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Original Research Article

Microsatellite Diversity in *Bos taurus*, *Equus caballus* and *Gallus domesticus* Breeds Reared in Ukraine

Key words

microsatellite;
diversity;
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Andriy V. Shelyov[1], Roman O. Kulibaba[2]*, Kyrylo V. Kopylov[1], Kateryna V. Kopylova[1]

[1]M. V. Zubets Institute of Animal Breeding and Genetics, the NAAS 1, Pohrebniaka Str, Chubynske village, Kyiv region, 08321, Ukraine, [2]National University of Life and Environmental Sciences of Ukraine, 15, Heroyiv Oborony Str., Kyiv, 03041, Ukraine

***Corresponding author:** romankx37@gmail.com

Abstract: This study is dedicated to the comparative analysis of the main parameters of microsatellite variability in the populations of animals from different taxa (*Bos taurus*, *Equus caballus*, and *Gallus domesticus*) of different breeds, reared in Ukraine. To investigate microsatellite variability, the following SSR-markers were used: for *Bos taurus* – TGLA126, TGLA122, INRA023, ETH003, ETH225, BM1824, TGLA227, BM2113, ETH10 and SPS115; for *Equus caballus* – HTG04, HMS06, AHT04, ASB23, HTG07, HTG06, CA425, VHL20, HMS03, HMS07 and ASB17; for *Gallus domesticus* – ADL0268, ADL0278, MCW0248, LEI0094 and MCW0216. The results of analyzing the parameter of the average number of alleles per locus (A) were used to determine their least amount in *Gallus domesticus* (6.56) and the highest one – in *Equus caballus* (10.76). The observed data are in agreement with the standardization procedure results, based on the rarefaction analysis on the level of 25 animals for each specific species of animals. The highest values of the total genetic diversity (uHe) were notable for *Bos taurus* (0.835), and the lowest ones – for *Gallus domesticus* (0.690). These results were confirmed by the Shannon's index values (1.940 for *Bos taurus*, 1.886 for *Equus caballus* and 1.420 for *Gallus domesticus*) as well as by the number of effective alleles (6.166; 5.614 and 3.848, respectively). The value of genetic subdivision (differentiation) according to F_{st} values fluctuated depending on the taxon and amounted to 0.119 for *Gallus domesticus*; 0.043 for *Equus caballus* and 0.03 for *Bos taurus*. Genetic differentiation between the populations, evaluated by the analysis of molecular variance (AMOVA), was in the range from 3 to 14 % for different taxa.

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Introduction

Genetic monitoring of gene pools of farm animals is one of the most relevant tasks of modern genetics as, in addition to providing general information about the diversity parameters, it also allows for evaluating the microevolutionary processes in the experimental populations. As Groeneveld *et al.* noted, efficient management of genetic resources of farm animals requires comprehensive knowledge about the characteristics of different breeds, including information about the specificities of genetic structure and the level of genetic diversity (1). From the standpoint of the tasks regarding the evaluation of biological diversity, one of the most efficient and highly informative research instruments is found in microsatellites (2).

For over thirty years, many genetic laboratories have been conducting numerous studies, aimed at the investigation of microsatellite variability in the populations of animals from different breeds and breed groups (3–5). There are studies on the specificities of genetic and population structure of the breeds of the most common farm species, such as cattle, horses, and chickens (6-8). Noteworthy is the tendency towards investigating not only commercial lines/breeds of animals but also the analysis of aborigine, local populations

(9–13). Microsatellites are successfully applied to solve the tasks of passportization and general genetic and population characterization of specific breeds of animals and also to analyze the origin and conduct comparative phylogenetic studies (14–16). It should be noted that genetic and population studies using microsatellite markers are getting more and more popular and involve many different countries from different regions of the world (17–20).

In Ukraine, the issues of research on microsatellite variability are gradually becoming more urgent, which triggers a consistent increase in the number of publications in this field (21–23). However, despite positive dynamics, most articles are aimed at studying specific populations and breeds of farm animals, without any attempt at the general comparative analysis of microsatellite variability indices showing different taxa. Also, the domestic publications (the articles of Ukrainian authors) do not have the only unified approach (some articles do not contain the results regarding Wright's F-statistics, AMOVA, etc.) to analyzing the indices of genetic diversity for different populations, which often does not allow for conducting comparative analytic studies. Therefore, it is not deemed possible to evaluate the general degree of genetic diversity and the specificities of microevolutionary processes in the experimental populations/breeds of farm animals, bred in Ukraine, which substantiates the urgency of the set task in many aspects.

In Ukraine, some of the most typical representatives of domestic animals of two classes (mammals and birds) are cattle, horses, and chickens. While practical significance is more attributed to the breeds of cattle and horses, reared in Ukraine (Ukrainian Black-and-White dairy breed of cows, Ukrainian Red-and-White dairy breed of cows, Ukrainian Saddle Horse, etc.), the situation with chickens is completely opposite. As compared to other taxa, *Gallus domesticus* belongs to one of the most common and diverse species in the world, including Ukraine (24). However, the industrial significance in the context of productivity indices (meat and eggs) is actually noted only for commercial breeds and crossbreds of chickens (Hy-Line, Hisex, etc.), which makes this situation completely different from the one described above regarding the mammals.

This article aims to feel the gap in presenting the general parameters of microsatellite variability in different taxa from the comparative aspect. Previously we have conducted the studies on microsatellite variability of the populations of different breeds and species of farm animals, highlighting the specificities of polymorphism and its characterization for specific groups by the combination of SSR-markers (25, 26). However, these studies were directed only at particular issues of the genetics of some animal species without analyzing the general parameters of genetic diversity of different taxa.

Therefore, the aim of the study, described in this article, is to conduct the comparative analysis of the main parameters of microsatellite variability in the populations of animals of different taxa (*Bos taurus*, *Equus caballus*, and *Gallus domesticus*) of different breeds, reared in Ukraine. In this work, we used only the results of typing at microsatellite loci obtained using the ABI Prism 3130 Genetic Analyzer. In previous years, we obtained results on microsatellite variability in populations of animals of different breeds using electrophoresis in polyacrylamide gel, which we used only for comparison in this publication due to differences in the resolution of each method.

Materials and methods

Sample collection

Different species of farm animals of different taxa were used as the objects of the study: *Bos taurus* – Ukrainian Black-and-White dairy breed (n=43), Ukrainian Red-and-White dairy breed (n=45), Ukrainian Grey cattle breed (n=45); *Equus caballus* – Hutsul breed (n=78), Thoroughbred breed (n=51), Ukrainian Saddle Horse (n=152); *Gallus domesticus* – cross Lohmann LSL (n=100), Lohmann Brown (n=83), Hisex White (n=122), Hy-Line W-98 (n=22), Hisex Brown (n=81). All the populations of animals were from different farms in different regions of Ukraine.

DNA Extraction and Microsatellite Analysis

Whole blood was used as the source of biological material. DNA was extracted using the commercial set of reagents "DNA-sorb-B" (Amplisense, RF) according to the manufacturer's recommendations. PCR was conducted using Applied Biosystems Veriti TM 96 Well Thermal Cycler (Applied Biosystems, USA) and the commercial reagent kit "DreamTaq PCR Master Mix" (Thermo Scientific). The volume of the final reaction mixture was 20 µl. The final concentration of primers in the reaction mixture was 0.2 µM. ABI Prism 3130 Genetic Analyzer (Applied Biosystems, USA) was used in typing the animals by microsatellite markers. To investigate microsatellite diversity, the following SSR-markers were used: for *Bos taurus* – TGLA126, TGLA122, INRA023, ETH003, ETH225, BM1824, TGLA227, BM2113, ETH10 and SPS115; for *Equus caballus* – HTG04, HMS06, AHT04, ASB23, HTG07, HTG06, CA425, VHL20, HMS03, HMS07 and ASB17; for *Gallus domesticus* – ADL0268, ADL0278, MCW0248, LEI0094 and MCW0216.

The sizes of alleles were determined using "GeneMapper 3.7" (Applied Biosystems, USA) based on the standard of GeneScan-500 LIZ™ (Applied Biosystems, USA).

Data analysis

The average number of alleles per locus (A), the average number of unique alleles per locus (A_{unq}), sample size over all loci (N), number of effective alleles (N_e), Shannon's information index (I), observed heterozygosity (H_o), expected

heterozygosity (H_e), unbiased expected heterozygosity (uH_e), number of migrants or gene flow (Hm) and AMOVA were calculated using GENALEX version 6.5 (27). The parameters of F-statistics (F_{is}, F_{it}, F_{st}) were estimated by F_{stat} version 2.9.4 (28). HP-Rare 1.1 software was used to perform the rarefaction on the measures of allelic richness (29).

Results

Due to the impossibility of direct comparison of the results, obtained from the study on the genetic structure specificities regarding the detected variants and genotypes in the populations of representatives from different taxa, the results of the evaluation of the main parameters of microsatellite variability were analyzed by the average values, obtained for specific populations within each taxon. To ensure accurate comparison of the representatives of different taxa, the indices of the average number of alleles per locus and the average number of unique alleles per locus were used.

The data regarding the absolute values (the number of alleles per locus for specific populations of animals of different species, etc.) were presented in our previous publications (26, 30). The representatives of *Bos taurus* were analyzed by 10 microsatellite loci, *Equus caballus* – by 11, *Gallus domesticus* – by 5. According to the study results, all loci in each breed of different species of animals were found to be polymorphic (the percentage of polymorphic loci = 100 %).

The results of individual genotyping of animals allowed for conducting further estimations to detect the specificities of genetic diversity in the populations of the representatives of *Bos taurus*, *Equus caballus* and *Gallus domesticus*.

Tables 1 and 2 present the data regarding the main parameters of genetic diversity in terms of the average values of

Table 1: Allele polymorphism of different farm animal species

Species	A	A_{unq}	Rarefaction (n=25)	
			A (M ± SE)	Aunq (M ± SE)
Bos taurus	9.33	0.80	8.76 ± 1.938	0.74 ± 0.827
Equus caballus	10.76	1.42	10.15 ± 2.691	1.15 ± 1.453
Gallus domesticus	6.56	0.68	5.87 ± 2.077	0.57 ± 0.91

A – average number of alleles per locus; Aunq – average number of unique alleles per locus

Table 2: Descriptive statistics over all loci for different farm animal species

	N	N_e	I	H_o	H_e	uH_e
			Bos taurus			
Mean	44.333	6.166	1.940	0.760	0.826	0.835
SE	0.175	0.308	0.047	0.026	0.009	0.009
			Equus caballus			
Mean	93.667	5.614	1.886	0.746	0.791	0.796
SE	7.548	0.352	0.067	0.022	0.017	0.017
			Gallus domesticus			
Mean	81.600	3.848	1.420	0.625	0.684	0.690
SE	6.786	0.318	0.087	0.045	0.033	0.033

N – sample size over all loci; N_e – number of effective alleles; I – Shannon's information index; H_o – observed heterozygosity; H_e – expected heterozygosity; uH_e – unbiased expected heterozygosity.

Table 3: Estimates of F-statistics over all loci for different farm animal species

Species	F_{is}		F_{it}		F_{st}		Nm	
	Mean	SE	**Mean**	SE	**Mean**	SE	**Mean**	SE
Bos taurus	0.079	0.033	0.107	0.034	0.030	0.005	10.529	1.816
Equus caballus	0.058	0.015	0.098	0.015	0.043	0.009	9.483	2.407
Gallus domesticus	0.089	0.061	0.194	0.071	0.119	0.019	2.175	0.522

F_{is} – inbreeding coefficient; F_{it} – overall inbreeding coefficient; F_{st} – fixation index; Nm – number of migrants (gene flow)

Table 4: Analysis of molecular variance (AMOVA). Interpopulation and intrapopulation variance for different farm animal species

Source of variance	df	Sum of squares	Mean squares	Variance components	Percentage of variance
			Bos taurus		
Among populations	2	34.163	17.082	0.141	3
Among individuals	130	593.134	4.563	0.383	9
Within populations	133	505.000	3.797	3.797	88
Total	265	1132.297	-	4.321	100
			Equus caballus		
Among populations	2	88.017	44.008	0.234	5
Among individuals	278	1336.455	4.807	0.279	6
Within populations	281	1194.000	4.249	4.249	89
Total	561	2618.472	-	4.762	100
			Gallus domesticus		
Among populations	4	187.976	46.994	0.288	14
Among individuals	403	784.824	1.947	0.227	11
Within populations	408	609.000	1.493	1.493	74
Total	815	1581.800	-	2.008	100

df – degrees of freedom

the total number of alleles and the number of unique alleles per locus among the populations of animals from different taxa.

The analysis of the parameter of the average number of alleles per locus (A) demonstrated that their least amount was found in *Gallus domesticus* and the highest amount – in *Equus caballus* (Table 1).

The observed data are in agreement with the standardization procedure results, based on the rarefaction analysis on the level of 25 animals for each specific species of animals.

In terms of the parameter of the number of unique alleles per locus (A_{unq}), the situation is the same. The highest number of unique alleles was detected for *Equus caballus*, which corresponds to the value of the average number of alleles per locus as compared to *Bos taurus* (in the comparative

aspect, there was practically a twofold surplus over the index A_{unq}).

Using the parameters of the general genetic diversity, it was determined that the highest values of diversity (genetic diversity that is equal to uHe) were notable for *Bos taurus*, and the lowest – for *Gallus domesticus*. These results were confirmed by the Shannon's information index values and the index of the number of effective alleles (Table 2).

The indices of Wright's F-statistics were used to analyze the degree of the subdivision in the populations of different taxa representatives (F_{is}, F_{it}, F_{st}) (31). The genetic subdivision (differentiation) value in terms of the values of F_{st} expressly fluctuated depending on the taxon and amounted to 0.119 (which corresponded to the average degree of genetic differentiation according to Wright) for *Gallus domesticus* and 0.03 for *Bos taurus* (insignificant degree of differentiation) (Table 3).

The absence of the expressed excess of heterozygotes was also noted for all the taxa – the values of F_{is} index were positive. Among all the investigated taxa, the lowest value of the gene flow index was noted for *Gallus domesticus*, and the highest – for *Bos taurus*.

Genetic differentiation between the populations of different taxa, evaluated using the analysis of molecular variance (AMOVA), amounted to 3–14 % (Table 4). It should be noted that the highest value of the interpopulation variance was noted for *Gallus domesticus* (14 %), and the lowest – for *Bos taurus* (3 %).

Discussion

The highlight of the conducted investigations was the comparative analysis of genetic diversity parameters in the populations of domestic animals of different breeds (mammals and birds), which are reared in Ukraine's territory. The results of the studies have demonstrated that mammals are characterized by general features which distinguish them from birds.

Due to the application of different numbers of microsatellite markers for the analysis of genetic diversity parameters of experimental populations (10 for *Bos taurus*, 11 for *Equus caballus*, 5 for *Gallus domesticus*) as an index of group characterization, the value of the average number of alleles per locus was used (A). The same is true for the index of the average number of unique alleles per locus. This approach allowed for evaluating the general degree of diversity by the number of alleles of microsatellites per locus. The results of the studies demonstrated close values in terms of parameter A (average number of alleles per locus) regarding mammals which distinguishes them considerably from birds (in which the values of the average number of alleles per locus are practically 1.5 times lower). The results of the variability analysis by the index of the number of alleles per locus and the number of unique alleles per locus are confirmed by the results of the rarefaction method, used to unify the comparison of different populations with different samplings with the purpose of minimizing the impact of the sampling volume on the data regarding genetic diversity (in this case, genetic diversity is determined by the terms of the number of alleles per locus, according to Kalinowski S.T.) (32). Here the use of rarefaction was required due to a different number of animals of different breeds and populations both within one species and between species in general. The results of the comparison of observed values and the data, obtained by the rarefaction method, demonstrate close values of indices within taxa, which, in its turn, confirms the conclusions – mammals are characterized by a higher value of the average number of alleles per locus and less expressed differences among themselves in terms of the number of unique alleles. At the same time, all three taxa had a similar ratio of the number of unique alleles per locus (Aunq) and the average number of alleles per locus (A), which was ⩽10 %. In its turn, the minimal value was 8.57 for *Bos taurus*, and the maximal value (13.19) – for *Equus caballus*. *Gallus domesticus* had a medium result. The calculations based on the results of rarefaction yielded a similar final outcome. Therefore, a conclusion can be made that the ratio between the average number of unique alleles per locus and the average number of alleles per locus actually does not depend on the animal species under investigation (in the context of this study).

The obtained results are confirmed by the analysis of the main parameters of genetic diversity. In this case, mammals also demonstrate a higher value of the Shannon's index and the index of expected heterozygosity (genetic diversity). At the same time, despite the leading position in terms of the average number of alleles per locus, *Equus caballus* demonstrated lower values of the number of effective alleles and the indices of observed and expected heterozygosity as compared to *Bos taurus*. In its turn, *Gallus domesticus* was characterized by the lowest values of the number of observed and effective alleles, the value of the Shannon's index, and the minimum values of the observed and expected heterozygosity. Lower values of the indices of the general parameters of genetic diversity for *Gallus domesticus* can be explained by the use of the representatives of commercial chicken breeds (crosses) in the study. At the same time, for mammals, the representatives of local breeds, reared in Ukraine, were used. Therefore, the differences in genetic diversity indices between the representatives of mammals and birds can be explained due to the specificities of the breeding work.

The genetic diversity among populations (F_{st}), which is the measure of the genetic subdivision of populations, was found to be maximally expressed in *Gallus domesticus*. At the same time, in mammals, the values of F_{st} were within a relatively close range and about 3–4 times lower than those for birds. The analysis of the observed F_{st} values compared

to the standard values demonstrated weak divergence for mammals (F_{st} standard for a weak degree of divergence is 0.00–0.05) and the average degree for birds (F_{st} level for the average diversity is 0.06–0.15) (33). At the same time, inconsiderable excess (inbreeding) of homozygous invididuals was noted according to the index F_{is}. Its maximal value was found for *Gallus domesticus*, the minimal value – for *Equus caballus*, but the differences between species were not much expressed.

The index of genetic diversity (F_{st}) reached its maximal (compared to other taxa) value in chickens regardless of the fact that the populations of a similar (egg) productivity were analyzed for *Gallus domesticus*, whereas for *Bos taurus*, these were dairy (Ukrainian Black-and-White and Red-and-White dairy breeds) and combined (Ukrainian Grey cattle breed). Thus, in the case under study, the factor of productivity did not play the leading role in the genetic divergence of populations.

It is noteworthy that by the values of F_{is} index, the representatives of *Gallus domesticus* did not differ much from other taxa despite the fact that the study actually involved commercial crosses of chickens instead of the initial inbred lines (breeds).

In its turn, the comparison of the study results against the data, obtained while studying the microsatellite variability of local Ukrainian chicken breeds of different direction of productivity (egg-laying and combined), the values of F_{is} and F_{st} indices were appropriately lower (0.089 vs 0.110 and 0.119 vs 0.195) (34). In case of local Ukrainian breeds, the comparison of genetic diversity parameters of chicken breeds of different productivity made a considerable contribution to the study results and demonstrated the prevailing significance of origin as compared to the direction of productivity. A similar conclusion can also be predicted with the consideration of the neutral character of microsatellite markers in total (35).

The results of the analysis of F_{st} values were similarly reflected in the AMOVA results. In general, mammals were characterized by low values of the genetic diversity among populations (genetic differentiation between populations was from 3 to 5 % for both taxa, respectively) with considerably higher values of genetic differentiation between animals within populations. Thus, as for mammals (within the species under investigation), most of the detected genetic diversity fell on the component between populations.

At the same time, *Gallus domesticus* was notable for the maximal (as compared to other taxa) value of the genetic diversity among populations (14 %), which confirmed the assumptions, previously made in the article. Therefore, the general breeding work within the same productivity direction and the factor of origin introduces a considerable change into the values of genetic differentiation, which may exceed the divergence, occurring due to the differences in the direction of productivity of animals from other taxa (like in case of *Bos taurus*).

Conclusion

The results of the studies were used to analyze the main indices of genetic diversity by the combination of microsatellite markers in different taxa of farm animals, reared in Ukraine. It was demonstrated that the highest values of genetic differentiation were notable for *Gallus domesticus*, and the lowest – for *Bos taurus*. At the same time, different species of mammals (*Bos taurus* and *Equus caballus*) were remarkable for very close values of genetic diversity parameters, which were considerably different from those for *Gallus domesticus*.

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Mikrosatelitska raznolikost pri pasmah *Bos taurus*, *Equus caballus* in *Gallus domesticus*, vzrejenih v Ukrajini

A. V. Shelyov, R. O. Kulibaba, K. V. Kopylov, K. V. Kopylova

Izvleček: Raziskava je bila posvečena primerjalni analizi glavnih parametrov variabilnosti mikrosatelitov v populacijah živali različnih taksonov (*Bos taurus*, *Equus caballus* in *Gallus domesticus*) različnih pasem, ki se vzrejajo v Ukrajini. Za raziskovanje mikrosatelitske variabilnosti so bili uporabljeni naslednji SSR-označevalci: za *Bos taurus* – TGLA126, TGLA122, INRA023, ETH003, ETH225, BM1824, TGLA227, BM2113, ETH10 in SPS115; za *Equus caballus* – HTG04, HMS06, AHT04, ASB23, HTG07, HTG06, CA425, VHL20, HMS03, HMS07 in ASB17; za *Gallus domesticus* – ADL0268, ADL0278, MCW0248, LEI0094 in MCW0216. Na podlagi rezultatov analize parametra povprečnega števila alelov na lokus (A) smo njihovo najmanjšo količino določili pri *Gallus domesticus* (6,56) in največjo pri *Equus caballus* (10,76). Ugotovljeni podatki so v skladu z rezultati postopka standardizacije, ki temelji na analizi redkosti na ravni 25 živali za vsako posamezno živalsko vrsto. Najvišje vrednosti skupne genetske raznolikosti (uHe) so bile opazne za *Bos taurus* (0,835), najnižje pa za *Gallus domesticus* (0,690). Te rezultate so potrdili vrednosti Shannonovega indeksa (1,940 za *Bos taurus*, 1,886 za *Equus caballus* in 1,420 za *Gallus domesticus*) ter število učinkovitih alelov (6,166; 5,614 in 3,848). Vrednost genetske delitve (diferenciacije) glede na vrednosti F_{st} je nihala glede na takson in je znašala 0,119 za *Gallus domesticus*; 0,043 za *Equus caballus* in 0,03 za *Bos taurus*. Genetska diferenciacija med populacijami, ocenjena z analizo molekularne variance (AMOVA), je bila pri različnih taksonih v razponu od 3 do 14 odstotkov.

Ključne besede: mikrosateliti; raznolikost; polimorfizem; populacija; lokalne pasme; govedo; konj; piščanec