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Mitochondrial DNA Analysis in Aruba: Strong Maternal Ancestry of Closely Related Amerindians and Implications for the Peopling of Northwestern Venezuela

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ABSTRACT.—**The continental origin of human mtDNA can be identified by its haplogroup determination through restriction fragment length polymorphism (RFLP) analysis. Hair root samples from 16 Aruban informed volunteers were analyzed by this method. Thirteen samples had mtDNAs of Amerindian origin and the remainder had their origin in sub-Saharan Africa; thus suggesting a substantial Amerindian maternal ancestry in Arubans, and helping explain the high incidence of health-related conditions common in Amerindian populations. Nine of the thirteen Amerindian mtDNAs belonged to haplogroup D, suggesting that despite intense Indian population movements through historical times, most of the mtDNAs shared a recent common origin. Our results, combined with the pre- and post-Columbian history of Aruba and northwestern Venezuela, lead us to hypothesize that the frequency of haplogroup D mtDNAs should be high in the Caquetío tribe of coastal Falcón in Venezuela and at least moderate in the Guajiro Indians of eastern Colombia. In addition, if the native Aruban Indians contributed substantially to the current mtDNA pool of Arubans despite the repeated historical deportation events that presumably removed the complete populations from the island, they must also have possessed haplogroup D mtDNAs in high frequency. Our results do not rule out an origin for the Amerinds of northwestern Venezuela in ancient Amazonian ceramic cultures.**

INTRODUCTION

High-resolution restriction endonuclease analysis of the mitochondrial DNAs (mtDNAs) of people from various continents, followed by phylogenetic reconstruction analysis, has identified many groups of evolutionarily related haplotypes (haplogroups) that are mostly continent-specific. Thus, haplogroups can be defined by sets of evolutionarily stable restriction motifs that are common to all of their haplotypes. Furthermore, the continental origin of mtDNA obtained from a mixed population can be determined by its haplogroup identity. Hence, restriction fragment length polymorphism (RFLP) analyses have been used, sometimes in combination with control region sequences, to estimate the contribution of peoples from different continents to the mtDNA pool of mixed populations (Rando et al., 1998; Alves-Silva et al., 2000; Green et al., 2000).

Because of its strict maternal inheritance, mtDNA studies only measure female input. Thus, the results of such studies in populations that have emanated from a colonization process are frequently not in concordance with those obtained from studies based on the Y chromosome, which displays strict paternal inheritance patterns. The results of combining data from mtDNA and Y chromosome studies typically reveal a predominance of asymmetric matings between men from colonizing populations and women from colonized ones (Pinto et al., 1996; Batista Dos Santos et al., 1999; Carvajal-Carmona et al., 2000).

Aruban preceramic archaeological sites belong to the Meso-Indian I period (Rouse and Cruxent, 1963). The oldest dated site is no more than 4500 years old. The Archaic settlers were apparently fisher-hunter-gatherers organized in bands of 10 to 15 people who sustained themselves primarily on seafood (Versteeg, 1991). Polished axe-heads made of greenish-gray volcanic

material associated with the Meso-Indian industry of Venezuela, and a sherd of a pottery disc found at the Canashitu preceramic site (500 BC-500 AD), suggest that the Archaics traded with the ceramic cultures of Venezuela (Dahn, 1970). Because of their proximity, we believe that the most likely candidates for such trade lived in the Paraguaná peninsula, located approximately 30 km south of Aruba.

The most recent skeletal remains of Aruban Archaics have been found in Malmok (600 AD-900 AD). These remains bear clear distinctions when compared with those of the ceramic culture that arrived approximately at 1000 AD. The Archaics possessed high, narrow, and long dolichocephalic skulls, while those from the ceramic culture were low, wide, and short (brachycephalic). Furthermore, all Archaic skulls had shovel-shaped incisors while such teeth are rare in skulls from the ceramic culture (Versteeg, 1993; Tacoma, 1991).

The ceramic culture of Aruba belonged to the Dabajuran subtradition of the Dabajuroid tradition. Because of similar elements in their pottery, the Dabajuroid and Tierroid traditions of western Venezuela are believed to share a common ancestral culture that may have existed somewhere between the Turbio, Upper Cojedes, and Upper Yaracuy rivers (Oliver, 1995). The Dabajuroid tradition expanded in the Maracaibo basin after 445 BC; it occupied a large region that included the San Cristóbal area of the Andes, south of Lake Maracaibo, and coastal areas from Lake Maracaibo to Cumaná as well as the islands of Aruba, Curaçao, and Bonaire. Four subtraditions developed from this expansion (Oliver, 1989), of which the Dabajuran subtradition took over coastal Falcón, including the Paraguaná peninsula and the islands of Aruba, Curaçao, and Bonaire (Fig. 1). The Indians occupying this region were known as Caquetíos by the Spaniards, and their language (Caquetío) belongs to the Arawakan family of languages (Oliver, 1989).

The dominant hypothesis is that the Arawakan family of languages originated approximately 5500 years ago in the junction between the Negro and Amazon rivers (Rouse, 1986). Proto-Arawakan speakers migrated north, upstream along the Negro River, through the Casiquiare Canal, and downstream along the Orinoco River. Splits occurred at the Meta-Orinoco and the Apure-Orinoco junctions. Some of these ancient people migrated further downstream along the Orinoco River, all the way to the coast, and to the Lesser and Greater Antilles, while others migrated west, upstream along the Meta or the Apure River. Those that migrated up the Meta River reached the Andes. Some of those migrating up the Apure River diverged up the Cojedes River and eventually reached the coast migrating downstream along the Ya-

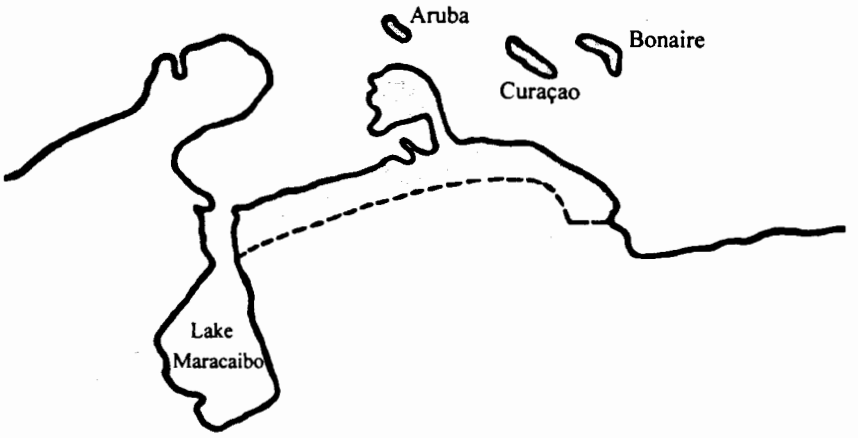

FIG. 1. Geographic distribution of the Dabajuran subtradition (shaded area).

racuy River. The latter may have given rise to the Macro-Dabajuroid tradition (Oliver, 1989).

The Dabajurans of Aruba developed an agrarian lifestyle. Thus, whereas preceramic sites have large shell middens, suggesting that the Archaics consumed many bivalves and oysters (Versteeg and Ruiz, 1995), ceramic sites have comparatively fewer shells (Versteeg and Rostain, 1997) among which those of the queen conch, *Strombus gigas*, predominate (Boerstra, 1983).

There is strong evidence that Aruban Dabajurans maintained close contacts with mainland cultures. For example, close to 700 chert flakes were found at the Tanki Flip ceramic site (Heidecker and Siegel, 1969), and since no chert source is found in Aruba, these cherts must have been imported. In addition, the avemorphic motifs painted in pottery found in Aruba seem to have originated in non-Dabajuroid cultures. These motifs likely came from the La Guajira peninsula of Colombia, where the oldest site containing artifacts with these motifs occurs in the Ranchería Valley. Apparently, only later did the avemorphic motifs made it to the Venezuelan Dabajuran site of Los Médanos (Oliver, 1989), suggesting that the Aruban Indians traded not only with their Dabajuran kins in Venezuela, but with other cultures as well.

It is estimated that some 450 to 600 Indians lived in Aruba at the time of the Spanish discovery (Dijkhoff, 1997) in 1499. Together with Curaçao and Bonaire, Aruba was declared an island without use in 1513, and two years later some 2000 Caquetío Indians from the three islands combined were transported to Hispaniola to work in mines (Hartog, 1961). These Indians presumably comprised the entire population of the islands (Versteeg and Ruiz, 1995), but 150 to 200 were returned to Aruba and Curaçao starting in 1526 to work on the exportation of brasilwood, kwihi, and divi-divi. The Indians returned to Aruba and Curaçao were mainly Caquetíos, but some Arawaks from other Caribbean islands were included in the group (Hartog, 1961).

Upon their return, the Spaniards noted the presence of Indians on the islands (Versteeg and Ruiz, 1995). Because of the complexity of the Aruba cave labyrinths, it is possible that the Indians were mostly natives who had escaped deportation, but they could have been recent migrants from the mainland. In addition, substantial mainland to Aruba migrations of Indian escapees occured from 1529 to 1556 during the development of the Venezuelan colony (Haviser, 1991).

Aruba was neglected by the Spaniards from 1533 until the Dutch conquest of 1636, when Spanish and Amerindian languages (especially Caquetío) were widely spoken. Upon the Dutch conquest the Spaniards fled, and all the Indians were deported to the mainland because they were regarded as sympathetic to the Spaniards (Hartog, 1961). However, in that same year the Dutch West Indian Company assigned Aruba the duty of breeding horses and cattle, and Indians were chosen for these endeavors because they had a good reputation as wild-horse hunters. Thus, Indian migration to Aruba was stimulated. Also, some Indians in war with Spaniards west of Maracaibo fled to Aruba (Hartog, 1961).

The importance of Aruba diminished after the 1648 Dutch Peace Treaty with Spain and the island was neglected again. In 1655, the Dutch West Indian Company recognized free Indians in Aruba as trade partners. These Indians were assigned a piece of land on which to maintain themselves through cultivation; they also cut and sold wood and exploited marine resources (Versteeg and Ruiz, 1995). In his description of the Aruban way of life during the second half of the 17th century, A.O. Exquemelin points out that the Indians spoke Spanish, were Catholic, and were visited frequently by Spanish priests from the mainland (Hartog, 1961). As an example of their strong links with the mainland, some 200 Indians agreed to leave Aruba in 1723 to raise the Venezuelan town of El Carrizal under the ecclesiastic jurisdiction of the city of Coro. These Indians were described by the Spaniards as Caquetíos (Fortique, 1989).

The migration of non-Indians to Aruba was scant. From 1640 to 1754, Aruba was to be colonized only with the permission of

the Dutch West Indian Company director. A.O. Exquemelin described the Lieutenant Governor of Aruba as the only white man living on the island, and he was presumably the only person who owned black slaves. African slaves are mentioned for the first time in the history of Aruba in 1750, when they revolted and killed four residents (Alofs, 1996). Aruban Indians had their own slaves. They raided or bought captive mainland Indian minors of both sexes called red slaves (Hartog, 1961).

The migration of non-Indians increased substantially under a 1785 land tax that made it easier to get a license to settle (Hartog, 1961). Settlers from various European countries moved to Aruba with their black slaves, but these migrations were stymied by a famine in 1800 that occurred as a consequence of drought and war. Aruba shifted between English and Dutch hands several times from 1803 to 1816, when the English recapitulated.

Migration and admixture increased dramatically during the 19th and 20th centuries. The Caquetío language disappeared from Aruba during the first half of the 19th century, and oral tradition holds that the last full-blooded Aruban Indian died around 1862 (Hartog, 1961). However, the red slave trade also increased during the 19th century until the abolition of slavery in 1863. Baptism records show that red slaves during this period were mainly Guajiro Indians (Nooyen, 1965). The 1816 census of Aruba reports 1732 inhabitants classified into 584 colored free people (probably mixed), 564 full-blooded Indians, 133 colored (red) slaves, 240 blacks, and 211 whites. Women in these categories were 313, 307, 48, 137, and 101, respectively (Hartog, 1961).

This historic data shows that Arubans are a mixed population, much like those of many Latin American nations. Rhesus and ABO blood group findings suggested that a trihybrid admixture pattern including Amerindian genes explains better the observed Aruban blood-group distributions than the bihybrid Afro-European model of the Caribbean. Rhesus-negative (*cde*) prevalences were never intermediate between African (9 %) and European (16 %) values but were consistently lower (i.e. 7 % of 2536 in 1957) (De Ruyter, 1957) and even 5 % of 474 in 1968 and 8 % of 1027 in 2000 (O.R. Wever, unpubl. data). Blood-group O prevalences were never intermediate between African (47 %) and European (44 %) values but were instead consistently higher (52 % of 474 in 1968, 54 % of 1577 in 1996 and 49 % of 1027 in 2000: O.R. Wever, unpubl. data). Hence, Aruban prevalences for Rhesus-negative (*cde*) and for blood-group O are intermediate between African values and Amerindian values (*cde* 0 %; blood-group O 100 %).

Although the 1816 census scores 33 % of the Aruba inhabitants as full-blooded Indians, after 1820 only whites, blacks and colored people are mentioned as ethnic components (Hartog, 1961; Alofs, 1996; Dijkhoff, 1997). We hope to show that this poorly defined category of colored people not only covered Afro-European mulattos but mostly included mestizos and Amerindians. The persistence of a substantial proportion of Amerindian genetic ancestry in Arubans may explain the current high prevalences of health-related conditions that are common in Amerindians, such as diabetes (Muneta et al., 1993; Ramachandran, 1994; Lee et al., 1995), pterygium (Hilgers, 1959), and lactase deficiency (Caskey et al., 1977; Newcomer et al., 1977).

We provide further evidence of a strong Amerindian ancestry among Arubans, showing that most Arubans have mtDNAs of Amerindian origin. We also show that the Amerindian mtDNAs have low genetic diversity and that their haplogroup distribution is highly structured, suggesting that the Amerindian mtDNA pool of Aruba is homogeneous despite the intense migration events that occurred during historical times. Finally, we compare our mtDNA results with those of various South American tribes, as well as with linguistic and archaeological evidence, in an attempt to advance our understanding on the pre-Columbian events that gave rise to the people found by the Spaniards in Aruba.

MATERIALS AND METHODS

Samples.—Hair follicle samples were obtained with informed consent from 16 un-

related Aruban patients at the medical office of Dr. Oswald R. Wever in Oranjestad, Aruba. The mothers and maternal grandmothers of 15 participants were born in Aruba, while those of the other participant were born in Cuba. The mothers of the participants were all born between 1903 and 1940 with a mean at 1923. It was not possible to date with precision the birthdays of the maternal grandmothers.

Experimental strategy.—The haplogroups found here were described initially as monomorphic at the 10394 *Dde*I and 10397 *Alu*I sites (Chen et al., 1995; Wallace, 1995). This fact combined with the proximity of these two restriction sites, which allows analysis of both sites produced from a single amplicon, makes their testing a convenient start in the haplogroup identification of mtDNAs obtained from a mixed population. By identifying the 10394 *Dde*I/10397 *Alu*I motif of an unknown mtDNA, the number of candidate haplogroups is quickly reduced. The major haplogroups classified by their 10394 *Dde*I/10397 *Alu*I motifs and their specific markers and geographic origins are presented in Table 1.

We grouped our samples by their 10394 *Dde*I/10397 *Alu*I motif and tested all the samples within each group for particular haplogroup-specific markers. Because some haplogroup I mtDNAs possess the haplogroup L3d-specific marker, the identification of mtDNAs as belonging to haplogroup L3d was confirmed by showing that they did not possess the haplogroup I-specific marker. Since one Italian haplogroup U mtDNA possessing the haplogroup A-specific marker has been found (Torroni et al., 1997), the identification of a mtDNA as belonging to haplogroup A was confirmed through the sequence determination of its HV-I region.

Experimental procedure.—Total nucleic acids were released from the hair follicles and specific mtDNA fragments were amplified and subjected to restriction analyses. Procedures were performed as in Martínez-Cruzado et al. (2001) with the following exceptions: 1. The amplification reaction mixtures contained 7 uL of sample DNA, bovine serum albumin at 0.3 mg/mL, and 2 units of *Taq* DNA polymerase. 2. Mixtures were heated at 95°C for 2.5 min before being subjected to 32 cycles of 30 s at 94 °C, 1 min at 54°C, and 70 s at 72°C. Three restriction sites not tested by Martínez-Cruzado et al. (2001) were tested in this study; these define the sub-Saharan African haplogroups L3d (–8616 *Dpn*II) and L3e (+2349 *Dpn*II) (Rando et al., 1998), which are equivalent to haplogroups L3b and L3a of Chen et al. (2000), respectively, and the Caucasian haplogroup I (–4529 *Hae*II). The 8616 and 2349 *Dpn*II sites were tested from a 140 bp fragment amplified using primers L8558 (5′ TCTGTTCGCTTCATTCATTG 3′) and H8657 (5′ TGATTAGTCATTGTTGGGTG 3′) and from a 187 bp fragment amplified using primers L2272 (5′ TCCTCACACCCAATTGGAC 3′) and H2420 (5′ ATGCCTGTGTTGGGTTGAC 3′), respectively. The 4529 *Hae*II site was tested from a 199 bp fragment amplified using primers L4462 (5′ AAAATGTTGGTTATACCCTTC 3′) and H4620 (5′ ATGGCAGCTTCTGTGGAAC 3′).

To confirm the haplogroup of the mtDNA identified as belonging to haplogroup A, the sequence of its HV-I region was determined. An 879 bp fragment containing the HV-I region was amplified using the primers L15766 (5′ ATTCTAACCTGAATCGGAGG 3′) and H34 (5′ ACCAAATGCATGGAGAGCTCC 3′). The amplicon was purified using the High Pure PCR Product Purification Kit (Roche Molecular Biochemicals) as per manufacturer's instructions. Six hundred nanograms of the product and 10 uL of each sequencing primer (1 μM concentration) were sent to the University of Medicine and Dentistry of New Jersey, New Jersey Medical School Molecular Resource Facility, for automated sequencing. An Applied Biosystems (ABI) model 3100 capillary sequencer was used after cycle sequencing with Dye Terminator mix version 2.0. The primers used for sequencing were L15854 (5′ CCTAATCCTAATACCAACTATC 3′) and H16526 (5′ GGGAACGTGTGGGCTATTTAGG 3′). Diversity for the Amerindian mtDNAs was calculated using the method of Tajima (1989), $h = [1 - \sum x_i^2]n/(n-1)$, where x_i is the frequency of each haplogroup and n is the population size.

TABLE 1. Major RFLP-defined haplogroups, their origins, 10394 DdeI/10397 AluI motifs, and haplogroup-specific markers.

10394 *Dde*I/10397 *Alu*I motif	Haplogroup	Origin[1]	Haplogroup-specific marker[2]	10394 *Dde*I/ 10397 *Alu*I motif	Haplogroup	Origin	Haplogroup-specific marker
(−/−)	A	Native American	+663 *Hae*III	(+/+)	C	Native American	+13262 *Alu*I
(−/−)	B	Native American	9 bp deletion Region V	(+/+)	D	Native American	−5176 *Alu*I
(−/−)	F	Asian	−12406 *Hpa*I	(+/+)	E	Asian	−7598 *Hha*I
(−/−)	H	Caucasian	−7025 *Alu*I	(+/+)	G	Asian	+4830 *Hae*II
(−/−)	K[3]	Caucasian	+12308 *Hin*fI/ −9052 *Hae*II	(+/−)	I	Caucasian	−4529 *Hae*II
(−/−)	T	Caucasian	+4216 *Nla*III	(+/−)	J	Caucasian	+4216 *Nla*III
(−/−)	U	Caucasian + African	+12308 *Hin*fI/ +9052 *Hae*II	(+/−)	K	Caucasian	+12308 *Hin*fI/−9052 *Hae*II
(−/−)	V	Caucasian	−4577 *Nla*III	(+/−)	L1 & L2	African	+3592 *Hpa*I
(−/−)	W	Caucasian	+8249 *Ava*II	(+/−)	L3b	African	+10084 *Taq*I
(−/−)	X	Caucasian + Native American	+14465 *Acc*I	(+/−)	L3d	African	−8616 *Dpn*II
				(+/−)	L3e	African	+2349 *Dpn*II

[1]The Native American haplogroups have their origin in Asia.
[2]Numbers refer to the nucleotide position according to the Cambridge Reference Sequence (Anderson et al., 1981).
[3]Haplogroup K is the only one with two 10394 *Dde*I/*Alu*I motifs in moderate frequencies.

RESULTS

A hierarchical strategy (Fig. 2) was used to identify the mtDNA haplogroups of the 16 samples. Each sample was first tested for the 10394 *Dde*I/10397 *Alu*I motif. Two samples lacked both restriction sites and each was tested for the markers specific for haplogroups A and B; one sample had the 663 *Hae*III site but not the 9 bp deletion at region V, and thus belonged to haplogroup A, while the other had the inverse profile and thus belonged to haplogroup B. The identity of the haplogroup A mtDNA was confirmed with the DNA sequence of its HV-I region. The sequence presented the transitions at positions 16111, 16223, 16290, and 16319 that characterize virtually all haplogroup A mtDNAs of Native American origin (Ward et al., 1991; Horai et al., 1993; Torroni et al., 1993; Santos et al., 1994; Starikovskaya et al., 1998).

Three samples had the 10394 *Dde*I site but lacked the 10397 *Alu*I site. These samples were tested first for the 3592 *Hpa*I site that characterizes haplogroups L1 and L2 (which represent over 70 % of all sub-Saharan Africans) but the three samples lacked the site. They were then tested for the 2349 *Dpn*II site that is specific for haplogroup L3e. Two of the samples, including the one of Cuban ancestry, had the site and thus belonged to haplogroup L3e. The three samples were also tested for the −8616 *Dpn*II motif that characterizes haplogroup L3d. Only the sample lacking the 2349 *Dpn*II site showed the −8616 *Dpn*II motif and thus belonged to haplogroup L3d. Because several of the mtDNAs belonging to the Caucasian haplogroup I that have been analyzed for their entire coding sequences show a transversion at position 8616 that eliminates the 8616 *Dpn*II site (Finnilä et al., 2001; Herrnstadt et al., 2002), it was necessary to confirm the identity of the mtDNA belonging to haplogroup L3d. This was done by testing and showing that it lacked the −4529 *Hae*II haplogroup I-specific marker.

Eleven samples possessed the 10394 *Dde*I and the 10397 *Alu*I sites and all were tested for the 13262 *Alu*I site that identifies haplogroup C and for the lack of the 5176 *Alu*I site specific for haplogroup D. Nine samples lacked both *Alu*I sites and thus belonged to haplogroup D; the other two possessed both sites and thus belonged to haplogroup C. The test for the 5176 *Alu*I site that identified eight of the haplogroup D samples is shown in Fig. 3. All results are summarized in Table 2.

DISCUSSION

The abundant literature available on the human mtDNA variation and continent-specific polymorphisms has allowed us to use a hierarchical strategy to quickly identify the haplogroup and the continental origin of each of the mtDNA samples. This exempted us from the need to perform tests on all samples for the presence of Cauca-

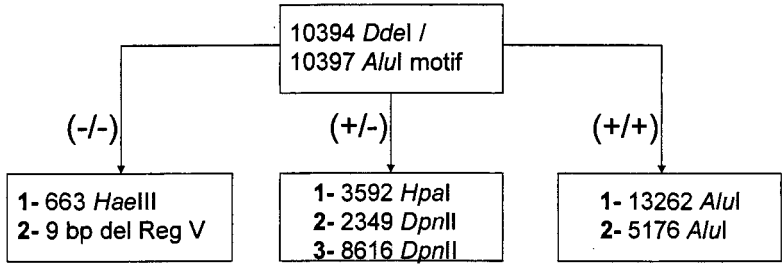

FIG. 2. Hierarchical strategy used to identify the haplogroups of the 16 samples. The 10394 *Dde*I/10397 *Alu*I motif was tested first. Samples (−/−) for the motif were tested for the markers specific for haplogroups A (+663 *Hae*III) and B (Region V 9 bp deletion). Those (+/−) for the motif were tested for the 3592 *Hpa*I site shared by haplogroups L1 and L2, and for the markers specific for haplogroups L3e (+2349 *Dpn*II) and L3d (−8616 *Dpn*II). The samples (+/+) for the motif were tested for the markers specific for haplogroups C (+13262 *Alu*I) and D (−5176 *Alu*I).

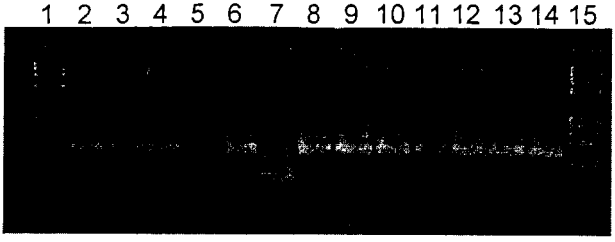

FIG. 3. Test for the 5176 *AluI* site. Fragments 149 bp long were amplified using primers L5121 and H5229 (Martínez-Cruzado et al., 2001), subjected to *AluI* digestion and fractionated on 3% agarose gels. Lanes 1 and 15: phiX174-*HaeIII* molecular weight marker (New England Biolabs). Lanes 2 to 4: 149 bp control fragments not subjected to *AluI* digestion. Lane 5: blank. Lanes 6 to 14: experimental fragments. Only the sample in lane 7 was cut by *AluI* at the 5176 site, producing two undistinguishable fragments of approximately equal size. All other samples lacked the *AluI* site and thus belong to haplogroup D.

TABLE 2. Haplogroup distribution

Haplogroup	Ancestry	Frequency
A	Native American	1 (6.3)[1]
B	Native American	1 (6.3)
C	Native American	2 (12.5)
D	Native American	9 (56.3)
L3d	sub-Saharan African	1 (6.3)
L3e	sub-Saharan African	2 (12.5)

[1]Numbers in parenthesis represent percent frequencies.

sian haplogroups HV, I, JT, and W, the sub-Saharan African haplogroup L3b, the Caucasian-Native American haplogroup X, and the Caucasian-African haplogroup U.

It could be argued that because of the relatively high mutation rate of the coding region of the human mtDNA, estimated at 2.2 to 2.9 % per million years (Torroni et al., 1994a), all markers used to define any haplogroup could arise by an independent mutation in mtDNAs belonging to other haplogroups, thus producing the misidentification of the mtDNAs. However, such events are very rare. In combination, Finnilä et al. (2001) and Herrnstadt et al. (2002) sequenced the complete coding region of 627 Caucasian, 69 Native American or Asian, and 56 African mtDNAs. In addition, 418 mtDNAs of Native American origin, 397 of Caucasian, and 214 of African origin have been subjected to high-resolution restriction endonuclease analysis (Torroni et al., 1993, 1994b, 1994c, 1996, 1997; Chen et al., 1995, 2000; Huoponen et al., 1997). We searched these mtDNAs for those possessing two of the haplogroup-specific markers reported here. MtDNAs with haplogroup-specific markers of two haplogroups which shared the same 10394 *DdeI*/10397 *AluI* motif were found in only three cases. Specifically, 9 haplogroup I mtDNAs were found to possess the −8616 *DpnII* motif (Torroni et al., 1994c, 1997; Herrnstadt et al., 2002) that defines haplogroup L3d. In addition, 4 haplogroup A mtDNAs had the 9 bp deletion (Torroni et al., 1993, 1994b) that defines haplogroup B. Finally, 1 haplogroup U mtDNA contained the +663 *HaeIII* haplogroup A-specific marker (Torroni et al., 1997). Thus, our results were confirmed by testing our haplogroup L3d mtDNA for the haplogroup I-specific marker, testing the two mtDNAs with a (−/−) motif for the markers specific for haplogroups A and B, and sequencing the HV-I region of the haplogroup A mtDNA.

From the interviews, we learned that 15 of the 16 mtDNAs studied were present in Aruba by the first decades of the 20th century (when the mothers of the participants were born) and that 13 (86.7 %) of these were of Amerindian origin, suggesting a substantial Amerindian maternal ancestry in Arubans. This is not surprising because the 1816 census category of colored free people probably consisted of people of mixed ancestry. Typically, admixtures in the New World have consisted of men from colonizing and women from colonized populations, thus producing mixed populations with Amerindian mtDNAs in very

high frequencies (Green et al., 2000; Carvajal-Carmona et al., 2000; Alves-Silva et al., 2000; Martínez-Cruzado et al., 2001). Thus, it is reasonable to speculate that the number of women with Amerindian mtDNAs in 1816 was equal or higher than the sum of the colored free people, the red slaves, and the full-blooded Indians. This adds to 668 out of 906 (73.7 %) women. The remainder was composed of 137 (15.1 %) "blacks" and 101 (11.1 %) "whites".

The absence of mtDNAs of Caucasian origin in our samples probably stems from sampling error. However, it is noteworthy that 90 of the 101 women counted in the 1816 census as whites were born in Aruba, leaving open the possibility that many of them were mixed. For comparison, while more than 80 % of the population of Puerto Rico was classified as "white" in the 2000 census, more than half and over a quarter of this same population has mtDNAs of Amerindian and sub-Saharan African origin, respectively (Martínez-Cruzado et al., 2001, and unpubl. data). Thus, a high Amerindian or sub-Saharan African mtDNA frequency among those women classified as white in the 1816 Aruba census cannot be ruled out.

Although our results show only a small window of the universe that existed in Aruba at the beginning of the 20th century, the observed frequency is very similar to what may have been expected from an equivalent study done in 1816. Hence, our results suggest that most women who migrated to Aruba during the 19th century had Amerindian maternal ancestry. This is supported by experiments showing that the Rhesus-negative and blood-group O prevalences in Aruba are consistently out of the ranges imposed by African and European prevalences, and more towards those imposed by Amerindian prevalences (O.R. Wever, unpubl. data). Thus, the mtDNA results presented here should be substantiated through a study using a sample set better representing the Aruba population.

The haplogroup distribution of the Amerindian mtDNAs of Aruba (Table 2) is highly structured, and thus their genetic diversity (calculated at 0.5256) is low. Highly structured distributions and low genetic diversities are common in tribes of the New World, suggesting that many of them were founded by small groups of closely related women. However, only 11 of the 55 New World tribes listed by Merriwether et al. (1995) have a haplogroup distribution (as measured by the frequency of the most common haplogroup) as highly structured as that of the Aruba population. Furthermore, only 9 of the 25 Colombian tribes studied by Keyeux et al. (2002) were calculated to have genetic diversities lower than that displayed in the Aruba study. These observations suggest that despite the intense movement of Indians in and out of Aruba during historical times, most Aruba Amerindian mtDNAs originate from one or from a few sibling tribes.

The most likely candidate is the Caquetío tribe. During the first years of colonization, the native Indians of Aruba were described by the Spaniards as Caquetíos (Hartog, 1961). In addition, the Caquetíos in the mainland were the tribe geographically closest to Aruba, and archaeological evidence points towards close ties between both groups during pre-Columbian times (Oliver, 1989). Thus, the most likely scenario is that most of the Aruban Indians that returned to Aruba from Hispaniola were Caquetíos, as were also most of those that were found in Aruba at this time. The latter were likely either deportation escapees or mainland migrants. This scenario is supported by the fact that the Indians that migrated from Aruba to the mainland in 1723 to found the town of El Carrizal were described as Caquetíos (Fortique, 1989).

Some of the mtDNAs studied could have a Guajiro Indian origin because many Guajiros were sent as slaves to Aruba during the 19th century (Nooyen, 1965). We may hypothesize that haplogroup D is predominant among Caquetíos and at least very common among Guajiros, suggesting that both tribes are genetically closely related. We expect that similar studies in Caquetíos and Guajiros, and in ancient remains from these two tribes and Aruba will reveal high haplogroup D frequencies.

Similar haplogroup distributions do not necessarily imply strong genetic relationships. Strictly speaking, the finding that

two Amerindians share a haplogroup D mtDNA only means that their most recent common maternal ancestor cannot be older than the age of the haplogroup D woman who crossed the Bering Strait some 29 000 years ago (Forster et al., 1996; Silva et al., 2002). However, we could expect a closer relationship between these tribes due to their geographic proximity and because, although distinct, the Caquetío and Guajiran languages belong to the Arawakan family (Rouse, 1986). It is noteworthy that among South American Indians, genetic variation is more strongly correlated with linguistic than with geographic variation (Fagundes et al., 2002). Median network analyses (Bandelt et al., 1995, 1999) of mitochondrial HV-I and HV-II region sequences are necessary to ascertain a common origin for the tribes.

Because of their language kinship, it could be postulated that all Arawakan speaking tribes north of the Amazon River share a recent genetic ancestry, and that repeated founder events stemming from a common ancestor population generated derived populations with the markedly different mtDNA haplogroup distributions found in some Arawakan speaking tribes. For example, haplogroup D is absent in Puerto Rico, where haplogroups A and C constitute over 91 % of the Amerindian mtDNAs (Martínez-Cruzado et al., 2001). Presumably, these mtDNAs derive mainly from the extinct Taínos, whose Taíno language belonged to the Arawakan family of languages. The Amazonian origin of most of the haplogroup C mtDNAs in Puerto Rico is supported by their –7013 *Rsa*I motif (Martínez-Cruzado, unpubl. data), which had been found only in Amazonian tribes (Torroni et al., 1993a). Although 69.2 % of the Amerindian mtDNAs of Aruba belong to haplogroup D, the common origin of both populations in the ancient proto-Arawakan populations of the Amazon is supported by the high frequencies of haplogroups C (45.5 %) and D (34.8 %) in tribes north of the Amazon River, such as the Makiritare, the Yanomama, the Macushi, the Marubo, and the Wapishana (Torroni et al., 1993), even when not all of these tribes speak Arawakan languages. Under this scenario, the Caquetíos would be located close to the northwestern edge of this expansion, as the Wayuu tribe in the Guajira peninsula of Colombia displays clear genetic differences. The latter tribe has haplogroups A and B at a 53 % combined frequency and lacks haplogroup D (Mesa et al., 2000; Keyeux et al., 2002).

It is intriguing that almost 70 % of the Amerindian mtDNAs in Aruba belong to the same haplogroup, despite strong archaeological evidence indicating that the ceramic people who came to Aruba ca. 1000 AD were physically distinct to the Archaics (Versteeg, 1991, 1993; Tacoma, 1991). A genetically homogeneous population would suggest the absence of any admixture between the ceramic and preceramic peoples, and thus the complete prehistoric replacement of one population by another. Detailed analyses of haplogroup D mtDNAs from Caquetíos might reveal two or more groups within haplogroup D that could represent independent populations whose admixture gave rise to the Caquetíos. In combination with mtDNA studies on ancient remains, these studies should lead us towards a better understanding of the peopling and culture development of the region.

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LITERATURE CITED

Alofs, L. 1996. Slaven zonder plantage. Slavernij en emancipatie op Aruba, 1750-1863. Charuba, Edicion Educativo no.2, Aruba.

Alves-Silva, J. et al. 2000. The ancestry of Brazilian mtDNA lineages. Am. J. Hum. Genet. 67: 444-461.

Anderson, S. et al. 1981. Sequence and organization of the human mitochondrial genome. Nature 290: 457-465.

Bandelt, H.J., P. Forster, B.C. Sykes, and M.B. Richards. 1995. Mitochondrial portraits of human populations using median networks. Genetics 141: 743-753.

Bandelt, H.J., P. Forster, and A. Rohl. 1999. Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 16: 37-48.

Batista Dos Santos, S.E., J.D. Rodrigues, A.K.C. Ribeiro-Dos-Santos, and M.A. Zago. 1999. Differential contribution of indigenous men and women to the formation of an urban population in the Amazon region as revealed by mtDNA and Y-DNA. Am. J. Phys. Anthropol. 109: 175-180.

Boerstra, E.H.J. 1983. Some of the soil marks in the Tanki Flip excavation, Aruba, N.A.: the ditches. Proceedings of the Ninth International Congress for the Study of the Pre-Columbian Cultures of the Lesser Antilles: 173-183.

Carvajal-Carmona, L.G. et al. 2000. Strong Amerind/white sex bias and a possible Sephardic contribution among the founders of a population in northwest Colombia. Am. J. Hum. Genet. 67: 1287-1295.

Caskey, D.A. et al. 1977. Effects of age on lactose malabsorption in Oklahoma Native Americans as determined by breath H_2 analysis. Digest. Diseases 22: 113.

Chen, Y.-S., A. Torroni, L. Excoffier, A.S. Santachiara-Benerecetti, and D.C. Wallace. 1995. Analysis of mtDNA variation in African populations reveals the most ancient of all human continent-specific haplogroups. Am. J. Hum. Genet. 57: 133-149.

Chen, Y.-S. et al. 2000. mtDNA variation in the South African Kung and Khwe - and their genetic relationship to other African populations. Am. J. Hum. Genet. 66: 1362-1383.

Dahn, J. 1970. Ecological and archaeological presentation of the Canashitu site in Aruba, Netherland Antilles. Rapport 14-9-1970.

De Ruyter, J.A.M. 1957. Analysis of some results in hospital obstetrics in Aruba. Ph.D. thesis, Leiden University, Holland.

Dijkhoff, R.A.C.F. 1997. Tanki Flip / Henríquez-I: an early Urumaco site in Aruba. Master thesis, Leiden University, Holland.

Fagundes, N.J.R., S.L. Bonatto, S.M. Callegari-Jacques, and F.M. Salzano. 2002. Genetic, geographic, and linguistic variation among South American Indians: possible sex influence. Am. J. Phys. Anthropol. 117: 68-78.

Finnilä, S., M.S. Lehtonen, and K. Majamaa. 2001. Phylogenetic network for European mtDNA. Am. J. Hum. Genet. 68: 1475-1484.

Forster, P., R. Harding, A. Torroni, and H.J. Bandelt. 1996. Origin and evolution of Native American mtDNA variation: a reappraisal. Am. J. Hum. Genet. 59: 935-945.

Fortique, J.R. 1989. Noticias sobre el Carrizal, pueblo de indios caquetíos. Boletín de la Academia Nacional de la Historia, 72: 57-80, Caracas, Venezuela.

Green, L.D., J.N. Deer, and A. Knight. 2000. mtDNA affinities of the peoples of North-Central Mexico. Am. J. Hum. Genet. 66: 989-998.

Hartog, J. 1961. Aruba past and present, from the time of the Indians until today. De Wit, Aruba, 451 pp.

Haviser, J.B. 1991. The first Bonaireans. Reports of the Archaeological-Anthropological Institute of the Netherland Antilles no.10, Curaçao.

Heidecker, L. and M.I. Siegel. 1969. Preliminary report on the excavation Henríquez-I Site, Tanki Flip, Aruba, Netherland Antilles. Fla. Anthropol. 22: 12-26.

Herrnstadt, C. et al. 2002. Reduced-median-network analysis of complete mitochondrial DNA coding-region sequences for the major African, Asian, and European haplogroups. Am. J. Hum. Genet. 70: 1152-1171.

Hilgers, J.H.C. 1959. Pterygium on the island of Aruba. Ph.D. thesis, Amsterdam.

Horai, S. et al. 1993. Peopling of the Americas, founded by four major lineages of mitochondrial DNA. Mol. Biol. Evol. 10: 23-47.

Huoponen, K. et al. 1997. Mitochondrial DNA and Y chromosome-specific polymorphisms in the Seminole tribe of Florida. Eur. J. Hum. Genet. 5: 25-34.

Keyeux, G., C. Rodas, N. Gelvez, and D. Carter. 2002. Possible migration routes into South America deduced from mitochondrial DNA studies in Colombian Amerindian populations. Hum. Biol. 74: 211-233.

Lee, E.T. et al. 1995. Diabetes and impared glucose tolerance in three American Indian populations aged 45-74 years: the strong heart study. Diabetes Care 18: 599-610.

Martínez-Cruzado, J.C. et al. 2001. Mitochondrial DNA analysis reveals substantial Native American ancestry in Puerto Rico. Hum. Biol. 73: 491-511.

Merriwether, D.A., F. Rothhammer, and R.E. Ferrell. 1995. Distribution of the four founding lineage haplotypes in Native Americans suggests a single wave of migration for the New World. Am. J. Phys. Anthropol. 98: 411-430.

Mesa, N.R. et al. 2000. Autosomal, mtDNA, and Y-chromosome diversity in Amerinds: pre- and post-Columbian patterns of gene flow in South America. Am. J. Hum. Genet. 67: 1277-1286.

Muneta, B., J. Newman, S. Wetterall, and J. Stevenson. 1993. Diabetes and associated risk factors among native Americans. Diabetes Care 16: 1619-1620.

Newcomer, A.D., P.J. Thomas, D.B. McGill, and A.F. Hofmann. 1977. Lactase deficiency: a common ge-

netic trait of the American Indian. Gastroenterology 72: 234.

Nooyen, R.H. 1965. Millefiori di Aruba. Stichting Aruba Nostra, 115 pp.

Oliver, J.R. 1989. The archaeological, linguistic and ethno-historical evidence for the expansion of Arawakan into northwestern Venezuela and northeastern Colombia. Ph.D. thesis, University of Illinois, Urbana-Champaign.

Oliver, J.R. 1995. The archaeology of Maticora, western Venezuela: a prehistoric longhouse structure in site Fal-7. Proceedings of the Fifteenth International Congress for Caribbean Archaeology: 1-52.

Pinto, F., A.M. González, M. Hernández, J.M. Larruga, and V.M. Cabrera. 1996. Genetic relationship between the Canary Islanders and their African and Spanish ancestors inferred from mitochondrial DNA sequences. Ann. Hum. Genet. 60: 321-330.

Ramachandran, A. 1994. Increasing prevalence of NIDDM. A public health problem of developing nations. Dialogue 11-15.

Rando, J.C. et al. 1998. Mitochondrial DNA analysis of northwest African populations reveals genetic exchanges with European, Near-Eastern, and sub-Saharan populations. Ann. Hum. Genet. 62: 531-550.

Rouse, I. 1986. Migrations in prehistory: inferring population movement from cultural remains. Yale University Press. New Haven, Connecticut, 202 pp.

Rouse, I. and J.M. Cruxent. 1963. Venezuelan archaeology. Yale University Press, New Haven, Connecticut, 179 pp.

Santos, M., R.H. Ward, and R. Barrantes. 1994. mtDNA variation in the Chibcha Amerindian Huétar from Costa Rica. Hum. Biol. 66: 963-977.

Silva, W.A. et al. 2002. Mitochondrial genome diversity of Native Americans supports a single early entry of founder populations into America. Am. J. Hum. Genet. 71: 187-192.

Starikovskaya, Y.B., R.I. Sukernik, T.G. Schurr, A.M. Kogelnik, and D.C. Wallace. 1998. mtDNA diversity in Chukchi and Siberian Eskimos: implications for the genetic history of ancient Beringia and the peopling of the New World. Am. J. Hum. Genet. 63: 1473-1491.

Tacoma, J. 1991. Precolumbian human skeletal remains from Curaçao, Aruba and Bonaire. Proceedings of the Thirteenth International Congress for Caribbean Archaeology, Part II: 802-813.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123: 585-595.

Torroni A. et al. 1993. Asian affinities and continental radiation of the four founding Native American mtDNAs. Am. J. Hum. Genet. 53: 563-590.

Torroni A., J.V. Neel, R. Barrantes, T.G. Schurr, and D.C. Wallace. 1994a. Mitochondrial DNA "clock" for the Amerinds and its implications for timing their entry into North America. Proc. Natl. Acad. Sci. USA 91: 1158-1162.

Torroni A. et al. 1994b. mtDNA and Y-chromosome polymorphisms in four Native American populations from southern Mexico. Am. J. Hum. Genet. 54: 303-318.

Torroni A. et al. 1994c. mtDNA and the origin of Caucasians: identification of ancient Caucasian-specific haplogroups, one of which is prone to a recurrent somatic duplication in the D-loop region. Am. J. Hum. Genet. 55: 760-776.

Torroni A. et al. 1996. Classification of European mtDNAs from an analysis of three European populations. Genetics 144: 1835-1850.

Torroni A. et al. 1997. Haplotype and phylogenetic analyses suggest that one European-specific mtDNA background plays a role in the expression of Leber Hereditary Optic Neuropathy by increasing the penetrance of the primary mutations 11778 and 14484. Am. J. Hum. Genet. 60: 1107-1121.

Versteeg, A.H. 1991. The Indians of Aruba. Publications of the Archaeological Museum of Aruba no.4, Aruba.

Versteeg, A.H. 1993. Malmok revisited: geometric layout and patterns in a preceramic cemetery. Proceedings of the Fourteenth International Congress for Caribbean Archaeology: 115-124.

Versteeg, A.H. and S. Rostain. 1997. The archaeology of Aruba: the Tanki Flip site. Publication of the Archaeological Museum of Aruba no.8, Aruba, 519 pp.

Versteeg, A.H. and A.C. Ruiz. 1995. Reconstructing brasilwood island: the archaeology and landscape of Indian Aruba. Publications of the Archaeological Museum of Aruba no.6, Aruba.

Wallace, D.C. 1995. Mitochondrial DNA variation in human evolution, degenerative disease and aging. Am. J. Hum. Genet. 57: 201-223.

Ward, R.H., B.L. Frazier, K. Dew-Jager, and S. Pääbo. 1991. Extensive mitochondrial diversity within a single Amerindian tribe. Proc. Natl. Acad. Sci. USA 88: 8720-8724.