

GENETIC DIVERSITY OF A BRAZILIAN CREOLE CATTLE BASED ON FOURTEEN MICROSATELLITE *LOC*

DIVERSIDAD GENÉTICA DEL BOVINO CRIOLLO BRASILEÑO UTILIZANDO CATORCE *LOC* DE MICROSATÉLITES

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PALABRAS CLAVE ADICIONALES

Recursos genéticos. Bovino Criollo brasileño.

SUMMARY

The Brazilian Creole cattle was introduced in America in the 16th century during colonization. Along four centuries, these animals have been developing through natural selection demonstrating high adaptation to environmental conditions. Nowadays they are at risk of extinction, with their population not exceeding 500 animals. The present work evaluated the genetic diversity of fourteen microsatellite *loci* (TGLA227, ETH225, UW53, MM12, URB002, BM1824, RM088, BM1074, BM6315, ILSTS002, IDVGA51, BM1500, BMS3004, BMS3013) in a sample of 73 animals, comparing it with other bovine populations. Compared to other two Brazilian herds, Creole breed presented 23 exclusive alleles. The comparison of these data with some Spanish and Portuguese herds that could have given origin to the Brazilian Creole would be very interesting to confirm or not the occurrence of these private alleles in the Brazilian Creole population.

América durante la colonización en el siglo XVI. A lo largo de cuatro siglos, estos animales se vienen desarrollando por selección natural, demostrando alta adaptación a las condiciones ambientales. Hoy se encuentra en riesgo de extinción, ya que su población no pasa de 500 animales. El presente trabajo evaluó la diversidad genética de catorce *loci* de microsatélites (TGLA227, ETH225, UW53, MM12, URB002, BM1824, RM088, BM1074, BM6315, ILSTS002, IDVGA51, BM1500, BMS3004, BMS3013) en una muestra de 73 animales, comparándola con otras poblaciones bovinas. En la comparación con otras dos razas Brasileñas, el ganado Criollo presentó 23 alelos exclusivos. La comparación de esos datos con algunas razas españolas y portuguesas que podrían haber originado al Criollo Brasileño sería de gran interés para confirmar la ocurrencia o no de estos alelos exclusivos en la población del Criollo brasileño.

RESUMEN

El bovino Criollo Brasileño fue introducido en

INTRODUCTION

The Brazilian Creole cattle was

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introduced in America by the Portuguese and Spanish settlers during the 16th century. Coming from the Iberian Peninsula, these animals seem to have originated in Egypt about 6000 - 4000 A.C. They probably descend from the old Hamitic cows (Inchausti and Tagle, 1964), having arrived in Europe through Gibraltar after coasting the African continent (Ortiz, 1997).

In Brazil, they were introduced in 1534, brought from Madeira and Cabo Verde islands. The animals had been brought from the Alentejo (Portugal) and the Extremadura (Spain) regions (Ortiz, 1999; Wilkins *et al.*, 1993; Primo, 1992). About 1555, some animals were sent from Brazil to Paraguay where they admixed with those coming from the north of Latin America, undergoing a population expansion (Cardellino, 2000; Holgado, 1993). In 1626 these Paraguayan cattle was brought again to Brazil by the Jesuits (Holgado, 1993) and gave origin to the herd here investigated. During the 19th century other European and Asian breeds, considered to be more productive, were imported to South Brazil and the Creole cattle was abandoned, being now restricted to some areas of difficult environmental adaptation with some particular farmers.

The Brazilian Creole breed is now considered to be at risk of extinction, with the population not exceeding 500 animals, and being found only in South Brazil, in Santa Catarina and Rio Grande do Sul states. These animals had developed along four centuries through natural selection, demonstrating high adaptation to environmental conditions. They present wide phenotype variability, with variable coat color,

long horns and angular conformation (Holgado, 1993; Inchausti and Tagle, 1964). They present also great resistance to parasitic and infectious diseases (Inchausti and Tagle, 1964), high longevity and high fertility rates (Bouzat *et al.*, 1998), with the females having good maternal ability (Holgado *et al.*, 1988).

Due to these aspects, preservation strategies for these animals should be developed, the first action being the knowledge of their genetic composition. This work analyzed fourteen microsatellite *loci* in a Brazilian Creole cattle sample in order to verify their degree of genetic diversity, comparing the data with those of other breeds.

MATERIAL AND METHODS

Fourteen microsatellite *loci* (STRs) distributed along seven bovine chromosomes (TGLA227, ETH225, UW53, MM12, URB002, BM1824, RM088, BM1074, BM6315, ILSTS002, IDVGA51, BM1500, BMS3004, BMS3013) were analyzed in 73 individuals (90 percent adults; 10 percent males) of a breed from the Brazilian Creole cattle. The animals comes from a breed nucleus and have been managed exclusively on native pasture in an extensive livestock system with natural breeding. No artificial selective procedure being applied and no migrants have been reported. Blood samples were obtained by puncture of the jugular vein, with ACD (0.8 percent citric acid, 2.2 percent trisodium citrate and 2.45 percent dextrose) as anticoagulant. Genomic DNA was extracted from whole blood

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Table I. Methodology information about the STRs analyzed in present study. (Información metodológica sobre los STRs analizados en el presente estudio).

STRs	Primers Sequences	AT (°C)	Chrom.	References
TGLA227	5' CCC TCC TCC AGG TAA ATC AGC 3' 3' AAT CAC ATG GAA ATA AGT ACA TAC 5'	60-48*	21	Crawford <i>et al.</i> (1995)
ETH225	5' GATCACCTTGCCACTATTTCT 3' 3' ACATGACAGCCAGCTGCTACT 5'	60	9	Steffen <i>et al.</i> (1993)
UW53	5' TCGGATATGACTGAGTGACTG 3' 3' TCTGACAAGGATGTTAAAGCTGTTAC 5'	60	6	Sun <i>et al.</i> (1996)
MM12	5' CAAGACAGGTGTTTCAATCT 3' 3' ATCGACTCTGGGGATGATGT 5'	50	9	Mommens <i>et al.</i> (1994)
URB002	5' AGCCCTGGCTGCCAACATATATC 3' 3' GCTGTCTTCTCCCTGTATCTTC 5'	60	17	Ma <i>et al.</i> (1996)
BM1824	5' GAGCAAGGTGTTTTTCCAATC 3' 3' CATTCTCCAAGTCTTCTTG 5'	58	1	Sun <i>et al.</i> (1996)
RM088	5' GAT CCT CTT CTG GGA AAA GAG GAC 3' 3' CCT GTT GAA GTG AAC CTT CAG AA 5'	63	4	Kossarek <i>et al.</i> (1995)
BM1074	5' CAGTAGCCAAGATATGGAAGCA 3' 3' AGCTCCTTGCTGCTACAAATG 5'	58	4	Stone <i>et al.</i> (1995)
BM6315	5' CCT GAA TGA ATA TGT GTG AGC A 3' 3' CTT GCC AGT GAT GTC ATG C 5'	58	4	Kappes <i>et al.</i> (1995)
ILSTS002	5' TCTATACACATGTGCTGTGC 3' 3' CTTAGGGGTGAAGTGACACG 5'	52	18	Kemp <i>et al.</i> (1992)
IDVGA-51	5' ATGGCAATATTTTGTCTTTTTC 3' 3' ATTCCTTGATGGTCTAATGGTTA 5'	58	4	Mezzelani <i>et al.</i> (1995)
BM1500	5' GAT GCA GCA GAC CAA GTG G 3' 3' CCC ATT GCT AGA ACC CAG G 5'	58	4	Fitzsimmons <i>et al.</i> (1998)
BMS3004	5' GGA CAG AGG AGC CTG GTT G 3' 3' AGT TGC GTT GTT CAT CAT TCC 5'	60	18	Stone <i>et al.</i> (1996)
BMS3013	5' CAT TTA CCA TCA CAG TTC CTG G 3' 3' CTG GTG GGC TAC AGT TCA GT 5'	58	4	Stone <i>et al.</i> (1996)

AT: annealing temperature; *touch down.

Table II. Allele frequencies and diversity parameters in 14 microsatellites of a Brazilian Creole cattle. (Frecuencias alélicas y parámetros de diversidad en 14 microsatélites en el bovino Criollo Brasileño).

TGLA227 n = 71		ETH225 n = 72		UW53 n = 73		MM12 n = 73		URB002 n = 73		BM1824 n = 71		BM3013 n = 73	
Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq
79	0.18	139	0.01	130	0.03	117	0.01	111	0.08	180	0.04	112	1.00
81	0.02	141	0.03	132	0.12	119	0.05	113	0.29	182	0.19		
83	0.13	143	0.05	134	0.38	121	0.20	115	0.06	184	0.26		
85	0.16	145	0.12	136	0.02	123	0.16	117	0.20	186	0.12		
87	0.02	147	0.15	138	0.01	125	0.20	119	0.27	188	0.11		
89	0.13	149	0.20	142	0.17	127	0.07	121	0.03	190	0.16		
91	0.09	151	0.15	144	0.09	129	0.03	123	0.03	192	0.12		
93	0.09	153	0.04	146	0.02	131	0.01	125	0.03				
95	0.10	155	0.12	148	0.14	133	0.23	127	0.01				
97	0.04	157	0.08	150	0.01	135	0.03						
99	0.02	159	0.04	152	0.01	139	0.01						
101	0.02	161	0.02										
Ho	0.97	Ho	0.97	Ho	0.75	Ho	0.99	Ho	0.99	Ho	0.88	Ho	0.00
He	0.89	He	0.88	He	0.79	He	0.84	He	0.80	He	0.84	He	0.00
F	-0.09	F	-0.10	F	0.04	F	-0.18	F	-0.20	F	-0.04	F	-
PIC	0.87	PIC	0.86	PIC	0.76	PIC	0.81	PIC	0.76	PIC	0.81	PIC	0.00
PI	0.03	PI	0.03	PI	0.07	PI	0.05	PI	0.07	PI	0.05	PI	1.00

Ho - observed heterozygosities; He - expected heterozygosities; F - fixation indexes; PIC - polymorphic information content; PI - probability of identity.

by the technique described for cattle by Plante *et al.* (1992). DNA fragments were amplified by the polymerase chain reaction (PCR) according to Stone *et al.* (1996) but with primers and annealing temperatures specific for each fragment (**table I**). The amplified fragments were separated by vertical electrophoresis in 7 percent polyacrylamide non-denaturing gel (Lahiri *et al.*, 1997). **Figure 1** shows the electrophoretic patterns here observed for one (BMS3004) of the investigated STRs.

Average heterozygosity (H) and the

polymorphic information content (PIC) were estimated by the methods of Nei and Kumar (2000) and Botstein *et al.* (1980), respectively. Hardy Weinberg equilibrium and fixation indices (F) for each system were evaluated according to Guo and Thompson (1992) and Slatkin (1995), respectively, using the Arlequin ver 2.0 software (Schneider *et al.*, 2000). The probabilities of identity of two animals, chosen at random in the herd were estimated following Van Zeveren *et al.* (1990).

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Table II continuación. Allele frequencies and diversity parameters in 14 microsatellites of a Brazilian Creole cattle. (Frecuencias alélicas y parámetros de diversidad en 14 microsatélites en el bovino Criollo Brasileño).

RM088 n=73		BM1074 n=73		BM6315 n=69		ILSTS002 n = 72		IDVGA51 n=73		BM1500 n=73		BMS3004 n = 73	
Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq
124	0.03	141	0.01	126	0.01	129	0.03	171	0.02	135	0.41	129	0.59
126	0.06	145	0.02	130	0.06	131	0.10	173	0.40	141	0.08	132	0.16
128	0.01	147	0.03	132	0.16	133	0.28	175	0.18	143	0.43	138	0.13
130	0.06	151	0.05	136	0.04	135	0.31	177	0.01	145	0.03	141	0.12
132	0.37	153	0.16	138	0.28	137	0.08	179	0.14	147	0.05		
134	0.09	155	0.39	140	0.34	139	0.17	181	0.23				
136	0.09	159	0.14	142	0.10	141	0.03	183	0.02				
138	0.03	161	0.19	148	0.01								
140	0.16												
142	0.01												
144	0.06												
146	0.02												
152	0.01												
<i>Ho</i>	0.81	<i>Ho</i>	0.63	<i>Ho</i>	0.71	<i>Ho</i>	0.75	<i>Ho</i>	0.79	<i>Ho</i>	0.80	<i>Ho</i>	0.51
<i>He</i>	0.81	<i>He</i>	0.76	<i>He</i>	0.76	<i>He</i>	0.78	<i>He</i>	0.74	<i>He</i>	0.65	<i>He</i>	0.59
<i>F</i>	0.01	<i>F</i>	0.18	<i>F</i>	0.10	<i>F</i>	0.04	<i>F</i>	-0.07	<i>F</i>	-0.25	<i>F</i>	0.15
<i>PIC</i>	0.79	<i>PIC</i>	0.73	<i>PIC</i>	0.72	<i>PIC</i>	0.74	<i>PIC</i>	0.69	<i>PIC</i>	0.57	<i>PIC</i>	0.55
<i>PI</i>	0.05	<i>PI</i>	0.09	<i>PI</i>	0.10	<i>PI</i>	0.08	<i>PI</i>	0.11	<i>PI</i>	0.20	<i>PI</i>	0.21

Ho - observed heterozygosities; He - expected heterozygosities; F – fixation indexes; PIC - polymorphic information content; PI - probability of identity.

RESULTS AND DISCUSSION

Allele frequencies and the genetic diversity parameters are presented in **table II**. Except for BMS3013 all markers were polymorphic, the number of alleles varying from 4 (BMS3004) to 13 (RM088). The population genotype frequencies do not agree with Hardy Weinberg expectations in relation to most systems, with the exception of IDVGA51 STR, but no indication of inbreeding or population

subdivision could be verified. The observed heterozygosities vary from 0.51 to 0.99, being in general higher than those expected whole values range from 0.59 to 0.89. Although the population is managed in a natural breeding system, with no artificial selection, it does not follow the Hardy Weinberg presuppositions, the number of males being several times smaller than those of females, as usual for livestock breeds. Otherwise the number of alleles of microsatellite *loci* are high

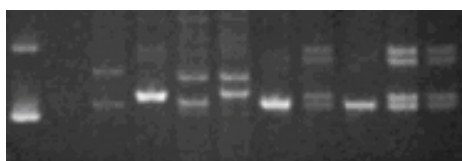


Figure 1. Electrophoretic patterns of BMS3004 STR: Lane 1: 25bp molecular marker; Lane 2: 129/141, Lane 3: 132/132, Lane 4: 129/138, Lane 5: 132/138, Lane 6: 129/129, Lane 7: 129/132, Lane 8: 129/129, Lane 9: 129/132, Lane 10: 129/132. (Padrones electroforéticos del STR BMS3004: Línea 1: marcador de peso molecular 25pb; Línea 2: 129/141; Línea 3: 132/132; Línea 4: 129/138; Línea 5: 132/138; Línea 6: 129/129; Línea 7: 129/132; Línea 8: 129/129; Línea 9: 129/132; Línea 10: 129/132.

and the sample size is relatively small, the estimates being therefore subjected to large sample errors.

Polymorphic information content varies from 0.55 (BM3004) to 0.87 (TGLA227). The high genetic diversity is also reflected in the probability of identity between two animals which is very small ranging from 0.03 to 0.21, the average value being almost zero (2×10^{-15}).

Results of genetic diversity of five STRs (BM1824, ETH225, ILSTS002, TGLA227 and URB002) could be compared with those of some European, African and South American breeds.

In relation to ILSTS002, the degree of variability was higher for Brazilian Creole than for Barrosã (Viana *et al.*, 1998). The heterozygosity level of URB002 here observed was similar to those of South Devon and Gelbvieh, but higher than those of Red Angus,

Saler, and Simmental (Heyen *et al.*, 1997). The diversity observed in TGLA227 was higher for Brazilian Creole than for Holstein Friesian, Belgian Red Pied, East Flemish, and Belgian Blue (Peelman *et al.*, 1998).

Table III shows the heterozygosity (H) and PIC values for BM1824 and ETH225 microsatellites for 10 populations. It can be seen that H and PIC are high for Brazilian Creole. Two populations have similar degree of diversity, the hybrid Brangus-Ibagé herd for ETH225 and *Bison bison*, for BM1824.

All the microsatellites could be compared with two other breeds from Brazil, a synthetic hybrid herd, Brangus-Ibagé (3/8 Aberdeen Angus X 5/8 Nelore) and an Aberdeen Angus breed (unpublished data from our laboratory).

According to this comparison, Brazilian Creole presented 23 exclusive alleles (*BM1074*141, BM6315*126, BM6315*130, BM6315*148, BMS3004*141, ETH225*161, IDVGA51*171, IDVGA51*173, MM12*135, MM12*139, RM088*124, RM088*126, RM088*128, RM088*152, TGLA227*97, URB002*123, URB002*127, UW53*130, UW53*134, UW53*138, UW53*148, UW53*150, UW53*152*). The comparison with data of some Spanish and Portuguese herds that could have given origin to the Brazilian Creole would be very interesting to confirm or not the occurrence of these private alleles in the Brazilian Creole population.

The high degree of variability verified in Brazilian Creole despite its reduced population size suggests that this herd is a rich reservoir of genetic

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Table III. *BM1824 and ETH225 heterozygosities and polymorphic information contents in 10 bovine populations.* (Heterozigosidad y contenido de información polimórfico en el BM1824 y ETH225 en 10 poblaciones bovinas).

		Brazilian Creole	Brangus Ibagé	Holstein Friesian	Belgian Red Pied	East Flemish	Belgian Blue	Bison bison	Argentine Creole	Berrenda en negro1	negro2
ETH225	H	0.88	0.88	0.73	0.76	0.78	0.74	0.46	0.64	0.53	0.45
	PIC	0.86	0.84	0.70	0.72	0.74	0.69	0.41	0.59		
BM1824	H	0.83	0.67	0.70	0.70	0.76	0.65	0.83	0.72	0.67	0.62
	PIC	0.81	0.59	0.64	0.65	0.72	0.58	0.80	0.67		
References	this invest.	1	2	2	2	2	3	4	5	5	

1- Inpublished data from our laboratory; 2- Peelman *et al.*, 1998; 3- Mommens *et al.*, 1998; 4- Zamorano *et al.*, 1998a; 5- Zamorano *et al.*, 1998b.

diversity. This fact plus its marked environmental adaptation reinforce the importance of its preservation as a pure breed, and/or its use in agricultural exploitation as a resource for animal improvement, to obtain more productive and resistant breeds.

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