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GENETIC POPULATION STRUCTURE OF BRAZILIAN BOVINE BREEDS INFERRED BY RAPD MARKERS

ESTRUTURA GENÉTICA DE POPULAÇÕES DE RAÇAS BOVINAS BRASILEIRAS INFERIDA POR MARCADORES RAPD

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ADDITIONAL KEYWORDS

Bovine native breeds. Populations structure. Conservation genetics.

PALAVRAS CHAVE ADICIONAIS

Raças bovinas nativas. Estrutura de populações. Conservação genética.

SUMMARY

Conservation and improvement strategies should be based on the association between genetic and phenotypic characteristics. In this study 10 different populations of five native Brazilian cattle breeds (Caracu, Mocho Nacional, Crioulo Lageano, Curraleira and Pantaneira) were characterized using RAPD techniques to estimate their genetic relationships and to verify the existence of possible regional differences within each breed. Two commercial breeds were used as control and outgroup respectively (Holstein and Nelore). 120 arbitrarily primers were screened, of which 22 were selected, and generated 122 polymorphic bands. When all groups were analysed in pairs, non-significant genetic variability ($p > 0.05$) observed only between two populations of Curraleiro breed located in States of Piauí (CUPI) and Minas Gerais (CUMG). Using Popgene program Nei's 1978 genetic distance was estimated. The smallest divergences were observed between groups of the same breed and the least distance between the two groups of Caracu breed (0.0159). In the four populations of Curraleiro the groups CUPI and CUMG presented the smallest difference (0.0285). These results could indicate that these

two populations have high genetic similarity. In the dendrogram generated by UPGMA method all groups clustered in their respective breed. In this study we could demonstrate the existence of regional genetic differences between the populations of native breeds. These estimates could be useful in crossbreeding and genetic sample exchange between different nuclei.

RESUMO

Estratégias para a conservação e melhoramento animal devem ser baseadas na associação de características fenotípicas e genéticas. Neste estudo realizou-se a caracterização genética de 10 diferentes populações de cinco raças bovinas nativas brasileiras (Caracu, Mocho Nacional, Crioulo Lageano, Curraleira e Pantaneira) através da técnica de RAPD, visando estimar a relação genética das populações e verificar a possibilidade de existir diferenças regionais dentro de cada raça. Duas raças comerciais foram utilizadas como controle e *outgroup* respectivamente (Holandesa e Nelore). Uma triagem com 120 *primers* foi realizada dos

quais foram selecionados 22, que geraram 122 bandas polimórficas. Quando todos os grupos foram confrontados em pares, a variabilidade genética não foi significativa ($p>0,05$) apenas para dois grupos da raça Curraleira localizados no estado do Piauí (CUPI) e no de Minas Gerais (CUMG). Utilizando o programa Popgene, foram estimadas as distâncias genéticas, através do método de Nei (1978). As menores divergências foram observadas entre os grupos da mesma raça e a menor delas foi encontrada entre os dois grupos da raça Caracu (0,0159). Entre as quatro populações da raça Curraleira, os grupos CUPI e CUMG apresentaram a menor diferença (0,0285). Este resultado indica que esses dois grupos possuem grande similaridade genética. Os grupos de cada raça se agruparam em *clusters* diferentes, pertencentes às respectivas raças no dendrograma gerado pelo método de UPGMA. Neste estudo pode-se demonstrar a existência de diferenças genéticas regionais nas populações das raças nativas. Estas estimativas podem ser utilizadas em cruzamentos e trocas de amostras genéticas entre os diferentes núcleos.

INTRODUCTION

Brazilian native bovine breeds have developed from those brought by the Portuguese soon after the discovery. With importation of some exotic breeds, mainly zebus, at the beginning of the past century, the native animals have gradually been substituted making most of them threatened with extinction. Although the exotic breeds are considered more productive, they do not necessarily possess the adaptation, resistance to illnesses and parasites found in the native breeds.

The techniques for the analysis of the genetic variability are essential ingredients for programs of rational

conservation and improvement, that these must be based on the combination of the phenotypic and genetic data.

Studies related to the genetic characterization of the native Brazilian bovine breeds are being carried out in the Laboratory of Animal Genetics - Brasilia, DF – using DNA molecular markers. One of those molecular markers used is the RAPD (random amplified polymorphic DNA). The RAPD methodology is being widely used in studies on conservation, as it is relatively simple, low cost and that does not require specific knowledge on the population to be studied, helping in the choice of strategies to preserve threatened species and breeds. Here these markers were used to study 10 populations of five native Brazilian bovine breeds in order to investigate the existence of regional genetic differences.

MATERIAL AND METHODS

In these study we analysed possible regional differences between Curraleiro (CU) from the Northeast and Central West States; Caracu (CA) from Central West and Southern Brazil and National Polled breed (MN) from Minas Gerais State, South-eastern Brazil. Two other naturalized Brazilian cattle breeds and two commercial breeds were also studied. These included the Pantaneiro (PAN, n=48) from the Pantanal region, South Western Brazil and Crioulo Lageano (CL, n=48) from Santa Catarina State, Southern Brazil. As all native cattle breeds are of taurine origin the Holstein-Friesian (HOL, n=48) was included as a control group

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and Nelore (NEL, n=48) (*Bos indicus*) as an outgroup.

The Caracu breed was divided in two groups, in accordance with the aptitude of the populations, the Caracu Caldeano (CA1, n=17) selected for milk and the other collected in the remaining portion of Brazil (CA2, n=31), usually selected for meat production. The Curraleiro was divided in four groups of origin and/or region of the herd. The first group (CUPI, n=12) was composed of animals from Piauí, the II group (CUMG, n=7) of Minas Gerais, and the other two were from Goiás, group III (CUGO1, n=9) of Maranhão origin and the IV group (CUGO2, n=2) from Natividade-GO. The National Mocho was divided in two groups, in the first group (MN1, n=13) from the Três Barras Farm - MG and the other (MN2, n=34) animals from the Bank of Animal Germplasm located in Embrapa Recursos Genéticos e Biotecnologia, Brasília-DF.

DNA extraction was based on a non-organic protocol described on Miller *et al.* (1988) with slight modifications. A total of 120 arbitrary primers (Operon Technologies Inc., Alameda, Calif.: A, B, E, J, K and AB) were screened using one sample of each breed.

Amplification reactions were performed in 50mM KCl, 20mM Tris-HCl (pH 8,4), 2,5mM MgCl₂, 200µM of each dNTP, 0,4µM primer, 9ng template DNA, 8 percent of BSA (2,5 mg/ml) and 1.5UI Taq DNA polimerase in a final volume of 13µl. 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 a cooling at 4°C. PCR products were separated by electrophoresis in 1.4 percent agarose gels in 1 x TBE, and were stained with ethidium bromide (0,6µg/ml) and observed under UV light.

The fragment data were entered in a computer file as a binary matrix: 0 coded for absence and 1 for presence of a band. The genetic distances between populations 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). All cluster analyses done in this work used UPGMA and the resulting clusters were expressed as dendrograms. The bootstrap analysis was carried out using the TFPGA program (Tools for Population Genetics Analyses, version 1.3) (Miller, 1997). The analysis of molecular variance (AMOVA) (Excoffier *et al.*, 1992) was used to analyze the variance between the populations.

RESULTS AND DISCUSSION

Of the 120 primers investigated, 72 were polymorphic (60 percent), 23 monomorphic (19,2 percent) and 25 did not amplify or had poor amplification (20.8 percent). Only those that presented at least 4 polymorphic bands were used, thus reducing the time and the cost of the experiments. Using these criteria, 22 primers had been selected and generated a total of 122

polymorphic bands (5.5 bands/primer).

In relation to the genetic variability between groups, when CUIPI and CUMG were studied, no significant difference was observed ($p>0.05$), therefore they cannot be considered distinct genetic groups, as observed by Martins (1996) who, using 37 polymorphic RAPD markers, found significant differences between individuals of the Curraleiro breed from Salto da Divisa - MG and Piauí, recommending that these individuals were considered distinct genetic entities. Although the number of individuals used in each group was small, in both studies, this difference may be due to the number of markers used. The distribution of the genetic variability within and between groups was carried through using the AMOVA. The genetic variabilities between the others groups were significant ($p<0.05$).

The estimates of genetic distances between the studied groups were calculated to investigate genetic relations

between them. These measures also express the degree of genetic divergence between the groups. The genetic distances indices corrected for small samples (Nei, 1978) were estimated for the pairs of studied groups using program POPGENE (**table I**).

The genetic distances for the 12 groups showed less divergence between groups of the same breed (**table I**). These values agree with those of Del Lama (1992), where the expected values of distances between local breeds are generally, between 0.00 and 0.05 and between subspecies and species these values can increase to between 0.02 and 0.20, varying usually from 0.10 to 1.00. The two Caracu groups showed the least divergence between groups of one breed (0.0159), which nevertheless was significant ($p<0.05$). Although group CA1 has been isolated for more than a century, when compared with the animals of group CA2, the distances were low. These findings may be due

Table I. Genetic distance matrix generated from 122 RAPD markers. (Matriz de distância genética gerada a partir de 122 marcadores RAPD).

	CABR	CAMG	CL	CUIPI	CUMG	CUGO1	CUGO2	HOL	MNV	MNTB	NEL
CAMG	0.0207										
CL	0.0878	0.0911									
CUIPI	0.0833	0.0978	0.0556								
CUMG	0.1238	0.1287	0.0712	0.0443							
CUGO1	0.0908	0.0968	0.0656	0.0555	0.0776						
CUGO2	0.0843	0.0835	0.0704	0.0421	0.0703	0.0674					
HOL	0.0984	0.1022	0.0883	0.1135	0.1334	0.1331	0.1052				
MNV	0.1006	0.1121	0.0634	0.0873	0.1008	0.0934	0.1060	0.0921			
MNTB	0.0940	0.1014	0.0537	0.0720	0.0924	0.0824	0.0842	0.0845	0.0324		
NEL	0.3929	0.4007	0.2571	0.3147	0.3284	0.3174	0.3229	0.4260	0.2767	0.2653	
PAN	0.1007	0.1066	0.0633	0.0674	0.0916	0.0828	0.0691	0.1133	0.0887	0.0818	0.2590

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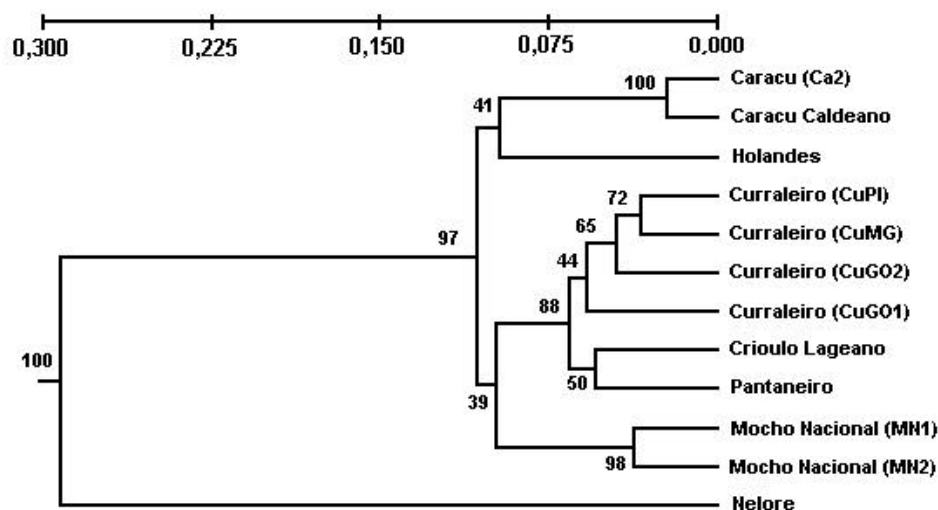


Figure 1. Dendrogram generated for the UPGMA method using Nei (1978) corrected for small populations for the data generated with 122 RAPD markers. The consistency of clusters was verified using the bootstrap (10000 permutations). (Dendrograma gerado pelo método de UPGMA a partir da matrix de distância genética de Nei (1978) gerada por 122 marcadores RAPD. A consistência dos clusters foi verificada por bootstrap (10000 permutações)).

to the work of conservation carried out on Chiqueirão Farm, MG, and in the recovery of the breed after being threatened with extinction.

In the groups of the Curraleiro breed, the least divergence was between CUPI and CUMG (0.0285). This can better be visualized in **figure 1** and is confirmed by the genetic similarity between these groups. But, as already cited, this result disagrees with those of Martins (1996) and needs to be confirmed using a larger number of individuals. The dendrogram generated by UPGMA from the genetic distance matrices (**figure 1**), show the breed populations clustering with their own breeds.

CONCLUSIONS

Based on the results obtained in this study we can conclude that the RAPD technic is an efficient method for the study of genetic similarity between populations. All the populations studied (Curraleiro, Caracu and National Polled breed, Pantaneiro and Crioulo Lageano), can be considered as distinct genetic entities, only two populations of Curraleiro breed (located in States of Piauí (CUPI) and Minas Gerais (CUMG)) were not significantly different ($p > 0.05$). These estimates could be useful in crossbreeding and genetic sample exchange between different nuclei.

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