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Genetic variability of dairy cattle breeds in Lithuania

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Large numbers of different scientific studies are conducted to preserve the breeds and improve the existing ones by introducing the variability of the plate genes, which best shows the phenotypic characteristics that can improve the health of dairy cattle and the quality of their production. The main purpose of this study was to perform an analysis of genetic variability of Lithuanian cattle breeds. Three subpopulation groups were studied: Lithuanian Black and White (95 individuals), Lithuanian Red (49), and Lithuanian White and Red (48). Bovine genetic material was genotyped using a total of 11 fluorescent microsatellite primers to estimate genetic variability. All loci presented a high degree of polymorphism and a total of 292 different alleles (Na) were detected. Thirty-two private alleles were detected in all evaluated subpopulations. After completing pairwise population assignment, which is based on the distribution of allelic frequencies, three populations showed the tendency to group into three separate clusters. However, the performed Principal Coordinates Analysis (PCoA), which is based on genetic distances, showed no anticipated clear grouping. Bayesian structure analysis revealed three genetic clusters. Analysis of FST (0.001–0.027) and Nei genetic distance (0.029–0.084) revealed that the genetic diversity of inter subpopulation in cattle groups was estimated to be lower than the genetic diversity of intra subpopulation.

Keywords: genetic variability, microsatellite, cattle

INTRODUCTION

Structured cattle development in Lithuania is observed since the beginning of the twentieth century (Skinkytė et al., 2005). Lithuanian Red, Lithuanian Red and White, Lithuanian Black and White are the main cattle breeds raised in Lithuania. To increase the phenotypic and genotypic parameters, they are bred with foreign species (Šveistienė, Jatkauskienė, 2008). The breed of the Lithuanian Black and White cattle was obtained by crossbreeding local cattle with Dutch Belted, German Black and White, and Swedish Black and White. It is important to mention that Lithuanian cattle are bred with Holstein cattle

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breed to increase the productivity (LBWCBA, 2011). The Lithuanian Red and Lithuanian Red and White cattle breeds were obtained by crossbreeding local cattle with Danish Red, Anglers, Swiss and Latvian Brown, Swedish Red and White, and sometimes with Simmentals. It is estimated that by increasing the percentage of the Lithuanian Red genotype in the individual, a negative effect on milk productivity is observed but it increases the lifetime of the individual (Japertienė et al., 2016). To increase the lifetime, crossbreeding with Anglers, Swedish Red and White, and Swiss Red is also suggested. To improve milk quality, crossbreeding with Ayrshire is advised, and to improve physical properties, Red and White Holstein, Danish Red are advised (Ministry of Agriculture of the Republic of Lithuania, 2003). The aim of the current study was to evaluate the genetic diversity among Lithuanian Black and White, Red and White, and Red cattle breeds in order to provide information for future breeding programmes.

MATERIALS AND METHODS

Lithuanian Black and White and Lithuanian Red Cattle Improvers' associations provided 192 ear tissue samples. DNA extraction was performed using Genomic DNA Purification Kit (Thermo Fisher Scientific, Lithuania) according to the manufacturer's recommendations and its concentration (ng/µl) was assessed using NanoDrop™ 2000 (Thermo Fisher Scientific™) spectrophotometer. DNA samples were stored at –20°C until use. A set of 11 microsatellite loci (BTJAB1, BOVIRBP, BM6438, BM2830, BM1225, BM1818, TGLA122, ETH10, HEL9, CSSM66, TGLA227) used in this study were chosen from part of a panel markers recommended by the International Society of Animal Genetics (ISAG/FAO, 2004).

The final volume of the PCR mix was 15 µL, which contained 7.5 µL of 2X PCR buffer, 2 µL of the target DNA, 1 µL of each primer pair, and ddH_2O, the volume of which was based on the missing final volume of the reaction mix. The forward primers for the screened polymorphic loci were labelled with fluorescent dyes (5-CY3 or 5-FAM) and their PCR products were separated by capillary electrophoresis using a SeqStudio Genetic Analyzer Sequencer (Applied Biosystems®).

The allele size was determined using GeneMapper™ 6.0 Software (Thermo Fisher Scientific, No. 4475073). The mean number of alleles per locus (Na), expected heterozygosity (H_E), and observed heterozygosity (H_O) were calculated using GeneAlEx v. 6.5 (Peakall, Smouse, 2012). The Markov chain algorithm was used to calculate the exact *P* value where the following parameters were used: 10,000 dememorization, 100 batches, and 1000 replications. Hierarchical molecular variance analysis (AMOVA) was performed using Arlequin v. 3.5.2.2 (Excoffier et al., 2010). The distribution of total genetic diversity between sample groups and individuals (excluding repetitive genotypes) was assessed using the FST (IAM) model. Groups of genetic clusters were identified by Bayesian cluster analysis according to the Markov chain algorithm (MCMC) for 200,000 iterations with 100,000 replications; each was run ten times with STRUCTURE 2.3.4 software (Pritchard et al., 2000). To summarise the obtained results, the CLUMPAK system package (Kopelman et al., 2015) analysis based on the formed Q – matrix was performed.

RESULTS AND DISCUSSION

A total of 88 different alleles were found in the group of Lithuanian Black and White (LBW) cattle breed, 98 in the Lithuanian Red (LR) cattle breed, and 106 in the Lithuanian Red and White (LRW) cattle breed. The observed heterozygosity (H_O) and the expected heterozygosity (H_E) ranged from 0.067 to 1.000 and from 0.065 to 0.887, respectively. BM2830 locus had the highest H_E and H_O in all three cattle breeds, except the CSSM66 locus, which had the highest expected heterozygosity rates in the LRW cattle breed. On the other hand, BOVIRBP had the lowest rates (0.067 and 0.065, respectively) in LBW, LR, and LRW cattle breeds (Table 1).

Table 1. **Values of the individuals studied at the locus (N), the number of different alleles (Na), the number of effective alleles (Ne), the observed heterozygosity (H_O), and the expected heterozygosity (H_E)**

	Lithuanian Black and White					Lithuanian Red					Lithuanian Red and White				
Locus	N	Na	Ne	H_O	H_E	N	Na	Ne	H_O	H_E	N	Na	Ne	H_O	H_E
BTJAB1	88	5	3.942	0.989	0.746	46	7	4.31	0.957	0.768	48	5	3.61	0.958	0.723
BOVIRBP	90	4	1.070	0.067	0.065	47	5	1.30	0.234	0.230	47	4	1.14	0.128	0.122
BM6438	90	6	2.098	0.622	0.523	47	5	2.18	0.532	0.542	46	9	3.36	0.674	0.703
BM2830	95	11	5.648	1.000	0.823	46	12	8.82	1.000	0.887	46	15	8.33	1.000	0.880
BM1225	95	6	2zzz.907	0.684	0.656	49	7	3.12	0.653	0.679	48	9	2.93	0.479	0.658
BM1818	95	6	2.633	0.621	0.620	48	6	3.56	0.792	0.719	45	8	4.15	0.867	0.759
TGLA122	95	11	4.153	0.684	0.759	49	12	3.34	0.592	0.701	48	14	3.69	0.542	0.729
ETH10	95	8	4.265	0.958	0.766	49	13	5.73	0.959	0.825	48	11	5.81	0.917	0.828
HEL9	90	9	6.004	0.933	0.833	46	9	4.00	0.826	0.750	45	8	5.93	0.933	0.831
CSSM66	90	12	5.389	0.822	0.814	45	13	7.32	0.822	0.863	44	12	8.78	0.841	0.886
TGLA227	84	10	4.040	0.798	0.752	43	9	4.71	0.744	0.788	45	11	6.32	0.822	0.842
Total	–	**88**	42.15	**0.743 ± 0.080**	**0.669 ± 0.067**	–	**98**	**48.38**	**0.705 ± 0.056**	**0.712 ± 0.056**	–	**106**	**54.05**	**0.724 ± 0.064**	**0.732 ± 0.065**

Thirty-two private alleles were detected in all cattle breeds (15 private alleles in LRW, nine private alleles in LBW, eight private alleles in LR). Private alleles were not detected in the HEL9 loci (Table 2). Private alleles usually appear in big populations due to random mutations, gene drift, or individuals' migration between populations. These alleles are specific and may have a crucial role in future breeding (Stolpovsky et al., 2020).

There is a clear tendency to form three groups of subpopulations based on allelic frequencies (Fig. 1). However, after executing Principal coordinates analysis (PCoA), which is based on genetic distances, no clear clustering was observed (Fig. 2).

Table 2. **Distribution of private alleles in the populations of Lithuanian Black and White (LBW), Lithuanian Red (LR), and Lithuanian Red and White (LRW) cattle**

Locus	LBW	LR	LRW	Number of private alleles
BTJAB1	–	216 (0.011); 230 (0.011)	–	2
BOVIRBP	–	141 (0.011)	–	1
BM6438	–	–	262 (0.011); 264 (0.011); 280 (0.011)	3
BM2830	165 (0.005)	–	138 (0.011); 140 (0.011); 150 (0.033)	4
BM1225	235 (0.005)	–	231 (0.010); 247 (0.010); 259 (0.021)	4
BM1818	–	270 (0.010)	240 (0.011); 256 (0.011)	3
TGLA122	164 (0.015); 178 (0.005); 182 (0.005)	134 (0.010)	146 (0.011); 168 (0.010)	6
ETH10	223 (0.005)	207 (0.005); 211 (0.042);	220 (0.010)	4
CSSM66	70 (0.005); 104 (0.027)	82 (0.011)	–	3
TGLA227	204 (0.006)	–	194 (0.022)	2
Total	**9**	**8**	**15**	**32**

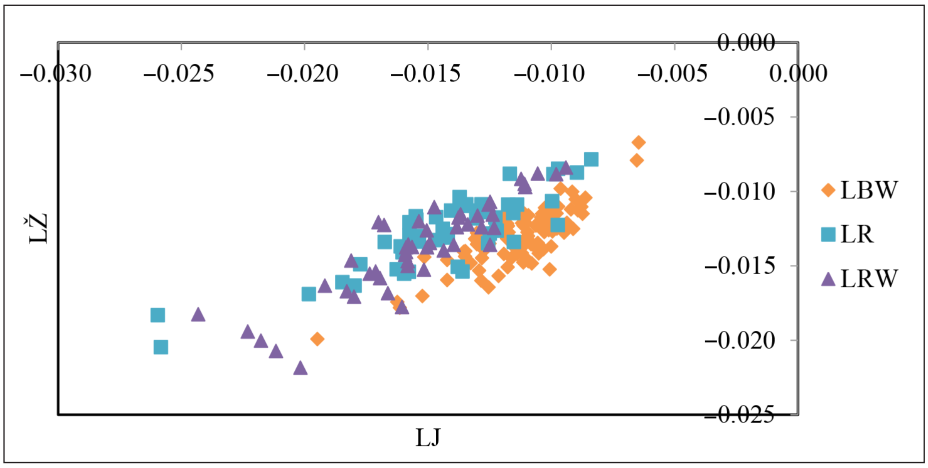

Fig. 1. Assignment of Lithuanian dairy cattle to subpopulations based on allele frequencies

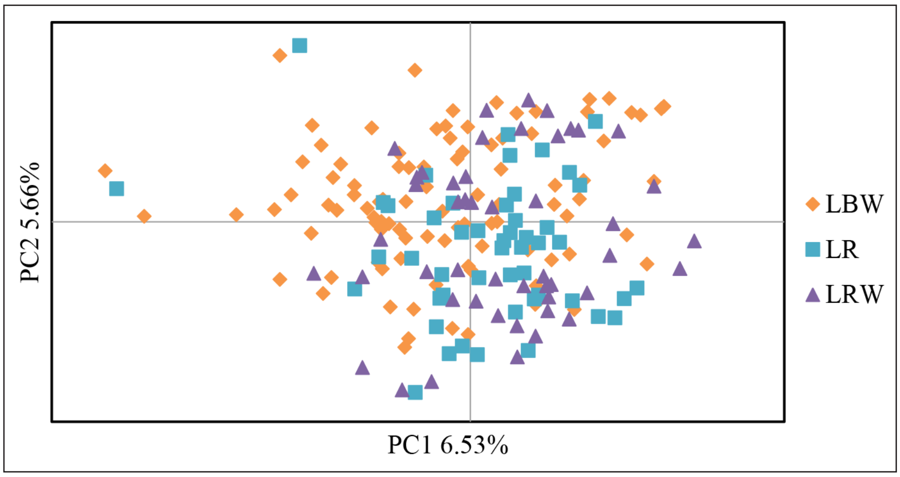

Fig. 2. Principal component analysis based on genetic distances of Lithuanian dairy cattle

Model-based clustering analysis revealed that Lithuanian dairy cattle had the highest ΔK when K was set to 3; that provided an explanation for the genetic structure and levels of admixture for the populations. By completing CLUMPAK analysis, results were presented in the dispersed bar (Fig. 3).

To identify genetic differentiation between different cattle breeds, Nei genetic distances and FST values were obtained. Both parameters revealed low genetic differentiation between cattle breeds (the total mean value of FST was 0.019). Also, FST and Nei values were calculated for breed groups, where the highest value was observed between LBW and LRW (FST = 0.027; Nei = 0.084) (Table 3).

Moreover, the analysis of molecular variance (AMOVA) was performed, which confirmed low genetic variability between subpopulations; however, it showed high genetic differentiation between individuals in subpopulations (Table 4).

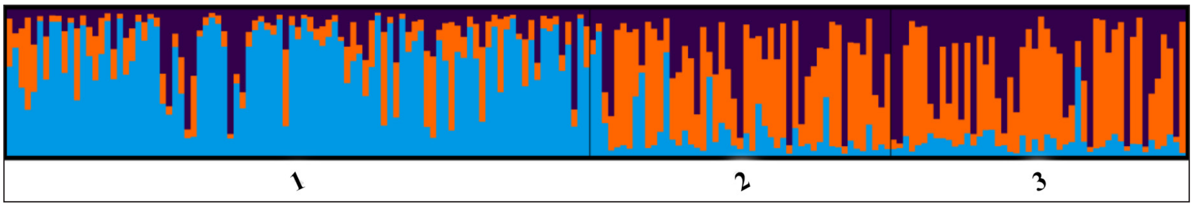

Fig. 3. Population structure of the three analysed cattle breeds obtained by using a model-based clustering method implemented in STRUCTURE for K = 3. Each column represents the proportion in which an individual belongs to a different-coloured cluster. 1 – Lithuanian Black and White; 2 – Lithuanian Red; 3 – Lithuanian White and Red

Table 3. **Values of F_{ST} (below diagonal) and Nei (above diagonal) genetic distance between the subpopulations dairy cattle studied in Lithuania. Maximum values are highlighted**

LBW	LR	LRW	
0.000	0.071	**0.084**	**LBW**
0.021	0.000	0.029	**LR**
0.027	0.001	0.000	**LRW**

Table 4. **Values of degrees of freedom (d.f.), sum of squares (SS), and variance components expressed as** a **percentage of variability**

Source	d. f.	SS	Dispersion component	Variability %
Among populations	1	14.152	0.06321	**2.85**
Within the population	192	440.000	2.29167	**97.15**
Total	193	480.152	2.35488	100

DISCUSSION

Investigation into genetic variability of Lithuanian cattle breeds using microsatellite primers is very important for the preservation and improvement of their phenotypic and genotypic properties. After studying 192 individuals, from 88 (N = 95) to 106 (N = 48) different alleles were found in subpopulations (292 being the total number of different alleles). The study conducted by Svishcheva et al. (2020) focused on cattle breeds in the Eurasian area, where it was found that the number of different alleles in the breeds of the European region ranged from 79 (N = 49) to 113 (N = 48). Ozsensoy and Kurar (2014) determined that an average of 13.45 different alleles are found per locus; in the study of Snegin et al. (2019), it ranged between 6.5 and 13.6 alleles per population, whereas in our study this value was 26.55. These differences are due to the number of individuals in different populations (subpopulations), the values of the range of the markers used, and differences in breeds.

The H_O values analysed in this study ranged from 0.705 ± 0.056 to 0.743 ± 0.080 and the H_E values from 0.669 ± 0.067 to 0.732 ± 0.065. The values of the observed heterozygosity in the Swiss Brown breed (the breed used for Lithuanian Red crossbreeding) studied by Svishcheva et al. (2020) were 0.72 and 0.71, which fully correlates with the data obtained in our study into the subpopulation of Lithuanian Black and White cattle (0.74 and 0.67).

During the analysis of private alleles, we found as many as 15 private alleles in our study into the subpopulation of the Lithuanian Red cattle breed (N = 48). Van der Westhuizen et al. (2020) conducted a study with a larger population sample (N = 550) and found 2–9 private alleles. Snegin et al. (2019) also found a lower number of private alleles (1–5 alleles per breed) in his study (N = 752). In our study, most of the private alleles (6) were found in the TGLA122 locus. In Snegin et al. (2019), the majority of private alleles were also found in the TGLA122 locus (4), and Svishcheva et al. (2020) found the largest number of private alleles in the TGLA227 locus (3) (N = 1168), whereas in our study, two (one in LJ and one in LJM subpopulations) were found (N = 192). A review of the overall trend and a comparison of the number of private alleles and the number of individuals tested suggest that the primers selected in this study were suitable for the identification of private alleles for the selected breeds.

The results of this study showed that the variation is 2.85% between subpopulations and 97.15% within a population. Ozsensoy and Kurar (2014) examined native Turkish varieties and obtained a 2% variation between populations and 98% within a population. Meanwhile, Prusak et al. (2015) showed a 26.5% variation between populations and 73.5% within the population when studying local Polish cattle breeds.

The FST analysis among dairy cattle breeds (LBW, LR, LRW) resulted in overall FST = 0.019. Among bovine subpopulations, the highest FST and Nei values (FST = 0.027; Nei = 0.084) were low compared to the results obtained from different studies. In a study conducted in Poland among the Black and White, Polish Black-Backed, and Polish Red populations, FST ranged from 0.247 to 0.941 (Prusak et al., 2015). According to Zatoń-Dobrowolska et al. (2007), FST values varied between 0.173 and 0.197 in Red cattle breeds; Nei values were 0.112–0.157.

Meanwhile, Svishcheva et al. (2020) obtained the FST value of 0.153 value. In Snegin et al. (2019), the highest FST value among breeds was 0.469 and Nei 0.679.

CONCLUSIONS

Our results provide new information about the genetic variability of the Lithuanian cattle breeds: Lithuanian Red, Lithuanian Red and White, Lithuanian Black and White. The results of the present study demonstrate low genetic variability between subpopulations; however, it showed high genetic differentiation between individuals within subpopulations. It is recommended to use these results for further Lithuanian cattle breeding to maintain high phenotypic and genotypic performance.

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Romualdas Lapickis, Loreta Griciuvienė, Asta Aleksandravičienė, Indrė Lipatova, Algimantas Paulauskas

PIENINIŲ GALVIJŲ VEISLIŲ GENETINIS KINTAMUMAS LIETUVOJE

Santrauka

Pagrindinis šio tyrimo tikslas – atlikti Lietuvos galvijų veislių genetinio kintamumo analizę. Tirtos trys veislės: Lietuvos juodmargiai (95), Lietuvos žalieji (49), Lietuvos žalmargiai (48). Genetiniam kintamumui įvertinti buvo naudojama 11 fluorescencinių mikrosatelitinių žymenų. Visi lokusai pasižymėjo dideliu polimorfizmo laipsniu. Iš viso buvo aptikti 292 skirtingi aleliai (Na). Visose vertintose subpopuliacijose buvo aptikti 32 privatūs aleliai. Sugrupavus individus pagal alelių dažnius, nustatyta trijų atskirų grupių tendencija. Tačiau atlikus pagrindinių komponenčių analizę (PCoA), pagrįstą genetiniais atstumais, aiškaus grupavimosi, kurio buvo tikimasi, nenustatyta. Bajeso struktūros analizė atskleidė tris genetines grupes. Išanalizavus FST (0,001–0,027) ir Nei genetinį atstumą (0,029–0,084), mažesnė genetinė įvairovė nustatyta tarp subpopuliacijų nei subpopuliacijų viduje.

Raktažodžiai: genetinis kintamumas, mikrosatelitai, galvijai