owned by collective farms and private owners of four breeding regions of the Vyatka breed: the Udmurt Republic, Kirov, Lipetsk and Moscow regions. DNA extraction from hair follicles was performed using ExtraGene DNA Prep 2000 ("Isogen", Moscow). When scanning for mutations in GYS1 and MSTN loci, DNA amplification was performed by allele-specific PCR (AS-PCR) using specially selected primers. DMRT3 gene polymorphism was detected by restriction fragment length determination (PCR-PRFL) using BstDE-I restrictase. Electrophoresis of the amplified samples was carried out in 2 % agarose gel with the addition of ethidium bromide and visualization of the results in UV radiation. CAST and PRLR analysis were performed by allele-specific PCR. The main studies were carried out on the basis of the HorseGen Independent Research Laboratory (Moscow), and partially at the Etalon Diagnostics laboratory (USA) with the great assistance of Dr. M. Wilkinson. Statistical calculations of the allele frequencies and the types of the studied loci were carried out using MS Excel 10 program.

Results

For the first time, a genomic intrapopulation analysis was carried out for a number of SNP markers of great breeding and economic importance (MSTN, DMRT3, PSSM1) in the Vyatka breed. For the first time in horse breeding, the genes of calpastatin (CAST) and prolactin receptor (PRLR) have been studied. Table 1 presents the results of Vyatka breed horse genotyping for the CAST, PRLR and MSTN genes in the context of lines.

CAST and PRLR genotypes were studied in seven out of ten male lines of the breed. The Vyatka breed demonstrated polymorphism of the CAST and PRLR genotypes, represented by three genotypes and two alleles at each locus. Heterozygous genotypes G/A

(CAST) and G/C (PRLR) with the indicators of 0.472 and 0.417 dominate by the frequency of occurrence. Intraline analysis of genotype occurrence frequency in the CAST locus showed a certain predominance of the homozygous genotype A/A and G/G in the Bob line and the A/A genotype in the Gabizon line. The heterozygous genotype CAST G/A prevails in the Buran and Dobrik lines. The prevalence of the homozygous G/G genotype was noted in the most numerous lines of Buran and Dobrik at the PRLR locus. The heterozygous G/C genotype predominates in the Gabizon line (Table 1).

Myostatin genes (MSTN) have been studied in all ten genealogical lines of the Vyatka breed. Almost all lines showed the highest cumulative frequency of MSTN T/C and T/T genotypes. C/C genotype, which is not typical for aborigines, was detected in only two lines, including the Znatok line, whose representatives, in terms of type, agility and quality of movements, deviate towards the "leptosome" (quickly agile) type. The predominance of the T/T genotype characteristic of local breeds was noted in Bob, Gabizon, Malakhit and Vorobey lines, among the studied representatives of the Buran, Znatok and Radius lines, the T/C and T/T genotypes are equally found, and there is a predominance of heterozygous T/C genotype in the lines of Dobrik and Kabur. In general, for the breed, the maximum frequency of occurrence was noted among the horses with the T/T genotype (0.579), the minimum – among individuals with the C/C genotype (0.079) (Table 1).

In order to identify potential genetic trends in the Vyatka breed, the laboratory of Etalon Diagnostics (USA) with the great assistance of Dr. M. Wilkinson conducted genomic studies of almost standard Vyatka stallion Blagovest by phenotype, obtained from the Dobrik × Gabizon cross line common in the Vyatka breed. Genotyping was carried out at 19 loci. In particular, genetic predisposition to immune system diseases (SLC5A3, DNAPK, OAS1), muscular (GYS1 (PSSM type 1), GBE1, SCN4A, RYR1, CLCN4), neurologi-

Table 2
The frequency of allele A occurrence in GYS1 (PSSM type 1) and DMRT3 mutations among Vyatka breed lines

Line	DMRT3			PSSM-1		
	N	n	P	N	n	P
Bob	5	0	0.000	4	1	0.250
Bubenchik	1	0	0.000	1	0	0.000
Gabizon	3	0	0.000	3	1	0.333
Buran	–	–	–	6	3	0.500
Znatok	6	2	0.333	4	0	0.000
Radius	1	0	0.000	3	0	0.000
Dobrik	2	0	0.000	10	1	0.100
Malakhit	3	0	0.000	2	1	0.500
Vorobey	2	0	0.000	1	0	0.000
Kabur	–	–	–	3	0	0.000
Total	23	2	0.087	37	7	0.189

cal (MUTYH, MYO5A), reproductive disorders (AR, FKBP6), and skin diseases (PPIB, LAMC2, LAMA3) was revealed among this stallion. Among the genes responsible for economically useful traits, the following have been studied: lordosis of horses – a predisposition to age-related spine collapse (ECA20), to non-standard gaits (DMRT3), the aforementioned myostatin (MSTN) and the “curiosity/vigilance” gene responsible for the good nature of a horse (DRD4), which genetically confirmed the good nature and human orientation of this horse. The latter gene is of particular interest for practical application, however, since the DRD4 isolation technique was not finally tested in the Russian laboratory “HorsGen”, where our genotyping was carried out, the studies of this gene of interest to us have not yet been continued on a large sample of animals.

The results of the studies revealed the absence of 14 out of 15 prototyped mutant alleles that cause hereditary diseases. Among the genes responsible for health, the stallion Blagovest turned out to be a carrier of the PSSM type 1 allele encoded by A, which gave us grounds to analyze the frequency of this allele occurrence in a given sample. Along with this, we typed a group of Vyatka horses for the mutation DMRT3_Ser301STOP, which is not typical for the Vyatka breed, also encoded by A (Table 2).

The studies of GYS1 allele (PSSM1) in Vyatka horses found that its frequency in the breed makes 0.189. The carriers of this allele were identified in the lines of Bob, Gabizon, Buran, Dobrik, and Malakhit (Table 2). We believe that this allele was introduced into the Vyatka breed through wide cross-breeding with the Russian draft horse. The ancestors of the lines with the highest frequency of occurrence of the PSSM-1 mutation (0.500) Buran and Malakhit were phenotypically characterized by a rather massive “airizome” physique, which suggests that they have a certain proportion of draft horse blood. Besides, Sobor, the only successor of the Buran line, who also had a mutation, was inbred to the Russian heavy draft stallion Gilita (III–IV). Homozygous individuals for this mutation with the GYS1A/A genotype were not found in the Vyatka breed. Two carriers of the mutant allele of DMRT3 gene were identified in the Znatok line, which, like the ancestor, represented a pronounced lightweight type by phenotype.

Discussion and Conclusion

Polymorphism of the CAST, PRLR and MSTN genotypes was revealed in the Vyatka breed, heterozygous genotypes G/A (CAST), G/C (PRLR) and the T/T genotype typical of local breeds (MSTN) dominate by the frequency of occurrence. The homozygous genotype MSTN T/T prevails in almost all lines of the Vyatka breed. They revealed the mutation of polysaccharides PSSM1 accumulation, which is characteristic of draft horses, and the mutation that determines the ability to amble DMRT3, noted in Oryol trotters, which indicates the presence of draft and trotting blood in the lines carrying these alleles. MSTN/C and DMRT3/A alleles, which are not typical for aborigines, are noted in the Znatok line, whose representatives are mainly characterized by a lighter type and productive movements. The carriers of the defective GYS1 allele (PSSM1) were noted in five lines (Bob, Gabizon, Buran-Sobor, Dobrik, Malakhit). The comparison of the above genetic and phenotypic factors gives grounds to assume the obvious influence of trotting blood in the origin of the Znatok line ancestor, as well as the possible influence of draft horses in the origin of the Buran and Malakhit line ancestors. Genotyping of all stallions used for the genes associated with economically useful traits will allow more efficient breeding of the Vyatka breed and prevent the spread of defective alleles, which is especially important for small populations.

The data on the genetic profile of the prototyped Vyatka horses is included in the list of bioinformatic data about horses entered into the electronic database of the Vyatka breed developed by us. The results of our research are applied already in drawing up a program of selection and breeding work with the Vyatka breed of horses. It is planned to continue and expand the studies of genotypic markers that are responsible for the economic and biological characteristics of a horse, as well as carrying undesirable alleles. Also, in order to increase the efficiency of breeding work, preserve and improve horse breeds, it is necessary to expand the list of DNA genotyping methods available in Russia for other unexplored animal markers, especially those with the most economic importance.

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