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STRUCTURE OF THE STR ALLELE POOL OF THE POPULATION OF KHOLMOGORY BREED BULLS, SAVED IN THE KOMI REPUBLIC OF THE BANK OF FROZEN SEMEN

© 2025 V. S. Matyukov*, A. S. Leychenko, Ya. A. Zharikov, and S. V. Nikolaev

Institute of Agrobiotechnologies, Federal Research Center of the Komi Scientific Center, Ural Branch of the Russian Academy of Sciences, Syktyvkar, Russia

**e-mail: nipti38mail.ru*

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Abstract. Microsatellite polymorphism was studied in 162 purebred Pechora-type bulls of the Kholmogory, Kholmogory, and Holstein breeds, as well as Holstein-Kholmogory crossbreeds. For 15 microsatellite loci, 132 alleles were identified, or 8.8 on average per locus. Of the total number of alleles, 78 (59.1%), or 5.2 alleles per locus on average, occurred with frequencies greater than 0.1 in at least one of the groups of bulls of different genealogy and breed. Twenty-one alleles in 13 loci occurred with frequencies of 0.15 and higher, regardless of the breed, genealogy, and breed of the group. The greatest number of alleles detected in animals in only one sample were found in the Pechora-Kholmogory-Holstein and Kholmogory-Holstein crossbreed groups. The maximum genetic distance was established between the crossed Pechora-Kholmogory bulls and Holstein bulls ($D_N = 0.237$, $F_{ST} = 0.045$). High genetic differentiation of Holstein bulls with crossed and purebred Pechora-type sires of the Kholmogory breed was confirmed by cluster analysis. The genetic difference between the classical Kholmogory breed and the Holstein was lower. Cluster analysis of the results of genotyping by microsatellites of Kholmogory bulls in one array with samples of Holstein animals and Kholmogory-Holstein crosses made it possible to obtain additional information for planning measures to maintain the genetic diversity of the seved herd of purebred Kholmogory cattle.

Keywords: *Kholmogory breed, Pechora-type bulls, cluster, probability, genetic differentiation, microsatellites*

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INTRODUCTION

Since the 1970s, cattle breeding in the Russian Federation has been overtaken by the widespread practice of absorption of domestic livestock by commercial breeds. This process reached its peak in the 21st century. As a result, our country has lost many domestic gene pools. In the Komi Republic, crossbreeding with imported breeds began somewhat later and less intensively than in many regions of the Russian Federation. Therefore, a small population of domestic Kholmogory cattle has still been preserved in some economically weak farms located in the Far North and Circumpolar regions with unfavorable feeding and housing conditions. The head breeding enterprise Republican State Unitary Agricultural Enterprise (RGUSP) "Komi" for artificial insemination and breeding work has preserved a bank of deep-frozen semen of purebred and crossbred Kholmogory breeding bulls, which has been destroyed in most regions.

Among domestic breeds, Kholmogory cattle for several centuries was considered one of the best in milk productivity and most adapted to breeding in the conditions of the Russian North. During the Soviet period, it was used for breeding some breeds and was distributed in many regions of the European part of Russia, Siberia, Chukotka, Kamchatka, and Sakhalin. Until the 2000s, the proportion of purebred livestock and low-blood Kholmogory-Holstein crossbreeds still occupied a significant share in the Kholmogory breed. In terms of milk productivity, it competed with purebred Holstein cattle. Thus, according to the averaged data from

the evaluation of dairy cattle in farms of all categories of the Russian Federation for 2006–2009, with milk yield levels of about 5000 kg of milk for 305 days of the last completed lactation, in terms of milk yield and milk fat production per one month of life, the Kholmogory breed was second only to the Ayrshire. The difference with the Black Pied and Holstein breeds was insignificant [1], and taking into account additionally obtained offspring (converted to milk), it exceeded the Holstein breed in milk fat + protein production per month of life by 0.23 kg or 6.15% [2].

The homeland of the Kholmogory breed is considered to be the floodplain of the lower reaches of the Northern Dvina River, populated in the 10th-13th centuries by settlers from Novgorod the Great and from the upper reaches of the Volga. It is known that the cattle of the lower Northern Dvina, long before its crossing with Western European breeds, stood out among the local northern cattle for their larger size and better productivity [3].

East of the Kholmogory cattle distribution area in the Pechora River basin, local Pechora northern polled cattle were bred. From the 16th century, and possibly earlier, Kholmogory cattle began to penetrate the middle and lower Pechora through trade routes from Kholmogory and the White Sea region. However, the methodical absorption and displacement of the northern polled cattle by Kholmogory cattle in the north of the Komi Republic began with the importation in 1931 of 55 Kholmogory bulls to farms in the Ust-Tsilemsky district of the Komi ASSR. Then the importation of Kholmogory cattle became constant in the subsidiary farms of the GULAG, and later everywhere [4]. Between supporters and opponents of the absorption of local cattle by Kholmogory cattle in the fifties and sixties of the last century, a heated discussion was conducted in the Komi Republic. The compromise solution was the development and approval of the zonal Pechora type of Kholmogory breed as a selective achievement. Animals of the Pechora type differed in some exterior and economic characteristics from the Kholmogory breed of leading breeding farms in other regions. In the conditions of the Polar region, they were characterized by a longer period of economic use, less frequently dropped out due to alimentary diseases and limb diseases. In the farms of the Komi Republic, the average milk yield of first-calf heifers of the Pechora type of Kholmogory breed for 305 days of the first lactation exceeded by 165.7 kg of milk or 4.9 kg of milk fat the similar indicators of peers of classic Kholmogory lines. The survey of herds unfavorable for leukemia showed that the infection with BLV of Pechora type cows was 4.8% lower than that of peers originating from bulls of classical lines of the Kholmogory breed ($p \leqslant 0.05$) [5]. Long-term veterinary practice (unfortunately not confirmed by official documents) indicated that according to hematological and post-mortem diagnostics, the frequency of clinical manifestations of bovine leukemia in the Pechora type of Kholmogory breed was lower than in animals originating from sires imported from outside the Komi Republic.

Currently, the Pechora type of Kholmogory breed, as well as the Kholmogory breed as a whole, are on the verge of extinction and are subject to protection and preservation as part of the implementation of the global program for conservation of farm animal genetic resources [6]. Therefore, to carry out effective measures for the preservation of the gene pool, a comprehensive assessment of the genetic diversity of the preserved population of Kholmogory cattle is necessary. Molecular markers are an effective tool and means for monitoring genetic diversity, individual and population identity [7, 8]. Microsatellites are among the most accessible and effective markers for genetic analysis [9–11].

The aim of the study is to examine intra- and intergroup genetic variability, as well as the cluster structure of subpopulations and groups of purebred and crossbred Kholmogory cattle, represented by the bank of cryopreserved bull semen from the Komi Republic, using genetic-statistical analysis and STR markers.

MATERIALS AND METHODS

Microsatellite polymorphism was studied in breeding bulls of the Kholmogory breed belonging to the RGUSP "Komi" for breeding work, whose bioproducts were stored in the cryobank of the main breeding enterprise. DNA for the study was isolated from cryopreserved semen of breeding bulls. Molecular genetic studies of microsatellite polymorphism were conducted on a commercial basis by the Laboratory of DNA Technologies of the All-Russian Research Institute of Breeding. DNA for the study was isolated from cryopreserved bull semen. Analysis of the microsatellite profile of the bulls was carried out for 15 loci: *SPS115, TGLA53, TGLA122, BM1818, ETH10, BM1824, INRA23, BM2113, TGLA227, CSRM60, TGLA126, ETH225, CSSM66, ETH3, ILSTS6.* Information about bull genotyping for microsatellite loci was borrowed from the SELEX program database.

The total size of the genotyped bull sample was 162 animals, of which 76 were purebred Kholmogor, including 18 of the Pechora type of Kholmogor breed (P), 38 of classical Kholmogor breed (Kh), and 20 crossed Pechora-Kholmogor (PKh). Crossbred Kholmogor bulls of classical lines with Holstein blood percentage from 3 to 50% — 39 animals (KhGl[1]), with blood percentage of 50% and above — 19 animals

(KhGl[2]), Pechora-Kholmogor-Holstein crossbreds (PKhGl) — 18 animals, purebred Holstein (Gl) — 10 animals. The blood percentage of crossbred Kholmogor-Holstein bulls is indicated in percentages after the breed and purity abbreviation. By "classical" Kholmogor breed and "classical" lines, as opposed to the Pechora type of Kholmogor breed, we mean the array of purebred Kholmogor cattle of farm breeding from the lines of Nailuchshiy, Tsvetok, Topol, Limon, Khlopchatnik, etc. without crosses with Pechora type lines and without blood infusion from other breeds for at least five generations.

The results of bull genotyping by STR loci were processed using GenAlEx 6.5[1] software [12]. The following parameters were calculated for groups of different breed purity: *N* — number of animals in the group; *Na* — average number of alleles per locus; *Ne* — average effective number of alleles per locus; *Ho* — estimate of average observed (actual) heterozygosity, *He* — estimate of average expected heterozygosity per locus, *uHe* — unbiased estimate of average expected heterozygosity per locus, fixation (inbreeding) index *Fis*. Pairwise genetic distances between bull groups were calculated using the following methods: D_N — genetic distance according to Nei M., *Fst* according to Wright S., uD_N — unbiased genetic distance according to Nei M. and estimates of *Fst', G'st(Nei), G'st(Hed), G"st(Hed), Dest*, obtained using the random sampling generation method in GenAlEx 6.5 software.

In the program "Structure Version 2.3.4" [9] we calculated the posterior probability (Q) of assigning individual multilocus *STR* genotypes to different clusters without introducing preliminary information about their classification by genealogy. For each genealogical and breed group, we calculated the average probability of individual membership (Q) in the k-th cluster, from k=2 to k=4. The distribution of average probabilities across clusters was compiled into tables and visualized graphically. To detail the genetic differences of individuals assigned to different clusters at k=3 and k=4, within each cluster we sorted individuals by membership probabilities and formed groups with a probability threshold of 0.75. Then, using the GenAlEx 6.5 program, we calculated genetic population characteristics and genetic distances between these groups. Statistical data processing and graphics were performed in Excel.

RESULTS

In the sample of breeding bulls genotyped by STR loci we identified 132 alleles or 8.8 on average per locus. Of the total number of alleles, 78 (59.1%) or an average of 5.2 alleles per locus occurred with frequencies greater than 0.1 in at least one of the breed groups of bulls. Twenty-one alleles from 13 loci occurred with frequencies of 0.15 and higher in all breed groups without exception.

The largest number of STR alleles that did not occur in other samples was found in PKhGl and KhGl. In the relatively numerous groups of purebred Kholmogory bulls, we identified only two such alleles, in the group of Pechora type bulls – two, and in the bulls of the crossed Pechora-Kholmogory group – one.

Different groups by breed purity did not significantly differ in genetic diversity parameters (Table 1). Although differentiation by combinations of certain indicators between groups was noticeable. Thus, despite a smaller sample size, purebred Holstein bulls exceeded the more numerous samples of Pechora-Kholmogor bulls in the effective number of alleles per locus and were virtually identical to groups Kh and P in this indicator. The most numerous samples of HGl[1] crossbreeds with

Table 1. Characteristics of bull groups by genetic population parameters

Samples	*N*	*Na*	*Ne*	*Ho*	*He*	*uHe*	*Fis*
		X±*sx*	*X*±*sx*	*X*±*sx*	*X*±*sx*	*X*±*sx*	*X*±*sx*
Gl	10	5.333±0.422	3.813±0.385	0.627±0.052	0.691±0.037	0.727±0.039	0.087±0.061
P	18	6.000±0.458	3.839±0.410	0.704±0.025	0.703±0.026	0.723±0.027	–0.012±0.037
PKhGl	18	6.467±0.533	4.138±0.405	0.721±0.039	0.726±0.027	0.749±0.028	0.014±0.027
PKh	20	5.867±0.389	3.579±0.283	0.724±0.030	0.698±0.021	0.715±0.022	–0.040±0.034
Kh	38	6.800±0.490	3.814±0.279	0.733±0.024	0.717±0.021	0.727±0.021	–0.024±0.026
KhGl[1]	39	7.133±0.601	4.112±0.386	0.701±0.023	0.729±0.023	0.738±0.023	0.035±0.023
KhGl[2]	19	6.400±0.349	3.972±0.299	0.688±0.033	0.725±0.023	0.745±0.024	0.054±0.027

Note. PKhGl – Pechora-Kholmogor-Holstein crossbreeds with an average blood percentage of 27.7% for Holstein breed; KhGl[1] – Kholmogor-Holstein crossbreeds with an average blood percentage of 25.9% and KhGl[2] – 60.5% for Holstein breed.

high indicators of absolute and effective number of alleles per locus, excluding the small Gl group, was inferior to other groups in the level of observed heterozygosity. In addition, this group showed a significant deviation from genetic equilibrium in genotypes of five loci out of fifteen (Table 1).

The highest genetic differentiation based on genetic distance estimates was found between crossbred Pechora-Kholmogor bulls and the Holstein breed. The maximum difference between them was confirmed by different methods (Table 2). A somewhat lower genetic distance was established between purebred groups of bulls Gl and Kh. Regardless of the method of calculating genetic distances, the correlation between their estimates in pairwise comparison of groups of bulls of different breeds was high and ranged from 0.894 to 1.0. A slight decrease in correlation coefficients was established between indicators *Fst* and *Fst'* with *G'st(Nei), G'st(Hed), G"st, Dest*. Relationships between estimates of *G'st(Nei), G'st(Hed), and G"st, Dest* were close to functional.

Division of the entire array into two clusters corresponding to two breeds (Holstein and Kholmogor) showed high genetic differentiation of Holstein bulls with crossbred and purebred bulls of the Pechora type of Kholmogor breed and to a lesser extent with Kholmogor breed sires (Fig. 1).

The average probability of membership in the first cluster for Holstein bulls was 0.107±0.039, in the second 0.893±0.039, for Pechora type of Kholmogor breed 0.828±0.052 and 0.172±0.052, for Pechora-Kholmogor 0.860±0.019 and 0.140±0.019, for Kholmogor breed bulls – 0.633±0.046, 0.367±0.046.

Compared to pure-line bulls of the Pechora type and cross-bred Pechora-Kholmogory sires, Kholmogory bulls showed a higher probability of membership in the second "Holstein" cluster (Fig. 1). The distribution of

Table 2. Genetic distances calculated by different methods in pairwise comparison of bull groups of different genealogy and breed composition

Estimates / Samples	D_N	uD_N	F_{ST}	F_{ST}'	G'_{ST} (NEI)	G'_{ST} (HED)	G''_{ST}	De_{ST}
Gl*P	0.180	0.082	0.033	**0.035**	**0.026**	**0.083**	**0.095**	**0.071**
Gl*PKhGl	0.132	0.028	0.024	0.025	0.007	0.022	0.026	0.019
P*PKhGl	0.074	0.000	0.014	0.014	–0.001	–0.002	–0.003	–0.002
Gl*PKh	0.237	0.145	0.045	**0.045**	**0.049**	**0.152**	**0.173**	**0.130**
P*PKh	0.049	0.000	0.010	0.010	–0.006	–0.018	–0.021	–0.015
PKhGl*PKh	0.065	0.000	0.013	0.013	–0.001	-0.002	–0.002	–0.001
Gl*Kh	0.185	0.106	0.034	**0.035**	**0.034**	**0.111**	**0.126**	**0.095**
P*Kh	0.078	0.025	0.014	**0.015**	**0.009**	**0.029**	**0.033**	**0.024**
PKhGl*Kh	0.058	0.000	0.010	0.011	0.000	0.001	0.001	0.001
PKh*Kh	0.063	0.016	0.013	0.013	0.007	0.020	0.024	0.017
Gl*KhGl[1]	0.141	0.061	0.028	**0.027**	**0.018**	**0.059**	**0.068**	**0.051**
P*KhGl[1]	0.072	0.019	0.014	0.014	0.006	0.020	0.023	0.017
PKhGl*KhGl[1]	0.058	0.000	0.011	0.010	–0.001	–0.002	–0.003	–0.002
PKh*KhGl[1]	0.079	0.033	0.016	**0.016**	**0.012**	**0.038**	**0.044**	**0.032**
Kh*KhGl[1]	0.058	0.023	0.010	**0.011**	**0.008**	**0.026**	**0.030**	**0.022**
Gl*KhGl[2]	0.121	0.021	0.023	0.023	0.003	0.011	0.012	0.009
P*KhGl[2]	0.110	0.038	0.020	**0.021**	**0.012**	**0.040**	**0.046**	**0.034**
PKhGl*KhGl[2]	0.096	0.018	0.016	0.017	0.005	0.016	0.019	0.014
PKh*KhGl[2]	0.146	0.080	0.027	**0.028**	**0.028**	**0.090**	**0.102**	**0.077**
Kh*KhGl[2]	0.130	0.076	0.022	**0.023**	**0.025**	**0.084**	**0.096**	**0.072**
KhGl[1] *KhGl[2]	0.065	0.011	0.012	0.012	0.002	**0.008**	0.010	0.007

Note. Statistically significant values of genetic distances at significance level of $P \leqslant 0.05$ are shown in bold.

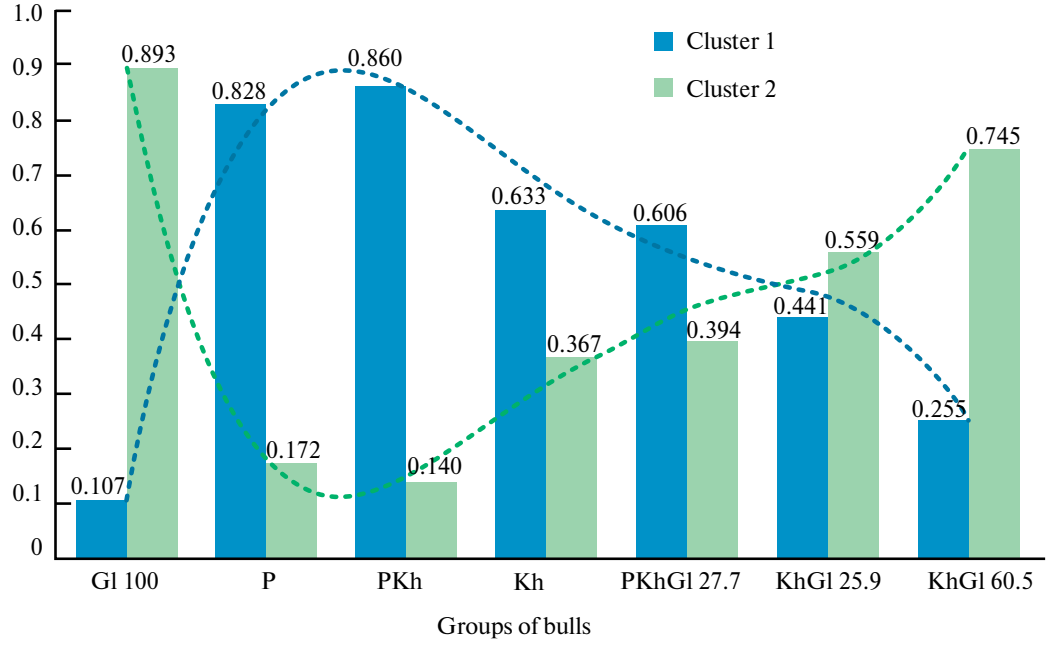

Fig. 1. Distribution of average probabilities of individuals from groups of different breed composition belonging to clusters at $k = 2$

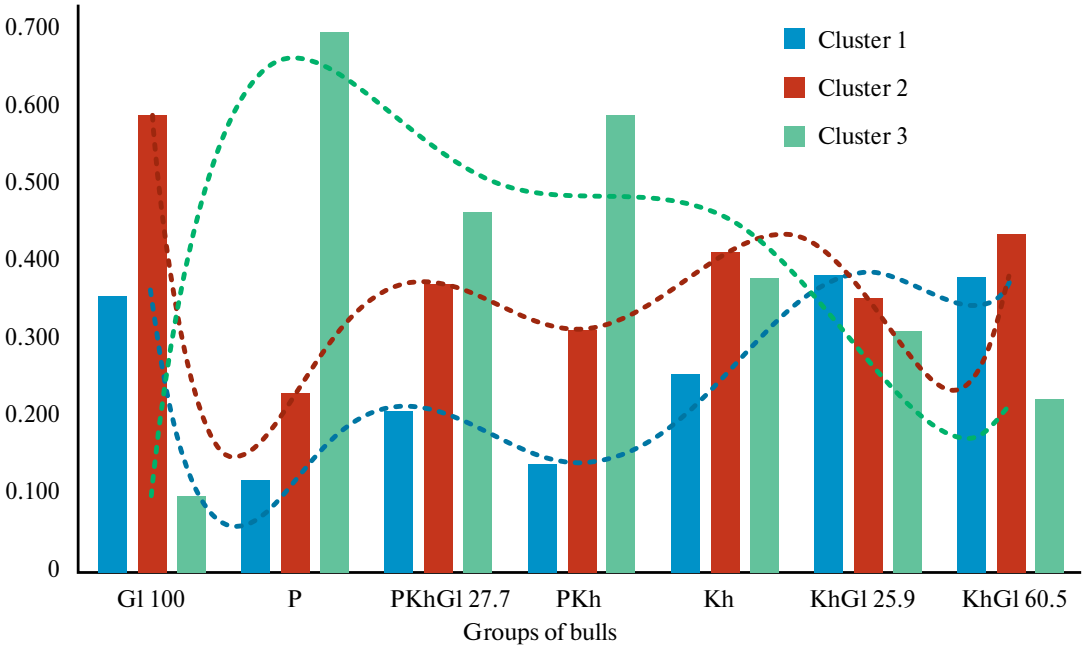

Fig. 2. Distribution of average probability estimates of individuals from groups of different breed composition belonging to clusters at $k = 3$

average membership probabilities of classic Kholmogory breed bulls across individual clusters was similar to the probability distribution in the PKhGl crossbred group with an average Holstein bloodline of 27.7%. In Kholmogory-Holstein crossbreds, the probability of belonging to the second cluster increased with higher Holstein bloodline percentage.

When dividing the entire array into three clusters, components of all three clusters were present in all groups in different proportions, regardless of their genealogy and breed composition. In the Holstein bull group, the average probabilities of individuals belonging to clusters were distributed as follows: first – 0.343±0.087, second – 0.564±0.095, third – 0.093±0.019 (Fig. 2).

Bulls of the Pechora type had the highest probability of belonging to the third cluster – 0.666±0.102 and the minimum to the first (0.115±0.135). The correlation coefficient between distributions of average membership probabilities of individuals in separate clusters for these two groups was negative (–0.785±0.122). Compared to the Pechora type, the Kholmogory breed had more evenly distributed average membership probabilities across clusters: 0.245±0.087, 0.394±0.040, 0.361±0.044 (Fig. 2). With k=3, the correlation coefficient of distribution of average membership probabilities of classic Kholmogory and Holstein breed bulls across clusters was 0.175±0.145, between the Kholmogory breed and the Pechora type of Kholmogory breed 0.473±0.120.

Thus, the distribution of average membership probability of individuals in clusters for the Kholmogory breed simultaneously positively correlated with the probability distributions across clusters for both the Pechora type and Holstein breed.

Genetic distances F_{ST} between clusters, estimated based on groups of individuals formed with a cluster membership probability threshold of 0.75 and higher at k=3 are as follows: 1*2 – 0.055, 1*3 – 0.044, 2*3 – 0.027; at k=4: 1*2 – 0.056, 1*3 – 0.080, 1*4 – 0.077, 2*3 – 0.042, 2*4 – 0.032, 3*4 – 0.053. From these data, it is evident that some estimates of genetic distances between different clusters approached or reached the values of inter-breed differences [12]. However, only the genetic difference between animals of the first clusters and the whole bull array reached F_{ST} values of 0.033 and 0.046. For the remaining clusters, F_{ST} estimates were significantly lower: at k=3 – 0.008 – 0.012, at k=4 – 0.010 – 0.023.

Analysis of individual membership probability profiles of bulls of the Pechora type of Kholmogory breed in clusters showed that at k =2, they were distributed within the range from 0.146 to 0.969. For 86.8% of animals, the probability of assignment to the first cluster reached and exceeded the probability threshold of 0.75. Only one bull (2.6%) was assigned to the second cluster with a probability of 0.75 and higher. Among the animals of the classical Kholmogory breed, 36.8% of individuals with a probability of 0.75 and higher were assigned to the first cluster and 13.2% to the second. At k=3 for bulls of the classical Kholmogory breed, the individual value of Q in the third cluster varied from 0.027 to 0.847 with only 10.5% of animals reaching and exceeding the threshold value of 0.75, 18.4% of animals were assigned to the second cluster at the membership probability threshold of 0.75, and three animals (7.9%) to the first cluster. For the Pechora type of the Kholmogory breed, membership probability values in the third cluster were distributed within the range of 0.125-0.924. For 50.0% of individuals, the probability threshold of 0.75 for assignment to the third cluster was exceeded, for 5.2% to the second cluster, and no animals to the first cluster.

DISCUSSION

It is believed that microsatellites, due to their high polymorphism, allow obtaining reliable estimates of genetic differentiation between populations and intrapopulation subunits [13]. For this purpose, genetic distances by S. Wright – F_{ST} [12], M. Nei – D_N (G_{ST}) and its modifications [14] are widely used as measures of genetic differentiation. However, the indicators of F_{ST} by S. Wright and G_{ST} by M. Nei, obtained using multi-allelic marker systems, are influenced by the level of heterozygosity within individual populations. Therefore, P. W. Hedrick proposed calculating the standardized value *G"ST(HED)* and L. Jost developed the statistic DE_{ST}, based on the variability of the effective number of alleles [15]. In our study, the correlation coefficients of genetic distance estimates obtained by different methods were essentially equally informative.

Regardless of the methods for estimating genetic distances, the differentiation of the Kholmogory breed with Holstein was slightly higher than with the Pechora type of the Kholmogory breed, but lower than between Gl and PKh. Between the groups of bulls P*PKh, PGl*KhGl[1], PKh*PGl, P*PGl, the genetic distances according to most estimates were close to zero (see Table 2).

The reduction of heterozygotes, which was observed in groups of purebred Holstein bulls and their crossbreeds with the Kholmogory breed (see Table 1), has not yet been explained. Most likely, the lack of heterozygotes in these groups was a consequence of the inbreeding of the Holstein sires used, which was not compensated by an increase in heterozygosity due to differences in gene frequencies between the crossed Holstein and Kholmogory breeds. This assumption is supported by the

minimization of heterozygote deficiency in the genetically most heterogeneous group PKhGl.

S. V. Nikolaev and V. L. Yaluga [16] also established an increase in heterozygote deficiency in crossbred Kholmogory cattle with an increase in Holstein blood percentage. In the group of crossbreeds with 3–24% Holstein blood, they observed genetic equilibrium between hetero- and homozygotes (F_{is} 0.005±0.017), while in the group with 75–91% blood, heterozygote reduction reached 22.4% (F_{is} 0.224±0.045). Simultaneously, in this group, the observed heterozygosity significantly decreased compared to other groups. V. V. Volkova et al. [17] established that based on STR markers, the Kholmogory breed compared to Holstein was characterized by a higher level of allelic diversity in terms of the average number of alleles per locus, the number of informative alleles, and the effective number of alleles. The tendency of decreasing allelic diversity with increasing Holstein blood percentage in Kholmogory-Holstein crossbreeds was also noted in [16]. In our study, this tendency is not obvious and may only apply to the dynamics of the effective number of alleles. Nevertheless, regardless of the use of different microsatellite panels in the above-cited studies [17, 18], both our estimates of allelic diversity, heterozygosity, and data on lower allelic diversity in Pechora type animals compared to the classic Kholmogory breed were in good agreement with [17, 18].

As a result of genogeographic studies of mtDNA polymorphism, Y-chromosome haplotypes, and STR markers, new data on the genesis of Eurasian cattle and, in particular, Kholmogory cattle were obtained [19, 20]. The study of Y-chromosome haplotypes established similarities between cattle inhabiting the territories of northern England, Baltic lowlands, Scandinavia, and the European North of Russia. These data suggested that Kholmogory cattle of the Arkhangelsk region and the territory of the Komi Republic, occupied by the Pechora type of the Kholmogory breed, were actively colonized by Western European breeds of black-and-white root. Simultaneously, the study of mtDNA showed similarities between Yaroslavl and Kholmogory cattle, including the Pechora type, with the northern local cattle of Finland [19].

At the same time, based on 30 microsatellite loci, it was not possible to identify gene flow from Black Pied cattle to the Kholmogory breed [20]. At first glance, the results of these studies may seem contradictory. However, it is known that the Y-chromosome is inherited only through the male line. Therefore, if the local males in a population are replaced even once with migrants, with subsequent selection of their male offspring from local females for reproduction, then even without repeated imports during population reproduction, the Y-chromosome of males in the local population will be displaced by the Y-chromosome of migrants. The situation for the X-chromosome can be directly opposite, and for genes localized in autosomes, the population can restore the original allele pool according to the maternal breed. The history of Kholmogory cattle breeding and DNA polymorphism studies have confirmed the correctness of this assumption [2].

In the present study, the Holstein and classical Kholmogory breeds, the Pechora type of the Kholmogory breed, and groups of Kholmogory-Holstein crossbreeds of different blood proportions were presented, which allowed cluster analysis of each of these groups at k=2...4. As a result, it was established that with an increase in the number of clusters, the values and distributions of the average probability of individual membership in the corresponding clusters changed. From the comparison of diagrams and trend lines (see Fig. 1 and Fig. 2), it can be seen that with an increase in the number of clusters, the similarity (difference) between the probabilistic profiles of groups of different breeds is manifested in more detail, characterizing the features of the structure of their allele pools. At k=2 (see Fig. 1), there was a clear distinction between the probabilistic profiles of the Holstein breed (Gl) with the P and PKh groups, and compared to them, a significant drift of the classical Kholmogory breed toward the Holstein breed. At the same time, according to genetic distances, Gl with P, PKh, and Kh did not show such clear differentiation.

When dividing the groups into three clusters (see Fig. 2), the maximum differentiation in the probabilistic profile was established between Gl and P. In group P, the average probability of individual membership in the second cluster was 0.220 (minimum), in the Holstein breed 0.564 (maximum), and in the third cluster 0.666 and 0.093 respectively. The correlation between the distributions of average probabilities of individual membership in clusters was highly negative(–0.785).

The use of cluster analysis proved to be effective for studying the genetic differentiation of related crossbred groups. Thus, in the PKhGl and KhGl groups with similar Holstein blood percentage, the distribution of the average probability of individual membership in the first, second, and third clusters was as follows: 0.200, 0.355, 0.445 and 0.369, 0.335, 0.296 respectively. These data show that the average probabilities of membership in the second cluster (conditionally "Holstein") in the compared groups are almost equal, while the differentiation between the genetic structure of the groups is due to the difference between the probabilities of individual membership in the third cluster (conditionally "Pechora type") and the first

(conditionally "transitional"). The data shows that in the PKhGl group, the proportion of the Pechora type allele pool is higher than in the KhGl group. This difference is due to the higher genetic (genealogical) contribution (regardless of the sex of ancestors) of the Pechora type to PKhGl through a reduction in the contribution of the Kholmogory breed relative to KhGl.

Thus, cluster analysis of the microsatellite genotyping results of Kholmogory bulls in a single dataset with samples of Holstein animals and Kholmogory-Holstein crossbreeds provided additional information for planning and implementing measures to preserve and reproduce the disappearing gene pool. In particular: a) it was possible to show that the differentiation of the Pechora type of the Kholmogory breed with the Holstein breed by allele pool components is significantly higher than that of classical Kholmogory cattle; b) to establish the difference in intragroup genetic structure between different genealogical and breed groups, whose genetic differentiation could not be identified by other methods; c) to show genetic differentiation between groups of individuals whose probability of membership in individual clusters was equal to or exceeded the threshold value.

The practical value of the obtained information is that it will facilitate the individual and group search, selection, and matching of multilocus STR genotypes for reproduction when planning measures to preserve genetic diversity in the reproduction generations of the endangered breed.

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ETHICS DECLARATIONS

The study was approved by the Ethics Committee of the Institute of Agrobiotechnologies of the Federal Research Center of Komi Scientific Center of the Ural Branch of the Russian Academy of Sciences, 01.07.2024, protocol No. 2.

STATEMENT OF COMPLIANCE WITH ETHICS REQUIREMENTS

All applicable international, national, and/or institutional principles for the care and use of animals were observed.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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