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ORIGIN OF CUBAN CREOLE CATTLE INFERRED BY PATRI- AND MATRILINEAGES

INFERENCIA DEL ORIGEN DEL BOVINO CRIOLLO CUBANO A TRAVÉS DEL ANÁLISIS DE PATRI- Y MATRILINAJES

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PALABRAS CLAVE ADICIONALES

ADN mitocondrial. Cromosoma Y. Polimorfismo.

SUMMARY

Cattle was absent from America before the discovery. Initially, bovine were brought to Greater Antilles (La Española, Puerto Rico, Jamaica and Cuba islands), and in the course of a few years, they were taken from Caribbean islands to the rest of Latin America. Nowadays, Cuban Creole cattle population is about 1300 heads, mainly located in the eastern region of the island. With the aim of analyzing the maternal origin of Cuban Creole cattle and detect possible contemporaneous, male mediated, gene flow, a 240 pb fragment of mitochondrial D-loop (mtDNA) and five microsatellites of Y chromosome (BTY) were studied in 36 dams and 21 sires, respectively. Genetic diversity was evaluated through number of haplotypes, mean number of pairwise differences and nucleotide diversity. The phylogenetic analysis was performed using a median joining. A total of 15 mtDNA haplotypes were detected in the studied population (10 from the European haplogroup T3, 3 from the African T1, 1 from the Nearern East T2, and 1 ambiguous T1-T3). The number of polymorphic sites, the mean nucleotide diversity, and the mean number of pairwise differences were 23, 0.014 and 3.36, respectively. Two patrilinages were detected, both belonging to the Y3 Zebu haplogroup. In conclusion, Cuban Creole cattle population had a mtDNA haplotypic composition similar to the observed in Creole and Mediterranean breeds, what is in concordance with its historical origin. Y chromosome analysis evidenced a male mediated process of zebu introgression.

RESUMEN

Antes de descubrimiento, no existían bovinos en América. Los primeros, fueron introducidos en la Antillas Mayores (La Española, Puerto Rico, Jamaica y Cuba), y desde allí trasladados al resto de Latinoamérica. Actualmente, existen en Cuba alrededor de 1300 bovinos Criollos, concentrados principalmente en la región oriental. Con el objetivo de analizar el origen materno de esta raza y detectar eventos contemporáneos de flujo génico por vía paterna, se analizó un fragmento de 240 pb del D-loop mitocondrial (mtADN) y 5 microsatélites del cromosoma Y (BTY), en 36 hembras y 21 machos respectivamente. La diversidad genética se estimó mediante el número de haplotipos, el número de sitios polimórficos, el número de diferencias nucleotídicas entre pares de secuencias y el índice de diversidad nucleotídica, mientras que el análisis filogenético se realizó utilizando el método de *median joining network*. Dicho análisis permitió detectar 15 haplotipos mitocondriales (10 del haplogrupo europeo T3, 3 del africano T1, 1 del cercano oriente T2 y 1 ambiguo T1-T3) y 3 haplotipos

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en el BTY, ambos del haplogrupo cebuíno Y3. En el mtADN se detectaron 23 sitios polimórficos con una diversidad nucleotídica de 0,014 y 3,36 diferencias medias entre pares de secuencias. En conclusión, la población de bovinos Criollos Cubanos presentó una composición haplotípica mitocondrial comparable a la de otras razas criollas y mediterráneas, hecho que concuerda con su origen histórico. El BTY evidenció altos niveles de introgresion paterna de genes del zebú.

INTRODUCTION

Anthropological, paleontological and historical evidences show that cattle was absent from America before the discovery. First bovines were brought by Spanish settlers in 1493 (Primo, 1992), and during the next 50 years, Creole cattle founder populations were established by Spanish and Portuguese settlers. Initially, cows were brought to Greater Antilles (La Española, Puerto Rico, Jamaica and Cuba islands), and in the course of a few years, they were taken from Caribbean islands to Central and South America and to the south of the modern United States. Simultaneously, there were direct shipments of Portuguese cattle to Brazil (De Alba, 1978, Primo, 1992). In only few decades, the Creole cattle spread over Latin America, being the only bovine for more than 300 years until the introduction of selected European and Indian breeds.

Nowadays, a number of distinct local Creole breeds are found throughout the Americas (Wilkins *et al*., 1982; Primo, 1992). This cattle shows great phenotypic heterogeneity and is adapted to a wide range of environments, with a moderate human intervention. In Cuba, Creole cattle, the descendant of bovine introduced into the island by Spanish settlers during the first years of the colonization, are named Cuban Creole. It has been suggested that Cuban Creole is related to the Spanish breeds Rubia Gallega, Asturiana, and Andaluza (Uffo, 2003). Furthermore, Cuban Creole was influenced by animals, with *Bos indicus* blood, introduced from Jamaica. This Zebu introgression was studied by cytogenetic and microsatellite analysis (Sánchez *et al.*, 1977; Uffo *et al.*, 2006). Cuban Creole is a hump-less bovine (*Bos taurus*) with horns; and is double purpose breed (meat and milk), and also an exceptional working animal. It has been adapted to the Cuban tropical environment for over 500 years. Due to its high adaptability and versatility, are of great importance in this Cuban region. Even though, the conservation of this breed has been affected by the introduction of foreign breeds, especially due to economical aspects (Uffo, 2003). Cuban Creole cattle suffered a severe reduction in population size during the 20th century as a result of the introduction and massive production of highly selected European and Zebu commercial breeds. As a consequence, the distribution of Creole populations which originally covered most of Cuban territory became mainly restricted to eastern region of the Island. Currently, the most abundant dairy and beef breed in Cuba are Cuban Zebu, Brahman, Siboney (5/8 Holstein and 3/8 Cuban Zebu) and Holstein; while there are around 1320 heads of female Creole cattle mainly located in the eastern region of the island (DNG, 2010).

Mitochondrial DNA (mtDNA) and Y chromosome DNA uniparental markers and autosomal markers (DNA sequences, microsatellites, AFLP, SNPs) have been widely and successfully used to study the origins of domestic cattle as well as breed relationships (Bruford *et al*., 2003; Giovambattista *et al*., 2010). Although the mtDNA, Y chromosome and autosonal markers are informative, they account for different events of the population history. In this sense, mtDNA and Y chromosome analysis retrieve exclusively the genetic matrilineal and patrilineal relationships, respectively. In consequence, they have been a valuable tool, aimed to phylogeographical studies, to understand the origin and domestication of livestock. In the particular case of Creole cattle studies, Y

chromosome markers have a powerful resolution to detect contemporaneous gene flow, because of the predominant asymmetric mating occurring during the last century, between Creole dams and foreign sires, to improve livestock (Giovambattista *et al*., 2000; Lirón *et al.*, 2006b, 2008; Ginja *et al*., 2010).

MtDNA control region polymorphisms have been reported in American Creole cattle breeds from several North, South, Central and Caribbean countries (Magee *et al.*, 2002; Miretti *et al.*, 2002, 2004; Carvajal-Carmona *et al.*, 2003; Mirol *et al.*, 2003; Lirón *et al.*, 2006a; Ginja *et al.*, 2010). These studies reported the existence of European T3, African T1, and Middle Eastern T2 haplotypes. African haplotypes in America, were also identified as belonging to two different sub-haplogroups, T1* and T1a (AA1 in Miretti *et al*., 2002 nomenclature; Miretti *et al.*, 2002, 2004; Lirón *et al.*, 2006a). In addition, the patrilinages present in Creole cattle were analyzed through Y chromosome microsatellites and single nucleotide polymorphisms (SNPs) (Giovambattista *et al*., 2000; Ginja *et al*., 2010). The aim of the present work was to analyze mitochondrial and Y chromosome polymorphisms in Cuban Creole cattle to investigate the maternal origins and possible contemporaneous male mediated gene flow. Results of this analysis are discussed in the context of previous genetic and historical data obtained in Creole breeds.

MATERIAL AND METHODS

A total of 36 Cuban Creole dams were sampled from the eastern region of Cuba for matrilineage analyses. Additionally, 21 sires, that represent the whole set of males from the Artificial Insemination Centre from Cuban National Direction of Genetics, were sampled for patrilineage analysis. All studied animals were phenotypically hump-less bovine (*Bos taurus*) with horns and tan-coloured. Available pedigree information was used to select individuals in order to sample the whole genetic diversity in Cuban Creole cattle.To check Taurine or Zebu Y chromosome lineage haplotypes, data from 38 DNA samples [Angus (AA)= 10, Hereford (He)= 10, Argentine Creole (ArC)= 5, Bolivian Chaqueño Creole (ChC)= 2, Bolivian Valles Creole (VaC)= 2, Nellore (Ne)= 6, Brahman (Br)= 3,], were included. These samples have been previously analysed for Y chromosome morphology (acrocentric or submetacentric) by conventional cyto-genetic methods and genotyped for Y chromosome STRs (Giovambattista *et al.*, 2000; Rogberg-Muñoz *et al.*, 2009).

The mtDNA hypervariable region I of the control region (nucleotide positions 16023-16262 in the complete mitochondrial genome sequence, Accession No. V00654; Anderson *et al*., 1982) was sequenced using primers L15960 and H16334 as described by Troy *et al.* (2001). Amplification products were purified with polyethylene glycol 8000 and sequenced in an automatic DNA sequencer MegaBACE 1000 (GE Healthcare) by using DYEnamic ET Terminator Kit (GE Healthcare) and PCR primers. Raw sequences were edited by using Sequence Analyzer (GE Healthcare).

Five Y chromosome microsatellites (BYM1, INRA124, INRA189, INRA057 and UMN307A (**table I**) were amplified in two multiplex in a total volume of 12.5 ml using a DNA Engine Thermal Cycler (Bio-Rad Laboratories Inc.). Each reaction contained: 1X reaction buffer with 1.5 mM of $MgCl_2$ provided by the supplier, 0.2 mM of each dNTP, 0.25 U Taq polymerase (Metabion International AG, Martinsried, Germany), 0.2 to 0.8 μM of each primer, and 40 ng of DNA. The cycling conditions were: a denaturation step of 2 min at 94ºC, followed by 10 cycles of 1 min at 94 ºC, 45 sec at 60ºC, and 45 sec at 72ºC, and followed by 25 cycles of 1 min at 94ºC, 45 sec at 58, and 45 sec at 72ºC, with a final elongation step of 15 min at 72 ºC. For BYM1, annealing temperatures were 66ºC in the first 10 cycles and 64ºC in

the next 25 cycles. Fragments were separated by electrophoresis in an automatic DNA sequencer MEGABACE 1000 (GE Healthcare, USA) and analyzed with MegaBase fragment profiler (version 1.2; GE Healthcare). ET-ROX 550 was used as internal size standard (GE Healthcare).

Variations in the D-loop region were defined by direct comparison with the reference bovine mtDNA sequence (Accession No.V00654, Anderson *et al.* (1982). Number of mtDNA and BTY haplotypes were calculated directly, while mean number of pairwise nucleotide differences (Tajima, 1993) and nucleotide diversity (Nei, 1987; Tajima, 1993) were calculated using the algorithms implemented in Arlequin 3.5 (Schneider *et al.*, 2000; http://cmpg.unibe.ch/software/arlequin3), using the default general setting. A median joining network was constructed using the methodology described in Bandelt *et al.* (1995) using Network 4.516 (http://www.fluxus-technology.com/sharenet.htm, consulted 06/01/10), using default setting and the same weight (10) for transitions and transversion. The nucleotide sequences obtained were compared by applying blastn 2.2.24 against cattle D-loop DNA sequences previously reported in the GenBank database.

RESULTS AND DISCUSSION

D-loop analysis in 36 Cuban Creole dams showed a total of 23 polymorphic sites characterizing 15 haplotypes (**table II**). These polymorphisms included 21 transitions and two transversions by comparison with the bovine reference sequence (Anderson *et al.*, 1982). As expected, haplogroup T3, the most prevalent in Western Europe, was also the most commonly found with a incidence of 69.4%. This haplogroup was represented by ten haplotypes that diverge from the consensus sequences in one or two mutations. The African haplogroup T1 was found 8 times (22.2%), and comprised 3 haplotypes. Remarkably, one T1 haplotype has 3 out of the 4 mutations that defined the sub-haplogroup T1a. Haplogroup T2, which is frequently observed in the Near East with a minor presence in European breeds, was detected in only two individuals (5.5%) **(figure 1)**. The remaining haplotype is ambiguous because it has only two of the three T1 diagnostic sites (T at position 16050 and C at position 16113). None of the analyzed Cuban Creole cattle presented

Table I. *Details of the Y chromosome microsatellites panel.* (Detalles del panel de microsatélites del cromosoma Y).

Locus	Primer	Range size[1]	Dye	Reference
BYM1	Forward 5´-CCTTGTTTGAGCTTGACCAGT-3´			
	Reverse 5´-TTGCAGGCACAGAAACGGA-3´	157-159	HEX	Ward *et al.*, 2001
INRA124	Forward 5´-GATCTTTGCAACTGGTTTG-3´			
	Reverse 5´-AGGACACAGGTCTGACAATG-3´	130-132	FAM	Vaiman *et al.*, 1994
INRA189	Forward 5´-TTTTGTTTCCCGTgCTGAG-3´			
	Reverse 5´-GAACCTCGTCTCCTTGTAGCC-3´	147	FAM	Kappes *et al.*, 1997
INRA057	Forward 5´-CCTAGCGACTGTCCAAGCG-3´			
	Reverse 5´-CACGGCTGAGAATTCAAAC-3´	126	HEX	Vaiman *et al.*, 1994
UMN307	Forward 5´-GATACAGCTGAGTGACTAAC-3´			
	Reverse 5´-GTGCAGACATCTGAGCTGTG-3´	149	HEX	Liu *et al.*, 2002

[1]The allele range detected in this study.

Zebu maternal haplotypes. The mean nucleotide diversity was 0.014 (SD 0.008) and the mean number of pairwise differences was 3.36 (SD 1.76). All sequences have been submitted to GenBank (accession numbers FJ611967-FJ611986, FJ799719-FJ799722, FJ857930-FJ857932, and HM448433-HM448440). Regarding Y chromosome results, only three patrilineages were detected, all belonging to Zebu Y3 type (**table II**).

From a maternal perspective, Cuban Creole cattle shared the main characteristics previously reported for Creole and Mediterranean breeds. In this sense, the mitochondrial diversity was 100% taurine, and it was composed mainly by T3 haplogroup, followed by T1 haplogroup, and T2 haplogroup represented at low frequency. The consensus T3 haplotype, predominant in Europe (including the Iberian Peninsula), was the most frequent in this native breed (36.1%). Furthermore, a considerable percentage of private sequences (specific allele of a population/breed) for Cuban (four out of fifteen) or American Creole cattle (one out of fifteen) was found. The remaining eight haplotypes, excluding the T3 and T1 nodal sequences, have a wide geographical distribution in Europe, all of them were detected in Iberian breeds, including South Spanish and Portuguese ones like Retinta, Mostrenca, Berrenda. Furthermore, several of these haplotypes were also shared with other Creole cattle populations, including the T2 haplotype previously detected in Colombia. Outstandingly, Cuban population shared haplotypes

Table II. *Variable positions in the D-loop sequences of Cuban Creole cattle.* (Posiciones variables en la secuencias del D-loop de los bovinos Criollos Cubanos).

Ht	N	16042	16050	16053	16057	16074	16084	16109	16112	16113	16121	16122	16135	16139	16141	16146	16185	16196	16231	16247	16248	16250	16255	16260	
Ref. seq.		T	C	T	G	T	C	T	T	T	G	T	T	C	T	A	G	G	C	C	C	A	T	C	H
CCu1	1													T								G			T3
	2																								T1
CCu2			T	C						C		C						A					C		(T1*)
	1																								T3
CCu3	2																								
CCu4	3		T							C													C		T1
CCu5	1										A														T3
CCu6	2				C					C			C				A			T			C		T2
CCu7	3																			T					T3
	3																								T1
CCu8			T							C						G			T	T			C		(T1a)
CCu9	1	C																							T3
CCu10	1														C										T3
CCu11	2							C			A														T3
CCu12	2																							T	T3
CCu13	1						T																		T3
CCu14	1	C							C																T3
CCu15	1					C				C											T		C		T3

Ht: Haplotype; H: Haplogrup; Ref. seq.: reference sequence (Anderson *et al.,* 1982); N: number of detected haplotypes.

with neighbouring Creole breeds distributed in the Minor Antilles (Antigua, Guadalupe, St. Lucia); Central and south of North America (Mexico and USA), and northern countries of South America (Colombia and Ecuador). In contrast, they have only one haplotype in common with Creole breeds from the maridional countries of South America (Argentine, Bolivia, Brazil, Paraguay and Uruguay). None of detected haplotypes, with the exception of T3 and T1 nodal sequence, was shared with Canarian Islands native breeds, despite these islands were a common intermediate port used by Spanish ships. Within T1 haplogroup, Cuban Creole cattle presented a haplotype displaying had three out of the four mutations that define the T1a subhaplo-

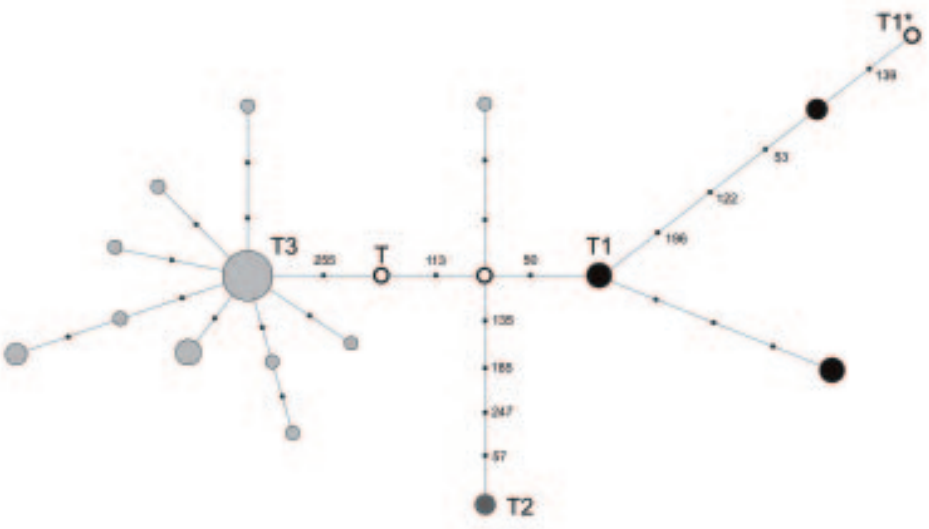

Figure 1. *A median joining (MJ) network of mtDNA was constructed using the median algorithm reported by Bandelt* et al. *(1995). The relationship between European (T3, light grey circles) and African (T1, black circles) central haplotypes is defined by transitions at nucleotide positions 16255, 16113, and 16050. Haplogroup T (bold white circle) was defined by transitions at 16255, T2 (dark grey circles) was defined by transitions at 16185 and 16255 plus a transversion at 16057, while T1* (bold white circle) was defined by transitions at 16053, 16122, 16139 and 16196 from T1 consensus. Circle areas are proportional to the frequency of each haplotype. Line length is proportional to the number of nucleotide substitutions (small square). Bold white circles denoted unsampled theoretical haplotypes.*(El median joining (MJ) network del ADNmt se construyó utilizando el algoritmo reportado por Bandelt *et al.* (1995). La relación entre los haplotipos nodales europeos (T3, círculos grises claro) y africanos (T1, círculos negros) es definido por las transiciones en las posiciones nucleotídicas 16255, 16113 y 16050. El haplogrupo T (círculo blanco con borde remarcado) es definido por la transición en la posición 16255, el T2 (círculos gris oscuro) es definido por las transiciones en las posiciones 16185 y 16255 junto a la transversión en el sitio 16057, mientras que T1* (círculo blanco con borde remarcado) es definido por las transiciones en los sitios 16053, 16122, 16139 y 16196 del consenso T1. Las áreas de los círculos son proporcionales a la frecuencia de cada haplotipo. El largo de las líneas es proporcional al número de sustituciones nucleotídicas (cuadrados pequeños). Los círculos blancos con borde remarcado indican haplotipos no muestreados).

group, mainly characteristic of Brazil and Minor Antilles. Despite T1a haplogroup has not been yet detected in Africa, intermediate haplotypes between T1 nodal and T1a have also been identified in Tunisian and Southern Portugal breeds (Beja-Pereira *et al*., 2006; Ginja *et al.*, 2010).

Different explanations have been proposed for the geographical origin of African lineages in American Creole cattle. Carvajal-Carmona *et al.* (2003) explained the presence of haplogroup T1* in Colombia as originating from North Africa, probably as the result of the Arab occupation of Iberia prior to European migration to the New World; while Magee *et al.* (2002) suggested that the African influence in Creole is, at least in part, attributable to the direct historical importation of West African cattle to the Caribbean. Miretti *et al.* (2004) postulated that haplogroup T1a (AA1 in their nomenclature) would have originated in Spain, while T1* would have arrived in America from West Africa. By contrast, an alternative hypothesis suggested the possibility of introgression of at least part of the African mtDNA haplotypes (including the T1a) from somewhere in mainland Africa, perhaps following the slave trade routes (Lirón *et al*., 2006a, 2008). In conclusion, the African haplogrupes present in Cuban Creole cattle, as well as in other Creole populataions, could be introduced directly from Iberia and alternatively following slave trading route.

Regarding Y chromosome results, the studied population exhibited two haplotypes, both belonging to Zebu Y3

Table III. *Y chromosome haplotypes defined by 5 microsatellites loci and their overall frequency in Cuban Creole cattle (CCu). Control DNA samples were included: Angus (AA), Hereford (He), Argentine Creole (ArC), Bolivian Chaqueño Creole (ChC), Bolivian Valles Creole (VaC), Nellore (Ne) and Brahman (Br).* (Haplotipos del cromosoma Y definidos mediante el análisis de 5 microsatélites y sus frecuencias en el bovino Criollo Cubano (CCu). ADN de muestras control fueron incluidas: Angus (AA), Hereford (He), Criollo Argentino (ArC), Criollo Chaqueño Boliviano (ChC), Criollo de los Valles Bolivianos (VaC), Nelore (Ne) y Brahman (Br)).

Haplotype	F	BYM1	INRA124	INRA189	INRA057	UMN307	Y chromosome morphology[2]
CCu1	0.71	257	130	147	126	149	ND
CCu3	0.29	259	130	147	126	149	ND
Ne[1]	6	ND	130	147	126 / 130[1]	149	Acrocentric
Br[1]	3	ND	130	147	126 / 130[1]	149	Acrocentric
AA[1]	10	259	132	157	126 / 130[1]	151	submetacentric
		259	132	159	130	151	Submetacentric
		259	132	161	130	155	submetacentric
He[1]	10	ND	132	159	130	149	Submetacentric
		ND	132	159	130	155	Submetacentric
		ND	132	161	130	155	submetacentric
ArC[1]	5	ND	132	157	130	155	Submetacentric
		ND	132	159	130	155	Submetacentric
		ND	132	161	130	149	submetacentric
	-	259	132	163	130	149	submetacentric
ChC[1]	2	ND	130	147	126 / 130[1]	149	Acrocentric
VaC[1]	2	ND	132	161	130	149	submetacentric

[1]Two fragments amplified in *Bos indicus*.
ND: not determined, [1]Rogberg-Muñoz *et al.*, 2009; [2]Giovambattista *et al*., 2000.

haplogroup despite Cuban Creole is phenotypically hump-less cattle. Taurine or Zebu haplotype origin was defined by INRA124 and INRA057. This genetic diversity of patrilineages seems to be low compared with other species, such as human. However, previous works in bovine and other domestic animals, showed a similar reduced number of within-breed haplotypes observed at high frequency (Li *et al.*, 2007; Ginja *et al.*, 2009, 2010; Kantanen *et al*., 2009; Pérez-Pardal *et al.*, 2009). This situation is even more extreme in highly selected breeds where one haplotype is often fixed or close to be fixed. This low level of patrilineages is probably related to the reduced effective male population size characteristic of domestic animal species, as well as intensive breeding programs and artificial insemination practice. Different studies have been published using different genetic markers and different primers, in consequence microsatellites molecular sizes and haplotypes are difficult to be compare without standardization. Even though, the haplotypes detected in Cuban Creole cattle could correspond to any of the haplotypes H1 to H11 (INRA124*130 – INRA189*88 - BYM1*256) reported by Li *et al*. (2007), and/or H18Y3 to H21Y3 (INRA124*130 – INRA189*88 – UMN307*149) reported by Ginja *et al*. (2010). Several Latin American Creole cattle reflect a high degree of Zebu introgression mediated by males. It is notable that these introgressions appear to be higher in tropical and lowland regions, than in temperate and highland cattle (Giovambattista *et al.*, 2000; Lirón *et al*., 2006b). Cuban Creole Y chromosome variation is in agreement with the cytogenetic study performed by Sánchez *et al.* (1977), which shows the precence of Zebu acrocentric Y chromosome. Furthermore, the historical data propose that the cattle arrived from Jamaica to Cuba had *Bos indicus* influence. The introduction of Zebu animals could have increased the degree of Zebu introgression during the last century. In concordance patrilineage data, Uffo (2003) and Uffo *et al*. (2006) found high frequencies of Zebu-specific alleles in microsatellites and milk proteins (CASA1C, CASBA, LAAB) study of this population.

In conclusion, Cuban Creole cattle population present a pattern of genetic admixture between Taurine and Zebu. Discrepancies are observed between mitochondrial and Y chromosome markers, with a Taurine mtDNA haplotypic composition comparable with that observed in other Creole and Mediterranean breeds (as well as European), while Y chromosome evidenced a probably recent male mediated process of Zebu introgression. These different pictures are in concordance with the historical origin of this breed. The genetic characterization, using molecular markers, of indigenous breeds, such Cuban Creole cattle, it is useful and necessary information for define the conservation units and it is one of the previous recommended steps for design conservation plan of these important reservoirs of genetic diversity for commercial domestic species (Delgado Bermejo *et al.*, 2010).

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