Evaluation of current gene pool of Kholmogor and Black-and-white cattle breeds based on whole genome SNP analysis

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Conservation of local cattle genetic resources is an important strategy for achieving Russia’s food security. During last decades, in the Russian Federation, local livestock populations were either crossbred or replaced by highly productive imported breeds, which led to a loss of the major part of original breeds identities. The objective of our study was to identify genetic differences between the populations of Kholmogor and Black-and-white cattle with varying degrees of admixture with the Holstein breed. The aforementioned breeds were studied using their whole-genome single nucleotide polymorphism (SNP) genotypes. The Kholmogor breed was subdivided into three groups: purebred (KHLM, n = 3), admixed with Holsteins (KHLM-HLST, n = 4) and representatives of old-type breed (KHLM-INTA, n = 15). Black-and-white was subdivided into four groups: purebred (BLWT, n = 9), with a low (BLWT-75, n = 8) and a high (BLWT-HLST, n = 10) level of admixture with Holstein, and represented by archival samples from the 1970s and 1980s (BLWT-OLD, n = 15). The Holsteins genetic profiles (HLST, n = 27) were taken as a comparison group. PLINK 1.07, Admixture 1.3, SplitsTree 4.1.4.6 and R package STAMPP were used to infer genetic relationship between the studied groups. After quality control, 29,688 SNPs were selected for analysis. Multidimensional scaling (MDS), Admixture analysis and a dendrogram constructed using the Neighbor-Net method, revealed the presence of three clusters belonging to the Kholmogor, Black-and-white and Holstein breeds. The first one included KHLM and KHLM-INTA, the second – BLWT and BLWT-OLD, and the third – HLST, KHLM-HLST and BLWT-HLST. The BLWT-75 samples were placed between HLST and BLWT. Thus, our results showed that currently the populations of native cattle breeds with valuable genotypes still exist. The populations with a high level of admixture with Holsteins could be considered neither as the Kholmogor nor as Black-and-white breeds, and would rather be referred as the Holstein breed of local breeding.

Key words: cattle; SNP; markers; whole-genome analysis; genetic resources conservation.
For centuries, groups of cattle well adapted to local environmental and climatic conditions have been forming in various regions around the world. Artificial and natural selection led to fixation of traits meeting the economic needs of people, while making animals better adapted to the environmental conditions. Conservation of these genetic resources is a basis for effective livestock breeding in the future, since the availability of a rich gene pool may be used for deriving new breeds and improving the already existing ones.

There is a series of valuable breeds in the Russian Federation, which combine high productivity, adaptiveness to local climatic conditions, resistance to various diseases, and simplicity of handling. In particular, these include the Kholmogor and Black-and-white breeds.

The Kholmogor breed was established in late 17th century in Kholmogor District of Arkhangelsk Oblast due to favorable feeding conditions and selection of the best individuals for breeding. Following the decree by Peter the Great, several dozens of animals of Dutch breed were imported after 1725 to improve the Kholmogor breed. Cattle import from Holland, Holstein, and England continued from 1765 to 1898 (Ernst et al., 1977). Special breeding efforts to improve the breed were also undertaken in the 1920s (Kuznetsov, 2016). The studbook of Kholmogor breed was issued in 1927 and 1934 saw the establishment of the state breeding center. To improve the breeding stock, Holstein bulls were introduced in the 1980s (Dmitriev, Paronyan, 1994).

The Black-and-white breed was established in the USSR in the 1930–1940s by crossing local breeds with the Dutch ones and was consolidated in 1959. Breeding bulls imported from Germany, Holland, Estonia, Lithuania, and Sweden were a major influence on breed formation. Since the consolidation of the breed occurred on a large territory with local cattle involved, five populations were distinguished for the following regions: Central (middle-Russian), Baltic, Ural, Siberian, other regions of the USSR (Ukraine, Belarus, Central Asia, and Transcaucasia) (Dmitriev, 1978; Kuznetsov, 2016). High milk yield and good adaptive capacities have made the Black-and-white one of the widest spread in the country (Ernst et al., 1977). Along with other breeds, the cattle have been subjected to large-scale admixture with Holstein breed since the 1980s.

Genetic research is a critical step in preserving the gene pool of farm animals’ breeds. Simultaneous analysis of several tens of thousands single nucleotide polymorphisms (SNPs) distributed throughout the whole genome currently appears to be the best suited method for this research (Decker et al., 2009; Kuehn et al., 2011; McTavish et al., 2013). The studies on genetic variety and population structure of Russian cattle breeds performed using genome-wide SNP scanning showed uniqueness of their allele pool (Zinovieva et al., 2009; Kuehn et al., 2011; McTavish et al., 2013). The studies showed that the Black-and-white breed has a number of specific genetic markers, which are not found in other breeds.

The objective of the present study was to identify genetic differences between the Kholmogor and Black-and-white cattle breed populations having varying degrees of admixture with the Holstein breed.

Material and methods
Cattle biological material samples (sperm and blood) from the Kholmogor, Black-and-white and Holstein breeds were studied. The Kholmogor breed individuals were divided into three groups: purebred bulls (KHLM, n = 3), bulls admixed with Holsteins (KHLM-HLST, n = 4), with the degree of admixture with original breed varying from 1 to 20 %, and representatives of old-type breed from the “Inta” breeding farm from Komi Republic (KHLM-INTA, n = 15). Black-and-white breed individuals were divided into four groups: purebred bulls (BLWT, n = 9), bulls with degrees of admixture with Holsteins varying from 6 to 25 % (BLWT-75, n = 8) and from 80 to 99 % (BLWT-HLST, n = 10), and represented by archival samples from the 1970–1980s (BLWT-OLD, n = 15). The Holstein breed specimens were used as a reference group (HLST, n = 27).

Genomic DNA was isolated using the Nexttce columns (Nexttec Biotechnology GmbH, Germany) in accordance with the manufacturer's instructions. Genome-wide SNP screening was performed using Bovine SNP50 BeadChip (Illumina Inc., USA) and the GGP HD150K
chips (Neogene/GeneSeek, USA). To carry out all the analyses, common loci for these chips were used, which were genotyped in at least 90% of individuals with minor allele frequencies of at least 5% and fulfilling the Hardy–Weinberg equilibrium \((p > 1e^{-6})\). SNPs with an unknown position, SNPs that are localized on sex chromosomes and SNPs that are being in a linkage disequilibrium (LD) were excluded as well. A ratio of successfully genotyped SNPs in all individuals was at least 90%.

The PLINK 1.07 software was used to search for markers fulfilling the requirements above (Purcell et al., 2007). The same software was used to perform multidimensional scaling (MDS) based on identical-by-state distances (IBS). MDS was visualized using R software package ‘ggplot2’ (Wickham, 2009). Cluster analysis to identify population structure was carried out using the Admixture 1.3 software (Alexander et al., 2009) and visualized using R package ‘pophelper’ (Francis, 2017). Pairwise values of fixation index \((F_{ST})\) were calculated using R package ‘StAMPP’ (Pembleton et al., 2013). \(F_{ST}\) values obtained were used to plot a phylogenetic tree based on the Neighbor-Net algorithm in SplitsTree 4.14.5 software (Huson, Bryant, 2006). The initial files were created in R 3.5.0 software environment (R Core Team, 2012).

**Results**

Following the quality control, 29 688 SNP were selected for further analysis.

MDS results showed that all the groups under study were divided into three clusters corresponding to the Kholmogor, Black-and-white, and Holstein breeds (Fig. 1, \(a\)). Here, a distinction between domestic breeds \((C < 0)\) and the Holstein breed \((C > 0)\) was observed in terms of first component \(C_1\), which accounts for 9.71% of variability. Black-and-white breed individuals with Holstein admixture were localized between two parent forms in accordance with the degree of admixture, i.e. BLWT-75 cluster overlapped with BLWT, and BLWT-HLST cluster – with HLST. The purebred representatives of the Black-and-white breed (BLWT) were clustered together with archival samples (BLWT-OLD).

A dividing of the latter breeds was only observed in the third component (Fig. 1, \(b\)). No significant differences were observed between the KHLM and KHLM-INTA groups. Individuals of Kholmogor breed with Holstein admixture (KHLM-HLST) were assigned to HLST cluster.

Population structure analysis (Fig. 2) showed different historical origin of the Holstein and Russian breeds. Here, the Kholmogor and Black-and-white breeds separated from the Holstein breed at \(K = 2\), while the differences between two Russian local breeds were observed at \(K = 3\). The percentage of Holstein genomic component in the purebred Kholmogor and Black-and-white breeds (KHLM, KHLM-INTA, BLWT and BLWT-OLD) was insignificant. At the same time, Russian breeds with high degree of Holstein admixture (KHLM-HLST and BLWT-HLST) were almost identical to purebred Holstein breed specimens.

It should be noted that, according to the cross-validation (CV) error calculations, the most probable number of clusters in the studied sample was three (Fig. 3). The results of cluster analysis were validated with calculations of pairwise values of fixation index \((F_{ST})\), according to which no differences were found between the HLST, KHLM-HLST, and BLWT-HLST groups. Among the Kholmogor breed groups, KHLM-INTA had slightly higher genetic distance from Holstein breed than KHLM: \(F_{ST} = 0.101\) and 0.087, respectively. Genetic distances between BLWT and BLWT-OLD Black-and-white breed groups and the Holstein breed were similar \((F_{ST} = 0.081\) and 0.084\) and were higher than for the BLWT-HLST group with Holstein admixture \((F_{ST} = 0.059)\).

To visualize pairwise values of fixation index, a dendrogram for this parameter based on the Neighbor-Net
algorithm was constructed (Fig. 4). Here, a clear distinction between three branches corresponding to the Kholmogor (KHLM, KHLM-INTA), Black-and-white (BLWT, BLWT-OLD, BLWT-75), and Holstein (HLST, KHLM-HLST и BLWT-HLST) breeds was observed.

**Discussion**

In recent years, active efforts have been made in the Russian Federation to improve domestic cattle breeds by crossbreeding with foreign commercial breeds, primarily the Holstein one. These animals typically strongly outperform ‘not-improved’ individuals in terms of milk yield, which is why stock breeders keep increasing the degree of Holstein admixture even up to 99 % (Saksa, Barsukova, 2013; Ruhlova et al., 2014). Thus, productivity of indigenous cattle is in fact improved due to its replacement by imported breeds. As a result, the valuable gene pool formed for centuries in indigenous breeds that allowed cattle to adapt to harsh local natural and climatic conditions can be lost (Baranov, 2011; Matyukov, Zharikov, 2012; Matyukov et al., 2013, 2018).

It should be noted that overuse of imported foreign cattle breeds took place as early as late 19th century. For example, academician A.Sh. Middendorf believed that the primary way to improve cattle was to replace indigenous breeds with imported stud breeds or use crossbreeding on a large scale (Vereshchagine, 1889). At the same time, a major team of scientists headed by N.V. Vereshchagine and Av.A. Kalantar believed that it would be more reasonable to improve indigenous breeds, while providing better feeding and maintaining. They stated in their works that this way, although lengthier, was more reliable (Kalantar, 1927а, b).

Advances in genetics in the 21st century after cattle genome sequencing in 2009 (Elsik et al., 2009; Zimin et al., 2009) made it possible to carry out whole genome studies based on SNPs. This method makes it possible not only to investigate the origin of breeds and evaluate genetic links between them, but also to perform genome-wide association studies (GWAS) to reveal the links between genetic variants and phenotypic traits. Thus, an opportunity arises to identify valuable alleles in domestic breeds that are associated with stability against disease, simplicity of maintaining, etc. This information will be useful for developing preservation and improvement programs for Russian cattle populations. The present paper is the first step towards solving these problems.

In our study, we used about 30 thousand genetic markers to show that Kholmogor and Black-and-white breed individuals with high degree of Holstein admixture formed a common cluster with purebred Holstein breed animals in MDS plot, with no differences revealed by cluster analysis using the Admixture software and calculations of pairwise $F_{ST}$ values. In addition, purebred Kholmogor and Black-and-white cattle groups had their own separate clusters. It shows that there still are individuals carrying unique alleles. Distinction between the
indigenous breeds studied and the Holstein breed in the first component (C1) of the MDS analysis and their common structure at \( K = 2 \) in the Admixture plot shows that the Kholmogor and Black-and-white breeds are genetically closer to each other than to the Holstein breed, which is probably caused by their common origin from the northern Great Russian cattle.

Thus, we believe that the Kholmogor and Black-and-white breed individuals whose degrees of admixture with the Holstein breed equals 80 % and more, cannot be considered as representatives of the aforementioned breeds. It seems more reasonable to attribute them to the locally bred Holstein cattle. To preserve the gene pool of Russian breeds, it is necessary to evaluate them using whole genome SNP analysis and identify herds that managed to preserve their authenticity. Improvement of indigenous breeds with foreign ones is to be implemented in accordance with breeding programs, which imply the use of genome-based techniques. This approach will make it possible to preserve adaptive advantages of local cattle, along with valuable alleles of the improving breed.

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Conflict of interest
Authors declare no conflict of interest.

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