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Analysis of genetic diversity of Romanian buffalo – a preliminary study

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Abstract

Romanian Buffalo (Bubalus bubalis, LINNAEUS, 1758) was an important resource for meat and milk, plus a number of traditions associated with this species. Currently in our country buffalo herd is continuously decreasing. In this context, conservation of genetic resources represents an extremely important action. Loss of genetic diversity is the main cause of the disappearance of populations or their entrance in the area of vulnerable or endangered risk status. The goal of this study is to evaluate the genetic diversity within Romanian Buffalo population from Şercaia Research and Development Station for Buffalo Breeding, using information provided by microsatellites. For the investigated population, the results suggest the existence of crosses in the past of population, without being able to specify the type of the cross. Only genealogical analysis will be precisely this, but the fact that molecular genetics senses this is an advantage. Also, a bottleneck effect was underlined. There is no question of inbreeding in case of studied loci, but the problem also requires genealogical studies. Heterogeneity is an asset for the future of population.

Key words: Romanian Buffalo, genetic history, genetic diversity, microsatellite markers.

1. Introduction

In Romania, the buffalo entered with the invasion of the Huns and Avars in the Carpatho-Danubian area. It found the good pedo-climatic conditions and so, in our country, a buffalo population perfectly distinct from other such populations has developed, which had its own evolutionary path because of reproductive isolation (VIDU & al, 2008 [1]).

Compared with cows, buffalo milk has quality parameters with higher values. The fat percentage range between 6.87 to 8.59% (ROSATI and VANVLECK, 2002 [2]; TONHATI & al, 2000 [3]), and protein percentage between 4.13 to 4.55% (MACEDO & al, 2001 [4]; ROSATI and VANVLECK, 2002 [2]). In Romania, VELEA & MĂRGINEAN (2004) [5] specifies that buffalo's milk production falls into the following parameters: average milk yield: 1111.11 kg/lactation, average fat yield 82.10 kg (7.39%), and average protein yield 46.21 kg (4.23%).

In Europe, the main country that exploits buffalo is Italy, the main production being mozzarella type soft cheese. In 2004, Romania ranked 2 in Europe in terms of buffalo breeding, with 100,000 heads (VIDU & al, 2008 [1]).

Romanian Buffalo was an important resource for meat and milk, plus a number of traditions associated with this species, characteristic for growth areas. As a consequence of the lack of supportive policies in the area of buffalo, herd showed a decreasing trend in Romania, FAO estimating that there are 70,000 heads in 2006 whereas VIDU (2007 [6]) estimated a

population of about 64,000 heads. Romania, along with other countries in the region, faced with a decrease in economic value of this species. As a result of increased milk demand, Romanian farmers have preferred exploitation of other species, more productive and economically efficient. In this respect, BORGHESE (2005 [7]) underlined that in some countries form EE (Bulgaria, Romania, Turkey), the decrease number of buffaloes is associated with three factors: holsteinization, mechanization, and poor market demand for buffalo product.

In this context, conservation of genetic resources represents an extremely important action. Loss of genetic diversity is the main cause of the disappearance of populations or their entrance in the area of vulnerable or endangered risk status.

Our country has some problems regarding sustainable utilization of genetic resources and for this reason special efforts for development of sustainable programs for genetic resources management, according to international organizations requirements has to be made. The reasons for these problems are: genetic evaluation procedures according to the '80, are utilized and on the other hand the elaboration of national breeding programs has a general character (without mathematical and molecular determination of population risk status). Analysis of genetic diversity within population is a critical parameter to asses for all researches which are implicated in conservation activity. An approach for genetic diversity study is analysis of genetic history. This activity involved a genealogical analysis in order to obtain some important parameters for risk status evaluation. Though they have an auxiliary role, not a fundamental one, information provided by molecular genetics are very useful. In order to elaborate consistent breeding strategies, for optimal use and conservation of genetic resources, it is essential to understand the genetic structure and degree of relatedness (genetic relationship) between buffalo populations. Thus, the study of genetic structure using microsatellites became very important (GONCALVES & al, 2004 [8]). On the other hand, DNA polymorphism are useful tool for understand evolution and genetic diversity (NAVANI & al, 2001 [9]), genome mapping, paternity testing and conservation of genetic resources (JARNE and LAGODa, 1996 [10]; KAPPES & al, 1997 [11]; KIM & al, 2001 [12]). In this context, VENANCIO & al (2007) [13] studied and introduced in the database 6 microsatellites using selective hybridization method at river buffalo.

Microsatellites are short repetitive sequences dispersed throughout the entire genome and present in all vertebrates. Microsatellites loci represent an important source of information about population histories and evolutionary processes (GOLDSTEIN & SCHLÖTTERER, 1999 [14]) and they are useful in estimating the genetic distance among different individuals or populations (TAKEZAKI & NEI, 1996 [15]). Few studies of genetic relationships between different buffalo populations or breeds using microsatellites were done and these provide useful information about breeds evolution and history, gene pool structure and the degree of genetic differentiation (EL-KHOLY & al, 2007 [16]; ZHANG & al, 2007 [17]; AMINAFSHAR & al, 2008 [18]; ELBELTAGY & al, 2008 [19]; BABAR & al, 2009 [20]; GARGANI & al, 2009 [21]; KATARIA & al, 2009 [22]; JAAYID & al, 2013 [23]; UNAL & al, 2014 [24]).

Unfortunately, in Romania are few scientists who understand the importance of genetic analyzes and for this reason these are either neglected or constitute the subject of serious confusions. The genetic history is an obligatory action in management of genetic resources, represented by a sum of analyzes used for selection effect and genetic trend estimate. All of these actions have as a main goal to establish the population risk status necessary for strategy elaboration (POPA, 2009 [25]).

This paper is a part of an ample project which aims to develop a sustainable program for genetic resources management represented by indigenous buffalo. The goal of this study is to evaluate the genetic diversity within Romanian Buffalo population from Șercaia Research

and Development Station for Buffalo Breeding, using information provided by microsatellites. We highlight that Şercaia Research Station has a tradition of over 35 years in the field, it is the owner of an important livestock and the main, if not the only supplier of biological material.

2. Materials and Methods

Sampling and DNA extraction

Fresh blood samples were collected from 22 domestic water buffalo (*Bubalus bubalis*), from Şercaia Research and Development Station for Buffalo Breeding, Brasov County, Romania. The isolation of genomic DNA from the white blood cells was performed with Genomic DNA Extraction Kit (Promega).

Microsatellite analyses

The individuals were genotyped for 6 microsatellite markers specific to cattle: TGLA227, BM2113, TGLA53, SPS115, TGLA126, and ETH3. Cross-amplification of the microsatellites was achieved using StockMarks® for Cattle Bovine Genotyping Kit (Applied Biosystems), according to procedure recommended by the manufacturer.

The multiplex PCR was performed in a 15µl final volume with 20 ng template DNA. PCR amplifications were carried out in GeneAmp PCR System 9700 (Applied Biosystems) in 31 cycles containing denaturation at 94ºC (45 s), annealing at 61°C (45 s) and extension at 72°C (60 s). The first denaturation step was performed at 95ºC (10 min) and the last extension was 60 min at 72°C. Additionally, we performed one cycle at 25°C for 2 hours.

For genotyping the samples, PCR products were detected by capillary electrophoresis using ABI Prism 310 DNA Genetic Analyser (AppliedBiosystems). The size of alleles was determined by using GeneScan-500 ROX Size Standard (Applied Biosystems) and the results were processed with the GeneMapper v3.0 Software (AppliedBiosystems).

Statistical analysis

The Polymorphic Information Content (PIC) for each locus was calculated by using the PIC calculator (https://www.liverpool.ac.uk/~kempsj/pic.html).

Allele frequencies, the number of alleles (n_a), observed heterozygosity (H_o) and (H_e) and D_a genetic distance (NEI & al., 1983 [26]) between individuals using 1000 permutations were calculated using GENETIX version 4.05.2 (BELKHIR & al., 2004 [27]). The estimation of inbreeding per locus and in population was calculated with the same software by using 1000 permutations.

To infer the genetic relationship between all of the individuals a Neighbor Joining (NJ) tree was built on the basis of D_a distance matrix by using Populations v1.2.31 software (LANGELLA, 2002 [28]). The phylogenetic tree was visualized with TREEVIEW v1.6.6 (PAGE, 1996 [29]).

3. Results and Discussion

The number of alleles (n_a), allele size, allele frequency, Polymorphic Information Content (PIC), expected heterozygosity (H_e), observed heterozygosity (H_o) and Wright's inbreeding index (Fis) are summarized in Table 1.

For the investigated population of *Bubalus bubalis* a total of 18 alleles were identified. The most polymorphic locus is TGLA227 with five alleles, while the loci TGLA53 and TGLA126 present a fixed allele. The mean number of alleles per locus is 3.

The PIC value ranged from 0 for the loci TGLA53 and TGLA126 to 0.632 for TGLA227. The PIC index gives information about a genetic marker's usefulness (BOTSTEIN & al., 1980 [30]) and for codominant markers which are the case of microsatellites have values from 0 to 1. The values lowest than 0.25 indicates a low diversity, while the values higher than 0.5 indicates a significant diversity. Our results showed that among the investigated loci, four

present a high diversity and are useful in population genetics studies for quantifying the genetic diversity, while two loci showed no variation.

Table 1. Genetic variability estimators in the analyzed water buffalo population

Locus	n_a	Allele size	Allele frequency	PIC	H_e	H_o	Fis
TGLA227	5	69	0.0227	0.632	0.6942	0.9091	-0.288
		71	0.3182				
		73	0.0227				
		75	0.2955				
		77	0.3409				
BM2113	4	118	0.0909	0.5369	0.6126	0.6364	-0.016
		124	0.4773				
		133	0.3864				
		136	0.0455				
TGLA53	1	197	1	0	0	0	-
SPS115	4	248	0.4091	0.4335	0.5341	0.5455	0.002
		254	0.5455				
		256	0.0227				
		264	0.0227				
TGLA126	1	103	1	0	0	0	-
ETH3	3	102	0.3864	0.5028	0.5878	1.0000	-0.689
		104	0.1136				
		122	0.5000				
Overall					0.4048	0.5152	-0.25099

n_a – number of alleles
PIC = Polymorphic Information Content
H_e – Expected heterozygosity
H_o – Observed heterozygosity
Fis – Wright's index for estimate inbreeding (after WEIr & COCKERHAM, 1984 [31])

Heterozygosity estimates the most accurate the genetic diversity within population. For every locus H_o has a higher value than H_e and also the mean value of H_o (0.5152) is higher than H_e (0.4048). If heterozygosity is higher than expected, this might be an indication of an isolate-breaking effect caused by the mixing of two previously isolated populations. Also, the H_o being upper than H_e suggest a bottleneck that was experienced by the studied population.
The Wright's inbreeding index Fis is negative (-0.25099) which indicates no inbreeding has been depicted in this water buffalo population.
The genetic distance between the analyzed individuals was highlighted by a distance matrix of D_a (Nei & al, 1983 [26]). The values ranged from 0 (for individuals showing identical genotypes) to 1 (for individuals showing completely different genotypes), but the most of the individuals showed intermediate values (Figure 1).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
1		0.431	0.833	0.931	0.362	0.833	0.362	0.931	0.529	0.764	1	0.695	0.931	0.362	0.167	0.362	0.264	0.764	0.833	1.167	0.598	0.195	1
2			0.598	0.5	0.264	0.598	0.264	0.529	0.098	0.333	0.431	0.264	0.431	0.264	0.264	0.264	0.362	0.333	0.598	0.764	0.362	0.529	2
3				0.529	0.195	0.167	0.362	0.667	0.362	0.264	0.264	0.764	0.529	0.195	0.833	0.362	0.431	0.598	0.167	0.598	0.098	0.529	3
4					0.5	0.529	0.5	0.431	0.333	0.264	0.431	0.167	0.333	0.5	0.764	0.5	0.598	0.333	0.529	0.362	0.598	0.764	4
5						0.362	0.167	0.598	0.167	0.264	0.431	0.5	0.5	0	0.362	0.167	0.098	0.5	0.362	0.764	0.098	0.195	5
6							0.195	0.833	0.362	0.264	0.264	0.764	0.529	0.362	0.667	0.195	0.598	0.598	0	0.362	0.264	0.529	6
7								0.764	0.167	0.264	0.431	0.5	0.5	0.167	0.195	0	0.264	0.5	0.195	0.529	0.264	0.195	7
8									0.598	0.764	0.667	0.264	0.431	0.598	0.931	0.764	0.833	0.598	0.833	0.764	0.5	1	8
9										0.098	0.264	0.333	0.333	0.167	0.362	0.167	0.264	0.333	0.362	0.529	0.264	0.431	9
10											0.195	0.5	0.431	0.264	0.598	0.264	0.362	0.333	0.264	0.431	0.362	0.529	10
11												0.598	0.431	0.431	0.833	0.431	0.667	0.264	0.264	0.46	0.333	0.833	11
12													0.333	0.5	0.529	0.5	0.598	0.333	0.764	0.598	0.598	0.764	12
13														0.5	0.764	0.5	0.667	0.667	0.529	0.362	0.431	0.833	13
14															0.362	0.167	0.098	0.5	0.362	0.764	0.098	0.195	14
15																0.195	0.264	0.598	0.667	0.931	0.598	0.195	15
16																	0.264	0.5	0.195	0.529	0.264	0.195	16
17																		0.598	0.598	0.931	0.333	0.098	17
18																			0.598	0.764	0.598	0.764	18
19																				0.362	0.264	0.529	19
20																					0.695	0.931	20
21																						0.431	21
22																							22

Figure 1. Matrix of genetic distance D_a (Nei & al., 1983 [26]) between the analyzed individuals

The genetic relatedness between the individuals was illustrated by a NJ tree based on the distance matrix (Figure 2).

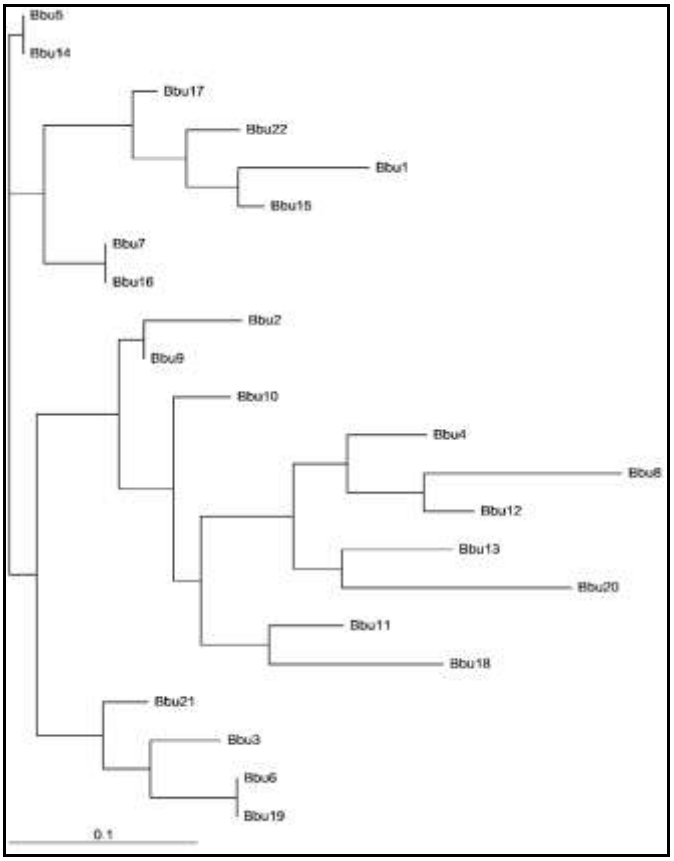

Figure 2. Neighbor Joining (NJ) tree based on D_a distance matrix illustrating the relationships between the analyzed individuals

The tree topology showed that despite that some individuals exhibit a high genetic resemblance there is a relative heterogeneity within population.

4. Conclusion

Conservation activity represent an important action in management of genetic resources. An extinct genetic structure is impossible to be restore. On the other hand, loss of genetic

diversity lead to genetic drift and disappearance of populations. In this respect, information provide by molecular genetics are very useful. For the investigated population, the results suggest the existence of crosses in the past of population, without being able to specify the type of the cross.
Only genealogical analysis will be precisely this, but the fact that molecular genetics senses this is an advantage. Also, a bottleneck effect was underline. There is no question of inbreeding in case of studied loci, but the problem also requires genealogical studies.
Heterogeneity is an asset for the future of population.

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