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Research Article

Mitochondrial control region genetic diversity and maternal ancestry of a Brangus-Ibage cattle populations

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Abstract

The genetic diversity of 277 nucleotides in the mitochondrial DNA control region (nt 15,964 to 16,240 in reference sequence) was analyzed in crossbreed beef cattle (Brangus-Ibage, 5/8 *Bos primigenius taurus* x 3/8 *Bos primigenius indicus*) as well as in some Nellore samples (*B. p. indicus*). Fifty-seven mutations were found in Brangus-Ibage comprising 18 haplotypes (haplotype diversity, h = 0.851 ± 0.041 and nucleotide diversity, ntd = 0.009 ± 0.006) and 66 in Nellore (h = 1.00 ± 0.27, ntd = 0.014 ± 0.012). These data indicated sequence identities of 99.6 and 92.1% between the *B. p. taurus*' reference sequence and Brangus-Ibage and Nellore, respectively. The comparison of our data with sequence data for 612 individuals recovered from GenBank showed a total of 205 haplotypes defined by 99 polymorphic sites. Most of the variability (53%) was due to differentiation within breeds. The phylogenetic tree constructed using the neighbor-joining method showed clearly the well-known dichotomy between *B. p. taurus* and *B. p. indicus.* The Brangus-Ibage clustered with *B. p. taurus* lineages; however, the displacement of Nellore from *B. p. indicus* branch probably indicates a substantial *B. p. taurus* maternal ancestry in some Nellore samples (obtained from GenBank) and reflects the primarily male-driven introduction of this breed in Brazil.

Key words: bovine mtDNA, maternal lineage, sequence analysis, beef cattle, genetic diversity.

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Introduction

Mitochondria are maternally inherited organelles of eukaryotic cells, which play an important role in the cell energy provision. Vertebrate mtDNA includes, in addition to coding regions, a non-coding segment: the displacement loop (D-loop), which is the major control region for mtDNA expression (Taanman, 1999). Therefore, sequence differences in mtDNA D-loop may alter the transcription and/or replication rates (Schutz *et al.*, 1994). However, despite its functional importance, this region has a rate of nucleotide substitution five to ten times higher than that of nuclear DNA (Brown *et al.*, 1979).

The rapid rate of sequence divergence of mtDNA makes it suitable for the analysis of short-term evolutionary phenomena, while the maternal mode of inheritance allows the evolutionary relationships between lineages to be defined in terms of their phylogenetic divergence without the ambiguities caused by recombination. Therefore, mtDNA polymorphisms have been widely used to investigate the structure of populations, interspecies variability, the evolutionary relationships between populations or species and for the identification of maternal lineages (Bradley and Cunningham, 1999; Cymbron *et al.*, 1999; Magee *et al.*, 2002; Troy *et al.*, 2001). Additionally, there has been an increasing interest in its potential use in the development of new biotechnologies (Smith *et al.*, 2000).

In livestock species, mtDNA variability has been studied in connection with maternally inherited physiological parameters and it has been suggested that bovine mtDNA may affect milk production as well as some other productive traits (Schutz *et al.*, 1992; Schutz *et al.*, 1994; Mannen *et al.*, 2003; Henkes *et al.*, 2004).

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In this study, we provide mtDNA D-loop sequencing data for a Brangus-Ibage cattle population (5/8 *Bos primigenius taurus* x 3/8 *Bos primigenius indicus*) as well as for some Nellore samples (*B. p. indicus*). We also analyzed these data pooled with the majority of cattle D-loop sequences available in GenBank in an attempt to advance our understanding of the origins of the maternal lineages that contributed to the formation of this Brangus-Ibage population.

Material and Methods

Brangus-Ibage is a composite beef cattle breed resulting from the crossing of Aberdeen Angus cows (ABG) and Nellore bulls (NEL). In Brazil, the early work crossing Nellore and Aberdeen Angus cattle was done by the Brazilian Agricultural Research Corporation (EMBRAPA - CPPSUL) and started about 1945 (Oliveira *et al.*, 1998).

Samples of whole blood were collected from 49 Brangus-Ibage cows from the Brazilian Agricultural Research Corporation and DNA was extracted by the method of Plante *et al.* (1992). To validate the supposed *B. p. taurus* origin of the Brangus-Ibage mtDNA, three DNA samples of Nellore (*B. p. indicus)* animals were also investigated. These samples were kindly provided by Dr. F.V. Meirelles (for details of these samples see Meirelles *et al.*, 1999).

A 277 nt fragment of the control region (positions 15964-16240) was analyzed from an amplified product obtained using primers designed from the reference sequence of *B. p. taurus* mtDNA (Anderson *et al.*, 1982) as follows:

MTR 11, 5' CCT ACG CAA GGG GTA ATG TA 3' (positions 15,949-15,968) and

MTR 12, 5' CCT GAA GAA AGA ACC AGA TG 3' (positions 16,265-16,285).

PCR amplification was carried out by two reactions: the first used 100 ng of DNA in 25 µL reaction volume, with 1.25 mM of each deoxynucleotide, 1.25 µM of each primer and 1 unit of Taq DNA Polymerase (Pharmacia Biotech) for 30 cycles. Each cycle consisted of denaturation at 95 °C for 1 min, annealing at 45 °C for 50 s and extension at 72 °C for 1 min. These double-stranded amplification products were then purified with the Wizard PCR Preps DNA Purification System (Promega Corporation). Aliquots of these products were later used for asymmetric amplification (Ward *et al.*, 1991) using a 1:10 ratio of the same primers. The single-strand amplification products were then purified using a SEPHADEX column and phenol/chloroform (1:1) extraction. The purified fragments were sequenced using a T7SequencingTM Kit (Pharmacia Biotech) according to the manufacturer's instructions. Reaction products were separated by electrophoresis on 6% polyacrylamide gels containing 8 M urea. Gels were fixed in 5% acetic acid and 15% methanol for 10-15 min, dried and exposed to a Kodak XAR film for 24-48 h. Sequences were analyzed in at least two independent gels, aligned and compared using the BIOEDIT computer package (Hall, 1999). Insertions/deletions were introduced in order to minimize substitutions. The variant sequences observed in this study were submitted to GenBank under accession numbers AF308591 and AF309100-AF3091113 (Brangus-Ibage) and AF309097-AF309099 (Nellore).

Intra-populational variation was estimated by computing haplotype and nucleotide diversities (Nei and Tajima, 1981; Nei, 1989). The last analysis was corrected for among-site heterogeneity (Tamura and Nei, 1993).

The present sequence data were compared to those of all other breeds for which, at that time, there were at least three available sequences in GenBank (n = 612, GenBank accession numbers AB003793-AB003801, AB044587-AB044592, AB065119-AB065131, AB079300-AB079365, AB085922, AB085923, AF016060-AF016071, AF016079-AF016097, AF022916, AF022918-AF022924, AF034439, AF034441, AF034442, AF034444, AF034445, AF083354, AF209124, AF209126, AF308591, AF309095, AF309096, AF336383-AF336744, AF516713, AF516714, AF531383, AF531412, AF531413, AJ295936, AY119666, AY235731-AY378140, L27720, L27721, L27732, L27733, U51806-U51842 and U92230-U92244). This comparison was restricted to 210 nucleotides to accommodate the shorter published sequences. Data for all individuals were available only between nucleotides 16,031 and 16,240. The total analysis was comprised of 664 individuals (49 Brangus-Ibage, 3 Nellore and 612 from GenBank) from 54 different cattle breeds.

Population genetic structure indexes were estimated by analyses of molecular variance (AMOVA, Excoffier *et al.*, 1992) using the substitution model of Tamura and Nei (1993). The significance of these analyses was tested using a non-parametric permutation procedure (Excoffier *et al.*, 1992). All of these analyses were performed using the Arlequin software (Schneider *et al.*, 2000).

The phylogenetic relationships between populations were determined using the neighbor-joining (NJ) algorithm (Saitou and Nei, 1987). The robustness of the tree was evaluated by resampling the data by the bootstrap test (Felsenstein, 1985) with 2,000 replicates (Hedges, 1992). These analyses were conducted using MEGA version 2.1 (Kumar *et al.*, 2001). The relatedness of the Brangus-Ibage mtDNA haplotypes was assessed through reduced median networks (Bandelt *et al.,* 1995) constructed with Network software (www.fluxus-engineering.com).

Results

Sequence analysis of mtDNA was based on a 277 bp fragment of the D-loop region (nt 15,964 to 16,240 in reference sequence). Sixteen polymorphic sites were identified in Brangus-Ibage mtDNA sequences (Table 1). A total of 18 mitochondrial lineages were verified; the most frequent occurred in 17 individuals and was equal to the *B. p. taurus*' reference sequence (Anderson *et al.*, 1982). Most

Table 1 - Polymorphic nucleotide sites observed in Brangus-Ibagé and Nellore cows. Only one animal of each haplotypes is presented.

	Nucleotide position																																		
	15995	16022	16049	16050	16053	16057	16058	16074	16082	16084	16102	16109	16110	16113	16116	16117	16119	16121	16122	16130	16137	16138	16139	16143	16147	16156	16177	16191	16195	16196	16200	16200	16221	16229
Iba-1*	A	G	C	C	T	G	C	T	G	C	G	T	C	T	T	G	T	G	T	T	T	T	C	A	T	G	T	C	A	G	G	+	G	A
Iba-2	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Iba-3	.	.	.	T	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Iba-4	.	.	.	T	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.
Iba-5	.	.	.	T	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	T	.	C	.	.	.	.	.	.	.	.	.
Iba-6	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Iba-7	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.
Iba-8	.	.	.	.	.	.	.	C	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Iba-9	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Iba-10	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Iba-11	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.
Iba-12	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	T	.	C	.	.	.	.	.	.	.	.	.
Iba-13	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.
Iba-14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	-	G	.	.	.	.	.
Iba-15	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.
Iba-16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	C	.	.	.	.	.	.	.
Iba-17	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	A	.	.	.
Iba-18	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	A	.	.	.
Nel-1	G	A	T	.	.	A	T	C	A	T	A	C	.	C	.	A	C	A	C	C	C	C	.	-	C	.	.	.	.	A	.	A	.	.
Nel-2	G	A	T	.	.	A	T	C	A	T	A	C	.	C	.	A	C	A	C	C	C	C	.	-	.	.	.	.	.	A	.	A	.	G
Nel-3	.	.	T	.	.	A	T	C	A	T	A	C	.	C	C	A	C	A	C	C	C	C	.	-	C	.	.	.	.	A	.	A	A	.

*equal to the *B. p. taurus*' reference sequence (Anderson *et al.*, 1982; n = 17); Iba: Brangus-Ibage; Nel: Nellore; +: insertion; -: deletion.

haplotypes were scored only once or twice (Table 2), resulting in a high total haplotype diversity, h = 0.851 ± 0.041. The nucleotide diversity was estimated at 0.009 ± 0.006. Most of the variable sites were transitions (transition/transversion rate of 15) and deletion/insertion was identified in one position only (16,191). The mean number of pairwise differences among sequences was 1.91 ± 1.11. Nellore samples showed 3 different mitochondrial lineages defined by 25 polymorphic sites (Table 1), when compared with *B. p. taurus'* sequence (Anderson *et al.*, 1982). One deletion was identified at position 16,143 and an A insertion was observed at one site (16,200). The Nellore haplotype diversity was estimated at 1.00 ± 0.27 and the nucleotide diversity was 0.014 ± 0.012. The mean number of pairwise differences among sequences was 4.00 ± 2.72.

In the whole sample, 57 mutations were found in Brangus-Ibage and 66 in Nellore. These data showed average sequence identities of 99.6% between the *B. p. taurus'* reference sequence (Anderson *et al.*, 1982) and Brangus-Ibage and of 92.1% between *B. p. taurus'* sequence (Anderson *et al.*, 1982) and Nellore.

Pooling our mitochondrial sequences with those obtained from GenBank defined 99 polymorphic sites (Table 3) and a total of 205 haplotypes. Eighty-seven of the substitutions were transitions, 11 were transversions (transitions/transversions rate = 7.9) and insertions/deletions were identified at 9 positions. More than two nucleotides were detected only at positions 16,057, 16118, 16156 and 16230 (Table 3). The average pairwise sequence divergence within breeds varied from 0.67 ± 0.67 (Charolais and Friesian) to 4.00 ± 2.73 (Tharparkar, *B. p. indicus*). Two mutations are being described here for the first time, at position 16177 (T $\rightarrow$ C) in Brangus-Ibage and at site 16221 (G $\rightarrow$ A) in Nellore.

Hierarchical analysis of haplotype diversity (Table 4) indicated that 53% of the variability was due to differentia-

Table 2 - Haplotype frequencies in a Brangus-Ibagé cattle population.

N.	Freq.	s.d.	Site(s) of mutation(s)
1	0.347	0.069	None*
2	0.041	0.029	16022 (G → A)
3	0.020	0.020	16050 (C → T) 16113 (T → C)
4	0.020	0.020	16050 (C → T) 16113 (T → C) 16139 (C → T)
5	0.020	0.020	16050 (C → T) 16113 (T → C) 16139 (C → T) 16147 (T → C)
6	0.041	0.029	16053 (T → C)
7	0.020	0.020	16057 (G → T) 16139 (C → T)
8	0.020	0.020	16074 (T → C) 16110 (C → T)
9	0.041	0.029	16113 (T → C)
10	0.163	0.053	16113 (T → C) 16119 (T → C)
11	0.020	0.020	16113 (T → C) 16139 (C → T)
12	0.020	0.020	16113 (T → C) 16139 (C → T) 16147 (T → C)
13	0.020	0.020	16119 (T → C) 16195 (A → G)
14	0.041	0.029	16119 (T → C) 16191 (DEL C) 16195 (A → G)
15	0.041	0.029	16139 (C → T)
16	0.020	0.020	16156 (G → A) 16177 (T → C)
17	0.020	0.020	16196 (G → A)
18	0.082	0.040	16200 (G → A)

*identical to *B. p. taurus'* reference sequence (Anderson *et al*., 1982).

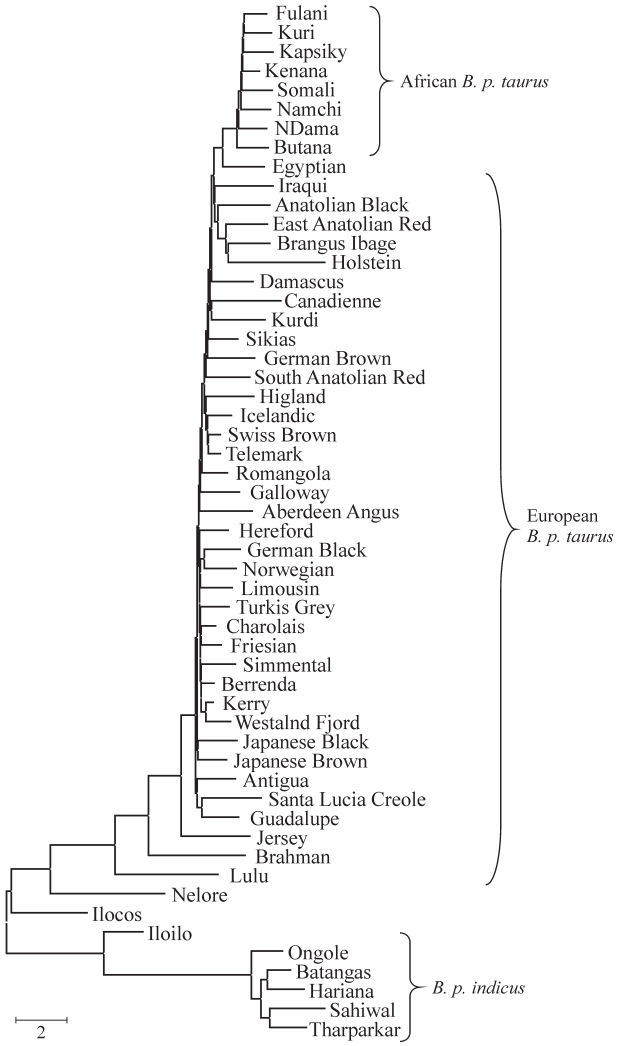

Figure 1 - Phylogenetic relationships among 54 cattle populations.

tion within the breed. The validity of this partition tested by the permutation test was highly significant ($p < 0.001$).

The phylogenetic tree constructed using the neighbor-joining method (Figure 1) shows clearly the two main branches separating *B. p. taurus* and *B. p. indicus*. Brangus-Ibage is clustered with *B. p. taurus* lineages. The reduced mean network (Figure 2) shows that all haplotypes in Brangus-Ibage root back to the phylogeny through the primary *B. p. taurus* haplotypes (Troy *et al.*, 2001). The Nellore haplotypes from *B. p. indicus* origin are clearly dispersed from the main node.

Discussion

As expected Brangus-Ibage sequences were similar to those of *B. p. taurus* animals (99.6% of sequence identity) and distinct from those of Nellore. These data confirm no female contribution of *B. p. indicus* to the composition of the maternal lineages of Brangus-Ibage. However, we have detected some samples displaying mutations at positions 16050 and 16113 which, together with the substitution at position 16255, would be an indication of the presence of haplogroup T1, characteristic of African *B. p. taurus* (Troy *et al*., 2001). It is well-known that most European modern cattle breeds were introduced to South America at the beginning of the last century and most imported animals were males, which were mated to local cows (cows previously introduced in South America). The Brazilian Aberdeen Angus did not escape this rule (http://www.angus.org.br). Some cows of our herd might be descendants from cattle introduced in America by the first Portuguese and Spanish settlers. Since there is evidence of African *B. p. taurus* influence in Portuguese cattle (Cymbron *et al.*, 1999) and since we found three haplotypes that could be of African origin (Iba-3, Iba-4 and Iba-5), we extended our analysis of these three haplotypes in order to investigate the position 16255. Only one of these haplotypes (Iba-3) presented the mutation T → C at this position, characteristic of African *B. p. taurus*. The presence of an African *B. p. taurus* haplotype that had survived might suggest some adaptive value in this specific environment. Interestingly, Brangus-Ibage is situated at an intermediate position between the European and African grouping on the phylogenetic tree (Figure 1).

Table 3 - Polymorphic sites and nucleotide frequencies in a 210 bp D-loop fragment from 664 individuals of 54 different cattle breeds.

Site	State	State frequencies				
		A	C	G	T	DEL
16042	2		0.0600		0.9400	
16044	2		0.9985		0.0015	
16047	2	0.0059		0.9941		
16047+1	2			0.0029		
16049	2		0.9239		0.0761	
16050	2		0.8155		0.1845	
16051	2		0.0059		0.9941	
16052	2		0.9985		0.0015	
16053	2		0.0059		0.9941	
16055	2		0.0132		0.9868	
16056	2	0.9941		0.0059		
16057	4	0.0966	0.0454	0.8565	0.0015	
16058	2		0.9092		0.0908	
16062	2	0.9927		0.0073		
16063	2		0.9985		0.0015	
16066	2	0.9985		0.0015		
16067	2	0.9883		0.0117		
16068	2		0.0059		0.9941	
16069	2	0.9956		0.0044		
16073	2	0.9956		0.0044		
16074	2		0.1010		0.8990	
16075	2	0.9971		0.0029		
16076	2	0.9941		0.0059		
16079	2	0.9956		0.0044		
16082	2	0.0630		0.9370		
16083	2	0.9985		0.0015		
16084	2		0.9327		0.0673	
16085	2		0.0337		0.9663	
16086	2	0.0015		0.9985		
16088	2	0.9927		0.0073		
16092	2	0.9971		0.0029		
16093	2	0.0395		0.9605		
16094	2		0.0015		0.9985	
16099	2		0.0029		0.9971	
16101	2		0.0029		0.9971	
16102	2	0.0630		0.9370		
16104	2		0.9956		0.0044	
16107	2	0.9985			0.0015	
16108	2		0.0044		0.9956	
16109	2		0.0717		0.9283	
16110	2		0.9868		0.0132	
16112	2		0.0029		0.9971	
16112+1	2				0.0015	
16113	2		0.2606		0.7394	
16116	2		0.0644		0.9356	
16117	2	0.0630		0.9370		
16118	3	0.9970	0.0015	0.0015		
16119	2		0.1083		0.8917	
16121	2	0.0717		0.9283		
16122	2		0.1025		0.8975	
16123	2	0.9985	0.0015			
16124	2		0.0015		0.9985	
16126	2		0.0088		0.9912	
16127	2		0.9868		0.0132	
16128	2		0.0015		0.9985	
16129	2	0.9985		0.0015		
16130	2		0.0630		0.9370	
16131	2		0.0088		0.9912	
16133	2		0.0102		0.9898	
16135	2		0.0073		0.9927	
16136	2	0.9985			0.0015	
16137	2		0.0703		0.9297	
16138	2		0.0864		0.9136	
16139	2		0.9707		0.0293	
16140	2		0.9971		0.0029	
16141	2		0.0278		0.9722	
16142	2		0.0073		0.9927	
16143	3	0.9881		0.0059		0.0060
16147	2		0.0673		0.9327	
16148	2		0.0044		0.9956	
16154	2	0.9956		0.0044		
16156	3	0.0015		0.9970	0.0015	
16164	2		0.0044		0.9956	
16165	2		0.0015		0.9985	
16167	2		0.9971		0.0029	
16175	2		0.9985	0.0015		
16177	2		0.0015		0.9985	
16183	2		0.9971			0.0029
16185	2	0.0469		0.9531		
16191	2		0.9941			0.0059
16193	2		0.9985			0.0015
16195	2	0.9883		0.0117		
16196	2	0.0688		0.9312		
16197	2	0.0015		0.9985		
16200	2	0.0249		0.9751		
16201	3	0.0586		0.9370		0.0044
16201+1	2			0.0644		
16204	2		0.0044		0.9956	
16206	2		0.9985		0.0015	
16208	2		0.0015		0.9985	
16209	2		0.9971		0.0029	
16219	2		0.9985		0.0015	
16221	2	0.0015		0.9985		
16225	2	0.0015	0.9985			
16226	2	0.9985			0.0015	
16227	2	0.0015			0.9985	
16228	2	0.9985		0.0015		
16229	2	0.9327		0.0673		
16230	3	0.9970	0.0015	0.0015		
16231	2		0.9780		0.0220	
16232	3		0.9853		0.0132	0.0015

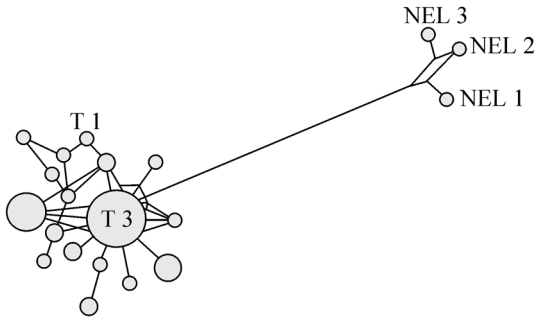

Figure 2 - Reduced median network relating mtDNA haplotypes detected in Brangus-Ibage and Nellore samples. The T1 and T3 reflects ancestral haplotypes of African *B. p. taurus* and European *B. p. taurus* as defined by Troy *et al*. (2001).

The degree of mtDNA genetic diversity of this sample is equal to that of Aberdeen Angus sequences and is between the range of variation observed for the other cattle samples whose nucleotide diversity ranges from 0.003 (Charolais and Friesian) to 0.019 (*B. indicus* Sahiwal and Tharparkar).

The three Nellore sequences we reported are unique and did not match any sequence of this breed so far described in GenBank. Our three sequences clustered with *B. p. indicus* branch on the phylogenetic tree. However, polled with the GenBank published Nellore sequences, this breed clustered in an intermediate position between the major *B. p. indicus* and *B. p. taurus* branches, corroborating earlier findings that Brazilian Nellore has a substantial *B. p. taurus* maternal ancestry (Meirelles *et al.*, 1999).

The strong transitional bias verified here is a characteristic of mtDNA evolution and has been observed not only in cattle but also in other mammalian species (Loftus *et al.*, 1994; Simonsen *et al.*, 1998; Wood *et al.*, 1996).

Three new mutations were observed in Brangus-Ibage and two in Nellore. Among them, the C deletion at position 16,191 and the transition T → C at 16,177 are in Box F of the conserved sequence box, a region of remarkable sequence identity between vertebrates (Steinborn *et al.*, 1998). According to these authors, mutations in this region might be associated with functional constraint. However, we verified that maternal lineages presenting the 16,191 deletion showed significantly higher calf birth weight than animals without this mutation (Henkes *et al.*, 2004). Furthermore, considering the high variation in Nellore mtDNA, it might be interesting to compare the performance of Brangus originated from an opposite crossing (ABG bulls and Nellore cows), carrying *B. p. indicus* mitochondria with the performance of the Brangus-Ibage investigated here (originating from the crossing between Nellore bulls and ABG cows) in different environments in order to uncover any specific mitochondrial influence.

Table 4 - Hierarchical analysis of molecular variance.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among breeds	53	1035.79	1.4264 Va	46.59
Within breeds	610	1028.35	1.6349 Vb	53.41
Total	663	2064.14	3.0613	

Loci with large mutation rates such as D-loop mtDNA frequently exhibit higher population gene diversities than loci with low mutation rates (Chakraborty and Jim, 1992). In the present analysis, 47% of the diversity is due to differentiation among breeds, but this value drops to 14% if only *B. p. taurus* samples are compared. In this last case the variability due to differentiation within population is as high as those verified in other species (Bortolini *et al.*, 1998; Simonsen *et al.*, 1998). Therefore, this very high level of differentiation among breeds verified herein results from the highly divergent *B. p. indicus* sequences.

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References

Anderson S, Bruijn MH, Coulson AR, Eperon IC, Sanger F and Young IG (1982) Complete sequence of bovine mitochondrial DNA. Conserved features of the mammalian mitochondrial genome. J Mol Biol 156:683-717.

Bandelt HJ, Forster P, Sykes BC and Richards MB (1995) Mitochondrial portraits of human populations. Genetics 141:743-753

Bortolini MC, Baptista C, Callegari-Jacques SM, Weimer TA and Salzano FM (1998) Diversity in protein, nuclear DNA, and mtDNA in South Amerinds - Agreement or discrepancy? Ann Hum Genet 62:133-145.

Bradley DG and Cunningham EP (1999) Genetic aspects of domestication, common breeds and their origins. In: Fries R and Ruvinsky A (eds) The Genetics of Cattle. CABI Publishing, Oxon, UK, pp 15-31.

Brown WM, George M and Wilson AC (1979) Rapid evolution of animal mitochondrial DNA. Proc Natl Acad Sci USA 76:1967-1971.

Chakraborty R and Jin L (1992) Heterozygote deficiency, population substructure and their implications in DNA fingerprinting. Hum Genet 88:267-272.

Cymbron T, Loftus RT, Malheiro MI and Bradley DG (1999) Mitochondrial sequence variation suggests an African influ-

ence in Portuguese cattle. Proc R Soc Lond B Biol Sci 266:597-603.

Excoffier L, Smouse PE and Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics 131:479-491.

Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783-791.

Hall T (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41.

Hedges SB (1992) The number of replications needed for accurate estimation of the bootstrap P value in phylogenetic studies. Mol Biol Evol 9:366-369.

Henkes LE, Benavides MV, Oliveira JFC, Moraes JCF and Weimer TA (2004) Evaluation of cytoplasmic genetic effects on reproductive traits in a beef cattle herd. Ciencia Rural 34 (in press).

Kumar S, Tamura K, Jakobsen IB and Nei M (2001) MEGA2: Molecular evolutionary genetics analysis software. Bioinformatics 17:1244-1245.

Loftus RT, MacHugh DE, Bradley DG, Sharp PM and Cunningham P (1994) Evidence for two independent domestications of cattle. Proc Natl Acad Sci USA 91:2757-2761.

Magee DA, Meghen C, Harrison S, Troy CS, Cymbron T, Gaillard C, Morrow A, Maillard JC and Bradley DG (2002) A partial African ancestry for the Creole cattle populations of the Caribbean. J Hered 93:429-432.

Mannen H, Morimoto ML, Oyamat K, Mukai F and Tsuji S (2003) Identification of mitochondrial DNA substitutions related to meat quality in Japanese Black cattle. J Anim Sci 81:68-73.

Meirelles FV, Rosa AJM, Garcia JM, Lobo RB, Smith LC and Duarte FAM (1999) Is the American Zebu really a *Bos indicus*? Genet Mol Biol 22:543-546.

Nei M (1989) Molecular Evolutionary Genetics. Columbia University Press, New York, 512 pp.

Nei M and Tajima F (1981) DNA polymorphism detectable by restriction endonucleases. Genetics 97:145-163.

Oliveira NM, Salomoni E, Leal JJB, Moraes JCF and Del Duca LOA (1998) Genetic and environment effects on growth of ??? Nellore x ??? Aberdeen Angus beef cattle derived from different crossbreeding schemes. Arch Lat Prod Anim 6:173-188.

Plante Y, Schmutz S and Lang K (1992) Restriction fragment length polymorphism in the mitochondrial DNA of cloned cattle. Theriogenology 38:897-904.

Saitou N and Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406-425.

Schneider S, Roessli D and Excoffier L (2000) Arlequin: A software for population genetics data analysis. Ver 2.000. Genetics and Biometry Lab, Dept. of Anthropology, University of Geneva.

Schutz MM, Freeman AE, Beitz DC and Mayfield JE (1992) The importance of maternal lineage on milk yield traits of dairy cattle. J Dairy Sci 75:1331-1341.

Schutz MM, Freeman AE, Lindberg GL, Koehler CM and Beitz DC (1994) The effect of mitochondrial DNA on milk production and health of dairy cattle. Livestock Production Science 37:283-295.

Simonsen BT, Siegismund HR and Arctander P (1998) Population structure of African buffalo inferred from mtDNA sequences and microsatellite loci: High variation but low differentiation. Mol Ecol 7:225-237.

Smith LC, Bordignon V, Garcia JM and Meirelles FV (2000) Mitochondrial genotype segregation and effects during mammalian development: Applications to biotechnology. Theriogenology 53:35-46.

Steinborn R, Muller M and Brem G (1998) Genetic variation in functionally important domains of the bovine mtDNA control region. Biochim Biophys Acta 1397:295-304.

Taanman JW (1999) The mitochondrial genome: Structure, transcription, translation and replication. Biochim Biophys Acta 1410:103-123.

Tamura K and Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 10:512-526.

Troy CS, MacHugh DE, Bailey JF, Magee DA, Loftus RT, Cunningham P, Chamberlain AT, Sykes BC and Bradley DG (2001) Genetic evidence for Near-Eastern origins of European cattle. Nature 410:1088-1091.

Ward RH, Frazier BL, DewJager K and Paabo S (1991) Extensive mitochondrial diversity within a single Amerindian tribe. Proc Natl Acad Sci USA 88:8720-8724.

Wood NJ and Phua SH (1996) Variation in the control region sequence of the sheep mitochondrial genome. Anim Genet 27:25-33.

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