GENETIC POTENTIAL OF THE URUGUAYAN CREOLE CATTLE AND COMPARISON WITH ARGENTINIAN AND BRAZILIAN CREOLE POPULATIONS USING MOLECULAR MARKERS.

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INTRODUCTION

The genetic reserve of Uruguayan Creole cattle (UCC) was created around 1940 from 35 Creole cows, bulls and calves brought from isolated areas of Treinta y Tres and Maldonado departments (Arredondo, 1958; Postiglioni *et al.,* 2002). Today, it consists of 575 animals adapted to a natural environment of ridges, woods and native vegetation and is located in San Miguel National Park (33°40'S y 53°38'W) (Postiglioni and Armstrong, 2005).

This reserve is remnant of the huge cattle population that once thrived over all the country, originated from the cattle brought from the Iberian peninsula during the XVI and XVII centuries (Wilkins *et al*., 1989; Primo, 1992). It developed without artificial selection and turned semi-wild before the introduction of commercial cattle breeds in the XIX century. At that time, in order to improve meat quality, crosses between Hereford and Creole cattle were common, related with the growing meat industry (Seoane, 1928). This genetic introgression process greatly reduced the distribution of the Creole population.

Population viability analysis of the reserve using VORTEX v.8.31 software demonstrated that its demographic structure and high genetic diversity are adequate for its future development. The creation of sub-populations located in different regions and connected by gene flow was proposed as a viable management option (Armstrong *et al*., in press, a).

Different molecular markers related to productive characteristics were studied in this animals, such as major dairy genes of economic interest (*κ, β, α_{S1}-casein, β-lactoglobulin, α-lactalbumin* and *DGAT1*), and the BoLa DRB3 gene. The genetic diversity of the reserve was analyzed with randomly distributed markers (RAPDs) and a set of 18 microsatellites recommended by ISAG/FAO in a sample of bulls (Armstrong *et al*., in press, b). These markers revealed a high genetic diversity (He > 0.50) and a low inbreeding coefficient (F < 0.05) (Postiglioni and Armstrong, 2005).

In this communication we analyze the genetic potential and diversity of the UCC genetic reserve with molecular markers, in relation to productive management and in comparison with two Argentinian Creole cattle samples (ACC and ACCP, the latter from Patagonia) and the Brazilian Creole cattle (BCC).

MATERIALS AND METHODS

Data from previous studies on this population with milk major genes was used for comparison with Argentinian Creole populations (Rincón *et al.,* in press).

Two microsatellite markers (MM12 and TGLA227) were analized in a sample of the whole UCC population. Genomic DNA was extracted from blood samples of 64 animals by the phenol-chlorophorm technique. The amplification and genotyping of the microsatellites was

performed by PCR and authomatic sequencing as described in Armstrong *et al.* (in press, b). Allele size was standardized using reference samples distributed by ISAG for comparison tests.

Allelic frequencies and Hardy-Weinberg equilibrium analysis were performed using GENEPOP v3.1c (Raymond and Rousset, 1995). GENETIX v4.02 was used to calculate the observed and expected heterozygosity and F_{IS} statistic (Belkhir *et al,* 1998). PIC values were calculated according to Botstein *et al.* (1980).

Genetic distances between UCC and Creole cattle populations of neighbour countries were analyzed using the protein and microsatellite data. Milk protein data from the Argentinian Creole cattle population of Patagonia (ACCP) and from a unified sample of several other Argentinian Creole populations (ACC) were obtained from Lirón et al., 2002. Microsatellite data from the ACCP were obtained from Martinez *et al.* (2005). Data from the Brazilian Creole cattle (BCC) of Southern Brazil were obtained from Steigleder *et al.* (2004). Nei´s genetic distance, Cavalli-Sforza and Edwards chord measure and Reynolds, Weir and Cockerham genetic distance were calculated by using PHYLIP 3.6 (Felsenstein, 2004).

RESULTS AND DISCUSSION

Milk protein data.

Different analyses performed in dairy cattle showed that B alleles in most of the proteins of the casein cluster as well as in the *b-lactoglobulin* are related to a high quality of cheese as they increase the rate of curd formation, rennet clotting time and coagulum strength (Van Eenennam and Medrano, 1990). The UCC showed similar or higher frequencies of the B allele for the three genes (Table 1). As it can be seen in Table 1, the UCC allelic distribution for α_{S1}*-casein* is similar to the ACCP, a semi-wild unselected population. On the other hand, UCC´s allele frequencies for *b-lactoglobulin* are more similar to ACC. These genes should be considered as an important target to preserve in this population for future dairy management.

Table 1: Comparison of allelic frequencies for milk protein genes (*κ-casein, α_{S1}-casein, β-lactoglobulin*) between Uruguayan Creole cattle and two populations of Argentinian Creole cattle. N_{UCC} = 115; N_{ACC} = 230; N_{ACCP} = 25.

	κ-casein		*αS1-casein*		*β-lactoglobulin*	
alleles	A	B	B	C	A	B
UCC[1]	0.500	0.500	0.865	0.135	0.493	0.507
ACC[2]	0.609	0.391	0.653	0.347	0.346	0.654
ACCP[2]	0.605	0.395	0.917	0.083	0.845	0.155

1: This work. 2: Lirón *et al*., 2002.

Table 2: Genetic distance of Nei calculated from the milk proteins allelic frequencies.

	UCC	ACC
ACC	0.047	0
ACCP	0.069	0.179

Nei´s genetic distance indicate that UCC has less divergence with ACC and ACCP than both Argentinian populations between them, in accordance with Golijow et al., 1999, who reported a strong differentiation between herds of Argentinian Creole with a similar set of dairy markers. Both UCC and ACCP have never submitted to artificial selection, while some ACC populations did have. This fact together with a similar origin and a close geographic distribution may account for these divergence levels.

Microsatellite data.
Five alleles were detected for marker MM12 and eight for marker TGLA227 in the Uruguayan Creole cattle sample. These frequencies are shown in Table 3, and are compared with the ones of the Argentinian Creole Cattle of Patagonian and Brazilian Creole cattle.

Table 3: **Microsatellite allelic frequencies. $N_{UCC} = 64$; $N_{ACCP} = 36$; $N_{BCC} = 73$.**

Marker *MM12*				Marker *TGLA227*			
allele	UCC [1]	ACCP [2]	BCC [3]	allele	UCC [1]	ACCP [2]	BCC [3]
115	0.08	0.24	0	79	0	0	0.18
117	0.02	0	0.01	81	0	0	0.02
119	0.25	0.15	0.05	83	0.01	0.04	0.13
121	0	0.05	0.20	85	0.19	0.28	0.16
123	0.05	0	0.16	87	0	0	0.02
125	0	0	0.20	89	0.13	0.08	0.13
127	0	0	0.07	91	0.06	0.07	0.09
129	0	0	0.03	93	0.36	0.15	0.09
131	0.60	0.56	0.01	95	0.21	0.06	0.10
133	0	0	0.23	97	0.01	0	0.04
135	0	0	0.03	99	0.03	0.31	0.02
139	0	0	0.01	101	0	0	0.02

1: This work. 2: Martínez et al., 2005. 3: Steigleder et al., 2004.

The UCC has three alleles in common with the ACCP and four with the BCC for marker MM12. Marker TGLA227 shows more allelic diversity, being most alleles present in the three populations.

Table 4: Microsatellite population parameters.

		UCC [1]	ACCP [2]	BCC [3]
MM12	Ho	0.571	0.667	0.99
	He	0.563	0.609	0.84
	F_{IS}	0.030	-0.08	-0.18
	PIC	0.51	0.56	0.81
	HW	P > 0.05	P > 0.05	P < 0.05
TGLA227	Ho	0.778	0.778	0.97
	He	0.765	0.788	0.89
	F_{IS}	-0.014	0.027	-0.090
	PIC	0.73	0.76	0.87
	HW	P > 0.05	P > 0.05	P < 0.05

1: This work. 2: Martínez *et al.,* 2005. 3: Steigleder *et al.,* 2004.

The heterozygosity and PIC of MM12 is medium to high in UCC and ACCP, and high in BCC, related to the number of alleles that were detected in each population. Marker TGLA227 shows high genetic diversity indexes in the three samples, and similar values for all parameters in UCC and ACCP. These two populations showed no significant deviations from Hardy Weinberg expectations, as opposed to BCC. According to Steigleder *et al.* (2004), this could be caused by the reproductive management of the BCC. FIS values reflect this trend, being low in UCC and ACCP, but medium in BCC with a tendency to heterozygote excess.

The genetic diversity levels of these populations are similar to those found in Spanish breeds proposed as ancestrals, like the Berrenda (Zamorano *et al*., 1998) and Mostrenca (Martínez *et al*., 2005) breeds.

Table 5: Genetic distance of Nei, calculated from MM12 and TGLA227 allelic frequencies.

	UCC	ACCP
ACCP	0.169	0
BCC	1.176	1.291

Table 6: **Genetic distances of Cavalli-Sforza and Edwards (1967) (upper table) and of Reynolds, Weir and Cockerham (1983) (lower table), calculated from MM12 and TGLA227 allelic frequencies.**

	UCC	ACCP	BCC
UCC	0	0.042	0.168
ACCP	0.068	0	0.182
BCC	0.183	0.177	0

As Nei´s distance is based in the IAM neutral mutation model and assumes a constant Ne over time, it may be less accurate in this case, and so alternative distances were calculated. The SMM mutation model has been proposed as more appropriate for microsatellites (Takezaki and Nei, 1996). Cavalli-Sorza and Reynold´s distances assume that the divergence is only due to genetic drift and account for changes in the Ne, such as bottleneck events that have most probably affected these relictual populations (Felsenstein, 1984, 2004).

The genetic distances show that UCC has lower divergence from ACCP than from BCC. With the exception of Reynold´s distance, BCC is closer to UCC than to ACCP. These findings are in accordance with geographic distances and historical data, as well as with the protein genetic distance analysis presented above. ACCP and UCC have very similar origins and were distributed over adjacent regions in past centuries (Primo, 1992). The studied population of BCC is in the Southern region of Brazil, close to the Uruguayan North-East border, where the present UCC reserve remains. The three populations were originated by the cattle brought from the Iberian Peninsula in the XVI and XVII centuries.

More calculations should be made including more markers in order to assess with better accuracy the genetic relationships between American Creole cattle populations, as well as with Iberian ancient breeds. The present work contributes to the elucidation of this issue and supports the productive potential of the Uruguayan Creole cattle.

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