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ASSESSMENT OF GENETIC DIVERSITY IN POPULATIONS OF DAIRY CATTLE BREEDS OF UKRAINIAN SELECTION USING MICROSATELET MARKERS

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The article presents the results of a study of the genetic and population structure in herds of Ukrainian Black-and-White and Red-and-White dairy breeds kept in the Kharkiv region (DPDH "Hontarivka"). The analysis of genetic variability in the experimental groups of animals was carried out using 10 microsatellite loci recommended by FAO-ISAG: ETH225, BM2113, ETH3, BM1818, BM1824, ILSTS006, INRA023, TAGLA053, TAGLA12, ETH10. The amplification products were separated in native polyacrylamide gels of different concentrations (5 – 8 %). All studied loci were found to be polymorphic. The number of detected alleles per locus ranged from 4 to 8 (on average 5 alleles per locus) the size of which ranged from 115 bp (ETH3) to 307 bp (ILSTS006). The vast majority of studied loci belong to informatively valuable markers (PIC > 0.5). The most polymorphic loci for both breeds were TGLA053 (8 alleles), BM2113 (6) and ETH3(6). The main population genetic parameters were calculated for the studied loci. The highest values of heterozygosity indices (He) and effective number of alleles (ne) were characteristic of the BM2113 locus (He=0.80-0.81, ne=5.1-5.3). The minimum values of expected heterozygosity were established for the ETH3 loci (0.53-0.55; Ukrainian Black-and-White and Red-and-White dairy breeds) and BM1818 (0.59, Ukrainian Black-and-White dairy breed).

For most microsatellite loci, an equilibrium state between the actual and expected genotype frequencies is characteristic. A probable deviation in the form of a deficit of heterozygotes was established only for the BM1818 locus in both experimental populations (Fis = 0.37; p < 0.05).

Changes in the genetic structure of the experimental cattle population (Kharkiv region) were analyzed in comparison with data from previous years of research, other regions, and with data from the initial forms involved in the creation of these breeds. Analysis of genetic changes that occurred during the reproduction of experimental cattle populations indicates a narrowing of genetic variability and the need to control genetic processes in breeding work.

Keywords: *microsatellites, polymorphism, population, cattle, allele, genotype, heterozygosity.*

ОЦІНКА ГЕНЕТИЧНОГО РІЗНОМАНІТТЯ В ПОПУЛЯЦІЯХ МОЛОЧНИХ ПОРІД УКРАЇНСЬКОЇ СЕЛЕКЦІЇ ЗА ВИКОРИСТАННЯ МІКРОСАТЕЛІТНИХ МАРКЕРІВ

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У статті наведені результати дослідження генетико-популяційної структури в стадах корів українських чорно-рябої та червоно-рябої молочних порід, які утримуються в Харківській області (ДПДГ «Гонтарівка»). Аналіз генетичної мінливості в дослідних групах тварин проводили з використанням 10 мікросателітних локусів рекомендованих FAO-ISAG: ETH225, BM2113, ETH3, BM1818, BM1824, ILSTS006, INRA023, TAGLA053, TAGLA12, ETH10. Продукти ампліфікації розділяли в нативних поліакриламідних гелях різних концентрацій (5 – 8 %). Всі досліджені локуси виявились поліморфними. Кількість виявлених алелів на локус коливалась від 4 до 8 (у середньому 5 алелів на локус) розмір яких знаходився в межах від 115 п.н. (ETH3) – до 307 п,н,(ILSTS006), Переважна більшість досліджених локусів належить до інформативно цінних маркерів (PIC > 0,5). Найбільш поліморфними для обох порід виявилися локуси TGLA053 (8 алелів), BM2113 (6) та ETH3(6). Розраховано основні популяційно-генетичні параметри за досліджуваними локусами, Найвищі значення показників гетерозиготності (Не) і ефективної кількості алелів (ne) були властиві локусу BM2113 (He=0,80-0,81, ne=5,1-5,3). Мінімальні значення очікуваної гетерозиготності встановлені для локусів ETH3 (0,53-0,55; УЧР, УЧеР) і BM1818 (0,59, УЧР).

Для більшості мікросателітних локусів властивим є рівноважний стан між фактичними і очікуваними показниками частот генотипів, Вірогідне відхилення у вигляді дефіциту гетерозигот встановлено лише для локусу BM1818 в обох дослідних популяціях (Fis = 0,37; p < 0,05).

Проаналізовано зміни в генетичній структурі дослідних популяції корів (Харківська обл.) порівняно з даними попередніх років досліджень, інших регіонів та з даними вихідних форм, задіяних у створенні цих порід. Аналіз генетичних змін, які відбувалися в процесі відтворення дослідних популяцій ВРХ свідчить про звуження генетичної мінливості та необхідність контролю генетичних процесів в селекційно-племінній роботі.

Ключові слова: *мікросателіти, поліморфізм, популяція, корови, алель, генотип, гетерозиготність*

Introduction. To solve a number of tasks related to the scientific support of breeding work, in particular regarding the certification of animal breeds, determining the level of consolidation of created groups and the degree of genetic differentiation of populations, a separate class of molecular genetic markers - microsatellites - is widely used (Debrauwere H. et al., 1997, Senan S. et al., 2014). Due to the high level of polymorphism of microsatellite markers (SSR, Simple Sequence Repeat), which is

reflected in a larger number of alleles per locus compared to classical biallelic systems, microsatellites can be used as a rather subtle and effective tool for studying genetic variability, which allows successfully solving the entire range of these issues (Shelyov, 2015; Mishra S. et al., 2017, Zhao J. et al., 2017). SSR markers are considered to be selection-neutral and, due to their wide localization in the genome, are of interest for controlling genetic processes that occur in artificially reproduced animal populations (Shelyov, 2017, Al-Jubori & Senkal, 2023). Given the importance of this issue in the context of preserving the biodiversity of animal breeds, the international organizations FAO and ISAG have proposed microsatellite panels for the main species of farm animals and recommendations for their use in scientific research (ISAG/FAO, 2004, FAO, 2011). According to the FAO recommendations for cattle, 30 microsatellite loci have been identified with localization in each chromosome. ISAG proposes the use of the 12 most polymorphic of them. The first publications on the polymorphism of microsatellite loci in cattle (Bostaurus, Bovidae) appeared in the early 90s of the 20th century, and to date, a considerable amount of information has been accumulated on the genetic variability of individual breeds within this type of farm animals.

In Ukraine, studies of microsatellite variability in cattle have mainly concerned local herds of indigenous breeds of domestic selection, which are valuable as carriers of specific biological and economic characteristics for specific geoclimatic breeding conditions. These are breeds such as Ukrainian gray (Shkavro N. et al., 2010), Lebedynskaya (Shkavro et al., 2018, Ladyka et al., 2019), red steppe (Kramarenko et al., 2018), southern meat (Kramarenko, 2019), buffaloes (Dzitsiuk et al., 2020).

Purpose of the study. Changes occurring in the genetic structure of populations of factory-type breeds used in intensive milk and meat production are also of great interest from the point of view of optimizing the selection and breeding process in the direction of maintaining biological diversity.

In ukraine, the vast majority of dairy cattle are represented by two breeds - ukrainian black-and-white and ukrainian red-and-white dairy breeds (glady m.v. et al., 2015, vyshnevskyi et al., 2019). more than 28 years have passed since the official registration of these breeds (black-mottled (1996), red-mottled (1993)). there are data on the number of allelic variants of microsatellite loci of cattle of 24 breeds bred in ukraine, including the ukrainian black-and-white and red-and-white dairy breeds (podoba b.e. et al., 2013). among the publications over the past 5-7 years, one can note the work of shelyov a.v. et al. (2017) on the study of microsatellite variability in herds of ukrainian black-and-white and red-and-white dairy breeds of the kyiv region. another joint publication concerned microsatellite variability in cattle populations of ukrainian and russian origin (snegin et al., 2019).

Given the wide distribution area of Ukrainian black-and-white and red-and-white dairy breeds, our goal was to analyze changes in the genetic structure of the experimental cow population in the Eastern region of Ukraine (Kharkiv region) compared with data from previous years of research, other regions, and data from the initial forms involved in the creation of these breeds.

Materials and methods of research. The population of cows of Ukrainian black-and-white and red-and-white dairy breeds (DPDH “Hontarivka” of Vovchansky district of Kharkiv region) was used as the object of research. The sample consisted of 30 individuals for each experimental population. DNA isolation was performed from hair follicles using the “NeoPrep DNA” reagent set (Lab Neogene P.C., Ukraine).

According to the recommendations of FAO-ISAG, 10 microsatellite loci were selected for research: ETH225, BM2113, ETH3, BM1818, BM1824, ILSTS006, INRA023, TAGLA053, TAGLA122, ETH10 (Table 1).

Table 1

Nucleotide sequences of primers for microsatellite loci

№	Microsatellite	Primers	Annealing, °C	Amplicon, bp
1	ETH225 (Chromosome 9)	gatcaccttgccactatttcct; acatgacagccagctgctact	58	131-159
2	BM2113 (Chromosome 2)	gctgccttctaccaaataccc; cttcctgagagaagcaacacc	58	122-156
3	ETH3 (Chromosome 19)	gaacctgcctctcctgcattgg; actctgcctgtggccaagtagg	60	103-133
4	BM1818 (Chromosome 23)	agctgggaatataaccaaagg; agtgctttcaaggtccatgc	58	248-278
5	BM1824 (Chromosome 1)	gagcaaggtgtttttccaatc; cattctccaactgcttccttg	56	176-197
6	ILSTS006 (Chromosome 7)	tgtctgtatttctgctgtgg; acacggaagcgatctaaacg	56	277-309
7	INRA023 (Chromosome 3)	gagtagagctacaagataaacttc; taactacagggtgttagatgaactc	58	195-225
8	TGLA53 (Chromosome 16)	gctttcagaaatagtttgcattca; atcttcacatgatattacagcaga	58	143-191
9	TGLA122 (Chromosome 21)	ccctcctccaggtaaatcagc; aatcacatggcaaataagtacatac	58	136-184
10	ETH10 (Chromosome 5)	gttcaggactggccctgctaaca; cctccagcccactttctcttctc	62	207-231

Amplification of fragments of the studied loci was carried out using a thermocycler "Amply-4" (Biocom, Russia) using the appropriate program: 1 cycle - denaturation 94°C 3 min; 35 cycles - denaturation 94°C 30 s, annealing 30 s (56-62 °C depending on the locus), elongation 72°C 50 s; 1 cycle - final elongation 72 °C 10 min. The volume of the reaction mixture was 10 μL, which included 5 μL of Mastermix (2×buffer with 4 mM MgCl2, 0.4 mM DNTP mixture and 0.5 units of DreamTaq DNA polymerase (Thermo Scientific), 2.5 μL of 1 mM primer and 2.5 kl of DNA template.

The amplification products were separated in polyacrylamide gels of different concentrations (5-8%), both native and denaturing. Gels were stained using ethidium bromide (visualization was performed in the ultraviolet spectrum) or silver nitrate. The size of the fragments was determined using molecular mass markers pUC19 and O'RangeRuler 20 bp («Thermoscientific», USA). Calculation of molecular masses of amplification products was performed using the GelAnalyzer program (Version 2010a freeware).

Genotyping of individuals by a set of microsatellite markers in native polyacrylamide gels was performed according to the authors' method (Kulibaba & Liashenko, 2016).

Based on the obtained data, genotype and allele frequencies, actual (H_o) and expected (H_e) heterozygosity, effective number of alleles (n_e), Wright fixation index (F_{is}) was calculated, and the Hardy-Weinberg genotype distribution was checked using the GenAlEx 6.503 add-in integrated into Excel (Peakall & Smouse, 2012.) (https://biology-assets.anu.edu.au/GenAlEx/Download.html).

Research results. According to the results of the studies, it was found that all microsatellite loci used in the experimental animal populations are polymorphic (the proportion of polymorphic loci was 100%).

The number of detected alleles per locus ranged from 4 (ETH10, etc.) to 8 (TGLA53). The analysis of the obtained results of genotyping of individuals allowed to detect a total of 51 alleles for 10 microsatellite loci, the size of which was in the range from 115 bp (ETH) to 307 bp (ILSTS006) (Table 2).

Table 2

Allele frequencies of the studied breeds for 10 loci

Locus	Allele, bp	Black-and-White	Red-and-White	Red-and-White	Red-and-White	Red-and-White	Red-and-White
ETH 225	140	0.41	0.37	*TGLA 053*	160	0.03	0.03
	146	0.15	0.18				
	150	0.05	0.04		168	0.06	0.06
	152	0.19	0.16		170	0.54	0.46
	154	0.2	0.25		174	0.07	0.1
TGLA 122	148	0.14	0.11		178	0.06	0.07
	152	0.35	0.28		180	0.15	0.11
	156	0.02	0.06		182	0.03	0.1
	160	0.40	0.45		190	0.06	0.07
	172	0.09	0.10	*ETH 3*	115	0.61	0.26
BM 2113	125	0.16	0.23		117	0.02	0.02
	127	0.25	0.15		119	0.03	0.02
	135	0.06	0.13		121	0.03	0.04
	137	0.12	0.07		125	0.03	0.03
	139	0.26	0.17		127	0.28	0.63
	141	0.15	0.25	*ILSTS 006*	291	0.26	0.07
BM 1824	190	0.35	0.42		295	0.17	0.22
	192	0.05	0.07		301	0.22	0.31
	194	0.24	0.29		307	0.35	0.40
	196	0.36	0.22	*BM 1818*	266	0.30	0.48
INRA 023	199	0.02	0.03		268	0.13	0.13
	203	0.07	0.17		276	0.55	0.35
					278	0.02	0.04
	211	0.52	0.33	*ETH 10*	216	0.14	0.17
	215	0.05	0.06		218	0.23	0.25
	219	0.34	0.41		222	0.17	0.43
					224	0.46	0.15

The most polymorphic loci in terms of the number of alleles were the TGLA53 loci (8 alleles) and 2 loci that had 6 alleles (BM2113 and ETH3). The studied microsatellite loci differed significantly in terms of the number of detected genotypes. Thus, for the most polymorphic locus TGLA53, out of 32 possible genotypes for the black and red-and-pigmented breeds, only 15-16 were detected. At the same time, the frequency of occurrence of two genotypes out of all detected was about 50%, and for 11 genotypes the frequency of occurrence was less than 5%. This distribution of genotypes affected the value of the effective number of alleles (n_e), which was 3.0-3.9 (37-48%) (Table 3)

Table 3

Main genetic and population indicators of the studied breeds by microsatellite loci

Locus	Parameter											
	N_a		n_e		H_o		H_e		F_{is}		PIC	
	I	II	I	II	I	II	I	II	I	II	I	II
ETH225	5	5	3,71	3,86	0,69	0,71	0,73	0,74	0,05	0,04	0,70	0,72
BM2113	6	6	5,10	5,30	0,82	0,77	0,80	0,81	-0,02	0,05	0,79	0,80
ETH3	6	6	2,20	2,14	0,59	0,57	0,55	0,53	-0,07	-0,08	0,50	0,49
BM1818	4	4	2,44	2,69	0,37	0,40	0,59	0,63	0,37	0,37	0,55	0,59
BM1824	4	4	3,20	3,19	0,66	0,68	0,69	0,69	0,04	0,01	0,65	0,66
ILSTS006	4	4	3,74	3,23	0,70	0,66	0,73	0,69	0,04	0,04	0,70	0,65
INRA023	5	5	2,54	3,22	0,63	0,65	0,61	0,69	-0,03	0,06	0,57	0,67
TGLA53	8	8	3,02	3,88	0,64	0,72	0,67	0,74	0,04	0,03	0,68	0,72
TGLA122	5	5	3,22	3,26	0,67	0,65	0,69	0,69	0,03	0,06	0,66	0,65
ETH10	4	4	3,19	3,35	0,70	0,74	0,69	0,70	-0,01	-0,06	0,67	0,68
Mean	5,1± 0,41	5,1± 0,41	3,24 ± 0,26	3,41 ± 0,26	0,65 ± 0,04	0,66 ± 0,03	0,67 ± 0,02	0,69 ± 0,02	0,04 ± 0,04	0,05 ± 0,04	0,65 ± 0,03	0,66 ± 0,03

Notes: I – Ukrainian Black-and-White dairy breed; II – Ukrainian Red-and-White dairy breed; N_a – number of alleles; n_e – effective number of alleles; H_o – observed heterozygosity; H_e – expected heterozygosity; F_{is} – Wright's fixation index; PIC – Polymorphic Information Content.

Among the 6-allelic loci for dairy breeds, the most balanced in terms of allele frequencies (0.17 ± 0.03) and the maximum value of their effective number (n_e = 5.2; 87%, Table 3) was BM2113. Among the loci with 5 alleles, the most uniform distribution of allele frequencies was observed for the ETH225 locus (0.2 ± 0.05), of which 74-77% can be considered effective.

Analysis of the distribution of expected heterozygosity (H_e) in the studied cattle breeds by the set of loci revealed an average level of genetic variability in dairy cattle populations (H_e = 0.67 ± 0.023 and 0.69 ± 0.023. The highest expected heterozygosity indices were characteristic of the studied populations at the BM2113 locus (0.80 and 0.81, respectively). For the remaining loci, the minimum estimated number of heterozygous individuals was at the level of 53 (ETH3, Ukrainian Red-and-White dairy breed) -59 (BM1818, Ukrainian Black-and-White dairy breed) %.

For most microsatellite loci within the studied cattle populations, an equilibrium state between the actual and expected indices is characteristic (Table 4).

Checking the nature of the distribution of genotype frequencies according to Hardy-Weinberg revealed a probable deviation in the form of a deficit of heterozygotes for the VM1818 locus in only 2 cases (F_{is} = 0.37; χ^2 = 8.0-8.3; $p < 0.05$). The average value of the Wright fixation index gives grounds to believe that in the study populations there is an independent state of genotype distribution with a certain tendency to increase the number of homozygous individuals (F_{is} = −0.04 ÷ 0.13 (black-pigmented) and F_{is} =−0.03 ÷ 0.14 (red-pigmented).

The obtained analysis results prove the possibility of using 9 out of 10 SSR markers for passporting, identification and confirmation of the origin of individual individuals within the studied cattle populations.

Table 4

Results of the assessment of the reliability of deviations in the distribution of genotype frequencies according to Hardy-Weinberg

Locus	χ^2	
	Ukrainian Black-and-White dairy breed	**Ukrainian Red-and-White dairy breed**
ETH225	0,150	0,099
BM2113	0,037	0,146
ETH3	0,317	0,342
BM1818	8,342*	7,997*
BM1824	0,113	0,013
ILSTS006	0,101	0,113
INRA023	0,064	0,202
TGLA53	0,120	0,044
TGLA122	0,050	0,202
ETH10	0,013	0,196

*Note. * – $\chi^2 > \chi^2_{cr}$. level of significance $p < 0,05$*

According to the results of the conducted studies, it was found that the vast majority of the studied loci belong to informatively valuable markers (PIC > 0.5). The exception is the ETH3 locus (PIC = 0.50) for both breeds.

Discussion. The obtained data on the genetic structure of the studied cattle population according to the complex of microsatellite loci are a valuable source of information in terms of both the preservation of the gene pool of breeds and for the control of genetic processes in artificially reproduced animal populations. Given the potential value of the studied dairy breeds as carriers of specific biological and economic characteristics for specific geoclimatic breeding conditions, it would be advisable to analyze changes in their genetic structure according to microsatellite markers compared with data from previous years of research, with data from the initial forms involved in the creation of the breed. Unfortunately, in the available literary sources there is little information on the subject of the study, which could be correctly used for analysis.

Similar studies on the determination of allelic polymorphism of microsatellite loci of biological objects, starting from the end of the 90s of the last century, are carried out on appropriate equipment. We are talking about DNA analyzers (sequencers), which allow to unify the fragmentary analysis of amplified fragments and, thereby, minimize the influence of the human factor on the decision-making process regarding the number and size of microsatellite alleles. This allows, taking into account the recommended FAO-ISAG list of SSR markers, to correctly conduct a comparative analysis of genetic polymorphism both within one and several animal populations.

However, most domestic scientists are deprived of the opportunity to use such equipment and, at best, send samples for analysis abroad. We have accumulated considerable experience in studying microsatellite variability in various animal species and have developed a method for assessing the conformational structure of DNA under native PAGE electrophoresis conditions based on the use of available equipment (Kulibaba & Liashenko, 2016). This allows us to accurately determine the allelic spectrum (number of alleles) of the studied SSR loci. The disadvantage of this approach is certain inaccuracies in determining the sizes of amplified fragments. In the case of a DNA analyzer, a molecular mass marker for each individual sample and software data processing based on a given mathematical regression equation are used to determine the

size of the allele. We use one molecular marker for several samples and unlicensed GelAnalyzer software (Version 2010a freeware) with manual selection of the appropriate approximation equation for electrophoresis analysis and a regular millimeter ruler for control. This may give certain inaccuracies and deviations from the data obtained on the DNA analyzer. Considering the above, we will conduct a comparative analysis obtained by other authors based on the assessment of the total number of alleles per locus, their effective number, heterozygosity indicators, Wright's F-statistics and deviation from the Hardy-Weinberg equilibrium. We found three options for analysis. The first source that provides information on microsatellite variability of breeding bulls of different breeds of cattle from the Genetic Resources Bank is the work of scientists of the Institute of Genetic Resources of the National Academy of Sciences of Ukraine (Podoba et al., 2013). The studies were conducted using a microsatellite panel (10 loci) on an ABI Prism 3130 DNA analyzer. This work presents only data on the total number of allelic variants detected. It should also be noted that the comparative analysis was performed for 6 of the 10 microsatellite loci that are common to both studies, namely the loci: ETH3, ETH10, BM2113, BM1824, INRA023, TAGLA122 (Table 5).

Table 5

Total number of alleles detected in the studied cattle breeds

Cattle breed	Locus					
	BM1824	**BM2113**	**ETH10**	**ETH3**	**INRA023**	**TGLA122**
Holstein	13	9	9	9	12	18
Simmental	7	8	4	6	9	9
Ukrainian Black-and-White (genbank)	5	5	5	6	7	7
Ukrainian Red-and-White (genbank)	10	10	8	6	11	10
Ukrainian Black-and-White	4	6	4	6	5	5
Ukrainian Red-and-White	4	6	4	6	5	5

The largest number of alleles was observed in Holstein cattle, which is a component breed in the creation of Ukrainian dairy breeds. The maximum number of allelic variants in this breed was recorded at the TGLA122 locus (n=18). The magnitude of allelic polymorphism in breeding herds of black and red-and-white breeds of the experimental farm of the NAAS network "Hontarivka" is significantly lower (5 alleles per locus) compared to Holstein (11.7), Simmental (7.2), Ukrainian Red-and-White (9.2) and Ukrainian Black-and-White (5.8) breeding herds.

Another study of microsatellite variability in herds of cows of Ukrainian Black-and-White and red-and-white dairy breeds was dated 2017 (Shelyov et al.). The authors of the article analyze population genetic processes in experimental groups of cows from the Boryspil district of the Kyiv region (Voronkiv village). Unfortunately, this work presents data only on the level of heterozygosity (No and No). Of the 10 microsatellite loci used, we present values for 7 (ETH3, , ETH10, ETH225, BM2113, BM1824, INRA023, TAGLA122, Table 6).

Table 6

Level of genetic variability in experimental herds of dairy cows

Locus	Kyiv region				Kharkiv region			
	I		II		I		II	
	H_o	H_e	H_o	H_e	H_o	H_e	H_o	H_e
TGLA122	0,76	0,86	0,74	0,86	0,67	0,65	0,69	0,69
INRA23	0,73	0,86	0,88	0,88	0,63	0,65	0,61	0,69
ETH3	0,84	0,77	0,86	0,77	0,59	0,57	0,55	0,53
ETH225	0,78	0,80	0,91	0,80	0,69	0,71	0,73	0,74
BM1824	0,80	0,84	0,81	0,86	0,66	0,68	0,69	0,69
BM2113	0,87	0,86	0,77	0,83	0,82	0,77	0,80	0,81
ETH10	0,82	0,77	0,81	0,77	0,70	0,74	0,69	0,70
Середнє	0,80± 0,018	0,82± 0,016	0,83± 0,023	0,82± 0,017	0,68± 0,027	0,68± 0,025	0,68± 0,030	0,69± 0,032

Notes: I – Ukrainian Black-and-White dairy breed; II – Ukrainian Red-and-White dairy breed; H_o – observed heterozygosity; H_e – expected heterozygosity.

Analysis of actual and expected heterozygosity in dairy herds of Kyiv region indicates a relatively high level of genetic variability. (H_e=0.82). Some cases of consanguineous inheritance (excess homozygotes) were noted at loci TGLA122 and INRA23 for the black and white breed (F_{is} =0.12 and 0.15, respectively) and for TGLA122 of the red and white breed (Fis =0.14). A significantly lower level of heterozygosity occurred in the experimental groups of animals of Kharkiv region (H_e=0.68-0.69), although without significant deviations from the equilibrium state in the distribution of genotypes.

Another option for analysis was the article by Snegin, Kramarenko et al., (2019) in which the authors assess the genetic diversity and relationships among eight Russian and Ukrainian breeds of cattle using 10 microsatellite markers. The object of the study was two Ukrainian (Southern Beef and Red Steppe, Kherson, Mykolaiv regions) and four breeds kept in the Belgorod region (Ayrshire, Simmental, Black and Piebald Holstein, Swiss, Russian Black and Piebald and Red and Piebald). First of all, we were interested in data on the genetic structure of the populations of the Ayrshire, Simmental, Holstein breeds, which were the initial forms in the creation of the Ukrainian Black and Piebald and Red and Piebald dairy breeds. It was also possible to compare our data with the results obtained in herds of black-and-white and red-and-white dairy breeds from a neighboring region (Bilgorod region).

It should be noted that the comparative analysis was carried out for 8 out of 10 microsatellite loci that are common to both studies, namely for the loci: ETH3, , ETH10, BM2113, BM1818, BM1824, INRA023, TAGLA053, TAGLA122. Analysis of the total allele pool for these loci indicates a narrower interval of variation of allele sizes for our experimental populations of dairy breeds. This applies to both the upper and lower limits of variation in the lengths of amplification fragments for most microsatellite loci (Table 7).

Among the studied breeds, the smallest number of alleles per locus was observed in the herds of Ukrainian Black and Red-motley cows. In particular, it is almost half as low as in the Ayrshire, Holstein, Simmental breeds of foreign selection and the Black and Red-motley breeds from the neighboring Belgorod region (Table 8).

Table 7

Allele sizes of microsatellite loci in the compared cattle breeds

Locus	Alleles, bp	
	BPua-RPua	Ayr, BPH, Sim, Sw, RPru, BPru
BM2113	125-141	122-156
ETH3	115-127	103-133
BM1818	266-278	248-278
BM1824	190-196	176-197
INRA023	199-219	195-225
TGLA53	160-190	142-191
TGLA122	148-172	129-184
ETH10	216-224	206-231

Notes: Ayr – Ayrshire; BPH – Holstein black-and-white; Sim – Simmental; Sw – Swiss Brown; RPru – Russian red-and-white; BPru – Russian black-and-white; BPua – Ukrainian black-and-white (Hontarivka); RPua – Ukrainian red-and-white (Hontarivka).

Table 8

Indicators of genetic variability in the studied cattle populations

Cattle breed	Parameter				
	N_a	n_e	H_o	H_e	F_{is}
Ayr	10,3±1,2	5,2±0,6	0,870±0,046	0,775±0,034	–0,120±0,030
BPH	13,6±1,3	5,6±0,6	0,823±0,037	0,800±0,024	–0,033±0,044
Sim	10,2±1,3	4,0±0,5	0,808±0,059	0,698±0,051	–0,161±0,033
Sw	6,0±1,0	3,3±0,7	0,619±0,074	0,597±0,067	–0,042±0,062
RPru	9,4±0,9	4,5±0,5	0,824±0,047	0,745±0,035	–0,104±0,024
BPru	9,6±1,0	4,8±0,6	0,794±0,044	0,735±0,035	–0,064±0,056
SMua	10,4±0,8	4,7±0,2	0,673±0,047	0,780±0,012	0,138±0,056
RSua	6,4±0,6	3,8±0,4	0,603±0,094	0,700±0,037	0,185±0,116
BPua	5,1±0,4	3,24±0,3	0,647±0,036	0,675±0,023	0,044±0,038
RPua	5,1±0,4	3,41±0,3	0,655±0,033	0,691±0,023	0,052±0,039

Notes: Ayr – Ayrshire; BPH – Holstein black-and-white; Sim – Simmental; Sw – Swiss Brown; RPru – Russian red-and-white; BPru – Russian black-and-white; SMua – Ukrainian Southern Meat cattle; RSua – Ukrainian red steppe; BPua – Ukrainian black-and-white (Hontarivka); RPua – Ukrainian red-and-white (Hontarivka).

In terms of the number of alleles per locus (N_a), effective alleles (n_e) and the level of heterozygosity (H_e), the populations of dairy breeds studied by us are similar to the indicators obtained in the population of the Ukrainian Red Steppe breed (N_a=6.4; n_e=3.8; H_e=0.7). Among the breeds of foreign selection, the lowest level of genetic variability was found in the herd of cows of the Swiss breed (N_a=6.0; n_e=3.3; H_e=0.6). The maximum values of the indicators of genetic diversity were characteristic of the populations of the Ayrshire and Holstein breeds (N_a=10.3-13.6; n_e=5.2-5.6; H_e=0.78-0.8).

In relation to the Hardy–Weinberg Equilibrium (HWE), a characteristic feature of Ukrainian breeding breeds is the deficit of heterozygous individuals (4.4-13.8%), which is reflected in the magnitude and sign of Wright's fixation index (F_{is} (SMua) = 0.138; F_{is} (RSua) = 0.185; F_{is} (BPua) = 0.185; F_{is} (RPua) = 0.185; Table 8). A significant deviation from HWE towards the excess of homozygotes was established at the BM1818 locus for

Ukrainian black and red-motley breeds, ETH3, INRA023, TGLA053 for southern meat breeds and INRA023 and TAGLA053 for red steppe breeds (Table 9).

Table 9

Results of Hardy-Weinberg Equilibrium (HWE) testing in cattle populations for 8 microsatellite loci

Locus	Cattle breed									
	Ayr	**BPH**	**Sim**	**Sw**	**RPru**	**BPru**	**SMua**	**RSua**	**BPua**	**RPua**
BM1818	NS	E	NS	NS	NS	E	NS	NS	D	D
BM1824	E	NS	E	D	E	NS	NS	NS	NS	NS
BM2113	NS	NS	E	NS	E	NS	NS	NS	NS	NS
ETH10	E	NS	NS	NS	NS	E	NS	NS	NS	NS
ETH3	E	D	NS	NS	E	D	D	NS	NS	NS
INRA023	E	E	NS	NS	NS	NS	D	D	NS	NS
TGLA122	NS	NS	NS	NS	E	NS	NS	NS	NS	NS
TGLA53	NS	NS	NS	NS	NS	E	D	D	NS	NS

For breeds of foreign origin, out of 18 cases of deviation from HWE, only 3 showed a deficit of heterozygotes.

Thus, the results of the comparative analysis of microsatellite variability give reason to believe that in the herds of Ukrainian Black and Red-motley dairy breeds kept in the DPDG "Hontarivka" of the Kharkiv region. a significant narrowing of genetic variability is observed. First of all, this is manifested in a significant decrease in the number of allelic variants for all studied loci (an average of 5 alleles per locus), as well as the number of heterozygous individuals (H_o=0.65-0.67). The reason for this may be several factors. First of all, this is the selection of individuals for crossing in artificially reproduced animal populations and, as a consequence, gene drift. On the example of artificial populations, we have an example of the action of the main factors of the evolutionary process, which arise in nature by chance. A person himself isolates individuals, preventing free crossing, purposeful selection of desired genotypes, as a result of which changes in allele frequencies occur primarily in small populations. According to reported data as of 01.01.2022, there were 714 dairy cows in the experimental farm (370 Red-mottled and 344 Black-mottled dairy breeds). Is this a lot or a little for the normal functioning of the genetic potential of an artificial population of animals. If we draw an analogy with natural populations, it is believed that the threshold values of the effective population size (N_e) are 50 individuals, which is an insufficient number to prevent inbreeding depression. Even 500 individuals are too few to fully preserve the evolutionary potential of a given species of animals (the best approximation is $N_e \geq$. 1000, Frankham et al., 2014). In our case, when there are objective reasons for limiting the number of animals, the first priority should be the control of genetic processes in artificially reproduced animal populations, which must be taken into account in selection and breeding work.

Conclusions

1. Analysis of polymorphism of 10 microsatellite loci in experimental populations of Black-and-White and Red-and-White dairy breeds allowed to detect a total of 51 alleles for each of the populations. The number of detected alleles per locus ranged from 4 (ETH10 and others) to 8 (TGLA53) (average 5.1).

1. The average level of genetic variability was established for the set of microsatellite loci in dairy cattle populations (H_e = 0.68±0.025).

2. For most microsatellite loci, an equilibrium state between the actual and expected genotype frequencies is characteristic. A probable deviation in the form of heterozygote deficiency was established only for the BM1818 locus in both experimental populations (F_{is} = 0.37; χ^2 = 8-8.3; $p < 0.05$).

3. The vast majority of the studied loci belong to informatively valuable markers (PIC > 0.5), The exception is the ETH3 locus (PIC = 0.50) for both breeds.

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