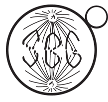

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Research Article

Microsatellite analysis of a sample of Uruguayan Creole bulls (*Bos taurus*)

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Abstract

The Uruguayan Creole cattle genetic reserve consists of a herd of about 600 animals (bulls, cows and calves) located in an indigenous habitat of 650 hectares. In a previous study, a random sample from this herd showed high heterozygosity and *a* Hardy-Weinberg equilibrium for markers of major genes related to milk production. To study its genetic diversity we genotyped a sample of bulls (N = 19 out of 23 for the whole herd) using the PCR reaction with a set of 17 microsatellite markers. Between two and seven different alleles were identified per microsatellite in a total of 73 alleles. The expected mean heterozygosity (He) per locus was between 0.465 and 0.801, except for microsatellite HEL13 which gave a He value of 0.288. The expected mean heterozygosity was 0.623 and the polymorphic information content (PIC) was between 0.266 for HEL13 and 0.794 for CSSM66. The genetic diversity found in polymorphic markers in the breeding bulls of this Creole cattle population supports previous genetic analyses using major production genes and indicate that further studies should be carried out on this population to provide data of interest to cattle production.

Key words: Uruguayan Creole cattle, genetic diversity, microsatellites.

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Introduction

Creole cattle were introduced into America in 1493 by the Spanish and Portuguese conquerors, these cattle being considered the product of multiple admixtures of Iberian and other European cattle. The favorable environment into which the cattle were introduced promoted their reproduction and they soon spread through the entire Central and South American continent. Their adaptation to different environments allowed the expression of a high level of genetic variability but currently only few semi-wild Creole cattle populations remain in South America, examples being the Patagonian Creole cattle of Argentina and the Pantaneiro cattle of Brazil (Primo, 1992). Such semi-wild cattle populations are important because they may be a source of hidden alleles which have potential use in breeding programs, a major reason for locating and conserving such herds (Rendo *et al*., 2004).

The first introduction of cattle into Uruguay was carried out by Hernando Arias de Saavedra at the beginning of the seventeenth century, and later by the Jesuit Missions of Alto Uruguay. By the end of the nineteenth century many commercial cattle breeds were introduced, including Holstein-Friesian, Hereford and Aberdeen Angus, with the aim of improving cattle production and the Uruguayan economy. These introductions reduced the huge population of Creole cattle to small and sparse subpopulations throughout the country and there is now just a single semi-wild population of about 600 head in southeastern Uruguay in an area of about 650 hectares of native woods, ridges and wetlands. In fact, the population of this herd had reached 1000 animals in recent years but had to be adjusted to a more limited area. Arredondo (1958) documented the creation of the population about 70 years ago from a foundation stock consisting of 35 Creole bulls, cows and calves brought from different locations with similar environments.

Genomic studies using random amplified polymorphic DNA (RAPD) on samples of Creole, Hereford and Uruguayan Holstein-Friesian cattle showed particular genetic distances in terms of band sharing frequencies (Hereford - Creole: 0.77; Holstein - Creole: 0.78; Hereford - Holstein; 0.81). In spite of possible genetic introgression events from commercial breeds in the past, band sharing frequencies were higher among commercial breeds but

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lower between commercial breeds and Creole cattle, suggesting that the Creole population has developed mainly in reproductive isolation (Rincón *et al*., 2000).

Preliminary research on a random sample of cattle from the Uruguayan Creole cattle genetic reserve using the CYP21 and BM2113 polymorphic microsatellites and diallelic sequences of interest to dairy production showed genetic equilibrium and high expected heterozygosity (He = 0.800) (Postiglioni, *et al*., 2002). In the present study of the same population we analyzed only breeding bulls with a set of 17 microsatellites included in the list recommended by the Food and Agricultural Organization (FAO) for genetic diversity studies in domestic animals that justify their conservation as a sustainable genetic resource (ww.fao.org/DAD-IS; www.ri.bbsrc.ac.uk). In this population there are around 600 individuals but only 23 breeding males, so in such a small population it is important to analyze the diversity of the males as a single category because they contribute half of the genetic variability of future generations.

Material and Methods

Blood samples and markers analyzed

The population of Uruguayan Creole cattle of San Miguel National Park consists of 23 bulls and about 445 cows and 105 calves of both sexes. Genomic DNA was extracted from blood samples of 19 bulls by the phenol-chloroform technique (John *et al*., 1991). The genomic DNA is stored in the genetic bank of the Genetic Laboratory of Facultad de Veterinaria, Uruguay.

The 17 microsatellites analyzed were all dinucleotidic (Table 1) selected based on the following criteria: inclusion in the microsatellite list proposed by the FAO/IDAD (Initiative for Domestic Animal Diversity) program and/or the European Union Cattle Diversity Data Base of the bovine diversity project (ww.fao.org/DAD-IS; www.ri.bbsrc.ac.uk); present a high level of polymorphism; are widely used in the bibliography to allow comparative studies with other breeds; are relatively easy to work with and can be genotyped by multiplex reactions; are evenly-distributed throughout bovine genome.

Microsatellite genotyping

The PCR genotyping of the 17 microsatellite sequences was performed in a final volume of 20 μL: 50 ng μL^{-1} genomic DNA, 10X PCR buffer, 2.5 mM $MgCl_2$, 2.5 mM dNTPs, 0.15 μM primers and 1 U μL^{-1} of Taq polymerase. Amplification was carried out in three multiplex reactions (M1, M2 and M3) using different fluorochromes for similar-sized microsatellites: M1 = BM1314, CSSM66, ILSTS011, INRA37 and ETH10; M2 = BM1818, BM2113, BM8125, INRA32 and MM12; and M3 = HAUT27, HEL13, HEL9, CSRM60, ILSTS006, INRA63 and TGLA227. Amplification was carried out in a PTC 100 thermocycler (MJ Research Inc, USA) using an amplification protocol consisting of denaturalization at 95 °C for 30 s, followed by 35 cycles of 95 °C for 30 s, 55 °C for 45 s and 72 °C for 30 s, with a final extension at 72 °C for 30 min. The amplified fragments were separated on 6% polyacrylamide gel electrophoresis in an ABI377XL automatic sequencer and the gels read using the GENESCAN ANALYSIS v3.2.1 software, the GENOTYPER v2.5 program being used to assign an allele to each detected peak or band (both softwares and sequencer are from Applied Biosystems, Forster city Ca. USA). Allele size was standardized using reference samples distributed by ISAG (International Society of Animal Genetics) for comparison tests.

Statistical analysis

Allele and genotype frequencies of the 17 microsatellite loci were calculated using GENEPOP v3.1c (updated version of GENEPOP v1.2 described in Raymond and Rousset, 1995) and GENETIX v4.02 (Belkhir *et al*., 1998), this last program also being used to calculate the expected heterozygosity (He), observed heterozygosity (Ho) and expected unbiased heterozygosity (He_u) according to the formula developed by Nei (1973) and Nei and Roychoudhury (1974). The polymorphic information content (PIC) index for each marker was calculated according to Botstein *et al*. (1980).

Results and Discussion

Of the 169 alleles described for the markers used (http://www.marc.usda.gov/genome/genome.html) 73 alleles were detected in our sample of bulls. The most polymorphic microsatellites were CSSM66 and TGLA227 with seven alleles, while the least polymorphic was BM8125 with two alleles (Table 2). The level of polymorphism detected in each microsatellite was similar to that stated in the literature (*q.v.* Table 1). In a study of six native Spanish breeds, Martin-Burriel *et al*. (1998) also found TGLA227 to be the most polymorphic marker.

Regarding measures of genetic diversity (Table 3), the marker with the highest unbiased heterozygosity was CSSM66, followed by HEL9, TGLA227 and BM2113 while the marker with the lowest unbiased heterozygosity was HEL13, followed by BM8125. With the exception of these last two markers, all the microsatellites showed levels of expected unbiased heterozygosity higher than 0.500 (Table 3). Table 3 also shows the mean heterozygosity for the total sample, the fact that there is a difference between the expected (He) and observed (Ho) heterozygosity suggesting a tendency towards heterozygote deficiency.

The PIC values were between 0.266 for HEL13 and 0.794 for CSSM66 (Table 3). A high PIC value depends on the number and frequency distribution of the alleles measured; markers with PIC values exceeding 0.500 being considered more informative (Botstein *et al*., 1980). In our

Table 1 - Description of the 17 molecular markers analyzed.

Name[1]	Chromosome	Primers	Size (bp)
BM8125	17	Forward = CTCTATCTGTGGAAAAGGTGGG Reverse = GGGGGTTAGACTTCAACATACG	109-125
BM1314	26	Forward = TTCCTCCTCTTCTCTCCAAAC Reverse = ATCTCAAACGCCAGTGTGG	143-167
BM1818	23	Forward = AGCTGGGAATATAACCAAAGG Reverse = AGTGCTTTCAAGGTCCATGC	258-272
BM2113	2	Forward = GCTGCCTTCTACCAAATACCC Reverse = CTTCCTGAGAGAAGCAACACC	123-143
CSSM66	14	Forward = ACACAAATCCTTTCTGCCAGCTGA Reverse = AATTTAATGCACTGAGGAGCTTGG	180-200
ETH10	5	Forward = GTTCAGGACTGGCCCTGCTAACA Reverse = CCTCCAGCCCACTTTCTCTTCTC	212-224
ILSTS011	14	Forward = GCTTGCTACATGGAAAGTGC Reverse = CTAAAATGCAGAGCCCTACC	262-276
INRA032	11	Forward = AAACTGTATTCTCTAATAGCAC Reverse = GCAAGACATATCTCCATTCCTTT	161-187
INRA037	10	Forward = GATCCTGCTTATATTTAACCAC Reverse = AAAATTCCATGGAGAGAGAAAC	112-148
MM12	9	Forward = CAAGACAGGTGTTTCAATCT Reverse = ATCGACTCTGGGGATGATGT	105-145
CSRM60	10	Forward = AAGATGTGATCCAAGAGAGAGGCA Reverse = AGGACCAGATCGTGAAAGGCATAG	90-110
HAUT27	26	Forward = TTTTATGTTCATTTTTTGACTGG Reverse = AACTGCTGAAARCTCCATCTTA	128-156
HEL13	11	Forward = TAAGGACTTGAGATAAGGAG Reverse = CCATCTACCTCCATCTTAAC	177-197
HEL9	8	Forward = CCCATTCAGTCTTCAGAGGT Reverse = CACATCCATGTTCTCACCAC	143-167
ILSTS006	7	Forward = TGTCTGTATTTCTGCTGTGG Reverse = ACACGGAAGCGATCTAAACG	281-299
INRA063	18	Forward = ATTTGCACAAGCTAAATCTAACC Reverse = AAACCACAGAAATGCTTGGAAG	178-188
TGLA227	18	Forward = CGAATTCCAAATCTGTTAATTTGCT Reverse = ACAGACAGAAACTCAATGAAAGCA	76-102

[1]References: ETH10 = SolinasToldo *et al*. (1993); CSSM66 = Barendse *et al*. (1997); MM12 = Mommens *et al*. (1994); CSRM60 = Moore *et al*. (1994); HAUT27 = Anon (1999); and all the others from Kappes *et al*. (1997). bp = basepairs.

study, the highest PIC values were obtained for those markers with a high number of alleles (*e.g.* BM2113, CSSM66 and TGLA227) or which showed a more homogeneous allele frequency distribution even when the number of alleles detected were low (*e.g.* ILSTS011, INRA32 and ILSTS006) and for those alleles presenting both of these characteristics (*e.g.* HEL9).

Our study shows that the microsatellite markers with highest heterozygosity and PIC values were BM2113, CSSM66, HEL9 and TGLA227, which should be included in future genetic diversity studies of this and other cattle populations.

The average diversity levels detected were high (He = 0.623; He_u = 0.644; Ho = 0.584; mean PIC = 0.589; mean number of alleles per locus: 4.294). If the sample is representative of the population, the observed values may be related to the demographic history of the reserve. To support this idea, a larger sample of the population that includes other age-sex categories should be analyzed.

Uruguayan Creole cattle developed from the admixture of many breeds in a process that generates high levels of genetic diversity (Kantanen *et al*., 2000). Random mating over four centuries appears to have contributed to maintain a high level of diversity in the population studied, aided by the fact that this population was created from 35 Creole cattle that came from different parts of Uruguay, the fusion of small previously isolated populations being known to result in increased heterozygosity due to a reduction in the frequency of homozygotes (Hartl, 1988).

The heterozygosity detected in this study was similar to that found previously in this population (Rincón *et al*., 2000; Postiglioni *et al*., 2002). Our results for Uruguayan

Table 2 - Marker name, alleles detected (in base pairs) and their frequencies.

Marker name and number of alleles (bp)	Frequency	Marker name and number of alleles (bp)	Frequency	Marker name and number of alleles (bp)	Frequency
BM8125		BM1314		BM1818	
116	0.6316	155	0.0294	260	0.2857
122	0.3684	157	0.4118	262	0.1071
		159	0.4412	264	0.5357
		161	0.1176	268	0.0714
BM2113		CSSM66		ETH10	
126	0.3611	179	0.0294	213	0.0938
128	0.0556	181	0.2059	217	0.4375
134	0.1111	183	0.0882	219	0.4688
136	0.1389	187	0.3235		
138	0.2778	189	0.1471		
140	0.0556	195	0.0882		
		197	0.1176		
ILSTS011		INRA32		INRA37	
264	0.1000	180	0.2727	114	0.1176
268	0.4000	182	0.2727	126	0.0882
270	0.3000	184	0.4545	128	0.0294
272	0.2000			132	0.3824
				136	0.3824
MM12		CSRM60		HAUT27	
115	0.1053	93	0.5526	140	0.0417
119	0.2632	97	0.1579	144	0.0417
131	0.6316	99	0.0526	148	0.6250
		103	0.1842	150	0.1667
		105	0.0526	154	0.1250
HEL13		HEL9		ILSTS006	
184	0.0417	151	0.2368	289	0.5000
188	0.1250	159	0.2368	291	0.1071
192	0.8333	161	0.1579	295	0.0714
		163	0.1579	297	0.3214
		165	0.2105		
INRA063		TGLA227			
173	0.3529	85	0.1053		
175	0.0882	89	0.3158		
181	0.5588	91	0.0526		
		93	0.3421		
		95	0.1316		
		97	0.0263		
		99	0.0263		

Creole cattle are similar to those from other American Creole and Iberian cattle breeds studied with microsatellite markers from the same FAO and ISAG references lists. For example, Zamorano *et al*. (1998a) studied Argentinean Creole cattle from Patagonia and found that the expected heterozygosity per locus was between 0.46 and 0.72 while in a different study the same workers (Zamorano *et al*., 1998b) found an expected heterozygosity of 0.60 for the Andalusian breed `Berrenda en Negro', a proposed ancestral breed of American Creole cattle. In an analysis of six Spanish native breeds, Martín-Burriel *et al*. (1998) found an average expected heterozygosity between 0.56 and 0.68, depending on the breed. In addition, Rendo *et al*. (2004) found an expected heterozygosity of between 0.69 and 0.76 in four Western Pyrenean cattle breeds, while a study by Mateus *et al*. (2004) of 15 Portuguese cattle breeds found

Table 3 - Marker name, number of alleles, heterozygosity and polymorphic information content (PIC) of the *a*markers studied.

		Heterozygosity			
Marker name	Number of alleles	Expected (He)	Expected unbiased (He_u)	Observed (Ho)	PIC
BM8125	2	0.465	0.478	0.316	0.357
BM1314	4	0.621	0.640	0.647	0.549
BM1818	4	0.615	0.638	0.500	0.585
BM2113	6	0.755	0.776	0.778	0.750
CSSM66	7	0.801	0.825	1.000	0.794
ETH10	3	0.580	0.599	0.563	0.493
ILSTS011	4	0.700	0.737	0.500	0.661
INRA32	3	0.645	0.675	0.727	0.603
INRA37	5	0.685	0.706	0.706	0.642
MM12	3	0.521	0.535	0.526	0.464
CSRM60	5	0.630	0.647	0.632	0.615
HAUT27	5	0.563	0.587	0.417	0.539
HEL13	3	0.288	0.301	0.333	0.266
HEL9	5	0.794	0.815	0.737	0.781
ILSTS006	4	0.630	0.653	0.286	0.623
INRA63	3	0.555	0.572	0.412	0.549
TGLA227	7	0.751	0.771	0.842	0.743
Mean	4.294	0.623	0.644	0.584	0.589

an average expected heterozygosity of between 0.63 and 0.74.

However, the heterozygosity found in our sample of Uruguayan Creole bulls is considerably higher in comparison to that found in studies on commercial breeds that used similar microsatellites. For example, Hanslik *et al*. (2000) found an average expected heterozygosity of 0.43 in the Holstein-Friesian population of the United States and of 0.48 in the original Netherlands population, while MacHugh *et al*. (1994) detected average heterozygosity levels of between 0.40 and 0.49 in six European commercial breeds (Aberdeen Angus, Charolais, Holstein-Friesian, Hereford, Jersey and Simmental) using a set of 12 microsatellite markers. These studies show that highly selected commercial breeds are much less diverse and more inbred than local breeds, what reinforces the importance of local breeds as reserves of genetic diversity for a sustainable agriculture.

Microsatellites give more exact and unbiased estimations of populational genetic diversity than other molecular markers with less polymorphism (Kantanen *et al*., 2000; Lirón *et al*. 2002). Our present analysis contributes to the genetic characterization and conservation management strategies of the Uruguayan Creole cattle population.

In conclusion, the sample of Uruguayan Creole breeding bulls in the genetic reserve showed high levels of genetic diversity. Since the bulls studied represent the male parents of future generations, it should be possible to maintain an adequate level of genetic diversity in the reserve for the next few years. Future population viability analysis (Lacy, 1993) will help determine if this number of males is appropriate for sustaining the development of the reserve. Further analysis of other age-sex categories and of the reserve as a whole will reveal the diversity and genetic structure of the population and will yield the necessary data for achieving characterization and conservation goals.

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