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Uniparental (mtDNA, Y-chromosome) Polymorphisms in French Guiana and Two Related Populations – Implications for the Region's Colonization

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Summary

Blood samples collected in four Amerindian French Guiana populations (Palikur, Emerillon, Wayampi and Kali'na) in the early 1980s were screened for selected mtDNA and Y-chromosome length polymorphisms, and sequenced for the mtDNA hypervariable segment I (HVS-I). In addition, two other Amerindian populations (Apalaí and Matsiguenga) were examined for the same markers to establish the genetic relationships in the area. Strong dissimilarities were observed in the distribution of the founding Amerindian haplogroups, and significant p-values were obtained from F_{ST} genetic distances. Interpopulation similarities occurred mainly due to geography. The Palikur did not show obvious genetic similarity to the Matsiguenga, who speak the same language and live in a region from where they could have migrated to French Guiana. The African-origin admixture observed in the Kali'na probably derives from historical contacts they had with the Bushinengue (Noir Marron), a group of escaped slaves who now lead independent lives in a nearby region. This analysis has identified significant clues about the Amerindian peopling of the North-East Amazonian region.

Keywords: genetic markers, Amerindian populations, French Guiana colonization

Introduction

French Guiana is located on the Atlantic coast of South America, north of the mouth of the Amazon River. This French department is mostly covered by the Amazonian rainforest and is presently inhabited by five non-acculturated Native American populations whose members speak languages from three large families: Arawak (Palikur), Karib (Kali'na, Wayana), and Tupi-Guarani (Emerillon, Wayampi). The first groups arrived in the region in the first centuries of the present era, occupying distinct places in the littoral and interior along the years; culturally they show a series of clear distinctions, both in relation to their material tools and social organization (Nimuendajú, 1926; Grenand & Grenand, 1985; Campbell, 1997). Their genetic relationships were initially examined by the study of several hemato-immunologic systems (Larrouy et al. 1964a,b; Daveau et al. 1975; Tchen et al. 1978a,b, 1981; Dugoujon et al. 1994). The results obtained for the Apalaí-Wayana, a neighbouring Amerindian tribe living in the Brazilian state of Amapá (Salzano et al. 1988), mainly revealed a genetic distinction between the hinterland Emerillon and the coastal Palikur, in accordance with archaeological, ethnological and contemporary demographic data (Mazières et al. 2007). These authors stressed the usefulness of DNA investigations to better understand French Guiana's early settlement. In particular, the Palikur remain one of the most enigmatic populations. Linguistic approaches placed the putative origin of this Maipurean-speaking group in a remote area in the North-Central Peruvian region, from where speakers of the Maipurean branch of the Arawak family could have expanded 3,000 years ago (Urban, 1992; Campbell, 1997).

For more than two decades Native American Indians have been widely examined using their mitochondrial DNA (mtDNA) and the nonrecombining portion of the

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Y-chromosome (NRY), two uniparentally inherited genetic systems that are intensively screened in human populations (Horai et al. 1995; Ingman et al. 2000; Underhill et al. 2000, 2001). The Amerindian populations relationships and migration patterns were investigated (Torroni et al. 1992; Horai et al. 1993; Merriwether et al. 1995; Underhill et al. 1996; Bonatto & Salzano, 1997; Salzano, 2002; Schurr & Sherry, 2004). These and other studies showed that most of the Y-chromosome and mtDNA diversity fell into major haplogroups defined by specific single nucleotide polymorphisms (SNPs) and other types of markers (Wallace et al. 1985; Schurr et al. 1990; Bailliet et al. 1994; Forster et al. 1996; Underhill et al. 1996; Brown et al. 1998; Bergen et al. 1999; Bortolini et al. 2003; Jobling & Tyler-Smith, 2003; Seielstad et al. 2003). In South America these studies have dealt with extinct and extant populations (Ribeiro-dos-Santos et al. 1996; Lalueza et al. 1997; Bert et al. 2001, 2004; Garcia-Bour et al. 2004; Moraga et al. 2005; Lewis et al. 2005; Marrero et al. 2007a; Torres et al. 2006). However, data derived from French Guiana are still nonexistent.

In the present work four native French Guianan populations (Palikur, Kali'na, Emerillon, Wayampi) were studied for three RFLP sites and a 9-bp deletion which are diagnostic for the major mtDNA haplogroups, as well as for the HVS-I region of the mtDNA; males were also tested for eight NRY biallelic markers. They were compared, using the same markers, with the neighbouring Brazilian Apalaí and the Peruvian Matsiguenga, a Maipurean-speaking tribe linguisticaly related to the French Guiana Palikur. The following questions were addressed: (1) are the genetic relationships mainly associated to geographic or linguistic factors; (2) by comparing the two Maipurean-speaking tribes, can genetics link the Palikur to the putative region from where they could have originated; (3) are any outside (non-Amerindian) influences mainly due to recent or past events; and (4) do the historical data agree with the genetic results? Finally, what inferences can be drawn from the genetic data about French Guiana's Amerindian colonization?

Subjects and Methods

Population Samples, DNA Extraction and Typing

A total of 892 individuals from the six populations (Fig. 1) were previously sampled during the 1971–1985 missions led by two of us (G.L. and F.M.S.), as well as by E. B., under the auspices of the Centre National de la Recherche Scientifique (Centre d'Hémotypologie, Toulouse), Institut National de la Santé et de la Recherche Médicale (Paris) and the Universidade Federal do Rio Grande do Sul (Porto Alegre). Populations, sample collection and preservation were described in Salzano et al. (1988, 1997), Dugoujon et al. (1995), and reviewed in Mazières et al. (2007). Briefly, blood samples were refrigerated shortly after collection, and at the laboratory the red cells separated from the plasma, the material tested, and afterwards stored at $-20°$C. During the French Guiana collections up to six-generation pedigrees were recorded. They indicated the presence of 347 maternal and 205 paternal unrelated lineages.

Genomic DNA from the Palikur, Kali'na, Emerillon, Wayampi and Matsiguenga samples was extracted from sera using the NucleoSpin Blood QuickPure kit (Macherey-Nagel), and the phenol-chloroform method coupled with the Cleanmix kit (Talent). The Apalaí DNA was obtained with the QIAmp DNA Minikit (Qiagen) from glycerolized red cells on which white cells were still adsorbed.

mtDNA and Y-chromosome Analyses

Fragments enclosing the *Hae*III np663, 9bp-deletion, *Hinc*II np13259, and the *Alu*I np5176 polymorphisms, which distinguish four (A–D) of the five major Amerindian

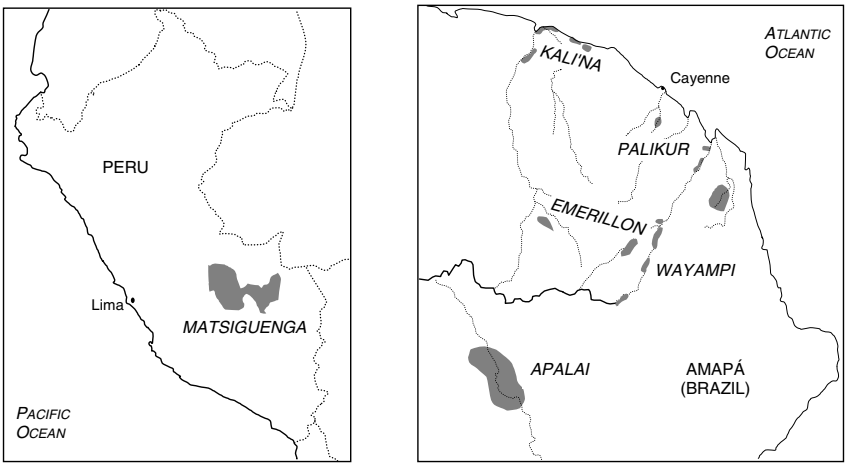

Figure 1 Partial maps of Peru and French Guiana showing the geographic location of the studied populations.

mtDNA haplogroups (Torroni et al. 1992) were directly amplified using polymerase chain reaction (PCR) with the following primers (Invitrogen): Haplogroup A, F570 (5′-CAACCAAACCCCAAAGACAC-3′) and R741 (5′-ATGCTTGTCCCTTTTGATCG-3′); haplogroup B, F8223 (5′-CATGCCCATCGTCCTAGAAT-3′) and R8383 (5′-TATGGTGGGCCATACGGTAG-3′); haplogroup C, F13229 (5′-CGCCCTTACACAAAATGACA-3′) and R13353 (5′-GGACCCGGAGCACATAAATA-3′); haplogroup D, F5074 (5′-CCGTACAACCCTAACATAACCA-3′) and R5219 (5′-GAGAGGAGGGTGGATGGAAT-3′). Since there is abundant evidence that haplogroup X is present in North America only (Dornelles et al. 2005) we did not try to test for it. The nucleotide sequence of the first mtDNA hypervariable segment (HVS-I) was sequenced between nucleotide positions 16021 and 16422 of the Cambridge Reference Sequence (CRS; Anderson et al. 1981; Andrews et al. 1999) with the F16021 (5′-CTGTTCTTTCATGGGGAAGC-3′) and R16422 (5′-ATTGATTTCACGGAGGATGG-3′) primers.

Amplifications were carried out in a reaction mix containing 1 × buffer, 3mM magnesium ions, 0.05mM of each dNTP, 0.2μM of each primer and 1.25 U *Taq* polymerase. After an initial 10-minutes (min.) denaturation step, 35 cycles of amplification were performed. The temperature profile was 94°C for 1 min., 60°C for 1 min., 72 °C for 1:30 min., and a final extension step of 10 min at 72°C.

PCR products were digested with the appropriate endonucleases. The resulting fragments, as well as the PCR products containing the 9pb-deletion, were screened through electrophoresis on 3% NuSieve-Agarose (1:2) (Tebu-bio) gels stained with ethidium bromide. After purification of the HVS-I fragments with the QIAquick spin columns (Qiagen), both strands were sequenced using the BigDye^{TM} terminator Cycle Sequencing Ready Reaction v1.1 (AB Applied Biosystems). The runs were carried out in Toulouse using an automatic ABI Prism 310 sequencer. The Apalaí samples were purified using the polyethylenoglycol (PEG) method and sequenced in Porto Alegre using the DYEnamic^{TM} ET Dye terminator Cycle Sequencing kit, as required for the MegaBACE^{TM} DNA Analysis System (Amersham Biosciences).

For the Y-chromosome eight biallelic markers (M3, M242, M9, 92R7, YAP, M2, RPS4Y$_{711}$ and M19) were typed using the methods described in Bortolini et al. (2003) and Marrero et al. (2005). Haplogroups defined by these polymorphisms were named following the nomenclature suggested by the last Y Chromosome Consortium release (Jobling & Tyler-Smith, 2003). A designation such as Q* defines all chromosomes that do not possess the derived allele, in this case describing all chromosomes in clade Q except those in Q3. Haplogroup Y* indicates the presence of the ancestral alleles for the eight markers investigated here.

Preventing Contamination and Artifacts

DNA extractions and PCR determinations were performed following a series of standard practices to avoid laboratory contamination. Negative controls consisting of mock extractions (sample omitted) and PCR blanks without DNA were used throughout the testing procedures. To prevent the introduction of exogenous DNA, laboratory rooms were routinely ultraviolet (UV)-irradiated. Nucleic acid extractions, PCRs and post-PCR handling were performed in isolated work areas using sterile equipment. The instruments were subjected to extensive rinsing in sterile μQH_2O. Aerosol-resistant barrier pipette tips were employed. All equipments, disposables, and reagents were UV-irradiated before use, and frequent bleaching of working surfaces was adopted. Finally, all researchers and laboratory staff were typed for the markers of study.

Stringent criteria were employed for authentication. As far as possible, related individuals were typed or sequenced. The genotypic results of their lineages were then validated after comparison with the genealogical data; only unrelated individuals, however, were included in the analysis. To ensure that no systematic artifacts were introduced in the course of the sequencing process that could have produced "phantom" mutations we applied the filtering process described by Bandelt et al. (2002). This analysis filters speedy transitions and thus scores weighty mutations only.

Genetic Analysis

The mtDNA and Y-chromosome haplogroups were assigned following published criteria (Torroni et al. 1992; Salas et al. 2002; Bandelt et al. 2003, Jobling & Tyler-Smith 2003). Haplogroup frequencies were calculated by counting. Genetic distances were obtained with the DISPAN (Ota, 1993) package. The distance employed was the modified Cavalli-Sforza D_A distance (Nei et al. 1983) since it has more discriminatory power for closely related groups (Nei & Roychoudhury, 1993; Nei & Takezaki, 1996). The relationships among the populations were then displayed through neighbour-joining (NJ) trees (Saitou & Nei, 1987) with the PHYLIP (Felsenstein, 2002) software, using D_A matrices as input files, and visualized with TREEVIEW (Page, 1996).

HVS-I sequence evaluation was manually performed with the SEQUENCING ANALYSIS (ABI Prism v.3.7) and CHROMAS v2.3 reading programs. Sequence alignment was accomplished using the BIOEDIT (Hall, 1999) software. F_{ST} distances were calculated with the ARLEQUIN ver 3.1 (Excoffier et al. 2005) program, using the pairwise difference method. The reliability of the F_{ST}-based tree was tested by bootstrap replications, following Hedges (1992), considering each polymorphic site as a system.

Results

mtDNA Analysis

Three hundred of the 349 samples available (86%) were successfully typed by both the RFLP and sequencing methods. Considering that the samples were collected at different times, under diverse field conditions, this level of success should be considered favourably. Tables 1 and 2 summarize the mtDNA sequence variation observed among the six populations examined. Fifty-one polymorphic sites

Table 1 HVS-I sequence variation in four French Guiana and two related populations[1]

	Nucleotide variable positions																																																			Populations					
				1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3							
	5	9	9	1	2	2	4	4	5	6	6	7	7	8	8	8	8	0	1	2	2	3	5	5	5	5	6	6	6	6	7	7	8	9	9	9	9	9	9	9	0	1	1	2	2	4	5	5	5	6	6	Hgp						
	1	2	3	1	6	9	2	5	5	8	9	8	9	2	3	4	9	9	7	3	4	9	6	7	8	9	1	5	6	7	4	8	8	0	1	2	3	4	5	8	3	1	9	5	7	2	5	6	7	2	8		Pal	Kal	Em	Wyp	Ap	Mts
CRS	A	T	T	C	T	G	C	G	A	C	C	T	C	A	A	C	T	T	T	C	T	C	C	C	A	C	C	A	C	C	G	C	T	C	C	C	A	C	C	T	G	T	G	T	C	T	C	T	T	T	T							
1	G	.	.	T	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	C	.	A2		2				
2	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	C	.	A2			9	5	11	
3	N	.	.	T	.	A	.	.	.	.	.	.	.	C	C	.	C	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	C	A	.	.	.	.	.	.	C	.	A2				4		
4	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	C	.	C	.	A2				13		
5	.	.	.	T	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	C	.	A2					7	
6	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	C	C	.	A2					7	
7	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	A	.	.	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	C	.	A2					12	
8	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	T	.	T	.	.	.	T	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	C	.	A2					1	
9	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	C	A	.	.	.	.	.	.	C	.	A2					1	
10	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	C	.	A4				3		
11	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	C	C	.	A4					1	
12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	C	.	A4						1
13	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C	.	C	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	C	.	A4						1
14	N	.	.	.	.	.	.	.	.	.	.	.	.	C	C	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	B4	1					
15	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	B4	3	2				
16	N	.	.	.	.	.	.	.	.	.	T	.	.	C	C	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	B4		1				
17	.	C	.	.	.	.	.	.	.	.	.	.	.	C	C	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	B4					1	
18	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	B4						8
19	N	.	C	.	.	.	.	.	.	.	.	.	.	C	C	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	B4						1
20	N	.	.	.	.	.	.	.	.	.	.	C	.	.	C	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	B4	2					
21	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	B4	2	1	19			3
22	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	B4	16					
23	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	C	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	B4	2					
24	N	N	N	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	B4	1					
25	.	C	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	A	.	.	.	.	.	.	.	.	B4		4				
26	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	B4		1				
27	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	C	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	B4			1	7		
28	N	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	B4			1			
29	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	C	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	B4						4
30	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	B4						1
31	N	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	B4				4		16
32	N	.	.	.	.	A	.	.	.	T	.	.	.	.	C	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	B4						1
33	N	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	B4						1
34	N	N	N	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	B		1				
35	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	T	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	B		2				

Table 1 (Cont.)

	Nucleotide variable positions																																																			Hgp	Populations					
				1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3							
	5	9	9	1	2	2	4	4	5	6	6	7	7	8	8	8	8	0	1	2	2	3	5	5	5	5	6	6	6	6	7	7	8	9	9	9	9	9	9	9	0	1	1	2	2	4	5	5	5	6	6							
	1	2	3	1	6	9	2	5	5	8	9	8	9	2	3	4	9	9	7	3	4	9	6	7	8	9	1	5	6	7	4	8	8	0	1	2	3	4	5	8	3	1	9	5	7	2	5	6	7	2	8	Hgp	Pal	Kal	Em	Wyp	Ap	Mts
CRS	A	T	T	C	T	G	C	G	A	C	C	T	C	A	A	C	T	T	T	C	T	C	C	C	A	C	C	A	C	C	G	C	T	C	C	C	A	C	C	T	G	T	G	T	C	T	C	T	T	T	T							
36	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	T	.	.	.	.	.	.	C1					2	
37	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	C	T	.	.	.	.	.	.	C1		3				
38	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	C	T	.	.	.	.	.	.	C1		4				
39	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	C	T	.	.	.	.	.	.	C1		4				
40	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	C	.	.	.	C	T	.	.	.	.	.	.	C1					15	
41	G	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	C	T	.	.	.	.	.	.	C1					1	
42	G	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	C	T	.	.	.	.	.	.	C1					3	
43	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	C	.	.	.	C	T	.	.	.	.	C	.	C1					9	
44	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	D1	11	1				
45	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	D1	1					
46	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	D1				8		
47	G	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	D1	9					
48	N	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	C	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	D1				1		
49	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	C	.	.	.	.	.	C	.	D1		1		1	27	
50	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	C	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	C	.	.	.	.	.	C	.	D1				5		
51	N	.	.	.	.	.	T	.	G	.	.	.	T	.	.	.	C	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	C	.	.	.	.	.	C	.	D1				1		
52	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	D1				1		
53	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	D1						1
54	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	D1					1	
55	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.	.	C	.	D1					3	
56	.	.	.	A	.	.	.	A	.	.	.	.	.	.	.	T	C	.	.	T	.	T	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	L2d2		2				

Note: [1]Nucleotide positions are numbered after 16,000, following the Cambridge Reference Sequence (Anderson et al. 1981; Andrews et al. 1999). Haplogroup (Hgp) assignation according to Bandelt et al. (2003). Populations: Pal: Palikur; Kal: Kali'na; Em: Emerillon; Wyp: Wayampi; Ap. Apalaí; Mts: Matsiguenga. N: Not determined.

Populations	N[1]	Haplogroups A2	A4	B2	B4	C1	D1	L2d2
Palikur	48				56.2		43.8	
Emerillon	30	30.0			70.0			
Kali'na	29	6.9		10.4	31.0	37.9	6.9	6.9
Wayampi	53	41.5	5.7		20.7		32.1	
Apalaí	102	38.2	1.0		1.0	29.4	30.4	
Matsiguenga	38		5.3		92.1		2.6	

Table 2 mtDNA haplogroup frequencies (%) in four French Guianan and two related populations

Note: [1]N: Number of individuals studied; [2]The HVS-I markers alone are not sufficient for subhaplogroup assignment.

defining 56 haplotypes were observed. Almost all of them could be classified into the four major founding mtDNA haplogroups. A non-Amerindian, African mtDNA lineage (L2d2) was found in the Kali'na (haplotype n°56; it also shows mutations 16390 and 16399, not indicated in Table 1 because these sites do not vary among Amerindians). Only two major haplogroups were found in the Emerillon (A: 30%, B: 70%) and Palikur (B: 56%, D: 44%), while three or more were observed in the other four populations examined. Finally, only seven HVS-I haplotypes (n°s. 2, 15, 21, 27, 31, 44, and 49) were shared by the populations.

The median joining network obtained with the 300 HVS-I sequences is displayed in Fig. 2. Four clusters could be visualized, corresponding to the B/B4 (left), A2/A4,

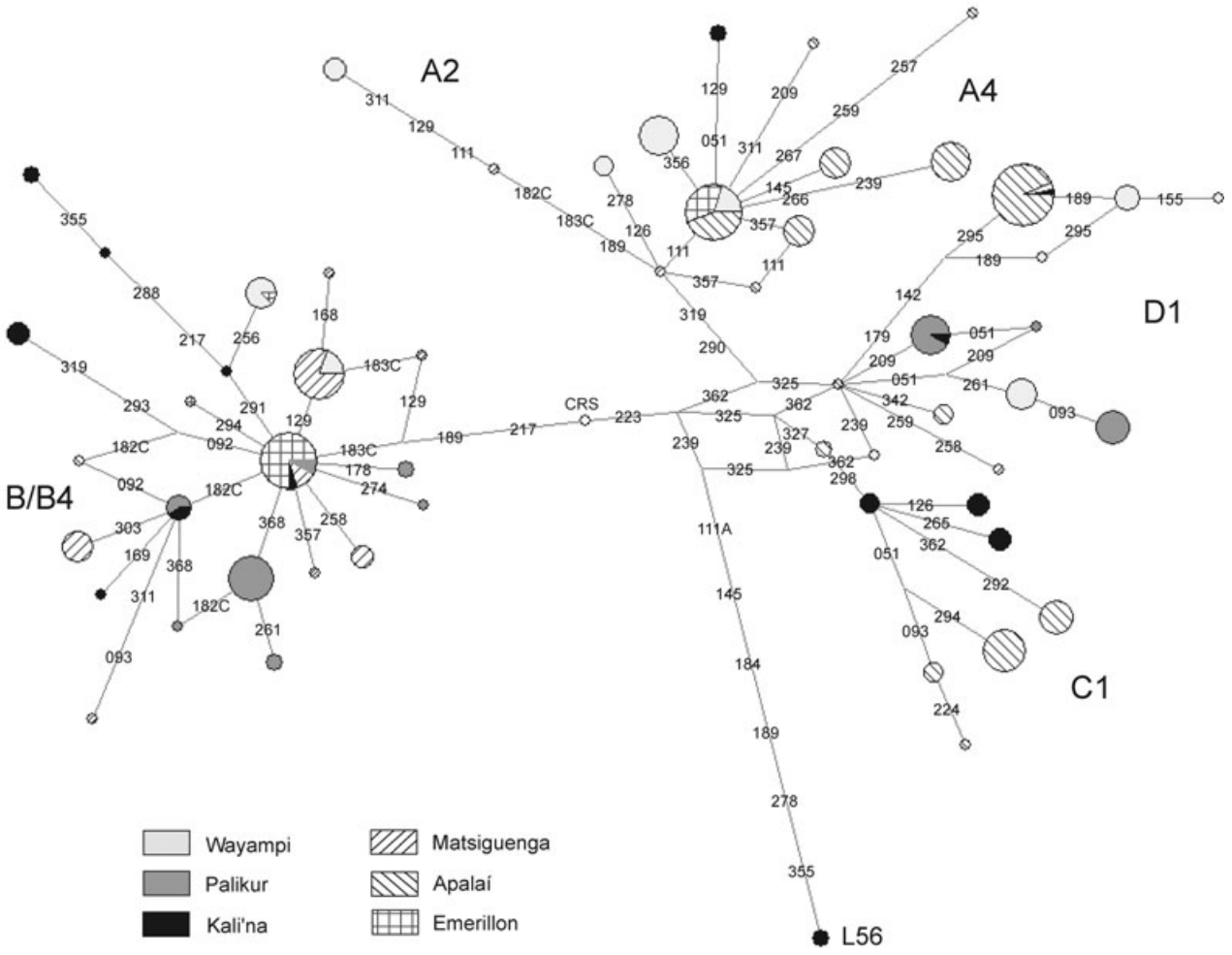

Figure 2 Median joining network showing the relationships among the 56 mtDNA HVS-I haplotypes. The numbers represent the nucleotide position (-16000). Tranversions are indicated by letters after the numbers.

Table 3 Y-chromosome haplogroup frequencies (%) in four French Guianan and two related populations

		Haplogroups								
Populations	N[1]	Q3a	Q3*	Q*	P*	K*	C*	E3a*	DE*	Y*
Palikur	35		91.4	2.9		5.7				
Emerillon	9		100							
Kali'na	21		81			4.8		9.4		4.8
Wayampi	38		100							
Apalaí	48		100							
Matsiguenga	28		80.7	9.7		3.2		3.2		3.2

Note: [1]N: Number of individuals studied.

D1, and C1 (right) haplogroups. The mutations leading to the different haplotypes are shown, together with their frequencies (as depicted by the sizes of the circles and their relative shading). The distinctiveness of the African lineage no. 56 could be clearly observed, its connection with the network being mediated by seven mutations. Exclusive, relatively frequent Apalaí and Wayampi haplotypes were found in A2, A4 and D1, but no clear pattern appeared in relation to the populations in which the other haplotypes occur.

Y-chromosome

Successful typing for the eight Y-chromosome markers was obtained for 179 of the 203 male samples tested. Y-haplogroup frequencies are presented in Table 3. Amerindian lineages Q* and Q3* were predominant, and the Emerillon, Wayampi, and Apalaí were monomorphic for Q3*. There has been no previous report of the K* occurrence in Amerindians; this haplogroup is especially frequent in Asiatics (about 30%), but also occurs (generally in frequencies of less than 5%) in Europeans (Bortolini et al. 2003). It is possible, therefore, that its presence in three of the six populations sampled may be due to non-Amerindian heritage. The sub-Saharian E3a lineage was found in the Kali'na (9.4%; who also presented evidence of African admixture in the mtDNA data) and Matsiguenga (3.2%).

Population Relationships

Interpopulation genetic distances based on Y-chromosome and mtDNA haplogroups were calculated (data not shown), but this comparison was hampered by the lack of variability at the Y-chromosomal level. In this case the only point that can be stressed is the separation of the Palikur from the three other Guianese tribes that are monomorphic for Q3*. Table 4 presents the F_{ST} distances matrix based on the mtDNA HVS-I sequence variability. They varied from 0.11 (Apalaí-Wayampi) and 0.51 (Apalaí-Matsiguenga), but almost half (7 in 15) occurred in the 0.11–0.19 interval. The derived relationships are displayed in the tree of Fig. 3. No clear north-south gradient was observed, but the littoral groups (Kali'na, Palikur) were near each other, as well as the southern Apalaí and Wayampi. Also, no clear relationships according to languages could be discerned. The two Karib (Apalaí, Kali'na), two Tupi-Guarani (Wayampi, Emerillon), and two Arawak (Palikur, Matsiguenga) were all separated in both mtDNA trees.

Genetic Intrapopulation Diversity

The Y-chromosome data was not appropriate for intrapopulation diversity comparisons because the variability was low and may be heavily influenced by non-Amerindian admixture. The mtDNA results, on the other hand, could be profitably used for this purpose, and results of the

Table 4 F_{ST} distances matrix based on the mtDNA HVS-I sequence variability[1]

	Palikur	Emerillon	Kali'na	Wayampi	Apalaí	Matsiguenga
Palikur						
Emerillon	0.15349					
Kali'na	0.13189	0.14991				
Wayampi	0.20324	0.18076	0.15186			
Apalaí	0.31340	0.34974	0.20315	0.11077		
Matsiguenga	0.26139	0.18964	0.28641	0.38321	0.51551	

Note: [1]All distances are statistically significant at the 5% level.

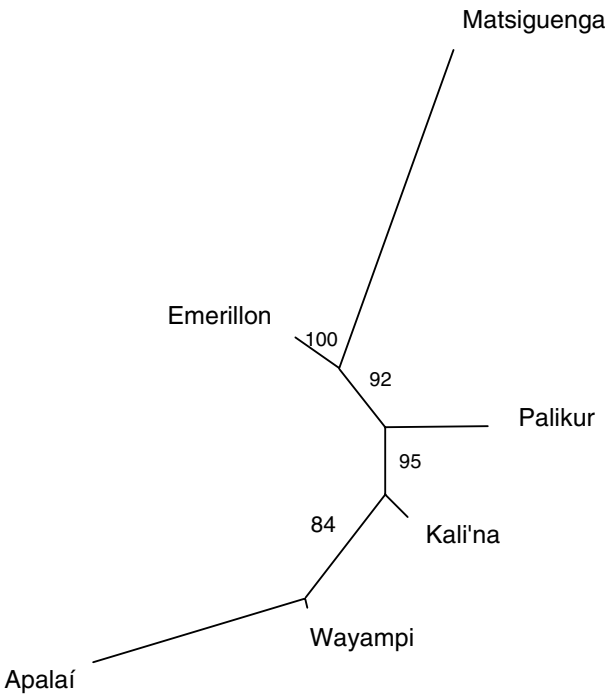

Figure 3 Neighbour-joining tree obtained from pairwise differences between HVS-I haplotypes. Numbers are percentage bootstrap values based on 2,000 replications.

analyses made are displayed in Table 5. The amount of diversity found in the present study showed values that are generally within the range (with two exceptions) observed in a survey of other South Amerindians. The Kali'na, Wayampi and Apalai revealed values above the South Amerindian averages, with the opposite occurring with the Emerillon and Matsiguenga; the Palikur showed a mixed pattern.

Discussion

We can now examine the questions asked in the Introduction in light of the results obtained. In relation to the first question, it is clear that geographic factors should have played a more important role than linguistic similarity in French Guiana, since the mtDNA relationships associated mainly the two littoral (Kali'na, Palikur) and two southern (Apalaí, Wayampi) tribes, who speak languages from different families (Karib, Tupi-Guarani).

The second question is concerned with the Arawak Maipurean-speaking tribes, the Matsiguenga and Palikur. There have been several hypotheses in relation to the possible centre of Maipurean dispersion. The Maipurean language cluster's chronological depth is estimated to be three thousand years, and Payne (1991) subdivided it into five groups according to the location of the tribes which speak versions of the language. Two of the five have representatives in north-central Peru, and Urban (1992) suggested that this area could represent the Maipurean centre of dispersion. The Palikur of French Guiana represent the most extreme eastern speakers of this language, and we therefore decided to verify whether they showed any genetic resemblance to another Peruvian Maipurean-speaking group. As was previously indicated the answer is negative, since they

		Diversity parameters				
Populations	No. indiv.	H	k	D (SE)	P_w	π (SE)
Palikur	48	0.503	10	0.808 (0.034)	4.9	0.015 (0.008)
Emerillon	30	0.434	4	0.524 (0.074)	3.3	0.010 (0.006)
Kali'na	29	0.695	14	0.938 (0.021)	7.4	0.023 (0.012)
Wayampi	53	0.640	12	0.882 (0.022)	5.9	0.020 (0.011)
Apalaí	102	0.681	16	0.872 (0.017)	6.1	0.019 (0.010)
Matsiguenga	38	0.234	11	0.777 (0.054)	2.6	0.008 (0.005)
South Amerindians[2]	986					
Minimum	-	0.165	3	0.204	1.1	0.003
Average	-	0.511	14	0.767	4.3	0.013
Maximum	-	0.756	42	0.968	6.5	0.018

Table 5 Mitochondrial DNA diversity measurements (haplogroups and HVS-I sequences) in the populations studied here compared to other South Amerindians[1]

Notes: [1]The numbers of individuals studied for H (haplogroup heterozygosity) were slightly different from those given in the table: Wayampi, 54; Apalaí, 103; Matsiguenga, 40. k: number of haplotypes; D: haplotype diversity; P_w: number of differences between pairs of haplotypes; π: nucleotide diversity; [2]Included 26 population samples, but the values shown are only for 13 tribal samples with at least 30 individuals tested. Tribes with extreme values were the Aché, with uniformly low indices (Schmitt et al. 2004); and the Cayapa with high H, P_w and π (Rickards et al. 1999); Yanomama, with high k (Easton et al. 1996); and Mapuche, with high D (Ginther et al. 1993).

Table 6 Historical and linguistic data about the French Guianan, and one neighbouring, tribes

Characteristic	Kali'na	Palikur	Emerillon	Wayampi	Apalaí
Language	Karib	Arawak	Tupi-Guarani	Tupi-Guarani	Karib
Date of colonization of present territory (AD)	900	800	1400	1400	1890
Origin	Amazon headwaters	Amazon headwaters	Tapajós headwaters	Amazon headwaters	Paru de Leste river
Population estimates					
First, Number	5,500	4,000	400	6,000	3,000
Year	1604	1604	1767	1824	1790
Nadir, Number	250	220	52	212	280
Year	1848	1840	1953	1947	1890
Recent, Number	1,550	866	218	910	415
Year	1978	1998	1985	1994	1998

Note: Sources: Grenand & Grenand (1979); Salzano et al. (1988); Campbell (1997); Ricardo (2000); http://www.pegue.com/indio/palikur.htm.

did not show any special type of similarity. At this juncture, Rostain's (1994) suggestion that the Palikur may be the Arawak descendants of people who developed the Aristé archeological complex in Amapá (northern Brazil) seems the most likely explanation, since the Aristé sites overlap with the present Palikur distribution and this complex seems to represent an ancient centre of population diversification.

Thirdly, the question of non-Amerindian influence was addressed. The mtDNA results indicated only one non-Amerindian, African haplogroup, present in two Kali'na subjects, was detected. The Y-chromosome data, as expected for the asymmetrical mating patterns that characterized Colonial America (predominantly European-derived men with Amerindian- or African-derived women; Bortolini et al. 2004; Campos-Sánchez et al. 2006; Marrero et al. 2007b) showed additional indications of admixture. In relation to the E3a* lineage findings, while the Matsiguenga result may have derived from recent interbreeding, its presence in the Kali'na could be most easily explained by historical contacts they had with the Bushinengue (Noir-Marron), an African-derived group which lives nearby and is composed of escaped slaves who established a free, independent life at the mouth of the Maroni river (Price & Price, 2004).

Historical and anthropological data have provided a fairly good picture of French Guiana's Amerindian colonization (review in Grenand & Grenand, 1985). Table 6 shows some selected results which may be correlated with the genetic findings reported here. The northern littoral populations (Kali'na, Palikur) were the first to enter the region in 800–900 AD. The Tupi-Guarani colonization (Emerillon, Wayampi) occurred half-a-century later, and the two tribes may have followed different migration routes. The mtDNA results agree with this by showing a good relationship between the Kali'na and Palikur, but clear differences between the Emerillon and Wayampi. As for the demographic-anthropological population estimates, the fact that all four tribes experienced strong population bottlenecks, the Kali'na and Palikur at the middle of the 19th century and the Emerillon and Wayampi one hundred years afterwards, is reflected especially in the low haplotype mtDNA diversity found among the Emerillon (who experienced a very strong bottleneck, with a nadir population size of 52).

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