Animal Genetics, 2000, **31**, 302–305

Male-mediated introgression of *Bos indicus* genes into Argentine and Bolivian Creole cattle breeds

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Summary

The geographic distribution and frequency of *Bos taurus* and *Bos indicus* Y chromosome haplotypes amongst Argentine and Bolivian Creole cattle breeds were studied, using cytogenetic and molecular genetic techniques. A complete correspondence between Y chromosome morphology and the haplotype of the Y-linked microsatellite marker *INRA 124* was found in all males examined. The taurine and indicine haplotypes were detected in 85·7 and 14·3% of the males studied, respectively, although these frequencies varied amongst the different breeds examined. The geographic distribution of this polymorphism suggests a pattern of zebu introgression in South America. The highest frequencies of the Zebu Y-chromosome are found in Brazilian populations (43–90%), in the eastern part of the continent, while it is absent in the southermost breeds from Uruguay and Argentina. Bolivan breeds, at the centre of the continent, exhibit intermediate values (17–41%). This east/west and north/south gradient of male Zebu introgression could be explained by historical events and environmental factors.

Keywords: Creole cattle, genetic introgression, molecular markers, Y chromosome polymorphism

Introduction

Domestic cattle are usually divided into two major morphological groups, the humped zebu and the humpless taurine types. During the 1970s, a large number of studies of protein loci demonstrated that *Bos indicus* and *Bos taurus* were relatively divergent (for a review, see Manwell & Baker, 1980). MacHugh *et al.* (1997) confirmed this divergence through the analysis of autosomal microsatellites. Loftus *et al.* (1994) reported mtDNA sequence data consistent with divergence from a common ancestor 200 000 to 1 million years BP, well before the domestication of cattle, suggesting an independent origin of the two domestic types.

At the cytogenetic level, the taurine and zebu cattle could only be differentiated through differences in Y chromosome morphology (Kieffer & Cartwright, 1968). The acrocentric morphology of this chromosome has been detected in zebu breeds from Asia and Africa, while the submetacentric type has been found in taurine breeds. However, some phenotypically defined taurine and zebu breeds exhibit both Y chromosome morphologies in their populations (Bradley *et al.*, 1994).

Recently, techniques capable of detecting Y-chromosome polymorphisms at the DNA level have been developed (Bradley *et al.*, 1994; Gwakisa, Kemp & Teale, 1994; Teale *et al.*, 1996; Nijman *et al.*, 1999). Hanotte (1997) and Hanotte *et al.* (1998) reported one specific allele for *B. taurus* and another variant for *B. indicus* in the microsatellite *INRA 124* (*DYS6*) (Vaiman *et al.*, 1994).

Cattle were introduced for the first time into America by the Spanish conquerors in the Caribbean islands in 1492. In the course of a few years cattle spread all over the continent. Since then, these animals have evolved under wild and semi-wild conditions and as a result of natural selection, they have adapted to different environments in the continent, such as tropical rainforest, subtropical dry forests, highland steppe, and Patagonian steppe. Nowadays, almost all North and South American countries have Creole cattle, i.e. native breed descendants of Iberian cattle.

The Argentinian Creole cattle breed (ACc) has a widespread geographical distribution and the existence of different strains has been suggested (Bouzat *et al.*, 1998). In Bolivia, there are four main breeds of Creole cattle: the Chaqueño Boliviano Creole cattle (ChBc), the Altiplano Creole cattle (AlCc), the Yacumeño Creole cattle (YCc) and the Saavedreño Creole cattle (SCc). Each one of these breeds has a different origin and is adapted to particular environmental conditions (Lotfus & Scherf, 1993).

In the present work, the geographic distribution and frequency of the *B. taurus* and *B. indicus* Y chromosome haplotypes amongst Argentine and Bolivian Creole cattle breeds were studied using cytogenetic and molecular techniques. The breeds analysed are shown in Fig. 1 and Table 1.

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Accepted 29 May 2000

Fig. 1. Frequency of Zebu and Taurine Y-chromosome in South American Creole breeds. In black, indicine Y-chromosome, and in white, taurine. ACc, Argentinian; ChBc, Chaqueño Boliviano; YCc, Yacumeño; SCc, Saavedreño; UCc, Uruguayan; CCc, Caracu; CuCc, Curraleiro; LCc, Lageano; I, Iberic taurine cattle; Z, zebu cattle. Asterix corresponds to the only animal sampled in the Bolivian Altiplano, with a taurine Y-chromosome.

Blood and semen samples were collected from males belonging to nine populations of ACc ($n = 56$), one population of SCc ($n = 42$), one herd of ChBCc ($n = 22$), one population of YCc ($n = 18$), and one of AlCc ($n = 1$). As a control, microsatellite size and Y chromosome morphology of zebu, Holstein and Aberdeen Angus were established by molecular and cytogenetic methods. Lymphocyte cultures and karyotypes were perfomed as described previously by De Luca *et al.* (1997).

Genomic DNA was isolated from blood or semen samples by DNAzol (GIBCO BRL-Life Technologies), following the manufacturer's recommendations. The *INRA124* (*DYS6*) microsatellite was amplified using the primers designed by Vaiman *et al.*, (1994). The 25 μL reaction mix contained 5 μL of the DNAzol extract, 0.5 μM of each primer, dNTPs 0.1 mM and 1 U *Taq* polymerase (Gibco BRL-Life Technologies) in 10 mM Tris–HCl (pH = 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, under mineral oil. PCR amplification consisted of a first denaturation step at 96 °C for 2 min followed by 10 cycles of 1 min at 94 °C, 45 s at 55 °C and 50 s at 72 °C, and followed by 25 cycles of 1 min at 90 °C, 45 s at 55°C, 50 s at 72 °C with a final elongation step of 5 min at 72°C.

The specific taurine (124 bp) or indicine (126 bp) variants were distinguished by denaturing polyacrylamide gel (6%) electrophoresis and visualized using silver stain. Gene frequencies were determined by direct counting.

The Argentine and Bolivian Creole cattle are phenotypically humpless breeds. However, acrocentric and submetacentric Y chromosome morphologies, and both *INRA124* alleles were observed in the sample studied. All Creole males that had a typically *B. indicus* acrocentric Y chromosome had the 124-bp allele for the *INRA124* microsatellite. In contrast, all bulls that had submetacentric Y chromosomes had the 126 bp allele at this locus. No discrepancy was found between cytogenetic and molecular techniques. These results are consistent with the correlation between karyotype and a different molecular (RAPD) marker genotype previously reported in African breeds by Teale *et al.* (1996).

Table 1. Main features of Argentine Creole cattle (ACc), Saavedreño Creole cattle (SCc), Chaqueño Boliviano Creole cattle (ChBCc), Yacumeño Creole cattle (YCc) and Altiplano Creole cattle (AlCc) breeds. The last two columns indicate the frequencies of taurine (124) and indicine (126) INRA 124 haplotypes found

Breed	Geographical distribution and environment	Population size	Production purpose	Haplotype frequency	
ACc	Widespread distribution in Argentine in a wide range of environments	300 000	beef	1·00	0
SCc	Saavedra Experimental Station in the tropical plain of Santa Cruz Department, Bolivia.	300 adults	dairy	0·80	0·20
ChBCc	'del Chaco El Salvador' Experimental Station in the Southeast dry forest of Bolivia	1200 adults	beef	0·59	0·41
YCc	'Espiritu' farm in the tropical and humid flooded plain of El Beni, Northern Bolivia.	1200 adults	beef	0·83	0·17
AlCc	Altiplano highland plain, Western Bolivia.	200 000	beef	1·00	0

Over a total of 139 bulls studied, the taurine haplotype was detected in 119 males (85·6%), while the indicine one was observed in 20 sires (14·4%). However, the geographic distribution and frequency of taurine *B. taurus* and zebu *B. indicus* Y chromosome haplotypes amongst Argentinan and Bolivian Creole cattle breeds were not homogeneous. The taurine haplotype was observed in 100% of the ACc bulls studied, in 83% of the YCc, in 80% of the SCc males, and in 59% of the ChBCc animals, while a single male from the Altiplano also showed this haplotype (Fig. 1).

Previous cytogenetic studies on other South American Creole cattle breeds showed that the zebu Y chromosome was present in 90% of Caracu, Mocho Nacional and Curraleiro bulls, and in 43% of Lageano males, while it was absent in Uruguayan (UCc), Argentinian and Venezuelan Creole cattle breeds (Tambasco, Trovo & Barbosa, 1985; Muñoz *et al.*, 1994; Postiglioni *et al.*, 1996; De Luca *et al.*, 1997). The comparison between our results and the previously reported data shows that the percentage of indicine Y chromosome within breeds ranges from 0 to 90%. As shown in Fig. 1, the geographic distribution of this polymorphism reveals a clear pattern of zebu introgression in South America. The zebu Y chromosome has penetrated to a high proportion into Brazilian populations in the east of the continent, while its lowest level was found in the southernmost breeds (Uruguayan and Argentinian). On the other hand, Bolivian breeds and Lageano (the Southern Brazilian breed) exhibit intermediate values between Brazilian and populations in the South.

The declining east to west and north to south gradient of male Zebu introgression could be explained through historical data and environmental conditions. In this sense, the highest percentages observed in Brazilian breeds agree with the massive introduction of zebu animals into Brazil during the eighteenth and nineteenth centuries, in order to improve native breeds in tropical regions. Furthermore, it has been suggested that the ancestral indicine Y chromosome of the Brazilian native breeds could have reached the area before the extensive introduction of zebu breeds into Brazil, as a result of the introduction of zebu cattle by the Portuguese from their Asian and African colonies since 1534.

During the second half of the twentieth century, Brazil served as a centre for zebu distribution in South America. At that time, zebu cattle were exported from Brazil to neighbouring countries like Bolivia, where indiscriminate crossbreeding between Creole dams and zebu sires was extensively practised, reducing the number of pure Creole cattle. Amongst Bolivian breeds the percentage of the zebu haplotype varied from 17 to 41%. The lowest value observed in the YCc could be a result of the longer isolation period of this breed. In contrast, the CHCc exhibited the highest percentage of zebu haplotype and the shortest isolation period.

Temperate weather constitutes an obstacle to zebu breeding. This is probably the reason for the observed complete absence of the indicine Y haplotype in ACc and UCc cattle. The altitude of the ‘Altiplano’ also constitutes a barrier to zebu introduction, and so we expected to find the submetacentric Y chromosome in 100% of males. Unfortunately, only one bull from the Altiplano, which exhibited taurine haplotype, was examined.

As mentioned above, the founder group of SCc was established using animals from four lineages. Interestingly, the analysis of SCc paternal pedigree revealed that all acrocentric Y chromosomes were detected in bulls belonging to the Caracu lineage. This result constitutes an example of the sex-mediated process of genetic introgression that took place between Bolivian females and Brazilian males.

This work established a pattern of introgression of zebu into taurine creole cattle based on Y chromosome morphology and haplotype. However, because this marker only detects male-mediated introgression, and is strongly affected by genetic drift and breeding strategy, a more accurate estimation of the genetic admixture should be revealed using autosomal and mitochondrial markers.

Acknowledgements

This paper was supported by the National Research Council (CONICET), Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC), Universidad Nacional de La Plata and The Japanese International Cooperation Agency (JICA).

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