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FORMATION OF THE GENETIC STRUCTURE OF CATTLE POPULATIONS BY SINGLE LOCUS DNA FRAGMENTS DEPENDING ON THEIR PRODUCTIVITY DIRECTION AND ORIGIN

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Aim. Our work was aimed at investigating the specificities in the formation of the genetic structure of populations depending on the productivity direction of cattle, bred in Ukraine, using single locus DNA fragments, and studying the impact of the parental form on genetic polymorphism of modern intensive specialized breeds as a factor. **Methods**. The following methods were used in the work: *veterinary methods* (peripheral blood sampling); *molecular-genetic methods* (the isolation and genotyping of DNA samples of dairy (83 animals), meat (192 animals) and aboriginal (43 animals) cattle, bred in Ukraine, were performed by 10 microsatellite loci from the list, recommended by the International Society for Animal Genetics (ISAG); the complex of statistics methods was used for *mathematic-statistical analysis,* using modern software. **Results.** The analysis by 10 microsatellite loci demonstrated the specificities of genetic differentiation and the similarities between the investigated cattle populations, bred in Ukraine. Our results provide new information about the impact of artificial selection factors on single locus DNA fragments under the specialization of cattle breeds. The impact of the factor of ancestral form on the genetic structure was determined and confirmed by the same polymorphism spectra of the investigated DNA fragments in the maternal and derivative breeds. Another confirmation was found in the differences, observed in animals of different productivity directions, which are a probable result of the breed-forming process, demonstrated by the results of the mathematic calculations of the data obtained. It was shown that microsatellite DNA loci are highly informative markers of genetic processes, occurring in domestic cattle populations. **Conclusions.** The specificities in the formation of the genetic structure of populations depending on the productivity direction of animals were determined. The impact of the parental form on genetic polymorphism of modern intensive specialized breeds was noted. It was found that among 10 microsatellite loci, used by us, there were loci in each group of animals, regarding which the hypothesis about their neutrality was reliably rejected according to the results of Ewens-Watterson test: for dairy cattle (*INRA023, ETH3, ETH225, BM1824, BM2113, ETH10* and *SPS115*), for meat cattle (*TGLA122* and *ETH225*), and for aboriginal cattle (*TGLA126, INRA023* and *TGLA227*). We determined a high level of genetic diversity, remarkable for each investigated cattle population, bred in Ukraine, and general tendencies of differentiation in the selected populations depending on the targeted breeding work, on the level of polymorphism of microsatellite DNA loci (Friedman's test: $P < 0.01$), and a similar genetic picture for a number of loci of investigated DNA fragments, which may be related to the history of creating these breeds.

Key words: cattle, DNA-markers, microsatellites, polymorphism, productivity direction.

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INTRODUCTION

The improvement of domestic animals for human needs depends on genetic diversity of the species (Weigend S et al, 2002). The criterion for such diversity is the availability of different species which are the main material for breeders and the foundation for the adjustment of domestic animals to our needs (Mackowski M

et al, 2015). Each breed has its own set of genes, formed under the impact of different factors of artificial and natural selection (Putnová L et al, 2019). Recently the problem of preserving domestic breeds and sustainable use of genetic resources has become one of the most relevant ones for most countries (Oppermann M et al, 2015; Sunnucks P, 2000; Pamilo P, Nei M, 1988).

To estimate the genetic structure and to study the dynamics of population genetic processes in the populations of domestic animals, many countries use the advantages of the methods of molecular-genetic analysis. Microsatellites – highly polymorphic genetic markers – are the main instrument for European investigators (Teneva A et al, 2018).

The analysis of microsatellite DNA sequences in different breeds is of interest because the study of this issue may facilitate the understanding of the evolution mechanisms, divergence dynamics for both wild and domesticated species, including the processes of breed-formation (Li MH et al, 2009). Microsatellite loci have non-randomized character of distribution, but their functional relevance, genetic and evolutionary mechanisms of formation are yet to be determined. Microsatellites are believed to be selectively neutral DNA fragments, not related to productive features. However, recently there is more and more information about the association between some microsatellite loci and specific productivity indices in domestic animals (Ciampolini R et al, 2002; Bressel RMC et al, 2003; Andrade PC et al, 2008; Komatsu M et al, 2011).

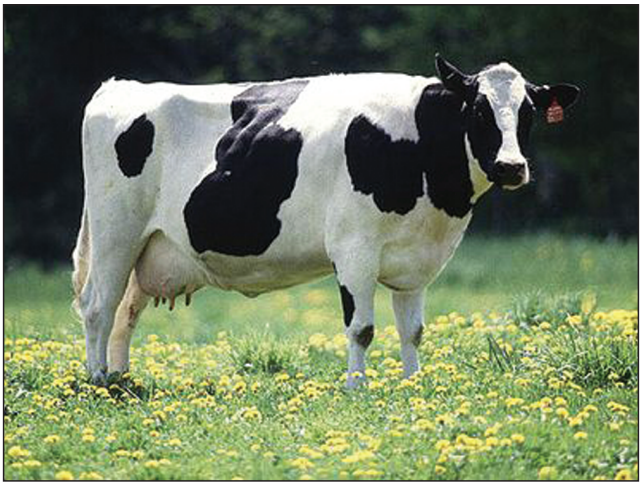
Contrary to our previous studies (Shelyov AV et al, 2017; 2018) this publication highlighted the specificities in the formation of the genetic structure of populations depending on the productivity direction of animals, which allowed us to combine animals of two Ukrainian dairy breeds into one population according to the productivity direction. The second important aspect of this study was to estimate the impact of the parental form on genetic polymorphism of modern intensive specialized breeds.

MATERIALS AND METHODS

The material of our study was the herd (n = 318 animals) of Ukrainian cattle, which belongs to dairy cattle (n = 83 animals), represented by Ukrainian Red-and-White dairy breed (n = 42 animals) and Ukrainian Black-and-White dairy breed (n = 41 animals), kept at Voronkiv farm, Boryspil District, Kyiv Region; meat cattle (n = 192 animals), represented by Southern Beef breed, kept at SE DG Askaniyske farm of the Askanian State Agricultural Experimental Station of the Institute of Irrigated Agriculture, the NAAS, in Kakhovka District, Kherson Region; and aboriginal cattle (n = 43 animals), represented by Gray Ukrainian breed, kept at Voronkiv farm, Boryspil District, Kyiv Region.

Ukrainian Red-and-White dairy breed

The molecular-genetic analysis was conducted in the Department of Genetics and Biotechnology of the M.V. Zubets Institute of Animal Breeding and Genetics, the NAAS of Ukraine, the Mykolayiv National Agrarian University, and the experimental part – at the Ukrainian Laboratory of Quality and Safety of Agricultural Products of the National University of Life and Environmental Sciences of Ukraine.



Ukrainian Black-and-White dairy breed

The following methods were used in the study.

Veterinary methods. The blood was sampled from the jugular vein using double-ended needles Venoject and vacuum tubes and holders Venosafe (Terumo, Belgium)

following the standard method in accordance with the manufacturer's recommendations in sterile conditions.

Southern Beef breed

Molecular-genetic methods. DNA isolation from blood samples was conducted using DNA-sorb-B kit (Amplisense, Russia) according to the manufacturer's recommendations. The microsatellite analysis was performed using 10 loci, recommended by the International Society for Animal Genetics (ISAG). The polymerase chain reaction (PCR) was conducted using AB 2720 Thermal Cycler (Applied Biosystems, USA). The reaction mixture for PCR was prepared according to the protocol, recommended by the manufacturer of the test-system (Stock Marcs, 2010). The amplified DNA was separated by the method of capillary gel electrophoresis at ABI Prism 3130 Genetic Analyzer (Applied Biosystems, USA). The registration of the obtained graphic results was done using programs Run 3130 Data Collection v.3.0 (Applied Biosystems, USA) and GeneMapper 3.7 (Applied Biosystems, USA).

Gray Ukrainian breed

Methods of mathematic-statistical analysis. The incidence of genotypes and alleles (*Na*), the effective number of alleles (*Ae*), observed (*Ho*) and estimated (*He*) heterozygosity and the inbreeding index (*Fis*) for specific microsatellite DNA loci were assessed for each sampling using *GenAIEx v. 6.5* (Peakall R et al, 2012). In addition, M-ratio was calculated for each breed and locus of microsatellites (Garza JC et al, 2001). The hypothesis on the absence of significant differences between the investigated groups of animals in terms of the incidences of rare and most common alleles was checked using Pearson's chi-square in *PAST* program (Hammer Ø et al, 2001). *PopGen* program was used to check the correspondence of the distribution of genotypes of each microsatellite DNA locus in each breed to the state of Hardy-Weinberg genetic equilibrium (*HWE*) based on the algorithm of *G*-test of maximal likelihood (Yeh FC et al, 1999). To check the hypothesis on the absence of significant differences in the applied indices of genetic diversity, we conducted Friedman's non-parametric disperse test using *PAST* program (Hammer Ø et al, 2001) for several breeds. The estimation of Wright's *F*-statistics (*Fis* and *Fst*) for each microsatellite DNA locus and each breed was done using *GenALEx v. 6.5* (Peakall R et al, 2012). The significance value of the deviation of the obtained estimates from zero was calculated using the exchange test with 999 exchanges. The assignment test, based on the distribution of the incidences of microsatellite multilocus genotypes (Paetkau D et al, 1995), was conducted for several groups of animals using *GenALEx* program (Komatsu M et al, 2011). The hypothesis on the presence of the "bottleneck effect" for different breeds in the past based on the use of three models (*IAM*, *SMM* and *TPM*) was checked using *BOTTLENECK v.1.2.03* program (Cornuet JM et al, 2001). The estimation of the mean correlation between alleles (r) and the number of reliable cases of linkage disequilibrium (*NLD*) between some alleles in 10 microsatellite DNA loci for different breeds, as well as the results of Ewens-Watterson test in terms of their neutrality was conducted using *PopGen* (Yeh FC et al, 1999). The effective number of the population for some groups of animals (*Ne/Neb*) was estimated using *NeEstimator v. 2.0* (Do C et al, 2014).

RESULTS

The analysis of animals from different cattle breeds by 10 microsatellite DNA loci demonstrated 138 allelic variants. The rate of allelic diversity of different breeds is presented in Table 1.

For different breeds, from one third to almost a half of the number of noted alleles were presented by very rare alleles (with the share of ≤ 0.050). Here the reliable differences (Pearson's chisquare: $\chi^2 = 8.79$; $df = 2$; $P = 0.013$) were determined between the breeds only by the share of the most common alleles, the estimates of which fluctuated from 0.055 (dairy productivity direction) to 0.176 (southern meat productivity) (Table 1).

The indices of genetic diversity and M-ratio estimation for 10 microsatellite DNA loci of cattle of different selection (per one locus, on average) are presented in Table 2. The reliable association between a breed and a microsatellite locus was noted only regarding the estimates of the effective number of alleles (*Ae*) and the expected heterozygosity (*He*) (Friedman's test: in both cases $P < 0.01$). Therefore, the patterns of genetic variability for some loci by these indices were considerably different for animals, belonging to different groups of cattle.

The average number of alleles was the highest for the dairy cattle (11.00 alleles per locus), meat cattle (10.20 alleles per locus), and the lowest – for Ukrainian Grey breed (9.40 alleles per locus). The lowest index of the effective number of alleles (4.74 alleles per locus) was noted for meat cattle which was more than twice lower as compared with the average number of alleles. It may be the result of a high number of alleles in this breed with both very low and very high incidence (18 alleles out of 102 registered ones). In general, almost two thirds of alleles within this breed had either very low or very high incidence whereas the share of such alleles for dairy cattle was a little over 1/3 (Table 1).

The average estimates of the observed heterozygosity varied from 0.642 (meat cattle) to 0.802 (dairy cattle), whereas the average estimates of the expected heterozygosity varied from 0.773 (meat cattle) to 0.861 (dairy cattle). The prevalence of the expected heterozygosity indices over the observed ones was noted in all the cases which led to obtaining positive and relatively high indices of heterozygosity index – from 0.069 (dairy cattle) to 0.185 (aboriginal cattle) per locus on average. It demonstrated a considerable deficit of heterozygosity among the animals from the investigated breeds, especially among dairy cattle, which may serve as a manifestation of active breeding work, conducted with these breeds.

It is remarkable that on the background of the decreased rate of allelic diversity (noted for meat cattle) there was no narrowing of the spectrum of this diversity, that was the same for the animals of two other groups, which was confirmed by M-ratio indices, very similar for all three groups of animals (Table 2), that were much higher than the critical value of 0.600.

Table 1. The total number of alleles (*NAT*), number/share of rare (≤ 0.050) and common (≥ 0.200) alleles for 10 MC-DNA loci

Cattle	*NAT*	Incidence/share of alleles with this incidence	
		≤ 0.050	≥ 0.200
Dairy	110 (79.7 %)	36/0.327	36/0.327
Aboriginal	94 (68.1 %)	35/0.372	35/0.372
Meat	102 (73.9 %)	48/0.471	48/0.471
χ^2 (*P*)	–	4.73 (ns)	4.73 (ns)

Note. ns – $P > 0.05$.

Table 2. Indices (*Mean* ± *SE*) of genetic diversity and estimation of *M-ratio* for 10 MC-DNA loci (per locus on average)

Cattle	Index					
	Na	*Ae*	*Ho*	*He*	*Fis*	*M-ratio*
Dairy	11.00 ± 1.05	7.56 ± 0.55	0.802 ± 0.021	0.861 ± 0.010	0.069 ± 0.022	0.895 ± 0.053
Aboriginal	9.40 ± 0.78	6.02 ± 0.50	0.672 ± 0.064	0.824 ± 0.013	0.185 ± 0.078	0.898 ± 0.041
Meat	10.20 ± 0.83	4.74 ± 0.49	0.642 ± 0.060	0.773 ± 0.019	0.160 ± 0.080	0.874 ± 0.053
Friedman's test χ^2 (*P*)	4.638(ns)	9.604(0.008)	5.446(ns)	10.612(0.005)	0.495(ns)	1.202 (ns)

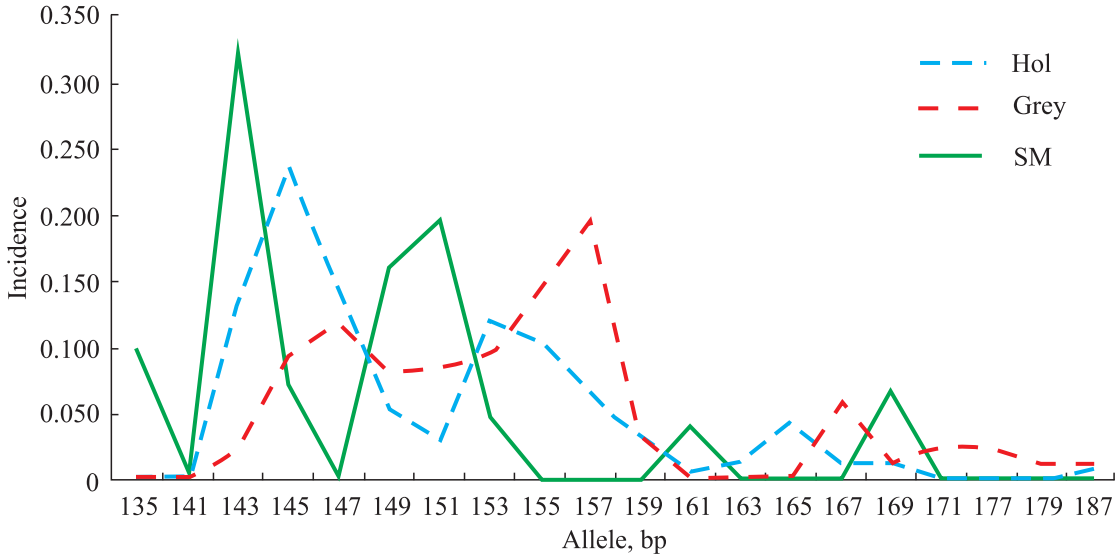


Fig. 1. The distribution by the incidence of alleles in locus *TGLA122*

In terms of some loci, we detected very low indices of M-ratio (0.500–0.652) only for locus *TGLA122*, which demonstrated faster loss of allelic diversity by this locus than on average for 10 investigated microsatellite DNA loci. It was especially notable for meat cattle, where a very high number of alleles were absent in the investigated animals (*TGLA122*^{147}, *TGLA122*^{155}, *TGLA122*^{157}, *TGLA122*^{159}, *TGLA122*^{163}, *TGLA122*^{165} and *TGLA122*^{167}) (Fig. 1).

As for the multilocus heterozygosity (MLH), the highest indices were noted for dairy cattle (0.802 ± 0.015), where 8 out of 10 investigated microsatellite DNA loci (on average) were in the heterozygous state (Fig. 1). As for aboriginal and meat cattle, these indices were significantly lower – 0.672 ± 0.021 and 0.677 ± 0.011, respectively (one-way ANOVA: $F_{2;315} = 23.63$; $P < 0.001$).

No significant deviation from Hardy-Weinberg equilibrium was found for three out of 10 microsatellite DNA loci, used in the analysis (*TGLA122, INRA023* and *ETH10*) (Table 3). In general, the significant deviation from the state of genotype equilibrium was noted for 4–5 loci in different breeds.

The estimation of the inbreeding index, *Fis* (as a total for three groups) varied from –0.040 (locus *SPS115*) to 0.262 (locus *ETH225*) and was 0.138 ± 0.030 (per locus on average), which demonstrated some deficit of heterozygosity among the investigated animals (Table 4).

The estimation of the genetic differentiation index between breeds (*Fst*) also varied within a considerable range – from 0.032 (locus *ETH225*) to 0.093 (locus

Table 3. The results of checking the Hardy-Weinberg genotypic equilibrium for 10 microsatellite DNA loci based on the likelihood ratio G-test

Locus	Cattle		
	Dairy	Aboriginal	Meat
TGLA126	0.041	< 0.001	ns
TGLA122	ns	ns	ns
INRA023	ns	ns	ns
ETH3	0.009	ns	< 0.001
ETH225	0.001	ns	< 0.001
BM1824	0.009	ns	0.036
TGLA227	ns	0.050	ns
BM2113	0.004	< 0.001	ns
ETH10	ns	ns	ns
SPS115	ns	< 0.001	0.028

TGLA126), and was 0.060 ± 0.007 on average. Significant differences between breeds were found in terms of the distribution of allele incidences for all microsatellite loci and for the mean value of *Fst* (Table 4).

A high specificity rate was confirmed by the results of the assignment test based on the distribution of incidences of multilocus genotypes by 10 microsatellite DNA loci (Table 5). The genetic uniqueness of the in-

Table 4. The estimates of Fisher's indices (*Fis* and *Fst*) for 10 MC-DNA loci

Locus	*Fis*	*P*	*Fst*	*P*
TGLA126	0.186	ns	0.093	< 0.001
TGLA122	0.186	0.038	0.051	< 0.001
INRA023	0.070	ns	0.079	< 0.001
ETH3	0.191	ns	0.044	< 0.001
ETH225	0.262	ns	0.032	0.002
BM1824	0.113	ns	0.060	< 0.001
TGLA227	0.070	ns	0.064	< 0.001
BM2113	0.252	< 0.001	0.057	< 0.001
ETH10	0.090	ns	0.087	< 0.001
SPS115	–0.040	ns	0.033	< 0.001
On average ± SE	0.138 ± 0.030	< 0.001	0.060 ± 0.007	< 0.001

Table 5. The results of the assignment-test between different cattle breeds based on the distribution of incidences of multilocus genotypes by 10 MC-DNA loci

Cattle (observ.)	Breed (expected)			Precision of referring an animal to its breed, %
	Dairy	Aboriginal	Meat	
Dairy	**78**	5	0	94.0
Aboriginal	4	**39**	0	90.7
Meat	0	0	**192**	100.0

Table 6. The results of Ewens-Watterson neutrality test for 10 MC-DNA loci (only the loci, for which the hypothesis about neutrality was reliably rejected, are presented)

Cattle	Locus	*Obs. F*	*L95–U95*
Dairy	*INRA023*	0.1088	0.1395–0.5182
	ETH3	0.1495	0.1682–0.6210
	ETH225	0.1555	0.1957–0.6884
	BM1824	0.1353	0.1567–0.5398
	BM2113	0.1008	0.1489–0.5292
	ETH10	0.1585	0.2167–0.7683
	SPS115	0.1542	0.1761–0.6511
Aboriginal	*TGLA126*	0.2155	0.2299–0.7480
	INRA023	0.1652	0.1668–0.5489
	TGLA227	0.1214	0.1374–0.4321
Meat	*TGLA122*	0.1891	0.1977–0.7205
	ETH225	0.1150	0.1200–0.3882

Note. *Obs. F* – observed sum of squares of allele incidences; *L95*, *U95* – lower and upper thresholds of 95 % confidence interval of the estimate of *Obs. F*, calculated as based on 1000 simulations.

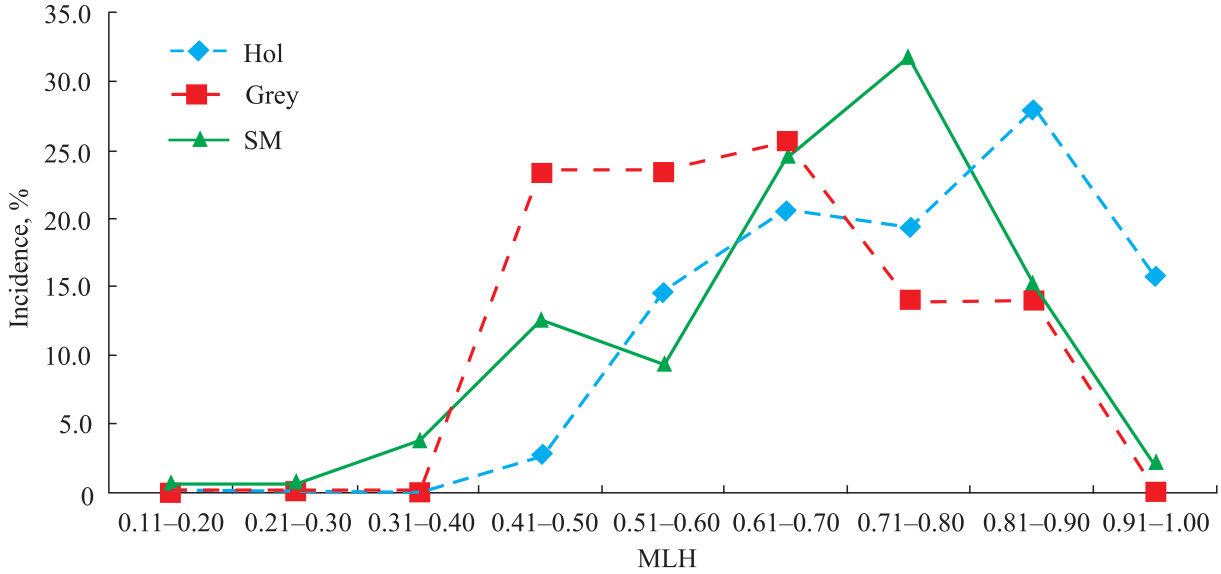


Fig. 2. The distribution by the estimates of multilocus heterozygosity (MLH) for 10 loci of microsatellite DNA Змінити Frequency на Incidence

vestigated cattle varied from 90.7 % (Ukrainian Grey) to 100 % (southern meat cattle).

To analyze possible impact of targeted breeding work on the formation of genetic structure of different breeds (Shelyov AV et al, 2017; 2018) by single locus DNA fragments, we conducted the population genetic evaluation of the groups of animals, different in the productivity direction. Although *a priori* microsatellite DNA loci are neutral molecular-genetic markers, we determined that among 10 loci, used by us, there were some loci, demonstrating that the hypothesis on their neutrality was reliably refuted based on the results of Ewens-Watterson test (Table 6). As could be expected considering active breeding work with these breeds, the highest number of these loci (7 out of 10) were registered for dairy cattle. The number of such loci was considerably lower for aboriginal and meat cattle (three and two), and half of them had the indices of *Obs. F* were close to the lower threshold (95 %) of the confidence interval (Table 6).

Table 7. The results of checking the hypothesis on the manifestation of the "bottleneck effect" in the past based on the estimates of heterozygosity for 10 MC-DNA loci (the expected and observed numbers of loci with excessive heterozygosity are presented)

Cattle	Model		
	Model	TPM	SMM
Dairy	6.01/9 (ns)	5.90/5 (ns)	5.85/4 (ns)
Aboriginal	5.99/5 (ns)	5.92/3 (ns)	5.89/2 (0.015)
Meat	5.96/5 (ns)	5.97/5 (ns)	5.98/0 (0.001)

Table 8. The estimates of the average correlation between alleles (*r*) and the number of reliable cases of linkage disequilibrium (N_{LD}) between some alleles of 10 DNA loci

Cattle	*r*	*Df*	χ^2	*P*	N_{LD}
Dairy	0.127	45	38.01	ns	13
Aboriginal	0.262	45	66.66	0.020	24
Meat	0.138	45	102.03	< 0.001	32

Table 9. The estimates of effective population number, calculated as based on LD- and MC-methods using 10 MC-DNA loci (the thresholds of 95 % confidence interval are presented)

Cattle	Method	
	LD (*Ne*)	Molecular Coancestry (*Neb*)
Dairy	709.0 (299.9–∞)	26.2 (5.4–63.2)
Aboriginal	61.1 (40.3–109.3)	12.0 (4.4–23.4)
Meat	78.4 (52.5–127.2)	10.7 (5.9–16.8)

On the other hand, the reliable manifestation of "bottleneck effect" was noted for these two groups in the past, based on the heterozygosity indices for 10 microsatellite loci (Table 7).

Similarly, the negative consequences of population demographic processes within aboriginal and meat cattle were also noted regarding their estimates of mean correlation between alleles (*r*), used in the analysis of loci, which exceeded 0 reliably, and a considerable number of significant cases of linkage disequilibrium (N_{LD}) between some alleles of 10 microsatellite DNA loci (Table 8).

These processes, related to the limited number of animals, bred in Ukraine, and a low rate of their heterozygosity (Table 2, Fig. 2), resulted in very low estimates of effective population number (*Ne*) and the effective number of inseminators (*Neb*) for aboriginal and meat cattle (Table 9).

In general, the estimated effective population number of the investigated cattle was 61–78 animals (with 95 % confidence interval from 40 to 127 animals) while the estimate of *Ne* for dairy cattle was 709 animals (with open higher threshold of 95 % confidence interval). The estimate for the effective number of inseminators (*Neb*) for these animals was also 2.0–2.5 times higher as compared with aboriginal and meat breeds (Table 9).

DISCUSSION

In our work, we determined a high rate of genetic diversity in all investigated populations of Ukrainian cattle. Despite the decreased number of the registered allelic variants, their spectrum did not narrow (*M-ratio > 0.85*). The animals under investigation were characterized by specificities of their genetic structure, confirmed by the results of the assignment test (the range of the results varied from 90.7 % (aboriginal cattle) to 100 % (meat cattle) using 10 STR. If the number of microsatellites is increased up to 12, the results of testing allow referring an animal to a specific breed correctly with the likelihood of >98 % (Opara A et al, 2012), if 19 microsatellite markers are applied for the analysis, the likelihood rises up to 99.5 %, and even for rather close breeds (*Fst* = 0.041) this likelihood may reach as high as 96.3 % (Ciampolini R et al, 2006). This makes the assignment test, based on multilocus genotypes, a powerful method, which allows referring an animal to a certain breed with rather high likelihood. The urgency of this issue is conditioned by more detailed attention to the matter of confirming the origin and quality of the products of animal breeding in recent years.

The highest number of alleles, found in dairy cattle as compared with meat and local (aboriginal) breeds in our study, was also noted in numerous studies of other authors. For instance, the highest number of detected alleles in dairy cattle (Holstein) (110) as compared with meat cattle (Simmental) (95) and local (Italian Aosta Black Pied (76) and Swiss Evolene (61) breeds by 17 microsatellite loci was noted for Alpine populations (Del Bo L et al, 2001). A higher level of allelic diversity for dairy cattle as compared with meat cattle was registered in the studies of Polish (Holstein-Friesian (76 alleles) and Hereford (61)) scientists (Radko A et al, 2005). Slovakian researchers also noted a prevalence of meat breeds over local ones by this index (Czerneková V et al, 2006). It was remarkable that the estimate of the mean number of alleles for Czech population of Holstein animals was much lower than for local Slovakian Pinzgau (a traditional breed for mountainous districts of Slovakia) – 5.8 and 9.0 alleles per locus, respectively. However, it should be noted that in this study the dairy cattle had the lowest rate of variability.

Our data on a higher rate of genetic variability, observed in dairy cattle (NAT = 110 (79.7 %), Na = 11.0, Ae = 7.56, Ho = 0.802, He = 0.861 and MLH = 0.802) were confirmed by the results of highly productive dairy breeds as compared with the local ones in European (Peelman LJ et al, 1998) and Asian populations (Kim KS et al, 2002).

In our opinion, it may be related to the breeding work, targeted at increasing the dairy productivity indices, and the factor of admixture of genetic material of different breeds during their creation.

The range of genetic differentiation (*Fst*) was from 0.032 (locus *ETH225*) to 0.093 (locus *TGLA126*), on average amounting to 0.060 ± 0.007 ($p < 0.001$). It

corresponded to the mean values for European (Ciampolini R et al, 2006; Dalvit CC et al, 2008), Indian (Chaudhari MV et al, 2009), and African breeds (Adido MS et al, 2019; Kassa SK et al, 2019).

We determined a significant difference between animals in terms of such indices as the effective number of alleles (*Ae*) and expected heterozygosity (*He*) for several loci depending on the productivity direction, which was demonstrated by a reliable association between the direction of breeding work and microsatellite loci (Friedman's test: $P < 0.01$). This is also confirmed by the data of German scientists (Brenig B, Schütz E, 2016), who demonstrated the association of nine microsatellites and the yield of protein and milk fat, the bodyweight at birth and weaning, and the index of somatic cells, the percentage of milk fat and the area of long muscles.

Moderate intensity of the selection pressure is observed in all the populations under investigation, which is demonstrated by the deficit of heterozygotes ($Ho < He$), the presence of moderate inbreedness ($Fis = 0.138$) and reliable deviation from the state of Hardy-Weinberg genetic equilibrium for only 4–5 microsatellite loci out of 10. It is in agreement with the tendencies, registered for European cattle populations of different selection (*Bos taurus*) (Gamarra D et al, 2017), and different populations of Indonesian (*Bos javanicus*) (Agung P et al, 2019) and zeboid (*Bos indicus*) cattle of different selection (Chaudhari MV et al, 2009; Sodhi M et al, 2005).

A wide range of allelic variants was noted for southern meat breed despite their lowest number. Two thirds of alleles had very low or very high incidences (18 alleles out of 102 registered ones), while the share of these alleles in others was considerably lower which may be related to the history of creating this breed and involving zebu on the initial stages.

We determined high informative value of the chosen microsatellite loci; rather low indices of M-ratio (0.500–0.652) were found only for locus *TGLA122* which demonstrated faster loss of allelic diversity in domestic breeds by this locus.

The application of Ewens-Watterson test allows estimating the neutrality of some DNA fragments in terms of the impact of paratypical factors. For instance, in the work (Li MH et al, 2010) 13 loci were determined for cattle from 10 northern European populations (represented by Finnish Ayrshire, Finnish Holstein-Friesian and Finncattle breeds), and the test for their neutrality was reliably refuted. Among these, there were four loci (*BM2113, ETH10, ETH225* and *TGLA227*), for which the hypothesis of neutrality was reliably refuted regarding the animals, investigated by us (Table 6).

No neutrality of locus SSM66 was registered in local Scandinavian and globally common cattle breeds of northern European selection (Kantanen J et al, 2000). MC-DNA loci, the neutral character of which was dismissed based on the results of Ewens-Watterson test, were also noted for other representatives of Bovidae family, for instance, for buffalo (*Bubalus bubalis)* these were loci ILSTS17 (Bhuyan DK et al, 2010), ILSTS089 and ILSTS036 (Kathiravan P et al, 2009). For zebu (*Bos indicus*), these were loci TGLA122 and TGLA227 (Vohra V et al, 2017), also noted in our research. The neutrality of all the investigated microsatellite loci was also noted in the studies of such local populations as Arunachali in India (Sharma H et al, 2018), and 12 local (aboriginal) breeds in Portugal (Bastos-Silveira C et al, 2009), as well as Creole local breed La Angelica (Giovambattista G et al, 2001).

Similar to our study, the manifestation of the "bottleneck effect" in the past, found in the analysis of MC-DNA loci was also noted for the dairy breed, Danish Red (Kantanen J et al, 2000), and Red Steppe breed (Kramarenko AS et al, 2018), as well as the meat cattle – Japanese Black breed (Sasazaki S et al, 2004). In addition, this effect was registered in the populations of other representatives of Bovidae family – zebu-like (*Bos indicus*) cattle Nellore in Brazil (Barbosa ACB et al, 2013) and Bargur in India (Ganapathi P et al, 2012), as well as banteng (*Bos javanicus*) in Australia (Bradshaw CJ et al, 2007).

It is not always possible to prove statistically that the observed rate of genetic diversity in the investigated populations was conditioned by negative consequences of some population genetic processes, which took place in the past, but it was proven for local breeds in Turkey (Anatolian Black, Anatolian Grey, South Anatolian Red, Native Southern Anatolian Yellow, East Anatolian Red, Zavot cattle) (Özšensoy Y, Kurar E, 2014; Semen Z et al, 2019), a local Icelandic breed (Icelandic cattle) (Asbjarnardottir MG et al, 2010), a local Croatian breed (Istrian cattle) (Ivankovic A et al, 2011), local Austrian breeds (Carinthian Blond and Waldviertler Blond) and a Hungarian breed (Hungarian Grey) (Manatrinon S et al, 2008), as well Romanian breeds (Ilie DE et al, 2015).

This indefiniteness may be explained by the fact that the obtained results may sometimes be ambiguous – when using one model (e.g. IAM) the hypothesis about

the manifestation of the "bottleneck effect" in the past is refuted, whereas during the application of another model (SMM) this hypothesis cannot be refuted, like what happened in our case (Table 7). A similar situation was described while studying 17 MC-DNA loci of the Creole breed from Uruguay (Armstrong E et al, 2013).

As for widely common dairy and meat breeds, in general the estimates of the effective number of the population are on a relatively high level, for instance, for such breeds as Holstein (Ne = 100–150 animals) (Qanbari S et al, 2010), Hayes BJ et al, 2003), Charolais and Limousine in France (Ne = 501 (198–958) and Ne = 376 (168–740), respectively (Leroy G et al, 2013), for American Red Angus – Ne = 429 (369–459) (Marquez GC et al, 2010), although critical values were obtained in several studies – (Holstein Ne = 39) (Weigel KA, 2001); Hereford Ne = 85 (Cleveland MA et al, 2005) and 64 (Mc Parland S et al, 2007); Aberdeen Angus Ne = 30 animals (Falleiro VB et al, 2014). Previously we obtained the values for Ukrainian dairy breeds (Ukrainian Black-and-White and Ukrainian Red-and-White) which demonstrated no threat of losing genetic diversity in their populations, as Ne values were 397 and 505 animals, respectively (Shelyov AV et al, 2017). The values of Ne = 709.0 (299.9 – ∞) animals, obtained in this study, are very close to these indices (Table 9).

On the other hand, there have been threats for aboriginal dairy breeds, when the estimates of effective population number were within the range of 50–100 animals, for instance, for such French local breeds as Montbeliarde (Ne = 57 animals) and Normande (Ne = 64 animals) (Leroy G et al, 2013). The corresponding estimate for Red dairy breed was even lower – Ne = 23 (11–74) animals (Kramarenko AS et al, 2018). Yet, among cattle breeds probably the most dangerous threat is faced by a dairy breed Wagyu cattle in the USA, the estimate of effective population of which is only 17 animals (with the range from 2 to 43 animals) (Scraggs E et al, 2014). Similarly, there are very low estimates of effective population number for the populations of local meat cattle breeds, for instance, a Japanese aboriginal breed, Japanese Black – Ne = = 30 (13–52) (Nomura T et al, 2001) and a Portuguese aboriginal breed, Merto-lenga – Ne = 25 animals (Carolino N, Gama LT, 2008).

We determined that the estimate of average correlation between alleles was the highest for Grey Ukrainian breed (r = 0.262) which demonstrated a considerable manifestation of linkage disequilibrium between the investigated MC-DNA loci. Previously, the manifestation of linkage disequilibrium was noted for the animals of this breed between loci *INRA037* and *CSRM60* (Kiselyova TY et al, 2014).

As for the aboriginal breeds of Spanish cattle under the threat of extinction, the share of the pairs of markers, between which a reliable manifestation of linkage disequilibrium was noted, may vary from 6.2 % (for Casta Navarra breed) to 80.9 % (for Betizu) (Martín-Burriel I et al, 2007).

It is believed that the share of pairs of loci, between which a reliable manifestation of linkage disequilibrium is noted, is in inverse relation to the recombination frequency and thus the distance between markers (Thevenon S et al, 2007). There are also data, stating that most linkage disequilibrium cases are conditioned by the random genetic drift, especially in the populations with low indices of effective population number (Farnir F et al, 2000).

In general, the consequences of population demographic processes in the populations of Gray Ukrainian and southern meat cattle are manifested in their estimates of mean correlation between alleles (*r*), used in the analysis of loci, which exceeded 0 reliably, and a considerable number of significant cases of linkage disequilibrium (N_{LD}) between some alleles of 10 loci. The consequences of these processes, associated with a limited number of animals from these breeds and a low rate of their heterozygosity, were low estimates of effective population number (*Ne*) and effective number of inseminators (*Neb*).

In general, the results obtained reflect the history of creating the investigated Ukrainian cattle breeds, namely, Grey Ukrainian is a long-standing breed, the product of long-term selection of local Grey Steppe cattle (Podolian type), which inhabited a wide steppe of Mediterranean and Black Sea regions in the nineteenth century and originated from one of the forms of wild buffalo (*Bos taurus primigenius*). In early 1900s they received the name "Grey Ukrainian". The substitution of Grey Ukrainian cattle with productive breeds started at the end of the 19th – the beginning of the 20th century. This breed was the basis for the formation of local and first domestic breeds, which later became maternal breeds for modern meat and dairy breeds. Ukrainian Red-and-White dairy breed was created by the method of complicated reproductive crossing of Ukrainian Simmental breed, which originated from the accumulation cross-breeding of local cattle (Grey Ukrainian) and Swiss Simmental with simultaneous breeding of desired hybrids "in itself", with Red-and-White Holstein and,

in some areas, additionally with Ayrshire and Montbeliarde breeds; Ukrainian Black-and-White dairy breed was created by reproductive crossing of Holstein and Dutch Black-and-White breeds, and local Black-and-White, Simmental, and Whiteheaded Ukrainian breeds, used for improvement (Yefimenko M.Ya. et al (2013). The southern meat breed was created by the method of complicated reproductive crossing and hybridization of Red Steppe breed (created by crossing the local Grey Ukrainian breed and Red Ostfriesen, and later – Angler, Wilstermarsh and some other breeds from the Middle European hollow) with the animals of Charolais, Hereford, Santa Gertrudis, and Cuban zebu (Vdovychenko YuV et al, 2014). The breeds, investigated in this study, preserved relative blood relationship to Grey Ukrainian breed approximately within the range of 1/32–1/64.

CONCLUSIONS

The specificities in the formation of the genetic structure of populations depending on the productivity direction of animals were determined using the results of our studies. The impact of the parental form on genetic polymorphism of modern intensive specialized breeds was noted. Among 10 microsatellite loci, used by us, there were loci in each group of animals, regarding which the hypothesis about their neutrality was reliably refuted according to the results of Ewens-Watterson test: for dairy cattle (*INRA023, ETH3, ETH225, BM1824, BM2113, ETH10* and *SPS115*), for meat cattle (*TGLA122* and *ETH225*), and for aboriginal cattle (*TGLA126, INRA023* and *TGLA227*). A significant difference was observed between animals in terms of such indices as the effective number of alleles (*Ae*) and expected heterozygosity (*He*) for several loci depending on the productivity direction, which was demonstrated by a reliable association between the direction of breeding work and some microsatellite loci (Friedman's test: $P < 0.01$). The highest rate of genetic variability was observed for dairy cattle (*NAT* – 110 (79.7 %)), *Na* – 11.0, *Ae* – 7.56, *Ho* – 0.802, *He* – 0.861 and MLH – 0.802, which may be related to the selection direction. We determined a high diversity rate for the chosen microsatellite loci, except for locus *TGLA122*, for which rather low indices of M-ratio (0.500–0.652) were found which demonstrated faster loss of allelic diversity in domestic breeds by this locus.

Adherence to ethical principles. All procedures performed in the studies involving animal participants were in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, Strasbourg, 1986.

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Формування генетичної структури популяцій великої рогатої худоби за монолокусними ділянками ДНК в залежності від напряму продуктивності та походження

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Мета. Вивчення особливостей формування генетичної структури популяцій залежно від напряму продуктивності великої рогатої худоби української селекції за монолокусними ділянками ДНК та оцінювання фактора впливу батьківської форми на генетичний поліморфізм сучасних інтенсивних спеціалізованих порід. **Методи**. В роботі використовувалися такі методи: ветеринарні (відбір периферійної крові); молекулярно-генетичні (виділення та генотипування зразків ДНК молочної (83 голови), м'ясної (192 голови) та аборигенної (43 голови) великої рогатої худоби української селекції здійснювали за 10 мікросателітними локусами, що входять до переліку рекомендованих Міжнародним товариством з генетики тварин (ISAG). Для математико-статистичного аналізу застосовано комплекс статистичних методів за використання сучасного програмного забезпечення. **Результати**. В результаті проведеного аналізу за 10 мікросателітними локусами встановлено особливості генетичної диференціації та подібності досліджених популяцій великої рогатої худоби української селекції. Одержані результати надають нову інформацію щодо впливу на монолокусні ділянки ДНК факторів штучного відбору за спеціалізації порід великої рогатої худоби. Встановлено вплив на генетичну структуру популяцій фактора предкової форми, що підтверджується однаковими спектрами поліморфізму досліджуваних ділянок ДНК у материнської та похідних порід, а також відмінностями котрі спостерігаються між тваринами різних напрямів продуктивності й, безумовно, є результатом породотворчого процесу, на що вказують результати математичних обрахунків одержаних даних. Показано, що мікросателітні локуси ДНК є високоінформативними маркерами генетичних процесів, які мають місце в популяціях свійської худоби. **Висновки.** Встановлено особливості

формування генетичної структури популяцій залежно від напряму продуктивності тварин. Зафіксовано вплив фактора батьківської форми на генетичний поліморфізм сучасних інтенсивних спеціалізованих порід. Виявлено, що серед використаних нами 10 мікросателітних локусів, у кожній групі тварин були виявлені локуси для яких гіпотеза щодо їхньої нейтральності достовірно відхилялась згідно результатів тесту Евенса-Ваттерсона: для молочної худоби (INRA023, ETH3, ETH225, BM1824, BM2113, ETH10 та SPS115), для м'ясної (TGLA122 та ETH225) й для аборигенної худоби (TGLA126, INRA023 та TGLA227). Встановлено високий рівень генетичного різноманіття, характерний для кожної з досліджених популяцій великої рогатої худоби української селекції та загальні тенденції диференціації обраних популяцій залежно від спрямованої селекційної роботи, на рівні поліморфізму мікросателітних локусів ДНК (критерій Фрідмана: $P < 0,01$) та подібну генетичну картину за низкою локусів досліджених ділянок ДНК, що може бути пов'язано з історією створення цих порід.

Ключові слова: ВРХ, ДНК-маркери, мікросателіти, поліморфізм, напрям продуктивності.

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