

## Genetic diversity and population structure of Brazilian native bovine breeds

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Abstract – Conservation and improvement strategies should be based on the association between genetic and phenotypic characteristics. The objective of this work was to characterize five native Brazilian cattle breeds (Caracu, Crioulo Lageano, Curraleiro, National Polled and Pantaneiro) and two commercial breeds (Holstein and Nellore) using RAPD technique to estimate genetic distances and variability between and within breeds. Genetic relationships were investigated using 22 primers which generated 122 polymorphic bands. Analysis of molecular variance indicated that most of the genetic variation lay among individuals within populations. The genetic variabilities between pairs of breeds were statistically significant. The smallest genetic divergence was between Crioulo Lageano and Curraleiro. The National Polled, although historically considered to be of *Bos taurus aquitanicus* origin, similar to the Caracu, was grouped together with the other breeds of *Bos taurus ibericus* origin. Generally, the individual breeds formed distinct clusters except the National Polled. The RAPD technique was capable to distinguish genetically between the breeds studied; the Caracu, Crioulo Lageano, Curraleiro and Pantaneiro may be considered distinct genetic entities thereby proving the uniqueness of the populations; the National Polled has not been able to re-establish itself after its decline in the 1950s, thereby losing its genetic identity.

Index terms: animal conservation, cattle, genetic characterization, RAPD.

### Diversidade genética e estrutura de população de bovinos nativos

Resumo – Estratégias para conservação e melhoramento animal devem ser baseadas na associação de características fenotípicas e genéticas. O objetivo deste trabalho foi caracterizar cinco raças nativas brasileiras (Caracu, Crioulo Lageano, Curraleiro, Mocho Nacional e Pantaneiro) e duas comerciais (Holandês e Nelore) pela técnica de RAPD para estimar a distância genética e a variabilidade genética entre e dentro das raças. As relações genéticas foram investigadas utilizando-se 22 primers, que geraram 122 bandas polimórficas. A análise de variância molecular revelou que a maior parte da variabilidade genética total ocorreu em virtude das diferenças de indivíduos dentro das populações. Na comparação da variabilidade genética entre os pares de raças, observou-se que todos os valores obtidos foram estatisticamente significativos. A menor divergência genética foi observada entre Crioulo Lageano e Curraleiro. A raça Mocho Nacional, apesar de historicamente ser considerada de origem *Bos taurus aquitanicus*, mesma origem da raça Caracu, agrupou-se com as demais raças nativas consideradas de origem *Bos taurus ibericus*. De modo geral, as raças estudadas se agruparam em clusters distintos, com exceção da Mocho Nacional. A técnica de RAPD foi capaz de distinguir geneticamente as raças estudadas; Caracu, Crioulo Lageano, Curraleiro e Pantaneiro podem ser consideradas entidades genéticas distintas, comprovando assim, a unicidade de suas populações; a Mocho Nacional não conseguiu se restabelecer, após seu declínio na década de 50, perdendo, sua identidade genética.

Termos para indexação: conservação animal, bovino, caracterização genética, RAPD.

### Introduction

Contrary to phenotypic characterization, genetic characterization is free of environmental influences, which means greater accuracy in the data generated. This is fundamental when decisions have to be made in

conservation programs or on the utilization of animal genetic resources. Thus, techniques used in the analysis of genetic variability are essential ingredients for conservation programs and in making rational breeding decisions (Egito et al., 1999).

In Brazil, few studies can be found on the characterization of native/naturalized cattle breeds. A study of these breeds could help in rational development of future breeding programs, as well as in the preservation and conservation of these germplasm. Gains in economic efficiency, which could result from the use of this genetic material, may be greater than the cost of conservation of these animals. Many breeds, which were once economically important, are now rare but may have traits with economic potential (Hall & Bradley, 1995).

Brazilian bovine breeds were developed from breeds brought by the colonizers soon after the discovery of Brazil. Since then those breeds underwent natural selection in specific environments, with adaptation of traits suited to those conditions (Mariane & Cavalcante, 2000).

Portuguese purebred cattle belonged to one of three main bloodlines: Batavian (*Bos taurus batavicus*), represented by the Barrosa and Turino breeds; Aquitanian (*Bos taurus aquitanicus*), represented by the Galega, Arouquesa, Alentejana, Mertolenga, Algarvia, and Minhota breeds; and Iberian (*Bos taurus ibericus*), represented by the Mirandesa and Brava breeds.

According to Athanassof (1957), the Brazilian cattle breeds that belong to the *Bos taurus ibericus* are small to medium sized; those of the *Bos taurus aquitanicus* are above average size, while those that belong to the *Bos taurus batavicus* are, generally, crossbreeds with dual purpose breeds and present size below average. Based on historic information, Primo (1993) stated that Curraleiro, Crioulo Lageano and Pantaneiro breeds have their origin in breeds from the *Bos taurus ibericus* type, while the Caracu and National Polled breeds belong to the *Bos taurus aquitanicus* type.

Because of the importation, at the beginning of the 20<sup>th</sup> century, of some exotic breeds, especially zebu, native breeds were gradually substituted. Thus, today the greater part of the native breeds are threatened with extinction, making the study of the genetic variability of these breeds of prime importance (Egito et al., 1999). Although the exotic breeds are considered more productive, they lack adaptive, disease and parasite resistance traits found in the native breeds.

Modern strategies for bovine genetic improvement and conservation require sets of molecular markers that characterize different populations (Hetzl & Drinkwater, 1992). An efficient and low-cost technique for detecting DNA polymorphisms uses PCR and primers of arbitrary sequence to amplify genomic DNA (Williams et al.,

1990), in the Random Amplified Polymorphic DNA (RAPD) assay.

This technique is widely used to help in the choice of strategies for preservation of species and animals threatened with extinction (Apostolidis et al., 2001).

However, only few studies have been conducted with this technique on bovine germplasm, focusing on subspecies or breed identification and identification of chromosome markers (Teale et al., 1995).

The objective of this work was to characterize five native Brazilian cattle breeds and two commercial breeds using RAPD technique to estimate genetic distances and variability between and within breeds.

## Material and Methods

### Animals

Five naturalized Brazilian cattle breeds were studied. These included the Pantaneiro (PAN) from the Pantanal region, South Western Brazil; Crioulo Lageano (CL) from Santa Catarina State, Southern Brazil; Curraleiro (CU) from the Northeast and Central West States; National Polled (MN) from Minas Gerais State, Southeastern Brazil; and Caracu (CA) from Central West and Southern Brazil. Two established and well defined breeds in Brazil were included: the Holstein-Friesian (HOL) (*Bos taurus*) as a control group, and the Nellore (NEL) (*Bos indicus*) as an outgroup.

To obtain a representative sample size for each native breed, depending on its occurrence, animals from different herds and regions of Brazil were collected (Table 1). The total sample size was 335 individuals, which included 48 animals of each breed (the number recommended by FAO for characterization studies), except for the National Polled for which only 47 samples were available.

### DNA extraction and amplification

Genomic DNA was obtained from blood samples or frozen semen samples provided by private breeders, Conservation Nuclei and Animal Germplasm Bank of Embrapa Recursos Genéticos e Biotecnologia. Semen DNA extraction was based on a phenol protocol described by Egito (1995) and blood DNA extraction was based on Miller et al. (1988) with slight modifications (non-organic protocol).

A total of 120 arbitrary primers (Operon Technologies Inc., Alameda, Calif.: A, B, E, J, K and AB) was screened using one sample of each breed. A total of 22 primers was selected and used to amplify DNA from all

individuals (OPA 2, OPA 4, OPA 8, OPA 9, OPA 11, OPA 12, OPA 15, OPA 16, OPA 17, OPA19, OPB 1, OPB 8, OPB 12, OPE 12, OPJ 7, OPJ 8, OPJ 12, OPJ 14, OPJ 17, OPAB 1, OPAB 4 and OPAB 5).

Amplification reactions were performed in 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 2.5 mM MgCl<sub>2</sub>, 200 mM of each dNTP, 0.4 μM primer, 9 ng template DNA, 8% of BSA (2.5 mg/mL) and 1.5 UI Taq DNA polymerase in a final volume of 13 μL. DNA amplification was performed in a thermal cycler programmed as follows: 94°C for 5 min followed by 40 cycles consisting of 94°C for 1 min, 36°C for 1 min, 72°C for 2 min. A final extension at 72°C was carried out for 7 min followed by cooling at 4°C. PCR products were separated by electrophoresis in 1.4% agarose gels in 1 x TBE, and were stained with ethidium bromide (0.6 μg/mL) and observed under UV light.

#### Data scoring and analysis

Although a large number of fragments were generated from each primer, only clearly distinguishable and reproducible polymorphic bands were selected for analysis. Sex specific or individual bands were excluded. Fragment data were entered in a computer file as a binary matrix: "0" coded for absence and "1" for presence of a band.

**Table 1.** Estimated population size of Brazilian naturalized cattle breeds, herds collected for genetic characterization and number of analyzed animals, by breed and herd.

Breed	Estimated pop. size	Origin of the animals sampled for genetic characterization (number of animals analyzed)
Caracu	30,000	Chiqueirão Farm - Poços de Caldas, MG (17) São Miguel Farm - Conceição da Aparecida, MG (5) São Francisco Farm - Bambuí, MG (8) Belém Farm - Passos, MG (6) São Bento Farm - São Luiz dos Montes Belos, GO (5) Recreio Farm - São Sebastião da Gra, SP (5) Douradinha Farm - Uberlândia, MG (2)
Crioulo Lageano	100-1,000	Canoas Farm, Crioulo Lageano Nuclei - Lages, SC (48)
Curraleiro	500-1,000	Curraleiro Nucleus - Embrapa Meio Norte PI (12) Salto da Divisa, MG (7) Lagoa Branca Farm - Mara Rosa, GO (9) <sup>(1)</sup> Santa Mônica Farm - Mara Rosa, GO (20) <sup>(2)</sup>
National Polled	400	Brazilian Animal Gene Bank (Embrapa Genetic Resources and Biotechnology) - Brasília, DF (13) Três Barras Farm - Uberaba, MG (34)
Pantaneiro	8,300	Nhumirim Experimental Farm - Embrapa Pantanal, Corumbá, MS (48)

<sup>(1)</sup>Animals originally from the State of Maranhão. <sup>(2)</sup>Animals originally from the State of Tocantins.

Data analyses were conducted using NTSYS-pc, version 2.0. Similarities between individuals were estimated using Jaccard coefficients of similarity (Sneath & Sokal, 1973) and the resulting pairwise similarities were expressed as distance matrices.

The genetic distances between breeds were calculated using the POPGENE program (Population Genetic Analysis) version 1.31 (Yeh et al., 1999). This program establishes standardized genetic distance matrices (Nei, 1972) and matrices of genetic distances corrected for small samples (Nei, 1978). The method proposed by Nei (1972) is one of the most used to obtain genetic distances between populations (Lynch & Milligan, 1994) and its use is recommended with RAPD data (Apostolidis et al., 2001). All cluster analyses done in this work were conducted using UPGMA and the resulting clusters were expressed as dendrograms. The bootstrap analysis was conducted using the TFPGA program (Tools for Population Genetics Analyses, version 1.3) (Miller, 1997). The POPGENE program also generated gene diversity indices (h) for each breed based on Nei (1973). According to Weir (1996) this is the most adequate method to study unique populations.

The analysis of molecular variance (AMOVA) (Excoffier et al., 1992) was used to separate the variance between and within populations using the ARLEQUIN program (Schneider et al., 2000). Although this test was not originally designed for analysis of RAPD data, it has been used in other similar studies (Tansley & Brown, 2000).

## Results and Discussion

### Genetic structure of the breeds

When all breeds were used, the greater part of total genetic variability (70.04%) was due to differences between individuals within populations while the remaining 29.96% was due to differences between breeds (Table 2). When only the native breeds were used, the within breed variability increased to 83.73%. Therefore, the genetic variability between the native breeds was much lower than between all breeds, confirming their more restricted genetic base. These results agree with other studies using genetic markers (Tansley & Brown, 2000), where the greater part of genetic variance is due to differences between individuals within breeds.

The gene diversity index was calculated for all breeds, considering them as unique populations (Table 3). This

analysis was based on the mean allelic frequency of the 122 RAPD markers, which varied from 300 bp to 2,000 bp. The Caracu presented lowest gene diversity (0.1904) when compared to the other native breeds. These results agree with Poli (1985) who, using blood factor phenograms, also observed lower gene diversity in this breed. Until the year 2000 this was the only native breed that had a Breeder's Association, which may explain these observations.

#### Estimation of genetic distance

The estimates of genetic distances between the breeds were calculated to help in the study of genetic

relationships and genetic divergence between pairs of populations for standard genetic distances (Dp) (Nei, 1972) and those corrected for small populations (Dc) (Nei, 1978) (Table 4).

Native breeds closest to the Nelore were the Crioulo Lageano (Dc = 0.2536) and Pantaneiro (Dc = 0.2550) and the furthest was the Caracu (Dc = 0.3879). The first observation may be explained by the historical data from the breed in which, in the past, Nelore or crossbred Nelore bulls were used. The lowest divergence was observed between the Crioulo Lageano and Curraleiro breeds (Dc = 0.0424). The phenotypes of these breeds are different and they inhabit distinct regions of Brazil (extreme south and central west, respectively). Poli (1985) found the smallest difference between Crioulo Lageano and Pantaneiro breeds. The difference found here might be due to the different techniques used. Poli (1985) compared the breeds based on three blood group frequencies and the mean number of blood factors per animal in each group. In spite of these different results, Primo (1993) stated that the probable ancestor of the Curraleiro, Crioulo Lageano and Pantaneiro breeds was the *Bos taurus ibericus*, while the Caracu and National Polled had the *Bos taurus aquitanicus* as their common ancestor. The mean Dp and Dc values between native breeds were 0.06887 and 0.06466, respectively.

The dendrogram of the genetic distance matrix generated by the UPGMA method (Nei, 1978) as well as bootstrapping proportions are shown in Figure 1. The *Bos taurus* breeds are grouped in one cluster separated from the Nelore (*Bos indicus*). The native breeds are in a cluster separated from the Holstein-Friesian, reconfirming the hypothesis that they share a common ancestor. Within the native breed cluster, the smallest genetic divergence was shown between the Crioulo Lageano and Curraleiro breeds and the National Polled

**Table 2.** Analysis of molecular variance (AMOVA) of the seven populations (breeds) studied, using 122 RAPD markers<sup>(1)</sup>.

Source of variation	DF	SS	MS	VC	% Total
Between breeds	6	1709.453	284.901	5.676	29.96*
Within breeds	328	4352.064	13.26849	13.268	70.04 <sup>ns</sup>
Between native breeds <sup>(2)</sup>	4	557.652	139.413	2.633	16.27*
Within native breeds	234	3170.172	12.54775	13.548	83.73 <sup>ns</sup>

<sup>(1)</sup>DF: degrees of freedom; SS: sum of squares; MS: mean square; VC: variance components. <sup>(2)</sup>Only native breeds were considered.

<sup>ns</sup>No-significant. \*Significant at 5% level of probability.

**Table 3.** Nei's (1973) gene diversity index (h) of breeds, using the POPGENE program, considering them as unique populations.

Breed	(h)
Caracu	0.1904
Crioulo Lageano	0.2409
Curraleiro	0.2492
Holstein Friesian	0.1342
National Polled	0.2449
Nelore	0.2524
Pantaneiro	0.2464

**Table 4.** Genetic distances between seven cattle breeds estimated using Nei (1972, 1978) methods, from RAPD data, using the POPGENE program. The standard genetic distances (Nei, 1972) are below the diagonal, and corrected distances (Nei, 1978), above the diagonal.

Cattle breed	Caracu	Crioulo Lageano	Curraleiro	Holstein Friesian	National Polled	Nelore	Pantaneiro
Caracu	-	0.0813	0.0650	0.0930	0.0847	0.3879	0.0951
Crioulo Lageano	0.0842	-	0.0424	0.0857	0.0464	0.2536	0.0599
Curraleiro	0.0680	0.0458	-	0.0940	0.0566	0.2971	0.0510
Holstein Friesian	0.0950	0.0883	0.0965	-	0.0766	0.4234	0.1108
National Polled	0.0877	0.0499	0.0600	0.0801	-	0.2585	0.0738
Nelore	0.3909	0.2571	0.3006	0.4260	0.2621	-	0.2550
Pantaneiro	0.0981	0.0633	0.0544	0.1133	0.0773	0.2590	-

was grouped with animals considered of *Bos taurus ibericus* origin (Curraleiro, Crioulo Lageano and Pantaneiro). According to Athanassof (1957), these animals were small to medium sized. This puts the affirmation of the common ancestor of the Caracu and National Polled as being the *Bos taurus aquitanicus* (large framed animals) in question.

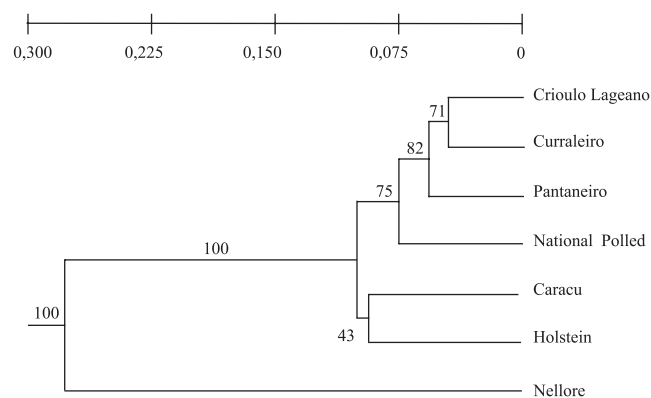
**Genetic similarity**

The genetic similarity indices were used to generate, using UPGMA, a dendrogram based on all the samples used. Jaccard similarity coefficients were calculated from the binary data matrix from the 122 markers obtained by RAPD (Figure 2).

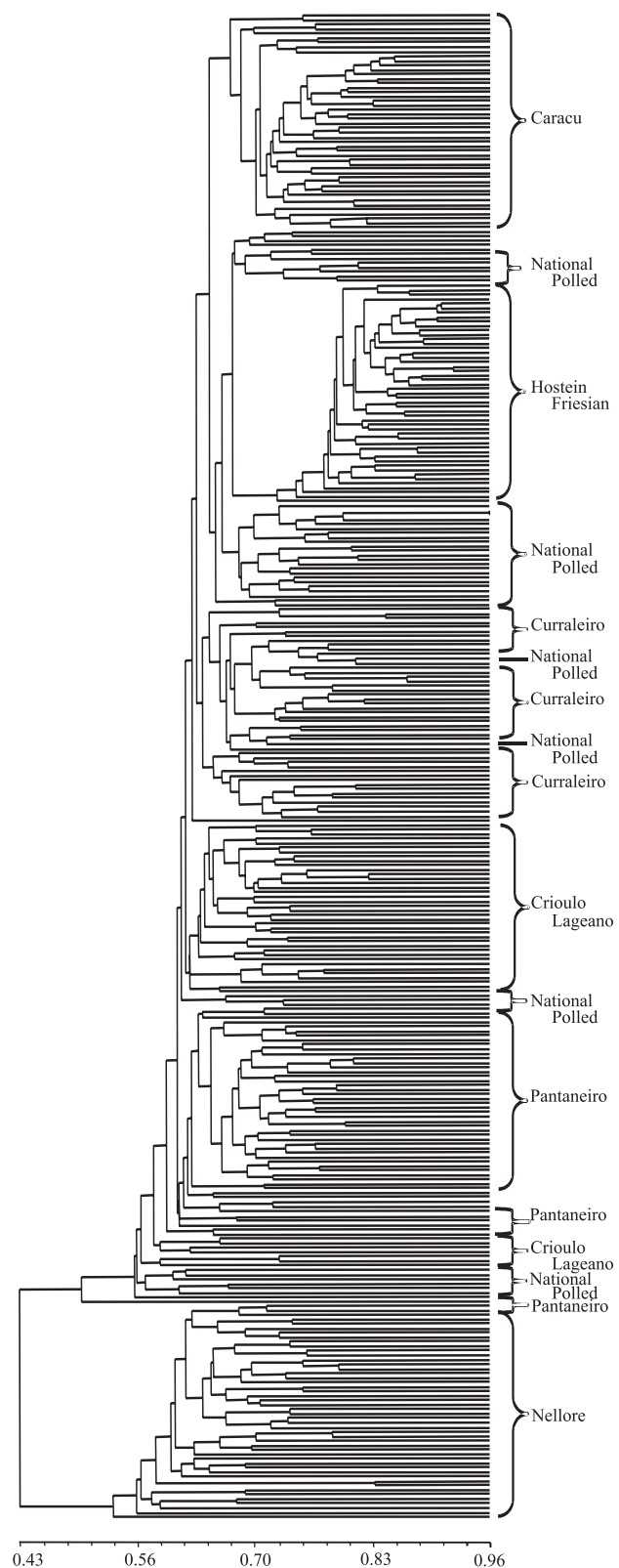
The Caracu has the best genetic standard defined within the native breeds, as its animals are all within the same cluster. This may be due to the selection that has been carried out, as well as the work carried out by the Breeder's Association to maintain this breed. The Nellore and Holstein Friesians also are grouped in single clusters.

Generally, the native breeds are grouped in clusters, except for the National Polled, in which 29 animals are grouped beside the Holstein Friesian cluster and the rest are distributed within the clusters of the other breeds. This may reflect the genetic dilution process to which this breed has been submitted over the last decades. The National Polled was considered practically extinct in the 1970s and various breeders crossed the remaining animals with other breeds, which justifies their distribution throughout the dendrogram.

Its phenotypic similarity to the Caracu led many technicians and breeders to believe that this was a polled



**Figure 1.** Dendrogram generated by the UPGMA method for Nei (1978) genetic distance using TFPGA program. The confidence value of each node was estimated from 10.000 permutations.



**Figure 2.** Individual dendrogram constructed from Jaccard similarity coefficient using NTSYS-pc program.

version of the Caracu, and the Caracu Breeder's Association registers it as "Caracu, polled variety". This study shows that, probably, these breeds were not genetically similar in the past. By means of the breeding strategies being carried out on this breed, the tendency is that they become genetically similar to the Caracu, and the latter will absorb the National Polled genes.

Various small clusters were formed near the Nelore group, which include Pantaneiro, Crioulo Lageano and National Polled animals separated from the general *Bos taurus* cluster. These may represent miscegenation of these animals due to crossbreeding with zebu breeds. It is well known that since the introduction of zebu animals in Brazil, these were widely used in grading up crossbreeding schemes with native breeds (Mariane, 1993). Lara (1998) found a considerable contribution of zebu genes in a population of Pantaneiro cattle from the Poconé region of Mato Grosso State, which therefore was grouped together with the *Bos indicus* cluster. The animals from the Pantanal Conservation Nucleus in Corumbá, Mato Grosso do Sul State, grouped with the *Bos taurus* cluster. The Crioulo Lageano nucleus, according to information from the breeder, underwent some introduction of Nelore genes for a short period in the 1980s.

The Brazilian native breeds, with the exception of the Caracu, lack standardization and control. Crossbreds of these breeds with zebu or other commercial breeds, or even with animals that are phenotypically but not genetically similar, should be identified and excluded from conservation programs.

### Conclusions

1. The native Caracu, Crioulo Lageano, Curraleiro and Pantaneiro breeds can be considered distinct genetic entities, thereby proving the uniqueness of their populations and the importance of their conservation.
2. Among the native breeds, Caracu has the best genetic standard, showing the importance of the Herdbook and Breeder's Association for maintenance and conservation of the breed, while the National Polled probably has not managed to re-establish itself after its decline in the 1950s, losing its genetic identity.
3. Zebu genes have been introduced into some of the animals of the native breeds, especially Crioulo Lageano, National Polled and Pantaneiro. Control of crossbreeding is fundamental if the preservation of native breeds, threatened with extinction, is to be successful.

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