ANALYSES OF GENETIC MARKERS IN ROMANIAN BLACK SPOTTED CATTLE BREED

Nicoleta Isfan[1*], S.E. Georgescu[2], Mariana Rebedea[2], Ioana Nicolae[3], Monica Marin[1], Marieta Costache[2], Dana Popa[1]

[1] University of Agricultural Sciences and Veterinary Medicine of Bucharest, Faculty of Animal Science, Bucharest, Romania
[2] University of Bucharest, Faculty of Biology, Department of Biochemistry and Molecular Biology, Bucharest, Romania
[3] Research and Development Institute for Cattle Breeding, Balotesti, Romania

Abstract

The genetic diversity found in domestic breeds allows farmers to develop new characteristics in response to changes in the environment, the appearance of diseases, or of modifications in market conditions. Microsatellite markers are more likely than other methods to be used in order to detect small differences between populations due to their high levels of allelic variation, due to their capacity to differentiate between overall heterozygosity and the mean number of alleles. Genetic characterization of Romanian Black Spotted population was made using eleven microsatellites: TGLA227, BM2113, TGLA53, ETH10, SPS115, TGLA126, TGLA122, INRA23, ETH3, ETH225, and BM1824. They were chosen based upon the polymorphism detected in other breeds. The observed heterozygosity was 0.664 and the expected heterozygosity 0.749. Hardy-Weinberg equilibrium was tested and for TGLA53 locus we observed a significant deviation. The study of eritrocitary antigenic configuration is mainly used for establishing the animal identity and genetic characterization. The interpretation of electrophoresis graphs had detected two categories of individuals: homozygous for genes TfD and heterozygous TfA/TfD have been identified on seric transferring locus. The two genetic categories are genetically determined by the presence, at the Haemoglobin locus, of two categories of genes, HbA and HbB.

Key words: microsatellites, allele frequency, blood phenogroups, transferring, haemoglobin

INTRODUCTION

During the last three decades, new methods have been developed and applied to investigating the genetics of the cattle and to improve its performance. Local cattle breeds often possess gene combinations and special adaptations not found in other breeds. Conservation policies of native breeds will depend to a large extent on our knowledge of historic and genetic relationships among breeds. Microsatellites are new powerful polymorphic markers used for gene mapping, individual identification and parentage verification. They can also be used to screen for markers linked to performance traits or for diagnosis of genetic disorders. The study of the genetic markers and identifying new markers involves an increasing number of research projects in the fields of genetics of immunology, biochemical genetics, molecular genetics, quantity genetics and the genetic improvement of animals [1].

Some studies on genes frequency determining the red cells specificity and for whey haemoglobin are approached in the present report. In this way, some blood factors, most of them belonging to B system (the most complex system in cattle) have been evidenced.

MATERIAL AND METHODS

Sampling

Fresh blood from Romanian Black Spotted cattle was collected. The individuals were chosen at random and we avoided closely related animals.

DNA Extraction

The isolation of genomic DNA from fresh blood was performed with Wizard Genomic DNA Extraction Kit (Promega).

*Corresponding author: ndefta@yahoo.com
The manuscript was received: 09.04.2012
Accepted for publication: 19.11.2012

Microsatellites Analysis

The animals were genotyped for 11 microsatellite markers: TGLA227, BM2113, TGLA53, ETH10, SPS115, TGLA126, TGLA122, INRA23, ETH3, ETH225, and BM1824. Amplification of the microsatellite loci was realized by multiplex PCR using StockMarks® for Cattle Bovine Genotyping Kit (AppliedBiosystems) and PCR products were detected by capillary electrophoresis using an ABI Prism 310 DNA Genetic Analyzer (AppliedBiosystems). Thirty-one amplification cycles were performed in GeneAmp® PCR System 9700: 94ºC/45 sec; 61°C/45 sec; 72°C/60 sec. The first denaturation step was performed at 95ºC/15 min and the last extension was 72°C/60 min. The size of alleles was determined by using GeneScan-500 ROX Size Standard and the results were processed with the GeneScan®3.1.2 and Genotyper®2.5.2 Software (AppliedBiosystems). The allelic frequencies, observed and expected heterozygosities (Ho and He) were estimated using the CERVUS 2.0 program [5].

Blood phenogroups

The blood samples collection was accomplished in heparinised, standard test tubes. Determination of blood phenogroups was realized according to the standard methodology, by using the set of 40 reagents existing in the immune-serology laboratory. The various degrees of haemolysis realizing were estimated by reading:

-negative reaction: all erythrocytes are deposited, the above liquid is clear;

-positive reaction: it was appreciated according to the lysed red cell utilized in the following four values scale: light haemolysis, accentuated haemolysis, net haemolysis, complete haemolysis.

For establishing the types of transferrin and haemoglobin, we used the technique of vertical electro-phoresys, using polyacryl amidae as a migration support; the same technique as used by Meriaux J.C. [4], adapted to the conditions in the bio-chemistry laboratory the Faculty of Biology of The University of Bucharest.

RESULTS AND DISCUSSIONS

Microsatellites variations

In our study all eleven loci are di-nucleotide repeats, so successful amplification yields allele peaks with the associated PCR stutter bands within a maximum range of eight base pairs from the allele peak. The number of allele peaks depends on whether the individual tested is a heterozygote or homozygote. Allele frequencies are presented in Table 1.

Table 1 The allele frequency of the 11 microsatellites in Romanian Black Spotted population

Loci	**Alleles/Frequencies**										
BM 1824	**179**	**181**	**183**	**189**	**191**						
	0.287	0.256	0.277	0.170	0.011						
BM 2113	**120**	**122**	**124**	**126**	**128**	**130**	**132**	**134**	**136**	**140**	
	0.011	0.245	0.149	0.011	0.011	0.043	0.234	0.170	0.085	0.043	
ETH3	**113**	**115**	**117**	**121**	**123**	**125**	**127**	**129**			
	0.489	0.064	0.043	0.202	0.074	0.085	0.032	0.011			
ETH 10	**210**	**212**	**214**	**216**	**218**	**220**	**222**				
	0.136	0.011	0.091	0.398	0.080	0.159	0.125				
ETH 225	**134**	**136**	**138**	**140**	**142**	**144**	**146**	**152**			
	0.255	0.011	0.064	0.011	0.224	0.255	0.170	0.011			
INRA23	**197**	**199**	**201**	**203**	**205**	**207**	**209**	**211**	**213**		
	0.011	0.160	0.149	0.011	0.330	0.096	0.106	0.064	0.074		
SPS 115	**242**	**244**	**246**	**248**	**250**	**252**					
	0.628	0.026	0.192	0.051	0.090	0.013					
TGLA53	**147**	**149**	**151**	**155**	**157**	**159**	**161**	**163**	**167**	**183**	
	0.032	0.032	0.085	0.011	0.521	0.202	0.032	0.032	0.043	0.011	
TGLA122	**138**	**140**	**148**	**150**	**152**	**154**	**160**	**162**	**172**		
	0.021	0.244	0.351	0.223	0.021	0.011	0.064	0.053	0.011		
TGLA126	**116**	**118**	**120**	**122**	**124**	**126**					
	0.404	0.394	0.053	0.074	0.053	0.021					
TGLA227	**75**	**79**	**81**	**83**	**87**	**89**	**91**	**93**	**97**	**99**	**103**
	0.043	0.032	0.085	0.011	0.032	0.107	0.234	0.021	0.383	0.021	0.032

The size range of alleles at the individual loci varied between 75 and 252 bp. High levels of polymorphism were observed for the Romanian Black Spotted cattle population studied. Among the 11 microsatellites, three were highly polymorphic (TGLA227, BM2113, TGLA53) and two (SPS115 and BM1824) present low polymorphism (Table 2).

Table 2 Number of alleles and their sizes per each locus for Romanian Black Spotted population

Locus	Size obtained (bp)	Number of alleles
TGLA227	75-103	11
BM2113	120-140	10
TGLA53	147-183	10
ETH10	210-222	7
SPS115	242-252	5
TGLA126	116-126	6
TGLA122	138-172	9
INRA23	197-213	9
ETH3	113-129	8
ETH225	134-152	8
BM1824	179-191	5

Observed and expected heterozygosities (H_o and H_e) ranged from 0.362 to 0.765 and from 0.565 to 0.843, respectively (Table 3). Hardy-Weinberg equilibrium was tested and for TGLA53 locus we observed a significant deviation. This might be caused by hybridisation between breeds, or a null allele segregating in the population.

Table 3 Statistical analysis for Romanian Black Spotted populations

Locus	H_o	H_e	PIC (Polymorphic Information Content)
TGLA227	0.744	0.793	0.761
BM2113	0.638	0.843	0.814
TGLA53	0.362	0.684	0.647
ETH10	0.751	0.776	0.741
SPS115	0.513	0.565	0.518
TGLA126	0.617	0.677	0.614
TGLA122	0.681	0.779	0.739
INRA23	0.765	0.832	0.805
ETH3	0.702	0.718	0.684
ETH225	0.746	0.820	0.786
BM1824	0.787	0.759	0.708

The Locus of Blood Phenogroups

In this effective there were not emphasized individuals with the I_2, E'_3, G'', F_2 and H'' factors.

The M factor is associated with a low milk production. This factor was noticed at 2,6% of individuals.

For 9% from the emphasized erythrocytary, a net haemolysis was obtained. The most positive reactions (87%) were of complete haemolysis. For the rest of positive reactions (4%) it was found that the haemolysis was of 50%, the above liquid being pink-reddish colored [3].

The most reactions were observed within system B, known as the most complex blood group system at cattle (figure 1)

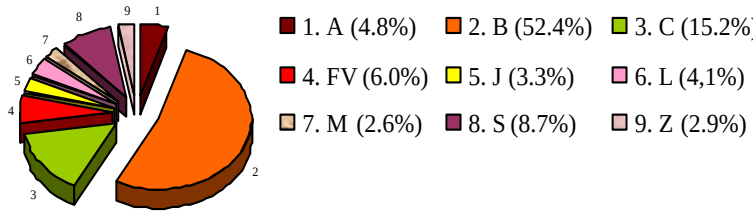

Fig. 1 Share of blood group systems category

The most relevant was the complex BGK. These tree factors were observed in five different combinations, namely BGK, BG, B, G, and their total absence. Another type of relations, namely the linear relations were observed between the factors of systems A (A_1, A_2), B (G_1, G_2, O_1, O_2), C (C_1, C_2; X_1, X_2). The genetic explanation of these subtypes of blood group factors consists of the different antigenic structure of the respective factors [2]. Between the subtypes of the same factor crossed reactions may appear, these being characteristic for each system.

The Locus of Serum Transferines

The interpretation of electrophoresis graphs has detected two categories of individuals: homozygous for genes Tf^D and heterozygous Tf^A/Tf^D (figure 2).

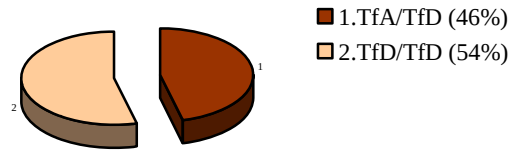

Fig. 2 The weight (%) of genotypic categories on seric transferine locus

The presence of two genotype categories in the group demonstrates the presence of two categories of genes, Tf^A and Tf^D identified with different frequency.

Since no Tf^ATf^A individuals have not been identified, Tf^A low gene frequency occurred, namely 50% lower than Tf^D(figure 3).

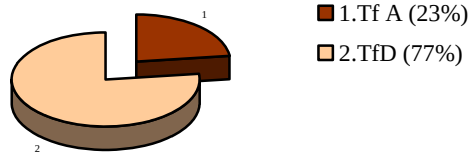

Fig. 3 The weight (%) of genes categories on seric transferine locus

Determining the ratios of the genotype categories and, as a result, the genetic structure of the sample in this study allowed building an estimate for the status of genetic equilibrium, for locus analyzed. The analysis of genetic equilibrium was made using χ^2 test, and it led to the conclusion that the studied sample express genetic equilibrium for transferine locus (χ^2_{calc}= 3.047 and χ^2_{tab}= 5.99).

The Locus of Haemoglobin

Statistical analysis of the studied sample revealed two types of haemoglobin, namely A and AB genetically determined by the following genotypes: homozygous Hb^A/Hb^A and heterozygous Hb^A/Hb^B. Identification of genotypic categories on haemoglobin locus allowed establishing genetic structure within this sample. Homozygous Hb^A/Hb^A individuals presents the highest occurrence within genetic structure, namely ten times higher than heterozygous ones (table 5). Both genotypic categories are genetically controlled by two alleles, different from frequency point of view, Hb^A şi Hb^B.

Table 5 Distribution of gene and genotype categories at haemoglobin locus

Genotype categories	N	The ratio of genotype categories (%)	The distribution of gene categories (%)	
			Hb^A	Hb^B
Hb^A/Hb^A	32	91	95.5	4.5
Hb^A/Hb^B	3	9		

Determining the ratios of the genotype categories and, as a result, the genetic structure of the sample in this study allowed building an estimate for the state of genetic equilibrium. Hardy-Weinberg equilibrium was tested; the sample studied has found to be genetically balanced (χ^2_{calc}= 0.071 and χ^2_{tab}= 5.99).

CONCLUSIONS

Microsatellite markers are more likely to detect differences between populations than other methods, being able to discriminate in both overall heterozygosity and mean number of alleles. Our data demonstrate an important level of polymorphism detectable with microsatellite loci within the Romanian Black Spotted cattle population. In cattle breeding this technology has the potential to be of great use in monitoring levels of genetic variation within stocks as well as for parentage and relatedness purposes.

It is possible that by a series of biochemical or immuno-serological processes should be identified certain gene markers and their evolution within the selection process should be analyzed and also, it should be established the extent of correlation with some traits which present bio-economic importance. Presently, the amelioration staff request that population (livestock) genetics should offer some simpler and more direct means in order to estimate general genetically variability. One of these means is represented by molecular genetics, by one of its major contribution to animal husbandry progress, such as the possibility of introspection within the inner structure of breeds.

ACKNOWLEDGEMENTS

This work (immuno-biochemical markers) was co-financed from the European Social Fund through Sectoral Operational Programme Human Resources Development 2007-2013, project number POSDRU/89/1.5/S/63258 ”Postdoctoral school for zootechnical biodiversity and food biotechnology based on the eco-economy and the bio-economy required by eco-san-genesis”.

REFERENCES

[1] Bacila V, Vladu Marius, Bacila Anamaria, 2010, Animal molecular marker assisted selection, The sixth edition of “Scientific Conferences – Durable Agriculture-Agriculture of Future”, vol. XL/1, p. 11.

[2] Bell, K., 1983, The blood groups of domestic mammals. In Agar, N.S. and doard, P.G. (eds)Red Blood Cell of Domestic Mammals. Elsevier Science Publishers B.V. Amsterdam, pp. 133-164

[3] Hines, H.C., Blood groups and biochemical polymorphisms. The genetics of cattle, 1999, pp. 77-108.

[4] Mariaux, J.C., Control de filiation et marquers sanguines chey les equides. Rec.Med.Vet. Special Reprod.des Equides, 1992, pp.17-24

[5] Marshall T.C., Slate-Kruuk L., Pemberton J.M., 1998, Statistical Confidence for Likelihood-Based Paternity Inference in Natural Populations. Mol. Ecol., 7, 639-655.