UDC 636.2:[575.17:577.213.3]

ANALYSIS OF POPULATION-GENETIC PROCESSES IN DIFFERENT CATTLE BREEDS BY MICROSATELLITE LOCI OF DNA

A.V. Shelyov [1], K.V. Kopylov [1], S.S. Kramarenko [2], O.S. Kramarenko [2]

[1] Institute of Animal Breeding and Genetics NAAS,
v. Chubynske, 1, Pohrebniaka Str., Kyiv region, Ukraine, 08321
[2] Mykolayiv National Agrarian University, 9, Georgiy Gongadze Str., Mykolayiv, Ukraine, 54020

e-mail: shelyov@gmail.com

Received on April 27, 2017

Aim. The aim of our research was to analyze the population-genetic processes in different dairy breeds using highly polymorphic molecular and genetic markers (DNA microsatellites). **Methods.** We used 10 loci recommended by the International Society for Animal Genetics (ISAG) to analyze 88 DNA samples of the two most abundant dairy breeds of cattle in Ukraine – Ukrainian red-motley dairy and Ukrainian black-and-white dairy breeds. Using formulae that related the expected linkage disequilibrium (LD) to the effective population size (Ne), Ne was estimated. **Results.** The work presents the results of the study on genetic processes in the populations of Ukrainian red-and-motley breed using 10 microsatellite loci of DNA. It was shown that, being highly polymorphic multilocus genetic systems, microsatellites of DNA are highly informative markers of population-genetic processes, occurring in the populations of cattle. **Conclusions.** The studied populations of Ukrainian dairy cattle breeds are impacted by population-genetic and genetic-automatic processes. In particular, the effect of the latter on Ukrainian red-and-motley dairy breed was noted. These animals had notable significant loss of rare alleles and the manifestation effect of "bottle neck". The values obtained testify to a low level of inbredness in these populations. The effective population size in the studied Ukrainian dairy cattle breeds was estimated in the approximate range of 397–555 heads which testified to a favorable condition of the population of Ukrainian red-and-motley dairy cattle and a critical condition of the Ukrainian black-and-white dairy breed.

Keywords: dairy cattle, DNA markers, microsatellite, biodiversity, population genetic.

DOI: 10.15407/agrisp4.01.074

INTRODUCTION

The study on the structure of populations and genetic processes, occurring therein, surveillance of the genetic information flow from generation to generation as well as the determination of relations between separate markers and selective features constitute a necessary database for the analysis of genotypes of specific animals with the purpose of comprehensive evaluation of their pedigree value.

The populations of farm animals have been subject to various evolutionary factors in effect throughout the whole history of their existence. The cumulative effect of "genetic drift", related to the "founder effect", considerable level of population consolidation, leading to the "bottleneck effect" in combination with the action of natural and artificial selection lead to the formation of genetically unique and isolated breeds [1]. Numerous studies have proven that there are considerable differences between different cattle breeds on the genetic level, which may be registered using molecular and genetic markers, including microsatellites of DNA [2, 3, 4]. The quantitative evaluation of these processes and the monitoring of actual conditions of the populations in time and space are of utmost relevance for the maintenance of genetic diversity and prevention of further loss of important animal genetic resources [5, 6].

The main purpose of our study was to analyze population-genetic processes in different dairy cattle breeds using highly polymorphic molecular and genetic markers (microsatellites of DNA).

MATERIALS AND METHODS

The study was conducted using the population (n = = 88 heads) of Ukrainian red-and-motley dairy cattle breed (URM; n = 45 heads) and Ukrainian black and white dairy breed (UBW; n = 43 heads), kept at Voronkiv farm in Boryspil district, Kyiv region.

The blood was sampled from the jugular vein using double-ended Venoject needles and Venosafe vacuum tubes and holders ("Terumo", Belgium), following the standard method in accordance to the manufacturer's recommendations in sterile conditions.

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 amplifier ("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).

All the statistical calculations were done by common methods [7] using special software for population-genetic analysis – GenAIEx [8], BOTTLENECK [9], PopGene [10], and NeEstimator [11].

RESULTS AND DISCUSSION

It was demonstrated in [12] that rather an adequate marker of the manifestation of genetic-automatic processes was found in the ratio of the number of the determined alleles (by a specific microsatellite locus) and the difference in length between the farthest alleles by this locus, which was called *M-ratio* by the authors. This index characterizes the intensity of the decrease in the degree of genetic diversity due to the action of genetic-automatic processes (first of all, population fluctuation, inbreeding, and bottleneck effect). Low values of *M-ratio* testify to a higher susceptibility of the subpopulation of the studied animals to the effect of the abovementioned processes.

The lowest indices of *M-ratio* (0.244 and 0.268) among the studied cattle were registered for loci *TGLA122* and *BM1824*, respectively. In other loci, the values of this index fluctuated in the range of 0.412 (*SPS115*) – 0.538 (*ETH3* and *ETH10*). As for the distribution of the values of this characteristic by breeds, it was less or more even. For instance, by locus *TGLA126* the lowest value (0.462) was notable for UBW animals, while for URM animals it was 0.533, by locus *TGLA122* the lowest value (0.244) was registered for URM, and the highest one (0.478) – for UBW, by locus *INRA23* – the minimal value (0.480) was found in UBW, and the maximal one (0.524) – in URM, by locus *ETH3* – the lowest value (0.412) was registered for URM, and in UBW – 0.538, by locus *BM1824* – the lowest value (0.268) was in UBW, and in URM – 0.526, by locus *TGLA227* the lowest value (0.400) was noted for UBW animals, while in URM it was 0.419, locus *BM2113* was remarkable for the lowest value of *M-ratio* (0.474) in UBW breed and the highest (0.524) in URM, by *SPS115* the lowest value of this index was 0.412 (URM), and for UBW – 0.474, and, finally, for loci *ETH225* and *ETH10* in both breeds, similar values of this feature were registered – 0.533 and 0.538, respectively.

Thus, there were no considerable differences by *M-ratio* registered among the studied breeds which testified to almost the same level of genetic-automatic processes in the investigated populations. It is likely that a sharp decrease in the population number, first of all, in the population gene pool, may lead to the loss of rare alleles, but these may not always be the longest or shortest ones [12]. Therefore, allele diversity would be reduced faster than the sizes of alleles which would lead to the reduction in *M-ratio* estimations.

Table 1. Heterozygosity status for microsatellite loci of different cattle breeds

Locus	URM		UBW	
	Ho	*Heq*	*Ho*	*Heq*
TGLA126	0.667	0.803	0.628	0.728
TGLA122	0.756	0.861	0.744	0.861
INRA23	0.733	0.861	0.884	0.876
ETH3	0.844	0.772	0.860	0.773
ETH225	0.778	0.802	0.907	0.803
BM1824	0.800	0.844	0.814	0.862
TGLA227	0.778	0.886	0.930	0.896
BM2113	0.867	0.862	0.767	0.828
ETH10	0.822	0.769	0.814	0.772
SPS115	0.800	0.824	0.860	0.771
Sign test (*p*)	0.066		0.605	

Note. *Ho* – actual heterozygosity; *Heq* – heterozygosity at equilibrium between the mutation process and gene drift.

On the other hand, the action of genetic-automatic processes was manifested in the decrease in the heterozygosity level, and thus in higher level of inbredness among animals. In this case, the actual heterozygosity would be much lower than the equilibrium one (*Heq*), as the gene drift would prevail over the effect of the mutation process [9].

The estimations of actual and equilibrium heterozygosity were different for the investigated breeds (Table 1). There was a considerable difference, noted for URM breed animals. The prevalence of the equilibrium heterozygosity over the actual one was found for seven loci and the contrary situation – for two loci. The same ratio was four to five for UBW breed.

The obtained results allowed for the conclusion that the actual tendency in the manifestation of bottleneck effect was remarkable only for URM animals with some allowance, as the zero hypothesis in their case may be dismissed with the relevance level p = 0.066 (sign test). According to the results of Wilcoxon test, URM breed was statistically ($P < 0.05$) notable for heterozygote deficiency. Here there were noted cases of related inheritance of different loci in animals of all the investigated cattle breeds.

The evaluation of the degree of nonrandom union of gametes (*HWD*) did not demonstrate any reliable prevalence of any breed, the value of this index for UBW animals was close to zero (–0.021), which testified to the fact that nonrandom union of gametes in this population was almost random (Table 2).

Table 2. Results of analysis on *LD* of the studied breeds using the polymorphism of microsatellite loci

Breed	N_{LD}	*HWD*	*df*	χ^2	*p*
URM	12	0.117	10	8.56	0.575
UBW	7	–0.0217	10	7.30	0.696

Note. N_{LD} – number of cases of adherence between alleles of different microsatellite loci. *HWD* – Measuring nonrandom union of gametes

Table 3. Effective population of different cattle breeds by the polymorphism of microsatellites of DNA, (heads)

Breed	Evaluation of *Ne*	95 % confidence interval
URM	554.6	132.8-∞
UBW	397.1	93.2-∞

Note. *Ne* – the effective population size

Relatively low values N_{LD} for the studied cattle breeds (7 and 12 cases out of 45) testified to a low degree of inbreeding for the investigated populations.

According to the rule, "50: 500", if the effective population size (*Ne*) exceeds 500 animals – the population is in a favorable condition, if it is in the range of 50–500 animals – it is in a vulnerable condition, and, finally, if it drops below 50 – it is at the edge of extinction [13, 14].

Taking into consideration the results of the calculations (Table 3) we can say that URM breed is in the favorable conditions (*Ne* = 554.6 animals, with 95 % confidence interval – 132.8-∞), and UBW breed – in the critical conditions (*Ne* = 397.1 animals, with 95 % confidence interval – 93.2-∞). On the other hand, the estimate of effective population even for such a common beef breed as Hereford is only 60–90 animals [15, 16], and that for Aberdeen-Angus breed in the USA – as low as 30 heads [17].

The analysis of the results of estimating genetic distance and genetic identity according to M. Nei [18] provided for the assumption that the cattle breeds, investigated by us, were rather alike: genetic distance $D = 0.170$, and genetic identity $I = 0.844$.

The calculations testify to a considerable level of genetic consolidation of the investigated populations. The animals of the studied breeds were characterized by insignificant yet reliable level of genetic differentiation.

CONCLUSIONS

The evaluation of genetic-automatic processes in the populations made it possible to determine a low level of inbreeding of the populations and approximately the same level of genetic-automatic processes in these populations. The manifestation of the action of genetic-automatic processes was the decrease in the heterozygosity level and respective higher level of inbreeding only among representatives of URM breed. The results obtained demonstrated the progressing tendency in the manifestation of the "bottleneck effect" for URM cattle breed. Also, URM breed statistically had heterozygote deficiency. The evaluation of the degree of non-random zygosis (*HWD*) in animals of the investigated breeds was unreliable, and the value of this index for UBW breed animals testified to the fact that union of gametes in the investigated population was almost random. The obtained

values of N_{LD} testified to the low level of inbreeding of these populations. The results of the calculations of effective population size for the investigated breeds demonstrated that URM breed was in the favorable conditions, and UBW – in the critical state.

Аналіз популяційно-генетичних процесів
у різних порід великої рогатої худоби
за мікросателітними локусами ДНК

А.В. Шельов [1], К.В. Копилов [1],
С.С. Крамаренко [2], О.С. Крамаренко [2]

[1] Інститут розведення і генетики тварин НААН,
с. Чубинське, Київська область, вул. Погребняка 1,
Україна, 08321
[2] Миколаївський національний аграрний університет,
м. Миколаїв,
вул. Георгія Гонгадзе 9, Україна, 54020

Мета. Метою нашого дослідження був аналіз популяційно-генетичних процесів у різних порід молочної худоби з використанням високо поліморфних молекулярно-генетичних маркерів (мікросателітів ДНК). **Методи**. Було використано 10 локусів, рекомендованих Міжнародним товариством з генетики тварин (ISAG), для аналізу 88 зразків ДНК двох найчисельніших в Україні молочних порід великої рогатої худоби – української червоно-рябої молочної та української чорно-рябої молочної. Було застосовано формули, що пов`язують очікувану неврівноваженість зчеплення (*LD*) з ефективним розміром популяції (*Ne*). **Результати.** У роботі викладено результати дослідження генетичних процесів, що мають місце в популяціях українських червоно- та чорно-рябої молочної худоби, за використання 10 мікросателітних локусів ДНК. Показано, що мікросателіти ДНК, як високо поліморфні мультилокусні генетичні системи, є високо інформативними маркерами популяційно-генетичних процесів, які мають місце в популяціях свійських тварин. **Висновки.** Досліджені популяції українських молочних порід великої рогатої худоби зазнають впливів популяційно-генетичних та генетико-автоматичних процесів. Їхній негативний вплив було зареєстровано на українську червоно-рябу молочну породу. У цих тварин відмічено значну втрату рідкісних алелів і прояв ефекту «пляшкового горлечка». Одержані значення свідчать про низький ступінь інбредності цих популяцій. Ефективний розмір популяцій у досліджених українських молочних порід великої рогатої худоби оцінювався приблизно у 397–555 голів, що свідчить про сприятливий стан популяції української червоно-рябої молочної худоби та загрозливий української чорно-рябої.

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

Анализ популяционно-генетических процессов у
разных пород крупного рогатого скота
по микросателлитным локусам ДНК

А.В. Шелёв [1], К.В. Копылов [1],
С.С. Крамаренко [2], А.С. Крамаренко [2]

e-mail: shelyov@gmail.com

[1] Институт разведения и генетики животных НААН,
с. Чубинское, Киевская область, ул. Погребняка 1,
Украина, 08321
[2]Николаевский национальный аграрный университет,
г. Николаев,
ул. Георгия Гонгадзе, 9, Украина, 54020

Цель. Целью нашего исследования был анализ популяционно-генетических процессов в разных породах молочного скота с использованием высоко полиморфных молекулярно-генетических маркеров (микросателлитов ДНК). **Методы**. Были использованы 10 локусов, рекомендованных Международным обществом генетики 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. Были использованы формулы, позволяющие связать ожидаемую неравновесность сцепления (*LD*) с эффективным размером популяции (*Ne*). **Результаты**. В работе представлены результаты исследования генетических процессов, происходящих в популяциях украинских красно- и черно-пестрой молочных пород крупного рогатого скота с использованием 10 микросателлитных локусов ДНК. Показано, что микросателлиты ДНК, как высоко полиморфные мультилокусные генетические системы, являются высоко информативными маркерами популяционно-генетических процессов, происходящих в популяциях домашних животных. **Выводы**. Исследованные популяции украинских молочных пород крупного рогатого скота подвергаются воздействиям популяционно-генетических и генетико-автоматических процессов. Их негативное влияние было зарегистрировано на украинскую красно-пеструю молочную породу. Для этих животных отмечена значительная элиминация редких аллелей и проявление эффекта «бутылочного горлышка». Полученные значения свидетельствуют о низкой степени инбредности данных популяций. Эффективный размер популяций в исследованных украинских молочных породах крупного рогатого скота оценивался примерно в 397–555 голов, что свидетельствует о благополучном состоянии популяции украинского красно-пестрого молочного скота и угрожающем – украинского черно-пестрого.

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

REFERENCES

1. *Kramarenko A*. Analysis of genetic and demographic processes in the population of the Southern Meat cattle breed. *Ukrainian Black Sea Region Agrarian Science*. 2015;**82**(1):203–9.
2. *Blott SC, Williams JL, Haley CS.* Genetic relations among European cattle breeds. *Animal Genetics*. 1998; **29**(4):273–82.
3. *Machugh DE, Loftus RT, Bradley DG, Sharp PM, Cunningham P.* Cattle Breeds Microsatellite DNA Variation within and among European. *Proceedings: Biological Sciences*. 1994;**256**(1345):25–31.
4. *Delgado JV, Martínez AM, Acosta A, Alvarez LA, Armstrong E, Camacho E, Cañón J, Cortés O, Dunner S, Landi V, Marques JR, Martín-Burriel I, Martínez OR, Martínez RD, Melucci L, Muñoz JE, Penedo MC, Postiglioni A, Quiróz J, Rodellar C, Sponenberg P, Uffo O, Ulloa-Arvizu R, Vega-Pla JL, Villalobos A, Zambrano D, Zaragoza P, Gama LT, Ginja C*. Genetic characterization of Latin-American Creole cattle using microsatellite markers. *Animal Genetics*. 2012;**43**(1):2–10.
5. *Hall SJG, Ruane J.* Livestock breeds and their conservation: A global overview. *Conservation Biology*. 1993;**7**(4):815–25.
6. *Hall SJG., Bradley DG*. Conserving livestock breed biodiversity. *Trends Ecol Evol*. 1995;**10**(7):267–70.
7. *Weir B*. Analysis of genetic data. Moskva, *Mir*. 1995; 400 p.
8. *Peakall R, Smouse PE*. GenAIEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. *Bioinformatics*. 2012;**28**(19): 2537–9.
9. *Piry S, Luikart G, Cornuet JM*. BOTTLENECK: a computer program for detecting recent reductions in the effective size using allele frequency data. *J Hered*. 1999;**90**(4):502–3.
10. *Yeh FC, Yang RC, Boyle T.* PopGene. Microsoft Windows based freeware for population genetic analysis: Quick User Guide. University of Alberta, 1999;28 p.
11. *Peel D, Ovenden JR, Peel SL*. NeEstimator: Software for estimating effective population size. Queensland Government, Department of Primary Industries and Fisheries, Queensland, Australia. 2004.
12. *Garza JC, Williamson EG*. Detection of reduction in population size using data from microsatellite loci. *Mol Ecol*. 2001;**10**(2):305–18.
13. *Frankham R, Bradshaw CAJ, Brook BW*. Genetics in conservation management: revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. *Biological Conservation*. 2014;**170**:56–63.
14. *Frankham R, Briscoe DA, Ballou JD.* Introduction to conservation genetics. Cambridge University Press. 2010;644 p.
15. *Cleveland MA, Blackburn HD, Enns RM, Garrick DJ.* Changes in inbreeding of U.S. Herefords during the twentieth century. *J Anim Sci*. 2005;**83**:992–1001.
16. *Parland SMc, Kearney JF, Rath M, Berry DP.* Inbreeding trends and pedigree analysis of Irish dairy and beef cattle populations. *J Anim Sci*. 2007;**85**:322–31.
17. *Falleiro VB, Malhado CHM, Malhado ACM, Carneiro PLS, Carrillo JA, Song J.* Population structure and genetic variability of Angus and Nellore herds. *J Agric Sci*. 2014;**6**(12):276–85.
18. Nei M. Genetic distance between populations. *The American Naturalist*. 1972; **106** (949): 283–92.