Abundant mtDNA Diversity and Ancestral Admixture in Colombian *criollo* Cattle (*Bos taurus*)

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ABSTRACT

Various cattle populations in the Americas (known as *criollo* breeds) have an origin in some of the first livestock introduced to the continent early in the colonial period (16th and 17th centuries). These cattle constitute a potentially important genetic reserve as they are well adapted to local environments and show considerable variation in phenotype. To examine the genetic ancestry and diversity of Colombian *criollo* we obtained mitochondrial DNA control region sequence information for 110 individuals from seven breeds. Old World haplogroup T3 is the most commonly observed CR lineage in *criollo* (0.65), in agreement with a mostly European ancestry for these cattle. However, *criollo* also shows considerable frequencies of haplogroups T2 (0.9) and T1 (0.26), with T1 lineages in *criollo* being more diverse than those reported for West Africa. The distribution and diversity of Old World lineages suggest some North African ancestry for *criollo*, probably as a result of the Arab occupation of Iberia prior to the European migration to the New World. The mtDNA diversity of *criollo* is higher than that reported for European and African cattle and is consistent with a differentiated ancestry for some *criollo* breeds.

THE analysis of mtDNA sequence diversity has provided important insights into the origin and diversification of modern cattle populations. The main phenotypic subdivision of cattle into *Bos taurus* and *Bos indicus* has been shown to correlate with a marked sequence differentiation at the mtDNA level (Loftus *et al.* 1994). This sequence divergence seems to predate archeological estimates of cattle domestication (~12,000 years), in agreement with independent domestication events for *B. taurus* and *B. indicus* (Loftus *et al.* 1994). More recently, it has been observed that mtDNA lineages of European *B. taurus* are a subset of those found in the Near East, an observation consistent with a Near Eastern origin for European cattle (Troy *et al.* 2001). Interestingly, African cattle have mtDNA lineages that are phylogenetically differentiated from other *taurus* populations and these lineages are rare in the Near East, suggesting a separate domestication center for African *B. taurus* (Bradley *et al.* 1996; Troy *et al.* 2001).

Before the arrival of Europeans, there were few domesticated animals in the Americas (Primo 1992; Del Rio Moreno and López y Sebastián 1998). The "cattling" of the continent closely followed the routes of dispersion of immigrants and was accompanied by a gradual phenotypic differentiation of cattle populations (Pinzón Martínez 1984). A number of distinct local *criollo* breeds are now found throughout the Americas. These cattle show great phenotypic heterogeneity and have adapted to a wide range of environments with little human intervention (Pinzón Martínez 1984). Traits of potential interest for breeding that have been reported in Latin American *criollo* include tolerance to heat and humidity, resistance to certain infectious diseases, and a high longevity and fertility (Pinzón Martínez 1984; Primo 1992; Giovambattista *et al.* 2001; Miretti *et al.* 2001). Despite the potential of *criollo* as a genetic resource, little is known about the genetic makeup and population structure of these breeds.

Here we examine the mtDNA control region sequence diversity of seven Colombian *Criollo* breeds. The mtDNA diversity of these cattle is higher than that in European or African breeds and is comparable to that reported for the Near East. About 26% of Colombian *criollo* mtDNA lineages have an African ancestry, most likely due to population exchanges between North Africa and Iberia prior to the introduction of cattle to the Americas. Findings in *criollo* suggest a differentiated ancestry for some breeds and are consistent with a domestication center for cattle in North Africa.

CR sequences from this article have been deposited with the GenBank Data Library under accession nos. AY4444383–AY444492.

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MATERIALS AND METHODS

Cattle samples: A total of 110 blood samples were collected from seven Colombian *criollo* breeds (Figure 1): Blanco Oreji-

FIGURE 1.—Approximate geographic location of the seven Colombian *criollo* breeds examined.

negro (Bon, $n = 19$), Chino Santandereano (Chino, $n = 8$), San Martinero ($n = 19$), Casanareño ($n = 4$), Hartón del Valle (Hartón, $n = 21$), Costeño con Cuernos (Costeño, $n = 19$) and Romosinuano (Romo, $n = 20$). Samples for five of these breeds (Bon, San Martinero, Casanareño, Costeño, and Romo) were obtained from herds maintained by the Colombian Ministry of Agriculture (CORPOICA). Chino and Hartón were sampled in private herds. Available genealogical records were examined to avoid the sampling of closely related animals. DNA was extracted using the QIAamp DNA mini kit (QIAGEN, Valencia, CA), following the manufacturer's instructions.

DNA sequencing: The mitochondrial control region (CR) was sequenced between positions 15,969 and 16,324 (~350 bp). PCR reactions were performed in a final volume of 50 μl containing 50 ng of DNA, 200 nmol of primers AN4 (5′-GGT AATGTACATAACATTAATG-3′) and AN3 (5′-CGAGATGT CTTATTTAAGAGG-3′; CYMBRON *et al.* 1999), 200 μM dNTPs, 10 mM Tris-HCl (pH 9.0), 50 mM KCL, 2.5 mM $MgCl_2$, and 1 unit of Taq DNA polymerase (GIBCO). A total of 35 cycles of 94° for 1 min, 55° for 1 min, and 72° for 1 min were carried out in an MJ PTC200 PCR machine. PCR products were purified using Centricon columns following the supplier's instructions. Sequencing was carried out from both ends of the PCR products using the ABI (Foster City, CA) Big Dye cycle sequencing kit and protocol. Sequencing products were run on an ABI 3700 genetic analyzer. Sequence editing and alignment were carried out using CHROMAS (Technelyium pty, Queensland, Australia) and GeneDoc (NICHOLAS and NICHOLAS 1997) software, respectively.

For comparison with the *criollo* data, published *B. taurus* CR sequences from Europe ($n = 208$), Africa ($n = 92$), and the Near East ($n = 80$) were collated (BRADLEY *et al.* 1996; TROY *et al.* 2001). These comparisons were limited to the region of overlap across data sets between positions 16,023 and 16,262 of the reference mtDNA sequence (ANDERSON 1982).

Data analysis: Haplotype diversity, mean number of pairwise differences (MNPD), F_{ST} (NEI 1987), and the pairwise mismatch distribution between CR sequences were obtained using the Arlequin 2000 computer package (SCHNEIDER *et al.* 2000). Reduced median networks (BANDELT *et al.* 1995) were generated using the NETWORK 3.0 program (http://www.fluxus-technology.com/). A principal component analysis (RENCHER 2002) of the frequency of mtDNA sequences in the *criollo* breeds was performed with the Ntsys package (version 2.1; ROHLF 2001).

RESULTS

mtDNA lineages in Colombian *criollo*: The 110 *criollo* mtDNA CR sequences obtained yield 29 different haplotypes defined by 33 polymorphic sites (30 transitions and 3 transversions; Figure 2). On the basis of the nucleotide present at defining positions, the *criollo* sequences can be assigned to three of the four major Old World mtDNA lineages defined by TROY *et al.* (2001): 71 to haplogroup T3 [corresponding to the European reference sequence (ANDERSON 1982)], 29 to haplogroup T1 (defined by positions 16050, 16113, and 16255), and 10 to haplogroup T2 (defined by positions 16185, 16255, and 16057). Eighteen of the *criollo* lineages were detected more than once with 11 of these being found in several breeds (Figure 2). Five of the Colombian *criollo* CR sequences have been previously detected in the Old World. Of these, Col1 and Col20 correspond to T3 and T1 sequences that are markedly predominant in European and African cattle, respectively (BRADLEY *et al.* 1996; TROY *et al.* 2001). Lineage Col1 was observed in all the breeds examined except Bon and this sequence has the highest overall frequency in *criollo* (~0.33; Figure 2).

Figure 3 shows a median-joining network relating the mtDNA sequences observed in Colombian *criollo*. Three major clusters can be recognized, corresponding to haplogroups T1, T2, and T3 of TROY *et al.* (2001). At the center of the T3 cluster is the modal sequence seen in *criollo* and European cattle (Col1). A similar star-shaped network has been observed in European breeds (TROY *et al.* 2001). By contrast, the T1 lineages found in Colombian *criollo* form a dispersed cluster and show no marked predominance of one sequence (Figure 3). This pattern differs markedly from findings in cattle from Africa (BRADLEY *et al.* 1996; TROY *et al.* 2001). In the African breeds studied to date (mostly sub-Saharan), one sequence (equivalent to Col20) accounts for >40% of haplogroup T1 lineages and is at the center of a tightly structured star-shaped cluster (BRADLEY *et al.* 1996; TROY *et al.* 2001). Figure 4 shows the pairwise mismatch distribution of CR sequences in Colombian *criollo* and in West African cattle, a potential source for *criollo* T1 lineages. This distribution is shifted to the right in the Colombian cattle, resulting in an MNPD for *criollo* of 2.12 ($n = 29$) *vs.* an MNPD of 1.60 ($n = 50$) in West Africa. Similarly, the haplotype diversity of T1 lineages in *criollo* is higher than that in West Africa (0.90 *vs.* 0.81, respectively).

Diversity of Colombian *criollo* breeds: Estimates of MNPD in *criollo* range from 1.99 in Romo to 4.70 in Hartón (Table 1). The MNPD is higher in Bon, Chino,

	Nucleotide Position																															Frequency							
	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	3								
	1	2	4	4	5	5	5	5	7	7	8	9	1	1	1	1	2	2	3	3	3	3	4	4	8	9	0	3	4	4	5	6	0							
	3	2	2	9	0	5	6	7	3	4	4	5	0	2	3	9	1	7	3	5	8	9	3	7	5	5	0	1	7	8	5	4	2							
BRS	A	G	T	C	C	T	A	G	A	T	C	A	C	T	T	T	G	C	T	T	T	C	A	T	G	A	G	C	C	C	T	G	G	BO	CA	CS	RO	HV	SM	CC	N
T3																																									
Col 1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		2	3	13	4	4	10	36
Col 2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.					3	2	1	6
Col 3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.						6		6
Col 4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3							3
Col 5	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.			1			2		3
Col 6	.	.	.	.	..	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3					1		4
Col 7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	3							3
Col 8	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2							2
Col 9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	1							1
Col 10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.				1				1
Col 11	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	1							1
Col 12	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		1						1
Col 13	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	T	.	.	.	.	.						1		1
Col 14	.	.	C	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.						1		1
Col 15	.	.	.	.	.	.	.	.	.	C	.	.	T	.	.	.	.	.	.	.	.	.	G	.	.	.	.	T	.	.	.	.	.			1					1
Col 16	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	T	.	.	.	.	.							1	1
T1																																									
Col 17	.	A	.	.	**T**	C	.	.	.	.	.	.	.	.	**C**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**C**	.	.	1				4			5
Col 18	.	.	.	.	**T**	.	.	.	.	.	.	.	.	.	**C**	.	.	.	.	.	.	T	.	C	.	.	.	.	.	.	**C**	.	.		1	1	1		1		4
Col 19	.	.	.	.	**T**	.	.	.	.	.	T	.	.	.	**C**	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	**C**	.	.			1				2	3
Col 20	.	.	.	.	**T**	.	.	.	.	.	.	.	.	.	**C**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**C**	.	.				1	1		1	3
Col 21	.	.	.	.	**T**	.	.	.	G	.	.	.	.	.	**C**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**C**	.	.				1			2	3
Col 22	.	A	.	.	**T**	.	.	.	.	.	.	.	.	.	**C**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**C**	.	.				3				3
Col 23	.	.	.	.	**T**	.	.	.	.	.	.	.	.	.	**C**	.	.	.	.	.	.	.	.	.	.	.	A	.	.	T	**C**	.	.					2			2
Col 24	.	.	.	.	**T**	.	.	.	.	.	.	.	.	.	**C**	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	**C**	.	.						1	1	2
Col 25	.	.	.	.	**T**	.	.	.	.	.	.	.	T	.	**C**	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	**C**	.	.	2							2
Col 26	.	.	.	.	**T**	.	.	.	.	.	.	.	.	.	**C**	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	**C**	.	.	1							1
Col 27	.	.	.	.	**T**	.	.	.	.	.	T	.	.	.	**C**	.	.	.	.	.	.	T	.	.	.	.	.	.	T	.	**C**	.	.					1			1
T2																																									
Col 28	.	.	.	.	.	.	.	**C**	.	.	.	.	.	.	.	.	.	.	C	C	.	.	.	**A**	.	.	.	.	.	**C**	.	.	2			1		6			9
Col 29	.	.	.	.	.	.	.	**C**	.	.	.	.	.	.	C	.	.	.	.	C	.	.	.	**A**	.	.	.	.	.	**C**	.	.								1	1
																																		19	4	8	20	21	19	19	110

FIGURE 2.—mtDNA CR sequences and their frequency in seven Colombian *criollo* breeds. Only variant positions relative to the bovine reference sequence (BRS) of ANDERSON (1982) are shown. Identities are indicated by a dot and the prefix "16" has been omitted from the nucleotide position. The sequences have been grouped into lineages T1, T2, and T3 on the basis of diagnostic changes (shown in boldface and described in the text). BO, Blanco Orejinegro; CA, Casanareño; CC, Costeño con Cuernos; CS, Chino Santandereano; HV, Hartón del Valle; RO, Romosinuano; and SM, San Martinero.

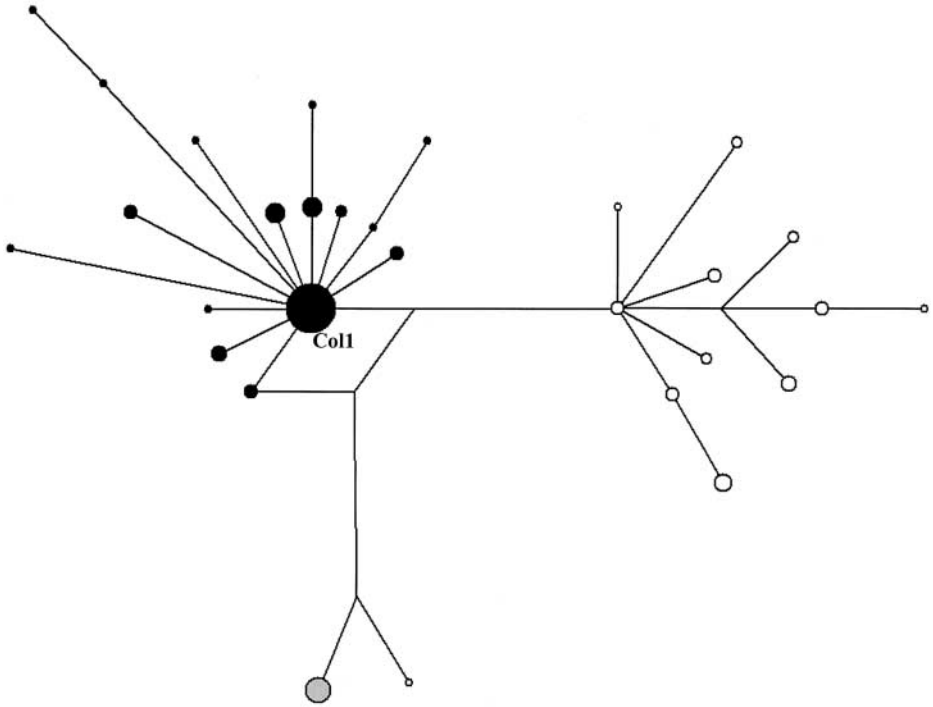

FIGURE 3.—Median-joining network (BANDELT *et al.* 1995) relating the mtDNA CR sequences observed in seven Colombian *criollo* breeds. T1 lineages, open circles; T2, shaded circles; and T3, solid circles. The position of sequence Col1 is indicated. Circle size is proportional to sequence frequency.

and Hartón (MNPD 4.46–4.70), which show the most uniform distribution of the three Old World haplogroups. Lineages T1 and T3 were observed in all the breeds examined (Table 1). In Hartón, T1 lineages predominate (0.38) while in the other breeds T3 lineages are the most common. Hartón is also notable in that it shows the highest frequency of the T2 lineage (0.29). In three breeds (San Martinero, Casanareño, and

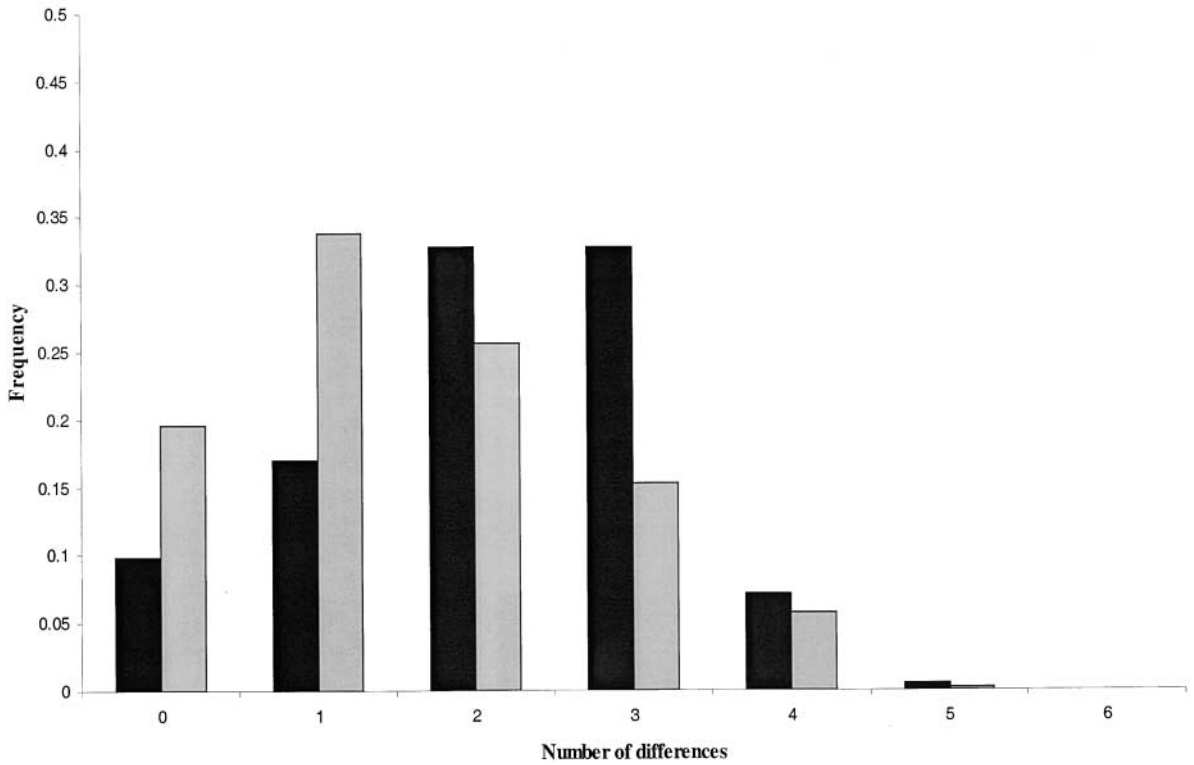

FIGURE 4.—Pairwise mismatch distribution of haplogroup T1 CR sequences in Colombian *criollo* ($n = 29$, solid bars) and in cattle from West Africa ($n = 50$, shaded bars). Data for Africa are from BRADLEY *et al.* (1996) and TROY *et al.* (2001).

TABLE 1

Relative frequency of three Old World mtDNA haplogroups (T1, T2, and T3) and mean number of pairwise differences in CR sequences from seven Colombian *criollo* breeds

Breed (*n*)	T1	T2	T3	MNPD (SE)
Bon (19)	0.21	0.11	0.68	4.46 (2.30)
Casanareño (4)	0.25	0.00	0.75	3.00 (1.96)
Chino (8)	0.25	0.13	0.63	4.57 (2.51)
Costeño (19)	0.32	0.05	0.63	2.88 (1.58)
Hartón (21)	0.38	0.29	0.33	4.70 (2.39)
Romo (20)	0.30	0.00	0.70	1.99 (1.17)
San Martinero (19)	0.11	0.00	0.89	2.20 (1.27)
Colombian *criollo* (110)	0.26	0.09	0.65	3.49 (1.79)

Romo) the T2 lineage was not observed and these breeds also show the highest frequency of the T3 lineage (0.70–0.85). On aggregate, the frequencies of lineages T1, T2, and T3 in *criollo* are 0.26, 0.09, and 0.65, respectively (Table 1).

The variation in frequency of CR sequences across breeds results in an F_{ST} for Colombian *criollo* of 0.20. Figure 5 shows the relatedness of the seven *criollo* breeds examined on the basis of a principal component analysis of CR sequence frequency. Bon, San Martinero, and Hartón appear relatively differentiated from the other four breeds and a close affinity is observed between Romo and Costeño.

DISCUSSION

The advanced agricultural communities of Central and South America domesticated a large number of plants but only a few animals (Diamond 2002). The near absence of livestock facilitating transportation and food production was an important hurdle for the Spanish colonization of America. Consequently, as early as Columbus' second trip (1493), horses, cattle, and sheep were brought to the New World from Europe. Such imports arrived initially to islands in the Caribbean and around two decades later, the first herds were taken to the American mainland (Pinzón Martínez 1984; Primo 1992; Del Rio Moreno and López y Sebastián 1998). The first documented arrival of livestock into current Colombia dates from 1523 when conquistador Rodrigo de Bastidas brought 200 cattle, 300 pigs, and 25 horses from Spain to the Caribbean port of Santa Marta (Pinzón Martínez 1984). Other than the direct arrival of livestock to Colombian Caribbean ports, some additional cattle were introduced by land from neighboring Venezuela and Ecuador. Available documents indicate that Colombian *criollo* descend from these early colonial imports (Pinzón Martínez 1984).

Old World mtDNA lineages in Colombian *criollo* breeds: The population surveys carried out by Bradley *et al.* (1996) and Troy *et al.* (2001) indicate that CR haplogroups T3 and T2 are common in the Near East while haplogroup T1 is restricted mostly to African cattle. In Europe, haplogroup T3 is markedly predominant (0.94, $n = 208$) and shows a reduced diversity relative

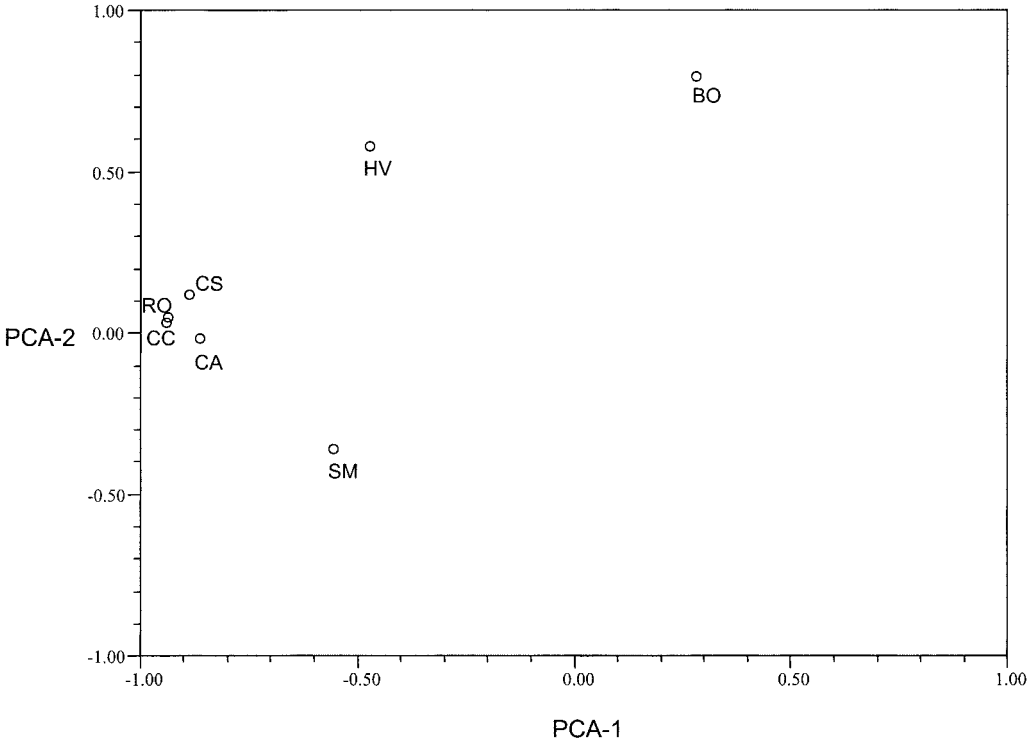

Figure 5.—Principal component analysis of the frequency of mtDNA CR sequences in seven Colombian *criollo* breeds (shown in Figure 2). The two components displayed account for 71% of the variance in sequence frequency. BO, Blanco Orejinegro; CA, Casanareño; CC, Costeño con Cuernos; CS, Chino Santandereano; HV, Hartón del Valle; RO, Romosinuano; and SM, San Martinero.

to Near Eastern cattle, consistent with an origin of European cattle in the Near East (Troy *et al.* 2001). In agreement with the documented European origin of Colombian *criollo*, the most common haplogroup in these cattle is T3 (0.65) and the same sequence (Col1) predominates in *criollo* as in European breeds. However, Colombian *criollo* also shows a relatively high frequency of lineages T1 (0.26) and T2 (0.09; Table 1). This observation suggests a substantial non-European ancestry in *criollo*. The admixed origin of these cattle could relate to ancestral gene flow between North Africa and Iberia prior to the introduction of cattle to Colombia. Alternatively, although there is no documentary evidence, it is possible that African cattle could have arrived in Colombia on the ships transporting slaves from West Africa (Curtin 1969).

An assessment of the North African *vs.* West African ancestry of Colombian *criollo* is complicated by the fact that available CR sequence data refer mostly to cattle from Northwestern Europe and sub-Saharan Africa. In the survey of Troy *et al.* (2001) only one breed from Spain (Berrenda, $n = 10$) and one breed from North Africa (Egyptian, $n = 11$) were examined. However, several observations indicate that the non-European ancestry of *criollo* is most likely the result of cattle admixture in Iberia rather than in Colombia. First, although Troy *et al.* (2001) did not detect T1 lineages in the European breeds they examined, another study has documented the existence of T1 lineages in Portuguese cattle, thus demonstrating some level of African admixture in Iberia (Cymbron *et al.* 1999). Second, a North African ancestry for *criollo* is consistent with the relatively high frequency (0.09) of lineage T2 in Colombian cattle. The T2 lineage has not been detected in sub-Saharan Africa ($n = 92$), is rare in Europe (0.03, $n = 208$), and has maximal frequency in the Near East (0.23, $n = 80$). Third, an important direct introduction of cattle from West Africa to Colombia would be expected to result in a pattern of diversity of T1 lineages in *criollo* similar to that seen in West African breeds (*i.e.*, a marked predominance of lineage Col20). However, the sequence diversity of the T1 haplogroup in Colombian *criollo* is in fact higher than that in West Africa and there is no clear predominance of one lineage (Figures 3 and 4). Finally, the frequency and diversity of the T1 haplogroup in *criollo* would suppose the introduction of a substantial number of animals from West Africa, particularly since most of the slave trade occurred about two centuries subsequent to the first arrival of cattle to South America (Curtin 1969), at a time when *criollo* populations would have already expanded. A large importation of cattle from West Africa seems unlikely to have gone unrecorded.

Gene flow between cattle populations of Iberia and North Africa could have occurred at various times throughout history (Cymbron *et al.* 1999). Exchanges between these two regions were particularly frequent throughout the long period of Arab occupation of the peninsula (A.D. 711–1492), during which the Arabs developed important cattle and sheep farms in the peninsula (Del Rio Moreno and López y Sebastián 1998; Baja-Pereira *et al.* 2002). The likely North African ancestry of *criollo* and the observation that T1 lineages in these cattle are more diverse than those reported for sub-Saharan Africa indicate a higher mtDNA diversity in North African cattle relative to sub-Saharan breeds, consistent with the high mtDNA diversity of Egyptian cattle relative to sub-Saharan breeds (respectively, MNPD 3.71 *vs.* an average MNPD for eight breeds of 1.72, SE ±0.27). A greater mtDNA diversity of North African cattle is to be expected on the basis of archeological evidence of a domestication center for cattle in North Africa (Smith 1986; Wendorf and Schild 1994) and with proposed models for the spread of pastoralism in Africa (Hanotte *et al.* 2002).

Within and between breed diversity in criollo: A high level of mtDNA diversity was observed in Colombian cattle (Table 1). The average MNPD for the seven *criollo* breeds (3.18, SE ±0.43) is markedly higher than that in European (MNPD 1.8, SE ±0.24, $n = 18$) and African (MNPD 1.94, SE ±0.33, $n = 9$) populations (Bradley *et al.* 1996; Troy *et al.* 2001). The diversity of Colombian cattle approaches that of Anatolian/Near Eastern breeds (MNPD 3.77, SE ±0.25, $n = 8$; Troy *et al.* 2001). Diversity is particularly elevated in the three Andean populations (Bon, Chino, and Hartón), in which the three Old World lineages (T1–T3) are present at relatively high frequencies (>10%). The lowest mtDNA diversity is observed in Romo (MNPD 1.99; Table 1), and the principal component analysis (PCA) of the CR data indicates that this breed is closely related to Costeño (Figure 5). Autosomal microsatellites also show a lower genetic diversity for Romo relative to other Colombian *criollo* breeds and a close affinity between this breed and Costeño (L. G. Carvajal-Carmona and A. Ruiz-Linares, unpublished data). These findings are consistent with the proposed origin of Romo from Costeño (Pinzón Martínez 1984). Other than the lack of horns in Romo (Romo Sinuano means "hornless from the Sinú"), the phenotype of these two breeds is very similar and they are both distributed in the same areas of Caribbean Colombia (Figure 1). Among the Colombian breeds examined, San Martinero shows the highest European ancestry, as indicated by the marked predominance of T3 lineages (0.89) and the observation that two of the three CR sequences shared between Colombian *criollo* and European breeds are seen only in San Martinero (data not shown). Phenotypically, San Martinero shares many features with the Pirenaica breed from Northern Spain (Pinzón Martínez 1984). Bon is peculiar in that it lacks the most common T3 sequence seen in European cattle and in all the other *criollo* breeds examined (Col1). Furthermore, five of the six T3 lineages observed in Bon are unique to this breed, includ-

ing the only sequences with transversions (Figure 2). The genetic distinctness of Bon is also manifest in the PCA of CR sequence frequency (Figure 5). At the phenotypic level Bon is unique among the breeds examined in having a white coat and black ears (Blanco Orejinegro means "white with black ears"). The high within-breed CR diversity and the important genetic and phenotypic differentiation of Bon, Harton, and San Martinero relative to the other populations examined suggest a differentiated ancestry for some *criollo* breeds. In conclusion, the high mtDNA and phenotypic diversity observed in Colombian *criollo* underscore the significance of these populations as an important genetic resource.

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