A Partial African Ancestry for the Creole Cattle Populations of the Caribbean

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Seventy-eight cattle samples from three Creole Caribbean islands and one Brazilian breed were analyzed for sequence variation in the hypervariable segment of the mitochondrial DNA control region. Seventy-three samples displayed *Bos taurus* haplotypes, and five samples exhibited haplotypes that were of *Bos indicus* ancestry. Phylogenetic analysis revealed that all sampled *B. taurus* sequences fell into two distinct clusters with separate African and European origins. European sequences were encountered in each population; however, the distribution of African haplotypes was uneven, with the highest proportion of African influence found in the Guadeloupe Creole. The reduced levels of African haplotypic variation within the Caribbean and Brazilian are consistent with prior founder effects. Additionally, genetic variation at three microsatellite loci illustrated African influence uniquely in the Guadeloupe Creole. Collectively, the data suggest that this African influence is, at least in part, attributable to the historical importation of African cattle to the Americas. Furthermore, alleles of *B. indicus* ancestry were detected at appreciable frequencies in all Caribbean Creole populations and may reflect zebu introgressions from either West Africa or the Indian subcontinent.

Examination of variation in mitochondrial DNA (mtDNA) control region sequences has been pivotal in the elucidation of bovine phylogeography. Initial studies have demonstrated a deep bifurcation in bovine mtDNA phylogeny, which indicates a predomestic divergence between the two major taxa of cattle, humped zebu (*Bos indicus*) and humpless taurine (*Bos taurus*; Loftus et al. 1994). Subsequent genetic investigations have yielded further inference regarding origins within the *B. taurus* lineage. *B. taurus* mtDNA sequences fall into one of five ancestral star-like haplotypic clusters, which are geographically distributed (Mannen et al. 1998; Troy et al. 2001). Just one of these clusters, T3, predominates in Western Europe. Symmetrically, diversity within Africa is composed almost exclusively of members of a separate haplotypic cluster, T1, which is rarely detected elsewhere. The almost mutually exclusive geographic distribution of these two haplotypic clusters allows geographical exceptions to be securely identified as secondary introductions, such as the African mtDNA haplotypes encountered in southern Portuguese breeds (Cymbron et al. 1999).

Cattle were first introduced to the Caribbean by Spanish explorers in 1493 (Wilkins 1984), and by 1525 Spanish cattle had spread throughout the Caribbean and much of Central and South America (Felius 1995). Direct shipments of Portuguese cattle to Brazil have also been reported (de Alba 1978; Primo 1992). West African cattle are thought to have entered the continent during the 16th and 18th centuries, presumably as a consequence of slave trade routes, whereas zebu animals were later imported to improve the adaptability of local herds to tropical conditions (Felius 1995; Maillard et al. 1993; Rouse 1973).

To elucidate the genetic ancestry of Creole cattle, we have analyzed molecular diversity in three cattle populations from the Caribbean and one from the South American mainland, using mtDNA sequence and microsatellite allelic variation. The data presented in this study suggest a partial African ancestry for Caribbean Creole populations, the extent of which greatly varies between Caribbean island populations. Although it is probable that a portion of this African genetic contribution originates from the historical African influence associated with the breeds of the Iberian Peninsula, the detection of zebu-specific microsatellite alleles in the modern Caribbean samples suggests some direct importation of West African cattle to the region.

Materials and Methods

Cattle Samples and DNA Extraction

A total of 78 samples from three Caribbean Creole populations—Antigua (21), St. Lucia (13), and Guadeloupe (25)—together with a Brazilian zebu breed, Nelore (19), were analyzed for mtDNA variation. DNA was extracted from blood or serum according to standard protocols previously outlined by Sambrook et al. (1989).

MtDNA Amplification and Sequencing

Molecular diversity was assessed within the most variable 240 base pair (bp) region of the bovine control region (nucleotide positions 16023–16262), which has been widely examined in previous surveys of bovine mtDNA variation (Cymbron et al. 1999; Troy et al. 2001). Partial mitochondrial control regions were amplified, purified, and sequenced with the primers and conditions described by Cymbron et al. (1999). Variations in the control region were identified by direct comparison with the predominant European T3 mtDNA haplotype as previously defined by Troy et al. (2001).

MtDNA-Based Phylogenetic Reconstruction

MtDNA sequences were aligned by eye, and a reduced median network was

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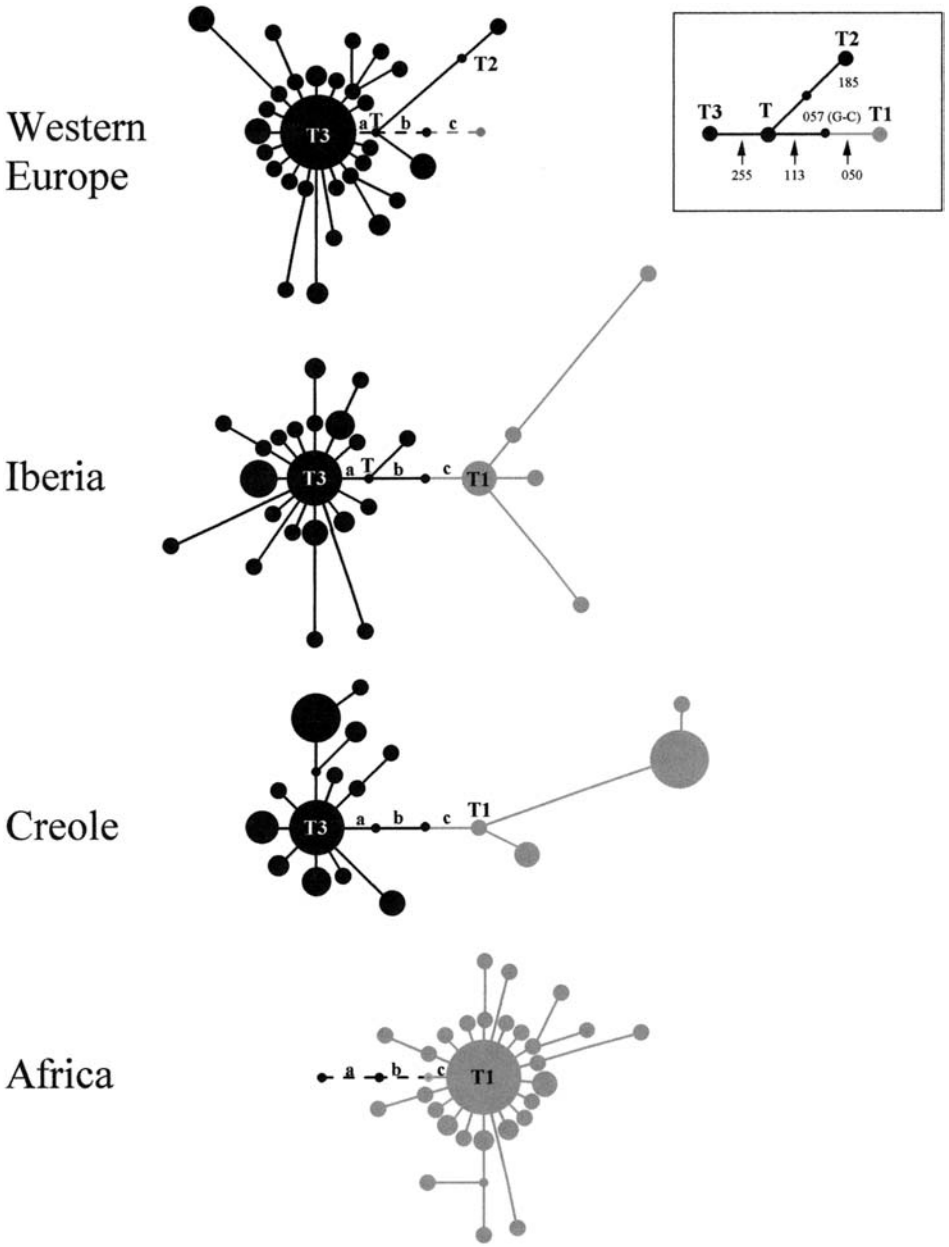

Figure 1. A reduced median network (Bandelt et al. 1995) featuring 73 *Bos taurus* mtDNA lineages sampled in the Caribbean and Brazil. For comparison, reduced median networks are shown for Western Europe (67 samples comprising Charolais, Friesian, German Black, Limousin, Romagnola, and Simmental), Iberia (60 samples comprising Alentejana, Aroquesa, Barrosa, Barrenda, Maronesa, Mertolenga, and Preta), and Africa (61 samples comprising Kapsiki, Kuri, Namchi, N'Dama, Somba, and White Fulani) from previously published data (Cymbron et al. 1999; Troy et al. 2001). The four ancestral *B. taurus* haplotypes, T, T1, T2, and T3, as defined by Troy et al. (2001), are shown (inset). Almost all European variation is composed of members of the T3 haplogroup, whereas continental African sequences cluster around the T1 ancestral variant. The relationship between European (T3) and African (T1) central haplotypes is defined by transitions at nucleotide positions 16255, 16113, and 16050, denoted a, b, and c, respectively. Haplotypes encountered in each region (shaded circles) and unsampled intermediate nodes (small points) are shown. Circle areas are proportional to the frequency of each haplotype, and shading indicates to which of the two skeleton network haplotypes they root. Lines connecting circles denote nucelotide substitutions.

constructed manually according to the methods outlined by Bandelt et al. (1995). With use of 188 previously published *B. taurus* mtDNA sequences (Cymbron et al. 1999; Troy et al. 2001), the resulting phylogeny was compared directly to networks constructed for continental European, Iberian, and African populations (Figure 1). The Creole mtDNA sequences reported in this study have been submitted to GenBank.

Microsatellite Markers and Genotyping

All three Caribbean Creole populations, plus three Portuguese populations, were assayed for allelic length variation at three autosomal microsatellite loci: *BM2113* (Sunden et al. 1993), *HEL1* (Kaukinen and Varvio 1993), and *ILSTS001* (Brezinsky et al. 1992). Microsatellite allele typing was performed according to Loftus et al. (1999). Allele frequencies for each locus were determined by direct counting. The *BM2113* and *ILSTS001* loci have been described previously as displaying a total of four alleles specific to West African *B. taurus* breeds, whereas three alleles across the *HEL1* and *ILSTS001* loci are considered to be exclusive to populations exhibiting *B. indicus* ancestry (MacHugh et al. 1997). The percentages of both allele groups were averaged to estimate the proportion of West African taurine and zebu admixture for each Caribbean Creole population (Table 1).

Results

MtDNA Control-Region Sequence Variation and Phylogenetic Network Reconstruction

In total, 73 samples displayed *B. taurus* mtDNA sequences. The remaining five sequences were typical of *B. indicus* lineages and were detected exclusively in the Nelore breed. Comparison of all Creole *B. taurus* mtDNA sequences revealed a total of 17 haplotypes, differentiated at 20 polymorphic sites, all of which displayed transitions. This result conforms to the strong transitional bias previously described in the bovine mtDNA control region (Bradley et al. 1996; Cymbron et al. 1999; Loftus et al. 1994; Troy et al. 2001).

Figure 1 places each of the 73 *B. taurus* Creole sequences in a reduced median network. For comparative purposes, networks were also constructed from previously published Western European, Iberian, and African mtDNA sequences (Cymbron et al. 1999; Troy et al. 2001). Essentially, almost all mtDNA sequences sampled in Europe clustered around the predominant, centrally positioned haplotype T3, through which derivative haplotypes rooted. In Africa mtDNA sequences coalesced to an alternative, predominant variant, T1. The T3 and T1 central variants were separated by three transitions, and the star-like phylogeny of both clusters was consistent with past population expansions, presumably

associated with the domestication process (Bradley et al. 1996; Troy et al. 2001). Within Iberia sequences fell into either the European (T3) or African (T1) haplotypic clusters, indicating that these Iberian cattle samples share a partial African ancestry, which is most likely a legacy of historical North African influence in the peninsula (Cymbron et al. 1999).

All Creole *B. taurus* sequences fell into one of the two distinct European and African continental clusters. Here, the European central mtDNA variant was encountered 16 times, whereas only one sample possessed the central African haplotype. Three additional haplotypes shared all three African T1 haplogroup-defining substitutions, while the remaining 12 haplotypes were typically European. Sequences of European origin revealed patterns of diversity which are consistent with those noted for the European parental population. In contrast, the Creole T1 cluster was atypical of the patterns encountered within the parental African continental population. The most frequently represented African sequence (18 samples) was differentiated from the ancestral T1 haplotype by a total of four nucleotide substitutions and has not been reported in previous surveys of bovine mtDNA phylogeography.

Mitochondrial Admixture Analysis

The proportion of sequences possessing all three T1 haplogroup-defining substitutions were tabulated for each Caribbean Creole population and are presented in Table 1. For *B. taurus* mtDNA sequence data, the proportion of African mtDNA haplotypes was highest in the Guadeloupe Creole and Nelore populations. Conversely, the incidence of African mtDNA haplotypes in the St. Lucian and Antiguan Creole populations was low.

Microsatellite Allelic Variation and Admixture Analysis

A total of 30 microsatellite alleles were detected in the Caribbean Creole and Iberian samples assayed across all three autosomal microsatellite loci. The mean percentage of West African taurine-specific alleles in three African *B. taurus* populations was estimated at 39.4%, with low frequencies detected within Iberia. West African taurine-specific alleles were not detected within continental European populations. The Guadeloupe Creole was the only Caribbean population to exhibit West African–specific alleles. *B. indicus*–specific alleles, the average frequency of which was estimated here as 67.4% in an Indian zebu sample, were also detected at appreciable frequencies within each assayed Caribbean Creole population.

Table 1. The proportion of African mtDNA haplotypes and West African *Bos taurus*-specific and *Bos indicus*-specific microsatellite alleles detected in each assayed Caribbean Creole population

	MtDNA[a]	Microsatellite data	
Population	African T1 haplotypes (%)	West African taurine-specific alleles (%)	South Asian zebu-specific alleles (%)
Continental Europe[b]	0.0	0.0	0.0
Iberia[c]	18.3	1.5	0.0
Antiguan Creole (AC)	4.8	0.0	21.0
Guadeloupe Creole (GC)	60.0	6.6	22.5
Nelore (NE)	42.8	—	—
St. Lucian Creole (SC)	7.7	0.0	11.9
West African taurine[d]	100.0	39.4	2.6
Indian zebu[e]	0.0	—	67.4

[a] European, African, Iberian *B. taurus* mtDNA data taken from Cymbron et al. (1999) and Troy et al. (2001). Indian zebu mtDNA data taken from Loftus et al. (1994).

[b] Source European population for microsatellite analysis consisting of Charolais (n = 36), Friesian (n = 40), and Simmental (n = 36) samples. Data taken from MacHugh et al. (1997).

[c] Iberian sample population for microsatellite analysis consisting of Alentejana (n = 30), Aroquesa (n = 34), and Mertolenga (n = 37) samples. Data reported for the first time here.

[d] West African *B. taurus* population for microsatellite analysis consisting of N'Dama populations sampled from Guinea (n = 63), Guinea Bissau (n = 54), and Mali (n = 44). Data taken from MacHugh et al. (1997).

[e] Indian zebu population for microsatellite analysis consisting of Hariana (n = 10), Sahiwal (n = 13), and Tharparker (n = 10) samples. Data taken from MacHugh et al. (1997).

Discussion

The data presented in this study suggest a partial African ancestry for the American Creole cattle. MtDNA phylogentic analysis demonstrates that all Creole *B. taurus* sequences fall into two separate haplotypic clusters, which are representative of European and African continental lineages, as previously described by Troy et al. (2001). The contribution of African mitochondrial sequences to the total *B. taurus* Creole mtDNA pool is 31.5%, peaking in the Guadeloupe Creole (60.0%) and Brazilian Nelore (42.8%) populations. Conversely, the mtDNA pools of the St. Lucian and Antiguan Creole populations are predominantly European, with African mtDNA admixture proportions of 7.7% and 4.8%, respectively. The Guadeloupe Creole samples also display an average of 6.6% microsatellite alleles that are West African taurine-specific in origin, with no such alleles detected in the Creole populations sampled in St. Lucia and Antigua. Genetic contributions from introgressing zebu cattle are also observed in each of the assayed Caribbean Creole populations, with mean zebu-specific allele frequencies of 22.5% in Guadeloupe, 21.0% in Antigua, and 11.9% in St. Lucia.

B. taurus mtDNA phylogeographic analysis has identified African mtDNA haplotypes in Iberian cattle (Cymbron et al. 1999). Here, 18.3% of the Iberian mtDNA sample was of African origin, while an average of 1.5% of Iberian microsatellite alleles were diagnostic of West African taurine ancestry. It is therefore conceivable that a portion of the African influence noted in Caribbean Creole cattle, particularly that in Guadeloupe, may have originated from either Spanish or Portuguese introductions.

However, two lines of genetic evidence suggest that this African influence is partially attributable to the direct importation of West African cattle to the Caribbean. First, the proportion of African ancestry in the Caribbean Creole cattle, particularly in Guadeloupe, is substantially higher than that reported for the Iberian populations considered in this study. Second, the detection of *B. indicus*–specific alleles within each Caribbean Creole population, plus the absence of *B. indicus* mtDNA haplotypes, which is a feature of African zebu populations, suggest West African zebu cattle importations to the region (Hanotte et al. 2002; MacHugh et al. 1997; Maillard et al. 1993).

It is also possible that some of this *B. indicus* ancestry reflects male-mediated introgression from the Indian subcontinent to the Americas (Felius 1995; Giovambattista et al. 2000; Rouse 1973). Furthermore, the introduction of zebu dams from the Indian subcontinent to

Brazil is also supported by the occurrence of five *B. indicus* mtDNA sequences in the Nelore samples analyzed in this study.

Troy (1998) has demonstrated that approximately five mtDNA haplotypes are encountered in a typical European breed sample size of 12 individuals. A similar value was obtained for the Creole T3 cluster, in which 13 haplotypes were encountered in 50 sequences from one Brazilian and three Caribbean cattle populations. Additionally, the topology of the Creole T3 haplogroup is consistent with that observed for European populations, suggesting that this diversity is a relatively even sample of that encountered within continental Europe. In contrast, the diversity within the Creole T1 haplogroup is markedly different from that found within continental Africa. Here, only four haplotypes were encountered in a total of 23 samples, with a previously undescribed T1 derivative haplotype separated by four substitutions from the T1 central variant, comprising 78.3% of all the African haplotypic variation. Notably, the T1 central haplotype was represented only once. Typically, a continental African breed consisting of 12 samples displays an average of five haplotypes (Troy 1998). This overall reduction in African haplotypic diversity and numerical predominance of a peripheral T1 derivative haplotype within the Creole T1 haplogroup may suggest only a genetic founder effect, in which a restricted subset of the variation within the African parental population has survived and is displayed in modern populations.

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