GENETIC VARIATION DETERMINATION AND INTERBREED DIFFERENTIATION OF TWO UKRAINIAN DAIRY CATTLE BREEDS USING MICROSATELLITE LOCI OF DNA

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Aim. The aim of our work was to investigate the interbreed differentiation of Ukrainian Red-and-Motley and Black-and-White dairy cows based on the results of the analysis of allelic and genotypic polymorphism of DNA microsatellites. **Methods.** Genotyping of 88 DNA samples of two most numerous dairy cattle breeds in Ukraine – Ukrainian Red-and-Motley and Black-and-White dairy cows – was conducted by 10 loci, recommended by the International Society for Animal Genetics (ISAG). The analysis of allelic and genotypic polymorphism was performed using parametric and non-parametric methods. **Results.** Informative value of DNA microsatellites as markers of genetic processes, which take place in the populations of domestic animals, was assessed. **Conclusions.** The investigated breeds demonstrate a reliable level of genetic differentiation with a high level of similarity.

Keywords: cattle, DNA-markers, microsatellites, biodiversity, populational genetics.

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INTRODUCTION

The estimation of interbreed differentiation of the most numerous cattle breeds in Ukraine is one of the most relevant elements of breeding in domestic animal farming and the preservation of local breeds. One of the most conclusive directions of estimating this characteristic is the analysis of genetic diversity.

DNA microsatellites – highly polymorphic multilocus genetic systems – are highly informative markers of the degree of genetic differentiation for the populations of domestic animals [1, 2, 4]. Almost all the microsatellites are located in qualitative trait loci (QT) or are related to genes, connected to reproduction processes [3].

Microsatellites of DNA have been used for the analysis of genetic diversity of many cattle breeds including Northern breeds [5], breeds of Central [6] and Eastern Europe [7], breeds of India [8], Africa [9], Korea [10] and Southern-Eastern Asia [4], including those of Indonesia [11].

The most common cattle breeds in Ukraine are Ukrainian Red-and-Motley (URM) and Ukrainian Black-and-White (UBW) breeds. The main aim of this study was to analyze the genetic structure of the mentioned breeds and to determine the degree of their affinity and differentiation.

To this end, an analysis was made of allelic and genotypic polymorphism of microsatellites.

MATERIALS AND METHODS

The study was conducted using a population ($n = 88$ heads) of Ukrainian Red-and-Motley dairy cattle breed (URM; $n = 45$ heads) and Ukrainian Black-and-White dairy cattle breed (UBW; $n = 43$ heads), kept at the Voronkiv farm in Boryspil district, Kyiv region.

Blood was sampled under sterile conditions from the jugular vein using double-ended needles Venoject

and vacuum tubes and holders Venosafe (Terumo, Belgium) following the standard method in accordance to the manufacturer's recommendations.

DNA isolation from blood samples was conducted using the DNA-sorb-B kit (Amplisense, Russia) according to the manufacturer's recommendations. The microsatellite analysis was performed using 10 loci (Table 1), recommended by the International Society for Animal Genetics (ISAG).

The polymerase chain reaction (PCR) was conducted using an ABI 2720 Thermal Cycler (Applied Biosystems, CA, USA). The reaction mixture for PCR was prepared according to the protocol, recommended by the manufacturer of the test-system (StockMarcs, Cattle Bovine Genotyping Kit (Applied Biosystems, CA, USA). The amplified DNA was separated by the method of capillary gel electrophoresis on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems, CA, USA). Data registration and mapping (genotyping) was performed using programs Run 3130 Data Collection v.3.0 and GeneMapper 3.7 of Applied Biosystems, CA, USA.

The frequencies of alleles and the genotypes were estimated, including one-time (N1), two-times (N2) observed and unique ones (Nunik). The comparison of breeds in terms of frequencies of alleles and genotypes was performed using the criterion χ^2 of K. Pearson (by Monte Carlo method for low frequencies) [12] using special software for population-genetic analysis – Ge-

nAIEx [13], BOTTLENECK [14], PopGene [15] and NetEstimator [16].

RESULTS AND DISCUSSION

The analysis of the allelic diversity of 10 microsatellite loci demonstrated that the highest average number of the allelic variants was found in the population of URM breed – 9.5 alleles/locus, whereas the same index for UBW breed was 9.2 alleles/locus. The values of the effective number of alleles were 6.757 and 6.023 alleles/locus, respectively. In our study each breed was characterized by the highest number of alleles in three microsatellite loci out of the 10 investigated ones, namely, URM – in loci *TGLA126*, *BM2113* and *SPS115*, and URM – in loci *INRA23, BM1824* and *TGLA227*. The differences between breeds did not exceed two alleles. In the remaining loci, the number of allelic variants was the same for the two breeds.

The broadest allelic spectrum was observed for the breed with the highest number of alleles. Loci with a similar number of allelic variants had the same allelic spectrum, except for locus *ETH3* that had a wider one. The obtained estimates of the criterion χ^2 of K. Pearson allow stating highly reliable (P < 0.001) differences in the distribution of the investigated population by the allelic frequencies in all the investigated loci.

Unique alleles were registered in seven out of ten investigated microsatellite loci, seven (30 %) for URM breed and five (22 %) – for UBW. A quite similar distribution was found for the number of loci with

Table 1. The number of alleles, including rare ones, calculated using the rarefaction-method ($n = 25$) for 10 microsatellite loci of two cattle breeds, Ukrainian Red-and-Motley (URM) and Ukrainian Black-and-White (UBW)

Locus	Breed			
	URM		UBW	
	number of alleles	inc. rare ones	number of alleles	inc. rare ones
TGLA126	7.45	0.14	5.83	0.04
TGLA122	10.39	1.43	10.11	0.65
INRA23	10.50	0.20	11.26	0.99
ETH3	6.80	1.00	6.92	0.45
ETH225	7.96	0.002	7.89	0
BM1824	9.78	0.05	9.95	0.58
TGLA227	11.67	1.85	13.40	2.45
BM2113	10.82	1.45	8.44	0.09
ETH10	6.98	0	6.99	0
SPS115	8.55	1.84	6.99	0.001
M e a n	9.09 ± 0.555	0.80 ± 0.251	8.78 ± 0.746	0.53 ± 0.240
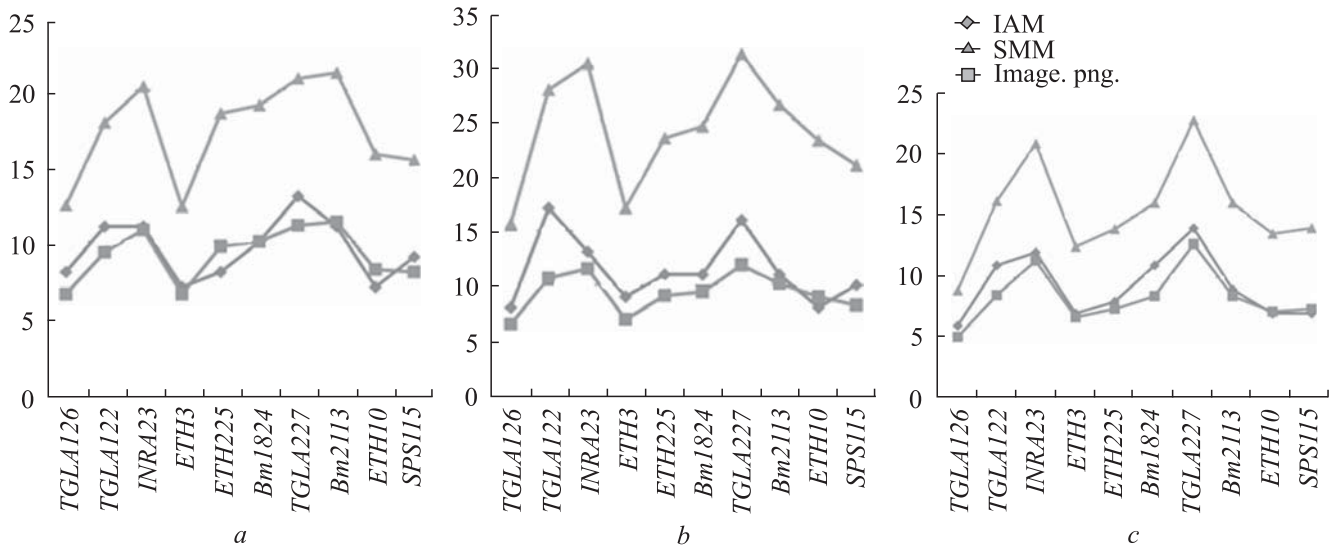
Fig. 1. Actual number of found alleles by DNA microsatellite loci, total (*a*) and for URM (*b*) and UBW (*c*), calculated using different mathematical models

unique alleles: the highest number (five, or 33 %) was found for URM breed, and the lowest number (four, or 27 %) – for UBW. In the populations of URM and UBW, two unique alleles were found in two and one loci (*TGLA227, SPS115* and *TGLA227*), respectively. It should be noted that only in loci *TGLA122* and *TGLA227* unique alleles were found in both investigated breeds. Unique alleles in loci *ETH3, BM2113* and *SPS115* were recorded for URM breed, and loci *INRA23* and *BM1824* – for UBW. In general, the frequency of unique alleles in URM breed (0.049) was almost twice higher than for UBW breed (0.026).

Two main models were used to analyze the character of the distribution of alleles of microsatellite DNA by specific loci – the infinite alleles model (IAM) [17, 18, 19] and the stepwise mutation model (SMM) [20, 21].

SMM was more adequate for both investigated breeds with the approximation of the level of allelic diversity in all the investigated microsatellite loci without exceptions, compared to IAM model (P < 0.0001). The latter exaggerates the actual values greatly both in general and for each breed, in particular (Fig. 1).

Taking into account our limited number of samples and limited number of loci studies where direct comparison of the obtained results is impossible, we used the rarefaction procedure. The number of alleles (calculated per 25 randomly selected diploid animals) varied greatly both for animals of different breeds and for different loci (Table 1). In general, URM breed had a higher total of alleles and more unique alleles were found than in UBW breed. The highest level of

allelic diversity, however, was observed for UBW in locus *TGLA227* (13.40 alleles), and the lowest – for the same breed in locus *TGLA126* (5.83 alleles). The highest number of unique alleles (2.45) was also found for UBW in locus *TGLA227*, and the lowest – for the same breed in the locus *SPS115* (0.01). The estimates, made using the rarefaction-procedure, prove the absence of unique alleles in both populations for locus *ETH10*, and as for UBW – for locus *ETH225* as well.

The highest number of genotype variants was found in URM, viz. four microsatellite loci out of ten investigated ones (*INRA23, ETH225, TGLA227, BM2113*), while for UBW this was found only for three loci – *TGLA122, ETH3* and *BM1824*. The number of genotype variants in loci *TGLA126, ETH10* and *SPS115* was the same for representatives of both breeds.

The analysis of the distribution character of the found genotype frequencies allows stating that URM and UBW breeds demonstrate a high level of similarity by this index (Table 2). There is also a high demonstrated level of polymorphism by the number and character of distribution of the frequencies of the established genotypes, which is proven by the value of the approximating model (the selected exponential function) and the degree of its adequacy and determination coefficient (Fig. 2).

In general, the number of rare genotypes in the representatives of the investigated breeds fluctuated from 11 (in locus *TGLA126*) to 45 (in locus *TGLA227*) (Table 2). The tendency of frequency distribution (the same loci were the most and the least polymorphic, *etc.*) was

found for the total number of genotypes, which were observed only once – from five (in locus *TGLA126*) to 33 (in locus *TGLA227*). The lowest number of genotypes, observed only twice each time (four genotypic variants), were found in locus *SPS115*, and the highest (15) – in locus *INRA23*. The lowest number of rare genotypes (two variants) determined for the representatives of URM in locus *ETH3*, and for UBW – in the locus *ETH225*. The highest number of rare genotypes (10 variants) was found for URM (in locus *TGLA122*), and as for UBW, this index was eight variants (in locus *BM1824*). The highest number of genotypes, observed once each time (24 variants), was found for locus *TGLA227* in URM, and the lowest (six variants) – in the same breed in the loci *TGLA126, TGLA122* and *ETH3*.

The analysis of the frequencies of unique genotypes showed that the lowest number of such genotypes (four genotype variants) was found in the loci *TGLA126* and *BM1824* in animals of both breeds and in the loci *ETH3* and *ETH225* – in UBW, and the highest (20 variants) – in locus *BM2113* in URM. The intrabreed analysis demonstrated that the average number of unique genotypes per locus in URM was 9.0 and fluctuated from four (in loci *TGLA126* and *BM1824*) to 20 (in locus *BM2113*). For UBW the average was 8.1 and fluctuated from four (in loci *TGLA126, ETH3, ETH225* and *BM1824*) to 17 (in locus *TGLA227*). It is noteworthy that no differences were found between the breeds in

the ratio of three out of ten investigated microsatellite loci, and there was only one genotype variant found in three more.

In general, the analysis of genetic variation in the two Ukrainian breeds of *Bos taurus* species demonstrated that all the investigated microsatellite loci were characterized by a high number of incongruous genotype variants, for instance, TGLA126$^{116/116}$, TGLA126$^{116/118}$, TGLA126$^{116/120}$, TGLA126$^{118/118}$, TGLA126$^{118/120}$, etc. Their total number in the animals of this species fluctuated from 21 (in loci *TGLA126*) to 53 (in locus *TGLA227*). The highest level of genotype polymorphism was found in loci *INRA23* and *TGLA227* in URM (32 variants) and UBW (31 variant). Locus *ETH3* was found to be the least polymorphic in URM (15 variants), and for UBW it was *TGLA126* (16 variants).
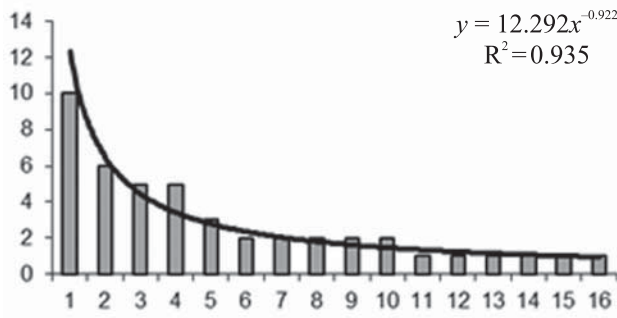
According to the *nonparametric* method of A. Chao, the use of which ensures more accurate comparison of the populations (groups of animals) of different size [22] in general, the distribution for the number of genotypes in the animals of different breeds is incongruous. For instance, in loci *TGLA126, INRA23, ETH3, ETH225, TGLA227, ETH10* and *SPS115* no reliable difference was found between the breeds, and in locus *BM2113* in URM, the potential number of genotypes was much higher than that for the representatives of the other breed.

The parametric rarefaction-procedure was also used to estimate the "possible genotype diversity". The ob-
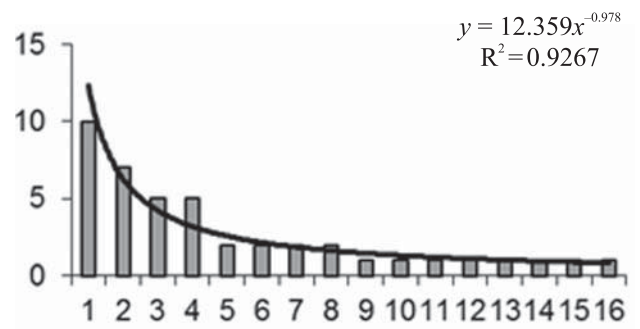
Table 2. The total number of genotypes, determined, rare and unique genotypes for 10 microsatellite loci of two Ukrainian cattle breeds

Locus	Breed							
	UR	URM	UR	URM	UR	URM	UR	URM
	N_a		N_1		N_2		N_{unik}	
TGLA126	16	16	6	8	5	3	4	4
TGLA122	21	25	6	14	10	6	11	12
INRA 23	32	31	23	22	7	6	12	12
ETH 3	15	18	6	8	2	5	5	4
ETH225	25	19	14	12	6	2	8	4
BM1824	24	25	15	13	3	8	4	4
TGLA227	32	31	24	22	5	7	14	17
BM2113	31	20	23	8	4	7	20	8
ETH10	22	22	10	12	5	4	5	10
SPS115	22	22	11	12	3	5	7	6
M e a n	24	22.9	13.8	13.1	5	5.3	9	8.1

$y = 12.292x^{-0.922}$
$R^2 = 0.935$

in locus *TGLA126* URM

$y = 12.359x^{-0.978}$
$R^2 = 0.9267$

UBW

$y = 6.2974x^{-0.555}$
$R^2 = 0.8249$

in locus *TGLA122* URM

$y = 5.5989x^{-0.566}$
$R^2 = 0.8686$

UBW

$y = 3.9335x^{-0.444}$
$R^2 = 0.83$

in locus *INRA23* URM

$y = 3.6667x^{-0.426}$
$R^2 = 0.819$

UBW

$y = 12.292x^{-0.922}$
$R^2 = 0.935$

in locus *ETH3* URM

$y = 12.359x^{-0.978}$
$R^2 = 0.9267$

UBW

$y = 6.4477x^{-0.618}$
$R^2 = 0.8967$
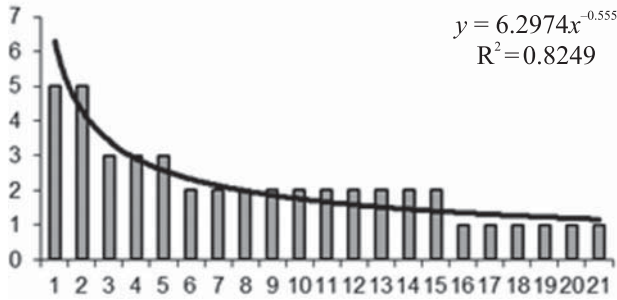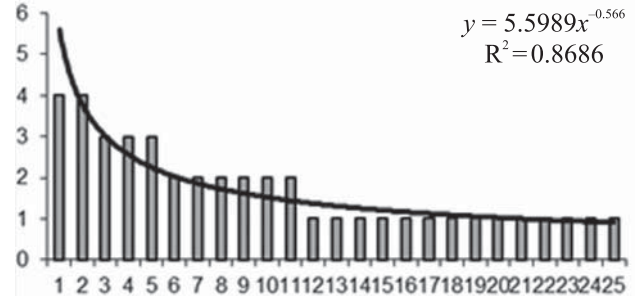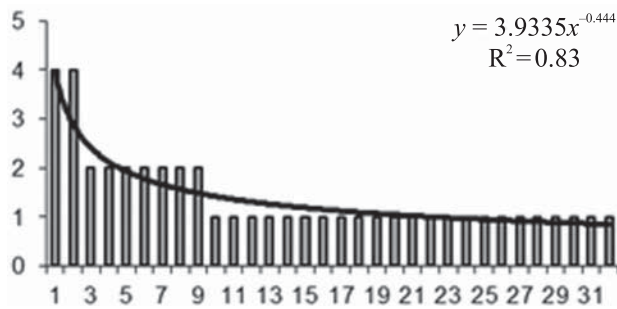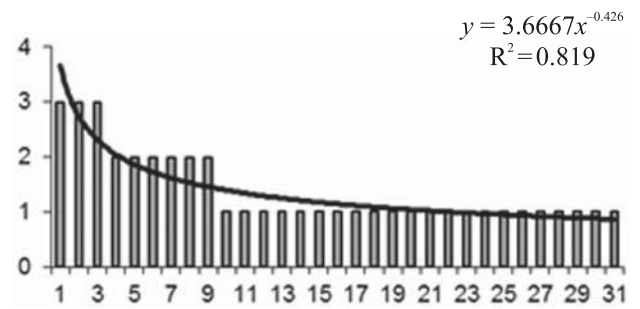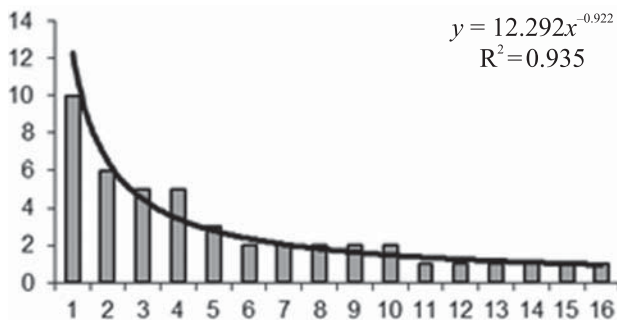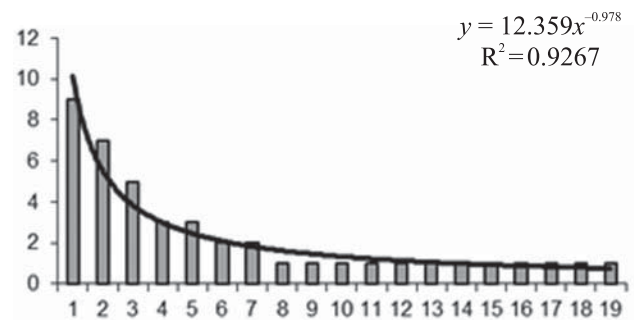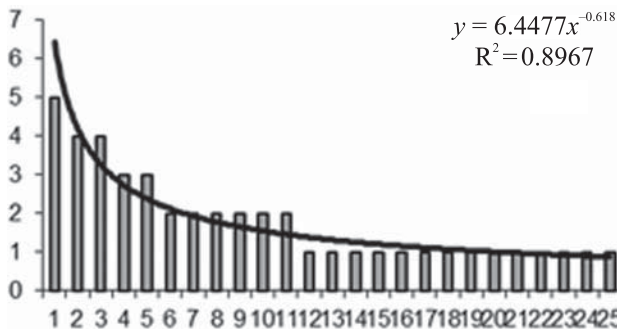
in locus *ETH225* URM

$y = 10.116x^{-0.88}$
$R^2 = 0.9103$
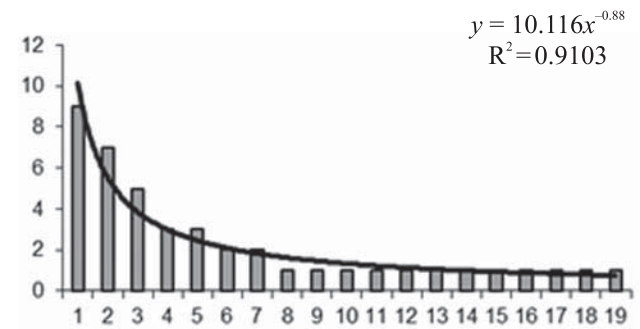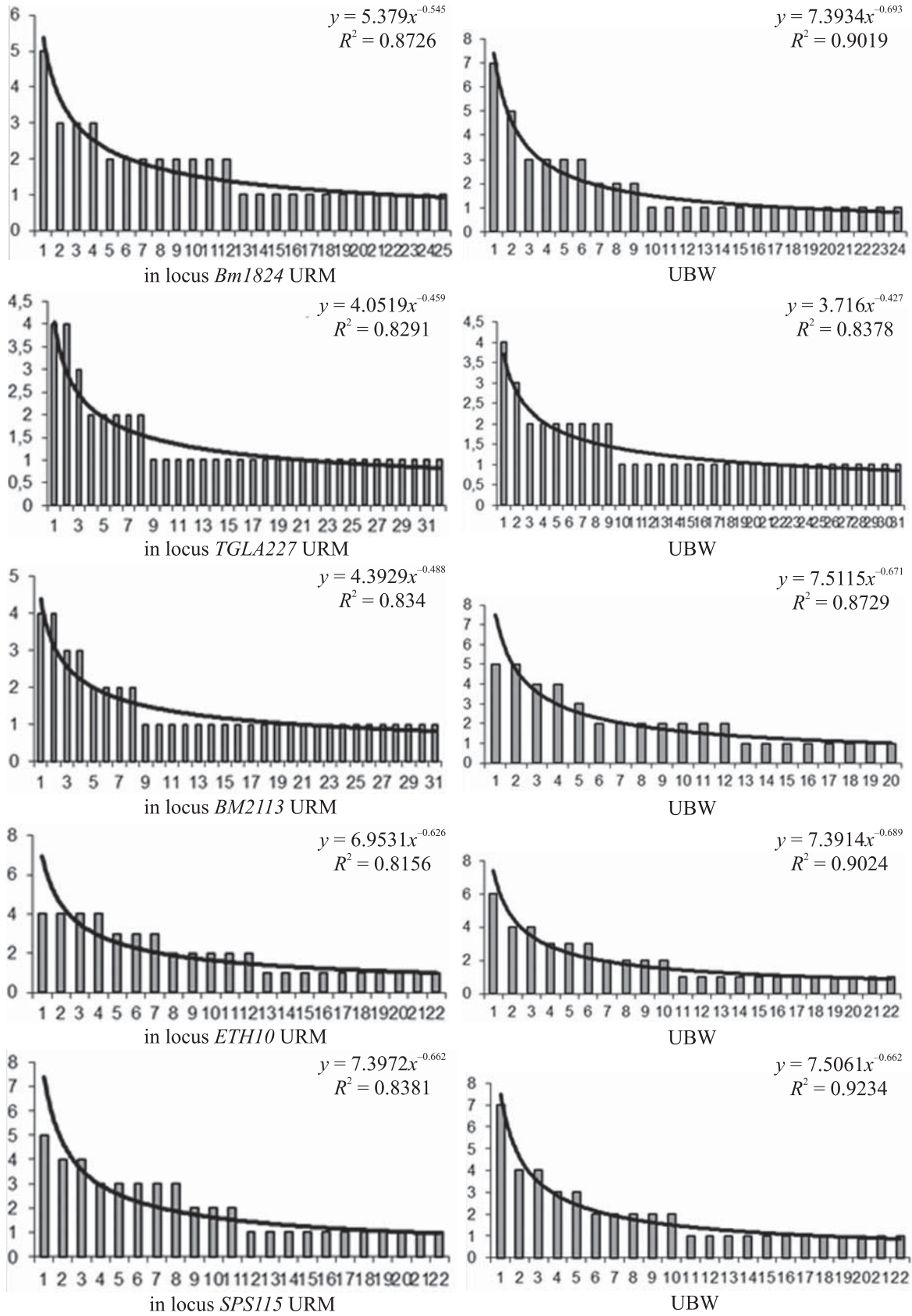
UBW

Fig. 2. Character of distribution for genotype frequencies in the investigated cattle breeds in 10 loci of microsatellite DNA, where *y* – approximating model (selected exponential function) and its adequacy level, *R*² – determination coefficient

tained data allow for the assumption that by the majority of loci both URM and UBW demonstrate high similarity in the distribution of genotype frequencies. For loci *TGLA126, INRA23, TGLA227, ETH10* and *SPS115* there was an observed tendency, similar to the general one, *i.e.* the distribution of frequencies in URM and UBW breeds was practically identical, in locus BM1824 it was very similar, and only for four loci (*TGLA122, ETH3, ETH225* and *BM2113)* the investigated animals demonstrated breed-specific character of the distribution of genotype frequencies in locus *TGLA126.*

CONCLUSIONS

Our analysis of the presented data allows the assumption that the investigated breeds (populations) demonstrated high level of identity with a simultaneous reliable level of genetic differentiation. This is confirmed with the observed differences by the character of distribution of frequencies of the found allelic variants (profiles of allelic polymorphism) (P < 0.001) and by the distribution of genotypes, which is proven by the estimates of the approximating model (the model of the exponential function) and the degree of its adequacy and determination coefficient.

High level of similarity between the investigated breeds, determined by the results of estimating «potential genotype diversity», obtained on the basis of the *rarefaction*-procedure, allows stating that by the prevailing majority of loci the animals of both breeds demonstrate high similarity regarding the distribution of genotype frequencies. For both breeds, the character of variability in the investigated microsatellite loci for both the ratio of observed alleles and the character of their distribution corresponds to the stepwise mutation model (*SMM*).

The results of estimating the indices of allelic (the highest number of found alleles, the number and distribution of breed-specific alleles) and genotypic variability (the number of found rare and breed-specific genotypes), confirmed by nonparametric methods (rarefaction-method and the method of A. Chao) demonstrate high level of genetic differentiation of the investigated breeds.

**Визначення генетичної варіативності
та міжпородна диференціація двох українських
молочних порід великої рогатої худоби
за мікросателітними локусами ДНК**

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Мета. Метою нашої роботи було дослідження між-породної диференціації українських червоно- та чорно-рябої порід молочної худоби за результатами аналізу алельного й генотипового поліморфізму мікросателітів ДНК. **Методи.** Генотипування 88 зразків ДНК двох найчисельніших в Україні молочних порід великої рогатої худоби – української червоно-рябої молочної та української чорно-рябої молочної – проводили за 10 локусами, рекомендованими Міжнародним товариством з генетики тварин (ISAG). Було здійснено аналіз алельного та генотипового поліморфізму із застосуванням параметричних та непараметричних методів. **Результати.** Вперше в Україні викладено результати аналізу алельного та генотипового поліморфізму популяцій українських червоно- та чорно-рябої молочної худоби за використання 10 мікросателітних локусів ДНК. Показано, що мікросателіти ДНК, як високополіморфні генетичні системи, є надзвичайно інформативними маркерами генетичних процесів, які мають місце в популяціях свійських тварин. **Висновки.** Досліджені породи демонструють достовірний рівень генетичної диференціації й, одночасно, високий рівень подібності, що, безсумнівно, пояснюється однаковими цілями та методами селекційної роботи з ними.

Ключові слова: велика рогата худоба, ДНК-маркери, мікросателіти, біорізноманіття, популяційна генетика.

**Определение генетической вариативности
и межпородная дифференциация двух украинских
молочных пород крупного рогатого скота
по микросателлитным локусам ДНК**

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Цель. Целью нашей работы было исследование межпородной дифференциации украинских красно- и черно-пестрой пород молочного скота по результатам анализа аллельного и генотипического полиморфизма микросателлитов ДНК. **Методы.** Было использовано 10 локусов, рекомендованных Международным обществом по генетике животных (ISAG), для анализа

88 образцов ДНК двух наиболее многочисленных в Украине молочных пород крупного рогатого скота – украинской красно-пестрой молочной и украинской черно-пестрой молочной. Был проведен анализ аллельного и генотипического полиморфизма с применением параметрических и непараметрических методов. **Результаты.** В работе изложены результаты анализа аллельного и генотипического полиморфизма популяций украинского красно- и черно-пестрого молочного скота с использованием 10 микросателлитных локусов ДНК. Показано, что микросателлиты ДНК, как высоко полиморфные мультилокусные генетические системы, являются сверхинформативными маркерами генетических процессов в популяциях домашних животных. **Выводы.** Исследованные популяции украинских молочных пород крупного рогатого скота показывают достоверный уровень генетической дифференциации и, одновременно, высокий уровень идентичности, что, несомненно, объясняется схожими целями и методами селекционной роботы с ними.

Ключевые слова: крупный рогатый скот, ДНК-маркеры, микросателлиты, биоразнообразие, генетика популяций.

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