THE ORIGINS OF THE AFRICAN ANCESTRY IN THE PUERTO RICAN POPULATION ACCORDING TO RESTRICTION ANALYSIS OF THE MITOCHONDRIAL DNA

by

Jorge Viera-Vera

A thesis submitted in partial
fulfillment of the requirements for the
degree of

MASTER OF SCIENCE

in

Biology

UNIVERSITY OF PUERTO RICO
MAYAGÜEZ CAMPUS
2006

Approved by:

_____	_____
Rafael Montalvo-Rodríguez, Ph.D. Member, Graduate Committee	Date
_____	_____
Carlos Ríos-Velázquez, Ph.D. Member, Graduate Committee	Date
_____	_____
Juan C. Martínez-Cruzado, Ph.D. President, Graduate Committee	Date
_____	_____
Elsie I. Parés-Matos, Ph.D. Representative of Graduate Studies	Date
_____	_____
Lucy Williams, Ph.D. Chairperson of the Department	Date

Abstract

The matrilineal genetic composition of 79 Puerto Rican samples with proven Sub-Saharan African ancestry was examined by restriction fragment length polymorphism (RFLP) analysis. The resulting data were combined with published RFLP and control region sequence information of 1931 samples with Sub-Saharan African ancestry from Puerto Rico, Dominican Republic, Brazil, and various African populations to explore the extent to which different regions in Africa have contributed to the present day composition of Puerto Rican maternal lineages. The results show that almost 60% of the Sub-Saharan African haplogroups present in the island are exclusive of or more common in the West African populations, and 26.5% in the West-Central African region. These results are in complete agreement with historical literature that strongly limits the participation of eastern African populations during the Atlantic slave trade, while supporting the western and west-central contributions.

Resumen

La composición genética matriarcal de 79 muestras puertorriqueñas con ascendencia africana del sur del Sahara fue examinada utilizando análisis de restricción (RFLP por sus siglas en inglés). Los datos obtenidos fueron combinados con información publicada de RFLP y secuenciación de 1931 muestras con ascendencia africana de Puerto Rico, República Dominicana, Brasil y varias poblaciones africanas para explorar hasta qué punto las regiones geográficas del continente africano han contribuido a la composición de linajes maternales en la población puertorriqueña actual. Los resultados demuestran que casi el 60% de los haplogrupos africanos sub-saharianos en la isla son exclusivos o más comunes en poblaciones del oeste de África y que el 26.5% lo es en la región occidental de centro África. Estos resultados concuerdan con la literatura histórica que limita la participación de poblaciones del este de África durante la trata de esclavos africanos, mientras establece una contribución significativa del oeste y centro-oeste del continente africano.

Acknowledgments

A mi comité graduado, los doctores Juan C. Martínez-Cruzado, Carlos Ríos Velásquez y Rafael Montalvo Rodríguez, gracias por los consejos, la motivación y el apoyo brindado antes y durante el desarrollo de este documento. En especial deseo reconocer a Juan Carlos, doctor, mil gracias por estimular mi interés en la investigación, por ser modelo y guía durante todos estos años y por creer en mi trabajo y habilidades.

A mis padres, Roberto Viera Suárez y Mildred Vera Ríos, cómplices de mi enseñanza y promotores de mis deseos de continuar los estudios graduados. Gracias por infundir la importancia de la educación y por el apoyo y motivación siempre brindados. Mami, mil gracias, ya que además de ser la mejor madre que cualquier hijo podría desear, también serviste de editora oficial de este trabajo, estoy seguro que no hay otra psicóloga en el mundo que sepa más de genética molecular que tú.

A mis hermanos, Ricardo Viera Vera y Michelle Viera Vera, gracias por su amor, apoyo y comprensión. Ambos, grandes mentores de la vida colegial.

A mis compañeros de clases, ustedes saben quienes son, gracias por compartir arduos días de estudios y relajantes días de playas, eso sí, siempre acompañados por el néctar azul o rojo o de cualquier otra región.

A Inés Rosario Méndez, gracias por el amor y la ternura que has traído a mi vida.

Table of Contents

List of Tables

Page

List of Figures

Page

Publication

Reconstructing the Population History of Puerto Rico by means of mtDNA Phylogeographic Analysis

Juan C. Martínez-Cruzado[1], Gladys Toro-Labrador[1], Jorge Viera-Vera[1], Michelle Y. Rivera-Vega[1,2], Jennifer Startek[1], Magda Latorre-Esteves[1,3], Alicia Román-Colón[1,4], Rebecca Rivera-Torres[1,5], Iris Y. Navarro-Millán[1,2], Enid Gómez-Sánchez[1], Héctor Y. Caro-González[1,6], and Patricia Valencia-Rivera[1,7]

[1]Department of Biology, University of Puerto Rico at Mayagüez, Mayagüez, PR 00680; [2]Escuela de Medicina, Universidad Autónoma de Guadalajara, Guadalajara, Jalisco, CP45110, MÉXICO; [3]Department of Pathology, Harvard Medical School, Harvard University, Boston, MA 02115; [4]Medical Sciences Campus, University of Puerto Rico, San Juan, PR 00936; [5]Department of Comparative Pathology, University of California at Davis, Davis, CA 95616; [6]Department of Biological Sciences, Stanford University, Stanford, CA 94305; [7]Department of Cell Biology, Harvard Medical School, Harvard University, Boston, MA 02115

Total number of pages: 82

Abbreviated title: Puerto Rico mtDNA phylogeography

Key words: mtDNA haplogroups; Taínos; principal component analysis

Address for correspondence: Dr. Juan C. Martínez-Cruzado, Department of Biology, University of Puerto Rico at Mayagüez, PO Box 9012, Mayagüez, Puerto Rico 00681-9012, USA. Tel: (787) 832-4040 X-3357, 3751, 2405. Fax: (787) 265-3837. E-mail: jumartin@uprm.edu

Grant sponsorship: NSF Physical Anthropology Program grant SBR-9904252 to JCMC.

The haplogroup identities of 800 mtDNAs randomly and systematically selected to be representative of the population of Puerto Rico were determined by AFLP, revealing maternal ancestries in this highly mixed population of 61.3% Amerindian, 27.2% Sub-Saharan African, and 11.5% West Eurasian. West Eurasian frequencies were low in all 28 municipalities sampled and displayed no geographic patterns. Thus, a statistically significant negative correlation was observed between the Amerindian and African frequencies of the municipalities. In addition, a statistically highly significant geographic pattern was observed for Amerindian and African mtDNAs. In a scenario in which Amerindian mtDNAs prevailed on either side of longitude 66°16' W, Amerindian mtDNAs were more frequent west of longitude 66°16' W than east of it, and the opposite was true for African mtDNAs. Haplogroup A had the highest frequency among Amerindian samples (52.4%), suggesting its predominance among the native Taínos. Principal component analysis showed that the Sub-Saharan African fraction had a strong affinity to West Africans. In addition, the magnitudes of the Senegambian and Gulf of Guinea components in Puerto Rico were in between of those of Cape Verde and São Tomé. Furthermore, the West Eurasian component did not conform to European haplogroup frequencies. HVR-I sequences of haplogroup U samples revealed a strong North African influence among West Eurasian mtDNAs and a new Sub-Saharan African clade.

Recent technical advances have facilitated the discovery of genetic polymorphisms in the human population, many of which are useful as markers for prehistorical migrations that gave rise to continental and regional populations. Continental-population histories have been reconstructed using Y-chromosome markers, which are paternally inherited (Hurles et al., 1998; Rosser et al., 2000; Bamshad et al., 2001; Hammer et al., 2001; Karafet et al., 2001; Kayser et al., 2001; Malaspina et al., 2001; Underhill et al., 2001; Bortolini et al., 2002, 2003; Cruciani et al., 2002; Lell et al., 2002; Pereira et al., 2002; Semino et al., 2002; Zerjal et al., 2002, 2003; Zegura et al., 2004), and mtDNA markers, which are inherited maternally (Merriwether and Ferrell, 1996; Comas et al., 1998; Starikovskaya et al., 1998; Richards et al., 2000, 2002; Forster et al., 2001; Kaestle and Smith, 2001; Malhi et al., 2001; Torroni et al., 2001a,b; Keyeux et al., 2002; Oota et al., 2002; Salas et al., 2002; Schurr and Wallace, 2002; Yao et al., 2002a,b; Kong et al., 2003), usually finding remarkable differences in sex migration histories.

In this study, we developed a hierarchical strategy that makes use of haplogroup-defining mtDNA restriction markers to identify maternal biological ancestries in a sample set randomly and systematically selected to be representative of the Puerto Rico population, a mixed Caribbean population of three principal components: Amerindian, Sub-Saharan African, and West Eurasian. With some notable exceptions, most haplogroups are regarded as being continent-specific. Thus, determining the haplogroup to which a mtDNA belongs usually identifies the mtDNA biological ancestry. The HVR-I sequence was used when biological ancestry could not be determined through restriction marker analysis.

The biological ancestries of a mixed people have implications in their population genetics and thus in public health. In terms of mtDNA ancestry, studies on European and North American populations have related particular West Eurasian haplogroups to higher frequencies of some diseases such as Alzheimer's (Hutchin and Cortopassi, 1995), Leber Hereditary Optic Neuropathy (Johns and

Berman, 1991; Brown et al., 1997; Hofmann et al., 1997; Lamminen et al., 1997; Torroni et al., 1997; Howell et al., 2003), Wolfram Syndrome and Sudden Infant Death Syndrome (Hoffman et al., 1997), and of some conditions such as asthenozoospermia and nonasthenozoospermia (Ruiz-Pesini et al., 2000). Furthermore, it has been shown that the +10394 *Dde*I state plays a protective role against Parkinson's disease, and that its effect is stronger when it is combined with other polymorphisms that are specific to haplogroups J and K (van der Walt et al., 2003). Thus, the characterization of the mtDNA pool of any population may be instrumental in determining risk factors for various diseases and conditions.

In addition, biological ancestries imply human migration routes that shed light on the possible origins of introduced fauna and flora, including agricultural varieties. Moreover, biological ancestries play a fundamental role in population history which, as one of the main categories of cultural history, is essential to explain the social systems and behavioral guidelines that rule all aspects of social life. Population history considers population growth in relation to geographic regions, biological ancestries, and admixture, and thus plays a central role in the cultural development of a people (Fernández-Méndez, 1970).

It is estimated that from 60,000 to 600,000 Arawak-speaking Taíno Indians lived in Puerto Rico when it was discovered for the Europeans by Christopher Columbus in 1493 (Abbad, 1959; Fernández-Méndez, 1970). Traditional history tells us that they were decimated by war, hunger, disease, and emigration, such that they had totally disappeared by the end of the 16th century. The vast majority of Spanish settlers were single men, and mixing with Indian women commenced fully upon colonization in 1506. The Spanish Crown took measures to increment the number of "white" people in the Island, including ordering "white" Christian female slaves to be sent to Puerto Rico in 1512. However, the 1530 census reported that only 57 of the 369 "white" men in the Island were married to "white" women. Such "whites" were a minority. The census reported 335 "black" female slaves and 1168 "black" male slaves, and a total of 1148 Indians, both genders included (Brau, 1904). By this time, the base of the Puerto Rican economy was shifting from gold mine exploitation to sugar cultivation. African slaves became the cornerstone of the sugar industry.

Traditional history includes abundant evidence of the widely dispersed geographic origins of the Sub-Saharan African peoples who were brought to the Americas, spanning from Cape Verde on the northwest edge of Sub-Saharan Africa to Mozambique and the island of Madagascar in the southeast. The arrival in Puerto Rico of people from various African regions can be confirmed by traditional festivals and other activities held in the name of African gods and by the use of words that can be found only in particular African regions. However, the lack of a classification system for slaves by tribe or even by geographic region during the Atlantic slave trade leaves doubts concerning the relative contribution of the different continental regions (Álvarez-Nazario, 1974).

Slaves were first brought to Puerto Rico in 1508 by her conquistador, Juan Ponce de León. These were residents of the Iberian Peninsula, many of North African, Senegambian or Guinean origin (Álvarez-Nazario, 1974); others were Greek, Slavic, or Turkish (Thomas, 1997), and others Jewish (Díaz-Soler, 2000). The capture of Sub-Saharan Africans with the goal of providing Spanish and Portuguese colonies in the Americas with labor force, first in the search for gold and later in sugar plantations, started in 1518 (Díaz-Soler, 2000). Up to the beginning of the second half of the 16th century, almost all slaves originated in Senegambia and Guinea (Alegría, 1985). The island of São Tomé, with slaves acquired mainly from the Gulf of Guinea, was an important supplier thereafter. Throughout the 16th century and with the exception of one in the west coast, all 13 sugar mills in Puerto Rico worked east of La Plata River, which streams along longitude 66^{o}16' W (Gelpí-Baíz, 2000).

The Portuguese were the legal source of African slaves until 1640, at which time Spain suspended all contracts in retaliation for the revolution that removed their Spanish rulers. The resulting shortage of slave labor provoked the collapse of the sugar industry, starting a period of subsistence economy that lasted for a century and a half until the Crown suspended all taxes and source restrictions on the slave trade in 1789. The poor state of the economy hindered the importation of slaves, and the tax collected upon their sale made the illegal trade their main source. The illegal slave trade was circuitous in that the main slave sources were the Dutch colony of Curaçao and the English colony of Jamaica, in that order. Slaves brought from the Gold Coast (Ghana) were the most common in these colonies at the time. The illegal Puerto Rican harbors were located on the west and south coasts, where most of the island population lived (Álvarez-Nazario, 1974). The only legal harbor was far away in San Juan, the capital, and few legal immigrants made it to Puerto Rico during these times.

The importation of slaves increased dramatically as a consequence of the land and tax reforms of the last decades of the 18th century, and approximately two-thirds of all slaves ever brought to Puerto Rico arrived from that point in time until the abolition of slavery in 1873 (Álvarez-Nazario, 1974). By then, the African harbors most used by slave traders extended from the Gold Coast to Angola (Thomas, 1997).

This new wave of slaves found Puerto Rico mainly inhabited by *criollos*, Puerto Rico natives that were the product of centuries of admixture and generations living under a subsistence economy with little or no Spanish government intervention (Fernández-Méndez, 2000).

The Spanish Empire started to crumble at the beginning of the 19th century, and an 1815 royal decree permitted the settlement in Puerto Rico of foreign Catholics with their wealth and slaves. Thus, wealthy "white" refugees and other immigrants from Europe and the Americas made it to Puerto Rico in great numbers, stimulating the economy by developing the sugar industry in the coastal plains and the coffee and tobacco industries in the mountains. International treaties banned importation of slaves directly from Africa north of the Equator in 1817 and south of it in 1820. However, the enforcements of the treaties were ineffective south of the Equator where the Portuguese had bountiful slave factories. Thus, the illegal Angolan trade became substantial in the

19th century. Larger sources of Africans were probably the West Indies, because trade within the Caribbean was not banned and because escapees arriving to Puerto Rico were granted freedom. In this respect, migrations from the then Danish-ruled island of Saint Thomas, which acquired its slaves mainly from the Gold Coast to the Slave Coast in the Bight of Benin (Thomas, 1997), were a major source (Álvarez-Nazario, 1974).

Our results conform to most accounts of traditional history, but not at all with the extermination of the Taíno people as early as the 16th century, thus showing that population genetics has a lot to offer to studies on Caribbean population history. It is important to note that people neglected rarely contribute to traditional history, and a great part of the cultural development of the Puerto Rican people occurred in the "darkness" of history, far away from the capital, as did the illegal trade that kept their subsistence economy alive.

In the interest of greater clarity, we often refer to Puerto Ricans carrying mtDNAs of Amerindian, African, or West Eurasian origin in such terms as Amerindians, Africans, and West Eurasians. However, it is important to keep in mind that we are referring to a thoroughly mixed population composed of people of a single culture and whose phenotypes do not help to predict individual mtDNA ancestries.

SUBJECTS, MATERIALS AND METHODS

Subjects

A random sample of 872 housing units representative of the island of Puerto Rico was selected using a sampling frame developed by the Center for Applied Social Research (University of Puerto Rico at Mayagüez) for survey research in Puerto Rico, based on the 1990 Census of Population and Housing. Excluding the island municipalities of Vieques and Culebra from the sampling frame, 28 of the 76 municipalities in Puerto Rico were selected (Fig. 1) as per the following description. The eight most populated municipalities were selected with probability equal to one. Each was assigned a number of housing units proportional to its estimated population size based on a total of 872 housing units for the entire island. To select the remaining 20 municipalities, the remainder of the island was divided into five geographical regions. Four municipalities from each region were selected at random with probability proportional to estimated population size while stratifying by estimated population size. They were assigned an equal number of housing units, proportional to the estimated population size of the geographic region they represented.

Thirty percent of the census tracts within each municipality were selected at random with probability proportional to estimated population size. Having established an estimated number of housing units for each census tract based on the number of housing units for the municipality and the relative population sizes of the selected census tracts, census blocks were selected within them so that each would contribute an expected eight households to the sample. On the field, housing units were chosen by systematic sampling with a pre-established random starting point for each block. This means that the actual number of housing units obtained from each block could be greater or smaller than initially expected depending on how the number of housing units in it had changed since 1990. An adult was selected at random from each housing unit. Participation in the project was agreed to by appropriate informed consent.

DNA manipulation

The sample collection and DNA extraction were performed as in Martínez-Cruzado et al. (2001). Thereafter, a 200 µl aliquot from each 500 µl sample was purified using the QIAamp DNA Mini Kit (QIAGEN). To each aliquot, 36 µl of 60 mM Tris-HCl pH 8.0, 60 mM Na_2EDTA pH 8.0, 0.6 M NaCl, 0.24 mM DTT and 12% SDS was added, followed by 250 µl of Buffer AL and 250 µl of 100% ethanol. The aliquots were vortexed thoroughly, transferred to a spin column, and spun at 8000 rpm for one minute. The filter was washed by adding 300 µl of Buffer AW1, spinning at 8000 rpm for one minute, adding 300 µl of Buffer AW2, and spinning at 14000 rpm for five minutes. The DNA was eluted from the filter into two 100 µl aliquots. The eluate aliquots were kept at -80°C as backups until the end of the study.

Except for the cycling conditions (see below) and that 1.5 U of *Taq* DNA polymerase were used in each amplification reaction, the DNA amplification, restriction digestion, and agarose gel electrophoresis procedures were performed as in Martínez-Cruzado et al. (2001). The amplification reactions were usually subjected to one cycle of 2.5 min at 94°C, 32 cycles of 30 sec at 94°C, 1 min at 54°C, and 70 sec at 72°C, and one cycle of 10 min at 72°C. Primer annealing was achieved at 52°C to amplify the diagnostic site for macroparagroup L and at 56°C to amplify the sites diagnostic for haplogroups G and L3d.

Haplogroup identification strategy and quality-control estimates

Studies involving high resolution restriction analysis (Ballinger et al., 1992; Torroni et al., 1992, 1993a,b, 1994a,b,c,d, 1996, 1997; Chen et al., 1995, 2000), analyses of the complete sequence of mitochondrial chromosomes (Kong et al., 2003; Reidla et al., 2003), or complete (Herrnstadt et al., 2002) or partial (Silva et al., 2002) sequences of their coding region have shown that all haplogroups are virtually monomorphic for the 10394 *Dde*I and 10397 *Alu*I sites, with the exception of haplogroup K. Thus, the *a priori* determination of the state of these sites quickly reduces the number of candidate haplogroups to which an unknown mtDNA may belong. Because these sites are close to each other, the 10394 *Dde*I/10397 *Alu*I motif (hereafter referred to as *the motif*) can be easily determined from a single amplicon.

Thus, each mtDNA sample was first tested for its motif. Depending on the result, each sample was then tested for the markers diagnostic for all the haplogroups known to share its motif. The haplogroups, their motifs, their defining markers, and the primers used are shown in Table 1. Haplogroups that are defined by two or more markers invariably share at least one of them with some other haplogroup. Thus, tests on the unshared haplogroup markers were performed only when the samples showed the shared ones. The two markers that define haplogroup L1b were tested on all (+/-) motif samples as each by itself

defines another (+/-) motif haplogroup. L is a macroparagroup, a large group of mtDNAs including several haplogroups and other paraphyletic mtDNAs (Chen et al., 1995; Salas et al., 2002). Among others, it includes haplogroup L2 (here further subdivided into L2a and L2* to pool subhaplogroups L2b, L2c, and L2d), and subhaplogroups L1b and L1c. All other L haplogroups and paraphyletic mtDNAs were included in paragroup L0 (Mishmar et al., 2003).

The testing of markers for all haplogroups within each motif group served as a quality-control measure, as it allowed us to detect false positives. In the few instances in which the mtDNA tested positive for no haplogroup-defining markers, its identity was determined by the sequence of its HVR-I and confirmed by restriction analysis. Thus, false negatives were also detected, and the likelihood of any error involving false haplogroup positives, false haplogroup negatives, or motif group misdiagnoses could be estimated experimentally. Such estimates were used to calculate the probabilities of any number of samples being misdiagnosed. Because all tests were performed independently, the likelihood that any two errors were committed in analyzing the same sample could be calculated based on the multiplicative rule of probability.

Amplicons to be sequenced were purified using the High Pure PCR Product Purification Kit (Roche Molecular Biochemicals) as instructed by the manufacturer. Automated sequencing was performed at the University of Medicine and Dentistry of New Jersey, New Jersey Medical School Molecular Resource Facility using an Applied Biosystems (ABI) model 3100 capillary sequencer after cycle sequencing with Dye Terminator mix version 2.0.

Biological ancestry determination and data analysis

Biological ancestries were inferred from haplogroup identity. Because only nine women of Asian ancestry were reported living in Puerto Rico in 1899 (Sanger et al., 1900), mtDNAs of haplogroups belonging to both the New World and Asia were assumed to be of Amerindian origin unless participant interviews revealed otherwise.

Data analysis was performed using the program SPSS 10.0.5 for Windows. To determine whether variations in participation rates or changes in population size occurring since 1990 in the sampled municipalities would lead to biased estimates of the parameters, we devised a weighting scheme. Through these weights, the number of samples provided by each municipality was adjusted so that it would be equal to the number expected by applying the original sampling proportions to the final sample size. The weights for the municipality samples (W_m) were a function of the sampling proportion of the municipality (P_m), the final total obtained sample size (n), and the number of samples provided by the municipality (n_m) so that

$W_m = (P_m \times n)/n_m$.

A triangular graphic of ancestry distribution among municipalities was constructed using MATLAB. A projected plane representing a linear function of form w = f(X, Y, Z), in which plotted population dots were defined as the end of vectors with form w = Xi + Yj + Zk, where X, Y, and Z represented Amerindian, African, and West Eurasian frequencies, respectively, was produced. The sum of X, Y, and Z was equal to one. Their magnitudes were a function of the 30 and 60 degree angles. Vectors i, j, and k were their respective unit vectors in the positive directions of the coordinate axes x, y, and z.

To illustrate the geographic distribution of Amerindian mtDNA frequencies, municipalities were listed in order according to such frequencies and divided into 12 categories by creating a new category every time that the difference between two municipalities was 1.6% or more. Divisions were drawn halfway between the frequencies of such municipalities.

Principal component (PC) analyses were performed using the POPSTR program of Henry Harpending (U of Utah). They were based on population haplogroup frequencies and included only populations with 17 samples or more. Sub-Saharan African mtDNAs were classified as follows. Macroparagroup L was divided into haplogroup L2 (further subdivided into L2a and L2*), subhaplogroups L1b and L1c, and paragroup L0 to pool all other haplogroups and paraphyletic mtDNAs within the macroparagroup. Paragroup L3A (Salas et al., 2002) was divided into L3b, L3d, L3e, L3f, L3g and L3*. We designate U5b2 as a Sub-Saharan African clade with the HVR-I sequence 16189-16192-16270-16320. Taken from one source were Shona (n = 17), Tongas (20), Shangaan (22), Chopi (27), Chwabo (20), Lomwe (20), Makonde (19), Makhwa (20), Ndau (19), Nyungwe (20), Nyanja (20), Ronga (21), Sena (21), and Tswa (19) from Mozambique (Salas et al., 2002), Brazil (65) (Alves-Silva et al., 2000), Bubi (45), São Tomé (49) (Mateu et al., 1997), Mandenka (118) (Graven et al., 1995), Serer (23), a group of other Senegalese tribes (48), a pool of Mauritanian and West Saharan tribes (24) (Rando et al., 1998), Tuareg (22), Yoruba (33), Hausa (20), Fulbe (60), Turkana (37), Somalia (27), Kikuyu (22) (Watson et al., 1997), Nubia (46) (Krings et al., 1999), Khwe (31) (Chen et al., 2000), and the southeastern islands of the Cape Verde Archipelago (169) (Brehm et al., 2002). From two sources were Biaka (34) and Mbuti (35) Pygmies (Chen et al., 1995; Watson et al., 1997), Wolof (66) (Chen et al., 1995; Rando et al., 1998), and !Kung (62) (Watson et al., 1997; Chen et al., 2000). For West Eurasians, mtDNAs were classified as belonging to H, V, HV, (pre-HV)1, J, T, I, W, X, M, N, R, K, U*, U2, U5*, U5(a+b), U6, and U(others) to pool the remaining clades (U1, U3, U4 and U7). Populations were obtained from Rando et al. (1998) (23 Moroccan non-Berbers and 58 Moroccan Berbers), Brakez et al. (2001) (37 Moroccan Souss Valley inhabitants), and Richards et al. (2000). This last group of authors compiled data from several authors concerning 13 populations from North Africa and the Near East, as well as several populations from Europe. They classified the European populations into 10 geographic regions and we observe those same classifications here. Amerindian populations were divided into 12 geographic regions and "Others". These were three from eastern North America [Mohawk (123) (Merriwether and Ferrell, 1996) and Ojibwa from Manitoulin Island (33) and northern Ontario (28) (Scozzari et al., 1997)], five from the Great Plains [Cheyenne/Arapaho (35), Sisseton/Wapheton

Sioux (45), Turtle Mountain Chippewa (28) and Wisconsin Chippewa (62) (Malhi et al., 2001), and Siouan (34) (Lorenz and Smith, 1996)], six from the North American Southeast [Choctaw (27) (Lorenz and Smith, 1996), Creek (39) and Seminole (40) (Weiss and Smith, 2003), Oklahoma Muskoke (70) (Merriwether and Ferrell, 1996), and Oklahoma Red Cross Cherokee (19) and Stillwell Cherokee (37) (Malhi et al., 2001)], 15 from the North American Southwest [Akimal O'odham (43), Apache (38) Delta Yuman (23), Navajo (64), North Paiute/Shoshoni (94), Pai Yuman (27), River Yuman (22), Tauno O'odham (37) and Zuni (26) (Malhi et al., 2003), and California Penutian (17), Havasupai/Hualapai/Yavapai/Mojave (18), Jemez (36), Pima (37), Quechuan/Cocopa (23) and Washo (28) (Lorenz and Smith, 1996)], four from Mesoamerica [Maya (26), Mixtec (29) and Nahua/Cora (32) (Lorenz and Smith, 1996), and North Central Mexico (199) (Green et al., 2000)], eight from eastern Central America [Bribri-Cabecar (24) (Torroni et al., 1993a), Emberá (Panamá) (44) and Wounan (31) (Kolman and Bermingham, 1997), Guatuso (20) and Teribe (20) (Torroni et al., 1994d), Huetar (27) (Santos et al., 1994), Kuna (63) (Batista et al., 1995), and Ngöbé (46) (Kolman et al., 1995)], 14 from western Colombia and Ecuador including the Andes [Cayapa (94) (Rickards et al., 1999), Chimila (34), Guambiano (23), Guane-Butaregua (33), Ijka-Arhuaco (40), Kogui (30), Paez (31), Tule-Cuna (29), Waunana (30) and Yuco-Yukpa (88) (Keyeux et al., 2002), and Emberá (Colombia) (41), Ingano (52), Wayuu (59) and Zenu (69) (Mesa et al., 2000; Keyeux et al., 2002)], nine from Colombia east of the Andes [Coreguaje (19), Curripaco (17), Guahibo-Sikuani (23), Guayabero (24), Huitoto (22), Murui-Muinane (18), Nukak (20) and Piaroa (18) (Keyeux et al., 2002), and Tucano (71) (Mesa et al., 2000; Keyeux et al., 2002)], seven from the Amazon [Belén (Brazil) (81) (Batista dos Santos et al., 1999), Brazilian North (26) (Alves-Silva et al., 2000), Gaviao (27), Xavante (25) and Zoró (30) (Ward et al., 1996), Ticuna (28) (Torroni et al., 1993a), and Yanomami (97) (Merriwether and Ferrell, 1996)], nine from the Perú, Bolivia and Chile highlands around Lake Titicaca [Atacameño (50) (Merriwether et al., 1995), Chimane (40), Ignaciano (21), Mosetén (19), Movima (22), Trinitario (33), Yuracaré (27) (Bert et al., 2001), and Aymara (98) and Quechua (51) (Merriwether and Ferrell, 1996; Bert et al., 2001)], six from northern Argentina [Mataco from the provinces of Chaco (28), Formosa (44) and Salta (55), Pilaga (40), and Toba from the provinces of Chaco (28) and Formosa (26) (Demarchi et al., 2001)], and five from southern South America [Huilliche (89) (Merriwether and Ferrell, 1996), Mapuche-Argentina (50) (Bailliet et al., 1994), Mapuche-Chile (156) (Merriwether et al., 1995; Moraga et al., 2000), Pehuenche (204) (Merriwether and Ferrell, 1996; Moraga et al., 2000) and Yaghan (21) (Moraga et al., 2000)]. Two "Other" populations were Bella Coola (36) (Lorenz and Smith, 1996), and Brazilian Southeast (33) (Alves-Silva et al., 2000).

Haplogroup diversity for the Amerindian mtDNAs was calculated using the method of Tajima (1989), $h = [1-\mathrm{E}x_i^2]n/(n-1)$, where x_i is the frequency of each haplogroup and n is the sample size.

RESULTS

Response rate

All selected housing units were identified between August 6, 1999 and March 19, 2000. Based on the 1990 Census of Population and Housing, a total of 872 housing units was selected. This translated into 1,067 because of housing growth through the decade. Eighty-one of the housing units were uninhabited. From the 986 remaining housing units 876 selected individuals were contacted. Exactly 800 of these agreed to participate, for a response rate of 81.1% based on the 986 selected individuals. The sampling procedure results for each municipality and region are detailed in Table 2.

Haplogroup identification data quality

The haplogroup identification strategy described above allowed the detection of misdiagnoses of both motif and haplogroup-defining marker identities, and thus an estimation of the probability that any misdiagnosis may have gone undetected. The largest margin of error lies within the (+/-) motif group. Initially, all (+/-) samples were tested, among others, for the +3592 *Hpa*I and +2349 *Dpn*II markers but not for markers +9070 *Taq*I and +16389 *Hin*fI, which are necessary to discriminate L1c and L2, respectively, from all other mtDNAs within L (Table 1). Thus, the samples belonging to L1b (+3592 *Hpa*I / +2349 *Dpn*II) and L3e (-3592 *Hpa*I / +2349 *Dpn*II) were quickly identified, while the samples with the +3592 *Hpa*I / -2349 *Dpn*II profile had to be subjected to a second round of tests for markers +9070 *Taq*I and +16389 *Hin*fI. HVR-I sequencing of those samples with no haplogroup-defining markers showed that the +3592 *Hpa*I motif of one of the 79 samples with the +3592 *Hpa*I / -2349 *Dpn*II profile initially went undetected. This gave us an experimental estimate of 1/79 for the frequency with which the +3592 *Hpa*I motif went undetected. Using such frequency and a base of 49 L1b samples, we calculated a probability of 53.6% that none of the 38 samples identified as belonging to haplogroup L3e (-3592 *Hpa*I / +2349 *Dpn*II) may actually belong to L1b (+3592 *Hpa*I / +2349 *Dpn*II). Using bases of 50, 51, and 52 L1b samples, we calculated probabilities of 33.9%, 10.9%, and 2.4% that one, two, or three samples identified as L3e actually belong to L1b.

There are two other scenarios by which misdiagnoses could occur. One is the combination of a misdiagnosis of the sample motif group with a false positive for a haplogroup-defining marker. The other is the occurrence of both a false negative and a false positive for haplogroup-defining markers with the same sample. Based on the detection of 11 motif misdiagnoses [three samples misdiagnosed as (-/-), seven as (+/-), and one as (+/+)], six false positives (one each for the markers corresponding to A, D, HV, L, L3b and J/T), 11 false negatives (three each for the markers of A and J/T, two for that of C, and one each for those of HV, L and L3b), the number of samples belonging to each motif group [377 (-/-), 233 (+/-), and 190 (+/+)], and the number of samples belonging to each haplogroup (Table 3), we estimate that the probability that no misdiagnoses were made under either of these two scenarios is 86.0%, and that the probability that two or more misdiagnoses were made is insignificant.

Haplogroup identities

Table 3 shows the distribution by municipality of all haplogroups found, their frequencies, and their biological origin. Only six of the 800 samples were confirmed, through HVR-I sequencing, as having 10394 *Dde*I/10397 *Alu*I motifs different from those corresponding to their haplogroups (Table 1). Specifically, two L3e, one L2*, one L2a, and one L1c samples were found to have (-/-) instead of (+/-) motifs. In addition, one haplogroup C sample had a (-/-) motif instead of the (+/+) expected.

On six occasions samples were confirmed as having more than one haplogroup-defining marker. The -8616 *Dpn*II marker that characterizes haplogroup L3d was found in one L0 and one L1c sample. Furthermore, two L1b samples had the +4216 *Nla*III marker that characterizes haplogroups J and T. These were all regarded as belonging to macroparagroup L because of the known stability of the L-defining +3592 *Hpa*I marker. The true haplogroup identities of the remaining two samples were determined from their HVR-I sequences. One haplogroup H sample had the 9 bp deletion between the $tRNA^{Lys}$ and COII genes that characterizes haplogroup B, and one haplogroup C sample had the -7598 *Hha*I mutation that characterizes Asian haplogroup E. Their respective HVR-I sequences were 16093-16362 and 16221-16223-16261-16298-16325-16327. Thus, the first lacked the transitions at positions 16189 and 16217 that characterize haplogroup B (Ginther et al., 1993; Horai et al., 1993), and the second possessed the haplogroup C-specific transitions at positions 16298 and 16327 as well as the Amerindian-specific transition at 16325 (Torroni et al., 1993b).

Samples that did not test positive for any haplogroup-defining marker were identified by sequencing their HVR-I as well as specific sites in their coding regions. Nine (+/-) mtDNAs were classified as L3* for having transitions at sites 10873 and 12705. The HVR-I sequences of two (-/-) mtDNAs not having transitions at 10873 or 12705 were 16288-16311 and 16126-16189-16362. The first (-/-) mtDNA had a transition at site 11719 but not at 16223, and was thus classified as belonging to R. The transitions at sites 16126 and 16362 showed that the second (-/-) mtDNA belonged to JT or (pre-HV)1 (Macaulay et al., 1999). The absence of a transition at 11719 showed that it belonged to (pre-HV)1 (Richards et al., 2000). Finally, the HVR-I sequence of one (+/+) sample that did not exhibit any haplogroup-defining marker was 16086-16183-16189-16223-16278-16298-16325-16327. Thus, it contained the 16223, 16298, 16325 and 16327 transitions specific for Native American haplogroup C, and transitions 16183, 16189, 16223, 16278, which are found in most haplogroup X mtDNAs (Brown et al., 1998). However, it possessed the +10397 *Alu*I motif specific of macrohaplogroup M, to which haplogroup C but not haplogroup X belongs. This motif has been shown to be very stable (Kivisild et al., 2002; Kong et al., 2003), and we thus regarded this mtDNA as belonging to haplogroup C. One haplogroup C mtDNA lacking the +13262 *Alu*I marker had been previously described from the Amazonian Makiritare (Torroni et al., 1993a), and (+/+) mtDNAs lacking defining markers for haplogroups C and D seem to be common in Colombia (Keyeux et al., 2002; Rodas et al., 2003). No mtDNAs belonging to haplogroups E, F, G, I, M, N, JT, W or X were found in our set of 800 samples.

Haplogroup U subdivisions

Among all haplogroups found here, U is the only one that has been reported in significant numbers in more than one continental region (Torroni et al., 1996). It was thus necessary to study such mtDNAs in more detail to identify their biological origin. The HVR-I sequence of the 27 samples belonging to haplogroup U segregates them into 10 types (Table 4). Although haplogroup U is mostly regarded as a West Eurasian haplogroup, it is apparent that nine of these samples originate from Sub-Saharan Africa. All share the same sequence type, which has not been found in Europe or the Near East despite the thousands of samples from these areas for which the HVR-I has been sequenced (Alves-Silva et al., 2000; Richards et al., 2000; Finnilä et al., 2001; Malyarchuk et al., 2002). However, it has been found in one out of 60 Fulbe sequences (Watson et al., 1997), and in one of 38 and 23 Wolof and Serer sequences, respectively (Rando et al., 1998). We classify it as a member of clade U5b* because of its 16189, 16192, 16270 motif (Richards et al., 2000). Its distinction is the addition of a transition at position 16320. We designate it as clade U5b2 to represent a Sub-Saharan African clade with a transition at 16320 as its signature.

Eleven samples seem to originate from North Africa and the Canary Islands. Two samples sharing the same sequence exhibit the 16163 motif, which is diagnostic for the Native Canarian-specific clade U6b (Rando et al., 1999). Nine samples segregate into three North African sequence types. The most common type (16224-16270), comprising seven samples, may correspond to the 16093-16224-16270 type of apparently North African ancestry that has been found in two Canarian Islands (Pinto et al., 1996; Rando et al., 1999), because our sequencing reactions did not extend to the left of the 16154 site in these samples. No other mtDNAs have been found with the 16093-16224-16270 or the 16224-16270 sequence types elsewhere. Of the two remaining North African sequence types, one (16224-16261-16270) may have derived directly from the most common type, as it differs from it at only one site. The remaining one has been found mainly in North Africa, but also in the Near East and Sub-Saharan Africa. Its highest frequency has been reported in the Berber-speaking Mozabites of northern Algeria: 10 out of 85 samples (Côrte-Real et al., 1996). Other populations with lower frequencies are Moroccan Berbers and non-Berbers (Pinto et al., 1996; Rando et al., 1998), Egyptians (Krings et al., 1999), Syrians (Richards et al., 2000), and some East and West African tribes (Watson et al., 1997). It has also been found in two of 54 samples from Portugal (Côrte-Real et al., 1996), but we believe its presence in the Iberian Peninsula is due to migrations related to the slave trade.

Two samples share the motif 16189-16362. They likely belong to the U2 clade, which is characterized by the 16051 motif (Kivisild et al., 1999; Macaulay et al., 1999), a site to which our sequencing reaction did not extend. However, most West Eurasian U2 mtDNAs, but not other haplogroup U clades, present substitutions at positions 16129 and 16362. Since these samples do not present motifs that would classify them under any other clade, but possess the 16362 transition, they likely belong to clade

U2. Clade U2 is virtually absent in North Africa and is found in the Near East at somewhat higher frequencies than in Europe. However, the precise sequence type is found at a higher frequency in the Iberian Peninsula than in any Near Eastern population except the Kurdish (Richards et al., 2000). PC analysis does not assign the Puerto Rican West Eurasian population a decisively higher affinity to the Kurdish or the European Mediterranean Western Region population (see below). Thus, we can only conclude that these samples should originate either in the Iberian Peninsula or the Near East.

The remaining four sequence types, encompassing only five samples, likely are of European origin. One of them differs from the CRS only by a transition at position 16192. Although the exact sequence type has not been reported elsewhere, it is regarded as European in origin because of the instability of the 16192 site in haplogroup U mtDNAs and the fairly high frequency of the otherwise resulting sequence type (CRS) in the Iberian Peninsula. The remaining three European sequence types are either particular U5b* types common only throughout Europe or belong to subclade U5a1a, which evolved in Europe (Richards et al., 2000).

L3* subdivisions

The HVR-I sequences of the nine (+/-) samples for which no haplogroup-specific markers were found are shown in Table 5. They segregate into eight sequence types sharing the 16223-16311 motif. Three of the sequence types possess the 16209 transition diagnostic of the L3f clade and the 16292 transition of subclade L3f1 (Salas et al., 2002). Only these three sequences showed a 1 bp deletion in the 5 bp T-stretch that runs from 15940 to 15944 in the CRS. This deletion is not the result of errors in the CRS (Andrews et al., 1999); it may play a significant role in RNA translation efficiency, as it makes the T*C arm loop of the $tRNA^{Thr}$ only two nucleotides long, and may become a useful phylogenetic marker to group L3* clades or to further subdivide subclade L3f1.

Another sequence type contains the 16293T-16355-16362 motif of clade L3g. The four remaining sequence types encompass five samples and cannot be grouped into any L3* clade. These thus remain classified as L3*. However, four of these five samples seem to be not too distantly related, as they all share the 16256A transversion. Two of them also share transitions at positions 16129 and 16362.

Geographic distribution of mtDNAs by biological ancestry

Little change is observed when the biological ancestry frequencies are corrected by sample weight. Frequencies and 95% confidence intervals of 61.0 $\pm$ 3.4% Amerindian, 27.5 $\pm$ 3.1% African, 11.4 $\pm$ 2.2% West Eurasian, and 0.1 $\pm$ 0.2% Asian (Table 3) are corrected to 61.3 $\pm$ 3.4% Amerindian, 27.2 $\pm$ 3.1% African, 11.5 $\pm$ 2.2% West Eurasian, and 0.0% Asian (Table 6).

Amerindian mtDNAs are the most common in all municipalities except Loíza, where African mtDNAs are more frequent, and Cayey, where the population is equally divided into African and Amerindian mtDNAs. Amerindian mtDNA frequencies are 50% or higher in all municipalities except Loíza, San Juan, and Carolina (Table 3).

In addition, West Eurasian frequencies are low in all municipalities (0 – 17.9%). Thus, in a triangular graph with axes representing biological ancestries, ancestry frequencies cluster close to the vertex where the Amerindian frequency equals one, and scatter, next to the side defined by zero West Eurasian frequency, towards the vertex where African frequency equals one (Fig. 2). A negative Pearson correlation (-9.19) between African and Amerindian frequencies is observed that is significant at the 0.01 level (2-tailed test). That is, the biological ancestry frequency of municipalities can be virtually described by stating only their African or Amerindian frequencies.

Figure 3 divides the 28 sampled municipalities into 12 categories according to their Amerindian mtDNA frequencies, and divides Puerto Rico by longitude 66°16' W, as 12 of the 13 sugar mills that worked throughout the 16th century were built east of it. It can be observed that the three municipalities with the lowest Amerindian frequencies are next to each other in San Juan and further east. Furthermore, all 11 municipalities east of longitude 66°16' W are among the 14 municipalities with the lowest Amerindian frequencies. There is a highly significant deviation from the null hypothesis that frequencies for all ancestries are the same east and west of longitude 66°16' W (Pearson $X^2 = 43.70$, df = 2, $p << .001$). X^2 tests also show highly significant deviations from null hypotheses of equal frequencies on each side of longitude 66°16' W for Amerindian (Pearson $X^2 = 41.72$, df = 1, $p << .001$) and African (Pearson $X^2 = 34.40$, df = 1, $p << .001$) mtDNAs. African mtDNAs are more frequent in the east than in the west; the reverse is true for Amerindian mtDNAs. No significant difference is found for West Eurasian mtDNAs.

Interestingly, the geographic distribution by biological ancestry does not fit expectations based on traditional history that place Amerindians fleeing to the mountains and African slaves working in sugar plantations on the coasts. The three municipalities with the highest Amerindian frequencies are coastal (Fig. 3), and X^2 tests show that Amerindian frequencies in noncoastal municipalities are not significantly higher than those in coastal ones, and that African frequencies are not significantly higher in coastal than noncoastal municipalities.

Principal component analyses

To learn more about the origins of African mtDNAs in Puerto Rico, their weighted haplogroup frequencies (Table 7) were subjected to principal component (PC) analysis. Figure 4 plots the Puerto Rico African haplogroup frequencies with those of various contemporaneous African populations for the first two PCs (panel A), and the first and third PCs (panel B). The three PCs account for 28%, 23% and 15% of the variation, respectively.

Little difference is found between the plots, which separately group the populations of West Africa and Mozambique, and scatter the East African populations in the lower side of PC2 and PC3. The Bubi from the island of Bioko are well separated from all these groups. Three of four very old populations also separate well from these

clusters. These are the Mbuti and Biaka Pygmies and the !Kung from South Africa. The South African Khwe fall within the Mozambique cluster. The ends of the West Africa cluster defined by PC1 are composed of populations located on the western coast to the left and populations located inland or close to the Gulf of Guinea to the right.

PC1 locates the population of Brazil outside of both the West African and Mozambican clusters. All other populations created by the slave trade are placed within the West African cluster. The southeastern Cape Verde Islands, which were uninhabited when discovered by the Portuguese and populated with slaves from the western coast of West Africa and inland thereafter (Thomas, 1997), fall in the middle of the West African cluster. To the right are located the Puerto Rico and São Tomé populations in that order, the latter lying at the edge of the West African cluster. Thus, a mainly West African origin is suggested for the African mtDNA fraction of Puerto Rico.

Figure 5 plots the Puerto Rico West Eurasian haplogroup frequency (Table 8) with those of European, Near Eastern and Moroccan populations for the first two PCs (panel A), and for the first and third PCs (panel B). The three PCs account for 38%, 16% and 13% of the variation, respectively. European and Moroccan clusters can be distinguished in the first plot but fuse together in the second plot. The results are similar in many aspects to those obtained by Richards et al. (2002). The European Southeastern, Mediterranean East, and Mediterranean Central populations cluster a bit separately from the rest of the European populations, showing affinities to the Near Eastern populations, and the long-isolated Basques lie the farthest away from the Near Eastern populations. Another similarity is the scattering of Near Eastern populations, with the exception that the Kurdish, Armenian, and Turkish populations cluster together in both plots.

As Puerto Rico was a Spanish colony for four centuries, it is perhaps expected that its West Eurasian mtDNA population would lie close to the Mediterranean West population; it is noteworthy that on both plots the Mediterranean West population is the European population closest to Puerto Rico, but the Puerto Rico population lies outside the European cluster. It lies closest to and about equidistant between the European and Moroccan clusters in the first plot, and closest to the Turkish-Armenian-Kurdish cluster in the second plot, suggesting that the West Eurasian women that migrated to Puerto Rico had multiple origins.

The resolution power of the PC analysis of the Amerindian weighted haplogroup frequencies (*n* for haplogroups A-D and X in Puerto Rico being 252, 42, 173, 14 and zero, respectively) is limited by the small number of haplogroups employed (Fig. 6). However, its frequent failure to group populations from particular geographic regions can often be explained by prehistoric demographic events. PC1 accounts for 38% of the variation, and although PC2 accounts for more variation (28%) than PC3 (20%), it is less effective at resolving most populations, as its resolution power lies on separating well Bella Coola from all other populations (Fig. 6 insert). The populations of five of the six North, Meso and Central American regions, as well as those around Lake Titicaca and southern South America, lie generally closer to each other. Those from Colombia and the Amazon are widely dispersed. The Puerto Rico population is found in the bottom-left quadrant, next to the Western Colombian population of Paez.

DISCUSSION

Data quality and representativeness of the sample set

Our results can be deemed as being representative of the Puerto Rico population because we used a sampling frame developed for survey research in Puerto Rico that produced a sample set representative of the population based on the 1990 Census of Population and Housing, and obtained a response rate (81.1%) that complies with the standards and experience for survey research in the Island (Alegría et al., 2001; Colón et al., 2001). Furthermore, no municipality had a particularly low response rate, San Juan (69.0%) having the lowest as the result of both the difficulty of contacting the selected people and a relatively high declination rate (Table 2). In addition, the biological ancestries among municipalities were not highly variable except for Loíza (Fig. 2), the response rate of which was good (82.2%). Thus, all significant variants that may exist in the population should have been adequately represented in the sample set. The very small differences observed between weighted and unweighted results add confidence to the assertion that the sample set is indeed representative.

We estimated a rather high likelihood (33.9%) that one, and a fair one (10.9%) that two L1b samples may have been misdiagnosed as belonging to haplogroup L3e. Because both haplogroups are Sub-Saharan African, the effects on our analyses of these possible misdiagnoses would be limited to the PCs (Fig. 5). In such cases, the Puerto Rico population would have plotted a little further to the left, as L1b is the haplogroup that contributes most significantly to the left side of PC1 (not shown). It would have plotted closer to the populations of Cape Verde and others found near the West Africa western coast, but still between Cape Verde and São Tomé. In addition, there is a fair possibility (14%) of other kinds of misdiagnoses occurring, but just once, and the effects of a single misdiagnosis in a set of 800 samples should be negligible.

Haplogroup-specificity of the 10394 *Dde*I/10397 *Alu*I motif

Table 3 shows that five of the 211 African samples belonging to (+/-) haplogroups instead possessed (-/-) motifs. Chen et al. (1995) studied 96 Senegalese and 39 Biaka Pygmies belonging to (+/-) haplogroups, finding the (-/-) motif in five L1c Biaka Pygmies but none in the Senegalese. Another study on South African tribes found the (-/-) motif in one L3e and five L0 out of 31 Khwe samples and in two L0 of 43 !Kung, for a total of 13 (-/-) motifs in 209 samples (Chen et al., 2000). Our five (-/-) samples were found in two L3e and in one each of L1c, L2*, and L2a. It is thus clear that the (-/-) motif has arisen independently several times in Africa as well as in Europe (Finnilä et al., 2001; Herrnstadt et al., 2002), but it seems to be found in African tribes at uneven frequencies, being more prevalent in old tribes of South Africa and Central African Pygmies, and rare elsewhere. Our review of traditional history found no evidence of Pygmies being brought to the Americas, and suggests that none or very

few members of South African tribes were brought to the Caribbean. Hence, our results may be reflecting what is found in more recent and widespread African tribes, most of which are related to Bantu expansions, and suggest that the (-/-) motif in (+/-) haplogroups is present at frequencies of 2% to 3% throughout most of Sub-Saharan Africa.

The 10394 *Dde*I/10397 *Alu*I motif behaves more consistently among Amerindian mtDNAs, probably because these form a more recent group. Only one Amerindian mtDNA showed a motif inconsistent with its haplogroup identity. It belonged to the (+/+) haplogroup C, and probably suffered a back-mutation at position 10398, thus losing both restriction sites simultaneously. Because, with the exception of haplogroup U, all African haplogroups are (+/-) whereas none of the Amerindian haplogroups are, the few motif inconsistencies found here suggest that testing the 10394 *Dde*I/10397 *Alu*I motif is an excellent starting point for sorting out African and Amerindian samples from mixed populations with high African and Amerindian affinities, such as those in the Caribbean.

Non-specificity in haplogroup-defining markers

The 3592 *Hpa*I site that defines macroparagroup L has proven to be highly stable, being found strictly on mtDNAs belonging to haplogroups L1 or L2 in Sub-Saharan Africa and elsewhere (Huoponen et al., 1997; Torroni et al., 1997). The story is not the same for the +4216 *Nla*III and -8616 *Dpn*II markers that define haplogroups J/T and L3d, respectively. Both, more often -8616 *Dpn*II than +4216 *Nla*III, have been found occasionally in mtDNAs belonging to other African and West Eurasian haplogroups (Finnilä et al., 2001; Herrnstadt et al., 2002). Thus, we deemed it unnecessary to determine the HVR-I sequence of the two L1b samples that exhibited the +4216 *Nla*III motif and of the single L0 and L1c samples that had the -8616 *Dpn*II motif (Table 3).

Other haplogroup-defining markers that were found to occur out of their expected haplogroups through HVR-I sequencing were the 9 bp deletion between the COII and $tRNA^{Lys}$ genes that defines haplogroup B and the -7598 *Hha*I marker of haplogroup E. The multiple independent emergences of the 9 bp deletion in Africa (Soodyall et al., 1996), India (Watkins et al., 1999; Clark et al., 2000), East Asia (Wrischnik et al., 1987; Yao et al., 2002a), Europe (Torroni et al., 1997), the New World (Torroni et al., 1993a) and Australia (Betty et al., 1996) are well known. To our knowledge, this is the first time that it has been reported in a haplogroup H background. However, the -7598 *Hha*I motif that defines haplogroup E is much more stable. Hitherto, it had been found outside of a haplogroup E background only in Finnish belonging to haplogroup K (Finnilä et al., 2001), and in all reported Han Chinese belonging to haplogroup G (Yao et al., 2002a). It was found here in a haplogroup C background.

Designation of clade U5b2

Mitochondrial DNAs from northern Sub-Saharan Africa with the (-/-) motif were first described by Chen et al. (1995). These turned out to belong to haplogroup U, as defined by the acquisition of a *Hin*fI site at position 12308 when using a mismatched primer (Torroni et al., 1996). Studies in northern Sub-Saharan populations have often found mtDNAs whose HVR-I sequence circumscribe their identity to haplogroups H or U, suggesting the reintroduction to Sub-Saharan Africa of West Eurasian mtDNAs through North Africa (Macaulay et al., 1999). Our results show that the sequence type 16189-16192-16270-16320 (Table 4) found in three African tribes but not in Eurasia belongs to haplogroup U. Its absence in all other haplogroup U sequences described to date strongly suggests that these mtDNAs have a very recent common origin in northern Sub-Saharan Africa. Insofar as no coding region analysis other than at the 12308 site has been performed on these mtDNAs, we must depend on their HVR-I sequence for their classification. Haplogroup U has been subdivided into eight clades plus haplogroup K, clade U5 being distinguished by its 16270 motif. U5 has been further subdivided into U5a and U5b, and since only one clearly unrelated haplogroup U mtDNA possessing the 16320 has been reported (Malyarchuk et al., 2002), we depend on its transitions at sites 16189 and 16192, both unstable (Macaulay et al., 1999; Finnilä et al., 2000), to classify the African clade. The combination 16189, 16192 has been found in both U5a and U5b clades, but it has always been accompanied by a transition at position 16256 within U5a, and by no substitution in particular within U5b (Richards et al., 2000). Thus, the parsimony rule guides us to classify sequence type 16189-16192-16270-16320 as a U5b clade. As clade U5b1 with the 16144 signature has already been described, we describe clade U5b2 with the 16320 signature. Coding region analysis must be performed to confirm the assignment of these mtDNAs to subhaplogroup U5b.

L3 clades with no defining restriction markers

Within Africa, paragroup L3 is found at its highest frequencies in East Africa, where it originated and gave rise to those mtDNAs that migrated out of Africa (Quintana-Murci et al., 1999; Maca-Meyer et al., 2001). Salas et al. (2002) described haplogroups L3f and L3g, both virtually restricted to East Africa except subclade L3f1, which is common in West Africa and thus not surprisingly the only subclade of L3f found in Puerto Rico. Similarly, L3* is far more common in East than in West Africa, but the C-to-A transversion at position 16256 that appeared in four of the five Puerto Rican L3* mtDNAs (Table 5) has appeared just once in the continent, among the West African Tuareg (Watson et al., 1997). It may thus represent the signature of a small West African clade. However, the presence of one L3g mtDNA in our sample strongly suggests that even some East Africans were victims of the slave trade.

Amerindian mtDNA frequencies and traditional history

The combination of the high Amerindian mtDNA frequency found and the representativeness of the sample set leaves no doubts that the mtDNA pool of Puerto Ricans is predominantly Amerindian. Such preponderance extends throughout the island with the notorious exception of Loíza (Fig. 2). Many of the slaves in the San Juan region emancipated upon the abolition of slavery in 1873 and not staying with their former masters were placed in Loíza. Because San Juan had an unusually high proportion of

female domestic slaves (Negrón-Portillo and Mayo-Santana, 1992), their transference to this lightly populated municipality increased its African maternal ancestry out of the range of all other municipalities.

Our results are in contrast with those based on genetic markers that estimate the contribution of Amerindians to the Puerto Rican gene pool at 18% (Hanis et al., 1991). This may be explained by the predominance of men among the Spaniards that arrived to the Island. Population studies based on mitochondrial and Y-chromosome markers have found strong sexual asymmetries in South America, mtDNAs usually being inherited from Amerindian women and Y-chromosomes from European men (Batista dos Santos et al., 1999; Alves-Silva et al., 2000; Carvajal-Carmona et al., 2000; Carvalho-Silva et al., 2001). Our preliminary results from Y-chromosome studies (unpublished) strongly suggest a similar scenario for Puerto Rico. The overall meaning of our mtDNA data is that the cumulative effect of female migrations to Puerto Rico for the last five centuries has been the reduction of the Amerindian mtDNA frequency from 100% to 61% and, assuming equal reproductive rates, that about seven African women arrived in Puerto Rico for every three West Eurasians who did.

Remarkably, our mtDNA frequencies mimic those obtained from a study in which JC virus strains excreted by two groups of Puerto Rico residents were identified: 61% carried a JCV strain of Asian and Amerindian origin, 26% of African origin, and 13% European (Fernández-Cobo et al., 2001). The frequency distribution of the strains might be explained by fitness differences among viral strains. However, our results support an alternate explanation: that the viral transmission mechanism may favor the mother as vector. The combined identification of JCV strains and mtDNA ancestries should be undertaken to test for a possible correlation between them.

Traditional history provides abundant evidence that Amerindian slaves were brought to Puerto Rico during colonial times from Iberian possessions such as the islands of Margarita, Trinidad, Aruba, Bonaire, the Venezuelan coast (Fernández-Méndez, 1970), the Yucatán peninsula (Zavala, 1948), and Brazil (Tapia, 1945). However, the haplogroup diversity of Amerindian mtDNAs, calculated with weighted samples at 0.5934, is not high, as may have been expected if the Amerindian predominance in Puerto Rico was the consequence of post-Columbian migrations. Specifically, the Puerto Rico haplogroup diversity is higher than in 16 of 19 tribes in the American Southwest and Baja California (O'Rourke et al., 2000; Malhi et al., 2003), where recent population replacement and expansion events may have reduced the haplogroup diversity of the population (Kaestle and Smith, 2001). However, it is higher than in only eight of 29 tribes in the remainder of the contiguous United States and subarctic Canada (O'Rourke et al., 2000; Malhi et al., 2001; Weiss and Smith, 2003). Haplogroup diversities in South America are generally lower than in North America, but the haplogroup diversity in Puerto Rico is higher than in only 12 of 31 South American tribes outside Colombia (Demarchi et al., 2001). In Colombia, the Andes function as a barrier dividing tribes of haplogroup diversities higher to the east, and lower in the Andes and further west (Keyeux et al., 2002). Hence, the Puerto Rico haplogroup diversity is higher than that of only five of 10 tribes to the east, but of 11 of 15 tribes in the Andes and further west. West of Colombia in eastern Central America, where recent population bottlenecks followed by population expansion events have drastically reduced haplogroup diversity, all five tribes examined have haplogroup diversities lower than Puerto Rico (Santos et al., 1994; Batista et al., 1995; Kolman et al., 1995; Kolman and Bermingham, 1997). In conclusion, the Puerto Rico haplogroup diversity is within the lower half of tribes in all regions of the subarctic New World, except for those regions were demographic histories have been affected by strong and recent population expansion events. It is unlikely that the very large number of post-Columbian migrants needed to catapult the Amerindian frequency in Puerto Rico to 61% after the putative total extermination of the native population, arriving from various Spanish and Portuguese colonies, would by chance form a population of approximately median haplogroup diversity. More likely, most Amerindian mtDNAs in Puerto Rico have their origin in the native Taíno population.

The scarcity and the geographic distribution of haplogroup D mtDNAs in Puerto Rico suggest that it may have been imported during colonial times. Its geographic distribution is similar to that of Sub-Saharan African mtDNAs, with a higher frequency on the eastern side of longitude 66°16' W. It is known that, for centuries, one of the main Indian slave harbors in the Americas was located in Coro, northwestern Venezuela (Sued-Badillo, 1995). It provided plenty of Indian slaves to Aruba in the 19th century (Nooyen, 1965), where haplogroup D is predominant (Toro-Labrador et al., 2003), and it is possible that it provided slaves to Puerto Rico in that period of time. However, haplogroup D was found in six of 24 Taíno bone samples from the La Caleta archaeological site in the Dominican Republic (Lalueza-Fox et al., 2001), suggesting that genetic differences between Taínos on both islands were larger than the few cultural differences that have been acknowledged (Veloz-Maggiolo, 1991; Rouse, 1992).

Indian slave trade notwithstanding, the predominance of Amerindian mtDNAs in modern Puerto Rico coupled with the very large Atlantic slave trade strongly suggests that most of the Amerindian mtDNAs found are native, and that haplogroup A was predominant among the Puerto Rico Taínos. Thus, it is intriguing that ancient DNA studies on a total of 43 ancient remains from three archaeological sites in Cuba and one each in Puerto Rico and the Dominican Republic have found only one remain belonging to haplogroup A (Sánchez-Crespo, 1999; Lalueza-Fox et al., 2001, 2003). One explanation for this is that genetic diversity in Taínos may have existed mostly between and not within settlements. This could be explained by the matrilineal descent system of the Taínos, in which newborns would join their maternal clan (Keegan, 1997).

PC analysis was performed in an attempt to associate the Taínos to some continental region. The general picture that emerged in the PC1-PC3 plot (Fig. 6) was that of regional continuity affected frequently by population demographic events such as mass population movements, admixture, and genetic bottlenecks. The populations of five

of the six North, Meso and Central American regions showed some clustering, as did the populations around Lake Titicaca and further south. All three Eastern North American and four of the five Great Plains populations, which have experienced population movement and admixture associated with the eastward migration of Algonquian-speaking groups (Malhi et al., 2001), fell in the bottom-left quadrant. Nine of the 15 North American Southwest populations clustered in the bottom-right quadrant, the two recently arrived (after A.D. 1000) Na-Dene-speaking populations (Navajo and Apache) being among those scattered. In addition, the four Mesoamerican populations fell close to the axes intersection. In a fashion similar to that of North American Southwest populations, five of the eight populations of eastern Central America were found in the upper-right quadrant, the two populations strongly influenced by recent migrations from Colombia (Emberá and Wounan) (Kolman and Bermingham, 1997) being among the three scattered. Not showing any cohesiveness were the populations of the North American Southeast, whose populations may have experienced genetic bottlenecks produced by drastic population declines after contact (Weiss and Smith, 2003), and those from Colombia, the Amazon, and northern Argentina. Genetic isolation of small populations may have resulted from the extreme topography of the northern Andes. Larger genetic drift as a consequence of the smaller effective population sizes that may have resulted from restricted mobility in this topography, and later through the dense tropical forests of the Amazon, may have been translated into widely scattered populations in the PC plot for Colombia, the Amazon, and northern Argentina. By contrast, most of the populations in the highlands around Lake Titicaca and further south that have been found through control region sequence analysis to have had large long-term effective population sizes (Fuselli et al., 2003), plotted close to each other. Five of the nine Lake Titicaca populations formed a cluster in the upper-right quadrant, and all five southern South American populations fell in the upper-left quadrant.

Our PC analysis failed to give an indication of the possible continental origins of the Taínos. Their sophisticated social and political organization, their agroceramic culture, religious rituals and Arawak language unmistakenly reveal the South American origin of their culture. However, the scattering of Colombian and Amazonian populations in the plot, coupled with the fact that the Taínos may have developed from the admixture of agroceramic South American cultures with nomads originating elsewhere (Veloz-Maggiolo, 1991; Rouse, 1992), further complicated the analysis. Clearly, expanded ancient DNA studies must be undertaken to better understand the prehistoric migrations that gave rise to the Taínos. Studies on modern populations, although affected by postcolonization migrations, may be useful in guiding and complementing ancient DNA studies.

The results presented here led us to reinterpret previous results. The 72.5% Amerindian mtDNA frequency found west of longitude 66°16' W is very similar to the 69.6% reported by Martínez-Cruzado et al. (2001) for a group composed of 56 people living in communities known historically for their strong Indian background or volunteers having a mother or maternal grandmother with Indian-like phenotypic traits. Only four of these 56 people traced their maternal lineage to the east of longitude 66°16' W, the remainder tracing it to the west. Thus, their relatively high Amerindian maternal ancestry can be explained simply by the geographical location of their maternal lineage west of longitude 66°16' W. That is, if they share the location of their maternal lineages west of longitude 66°16' W, a Puerto Rican with maternal ancestors with Indian-like phenotypic traits or living in communities known historically for their strong Indian background is not more likely to have an Amerindian mtDNA than another who does not. The significantly reduced Amerindian mtDNA frequency (52.6%) obtained from a random sample of 38 Puerto Ricans (Martínez-Cruzado et al., 2001) can be explained by the high number of participants that stemmed from east of longitude 66°16' W. If only those tracing their maternal lineage west of longitude 66°16' W are considered, the difference with the general population west of longitude 66°16' W turns out to be insignificant (not shown).

Traditional history holds that Indians became extinct from disease, war and slavery, leaving the whole island for the development of the Spanish colony. However, a new current of thought holds that although the number of Indians was lowered considerably by the conquest, and that their political, cultural and religious systems disappeared in one or two generations, many survived and adapted to the conditions imposed by the colonial order, most of them living away from the colonial towns and plantations, but not in the hostile environment of the mountain chains (Sued-Badillo, 1995). The numbers of colonial settlers and the lands occupied by them were small enough to leave sufficient fertile cattle and crop land for Indians and mixed people of Indian ancestry whose presence was largely ignored by the colonial government, and who developed into the so-called *criollos* of the late 18th and 19th centuries. Our overall results and the observed genetic bipartition defined by longitude 66°16' W strongly support this view. Twelve of the thirteen sugar-producing mills in Puerto Rico during the 16th century were located east of longitude 66°16' W (Gelpí-Baíz, 2000). It is thus to be expected that most of the people related in any way to the colonial society established themselves in this region. However, and by no reason that could be related to any initiative from Spain, the majority of the population lived west of longitude 66°16' W by 1776. Our work suggests that these people had a very high Amerindian ancestry. Furthermore, with the exception of San Juan and surrounding areas, the eastern side of Puerto Rico was virtually uninhabited (Abbad, 1959). Thus, land availability may have driven most of the 19th century immigrants to settle east of longitude 66°16' W. Because most of the immigrants were of non-Amerindian origin, the result was a stronger reduction of the Amerindian frequency east than west of longitude 66°16' W.

Sub-Saharan African and West Eurasian origins

It is apparent from a review of the literature on the Atlantic slave trade and Puerto Rico that the coast that extends from Ghana to Cameroon was the largest source of slaves to Puerto Rico, but that large numbers were also brought from the stretch extending from Senegambia to Sierra Leone, and from Congo and Angola, perhaps in that

order, in addition to smaller contributions from other African regions.

Our PC analysis succeeds in distinguishing the West African populations from those of Mozambique. Furthermore, it plots the Brazilian population, which must have a very large Angolan component (Thomas, 1997), separate from both and next to the Bubi population that lives in the island of Bioko close to the Cameroon coast. This suggests that when sufficient data from Angola and Congo become available, PC analyses may be able to distinguish clusters corresponding to West, Southeast, and Central-West Africa.

A geographic gradient can be observed within the West Africa cluster at the left half of PC1 (Fig. 4), where the frequencies of L1b, L2* and L3b (all more frequent in West Africa than in other African regions) are the main contributors in that order. Those populations in or very close to Senegambia are found at the left edge of the cluster and those in or closer to the Gulf of Guinea toward the right edge. The PC1 order of the populations created by the slave trade is consistent with historical accounts. The Cape Verde Islands, composed mainly of Senegambians but having also received people from other African regions, plot at the right edge of the Senegambian group. São Tomé, located in the Gulf of Guinea, and receiving mainly people from Ghana to northern Gabon (Tomás et al., 2002), lies at the right edge of the West African cluster. Brazil, with the largest Angolan and smallest Senegambian components (Salas et al., 2004), lies to the right of the West African cluster. The Puerto Rico population lies between the Cape Verde and São Tomé populations, suggesting that it contains a Senegambian component smaller than that of Cape Verde but larger than that of São Tomé, and Gulf of Guinea, Congo and Angolan components smaller than those of São Tomé but larger than those of Cape Verde.

The PC analysis of West Eurasian mtDNAs suggests that many of the West Eurasian women arriving to Puerto Rico were non-European in origin. The European populations cluster tightly, but the Puerto Rico population falls out of the European cluster, and is as close to it as to the Moroccan cluster in the first plot, and closer to the Turkish-Armenian-Kurdish cluster in the second (Fig. 5). In addition, 11 of the 18 haplogroup U West Eurasian mtDNAs found in Puerto Rico are likely not of European origin. This should not be surprising, as the vast majority of the early colonizers were single men. This fact not only accounts for the disparity between West Eurasian Y-chromosome and mtDNA frequencies in Puerto Rico (unpublished results), but generated in the first decades of colonization a demand for white female slaves to the New World. Historical documents show that in 1512 the Spanish Crown encouraged the exportation of white female slaves to San Juan (Fernández-Méndez, 1970), and that in 1530 licenses were granted to export white Moorish slaves to the Americas, including to people with strong ties to Puerto Rico (Thomas, 1997). Raids of Arab towns produced many slaves for the Spaniards through the 16th century, and Moorish and Berbers (Álvarez-Nazario, 1974), as well as Slavs and Turkish (Thomas, 1997), could still be found among slaves late in the 17th century. Moreover, in the 17th century, poor Canarian farmers moved with their families to Puerto Rico seeking incentives that were granted as part of a concerted effort to increase the white population of Puerto Rico (Fernández-Méndez, 1970). The location of the Puerto Rico population outside of the European cluster in the PC plot suggests that the combined input to Puerto Rico of all these sources was substantial as compared to the European input.

It is noteworthy that the motif of sequence type 16224-16270, which represents eight samples and is by far the most frequent among our West Eurasian U mtDNAs (Table 4), has not been found elsewhere except for one sample each in the Canarian islands of Tenerife and Lanzarote that also possessed a transition at 16093, a site that was not sequenced in our samples. It is thus evident that the samples possessing the 16224, 16270 motif are related to North Africa, probably to the Canary Islands, and maybe to the *guanches* (Canarian natives), because Lanzarote exhibits a strongly autochthonous genetic makeup (Rando et al., 1999). Furthermore, two other samples were identified as belonging to the Canarian-specific U6b clade, and one other to the North African-specific U6a. Thus, 11 of the 18 West Eurasian U mtDNAs may be related to North Africans. Only the remaining seven samples could be of European origin. Combined with the PC analysis, this observation suggests that the European contribution to the West Eurasian mtDNA pool of Puerto Rico could be less than half. That North African and Sub-Saharan African haplogroup U sequence types can be found at copy numbers of seven and nine, while those of likely European origin are never found at more than two, suggests that most of the European mtDNAs were introduced in Puerto Rico late in her history.

CONCLUSIONS

The hierarchical strategy employed here for RFLP mtDNA haplogroup identification was shown to be effective in the highly admixed population of Puerto Rico. The 10394 *Dde*I/10397 *Alu*I motif of each sample was determined first, followed by testing the haplogroup-defining markers of all haplogroups within the predetermined motif, and using HVR-I sequences to resolve inconclusive results. The effectiveness of the strategy depended largely on the stability of the motif, which was found to be inconsistent with the mtDNA haplogroup in only 6 of the 800 samples. Because, with the exception of haplogroup U, all African haplogroups have (+/-) motifs whereas none of the Amerindian haplogroups are, these results suggest that testing the 10394 *Dde*I/10397 *Alu*I motif is an excellent starting point for sorting out African and Amerindian samples from mixed populations with high African and Amerindian affinities, such as those in the Caribbean.

The findings here reported are consistent with most historical accounts, with the notable exception of the high frequency (weighted, 61.3%) of Amerindian mtDNAs found currently in Puerto Rico, among which haplogroups A (52.4%) and C (36.0%) are the most common. Their high frequency lead us to the conclusion that most Amerindian mtDNAs in Puerto Rico should stem from the indigenous Taínos, and that haplogroups A and C were predominant among them. These results are in agreement with ancient DNA studies only in the high frequency of haplogroup C. Ancient DNA studies have so far found only one haplogroup A mtDNA among 15 Ciboney remains in Cuba (Lalueza-Fox

et al., 2003), and none in the remains of 24 Taínos in the Dominican Republic (Lalueza-Fox et al., 2002), and four Pre-Taínos in Puerto Rico (Sánchez-Crespo, 1999). Furthermore, the low frequency of haplogroup D (2.9%) currently in Puerto Rico and its concentration in the areas of higher Sub-Saharan African ancestry suggests that it may have been absent in pre-Columbian Puerto Rico, but it was found in six of the 24 Taíno remains of the Dominican Republic and in five of the 15 Cuban Ciboney remains. Poor sampling and large genetic differences between archaeological sites could explain the virtual absence of haplogroup A in ancient samples. Substantial genetic differences between the islands, consisting of the absence in Puerto Rico of haplogroup D but its presence elsewhere, may account for the disparity in haplogroup D frequencies.

Because of their Arawakan culture, Taínos would be expected to be closely related to South American tribes. A haplogroup frequency-based principal component analysis shows some compartmentalization of regional populations, but not for Colombian or Amazonian populations, which tend to show lower effective population sizes, lower haplogroup diversities, and be more susceptible to genetic drift. Hence, these populations scatter widely throughout the plot, and the Puerto Rico population cannot be related to any particular region.

A principal component plot identifies West Africa as the main supplier of Sub-Saharan African mtDNAs to Puerto Rico and to other populations created by the slave trade such as São Tomé and Cape Verde, but not Brazil. The main contributors to the first principal component of this plot are haplogroups considered West African-specific. This component generates a geographic gradient in which the westernmost populations of West Africa are placed at the left end of the plot, and populations further to the east up to the Gulf of Guinea are placed further to the right. Along this component, Puerto Rico falls between Cape Verde to the left, and São Tomé to the right, suggesting a West African composition for Puerto Rico intermediate between these two populations. The strong West African composition of the Sub-Saharan African fraction of Puerto Rico becomes evident by the relatively high frequency of a U5b HVR-I sequence type previously found hitherto only in West Africa, and defined here as clade U5b2.

For having been an European colony for 405 years, it would be expected that the West Eurasian fraction of Puerto Rico would show strong affinities with Europe. Principal component analysis shows that this is not the case. While European populations tend to cluster tightly, the Puerto Rico population remains separated from all populations, being approximately equally close to the European, Moroccan, and Turkish-Armenian-Kurdish clusters. HVR-I sequences of Puerto Rican haplogroup U mtDNAs show sequence types closely related to North Africa and the Canary Islands, as well as to Europe. North African and Canarian sequence types are not more varied that those European but have a higher average copy number, suggesting that their presence in Puerto Rico preceded that of most Europeans.

Within Puerto Rico, the expected difference in African mtDNA frequencies between coastal and noncoastal municipalities was found to be statistically insignificant. However, a highly statistically significant difference was found between the eastern and western sides of La Plata River, east of which most of the 16th century sugar mills were established. The weighted Amerindian frequency was higher in the western (72.3%) than in the eastern side (50.0%). In addition, pockets of relatively low Amerindian frequencies were found surrounding harbors.

As the area of the island of Puerto Rico is only 9,104 km^2, this work shows how much population structuring can occur within small regions, especially between metropolitan-cosmopolitan areas and nearby mountains which may hold old, traditional populations. This observation suggests that studies based on careful sampling in traditional populations, unaffected by frequent migrations common in metropolitan areas, may offer further information on the prehistorical migrations that gave rise to continental populations around the world.

ACKNOWLEDGMENTS

We are deeply indebted to the 800 people who were randomly selected, received us at their homes, and willingly participated in the project with informed consent. We are very thankful to T. Arroyo-Cordero, A. Ayala-Rodríguez, H. Cerdá-Ramos, L. Colón-Negrón, J. Henry-Sánchez, R. Hernández-Rodríguez, J. Irizarry-Ramos, D. Knudson-González, M. Lacourt-Ventura, H. López-López, R. Lugo-Sánchez, F. Maldonado-Chamorro, N. Martínez-Aquino, A. Merced-Alejandro, C. Montalvo-Vélez, P. Ochoa-Méndez, J.C. Ortiz-Santiago, T. Pagán-Ortega, J. Pérez-Sepúlveda, E. Ríos-Santiago, R. Rodríguez-Ramos, F. Rosas-Rivera, A. Seda-Martínez, C. Varela-Valentín, and L. Vélez-Ortiz, students of the University of Puerto Rico at Mayagüez (UPRM) who undertook the strenuous task of identifying the selected households and adults, contacting the selected adults, educating them on the project, interviewing them, and collecting their participating signatures and samples. The significance of this project was greatly enhanced by the contribution of Walter Díaz and the Center for Applied Social Research (UPRM) in terms of its sampling frame for survey research and the facilities and resources it provided for data analysis. Melissa Rodríguez-Sánchez and Mónica Vega-Hernández did a great job entering the data in the SPSS program. We thank Robert Donnelly for his automated sequencing and oligonucleotide synthesis services at the Molecular Resources Facility of the University of Medicine and Dentistry of New Jersey. We are indebted to Julio Quintana, who constructed the triangular graphic of ancestry distribution among municipalities, Roy Ruiz, who worked Figure 3, and Martin Richards, who provided us the PC computer package. We also thank Duane Kolterman and John Uscian for reviewing our manuscript before submission, and two excellent anonymous reviewers for their superb job. This project was supported by the National Science Foundation Physical Anthropology Program grant SBR-9904252 to JCMC.

LITERATURE CITED

Abbad FI. 1959. Historia geográfica, civil y natural de la Isla de San Juan Bautista de Puerto Rico. San Juan, Puerto Rico: Ediciones de la Universidad de Puerto Rico.

Alegría RE. 1985. Notas sobre la procedencia cultural de los esclavos negros de Puerto Rico durante la segunda

mitad del siglo XVI. La Revista del Centro de Estudios Avanzados de Puerto Rico y el Caribe 1:58-79.

Alegría M, McGuire T, Vera M, Canino G, Matías L, and Calderón J. 2001. Changes in access to mental health care among the poor and nonpoor: results from the health care reform in Puerto Rico. Am J Public Health 91:1431-1434.

Álvarez-Nazario M. 1974. El elemento afronegroide en el español de Puerto Rico. San Juan, Puerto Rico: Instituto de Cultura Puertorriqueña.

Alves-Silva J, da Silva Santos M, Guimarães PEM, Ferreira ACS, Bandelt HJ, Peña SDJ, and Ferreira Prado V. 2000. The ancestry of Brazilian mtDNA lineages. Am J Hum Genet 67:444-461.

Anderson S, Bankier AT, Barrell BG, de Bruijn MHL, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJH, Staden R, and Young IG. 1981. Sequence and organization of the human mitochondrial genome. Nature 290:457-465.

Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, and Howell N. 1999. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nature Genetics 23:147.

Bailliet G, Rothhammer F, Carnese FR, Bravi CM, and Bianchi NO. 1994. Founder mitochondrial haplotypes in Amerindian populations. Am J Hum Genet 54:27-33.

Ballinger SW, Schurr TG, Torroni A, Gan YY, Hodge JA, Hassan K, Chen KH, and Wallace DC. 1992. Southeast Asian mitochondrial DNA analysis reveals genetic continuity of ancient Mongoloid migrations. Genetics 130:139-152.

Bamshad M, Kivisild T, Watkins WS, Dixon ME, Ricker CE, Rao BB, Naidu JM, Prasad BVR, Reddy PG, Rasanayagam A, Papiha SS, Villems R, Redd AJ, Hammer MF, Nguyen SV, Carroll ML, Batzer MA, and Jorde LB. 2001. Genetic evidence on the origins of Indian caste populations. Genome Res 11:994-1004.

Batista O, Kolman CJ, and Bermingham E. 1995. Mitochondrial DNA diversity in the Kuna Amerinds of Panamá. Hum Mol Genet 4:921-929.

Batista dos Santos SE, Rodrigues JD, Ribeiro dos Santos AKC, and Zago MA. 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.

Bert F, Corella A, Gené M, Pérez-Pérez A, and Turbón D. 2001. Major mitochondrial DNA haplotype heterogeneity in highland and lowland Amerindian populations from Bolivia. Hum Biol 73:1-16.

Betty DJ, Chin-Atkins AN, Croft L, Sraml M, and Easteal S. 1996. Multiple independent origins of the COII/tRNALys intergenic 9-bp mtDNA deletion in aboriginal Australians. Am J Hum Genet 58:428-433.

Bortolini MC, Salzano FM, Bau CHD, Layrisse Z, Petzl-Erler ML, Tsuneto LT, Hill K, Hurtado AM, Castro-de-Guerra D, Bedoya G, and Ruiz-Linares A. 2002. Y-chromosome biallelic polymorphisms and Native American population structure. Ann Hum Genet 66:255-259.

Bortolini MC, Salzano FM, Thomas MG, Stuart S, Nasanen SPK, Bau CHD, Hutz MH, Layrisse Z, Petzl-Erler ML, Tsuneto LT, Hill K, Hurtado AM, Castro-de-Guerra D, Torres MM, Groot H, Michalski R, Nymadawa P, Bedoya G, Bradman N, Labuda D, and Ruiz-Linares A. 2003. Y-chromosome evidence for differing ancient demographic histories in the Americas. Am J Hum Genet 73:524-539.

Brakez Z, Bosch E, Izaabel H, Akhayat O, Comas D, Bertranpetit J, and Calafell F. 2001. Human mitochondrial DNA sequence variation in the Moroccan population of the Souss area. Ann Hum Biol 28:295-307.

Brau S. 1904. Historia de Puerto Rico. New York: Appleton and Co.

Brehm A, Pereira L, Bandelt HJ, Prata MJ, and Amorim A. 2002. Mitochondrial portrait of the Cabo Verde archipelago: the Senegambian outpost of Atlantic slave trade. Ann Hum Genet 66:49-60.

Brown MD, Hosseini SH, Torroni A, Bandelt HJ, Allen JC, Schurr TG, Scozzari R, Cruciani F, and Wallace DC. 1998. mtDNA haplogroup X: an ancient link between Europe/Western Asia and North America? Am J Hum Genet 63:1852-1861.

Brown MD, Sun F, and Wallace DC. 1997. Clustering of Caucasian Leber Hereditary Optic Neuropathy patients containing the 11778 or 14484 mutations on an mtDNA lineage. Am J Hum Genet 60:381-387.

Carvajal-Carmona LG, Soto ID, Pineda N, Ortiz-Barrientos D, Duque C, Ospina-Duque J, McCarthy M, Montoya P, Álvarez VM, Bedoya G, and Ruiz-Linares A. 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.

Carvalho-Silva DR, Santos FR, Rocha J, and Peña SDJ. 2001. The phylogeography of Brazilian Y-chromosome lineages. Am J Hum Genet 68:281-286.

Chen YS, Olckers A, Schurr TG, Kogelnik AM, Huoponen K, and Wallace DC. 2000. mtDNA variation in the South African Kung and Khwe-and their genetic relationships to other African populations. Am J Hum Genet 66:1362-1383.

Chen YS, Torroni A, Excoffier L, Santachiara-Benerecetti AS, and Wallace DC. 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.

Clark VJ, Sivendren S, Saha N, Bentley GR, Aunger R, Sirajuddin SM, and Stoneking M. 2000. The 9-bp deletion between the mitochondrial lysine tRNA and COII genes in tribal populations of India. Hum Biol 72:273-285.

Colón HM, Robles RR, Canino G, and Sahai H. 2001. Prevalence and correlates of DSM-IV substance use disorders in Puerto Rico. Bol Asoc Med PR 93:12-22.

Comas D, Calafell F, Mateu E, Pérez-Lezaun A, Bosch E, Martínez-Arias R, Clarimon J, Facchini F, Fiori G, Luiselli D, Pettener D, and Bertranpetit J. 1998. Trading genes along the silk road: mtDNA sequences and the origin of Central Asian populations. Am J Hum Genet 63:1824-1838.

Côrte-Real HBSM, Macaulay VA, Richards MB, Hariti G, Issad MS, Cambon-Thomsen A, Papiha S, Bertranpetit J, and Sykes BC. 1996. Genetic diversity in the Iberian Peninsula determined from mitochondrial sequence analysis. Ann Hum Genet 60:331-350.

Cruciani F, Santolamazza P, Shen P, Macaulay V, Moral P, Olckers A, Modiano D, Holmes S, Destro-Bisol G, Coia V, Wallace DC, Oefner PJ, Torroni A, Cavalli-Sforza LL, Scozzari R, and Underhill PA. 2002. A back migration from Asia to Sub-Saharan Africa is supported by high-resolution analysis of human Y-chromosome haplotypes. Am J Hum Genet 70:1197-1214.

Demarchi DA, Panzetta-Dutari GM, Motran CC, López de Basualdo MA, and Marcellino AJ. 2001. Mitochondrial DNA haplogroups in Amerindian populations from the Gran Chaco. Am J Phys Anthropol 115:199-203.

Díaz-Soler LM. 2000. Historia de la esclavitud negra en Puerto Rico. San Juan, Puerto Rico: Editorial de la Universidad de Puerto Rico.

Fernández-Cobo M, Jobes DV, Yanagihara R, Nerurkar VR, Yamamura Y, Ryschkewitsch CF, and Stoner GL. 2001. Reconstructing population history using JC virus: Amerinds, Spanish, and Africans in the ancestry of modern Puerto Ricans. Hum Biol 73:385-402.

Fernández-Méndez E. 1970. Historia cultural de Puerto Rico. San Juan, Puerto Rico: Ediciones El Cemí.

Fernández-Méndez. 2000. Desarrollo histórico de la sociedad puertorriqueña. In: Padilla-Rodríguez C, editor. ¿Qué somos? San Juan, Puerto Rico: Instituto de Cultura Puertorriqueña. p 51-80.

Finnilä S, Hassinen IE, Ala-Kokko L, and Majamaa K. 2000. Phylogenetic network of the mtDNA haplogroup U in northern Finland based on sequence analysis of the complete coding region by conformative-sensitive gel electrophoresis. Am J Hum Genet 66:1017-1026.

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

Forster P, Torroni A, Renfrew C, and Röhl A. 2001. Phylogenetic star contraction applied to Asian and Papuan mtDNA evolution. Mol Biol Evol 18:1864-1881.

Fuselli S, Tarazona-Santos E, Dupanloup I, Soto A, Luiselli D, and Pettener D. 2003. Mitochondrial DNA diversity in South America and the genetic history of Andean highlanders. Mol Biol Evol 20:1682-1691.

Gelpí-Baíz E. 2000. Siglo en blanco. San Juan, Puerto Rico: Editorial de la Universidad de Puerto Rico.

Ginther C, Corach D, Penacino GA, Rey JA, Carnese FR, Hutz MH, Anderson A, Just J, Salzano FM, and King MC. 1993. Genetic variation among the Mapuche Indians from the Patagonian region of Argentina: Mitochondrial DNA sequence variation and allele frequencies of several nuclear genes. In: Peña SDJ, Chakraborty R, Epplen JT, Jeffreys AJ, editors. DNA fingerprinting: state of the science. Switzerland: Birkhauser Verlag Bassel. p 211-219.

Graven L, Passarino G, Semino O, Boursot P, Santachiara-Benerecetti S, Langaney A, and Excoffier L. 1995. Evolutionary correlation between control region sequence and restriction polymorphisms in the mitochondrial genome of a large Senegalese Mandenka sample. Mol Biol Evol 12:334-345.

Green LD, Derr JN, and Knight A. 2000. mtDNA affinities of the peoples of North-Central Mexico. Am J Hum Genet 66:989-998.

Hammer MF, Karafet TM, Redd AJ, Jarjanazi H, Santachiara-Benerecetti S, Soodyall H, and Zegura SL. 2001. Hierarchical patterns of global human Y-chromosome diversity. Mol Biol Evol 18:1189-1203.

Hanis CL, Hewett-Emmett D, Bertin TK, and Schull WJ. 1991. Origins of US Hispanics: implications for diabetes. Diabetes Care 14:618-627.

Herrnstadt C, Elson JL, Fahy E, Preston G, Turnbull DM, Anderson C, Ghosh SS, Olefsky JM, Beal MF, Davis RE, and Howell N. 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.

Hofmann S, Jaksch M, Bezold R, Mertens S, Aholt S, Paprotta A, and Gerbitz KD. 1997. Population genetics and disease susceptibility: characterization of central European haplogroups by mtDNA gene mutations, correlation with D loop variants and association with disease. Hum Mol Genet 6:1835-1846.

Horai S, Kondo R, Nakagawa-Hattori Y, Hayashi S, Sonoda S, and Tajima K. 1993. Peopling of the Americas, founded by four major lineages of mitochondrial DNA. Mol Biol Evol 10:23-47.

Howell N, Oostra RJ, Bolhuis PA, Spruijt L, Clarke LA, Mackey DA, Preston G, and Herrnstadt C. 2003. Sequence analysis of the mitochondrial genomes from Dutch pedigrees with Leber Hereditary Optic Neuropathy. Am J Hum Genet 72:1460-1469.

Huoponen K, Torroni A, Wickman PR, Sellitto D, Gurley DS, Scozzari R, and Wallace DC. 1997. Mitochondrial DNA and Y chromosome-specific polymorphisms in the Seminole tribe of Florida. Eur J Hum Genet 5:25-34.

Hurles ME, Irven C, Nicholson J, Taylor PG, Santos FR, Loughlin J, Jobling MA, and Sykes BC. 1998. European Y-chromosomal lineages in Polynesians: a contrast to the population structure revealed by mtDNA. Am J Hum Genet 63:1793-1806.

Hutchin T, and Cortopassi G. 1995. A mitochondrial DNA clone is associated with increased risk for Alzheimer disease. Proc Natl Acad Sci USA 92:6892-6895.

Johns DR, and Berman J. 1991. Alternative, simultaneous Complex I mitochondrial DNA mutations in Leber's Hereditary Optic Neuropathy. Biochem Biophys Res Commun 174:1324-1330.

Kaestle FA, and Smith DG. 2001. Ancient mitochondrial DNA evidence for prehistoric population movement: the Numic expansion. Am J Phys Anthropol 115:1-12.

Karafet T, Xu L, Du R, Wang W, Feng S, Wells RS, Redd AJ, Zegura SL, and Hammer MF. 2001. Paternal population history of East Asia: sources, patterns, and microevolutionary processes. Am J Hum Genet 69:615-628.

Kayser M, Brauer S, Weiss G, Schiefenhövel W, Underhill PA, and Stoneking M. 2001. Independent histories of human Y chromosomes from Melanesia and Australia. Am J Hum Genet 68:173-190.

Keegan WF. 1997. "No man [or woman] is an island": elements of Taíno social organization. In: Wilson SM, editor. The indigenous people of the Caribbean. Gainesville: University Press of Florida. p 111-117.

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

Kivisild T, Bamshad MJ, Kaldma K, Metspalu M, Metspalu E, Reidla M, Laos S, Parik J, Watkins WS, Dixon ME, Papiha SS, Mastana SS, Mir MR, Ferak V, and Villems R. 1999. Deep common ancestry of Indian and western-Eurasian mitochondrial lineages. Curr Biol 9:1331-1334.

Kivisild T, Tolk HV, Parik J, Wang Y, Papiha SS, Bandelt HJ, and Villems R. 2002. The emerging limbs and twigs of the East Asian mtDNA tree. Mol Biol Evol 19:1737-1751.

Kolman CJ, and Bermingham E. 1997. Mitochondrial and nuclear DNA diversity in the Chocó and Chibcha Amerinds of Panamá. Genetics 147:1289-1302.

Kolman CJ, Bermingham E, Cooke R, Ward RH, Arias TD, and Guionneau-Sinclair F. 1995. Reduced mtDNA diversity in the Ngöbé Amerinds of Panamá. Genetics 140:275-283.

Kong QP, Yao YG, Sun C, Bandelt HJ, Zhu CL, and Zhang YP. 2003. Phylogeny of East Asian mitochondrial DNA lineages inferred from complete sequences. Am J Hum Genet 73:671-676.

Krings M, Salem AH, Bauer K, Geisert H, Malek AK, Chaix L, Simon C, Welsby D, Di Rienzo A, Utermann G, Sajantila A, Pääbo S, and Stoneking M. 1999. mtDNA analysis of Nile River Valley populations: a genetic corridor or a barrier to migration? Am J Hum Genet 64:1166-1176.

Lalueza-Fox C, Gilbert MTP, Martínez-Fuentes AJ, Calafell F, and Bertranpetit J. 2003. Mitochondrial DNA from Pre-Columbian Ciboneys from Cuba and the prehistoric colonization of the Caribbean. Am J Phys Anthropol 121:97-108.

Lalueza-Fox C, Luna-Calderón F, Calafell F, Morera B, and Bertranpetit J. 2001. MtDNA from extinct Taínos and the peopling of the Caribbean. Ann Hum Genet 65:137-151.

Lamminen T, Huoponen K, Sistonen P, Juvonen V, Lahermo P, Aula P, Nikoskelainen E, and Savontaus ML. 1997. mtDNA haplotype analysis in Finnish families with Leber Hereditary Optic Neuropathy. Eur J Hum Genet 5:271-279.

Lell JT, Sukernik RI, Starikovskaya YB, Su B, Jin L, Schurr TG, Underhill PA, and Wallace DC. 2002. The dual origin and Siberian affinities of Native American Y chromosomes. Am J Hum Genet 70:192-206.

Lorenz JG, and Smith DG. 1996. Distribution of four founding mtDNA haplogroups among native North Americans. Am J Phys Anthropol 101:307-323.

Maca-Meyer N, González AM, Larruga JM, Flores C, and Cabrera VM. 2001. Major genomic mitochondrial lineages delineate early human expansions. BMC Genetics 2:13.

Macaulay V, Richards M, Hickey E, Vega E, Cruciani F, Guida V, Scozzari R, Bonné-Tamir B, Sykes B, and Torroni A. 1999. The emerging tree of West Eurasian mtDNAs: a synthesis of control-region sequences and RFLPs. Am J Hum Genet 64:232-249.

Malaspina P, Tsopanomichalou M, Duman T, Stefan M, Silvestri A, Rinaldi B, García O, Giparaki M, Plata E, Kozlov AI, Barbujani G, Vernesi C, Papola F, Ciavarella G, Kovatchev D, Kerimova MG, Anagnou N, Gavrila L, Veneziano L, Akar N, Loutradis A, Michalodimitrakis EN, Terrenato L, and Novelletto A. 2001. A multistep process for the dispersal of a Y chromosomal lineage in the Mediterranean area. Ann Hum Genet 65:339-349.

Malhi RS, Mortensen HM, Eshleman JA, Kemp BM, Lorenz JG, Kaestle FA, Johnson JR, Gorodezky C, and Smith DG. 2003. Native American mtDNA prehistory in the American Southwest. Am J Phys Anthropol 120:108-124.

Malhi RS, Schultz BA, and Smith DG. 2001. Distribution of mitochondrial DNA lineages among Native American tribes of northeastern North America. Hum Biol 73:17-55.

Malyarchuk BA, Grzybowski T, Derenko MV, Czarny J, Wo□niak M, and Mi□cicka-□liwka D. 2002. Mitochondrial DNA variability in Poles and Russians. Ann Hum Genet 66:261-283.

Martínez-Cruzado, JC, Toro-Labrador G, Ho-Fung V, Estévez-Montero M, Lobaina-Manzanet A, Padovani-Claudio DA, Sánchez-Cruz H, Ortiz-Bermúdez P, and Sánchez-Crespo A. 2001. Mitochondrial DNA analysis reveals substantial Native American ancestry in Puerto Rico. Hum Biol 73:491-511.

Mateu E, Comas D, Calafell F, Pérez-Lezaun A, Abade A, and Bertranpetit J. 1997. A tale of two islands: population history and mitochondrial DNA sequence variation of Bioko and São Tomé, Gulf of Guinea. Ann Hum Genet 61:507-518.

Merriwether DA, and Ferrell RE. 1996. The four founding lineage hypothesis for the New World: a critical reevaluation. Mol Phylogenet Evol 5:241-246.

Merriwether DA, Rothhammer F, and Ferrell RE. 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 NR, Mondragón MC, Soto ID, Parra MV, Duque C, Ortiz-Barrientos D, García LF, Vélez ID, Bravo ML, Múnera JG, Bedoya G, Bortolini MC, and Ruiz-Linares A. 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.

Mishmar D, Ruiz-Pesini E, Golik P, Macaulay V, Clark AG, Hosseini S, Brandon M, Easley K, Chen E, Brown MD, Sukernik RI, Olckers A, and Wallace DC. 2003. Natural selection shaped regional mtDNA variation in humans. Proc Natl Acad Si USA 100:171-176.

Moraga ML, Rocco P, Miquel JF, Nervi F, Llop E, Chakraborty R, Rothhammer F, and Carvallo P. 2000. Mitochondrial DNA polymorphisms in Chilean aboriginal populations: implications for the peopling of the southern cone of the continent. Am J Phys Anthropol 113:19-29.

Negrón-Portillo M, and Mayo-Santana R. 1992. La esclavitud urbana en San Juan de Puerto Rico: estudio del registro de esclavos de 1872. Río Piedras, Puerto Rico: Ediciones Huracán.

Nooyen RH. 1965. Millefiori di Aruba. Aruba: Stitching Aruba Nostra.

Oota H, Kitano T, Jin F, Yuasa I, Wang L, Shintaroh U, Saitou N, and Stoneking M. 2002. Extreme mtDNA homogeneity in continental Asian populations. Am J Phys Anthropol 118:146-153.

O'Rourke DH, Hayes MG, and Carlyle SW. 2000. Spatial and temporal stability of mtDNA haplogroup frequencies in native North America. Hum Biol 72:15-34.

Pereira L, Gusmão L, Alves C, Amorim A, and Prata MJ. 2002. Bantu and European Y-lineages in Sub-Saharan Africa. Ann Hum Genet 66:369-378.

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

Quintana-Murci L, Semino O, Bandelt HJ, Passarino G, McElreavey K, and Santachiara-Benerecetti S. 1999. Genetic evidence of an early exit of Homo sapiens sapiens from Africa through eastern Africa. Nature Genetics 23:437-441.

Rando JC, Cabrera VM, Larruga JM, Hernández M, González AM, Pinto F, and Bandelt HJ. 1999. Phylogeographic patterns of mtDNA reflecting the colonization of the Canary Islands. Ann Hum Genet 63:413-428.

Rando JC, Pinto F, González AM, Hernández M, Larruga JM, Cabrera VM, and Bandelt HJ. 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.

Reidla M, Kivisild T, Metspalu E, Kaldma K, Tambets K, Tolk HV, Parik J, Loogväli EL, Derenko M, Malyarchuk B, Bermisheva M, Zhadanov S, Pennarun E, Gubina M, Golubenko M, Damba L, Fedorova S, Gusar V, Grechanina E, Mikerezi I, Moisan JP, Chaventré A, Khusnutdinova E, Osipova L, Stepanov V, Voevoda M, Achilli A, Rengo C, Rickards O, De Stefano GF, Papiha S, Beckman L, Janicijevic B, Rudan P, Anagnou N, Michalodimitrakis E, Koziel S, Usanga E, Geberhiwot T, Herrnstadt C, Howell N, Torroni A, and Villems R. 2003. Origin and diffusion of mtDNA haplogroup X. Am J Hum Genet 73:1178-1190.

Richards M, Macaulay V, Hickey E, Vega E, Sykes B, Guida V, Rengo C, Sellitto D, Cruciani F, Kivisild T, Villems R, Thomas M, Rychkov S, Rychkov O, Rychkov Y, Gölge M, Dimitrov D, Hill E, Bradley D, Romano V, Calì F, Vona G, Demaine A, Papiha S, Triantaphyllidis C, Stefanescu G, Hatina J, Belledi M, Di Rienzo A, Novelletto A, Oppenheim A, Nørby S, Al-Zaheri N, Santachiara-Benerecetti AS, Scozzari R, Torroni A, and Bandelt HJ. 2000. Tracing European founder lineages in the Near Eastern mtDNA pool. Am J Hum Genet 67:1251-1276.

Richards M, Macaulay V, Torroni A, and Bandelt HJ. 2002. In search of geographical patterns in European mitochondrial DNA. Am J Hum Genet 71:1168-1174.

Rickards O, Martínez-Labarga C, Lum JK, De Stefano GF, and Cann RL. 1999. mtDNA history of the Cayapa Amerinds of Ecuador: detection of additional founding lineages for the Native American populations. Am J Hum Genet 65:519-530.

Rodas C, Gelvez N, and Keyeux G. 2003. Mitochondrial DNA studies show asymmetrical Amerindian admixture in Afro-Colombian and mestizo populations. Hum Biol 75:13-30.

Rosser ZH, Zerjal T, Hurles ME, Adojaan M, Alavantic D, Amorim A, Amos W, Armenteros M, Arroyo E, Barbujani G, Beckman G, Beckman L, Bertranpetit J, Bosch E, Bradley D, Brede G, Cooper G, Côrte-Real HBSM, de Knijff P, Decorte R, Dubrova YE, Evgrafov O, Gilissen A, Glisic S, Gölge M, Hill EW, Jeziorowska A, Kalaydjieva L, Kayser M, Kivisild T, Kravchenko SA, Krumina A, Kučinskas V, Lavinha J, Livshits LA, Malaspina P, Maria S, McElreavey K, Meitinger TA, Mikelsaar AV, Mitchell RJ, Nafa K, Nicholson J, Nørby S, Pandya A, Parik J, Patsalis PC, Pereira L, Peterlin B, Pielberg G, Prata MJ, Previderé C, Roewer L, Rootsi S, Rubinsztein DC, Saillard J, Santos FR, Stefanescu G, Sykes BC, Tolun A, Villems R, Tyler-Smith C, and Jobling MA. 2000. Y-chromosomal diversity in Europe is clinal and influenced primarily by geography, rather than by language. Am J Hum Genet 67:1526-1543.

Rouse I. 1992. The Taínos: rise and decline of the people who greeted Columbus. New Haven, Connecticut: Yale University Press.

Ruiz-Pesini E, Lapeña AC, Diez-Sánchez C, Pérez-Martos A, Montoya J, Álvarez E, Díaz M, Urriés A, Montoro L, López-Pérez MJ, and Enríquez JA. 2000. Human mtDNA haplogroups associated with high or reduced spermatozoa motility. Am J Hum Genet 67:682-696.

Salas A, Richards M, De la Fe T, Lareu MV, Sobrino B, Sánchez-Diz P, Macaulay V, and Carracedo A. 2002. The making of the African mtDNA landscape. Am J Hum Genet 71:1082-1111.

Salas A, Richards M, Lareu MV, Scozzari R, Coppa A, Torroni A, Macaulay V, and Carracedo A. 2004. The African diaspora: mitochondrial DNA and the Atlantic Slave Trade. Am J Hum Genet 74:454-465.

Sánchez-Crespo A. 1999. Variación genética en una población indígena en Puerto Rico. MSc thesis. Mayagüez, Puerto Rico: University of Puerto Rico.

Sanger JP, Gannett H, and Wilcox WF. 1900. 1899 census report of Puerto Rico. Washington, DC: Government Printing Office.

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

Schurr TG, and Wallace DC. 2002. Mitochondrial DNA diversity in Southeast Asian populations. Hum Biol 74:431-452.

Scozzari R, Cruciani F, Santolamazza P, Sellitto D, Cole DEC, Rubin LA, Labuda D, Marini E, Succa V, Vona G, and Torroni A. 1997. mtDNA and Y chromosome-specific polymorphisms in modern Ojibwa: implications about the origin of their gene pool. Am J Hum Genet 60:241-244.

Semino O, Santachiara-Benerecetti AS, Falaschi F, Cavalli-Sforza LL, and Underhill PA. 2002. Ethiopians and Khoisan share the deepest clades of the human Y-chromosome phylogeny. Am J Hum Genet 70:265-268.

Silva WA, Bonatto SL, Holanda AJ, Ribeiro-dos-Santos AK, Paixao BM, Goldman GH, Abe-Sandes K, Rodriguez-Delfin L, Barbosa M, Pacó-Larson ML, Petzl-Erler ML, Valente V, Santos SEB, and Zago MA. 2002. Mitochondrial genome diversity of Native Americans supports a single early entry of founder populations into America. Am J Hum Genet 71:187-192.

Soodyall H, Vigilant L, Hill AV, Stoneking M, and Jenkins T. 1996. mtDNA control region sequence variation suggests multiple independent origins of an "Asian-specific" 9-bp deletion in Sub-Saharan Africans. Am J Hum Genet 58:595-608.

Starikovskaya YB, Sukernik RI, Schurr TG, Kogelnik AM, and Wallace DC. 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.

Sued-Badillo J. 1995. The theme of the indigenous in the national projects of the Hispanic Caribbean. In: Schmidt PR, Patterson TC, editors. Making alternative histories: the practice of archaeology and history in non-Western settings. Santa Fe, New Mexico: School of American Research Press. p 25-46.

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

Tapia A. 1945. Biblioteca histórica de Puerto Rico. San Juan, Puerto Rico: Instituto de Literatura Puertorriqueña.

Thomas H. 1997. The slave trade: the story of the Atlantic Slave Trade: 1440 - 1870. New York: Simon & Schuster.

Tomás G, Seco L, Seixas S, Faustino P, Lavinha J, and Rocha J. 2002. The peopling of São Tomé (Gulf of Guinea): origins of slave settlers and admixture with the Portuguese. Hum Biol 74:397-411.

Toro-Labrador G, Wever OR, and Martínez-Cruzado JC. 2003. Mitochondrial DNA analysis in Aruba: strong maternal ancestry of closely related Amerindians and implications for the peopling of northwestern Venezuela. Carib J Sci 39:11-22.

Torroni A, Bandelt HJ, Macaulay V, Richards M, Cruciani F, Rengo C, Martínez-Cabrera V, Villems R, Kivisild T, Metspalu E, Parik J, Tolk HV, Tambets K, Forster P, Karger B, Francalacci P, Rudan P, Janicijevic B, Rickards O, Savontaus ML, Huoponen K, Laitinen V, Koivumäki S, Sykes B, Hickey E, Novelletto A, Moral P, Sellitto D, Coppa A, Al-Zaheri N, Santachiara-Benerecetti AS, Semino O, and Scozzari R. 2001a. A signal, from human mtDNA, of postglacial recolonization in Europe. Am J Hum Genet 69:844-852.

Torroni A, Chen YS, Semino O, Santachiara-Benerecetti AS, Scott CR, Lott MT, Winter M, and Wallace DC. 1994a. mtDNA and Y-chromosome polymorphisms in four Native American populations from Southern Mexico. Am J Hum Genet 54:303-318.

Torroni A, Huoponen K, Francalacci P, Petrozzi M, Morelli L, Scozzari R, Obinu D, Savontaus ML, and Wallace DC. 1996. Classification of European mtDNAs from an analysis of three European populations. Genetics 144:1835-1850.

Torroni A, Lott MT, Cabell MF, Chen YS, Lavergne L, and Wallace DC. 1994b. 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, Miller JA, Moore LG, Zamudio S, Zhuang J, Droma T, and Wallace DC. 1994c. Mitochondrial DNA analysis in Tibet: implications for the origin of the Tibetan population and its adaptation to high altitude. Am J Phys Anthropol 93:189-199.

Torroni A, Neel JV, Barrantes R, Schurr TG, and Wallace DC. 1994d. Mitochondrial DNA "clock" for the Amerinds with its implications for timing their entry into North America. Proc Natl Acad Sci USA 91:1158-1162.

Torroni A, Petrozzi M, D'Urbano L, Sellitto D, Zeviani M, Carrara F, Carducci C, Leuzzi V, Carelli V, Barboni P, De Negri A, and Scozzari R. 1997. Haplotype and phylogenetic analysis 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.

Torroni A, Rengo C, Guida V, Cruciani F, Sellitto D, Coppa A, Luna-Calderón F, Simionati B, Valle G, Richards M, Macaulay V, and Scozzari R. 2001b. Do the four clades of the mtDNA haplogroup L2 evolve at different rates? Am J Hum Genet 69:1348-1356.

Torroni A, Schurr TG, Cabell MF, Brown MD, Neel JV, Larsen M, Smith DG, Vullo CM, and Wallace DC. 1993a. Asian affinities and continental radiation of the four founding Native American mtDNAs. Am J Hum Genet 53:563-590.

Torroni A, Schurr TG, Yang CC, Szathmary EJE, Williams RC, Schanfield MS, Troup GA, Knowler WC, Lawrence DN, Weiss KM, and Wallace DC. 1992. Native American mitochondrial DNA analysis indicates that the Amerind and the Nadene populations were founded by two independent migrations. Genetics 130:153-162.

Torroni A, Sukernik RI, Schurr TG, Starikovskaya YB, Cabell MF, Crawford MH, Comuzzie AG, and Wallace DC. 1993b. mtDNA variation of aboriginal Siberians reveals distinct genetic affinities with Native Americans. Am J Hum Genet 53:591-608.

Underhill PA, Passarino G, Lin AA, Shen P, Lahr MM, Foley RA, Oefner PJ, and Cavalli-Sforza LL. 2001. The phylogeography of Y chromosome binary haplotypes and the origins of modern human populations. Ann Hum Genet 65:43-62.

van der Walt JM, Nicodemus KK, Martin ER, Scott WK, Nance MA, Watts RL, Hubble JP, Haines JL, Koller WC, Lyons K, Pahwa R, Stern MB, Colcher A, Hiner BC, Jankovic J, Ondo WG, Allen Jr FH, Goetz CG, Small GW, Mastaglia F, Stajich JM, McLaurin AC, Middleton LT, Scott BL, Schmechel DE, Pericak-Vance MA, and Vance JM. 2003. Mitochondrial polymorphisms significantly reduce the risk of Parkinson Disease. Am J Hum Genet 72:804-811.

Veloz-Maggiolo M. 1991. Panorama histórico del Caribe precolombino. Dominican Republic: Banco Central de la República Dominicana.

Ward RH, Salzano FM, Bonatto SL, Hutz MH, Coimbra Jr CEA, and Santos RV. 1996. Mitochondrial DNA polymorphism in three Brazilian Indian tribes. Am J Hum Biol 8:317-323.

Watkins WS, Bamshad M, Dixon ME, Bhaskara R, Naidu JM, Reddy PG, Prasad BVR, Das PK, Reddy PC, Gai PB, Bhanu A, Kusuma YS, Lum JK, Fischer P, and Jorde LB. 1999. Multiple origins of the mtDNA 9-bp deletion in populations of South India. Am J Phys Anthropol 109:147-158.

Watson E, Forster P, Richards M, and Bandelt HJ. 1997. Mitochondrial footprints of human expansions in Africa. Am J Hum Genet 61:691-704.

Weiss DA, and Smith DG. 2003. Unexpected patterns of mitochondrial DNA variation among Native Americans from the southeastern United States. Am J Phys Anthropol 122:336-354.

Wrischnik L, Higuchi RG, Stoneking M, Erlich HA, Arnhelm N, and Wilson AC. 1987. Length mutations in human mitochondrial DNA: direct sequencing of enzymatically amplified DNA. Nucl Acids Res 15:529-542.

Yao YG, Kong QP, Bandelt HJ, Kivisild T, and Zhang YP. 2002a. Phylogeographic differentiation of mitochondrial DNA in Han Chinese. Am J Hum Genet 70:635-651.

Yao YG, Nie L, Harpending H, Fu YX, Yuan ZG, and Zhang YP. 2002b. Genetic relationship of Chinese ethnic populations revealed by mtDNA sequence diversity. Am J Phys Anthropol 118:63-76.

Zavala S. 1948. Estudios indianos. México: Publicaciones del Colegio de México.

Zegura SL, Karafet TM, Zhivotovsky LA, and Hammer MF. 2004. High-resolution SNPs and microsatellite haplotypes point to a single, recent entry of Native American Y chromosomes into the Americas. Mol Biol Evol 21:164-175.

Zerjal T, Wells RS, Yuldasheva N, Ruzibakiev R, and Tyler-Smith C. 2002. A genetic landscape reshaped by recent events: Y-chromosomal insights into Central Asia. Am J Hum Genet 71:466-482.

Zerjal T, Xue Y, Bertorelle G, Wells RS, Bao W, Zhu S, Qamar R, Ayub Q, Mohyuddin A, Fu S, Li P, Yuldasheva N, Ruzibakiev R, Xu J, Shu Q, Du R, Yang H, Hurles ME, Robinson E, Gerelsaikhan T, Dashnyam B, Mehdi SQ, and Tyler-Smith C. 2003. The genetic legacy of Mongols. Am J Hum Genet 72:717-721.

TABLE 1. Haplogroups with their motifs and defining markers, and primers used for their identification[1]

Motif[2]	Haplo-group	Defining markers	Primers[3]	Motif	Haplo-group[4]	Defining markers	Primers
(-/-)	A	+663 *Hae*III	L612 (22) H742 (23)	(+/-)	I	-4529 *Hae*II	L4462 (21) H4620 (19)
(-/-)	B	9bp del regV	L8216 (20) H8296 (20)	(+/-)	J	+4216 *Nla*III	L4160 (19) H4291 (21)
(-/-)	F	-12406 *Hpa*I	L12237 (21) H12485 (20)	(+/-)	K	+12308 *Hin*fI -9052 *Hae*II	L8921 (19) H9086 (21)
(-/-)	HV	-14766 *Mse*I	L14711 (20) H14885 (19)	(+/-)	L	+3592 *Hpa*I	L3517 (19) H3667 (21)
(-/-)	H	-14766 *Mse*I -7025 *Alu*I	L6958 (20) H7104 (19)	(+/-)	L1b	+3592 *Hpa*I +2349 *Dpn*II	As in L and L3e
(-/-)	V	-14766 *Mse*I -4577 *Nla*III	L4462 (21) H4620 (19)	(+/-)	L1c	+3592 *Hpa*I +9070 *Taq*I	L8921 (19) H9086 (21)
(-/-)	T	+4216 *Nla*III	L4160 (19) H4291 (21)	(+/-)	L2*	+3592 *Hpa*I +16389 *Hin*fI	L16348 (21) H16401 (20)
(-/-)	U	+12308 *Hin*fI	L12265 (21) H12308[4] (23)	(+/-)	L2a	As in L2* plus +13803 *Hae*III	L13643 (19) H113809 (19)
(-/-)	K	+12308 *Hin*fI -9052 *Hae*II	L8921 (19) H9086 (21)	(+/-)	L3b	+10084 *Taq*I	L9963 (21) H10127 (19)
(-/-)	W	+8249 *Ava*II	L8216 (20) H8296 (20)	(+/-)	L3d	-8616 *Dpn*II	L8558 (20) H8657 (20)
(-/-)	X	+14465 *Acc*I	L14390 (19) H14559 (19)	(+/-)	L3e	+2349 *Dpn*II	L2272 (19) H2420 (19)
(+/+)	C	+13262 *Alu*I	L13233 (24) H13392 (24)	(+/+)	D	-5176 *Alu*I	L5121 (20) H5229 (20)
(+/+)	E	-7598 *Hha*I	L7492 (20) H7641 (22)	(+/+)	G	+4831 *Hha*I	L4754 (22) H4919 (19)

[1]L3* (+/-), M (+/+), N(-/-), (pre-HV)1 (-/-), and JT (-/-) have no defining marker.
[2]Refers to the state of the 10394 *Dde*I/10397 *Alu*I sites.
[3]Letters "L" and "H" denote the extended mtDNA strand, light and heavy, respectively. Numbers indicate the first nucleotide to be extended from the primers, based on the Cambridge Reference Sequence (CRS) (Anderson et al., 1981). Numbers in parentheses indicate primer length. When the haplogroup is defined by more than one marker, primers shown belong to the last marker used in the procedure.
[4]H12308 is a mismatched primer with a G at position 12312, thus generating a *Hin*fI site when the transition at position 12308 that characterizes haplogroup U is present.

TABLE 2. Sampling procedure results categorized by region

Region	Municipality	Number of housing units	Uninhabited housing units	Inhabited housing units	Agreed to participate	Selected not contacted	Declined to participate
Metro	Arecibo	33	3	30	26	2	2 (6.7%)
	Bayamón	60	7	53	43	6	4 (7.5%)
	Caguas	40	3	37	30	7	0
	Carolina	51	3	48	39	4	5
	Guaynabo	23	2	21	16	5	0
	Mayagüez	33	3	30	26	0	4
	Ponce	37	3	34	27	0	7
	San Juan	118	5	113	78	22	13
	Subtotal	395	29	366	285	46	35 (9.6%)
North	Florida	39	6	33	29	4	0
	Toa Baja	28	1	27	22	1 (3.7%)	4
	Vega Alta	50	3	47	38	4 (8.5%)	5
	Vega Baja	41	6	35	25	7	3 (8.6%)
	Subtotal	158	14	142	114	16	12 (8.5%)
East	Humacao	72	4	68	51	11	6 (8.8%)
	Loíza	46	1	45	37	4 (8.9%)	4 (8.9%)
	Patillas	26	2	24	21	1 (4.2%)	2 (8.3%)
	San Lorenzo	43	3	40	31	7	2 (5.0%)
	Subtotal	187	10	177	140	23	14 (7.9%)
South	Guayanilla	24	6	18	17	0	1 (5.6%)
	Juana Díaz	23	1	22	19	2 (9.1%)	1 (4.5%)
	Peñuelas	13	4	9	9	0	0
	Yauco	27	2	25	22	0	3
	Subtotal	87	13	74	67	2 (2.7%)	5 (6.8%)
West	Aguadilla	26	2	24	23	1 (4.2%)	0
	Hormigueros	33	1	32	28	2 (6.3%)	2 (6.3%)
	Moca	27	2	25	23	0	2 (8.0%)
	San	29	2	27	23	1 (3.7%)	3
	Subtotal	115	7	108	97	4 (3.7%)	7 (6.5%)
Central	Barranquitas	38	2	36	30	5	1 (2.8%)
	Cayey	31	1	30	22	7	1 (3.3%)
	Corozal	29	1	28	23	4	1 (3.6%)
	Jayuya	27	2	25	22	3	0
	Subtotal	125	6	119	97	19	3 (2.5%)
Total	Total	1067	81	986	800 (81.1%)	110 (11.2%)	76 (7.7%)

TABLE 3. Haplogroup distribution by municipality

	Haplogroups																						
	Amerindian				Sub-Saharan African									West Eurasian									
Municipality	A	B	C	D	L0	L1b	L1c	L2*	L2a	L3*	L3b	L3d	L3e	U[1]	H	J	K	T	U	V	HV	R	pre-HV	Totals
Aguadilla	6	4	7						1	1	1	2									1			23
Arecibo	10	1	8	1					3							1		1				1		26
Barranquitas	12		5	3		1		1				1	2		1	3			1					30
Bayamón	13	1	10	2	1	2	3[2]		1			1	2[3]	1	3	1			1		1			43
Caguas	11	1	5		1	1			1	1			4		4				1					30
Carolina	9	2	6	2		1	2		1	2	3	2	3		1	1	2		1				1	39
Cayey	7		4			2		3[3]	1				4	1										22
Corozal	7		9			3			1			1				1			1					23
Florida	9	1	9	1	1	1		1			2			1	2			1						29
Guayanilla	3		5	2	2		1							1	1[4]	1		1						17
Guaynabo	4	4	2			2	1		1				1			1								16
Hormigueros	11	2	8[3]		1	2	1								1	2								28
Humacao	15	1[5]	10	3		5	4[3]	3		1	1		1		4	1			1		1			51
Jayuya	8	3	4			2			1			1		1	1		1							22
Juana Díaz	11		6[6]			1	1																	19
Loíza	5		2		3	7	3	3	8[3]			5							1					37
Mayagüez	10	3	6					2	1				2				1		1					26
Moca	8	2	8[7]								1		1		1	2								23
Patillas	9	2	2							1	2	1	1		1	1			1					21
Peñuelas	3		2					1	1		1		1											9
Ponce	13	2	6	1		1			1						1	1	1							27
San Juan	16	3	14		2[2]	10[8]	2		4	3		2	6[3]	2	4		2	4	3	1				78
San Lorenzo	7	1	7	1		5		1	1		1	1	1		1	3			1					31
San Sebastián	10	2	3			2						2	1		1	1			1					23
Toa Baja	6	1	6		1	1			1			2	1		1	1			1					22
Vega Alta	13	2	9	1			3	1			2		3	2		1		1						38
Vega Baja	12	2	7						1		1				1				1					25
Yauco	8	2	4				1						4			1			2					22
Totals	256	42	174	17	12	49	22	16	29	9	15	21	38	9	29	23	7	8	18	1	3	1	1	800

[1]Corresponds to Sub-Saharan African clade U5b2 (see text).

[2]Two samples (one each from Bayamón and San Juan) showed the markers defining haplogroup L3d and macroparagroup L. These were regarded as belonging to macroparagroup L.

[3]Six samples (two belonging to haplogroup L3e and one each belonging to C, L1c, L2*, and L2a) contained (-/-) 10394 *Dde*I/10397 *Alu*I motifs instead of those corresponding to their respective haplogroups.

[4]One sample exhibited the markers defining haplogroups H and B. Its HVR-I sequence showed that it belongs to haplogroup H.

[5]The Humacao participant belonging to haplogroup B was of Chinese maternal ancestry.

[6]One Juana Díaz sample exhibited the markers defining haplogroups C and E. Its HVR-I sequence showed that it belongs to haplogroup C.

[7]One Moca sample with the (+/+) motif did not exhibit any haplogroup-defining marker, but its HVR-I sequence revealed that it belongs to haplogroup C.

[8]Two San Juan samples exhibited the marker defining haplogroups J and T in addition to those defining haplogroup L1b. They were regarded as belonging to haplogroup L1b.

TABLE 4. Haplogroup U HVR-I sequence types

Clade	Sequence type[1]	*n* (27)	Min reg cov[2]	Most likely origin
U6b	163, 172, 219, 311	2	081 – 526	Canary Islands
U6a	172, 189, 219, 278	1	161 – 526	North Africa
U5b2	189, 192, 270, 320	9	154 – 379	Sub-Saharan Africa
U5b*	189, 270	1	184 – 391	Europe
U5b*	189, 270, 311	1	184 – 394	Europe
U2[3]	189, 362	2	184 – 391	Europe or Near East
U*	192	1	154 – 394	Europe
U5*	224, 261, 270	1	154 – 391	North Africa
U5*	224, 270	7	154 – 382	North Africa
U5a1a	256, 270	2	154 – 392	Europe

[1]Numbers represent positions at which transitions occur. They correspond to the CRS (Anderson et al., 1981) minus 16000. Transitions at the 16519 hypermutable site are ignored.
[2]Shows the minimum region covered with confidence in all samples in each sequence type.
[3]Their belonging to haplogroup U2 is likely but not definite (see text).

TABLE 5. L3 HVR-I sequence types*

Clade	Sequence type[1]	*n* (9)	Min reg cov[2]
L3g	15924, 114, 223, 293T, 311, 316, 355, 362, 399	1	15877 – 16400
L3*	086, 126, 179, 215, 223, 256A, 284, 311	2	15879 – 16395
L3*	129, 172, 174, 192, 218, 223, 256A, 311, 362	1	15860 – 16524
L3f1	129, 209, 223, 286, 292, 295, 311	1	15882 – 16395
L3*	129, 223, 256A, 311, 362	1	15860 – 16523
L3f1	145, 209, 223, 292, 295, 311	1	15882 – 16394
L3f1	209, 223, 274, 292, 311	1	15882 – 16384
L3*	223, 311, 362	1	16026 – 16400

[1]Numbers represent sites at which base substitutions occur. They correspond to the CRS (Anderson et al., 1981) minus 16000 if only three digits are shown. Transversions relative to the CRS are indicated by showing the base identity after the site number. Transitions at the 16519 hypermutable site are ignored.
[2]Shows the minimum region covered with confidence in all samples in each sequence type.

TABLE 6. Weighted biological ancestry frequencies per municipality

	Ancestry				
Municipality	Amerindian	Sub-Saharan African	West Eurasian	Total	Weight
Aguadilla	22[1] (75.9)[2]	6 (20.7)	1 (3.4)	29	1.2873
Arecibo	18 (75.0)	3 (12.5)	3 (12.5)	24	0.8898
Barranquitas	15 (65.2)	4 (17.4)	4 (17.4)	23	0.7492
Bayamón	28 (60.9)	12 (26.1)	6 (13.0)	46	1.0658
Caguas	17 (56.7)	8 (26.7)	5 (16.7)	30	0.9791
Carolina	21 (48.8)	15 (34.9)	7 (16.3)	43	1.0980
Cayey	10 (50.0)	10 (50.0)	0	20	0.9183
Corozal	16 (69.6)	5 (21.7)	2 (8.7)	23	0.9817
Florida	19 (67.9)	6 (21.4)	3 (10.7)	28	0.9421
Guayanilla	8 (57.1)	3 (21.4)	3 (21.4)	14	0.8369
Guaynabo	12 (63.2)	6 (31.6)	1 (5.3)	19	1.2371
Hormigueros	21 (75.0)	4 (14.3)	3 (10.7)	28	1.0459
Humacao	14 (58.3)	7 (29.2)	3 (12.5)	24	0.4898
Jayuya	16 (69.6)	5 (21.7)	2 (8.7)	23	1.0544
Juana Díaz	15 (88.2)	2 (11.8)	0	17	0.8733
Loíza	5 (17.9)	22 (78.6)	1 (3.6)	28	0.7666
Mayagüez	19 (73.1)	5 (19.2)	2 (7.7)	26	0.9565
Moca	22 (78.6)	2 (7.1)	4 (14.3)	28	1.2396
Patillas	18 (62.1)	7 (24.1)	4 (13.8)	29	1.3563
Peñuelas	8 (57.1)	6 (42.9)	0	14	1.5450
Ponce	29 (80.6)	3 (8.3)	4 (11.1)	36	1.2969
San Juan	42 (42.0)	40 (40.0)	18 (18.0)	100	1.2878
San Lorenzo	13 (52.0)	8 (32.0)	4 (16.0)	25	0.8201
San Sebastián	17 (65.4)	6 (23.1)	3 (11.5)	26	1.1541
Toa Baja	17 (58.6)	8 (27.6)	4 (13.8)	29	1.3123
Vega Alta	18 (66.7)	8 (29.6)	1 (3.7)	27	0.7349
Vega Baja	19 (82.6)	2 (8.7)	2 (8.7)	23	0.8962
Yauco	10 (62.5)	4 (25.0)	2 (12.5)	16	0.7439
Total	489 (61.3)	217 (27.2)	92 (11.5)	798	----

[1]Numbers correspond to the number of samples from each municipality times the sample weight values rounded to integers.
[2]Percent frequencies are in parentheses.

TABLE 7. Weighted data for Sub-Saharan African haplogroups

	Sub-Saharan African Haplogroups												
	L0	L1b	L1c	L2*	L2a	L3*	L3b	L3d	L3e	L3f	L3g	U5b2	Total
n	13	48	18	13	29	5	16	22	38	4	1	9	216
Frequency (%)	6.0	22.2	8.3	6.0	13.4	2.3	7.4	10.2	17.6	1.9	0.5	4.2	100

TABLE 8. Weighted data for West Eurasian haplogroups

Haplogroup	*n*	Frequency (%)
H	29	31.5
HV	3	3.3
(pre-HV)1	1	1.1
I	0	0
J	22	23.9
K	8	8.7
M	0	0
N	0	0
R	1	1.1
T	9	9.8
U*	1	1.1
U2	2	2.2
U5*	8	8.7
U5(a + b)	4	4.3
U6	3	3.3
U(others)[1]	0	0
V	1	1.1
W	0	0
X	0	0
Total	92	100

[1]Includes U1, U3, U4, and U7.

FIGURE LEGENDS

Fig. 1. Municipalities selected.

Fig. 2. Biological ancestry triangular graphic showing the biological ancestry frequencies of the 28 municipalities. Municipalities are numbered as in Figure 1. Municipalities cluster close to the vertex representing Amerindian frequencies equal to one, but with some degree of dispersion toward the African vertex. The Loíza dot lies apart from all other municipalities.

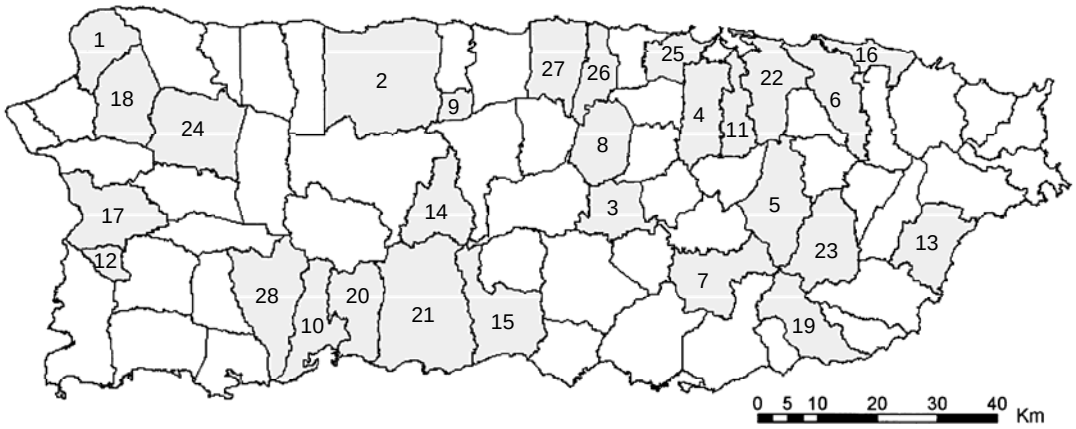
Fig. 3. Amerindian frequencies in the 28 surveyed municipalities distributed in 12 categories. Shown next to each category color indicator is the number of municipalities in the category and, in parenthesis, the Amerindian frequency range represented by the category. The Amerindian frequencies of the single municipalities that are in the first and last categories fall in the middle of the ranges represented by their categories. The 14 municipalities with the lowest Amerindian frequencies are the 11 located east of longitude 66°16' W and the three surrounding Guayanilla Bay. See text for categorization methodology.

Fig. 4. PC analysis of Sub-Saharan African populations based on mtDNA haplogroup frequencies. Crosslines indicate 0.1 units. Panel A: PC1 vs PC2. Panel B: PC1 vs PC3.

Fig. 5. PC analysis of West Eurasian populations based on mtDNA haplogroup frequencies. Crosslines indicate 0.1 units. Panel A: PC1 vs PC2. Panel B: PC1 vs PC3. Moroccan populations are designated by letters: a = non-Berber Moroccans, b = Berbers, c = Souss Valley. Populations of European regions are designated by numbers: 1 = Mediterranean East, 2 = Mediterranean Central, 3 = Mediterranean West, 4 = Basque, 5 = South East, 6 = Alpine, 7 = North East, 8 = North Central, 9 = North West, 10 = Scandinavia.

Fig. 6. PC analysis of Amerindian populations based on mtDNA haplogroup frequencies. Foreground: PC1 vs PC3. Insert: PC1 vs PC2. Crosslines indicate 0.05 units. Number of populations from each geographic region is indicated in parenthesis. Population codes are as follows. From eastern North America Mo = Mohawk, MO = Manitoulin Island Ojibwa, OO = Northern Ontario Ojibwa; from the Great Plains CA = Cheyenne/Arapaho, Cp = Turtle Mountain Chippewa, Si = Siouan, SW = Sisseton/Wapheton Sioux, WC = Wisconsin Chippewa; from the North American Southeast Ch = Choctaw, Cr = Creek, Mu = Muskoke, OK = Oklahoma Red Cross, SC = Stillwell Cherokee, Se = Seminole; from the North American Southwest AO = Akimal O'odham, Ap = Apache, CP = California Penutian, DY = Delta Yuman, HH = Havasupai/Hualapai/Yavapai/Mojave, Je = Jemez, Na = Navajo, Pi = Pima, PS = North Paiute/Shoshoni, PY = Pai Yuman, QC = Quechuan/Cocopa, RY = River Yuman, TO = Tauno O'odham, Wa = Washo, Zu = Zuni; from Mesoamerica Ma = Maya, Mi = Mixtec, Mx = North Central Mexico, NC = Nahua/Cora; from eastern Central America Br = Bribri-Cabecar, EP = Emberá-Panamá, Gt = Guatuso, He = Huetar, Ku = Kuna, Ng = Ngöbé, Te = Teribe, Wo = Wounan; from western Colombia Ci = Chimila, Cy = Cayapa, Gm = Guambiano, Gu = Guane-Butaregua, EC = Emberá-Colombia, IA = Ijka-Arhuaco, In = Ingano, Ko = Kogui, Pz = Paez, TC = Tule-Cuna, Wn = Waunana, Wy = Wayuu, YY = Yuco-Yukpa, Ze = Zenu; from eastern Colombia Cg = Coreguaje, Cu = Curripaco, Gb = Guayabero, GS = Guahibo-Sikuani, Ht = Huitoto, MM = Murui-Muinane, Nk = Nukak, Pa = Piaroa, Tu = Tucano; from the Amazon BB = Belén-Brazil, BN = Brazilian North, Gv = Gaviao, Ti = Ticuna, Xa = Xavante, Ya = Yanomami, Zo = Zoró; from around Lake Titicaca At = Atacameño, Ay = Aymara, Cm = Chimane, Ig = Ignaciano, Ms = Mosetén, Mv = Movima, Qu = Quechua, Tr = Trinitario, Yu = Yuracaré; from northern Argentina, MC = Mataco-Salta, MF = Mataco-Formosa, Mt = Mataco-Chaco, Pl = Pilaga, TF = Toba-Formosa, To = Toba-Chaco; from southern South America Hu = Huilliche, MA = Mapuche-Argentina, MC = Mapuche-Chile, Pe = Pehuenche, Yg = Yaghan. Code for Puerto Rico is PR, and for "Other" populations are BC = Bella Coola, BS = Brazilian Southeast.

Figure 1 *(printing size)*



1- Aguadilla	8- Corozal	15- Juana Díaz	22- San Juan
2- Arecibo	9- Florida	16- Loíza	23- San Lorenzo
3- Barranquitas	10- Guayanilla	17- Mayagüez	24- San Sebastián
4- Bayamón	11- Guaynabo	18- Moca	25- Toa Baja
5- Caguas	12- Hormigueros	19- Patillas	26- Vega Alta
6- Carolina	13- Humacao	20- Peñuelas	27- Vega Baja
7- Cayey	14- Jayuya	21- Ponce	28- Yauco

Figure 2 *(can be reduced to 80% of actual size)*

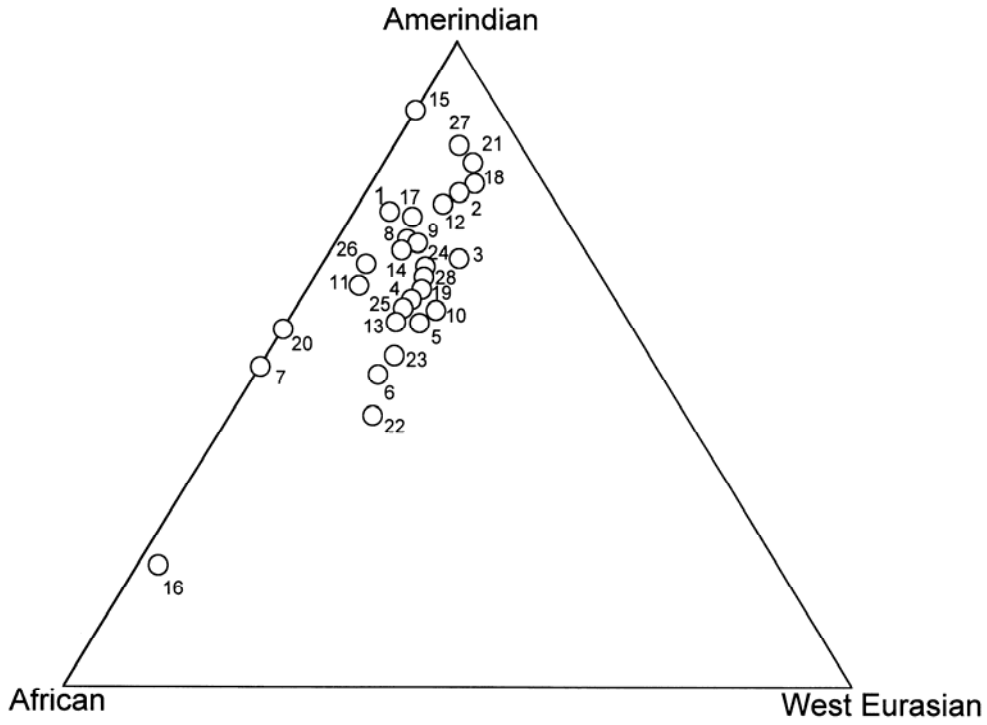


Figure 3 *(printing size)*

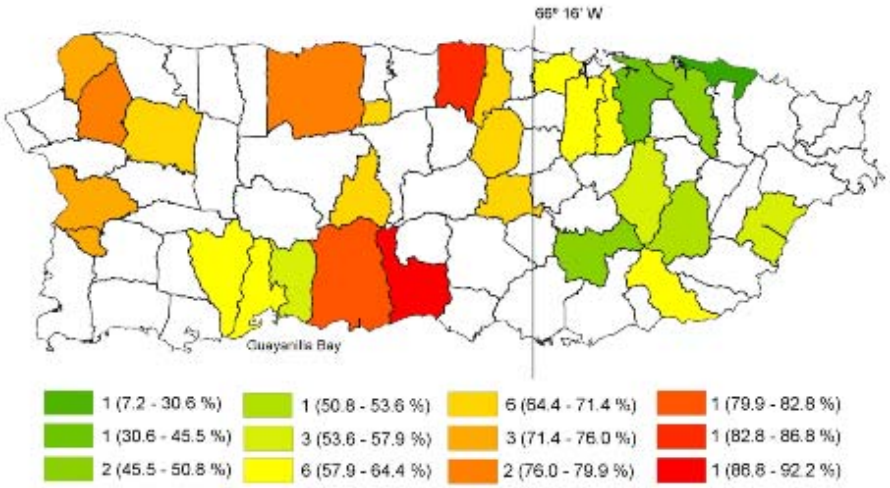


Figure 4 *(printing size)*

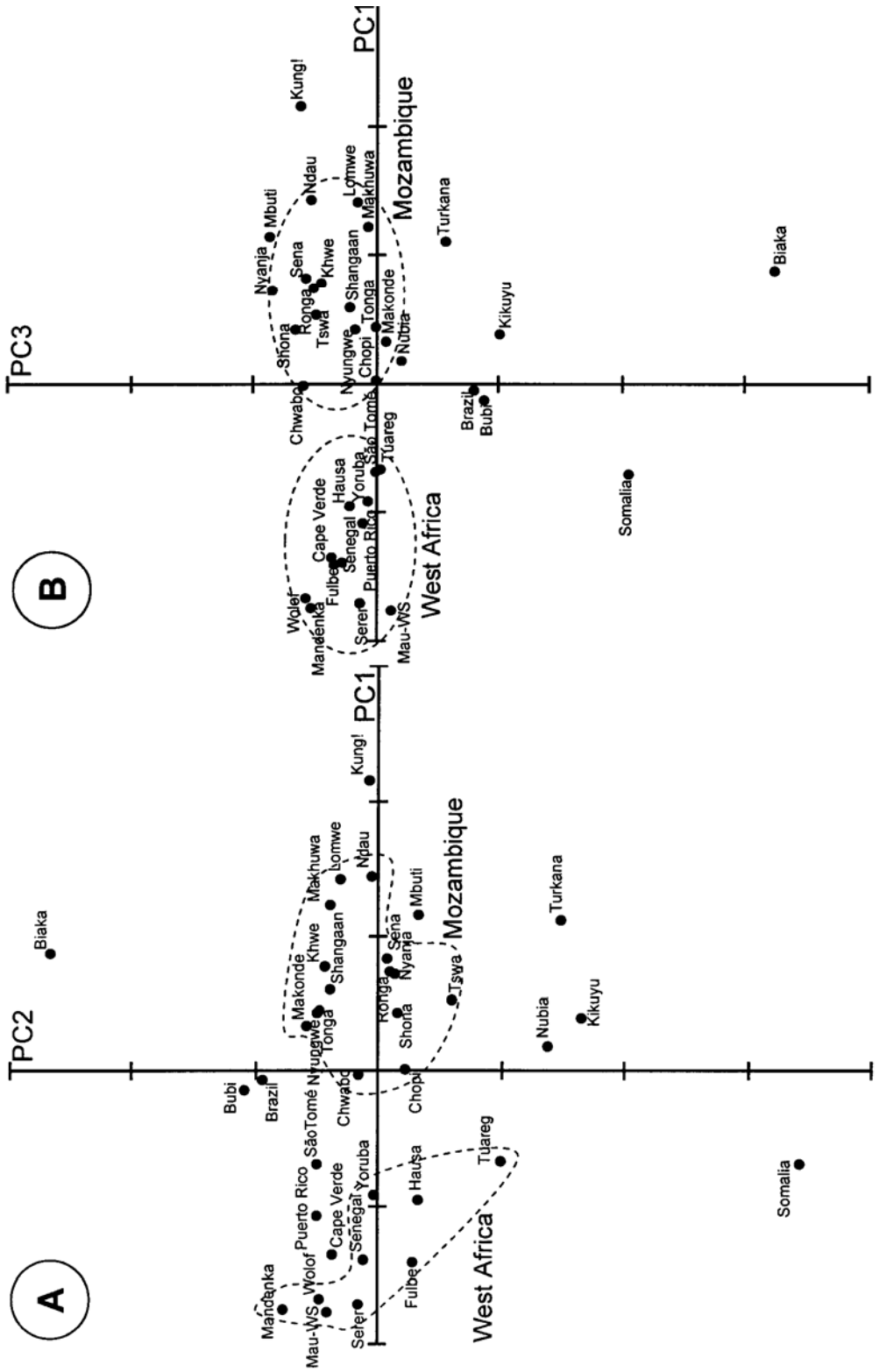


Figure 5 *(printing size)*

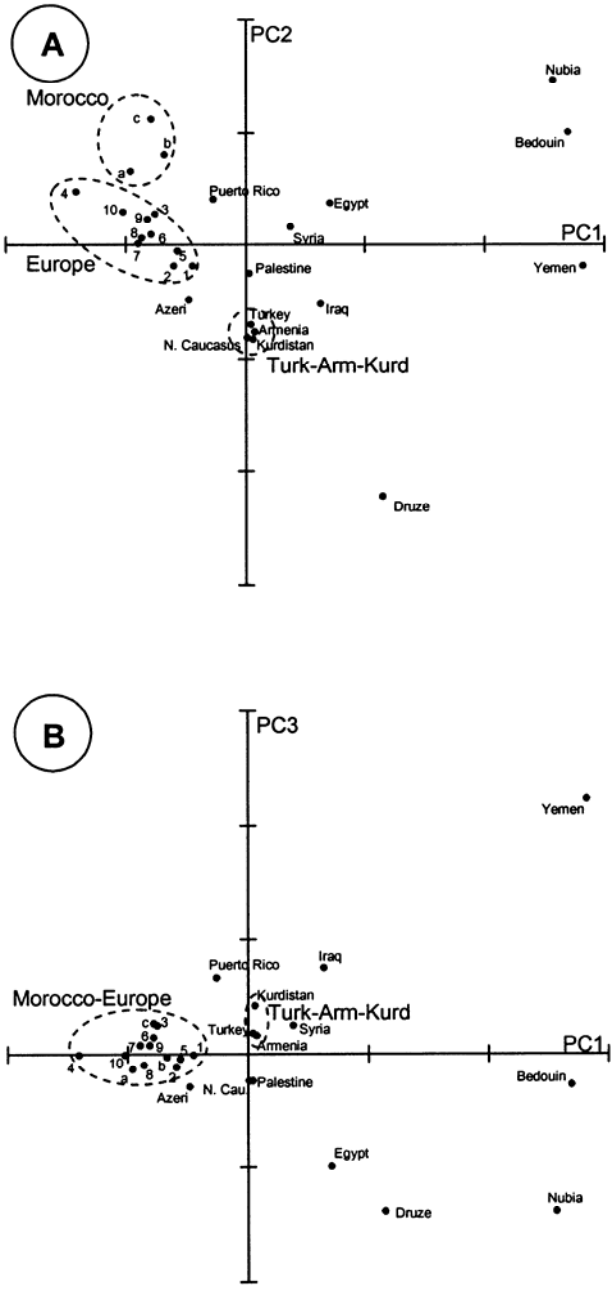


Figure 6 *(printing size)*

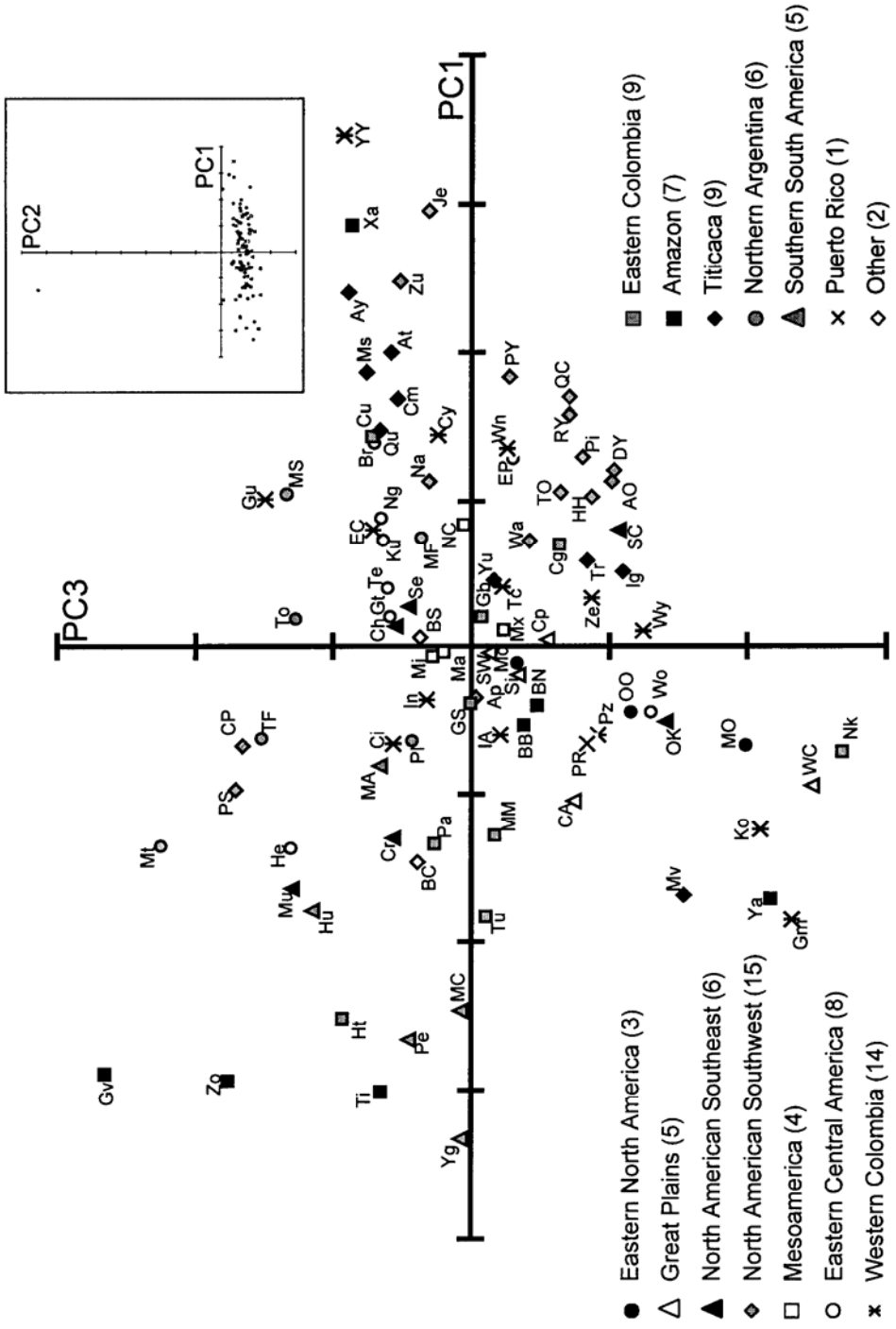
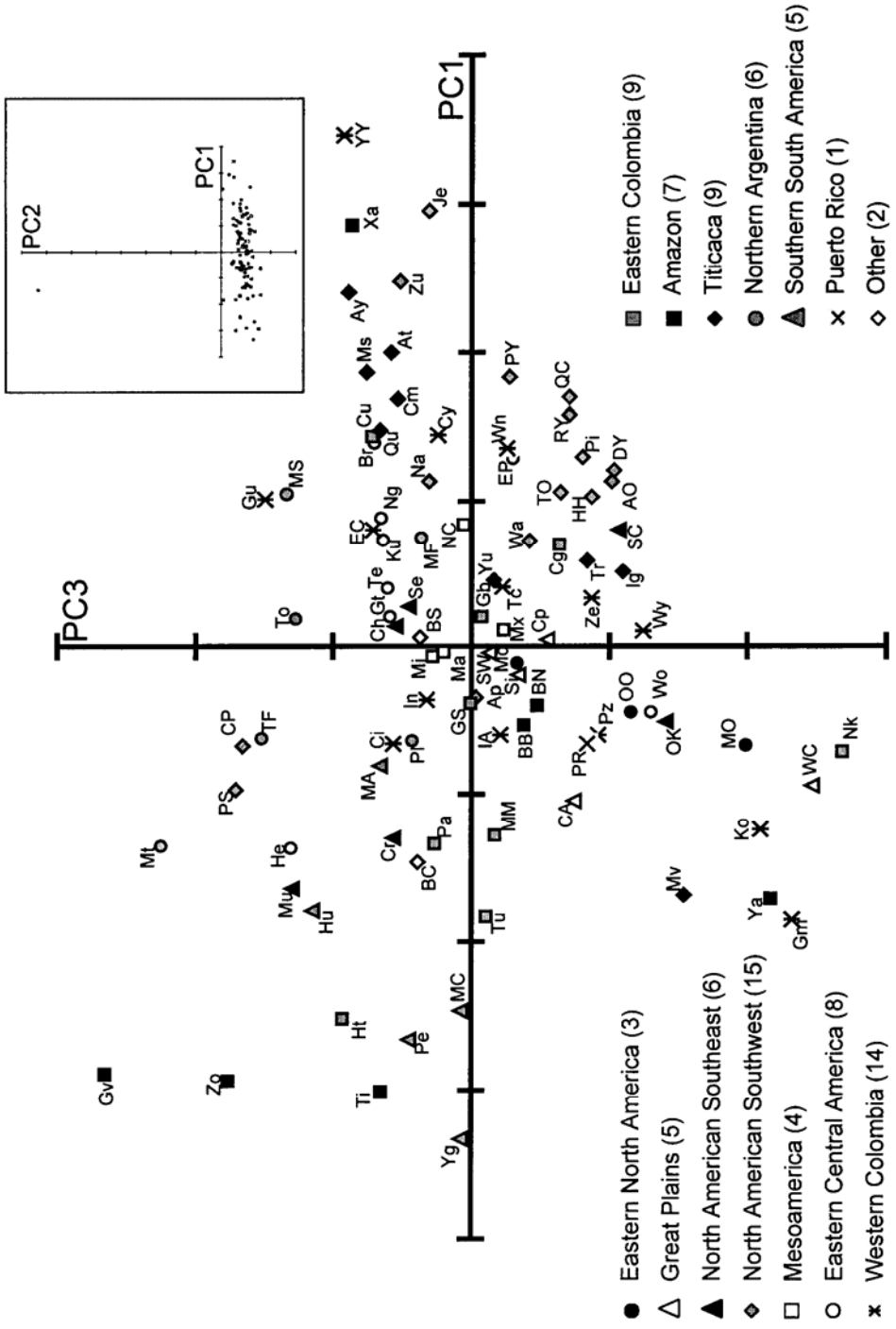


Figure 6 *(printing size)*



1. Introduction

Traditionally, scientists have sought to understand the origin and evolution of human populations through the examination of sediment samples and artifacts left by previous societies. For more recent societies, historical accounts of significant events that took place in a culture have also provided valuable information towards their origin. Recent advances in molecular technologies have allowed scientists to acquire a vast knowledge on the origins of human populations by studying their genetic composition. The genetic information stored within the DNA code provides a record of mutations that are inherited through generations, allowing researchers to compare the genetic code of different human populations to unravel the origins of a civilization. Overall, the combination of archaeological evidence, historical events, and variations in the genetic composition within populations allow researchers to trace more effectively the ancestry of particular groups of people.

1.1 Historical Background

Puerto Ricans compose a heterogeneous population which has undergone five centuries of admixture between three different population groups: Amerindians (Arawak-speaking Taíno Indians), Caucasians (mainly Spanish colonizers) and Sub-Saharan Africans. Admixture between Amerindians and Caucasians started soon after Christopher Columbus arrived to Puerto Rico in 1493. As the economic basis of the island was shifting from gold to sugar cultivation, African slavery became increasingly important to power this industry, thus becoming a relevant presence in the population. African slavery was justified by the state as a necessity towards the economic development of the island. The church also approved of this practice as a measure for civilizing and christianizing the African people (Díaz-Soler, 2000).

The epoch of slavery in Puerto Rico can be divided into two major periods in which the importation of Africans was relevant to the island. The first period took place during the first

century of the colonization as the need for men and women power increased due to the development of the sugar cane industry. During this period the region known as Senegambia and other regions of West Africa were the major outlets of Afro-Negroid slaves brought to the New World (Alegría, 1985). Decades later, slaves were also brought from regions to the south of Senegambia: Congo and Angola and eventually even from Mozambique. After the secession of Portugal from the Spanish crown in 1640, the slave supply to the Spanish colonies was drastically reduced, especially in those colonies with low acquisitive power, such as Puerto Rico. A smuggling system was developed which was characterized by illegal shipments from other Caribbean islands that were used as slave warehouses, such as Curaçao and Jamaica. History suggests that the Dutch and English took most of their slave stocks from the region to the south of Senegambia, from what today is known as Sierra Leone to the outlet of the Niger River. However, there were also incursions to other areas of the continent (Álvarez-Nazario, 1974).

The importation of slaves to Puerto Rico increased significantly after social reforms were imposed by the Bourbon dynasty at the end of the eighteenth century. The independence wars that characterized the first decades of the nineteenth century also fueled the migration of landlords with their slaves and their power to purchase slaves in the market. During this period the island experienced an economic upsurge through the financial incentive of these wealthy immigrants. Their arrival stimulated the economy by developing the sugar, tobacco, and coffee industries (Fernández-Méndez, 1970). A major portion of the slaves that came to work in the fields during this second wave originated from the region that extended from the Gold Coast to Angola (Díaz-soler, 2000).

The community of African slaves that populated the island contributed to the formation of a unique culture in which many African influences have been preserved in modern times. The

commemoration of African deities plus the use of certain names and the celebration of traditional festivals have established the presence of people from Central Africa, Angola, and Mozambique in modern Puerto Rico. However, the absence of a classification structure of the continental origin of the slaves leaves uncertainties on the relative contribution of these regions (Álvarez-Nazario, 1974).

1.2 Molecular Technology: A Mitochondrial DNA Overview

Genetics has provided scientists with resources through which the origins of the human species can be explored. The mitochondrial DNA (mtDNA) was the first polymorphic DNA structure examined in humans for evolutionary purposes and has proven to be a powerful mechanism for the study of the genetic history in human populations (Mateu *et al.*, 1997). The mtDNA is a circular double-stranded molecule with an extranuclear origin and about 1,000-10,000 copies in every somatic cell. Its genome consists of approximately 16.6 kbp (Anderson *et al*., 1981; Andrews *et al*., 1999) and encodes for 13 polypeptides, integral members of the mitochondrial respiratory chain, 22 distinct transfer RNAs, and 2 ribosomal RNAs (Lightowlers *et al*., 1997). The maternal mode of inheritance and the lack of recombination are some advantages of analyzing mtDNA for population studies. Another major advantage is its relatively high mutational rate (Stoneking, 1993), which accumulates mutations ten times faster than the nuclear genome (Brown *et al*., 1979; Ingman and Gyllesnsten, 2001). These properties are not found in any other locus in the human genetic makeup, thus making mtDNA the molecule of choice for the study of genetic relationships between populations.

It has been estimated that the mtDNA has undergone a mutation every 10,000 years since the first modern human, who lived approximately 150,000 – 200,000 years ago in Africa (Cann *et al*., 1987; Vigilant *et al*., 1991). Selectively neutral mutations have generated polymorphisms

that scientists have used to group mtDNA into clusters called haplogroups. These are groups of mtDNAs that share a common recent ancestor who suffered a mutation that is transmitted to all of her descendants, distinguishing them from all other mtDNAs. Most haplogroups are continent-specific. As a result, haplogroup allocations of a specific mtDNA lineage permit the identification of its continental origin, allowing the evaluation of matrilineal ancestry within mixed populations (Bravi *et al*., 1997; Rando *et al*., 1999; Green *et al*., 2000).

Most of the research efforts exploring mitochondrial genetic diversity have taken place during the last two decades. Initially, phylogeographic studies centered on low-resolution Restriction Fragment Length Polymorphisms (RFLP) of the complete molecule (Scozzari *et al*., 1988; Soodyall and Jenkins, 1992; 1993) or variations in one or both hypervariable segments, HVS-I and HVS-II (Vigilant *et al*., 1989; 1991). Subsequently, high-resolution RFLPs studies have been used to classify mtDNA haplogroups (Cann *et al*., 1987; Torroni *et al*., 1992; 2001; Chen *et al*., 1995; 2000). These phylogeographic studies of mtDNA ancestry have made possible the recognition of haplogroups that are specific to Africans, Caucasians, or Asians/Amerindians (Torroni *et al*., 1993; 1994; 1996; 1998; Chen *et al*., 1995; 2000; Richards *et al*., 1996, 2000).

1.3 Phylogeographic Analysis of African Populations

Some of the earliest studies employing the sequence and organization of the human mitochondrial genome were among South African Kung¡ populations (Denaro *et al*., 1981; Johnson *et al*., 1983). These studies employed a series of six rare cutting endonucleases (*Ava*II, *Bam*HI, *Hae*II, *Hinc*II, *Hpa*I, and *Msp*I) showing that ~90% to 95% of the mtDNAs examined were characterized by *Hpa*I site gain at nucleotide position (np) 3592 (numbering according to Anderson *et al*., 1981 and Andrews *et al*., 1999). Thereafter, studies by Scozzari and

collaborators (1988; 1994), Soodyall and Jenkins (1992; 1993), and Chen and colleagues (1995) revealed that the site gain at np 3592 is found in very high frequencies in other Sub-Saharan African populations and was not detected in non-African groups, with some exceptions (Cann *et al*., 1987). The African populations identified by the site gain at np 3592 define three clusters of related mtDNAs within haplogroup L. Because of the unsurpassed diversity within it, haplogroup L was recognized as the oldest lineage cluster, thus contributing to the hypothesis of a modern human mtDNA African origin (Johnson *et al*., 1983; Cann *et al*., 1987).

Four major clades within haplogroup L can now be recognized. These are paragroup L0 and haplogroups L1, L2, and L3 (Mishmar *et al*., 2003; Salas *et al*., 2004). The likely origin of all human mtDNAs lies between L0 and L1. L2 and L3 are two more recent macrohaplogroups that share a common origin within L1. Macrohaplogroup L3, the only without the np 3592 *Hpa*I site gain, gives rise to haplogroups M and N which encompass all mtDNAs that migrated out of Africa and spread all over the world. Each of these clades can be further divided into several subclusters (Figure 1).

The overall Sub-Saharan African phylogeny is divided into two main components, one having an *Hpa*I site gain at np 3592 and the other one lacking it. The component having the *Hpa*I site gain subdivides into two major branches: 1) L1 with a *Hin*fI site gain at np 10806 and 2) L2 having a combined *Hin*fI site gain at np 16389 and an *Ava*II site loss at np 16390. Based on more recent knowledge of African complete sequences, previously paraphyletic clade L1 is now split into two monophyletic units: L0 capturing formerly identified L1a, L1d, L1f, and L1k clades, and L1 now including only L1b, L1c, and L1e (Salas *et al*., 2002). The macrohaplogroup now known as L0 is branched into L0af, defined by a *Hae*III site gain at np16641 (Chen *et al*., 1995; Alves-Silva *et al*., 2000) and haplogroup L0a, which is defined by an *Alu*I site loss at np

4583(Chen *et al*., 2000). The L1 haplogroup branch is subdivided into L1b and L1c on the basis of the presence of an *Alu*I site at np 4310 and of a *Taq*I site at np 9070, respectively. L2 is subdivided into L2a, L2b, L2c, and L2d, based on the presence of a *Hae*III site at np 13803 for L2a, the presence of *Alu*I at np 4157 for L2b, the presence of *Hae*III at np 322 for L2c, and the presence of *Dpn*II at np 3693 for L2d.

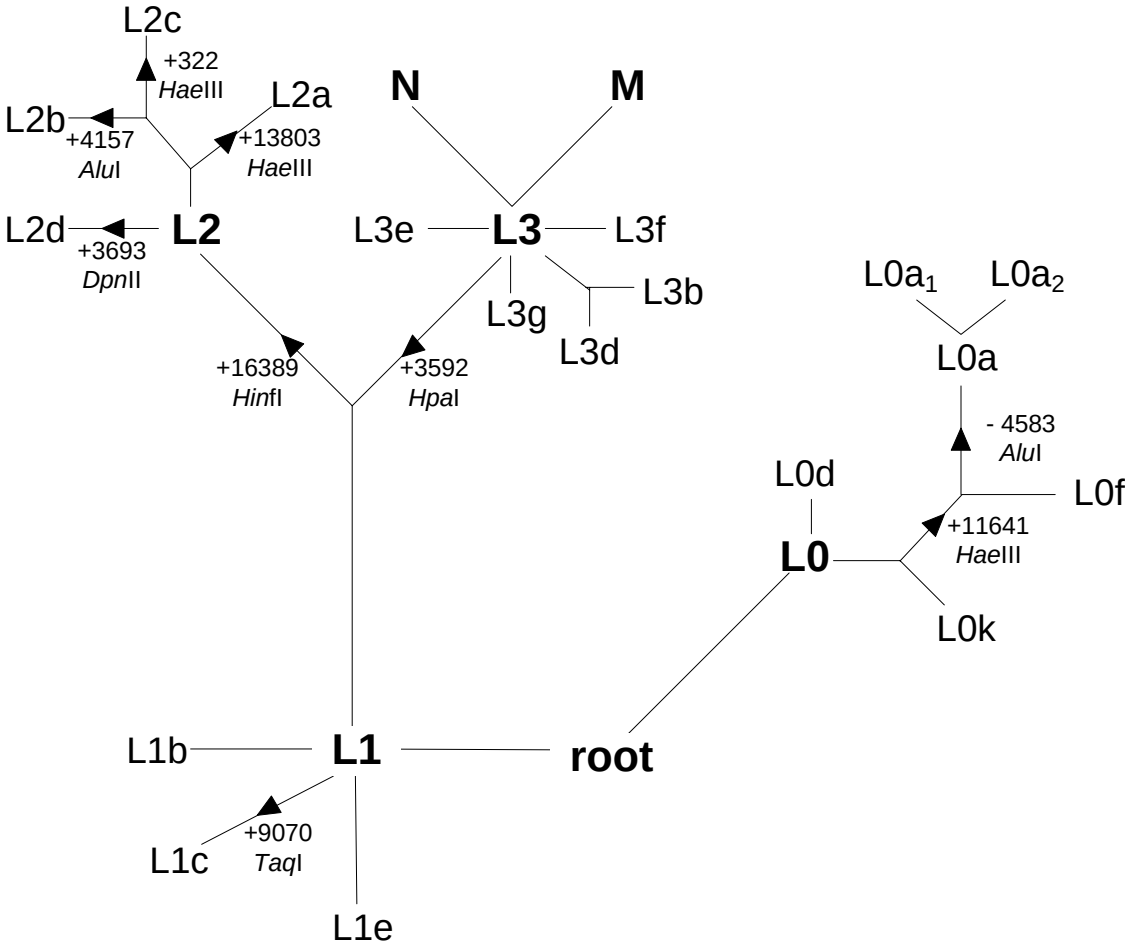


Figure 1 Scheme of global human mtDNA tree, haplogroups L, M, and N (based on Chen *et al*., 1995; Watson *et al*., 1997; Alves-Silva *et al*., 2000; Chen *et al*., 2000; Torroni *et al*., 2001; Hernstadt *et al*., 2002, Salas *et al*., 2002; 2004; and Tambets, 2004). Restriction sites shown here were analyzed in the present study.

The component lacking the *Hpa*I site at np 3592 is known as haplogroup L3. Haplogroup L3 is subdivided into six haplogroups: L3b, associated with a *Taq*I np 10084 site gain; L3d, associated with a *Mbo*I np 8616 site loss; and L3e, associated with a *Mbo*I np 2349 site gain. Haplogroups L3f, L3g, and L3h were identified through their control region sequence.

1.4 Phylogeographic Analysis of African Populations in Puerto Rico

Initial efforts to employ mtDNA analysis to elucidate the African origins of the modern population of Puerto Rico are presented by Martínez-Cruzado and collaborators (2005). Findings revealed that approximately 27% of the Puerto Rican population (sample size = 220) have Sub-Saharan African maternal ancestry. This first round of analysis aimed at identifying the haplogroup classification of participants with African mtDNA ancestry. Approximately two-thirds of this group was classified in the haplogroups within macrohaplogroup L3 or in haplogroup L1b, while the other third of the Sub-Saharan mtDNAs was pooled as macrohaplogroup L.

In the second round of analysis, presented in this study, we provide more specific RFLP typing for the samples characterized into macrohaplogroup L, subdividing them into the various haplogroups within L0, L1, and L2. In addition, we combine the data sets obtained from both analyses to offer an integrated view of the African phylogeny present in modern Puerto Ricans.

1.5 Medical and Social Implications

Mitochondrial DNA has been analyzed in human societies to trace the ancestry and geographic distributions of populations through time and to explore the role that mtDNA mutations play in human health (Torroni *et al*., 1996; Howell, 1999; Ingman *et al*., 2000; Herrnstadt *et al*., 2002). The study of human population history through mtDNA analysis has contributed to gain a better understanding of the roots and underlying elements upon which the

cultural history of a social group are sustained. Meanwhile, research on the mutations that occur within the mtDNA coding region has been linked to diseases in humans (Enriquez *et al*., 1995; Wallace, 1995; Schon *et al*., 1997). It appears that there may be a variety of mutations that go from benign to malignant and that nuclear-mitochondrial interactions may have a significant role in disease etiology (Herrnstadt *et al*., 2002). Several studies point toward a positive relationship between a clinical disorder and a specific mtDNA haplogroup: Leber Hereditary Optic Neuropathy (Johns and Berman, 1991; Torroni *et al*., 1997, Howell *et al*., 2003), Aminoglycoside-Induced and Nonsyndromic Deafness (Achilli *et al*., 2004; Zhao *et al*., 2004), Friedreich's Ataxia (Giacchetti *et al*., 2004), Sudden Infant Death Syndrome (Hoffman *et al*., 1999), asthenozoospermia and nonasthenozoospermia (Ruiz-Pesini *et al*., 2000). Based on the observed higher mutation rate between recently diverged lineages, other researchers have also indicated that many modern polymorphisms are more than just slightly deleterious and will thus be eliminated by natural selection (Ho *et al*., 2005). Furthermore, pathogenically benign polymorphisms, such as longevity (DeBenedictis *et al*., 1999; Rose *et al*., 2001), might be associated with particular mtDNA haplogroups. Thus, it is becoming important to extend our understanding of mtDNA haplogroups. Understanding the pathogenic role of the mitochondrial genome will require further research of the mtDNA sequences in prevalent community and patient groups. The identification of those haplogroups present in the Puerto Rican population is an important first step to understand the impact that genetic diseases mediated by mitochondria may have in the population of the island.

1.6 Aims of the Present Study

The main goal of the present study of mtDNA in the Puerto Rican population is to examine the phylogeographic affiliation of mtDNA lineages within the African continent.

Specifically, we aim to expand existing data on RFLP typings for the Sub-Saharan African macrohaplogroup L to gain a better understanding of the genetic variations of the Sub-Saharan African haplogroups in the Puerto Rican population. Then, we make an effort directed at tracing the Puerto Rican African-associated lineages back to the continent allowing us to explore the extent to which different regions in Africa have contributed to the present day composition of Puerto Rican maternal lineages. Overall, this study can become a rich source of information for understanding our population history, particularly the impact that the interactions with other cultures have had on our development as a group.

2. Methods

This study is a component of a research recently published by us (Martínez-Cruzado *et al*., 2005). Additional information about methods and results can be found in the Publication section.

2.1 Sampling Frame

To ensure conclusions reached were applicable to Puerto Rico as a whole, municipalities and census blocks were selected through a geographically segregated and stratified sampling scheme based on the 1990 Census of Population and Housing (Martínez-Cruzado *et al*., 2005). Thus, a random sample of 872 households, representative of the island population, was selected. Individuals living in institutions, group quarters or in military reservations were not eligible for the study population. The sampling framework included twenty eight of the seventy six municipalities in Puerto Rico (Table 1), including the eight most populated municipalities. The number of housing units selected from each of these eight municipalities was proportional to its population size. The total number was equal to approximately 40% of the total sample, as these eight municipalities encompass approximately 40% of the Puerto Rican population.

Table 1 Puerto Rican municipalities included in the sampling frame

Most populated municipalities	Regions				
	North	South	East	West	Central
Arecibo	Florida	Guayanilla	Humacao	Aguadilla	Barranquitas
Bayamón	Toa Baja	Juana Díaz	Loíza	Hormigueros	Cayey
Caguas	Vega Alta	Peñuelas	Patillas	Moca	Corozal
Carolina	Vega Baja	Yauco	San Lorenzo	San Sebastián	Jayuya
Guaynabo					
Mayagüez					
Ponce					
San Juan					

The remainder twenty municipalities were chosen by dividing the island into five geographical regions: north, south, east, west, and central. Each region was subdivided into four strata based on municipality population size, and one municipality within each stratum was selected at random with probability proportional to its population size. Equal amounts of housing units were assigned to each municipality in proportion to the population size of the geographical region they represented.

Within each municipality, thirty percent of the census tracks were randomly selected with a probability proportionate to its population size based on the 1990 census. Census blocks were chosen taking into consideration the estimated number of housing units that included an expected minimum of eight households per block. The actual number of housing units could be greater or smaller depending on structural changes made in each block since the 1990 census. The systematic selection of housing units was guided by a pre-established random starting point for each block. One adult from each housing unit was selected at random. We have identified a total of 1,067 housing units as a result of the sampling frame described above. Eighty one households were found to be uninhabited, and in 110 hoseholds the selected individual could not be contacted. Of the 876 individuals approached, 800 agreed to participate yielding a response rate of 81.1% based on the 986 households selected. After obtaining the informed consent, we collected the hair root specimen and conducted a private, face-to-face interview.

2.2 Molecular Analyses

We subjected hair root samples to high resolution restriction analysis, RFLP (Scozzari *et al*., 1988). In case of ambiguity in defining mtDNA haplogroups on the basis of the RFLP information, we analyzed data from the control region sequence (Vigilant *et al*., 1989; 1991).

2.2.1 Mitochondrial DNA Extraction

Hair roots provided by the participants were submerged in 0.5 mL of 5% Chelex (Sigma Chemical Co.) in a labeled 1.5 mL microtube. Tubes were stocked in coolers with ice packs until arrival to the laboratory the same day, where they where placed in a 58^{o}C incubator until the following day (12-16 hrs). The samples were vortexed, boiled for 8 minutes, vortexed again, and spun at 13,000 *g* for 3 minutes to liberate the DNA. The samples were subsequently stored at -20^{o}C for further use (Walsh *et al.* 1991).

2.2.2 Aliquot Preparation

A 200 µL aliquot was purified from each 500 µL sample using the QIAamp DNA Mini Kit (QIAGEN). In each aliquot, 36 µL of 60 mM Tris-HCl pH 8.0, 60 mM Na_2EDTA pH 8.0, 0.6 M NaCl, 0.24 mM DTT and 12% SDS were added, followed by 250 µL of Buffer AL and 250 µL of 100% ethanol. The aliquots were vortexed thoroughly and transferred to a spin column where they were spun at 8000 rpm for one minute. The filter was washed by adding 300 µL of a 125:95 mix of ethanol and Buffer AW1 concentrate, followed by spinning at 8000 rpm for one minute, then adding 300 µL of a 160:66 mix of ethanol and Buffer AW2 concentrate, and spinning at 14000 rpm for five minutes. The DNA was finally eluted from the filter into two 100 µL aliquots in the elution buffer (QIAGEN) and kept at -80^{o}C as backups for the study.

2.2.3 Polymerase Chain Reaction Amplification

The amplification reaction conditions were 2.5 µL 10X PCR buffer, 2.0 µL 25mM $MgCl_2$, 4 µL of 2.5 mM dNTP, 1.3 µL of each primer at 20 µM, 5 µL of the DNA sample, 1.0 µL of Red Taq DNA polymerase at 1U/µL, and filled with ddH_2O to a final volume of 25 µL. The primers used for such amplifications and the restriction sites tested in each amplified fragment are shown in Table 2. The amplification mixture was then heated at 94^{o}C for 2.5 minutes,

followed by 32 cycles consisting of 30 seconds at 94°C, 1 minute at 52°C, and 70 seconds at 72°C. The amplification procedure was completed after one last cycle of 10 minutes at 72°C.

Table 2 Primers and defining markers used to identify Sub-Saharan African mtDNA haplogroup distribution in the Puerto Rican population

Haplogroup[1]	*Defining markers*	*Primers*[2]	*Haplogroup*[1]	*Defining markers*	*Primers*[2]
L0af	+11641 *Hae*III	L11578 (24) H11719 (23)	L2a	+16389 *Hinf*I +13803 *Hae*III	As in L2 L13643 (19) H13809 (19)
L0a	+11641 *Hae*III -4583 *AluI*	As in L0af L4462 (21) H4620 (19)	L2b	+16389 *Hinf*I +4157 *Alu*I	As in L2 L4117 (19) H4375 (19)
L1c	+9070 *Taq*I	L8921 (19) H9086 (21)	L2c	+16389 *Hinf*I +322 *Hae*III	As in L2 L191 (21) H408 (20)
L2	+16389 *Hinf*I	L16219 (21) H16401 (20)	L2d	+16389 *Hinf*I +3693 *Dpn*II	As in L2 L3517 (19) H3775 (19)

[1]All haplogroups are defined as members of haplogroup L by the presence of restriction site 3592 *Hpa* I.

[2]Letters "L" and "H" denote the extended mtDNA strand, light and heavy, respectively. Numbers indicate the first nucleotide to be extended from the primers, based on the Cambridge Reference Sequence (CRS) (Anderson *et al*., 1981; Andrews *et al*., 1999). Numbers in parenthesis indicate primer length in bases.

2.2.4 Restriction Digestion

We performed site-specific endonuclease digestions according to manufacturer's conditions. It involved 1 μL of the restriction enzyme including 10 U to 20 U, 2 μL of the enzyme's buffer for every 10 μL to 15 μL of amplified mtDNA in the mixture, and filled with ddH_2O until 20 μL of the reaction mix was reached. Digested fragments were fractionated side-by-side to an undigested control and/or molecular weight marker in a 3% agarose gel. The gels

were stained with ethidium bromide at a final concentration of 5 μg/mL and photodocumented under UV light.

2.2.5 Mitochondrial DNA Sequencing

The hypervariable segment I (HVS-I) sequence of those samples that did not test positive for any of the haplogroup specific restriction sites was determined. Amplicons are generated through PCR using primers L15766 and H34 under the conditions used for RFLP fragment amplification. These amplicons were purified using the High Pure PCR Production Purification Kit (Roche Molecular Biochemicals), as instructed by the manufacturer. Automated sequencing was carried out at the University of Medicine and Dentistry of New Jersey, New Jersey Medical School Molecular Resource Facility using an Applied Biosystems (ABI) model 3100 capillary sequencer after cycle sequencing with Dye Terminator mix version 2.0. Sequencing primers were L1584 and H16526. We used the HVS-I motifs for haplogroup classification from Salas *et al*., 2004.

2.3 Haplogroup Identification Strategy

The haplogroup identification process conducted in the first set of analysis involved the following procedures (Martínez-Cruzado *et al.* 2005). First, the amplicon containing the 10394 *Dde*I / 10397 *Alu*I motif was analyzed since several studies have shown that nearly every haplogroup is monomorphic for it, with the exception of haplogroup K (Ballinger *et al*., 1992; Torroni *et al*., 1993; 1994; 1996; Chen *et al*., 1995; 2000; Herrnstadt *et al*., 2002; Silva *et al*., 2002; Kong *et al*., 2003; Reidla *et al*., 2003). Determining these restriction sites contributed to reduce the amount of candidate haplogroups to which unknown samples could belong. Depending on the results obtained from these two restriction sites, each sample was then tested for every haplogroup restriction marker known to share the same motif. This process allowed us

to identify Amerindian (A, B, C, and D), West Eurasian (H, J, K, T, U, V, HV, R, and pre-HV), and Sub-Saharan African (L, L1b, L3b, L3d, and L3e) haplogroups. Other Sub-Saharan African haplogroups (L3f, L3g, L3h, and U5b2) were identified by hypervariable segment I (HVS-I) sequencing.

Our findings showed that 220 participants had Sub-Saharan African maternal ancestry. In the first series of analyses, these samples were classified into the following haplogroups: L, L1b, L3*, L3b, L3d, L3e, L3f, L3g, L3h, and U5b2. In this study, we further analyzed the 79 samples classified in the L macrohaplogroup through a haplogroup identification strategy that allowed us to further subdivide this macrohaplogroup. All of the 79 samples were subjected to restriction analysis for L0af, L1c, and L2. Most samples proved to be positive for one of these three haplogroups. Those whose haplogroup was not recognized through this scheme were classified into paragroup L0*. The samples that tested positive for the slightly larger grouping L0af were analyzed for haplogroup L0a, and those which proved negative were classified into L0af*. All samples within haplogroup L2 were analyzed for every subgroup restriction marker: L2a, L2b, L2c, and L2d. The hypervariable segment I sequence was obtained from the samples that did not test positive for any of the L2 subgroups, allowing their classification into one of the L2 subgroups.

2.4 Data Analysis

Two sets of analyses are presented in this thesis. In the first set, we performed haplogroup frequency and principal component analyses with the 79 participants identified within the Sub-Saharan African haplogroup L. In the second set of analysis, we incorporated the 141 participants previously identified (Martínez-Cruzado *et al*., 2005) with mtDNA lineages within the African continent. Following we describe the analysis conducted for both data sets.

A weighting scheme was formulated to determine if biased estimates of the parameters were induced by variations in the participation or changes in population size that occurred since the 1990 census. With the use of the SPSS 10.0.5 for Windows program, the weighted values adjusted the number of samples per municipality so it would be equal to the number expected through the application of the original sampling proportions to the final sample size.

To gain a better understanding of the continental origins of the Sub-Saharan African groups present in modern Puerto Ricans we performed two sets of principal component analyses (PCA). Both sets were completed with haplogroup frequencies of various modern African populations (Figure 2), the weighted haplogroup frequency of the Puerto Rican population, and data sets from the Dominican Republic and Brazil (Table 3).

The PCA was undertaken using the POPSTR program, provided by Henry Harpending (University of Utah). The first set of PCAs encompassed the weighted data for the samples previously categorized in the L macrohaplogroup divided into the following haplogroups: L0*, L0af*, L0a, L1c, L2a, L2b, L2c, L2d. The second PCA incorporated the weighted data of participants previously identified in the haplogroups: L1b, L3*, L3b, L3d, L3e, L3f, L3g, L3h, and U5b2. The data of all Sub-Saharan African samples was broken down into the following haplogroups: L0*, L0af*, L0a, L1b, L1c, L0d / L1e, L2a, L2b, L2c, L2d, L3*, L3b, L3d, L3e, L3f, L3g, L3h, and U5b2.

Figure 2 Map of Africa showing the populations used in the present work.

Table 3 Ethnic groups used in the present study including their geographic origin, sample size, and reference

Ethnic group (code)	*Geographical origin*	*Sample Size*	*Reference*
Caribbean:			
Puerto Rico (Pr)	Puerto Rico	216	Present study and Martínez-Cruzado *et al*., (2005)
Dominican Republic (Dr)	Dominican Republic	112	Torroni, published data at Brehem *et al*., (2002)
South America:			
Brazil (Br)	Brazil	95	Bortoloni *et al*., (1997) and Alves-Silva *et al*., (2000)
West Africa:			
Hausa (Ha)	Nigeria, Níger	16	Wastson *et al*., (1997)
Kanuri (Ka)	Nigeria, Níger	13	Wastson *et al*., (1997)
Fulbe (Fu)	Nigeria, Níger, Benein, Cameroon, Burkin Faso	51	Wastson *et al*., (1997)
Songhai (Sg)	Nigeria, Níger, Mali	8	Wastson *et al*., (1997)
Tuareg (Tu)	Nigeria, Níger, Mali	10	Wastson *et al*., (1997)
Yoruba (Yo)	Nigeria	30	Vigilant *et al*., (1991)
Senegalese[1] (Sn)	Senegal	54	Scozzari *et al*., (1988) and Rando *et al*., (1998)
Serer (Sr)	Senegal	45	Rando *et al*., (1998)
Wolof (Wo)	Senegal	64	Chen *et al*., (1995) and Rando *et al*., (1998)
Mandenka (Mn)	Senegal	106	Craven *et al*., (1995)
Cape Verd (Cv)	Cape Verd	273	Brehem *et al*., (2002)
West-Central Africa:			
Bubi (Bu)	Equatorial Guinea	45	Mateu *et al*., (1997)
Sao Tome (St)	Sao Tome and Principe	153	Mateu *et al*., (1997) and Trovada *et al*., (2003)
Biaka / Western Pygmies (Bi)	Central African Republic	34	Vigilant *et al*., (1991) and Chen *et al*., (1995)
Mbuti / Eastern Pygmies (Mb)	Democratic Republic of Congo	33	Vigilant *et al*., (1991) and Chen *et al*., (1995)
East Africa:			
Turkana (Tk)	Kenya	27	Watson *et al*., (1997)
Somalia (So)	Somalia	17	Watson *et al*., (1997)
Kikuyu (Ki)	Kenya	14	Watson *et al*., (1997)
Southeastern Africa:			
Mozambique (Mo)	Mozambique	109	Salas *et al*., (2002)
Yao (Ya)	Mozambique (N), Tanzania (S)	10	Salas *et al*., (2002)
Tonga (To)	Mozambique (SE)	20	Salas *et al*., (2002)

Ethnic group (code)	*Geographical origin*	*Sample Size*	*Reference*
Shangaan (Sa)	Mozambique (SW), Zimbabwe (S), South Africa (NE), Swaziland	22	Salas *et al*., (2002)
Chopi (Ch)	Mozambique (SE)	27	Salas *et al*., (2002)
Chwabo (Cw)	Mozambique (NE)	20	Salas *et al*., (2002)
Lomwe (Lo)	Mozambique (NE)	20	Salas *et al*., (2002)
Makonde (Ma)	Mozambique (N), Tanzania (S)	19	Salas *et al*., (2002)
Makhuwa (Mk)	Mozambique (NE)	20	Salas *et al*., (2002)
Ndau (Nd)	Mozambique (C, E)	19	Salas *et al*., (2002)
Nguni (Ng)	Mozambique (W), Malawi (E)	11	Salas *et al*., (2002)
Nyungwe (Ny)	Mozambique (W), Zimbabwe (E)	20	Salas *et al*., (2002)
Nyanja (Na)	Mozambique (N), Malawi (E)	20	Salas *et al*., (2002)
Ronga (Ro)	Mozambique (S), Swaziland	21	Salas *et al*., (2002)
Shona (Sh)	Mozambique (C), Zimbabwe (E)	17	Salas *et al*., (2002)
Sena (Se)	Mozambique (C)	21	Salas *et al*., (2002)
Tswa (Ts)	Mozambique (SE)	19	Salas *et al*., (2002)
Southern Africa:			
Kung¡ (Ku)	Botswana, South Africa	61	Vigilant *et al*., (1991) and Chen *et al*., (2000)
Khwe (Kh)	South Africa	31	Chen *et al.* (2000)

[1]These tribes contribute to the Senegalese sample (*n*): Tukulor (11), Maure (3), Gambia (1), Bambara (6), Sosse (2), Manyake (5), Diola (8), Lebou (2), Peul (7), Balante (1), Soninke (3), Sarakhole (3), Bainouk (1), and Malinke (1).

3. Results

This section presents the results obtained for the two sets of data we analyzed. The first one included the samples initially categorized within macrohaplogroup L. Findings for this analysis were incorporated to the data set initially studied by Martínez-Cruzado et al. (2005). Results for the combined data sets are also presented.

3.1 HVS-I Sequence Analysis

DNA sequences of the HVS-I of the mitochondrial control region were determined for three L2 samples (Table 4). We used the HVS-I motifs for haplogroup classification from Salas *et al*., (2004) and determined one sample as a member of haplogroup L2a due to the point mutations at np 16233-16278-16294 , one as L2c due to mutations at np16233-16278-16390, and one as L2d due to the mutations at np 16233-16278-16390-16399.

Table 4 Sequence information of the mitochondrial control region from L2 samples

Sample Code	HVS-1 Sequence Motif (np – 16,000)	Haplogroup
JA6	214, 223, 250, 268, 278, 286, 294, 309	L2a
OA25	89, 223, 259, 274, 278, 390	L2c
PA2	184, 223, 278, 292, 355, 390, 399, 400	L2d

3.2 African mtDNA Haplogroup Frequencies in Puerto Rico

The mtDNA haplogroup frequencies were initially estimated for the weighted sample of 73 participants categorized in macrohaplogroup L. As shown in Table 5, approximately 67% of these samples are encompassed within haplogroups L2a (41.1%) and L1c (26.0%). Haplogroups L0a and L2d are the least frequent haplogroups among this sample.

Table 5 Weighted data for Sub-Saharan African samples categorized in the L macrohaplogroup

	Haplogroups								
	L0*	L0af*	L0a	L1c	L2a	L2b	L2c	L2d	*Total*
Sample size	2	9	1	19	30	5	6	1	73
Sample %	2.7	12.3	1.4	26.0	41.1	6.8	8.2	1.4	100.0

Next, we proceeded to combine the results obtained for those samples initially categorized in the L macrohaplogroup with the data from other samples with Sub-Saharan African phylogeny. Table 6 shows that almost 80% of the Puerto Rican Sub-Saharan African mtDNA pool is encompassed within haplogroups L1b, L3e, L2a, L3d, L1c, and L3b. The three largest haplogroups, L1b, L3e, and L2a, account for more than half (53.7%) of the Puerto Rican African gene pool. Meanwhile, haplogroups L0a, L2d, and L3g have a limited representation in the island. When we examined the frequency distribution amongst all Sub-Saharan African samples by municipality, we observed that most of the haplogroups under study distribute evenly (Table 7).

Table 6 Weighted data for Sub-Saharan African haplogroups present in a representative sample of Puerto Ricans

	Haplogroups								
	L0*	L0af*	L0a	L1c	L2a	L2b	L2c	L2d	L1b
Sample size	2	9	1	19	30	5	6	1	48
Sample %	0.9	4.2	0.6	8.8	13.9	2.4	2.7	0.4	22.1

	Haplogroups								
	L3*	L3b	L3d	L3e	L3f	L3g	L3h	$U5b_2$	**Total**
Sample size	1	16	22	38	4	1	4	9	216
Sample %	0.5	7.2	10.2	17.7	1.8	0.6	1.8	4.1	100.0

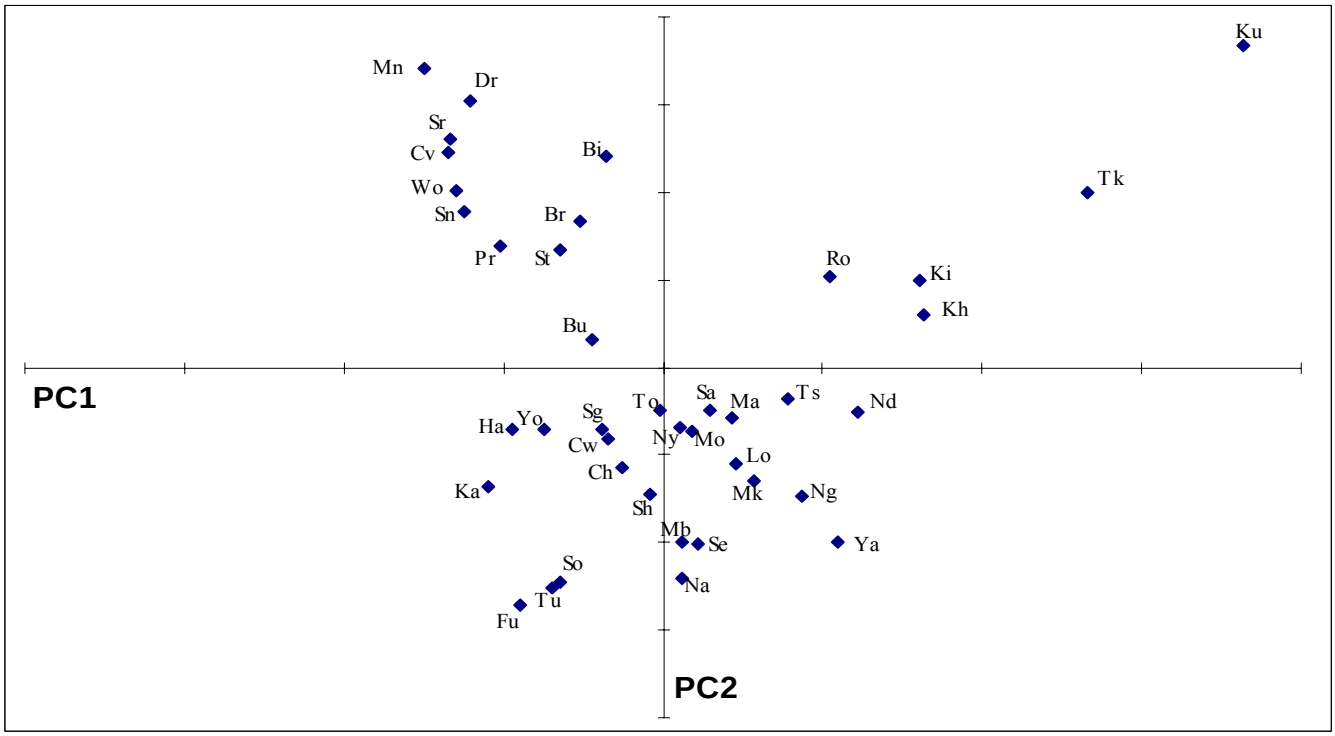
3.3 Principal Component Analysis

The first set of PCA, encompassing the weighted data of the 73 samples categorized in the L macrohaplogroup, shows two-dimensional plots which summarize the results obtained for this group. Figure 3, panel A, plots the first two principal components (PC) and Figure 3, panel B, the first and third PCs. The first principal component (PC 1), accounting for 29.4% of the genetic variance, separates the populations in both panels into a spectrum with West Africans, Puerto Rico, and Dominican Republic at one pole, followed by Central Africans, Brazilians, Southeast Africans, and East Africans with Kung¡ at the opposite pole.

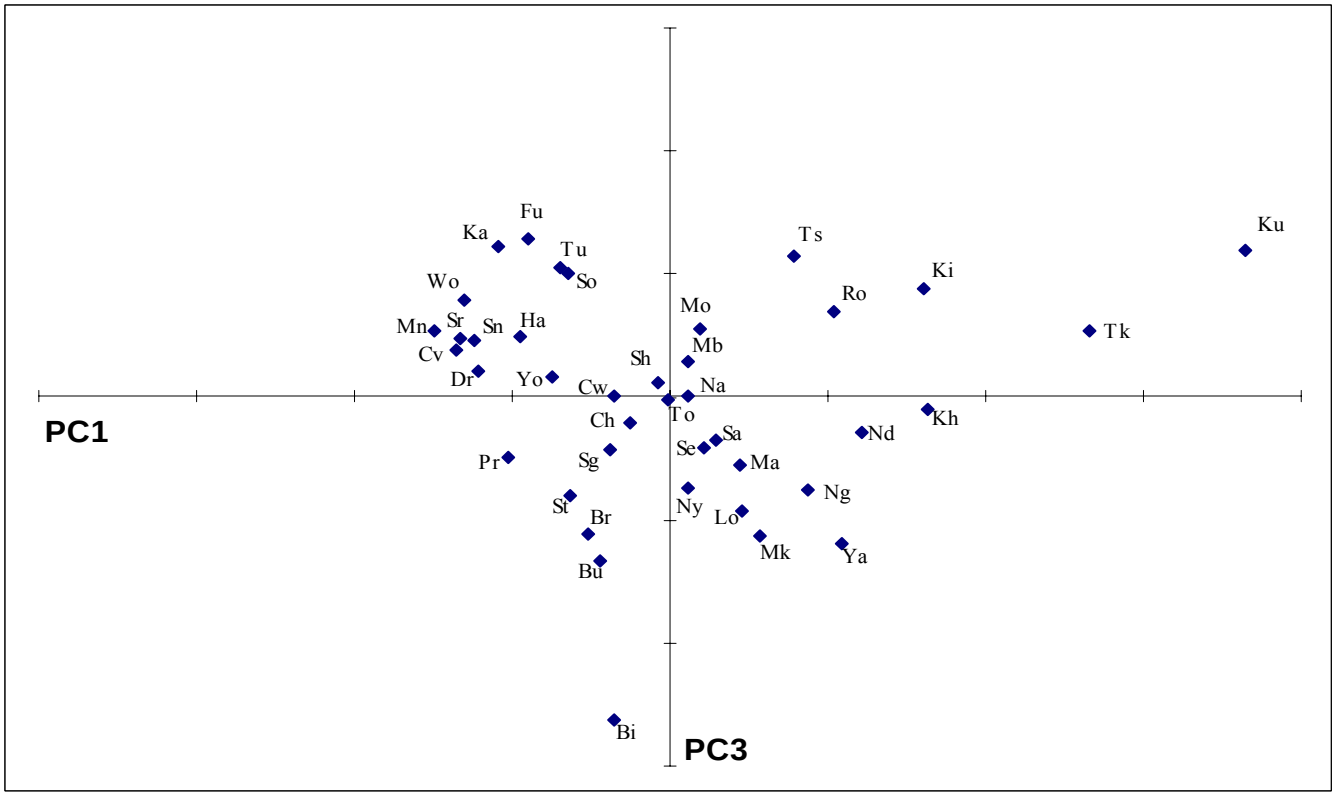
PC 2 (18.9% of the variance), by contrast, clusters Southeast Africans with West Africans on the lower segment while the top segment clusters other West African populations with Central Africans and East Africans. Again, West Africans tend to group with Puerto Rico and Dominican Republic while Central Africans cluster with Brazil. PC3, accounting for 16.9% of the genetic variance, clusters on the top segment West Africans and East Africans followed by Southeast Africans close to the center and finalizing with Central Africans at the opposite pole.

Table 7 Distribution of Sub-Saharan African haplogroups present in Puerto Rico by municipality

		Sub-Saharan African Haplogroups																	
		L0*	L0af*	L0a	L1b	L1c	L2*	L2a	L2b	L2c	L2d	L3*	L3b	L3d	L3e	L3f	L3g	L3h	$U5b_2$	Total
	NORTH																			
NORTH	Arecibo							3												3
	Bayamón		1		2	3		1						1	2				1	11
	Carolina				1	2		1				0	3	2	3			2		14
	Florida		1		1					1			2						1	6
	Guaynabo				2	1		1							1					5
	San Juan	1	1		10	2		4				0		2	6	2		1	2	31
	Toa Baja		1		1			1						2	1					6
	Vega Alta					3				1			2		3				2	11
	Vega Baja							1					1							2
	SOUTH																			
SOUTH	Guayanilla	1		1		1													1	4
	Juana Díaz				1	1														2
	Peñuelas							1	1			0	1		1					4
	Ponce				1			1												2
	Yauco					1									4					5
	EAST																			
EAST	Humacao				5	4	1		2			0	1		1			1		15
	Loíza		3		7	3		9		2				5						29
	Patillas											0	2	1	1	1				5
	San Lorenzo				5			1		1			1	1	1					10
	WEST																			
WEST	Aguadilla							1				0	1	2			1			5
	Hormigueros		1		2	1														4
	Mayagüez							1	2						2					5
	Moca												1		1					2
	San Sebastián				2									2	1					5
	CENTRAL																			
CENTRAL	Barranquitas				1				1					1	2					5
	Caguas		1		1			1				1			4					8
	Cayey				2			1		2	1				4				1	11
	Corozal				3			1						1						5
	Jayuya				2			1						1					1	5
	TOTAL	**2**	**9**	**1**	**49**	**22**	**1**	**30**	**6**	**7**	**1**	**1**	**15**	**21**	**38**	**3**	**1**	**4**	**9**	**220**



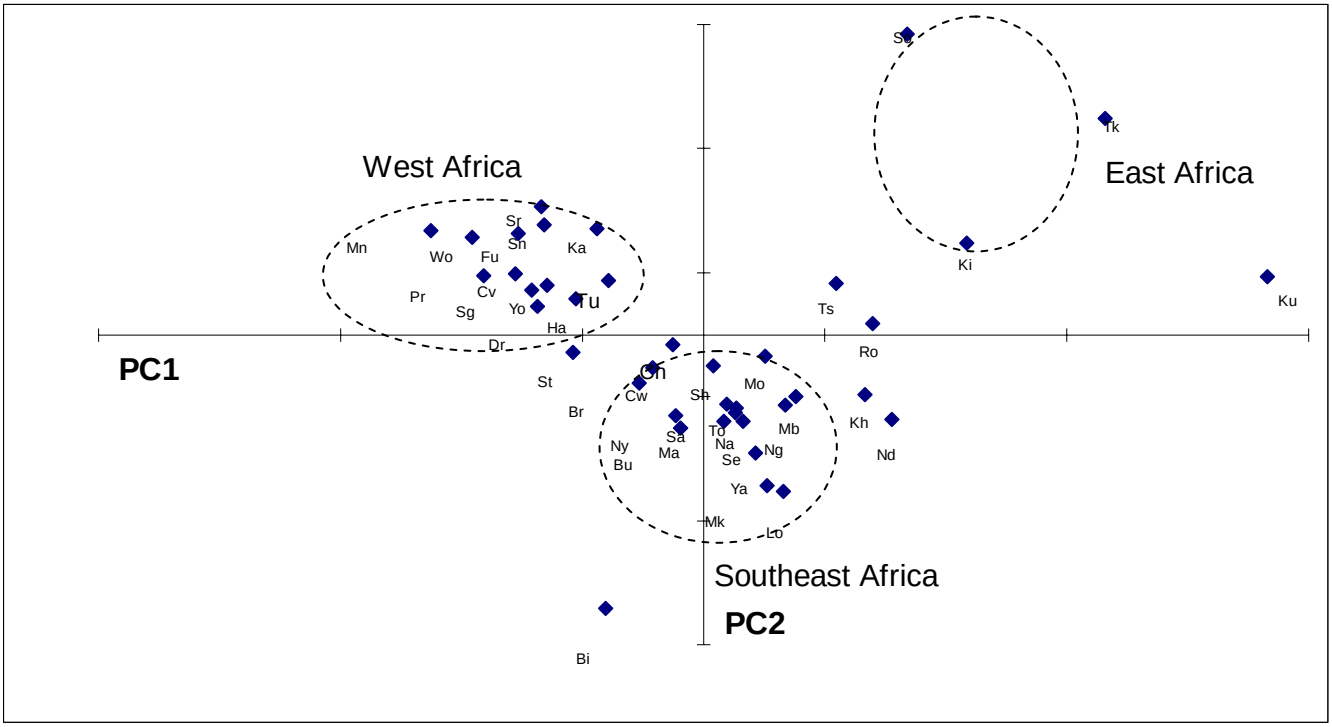
Panel A



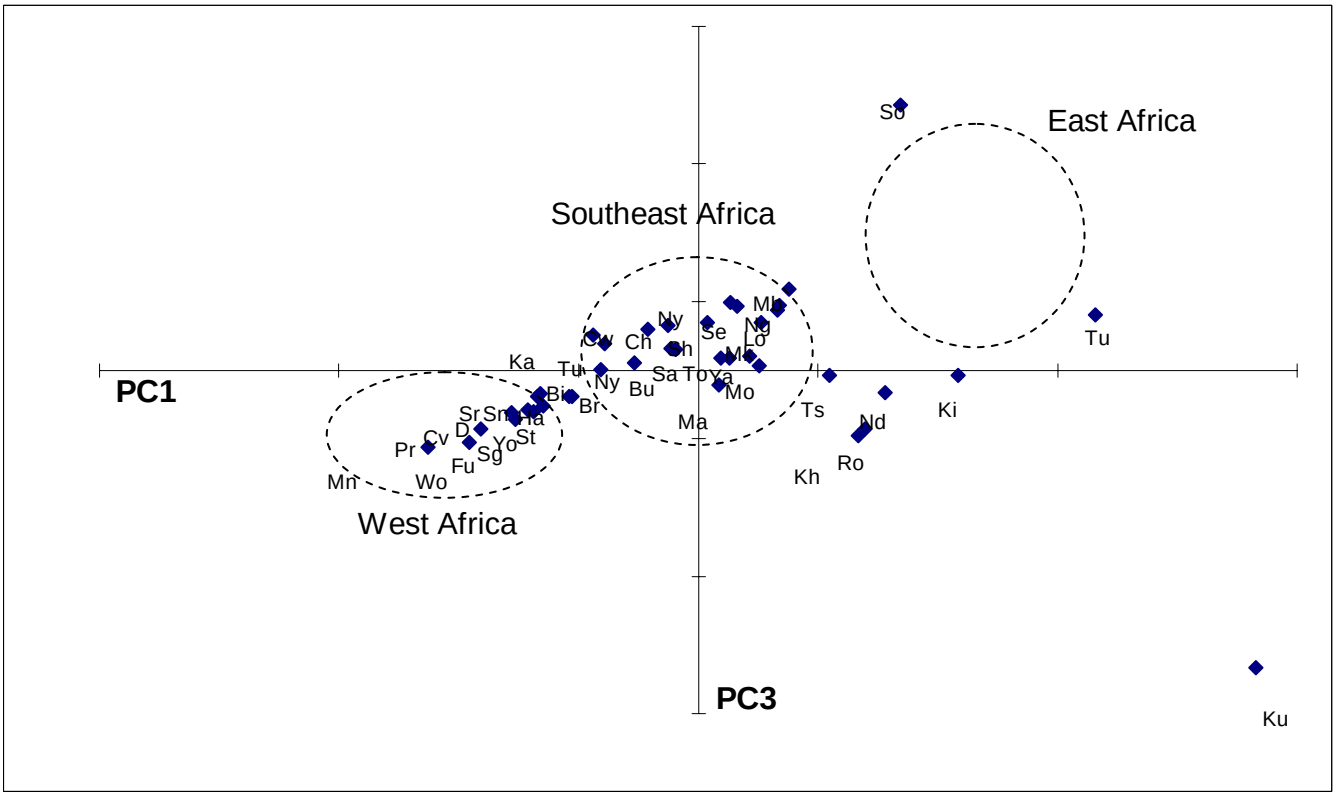
Panel B

Figure 3 PCA of Sub-Saharan African populations based on mtDNA haplogroup frequencies for the samples categorized in L macrohaplogroup. Population codes are defined at Table 3.

Next, we explored the effects of including all sub-Saharan African haplogroups in the analysis. The second set of PCAs includes the weighted data of all Sub-Saharan African samples from the Puerto Rican population. Again, panel A plots the first two PCs and panel B the first and third PCs (Figure 4). The most striking feature of PC1 and PC2 (encompassing 21.2% and 15.4% of the variance, respectively) is that two population clusters were clearly distinguished, one tightly grouping most West African populations with Puerto Rico, Dominican Republic, and Cape Verde and another cluster comprising the populations from Mozambique and Southeast Africa. Both panels show scattered East African populations positioned in the top-right quarter of the plot. PC3 comprehends 12.8% of the variance.



Panel A



Panel B

Figure 4 PCA of Sub-Saharan African populations based on mtDNA haplogroup frequencies for all samples. Population codes are defined at Table 3.

4. Discussion

In this study we depict the mtDNA variation in an attempt to trace Puerto Rican African-associated lineages back to the continent, allowing us to explore the extent to which different regions in Africa have contributed to the actual composition of Puerto Rican maternal lineages. Historical literature on the Atlantic slave trade and Puerto Rico suggests that the coast extending from Ghana to Cameroon was the largest outlet of slaves to the island, although significant contributions can also be attributed to the area from Senegambia to Sierra Leone, Congo, and Angola. Our results, which corroborate the historical picture, underline the overwhelming impact of western and west-central African regions to the Puerto Rican mtDNA composition. Almost 60% of the Sub-Saharan haplogroups in the island are exclusive to or are more common in the West African populations and 26.5% in the West-Central African region.

Predominantly western African haplogroups present in Puerto Rico include: L1b, L2b, L2c, L2d, L3b, and L3d. Haplogroup L1b, the most common of all Sub-Saharan African lineages in the island, with a frequency of 22.1%, is an exclusive West African haplogroup. Haplogroups L2b, L2c, and L2d, which account for 2.4%, 2.7%, and 0.4% of the Sub-Saharan African component in the Puerto Rican population respectively, are also much less frequent subgroups in the African continent. The geographic distribution of these three haplogroups is largely restricted to western Africa, not found in eastern Africa, and rarely present in Southeast Africa (Salas *et al*., 2004). L3b and L3d account for 7.2% and 10.2% of the African mtDNAs in Puerto Rico, respectively. While L3b mtDNAs are mainly West African, they are also present in North Africa and the Near East with very little dispersal into eastern or Central Africa (Salas *et al*., 2002). In addition, several L3d types are also found in southeastern Africa and in the southern populations of Khwe and Kung¡. Haplogroup L2a, the third (13.9%) most common

Sub-Saharan African haplogroup in the Puerto Rican population, is widely distributed in Africa. Although well represented in southeastern populations and present in some eastern ones, a West African origin is attributed to this haplogroup in America (Salas *et al*., 2002). Sequence haplotypes of many American L2a samples match or are close derivates of western African types (Salas *et al*., 2004). These studies also demonstrated matches with eastern and southeastern African types; however, all of them are also present in West Africa. Salas *et al*., 2004 concluded that a large western African origin, with a minor southeastern African contribution, is the simplest explanation for the continental origins of this clade in the Americas.

Haplogroups L1c and L3e, mainly West-Central African lineages, account for almost one-fourth of the genetic variance in the Puerto Rican population. Haplogroup L1c, ascribed to a west-central African origin (Salas *et al*., 2002; 2004; Trovoda *et al*., 2003), depicts 8.8% of the Sub-Saharan African mtDNAs in the Puerto Rican population. This haplogroup reaches particularly high frequencies amongst the Biaka Pygmies (~70%) from the Central African Republic, followed by the Bubi from the island of Bioko and the Angolans. Haplogroup L1c is also found in 8.0% of the Dominicans with Sub-Saharan African ancestry. This information reinforces the traditional history, which states similar Sub-Saharan African beginnings for Puerto Rico and the Dominican Republic, both Spanish colonies (Díaz-Soler, 2000). Haplogroup L3e, thoroughly analyzed by Bandelt *et al*., 2001, is the most widespread, frequent, and ancient of all African L3 clades. Bandelt proposed a Central African / Sudanese origin for this haplogroup around ~45,000 years ago. However, several L3e subclades appear to have spread into western Africa (Salas *et al*., 2004). While this haplogroup represents around one third of all the Sub-Saharan Africans mtDNAs found in Brazil (Alves-Silva *et al*., 2000), it accounts for significantly less of the African component in the Puerto Rican and Dominican (Torroni, published data at

Brehem *et al*., 2002) populations. The historical differences between Brazil and Puerto Rico and the Dominican Republic have become evident as the haplogroup distribution of the former differs from that of the other two nations. The mtDNA pool of modern Brazilians clearly reflects the imprints of Central Africa, including Cameroon and Angola, as haplogroups L1c and L3e constitute approximately one-half of its Sub-Saharan African fraction. Furthermore, haplogroups L1b and L3d, which are specific to West Africa, only occur in 10% of the Sub-Saharan African component of the Brazilian population (Alves-Silva *et al*., 2000).

The contributions of eastern, southeastern, and southern Africa to the Puerto Rican mtDNA gene pool appeared to be very small, again this concurs with historical research. History suggests that eastern African populations did not contribute significantly to the Atlantic slave trade (Díaz-Soler, 2000). This statement is reinforced by the total absence of East African haplogroups L1e and L1f in Puerto Rico and other American populations (Salas *et al*., 2004). Still, East African haplogroups, such as L3f (1.8%) and L3g (0.6%), are present in Puerto Rico. Although these haplogroups are mainly from East Africa, several researchers have found a small number of L3f types (Salas *et al*., 2002; Rosa *et al*., 2004) in West and Central Africa and L3g types (Bortoloni *et al*., 2004; Destro-Bisol *et al*., 2004) in Cameroon. Given that there is no strong historical evidence of significant slave trade from eastern Africa to Puerto Rico it is most likely that the presence of these two haplogroups in the island responds to gene flow from East Africa into western and Central Africa and then into Puerto Rico. Haplogroup L0a, typically found in southern and southeastern African populations (Watson *et al*., 1997, Pereira *et al*., 2001, Salas *et al*., 2002; 2004), is barely present in the Puerto Rican sample (0.6%). The research published by Salas *et al*., 2004 reveals that southeastern African L0a clades are also present in the Angolan population. Thus, a possible contribution of this haplogroup to the Puerto Rican

population could be attributed to gene flow from southern areas to other African regions, such as Angola.

The region-based PC analyses (Figuer 3 and Figure 4) allowed us to visualize the clustering of mtDNA haplogroup profiles in various Sub-Saharan African, Dominican, and Brazilian populations to gain a better understanding of the continental origins of the Sub-Saharan African haplogroups present in modern Puerto Ricans. Overall, our findings have shown that there is a significant geographical structuring, which distinguishes the West African populations from those of Southeast Africa. In its first component, our PC analysis (Figure 4, Panel A and Panel B) illustrates a west-east gradient in the West African cluster with populations in the vicinity of Senegal at the left edge of the West African cluster and those closer to Niger and Nigeria in the right edge. Haplogroups L1b, L3b, and L2c, all more common in western Africa, are the main contributors to the left pole of this component (Appendix A). PC1 also evidences that eastern African populations, Turkana, Somalia, and Kikuyu, are noticeably separated from the western and southeastern African clusters. Both, PC2 and PC3 (Figure 4, Panel A and Panel B, respectively) support these findings, which establish Sub-Saharan African groupings from populations in similar geographical regions.

The Puerto Rican population lies within the West African cluster and is surrounded by populations from the Senegambia region, Cape Verde being the closest one. The grouping of Puerto Rico with the Senegambian populations broadly corroborates historical reports identifying this region as one of the largest sources of slaves to the island. Furthermore, Senegambian mtDNAs were predominant in the 16th century and thus have spent more time reproducing in Puerto Rico than mtDNAs from more to the south, which were more common among slaves brought in the 19th century, according to history. The Dominican Republic, another population

historically associated to western African roots, also clusters within the West African grouping, in close proximity to the Cape Verde Islands. This archipelago was the Senegambian outpost of the Atlantic slave trade and was inhabited at first by slaves acquired along the Senegal River basin, the coast of the Gulf of Guinea, and later from the Coast of Angola (Barry, 1998; Brehm *et al*., 2002). Brazil, another American population largely influenced by the Atlantic slave trade, is positioned between the West African and southeastern African clusters, although relatively closer to the second one. These findings are in accordance with the historical documentation (Thomas, 1997) of a differential slave trade, with Central and Southeastern African slaves more likely to be taken to Brazil. Brazil is believed to have a significant Angolan mtDNA component (Alves-Silva *et al*., 2000); however the population in this Sub-Saharan African country must be further sampled to adequately attribute the phylogeographic origins of this American population.

5. Conclusion

The results obtained in our study reveal a strong matrilineal contribution of West African populations to modern Puerto Ricans, as well as secondary participation from West-Central African groups. This study also suggests that the involvement from southern and eastern African populations to the Puerto Rican gene pool has been minimal. These results are in complete agreement with historical sources that strongly limit the participation of East African populations during the Atlantic slave trade, while supporting the western and west-central contributions. We have also demonstrated that all Sub-Saharan African haplogroups present in the Puerto Rican population display an even distribution throughout the island's territory.

6. Recommendations

Upcoming mtDNA research must include coverage in other Sub-Saharan African regions not adequately studied to this date, such as Angola and Congo, so a complete data set, which includes a wider spectrum of African populations, can be used for future mtDNA analyses.

The present mtDNA study was conducted primarily with RFLP analysis. The inclusion of hypervariable segment analysis, at least for the most common haplogroups in the island (L1b, L2a, and L3e), could provide more specific information on the mtDNA similarities and differences among Puerto Ricans and Sub-Saharan African populations. Furthermore, the mtDNA data of the Puerto Rican population must be reinforced with detailed phylogeographic analyses of the African-specific Y-chromosome markers, which will ultimately provide a more precise picture of the phylogeographical landscape in this population.

However, it is important to keep in mind that mtDNA variation allows to trace maternal ancestry of Puerto Ricans to broad geographical regions within the African continent. As pointed out by Salas *et al*., 2005, caution must be exercised when dealing with promises made by testing companies about their ability to trace the ancestry of individuals to a specific African population or locale. Thus, mtDNA analysis will allow a better understanding of the regional African origins of the modern population of Puerto Rico, while the attribution of a more specific geographical Sub-Saharan African progenitor to any Puerto Rican African-associated mtDNA is a matter of conjecture.

7. References

Alegría RE (1985) Notas sobre la procedencia cultural de los esclavos negros de Puerto Rico durante la segunda mitad del siglo XVI. La Revista del Centro de Estudios Avanzados de Puerto Rico y el Caribe, San Juan, Puerto Rico.

Álvarez-Nazario M (1974) El elemento afronegroide en el español de Puerto Rico. Instituto de Cultura Puertorriqueña, San Juan, Puerto Rico.

Alves-Silva J, da Silva Santos M, Guimarães PEM, Ferreira ACS, Bandelt HJ, Peña SDJ, Ferreira Prado V (2000) The ancestry of Brazilian mtDNA lineages. Am J Hum Genet 67:444-461.

Anderson S, Bankier AT, Barrell BD, de Bruijin MH, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJ, Staden R, Young IG (1981) Sequence and organization of the human mitochondrial genome. Nature 290:457-465.

Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N (1999) Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet 23:147.

Ballinger SW, Schurr TG, Torroni A, Gan YY, Hodge JA, Hassan K, Chen KH, and Wallace DC (1992) Southeast Asian mitochondrial DNA analysis reveals genetic continuity of ancient Mongoloid migrations. Genetics 130:139-152.

Barry B (1998) Senegambia and the Atlantic slave trade. CUP, Cambridge, England.

Bravi CM, Sans M, Bailliet G, Martínez-Marignac VL, Portas M, Barreto I, Bonilla C, Bianchi NO (1997) Characterization of mitochondrial DNA and Y-chromosome haplotypes in a Uruguayan population of African ancestry. Hum Biol 69:641-652.

Brown WM, George M, Wilson AC (1979) Rapid evolution of animal mitochondrial DNA. Proc Natl Acad Sci 76:1967-1971.

Cann RL, Stoneking M, Wilson AC (1987) Mitochondrial DNA and human evolution. Nature 325:31-36.

Chen YS, Olckers A, Schurr TG, Kogelnik AM, Huoponen K, Wallace DC (2000) mtDNA variation in the South African Kung and Khwe-and their genetic relationships to other African populations. Am J Hum Genet 66:1362-1383.

Chen YS, Torroni A, Excoffier L, Santachiara-Benerecetti AS, Wallace DC (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.

Denaro M, Blanc H, Johnson MJ, Chen KH, Wilmsen E, Cavalli-Sforza LL, Wallace DC (1981) Ethnic variation in *Hpa*I endonuclease cleavage patterns of human mitochondrial DNA. Proc Natl Acad Sci USA 78:5768-5772.

Destro-Bisol G, Coia V, Boschi I, Verginelli F, Caglià A, Pascali V, Spedini G, Calafell F (2004) Tha analysis of variation of mtDNA hypervariable region 1 suggests that Eastern and Western Pygmies diverged before the Bantu expansion. Am Nat 163:212-226.

Díaz-Soler LM (2000) Historia de la esclavitud negra en Puerto Rico. Editorial de la Universidad de Puerto Rico, San Juan, Puerto Rico.

Enríquez JA, Chomyn A, Attardi G (1995) mtDNA mutation in MERRF syndrome causes defective aminoacylation of tRNA(Lys) and premature translation termination. Nat Genet 10:47-55.

Fernández-Méndez E (1970) Historia cultural de Puerto Rico. Ediciones El Cemí, San Juan, Puerto Rico.

Green LD, Derr JN, Knight A (2000) mtDNA affinities of the peoples of north-central Mexico. Am J Hum Genet 66:989-998.

Herrnstadt C, Elson JL, Fahy E, Preston G, Turnbull DM, Anderson C, Ghosh SS, Olefsky JM, Beal MF, Davis RE, Howell N (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.

Ho SYW, Phillips MJ, Cooper A, Drummond AJ (2005) Time dependency of molecular rate estimates and systematic overestimation of recent divergence times. Mol Biol Evol 22:1561-1568.

Howell N (1999) Human mitochondrial diseases: answering questions and questioning answers. Int Rev Cytol 186:49-116.

Ingman M and Gyllensten U (2001) Analysis of the complete human mtDNA genome: Methodology and inferences for human evolution. J Hered 92:454-461.

Ingman M, Kaessmann H, Pääbo S, Gyllensten U (2000) Mitochondrial genome variation and the origin of modern humans. Nature 408:708-713.

Johnson MJ, Wallace DC, Ferris SK, Rattazzi MC, Cavalli-Sforza LL (1983) Radiation of human mitochondrial DNA types analyzed by restriction endonuclease cleavage patterns. J Mol Evol 19:255-271.

Kong QP, Yao YG, Sun C, Bandelt HJ, Zhu CL, and Zhang YP (2003) Phylogeny of East Asian mitochondrial DNA lineages inferred from complete sequences. Am J Hum Genet 73:671-676.

Lightowlers RN, Chinnery PF, Turnbull DM, Howell N (1997) Mammalian mitochondrial genetics: heredity, heteroplasmy and disease. Trends Genet 13:450–455.

Martínez-Cruzado JC, Toro-Labrador G, Ho-Fung V, Estévez-Montero M, Lobaina-Manzanet A, Padovani-Claudio DA, Sánchez-Cruz H, Ortiz-Bermúdez P, Sánchez-Crespo A (2001) Mitochondrial DNA analysis reveals substantial Native American ancestry in Puerto Rico. Hum Biol 73:491-511.

Martínez-Cruzado JC, Toro-Labrador G, Viera-Vera J, Rivera-Vega MY, Startek J, Latorre-Esteves M, Román-Colón A, Rivera-Torres R, Navarro-Millán IY, Gómez-Sánchez E, Caro-González HY, Valencia-Rivera P (2005) Reconstructing the Population History of Puerto Rico by means of mtDNA Phylogeographic Analysis. Am J Hum Genet 128:131-155.

Mateu E, Comas D, Calafell F, Pérez-Lezaun A, Abade A, Bertranpetit J (1997) A tale of two islands: population history and mitochondrial DNA sequence variation of Bioko and São Tomé, Gulf of Guinea. Ann Hum Genet 61:507-518.

Rando JC, Cabrera VM, Larruga JM, Hernández M, González AM, Pinto F, Bandelt HJ (1999) Phylogeographic patterns of mtDNA reflecting the colonization of the Canary Islands. Ann Hum Genet 63:413-428.

Reidla M, Kivisild T, Metspalu E, Kaldma K, Tambets K, Tolk HV, Parik J, Loogväli EL, Derenko M, Malyarchuk B, Bermisheva M, Zhadanov S, Pennarun E, Gubina M, Golubenko M, Damba L, Fedorova S, Gusar V, Grechanina E, Mikerezi I, Moisan JP, Chaventré A, Khusnutdinova E, Osipova L, Stepanov V, Voevoda M, Achilli A, Rengo C, Rickards O, De Stefano GF, Papiha S, Beckman L, Janicijevic B, Rudan P, Anagnou N, Michalodimitrakis E, Koziel S, Usanga E, Geberhiwot T, Herrnstadt C, Howell N, Torroni A, and Villems R. (2003) Origin and diffusion of mtDNA haplogroup X. Am J Hum Genet 73:1178-1190.

Richards M, Côrte-Real H, Forster P, Macaulay V, Wilkinson-Herbots H, Demaine A, Papiha S, Hedges R, Bandelt HJ, Sykes B (1996) Paleolithic and Neolithic lineages in the European mitochondrial gene pool. Am J Hum Genet 59:185-203.

Rosa A, Brehm A, Kivisild T, Metspalu E, Villems R (2004) MtDNA profile of West Africa Guineans: towards a better understanding of the Senegambia region. Ann Hum Genet 68:340-352.

Ruiz-Pesini E, Lapeña AC, Diez-Sánchez C, Pérez-Martos A, Montoya J, Álvarez E, Díaz M, Urriés A, Montoro L, López-Pérez MJ, Enríquez JA (2000) Human mtDNA haplogroups associated with high or reduced spermatozoa motility. Am J Hum Genet 67:682-696.

Salas A, Richards M, De la Fé T, Lareu MV, Sobrino B, Sánchez-Diz P, Macaulay V, Carracedo A (2002) The making of the African mtDNA landscape. Am J Hum Genet 71:1082-1111.

Salas A, Richards M, Lareu MV, Scozzari R, Coppa A, Torroni A, Macaulay V, Carracedo A (2004) The African Diaspora: Mitochondrial DNA and the Atlantic Salve Trade. Am J Hum Genet 74:454-465.

Schon EA, Bonilla E, DiMauro S (1997) Mitochondrial DNA mutations and pathogenesis. J Bionerg Biomembr 29:131-149.

Scozzari R, Torroni A, Semino O, Sirugo G, Brega A, Santachiara-Benerecetti AS (1988) Genetic studies on the Senegal population. I. Mitochondrial DNA polymorphisms. Am J Hum Genet 43:534-544.

Silva WA, Bonatto SL, Holanda AJ, Ribeiro-dos-Santos AK, Paixao BM, Goldman GH, Abe-Sandes K, Rodriguez-Delfin L, Barbosa M, Pacó-Larson ML, Petzl-Erler ML, Valente V, Santos SEB, and Zago MA. (2002) Mitochondrial genome diversity of Native Americans supports a single early entry of founder populations into America. Am J Hum Genet 71:187-192.

Soodyall H, Jenkins T (1992) Mitochondrial DNA polymorphisms in Khoisan populations from southern Africa. Ann Hum Genet 56:315-324.

Soodyall H, Jenkins T (1993) Mitochondrial DNA polymorphisms in Negroid populations from Namibia: new light on the origins of the Dama, Herero, and Ambo. Ann Hum Biol 20:477-485.

Stoneking M (1993) DNA and recent human evolution. Evol Anthropol 2:60-73.

Tambets K (2004) Towards the understanding of post-glacial spread of human mitochondrial DNA haplogroups in Europe and beyond: A phylogeographic approach. Tartu University Press, Tartu, Estonia.

Thomas H (1997) The slave trade: The history of the Atlantic slave trade 1440 - 1870. Macmillan Publishers, London.

Torroni A, Bandelt HJ, D'Urbano L, Lahermo P, Moral P, Sellitto D, Rengo C, Forster P, Savontaus ML, Bonné-Tamir B, Scozzari R (1998) mtDNA analysis reveals a major late Paleolithic population expansion from Southwestern to Northeastern Europe. Am J Hum Genet 62:1137-1152.

Torroni A, Huoponen K, Francalacci P, Petrozzi M, Morelli L, Scozzari R, Obinu D, Savontaus ML, Wallace DC (1996) Classification of European mtDNAs from an analysis of three European populations. Genetics 144:1835-1850.

Torroni A, Lott MT, Cabell MF, Chen YS, Lavergne L, Wallace DC (1994) 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, Rengo C, Guida V, Cruciani F, Sellitto D, Coppa A, Luna Calderon F, Simionati B, Valle G, Richards M Macaulay V, Scossari R (2001) Do the four clades of the mtDNA haplogroup L2 evolve at different rates? Am J Hum Genet 69:1348-1356.

Torroni A, Schurr TG, Cabell MF, Brown MD, Neel JV, Larsen M, Smith DG, Vullo CM, Wallace DC (1993) Asian affinities and continental radiation of the four founding Native American mtDNAs. Am J Hum Genet 53:563-590.

Trovoda MJ, Pereira L, Gusmão L, Abade A, Amorim A, Prata MJ (2003) Pattern of mtDNA variation in three populations from São Tomé e Príncipe. Ann Hum Genet 68:40-54.

Vigilant L, Pennington R, Harpending H, Docher TD, Wilson AC (1989) Mitochondrial DNA sequences in single hairs from a southern African populations. Proc Natl Acad Sci USA 86:9350-9354.

Vigilant L, Stoneding M, Harpending H, Hawkes K, Wilson AC (1991) African populations and the evolution of mitochondrial DNA. Science 253:1503-1507.

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

Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10:506-513.

Watson E, Bauer K, Aman R, Weiss G, von Haeseler A, Pääbo S (1996) mtDNA sequence diversity in Africa. Am J Hum Genet 59:437-444.

Appendix A

Haplogroup contribution to the principal components

Principal Components (PC)			
Haplogroups	**PC1**	**PC2**	**PC3**
L0*	-0.0389	0.017	-0.0238
L0af*	0.0446	0.00159	0.0281
L0a	0.102	-0.0908	0.0778
L1b	-0.227	0.134	-0.113
L1c	-0.113	-0.24	-0.00432
L0d / L1e	0.426	0.0913	-0.304
L2a	-0.017	0.0137	0.161
L2b	-0.0803	0.0357	-0.0846
L2c	-0.148	0.0973	-0.0833
L2d	-0.0884	0.0316	-0.0215
L3*	0.0113	0.106	0.0735
L3b	-0.164	0.0984	-0.12
L3d	-0.0983	0.0317	0.00714
L3e	-0.021	-0.0847	-0.0125
L3f	0.0372	0.101	0.0317
L3g	0.174	0.292	0.248
L3h	-0.0346	0.0239	0.00033
U5b2	-0.0426	0.0188	-0.0257

The haplogroups with the highest numbers in each principal component influences on the positive side of that axl (component) while the haplgroups with the lowest numbers influences on the negative side of it. Thus, populations localized on either side of the component are more influenced by the haplogroups impacting that side of the axl.