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Genetic Studies on the Senegal Population. I. Mitochondrial DNA Polymorphisms

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Summary

The mtDNA of 186 Senegalese, mainly Wolof (110) and Peuls (47), were analyzed by means of six restriction enzymes: *Hpa*I, *Bam*HI, *Hae*II, *Msp*I, *Ava*II, and *Hinc*II. Two of the *Hpa*I, one of the *Hae*II, two of the *Msp*I, and one of the *Ava*II morphs had not been described before. The only enzymes which enabled Wolof and Peuls to be differentiated were *Hinc*II and, to a lesser extent, *Hae*II. Important differences emerge in the comparison of Senegalese with Bantu of South Africa and with Bushmen, the only other Africans who, as far as we know, were studied for the same genetic markers. Though Senegalese mtDNAs display typical African features (presence and frequency of *Hpa*I morph 3 and high incidence of *Ava*II morph 3), the distribution of *Msp*I and *Ava*II patterns markedly differentiates Senegalese from the others. The phylogeny of mtDNA types in Africa well portrays how the three African groups are clearly distinguishable genetic entities. Bushmen lie at one end of the range of variability, Senegalese being at the other end but still fairly closely related to Bantu. The information provided by individual restriction enzymes to the distinction among the three major ethnic groups is reviewed and discussed.

Introduction

Restriction-enzyme analysis of human mitochondrial DNA (mtDNA) has shown that the amount of variation among individuals may be very high (Brown and Goodman 1979; Brown 1980). Studies of mtDNA RFLPs have been carried out in different ethnic groups (Denaro et al. 1981; Blanc et al. 1983; Cann and Wilson 1983; Johnson et al. 1983; Cann et al. 1984; Horai et al. 1984; Wallace et al. 1985; Bonné-Tamir et al. 1986; Brega et al. 1986*a*, 1986*b*; Harihara et al. 1986; Horai and Matsunaga 1986; Cann et al. 1987; Santachiara-Benerecetti et al. 1988), and changes of mtDNA sequence have been used to construct phylogenies of mankind (Johnson et al. 1983; Cann and Wilson 1983; Wallace et al. 1985; Cann et al. 1987).

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These phylogenetic analyses confirmed previous suggestions (Brown 1980; Nei 1982) that blacks are the most divergent from others and that the mitochondrial differentiation among them is greater than in Caucasians and Orientals. However, the few data available so far either were obtained from African samples of relatively small sizes (Johnson et al. 1983) or were almost exclusively based on studies of a small group of American blacks (Cann and Wilson 1983; Cann et al. 1987).

To have a better understanding of mtDNA variation in Africans, an analysis of mtDNA polymorphisms was carried out in a sample of population from Senegal. Two different groups are most represented in this sample: Wolof and Peuls.

Wolof are negroid and together with Serere belong to the group of Senegambians (Murdoch 1959). They are confined to Senegal along the Atlantic coast and to its immediate hinterland, although their primitive habitat was farther north in the "sahel." From there they were pushed southward to near the mouth of the Senegal river by Berbers around the eleventh century (Mur-

doch 1959). With a population of about 2 million (Mbow 1983), they are the most important component (about 40%) of the Senegalese population and are mainly settled in the urban areas of the country.

Peuls represent about 15% of the total Senegalese population (Mbow 1983). They belong to a nomadic group scattered throughout western Africa, from Senegal in the west to Cameroon and Chad in the east. Because of their dispersion and their interpenetration with other peoples, they do not constitute an independent culture province (Murdoch 1959). The origin of this population is not clear. Murdoch (1959) is of the opinion that they, though mixed to a varying extent with Berbers, trace descent from the former inhabitants of the Senegal valley, the Tukolor of the present day, and are fairly closely related to their neighbors, the Wolof and Serere.

Material and Methods

The Sample

The sample consisted of 186 unrelated Senegalese, mostly patients from different wards of the Regional Hospital of Louga (fig. 1); it comprised 110 Wolof, 47 Peuls, 12 Tukolor, and 17 other Senegalese of various origin. Proper ethnic group of the sampled subjects and their parents was ascertained by interview data.

Methods

Blood specimens collected in EDTA were kept refrigerated until the buffy coats were separated (within 4–6 days) and stored in liquid nitrogen. DNA extraction and restriction analyses were performed according to Denaro et al. (1981), Blanc et al. (1983), and Johnson et al. (1983). Restriction enzymes–*Hpa*I, *Bam*HI,

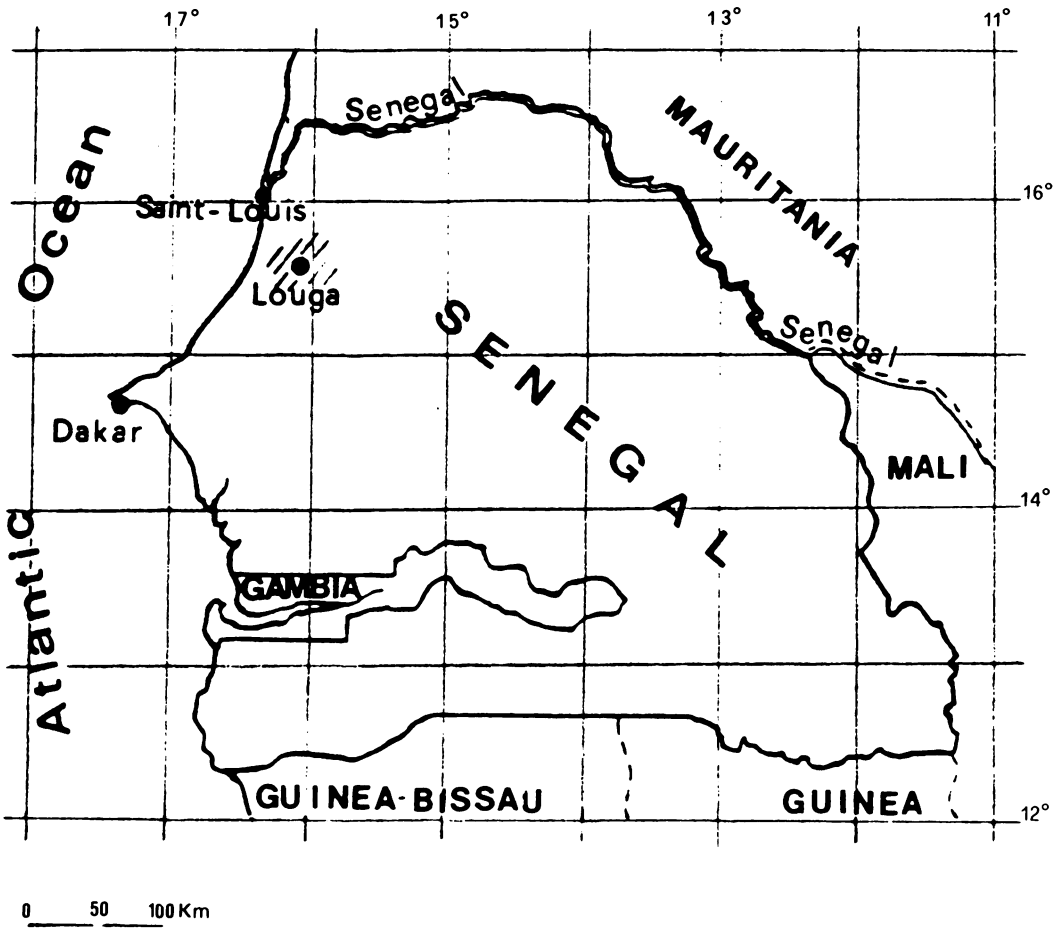

Figure 1 Map of Senegal showing the location of Louga, where blood samples were collected

Table 1

Frequencies of the Various mtDNA Morphs, Listed by Enzyme, in Senegalese

Morphs	Wolof (N = 110)	Peuls (N = 47)	Tukolor (N = 12)	Others (N = 17)	Total (N = 186)
*Hpa*I:					
1		2 (4.3 ± *2.9*)		1 (*5.9*)	3 (1.6 ± *.9*)
2	38 (34.5 ± *4.5*)	12 (25.5 ± *6.4*)	3 (25.0 ± *12.5*)	7 (41.2 ± *11.9*)	60 (32.3 ± *3.4*)
3	71 (64.5 ± *4.6*)	32 (68.1 ± *6.8*)	9 (75.0 ± *12.5*)	9 (52.9 ± *12.1*)	121 (65.1 ± *3.5*)
7^{Wolof}	1 (*.9*)				1 (*.5*)
8^{Peul}		1 (*2.1*)			1 (*.5*)
*Bam*HI:					
1	109 (99.1 ± *.9*)	47 (100.0)	12 (100.0)	17 (100.0)	185 (99.5 ± *.5*)
3	1 (*.9*)				1 (*.5*)
*Hae*II:					
1	100 (90.9 ± *2.7*)	45 (95.7 ± *1.9*)	11 (91.7 ± *8.0*)	15 (88.2 ± *7.8*)	171 (91.9 ± *2.0*)
2		1 (*2.1*)			1 (*.5*)
4	8 (*7.3* ± *2.5*)	1 (*2.1*)	1 (*8.3*)	1 (*5.9*)	11 (*5.9* ± *1.7*)
7				1 (*5.9*)	1 (*.5*)
12^{Wolof}	2 (*1.8* ± *1.3*)				2 (*1.1* ± *.8*)
*Msp*I:					
1	108 (98.2 ± *1.3*)	47 (100.0)	11 (91.7 ± *8.0*)	17 (100.0)	183 (98.4 ± *.9*)
12^{Wolof}	2 (*1.8* ± *1.3*)				2 (*1.1* ± *.8*)
$13^{Tukolor}$			1 (*8.3*)		1 (*.5*)
*Ava*II:					
1	61 (*55.5* ± *4.7*)	26 (*55.3* ± *7.3*)	5 (*41.7* ± *14.2*)	10 (*58.8* ± *11.9*)	102 (*54.8* ± *3.6*)
2	2 (*1.8* ± *1.3*)				2 (*1.1* ± *.8*)
3	41 (*37.3* ± *4.6*)	21 (*44.7* ± *7.3*)	7 (*58.3* ± *14.2*)	7 (*41.2* ± *11.9*)	76 (*40.9* ± *3.6*)
6	2 (*1.8* ± *1.3*)				2 (*1.1* ± *.8*)
9*[a]	1 (*.9*)				1 (*.5*)
17	2 (*1.8* ± *1.3*)				2 (*1.1* ± *.8*)
23^{Wolof}	1 (*.9*)				1 (*.5*)
*Hinc*II:					
1		2 (*4.3* ± *2.9*)		1 (*5.9* ± *5.7*)	3 (1.6 ± *.9*)
2	109 (99.1 ± *.9*)	40 (*85.1* ± *5.2*)	12 (100.0)	16 (*94.1* ± *5.7*)	177 (*95.2* ± *1.6*)
3		1 (*2.1*)			1 (*.5*)
7	1 (*.9*)				1 (*.5*)
10		4 (*8.5* ± *4.1*)			4 (*2.2* ± *1.1*)

NOTE.—Standard errors are in italics. They are not given for morphs observed only in one individual.

[a] See text.

*Hae*II, *Msp*I, *Ava*II, and *Hinc*II—were purchased from Boehringer and used according to manufacturers specifications. ^{32}P nick-translated (Rigby et al. 1977) human mtDNA purified from HeLa cells (Giles et al. 1980) was used as a probe.

Results

Table 1 shows the number and the frequency of the various mtDNA morphs obtained with the six restriction enzymes in the different groups analyzed.

*Hpa*I

Five *Hpa*I morphs were found among 186 individuals examined. As were other groups of African descent (Denaro et al. 1981; Cann et al. 1987), Senegalese were primarily morph 3. Morph 2 was also present at a frequency of about 30%. *Hpa*I morph 1, which shows a high frequency in Orientals (Denaro et al. 1981; Blanc et al. 1983; Brega et al. 1986*a*), was found in one Maure and two Peuls.

Two new *Hpa*I morphs were observed: *Hpa*I morphs 7^{Wolof} and 8^{Peul}. (As for hemoglobin variants, we mark the new morphs by an index indicating the country or the population in which they were first described.)

The *Hpa*I-7^{Wolof} differs from *Hpa*I morph 3 in the replacement of the 2.4- and 7.8-kb bands by a fragment of about 10.1 kb (fig. 2, lane 2), most likely ow-

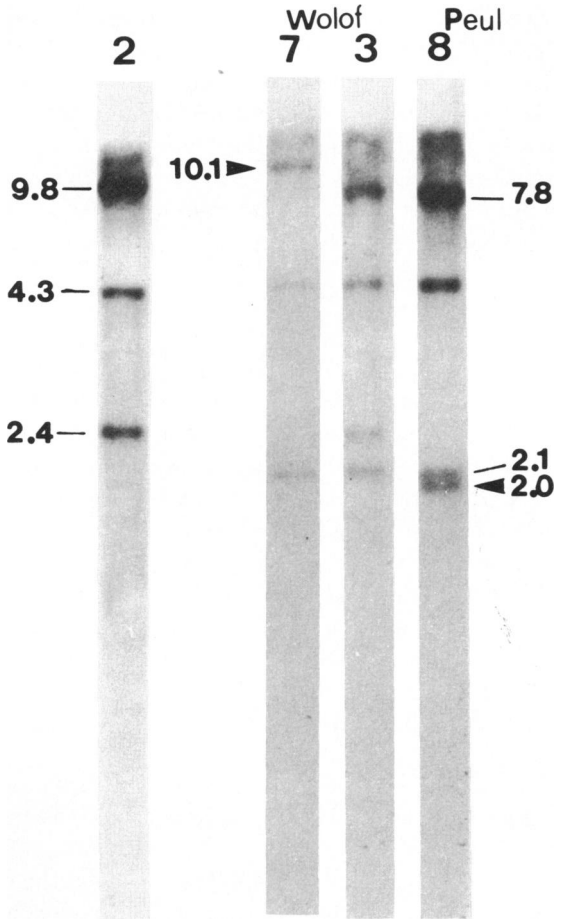

Figure 2 The new *Hpa*I morphs 7^{Wolof} (lane 2) and 8^{Peul} (lane 4), as compared with the two most common *Hpa*I morphs in Africa, *Hpa*I-3 (lane 3) and *Hpa*I-2 (lane 1). Note: Numbers at top correspond to each morph. The fragment sizes are given in kilobases; the new fragments are indicated by arrows.

ing to the loss of site *c* at 12406 np, as also observed in *Hpa*I morph 1 (Denaro et al. 1981). *Hpa*I morph 1 has always been observed in association with a *Hinc*II morph 1 (Denaro et al. 1981; Blanc et al. 1983), which is produced by loss of the same site (sites for *Hpa*I, GTTAAC, and for *Hinc*II, GTPyPuAC, are related). By contrast, the subject *Hpa*I morph 7^{Wolof} was a *Hinc*II morph 2, thus indicating that in this case the mutation which abolishes the site for *Hpa*I does not affect the *Hinc*II sensitivity of the same site. These data, therefore, show that site *c* has been lost in two different ways. The new morph can be derived from a *Hpa*I morph 3 by a transition T to C at 12408 np, which would not alter the amino acid sequence of the ND5 gene.

*Hpa*I morph 8^{Peul} can also be derived from *Hpa*I morph 3 by the acquisition of a new site in the 2.4-kb band, which is split into two new fragments of about 2.0 kb (fig. 2, lane 4) and 0.4 kb (not visible in the Figure). *Hpa*I/*Hin*dIII double digestion localized the mutation at about 12114 np, as in the case of *Hpa*I morph 4^{Sard} (Santachiara-Benerecetti et al. 1988). By *Hinc*II analysis, both *Hpa*I-8^{Peul} and *Hpa*I-4^{Sard} showed a *Hinc*II morph 3.

BamHI

With the exception of one Wolof with morph 3, Senegalese were monomorphic for *Bam*HI morph 1, as are the other African samples so far examined (Johnson et al. 1983).

HaeII

Senegalese were primarily *Hae*II morph 1. The second most common morph (about 6%) was the *Hae*II

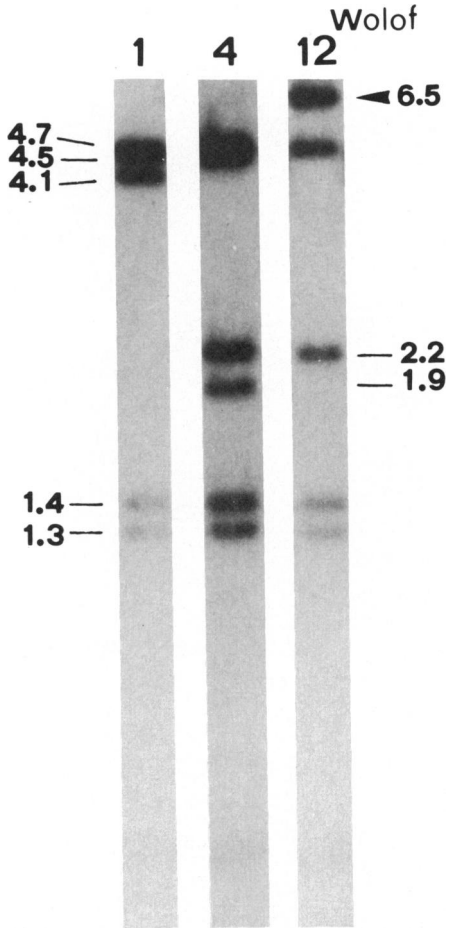

Figure 3 The new morph *Hae*II-12^{Wolof} (lane 3) aligned against morphs *Hae*II-1 (lane 1) and *Hae*II-4 (lane 2) for comparison. See note to fig. 2.

morph 4, previously observed in different groups but only as a rare variant (Johnson et al. 1983; Wallace et al. 1985; Bonné-Tamir et al. 1986; Brega et al. 1986*b*). Double digestion with *Hae*II/*Eco*RI confirmed that this is the same mutation as previously described, an A-to-G transition at 11002 np (Wallace et al. 1982; Johnson et al. 1983).

Morphs 2 and 7 were also seen in one individual each. Double digestion of *Hae*II morph 7 with *Hae*II/*Bam*HI confirmed that it is the same morph described by Johnson et al. (1983).

Table 2

Frequencies (in %) of the Various mtDNA Morphs Found in Senegalese, Together with Those Already Reported for Other Africans

Morphs	Senegalese (N = 186)	Bantu[a] (N = 40)[b]	Bushmen[a] (N = 34)[b]
*Hpa*I:			
1	1.6	4.2	
2	32.3	25.0	7.3
3	65.1	70.8	92.7
7^{Wolof}	.5		
8^{Peul}	.5		
*Bam*HI:			
1	99.5	100.0	100.0
3	.5		
*Hae*II:			
1	91.9	97.5	100.0
2	.5	2.5	
4	5.9		
7	.5		
12^{Wolof}	1.1		
*Msp*I:			
1	98.4	87.5	17.6
2		12.5	52.9
3			26.5
5			2.9
12^{Wolof}	1.1		
$13^{Tukolor}$	.5		
*Ava*II:			
1	54.8	40.0	11.8
2	1.1	12.5	58.8
3	40.9	37.5	2.9
4			5.9
5		5.0	20.6
6	1.1		
7		2.5	
9*[c]	.5		
11		2.5	
17	1.1		
23^{Wolof}	.5		

[a] Data are from Denaro et al. (1981) and Johnson et al. (1983).
[b] N = 48 Bantu and 41 Bushmen for *Hpa*I (Denaro et al. 1981).
[c] See text.

Two Wolof were found to have a new *Hae*II variant pattern: *Hae*II morph 12^{Wolof}. The *Hae*II-12^{Wolof} (fig. 3, lane 3) differs from morph 4 (fig. 3, lane 2) owing to the replacement of 4.5- and 1.9-kb fragments with a new band of about 6.5 kb. This morph is most likely derived from an *Hae*II morph 4 by loss of site *c* at 9052 np, but it could also originate from a *Hae*II morph 2 by gain of site *e* at 11001 np.

*Msp*I

With the exception of two new morphs, *Msp*I-12^{Wolof} and *Msp*I-$13^{Tukolor}$, found in two Wolof and one Tukolor, respectively, all Senegalese were *Msp*I morph 1. *Msp*I morph 2, which, as shown in table 2, has the highest frequency in Bushmen (52.9%) and is present in 12.5% of Bantu (Johnson et al. 1983), was not found among the 186 Senegalese examined, and the difference from the other two African groups is statistically highly significant ($P_{Yates} <<< .0001$ vs. Bushmen; $P_{Yates} < .001$ vs. Bantu). The absence of sites *a* and *b* at 8112 and 8150 np, respectively, which characterizes *Msp*I morph 2 (Johnson et al. 1983), is, however, reported for 1 of 20 individuals of African descent by Cann et al. (1987).

Like Bantu, Senegalese did not show the *Msp*I morph 3, which was observed in 26.5% of Bushmen (see table 2).

*Msp*I morph 12^{Wolof} (fig. 4*A*, lane 1) shows the disappearance of the 1.1-kb band and the presence of a new band of about 0.65 kb, the 0.5-kb band being reinforced. Double digestion with *Msp*I/*Pst*I (fig. 4*A*, lane 2) localized the new site at around 8646 np, where an A-to-G transition at 8649 np would produce the site without altering the amino acid sequence of ATPase 6.

*Msp*I morph $13^{Tukolor}$ (fig. 4*B*, lane 1) shows the replacement of the 2.4-kb band with a new band of about 1.9 kb, the 0.5-kb band being reinforced. Double digestion with *Msp*I/*Sst*I produced the replacement of the 1.9-kb band with a fragment of about 1.65 kb (fig. 4*B*, lane 2). This would localize the new site at approximately 11170 np. Several potential sites occur near this location, all found in ND4 gene. Either an A-to-G transition at nucleotide 11164 or 11179 or a transversion from A to C at 11194 np would create the new site without altering the amino acid sequence. It is worth noting that a mutation at 11164 creating an *Hpa*II/*Msp*I site has been also reported for two American blacks by Cann et al. (1987).

*Ava*II

Seven *Ava*II morphs were observed among Senega-

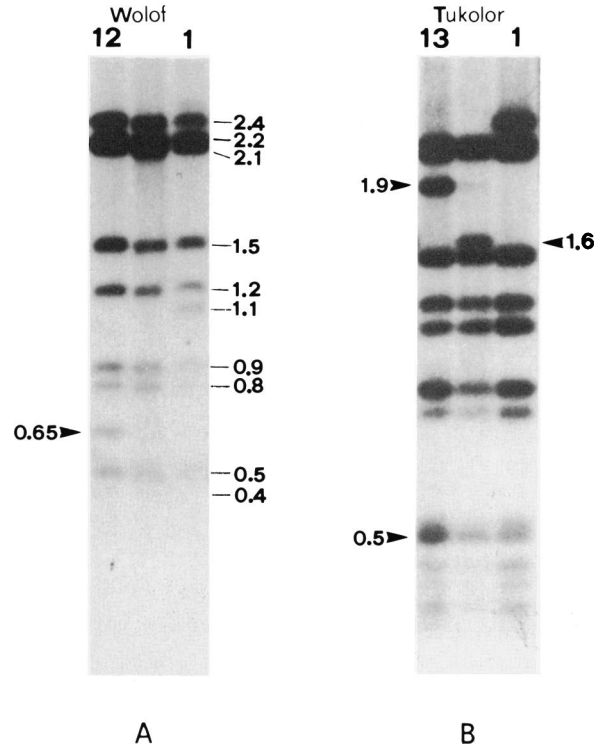

Figure 4 *A*, The new *Msp*I morph 12^{Wolof} (lane 1), in comparison with *Msp*I morph 1 (lane 3). In *Msp*I/*Pst*I double digestion of the *Msp*I morph 12^{Wolof} (lane 2), the disapperance of the 0.65-kb fragment localizes the new site at about 8650 np. *B*, The new *Msp*I morph $13^{Tukolor}$ (lane 1), together with its *Msp*I/*Sst*I double digest (lane 2) and *Msp*I morph 1 (lane 3). In lane 2 the 1.9-kb fragment is replaced by a band of about 1.6 kb, thus localizing the new site at around 11170 np. See note to fig. 2.

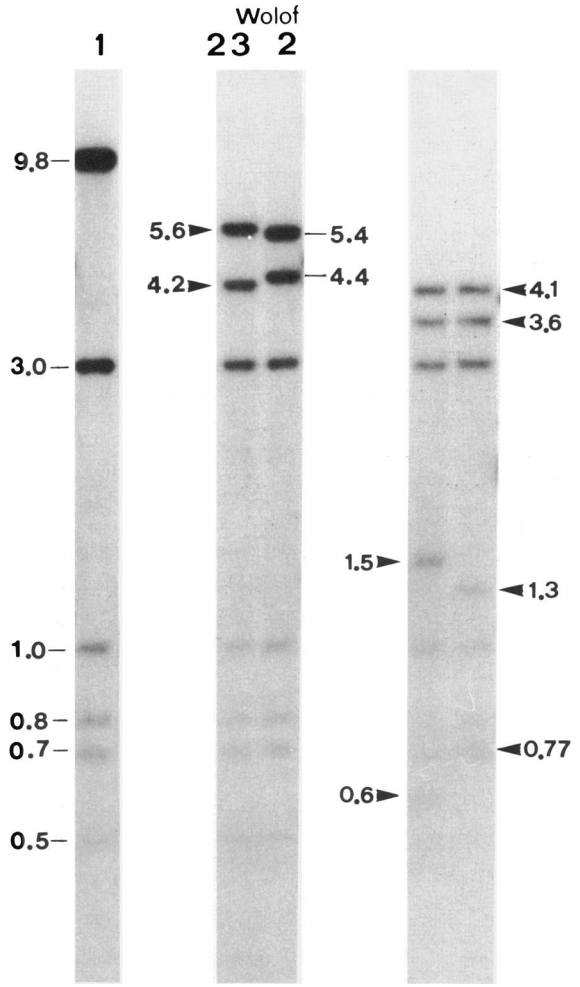

Figure 5 The new morph *Ava*II-23^{Wolof} (lane 2), as compared with *Ava*II morph 1 (lane 1) and *Ava*II-2 (lane 3). Lane 4 shows the two informative fragments of 1.5 and 0.6 kb obtained by *Ava*II/*Pst*I double digestion of *Ava*II-23^{Wolof}, as compared with the corresponding 1.3- and 0.77-kb fragments of the *Ava*II-2 double digest (lane 5). See note to fig. 2.

lese. One of these, *Ava*II-23^{Wolof}, had never been described before, and another two, *Ava*II-6 and *Ava*II-17, had not previously been observed in Africans.

*Ava*II morphs 1 and 3, both found in the two African groups previously examined (Johnson et al. 1983) but most represented in Bantu, were observed in this survey at frequencies (55% and 41%, respectively) even higher than those in Bantu (see table 2).

Two Wolof were found with the *Ava*II morph 2. *Ava*II/*Pst*I double digestion (fig. 5, lane 5) confirmed that the two *Ava*II morphs 2 were both due to the acquisition of site *c* at around 8250 np, as for the *Ava*II morph 2 already described (Johnson et al. 1983). The presence of an *Ava*II site at 8249 np has also been found in one subject among the 20 blacks of the survey by Cann et al. (1987). As shown in table 2, the frequency of the morph *Ava*II-2 in Senegalese is much lower than that in Bantu, which in turn is only about 20% of the Bushmen frequency. These differences are all highly significant (Senegalese vs. Bantu, $P_{Yates} \cong .001$; Senegalese vs. Bushmen, $P_{Yates} <<< .0001$; Bantu vs. Bushmen, $P_{Yates} < .001$).

Morph *Ava*II-6, previously found in 4.3% of Orientals (Johnson et al. 1983) and in one subject of the Italian population (Brega et al. 1986*b*), was observed in two Wolof. Double digestion with *Ava*II/*Bam*HI indicated that the mutation site is located at around 15890 np, as in the previous cases (Johnson et al. 1983; Santachiara-Benerecetti et al. 1988).

An *Ava*II morph resembling the rare *Ava*II morph

17^{Th}, described by Brega et al. (1986*a*) in the Tharu population of Terai (Nepal), was found in two Wolof. *Ava*II/*Sst*I double digestion placed the mutation at about 5229 np in the ND2 gene, as in the *Ava*II-17^{Th}. A C-to-G transversion at 5165 np would yield the new site without affecting the protein amino acid sequence.

One individual of the Wolof group showed an *Ava*II morph 9. Morph *Ava*II-9 is characterized by the loss of the *Ava*II site *d* at 13367 np. Since the *Ava*II morph 9 encountered in this study was found in association with a *Bam*HI morph 3, the mutation can be exactly identified as a G-to-A transition at 13368 np, which simultaneously creates a *Bam*HI site and abolishes an *Ava*II site (for a thorough discussion, see Santachiara-Benerecetti et al. 1988). Following the criterion already adopted (Santachiara-Benerecetti et al. 1988), this *Ava*II morph 9 is indicated by an asterisk. Previously, the mutation yielding *Bam*HI-3/*Ava*II-9* had been only observed in Caucasians, in 25 of about 350 individuals examined (Johnson et al. 1983; Bonné-Tamir et al. 1986; Brega et al. 1986*b*). But in all cases it was on an mtDNA carrying the variation responsible for *Msp*I morph 4.

Unlike above-described Caucasians, the individual with *Bam*HI-3/*Ava*II-9* of this survey was an *Msp*I morph 1. Therefore, a new mutation is most likely responsible for the presence of this mtDNA in Africans. It is worth noting that one mtDNA type from blacks was described (Cann et al. 1987) with the absence of the *Ava*II site at 13367 np in association with the *Hpa*II/*Msp*I site at 15925 np, the latter present, among others, in the *Msp*I morph 1 but not in the *Msp*I morph 4. However, since in the Cann et al. paper the nucleotide substitution causing the *Ava*II site loss was not identified, it is impossible to say whether the same mutation as that we observed in Senegalese is involved or not.

The new morph, *Ava*II-23^{Wolof} (fig. 5, lane 2), is characterized by the disappearance of the 9.8-kb band, which is split into two new bands of about 5.6 and 4.2 kb. *Ava*II/*Pst*I double digestion (fig. 5, lane 4)

Table 3

MtDNA Types[a] and Their Frequencies in the Different Groups of Senegalese Analyzed

Type No.	Enzyme Morphs[b]	Wolof (N = 110)	Peuls (N = 47)	Tukolor (N = 12)	Others (N = 17)
Old:					
1–2	(2.1.1.1.1.2)	23 (20.9)	11 (23.4)	1 (8.3)	6 (35.3)
2–2	(3.1.1.1.3.2)	38 (34.6)	15 (31.9)	7 (58.3)	6 (35.3)
2–7	(3.1.1.1.3.7)	1 (.9)			
2–10	(3.1.1.1.3.10)		4 (8.5)		
7–2	(3.1.1.1.1.2)	29 (26.4)	12 (25.5)	2 (16.7)	2 (11.8)
8–1	(1.1.1.1.1.1)		2 (4.3)		1 (5.9)
10–2	(3.1.1.1.2.2)	2 (1.8)			
27–2	(2.1.4.1.6.2)	2 (1.8)			
34–2	(3.1.2.1.3.2)		1 (2.1)		
39–2	(2.1.4.1.1.2)	5 (4.6)	1 (2.1)	1 (8.3)	1 (5.9)
52–2	(2.1.1.1.17.2)	2 (1.8)			
New:					
64–2	(3.1.7.1.3.2)				1 (5.9)
65–2	($2.1.12^{Wol}$.1.1.2)	2 (1.8)			
65–2	(7^{Wol}.1.1.1.3.2)	1 (.9)			
66–2	(2.3.1.1.9*.2)	1 (.9)			
67–2	(2.1.4.1.3.2)	1 (.9)			
68–2	($2.1.1.12^{Wol}$.1.2)	2 (1.8)			
69–3	(8^{Peul}.1.1.1.3.3)		1 (2.1)		
70–2	($2.1.1.13^{Tuk}$.1.2)			1 (8.3)	
71–2	($3.1.1.1.23^{Wol}$.2)	1 (.9)			
F[c]		.237	.232		

[a] mtDNA types are defined by combining morph data of the six restriction enzymes. Since *Hinc*II analysis was not carried out in all of the previous studies (Johnson et al. 1983; Bonné-Tamir et al. 1986), the classification for this enzyme is used as a subtyping.

[b] Enzyme morphs are indicated in the following order: *Hpa*I, *Bam*HI, *Hae*II, *Msp*I, *Ava*II, and *Hinc*II.

[c] F (the sum of the squares of the relative frequencies of each type) is a measure of homogeneity in the populations.

yielded two informative fragments of about 1.5 and 0.6 kb and placed therefore the new site (site *q*) at around 8400 np. Two potential sites are compatible with the size of these two fragments, at 8342 np in the tRNAlys and at 8391 np in the A6L gene. Mutation at the latter would change the amino acid from proline to serine or threonine.

HincII

With the exception of one individual with morph 7, Wolof as well as Tukolor were all *Hinc*II morph 2. Peuls, on the other hand, were rather polymorphic. Of 47 individuals, 85.1% were morph 2, 8.5% morph 10, 4.3% morph 1, and 2.1% morph 3. *Hinc*II morph 10, which is not rare in Peuls, so far has been found only in one Oriental (Horai et al. 1984) and in one Italian (Brega et al. 1986*b*).

mtDNA Types

By combining the data from the six enzymes, 20 different mtDNA types were recognized in the various groups analyzed. Their numbers and frequencies are reported in table 3. To have a measure of homogeneity, *F* values (Johnson et al. 1983) have been calculated from the data of table 3. These values—.237 and .232 for Wolof and Peuls, respectively—although higher than those already reported for Bantu and Bushmen (F = .192 and .195, respectively; Johnson et al. 1983), still show a greater heterogeneity in Africans than in Caucasians (F = .359 [Johnson et al. 1983]; F = .390 [Brega et al. 1986*b*]) and Orientals (F = .499 [Johnson et al. 1983]; F = .348 [Brega et al. 1986*a*]).

Preferential combinations between morphs have been observed. A strong association was found between *Ava*II morph 3 and *Hpa*I morph 3 and its derivatives (*Hpa*I-7^{Wolof} and *Hpa*I-8^{Peul}) (75 observed vs. 50.3 expected; $\chi^2_{1\ df,\ Yates}$ for disequilibrium = 58.4; $P << .0001$). Although a number of mtDNAs (48 of 123) have been found in this survey with the *Hpa*I-3 not associated with *Ava*II-3, only one mtDNA has been observed with *Ava*II-3 without *Hpa*I-3 or related morphs. Further, the Senegalese with morph *Hae*II-4 all were *Hpa*I morph 2 (11 observed vs. 3.55 expected; $\chi^2_{1\ df,\ Yates}$ for disequilibrium = 21.4, $P < .001$).

Discussion

This study is the most extensive survey of mtDNA polymorphisms in Africans. Different groups from Senegal, mainly Wolof and Peuls, have been analyzed for their mtDNA morphs and types by means of six restriction enzymes.

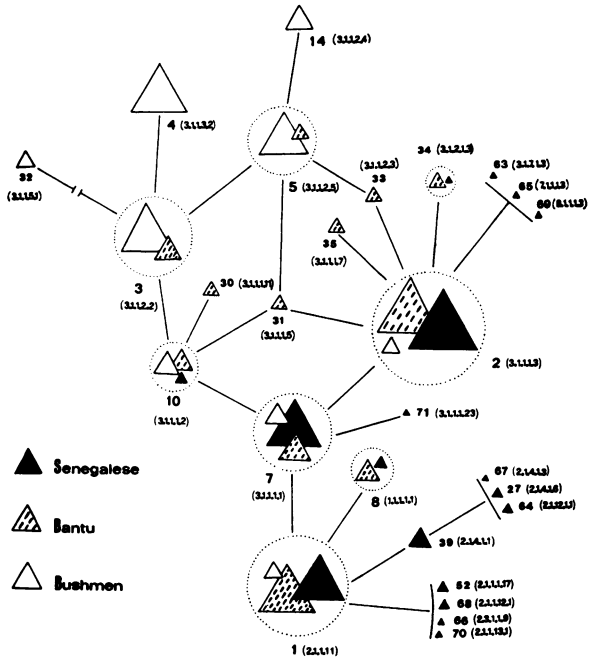

Figure 6 Phylogeny of mtDNA types in Africa, constructed according to Johnson et al. (1983). Data are from Johnson et al. (1983) and the present paper. The classification for *Hinc*II was disregarded, since *Hinc*II data were unavailable for the populations of Johnson et al. (1983). Since Wolof and Peuls could be differentiated almost exclusively by *Hinc*II analysis, all Senegalese were pooled together. Triangle areas are proportional to type frequencies. Numbers in parentheses denote enzyme morphs in the following order: *Hpa*I, *Bam*HI, *Hae*II, *Msp*I, and *Ava*II.

For both *Bam*HI and *Msp*I, Senegalese, as a whole, were virtually all morph 1. As for the fragment patterns produced by the other enzymes, features of interest emerge from the comparison between Wolof and Peuls (table 1). They have similar frequencies of *Hpa*I and *Ava*II morphs, though Wolof seem to be more variable for *Ava*II. By *Hinc*II analysis, on the other hand, there is a marked contrast between Peuls, who are rather polymorphic, and Wolof, who are almost monomorphic for *Hinc*II morph 2 ($\chi^2_{4\ df}$ = 17.44; $P \cong .002$). Even with *Hae*II could the two groups be distinguished ($\chi^2_{3\ df}$ = 8.60; $.05 > P > .02$).

*Ava*II morph 3 and *Hpa*I morph 3 call for special attention. The frequencies of these morphs, which are rare or absent in peoples not of African origin, are as high in Peuls as in Wolof and are very similar to the Bantu values (tables 1, 2). Whether or not Peuls, whose origin is still discussed, have acquired a caucasoid com-

ponent (Murdock 1959), present data indicate that they possess a substantial proportion of negroid mtDNAs. Unfortunately, we have no data on mtDNA variation among caucasoid populations of Northern Africa, and it is difficult to interpret these results in historical terms. It is, however, interesting to note that linguistic (Murdoch 1959) and genetic data agree in showing the Peuls from Senegal to have considerable negroid ancestry.

As seen in table 2, this African sample from Senegal shows, on the whole, similarities with the Bantu from South Africa, who are, together with Bushmen, the only other Africans analyzed for the same genetic markers (Denaro et al. 1981; Johnson et al. 1983). However, Senegalese can be clearly distinguished from Bantu for some features, such as the much lower frequency of *Ava*II morph 2 and the lack of variability for *Msp*I. These markers, on the other hand, can also well differentiate Bantu from Bushmen (Johnson et al. 1983), thus proving to be important in distinguishing from one another the major groups of negroids.

As for preferential combinations between morphs, the finding of the almost complete association *Ava*II-3/*Hpa*I-3 and derivatives (75 observed vs. 50.3 expected; $\chi^2_{1\ df,\ Yates}$ for disequilibrium = 58.4; $P << .0001$) is quite in line with that of Johnson et al. (1983) (28 observed vs. 23.1 expected; $\chi^2_{1\ df,\ Yates}$ for disequilibrium = 21.4, $P < .001$), whereas no significant association between the presence of *Hpa*I site *d* (yielding *Hpa*I morph 3) and the lack of *Ava*II site *f* (characterizing *Ava*II morph 3) was detected in the small sample of blacks of Cann et al. (1987) (5 observed vs. 3.3 expected). As previously discussed with reference to the combination between *Msp*I morph 4 and *Bam*HI morph 3 and its related *Ava*II morphs (those lacking site *d*) found in Caucasians (Santachiara-Benerecetti et al. 1988), the observed associations indicate that the high frequen-

Table 4

Frequencies (in %) of mtDNA Types Found in Senegalese, as Compared with Those Already Reported for Other Africans

Type No.	Enzyme morphs[a]	Senegalese (N = 186)	Bantu[b] (N = 40)	Bushmen[b] (N = 34)
1	(2.1.1.1.1)	22.1	25.0	2.9
2	(3.1.1.1.3)	38.2	32.5	2.9
3	(3.1.1.2.2)		7.5	26.5
4	(3.1.1.3.2)			26.5
5	(3.1.1.2.5)		2.5	20.6
7	(3.1.1.1.1)	24.2	10.0	5.9
8	(1.1.1.1.1)	1.6	5.0	
10	(3.1.1.1.2)	1.1	5.0	5.9
14	(3.1.1.2.4)			5.9
27	(2.1.4.1.6)	1.1		
30	(3.1.1.1.11)		2.5	
31	(3.1.1.1.5)		2.5	
32	(3.1.1.5.1)			2.9
33	(3.1.1.2.3)		2.5	
34	(3.1.2.1.3)	.5	2.5	
35	(3.1.1.1.7)		2.5	
39	(2.1.4.1.1)	4.3		
52	(2.1.1.1.17)	1.1		
63	(3.1.7.1.3)	.5		
64	($2.1.12^{Wol}$.1.1)	1.1		
65	(7^{Wol}.1.1.1.3)	.5		
66	(2.3.1.1.9*)	.5		
67	(2.1.4.1.3)	.5		
68	($2.1.1.12^{Wol}$.1)	1.1		
69	(8^{Peul}.1.1.1.3)	.5		
70	($2.1.1.13^{Tuk}$.1)	.5		
71	($3.1.1.1.23^{Wol}$)	.5		

[a] Enzyme morphs are listed in the following order: *Hpa*I, *Bam*HI, *Hae*II, *Msp*I, and *Ava*II. The classification for *Hinc*II was disregarded, since *Hinc*II data were unavailable for the population of Johnson et al. (1983).

[b] Data are from Johnson et al. (1983).

cies of the relevant haplotypes are due to diffusion of linked unique mutations rather than to repeated mutational events.

Table 4 shows the frequencies of mtDNA types in Senegalese as compared with those found in Bantu and Bushmen (Johnson et al. 1983). As already observed (Johnson et al. 1983), type 1 is the most common type among Caucasians and Orientals but not among Africans. Its frequency in Senegalese is about 20% and is very similar to that in Bantu, whereas it is only about 3% in Bushmen.

As for "African" types, those carrying the *Hpa*I morph 3, types 2 and 7 have high frequencies in Senegalese but are poorly represented in Bushmen. By contrast, types 3, 4, and 5, which are very common among Bushmen, were not found in Senegal and, when present, show low frequencies in Bantu.

From the data of table 4 a phylogeny of the mtDNA types in Africans was constructed by relating them through single site changes (fig. 6). As figure 6 well illustrates, Bushmen have, at rather high frequencies, unique types. For the shared types, there appears to be a general trend in decreasing or increasing frequency values from Bushmen to Bantu to Senegalese.

Considering all available data on the distribution of the mtDNA polymorphisms in different populations (Denaro et al. 1981; Blanc et al. 1983; Johnson et al. 1983; Horai et al. 1984; Wallace et al. 1985; Bonné-Tamir et al. 1986; Brega et al. 1986*a*, 1986*b*; Harihara et al. 1986; Horai and Matsunaga 1986; Santachiara-Benerecetti et al. 1988), some general conclusions can be drawn. As already stated, the outstanding feature of the mtDNA variation in Africa is the extremely high incidence of the *Hpa*I morph 3, a morph which appears to be confined to African peoples and peoples of African ancestry. The frequency of this morph is around 70% in Senegalese and Bantu, but it is over 90% in Bushmen. This suggests that the morph is a very ancient African characteristic which has spread from the ancestral proto-Khoisan, ultimately to reach the other African populations. The variability for *Bam*HI and the *Ava*II morph 9, which are virtually absent in the Africans as well as in the Orientals so far studied, allows us to consider these features, with more confidence than before, as caucasoid characteristics. *Ava*II morph 3, found at a low incidence in non-Africans, is fairly common (more than 30%) among Senegalese and Bantu, and it is found in an almost complete association with the "African" *Hpa*I morph 3. Africans, on the other hand, lack *Msp*I morph 4, which is instead common in Caucasians and Orientals. Further interesting information is provided by the analysis for *Hae*II: Bantu and Bushmen were found to be almost monomorphic for *Hae*II morph 1, whereas Senegalese, like Caucasians and Orientals, are rather polymorphic. However, different alleles seem to be frequent in different populations (*Hae*II-2 in Caucasians and Orientals, *Hae*II-4 in Senegalese). Finally, the greater heterogeneity of Africans as compared with other major ethnic groups (Johnson et al. 1983; Cann et al. 1987) is further confirmed by the data of the present study.

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