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Research Article

Mitochondrial DNA mapping of social-biological interactions in Brazilian Amazonian African-descendant populations

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Abstract

The formation of the Brazilian Amazonian population has historically involved three main ethnic groups, Amerindian, African and European. This has resulted in genetic investigations having been carried out using classical polymorphisms and molecular markers. To better understand the genetic variability and the micro-evolutionary processes acting in human groups in the Brazilian Amazon region we used mitochondrial DNA to investigate 159 maternally unrelated individuals from five Amazonian African-descendant communities. The mitochondrial lineage distribution indicated a contribution of 50.2% from Africans (L0, L1, L2, and L3), 46.6% from Amerindians (haplogroups A, B, C and D) and a small European contribution of 1.3%. These results indicated high genetic diversity in the Amerindian and African lineage groups, suggesting that the Brazilian Amazonian African-descendant populations reflect a possible population amalgamation of Amerindian women from different Amazonian indigenous tribes and African women from different geographic regions of Africa who had been brought to Brazil as slaves. The present study partially mapped the historical biological and social interactions that had occurred during the formation and expansion of Amazonian African-descendant communities.

Key words: African mtDNA, Amazon population, Amerindian mtDNA, HVS-I.

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Introduction

The Portuguese settled in the Amazon region between the sixteenth and eighteenth centuries and carried out economic activities targeted at the European market. Extractivism based on Amerindian slave labor initially prevailed but later agriculture and cattle raising was supported by the intense Sub-Sahara African slave trade (Porto, 1938; Bezerra-Neto, 2001). Historical records indicate that about three and a half million captives were brought to Brazil, mainly from western and central Africa (Curtin, 1969; Salzano and Freire-Maia, 1970; Ribeiro, 1995). African slaves were brought to the Brazilian Amazon principally in the second half of the eighteenth century and became the main work force in the region (Conrad, 1985; Salles, 1988; Ribeiro, 1995). Approximately 53,000 slaves disembarked in this area, and by the middle of the nineteenth century the slave population in the Brazilian state of Pará in the Amazon region was almost 34,000, of which 49% were women and 51% men (Salles, 1988).

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Slaves faced precarious living and working conditions in Brazil and their life expectancy was only 10 years after arrival. The most usual response to bad treatment was escaping, with many fugitive slaves founding independent communities called *quilombos* or *mocambos* in isolated areas, which are now known as African-descendant or African-derived communities (Ribeiro, 1995; Acevedo and Castro, 1998; Bezerra-Neto, 2001). Over last 20 years, these populations have been investigated by several groups of researchers such as anthropologists, linguists and geneticists, among others, in order to better understand the socio-biological formation and increment of such populations. Genetic studies based on classical genetic polymorphisms helped to reconstruct part of the history of these populations and also provided the first estimates concerning parental contributions, which demonstrated that these groups preserve a predominantly African genetic pool, notwithstanding the European and Amerindian contributions (Schneider *et al.*, 1987; Bortolini *et al.*, 1995, 1998; Guerreiro *et al.*, 1999).

During the last decade many studies have used lineage markers such as mitochondrial DNA (mtDNA) to describe the genetic variability and evolutionary processes of different world populations. Phylogenetic analyses of mtDNA lineages (matrilineages) have also been published and many geographic-specific haplogroups have been identified (Vigilant *et al.* 1991; Torroni *et al.*, 1993, 1996; Chen *et al.*, 1995, 2000; Santos *et al.*, 1996; Watson *et al.*, 1996, 1997; Bortolini *et al.*, 1997, 1999; Rando *et al.*, 1998; Bandelt *et al.*, 2001; Pereira *et al.*, 2001; Salas *et al.*, 2002, 2004; Yao *et al.*, 2002; Mishmar *et al.*, 2003; Rosa *et al.*, 2004; Shen *et al.*, 2004).

In this paper we provide information on the mtDNA haplogroup distribution of five African-descendant populations and evaluate the specific social mechanisms active in such communities, the aim being to better understand the genetic variability and the micro-evolutionary processes acting in human groups in the Brazilian Amazon. Additionally, our data were analyzed together with others recently published to determine the phylogeographic composition of the mtDNA lineages of African-descendant populations. We also discuss some aspects of the nature of the Atlantic slave trade to the Brazilian Amazon region.

Material and Methods

Sample population

Our population sample consisted of unrelated African-descendants (n = 159) living in five communities located in three states in the Brazilian Amazon region: Trombetas (n = 32), Marajó island (n = 34) and Pitimandeua (n = 29) in Pará state; ii) Tamauari (n = 31) in Maranhão state; and iii) Mazagão (n = 33) in Amapá state. The geographic locations of these communities, self-reported as being based on African-descendants, are shown in Figure 1. All individuals provided their prior informed consent and this research was approved by the Ethical Committee of the Universidade Federal do Pará.

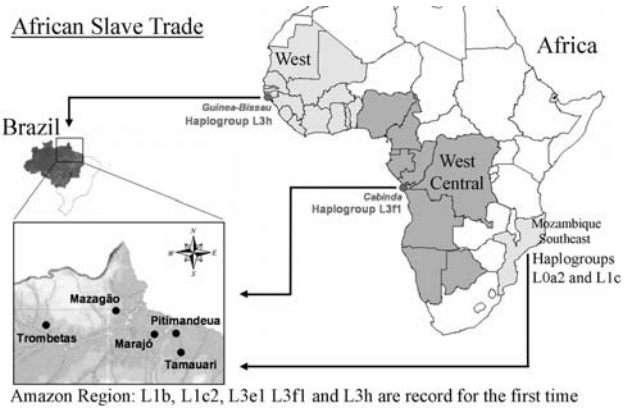

Figure 1 - Geographic localization of the Afro-descendant communities in the Brazilian Amazon region and the African probable origin of some mtDNA lineages observed in this study.

Mitochondrial DNA polymorphisms

The phenol-chloroform and ethanol methods (Sambrook *et al.*, 1989) were used to extract DNA from whole peripheral blood. After extraction the DNA was quantified in a Gene Quant RNA/DNA spectrophotometer (Amersham Biosciences, UK) and diluted to a working concentration of 100 ng mL^{-1}.

We Initially investigated the 9 bp deletion in region V of the CoII/tRNAlys intergenic region as well as the 663/*Hae* III, 13,259/*Hinc* II and 5,176/*Alu* I restriction fragment length polymorphisms (RFLP) which define Amerindian haplogroups (A-D) (Hertzberg *et al.*, 1989; Torroni *et al.*, 1992, 1993) along with the association of the 9 bp deletion with the 3,592/*Hpa* I RFLP which defines the African L0a2b subclade (Chen *et al.*, 2000). Next, we used the L15997 and H16401 primers (Pereira *et al.*, 2000) to amplify the first mitochondrial DNA hypervariable region (HVS-I) of samples from all five populations. The targets and the polymerase chain reaction (PCR) amplified segments were purified with a PureLink kit (Invitrogen, USA). The forward and reverse sequencing reactions were performed with the Big Dye™ Terminator Cycle Sequence kit (Applied Biosystems, USA). Vertical electrophoresis was performed on 5% (w/v) denaturing polyacrylamide gels using ABI prism 377 DNA Sequencer (Applied Biosystems, USA). To better classify some sequences that did not present informative HVS-I mutations for the geographically-specific haplogroups and Europeans lineages we also used a further 19 RFLP markers belonging to the L0a, L1b, L1c, L3b, L3d, L3e, K, H, W, T, X, U, V, I and J haplogroups (Torroni *et al.*, 1996; Alves-Silva *et al.*, 2000; Chen *et al.*, 2000).

The classification of each mtDNA lineage followed the nomenclature suggested for defining the African, Amerindian and European mitochondrial haplogroups (Torroni *et al.*, 1993, 1996; Pereira *et al.*, 2001; Salas *et al.*, 2002, 2004; Mishmar *et al.*, 2003). African mitochondrial haplogroups were characterized as L0a (*pro* L1a), L1b, L2a; L2b and other L3 haplogroups. Mutations at nucleotide positions (np) 16124, 16278 and 16362 were characterized as haplogroup L3b, and mutations at np 16124 and 16223 were characterized as haplogroup L3d. According to Bandelt *et al.* (2001), haplogroup L3e subdivides into L3e1 (16223 and 16327), L3e2 (16223 and 16320) and L3e3 (16223 and 16265T). Haplogroup L3f is characterized by transversions at np 16209 and 16311 (Salas *et al.*, 2002). Sub-clade L3f1 is characterized by mutations at 16209, 16218, 16256, 16292, and 16311. We classified as haplogroup L3h (Kivisild *et al.*, 2004; Rosa *et al.*, 2004) the haplotype characterized by mutations at np 16129, 16256 and 16362. Mutations at np 16224 and 16311 characterize European haplogroup K (Torroni *et al.*, 1996; Pereira *et al.*, 2000).

Data analysis

Global analysis was performed with the 159 samples. We carried out *Network* analysis separately for the Amerin-

dian and African lineage groups (Figure 2) using the median joining algorithm (Bandelt *et al.* 1999). Pairwise differences were obtained considering HVS-I for the same lineage groups according to the assumptions of Aris-Brosou and Excoffier (1996) using the DNAsp V 4.10 program (Rozas *et al.*, 2003). Haplotype and nucleotide diversities were estimated using ARLEQUIN 2.0 (Schneider *et al.*, 2000). Since the mtDNA haplogroups are geographically specific, the parental contributions for each population were obtained by direct counting.

Results and Discussion

Table 1 presents the 85 mtDNA haplotypes identified in the 159 samples from African-descendants defined by 78 variable nucleotides evaluated by network analysis (Figure 2). These HVS-I haplotypes have previously been reported by Ribeiro-dos-Santos *et al.* (2007) but we have reanalyzed all the haplotypes to better understand the genetic variability and the micro-evolutionary processes in the human groups in the Brazilian Amazon.

The five African-descendant populations investigated showed large genetic diversity and variability. The four main Amerindian haplogroups (A-D) and the African L1, L2, and L3 haplogroups were observed at different frequencies in these populations. The African L0 haplogroup was the least frequent and was observed only in the Trombetas and Curiaú populations, the latter having been previously studied by Ribeiro-dos-Santos *et al.* (2002).

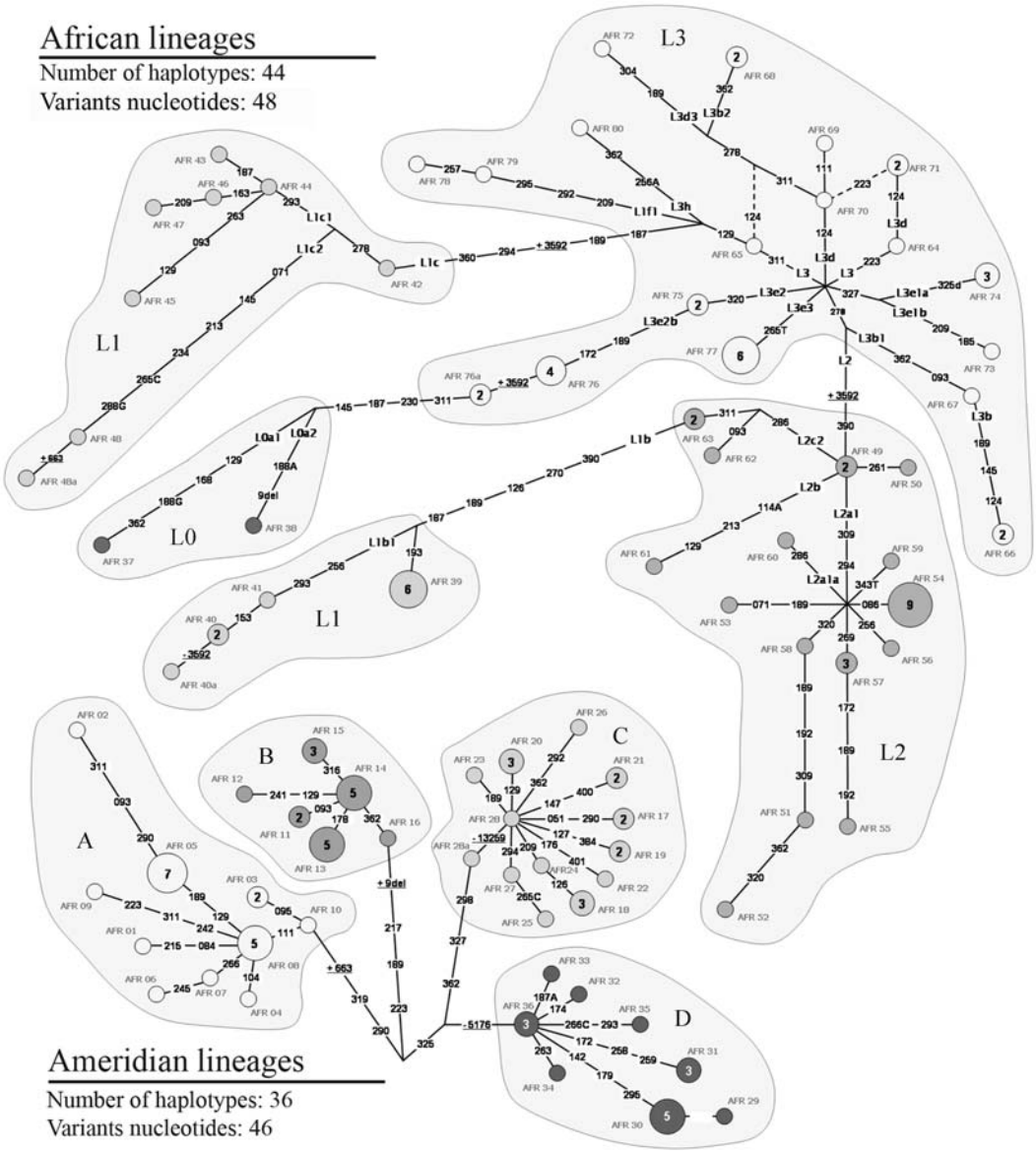

Figure 2 - Networks of African and Amerindian lineage groups. The first hypervariable region (HVS-I) variant nucleotides are indicated along the branches connecting the haplotypes. For the exact nucleotide position 16000 should be added to the numbers. Transversions are indicated by the nitrogen base and deletions by "d". The numbered haplotypes refer to multiple occurrences. The restriction sites and the 9 bp deletion are underlined.

Table 1 - Haplotypes defined by first hypervariable region (HVS-I) variant nucleotides in samples of Afro-descendant populations from the Brazilian Amazon. Haplotypes characterized only by HVS-I region nucleotide variability. Haplotypes followed by the letter "a" (*e.g.*, AFR 28a) also showed restriction fragment length polymorphism (RFLP) variation and the 9 bp deletion. The population sample consisted of unrelated African-descendants (n = 159) living in five communities located in three states in the Brazilian Amazon region: Trombetas (n = 32), Marajó island (n = 34) and Pitimandeua (n = 29) in Pará state; ii) Tamauari (n = 31) in Maranhão state; and iii) Mazagão (n = 33) in Amapá state. In the 'variable nucleotides' column the numbers indicate variable nucleotides after comparison with the Cambridge (CRS) reference sequence of the HVS-I region, 16000 must be added to these numbers for the exact nucleotide position. Transversions are indicated by the nitrogen base, and deletions by "del". The final classification is given in the last column. Results in parentheses correspond to the initial analysis of three RFLP and the 9 bp deletion. In the haplotype column 'n' represents the total number of individuals with the cited haplotype, while in the population column it represents the number of individuals per population.

Haplotypes (n)	Population (n)					Variable nucleotides	Haplogroups
	TRB	MRJ	PTD	TMR	MAZ		
AFR 01				1		084 111 215 223 290 319 362	A
AFR 02		1				093 111 129 189 223 311 319 362	A
AFR 03	2					095 223 290 319 362	A
AFR 04	1					104 111 223 290 319 362	A
AFR 05		3	1		3	111 129 189 223 290 319 362	A
AFR 06				1		111 223 245 266 290 319 362	A
AFR 07				1		111 223 266 290 319 362	A
AFR 08	1	1	1	1	1	111 223 290 319 362	A
AFR 09				1		111 242 290 311 319 362	A
AFR 10	1					223 290 319 362	A
Total for A (n = 21)	5	5	2	5	4		
AFR 11					2	093 189 217	B (+del.9pb)
AFR 12					1	129 189 217 241	B (+del.9pb)
AFR 13			1		4	178 189 217	B (+del.9pb)
AFR 14			2	1	2	189 217	B (+del.9pb)
AFR 15	1	2				189 217 316	B (+del.9pb)
AFR 16					1	189 217 362	B (+del.9pb)
Total for B (n = 17)	1	2	3	1	10		
AFR 17	2					051 223 325 327	C
AFR 18				3		126 209 223 298 325 327	C
AFR 19			1	1		127 223 298 325 327 384	C
AFR 20	1	2				129 223 298 325 327	C
AFR 21			1	1		147 223 298 325 327 400	C
AFR 22				1		176 223 298 325 327 401	C
AFR 23					1	189 223 298 325 327	C
AFR 24				1		209 223 298 325 327	C
AFR 25					1	223 265C 294 298 325 327	C
AFR 26				1		223 292 298 325 327 362	C
AFR 27			1			223 294 298 325 327	C
AFR 28				1		223 298 325 327	C
AFR 28a	1					223 298 325 327	C (+13.259/*Hinc*II)
Total for C (n = 20)	4	2	3	9	2		
AFR 29	1					142 179 192 223 295 325 362	D
AFR 30	3	1			1	142 179 223 295 325 362	D
AFR 31		3				172 223 258 259 325 362	D
AFR 32					1	174 223 325 362	D
AFR 33				1		187A 223 325 362	D
AFR 34		1				223 263 325 362	D
AFR 35		1				223 266G 293 325 362	D
AFR 36			1	1	1	223 325 362	D
Total for D (n = 16)	4	6	1	2	3		

Table 1 (cont.)

Haplotypes (n)	Population (n)					Variable nucleotides	Haplogroups
	TRB	MRJ	PTD	TMR	MAZ		
AFR 37	1					129 148 168 172 187 188G 189 223 230 311 320 362	L0a1
AFR 38	1					148 172 187 188A 189 223 230 311 320	L0a2 (+del.9pb)
Total for L0 (n = 2)	2	0	0	0	0		
AFR 39		6				126 187 189 193 223 264 270 278 311	L1b
AFR 40	2					126 153 187 189 223 256 264 270 278 293 311	L1b1
AFR 40a	1					126 153 187 189 223 256 264 270 278 293 311	L1b1 (-3.592/HpaI)
AFR 41	1					126 187 189 223 256 264 270 278 293 311	L1b1
AFR 42		1				129 187 189 223 294 311 360	L1c
AFR 43					1	129 187 189 223 278 293 294 311 360	L1c1
AFR 44					1	093 187 189 223 263 278 293 294 311 360	L1c1
AFR 45				1		129 163 187 189 209 223 278 293 294311 360	L1c1
AFR 46			1			129 163 187 189 223 278 293 294 311 360	L1c1
AFR 47					1	129 189 223 274 278 293 294 311 360	L1c1a
AFR 48			1			071 129 145 187 189 213 223 234 265C 278 286G 294 311 360	L1c2
AFR 48a			1			071 129 145 187 189 213 223 234 265C 278 286G 294 311 360	L1c2 (+663/HaeIII)
Total for L1 (n = 18)	4	7	3	1	3		
AFR 49			1			223 261 278 390	L2
AFR 50	1				1	223 278 390	L2
AFR 51	1					189 192 223 278 294 320 390	L2a
AFR 52		1				189 192 223 278 294 362 390	L2a
AFR 53		1				071 189 223 278 294 309 390	L2a1
AFR 54		7		2		086 223 278 294 309 390	L2a1
AFR 55					1	172 189 192 223 269 278 294 309 390	L2a1
AFR 56			1			223 256 278 294 309 390	L2a1
AFR 57					3	223 269 278 294 309 390	L2a1
AFR 58			1			223 278 294 309 320 390	L2a1
AFR 59					1	223 278 294 309 343T 390	L2a1
AFR 60			1			223 278 286 294 309 390	L2a1a
AFR 61				1		114A 129 213 223 278 390	L2b
AFR 62			1			093 223 264 278 390	L2c2
AFR 63			2			223 264 278 311 390	L2c2
Total for L2 (n = 27)	2	9	7	3	6		
AFR 64				1		223 311	L3
AFR 65					1	CRS	L3
AFR 66				2		093 124 145 189 223 278 362	L3b
AFR 67			1			093 223 278 362	L3b1 (+10.084/TaqI)
AFR 68	1				1	124 223 278 311 362	L3b2
AFR 69					1	111 124 223	L3d
AFR 70			1			124	L3d
AFR 71				2		124 223	L3d
AFR 72				1		124 189 223 278 304 311	L3d3
AFR 73				1		185 209 223 327	L3e1a
AFR 74	3					223 325del 327	L3e1b
AFR 75			2			223 320	L3e2
AFR 76		1	3			172 189 223 320	L3e2b
AFR 76a		1	1			172 189 223 320	L3e2b (+3.592/*Hpa*I)

Table 1 (cont.)

Haplotypes (n)	Population (n)					Variable nucleotides	Haplogroups
	TRB	MRJ	PTD	TMR	MAZ		
AFR 77	4		2			223 265T	L3e3
AFR 78	1					129 209 223 257 292 295 311	L3f1
AFR 79	1					129 209 223 292 295 311	L3f1
AFR 80				1		129 223 256A 311 362	L3h
Total for L3 (n = 33)	10	2	10	8	3		
AFR 81					1	224 311 322	K (+10.394/Dde I, -9.052/HaeII)
AFR 82		1				093 224 311	K (+10.394/Dde I, -9.052/HaeII)
Total for K (n = 2)	0	1	0	0	1		
AFR 83				1		093 223 355	Other
AFR 84					1	153 298	Other
AFR 85				1		223 288	Other
Total for Other (n = 3)	0	0	0	2	1		

Similar genetic diversity values were observed when the average nucleotide differences (k) and the haplotype diversity results (Hd) of Amazonian African-descendant Brazilians were compared with those of African groups from different regions of Africa (Mateu *et al.*, 1997; Salas *et al.* 2004) (Table 2). The same occurred when the genetic diversity values of the Amerindian stock identified in the African-descendant populations were compared with those of isolated indigenous communities of the Brazilian Amazon. Since these African-descendant populations have resulted from the miscegenation of Africans and Amerindians our results indicate that the remaining *quilombo* communities of the Amazon region are possibly an important reservoir of African and Amazonian indigenous mtDNA variability.

Pairwise difference and genetic diversity

Pairwise analyses of nucleotidic differences relative to HVS-I of the samples obtained from the 159 African-descendants of the Amazon region and the Amerindian (74 samples) and African (80 samples) lineage groups were performed separately. The charts showed a normal distribution curves, although the Tajima test results were non-significant ($p > 0.1$). The average numbers of nucleotide differences for the Amerindian and African lineage groups (see Table 2) were similar to those reported for other Brazilian Amazonian Amerindian populations (Ward *et al.*, 1991; Santos *et al.*, 1996) and native sub-Saharan African populations (Salas *et al.* 2002, 2004) as well as to haplotype and nucleotide diversity results.

Mitochondrial DNA variability and parental contribution estimates African fraction

Approximately half of the samples (80/159, 50.3%) were from African maternal lineages, which had the widest geographical contribution of the samples studied. The African lineage haplogroups detected by us were as follows:

Table 2 - Genetic diversity in Afro-descendant populations from the Brazilian Amazon. The Table shows number of samples (n), number of variable nucleotides (S), number of haplotypes (h), genetic diversity (H_d), standard deviation of the genetic diversity (SD_{Hd}); nucleotide diversity (π),standard deviation of the nucleotide diversity SD_π, average pairwise differences (k) and Tajima test value (D). NS = not significant.

Population	n	S	h	H_d	SD_{Hd}	π	SD_π	k	D
Tamauari	31	45	26	0.987	0.012	0.02033	0.00155	7,258	-1,36459 NS
Trombetas	32	40	21	0.966	0.016	0.02103	0.00210	7,486	-0,96574 NS
Marajó	34	38	16	0.920	0.027	0.02362	0.00076	8,433	-0,33288 NS
Mazagão	33	36	24	0.973	0.015	0.01980	0.00132	7,068	-0,73143 NS
Pitimandeua	29	38	21	0.973	0.017	0.02015	0.00220	7,192	-1,08894 NS
Afro-descendants	159	78	85	0.984	0.003	0.02098	0.00076	7,668	-1,56021 NS
Amerindian fraction	74	46	36	0.974	0.007	0.01823	0.00055	6,509	-1,06350 NS
African fraction	80	48	44	0.969	0.008	0.02088	0.00110	7,434	-0,95293 NS

L0, the least frequent (2/80, 2.5%) and containing sub-clades (also called variants) L0a1 and L0a2; L1, moderately frequent (18/80, 22.5%) and containing haplogroups L1b and L1c; L2, the second most frequent (27/80, 33.8%) and containing haplogroups L2a, L2b, and L2c; and L3, the most frequent (33/80, 41.2%) and containing sub-clades L3b, L3d, L3e, L3f and L3h.

The L0a1 sub-clade observed in our study is apparently absent from the northern region of the Brazilian Amazon (Silva-Junior *et al.*, 2006) but was previously identified in the African-descendant Curiaú population in the Brazilian Amazon (Ribeiro-dos-Santos *et al.*, 2002) and has also been described in populations from southeastern and southwestern Africa (Plaza *et al.*, 2004; Salas *et al.*, 2004; Beleza *et al.*, 2005). The L0a2 sub-clade HVS-I mutations detected by us, also identified by the 9 bp deletion in region V, have been reported at a low frequency of ~4.5% in Brazil (Silva-Junior *et al.*, 2006) but at ~19% in Mozambique in southeastern Africa (Salas *et al.*, 2002).

In our study, 10 of the African-descendants belonged to sub-clade L1b, the third most frequent haplogroup (10/80, 12.5%), which has been reported to be almost restricted to West Africa (Salas *et al.*, 2002, 2004). However, we found only eight lineages belonging to haplogroup L1c, which is a marker from western central Africa (Salas *et al.*, 2004; Beleza *et al.*, 2005). None of the HVS-I haplotypes belonging to the L1c haplogroup have been observed in Angola (Plaza *et al.*, 2004) but some have been found in Cabinda in western central Africa and in Mozambique. The L1c haplogroup, and more specifically L1c2, L0a1, L3e1 and L3e2, are known to be frequent in African descendants from different regions of Brazil (Alves-Silva *et al.*, 2000; Silva-Junior *et al.*, 2006). However, we found that the most frequent mtDNA lineages of African descendant in the Brazilian Amazon belonged to haplogroups L3e (19/80, 23.7%), L2a (18/80, 22.5%) and L1b (10/80, 12.5%), these three haplogroups together being found in a total of 47 individuals of the 80 individuals of African maternal lineage (58.7%). These results suggest the existence of dissimilarities in the geographical distribution of African lineages in Brazil, which may be directly associated with slave trade practices adopted in the eighteenth and nineteenth centuries.

We also found that of the two sub-clade L1c2 lineages detected, both with the same haplotype, one (AFR 48a) presented the *Hae* III restriction site at position 663, which is the marker for Amerindian lineages in haplogroup A. The isolated occurrence of this Amerindian mutation in a lineage of African background might indicate recurrent mutation events, as described for Amerindian lineages by various authors (Bailliet *et al.*, 1994; Santos *et al.*, 1996; Torres *et al.*, 2006). Because this lineage was of certain African origin and was the only one to exhibit a marker of a different geographic group (*i.e.*, an Amerindian marker) we classified it as a complex haplotype.

In our study, there were low frequencies of some other haplogroups and sub-clades, such as L1c2, L2b, L3f1 and L3h. Sub-clade L3f1 only matches the lineages described in the central African Cabinda population (Beleza *et al.*, 2005). The haplotype that represented haplogroup L3h (AFR 80) matched lineages described in the western African region of Guinea-Bissau where it was first described (Rosa *et al.*, 2004). The L1b, L1c2, L3e1, L3f1 and L3h sub-clades have not previously been reported in the Brazilian Amazon (Alves-Silva *et al.*, 2000; Silva-Junior *et al.*, 2006). It is important to emphasize that this is the first report of the L3h haplogroup in Brazil, although it has been reported in Guinea-Bissau in western Africa and Ethiopia in eastern Africa (Kivisild *et al.*, 2004; Rosa *et al.*, 2004) (Figure 1).

Regarding slave trade practices, historical data suggests that 30% of the Africans brought to the Brazilian Amazon came from western Africa (Klein, 2002). Although most of the mtDNA haplogroups and sub-clades are geographically nonspecific, some groups have different distributions in Sub-Saharan Africa. For example, if we consider haplogroups L1b, L2c and L3d as markers from western Africa (Bandelt *et al.*, 2001; Salas *et al.*, 2004) it is possible to estimate that the contribution made by this region to the formation of contemporary Brazilian Amazonian African-descendant populations is about 25%. On the other hand, other haplogroups or sub-clades are more frequent in western, central and southeastern Africa, regions related to the major Bantu linguistic branch. Studies of hemoglobin S related polymorphisms in Brazilian Amazonian African-descendant populations suggest a Bantu contribution of approximately 45% (Pante-de-Souza *et al.*, 1998). Adopting L0a, L2a, L1c, L3e1, and L3e2 as representative Bantu markers (Bandelt *et al.*, 1995; Watson *et al.*, 1997; Alves-Silva *et al.*, 2000) leads to an estimate of 50%. Therefore, the data obtained by us are in agreement with the historical sources and other previously published biological data.

Amerindian fraction

The second most important contribution to the mtDNA lineages of African-descendant populations were Amerindian-descendant (74/159, 46.6%). The four major Amerindian haplogroups (A, B, C and D) were well characterized by RFLP and the HVS-I region. Haplogroups A and C were the most frequent and presented similar distributions, followed by haplogroups B and D (Figure 2).

Haplogroup A lineages were defined by the 663/*Hae* III marker in association with the transitions at np 16111, 16290, and 16319 in the HVS-I region (Torroni *et al.* 1993). The mutation at np 16111, identified in Amerindian lineages, was not detected in two of the haplotypes (see Table 1). Similarly, Santos *et al.* (1996) also reported the absence of this transition in five Brazilian Amazonian Amerindian samples belonging to the same haplogroup. The 9 bp deletion and the absence of the 3592/*Hpa* I restriction

site occurred in all the 17 sequences identified for haplogroup B (Hertzberg *et al.* 1989; Torroni *et al.* 1993). Curiously, we found that in haplogroup C (present in 20 of our samples) the 13259/*Hinc* II site occurred in one sequence (AFR 28a), this also having been reported in other southern Amazonian Brazilian Amerindian populations by Santos *et al.* (1996) and Bailliet *et al.* (1994). A recent study of Colombian Amerindians (Torres *et al.* 2006) reported a haplogroup C revertant lineage (recurrent mutation recreated +13259 *Hinc* II) with the same HVS-I haplotype as that identified by us in lineage AFR 28a. Own genetic structure analyses are consistent with reverse mutation at an early stage during the tribalization process.

We found that haplogroup D was the least frequent haplogroup in African descendants from the Brazilian Amazon. All 16 samples classified in this group were marked by the absence of the 5176/*Alu* I site. The T → C transition at site 16325 of HVS-I identified in Amerindian lineages of haplogroup D in Native Americans was observed in all haplotypes (Ward *et al.*, 1991; Torroni *et al.*, 1993; Santos *et al.* 1996).

Amerindian lineage groups have been detected in Brazilian Amazon African-descendant communities by classical genetic analysis, which detected 14.7% Amerindian lineage groups, and molecular genetic analysis, which detected 19.1% Amerindian lineage groups (Table 3). However, the mtDNA data revealed an average Amerindian contribution of 50% in nine African-descendant populations located in former slave settlements the Brazilian Amazon. Different genetic markers (*e.g.*, nuclear DNA and Y-DNA) may have underestimated the assimilation of the indigenous element in the formation of African-descendant communities. Alternatively, the Amerindian contribution may have occurred through a larger introgression from Amerindian women than from Amerindian men.

Table 3 - Estimates of parental contributions for African-derived populations from the Brazilian Amazon, considering uniparental and biparental genetic markers.

Genetic system and population	African	Amerindian	European	References
Classical polymorphism				
Cajueiro	0.674	0.000	0.326	Bortolini *et al.* (1999)
Cametá	0.480	0.341	0.179	Bortolini *et al.* (1999)
Curiaú	0.736	0.000	0.264	Guerreiro *et al.* (1999)
Pacoval	0.443	0.283	0.274	Guerreiro *et al.* (1999)
Trombetas	0.620	0.110	0.270	Schneider *et al.* (1987)
Average	0.590	0.147	0.263	
Nuclear DNA				
Cajueiro	0.488	0.250	0.262	Bortolini *et al.* (1999)
Cametá	0.534	0.224	0.242	Bortolini *et al.* (1999)
Trombetas	0.576	0.099	0.325	Bortolini *et al.* (1999)
Average	0.533	0.191	0.276	
Y-markers				
Cajueiro	0.780	0.000	0.240	Bortolini *et al.* (1999)
Cametá	0.280	0.180	0.540	Bortolini *et al.* (1999)
Curiaú	0.570	0.060	0.370	Ribeiro-dos-Santos *et al.* (2002)
Trombetas	0.840	0.030	0.130	Bortolini *et al.* (1999)
Average	0.616	0.064	0.320	
Mitochondrial DNA				
Cajueiro	0.700	0.300	0.000	Bortolini *et al.* (1999)
Cametá	0.400	0.600	0.000	Bortolini *et al.* (1999)
Curiaú	0.523	0.473	0.000	Ribeiro-dos-Santos *et al.* (2002)
Marajó	0.530	0.440	0.030	Present study
Mazagão	0.364	0.576	0.030	Present study
Pitimandeua	0.690	0.310	0.000	Present study
Tamauari	0.387	0.548	0.000	Present study
Trombetas-I	0.340	0.660	0.000	Bortolini *et al.* (1999)
Trombetas-II	0.563	0.437	0.000	Present study
Average	0.500	0.482	0.018	

The high mtDNA estimate of Amerindian lineages in the composition of these communities was unexpected because there are no historical data on significant sexual interaction between African slaves and other ethnic groups in *quilombo* communities. However, the settlement of South America, and the Brazilian Amazon in particular, has involved complex ethnic-social interactions, especially when it comes to pre-existing Amerindian societies. Settlement has resulted in the formation of genetically mixed urban populations, with Alves-Silva *et al.* (2000) having demonstrated that 54% of the mtDNA lineages of individuals from northern Brazil who considered themselves white have an Amerindian descent. Furthermore, Santos *et al.* (1999) investigated the urban populations of Belém, the capital of the Brazilian state of Pará, and observed a high Amerindian matrilineal contribution of 57%.

The presence of Amerindian lineages in trihybrid urban populations in the Brazilian Amazon has resulted from Portuguese policy in the sixteenth and seventeenth centuries that encouraged marriages between Portuguese settlers and Amerindian women (Cruz, 1973, Salles, 1988). Since historical records concerning Amerindian miscegenation with African-descendant populations are scarce, we suggest that the "Amerindian-African" union results from a survival and social resistance strategy against the slavery policy adopted in Brazil until the mid nineteenth century.

European and other fractions

European lineages represented the smallest contribution, being detected in only two samples (1.3%). Haplogroup K occurred in Mazagão in Amapá state and Marajó island in Pará state and was defined after the analyses of 23 RFLP (9 bp deletion) and the sequencing of the first hypervariable region (Table 4). The participation of European groups in the formation of African-descendant communities has not been reported in historical records. Therefore,

Table 4 - Haplogroup classification on five individuals from the Brazilian Amazon Afro-descendant communities of Mazagão (denominated as samples "A" and "B"), Marajó (denominated as sample "C") and Tamauari (denominated as samples "D" and "E"). The classification was based on screening for 23 restriction fragment length polymorphisms (RFLP) and the 9 bp deletion plus the sequencing of the first hypervariable region (HVS-I). For the exact nucleotide position of the HSVI 16000 should bee added to the value for each group.

Polymorphism	Population				
	Mazagão (sample A)	Marajó (sample C)	Tamauari (sample D)	Tamauari (sample E)	Mazagão (sample B)
RFLP					
5176 / *Alu* I	+	+	+	+	+
663 / *Hae* III	-	-	-	-	-
13259 / *Hinc* II	+	+	+	+	+
3592 / *Hpa* I	-	-	-	-	-
1715 / *Dde* I	-	-	-	-	-
2349 / *Mbo* I	-	-	-	-	-
7055 / *Alu* I	+	+	+	+	+
9070 / *Taq* I	-	-	-	-	-
8616 / *Mbo* I	+	+	+	+	+
10084 / *Taq* I	-	-	-	-	-
10394 / *Dde* I	+	+	-	-	-
11641 / *Hae* III	-	-	-	-	-
12810 / *Rsa* I	-	-	-	-	-
4577 / *Nla* III	+	+	+	+	+
4529 / *Hae* II	+	+	+	+	+
7025 / *Alu* I	+	+	+	+	+
8249 / *Ava* II	-	-	-	-	-
8994 / *Hae* III	+	+	+	+	+
9052 / *Hae* II	-	-	-	-	-
10028 / *Alu* I	+	+	+	+	+
12308 / *Hinf* I	+	+	+	+	+
13366 / *BamH* I	-	-	-	-	-
13704 / *BstN* I	+	+	+	+	+
9 bp deletion	-	-	-	-	-
HVS-I variable nucleotides	224 311 322	093 224 311	093 223 355	153 298	223 288
Result	K	K	Inconclusive	Inconclusive	Inconclusive

such lineages may have resulted from recent interethnic miscegenation.

Even though the techniques used by us were sophisticated, the classification of three Tamauari and Mazagão lineages (AFR 83, 84, and 85) representing 1.9% of the total sample, remained inconclusive (Table 1).

According to the results observed, the present study partially mapped the social-biological interactions that had occurred during the formation and expansion of Amazonian African-descendant communities. The mtDNA approach reveals that these populations congregate two main genetic backgrounds: African and Native American lineages. Our results also indicate that these communities are an important reservoir of mtDNA variability and diversity for these human geographic groups.

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