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GENETIC EVALUATION OF HOLSTEIN CATTLE MAKES USE OF MICROSATELLITE DNA MARKERS

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Abstract. The results of a research of polymorphism of 12 microsatellite loci in Holstein cattle from an ordinal number of regions of Russia and external countries were presented. The average number of alleles per locus was 5.43±0.19, with variation in the range of 4–13 alleles, the average number of effective alleles was 3.26±0.11. A list of 29 most frequent alleles has been fixed. 22 private alleles were identified, and the frequency of private alleles was 0.004–0.033. It has been demonstrated that the amount of locally alleles in domestic herds is higher than in animals of external selection. The mean level of observed heterozygosity for all loci holds at 0.681±0.017 and varied in the range of 0.65–0.78 for a fixation index of −0.131±0.005. Genetic length between herds of domestic selection were <0.074. It was revealed that groups of cow herds come down into two clusters. The first cluster included animals from three areas of Russia, associated with bulls from Germany and the Netherlands, and the second cluster included individuals from other two provinces closest to the males of Canada, the USA and GB. At once, the oxen of Denmark and Finland founds themselves in a separate cluster. The basis of this work was to evaluate the allele reservoir of Holstein cattle of domestic selection and determine the genetic profile of the breed by STR markers.

Keywords: *genetic differentiation, Holstein breed, black-and-white cattle, microsatellites, markers, breed identity*

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INTRODUCTION

Holstein cattle breed belongs to the dairy type of productivity. It is characterized by high production indicators and the ability to adapt to various geographical conditions. In Russia, a domestic population of Holstein breed animals has been formed [1–3]. The relevance of the work lies in the fact that the results of individual genetic identification are used not so much for scientific purposes, but for practical purposes — determining the reliability of the origin of breeding material and for the genetic characterization of animal breeds [4]. It should be noted that this first-time work complies with the regulations on the procedure for conducting molecular genetic expertise of breeding products in accordance with the requirements of the EEC. Statistical analysis of genotyping results for microsatellite loci of nuclear DNA (STR, short tandem microsatellite repeats) made it possible to assess the level of kinship, genetic similarity, and distances between groups of animals. At the same time, the formation of kinship matrices is the basis of genomic evaluation [5].

Currently, in addition to the main method of establishing pedigree records according to herd book data, molecular genetic methods are used. In breeding work plans, STRs are used to determine the kinship of individuals within breeds and the breed affiliation of animal groups, which have a neutral character in relation to phenotypic traits and a high degree of polymorphism [6–8]. The criterion for assessing the general level of genetic diversity of Holstein breed animals is the polymorphism of STR sites, the list and frequency of allelic variants [9].

Table 1. Averaged indicators of genetic diversity

Genetic groups	$N_a \pm$ SD	N_a, $p \geqslant 5\% \pm$ SD	N_e *(avg.)* $\pm$ SD	$I \pm$ SD
BEL	7.91 ± 0.57	4.25 ± 0.47	3.65 ± 0.42	1.44 ± 0.10
PNZ	7.16 ± 0.72	4.50 ± 0.41	3.68 ± 0.39	1.43 ± 0.09
VLG	7.16 ± 0.61	4.25 ± 0.35	3.74 ± 0.38	1.44 ± 0.09
GBR	6.41 ± 0.39	4.50 ± 0.43	3.63 ± 0.37	1.41 ± 0.09
KUR	6.33 ± 0.55	4.50 ± 0.37	3.34 ± 0.31	1.36 ± 0.09
KRD	6.33 ± 0.55	4.08 ± 0.35	3.75 ± 0.36	1.41 ± 0.09
CAN	5.91 ± 0.54	4.25 ± 0.41	3.55 ± 0.39	1.37 ± 0.10
USA	5.66 ± 0.48	3.83 ± 0.38	3.34 ± 0.36	1.30 ± 0.09
DEU	4.75 ± 0.49	4.75 ± 0.49	3.30 ± 0.33	1.28 ± 0.10
NDL	4.08 ± 0.35	4.08 ± 0.35	2.97 ± 0.24	1.17 ± 0.09
DNM	4.00 ± 0.52	4.00 ± 0.52	2.87 ± 0.45	1.11 ± 0.14
SPA	2.66 ± 0.25	2.66 ± 0.25	2.46 ± 0.26	0.87 ± 0.11
FIN	2.25 ± 0.32	2.25 ± 0.32	2.02 ± 0.33	0.69 ± 0.13

In the world practice of breeding work in recent decades, molecular genetic methods based on STR and SNP markers have been recommended for solving identification issues [10, 11] (ICAR Genetics Guideline) [12]. One of the main problems in determining the genetic structure of a breed is the fact that expertise can only be carried out using the same panel of genetic markers with an identical method for determining genetic diversity. When using various closed commercial panels of STR loci, it is not possible to conduct comparative verifications [13–16].

The aim of the research was to evaluate the allele pool of Holstein cattle of domestic breeding and determine the genetic profile of the breed using microsatellite markers of genomic DNA. This work was conducted for the first time specifically with pedigree animals as part of breeding program plans, Holstein breed inventory, and bringing the breed into compliance with EEC requirements.

MATERIALS AND METHODS

The work was carried out in the DNA technology laboratory of the Federal State Budgetary Scientific Institution "All-Russian Research Institute of Breeding" (VNIIplem) of the Ministry of Agriculture of Russia. To characterize the gene pool of the Holstein breed of domestic and foreign breeding, the polymorphism of 12 microsatellite loci of nuclear DNA was determined (BM1824, SPS115, TGLA53, TGLA227, ETH3, BM2113, TGLA126, TGLA122, BM1818, INRA023, ETH10, ETH225) [12].

Then, the allele spectrum was determined for 722 heads of cattle from breeding farms in Kursk (KUR, 124 heads), Volgograd (VLG, 100 heads), Belgorod (BEL, 100 heads), Penza (PNZ, 100 heads) regions and Krasnodar Krai (KRD, 96 heads), as well as bulls of North American breeding from the USA (USA, 100 heads) and Canada (CAN, 35 heads) and European breeding — Great Britain (GBR), Netherlands (NDL), Germany (DEU), Denmark (DNM), Finland (FIN), Spain (SPA) (65 heads in total).

Primary processing of fragment analysis data obtained in multiplex PCR was carried out on an Applied Biosystems 3130 capillary sequencer using Genemapper software (version 6). Statistical data processing was calculated in the GenAIEx module (version 6.5). To identify inter-population differences within the breed, genetic distances between the studied groups were calculated using Mega software (version 6) [12].

RESULTS AND DISCUSSION

In this work, studies were conducted on the polymorphism of 12 STR loci, representing the parentage verification panel ISAG (Int. Society of Animal Genetics[1]) [3, 8, 16–18] of Holstein breed (especially breeding animals of the current gene pool) as a whole,

[1] www.isag.us

which forms the purpose of research as an inventory of the breed and significantly exceeds the volume of similar phylogenetic studies. The loci had high polymorphism (Table 1), the average number of alleles per locus N_a across all loci and genetic groups was 5.43±0.19 (in the Tyumen region 4.6±0.52 [19]), with variations in the range of 4–13 alleles, the mean number of effective alleles N_e –3.26 ± 0.11, and the average Shannon information index I reached 1.26 ± 0.03, which is also confirmed by previously obtained data from Tyumen breeders [19]. Previously obtained data on samples of Black Pied and Kholmogory cattle breeds showed 4 to 15 allelic variants per locus (in a 15-locus panel) [20]. For comparison, the average number of effective alleles for loci in the population of Khabarovsk Krai was 4.5 [21], and for the Ural population of Sverdlovsk region 4.02 [21].

Analysis of domestic herds of Holstein cattle in Russia showed a high level of polymorphism: the average number of observed alleles N_a exceeded 6 alleles per locus and varied in the range of 6.3–7.9.

The number of alleles with frequency $p > 5\%$ in domestic herds was in the range of 4.1–4.5, the number of effective alleles was in the interval 3.3–3.7, and the information index $I > 1.36$ (Table 1).

When considering animal polymorphism by loci, the highest overall N_a (avg.) was revealed for TGLA53 (8.15±0.88), the number of N_e (avg.) for the same locus reached 5.22±0.40, the highest value of the index I 1.74±0.11 was observed in TGLA53 (Fig. 1). The second highest index I was the TGLA227 locus (1.62±0.10) with N_a (avg.) 7.69±0.71 and N_e (avg.) 4.37±0.31 (in Tyumen ranking first, with a frequency of 0.905 [19]). According to similar studies conducted on a 13-locus panel, for the black-and-white breed, the value of N_a (avg.) was 6.57±0.32, and N_e (avg.) 3.74±0.20 [23]. Separately for the Holstein breed, the parameter values were about 1.8 and 2.1 with an index value I of about 1.5 respectively [24]. In the Ural population, the values of N_a (avg.) and N_e (avg.) were 10.9 and 4.03 respectively [22].

Comparative studies of allele frequencies (p) in domestic and foreign selection cattle groups were conducted (Fig. 1). Comparative analysis showed that the distribution of alleles within each locus in animals belonging to different herds and genetic groups has an uneven and similar character [25, 26]. The most frequent alleles were selected in each locus and a list of allelic variants with a mean value of $p > 0.5$ was compiled (Table 2). At a frequency of p(avg.) > 0.6[2] more than 70% of the herd animals have such alleles as part of homo- or heterozygous genotype. Based on the results of frequency distribution analysis in each locus, allelic variants that occur most frequently in Holstein breed animals were identified.

For comparison, in a small sample of Holstein cows from the Tyumen region, the highest frequency alleles were 117 (ETH3), 248 (SPS115) – 0.529–0.600, and overall, the frequency of major alleles reached 0.60 [19]. Notably, in herds of the related Black Pied cattle breed in Belarus, the highest efficiency in parentage control was observed for the TGLA53 locus (0.848), and the lowest for the ETH3 locus (0.470) [27]. In the Khabarovsk Krai population, the occurrence frequency varied from 0.020 to 0.587, with the highest frequency of 0.538–0.587 for alleles: 117/ETH3, 117/TGLA126, 248/SPS115. The number of effective alleles ranged from 2.4 in the SPS115 locus to 14.3 in the TGLA122 locus [21].

Private alleles were identified in 6 out of the 13 studied groups with a value of p 0.5–3%: VLG (allele 141 bp/locus BM2113, 179/TGLA122); PNZ (121/BM2113, 95/TGLA227, 180/TGLA53, 216/INRA023 (p 17.5%); BEL (186/BM1824, 123,147/BM2113, 138/ETH225, 113/113,109,125/TGLA126, 79/TGLA227, 156,178,182/TGLA53, 258/BM1818); KRD (159/TGLA122); CAN (145/ETH225); GBR (109/ETH3, 198/INRA023).

Thus, a total of 22 private alleles were identified in 10 loci out of 12 (Tables 3–4). The number of private alleles per locus varies from one to four (TGLA126), with the allele 216/INRA023 being more common. In the KRD group, only one allele 159/TGLA122 p 0.5% was found, in VLG —two 141/BM2113 and 179/TGLA122 $p < 0.4\%$, in PNZ — four 121/BM2113 p 0.5%, 95/TGLA227 p 1.5%, 180/TGLA53 p 3.3% and 216/INRA023 p 17.5%. The BEL herd revealed the highest number of alleles with $p < 2\%$.

These alleles likely originated from the maternal breed on which the herd was initially based, with subsequent absorptive crossbreeding with Holstein bulls. Since private alleles in the studied groups have low values of $p < 0.4$–3.3%, they cannot serve as a criterion for breed identification of animals. In comparison with the obtained data, the frequencies of conditionally private alleles in the Tyumen population were 0.014–0.043 [19].

When examining the group of Canadian bulls of North American breeding CAN, one private allele 145/ETH225 was identified p 1.4%, two alleles 109/ETH3 and 198/INRA023 — in GBR bulls p 1.3%, in the USA and DEU bull groups, no private alleles were detected. The reason for the absence of private alleles in the bull groups could be related to the fact that the bulls (or their close relatives) were used in the formation of domestic herds.

In a comparative analysis of the distribution of private alleles, it turned out that in domestic herds, the number

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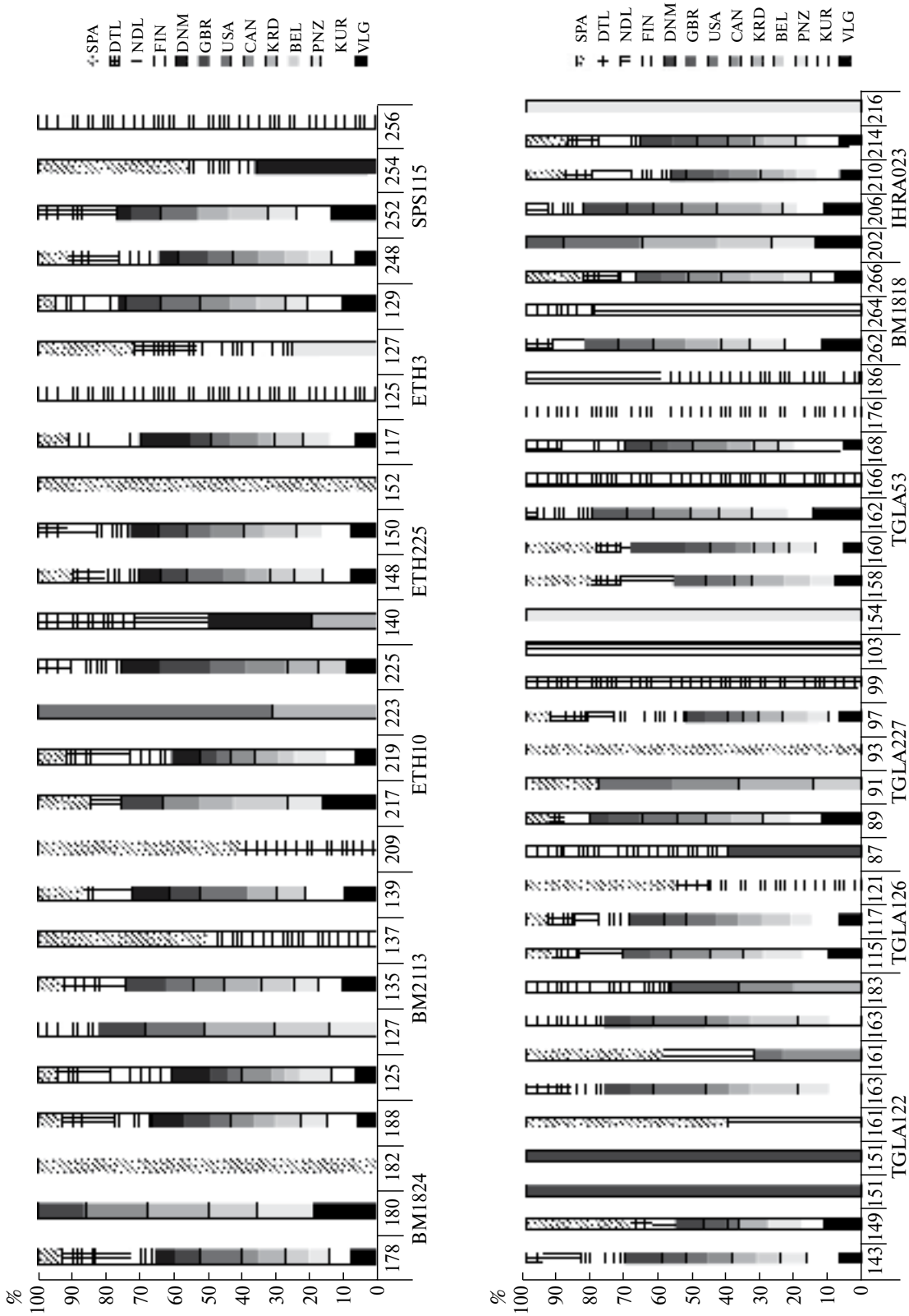

Fig. 1. Frequencies of microsatellite loci alleles

Table 2. Predominant alleles of microsatellite loci

Locus	Allele, bp	Genetic groups									
		VLG	KUR	PNZ	BEL	KRD	CAN	USA	GBR	NDL	DEU
BM1824	188	0.4	0.61	0.55	0.52	0.43	0.47	0.42	0.5	0.5	0.56
ETH10	219	0.39	0.52	0.56	0.3	0.4	0.43	0.27	0.29	0.64	0.5
ETH3	117	0.35	0.41	0.44	0.47	0.25	0.44	0.31	0.34	0.57	0.33
	129	0.47	0.48	0.29	0.4	0.37	0.4	0.56	0.48	0.21	0.39
SPS115	248	0.54	0.63	0.58	0.6	0.68	0.66	0.64	0.74	0.79	0.56
TGLA126	117	0.52	0.65	0.52	0.7	0.57	0.53	0.71	0.55	0.57	0.67
BM1818	266	0.46	0.42	0.48	0.59	0.54	0.59	0.48	0.44	0.29	0.64
INRA023	210	0.26	0.31	0.26	0.16	0.32	0.39	0.18	0.36	0.5	0.33

of alleles (found in < 25% of genetic groups) was higher than in animals of foreign breeding. The number of alleles varied from 0.33 to 0.42.

The number of alleles (found in < 50% of groups) in cows of domestic breeding was also higher than in animals of foreign breeding (range 1.08–1.34).

For example, in Holstein samples in the Syrian Arab Republic, with an average number of alleles per locus of 6.18, the number of private alleles was equal to four [28]. In several small herds in Kazakhstan, there were two private alleles, and the number of rare alleles in the breed was 14.9%, which is less than in the Black Pied breed — 14 and 32.8% respectively [29].

According to the analysis results, the average level of observed heterozygosity H_o in the groups was 0.68±0.02, which corresponds to the unbiased value of expected heterozygosity H_e 0.68±0.01. The average value of the fixation index F is negative –0.07±0.019, since unrelated crossing was observed [30].

Comparative analysis of group heterozygosity by loci revealed a high level of H_o in 5 loci: BM2113 0.78, TGLA122 0.80, TGLA227 0.81, TGLA53 0.75 and INRA023 0.78, and the level of expected heterozygosity H_e for these loci was 0.70, 0.74, 0.75, 0.77, and 0.70 respectively.

Previously, in the population of Khabarovsk Krai, the highest levels of observed and expected heterozygosity (0.857 and 0.930 respectively) were found in the TGLA122 locus, and the lowest (0.530 and 0.586 respectively) — in the SPS115 locus, while the average level of observed and expected heterozygosity was approximately 0.700 [21].

For comparison, in the sample of cows from the Tyumen region, the highest level of H_o was characterized

Table 3. List and frequency of private alleles

Genetic group	Locus	Allele, bp	Frequency, p
VLG	BM2113	141	0
	TGLA122	179	0
PNZ	BM2113	121	0.01
	TGLA227	95	0.02
	TGLA53	180	0.03
	INRA023	216	0.18
BEL	BM1824	186	0.01
	BM2113	123	0.02
	BM2113	147	0.01
	ETH225	138	0.01
	ETH3	113	0.02
	TGLA126	109	0.02
	TGLA126	125	0.01
	TGLA227	79	0.01
	TGLA53	156	0.01
	TGLA53	178	0.01
	TGLA53	182	0.01
	BM1818	258	0.01
KRD	TGLA122	159	0.01
CAN	ETH225	145	0.01
GBR	ETH3	109	0.01
	INRA023	198	0.01

by the TGLA227 locus — 0.905, the lowest — BM1824 –0.667. At the same time, H_e was the highest for the TGLA53 locus and amounted to 0.941 [19].

In individual samples of Holstein and Black-and-White breeds in Kazakhstan populations, the level of expected heterozygosity varied from 0.596 in the SPS115 locus to 0.867 in TGLA227, from 0.603 in the BM1818 locus to 0.844 in BM2113 respectively. The average expected heterozygosity for these breeds was 0.715 and 0.738 respectively [29], which is close to the average values of 0.721±0.036 and 0.662±0.081 obtained later [29], where the heterogeneity of the Black-and-White breed was lower.

In the Ural population in the Holsteinized Black-and-White breed, the average levels of H_o and H_e were 0.73 and 0.72 respectively, with an F index value of –0.004 [17].

The F index had low negative values: BM2113 –0.130, TGLA122 –0.091, TGLA227 –0.090, INRA023 –0.130,

Table 4. Distribution of private and local alleles

Genetic group	Private alleles, % (avg.) ± SD	Local alleles, % $p \leqslant$ (avg.) *25%* ± *SD*	Local alleles, % $p \leqslant$ (avg.) 50% ± SD
VLG	0.16 ± 0.11	0.42 ± 0.19	1.33 ± 0.33
KUR	–	0.33 ± 0.14	1.25 ± 0.33
PNZ	0.33 ± 0.14	0.33 ± 0.14	1.25 ± 0.33
BEL	1.00 ± 0.28	0.33 ± 0.19	1.08 ± 0.29
KRD	0.08 ± 0.08	0.33 ± 0.14	1.25 ± 0.33
CAN	0.08 ± 0.08	0.25 ± 0.13	1.08 ± 0.34
USA	–	0.17 ± 0.11	1.08 ± 0.36
GBR	0.17 ± 0.11	0.42 ± 0.19	1.33 ± 0.33
DNM	–	–	0.50 ± 0.23
FIN	–	–	0.08 ± 0.08
NDL	–	0.17 ± 0.11	0.58 ± 0.23
DEU	–	0.25 ± 0.13	0.92 ± 0.34
SPA	–	0.08 ± 0.08	0.33 ± 0.19

Table 5. Indicators of heterozygosity and fixation index values

Group	H_o	H_e	uH_e	F
VLG	0.708 ± 0.025	0.706 ± 0.025	0.709 ± 0.025	–0.005 ± 0.016
KUR	0.694 ± 0.033	0.673 ± 0.029	0.676 ± 0.029	–0.031 ± 0.021
PNZ	0.652 ± 0.029	0.697 ± 0.027	0.700 ± 0.028	0.062 ± 0.029
BEL	0.774 ± 0.041	0.685 ± 0.035	0.689 ± 0.035	–0.131 ± 0.025
KRD	0.699 ± 0.032	0.702 ± 0.031	0.706 ± 0.031	0.005 ± 0.014
CAN	0.663 ± 0.045	0.684 ± 0.031	0.695 ± 0.031	0.039 ± 0.039
USA	0.667 ± 0.033	0.664 ± 0.033	0.668 ± 0.034	–0.006 ± 0.017
GBR	0.657 ± 0.038	0.691 ± 0.033	0.700 ± 0.033	0.048 ± 0.033
DNM	0.650 ± 0.093	0.572 ± 0.069	0.635 ± 0.077	–0.119 ± 0.075
FIN	0.542 ± 0.096	0.427 ± 0.071	0.569 ± 0.095	–0.293 ± 0.096
NDL	0.700 ± 0.054	0.632 ± 0.036	0.682 ± 0.040	–0.121 ± 0.070
DEU	0.776 ± 0.070	0.664 ± 0.032	0.704 ± 0.033	–0.152 ± 0.073
SPA	0.667 ± 0.112	0.531 ± 0.060	0.708 ± 0.080	–0.248 ± 0.156

which also, as in the Urals, indicated non-related crossbreeding. For the most polymorphic locus — TGLA53, a positive value of F 0.080 was noted, which, compared with the value of H_e 0.84, indicated inbreeding (Table 5). The deficit of heterozygotes in Holstein cattle populations in other 11 out of 13 studies was observed in the range of 0.01–0.091 [31, 32].

Data analysis showed that the value of H_o (avg.) for all loci and groups of national selection varied from 0.652 in the PNZ herd to 0.774 in the BEL group. The observed level of H_o in domestic herds generally corresponds to the expected heterozygosity indicators. The H_e index varied in the range of 0.68–0.71, and the F index was –0.131–0.005. The greatest deviation of the F value in the negative direction indicated outbreeding in BEL farms (–0.139). In PNZ and KRD farms, values of F (0.062 and 0.005) close to zero positive indicated related crossbreeding.

In the groups of bulls of North American selection, the level of H_o was 0.66 (CAN) and 0.67 (USA) and corresponded to the expected values of 0.70 and 0.67 respectively. According to the value of F, the CAN bulls showed inbreeding (0.039), while the USA group showed outbreeding (–0.006).

In the DNM and FIN groups, negative values of F were more pronounced (–0.119 and –0.293), among them outbreeding was higher than in NDL and DEU bulls, where the value of F reached –0.151 and –0.152 respectively.

Pairwise analysis of Nei's genetic distances [33, 34] between groups allowed assessing the degree of separation of herds from different regions of Russia from groups of bulls from foreign countries (Table 6).

Between domestic Holstein herds, genetic distances (L) varied from 0.036 (VLG-KRD) to 0.074 (PNZ-KRD). It turned out that the values of L are closer when comparing animals from domestic herds and several foreign countries than when comparing domestic herds among themselves. The values of L between VLG and bulls from CAN, DEU were 0.022 and 0.018 respectively,

Table 6. Pairwise matrix of genetic distances, similarity, and herd subdivision

Group	VLG	KUR	PNZ	BEL	KRD	CAN	USA	GBR	DNM	FIN	NDL	DEU	SPA
VLG	–	0.96	0.96	0.96	0.97	0.98	0.94	0.99	0.77	0.76	0.95	0.98	0.97
KUR	0.05 *0.01*	–	0.95	0.96	0.94	0.98	0.95	0.97	0.84	0.84	0.99	0.99	0.96
PNZ	0.05 *0.01*	0.06 *0.01*	–	0.93	0.94	0.96	0.90	0.95	0.80	0.81	0.97	0.98	0.99
BEL	0.04 *0.01*	0.04 *0.01*	0.07 *0.02*	–	0.94	0.97	0.97	0.98	0.80	0.78	0.93	0.99	0.96
KRD	0.04 *0.01*	0.06 *0.02*	0.07 *0.02*	0.06 *0.02*	–	0.98	0.94	0.99	0.75	0.78	0.95	0.99	0.98
CAN	0.02 *0.01*	0.02 *0.01*	0.04 *0.01*	0.03 *0.01*	0.02 *0.01*	–	0.94	0.99	0.81	0.83	0.98	0.99	0.99
USA	0.06 *0.02*	0.06 *0.02*	0.11 *0.02*	0.04 *0.01*	0.06 *0.02*	0.07 *0.02*	–	0.97	0.76	0.75	0.90	0.96	0.92
GBR	0.01 *0.01*	0.03 *0.01*	0.06 *0.01*	0.03 *0.01*	0.01 *0.01*	0.01 *0.01*	0.03 *0.01*	–	0.78	0.81	0.98	0.99	0.99
DNM	0.26 *0.10*	0.18 *0.10*	0.22 *0.10*	0.22 *0.09*	0.29 *0.10*	0.22 *0.10*	0.27 *0.10*	0.25 *0.10*	–	0.83	0.86	0.83	0.81
FIN	0.28 *0.14*	0.18 *0.12*	0.22 *0.13*	0.25 *0.14*	0.24 *0.13*	0.19 *0.13*	0.29 *0.14*	0.21 *0.13*	0.19 *0.15*	–	0.89	0.86	0.78
NDL	0.05 *0.03*	0.01 *0.02*	0.03 *0.03*	0.07 *0.04*	0.05 *0.03*	0.02 *0.02*	0.11 *0.04*	0.03 *0.03*	0.16 *0.10*	0.12 *0.12*	–	0.99	0.99
DEU	0.02	0.01	0.02	0.01	0.01	0.01	0.05	0.01	0.19	0.15	0.01	–	0.99
SPA	0.03	0.04	0.01	0.04	0.02	0.01	0.09	0.01	0.21	0.25	0.01	0.01	–

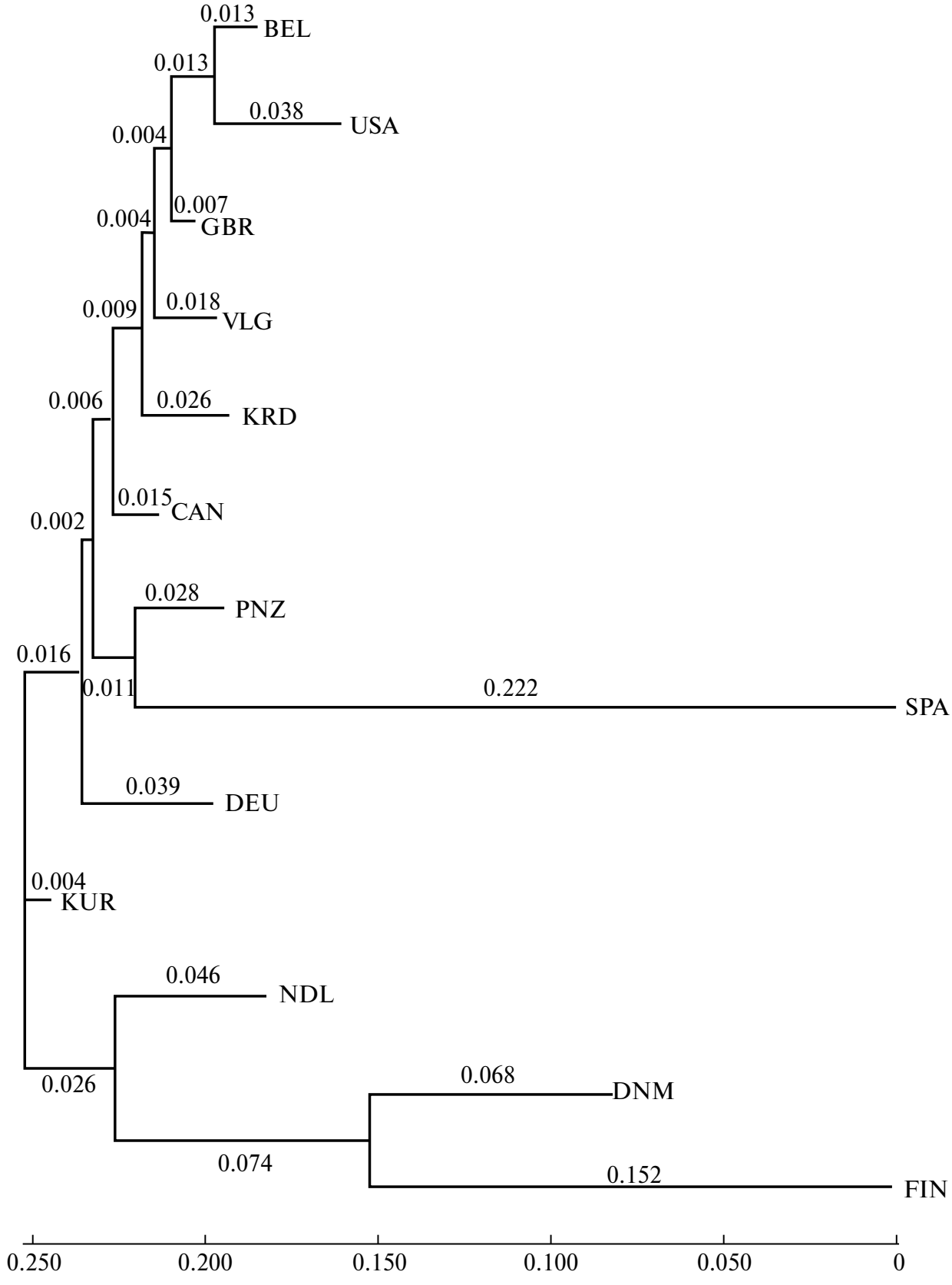

Fig. 2. Dendrogram of genetic distances

while between VLG and groups KUR, PNZ, BEL and KRD they presented values from 0.036 to 0.046. It is necessary to note significant distances between native herds and bulls from DNM and FIN, which differed by 10 or more times (0.175–0.287), as bulls from NDL, DNM and FIN were probably less used in the formation of domestic herds.

Analysis of molecular variance (AMOVA, analysis of mol. variance, σ^2_G) showed that 96% of the variance is represented by variability within domestic herds and 4% of dispersion provide differences between them. When considering the components of variance across the entire population of animals, the share of mathematical expectation increased to 98%, and 2% determined the differences between groups of different selection.

Analysis of Nei genetic identity indicators [30, 33] (L_i) showed that the level of genetic similarity [34, 35] among groups of domestic cattle ranged from 0.929 to 0.965. The similarity of domestic livestock with animals of foreign selection is confirmed by high values of L_i >0.9, with the exception of DNM and FIN bull groups, where the values of L_i for DNM and FIN bulls were 0.7–0.8 (Table 6). Apparently, the direction of selection for DNM and FIN bulls, as well as the intensity of their use in the formation of the Russian Holstein cattle population, differs from bulls of North American and German selection.

Genetic differences between and within groups related to the level of inbreeding are connected through the Fisher-Wright methodology via indicators of excess or deficiency of heterozygosity. The subdivision coefficient F_{st} within subgroups, in relation to the overall measured differentiation between them, is defined as the proportion of total genetic variability.

The coefficient F_{st} was >0 and for domestic cattle was in the range of 0.009–0.017. The F_{st} values for USA bulls were <0.025, and for DEU bulls <0.030. High values of F_{st} were observed in DNM bulls (0.080–0.101) and FIN bulls (0.121–0.156), indicating the difference of these groups from the rest of the population. Interbreed crossing of Black Pied and Holstein breeds contributes to the introduction of alleles and an increase in the level of genetic diversity [36], in contrast to purebred breeding, and a decrease in genetic differences between breeds was observed with F_{st} values from 0.058 to 0.026, and L from 0.306 to 0.123 [36].

To assess the genetic structure of the breed with the inclusion of bulls from other countries in the analysis, the method for evaluating genetic relationships L (Fig. 2) by principal coordinates PCoA was used, with two separate clusters identified between the groups. One

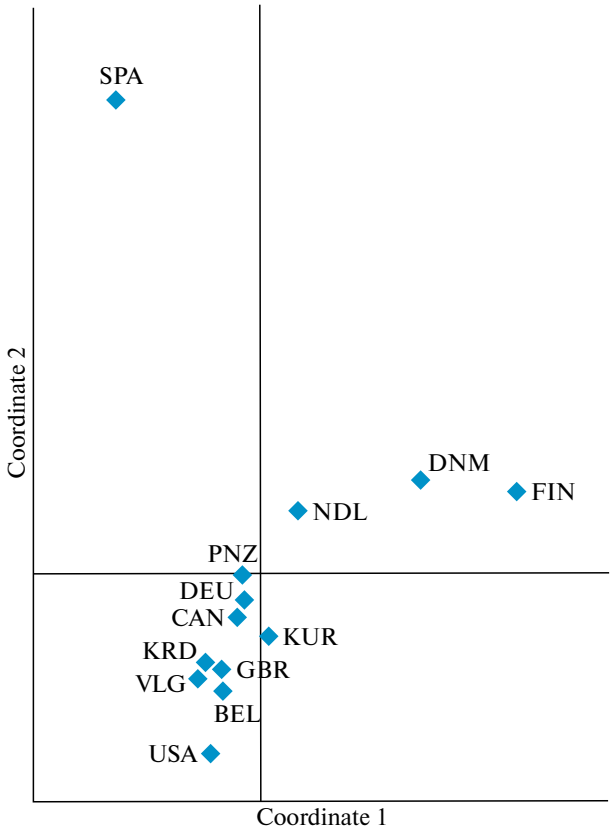

Fig. 3. Diagram of animal distribution in the system of principal coordinates

cluster included animals from VLG, KUR, and PNZ together with bulls from DEU, while the second cluster included groups BEL and KRD together with bulls from CAN, USA, and GBR — bulls from NDL, DNM, and FIN were separate. Based on the PCoA analysis, Holstein cattle of domestic selection were divided into two groups depending on the use of bulls of European or North American selection, which, along with common traits, have their own genetic characteristics. The results of the phylogenetic analysis according to Nei [33, 34] confirmed the arrangement of populations based on genetic distance indicators, reflecting the proportion of genetic variations (Fig. 3). The calculated genetic distances ranged from 0.002 to 0.222. Previously, genetic distances from 0.057 to 0.453 were identified [31, 32]. A decrease in genetic distances between the analyzed breeds due to the introduction of Holstein blood was shown.

The proportion of genetic variations was 55% for the first axis, 23% for the second, and 12% for the third. The group placement results indicated the genetic proximity of the national groups VLG, KUR, and PNZ with DEU bulls, while animals from BEL and KRD groups were genetically close to bulls of North American and

European selection — USA, CAN, DEU. Bulls from DNM and FIN (European selection) were genetically distant from domestic Holstein cattle.

CONCLUSION

Thus, for the first time, genotyping and profiling of the Holstein breed as a whole was carried out on a large number of livestock, significantly exceeding similar studies. It was confirmed that Holstein animals are closely related to each other regardless of the country of origin, meaning that the world's Holstein cattle population is a genetic unit. At the same time, calculations showed an internal subdivision of Holsteins depending on origin and breeding direction. Based on genotyping results, a list of alleles most frequently found in herds and present in most animals was established. The presence of these alleles is a typical characteristic of Holsteins and a genetic feature of the breed. Domestic herds differed from foreign ones by a greater degree of genetic diversity, the presence of private alleles characterizing the original breeding stock, and were differentiated depending on the use of bulls, adjoining the livestock from Germany, USA, and Canada.

Based on phylogenetic analysis, cattle of national selection were divided into two clusters depending on the intensity of coverage by bulls of European or North American selection. It was shown that bulls from Denmark and Finland are more genetically distant from domestic herds. Indicators of genetic distances and genetic identity were visualized by constructing phylogenetic trees and a principal coordinate system, revealing relationships between national herds and bulls of North American and European selection depending on the intensity of their use, subdividing the bulls of Denmark and Finland.

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ETHICS DECLARATIONS

The study was approved by the ethics committee of the Federal Budget Institution of Science (FBIS) of All-Russian Research Institute of Breeding of the Ministry of Agriculture of Russia on March 02, 2024, protocol No. 1.

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 of the article declare that they have no conflict of interest among themselves.

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