

Mitochondrial DNA Studies Show Asymmetrical Amerindian Admixture in Afro-Colombian and Mestizo Populations

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Abstract The origin of the African populations that arrived on the Colombian coasts at the time of the Spanish conquest and their subsequent settlement throughout the country and interaction with Amerindian and Spanish populations are features that can be analyzed through the study of mitochondrial DNA (mtDNA) markers. For this purpose, the present study investigates the admixture between these populations by analyzing the markers defining the main (*A, B, C, D*) and minor (*X*) founder haplogroups in Native Americans, the principal African haplogroup (*L*), and additional generic markers present in Caucasian (*I, J, K, H, T, U, V, W*) and minor African lineages (*L3*). As part of an interdisciplinary research program (the Expedición Humana, furthered by the Universidad Javeriana and directed by J.E. Bernal V.), 159 Afro-Colombians from five populations in which they are the majority and 91 urban Mestizos were studied. No Amerindian haplogroups (*A-D, X*) were detected in 81% of the Afro-Colombians. In those samples with Amerindian lineages (average 18.8%, with a range from 10% to 43%), haplogroup *B* predominated. When analyzed for the presence of African haplotypes, Afro-Colombians showed an overall frequency of 35.8% for haplogroup *L* mtDNAs, although with broad differences between populations. A few Afro-Colombian samples (1.9%) had mutations that have not been described before, and might therefore be considered as previously unsampled African variants or as new mutations arising in the American continent. Conversely, in Mestizos less than 22% of their mtDNAs belonged to non-Amerindian lineages, of which most were likely to be West Eurasian in origin. Haplogroup *L* mtDNAs were found in only one Mestizo (1.1%), indicating that, if present, admixture with African women would bring in other, rarer African lineages. On the other hand, in an accompanying paper (Keyeux et al. 2002) we have shown that Amerindians from Colombia have experienced little or no matrilineal admixture with Caucasians or Africans. Taken together, these results are evidence of different patterns of past ethnic admixture among Africans, Amerindians, and Spaniards in the geographic region now encompassing Colombia, which is also reflected in much of the region's cultural diversity.

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Mitochondrial DNA (mtDNA) is a maternally inherited genome that does not recombine (Giles et al. 1980), and its nucleotide sequence evolves 6 to 17 times faster than nuclear DNA sequences (Brown et al. 1979; Wilson et al. 1985). This rapid evolution has resulted in multiple restriction fragment length polymorphisms (RFLPs), and control (D-loop) and coding region nucleotide variants (Anderson et al. 1981; Wallace et al. 1987). These RFLPs and nucleotide variants are correlated with the ethnic and geographic origin of individuals, presumably because mtDNA mutations have accumulated along with radiation of maternal lineages as women migrated out of Africa into different continents (Cann et al. 1987; Stringer and Andrews 1988; Merriwether et al. 1991; Templeton 1992; Torroni et al. 1992; Horai et al. 1995; Penny et al. 1995; Stoneking and Soodyall 1996).

Since the 1970s, human geneticists have been analyzing human mtDNA diversity. Several studies have shown that between 65% and 100% of Africans harbor a nt3592 *HpaI* restriction site rarely seen in Asian and European populations, thus defining one major mtDNA African-specific cluster (haplogroup *L*) (Denaro et al. 1981; Scozzari et al. 1988, 1994; Soodyall and Jenkins 1993; Chen et al. 1995, 2000). This discovery suggested that African mtDNAs without the 3592 *HpaI* site could be the ancestral types from which European and Asian mtDNAs were derived (Johnson et al. 1983; Wallace 1995).

The characterization of the continent-specific mtDNA haplogroups revealed that each one originated by particular mutations. When these variants are analyzed, the continental origin of 75% of the African, European, Asian, and Native American mtDNAs can be determined (Johnson et al. 1983; Cann et al. 1987; Vigilant et al. 1991; Wallace 1995; MITOMAP 2001). Most Africans share a *DdeI* site at nt10394 with a group of Caucasian, Asian, and Amerindian lineages. As shown in Table 1, the branching of the continent-specific and African lineages involves this central marker plus an additional mutation creating an *AluI* site at nt10397.

The main sub-Saharan African haplotypes, thus, are characterized by a combination of 10394*DdeI*(+)/10397*AluI*(-)/3592*HpaI*(+) markers (haplogroup *L*, comprising the *L1* and *L2* lineages) (Chen et al. 1995, 2000). A less frequent group of haplotypes lacks the African-specific 3592 *HpaI* marker [10394*DdeI*(+)/10397*AluI*(-)/3592*HpaI*(-)] (Chen et al. 1995, 2000) and has been designated as haplogroup *L3* (Watson et al. 1997). A minority of African haplotypes (2.3% of Africans) lack all three of these mutations [10394*DdeI*(-)/10397*AluI*(-)/3592*HpaI*(-)]. Some align with the European lineage *U* (Chen et al. 2000), but a number of the mtDNAs belong to branches of the African haplogroup *L3*, itself derived from African haplogroup *L1* (Watson et al. 1997).

Attempts at reconstructing the population history of the American continent have revealed the presence of most of these haplogroups in the African-derived populations (Bravi et al. 1997; Parra et al. 1998; Bortolini et al. 1999) and the hybrid or "Mestizo" population (Torroni et al. 1995; Alves-Silva et al. 2000; Carvajal-Carmona et al. 2000; Green et al. 2000) from the countries so far studied, together with Caucasian and Amerindian haplogroups. However, little is known

about the last 500 years' admixture and diversification of the descendants of Africans in America since their importation by the Portuguese and other slave traders beginning in the 16th century (Curtin 1971; Friedemann and Arocha 1986). In Colombia, thousands of Africans disembarked in Buenaventura, Gorgona, and Barbacoas on the Pacific Coast, and Cartagena de Indias, Riohacha, Santa Marta, Tolú, and Darién on the Atlantic.

These ancestral populations originated from different African ethnic groups, especially from West and Central Africa. Each group of slaves was caught from a different clan, according to their place of origin in Africa: Congo, Bantu, Wolof, Yoruba, Fanti-Ashanti, and others (Friedemann 1993; Friedemann and Arocha 1995). They came from Senegambia; the Pepper, Ivory, and Gold Coasts; the Benin and Biafra Gulfs; Central Africa; Kongo; Mozambique; and Angola. To prevent rebellions, they were further mixed together in the mines and other places of labor (Friedemann 1993). Conservative estimates of the number of slaves that came to Colombia range between 100,000 and 200,000 (Colmenares 1979; Del Castillo 1981). Nowadays, their descendants call themselves Afro-Colombians.

In the last centuries, admixture has taken place, especially in the Afro-descendant and the Criollo (Spaniards born in the American colonies) populations. However, based on written records and reconstruction of family histories, it is difficult to evaluate the magnitude of Amerindian admixture within present-day Afro-American and Mestizo (the admixture of European, Amerindian, and African descendants with predominant Caucasian phenotype) populations in Colombia. On the other hand, in an accompanying paper (Keyeux et al. 2002), we have shown that Amerindian groups in Colombia have experienced almost no admixture, at least through matrilineal contribution, be it with contemporaneous Afro-Colombian or Mestizo individuals, or with their ancestors, the Spanish (and other European) colonizers and African slaves. Therefore, we decided to investigate the presence of founder Amerindian lineages in Afro-Colombian populations from representative geographical locations where they are predominant, and in an urban group of Mestizos.

The present study analyzes the markers that define the Amerindian haplogroups *A* (*HaeIII* site gain at nt663), *B* (9-base-pair [bp] deletion between COII/tRNALys), *C* (*HincII* site loss at nt13259), *D* (*AluI* site loss at nt5176), and *X* (*DdeI* site loss at nt1715) (Torroni et al 1992, 1993a; Forster et al. 1996). Additionally, we investigated the presence of the 3592 *HpaI* marker defining the African-specific macrohaplogroup *L* (Chen et al. 1995, 2000; Soodyall et al. 1996) and the 10394 *DdeI* and 10397 *AluI* generic sites separating haplogroups in European, Asian, and African populations (Table 1) (Schurr et al. 1990; Torroni et al 1992, 1993; Ballinger et al. 1992; Horai et al. 1993; Baillet et al. 1994; Bianchi et al. 1995).

Findings on differences and asymmetry in the mating between the three ancestral populations in the diverse regions of Colombia (present paper; Keyeux et al. 2002) will help to understand better the biology and the cultural diversity of

Table 1. Mutations Central to the Branching of the Principal Continental Mitochondrial DNA Haplogroups^a

| Population | Haplogroups | mtDNA Restriction Sites | | | |
|-----------------|---------------------------|-------------------------|-----------|----------|-------------|
| | | 10394DdeI | 10397AluI | 3592HpaI | 1715DdeI |
| African | <i>L (L1+L2)</i> | + | - | + | + |
| | <i>L3 (L3a, L3b, L3c)</i> | + | - | - | + |
| | <i>L3d</i> | - | - | - | + |
| European | <i>I, J, K</i> | + | - | - | -(I) +(J,K) |
| | <i>H, T, U, V, W</i> | - | - | - | + |
| Asian | <i>A, B, F</i> | - | - | - | + |
| | <i>C, D, E, G (M)</i> | + | + | - | + |
| Native American | <i>A, B</i> | - | - | - | + |
| | <i>C, D</i> | + | + | - | + |
| | <i>X</i> | - | - | - | - |

a. Sources: Torroni et al. 1992, 1993, 1994, 1996; Chen et al. 1995, 2000; Watson et al. 1997.

the populations, and might also shed some light on historical events that might not have been recorded properly or even have been lost.

Materials and Methods

Populations Studied. The present study analyzed 159 samples from five Afro-Colombian populations (Figure 1, Table 2), who identify themselves as descendants from African peoples with no clearly documented white (European) or Colombian Mestizo ancestry (Friedemann and Arocha 1986; Arocha 1992), and 91 Mestizos sampled in Bogotá, who are representative of most of the country's inner urban population. Of the five Afro-Colombian populations studied, three live on the Pacific Coast (Cauca, Nuquí, and Quibdó), one is a Caribbean coast population descended from slave refugees from the palenques in the 18th century (Palenque de San Basilio) (Friedemann and Patiño-Roselli 1983; Friedemann and Arocha 1986), and one is an island population (Providencia) of mixed African and Caucasian (English and Dutch) ancestry (Edwards 1970).

Sample Collection. Blood samples from unrelated individuals were collected under the auspices of "Expedición Humana" by members of the different field teams between 1990 and 1993. All participants gave their consent freely.

The blood samples were shipped or carried to Bogotá, where DNA was extracted by members of the Unidad de Genética Molecular at the Instituto de Genética Humana of the Universidad Javeriana, rendered anonymous, and stored at -20°C. DNA quality was assessed by electrophoresis through 0.7% agarose gels stained with ethidium bromide. Due to difficulties in the fieldwork storage conditions, some DNA samples were partially degraded and underwent total hydrolysis in the course of the studies, impeding further analyses in the present investigation.

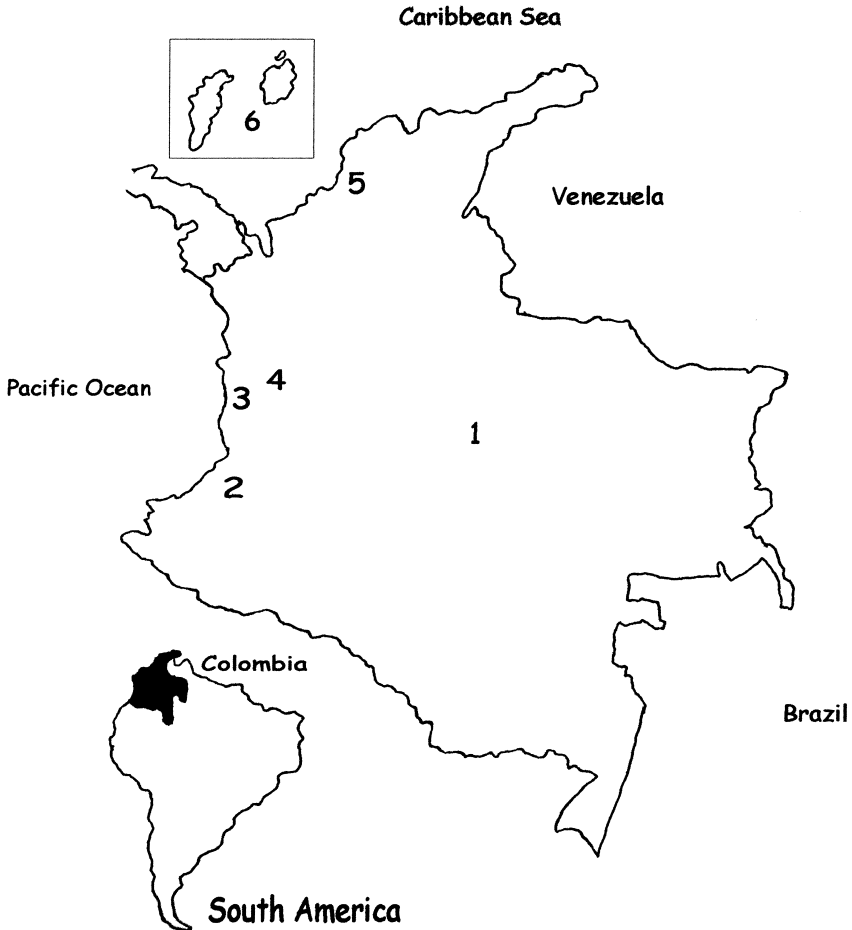


Figure 1. Map of Colombia showing the location of the Afro-Colombian groups. Mestizos were sampled in Bogotá (1). Map locations are: Cauca (2), Nuquí (3), Quibdó (4), Palenque de San Basilio (5), Providencia (6).

Table 2. Geographic Location and Sample Size of the Afro-Colombian Populations Studied

| <i>Area</i> | <i>Population</i> | <i>N</i> | <i>Geographic Location</i> |
|------------------------|-------------------------|----------|--------------------------------------|
| Atlantic Coast | Palenque de San Basilio | 41 | Bolívar Department |
| Pacific Coast | Nuquí | 33 | Chocó Department |
| | Quibdó | 28 | Chocó Department (Capital) |
| | Cauca | 20 | Cauca Dep. (Bocas del Guanguí) |
| San Andrés Archipelago | Providencia Island | 40 | Caribbean Sea, Northwest of Colombia |

Molecular Analyses. DNA was extracted from peripheral blood leukocytes as previously described (Keyeux et al. 1990). Total DNA (100–200 ng) was amplified by polymerase chain reaction (PCR). All the mitochondrial primers and reaction conditions for the markers defining haplogroups *A*, *B*, *C*, *D*, *L*, *X* and the markers 10394/10397 *AluI/DdeI* were the same as described in an accompanying paper (Keyeux et al. 2002).

Digestion of 25 μ L (30 μ L for *DdeI* 1715 marker) amplification product was carried out at 37°C overnight using 6 U (10 U *HpaI* and *DdeI*) enzyme. All restriction products were run on 3% (2% for *DdeI* 1715 marker) agarose gels at 120 V. The region V 9-bp repeat was analyzed by 6% polyacrylamide gel electrophoresis in a mini-protean II apparatus (1 hr at 150 V). The gels were stained with ethidium bromide and photographed under UV light. New mutation products were run on 8% or 12% polyacrylamide gels with appropriate size ladders in order to establish their length. Probable mutation sites were assigned according to product size and Anderson's sequence (Anderson et al. 1981; MITOMAP web site) location of putative new restriction sites.

Genetic Distances and Dendrograms. Distance matrixes using both Nei's (Nei 1972) and Prevosti's (Prevosti 1974) genetic distances were constructed and further used to build dendrograms using the UPGMA (unweighted pair group with arithmetic mean) clustering method implemented in PAUP (phylogenetic analyses using parsimony; Swofford 1999). All the haplogroups shown in Table 4 were used in the present analysis, even though the frequencies in columns I, II, and III (Other) might represent more than one haplogroup and continental lineage, as discussed later.

Results

To examine the proportion of Amerindian admixture in Afro-descendants from Colombia, 159 individuals from five different Afro-Colombian populations were tested for the four markers defining the *A-D* founder lineages in native Americans.

A significant proportion of Amerindian lineages were detected (Table 3), especially haplogroups *A* (mean frequency: 8.2%) and *B* (mean frequency: 10.6%) (Table 4). The samples with the 9-bp deletion in region V were further investigated for the 3592 *HpaI* marker defining African haplotypes (*AF60* or *AF61*) harboring a 9-bp deletion (Chen et al. 1995) and found to be negative, confirming its Asian/Amerindian ancestry in our samples.

Individual analysis of the Afro-Colombian populations shows that the Quibdó community bears the highest Amerindian mtDNA composition (42.9%), as opposed to the populations from San Basilio de Palenque (13.2%), Cauca (10%), and Providencia (10%), which show the lowest frequencies (Tables 3 and 4) (Rodas 1997).

The analysis of the combination of the 10394*DdeI*(-)/10397*AluI*(-) and 1715 *DdeI*(-) markers, characteristic of haplogroup *X* (Foster et al. 1996) (Table

Table 3. Compound Amerindian, African, and Other mtDNA Haplogroup Frequencies in Afro-Colombian and Mestizo Populations

| Colombian Populations | N | Amerindian Haplogroups | African Haplogroup L | Other Haplogroups | New Mutations | Unidentified |
|-----------------------|----|------------------------|----------------------|-------------------|---------------|--------------|
| Cauca | 20 | 0.1 | N.D. | 0 | 0.1 | 0.8 |
| Nuquí | 33 | 0.212 | 0.394 | 0.333 | 0 | 0.061 |
| Quibdó | 28 | 0.429 | 0.214 | 0.25 | 0 | 0.107 |
| Providencia | 40 | 0.1 | 0.525 | 0.325 | 0.025 | 0.025 |
| San Basilio | 38 | 0.132 | 0.447 | 0.341 | 0 | 0.079 |
| Mestizo | 91 | 0.78 | 0.011 | 0.187 | 0 | 0.022 |

Table 4. Main Amerindian and African mtDNA Haplogroup Frequencies in Afro-Colombian, Mestizo, and Amerindian Population from Columbia^a

| Colombian Populations | N | Haplo A | Haplo B | Haplo C | Haplo D | Haplo L | Other | | |
|-----------------------------|-----|---------|---------|---------|---------|---------|-------|-------|-------|
| | | | | | | | I | II | III |
| Cauca | 20 | 0 | 0.1 | 0 | 0 | N.D. | N.D. | N.D. | N.D. |
| Nuquí | 33 | 0.151 | 0.061 | 0 | 0 | 0.394 | 0.091 | 0.242 | 0 |
| Quibdó | 28 | 0.071 | 0.321 | 0.037 | 0 | 0.214 | 0 | 0.25 | 0 |
| Providencia | 40 | 0.1 | 0 | 0 | 0 | 0.525 | 0 | 0.325 | 0 |
| San Basilio | 38 | 0.053 | 0.053 | 0 | 0.026 | 0.447 | 0 | 0.316 | 0.026 |
| Mestizo | 91 | 0.374 | 0.264 | 0.077 | 0.066 | 0.011 | 0.165 | 0.022 | 0 |
| NW Amerindians ^b | 335 | 0.561 | 0.131 | 0.244 | 0.011 | 0.003 | 0.003 | 0 | 0.042 |
| SE Amerindians ^b | 346 | 0.072 | 0.473 | 0.323 | 0.118 | 0 | 0 | 0 | 0.02 |

a. Extended marker analyses of the samples without the founder Amerindian lineages (A, B, C, D, X) or African L haplogroup, grouped as “Other,” is as follows:

- I: 10394DdeI-/10397AluI-/3592HpaI-
- II: 10394DdeI+/10397AluI-/3592HpaI-
- III: 10394DdeI+/10397AluI+/3592HpaI-

b. Data are taken from Keyeux et al. (2002). Two groups of Amerindian populations are described in that paper, northwest Amerindians, and southeast Amerindians, according to their geographical, linguistic, and mtDNA haplogroup clustering.

1), shows that this Amerindian minor lineage is absent in the Afro-Colombian individuals (present study), as expected from the survey of 25 Colombian Amerindian groups, where no haplogroup X was detected (Keyeux et al. 2002).

African and possibly Caucasian lineages are present in 81.2% of the samples, with a range between 46% and 85% (Tables 3 and 4). The results from further restriction analyses of these are listed in Table 4: on average, 35.8% of the Afro-Colombian individuals bear the combination of the 10394DdeI(+)/10397AluI(-)/3592HpaI(+) restriction sites characteristic of haplogroup L, Quibdó being the population with the lowest frequency (21.4%) (Gelvez 1998). This macrohaplogroup contains two lineages, L1 and L2, and some of the L1 haplotypes are characterized by an AluI restriction site at nt10319 (Chen et al. 1995). Three in-

dividuals (7.5%) from Providencia (out of 21 belonging to haplogroup *L*) are 10394*DdeI*(+)/10319*AluI*(+)/3592*HpaI*(+), and thus belong to haplogroup *L1b* (Chen et al. 2000). No other Afro-Colombian populations exhibit this lineage.

Another 25.2% of all the individuals have 10394*DdeI*(+)/10397*AluI*(-)/3592*HpaI* (-) markers (Table 4, column Other-II), which could represent other more rare African haplotypes from the *L3* group (Watson et al. 1997), or Caucasian haplogroups *J* or *K* contributed by Mestizo or European admixture (haplogroup *I* was excluded because of the presence of a 1715 *DdeI* restriction site in all of these DNAs). Since the Mestizo population shows only 2.2% of these haplotypes (present study), it is unlikely that their presence in Afro-Colombians is due to non-African maternal admixture.

Three individuals (9.1%) from Nuquí are negative for all three markers (column I), indicating probable admixture with Caucasian haplogroup *H* or the less frequent *T*, *U*, *V*, and *W* (Torroni et al. 1996, 1998), which might be explained by admixture with Mestizos living close in the villages on the coast. Another possibility is that these individuals bear haplogroup *L3d* mtDNAs, a rare African lineage found in Senegal (Chen et al. 2000). Finally, only one individual (2.6%) from San Basilio shows a 10394*DdeI*(+)/10397*AluI*(+)/3592*HpaI*(-) extended haplogroup (column III), characteristic of haplogroup *M* in Asians and Amerindian *C* and *D* lineages (Wallace 1995). Since this individual does not have any of the markers characterizing the two Amerindian haplogroups, the most plausible hypothesis is that his mtDNA belongs to the Asian type *M* (Wallace 1995). Although admixture in the sample from San Basilio with an Amerindian mtDNA having undergone a reverse mutation either from a *C* or a *D* haplogroup could still explain the presence of this haplotype, this argument has been ruled out to explain the "other" [10394*DdeI*(+)/10397*AluI*(+)/3592*HpaI*(-)] lineages observed in a few of the Colombian Amerindian groups, due to their high incidence in some of these populations (up to 25%) (Keyeux et al. 2002).

We also found new mutations (Table 4). A length mutation seemingly corresponding to three copies of the intergenic COII/tRNA^{Lys} region 9-bp repeat is detected on a haplogroup *L* [10394*DdeI*(+)/10397*AluI*(-)/3592*HpaI*(+)] in one individual from Providencia Island. The COII/tRNA^{Lys} intergenic region has been shown to be a mutational hotspot in the mtDNA molecule, since deletions/insertions have repeatedly been found on different haplotypes in different populations (Ballinger et al. 1992; Chen et al. 1995; Soodyall et al. 1996; Lum and Cann 1998). Densitometric analysis of a 12% nondenaturing polyacrylamide gel using a molecular analyst software system (BioRad GS-700) and a reference 10-bp DNA ladder (Life Technologies) shows a difference in length of this fragment of roughly 9 bp (130 bp instead of 121 bp for the normal two copies). The probable insertion of one 9-bp repeat in one sample from Providencia, arising on an African haplogroup *L*, seems to be a parallel mutation of the one found in one Chukchi individual from Siberia (Schiels et al. 1992). Since it has not yet been reported in any other African group (Chen et al. 1995, 2000; Soodyall et al. 1996), we do not know, however, if it arose in Africa or is a new mutation in an African

descendant from the New World. Sequencing of this region should provide further insight as to whether it is a true CCCCTCTA insertion or a homopolymeric C-tract expansion following a transition mutation, as described in Pacific Islanders (Lum and Cann 1998).

The presence of one additional *AluI* restriction site is detected in two individuals (10%) from the Cauca population. Original fragments for the 5176 *AluI* site on the Cambridge Reference Sequence (Anderson et al. 1981) are 72 and 77 bp long; in the present case, densitometric analysis determined by imaging densitometry with the BioRad (GS-700) analyst software system (conditions as above) shows the 77-bp fragment is cleaved into two fragments 49 bp and 28 bp long, in agreement with a sequence for a putative *AluI* restriction site at nt5127 (Anderson et al. 1981; MITOMAP web site). This mutation is also new; it has not previously been found in African or Afro-descendant populations (Chen et al. 1995, 2000; Torroni et al 1995; Bravi et al. 1997; Parra et al. 1998; Alves-Silva et al. 2000). It is different from the nt5133 mutation found in a Cuban individual yielding two fragments differing in only 9 bp (Torroni et al. 1995), easily differentiable from those we observed (21-bp difference). Sequence typing should help defining if these mutations occurred independently on different haplotypes, or are one and the same ancestral mutation found in two currently non-related individuals.

The other population studied, the Mestizos, shows a high frequency of Amerindian lineages (78%) (Table 3), with predominantly A (37.4%) and B

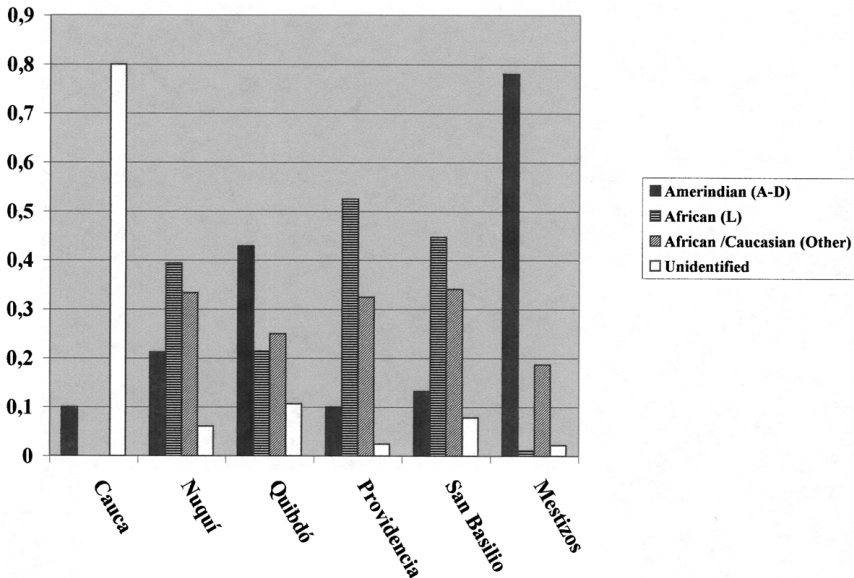


Figure 2. Histogram showing the compound frequencies of Amerindian (A-D), African (L), African or Caucasian (Other), and unidentified lineages in the six populations analyzed.

(26.4%) haplogroups (Table 4). As in Afro-Colombians (present paper) and Amerindians (Keyeux et al. 2002), no haplogroup *X* was found.

The other haplogroups (22%) represents in most cases European lineages: 16.5% of the individuals bear generic markers characteristic of the main European lineages (*H, T, U, V, W*) (column Other-I), and 2.2% could carry either *J* or *K* haplogroups (column II), or possibly rare African haplotypes from the *L3* group. Only one individual clearly shows African maternal admixture, since it has haplogroup *L1* [*10394DdeI(+)/10319AluI(+)*3592*HpaI(+)*].

Genetic distance analysis using Nei's method resulted in a topology with little anthropological significance (data not shown). We therefore used Prevosti's matrix in the construction of the tree and further analyses. The tree shows that the populations from Providencia, Palenque de San Basilio, and Nuquí are more closely related to each other than are the other three populations studied (Figure 3). Instead, the population from Quibdó, geographically close to Nuquí, sits on a branch apart from the previous ones, as does the Mestizo population, which is definitely not an Afro-descendant group. The population from Cauca, where very few molecular analyses could be performed, sits as an outgroup and does not change the topology of the tree when not included in the construction of the matrix (results not shown).

Although strict genetic relationships between human groups are difficult to assess when haplotype analyses are not available, present results are concordant with nuclear genes analyses performed in the same populations (Jaramillo-Correa et al. 2001).

Discussion

In order to study the extent of Amerindian gene flow in groups of Afro-Colombians living in close proximity with the first arrivals, we chose to study three communities from the Pacific Coast (Cauca, Nuquí, and Quibdó), one Caribbean coast population (Palenque de San Basilio), and one Caribbean island population (Providencia).

The presence mainly of *A* and *B* haplogroups in the Afro-Colombian population (present study) is interesting, since these constitute the principal lineages found in Amerindian groups inhabiting the western side of the Andes cordillera in

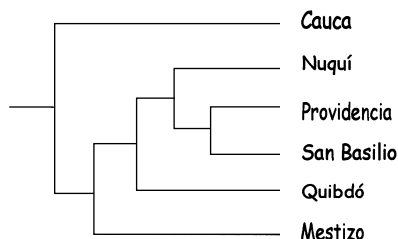


Figure 3. Phylogenetic analysis using the UPGMA clustering and Prevosti's genetic distance.

Colombia (Keyeux et al. 2002). The possibility that these could have arisen through mutations of some ancestral African haplotype(s) at nucleotide position (np) 663, np 13259, and np 5176, and hence, revert to haplogroups *A*, *C*, and *D*, respectively, is unlikely, since these mutations have not been found in any of the African populations so far studied (Chen et al. 1995, 2000; Soodyall et al. 1996). In the same way, the presence of haplotypes with the deletion of one COII/tRNALys 9-bp repeat in the Afro-Colombian populations is not due to the presence of African ancestral lineages from the subgroup *L1* mtDNAs characterizing Pygmies (Chen et al. 1995, 2000) and other sub-Saharan populations (Soodyall et al. 1996), but rather result from the presence of Amerindian lineages. Therefore, the high number of individuals in the Chocó population (Nuquí and especially Quibdó) carrying Amerindian haplogroups clearly shows that admixture with Amerindian women has been a common cultural practice, either during early Spanish colonial times between African slaves and native American women, or, more recently, between Afro-Colombian and Amerindian descendants or possibly Mestizos, and hence represents an important source of biological diversity.

Moreover, the distribution of the Amerindian haplogroups in Afro-Colombian populations follows more or less the pattern seen in the neighboring Amerindian communities studied, although with lower frequencies (Keyeux et al. 2002) (Table 4). This pattern is especially observed in areas where Amerindian and Afro-Colombian groups share their lands and resources and have cultural, economic, and social interchange as in the Chocó region (Nuquí, Quibdó). These findings further confirm anthropological studies documenting tight social and cultural relationships between some Amerindian and Afro-Colombian populations in areas of “conviviality,” which, in Colombia, is a term that defines a particular situation in the conflict of war and effort for peace (Friedemann and Arocha 1982, 1986; Ulloa 1996). The Embera Indians (one of the main Amerindian groups in the Chocó and Antioquia Departments) and their Afro-Colombian neighbors, for instance, are known to practice coparenthood (*compadrazgo*), meaning that familiar agreements are sealed when one Afro-Colombian individual stands as godfather at the baptism of an Amerindian child (Ulloa 1996).

In the case of Quibdó, an Afro-Colombian group that has no close contact with Amerindians, the high frequency of Amerindian lineages present in this group might be explained by the continuous displacement of isolated Afro-Colombian populations from their original point of settlement in the Chocó Department toward Quibdó, the capital. It is not surprising, then, that the two Embera communities from the Cauca (Keyeux et al. 2002) and Antioquia Departments (Mesa et al. 2000) that have high haplogroup *A* and *B* frequencies may have been an important source of haplogroup *B* mtDNAs into the Quibdó population.

In contrast, Palenque de San Basilio is one of the populations with the lowest frequencies of Amerindian mtDNA lineages (Table 3). Its condition of “palenque” community, created when rebel slaves escaped from their owners, generated an independent settlement consisting of commonalty properties, a unique Bantu-derived live Creole language (Del Castillo 1984; Schwegler 1989;

Ethnologue 2001), and almost no interaction with Amerindians (Friedemann and Arocha 1982, 1986; Friedemann 1993). In this case, one possible explanation for the Amerindian haplotypes found is admixture with Mestizos, who themselves have high frequencies of Amerindian lineages (present study; Carvajal-Carmona et al. 2000). This might also be the case in Providencia, where the insular isolation hindered contact with Amerindians from the rest of Colombia. It does not seem possible, either, that early admixture took place in Jamaica, before the English slave traders brought the African ancestors to San Andrés and Providencia (Edwards 1970), since Jamaicans do not themselves show evidence of Amerindian mtDNA lineages (Parra et al. 1998).

Compared to our results, Afro-Brazilians from the middle and south of the country show only 9.7% Amerindian lineages (Bortolini et al. 1999), whereas Afro-Uruguayans exhibit 31.7% Amerindian haplogroups (Bravi et al. 1997), indicating different patterns of admixture between Afro-descendants and Amerindian females in the different countries and regions. The small sample size and few locations in the two South American countries (Brazil: Rio Grande do Sul and Bahia; Uruguay: Melo) do not allow further comparisons with our survey in four clearly distinct regions (in terms of cultural and phenotypic traits) from Colombia. Nevertheless, they show a general trend of great variability in the crossing pattern between the populations that came in contact with each other several centuries ago, depending on cultural, religious, and political interests and constraints imposed by the dominant society and by the native inhabitants. This pattern is clearly seen in the almost total absence of Amerindian mtDNA gene flow in the Afro-descendant populations from North America and the West Indies (Jamaica) (Parra et al. 1998). In these areas, Anglo-Saxon political, territorial, and cultural policies regarding matings between Africans and native Americans seem to have been more strict than in the Spanish/Portuguese colonies. This pattern is also evident in the vocabulary established very early (17th century) in the Spanish colonies for assigning the degree of racial admixture between Africans and native Americans (guineos, criollos, zambos, zambaigos, mulattos, etc.) (Triana y Antorveza 1997).

From the expected high proportion of non-Amerindian lineages in Afro-Colombians, only 35.8% belong to the macro-haplogroup *L*, a major African lineage in west and central African populations (between 76% and 100% sub-Saharan Africans belong to this group) (Chen et al. 1995, 2000). Instead, presumably, the overall contribution of the more rare African *L3* lineages in Colombia is 25.2% (Table 4, column Other-II), thus indicating that slaves brought to Colombia came from diverse African ethnic sources. Other West Eurasian markers falling into this group (Others-II) have not been analyzed; in spite of this, the low percentage of these lineages in the Mestizo population (2.2%), where they could be expected to exist, is possibly an indirect argument for ruling them out in the Afro-Colombian populations studied. Group *L3* haplotypes are found in 27% of African populations from Senegal (Chen et al. 1995), 23% of Mandenkas from West Africa, and 49% of Yorubas from the Guinean Gulf (Watson et al. 1997), all

alleged geographical sources for the slave trade to Colombia and other Caribbean countries (Friedemann 1993).

Moreover, inside of Colombia itself, the distribution of these lineages is heterogeneous. The population from Quibdó exhibits almost equally low frequencies of *L* and *L3* haplotypes (21.4% and 25%, respectively), while Providencia's lineages belong mostly to the *L* macro-haplogroup (52.5%), thus showing significant differences in the African maternal ancestry in the present-day Colombian Afro-descendant populations. This heterogeneity is also apparent in other South American countries like Brazil, where the distribution of African lineages is quite similar to that seen in Providencia and San Basilio (present study), with 46.3% and 36.6% of *L* and *L3* haplogroups, respectively (Bortolini et al. 1999). By contrast, Afro-Uruguayans exhibit 36.6% haplogroup *L* and the lowest frequency of *L3* haplotypes (12.2%) (Bravi et al. 1997) in the South American populations so far studied.

The history of the Afro-Colombian populations is reflected in the topology of the tree (Figure 3), not only with respect to the early geographical distribution of the slaves with different African ancestry in the colonies, but also in terms of their degree of admixture both with Amerindian and Mestizo women, contributing different proportions of African (21%–52% haplogroup *L*) and Amerindian (10%–21.2%) maternal lineages. While Providencia, San Basilio, and Nuquí cluster together, the population from Quibdó, although geographically close to Nuquí, sits on a branch apart from the previous ones, probably due to the weight given by admixture with Amerindians, but also to the more diverse ethnic origin of the African ancestors (suggested by a low haplogroup *L* frequency).

The hybrid (Mestizo) population resulting from the admixture process between native Americans, Europeans, and Africans is predominant in Colombia (Census 1993). From our results (present study), it is clear that an asymmetrical gene flow has taken place, with an overwhelming contribution of maternal Amerindian mtDNA lineages. Others have shown that the input of the Y chromosome is predominantly of European ancestry, and both studies show that the African contribution to these Mestizo populations, either maternal (present study) or paternal (Carvajal-Carmona et al. 2000), is marginal. The question remains whether, in the Mestizo populations from the African-impregnated regions (Atlantic and Pacific Coast Departments), the African gene flow (both maternal and paternal) is higher and has a particular parental origin or asymmetry.

The presence of a high incidence of Amerindian haplogroups in the Mestizo populations (present study; Carvajal-Carmona et al. 2000) is interesting, since this population identifies itself, phenotypically as well as culturally, with its Spanish ancestors. This means that, while the paternal European ancestry has dominated the social and cultural horizon for 500 years, maternal Amerindian ancestors have left their biological imprint even after many generations. In fact, we presume that Amerindian women must have accounted for much more than 78% admixture in our Spanish-descent population (present study), since lineages transmitted through a male member of the families have been lost and, thus, underscore the

actual admixture process in Colombia. This is manifest in the population from Antioquia, which despite showing a more northern European phenotype (fair skin) than the population from the inner region of Colombia (this study), shows 89% Amerindian mtDNA lineages (Carvajal-Carmona et al. 2000).

Of the remaining haplogroups (22%) (present study), 75% probably consist mostly of the main (Caucasian) lineages (*H, T, U, V, W*), and 10% (2.2% of the total) might possibly represent other West Eurasian lineages (*J* or *K*) (Torroni et al. 1994) or African haplotypes from the *L3* groups (Chen et al. 1995). In any case, no admixture with African individuals who possess haplogroup *L* mtDNAs, the most frequent lineage in Afro-Colombians (present study), is evident.

The Amerindian haplogroup contribution to the gene pool in Mexican (Green et al. 2000) and Colombian Mestizos (present study; Carvajal-Carmona et al. 2000) is very similar. Interesting, too, is the fact that in both countries the Mestizo populations show a pattern distribution of the *A/D* haplogroups typical of the Chibcha-speaking native Americans (Schurr et al. 1990; Torroni et al. 1993; Merriwether et al. 1994; Santos and Barrantes 1994; Santos et al. 1994; Keyeux et al. 2002), consistent with the dual population settlement in South America postulated in an accompanying paper (Keyeux et al. 2002). In contrast, Cuba's Spanish ancestors seem to have mixed mostly with African women, since 46% of mtDNAs bear African haplogroups, and only 4% show native American lineages (Torroni et al. 1995). Brazil shows all possible situations, depending on the regional history of colonization: states in the Amazonian region exhibit the highest frequency of Amerindian mtDNA haplogroups, while those in the East, principally settled by African slaves with few Portuguese landowners, have the highest African lineages, and those in the South, the "European" states, exhibit the highest frequency of Caucasian maternal lineages (Alves-Silva et al. 2000).

In conclusion, our survey of five Afro-Colombian and one Mestizo populations shows that it is possible to bring to the fore different patterns of past ethnic admixture between Africans, Amerindians, and Europeans in the distinct geographic regions of Colombia, even if dominant cultural traits, in many cases, tend to fade away the real origins of many of these manifestations.

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