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## **ASSESSMENT OF GENETIC DIVERSITY IN THE POPULATION OF CHAROLAIS CATTLE OF UKRAINIAN SELECTION USING MICROSATELLITE MARKERS**

**Yuriy LYASHENKO**, Candidate of Agricultural Sciences, Senior Researcher,  
<https://orcid.org/0000-0003-2747-476X>

**Volodymyr MARCHUK**, post-graduate student

**Livestock Farming Institute of NAAS of Ukraine, Kharkiv, Ukraine**

*The article presents the results of a study of the genetic-population structure in a herd of beef-type Charolais breed cows of Ukrainian selection (SEEF "Hontarivka", Kharkiv region). Genetic variation analysis in the experimental group of animals was performed using 10 microsatellite loci recommended by FAO-ISAG: ETH225, BM2113, ETH3, BM1818, BM1824, ILSTS006, INRA023, TAGLA053, TAGLA122, ETH10. The amplification products were separated in native polyacrylamide gels. All the loci studied were polymorphic. The number of detected alleles per locus ranged from 2 (ETH10) to 10 (TGLA053) (an average of 5 alleles per locus), the size of which ranged from 117 bp (ETH3) to 307 bp (ILSTS006). The vast majority of the studied loci belong to informative and valuable markers ( $PIC > 0.5$ ). The most polymorphic loci were TGLA053 ( $PIC = 0.81$ ) and INRA023 ( $PIC = 0.72$ ). The main population and genetic parameters for the studied loci are calculated. The highest values of heterozygosity ( $H_e$ ) and effective allele count ( $n_e$ ) were inherent in the loci TGLA053 ( $H_e = 0.82$ ,  $n_e = 5.7$ ) and INRA023 ( $H_e = 0.73$ ,  $n_e = 3.8$ ). The minimum values of observed heterozygosity are set for loci ETH10 ( $H_o = 0.21$ ) and TGLA122 ( $H_o = 0.44$ ).*

*Most microsatellite loci are characterized by an equilibrium state between actual and expected genotype frequency indicators, and a likely deviation in the form of heterozygote deficiency was established only for the TGLA122 locus ( $F_{is} = 0.29$ ;  $p < 0.05$ ).*

*Changes in the genetic structure of the experimental population of Charolais cattle in comparison with data from previous years and populations from other regions of the world are analyzed. These results indicate a significant narrowing of genetic variability in the domestic Charolais population. This can have further negative consequences and requires replenishment of allelic diversity and control of genetic processes in breeding work using DNA markers.*

**Keywords:** microsatellites, polymorphism, population, Charolais, allele, genotype, heterozygosity



## ОЦІНКА ГЕНЕТИЧНОГО РІЗНОМАНІТТЯ В ПОПУЛЯЦІЇ КОРІВ ШАРОЛЕЗЬКОЇ ПОРОДИ УКРАЇНСЬКОЇ СЕЛЕКЦІЇ ЗА ВИКОРИСТАННЯ МІКРОСАТЕЛІТНИХ МАРКЕРІВ

**Юрій ЛЯШЕНКО**, кандидат сільськогосподарських наук, старший науковий співробітник, <https://orcid.org/0000-0003-2747-476X>

**Володимир МАРЧУК**, аспірант

**Інститут тваринництва НААН, Харків, Україна**

*У статті наведені результати дослідження генетико-популяційної структури в стаді корів м'ясного напрямку продуктивності шаролецької породи української селекції (ДПДГ «Гонтарівка», Харківської області). Аналіз генетичної мінливості в дослідній групі тварин проводили з використанням 10 мікросателітних локусів рекомендованих FAO-ISAG: ETH225, BM2113, ETH3, BM1818, BM1824, ILSTS006, INRA023, TAGLA053, TAGLA122, ETH10. Продукти ампліфікації розділяли в нативних поліакриламідних гелях. Всі досліджені локуси виявились поліморфними. Кількість виявлених алелів на локус коливалась від 2 (ETH10) до 10 (TAGLA053) (у середньому 5 алелів на локус), розмір яких знаходився в межах від 117 п.н. (ETH3) – до 307 п.н. (ILSTS006). Переважна більшість досліджених локусів належить до інформативно-цінних маркерів ( $PI > 0,5$ ). Найбільш поліморфними виявились локуси TAGLA053 ( $PI=0,81$ ) та INRA023 ( $PI=0,72$ ). Розраховано основні популяційно-генетичні параметри за досліджуваними локусами. Найвищі значення показників гетерозиготності ( $H_e$ ) і ефективної кількості алелів ( $n_e$ ) були властиві локусам TAGLA053 ( $H_e=0,82$ ,  $n_e=5,7$ ) та INRA023 ( $H_e=0,73$ ,  $n_e=3,8$ ). Мінімальні значення фактичної гетерозиготності встановлені для локусів ETH10 ( $H_o=0,21$ ) і TAGLA122 ( $H_o=0,44$ ).*

*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 TAGLA122 ( $F_{is} = 0,29$ ;  $p < 0,05$ ).*

*Проаналізовано зміни в генетичній структурі дослідної популяції корів породи шароле порівняно з даними попередніх років та популяцій з інших регіонів світу. Отримані результати свідчать про суттєве звуження генетичної мінливості у вітчизняній популяції шароле. Це може мати в подальшому негативні наслідки та потребує поповнення аельного різноманіття та контролю генетичних процесів в селекційно-племінній роботі за використання ДНК-маркерів.*

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

**Introduction.** Knowledge of the genetic diversity of cattle (cattle) is an important step for effective management of genetic resources in agriculture. Assessment of the genetic diversity of animals is carried out using molecular markers. Microsatellites are the most involved DNA markers for this type of research. Microsatellites (short tandem repeats, STR) are widely used for animal identification and genetic examination of origin, scientific support for breeding work, determination of the level of consolidation of created groups and the degree of genetic differentiation of populations (Debrauwere H. et al., 1997; Senan S. et al., 2014). Microsatellites can be used as a fairly subtle and effective tool for studying genetic variability, which allows monitoring processes, in particular, in artificially reproducible populations of farm animals (Shel'ov, 2017; Al-Jubori & Senkal,



2023) (Shel'ov, 2015; Mishra S. et al., 2017; Zhao J. et al., 2017).

To assess the biodiversity and genetic research of cattle, the International Society for animal genetics (ISAG) and the Food and Agriculture Organization of the United Nations (FAO) have proposed microsatellite panels and recommendations for their use in scientific research (ISAG/FAO, 2004; FAO, 2011). ISAG recommends 12 STR loci (TGLA53, TGLA122, TGLA126, TGLA227, ETH3, ETH10, ETH225, SPS115, INRA23, BM2113, BM1824, BM1818) based on dinucleotide repeats for testing and confirming paternity (Koskinen, 2006). According to the FAO recommendations, 30 microsatellite loci were identified for cattle (FAO, 2011).

The study of allelic polymorphism of cattle microsatellite loci began in the late 1990s. This development coincided with the introduction of specialized equipment, namely DNA analyzers such as Abi377, Abi3110, and their more modern versions. These analyzers enabled accurate fragment analysis of amplified segments. As a result, deviations in determining the number and size of microsatellite alleles were minimized. However, most domestic scientists of the NAAS system are deprived of the possibility of using such equipment and, at best, send samples for analysis abroad. An example of such works is the study of microsatellite variability of native cattle breeds of domestic selection, such as Ukrainian Gray (Shkavro et al., 2010), lebedinskaya (Shkavro et al.; 2018, Ladyka et al., 2019), Krasnaya stepnaya (Kramarenko et al., 2018), Southern Meat (Snegin et al., 2019, Kramarenko, 2019), Buffaloes (Dzitsiuk et al., 2020). Given the great desire to engage in research in this area, we searched for alternative methodological approaches. This possibility was found based on the use of native gel electrophoresis and detailed analysis of the conformational structure of the DNA molecules that are formed (Kulibaba & Liashko, 2016).

The paper presented for consideration is a continuation of the study of microsatellite polymorphism of cattle populations in the Kharkiv region (Liashko et al., 2024) and is devoted to the study of the genetic variability of one of the breeds of meat productivity – the Charolais breed. The versatility to adapt to a wide range of farming conditions has made Charolais a widely used cattle meat breed in the world. In Ukraine, populations of the Charolais breed were mainly concentrated in the experimental farm "Gontareva" of the Institute of animal husbandry of the National Academy of Sciences, LLC "Rachanskoe" of the Zhytomyr region and LLC "Agricor holding" of the Chernihiv region (Petrenko et al., 2016).

Purpose of the study. To assess the genetic diversity in the Charolais cattle herd *SEEF "Hontarivka"* (Kharkiv region), which is a specialized breeding farm for breeding this breed. To analyze the changes that occurred during the reproduction of the local Charolais population on the basis of reproductive and breeding management, as well as local isolation, data on the genetic diversity of the Charolais breed of the same farm, made in 2010, will be used (Shkavro et al.) and from other regions of the world (Sifuentes-Rincón et al., 2007; Putnova et al., 2011).

**Materials and methods.** The object of research was the number of cows of the meat direction of productivity of the sharolezh breed of Ukrainian selection, which was kept in the breeding reproducer of the State Enterprise Experimental Farm "Hontarivka" (Volchansky district, Kharkiv region) in 2018. The sample consisted of 30 individuals. DNA isolation was performed from hair follicles using the NeoPrep DNA reagent kit (Lab Neogene P.C., Ukraine).

10 microsatellite loci from the FAO-ISAG list were used to study microsatellite variability: ETH225, BM2113, ETH3, BM1818, BM1824, ILSTS006, INRA023, TAGLA053, TAGLA122, ETH10.

Amplification of fragments of the studied loci was performed using the Amply-4



thermal cycler (Biocom, Russia) according to the appropriate program: 1 cycle – denaturation 94°C 3 min; 35 cycles – denaturation 94°C 20 s, annealing 30 s (56-62 °C depending on the locus), elongation 72°C 55 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 MgCl<sub>2</sub>, 0.4 mM DNTP mixture and 0.5 units. DreamTaq DNA polymerase (Thermo Scientific)), 2.5 µL of 1 pM primer and 2.5 µL of DNA Matrix.

Amplification products were separated in native polyacrylamide gels of various concentrations (5-8 %). Gel staining was performed using ethidium bromide (visualization was performed in the ultraviolet spectrum) or silver nitrate. Genotyping of individuals based on a set of microsatellite markers was performed according to the method of assessing the conformational structure of DNA under the conditions of native PAAG electrophoresis based on the use of available equipment (Kulibaba & Liashko, 2016). This makes it possible 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 size of amplified fragments.

The fragment size was determined using molecular weight markers pUC19 and O'RangeRuler 20 bp (Thermo Scientific, USA). Molecular weights of amplification products were calculated using the GelAnalyzer program (version 2010a freeware). Control of the software calculation of the size of electrophoregram fragments was performed using a millimeter ruler on a monitor screen.

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

**Research results.** Based on the results of the conducted studies, it was found that all microsatellite loci that were used in experimental animal populations are polymorphic (the proportion of polymorphic loci was 100 %). The number of detected alleles ranged from 2 (ETH10) to 10 (TGLA053) per locus. The analysis of the obtained results of genotyping of individuals allowed us to identify a total of 50 alleles for 10 microsatellite loci (an average of 5 alleles per locus), the size of which ranged from 117 bp (ETH3) to 307 bp (ILSTS006) (table 1).

Table 1

**Allele frequencies in the study population at 10 loci**

locus	Allele frequency (allele (bp))-frequency)									
ETH10	216-0,89					218-0,11				
BM1824	190-0,15			194-0,46			196-0,39			
BM1818	266-0,08		268-0,63		276-0,19		278-0,10			
ILSTS006	291-0,11		295-0,28		301-0,11		307-0,50			
BM2113	125-0,22	127-0,09		135-0,17		137-0,50		139-0,02		
ETH3	117-0,65	119-0,10		121-0,04		125-0,17		127-0,04		
TGLA122	148-0,56		152-0,03		156-0,17		160-0,17		172-0,07	
ETH225	140-0,02	144-0,05		146-0,02		150-0,22	152-0,60		154-0,09	
INRA023	199-0,04		203-0,18		205-0,40		211-0,04	215-0,25		219-0,09
TGLA53	154-0,11	158-0,04	160-0,07	168-0,06	170-0,03	174-0,17	178-0,04	180-0,33	182-0,11	190-0,04



The highest level of polymorphism in the study population was found at the TGLA53 locus (10 alleles). However, the frequency of occurrence of only 2 alleles of this locus was 0.17 and 0.33, and for 6 out of 10 alleles, the frequency value was in the range of 4-7%, which affected the value of the effective number of alleles ( $n_e = 5.68$  (57) (table. 2).

Table 2

**Main genetic and population indicators in the experimental group of cattle by microsatellite markers**

Locus	Indicators						
	$N_a$	$n_e$	$H_o$	$H_e$	$F_{is}$	$\chi^2$	PIC
ETH225	6	2,38	0,60	0,58	-0,03	0.071	0,46
BM2113	5	2,98	0,63	0,66	0,05	0.124	0,62
ETH3	5	2,15	0,51	0,54	0,06	0.185	0,50
BM1818	4	2,23	0,57	0,55	-0,04	0.079	0,51
BM1824	3	2,59	0,58	0,61	0,05	0.145	0,54
ILSTS006	4	2,84	0,62	0,65	0,05	0.128	0,63
INRA023	6	3,76	0,75	0,73	-0,03	0.045	0,72
TGLA53	10	5,68	0,79	0,82	0,04	0.08	0,81
TGLA122	5	2,65	0,44	0,62	0,29	5.06*	0,58
ETH10	2	1,24	0,21	0,20	-0,05	0.15	0,18
Average	5,0	2,85	0,57	0,60	0,04	0.61	0,56
Error	0.68	0.37	0.05	0.05	0.03	1.56	0.05

Notes:  $N_a$  - number of alleles,  $n_e$  – effective number of alleles,  $H_o$  – observed heterozygosity,  $H_e$  – expected heterozygosity,  $F_{is}$  – Wright fixation index, PIC - information polymorphism Index, \* -  $P < 0.05$ .

For 6-allele loci, the most aligned in allele frequencies (0.17 to 0.06) and the maximum value of their effective number ( $n_e = 3.8$  (63 )) was locus INRA023. among loci with 5 alleles, the most uniform distribution of allele frequencies was observed for locus BM2113 (0.2 to 0.075), of which 60 % ( $n_e = 2.98$ ) can be considered effective. Higher  $n_e$  scores occurred for the less polymorphic loci ILSTS006 (2.84 and 71% of effective alleles) and BM1824 (2.59 and 86%, respectively) (Table 1). 2).

Analysis of the distribution of expected heterozygosity ( $H_e$ ), as one of the main indicators of genetic variation in the population, revealed the average level of the studied trait for a set of microsatellite loci ( $H_e = 0.6 \pm 0.05$ ). High values of expected heterozygosity were established for loci TGLA053 ( $H_e = 0.82$ ) and inra023 ( $H_e = 0.73$ ). The lowest level of variability in the study population was observed at the two-allele ETH10 locus ( $H_e = 0.2$ ), which coincides with the actual number of heterozygous individuals ( $H_o = 0.21$ ) and corresponds to the normal Hardy-Weinberg distribution ( $\chi^2 = 0.15$ ; table. 2).

The vast majority of microsatellite loci are characterized by an equilibrium state between actual and expected indicators ( $\chi^2 = 5.1$ ;  $p < 0.05$ ), which was caused by an excess of homozygous individuals ( $F_{is} = 0.29$ ). The average value of the Wright fixation index gives reason to believe that an independent state of genotype distribution occurs in the experimental cow population ( $F_{is} = 0.04 \pm 0.03$ ; table. 2).

Based on the results of the conducted studies, it was found that most of the studied loci belong to informatively valuable markers (average PIC value= $0.56 \pm 0.05$ ). The exception is loci ETH10 (PIC = 0.18) and ETH225 (PIC = 0.46).

The results of the analysis prove the possibility of using 9 out of 10 SSR markers



for certification, identification and confirmation of the origin of individual individuals within the studied cattle populations.

**Discussion.** To analyze the changes that occurred during the reproduction of the experimental Charolais population, we used data on the genetic diversity of the Charolais breed from the same farm, made in 2010 (Shkavro et al., 2010), as well as populations from other regions – Mexico (two populations, Sifuentes-Rincón et al., 2007) and the Czech Republic (Putnova et al., 2011). Table 3 shows data on indicators of genetic variability of Charolais populations (total number of alleles at the locus ( $N_a$ ) and expected heterozygosity ( $H_e$ )), the analysis of which allows us to draw certain conclusions regarding the assessment of genetic processes that occur in artificially reproduced animal populations.

Table 3

**Indicators of genetic variation in Charolais populations from different regions**

Locus	Population, variability indicators									
	Char1		Char2		Char3		Char4		Char5	
	$N_a$	$H_e$	$N_a$	$H_e$	$N_a$	$H_e$	$N_a$	$H_e$	$N_a$	$H_e$
<i>ETH225</i>	6	0,58	5	0.51	5	0.74	-	-	-	-
<i>BM2113</i>	5	0,66	5	0.67	7	0.82	8	0.80	9	0.78
<i>ETH3</i>	5	0,54	5	0.53	5	0.55	-	-	-	-
<i>BM1818</i>	4	0,55	-	-	7	0.78	-	-	-	-
<i>BM1824</i>	3	0,61	3	0.66	5	0.73	6	0.80	8	0.78
<i>ILSTS006</i>	4	0,65	-	-	10	0.88	-	-	-	-
<i>INRA023</i>	6	0,73	6	0.76	10	0.78	13	0.87	13	0.88
<i>TGLA53</i>	10	0,82	10	0.83	-	-	13	0.91	14	0.88
<i>TGLA122</i>	5	0,62	-	-	11	0.74	-	-	-	-
<i>ETH10</i>	2	0,20	2	0.19	4	0.15	3	0.48	5	0.40
<i>TGLA226</i>	-	-	3	0.53	5	0.58	-	-	-	-
<i>TGLA227</i>	-	-	6	0.81	9	0.84	-	-	-	-
<i>SPS115</i>	-	--	5	0.62	5	0.51	-	-	-	-

Notes. Char1-own data; Char2-Shkavro et al., 2010; Char3-Czech population, Putnova et al., 2011; Char4-Mexican (import from France), Char5 – Mexican (import from France, Great Britain and Ireland), Sifuentes-Rincón et al., 2007;  $N_a$ -number of alleles,  $H_e$ -expected heterozygosity

It should be noted that it was not possible to analyze polymorphism for all the studied loci due to the fact that the authors used different microsatellite panels. 7 common loci were found to compare Char1-Char2, 9 for Char1-Char3, 5 for Char1-Char2 (Char3). In addition, it is incorrect to compare the size of alleles and their frequencies for the Ukrainian Charolais population (Char1-Char2), but comparing data obtained using DNA analyzers (Char2-Char5) is of interest.

Analysis of the genetic structure of the Ukrainian population of Charolais cattle during 2010-2018 (possibly another period of time in the absence of information about the year of birth of cattle of the experimental livestock) revealed the absence of significant changes in the main indicators. The total number of Char1-Char2 alleles was 37 and 36 (average 5.3 and 5.1, respectively), and the average level of expected heterozygosity ( $H_e$ ) in both groups of cattle was 0.59. The absence of changes in the genetic variability of the experimental population may be due to the peculiarity of the reproduction scheme of this breed based on the use of a limited number of breeding bulls in the breeding core.

The results of assessing genetic variability in Charolais populations from other regions confirm our assumption. If we compare the data with the Czech population



(Char1-Char3, table.3), we have a significant advantage of Char3 both in the total number of alleles 64 versus 40 (Char1) (on average 7.1 and 4.4, respectively) and in the average level of heterozygosity ( $H_e^{Char3} = 0.69$ ;  $H_e^{Char1} = 0.57$ ).

For the Mexican population of Char4 (imported Charolais animals from France), the indicators of genetic variation at 5 common microsatellite loci have even higher values:  $N_a^{Char4} = 43$  (medium 8.6) and  $H_e^{Char4} = 0.77$  compared to the Ukrainian population ( $N_a^{Char1} = 26$  (5.2) and  $H_e^{Char1} = 0.60$ ). A similar trend is observed for another Mexican population of Char5, created on the basis of Charolaise animals imported from France, Great Britain and Ireland ( $N_a^{Char5} = 49$  (average 9.8),  $H_e^{Char5} = 0.74$ ).

Let's consider how the qualitative composition of alleles for the studied loci changed in the Ukrainian population of the Charolais breed. We are talking about the frequencies of the most common alleles inherent in the original forms of French breeding (Table 4).

*Table 4*

**The most common alleles in Charolais cow populations**

Locus	Population			
	Char2	Char3	Char4	Char5
<i>BM2113</i>	131, 135, <u>137</u>	131	131	<u>133</u> , 135
<i>TGLA53</i>	166, <u>170</u>	-	151, <u>153</u> , 157	155, <u>157</u> , 159
<i>INRA023</i>	200, <u>206</u> , 214	206	<u>203</u> , 205, <u>207</u>	199, <u>203</u> , 205
<i>BM1824</i>	178, 182, 188	182	178, 182	180, 182, 184

*Notes. Char2– Shkavro et al., 2010; Char3-Czech population, Putnova et al., 2011; Char4-Mexican (import from France), Char5 –Mexican (import from France, Great Britain and Ireland), Sifuentes-Rincón et al., 2007; lower underscore-allele with the highest frequency*

According to the BM1824 locus, the 182 bp allele is the most common in all populations. However, out of 5-8 alleles of BM1824, 3 remained in the Ukrainian population. a similar situation is observed for loci INRA023 (high frequency of occurrence of the INRA023<sup>206bp</sup> allele and a decrease in variability from 10-13 alleles to 6), BM2113 (predominance of the BM2113<sup>131bp</sup> allele, a decrease in the number of alleles from 7-9 to 5). The lowest genetic variability among all populations was observed for ETH10. Of the 6 known alleles of this locus, two ETH10<sup>217bp</sup> and ETH10<sup>219bp</sup> remained in the Ukrainian population and completely lost alleles, which were most common in the populations of French origin Char4 (ETH10<sup>215bp</sup>) and Char4 (ETH10<sup>211bp</sup>), as well as two alleles detected in the Czech Charolais population (ETH10<sup>221bp</sup> and ETH10<sup>223bp</sup>).

A significant decrease in the variability of these loci may be due to the peculiarities of reproductive and breeding management in the domestic Charolais population, which, according to Shkavro et al. (2010), for a long time bred "by itself" due to the lack of "blood flow", which usually occurs when purchasing new breeding bulls and using them in the herd reproduction system.

**Conclusions**

1. Data from the analysis of 10 microsatellite DNA markers in the population of cattle of the meat direction of productivity of the Charolais breed of Ukrainian selection are obtained. The proportion of polymorphic loci was 100 %. The number of detected alleles per locus ranged from 2 (ETH10) to 10 (TGLA053) and averaged 5 alleles per locus.

2. The average level of genetic variability for the set of microsatellite loci in the experimental population was established ( $H_e=0.6\pm0.05$ ). High values of expected heterozygosity are characteristic of loci TGLA053 ( $H_e = 0.82$ ) and INRA023 ( $H_e = 0.73$ ),



while low values are characteristic of locus ETH10 ( $H_e = 0.20$ ).

3. Most microsatellite loci are characterized by an equilibrium state in the distribution of genotype frequencies. The deviation was found only at the TGLA122 locus ( $\chi^2 = 5.1$ ;  $p < 0.05$ ) caused by the excess of homozygous individuals ( $F_{is} = 0.29$ ).

4. Most of the studied loci belong to informatively valuable markers (average value  $PIC = 0.56 \pm 0.05$ ), indicating the possibility of their use for certification, identification and confirmation of origin in the experimental animal population.

5. Analysis of genetic changes that occurred during the reproduction of experimental populations of the Charolaise breed in comparison with data from other regions of the world indicates a narrowing of genetic variability, which in the future may have negative consequences and requires replenishment of allelic diversity and control of genetic processes in breeding work using DNA markers.

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