

The Ancestry of Brazilian mtDNA Lineages

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We have analyzed 247 Brazilian mtDNAs for hypervariable segment (HVS)–I and selected restriction fragment-length–polymorphism sites, to assess their ancestry in different continents. The total sample showed nearly equal amounts of Native American, African, and European matrilineal genetic contribution but with regional differences within Brazil. The mtDNA pool of present-day Brazilians clearly reflects the imprints of the early Portuguese colonization process (involving directional mating), as well as the recent immigrant waves (from Europe) of the last century. The subset of 99 mtDNAs from the southeastern region encompasses nearly all mtDNA haplogroups observed in the total Brazilian sample; for this regional subset, HVS-II was analyzed, providing, in particular, some novel details of the African mtDNA phylogeny.

Introduction

Brazilians form one of the most heterogeneous populations in the world, the result of 5 centuries of inter-ethnic crosses between peoples from three continents: the European colonizers, represented mainly by the Portuguese; African slaves; and the autochthonous Amerindians. When the Portuguese arrived, exactly 500 years ago, there were ~2.5 million indigenous people living in the area of what is now Brazil (Salzano and Freire-Maia 1970; Bethell 1997). The Portuguese-Amerindian admixture started soon after the arrival of the first colonizers. Mating between European men and indigenous women became commonplace and later (after 1755) was even encouraged as a strategy for population growth and colonial occupation of the country (Mörner 1967). The Amerindian tribes underwent a drastic demographic decline due to conflicts with the European colonizers and diseases to which they were not adapted (Salzano and Freire-Maia 1967, 1970; Monteiro 1994; Ribeiro 1995). Today there are ~326,000 Amerindians in Brazil, living on land set aside for them by the federal government. Africans were introduced beginning in the middle of the 16th century, brought to Brazil as slaves to work on sugarcane farms and, later, in the gold and diamond mines and on coffee plantations. Historical records suggest that between 1551 and 1850 (when the slave trade

was abolished), ~3.5 million Africans arrived in Brazil (Salzano and Freire-Maia 1967; Curtin 1969; Ribeiro 1995). As to the European immigration, it is estimated that ~500,000 Portuguese arrived in the country between 1500 and 1808 (Salzano and Freire-Maia 1967). From then on, after the Brazilian ports were legally opened to all friendly nations, Brazil received increasing numbers of immigrants from several parts of the world. Portugal remained by far the most important source of migrants, followed by Italy, Spain, and Germany. In the 20th century, Asian immigration took place, mainly from Japan, as well as from Lebanon and Syria. According to Callegari-Jacques and Salzano (1999), 58% of the immigrants who arrived in Brazil between 1500 and 1972 were Europeans, 40% were Africans, and 2% were Asians. The question that arises is, How much did these different groups actually contribute to the gene pool of present-day Brazilians?

Several studies were performed during recent decades, in an attempt to characterize the genetic background of the non-Amerindian Brazilian population (for a review, see Salzano 1997; Callegari-Jacques and Salzano 1999; Dornelles et al. 1999; Guerreiro et al. 1999). These studies included mainly samples from the northern and southern regions of the country. On the basis of classical genetic markers, these studies demonstrated that all analyzed groups show some degree of admixture and that the extent of admixture varied, depending on the region analyzed. Recently, some Brazilian population samples have been analyzed for mtDNA (Bortolini and Salzano 1996; Santos et al. 1996*b*; Ward et al. 1996; Bortolini et al. 1997*b*; Batista dos Santos et al. 1999) and Y-chromosome polymorphisms (Batista dos Santos et al. 1999). The mtDNA studies have shown that Amerindian and African contributions to northern Brazilians

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are larger than those previously observed on the basis of classical markers (Batista dos Santos et al. 1999; Santos et al. 1999).

mtDNA analysis has been used extensively during the past 10 years, since the pioneering works of Vigilant (1990), Stoneking et al. (1991), and Vigilant et al. (1991). Phylogeographic analysis of mtDNA lineages from all over the world has led to the identification of mtDNA haplogroups that are specific to either Africans, Europeans, or Asians/Amerindians (Torroni et al. 1993, 1994, 1996, 1998; Chen et al. 1995; Richards et al. 1996; Watson et al. 1997; Kivisild et al. 1999a, 1999b; Macaulay et al. 1999; Metspalu et al. 1999). Haplogroup allocation of a given mtDNA lineage allows the assessment of its (sub)continental origin, so that the matrilineal ancestry of admixed populations can be evaluated well (Torroni et al. 1995; Bravi et al. 1997; Green et al. 2000; Rando et al. 1999).

In the present article, we follow this approach by sequencing part of the control region and by screening specific RFLP sites, to better understand the extent of the matrilineal genetic contribution of Europeans, Africans, Amerindians, and Asians to the gene pool of present-day Brazilians.

Subjects and Methods

Samples

We analyzed 247 unrelated Brazilian individuals (mainly classified as “white” in Brazil and belonging to the middle and upper-middle classes) who came from four of the five major geographic regions of the country (fig. 1). According to the Instituto Brasileiro de Geografia Estatística, responsible for the census in Brazil, 51.6% of Brazilians in 1996 classified themselves as white. In detail, there were 99 individuals from the southeastern (mostly from the state of Minas Gerais), 50 from the southern (states of Rio Grande do Sul, Santa Catarina, and Paraná), 48 from the northern (states of Amazonas, Pará, Rondônia, and Acre), and 50 from the northeastern (state of Pernambuco) regions. Thirty-seven individuals were students or staff in our laboratory, whereas 210 were randomly chosen unrelated participants in paternity-testing studies. Written consent was obtained from all participants, and all analyses were performed anonymously.

mtDNA Control-Region Amplification and Sequencing

The nucleotide sequence of mtDNA hypervariable segment I (HVS-I), between nucleotide positions (np) 16060 and 16362, was determined for all the individuals in the study (see GenBank accession numbers in the Electronic-Database Information section). PCR amplification of the mtDNA control region was performed in a 45- μ l vol-



Figure 1 Five major geographic regions of Brazil: N = northern, NE = northeastern, SE = southeastern, S = southern, and CW = central west. Brazilian states from which the mtDNA lineages of the present study have mainly been sampled are indicated by name.

ume. Each tube contained 0.8 μ M of primers L15926 5'-TCAAAGCTTACACCAGTCTTGTAACC-3' and H16498 5'-CCTGAAGTAGGAACCAGATG-3', 200 μ M dNTP, and 0.5 U of *Taq* DNA polymerase (Promega). Thirty cycles of denaturation at 94°C for 1 min, annealing at 55°C for 30 s, and an extension at 72°C for 1 min were done. Negative controls were run simultaneously, to detect reagent contamination. PCR products were visualized in 1% agarose-gel electrophoresis with ethidium bromide. Amplified segments were purified using Magic PCR Preps (Promega), and dideoxy sequencing was done with the Thermo Sequenase Sequencing Kit (Amersham Life Science) and a fluorescent-labeled primer L15996 5'-CTCCACCATTAGCACCC-AAAGC-3' or H16401 5'-TGATTTCACGGAGGATGGTG-3'. For the samples from the southeastern region, the HVS-II sequence between np 72 and 337 was also determined. Primers L29 5'-GGTCTATCACCCCTATTAACCAC-3' and H580 5'-TTGAGGAGGTAAGCTACATA-3' were used in PCR reactions, in the same conditions described above, and a fluorescent-labeled primer L48 5'-CTCACGGGAGCTCTCCATGC-3' or H408' 5'-CTGTTAAAGTGCATACCGCCA-3', was used in sequencing reactions.

Partial Restriction-Site Analysis

Several amplified segments, mainly in the mtDNA coding regions, were analyzed by RFLP tests, according

to the method described by Chen et al. (1995) and Torroni et al. (1996), to screen haplogroup-specific sites (table 1). PCR amplifications were performed using the primers and conditions described by Torroni et al. (1992, 1993, 1996). Digestions were carried out according to the conditions specified by the manufacturer (Gibco BRL). The resulting fragments were resolved by electrophoresis in 1% agarose gels and were visualized by UV-induced fluorescence after ethidium bromide staining. Depending on the number and length of resulting fragments, they were visualized in 8% acrylamide gels after silver staining. The 12308 *HinfI* polymorphic site was analyzed using the mismatched primer described by Torroni et al. (1996).

Phylogeographic Analysis

We build on the phylogenetic analyses of European (Richards et al. 1998; Macaulay et al. 1999) and African (Rando et al. 1998) mtDNA, which combine HVS-I and RFLP information. According to the nomenclature of those analyses, human mtDNAs are divided into three supergroups—L1 (+3592 *HpaI*, –10806 *HinfI*), L2 (+3592 *HpaI*, –16390 *HinfI*), and L3 (–3592 *HpaI*). L1 and L3 are further subdivided into haplogroups, which can be recognized by specific restriction sites (table 1). L1 and L2 are African specific, whereas L3 is ubiquitous but encompasses several haplogroups that are (nearly) continent specific. From HVS-I sequences

alone, the fine-grained haplogroup status can be read off only to some extent, and, therefore, their characteristic restriction site(s) need to be tested for confirmation. In the case of haplogroups that are shared between continents, HVS-I motifs or exclusive matches could further suggest the most plausible geographic origin. Figure 2 displays the hierarchy of haplogroups that is relevant for the present study. Note that haplogroup U includes haplogroup K. In the fine classification of mtDNA lineages, we employ the “asterisk notation” (Richards et al. 1998): an mtDNA lineage belongs to some haplogroup labeled with an asterisk if it is a member of that group but not of any otherwise-highlighted subgroup.

Results

mtDNA Composition of the Brazilian Population

The 247 Brazilian mtDNA lineages, yielding 170 different HVS-I haplotypes, can be perfectly allocated to the known haplogroups (tables 2 and 3 and fig. 2). Altogether, 82 mtDNA lineages fall into the Native American/Asian haplogroups, A–D (with one A lineage of confirmed western-Asian ancestry), whereas 69 belong to various African haplogroups and 96 belong to European haplogroups. The relative frequencies of these continental fractions of the mtDNA pool, though, vary considerably over the four Brazilian regions analyzed. In the northern region, the majority of the mtDNA line-

Table 1

RFLP Polymorphisms Used to Identify mtDNA Haplogroups and Geographic Origin

HAPLOGROUP	CHARACTERISTIC RESTRICTION SITE(S)	STATUS ^a		
		Sub-Saharan African	Native American	European
L1a	+3592 <i>HpaI</i> , +11641 <i>HaeIII</i>	+	–	–
L1b	+3592 <i>HpaI</i> , –7055 <i>AluI</i> , +2349 <i>MboI</i>	+	–	–
L1c	+3592 <i>HpaI</i> , +9070 <i>TaqI</i> , +12810 <i>RsaI</i>	+	–	–
L2	+3592 <i>HpaI</i> , +16389 <i>HinfI</i>	+	–	–
L3b	+10084 <i>TaqI</i>	+	–	–
L3d	–3592 <i>HpaI</i> , –8616 <i>MboI</i>	+	–	–
L3e	–3592 <i>HpaI</i> , +2349 <i>MboI</i>	+	–	–
A	+663 <i>HaeIII</i>	–	+	–
B	9-bp deletion	–	+	–
C	–13259 <i>HincII</i>	–	+	–
D	–5176 <i>AluI</i>	–	+	–
H	–7025 <i>AluI</i>	–	–	+
V	–4577 <i>NlaIII</i>	–	–	+
HV	–14766 <i>MseI</i>	–	–	+
U	+12308 <i>HinfI</i>	–	–	+
K	–9052 <i>HaeII</i>	–	–	+
J	–13704 <i>BstNI</i>	–	–	+
T	+13366 <i>BamHI</i> , +15606 <i>AluI</i>	–	–	+
I	–4529 <i>HaeII</i> , +8249 <i>AvaII</i> , +16389 <i>BamHI</i> , +10032 <i>AluI</i>	–	–	+
W	+8249 <i>AvaII</i> , –8994 <i>HaeIII</i>	–	–	+
X	–1715 <i>DdeI</i>	–	+	+

^a A plus sign (+) denotes that the haplogroup is indigenous; a minus sign (–) denotes that it is not.

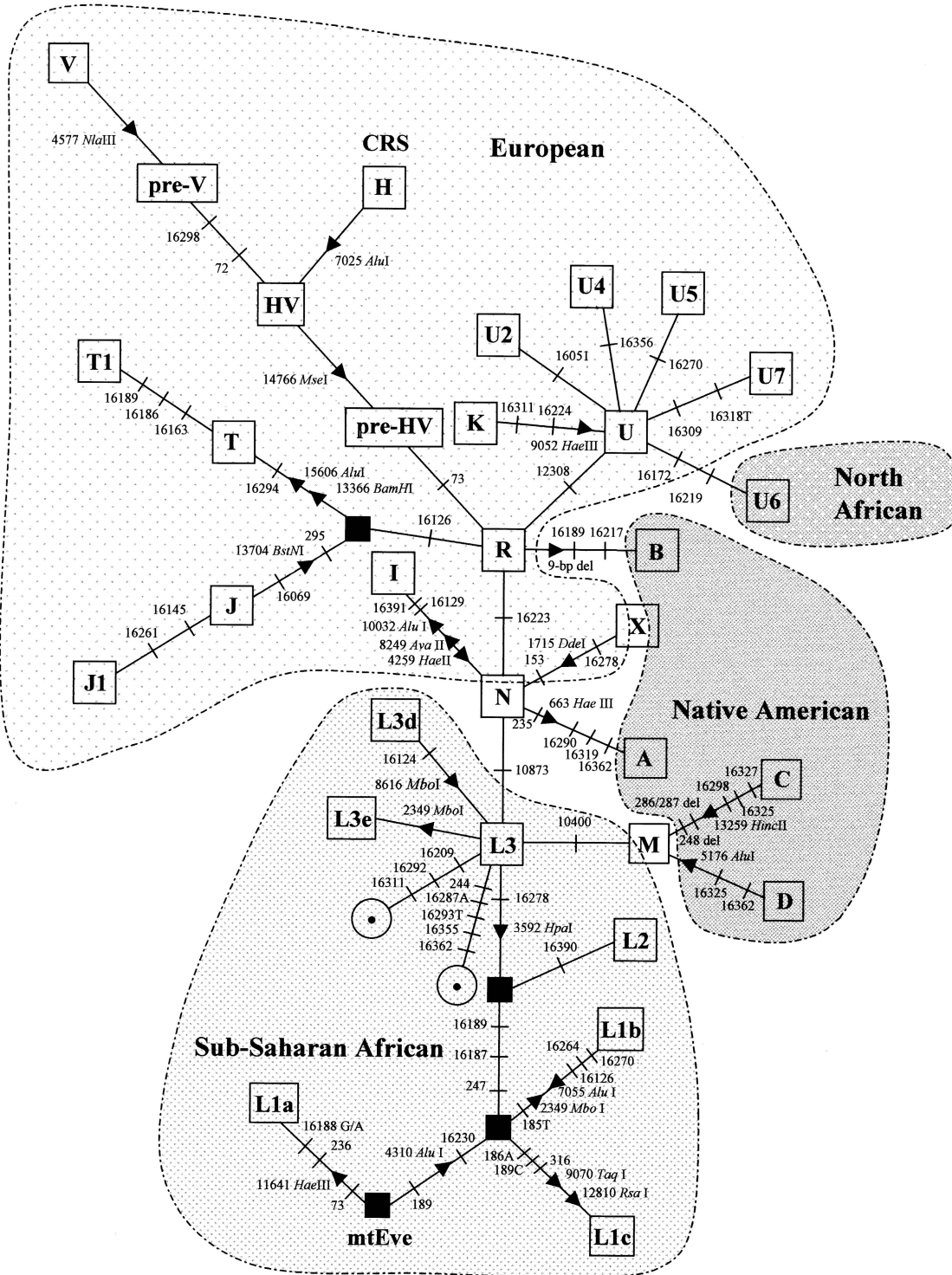


Figure 2 Classification tree highlighting selected diagnostic sites and positions for haplogroups present in the Brazilian sample (see tables 1 and table 6). Each square represents the root node of the respective haplogroup, with the acronym inscribed; two central/eastern-African haplogroups, represented by circles, are only partially characterized (T. Kisivild, personal communication). “CRS” indicates the revised reference sequence (Andrews et al. 1999). Numbers along links refer to RFLP sites (with arrows pointing to presence of sites) or transitions, unless a single-letter suffix indicates a transversion. Note that some diagnostic sites and positions, especially in the control region, have undergone recurrent mutations. The root of the tree, labeled “mtEve,” is inferred by employing the Neanderthal HVS-I and HVS-II sequences (Krings et al. 1997, 1999) and the coding-region sequences of bonobo and common chimpanzee (Horai et al. 1995) as outgroups; this corroborates the rooting of the Vigilant (1990) tree.

BR41	1		T.....G.....C.....CT.....	C
BR42	1	1	T.....A.....C.....CT.....T.	C
BR43		1	1T.....T.....C.....CT.....C	C
BR44	2		T.....C.....CT.....	C
BR45		2	T.....C.....C.....	D
BR46		1	T.....T.....T.....C.....C	D
BR47			1T.....T.....T.....C.....C	D
BR48	1	1	2T.....A.....T.....C.....C	D
BR49		1	T.....T.....C.....C.....C	D
BR50	1		C.....T.....C.....C.....C	D
BR51	1		C.....T.....C.....C.....C	D
BR52			1T.....C.....C.....C.....C	D
BR53	1		A.....T.....T.....G.....C.....T.....C.....C	D
African:					
BR54	1		T.....C.....TGC.....T.....G.....C.....T.....	L1a
BR55		2	A.....T.....TC.....TGC.....T.....G.....C.....T.....	L1a
BR56		1	A.....T.....TC.....TGC.....T.....G.....T.....C.....T.....C..	L1a
BR57		1	1A.....T.....TC.....TGC.....T.....G.....T.....G.....C.....T.....	L1a
BR58	1		C.....A.....T.....TC.....TGC.....T.....G.....T.....G.....C.....T.....	L1a
BR59		1	1C.....T.....C.....T.....C.....T.....T.....T.....T.....G.....C.....	L1b
BR60		1	T.....C.....T.....C.....T.....T.....T.....T.....G.....C.....	L1b
BR61	1		C.....T.....C.....T.....T.....T.....T.....T.....C.....	L1b
BR62		1	A.....T.....T.....C.....T.....T.....C.....TA.....T.....T.....C.....T.....	L1c
BR63		1	C.....A.....T.....T.....C.....T.....T.....C.....TG.....T.....C.....T.....	L1c
BR64	1		T.....C.....A.....A.....G.....T.....C.....A.....T.....T.....C.....TG.....T.....C.....T.....	L1c
BR65		1	A.....A.....G.....T.....C.....C.....T.....G.....T.....C.....T.....	L1c
BR66	2		A.....T.....G.....T.....C.....C.....T.....T.....T.....GT.....C.....T.....	L1c
BR67	1		T.....T.....G.....T.....C.....T.....T.....T.....GT.....C.....T.....	L1c
BR68			1T.....C.....T.....T.....T.....T.....GT.....C.....T.....	L1c
BR69	1		A.....T.....T.....C.....T.....T.....T.....GT.....C.....T.....	L1c
BR70	1		A.....T.....T.....C.....T.....T.....T.....GT.....C.....T.....	L1c
BR71	1		C.....A.....A.....T.....T.....C.....T.....G.....AT.....T.....GT.....C.....T.....	L1c
BR72			1A.....A.....G.....T.....C.....T.....T.....T.....GT.....C.....T.....	L1c
BR73	1		A.....T.....C.....T.....T.....T.....GT.....C.....T.....	L1c
BR74		1	C.....T.....T.....T.....T.....T.....G.....	L2
BR75		1	C.....TCT.....T.....T.....T.....G.....	L2
BR76	1		1CT.....T.....T.....T.....G.....	L2
BR77		1	T.....T.....T.....G.....	L2
BR78	1		T.....T.....T.....T.....	L2
BR79	1		T.....G.....T.....T.....	L2
BR80	1		A.....A.....T.....T.....T.....T.....T.....C.....T.....	L2
BR81	2		T.....T.....T.....T.....C.....	L2
BR82		1	T.....T.....T.....T.....	L2
BR83			1A.....A.....A.....T.....T.....T.....T.....	L2
BR84	1		A.....A.....A.....T.....T.....T.....	L2
BR85	1		TC.....T.....T.....	L2
BR86		1	A.....C.....T.....C.....	L3d
BR87		1	C.....C.....C.....T.....T.....T.....GT.....C.....C.....	L3d
BR88		1	C.....T.....T.....C.....	L3d
BR89		1	C.....C.....T.....T.....	L3d
BR90		1	T.....T.....	L3e

BR131	1		1T.....C...C.....	pre*V
BR132		1	T.....C.....	pre*V
BR133	1		C.....	V
BR134	1		C.....	V
BR135		1	A.....C.....	V
BR136		1	C.....C.....	V
BR137	2		C.....T....	V
BR138		1	A.....C.....	HV*
BR139	1		1C.....C.....C	U2
BR140	1		1T.....C.....C...	U4
BR141	1		C.....T..A.....C...C.....	U5b*
BR142		1	C.....T.....	U5b*
BR143		1	C.....T.C.....T.....	U5b1
BR144		1	G...T.....	U7
BR145	1		C.....	K
BR146		1	1C.....C.....C.....	K
BR147			1C.....C.....C.....	K
BR148	1		A.....C...C.....C.....	K
BR149			1C.....C.....C...A.....	K
BR150		1	C.....C.....C.....C...A.....	K
BR151		1	C.....C.....C.....C.....	pre*HV
BR152	1	2	1	2 .T.....C.....	J*
BR153		1		.T.....C.....T.....	J*
BR154			1	.T...C.....C.....T.....T.....	J*
BR155		1		.T.....C...A.....T.....T.....	J1*
BR156		2		.T.....C...A.....C.....T.....T.....	J1b1
BR157			1C.....C.....C.....T.T...C.....	T*
BR158	1	1	1C.....T.T...C.....	T*
BR159		1	C...T.....C.....T.TC...C.....C	T*
BR160	1		C.....T.T.....	T*
BR161	1		C.....T.T...C.....	T*
BR162		1	C...A.....T.T.....	T*
BR163			1C...A.....T.....T.....	T*
BR164			1	T.....T.C...A.....T.....T.....	T*
BR165		1	C.....T.T.....	T*
BR166		1	1C.....G.....T.C.....T.....	T1
BR167			1A.....T.....	I
BR168		1	C.....T.....T.....T.....	X
BR169		1	C.....T.....G.....G...T.....	X
BR170		1	C.....T.....T.....	X
Total	99	50	50	48	

^a BR13 and BR14 share the loss of 3534 *DdeI*, whereas controls BR4, BR5, BR15, and BR16 were +3534 *DdeI*. BR132 has C at np 72, and for BR139 the U2-characteristic transition at np 16051 has been confirmed.

^b SE = southeastern, S = southern, NE = northeastern, N = northern.

^c The prefix "16" has been deleted from all (three-digit) numbers. Length polymorphisms in the C run (np 16184 and 16193) are disregarded.

^d Confirmed by testing required restriction sites, listed in table 1, for each lineage (except for sites +9070 *TaqI* and +12810 *RsaI* in BR69 and BR70); more-extensive screening was performed for the lineages of status L3e*, pre*V, and pre*HV (see table 3).

^e Source: Andrews et al. (1999).

Table 3
RFLP Sites Screened in Some mtDNAs

POLYMORPHISM	STATUS OF SAMPLE ^a						
	BR107	BR108	BR109	BR131		BR132	BR151
				Southeastern	Northern		
9-bp deletion	-	-	-	-	-	-	-
663 <i>Hae</i> III	-	-	-	-	-	-	-
1715 <i>Dde</i> I	+	+	+	+	+	+	+
2349 <i>Mbo</i> I	-	-	-	-	-	-	-
3592 <i>Hpa</i> I	-	-	-	-	-	-	-
4216 <i>Nla</i> III	ND	ND	-	-	-	-	-
4529 <i>Hae</i> II	+	+	+	+	+	+	+
4577 <i>Nla</i> III	ND	ND	+	+	+	+	+
5176 <i>Alu</i> I	+	+	+	+	+	+	+
7025 <i>Alu</i> I	+	+	+	+	+	+	+
8249 <i>Ava</i> II	-	-	-	-	-	-	-
8616 <i>Mbo</i> I	+	+	+	+	+	+	+
8994 <i>Hae</i> III	ND	+	+	+	+	+	+
9052 <i>Hae</i> II	+	+	+	+	+	+	+
10032 <i>Alu</i> I	-	-	-	-	-	-	-
10084 <i>Taq</i> I	-	-	-	-	-	-	-
10394 <i>Dde</i> I	+	+	+	-	-	-	-
10397 <i>Alu</i> I	ND	-	-	-	-	-	-
12308 <i>Hin</i> fI	-	-	-	-	-	-	-
12406 <i>Hpa</i> I	-	-	+	-	+	+	+
13259 <i>Hin</i> cII	+	+	+	+	+	+	+
13366 <i>Bam</i> HI	-	-	-	-	-	-	-
13704 <i>Bst</i> NI	+	+	+	+	+	+	+
14766 <i>Mse</i> I	ND	+	ND	-	-	-	+
15606 <i>Alu</i> I	-	-	-	-	-	-	-
16389 <i>Bam</i> HI	-	-	-	-	-	-	-
Haplogroup	L3*	L3*	L3*	pre*V	pre*V	pre*V	pre*HV

^a A plus sign (+) denotes presence; a minus sign (-) denotes absence; and ND = not done.

ages are of Native American ancestry, whereas African ancestry is most prominent in the northeastern region, with the southeastern region being intermediate between the two former regions in this regard; finally, the southern region stands out, with a great majority of European mtDNA lineages (table 4).

Native American Fraction of the Brazilian mtDNA Pool

Haplogroup A is the most frequent haplogroup within the Native American fraction, closely followed by haplogroup B, with C coming next and D last (table 5). As to the regional distribution, the same order of frequencies is observed in the southern and southeastern regions, but C is the leading haplogroup in the northern region, whereas it is the least frequent in the northeastern region.

The major founder haplotypes of the Native American haplogroups A-D (Forster et al. 1996; Smith et al. 1999) are all present in Brazil and are shared with many Native American populations. Interestingly, there are several matches with derived haplotypes in other South American populations: haplotypes BR26, BR27, BR43, and BR47 have been observed elsewhere, in the Amazonian sample of Santos et al. (1996b); haplogroup BR14 has

also been identified, with the absence of the site 3534 *Dde*I (table 2, footnote a), in the Krahó from Goiás, Brazil (Torrioni et al. 1993); BR51 occurs in the Zoró from Mato Grosso, Brazil, and in the Gavião from Rondônia, Brazil (Ward et al. 1996); haplotype BR39 is found in Colombia (Horai et al. 1993); and BR49 is found in the Mapuche from Argentina (Ginther et al. 1993). Remarkably, one D lineage (BR53) constitutes a true outlier in our sample, since it differs by as many as seven transitions from the D founder haplotype, four of which are shared with a haplotype found in the Cayapa from Ecuador (Rickards et al. 1999).

African Fraction of the Brazilian mtDNA Pool

Haplogroups L3e and L1c together constitute approximately one-half (49%) of the African fraction (table 5). Nowhere in western Africa has such a high percentage been observed so far for this haplogroup pair: 7% is seen in the mtDNA pool of several Senegalese populations (Graven et al. 1995; Rando et al. 1998), and 17% has been seen in the joint mtDNA pool of the Songhai, Yoruba, Hausa, and Kanuri (Watson et al. 1997, table A1). The Bubi, from the island of Bioko in

Table 4
Frequency of Continent-Specific mtDNA Haplotypes in the Brazilian mtDNA Pool

CONTINENTAL FRACTION	FREQUENCY				
	Brazil	Northern	Northeastern	Southeastern	Southern
Native American	.33	.54	.22	.33 ^a	.22
African	.28	.15	.44	.34	.12
European	.39	.31	.34	.31	.66

^a Excludes the single lineage of confirmed Asian ancestry.

the Gulf of Guinea (central Africa), however, have an mtDNA contribution of 33% L3e and 22% L1c, yielding a joint percentage of 56% (as inferred from table 2 of Mateu et al. 1997). This exceeds even the corresponding relative frequency in our Brazilian sample. On the other hand, haplogroups L3d and L1b, which are quite specific to western Africa, are absent in the Bubi but together occur at a frequency of 10% in the African fraction of the Brazilian mtDNA pool. For this haplogroup pair, much higher frequencies are found in western Africa: 25% in Senegal and 17% among the Songhai, Yoruba, Hausa, and Kanuri. This suggests that the majority of the mtDNA lineages of African ancestry in the Brazilian sample had their origin in central Africa (which would include Cameroon, as well as Angola), although a substantial number must have come from western Africa. Only few mtDNA haplotypes in the Brazilian sample could potentially testify to southeastern-African origin. BR55 would be a good candidate since it perfectly matches the most frequent 9-bp-deletion L1a haplotype, found in Malawi and in southeastern Bantu speakers (Soodyall et al. 1996): the matching involves the 9-bp deletion, HVS-I (table 2), and HVS-II (table 6), even extending to position 64 (which, in this case, could be read with only one primer). The same HVS-I type (with the 9-bp deletion) has been found in São Tomé (Mateu et al. 1997), which served as an entrepôt for the Atlantic slave trade (Curtin 1969).

European Fraction of the Brazilian mtDNA Pool

The Brazilian sample includes mtDNA lineages from almost all the familiar European haplogroups (Torroni et al. 1996; Macaulay et al. 1999), except for some marginal ones, such as W and other quite-rare haplogroups related to haplogroup I (Kivisild et al. 1999b). The frequency of the dominant haplogroup H (44%; table 5) in the European fraction is somewhat higher, on average, than that observed in Europe but is well within the range of western-European H frequencies (Torroni et al. 1998). In particular, the relatively high frequency of the Cambridge reference sequence (CRS [Andrews et al. 1999]) haplotype (24%) and of haplogroup pre-V (9%) suggests predominantly western-European ancestry.

The majority of haplotypes from the European frac-

tion already have been recorded in the European mtDNA pool (Richards et al. 1996, 1998; Helgason et al. 2000) and, in many instances, match mtDNA lineages from the Iberian Peninsula. Nevertheless, there are a few sequences for which one would not predict southwestern-European ancestry; the most striking example is the U5b1 haplotype BR143, which bears the “Saami motif” (Sajantila et al. 1995) and is thus of northern Fennoscandian origin.

HVS-II Motifs for Classification of Brazilian mtDNA Lineages

To see to what extent a first sorting into haplogroups could be based on HVS-II sequences, we have sequenced all but one of the mtDNA lineages of the southeastern-Brazilian sample, for HVS-II (table 6). It turns out, by comparison with the data of Vigilant (1990), that haplogroups L1a, L1b, L1c, and an unnamed haplogroup (within L3*) are readily identified individually by one to three positions. L1 is separated from L2 and L3 by a transition at np 247 (fig. 2), which is also evident from the data of Graven et al. (1995). Curiously, np 247 was sorted into the class of most highly mutable positions in HVS-II, by Meyer et al. (1999), although it is known to be virtually unvaried in European mtDNA (see the data sets of Piercy et al. [1993] and Helgason et al. [2000]). The two major African haplogroups, L2 and L3e, represented in the southeastern-Brazilian data set exhibit only diagnostic positions that seem to be hyper-variable (np 146, 150, 152, and 195), although HVS-II certainly offers some information for internal classification of L2. Among the Native American haplogroups, only A and C can be detected using HVS-II alone: A is recognized by a transition at np 235, and C is recognized by two deletions (fig. 2). The major HVS-II polymorphism for European mtDNA is at np 73 (Côrte-Real et al. 1996): 73A characterizes pre-HV. Note that 73A is also characteristic of the African haplogroup L1a, but that, in general, np 73 seems to be fairly stable: no further parallel mutation from G to A has been observed, either in our data or in those of Graven et al. (1995), Brown et al. (1998), Macaulay et al. (1999), and Helgason et al. (2000), a result that is at variance with the findings of Salas et al. (1998), who reported J, K, and

Table 5
Haplogroup Frequencies within the Three Continental Fractions of Brazilian mtDNA Pool

HAPLOGROUP	FREQUENCY IN BRAZIL				
	Overall	Northern	Northeastern	Southeastern	Southern
Native American: ^a					
A	.30	.15	.37	.39	.27
B	.29	.31	.27	.30	.27
C	.24	.38	.09	.18	.27
D	.16	.15	.27	.12	.18
Total	1.00	1.00	1.00	1.00	1.00
African:					
L1a	.1018	.06	.17
L1b	.0405	.03	.17
L1c	.19	.29	.09	.23	.17
L2	.20	.14	.23	.23	...
L3d	.060933
L3e	.30	.43	.32	.32	...
L3*	.0405	.06	...
U6	.06	.1406	.17
Total	1.00	1.00	1.00	1.00	1.00
European:					
H	.44	.27	.65	.45	.39
pre*V	.03	.0703	.03
V	.0606	.13	.03
HV*	.0103
U	.16	.13	.18	.16	.15
pre*HV	.0103
J	.11	.20	.06	.03	.18
T	.14	.27	.06	.13	.12
I	.01	.07
X	.0306	.03
Total	1.00	1.00	1.00	1.00	1.00

^a Excludes the single A lineage (from the southeastern region) of confirmed Asian ancestry.

T lineages with 73A. The few back mutations from A to G that are observed in haplogroup H (Helgason et al. 2000) and L1a (Soodyall et al. 1996) may testify to a directional bias in substitution rates at np 73. Furthermore, only haplogroups pre-V, J, and X can be recognized by single positions in HVS-II; otherwise, HVS-II is not of much help for allocation to European haplogroups. In summary, even this rough HVS-II classification scheme would permit allocation of the majority of the Brazilian mtDNA lineages to the three continental fractions.

Discussion

We examined individuals from four different regions in Brazil (fig. 1), in an attempt to establish a portrait of the mtDNA variation throughout the country and to determine the relative matrilineal contributions of Europeans, Amerindians/Asians, and Africans to present-day white Brazilians. The total sample revealed as much as 33% Amerindian and 28% African contribution to the total mtDNA pool. In fact, these values are probably

minimum percentages, because, since our study group is primarily composed of middle- and upper-middle-class Brazilians, a bias toward a higher contribution of European mtDNA is to be expected.

Most of the studies involving the Brazilian population have been performed on Amerindian tribes, mainly from the Amazonian region (Schurr et al. 1990; Torroni et al. 1993; Bailliet et al. 1994; Bianchi et al. 1995; Bortolini and Salzano 1996; Easton et al. 1996; Santos et al. 1996b; Ward et al. 1996; Bortolini et al. 1998a), and predominantly African-derived groups (Schneider et al. 1987; Bortolini et al. 1992, 1995, 1997a, 1997b, 1998b; Arpini-Sampaio et al. 1999; Guerreiro et al. 1999). A small number of studies performed on white Brazilians (deemed to be mostly of European descent) that used mainly protein genetic systems focused on populations from the southern or northern regions (Franco et al. 1982; Rosa et al. 1984; Moraes et al. 1993; Santos et al. 1996a; Batista dos Santos et al. 1999; Dornelles et al. 1999). These studies showed that the amount of Amerindian ancestry in the white Brazilians varies widely and has distinct patterns in the different

regions of the country. The highest Amerindian influence was observed in populations of the Amazonian region, where a study analyzing 11 urban populations by use of nuclear markers observed an average of 41% Amerindian ancestry (Santos and Guerreiro 1995). Recent mtDNA analysis of another population from the northern region showed an even higher Amerindian contribution (59% [Batista dos Santos et al. 1999]). In our sample, we also observed a high Amerindian influence in the northern region (54%), corroborating the mtDNA data obtained by Batista dos Santos et al. (1999). In the other regions of Brazil, the genetic contribution from Amerindians is also markedly higher for mtDNA than for nuclear DNA: 22% (table 4) versus 13% (Krieger et al. 1965; Franco et al. 1982; Conceição et al. 1987) in the northeastern region and 22% (table 4) versus 11% (Dornelles et al. 1999) in the southern region. For the southeastern region, where we have detected 33% frequency of mtDNA lineages of Amerindian ancestry, not a single study with nuclear markers has yet been performed, probably because one did not anticipate measurable Amerindian genetic influence on urban populations (Salzano 1997). As for African admixture in the white Brazilian population, the picture is similar to what we have seen for the Amerindian genetic input: mtDNA analysis (table 4) suggests a higher contribution than that by nuclear markers, for which 12% in the northern region (Santos et al. 1996a), 36% in the northeastern region (Franco et al. 1982; Arpini-Sampaio et al. 1999), and 10% in the southern region (Dornelles et al. 1999) were reported; again, no nuclear data are available for the southeastern region.

The allocation of haplogroups to continents (as indicated in table 1) is, of course, not absolutely clear-cut. For instance, the European haplogroups H and U5 do occur in the sub-Saharan mtDNA pool, albeit in only two founder types (bearing transitions at np 16145 and 16222 for the H type and transitions at np 16189, 16192, 16270, and 16320 for the U5 type), possibly transmitted by Berbers or, even earlier, during the Saharan Neolithic age (Rando et al. 1999). None of these particular mtDNA lineages occur in our Brazilian sample. Similarly, northern-African U6 haplotypes have penetrated the Sahara and are found sporadically from the west (Senegal) to the east (Kenya). We consider it most plausible that the four U6 lineages in our sample have come from western Africa. On the other hand, African haplotypes were also transmitted, in low numbers, to Europe, especially to the Mediterranean area. African mtDNA lineages, then, constitute erratic outliers in the respective mtDNA samples, for instance, such as the L1c lineage in the British data of Piercy et al. (1993).

There is one caveat with regard to the distinction between European mtDNA haplotypes and Native

American ones: haplogroup X is shared by western Eurasia and North America (Brown et al. 1998; Smith et al. 1999), although there is as yet no compelling evidence for the occurrence of haplogroup X in Central or South America. The three X haplotypes that we detected in the Brazilian sample are certainly of European ancestry, since BR169 and BR170 do not bear the np-200 transition that is characteristic of (most of) the Native American haplogroup X (Brown et al. 1998), whereas BR168 (for which no HVS-II information is available) bears a transition (namely, at np 16248) already observed in Europe (Richards et al. 1998).

The distinction between Asian and Native American mtDNA haplotypes is more intricate inasmuch as haplogroups A–D are of Asian origin. Fortunately, the Native American A, C, and D founder HVS-I and HVS-II types can be distinguished from Asian haplotypes by mutations that are virtually absent, or at least rare, in Asia. The transition at np 16325 is (almost) diagnostic for Native American C and D haplotypes; the 2-bp deletion in HVS-II seems to be characteristic of Native American C (table 6; also see Ginther et al. 1993; Kolman and Bermingham 1997), since it has not been reported in Asian mtDNAs so far (Lee et al. 1997). The “Beringian” transition at np 16111 is seen in most Native American A lineages but is virtually absent in Asia (Horai and Hayasaka 1990; Horai et al. 1993; Torroni et al. 1993; Kolman et al. 1996; Lee et al. 1997). Thus, only haplotype BR16, which, incidentally, matches an mtDNA lineage from Hokkaido (Horai et al. 1996), would be a clear suspect for potential Asian ancestry. In fact, the lineage BR16 (from a student in our laboratory) turned out to be of Lebanese matrilineal ancestry, but its ultimate ancestry would be central/eastern Asian. Taking into account that the mtDNA haplotypes of seemingly Native American ancestry constitute only a small minority in the Asian mtDNA pool, we would realistically assume that no more than, say, one additional mtDNA lineage in our Brazilian sample was actually of eastern-Asian ancestry. The same could be said for western-Asian ancestry, inasmuch as, in our sample, we did not observe any U1, U3, R*, N*, M*, or non-Amerindian C haplotypes that would occur at considerable frequency in western Asia (Macaulay et al. 1999).

One lineage classified as haplogroup D, BR53, lacks the characteristic transition at np 16325 (very possibly because of back mutation) and is considerably different from most other Native American D lineages (Torroni et al. 1993; Horai et al. 1996; Santos et al. 1996b; Ward et al. 1996; Stone and Stoneking 1998); however, exactly as does the unique D lineage of the Mexican sample (Green et al. 2000), it shares three distinguishing mutations (at np 16241, 16301, and 16342) with a haplotype detected in the Cayapa from Ecuador (Richards et al. 1999). The Cayapa haplotype also bears the

Table 6 (Continued)

HAPLOTYPE ^a	NUCLEOTIDE POSITION ^b		
	11111111111111111122222222222222222222222233333333		
	779904555888888999900001222233344456667888990011122		
	233536012323568945890347756785694782035967579955657		HAPLOGROUP
BR103	.G...T.....C.....G.....C--...		L3e
BR105	.G...T.....C.....G.....---C...		L3e
BR106	.G...T.....G.C.....G.....---C...		L3e
BR107	.G...CT.C.....C..G..... <u>G</u>C--C...		L3*
BR108	.G...CT.C.....C..G..... <u>G</u>G.....---C...		L3*
BR110	.G...C.....A.....T.....G.....---C...		U6
BR111	.G.....T.....G.....C--C...		U6
European:			
BR112a	. <u> </u>G.....C--...		H
BR112b(3)	. <u> </u>G.....C--C...		H
BR112c	. <u> </u>G.....---...		H
BR112d(2)	. <u> </u>G.....---C...		H
BR112e	. <u> </u> T.....G.....C--C...		H
BR112f	. <u> </u> C.....G.....---C...		H
BR112g	. <u> </u>G.....CC--C...		H
BR115	. <u> </u>G.....C--...		H
BR120	. <u> </u>C.....G.....C--C...		H
BR124	. <u>G</u> .C.....---C...		H
BR130	. <u> </u>G.....C--C...		H
BR131	<u>C</u> ...T.....T.....G.....C--C...		pre*V
BR133	<u>C</u> .G.....C.....G.....C--C...		V
BR134	<u>C</u>G.....---C...		V
BR137(2)	<u>C</u>C.....G.....CC--...		V
BR139	.G.....C.....C.....G.....---...		U*
BR141	.G...T.....C.....G.....---C...		U4
BR142	.G...T.C.....C.....G.....CC--C...		U5b*
BR146	.G...C..C.....G.....---C...		K
BR149	.G.....C.....G.....---C...		K
BR153	.G.....A.G.....A.....G... <u>T</u> .C--C...		J*
BR159	.G...T.....G.....---C...		T*
BR161	.G.....G.....C--C...		T*
BR162	.G...T.....G.....C--C...		T*
BR166	.G...C..C.....C.G.C.....---C...		T*
BR170	.G..... <u>G</u>C.....G.....C--C...		X
BR171	.G..... <u>G</u>C.....A.G.....G.....---C...		X

^a Position 64 (which could be read for some sequences but only with one primer) is polymorphic in haplogroups L1a and A: BR3, BR8, and BR58 have 64C (like CRS), whereas BR1, BR7, BR9, BR10, BR11, BR12, BR14, BR16, and BR54 have 64T. BR15 could not be analyzed for HVS-II.

^b Haplogroup-diagnostic nucleotide positions are underlined.

transitions at np 16223 and 16362, which are typical of D haplotypes, but Rickards et al. (1999) claimed (without performing the necessary RFLP tests) that only haplogroups A–C were seen in the Cayapa sample. The related Brazilian haplotype was indeed classified as D, according to -5176 *AluI*, +10394 *DdeI*, and +10397 *AluI*, thus strongly suggesting that the “Cayapa-specific” lineages belong to haplogroup D as well.

In principle, it should be possible to narrow the matrilineal ancestry of Brazilians to a geographic scale narrower than that of the (sub)continents (e.g., see Alves-Silva et al. 1999). This, in general, requires extensive phylogeographic studies of the populations from the potential source areas, which, at present, are not avail-

able for Africa or most parts of Europe—in particular, Italy. Considering that 30% of the European immigrants (including the Portuguese colonizers) to Brazil came from Italy (Salzano and Freire Maia 1967; Callegari-Jacques and Salzano 1999), one can expect that a considerable number of mtDNA lineages in the Brazilian sample have Italian ancestry. In one case (a student in our laboratory), we could indeed confirm Italian matrilineal ancestry (BR137). Given the paucity of published Italian HVS-I mtDNA data (Francalacci et al. 1996; Torroni et al. 1996), we have practically no diagnostic haplotypes or characteristic haplogroup profiles at hand from which one could extrapolate the Italian mitochondrial input to the regional mtDNA pools

of Brazil. It needs to be emphasized that genetic distances, although trivial to compute, between the Brazilian mtDNA sample and mtDNA samples representing potential source populations would not allow the calculation of reliable admixture proportions, as demonstrated by Rando et al. (1999) in the case of the mixed population from the Canary Islands.

One could also use a reverse approach and infer the mtDNA profile of a source population from that of the target mixed population (given sufficient information on the other participating source populations). For instance, no mtDNA data for Angola are available—yet, since Angola was the major source of African slaves brought to Brazil, we can make inferences on how the mtDNA pool of Angolans would look: we should expect (i) a considerable number of L3e lineages—in particular, those bearing the np-16327 transition, also observed in the Herero and in other southern African populations (Vigilant 1990; Soodyall 1993); (ii) a possibly equal amount of L1c lineages, several of which will show the transversions at np 16265 and 16286, detected in Equatorial Guinea (Pinto et al. 1996) as well as in Namibia (Soodyall 1993); and, furthermore, (iii) some L2 lineages (which seem to be omnipresent in Bantu populations) but probably no Khoisan-specific mtDNA haplotypes from the L1 subgroup described by Bandelt and Forster (1997). The seven northeastern-Brazilian lineages (except for the U6 lineage) could therefore represent a typical Angolan minisample. The white Brazilian population, paradoxically, seems to be an excellent resource with which to study the phylogeny of western- and central-African mtDNA.

In conclusion, our mtDNA study of a random sample of white Brazilians has revealed an astonishingly high matrilineal contribution of Amerindians and Africans. Present-day Brazilians thus still carry the genetic imprint of the early-colonization phase: the pioneer-colonial population typically had Amerindian ancestry—and, after few generations, increasingly African ancestry—in the maternal line but Portuguese ancestry in the paternal line (as is reflected by Y-chromosome markers [D. R. Carvalho-Silva, F. R. Santos, and S. D. J. Pena, unpublished results]).

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

GenBank Overview, <http://www.ncbi.nlm.nih.gov/Genbank/GenbankOverview.html> (for HVS-I [accession numbers AF243627–AF243796] and HVS-II [accession numbers AF243539–AF243626])

Instituto Brasileiro de Geografia Estatística, <http://www.ibge.gov.br/>

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