

Artículo de investigación

## Characterization of the genetic structure of a Hereford breed herd based on STR loci

Характеристика генетической структуры стада герефордской породы по STR-локусам

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### Abstract

The authors present a characterization of the genetic structure of a Hereford breed herd based on 15 STR loci of nucleotide sequences of the DNA. Ear tissue from 30 heifers obtained from “Bizon” LLC (Tyumen Region, Russia) was used as the material for the study. The set of markers for analysis included 15 microsatellites: BM 1818, BM 1824, BM 2113, CSRM 60, CSSM 66, ETH 3, ETH 10, ETH 225, ILST 006, INRA 023, SPS 115, TGLA 53, TGLA 122, TGLA 126, TGLA 227. To achieve the goal, the authors determined allele frequencies, polymorphism, observed and expected heterozygosity and Wright’s fixation index. The survey found that 15 microsatellite loci included 104 alleles, the frequencies of which ranged from 0.017 to 0.683. The average number of informative alleles per locus was 6.93 and the number of effective alleles was 4.96, or 71.6%. An increase in the number of alleles in the locus was accompanied by an increase in the level of polymorphism, which was confirmed by the value of the positive correlation coefficient, which was 0.794 ( $p < 0.001$ ). The TGLA 53 locus had the highest polymorphism and the number of effective alleles was 10.0. The TGLA 126 locus had the lowest polymorphism and the number of effective alleles was 2.3. An analysis of genetic diversity demonstrated that the highest observed heterozygosity was

### Аннотация

В статье представлена характеристика генетической структуры стада герефордской породы по 15 STR – локусам нуклеотидных последовательностей ДНК. Материалом для исследования послужили ткани уха 30 тёлочек из ООО «Бизон» Тюменской области. Набор маркеров для анализа включал 15 микросателлитов - BM 1818, BM 1824, BM 2113, CSRM 60, CSSM 66, ETH 3, ETH 10, ETH 225, ILST 006, INRA 023, SPS 115, TGLA 53, TGLA 122, TGLA 126, TGLA 227. Для достижения поставленной цели были определены частоты встречаемости аллелей, уровень полиморфности, наблюдаемая и ожидаемая гетерозиготность, индекс фиксации Райта. В результате обследования установлено, что 15 микросателлитных локусов включали в себя 104 аллеля, частоты которых колебались в диапазоне от 0,017 до 0,683. Среднее число информативных аллелей на локус составляло 6,93, эффективных аллелей – 4,96 или 71,6%. Увеличение числа аллелей в локусе сопровождалось повышением уровня полиморфности, что подтверждает положительный коэффициент корреляции, величина которого составляла 0,794 ( $p < 0,001$ ). Наибольшим уровнем полиморфности характеризовался локус

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registered in the TGLA 53 locus and was 0.950 and the highest expected heterozygosity was recorded in the CSSM 66 locus and was 0.860. The average level of observed heterozygosity was 0.783, the average level of expected heterozygosity was 0.691 and Wright's fixation index was 0.133, indicating a high level of genetic diversity of Hereford herds.

**Keywords:** Microsatellites, polymorphism, genetic herd structure, locus, wright fixation index, allele, heterozygosity, hereford breed.

## Introduction

Cattle of the Hereford breed have been bred in the Tyumen Region (Russia) since 1996. The modern population of animals of this breed was formed from a breeding stock imported from stud farms of Western Siberia –the Novosibirsk Region, the Omsk Region and the Altai Territory. In 2012, in order to update the gene pool of purebred herds, Herefords from Sweden were brought into the region for genetic turnover (Bakharev et al., 2017; Sheveleva, 2008). Today, the animals of the Hereford breed are the most numerous among all beef breeds in the region – 7,789 animals, or 55% of the total number of beef cattle.

Selection and stock breeding with a certain breed are carried out through selection and breeding according to phenotypic characteristics, taking into account the lineage. However, the use of this method takes a long time to achieve high results in breeding. Until recently, the use of modern DNA technologies, such as genomic estimation, was not available in the animal breeding industry in the region. Nevertheless, DNA diagnostics of genes associated with milk productivity and hereditary diseases were used in the dairy cattle breeding in the region (Nesterenko et al., 2018; Nesterenko et al., 2019a; Koshchaev et al., 2019). A molecular genetic laboratory, which performs microsatellite DNA analysis of farm animals, was created in the Tyumen Region on the basis of the State Agrarian University of the Northern Trans-Urals.

The adoption of high-performance microsatellite typing methods will make it possible to assess the genetic diversity of breeds and populations at the

TGLA 53 с числом эффективных аллелей равным 10,0, а наименьшим локус TGLA 126 с числом эффективных аллелей – 2,3. Анализ генетического разнообразия показал, что наибольшая наблюдаемая гетерозиготность наблюдалась локусе TGLA 53 – 0,950, а наибольшая ожидаемая в локусе CSSM 66– 0,860. Средний уровень наблюдаемой гетерозиготности составлял 0,783, ожидаемой – 0,691, индекс фиксации Райта имел величину равную минус 0,133, что указывало на высокий уровень генетического разнообразия стада герефордского скота.

**Ключевые слова:** микросателлиты, полиморфизм, генетической структуры стада, локус, индекс фиксации райта, аллель, гетерозиготность, герефордская порода.

molecular level and use this information to increase the efficiency of genomic selection, which uses the connection between phenotype and genotype (Sedykh et al., 2014; Chasovshchikova et al., 2017). Microsatellites are DNA markers that are used to test paternity, verify the lineage of livestock and analyze the connection with economically significant traits and genetic diseases (Ashoory et al., 2015; Hayes et al., 2009). The popularity of microsatellite markers can be explained by their uniform distribution in the genome, high polymorphism, Mendelian codominant inheritance and high reproducibility of the results of their analysis (Stevanovic et al., 2009; Onischuk et al., 2016). Despite the fact that the microsatellite analysis is generally accepted for both individual and population classification of animals, Russian cattle breeds have almost no identification with the use of microsatellite loci (Gladyr et al., 2011a; Kenijz et al., 2018). Nevertheless, the work to remedy this situation has already begun and is successfully being carried out in different regions of Russia including the Tyumen Region (Koshchaev et al., 2018b; Sulimova et al., 2016; Anisimova et al., 2018).

**Aim.** Characterization of the genetic structure of a Hereford herd using STR loci of the DNA nucleotide sequences.

## Materials and methods

Heifers of the Hereford breed ( $n = 30$ ) were used as the object of the research. Studies of cross-satellite STR loci were carried out in 2017 at “Bizon” LLC, Tyumen Region. Samples of

animal ear tissue were used as the material for the study. Genomic DNA was isolated according to generally accepted methods (phenol, salt) and amplification was performed using a ProFlex™ 96-Well PCR System. Fragments were identified using PCR analysis of microsatellite loci, followed by detection of fluorescently labeled fragments with the use of capillary electrophoresis using an Applied Biosystems 3500 Thermo Fisher genetic analyzer. Analyses were carried out in the Molecular Genetic Laboratory of the State Agrarian University of Northern Trans-Urals (Tyumen).

We used a set of markers for analysis that included 15 microsatellites – BM 1818, BM 1824, BM 2113, CSRM 60, CSSM 66, ETH 3, ETH 10, ETH 225, ILST 006, INRA 023, SPS 115, TGLA 53, TGLA 122, TGLA 126, TGLA 227. During the study, the following indicators were calculated: the frequency of alleles ( $p$ ) (Starostina et al., 1997; Nesterenko et al., 2019b), the number of effective alleles or the level of polymorphism ( $A_e$ ) (Sulimova et al., 2016; Plutakhin et al., 2016), the observed heterozygosity ( $H_o$ ) (Sobol et al., 2017; Kenijz et

al., 2019), the expected heterozygosity ( $H_e$ ) (Koshchayev et al., 2018a, Chernykh et al., 2017), Wright's fixation index ( $F_{is}$ ) (Nesterenko et al., 2017a, Nesterenko et al., 2017b). The resulting digital material was processed using the methods of variation statistics on a personal computer with the use of Microsoft Excel software.

## Results and discussion

During our study, we found a total of 104 alleles in 15 microsatellite loci in heifers of the Hereford breed and the size range was 79-300 bp. We also identified from 4.0 to 11.0 alleles in the studied STR loci. The average number of alleles per locus was 6.93. Of the 15 microsatellite loci, the most informative for the studied herd were the loci with the highest number of alleles (9 to 11) – TGLA 122, TGLA 227, TGLA 53, and CSSM 66. The least informative were the loci with the lowest number of alleles (4 to 6) – VM 1824, INRA 023, TGLA 126, BM 1818, CSRM 60, ETH 3, ETH 225. The analysis demonstrated that the allele frequency varied from 0.017 to 0.683 (Table 1).

**Table 1.** Allele frequencies for 15 microsatellites,  $p$ .

Locus	Allele	Frequency	Locus	Allele	Frequency
BM1818	260	0.083	ILST006	286	0.017
	262	0.233		288	0.383
	264	0.050		292	0.217
	266	0.583		294	0.250
	268	0.033		296	0.083
	270	0.017		298	0.033
BM1824	178	0.167	INRA023	300	0.017
	180	0.067		206	0.117
	182	0.667		208	0.150
	188	0.100		214	0.683
	125	0.067		216	0.050
	127	0.017		246	0.017
BM2113	131	0.100	SPS115	248	0.350
	133	0.117		252	0.083
	135	0.100		254	0.050
	137	0.050		256	0.183
	139	0.417		258	0.017
CSRM60	141	0.133	TGLA53	260	0.300
	92	0.033		154	0.033

	96	0.083		160	0.200
	98	0.117		162	0.233
	100	0.067		164	0.067
	102	0.667		168	0.067
	104	0.033		170	0.133
	179	0.017		172	0.200
	181	0.033		176	0.033
	183	0.067		184	0.017
	185	0.217		186	0.017
	187	0.167		141	0.167
CSSM66	189	0.167		143	0.383
	191	0.033		147	0.017
	193	0.150		151	0.250
	195	0.050	TGLA122	153	0.017
	197	0.067		159	0.017
	199	0.033		161	0.033
	115	0.017		167	0.017
	117	0.517		183	0.100
ETH 3	119	0.350		79	0.017
	121	0.017		81	0.083
	125	0.033		83	0.067
	127	0.067		87	0.050
	211	0.017	TGLA227	89	0.267
	213	0.017		91	0.283
	215	0.083		93	0.150
ETH10	217	0.233		97	0.067
	219	0.200		103	0.017
	221	0.433		140	0.267
	225	0.017		144	0.117
	115	0.433	ETH225	146	0.167
TGLA126	117	0.417		148	0.217
	121	0.033		150	0.217
	123	0.117		152	0.017

We found that five alleles in five different loci – 117 (ETH 3), 266 (BM 1818), 182 (BM 1824), 102 (CSRM 60), 214 (INRA 023) – had the highest frequency (from 0.517 to 0.683) and 21 alleles in 11 loci – 270 (BM 1818); 127 (BM 2113); 179 (CSSM 66); 115, 121 (ETH 3); 211, 213, 225 (ETH 10); 152 (ETH 225); 286, 300 (ILST 006); 246, 258 (SPS 115); 184, 186 (TGLA 53); 147, 153, 159, 167 (TGLA 122); 79, 103 (TGLA 227) – had the lowest frequency (0.017).

To assess the level of polymorphism, the number of effective alleles was calculated. The smaller the number of effective or active alleles, the lower the genetic diversity of the population. Calculations demonstrated that the number of active alleles ranged from 2.3 in the TGLA 126 locus to 10.0 in the TGLA 53 locus. The average polymorphism level of the analyzed STR loci was 4.96 (Table 2).

**Table 2.** Characterization of polymorphism of STR locus microsatellites

Locus	Allele	Number of alleles per locus	Number of effective alleles per locus (Ae)
BM 1818	260-270	6.0	4.5
BM 1824	178-188	4.0	3.0
BM 2113	127-141	8.0	3.6
CSRM 60	92-104	6.0	4.5
CSSM 66	179-199	11.0	6.3
ETH 3	115-127	6.0	4.5
ETH 10	211-225	7.0	5.0
ETH 225	140-152	6.0	4.7
ILST 006	286-300	7.0	4.3
INRA 023	206-216	4.0	3.5
SPS 115	246-260	7.0	3.5
TGLA 53	154-186	10.0	10.0
TGLA 122	141-183	9.0	8.0
TGLA 126	115-123	4.0	2.3
TGLA 227	79-103	9.0	6.7
$\bar{x}$	–	6.93	4.96
$S_{\bar{x}}$	–	0.52	0.49

We found that in the analyzed controlled animals, the number of alleles for five of the 15 identified STR loci was higher than the average polymorphism level and for the remaining 10 it was lower than the average polymorphism level – from 2.3 to 4.7 alleles. In general, a high positive correlation was found between the total number of alleles in the locus and the number of effective alleles, the correlation coefficient was 0.794 ( $p < 0.001$ ). This implies that the more alleles in a locus, the higher the level of

polymorphism and the genetic diversity of the herd.

The degree of observed heterozygosity is an indicator of genetic variation in the population. This is due to the fact that heterozygotes carry different alleles. In addition to the observed heterozygosity, we calculated the expected heterozygosity, which more accurately describes the diversity of the studied population. The values of the observed and expected heterozygosity of the locus microsatellites in heifers of the Hereford breed are presented in Table 3.

**Table 3.** Heterozygosity of locus microsatellites

Locus	Observed heterozygosity (Ho)	Expected heterozygosity (He)	Fixation index (Fis)
BM 1818	0.750	0.595	-0.261
BM 1824	0.667	0.513	-0.301
BM 2113	0.688	0.767	0.104
CSRM 60	0.778	0.528	-0.474
CSSM 66	0.864	0.860	-0.051

ETH 3	0.778	0.604	-0.288
ETH 10	0.800	0.710	-0.126
ETH 225	0.909	0.793	-0.147
ILST 006	0.750	0.735	-0.020
INRA 023	0.714	0.495	-0.443
SPS 115	0.769	0.744	-0.034
TGLA 53	0.950	0.836	-0.136
TGLA 122	0.923	0.751	-0.230
TGLA 126	0.571	0.624	0.085
TGLA 227	0.833	0.807	-0.032
$\bar{x}$	0.783	0.691	-0.133
$S_{\bar{x}}$	0.026	0.031	-

The highest levels of observed heterozygosity were recorded for the following loci: TGLA 53 – 0.950, TGLA 122 – 0.923 and ETH 225 – 0.909; the lowest levels were recorded for the TGLA 126 locus – 0.571. At the same time, the highest expected heterozygosity was calculated for loci CSSM 66 – 0.860, TGLA 53 – 0.836 and TGLA 227 – 0.807 and the lowest expected heterozygosity was calculated for the INRA 023 locus – 0.495. The average observed heterozygosity was 0.783, while the expected heterozygosity was lower – 0.691.

To determine the deviation of the heterozygous genotypes from theoretically expected ones, Wright's fixation index was used, which had both positive and negative values – in the first case, it showed a shortage of heterozygotes and in the second, it indicated their excess. Analysis of the fixation index demonstrated that a shortage of heterozygotes was observed in the loci BM 2113 and TGLA 126, while the indices were low – 0.104 and 0.085, respectively. An excess of heterozygotes was observed in the remaining 13 loci, where the fixation index ranged from minus 0.474 for the CSRM 60 locus to minus 0.020 for the ILST 006 locus. Average fixation index value for 15 loci was minus 0.133, indicating an excess of heterozygotes.

Studies of microsatellite loci or STR (short tandem repeats) loci demonstrate that the DNA profiles are different for animals of the same breed in different herds. For example, according to A.T. Tyngoziyeva et al. (2017), the average number of alleles in 11 loci (that were analyzed in our study) in the Hereford populations of the

Republic of Kazakhstan was 11.8-12.2, while in one of the populations, there were 24 alleles in

the TGLA 122 locus and 15 alleles in the TGLA 227 locus.

In the studied Hereford herd, these loci were also the most informative, however, each had 9 alleles. At the same time, the average number of informative alleles and the number of alleles in the abovementioned loci in the animals of the Hereford breed brought to the Republic of Bashkortostan from Australia were more similar to the values recorded in the studied livestock. A.V. Garkovenko et al. (2018) reported that the number of alleles in the Australian animals of the Hereford breed was 7.0 in the TGLA 122 locus and 8.0 in the TGLA 227 locus and the average number of informative alleles per locus was 6.0. In turn, according to the number of alleles in the ETH10 and ETH225 loci, no significant differences between the studied the Hereford breed populations, including the one examined in this study, were observed (Koshchaev et al., 2016; Starostina et al., 1997; Tyngoziyeva et al., 2017).

The average level of polymorphism in the analyzed STR loci (which is an indicator of effective alleles) in the studied herd was 4.96 alleles. This is consistent with the data of E.A. Gladyr et al. (2011b) who reported that the average number of effective alleles in the Siberian Hereford breed population was 4.66. The genetic or allelic variation of the herd is characterized by the level of expected heterozygosity. Heterozygosity plays a



significant role in the adaptation of animals; the phenomenon of heterosis is associated with it. Heterozygosity in the studied herd was high—more than 0.691, in 9 out of 15 loci. Moreover, Wright's fixation index indicated a slight excess of heterozygotes (on average) for the analyzed STR loci, which shows a high level of genetic diversity in the studied herd of Hereford cattle.

### Conclusion

The Hereford breed was characterized by the presence of 104 alleles in 15 STR loci, with 4 to 11 alleles per locus. The average number of informative alleles per locus was 6.93, while the number of effective alleles was 4.96, or 71.6%. An increase in the number of alleles in the locus was accompanied by an increase in the level of polymorphism. The TGLA53 locus was characterized by the highest polymorphism level, the number of effective alleles was 10.0 and the TGLA 126 locus had the lowest polymorphism level with an of 2.3 effective alleles. Genetic diversity of the studied herd was high. The average level of observed heterozygosity was 0.783, the expected heterozygosity was 0.691 and Wright's fixation index was minus 0.133.

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