

Predominant African-Derived mtDNA in Caribbean and Brazilian Creole Cattle is also Found in Spanish Cattle (*Bos taurus*)

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African-derived mitochondrial DNA (mtDNA) have been described in South American and Caribbean native cattle populations, which could have been introduced into America from Iberia or by direct importation from Africa. However, the similarity among described haplotypes is not known. We examined mtDNA variation in Guadeloupe Creole and Spanish cattle in an attempt to identify African-derived mtDNA haplotypes and compare them with those previously described. Eleven haplotypes clustered into the European taurine haplogroup (T3), two haplotypes into the African taurine (T1) haplogroup, and three haplotypes into the African-derived American haplogroup (AA). The AA1 and *Eucons* haplotypes were the most frequently observed. The presence of the AA haplogroup in Spanish cattle confirms historical records and genetic evidence of Iberian cattle as the main source of American native cattle origin. The possible origin of African-derived mitochondrial haplotypes in Iberian and Creole cattle is discussed, and the accumulated evidence does not support a founder effect from African ancestral cattle by direct importations. The presence of taurine AA and T3 haplotypes in Brazilian Nelore may indicate introgression by local European-derived cattle. Data presented in this work will contribute to the understanding of the origin of Guadeloupe Creole cattle.

Analysis of mtDNA control region (CR) sequence variation is an important tool for understanding the phylogeography of domestic cattle. Thus far, two major taurine (*Bos taurus*) mtDNA haplogroups have been identified—European

taurine (T3) and African taurine (T1) cattle (Bradley et al. 1996; Loftus et al. 1994; Troy et al. 2001). The European consensus (*Eucons*) and the African consensus (*Afcons*) haplotypes represent the central haplotypes of a star-like phylogenetic network where a number of peripheral haplotypes coalesce.

Both African and European taurine haplotypes are present in Portuguese cattle breeds, which is consistent with historical records of the origins of Portuguese cattle (Cymbron et al. 1999). American Creole cattle breeds are descendant from animals introduced by Spanish and Portuguese conquerors during the first hundred years of colonization (Rabasa 1993), and they also contain African and European haplotypes (Carvajal-Carmona et al. 2003; Magee et al. 2002; Miretti et al. 2000; Miretti et al. 2002; Mirol et al. 2003). Another major haplogroup, AA (African-derived American), has been identified in some South American Creole cattle breeds. Its root, haplotype AA1, shares the three substitutions typical of *Afcons* (Miretti et al. 2002). Therefore, AA1 is more closely related to *Afcons* (diverging in four substitutions) than to the *Eucons* (seven substitutions). Members of the AA haplogroup have not been identified in Portuguese or African cattle breeds (Cymbron et al. 1999; Troy et al. 2001), and to our knowledge there have been no consistent investigations of mtDNA variation in Spanish cattle.

The aim of this work was to investigate the variation within the mtDNA CR of Spanish and Guadeloupe Creole cattle in order to reconstruct connections between American and Iberian cattle, particularly with interest in identifying the

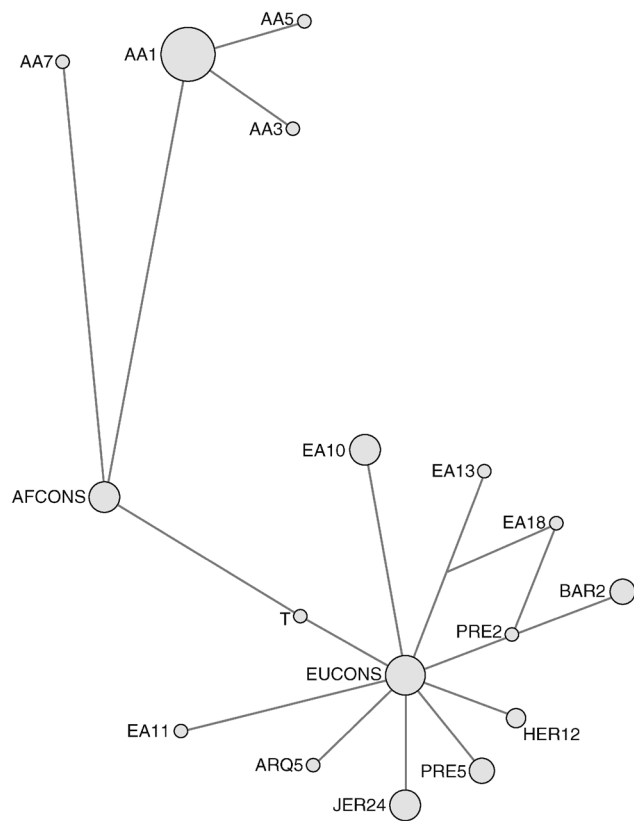


Figure 1. Median Joining Network constructed with mtDNA CR sequences (240 bp) observed in Guadeloupe Creole and Spanish cattle sampled. Nodes represent haplotypes where the circle size is equivalent to their frequency. Length of connections between haplotypes is relative to their divergence (substitution number). Haplotype identification as detailed in Table 1.

presence of AA haplotypes taurine cattle. In Guadeloupe Creole, we found mtDNA haplotypes of European and African taurine origin. The African influence was represented predominantly by the AA haplogroup. Members of the AA haplogroup are also present in Spanish cattle. Tracking the route of the AA haplogroup will contribute, in association with historical records and results from other markers, in elucidating the original source of American native cattle.

Materials and Methods

A 1-kb DNA fragment comprising the hypervariable region of the mtDNA CR was amplified with DNA from 27 samples of three Spanish cattle breeds—in essence, Retinta (RET, $n = 16$), Alistana (ALI, $n = 9$), and Pirenaica (PIR, $n = 2$)—and 28 samples of Guadeloupe Creole cattle (GC). Five Nellore (*Bos indicus*) individuals were assayed in order to determine to which haplogroups the taurine mtDNA found in Brazilian Nellore belongs. According to the restriction analysis (Meirelles et al. 1999), four of them carried taurine

mitochondria. Amplification reactions (200 ng of template DNA, 10 pmol each of primer 5'-ttccgaccactcagccaatg-3' and 5'-tgctgggtgctcaagatgc-3', 2.5 U *Taq* DNA polymerase, 1.5 mM MgCl₂) were subject to the amplification protocol: 94°C for 3 min, 30 cycles of 1 min at 94°C, 45 s at 57°C, 1 min at 72°C, and 4 min at 72°C. Purified polymerase chain reaction (PCR) products (20–40 ng), were used as template for sequencing reactions with primers 5'-aatccaataactcaacaca-3' and 5'-tgctgggtgctcaagatgc-3'.

The consensus sequence of each sample (~450 bp) was obtained by aligning at least one sequence read in the sense and complementary strands. Substitutions within a 240-bp fragment (16023–16262) of the mtDNA CR were checked by chromatogram inspection and polymorphisms were identified by direct comparison with the reference sequence (Anderson et al. 1982), which is the central haplotype (*Eucons*) of the European taurine (T3) cluster (Troy et al. 2001). Variations with respect to *Eucons* or to other root haplotypes (i.e., *Afcons* and AA1) were registered as follows. The *Afcons* haplotype is considered the root of African taurine T1 cluster, as its differences relative to *Eucons* concern C→T, T→C, and T→C transitions at positions 16050, 16113, and 16255, respectively; it can be identified as *Afcons*_(*Eucons* 050T-113C-255C). The AA1 haplotype, root of the AA cluster, shares the same three transitions as *Afcons* and also differs from it in four further substitutions, namely T→C, T→C, C→T, and G→A at positions 16053, 16122, 16139, and 16196, respectively. It could then be coded as AA1_(*Afcons* 053C-122C-139T-196A). Similarly, peripheral haplotypes of the AA haplogroup can be termed AA3_(AA1-115C). To make visible the relative positions of Guadeloupe Creole and Spanish cattle mtDNA haplotypes, a network was constructed with the software NETWORKS 4.0.0.0 (Bandelt et al. 1995; Bandelt et al. 1999, Bandelt et al. 2000).

Results and Discussion

Seventeen different haplotypes were identified by analysis of mtDNA CR sequences from Guadeloupe Creole and Spanish cattle breeds. Plotted in a phylogenetic network (Figure 1), these haplotypes fell into the three major clusters, namely three haplotypes into the African-derived haplogroup found in America (AA), two into the African taurine cluster (T1), and 12 into the European taurine (T3) haplogroup. Troy et al. (2001) have described two other minor haplogroups, T and T2; however, haplotype T can be considered a member of T3 within this context. Nine of the haplotypes reported in this study have been described previously by Bradley et al. (1996) and Cymbron et al. (1999); one, by Miretti et al. (2002). Seven have not been previously published (GenBank accession numbers AY426319–AY426325). Four out of the seven newly described haplotypes grouped within the T3 haplogroup; one, within T1; two, within AA (Table 1). The most frequently observed haplotype was AA1 (15 times), which was also the most common in both Guadeloupe and Retinta breeds (Table 1). Table 1 also lists positions and nucleotides involved in

Table 1. Number, frequency, and substitution details of mtDNA haplotypes found in Spanish and Guadeloupe Creole cattle

Haplotype/n	GC	RET	ALI	PIR	n	Haplotype substitutions	Reference
	28	16	9	2	55		
AA							
AA1	9	6	—	—	15	AA1 _(Afcons-053C-122C-139T-196A)	Miretti et al. (2002)
AA3	1	—	—	—	1	AA3 _(AA1-116C)	This work AY426319
AA5	1	—	—	—	1	AA5 _(AA1-074C)	This work AY426320
T							
Her20	—	—	—	1	1	Her20 _(Eucons-255C)	Troy et al. (2001)
T1							
Afcons	—	4	1	—	5	Afcons _(Eucons-050T-113C-255C)	Bradley et al. (1996)
AA7	1	—	—	—	1	AA7 _(Afcons-133C-164C-165C-195G)	This work AY426321
T3							
Eucons	2	4	2	—	8	Reference sequence	Bradley et al. (1996)
EA10	5	—	—	—	5	EA10 _(Eucons-058T-130C)	This work AY426322
EA11	—	1	—	—	1	EA11 _(Eucons-137C-231T)	This work AY426323
EA13	—	1	—	—	1	EA13 _(Eucons-119C-260T)	This work AY426324
Bar2	—	—	3	—	3	Bar2 _(Eucons-122A)	Cymbron et al. (1999)
Her12	—	—	2	—	2	Her12 _(Eucons-057A)	Troy et al. (2001)
Jer24	5	—	—	—	5	Jer24 _(Eucons-139T)	Troy et al. (2001)
Arq5	1	—	—	—	1	Arq5 _(Eucons-141C)	Cymbron et al. (1999)
Pre5	3	—	—	—	3	Pre5 _(Eucons-042C)	Cymbron et al. (1999)
EA18	—	—	1	—	1	EA18 _(Eucons-122C-260T)	This work AY426325
Pre2	—	—	—	1	1	Pre2 _(Eucons-122C)	Cymbron et al. (1999)
17					31		

GC = Guadeloupe Creole; RET = Retinta; ALI = Alistana; PIR = Pirenaica.

substitutions. The AA1 haplotype (AF517802) is identical to the “atypical African haplotype” (peripheral T1 derivative) reported in Guadeloupe Creole (Magee et al. 2002).

The mtDNA data obtained from Guadeloupe Creole is consistent with observations made in Caribbean native cattle breeds (Magee et al. 2002), as almost all the sequences cluster within two major haplogroups, 11 individuals within AA and 16 within T3. However, our results indicate a larger influence of European taurine mtDNA in Guadeloupe Creole. These results suggest an African influence in the Caribbean cattle, which is consistent with previous results (Magee et al. 2002; Miretti et al. 2000; Miretti et al. 2002) for Guadeloupe and Brazilian cattle.

The African incidence in Guadeloupe Creole in this study is mainly represented by the AA haplogroup. Interestingly, AA1 is also present in the Spanish cattle breed Retinta. This result is consistent with the widely accepted view of Iberian ancestry of American native cattle. The mtDNA data from Colombian Creole cattle also point towards the Iberian admixture of North African taurine mtDNA (Carvajal-Carmona et al. 2003). Alternatively, the African influence may be partially attributable to direct importation of West African cattle into the Americas, and the predominance of the peripheral T1 derivative haplotype may result from a genetic founder effect from the African parental population (Magee et al. 2002). Historical records suggest that direct introduction of African cattle in the French West Indies and Brazil could have occurred from West Africa because of the similar

slave trade routes from French and Portuguese African colonies (Maillard et al. 1993; Primo, 1992).

However, this assertion should be interpreted cautiously. First, no AA members were found in the available data from the “African parental” populations. Second, the common African-derived haplotype found in Caribbean Creole is distantly related to haplotypes found in African cattle; in essence, divergence of the AA1 haplotype and those of the T1 haplogroup found in Africa involves four or more substitutions (within the 240-bp fragment under study). Third, we did not detect any *Afcons* in Guadeloupe Creole. AA7 was considered as a T1 member, but it differs from the *Afcons* in four substitutions. In addition, although Magee et al. (2002) utilize atypical African haplotypes observed in Brazilian Nelore in the analysis of Caribbean cattle origin, it is not obvious why mtDNA from Brazilian Nelore animals would help explain the phylogeography of Caribbean Creole cattle. Notably, only one sample of Caribbean Creole ($n = 59$) studied by Magee et al. (2002) presented a haplotype already described in Africa. Finally, our small survey of Spanish cattle showed a higher proportion of African mtDNA (6 AA1 and 4 *Afcons* haplotypes found in 16 Retinta individuals), which supports Iberian cattle as the original source of American Creole cattle.

Studying the ancestry of the Guadeloupe Creole cattle, Magee et al. (2002) identified the AA1 haplotype in Brazilian Nelore animals. Among the Brazilian Nelore samples bearing taurine mitochondria analyzed in this work, two

mtDNA sequences matched with *Eucons* and two with the haplotype AA1. According to historical records, Brazilian breeders imported approximately 7,000 *Bos indicus* animals from India between the beginning of the 19th century and the 1960s (Magnabosco et al. 1997). Meirelles et al. (1999) demonstrated that Brazilian Nelore, Gir, and Brahman animals might actually carry taurine mitochondria and suggested that Nelore males imported from India mated with native cattle females (*B. taurus*), which gave rise through backcrossing to phenotypically zebuine animals with taurine mitochondria. This might indicate that taurine mtDNA haplotypes were introduced into Nelore animals in Brazil from Brazilian Creole breeds such as those we investigated (Miretti et al. 2002). AA1 could not have been introduced by Nelore importation from India, because Indian cattle have very dissimilar mtDNA sequence. In any case, the origin of AA in Brazilian native cattle still needs to be investigated.

In conclusion, we did not find compelling evidence in these samples to support direct contribution to maternal lineage from West Africa and a genetic founder effect from a parental African population. We identified the presence of AA1 in Spanish cattle, which is a plausible candidate for the origin of the AA haplogroup in Central and South America. Work is in progress to help explain the American native cattle phylogeography, based on a more comprehensive sampling. But, even then, the origin of the AA1 observed in Spanish cattle remains to be uncovered.

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