ELSEVIER

Research in Veterinary Science 74 (2003) 287–290

RVS

www.elsevier.com/locate/rvsc

Short communication

Patagonian Argentine Creole cattle polymorphism: comparison with North-West populations of this breed

R.D. Martinez [a], G. Giovambattista [b,*], M.V. Ripoli [b], J.C. De Luca [b], F.N. Dulout [b]

[a] *Laboratorio de Genética Animal, FCA, Universidad Nacional de Lomas de Zamora, Ruta 4 Km 2, 1823 Llavallol, Argentina*
[b] *Centro de Investigaciones en Genética Básica y Aplicada (CIGEBA), Facultad de Ciencias Veterinarias, UNLP, cc 296, B1900AVW La Plata, Argentina*

Abstract

The relict Patagonian Argentine Creole cattle population consist of a small feral population (Los Glaciares population) that is geographically isolated in the South-West of Patagonia. In order to determine the level of genetic variability of this population, the polymorphism of eight structural genes and two microsatellites loci were studied using the polymerase chain reaction (PCR). In addition, genetic characterisation was used to compare Los Glaciares population and the ACc breed of cattle. Results obtained in this study show that the value of average heterozygosity of the studied loci for the Los Glaciares were not significantly different from the ACc. Furthermore, the data of this report were consistent with the hypothesis that Los Glaciares originated from ACc brought to the area by colonialists in the last century. Such data may be useful in formulating management plans for Feral Patagonian Creole cattle populations.

Keywords: Patagonian Creole cattle; Domestic animal diversity; Polymorphism; Molecular marker

1. Introduction

Argentine Creole cattle (ACc) are descendents of animals brought by Spanish conquerors from 1555 until 1587. Within a few years, these cattle had spread all over the country and increased their population size to several million. After more than four centuries, ACc have dominated a wide range of environments, like tropical rainforest, subtropical dry forests, highland steppe, and Patagonian steppe.

Currently, the ACc is distributed throughout Argentina with the most numerous relict, about 200,000 head, situated in the subtropical region (Argentine North-West). For this reason, this breed was believed to be adapted mainly to this environment. By contrast, until the second half of last century 90% (about 20,000,000 in 1850) of the animals were located in the Pampa region (mild weather) (Lebedinsky, 1967).

The first report of Creole cattle in Southern Patagonia is from 1781 (Palermo, 1989). Since the end of 18th until the beginning of the 20 centuries, Creole cattle were introduced to Patagonia from the Pampa region. This occurred mainly by the end of 19th century, when the governor Moyano promoted the development of a stock farm (Moyano, 1968).

During the 20th century; Creole cattle were crossed with European breeds in an effort to improve the breed. For this reason, the population of pure Creole cattle decreased rapidly until it finally disappeared from the Pampa region. In 1989, a feral population of Creole cattle was found in the National Park Los Glaciares. This Park is located in the Southwest (50°20′S, 72°18′W) of the Patagonia and belongs to Andean Cold Forest. There is historical and geographical evidence suggesting this pure group remained isolated from others for at least 20 generations (Rodriguez, 1989). If this is the case, it would be logical that this group is unique and of descendants of the extinct Creole cattle from the Pampa. Nowadays, Los Glaciares population goes up to about 200 animals.

Since 1989, a project of conservation and recovery of the PACc population has been carried out by the

* Corresponding author. Tel./fax: +54-221-421-1799.
E-mail addresses: agrarias@unlz.edu.ar (R.D. Martinez), ggiovam@fcvt.unlp.edu.ar (G. Giovambattista).

0034-5288/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0034-5288(02)00190-X

Faculty of Agrarian Sciences National (University of Lomas de Zamora) and the National Park Administration. In order to perform the genetic characterisation, and analyse the level of genetic variability of Patagonian Creole cattle population, the polymorphism of eight structural genes and two microsatellites loci were studied by PCR. In addition, comparison between Los Glaciares and the ACc was carried out.

Genomic DNA was isolated from 30 blood samples corresponding to Los Glaciares Creole cattle population using DNAzol^{TM} method (Gibco Life Technologies, Rockville, MD). Pedigree records were unavailable for these bovines due to the fact that they were from wild conditions. DNA samples were typed for 10 genetic markers. These loci were selected because there are data available for other ACc populations.

Table 1
Comparison of gene frequencies and their standard errors estimated for each locus in the Los Glaciares Creole cattle population and Argentine Creole cattle

Allele	Patagonian Creole cattle	Argentine Creole cattle
κ-cas A	0.605 ± 0.079	0.648 ± 0.025
κ-cas B	0.395 ± 0.079	0.352 ± 0.025
α_{s1}-cas B	0.950 ± 0.028	0.695 ± 0.026
α_{s1}-cas C	0.050 ± 0.028	0.305 ± 0.026
β-lg A	0.845 ± 0.047	0.533 ± 0.053
β-lg B	0.155 ± 0.047	0.467 ± 0.053
F13A b	0.417 ± 0.014	0.224 ± 0.020
F13A B	0.583 ± 0.014	0.776 ± 0.020
GH A	1.000 ± 0.000	0.847 ± 0.036
GH B	0.000 ± 0.000	0.153 ± 0.036
PRL b	0.950 ± 0.028	0.958 ± 0.011
PRL B	0.050 ± 0.028	0.042 ± 0.011
DYA A1 A2	0.033 ± 0.023	0.026 ± 0.019
DYA A1 G2	0.300 ± 0.059	0.322 ± 0.004
DYA G1 A2	0.017 ± 0.016	0.035 ± 0.017
DYA G1 G2	0.650 ± 0.062	0.618 ± 0.045
MGTG7 306	0.233 ± 0.055	0.126 ± 0.023
MGTG7 302	0.000 ± 0.000	0.325 ± 0.033
MGTG7 292	0.767 ± 0.055	0.548 ± 0.035
TGLA53 169	0.158 ± 0.059	0.078 ± 0.034
TGLA53 167	0.105 ± 0.049	0.219 ± 0.052
TGLA53 163	0.000 ± 0.000	0.031 ± 0.022
TGLA53 161	0.026 ± 0.026	0.078 ± 0.034
TGLA53 159	0.237 ± 0.069	0.594 ± 0.062
TGLA53 157	0.237 ± 0.069	0.000 ± 0.000
TGLA53 155	0.079 ± 0.044	0.000 ± 0.000
TGLA53 153	0.158 ± 0.059	0.000 ± 0.000

The *BoLA-DRB3* and blood coagulation factor XIII, subunit A (*F13A*) loci were typed by hemi-nested PCR–RFLP using the methods as described by Van Eijk et al. (1992a) and Park et al. (1995), respectively. Genotyping of *BoLA-DYA*, κ-casein (*κ-cas*), β-lactoglobulin (*β-lg*), and growth hormone (*GH*) and prolactin (*PRL*) genes were performed by PCR–RFLP, according to the procedures described by Van Eijk et al. (1992b), Agrawala and Wagner (1992), Medrano and Aguilar-Cordoba (1990), Yao et al. (1996) and Lewin et al. (1992), respectively. α_{S1}-casein (α_{S1}-cas) was typed by PCR-SSP (sequence specific primers) (David and Deutch, 1992). A multiplex reaction was used to amplify the microsatellites *MGTG7* aid *TGLA53* (Kappes et al., 1997). The microsatellite variants were characterised in a polyacrylamide denaturing sequencing gel and stained with silver. Approximate size was determined by comparison with control animal DNA provided by H. Lewin at Biotechnology Laboratory (Illinois University, USA).

Gene and genotype frequencies for each locus were determined by the direct gene counting method. Genetic variation was measured by the number of alleles (n_a), the unbiased expected heterozygosity (h_e) and the average heterozygosity (He) (Nei, 1987). The comparison of the degree of genetic variation, measured through the parameter He, between ACc breed and Los Glaciares population was performed using Student test (Nei, 1987). Deviations from Hardy-Weinberg equilibrium (HWE) for each locus was estimated by F_{IS} statistics, using the GENEPOP package version 1.2 (Raymond and Rousset, 1995). The significance of the HWE probabilities across loci was determined using Fisher's method (Raymond and Rousset, 1995).

Allele frequencies and their standard errors are given in Tables 1 and 2. All loci were found to be polymorphic in the Los Glaciares population, with the single exception of *GH*. Furthermore, *PRL-b* and α_{S1}-cas-B variants were nearly fixed in this population.

The number of alleles detected at each locus for the Los Glaciares population are summarised in Table 3. These values ranged from one allele for *GH* to 10 variants for *BoLA-DRB3*. As shown in Table 3, expected heterozygosity and their standard errors were computed at each locus for the Los Glaciares population. The average heterozygosity and the standard error were also

Table 2
Gene frequencies and their standard errors estimated for *BoLA-DRB3* locus in Los Glaciares Creole cattle population

Allele	Frequencies ± SD	Allele	Frequencies ± SD
*BoLA-DRB3.2*01*	0.458 ± 0.072	*BoLA-DRB3.2*18*	0.017 ± 0.019
*BoLA-DRB3.2*07*	0.017 ± 0.019	*BoLA-DRB3.2*19*	0.035 ± 0.026
*BoLA-DRB3.2*08*	0.017 ± 0.019	*BoLA-DRB3.2*20*	0.193 ± 0.057
*BoLA-DRB3.2*11*	0.017 ± 0.019	*BoLA-DRB3.2*31*	0.035 ± 0.026
*BoLA-DRB3.2*15*	0.105 ± 0.044	*BoLA-DRB3.2*39*	0.017 ± 0.019

Table 3
Comparison of number of alleles (n_a), expected heterozygosity (h_e) and average heterozygosity (He) between Los Glaciares population and average values for ACc breed

Locus	Patagonian Creole cattle		Argentine Creole cattle		$t-p$ value[a]
	n_a	h_e	n_a	h_e	
κ-cas	2	0.491 ± 0.126	2	0.457 ± 0.015	-0.822, $p > 0.50$
α_{S1}-*cas*	2	0.096 ± 0.013	2	0.425 ± 0.020	3.360, $0.20 > p > 0.10$
β-lg	2	0.267 ± 0.047	2	0.503 ± 0.010	2.340, $0.50 > p > 0.20$
GH	1	0.000 ± 0.000	2	0.262 ± 0.051	5.175, $0.20 > p > 0.10$
PRL	2	0.096 ± 0.013	2	0.048 ± 0.026	0.426, $p > 0.50$
F13A	2	0.500 ± 0.129	2	0.348 ± 0.023	-3.360, $0.20 > p > 0.10$
BoLA-DRB3	10	0.753 ± 0.219	23	0.882 ± 0.008	2.296, $0.50 > p > 0.20$
BoLA-DYA	4	0.494 ± 0.127	4	0.518 ± 0.034	-0.202, $p > 0.50$
MGTG7	2	0.364 ± 0.081	3	0.580 ± 0.021	2.302, $0.50 > p > 0.20$
TGLA53	7	0.842 ± 0.25	5	0.596 ± 0.056	2.498, $0.50 > p > 0.20$
H_e	27	0.39 ± 0.026	47	0.462 ± 0.058	0.380, $0.8 > p > 0.7$

[a] Student test values for genetic diversity differences among Los Glaciares population and ACc breed.

calculated for this population across all studied loci. At polymorphic loci, the heterozygosities in the analysed sample ranged from 0.096 ± 0.013 for the *Prl* locus and α_{S1}-*cas* loci, to 0.842 ± 0.25 for the *TGLA53* locus with a mean of 0.390 ± 0.026. The He value obtained in Los Glaciares populations did not show significant difference from the value previously estimated in ACc breeds ($t = 0.380$, $0.8 > p > 0.7$).

The F_{IS} value provided evidence that the observed genotype frequencies did not deviate from those predicted by HWE at nine loci ($0.036 > F_{IS} > -0.167$, $1 > p > 0.41$) as well as all other loci that were considered ($\chi^2 = 9.3$, $p = 0.899$), while only the *BoLA-DYA* locus was in desequilibrium ($F_{IS} = 0.125$, $p = 0.040$).

Comparison of Los Glaciares and ACc gene frequencies for the structural low polymorphic genes showed that alleles with higher frequencies for each locus in the Los Glaciares population were also the most abundant in the ACc breed (Table 1). For example, the A variant for *κ-cas* was the most abundant in the Los Glaciares, in agreement with data previously reported for ACc (Poli and Antonini, 1992; Golijow et al., 1996). When the most common variants of the $\alpha_{S1}-cas$ (B), *F13A* (B), and *BoLA-DYA* (G1G2) from the Los Glaciares and ACc were compared, similar results were observed (Table 1). The *β-lg* gene was the only exception. While both alleles exhibited similar gene frequencies in the ACc breed (Poli and Antonini, 1992), in the Los Glaciares the alleles A of *β-lg* exhibited higher frequency than *β-lg* B.

In the Los Glaciares population only two alleles at the *MGTG7* locus were detected. Comparison between results of the *MGTG7* obtained for Los Glaciares and ACc breed showed that the *MGTG7 294* allele was the more abundant variant (gene frequency around 60%) in both groups, while the *MGTG7 306* allele variant exhibited a gene frequency value higher than 15%. The *MGTG7 302* allele was not detected in Los Glaciares population (being absent or present at low frequency), although this allele is common in ACc breed (aproximately 30%; Table 1). At locus *TGLA53*, seven alleles were detected in Los Glaciares population. Comparison between both groups showed that three of these alleles (*TGLA53 157*, *155* and *153*) were not detected in ACc. The *TGLA53 169*, *167*, *159*, *157* and *153* alleles were the more abundant variants (gene frequency higher than 10%) in Los Glaciares population, while two of them (*TGLA53 167* and *159*) were the most common alleles in ACc population.

The most striking difference between the Los Glaciares and the average value of ACc was observed in the gene *BoLA-DRB3*. At this locus, 10 out of the 23 previously reported alleles in ACc were detected in Los Glaciares population (Giovambattista et al., 1996). The *BoLA-DRB3.2*01* variant was present at high frequency in the Los Glaciares (more than 40%). However, this allele was either absent or present at very low frequency in other herds of ACc that have been studied (0–3.2%) (Giovambattista et al., 2001). Alleles *BoLA-DRB3.2*15* and *BoLA-DRB3.2*20* exhibited frequencies higher than 10% in both the Los Glaciares population and the ACc breed, while the other seven alleles were present at low frequency, in either population studied (< than 5%).

A small population, like Los Glaciares, without gene flow and geographical isolation for at least half century, could suffer a dramatic reduction of genetic diversity due to founding group, bottleneck, genetic drift, and inbreeding effects. In addition, natural selection could eliminate the unfavourable variants, decreasing the genetic variability.

However, when the data provided in the present study were compared with the genetic variability level previously reported for ACc, no significant differences were observed. These results would suggest that the degree of variability (measured through mean heterozygosity values) has been maintained within the Los Glaciares population. These results are consistent with data previously reported for other isolated populations

of ACc located in the North-West of Argentina (Giovambattista et al., 1995). Furthermore, effective avoidance of inbreeding has also been shown for a small populations of Camargue feral horses in South-Eastern France (Duncan et al., 1984) and Great Basin feral horses in Western USA (Bowling, 1994).

The general genetic concordance between Los Glaciares population and ACc breed is consistent with the hypothesis that the population of the Los Glaciares was a descendent of ACc population that lived in La Pampa region in the 19th century. If so, these results are in agreement with historical and morphological data. However, it is necessary to analyse additional genetic markers in order to confirm the origin and the degree of admixture of the analysed population.

Acknowledgements

The authors would like to thank Harris A. Lewin for providing control animal DNA. This work was supported by grants from JICA, CIC and CONICET.

References

Agrawala, P.L., Wagner, V.A., Geldermann, 1992. Sex determination and milk protein genotyping of preimplantation stage bovine embryos using multiplex PCR. Theriogenology 38, 969–978.

Bowling, A.T., 1994. Population genetics of Great Basin feral horses. Animal Genetics 25 (Suppl 1), 67–74.

David, V.A., Deutch, A.H., 1992. Detection of α_{s1}-casein genomic variants using the allele-specific polymerase chain reaction. Animal Genetics 23, 425–429.

Duncan, P., Feh, C., Gleize, J.C., Malkas, P., Scott, A.M., 1984. Reduction of inbreeding in a natural herd of horses. Animal Behavior 32, 520–527.

Giovambattista, G., Golijow, C.D., Ripoli, M.V., Roccia, I.L., Dulout, F.N.y., Lojo, M.M., 1995. Estudio de la variabilidad genética en una población de ganado Bovino Criollo Argentino aislada geográficamente en una pradera de altura. Analecta Veterinaria XV (1), 3–9.

Giovambattista, G., Golijow, C.D., Dulout, F.N., Lojo, M.M., 1996. Gene frequencies of DRB3.2 locus of Argentine Creole cattle. Animal Genetics 27, 55–56.

Giovambattista, G., Ripoli, M.V., Peral Garcia, P., Bouzat, J.L., 2001. Indigennous domestic breeds as reservoirs of genetic diversity: the Argentine Creole Cattle. Animal Genetics 32, 240–247.

Golijow, C.D., Giovambattista, G., Poli, M., Dulout, F.N., Lojo, M.M., 1996. κ-Casein gene frequencies support subdivision and historical origin of Argentine Creole cattle. Brazilian Journal of Genetics 19 (4), 583–588.

Kappes, S.M., Keele, J.W., Stone, R.T., Mcgraw, R.A., Sonstegard, T.S., Smith, T.P.L., Lopez-Corrales, N.L., Beattie, C.W., 1997. A second generation linkage map of the bovine genome. Genome Research 7, 235–249.

Lebedinsky, M., 1967. In: Estructura de la Ganaderia, Histórica y Actual. Editorial Quipo. Buenos Aires.

Lewin, H.A., Schmitt, K., Hubert, R., van Eijk, M.J.T., Arnheim, N., 1992. Close linkage between bovine prolactin and BoLA-DRB3 genes: genetic mapping in cattle by single sperm typing. Genomics 13, 44–48.

Medrano, J.F., Aguilar-Cordoba, E., 1990. Polymerase chain reaction amplification of bovine β-lactoglobulin genomic sequences and identification of genetic variants by RFLP analysis. Biotechnology 1 (1), 73–77.

Moyano, C., 1968. In: Carlos Moyano, el explorador de la patagonia. Editorial El Ateneo Bs. As.

Nei, M., 1987. In: Molecular Evolutionary Genetics. Columbia University press, New York, p. 512.

Palermo, M., 1989. Animales y cultivos llegan al sur. Ciencia Hoy 1 (4), 25.

Park, C.h., Russ, I., Da, Y., Lewin, H.A., 1995. Genetic mapping of F13A to BTA23 by sperm typing: difference in recombination rate between bulls in the DYA-PRL interval. Genomics 27, 113–118.

Poli, M.A., Antonini, A.G., 1992. Genetic structure of milk proteins in Argentinian Holstein and Argentinian Creole cattle. Hereditas 115, 177–182.

Raymond, M., Rousset, F., 1995. GENEPOP 1.2: a population genetics sotfware for exact test and ecumenicism. Journal of Hereditary 86, 248–249.

Rodríguez, C.A. et al., 1989. Bovinos Criollo Argentino – Biotipo Patagónico – Descripción y Conservación. In: Proceeding of XX Congreso Argentino de Genética, p. 104.

Van Eijk, M.J.T., Stewart-Haynes, J.A., Lewin, H.A., 1992a. Extensive polymorphism of BoLA-DRB3 gene distinguished by PCR–RFLP. Animal Genetics 23, 483–496.

Van Eijk, M.J.T., Russ, Y., Beever, J.E., Lewin, H.A., 1992b. Polymorphism in the exon 2 of the bovine lymphocyte antigen (BoLA)-DYA gene. Animal Genetics 23, 476.

Yao, J., Aggrey, S.E., Zadworny, D., Hayes, J.F., Kuhnlein, Y.U., 1996. Sequence variations in the bovine growth hormone gene characterized by single-strand conformation polymorphism (SSCP) anlysis and their association with milk production traits in Holsteins. Genetics 144, 1809–1816.