GENETIC CHARACTERIZATION OF THE CUBAN WATER BUFFALO POPULATION USING MICROSATELLITE DNA MARKERS

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ABSTRACT

The water buffalo (*Bubalus bubalis*) is an economically important livestock species. This study presents a molecular characterization of the Cuban water buffalo population on the basis of 16 bovine-specific microsatellite markers. The mean number of alleles across the 16 loci was 5.44, and the largest number of alleles for a locus was nine for the ETH225 marker and fewest alleles were two for the TGLA126 marker. Observed average heterozygosity was 0.46 ± 0.23 and expected heterozygosity was 0.54 ± 0.19. The overall polymorphic information content (PIC) value for these markers was 0.495. The observed value of inbreeding coefficient (F_{IS}) was of 0.148 and three loci were with significant reduction of heterozygosity (ETH3, HAUT24 and INRA032). Four loci were not in the Hardy-Weinberg equilibrium with a significant deficit heterozygote (ETH3, CSRM60, HAUT24 and INRA032).

Keywords: diversity analysis, *Bubalus bubalis*, microsatellite, polymorphism

INTRODUCTION

The domestic water buffalo (*Bubalus bubalis*) are broadly classified into two major categories based upon their phenotype, behavior and karyotype: river buffalo (2n=50) and swamp buffalo (2n=48) (Kumar *et al*., 2007). Buffalo milk is considered to be more economical for the production of casein, caseinates, whey protein concentrate, and fat-rich dairy products (Vijh *et al*., 2008).

The water buffalo was imported into Cuba from Australia and Trinidad and Tobago Island. A total of 2984 buffalos (2705 swamp buffalo and 279 river buffalo) were imported in order to contribute to the agricultural economy and food security of Cuba (Mitat, 2009).

In small populations, the lack of genetic variation is generally evaluated through inbreeding rate with a high sensitivity of parameter to pedigree quality as well as some population disadvantage about remaining closed for much time (Goyche *et al*., 2003).

Molecular markers like microsatellites

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are commonly used for the estimation of genetic diversity, calculation of genetic distances and detection of admixture, genetic bottlenecks, and inbreeding. Several studies have used the bovine microsatellite in study of characterization of buffalo (Barker *et al*., 1997; Van Hooft *et al*., 1999; Navani *et al*., 2002; Vijh *et al*., 2008). On the other hand, some have suggested that cattle microsatellite markers may not be optimal for genetic studies in *Bubalus bubalis* (Supajit *et al*., 2008). Two individuals with a common ancestor may both carry a copy of some gene possessed by their ancestor; if they mate, they may pass the same gene to their progeny (Miglior, 2000). Inbreeding does not affect all traits or all populations with the same intensity, so it is required that its effects be quantified for particular cases.

The objectives of this study were to assess the genetic diversity within the Cuban water buffalo population and to estimate the level of inbreeding using 16 bovine-specific microsatellite markers.

MATERIALS AND METHODS

Sampling and microsatellite loci

Blood samples of 50 adult females buffaloes were collected from different sections of the Western Region of Cuba (Pinar del Rio, La Habana and Matanzas). The animals were selected not closely related to one another and represented the populations. A total of 16 heterologous microsatellite loci (TGLA227, SPS115, ETH225, BM2113, ETH3, TGLA126, ETH152, CSRM60, HAUT24, INRA35, INRA037, CSSM66, MM12, INRA032, HEL1, and HEL13) were chosen for the study. These were analyzed to estimate various genetic diversity parameters. We used criteria similar to Navani *et al.* (2002) and Vijh *et al.* (2008)

for selection of the heterologous microsatellite loci, based on their polymorphism in buffaloes, polymorphism information content value, and number of alleles.

DNA extraction and PCR-based profiling

Genomic DNA was isolated from lymphocyte cells using the procedure described by Miller *et al*. (1988). The polymerase chain reaction (PCR) was carried out using the QIAGEN multiplex PCR kit with 2x QIAGEN multiplex PCR master mix (final concentration, 1x), Q-Solution 5x (final concentration, 0.5x), 0.1 to 0.5 µM of each primer, 20 ng of DNA and distilled water in a total volume of 6 µl. Microsatellite allele sizes were visualized by using the ABI PRISM 3130 Genetic Analyser (Applied Biosystems, Foster City, CA). The internal size standard GeneScan-500LYS (Applied Biosystems, Warrington, United Kingdom) was used for sizing alleles.

Computation and statistical analysis

The GENEPOP package Version 4.0.10 (Raymond and Rousset 1995; Rousset 2008) was used to calculate an exact test for deviation from Hardy-Weinberg equilibrium (HWE), allele frequencies, observed and expected heterozygosity.

Wright F-statistics (F_{IS}) and mean number of alleles per locus and overall were calculated using FSTAT (Goudet, 2002). Polymorphism information content was calculated as per Botstein *et al*. (1980). Inbreeding coefficient in water buffalo population was estimated according to the following equation (Wright, 1965).

$$F_{IS} = (H_e - H_o)/H_e$$

where (H_e) is expected heterozygosity and (H_o) is observed heterozygosity

RESULTS

A total of 87 alleles were observed in the Cuban water buffalo population. The average number of alleles per locus was 5.44. The number of alleles per locus ranged from two (TGLA126) to nine (ETH225) (Table 1).

The observed and expected heterozygosity ranged from 0.041 (TGLA126) to 0.806 (HEL1) and 0.040 to 0.791 in similar loci, respectively. The polymorphic information content (PIC) ranged from 0.039 (TGLA126) to 0.777 (HEL1), and the overall value for these markers was 0.495. The overall loci estimates of inbreeding showed that in Cuban water buffalo, there were three loci with significant reduction of heterozygosity (ETH3, HAUT24 and INRA032) both due to within population inbreeding (F_{IS}).

In the Hardy-Weinberg equilibrium test, it was observed that a total of four loci were not in equilibrium with a significant heterozygote deficit (ETH3, CSRM60, HAUT24 and INRA032).

The observed heterozygosity (H_o) was 0.46 ± 0.23 and the expected heterozygosity (H_e) was 0.54 ± 0.19 (Table 2).

DISCUSSION

Locus ETH225 is like a monomorphic marker in samples of the Murrah buffalo breed of Brazilian origin (Martínez et al., 2009). A similar result is reported by (Ángel-Marín et al., 2010) in five genetic groups (Brazilian Murrah, Bulgarian Murrah, Colombian Buffalo, crossbreeds of Colombian Buffalo, Bulgarian Murrah and Brazilian Murrah, and Murrah by absorption.

DNA microsatellites have found widespread application in gene mapping, pedigree determination and population genetics (Moore et al., 1995). The technique was employed in a study of genetic diversity and differentiation in domestic buffalo and of genetic differentiation between swamp and river buffalo based on 30 microsatellite markers (Zhang et al., 2007)

The polymorphic information content was smaller than other reported in two buffalo populations of northern India (Arora et al., 2004) and Colombia (Ángel-Marín et al., 2010). All loci were polymorphic microsatellite loci, it was an expected result and we wait due to selection of the heterologous microsatellite loci was based on their polymorphism in buffaloes (Moore et al., 1995; Navani et al., 2002; Vijh et al., 2008).

The coefficient of inbreeding is the probability that the two genes at any locus in an individual are identical by decent (Malécot, 1948). The effects of inbreeding is described as increased homozygosity (animal with a copy of the some allele at one locus), redistribution of genetic variances, decrease of homeostasis (inbred animals are less adaptive to environmental changes) and reduction of an animal´s performance, particularly in terms of reproduction, fertility and health (inbreeding depression) (Folconer, 1989). On average the inbreeding depression in bovine per each 1% increase in the inbreeding coefficient is -25 kg, -0.9 kg and -0.8 kg for milk, fat and protein yield, respectively (Miglior, 2000).

The inbreeding coefficient in Cuban water buffalo is consider high value if compared with other populations. For example Shokrollahi et al. (2009) reported a value of 0.047 in Iranian river buffalo.

Table 1. Descriptive statistics of the 16 microsatellite marker loci for Cuban water buffalo: # alleles (N), # Genotype (G), observed heterozygosity (H_O), expected heterozygosity (H_E), polymorphism information content (PIC), Wright F-statistics (F_{IS}), Exact test for Hardy-Weinberg equilibrium (P-value); statistical significance *=p<0.05; **=p<0.01; ***=p<0.001.

LOCUS	N	G	H_O	H_E	PIC	F_{IS}	P-value
TGLA227	4	50	0.560	0.616	0.605	0.092	0.443
SPS115	4	45	0.578	0.580	0.542	0.003	0.230
ETH225	9	44	0.341	0.310	0.305	-0.102	1.000
BM2113	5	49	0.224	0.210	0.201	-0.072	1.000
ETH3	4	32	0.313	0.553	0.465	0.439**	0.006**
TGLA 126	2	49	0.041	0.404	0.039	-0.011	1.000
ETH152	6	49	0.673	0.674	0.634	0.000	0.971
CSRM60	8	50	0.600	0.663	0.647	0.096	0.002**
HAUT24	4	38	0.211	0.501	0.491	0.583***	0.000***
INRA35	4	49	0.245	0.255	0.234	0.039	0.663
INRA037	7	50	0.540	0.627	0.611	0.140	0.090
CSSM66	6	50	0.640	0.724	0.703	0.117	0.353
MM12	3	50	0.260	0.263	0.260	0.011	1.000
INRA032	8	50	0.640	0.762	0.753	0.162*	0.0009***
HEL1	7	36	0.806	0.791	0.777	-0.018	0.295
HEL13	6	40	0.725	0.670	0.655	-0.084	0.658
Mean	5.438	45.688	0.462	0.537	0.495	0.087	0.482

Table 2. Summary statistics of genetic parameters for Cuban water buffalo. Estimates were obtained averaging over all 16 microsatellites: number of individuals (N); observed heterozygosity (H_O), expected heterozygosity (H_E); percent of polymorphic microsatellite loci ($P_{0.95}$) and inbreeding coefficient (F_{IS}). Standard errors in parentheses.

N	H_O	H_E	$P_{0.95}$	F_{IS}
50	0.46 (0.23)	0.54 (0.19)	100	0.148

All loci were polymorphic microsatellite loci ($P_{0.95}$). The observed value of inbreeding coefficient (F_{IS}) in Cuban water buffalo was of 0.148.

CONCLUSION

The overall polymorphic information content value for these markers was 0.495; therefore, these markers can be used in study of genetic diversity and differentiation of our population with others. The observed value of inbreeding coefficient if is compared with other population suggests to think in a high inbreeding accumulate. The present study on Cuban water buffalo population represents a much needed preliminary effort that could be include molecular characterization in this population.

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