

# Genetic Characterization of Argentine and Bolivian Creole Cattle Breeds Assessed through Microsatellites

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## Abstract

In the present report, the polymorphisms from 9 microsatellites were used to assess genetic diversity and relationships in 4 Creole cattle breeds from Argentina and Bolivia, 4 European taurine breeds, and 2 American zebu populations. The Creole populations display a relatively high level of genetic variation as estimated by allelic diversity and heterozygosity, whereas the British breeds displayed reduced levels of genetic diversity. The analysis of molecular variance indicated that 7.8% of variance can be explained by differences among taurine and zebu breeds. Consistent with these results, the first principal component (PC), which comprised the 40% of the total variance, clearly distinguishes these 2 groups. In addition, all constructed phylogenetic trees cluster together Nelore and Brahman breeds with robust bootstrap values. Only 1% of variance was due to difference between American Creole and European taurine cattle. Although this secondary split was supported by the classical genetic distance and the second PC (15%), the topology of trees is not particularly robust. The presence of zebu-specific alleles in Creole cattle allowed estimating a moderate degree of zebu admixture. When these data were compared with mitochondrial and Y chromosomal studies, a clear pattern of male-mediated introgression was revealed. The results presented here contribute to the understanding of origin and history of the American Creole cattle.

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Anthropological and paleontological evidence show that the first cattle were brought to America by Spanish conquerors from 1493 (Primo 1992). The founder populations of Creole cattle, introduced in America by the Spanish and Portuguese conquerors during the first 50 years of colonization, consisted of 300–1000 animals of Iberian origin (Wilkins and others 1982). In the course of a few years, these animals were taken to Central and South America and to the south of the current United States. In the course of few decades, the Creole cattle spread over Latin America, being the only bovine bred for more than 300 years until the introduction of selected European and Indian breeds.

Nowadays, a number of distinct local Creole breeds are found throughout the Americas. These cattle show great phenotypic heterogeneity and have adapted to a wide range of environments with little human intervention. In Bolivia, 4 different Creole cattle breeds can be recognized: 1) Yacumeño Creole, a breed adapted to the seasonal flood plain of the northern region (Department of El Beni) and raised primarily for beef; 2) Chaqueño Creole, a beef breed found on the dry forest environment at the southeastern parts of the country (Departments of Chuquisaca and Santa Cruz); 3) Saavedreño

Creole, a breed mainly found on a tropical plain at the east of Bolivian territory (Department of Santa Cruz), which is bred for dairy and beef; and 4) Chusco Creole, a beef breed adapted to the highland plain of western Bolivia (Department of La Paz). In Argentina, a single Creole cattle breed can be recognized with a broad geographical distribution, from subtropical region in the north to the Patagonian region in the south, adapted to a wide range of environments.

Microsatellites, which are abundant markers well dispersed in the genome and highly polymorphic, have been shown to be useful for a variety of purposes, such as genome mapping, parentage determination, legal medicine, disease research, cancer research, and determination of genetic variation (Goldstein and Schötterer 1999). Furthermore, microsatellites are currently the commonest markers used for genetic characterization of cattle breeds (Machugh and others 1994, 1997; Moazami-Goudarzi and others 1997; Loftus and others 1999; Kantanen and others 2000; Cañon and others 2001; Hanotte and others 2002; Beja-Perira and others 2003). Initially, it was difficult to group and compare the results obtained by different research groups because they do not use a common set of microsatellites.

The primary objective of the present work was to characterize the genetic diversity of Creole cattle from Argentina and Bolivia. The secondary goal was to clarify the genetic relationship of these indigenous cattle among them and with other common European and Indian cattle breeds. An additional aim was to assess the extent and pattern of gene admixture and the dynamics of the zebu genetic introgression in the studied Creole cattle populations. In order to perform these objectives, the most widespread European and Indian cattle breeds that are bred in Argentina and Bolivia were also analyzed.

## Materials and Methods

### Studied Populations

A total of 314 adult animals representing 10 Argentine and Bolivian breeds were analyzed. The breeds studied can be classified according to its origin in 3 groups: 1) Creole cattle represented by the Argentine Creole (Ac) and 3 Bolivian breeds, Saavedreño (SaC), Chaqueño (ChC), and Yacumeño (YaC); 2) European taurine group, represented by the Aberdeen Angus (AA), Hereford (He), Holstein (Ho), and Retinta (Re); and 3) American zebu group, including the Nelore (Ne) and Brahman (Bz). Table 1 summarizes the sampling strategies. When pedigree information was available, we selected individuals that have not shared a common ancestor for at least 2 generations.

### DNA Extraction

Genomic DNA was isolated from lymphocyte cells using the DNAzol reagent (Invitrogen, Carlsbad, CA) following the manufacturer's instructions.

### Microsatellite Markers

DNA typing was performed by polymerase chain reaction (PCR) using 9 markers. The microsatellites *MG1G7* and *TGLA53* were included in the Food and Agricultural Organization list for biodiversity studies, whereas the microsatellites *ETH225*, *INRA023*, *BM1824*, *BM2113*, *SPS115*, *TGLA122*, and *TGLA227* were also suggested by the International Society of Animal Genetics to be used for the International Comparison Test (Table 2).

**Table 1.** Summary of cattle breeds sampled

Breeds	Breed code	DNA samples	Country	Cattle group
Argentine Creole	AC	31	Argentina	Creole
Saavedreño	SaC	31	Bolivia	Creole
Yacumeño	YaC	25	Bolivia	Creole
Chaqueño	ChC	22	Bolivia	Creole
Aberdeen Angus	AA	59	Argentina	European taurine
Hereford	He	36	Argentina–Uruguay	European taurine
Holstein	Ho	33	Argentina	European taurine
Retinta	Re	20	España	European taurine
Nelore	Ne	26	Argentina–Bolivia	Zebu
Brahman	BZ	31	Bolivia	Zebu

### PCR Amplification and Genetic Analysis of PCR Products

PCR was carried out in a total volume of 12.5  $\mu$ l, containing 20 mM Tris-HCl (pH = 8.4), 50 mM KCl, 100 mM of each deoxynucleoside triphosphate, 0.5 U *Taq* polymerase (Invitrogen), 10–20 ng of DNA template, and both primers and  $MgCl_2$  at the appropriate concentrations (Table 2). The cycling conditions were an initial denaturation step of 2 min at 94 °C; followed by 10 cycles of 1 min at 92 °C, 45 s at specific annealing temperatures (Table 2), and 50 s at 72 °C; and followed by 25 cycles of 1 min at 90 °C, 45 s at specific annealing temperatures (Table 2), and 50 s at 72 °C with a final elongation step of 15 min at 72 °C. Alleles were identified (bp size) by gel mobility comparison that corresponded to previously typed DNAs that were included in the gel as standards. Two types of reference DNA were used that included reference DNA from the MoDAD and the International Comparison Test 2001–2002.

### Measures of Genetic Variability

Allele frequencies were determined for each population by direct counting. Levels of genetic variability were estimated using allelic diversity and the observed ( $H_o$ ) and unbiased expected ( $H_e$ ) heterozygosities. The  $H_o$  and  $H_e$  were computed according to Nei (1987). Initially, the number of

**Table 2.** Chromosomal location, PCR parameters, detected allele size range, and reference for 9 microsatellite markers

Locus	Chromosome	$T_a$ (°C)	$MgCl_2$ (mM)	Primer ( $\mu$ M)	Detected size range (bp)	Reference
<i>BM2113</i> ( <i>D2S26</i> )	2	63	1.5	0.8	122–144	Bishop and others (1994)
<i>BM1824</i> ( <i>D1S34</i> )	1	63	1.5	1.2	179–191	Bishop and others (1994)
<i>SPS115</i> ( <i>D15</i> )	15	63	1.5	0.4	234–256	Moore and Byrne (1993)
<i>INRA023</i> ( <i>D3S10</i> )	3	63	2	1.2	195–215	Vaiman and others (1994)
<i>ETH225</i> ( <i>D9S1</i> )	9	63	2	0.6	139–159	Steffen and others (1993)
<i>TGLA122</i> ( <i>D21S6</i> )	21	56	3	0.8	136–182	Georges and Massey (1992)
<i>TGLA227</i> ( <i>D18S1</i> )	18	56	3	1.6	79–105	Georges and Massey (1992)
<i>TGLA53</i> ( <i>D16S3</i> )	16	56	2.5	1.2	151–183	Georges and Massey (1992)
<i>MTG17</i> ( <i>D23S5</i> )	23	56	2.5	0.6	282–310	Georges and others (1995)

observed alleles ( $N_a$ ) was calculated by direct counting. The sample sizes were not identical for the breeds typed, ranging from  $n = 20$  for the Retinta to  $n = 56$  for the Aberdeen Angus. Consequently, to remove any sample bias, the number of alleles was calculated for a random sample of 20 animals. This procedure was repeated 1000 times for each breed, and the average of the estimated number of alleles ( $N_{\bar{a}}$ ) was calculated. Hardy–Weinberg equilibrium (HWE) for each locus within populations was estimated by  $F_{IS}$  statistics (Weir and Cockerham 1984), using the exact test included in the GENEPOP software, version 1.2 (Gou and Thompson 1992; Raymond and Rousset 1995a). Significance levels were adjusted using the sequential Bonferroni method to take into account multiple tests on the same data set (Weir 1996).

### Genic Differentiation and Population's Subdivision

The exact test proposed by Raymond and Rousset (1995b) was performed to assess breed differentiation based on genic differentiation. This test was evaluated for all 45 pairwise inter-population comparisons employing the GENEPOP package.

Population subdivision was evaluated with Wright's  $F_{ST}$  statistic, using the variance-based method of Weir and Cockerham (1984). A second estimator of gene differentiation,  $R_{ST}$ , was calculated, which accounts for variance in allele size and was defined for genetic markers undergoing a stepwise mutation model (Slatkin 1995).  $F$ -statistics were computed using FSTAT (Goudet 1995). After defining groups of breeds based on their historical origin, a hierarchical analysis of the variance was carried out using the analysis of molecular variance (AMOVA) software implemented in the ARLEQUIN package, version 2.0 (Schneider and others 2000).

Standard genetic distance ( $D_S$ ) of Nei (1972), Cavalli-Sforza and Edwards (1967) chord distance ( $D_C$ ), and stepwise-weighted genetic distance ( $D_{SW}$ ) (Shriver and others 1995) were calculated from allele frequencies. Dendograms were constructed from the distance matrix using the unweighted pair group method with arithmetic mean (UPGMA) (Sneath and Sokal 1973) and the Neighbor-Joining (NJ) (Saitou and Nei 1987) algorithms. Bootstrap values were calculated from 1000 replications of resampling loci. Distance and trees were computed using the POPULATIONS 1.2.28 software (Langela and others 1999). The trees were visualized using TREEVIEW (Page 1996).

To condense the genetic variation revealed for the 9 microsatellites, principal components analysis (PCA) was performed from all allele frequencies according to Cavalli-Sforza and others (1994). The PCA was carried out using the PAST (Paleontological Statistics; Hammer and others 2001) software.

### Analysis of Zebu Genetic Admixture

Two methods were used to estimate zebu gene introgression from the autosomal microsatellite markers in the 4 Creole cattle populations. The amount of *Bos Indicus* admixture was first estimated by zebu-diagnostic alleles. The criteria

used for determining an allele as zebu diagnostic was its presence at high frequency in the *B. Indicus* breeds (Nelore and Brahman) and absence in the European taurine populations (Aberdeen Angus, Hereford, and Holstein). The Spanish breed Retinta was excluded from the pooled European breed due to a possible introgression with *B. Indicus*. In the decades of 1960/1970, individuals from Nelore and St Gertrudis breed have been crossed with southern Iberian populations (Beja-Perira and others 2003). Five alleles corresponding to 3 loci (*ETH225.153*, *ETH225.157*, *ETH225.159*, *TGLA227.79*, and *TGLA122.136*) were classified as zebu diagnostic. In order to estimate the percentage of introgression, the frequencies for each diagnostic allele in the Creole populations were divided by the average frequency calculated for the indicine breeds.

A second method was used to estimate zebu admixture from the total data set based on the mean coalescent time of genes drawn either within or between admixed and parental populations. The statistic  $m_Y$  has been developed by Bertorelle and Excoffier (1998) and was calculated using the program ADMIX 1.0 (<http://www.unife.it/genetica/Giorgio/giorgio.html>).

## Results

A total of 104 alleles were detected across the 9 loci analyzed. At a threshold frequency of 5%, chosen to reduce the effects of sampling error, there were 3 private alleles (unique to one breed), one present in each one of Nelore, Retinta, and Holstein breeds. Allele frequency values for each marker and breed have been submitted to the MoDAD.

Measures of genetic variability are shown in Table 3. The average  $N_a$  and  $N_{\bar{a}}$  for all loci and all breeds were 7.2 and 6.6, respectively. The comparison between both estimators of the numbers of alleles was different in some breeds due to the variations in sample sizes. The least diverse were the British breeds (Aberdeen Angus and Hereford) with the lowest  $N_{\bar{a}}$  of 5.7 and 5.8, respectively. The population with greatest allelic diversity was the Chaqueño Creole from Bolivia with a  $N_{\bar{a}}$  of 7.5. The  $H_o$  and  $H_e$  per breed ranged from 0.61 (Nelore) to 0.86 (Saavedreño Creole) and from 0.64 (Nelore) to 0.79 (Holstein), respectively (Table 3).

There were a total of 90 HWE tests (9 loci in 10 populations). A total of 18 locus-population combinations were statistically significant ( $P \leq 0.05$ ) (Table 3). These deviations comprise 6 loci in Nelore; 4 loci in Hereford; 2 loci in Retinta, Holstein, and Aberdeen Angus; and 1 locus in Brahman and Saavedreño Creole. Nonsignificant deviations from HWE were observed for the other 3 Creole breeds.

When the results were pooled across breeds, the Nelore, Hereford, and Aberdeen Angus breeds gave significant deviations from Hardy–Weinberg proportions ( $P \leq 0.005$ , adjusted by the Bonferroni correction). Average  $F_{IS}$  values were significant in Nelore ( $F_{IS} = 0.036$ ,  $P < 0.0001$ ), Hereford ( $F_{IS} = -0.168$ ,  $P = 0.0018$ ), and Aberdeen Angus ( $F_{IS} = -0.024$ ,  $P < 0.005$ ). However, it is important to note that samples obtained from these breeds cannot be considered as a real population because they came from different herds.

**Table 3.** Summary statistics of genetic variability and Hardy–Weinberg disequilibrium. Total ( $N_a$ ) and mean ( $\bar{N}_a$ ) number of alleles, observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosities, and  $F_{IS}$  index and number of loci deviating from HWE

Breed code	$N_a$	$\bar{N}_a^a$	$H_o$	$H_e$	$F_{IS}$ ( $P$ value)	No. HWE deviations
AC	7.3	6.7	0.739	0.744	0.007 (0.1799)	0
SaC	7.7	7	0.851	0.781	-0.091 (0.3517)	1
ChC	7.8	7.5	0.806	0.771	-0.047 (0.2646)	0
YaC	6.6	6.2	0.724	0.722	-0.003 (0.0697)	0
AA	7.1	5.7	0.715	0.699	-0.024 (0.0018)*	2
He	6.4	5.8	0.827	0.710	-0.168 (0.0000)**	4
Ho	7.7	7	0.747	0.786	0.050 (0.0210)	2
R	6.2	6.2	0.771	0.745	-0.036 (0.0081)	2
Ne	7.0	6.6	0.615	0.637	0.036 (0.0000)**	6
BZ	7.7	6.8	0.700	0.676	-0.036 (0.1741)	1

<sup>a</sup> Average of 1000 random samples of 20 individuals per breed.

\* Statistical significance  $P \leq 0.006$ . Adjusted by the Bonferroni correction.

\*\* Highly significant.  $P \leq 0.0001$ .

An exact test for genic differentiation among populations was performed for the 45 pairwise population comparisons. Only 2 Creole breeds (Argentine and Bolivian Chaqueño) showed genetic similarity using this test ( $P = 0.0067$ , adjusted by the Bonferroni correction). In concordance, levels of apparent breed differentiation were considerable with  $F_{ST}$  values, indicating that 8.8% of the total genetic variation corresponded to differences among populations whereas the 91.2% was explained by differences among individuals. The average value obtained from  $R_{ST}$  statistic (14.4%) was almost 2-fold higher than the  $F_{ST}$  index. Moreover, the differences displayed among loci using  $R_{ST}$  were more accentuated than the ones showed by the  $F_{ST}$ , ranging from 2.3% (*MGTG7*) to 41.1% (*ETH225*).

The AMOVA permitted the partitioning of the genetic variability between different groups of breeds based on their historical origin. Levels of apparent breed differentiation were considerable when the breeds were grouped according to their taurine or zebu phenotypes. These analyses indicated that 7.8% of the total genetic variation corresponded to differences among the major types of cattle. When taurine breeds were divided in 2 groups according to their American (Creole cattle) or European origin, AMOVA showed that among groups variance accounts for only 1% of the total genetic variation, contrasting with the 6% of variance among populations within taurine groups.

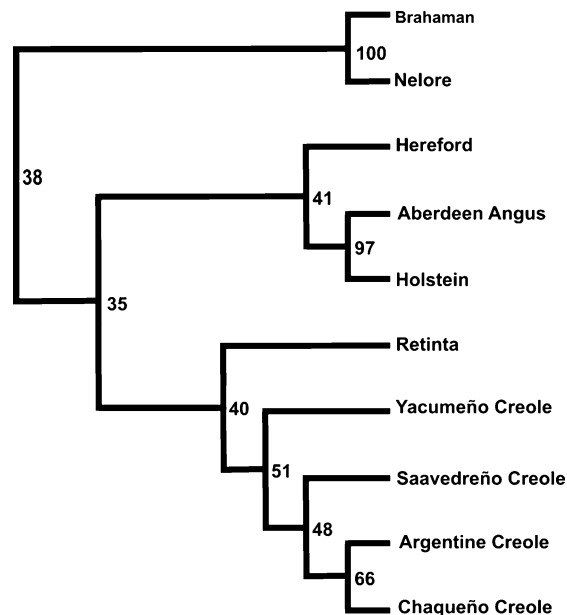
#### Genetic Distances and Relationships between Populations

Allele frequencies were used to generate the  $D_A$ ,  $D_C$ , and  $D_{SW}$  genetic distances for each pair of 10 cattle populations. Distances matrices were used in order to build phylogenetic trees using UPGMA and the NJ algorithms. Depending on whether the genetic distance or the clustering algorithm was used, different topologies and bootstrap values were obtained. Only the trees constructed with UPGMA using the classical genetic distance  $D_A$  and  $D_C$  exhibit similar topology consistent with historical and geographical data. As both trees retained the same structure, only the UPGMA tree constructed from a matrix of  $D_A$  distances is shown in Figure 1.

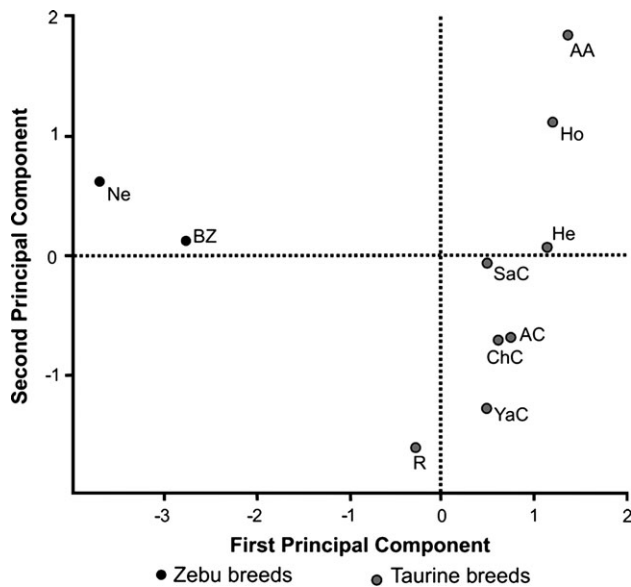
This tree illustrates the main divergence observed between the taurine and zebu clades. Taurine populations split into 2 clusters corresponding either to European breeds (Aberdeen Angus, Hereford, and Holstein) or to the other cluster that included the Creole breeds together with the Spanish Retinta breed. However, bootstrap values for these nodes are not significant.

#### Principal Components Analysis

The PCA results coincided with the topology of the phylogenetic trees constructed with UPGMA using the classical genetic distance  $D_A$  and  $D_C$ . Figure 2 illustrates the first and the second PCs for the 9 microsatellite allele frequency



**Figure 1.** UPGMA tree of genetic relationships among 10 cattle breeds using  $D_A$  genetics distances. The numbers on nodes are percentage bootstrap values in 1000 replications.



**Figure 2.** PCA of transformed allele frequencies from 9 microsatellites typed in 10 cattle breeds.

distributions in 10 cattle breeds. The first principal component (PC) accounts for 40% of the total variance and clearly distinguishes the taurine and the zebu groups. Among taurine breeds, the Creole populations and Retinta occupied an intermediate position between the zebu groups and the rest of the taurine breeds. This may be taken as evidence of different degrees of gene introgression (see Discussion). The second PC summarizes 15% of the variation and shows a differentiation pattern with the European breed on one side and the Creole plus Retinta on the other. The third PC, which accounts for 12% of the variance, was not represented in the figure because it only distinguished Hereford from the other breeds.

#### Analysis of Zebu Genetic Admixture

Table 4 shows the percentage of the 5 zebu-specific alleles at 3 diagnostic loci in 4 Creole cattle breeds, zebu breeds, and European taurine populations. It also shows the zebu admixture percentage calculated using the 5 zebu-specific alleles. The Creole cattle displayed a moderate degree of zebu admix-

ture, which ranged from 4% in the Argentine Creole to 10% in the Yacumeño. In concordance with these results, the mY statistic showed similar levels of zebu introgression, ranging from 2.6% in the Argentine Creole to around 10% in Bolivian Creole breeds (mY = 9.5%, 9.4%, and 10.3% for the Chaqueño, Saavedreño, and Yacumeño Creoles, respectively).

## Discussion

### Diversity Analysis

Our results showed that the Creole cattle populations display a relatively high level of genetic variation as estimated by allelic diversity and heterozygosity. On the other hand, the European cattle breeds and particularly those from Britain displayed reduced levels of genetic diversity. Despite the zebu breeds exhibiting an intermediate value of allelic diversity, they presented the lowest gene diversity.

Given that the higher degree of genetic diversity is located in the center of domestication and the level of variation decreases when the geographical distance from the center is increased, it is expected that Creole cattle would exhibit low levels of genetic diversity because introduction of bovine into America was one of the last steps of cattle dispersion over the world. Furthermore, the small size of the Creole founding groups and the drastic reduction and subdivision suffered by the population during the last century should have produced a further reduction in diversity. However, analysis of phenotypic and microsatellite data shows that Creole cattle have high degree of genetic diversity.

This apparent contradiction would be explained by the low levels of artificial selection suffered by Creole cattle as well as by demographic factors. mtDNA studies showed that Creole germoplasm includes European and African genes (Magee and others 2002; Miretti and others 2002, 2004; Carvajal-Carmona and others 2003; Mirol and others 2003; Lirón and others forthcoming), and this multiplicity of geographical origins could be responsible for the actual high levels of diversity. Adding a further and recent admixture of Creole breeds with commercial breeds such as zebu would also increase diversity even more. This last point is discussed in detail below.

### Genetic Relationship among Cattle Breeds

Mitochondrial DNA-, allozyme-, and microsatellite-based studies have demonstrated that the main subdivision of cattle

**Table 4.** Zebu-diagnostic alleles and zebu admixture proportions in Creole cattle breeds

	Zebu	Taurus	AC	ChC	SaC	YaC
<i>ETH225</i>	0.72 <sup>a</sup> (100%) <sup>b</sup>	0 (0%)	0 (0%)	0.03 (4.4%)	0.11 (15.5%)	0.08 (11.5%)
<i>TGLA227.79</i>	0.66 (100%)	0 (0%)	0.02 (3.2%)	0.06 (9.1%)	0.03 (4.4%)	0.03 (4.3%)
<i>TGLA122.136</i>	0.38 (100%)	0 (0%)	0.04 (9.8%)	0 (0%)	0.01 (3.7%)	0.03 (8.7%)
Average	0 (100%)	0 (0%)	0.02 (4.3%)	0.03 (4.5%)	0.05 (7.9%)	0.05 (8.2%)

<sup>a</sup> Gene frequency of zebu-specific alleles.

<sup>b</sup> Admixture proportion calculated by dividing the frequencies of each diagnostic allele by the average frequency calculated for the indicine breeds.

into *Bos taurus* (humpless) and *B. indicus* (humped) corresponded to a deep bifurcation (200 000–1 000 000 years ago), which predates archaeological estimates of cattle domestication (~12 000 year ago) (Manwell and Baker 1980; Lotfus and others 1994; Bradley and others 1996; Machugh and others 1997; Troy and others 2001). These results indicate independent domestication events for the 2 major taxa of cattle. By contrast, modern breeds were founded very recently, no more than 2 centuries ago.

Herein, we included 10 breeds that are representative of the 2 major bovine types. Assuming that we are working with a neutral polymorphism, 3 forces remain that can be used to explain the genetic diversity observed: mutation, genetic drift, and migration. Mutation is important only when studying long periods of time. The large divergence between the taurine and zebu genomes was reflected in the presence of some zebu-diagnostic alleles (Machugh and others 1997), which were present at high frequency in zebu populations but absent or present at low frequency in taurine populations. Furthermore, these alleles displayed dissimilar distribution in zebu and taurine breeds. These results are in agreement with previously reported data (Machugh and others 1997; Freeman and others 2004). Interestingly, these zebu-diagnostic alleles were present at low frequency in almost all the Creole cattle breeds.

Estimation of population subdivision evidenced that average proportion of genetic differentiation among breeds ( $F_{ST} = 9\%$ ) was equivalent to that reported for other cattle breeds (Machugh and others 1998; Kantanen and others 2000; Cañon and others 2001; Maudet and others 2002; Mateus and others 2004). Correspondingly with the differential allelic distribution displayed for some loci in zebu and taurine populations, the estimated  $R_{ST}$  were more than 2 times  $F_{ST}$ , suggesting that cattle differ in distributions of both allele frequency and allele size. In addition, the AMOVA indicated that 8% of the total genetic variation corresponded to differences between the 2 major types of cattle contrasting with the 1% observed between Creole cattle and European taurine breeds.

The considerable proportion of variance accounted for the differences among zebu and taurine groups was also clearly reflected in all constructed phylogenetic trees, where Nelore and Brahman breeds always cluster together, with robust bootstrap values. Consistent with these results, the first PC, comprising 40% of the total variance, clearly distinguishes these groups. The trees constructed with UPGMA, using the classical genetic distance  $D_A$  and  $D_C$ , although with low bootstrap values, suggested the divergence between European breeds (Aberdeen Angus, Hereford and Holstein) and the Iberia-America breeds (Creole cattle and Retinta). Furthermore, these data were supported by the second PC, which summarizes 15% of the variation, and showed a differentiation pattern with the European breed on one side and the Creole plus Retinta on the other. However, this apparent topology of taurine breeds is not particularly robust. This instability is reflected in low bootstrap values and branching orders, which vary depending on distance and constructed methods used. This weak structure was also reported in previous studies of European cattle phylogeny (Machugh and

others 1994, 1998; Moazami-Goudarzi and others 1997; Martín-Burriel and others 1999; Cañon and others 2001).

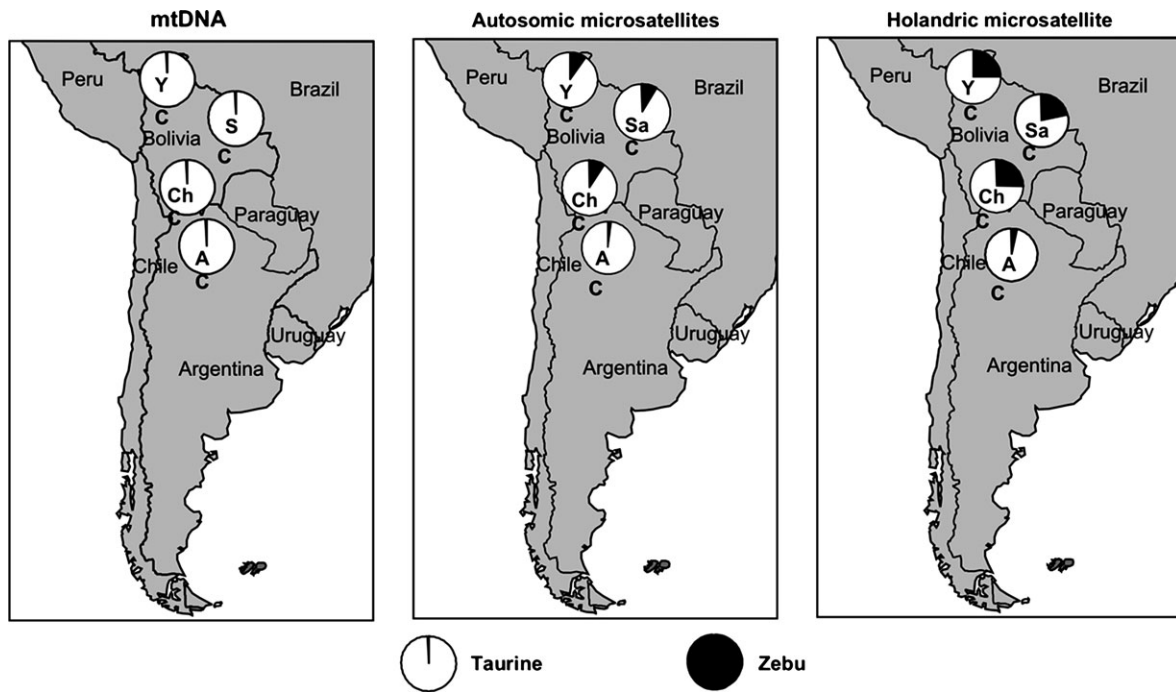
The lack of consistent topology of the taurine phylogenetic trees is commonly explained by several factors. First, the construction of trees using admixture population, such as Creole cattle, contradicts the principles of phylogeny reconstruction (Felsenstein 1982). The second issue that should be considered is the number of markers analyzed. Based on theoretical studies, Takezaki and Nei (1996) have shown that one of the important factors for analyzing the correct phylogenetic position of populations in a genetic study is the number of loci used. The number of markers genotyped here are probably insufficient for complete resolution. Nevertheless, various taurine phylogenetic analyses performed with a higher number of microsatellites also evidenced a weak topology in taurine breeds (Moazami-Goudarzi and others 1997; Martín-Burriel and others 1999; Cañon and others 2001).

As discussed by Moazami-Goudarzi and Laloe (2002), another way of understanding the lack of structure is assuming that populations have differentiated according to a radiative scheme of divergence. According to this model, it is expected that genetic distances between breeds would be equivalent, and any casual differences among them might be due to random genetic drift. Furthermore, this scheme anticipates discrepancies among topology exhibited by each marker. Increasing the number of loci does not necessarily enhance the reliability of the phylogeny. In contrast to the large divergence between the taurine and zebu cattle, the European breeds and American Creole, which originated around 4 centuries ago, could be considered to be closely related, and the main factor describing their genetic variability is random drift. Takezaki and Nei (1996) have demonstrated using computer simulations that Nei's distances and  $D_C$  are the most efficient means of obtaining the correct tree topology when within-species populations are being considered. Accordingly, our tree constructed using the classical  $D_A$  and  $D_C$  genetic distances recovered the most plausible topology consistent with historical and geographical data. A shallow inspection of the trees constructed with the NJ versus UPGMA algorithms revealed an apparently larger dependence of zebu admixture from UPGMA with respect to the correct tree topology than NJ method.

### Pattern of Genetic Admixture in Latin American Creole Cattle

Cattle were introduced to the American continent by the Spanish conquerors since 1493. Within a few years, these cattle had spread all over South America and increased their population size to several million. During the 19th and 20th centuries, as a result of the introduction of highly selected European and Indian commercial breeds, populations of Creole cattle suffered a severe reduction in number and their distribution became restricted to peripheral regions.

Here, we compared the patterns of genetic admixture displayed for mtDNA sequence, Y chromosome, and autosomal markers in the same populations of Argentine and Bolivian



**Figure 3.** The zebu introgression of the 3 different genomic components into Argentine and Bolivian Creole cattle.

Creole cattle. Mirol and others (2003) evidenced that all studied Creole cattle from Argentina and Bolivia possessed both taurine mtDNA haplogroups. In contrast to this, analysis of Y-specific INRA124 microsatellite and the Y chromosome morphology in the same Creole populations showed a considerable incidence of zebu Introgression (Giovambattista and others 2000).

It is not a coincidence that the autosomal genetic composition of Creole cattle populations presents an intermediate picture between the 2 uniparental extremes and represents the overall introgression of the zebu genome most accurately. Figure 3 shows a summary diagram of zebu genetic introgression for the 3 systems. Similar patterns of gene flow were also reported in indigenous African breeds (Machugh and others 1997; Freeman and others 2004). Kikkawa and others (2003) reported an analogous dynamic of genetic admixture in native breeds from Asia, although they described a taurine genetic introgression into the zebu extant populations. The results obtained in this work, as well as those reported for Africa and Asia native breeds, illustrate a general model for cattle genetic admixture, which is characterized by a male-mediated introgression.

The values of admixture observed in each region were not homogeneous. A clear pattern of zebu introgression in South America has been previously reported (Giovambattista and others 2000), with zebu allele frequencies decreasing from east to west and from north to south. In concordance, microsatellite data showed a higher zebu influence in Bolivian autosomal genomes than in Argentine Creole. In order to improve native breeds in tropical regions, male zebu cattle were extensively introduced into Brazil during the 18th

and 19th centuries. During the second half of the 20th century, Brazil was a center for zebu distribution in South America. Therefore, this gradient is compatible with historical and environmental data.

Although the 3 types of genetic markers used are informative, they account for different events of the Creole cattle history. On one hand, mtDNA marker is extremely informative to predict the conformation of the gene pool present in the Creole foundation group because dams were only introduced to America during the first decades of European colonization (Magee and others 2002; Miretti and others 2002, 2004; Carvajal-Carmona and others 2003; Mirol and others 2003; Lirón and others, in preparation). However, mtDNA is a poor predictor for estimating the overall genomic diversity and the level of admixture in cattle. On the other hand, despite that Y chromosome markers are much less variable within species than most other genomic sequences, these paternally inherited markers have a powerful resolution to detect contemporaneous gene flow in cattle because of the predominant asymmetric mating between Creole dams and foreign sires occurring during the last century to improve livestock. Finally, the autosomal microsatellite markers illustrate an intermediate admixture scenario and predict more accurately the overall genomic diversity because they have a biparental inheritance.

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## References

- Beja-Perira A, Alexandrino P, Bessa I, Carretero Y, Dunner S, Ferrand N, Jordana J, Laloe D, Moazami-Goudarzi K, Sanchez A, Cañon J. 2003. Genetic characterization of southwestern European bovine breeds: a historical and geographical reassessment with a set of 16 microsatellites. *J Hered* 94:243–50.
- Bertorelle G, Excoffier L. 1998. Inferring admixture proportions from molecular data. *Mol Biol Evol* 15:1298–311.
- Bishop MD, Kappes SM, Keele JW, Stone RT, Sunden SL, Hawkins GA, Solinas Toldo S, Fries R, Grosz MD, Yoo J, Beattie JW. 1994. A genetic linkage map for cattle. *Genetics* 136:619–39.
- Bradley DG, Machugh E, Cunningham P, Loftus RT. 1996. Mitochondrial diversity and the origins of African and European cattle. *Proc Natl Acad Sci USA* 93:5131–5.
- Cañon J, Alexandrino P, Bessa I, Carleos C, Carretero Y, Dunner S, Ferran N, Garcia D, Jordana J, Laloe D, Pereira A, Sanchez A, Moazami-Goudarzi K. 2001. Genetic diversity measures of local European beef cattle breeds for conservation purposes. *Genet Sel Evol* 33:311–32.
- Carvajal-Carmona LG, Bermudez N, Olivera-Angel M, Estrada L, Ossa J, Bedoya G, Ruiz-Linares A. 2003. Abundant mtDNA diversity and ancestral admixture in Colombian criollo cattle (*Bos taurus*). *Genetics* 165:1457–63.
- Cavalli-Sforza LL, Edwards AW. 1967. Phylogenetic analysis: models and estimation procedure. *Am J Hum Genet* 19:233–57.
- Cavalli-Sforza LL, Menozzi P, Piazza A. 1994. The history and geography of human genes. Princeton, NJ: Princeton University Press.
- Felsenstein J. 1982. How can we infer geography and history from gene frequencies? *J Theor Biol* 96:9–20.
- Freeman AR, Meghen CM, MacHugh DE, Loftus RT, Achukwi MD, Bado A, Sauveroché B, Bradley DC. 2004. Admixture and diversity in West African cattle populations. *Mol Ecol* 13:3477–87.
- Georges M, Massey J. 1992. Polymorphic DNA markers in bovidae (World Intellectual Property Org., Geneva). N 92/13102. Geneva, Switzerland: WO Publishers.
- Georges M, Nielsen D, Mackinnon M, Mishra A, Okimoto R, Pasquino AT, Sargeant LS, Sorensen A, Steele MR, Zhao X. 1995. Mapping quantitative traits loci controlling milk production in dairy cattle by exploiting progeny testing. *Genetics* 139:907–20.
- Giovambattista G, Ripoli MV, De Luca JC, Mirol PM, Lirón JP, Dulout FN. 2000. Male-mediated introgression of *Bos indicus* genes into Argentine and Bolivian Creole cattle breeds. *Anim Genet* 3:302–5.
- Goldstein DB, Schöterer JM, editors. 1999. Microsatellites, evolution and application. Oxford: Oxford University Press.
- Gou SW, Thompson EA. 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics* 48:361–72.
- Goudet J. 1995. FSTAT (version 1.2): a computer program to calculate F-statistics. *J Hered* 86:485–6.
- Hammer Ø, Harper DAT, Ryan PD. 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontol Electronica* 4:9. Available from: [http://palaeo-electronica.org/2001\\_1/past/issue1\\_01.htm](http://palaeo-electronica.org/2001_1/past/issue1_01.htm).
- Hanotte O, Bradley DG, Ochieng JW, Berjee Y, Hill EW, Rege EO. 2002. African pastoralism: genetic imprints of origins and migrations. *Science* 296:336–9.
- Kantanen J, Olsaker I, Holm LE, Lien S, Vilkkij J, Brusgaard K, Eythorsdottir E, Danell B, Adalsteinsson S. 2000. Genetic diversity and population structure of 20 North European cattle breeds. *J Hered* 91:446–57.
- Kikkawa Y, Takada T, Sutopo Nomura K, Namikawa T, Yonekawa H, Amano T. 2003. Phylogenies using mtDNA and SRY provide evidence for male-mediated introgression in Asian domestic cattle. *Anim Genet* 34:96–101.
- Langella O. 1999. POPULATIONS, version 1.2.28. Population genetic software. Available from: <http://www.pge.cnrs-gif.fr>.
- Lirón JP, Bravi CM, Mirol PM, Peral-García P, Giovambattista G. 2006. African matrilineages in American Creole cattle: evidence of two independent continental sources. *Anim Genet*. Forthcoming.
- Loftus RT, Ertugrul O, Harba AH, El-Barody MAA, MacHugh DE, Park SDE, Bradley DG. 1999. A microsatellite survey of cattle from a centre of origin: the Near East. *Mol Ecol* 8:2015–22.
- Loftus RT, MacHugh DE, Bradley DG, Sharp PM, Cunningham EP. 1994. Evidence for two independent domestications of cattle. *Proc Natl Acad Sci USA* 91:2757–61.
- Machugh DE, Loftus RT, Bradley DG, Sharp PM, Cunningham P. 1994. Microsatellite DNA variation within and among European cattle breeds. *Proc R Soc Lond B Biol Sci* 256:25–31.
- Machugh DE, Loftus RT, Cunningham P, Bradley DG. 1998. Genetic structure of seven European cattle breeds assessed using 20 microsatellite markers. *Anim Genet* 29:1–8.
- Machugh DE, Shriver MD, Loftus RT, Cunningham P, Bradley DG. 1997. Microsatellite DNA variation and the evolution, domestication and phylogeography of taurine and zebu cattle (*Bos taurus* and *Bos indicus*). *Genetics* 146:1071–86.
- Magee DA, Meghen C, Harrison S, Troy CS, Cymbron T, Gaillard C, Morrow A, Maillard JC, Bradley DG. 2002. A partial African ancestry for the Creole cattle populations of the Caribbean. *J Hered* 93:429–32.
- Manwell C, Baker MA. 1980. Chemical classification of cattle. 2. Phylogenetic tree and specific status of the zebu. *Anim Blood Groups Biochem Genet* 11:151–62.
- Martín-Burriel I, García-Muro E, Zaragoza P. 1999. Genetic diversity analysis of six Spanish native cattle breeds using microsatellites. *Anim Genet* 30:177–82.
- Mateus JC, Penedo MC, Alves VC, Ramos M, Rangel-Figueiredo T. 2004. Genetic diversity and differentiation in Portuguese cattle breeds using microsatellites. *Anim Genet* 35:106–13.
- Maudet C, Luikart G, Taberlet P. 2002. Genetic diversity and assignment tests among seven French cattle breeds based on microsatellite DNA analysis. *J Anim Sci* 80: 942–50.
- Miretti MM, Dunner S, Naves M, Contel EP, Ferro JA. 2004. Predominant African-derived mtDNA in Caribbean and Brazilian Creole cattle is also found in Spanish cattle (*Bos taurus*). *J Hered* 95:450–3.
- Miretti MM, Pereira HA, Poli MA, Contel EPB, Ferro JA. 2002. African-derived mitochondria in South American native cattle breeds (*Bos taurus*): evidence of a new taurine mitochondrial lineage. *J Hered* 93:323–30.
- Mirol PM, Giovambattista G, Liron JP, Dulout FN. 2003. African and European mitochondrial haplotypes in South American Creole cattle. *Heredity* 91:248–54.
- Moazami-Goudarzi K, Laloe D. 2002. Is a multivariate consensus representation of genetics relationships among populations always meaningful? *Genetics* 162:473–84.
- Moazami-Goudarzi K, Laloe D, Furet JP, Grousclaude F. 1997. Analysis of genetic relationships between 10 cattle breeds with 17 microsatellites. *Anim Genet* 28:338–45.
- Moore SS, Byrne K. 1993. Dinucleotide polymorphism at the bovine histamine H1 receptor locus. *Anim Genet* 24:150.



- Nei M. 1972. Genetic distance between populations. *Am Nat* 106: 283–92.
- Nei M. 1987. *Molecular evolutionary genetics*: New York: Columbia University Press 512 p.
- Page RDM. 1996. TREEVIEW: an application to display phylogenetic trees on personal computers. *Comput Appl Biosci* 12:357–8.
- Primo AT. 1992. El ganado bovino ibérico en las Américas: Quinientos años después. *Arch Zootec* 41:421–32.
- Raymond M, Rousset F. 1995a. GENEPOP (version 1.2): a population genetics software for exact test and ecumenicism. *J Hered* 86: 248–9.
- Raymond M, Rousset F. 1995b. An exact test for population differentiation. *Evolution* 49:1280–3.
- Saitou N, Nei M. 1987. The neighbour-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4: 406–25.
- Schneider S, Roessli D, Excoffier L. 2000. Arlequin version 2.000: a software for population genetics data analysis. Geneva, Switzerland: Genetics and Biometry Laboratory, University of Geneva.
- Shriver MD, Jin L, Boerwinkle E, Deka R, Ferrell RE, Chakraborty R. 1995. A novel measure of genetic distance for highly polymorphic tandem repeat loci. *Mol Biol Evol* 12:914–20.
- Slatkin M. 1995. A measure of population subdivision based on microsatellite allele frequencies. *Genetics* 13:983–93.
- Sneath PHA, Sokal RR. 1973. *Numerical taxonomy*. San Francisco, CA: W. H. Freeman.
- Steffen P, Eggen A, Dietz AB, Womack JE, Stranzinger G, Fries R. 1993. Isolation and mapping of polymorphic microsatellites in cattle. *Anim Genet* 24:1221–4.
- Takesaki N, Nei M. 1996. Genetic distance and reconstruction of phylogenetic trees from microsatellite DNA. *Genetics* 144:389–99.
- Troy C, Machugh DE, Bailey JF, Magee DA, Loftus RT, Cunningham P, Chamberlain AT, Sykes BC, Bradley DG. 2001. Genetic evidence of Near-Eastern origins of European cattle. *Nature* 410:1088–91.
- Vaiman D, Mercier D, Moazami-Goudarzi K. 1994. A set of 99 cattle microsatellites: characterization, synteny mapping, and polymorphism. *Mamm Genome* 5:288–97.
- Weir BS. 1996. *Genetic data analysis II*. Sunderland, MA: Sinauer Associates.
- Weir BS, Cockerham CC. 1984. Estimating F-statistics for the analysis of populations structure. *Evolution* 38:1358–70.
- Wilkins JV, Martinez L, Rojas F. 1982. *El Ganado Vacuno Criollo*. Documento 31. Santa Cruz, Bolivia: CIAT.

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