

Delaware Review of Latin American Studies

Vol. 1 No. 2 August 15, 2000

Juan Carlos Martínez Cruzado on some important Genetics research contributions to the study of Population History and Anthropology in Puerto Rico: An interview.

Juan Carlos Martínez Cruzado is Professor of Genetics at the University of Puerto Rico, Mayagüez Campus. We invited him to talk to us about his research project, funded by the National Science Foundation, to determine the continental origin of the mtDNA of Puerto Ricans--a project spurred by the surprise finding of a much larger-than-expected number of Puerto Ricans testing positive for Amerindian ancestry.

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Some time during the 1980s, the Institute of Puerto Rican Culture in San Juan, Puerto Rico, received four skeletal remains from a small burial ground that was accidentally discovered during the construction of a boardwalk in Arecibo, Puerto Rico. Having been disinterred out of context by a construction crew, these remains were of little archaeological value, so as a result, the Director of the Program of Archaeology of the IPRC, Juan José Ortiz Aguilú, gave the remains to Dr. Juan Carlos Martínez Cruzado, a Molecular Biologist at the University of Puerto Rico at Mayagüez (UPRM), to analyze for their mitochondrial DNA (mtDNA) content. Ortiz Aguilú's interest in mtDNA analysis of remains in burial sites was spurred by the peculiar positions in which he had found some skeletal remains, including one male interred holding a head in his hands, and another in which two skeletons were interred in one pit. An analysis of the mtDNA of these remains could shed light on the reasons for these peculiar positions by indicating whether the interred might have been parent and child, brothers, or, lacking a filial maternal relationship, victor and vanquished.

For Dr. Martínez Cruzado, the project had important administrative as well as research implications. Since returning to the UPRM in 1989 after receiving his Ph.D. at Harvard, he has worked with his departmental colleagues in order to develop initiatives designed to improve UPRM's pool of undergraduates who pursue Ph.D. degrees in the field of biomedical sciences. The dramatic success of these initiatives resulted in the need for improvement in the infrastructure of the Department of Biology since it could not meet the students' growing demand for research opportunities. The mtDNA project has helped ameliorate the situation by expanding the scope of the research projects in the department and by affording six students lab experience in identifying Puerto Rican mtDNA. Another six students from the Departments of Sociology and Psychology have also benefitted from the project, gaining field work experience by collecting genetic samples (hair roots) and interviewing donors.

Even more important, the identification of the Puerto Rican mtDNA could support or challenge--at least regarding the evolutionary contribution of females--the conventional wisdom that, because the indigenous population had disappeared by the end of the sixteenth century, there was little Amerindian contribution to the Puerto Rican gene pool. Should the results of the mtDNA analyses challenge the conventional wisdom, the stage would be set for Y-chromosome studies to assess the male contribution to the ethnic evolution of Puerto Ricans.

The results of Carbon 14 analysis, which dated the skeletal remains to approximately 645AD, was just the first of many exciting discoveries that this project has generated. The seventh century was a time of great change on the island because some great natural disaster paved the way for a fundamental change in the native population, most importantly, in the evolution of ceramic cultures. Before the disaster the inhabitants of the island were organized into egalitarian communities; but evidence indicates that, after the disaster, a hierarchical social structuralization of the native population evolved.

What exactly is mitochondrial DNA, and what does its analysis reveal?

An analysis of mitochondrial DNA can positively identify female ancestors because the mitochondrion is an organelle--a cell organ--that does not recombine as it passes from one generation to another down the female line; that is, it passes intact, without combining with the male mtDNA which is not transmitted from one generation to another. Nevertheless, the mitochondrion has a fast mutation rate, thus making it possible to trace ancestry within short periods of evolutionary time. These two characteristics of the mtDNA make it a highly informative genetic unit and the darling of human evolutionary geneticists.

What were the results of the mtDNA analyses of the skeletal remains that you received from Ortiz Aguilú?

The results were surprising: all 4 skeletons possessed identical mtDNA.

Why are these results surprising?

Though a high incidence of homogeneity within particular ethnic groups (referred to as the bottleneck effect) is not uncommon--and indeed, previous studies suggest the occurrence of just such an effect in the Pre-Columbian colonization of Puerto Rico through the Lesser Antilles--such homogeneity makes it impossible to identify the filial relationship of the interred. The results indicated that these people were definitely Amerindian, but it was not possible to determine whether a filial, as well as a cultural, relationship existed between them, even when the most hypervariable region of the mtDNA was analyzed. In order to determine that relationship we would have to examine the mtDNA of contemporary descendants of these people in search of variable sites in the mtDNA.

How could this be achieved?

Because the mitochondrion remains genetically intact through the maternal line, analysis of the mitochondria of contemporary Puerto Ricans who were likely to be of Amerindian ancestry could scientifically reveal such ancestry. Ideally, a study of a large group of Amerindian mtDNA should make it easier to determine the variable sites within that mtDNA, and so help us trace relationships back in time.

Considering that the history of Puerto Rico suggests that there were no Amerindians on the island by the end of the 16th century, how did you identify such descendants?

According to historian Salvador Brau, the censuses of 1777 and 1787 recorded the existence of some 2,000 Amerindians in the areas of Indiera Alta, Indiera Baja and Indiera Fría. These were descendants of a group of Tainos who, in 1570, decided to intern themselves in the mountainous regions of central Puerto Rico in order to protect themselves from Spanish colonization. Also, it is popular belief in the area around the city of Mayagüez that the barrio Miraflores of the town of Añasco was populated by many Indians and "negros cimarrones" fleeing slavery. We went to these areas and obtained a total of 23 samples of hair roots (18 from the Indieras, 5 from Miraflores) to analyze. We also sent a general e-mail to the staff, faculty and students of the UPRM requesting sample donations from anyone who had a mother or a grandmother who had Amerindian traits. This request resulted in 33 samples.

What did the analyses of these samples reveal?

More surprises. Of the 18 samples from the Indieras, 10 presented Amerindian mtDNA (55%); of the 5 samples from Miraflores, 4 were Amerindian (80%); of the 33 from the UPRM, 25 were Amerindian (76%). The high incidence of Amerindian mtDNA among these three groups was not in itself surprising because we had intentionally sought out those people who had reason to believe they were of Amerindian ancestry; but it was surprising to find that there was a higher incidence among the university students and personnel than among the inhabitants of the Indieras--who were considered "pure" Amerindians by the census of 1777 and 1787. This led us to request hair root samples from additional students regardless of their ancestry. Of the 38 samples obtained in this collection, 20 (53%) presented Amerindian mtDNA. Such a high incidence in the general student population suggested that, contrary to the prevailing view, some 53% of Puerto Ricans were of Amerindian ancestry exclusively through their maternal line. These findings made it clear that we needed to extend the study by analyzing a representative sample of the mtDNA of contemporary Puerto Ricans

It was at this point that you requested a grant from the National Science Foundation?

Yes. In August, 1999, I received a grant from the National Science Foundation to determine the continental origin of the mtDNA of Puerto Ricans through the analysis of a representative sample. To select the sample, we used a computer program that made a random selection of the total population of Puerto Rico based on the census of 1990. When corrected to take into account population growth in the last 10 years, the original 872 households chosen by the program became 1,073. To further insure the randomness of the sample, we requested hair root samples only from the adult in the household whose birthday most closely followed the date of the interview. We also interviewed the donors requesting information about their mothers, grandmothers, and great-grandmothers, as far back as they could remember, to learn of their origin. To date, 92% of the potential donors have agreed to participate, so that we have been able to collect hair samples from 781 residences.

What do the analyses of these samples suggest?

The results of the analyses of approximately 300 of these samples identify 62% as Amerindian, 30% as African blacks and 8% Caucasian.

So these results confirm your original findings and cast doubt on the notion that the Tainos disappeared from Puerto Rico by the end of the sixteenth century.

It seems so, for the moment, especially considering that similar studies in other countries have yielded similar results. In Belen, Brazil, for example, mtDNA analysis identifies 59% of the contemporary population as

Amerindian, while Y-chromosome analysis identifies less than 5% as Amerindian. This indicates that 59% of the population of Belen has an Amerindian mother somewhere down the ancestral line, while less than 5% of them have a male Amerindian ancestor.

Are any other traditional beliefs affected?

Yes. Our findings also indicate that the conventional wisdom that Amerindians would be concentrated in the mountains while African blacks would be concentrated in the coasts, is not accurate. A strong Amerindian presence has been found in the southern coastal city of Ponce, for example, while African black mtDNA is present in the central mountains of Puerto Rico. Undoubtedly, African slaves must have fled from the coasts to the mountains even though history does not record such a flight. Loiza Aldea, an area east of San Juan populated mostly by blacks, presents an interesting example. By a crown decree from Spain, the colonial government of Puerto Rico was instructed to place runaway slaves from the British colonies in what is today Loiza Aldea. This area was chosen by the Crown because it was the weakest flank of defense of the island, and they hoped that the freed blacks would help defend the island against British invaders. This is a historical fact, but what history cannot explain is the great quantity of fishermen among the blacks of Loiza Aldea. Fishing by blacks is considered an aberration because black slaves were traditionally taught a fear of the sea as a way to keep them enslaved. Some historians have argued that the blacks of Loiza developed their fishing skills through direct contact with the Tainos of Puerto Rico. The presence of Amerindian mtDNA in Loiza, supports this hypothesis. In general, the project underlines the fact that biology can help reveal ethnic origins as well as population growth and migration in the development of a people.

Sounds like a true meeting of the arts and sciences. What comes next?

Our findings to date are of great interest to historians and Puerto Ricans in general, but another important goal of our study of the continental origin of the mtDNA of Puerto Ricans is to determine the variability sites within the mtDNA so that the filial relationship among the remains found in Amerindian interments can be ascertained. A detailed characterization of Amerindian mtDNA will identify variable sites that will facilitate the design and execution of ancient DNA studies. Ancient DNA studies are necessary to relate the successive historic ceramic cultures found in Puerto Rico to Pre-Columbian migrations and population expansions. They may also be conducted to study the relationship of prehistoric Puerto Ricans to their neighbors, as well as their burial and religious practices. So, as you can see, we have only just begun our research. Fortunately, the large number of samples of contemporary Puerto Rican mtDNA that we have been able to collect is giving us a good basis for accomplishing our research goals; and the results of our analyses to date have set the stage for Y-chromosome studies that will allow us to estimate with precision the complete ethnic composition of the various geographic regions of Puerto Rico and define the contribution of both sexes to this composition. We have also found a number of variable sites in the mtDNA of Puerto Ricans, so we may some day be able to tell maternal relationships among our ancestors.