Kacike www.kacike.org
Journal of Caribbean Amerindian History and Anthropology

KACIKE: Journal of Caribbean Amerindian History and Anthropology ISSN 1562-5028
***Special Issue** edited by Lynne Guitar*
NEW DIRECTIONS IN TAINO RESEARCH
http://www.kacike.org/Current.html

The Use of Mitochondrial DNA to Discover Pre-Columbian Migrations to the Caribbean: Results for Puerto Rico and Expectations for the Dominican Republic

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In this study, we use Mitochrondrial DNA technology (mtDNA) to improve our understanding of the pre-Columbian migrations to the Antilles that gave rise to the Taínos. As a basis for our work, we need to review various studies, principally of the archaeological type, that have given us knowledge of the pre-Columbian migrations to the Greater Antilles.

It is known that more than 8,000 years ago the Greater Antilles were inhabited by nomads who depended for their survival upon the foods that they could collect and the animals that they could hunt. That era is known as the Lithic Era, which is distinguished by the stone tools that these people made.

Some 4,000 years later, we begin to note a great quantity of tools and adornments made of shells and some made of bone. That era is known as the Archaic Era, populated by nomads who appear to have subsisted principally on seafoods, but who also ate terrestrial products. It was not until some 2,200 years ago that a ceramic culture arrived in the Greater Antilles, consisting of agriculturalists who built permanent settlements near their areas of cultivation.

Little is known about the migrations from which the nomads originated, better known as the pre-ceramic culture. Three routes have been identified by which there could have been migratory waves to the Greater Antilles: proceeding from the Florida Peninsula by means of Cuba, proceeding from the Yucatan Peninsula also by means of Cuba, and proceeding from the Orinoco Delta by means of the Lesser Antilles. Dental studies done by Dr. Edwin Crespo, as in other studies, suggest that there were at least two migratory waves to the Greater Antilles. For this reason, confirmation of the use of the routes does not necessarily indicate that the other routes were not also used.

Distinct ceramic cultures existed in the Greater Antilles. Even before the time of Christ, on the island of Vieques, there already existed a Huecoid culture and Saladoid culture, clearly distinguishable by their ceramics, but also

through other cultural aspects. For example, while the burials of the Saladoids can be found relatively easily, the remains of Huecoid bones have never been found. All that has been found is one milk tooth. For this reason, it is believed that the religions of both cultures could have contained very different elements. Furthermore the Huecoids were specialists in working with semi-precious stones, which they frequently sculpted in the form of animals. Among them the figure of a bird stands out that many have identified as the Andean condor, for which reason they attach a continental origin to this region. Deposits with ceramic elements very similar to those of the Huecoids have been found near the mouth of the Guapo River in Venezuela. From there they would have taken the maritime route eastward toward Puerto Rico, Vieques, and other islands in the Northeastern Caribbean. The Saladoids, on the other hand, migrated from the region of Saladero near the mouth of the Orinoco River by means of the Lesser Antilles until they arrived in the Greater Antilles.

The Huecoid culture lasted some few hundred years, but the Saladoid culture, evolving with time, lasted until approximately the year A.D. 600. Certain evidence has been found in Puerto Rico that suggests great, natural events of a catastrophic nature that could have put an end to the Saladoid culture. What is certain is that a clearly distinct culture developed beginning from that date, the Ostionoid, which is divided into two stages known as pre-Taíno and Taíno. We do not know if the Ostionoid culture represents a marked cultural change of the Saladoid culture people due to the natural catastrophic events or some other type of event, or if it represents the arrival of a new migratory wave of Southamerican origin.

In conclusion, the archaeological evidence can identify four pre-Columbian migrations to the Greater Antilles: two pre-ceramic and two ceramic. The actual number of pre-Columbian migrations very well might have been only four, but there could have been many more.

We will see now what the mtDNA that we can extract from the contemporary inhabitants of the Major Antilles could offer us. The vast majority of our genetic material, perhaps better known as DNA, is found in the nucleus of the cell. The mtDNA, however, is not located in the nucleus of the cell but in an organelle known as the mitochondria. While nuclear DNA is inherited in equal parts from one's father and mother, mtDNA is inherited only from one's mother. It does not mix with that of the father and for that reason remains intact generation after generation, thus maintaining its original identity. That is to say, despite the intensive mestizaje (genetic "mixture") that has characterized our region over the centuries, we Caribbeans have mtDNAs that have maintained their original identity and that can be identified as African, Indian, or Caucasian. Their identity depends upon the women in our genetic tree at the end of the strictly maternal ancestral line. If this great-great-great grandmother were indigenous, then the corresponding Caribbean would have an indigenous mtDNA. He or she would have inherited it intact from that great-great-great grandmother who lived through those terrible first years of the colonization by means of his or her great-great grandmothers, great grandmothers, and maternal grandmother.

It was only a few months ago that we concluded a study of the ancestral

inheritance, through the strictly maternal line, of Puerto Ricans. Using the population information from the 1990 Census as well as a computer model, we randomly chose 1,067 residences in order to match the population density across Puerto Rico; therefore, these residences constituted a genuine representative sample of all of Puerto Rico's residences. Equally, one adult within each one of these residences that was inhabited was randomly chosen to guarantee the representation of our sample. Of those 1,067 residences, 985 were inhabited. Of the 985 inhabited residences, we could contact a selected adult in 875 of them. In exactly 800 of these 875 cases, the adults agreed to give us some samples of their hair roots in order to study their mtDNA. That is to say, 800 of the 985 selected adults (81.2%) participated in the study, and we could be satisfied that the 800 participants constituted a representative sample of the Puerto Rican population. Of the 800 participants, 489 (61.1%) had mtDNA of indigenous origin, 211 (26.4%) had mtDNA of African origin south of the Sahara, and exactly 100 (12.5%) had mtDNA of Caucasian origin.

There are two important things that have to be recalculated, confronted with these results. First, because inheritance is only through the mother, the mtDNA only traces the migrations of women. These results imply that there have been few migrations of women to Puerto Rico in post-Columbian times relative to the quantity of local women in the country, and that the cumulative effect of all those migrations after 500 years of colonial history has been to reduce the percentage of indigenous mtDNA from 100% to 61%.

Second, it is certain that the historical documentation reveals multiple occasions in which Indians from the Yucatan, from Hispaniola, from Margarita Island, from Brazil, and from other Spanish and Portuguese colonies were brought as slaves to Puerto Rico. Nonetheless, the historical documentation also reveals that the importation of African slaves greatly exceeded the importation of Indian slaves. Therefore, the greater frequency of indigenous mtDNA in Puerto Rico can only be explained from the basis of the mtDNA of Native Puerto Ricans, from whom must have come the major part of the indigenous mtDNA present in the country.

Although hair roots do not always provide sufficient material to make studies in great detail, many of the 489 samples that ended up being of indigenous origin had the necessary quality to let us study them more profoundly and to generate a hypothetical schematic of the pre-Columbian migrations to Puerto Rico. Owing to the affinity that existed between the Taínos of Quisqueya and those of Boriquén, we believe that this hypothetical schematic of the pre-Columbian migrations ought to apply in good measure to the Dominican Republic.

In order to better understand these detailed studies, it is necessary to know certain things in the field of molecular genetics and mtDNA. The molecule of mtDNA is the only DNA molecule in our cells that is circular. Also, it is the smallest. It was sequenced in its totality in Great Britain in 1981, determining that there were 16,569 long nucleotides. For those less expert in DNA, we can imagine the mtDNA molecule like a necklace of 16,569 pearls. Each pearl has a number and they are strung in order from 1 through 16,569. Furthermore, each pearl has a letter that could be A, T, C, or G, depending upon what its nitrogen base is.

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The change of one letter for another is known as a mutation.

Usually, the detection of mutations in the mtDNA is done by means of proof of restriction. An endonuclease restriction is an enzyme that selectively directs the DNA in a specific sequence. For example, the endonuclease *Alu*I cuts the DNA uniquely at those points where the DNA has sequential AGCT. If it were a molecule that had the AGCT sequence at some point, that molecule would be cut into two fragments by the endonuclease *Alu*I. If a mutation were to occur in one of the four nucleotides that form the sequence, the sequence would cease to exist, and *Alu*I would leave the molecule intact. This example illustrates one mutation that eliminates a restriction site known through the endonuclease. Other mutations create them. There are distinct restriction endonucleases that allow us to detect mutations easily at many points of the mtDNA. Thus, the mutations can be identified citing the number of the pearl where the mutation occurred in conjunction with the effect of the mutation due to the elimination or creation of a new restriction site.

Usually we think of a mutation as something very bad that has produced a clearly visible change and is unfavorable in the person, like producing a third eye or leaving him without a leg, but recently it has been demonstrated that the great majority of mutations do not produce a clearly visible effect in a person. Each one of us has approximately 175 mutations in the nucleus and nonetheless we consider ourselves normal. Well then, mtDNA is so small that a mutation occurs in it only once every 3,000 years. These mutations permit us to track the human migrations that have occurred throughout the world since human beings arose in Africa some 150,000 years ago. Frequently, migrations to unpopulated places have been accompanied by mutations. This causes the derived populations to have certain mutations that distinguish them from the original population. The mtDNA that composes a mutation that arose in an ancestral woman who has them in common with others, forms a family of mtDNAs known as a **haplogroup**. The mutation that all the mtDNAs have in common is known as the **marker of the haplogroup**. All of the mtDNAs that belong to a haplogroup have the marker of the haplogroup. Clearly, measured across the passage of time, there are some mtDNAs that belong to a haplogroup that develop particular mutations. It is said that two mtDNAs that belong to the same haplogroup but that are distinguished one from the other by additional mutations at some point of the molecule belong to distinct **haplotypes**.

The majority of the indigenous mtDNAs have Asian origins. Some 25,000 to 30,000 years ago, a group of Siberians crossed the Bearing Sttrait; thus human beings entered the New World for the first time. Among them were women who carried mtDNAs that belonged to the Asiatic haplogroups A, C, and D. It is possible that, by an alternative route closer to the sea, a haplogroup B simultaneously entered the New World, a group that was also Asiatic, but which is not found today in Siberia like haplogroups A, C, and D. Today, and possibly also in the past, haplogroup B is common from Central China through the Southeast in Indonesia, Polynesia, and Micronesia. A fifth indigenous haplogroup is haploid X. This is not found today in Asia, but in Europe, and could represent an independent migration from Europe via Greenland to the New World. Today within the New World, the

haplogroup X is found only in North America.

The indigenous haplogroups and their markers, those mutations that characterize the haplogroups, are: for haplogroup A, +665 *Hae*III, which is a mutation that creates a site of restriction known through the nuclease *Hae*III in pearl number 663; for haplogroup C, +13,262 *Alu*I; for goup D, -5,176 *Alu*I, which is a mutation that eliminates a site of restriction in the pearl number 5,176; and for haplogroup X, +14,465 *Acc*I. The marker of the haplogroup B is the only one that does not consist of a change of restriction. It consists of a deletion of nine pearls beginning in position 8,272, in a region of the mtDNA molecule known as region V.

The distribution of these five haplogroups in Puerto Rico was the following: Of the 489 samples of indigenous origin, 255 (52.!%) belonged to haplogroup A, 175 (35.8%) to haplogroup C, 42 (8.6%) to haplogroup B, 17 (3.5%) to haplogroup D, and zero to haplogroup X. This distribution structured around two haplogroups, specifically haplogroups A and C, which constitutes 88% of the indigenous samples, is typical of the New World tribes. It is additional evidence that the majority of the mtDNAs of indigenous origin in Puerto Rico originated from only one tribe that could not have been any other than the local tribe, the Taínos, because if it were otherwise we would have seen a distribution that was more equally structured, with all the haplogroups represented in comparable frequencies.

We conclude that the great majority of the Taínos of Puerto Rico belonged to the haplogroups A and C. We proceed now to explore the distinct migratory routes that could have given origin to the Taínos.

Studies made of other tribes on the continent show a clear dichotomy between the Indians who occupy the region from the north of Venezuela through the Amazon to Patagonia and the Indians who occupy the region to the west of the Andes, Central America, and North America. According to the theory of the Bering Strait, the settlements of human beings in the New World spread from north to south. Using this schematic, we can visualize the Columbian Andes as a great obstacle to the total occupation of South America on the part of the Indians who arrived from Central America. Few of the Indians would have crossed the Andes when it meant crossing them to enter the Amazon jungle. In biological evolution, this type of event is known as a founding event. Founding events are characterized by being susceptible to a genetic drift. Genetic drift increases the likelihood of a dramatic change in the frequency of haplogroups of a population that could occur fortuitously when the size of the population is dramatically reduced. The reduction in size of the population that crossed the Andes could have affected the frequency of the haplogroups of that population. As a consequence, while the populations west of the Andes have haplogroup A as the most frequent and haplogroup D as the least frequent, from Venezuela to Patagonia, haplogroup A is the least frequent and haplogroup D the second most frequent after haplogroup C. The frequencies of haplogroups from the Florida peninsula, Mexico, and Central America are very similar in that haplogroup A is the most frequent and D the least frequent. They differ only a little in that haplogroup C is a little more frequent.

Accepting that the Saladoid culture as well as the Huecoid had a

Southamerican origin, the present schematic has two explanations. One is a new genetic drift. The maritime route along the Greater Antilles could have been accompanied by a marked reduction in the size of the population, occasioning anew a drastic change in the frequency of the haplogroups of the population and producing one more similar to that of North and Central America than that of the original population in Venezuela. The second explanation is based on the principle in the field of population genetics that says that when a migratory population arrives at a place where there already exists another population and competes against it, the genetics of the native population will remain predominant. Based on this schematic, the predominant haplogroup, A, would belong to the original population of Puerto Rico, the pre-ceramic population, which very well might have originated on the Yucatan Peninsula or that of Florida. The less frequent haplogroup, C, would belong to the people of the ceramic culture who arrived later from Venezuela. That is to say, the Taínos would have been the product of a mixture between at least two ancestral indigenous cultures. The evidence that I am showing you suggests that the second explanation is the most probable.

All haplogroups can be divided into two subgroups according to the presence or absence of a site of *Hae*III restriction in position 16,517. This site of restriction is hypermutable, which means it has little filogenetic value. Nonetheless, its filogenetic value ought to be greater for the human migrations in the New World due to the recentness of those migrations.

Haplogroup A can be divided into groups A1 and A2 according to the presence or absence of the site of *Hae*III restriction in position 16,517, respectively. The proportion of A1 above A2 in Puerto Rico is practically identical to that of Florida, like that of Mexico and Central America, and is distinct from that of the Amazon. The theory of genetic drift by means of the Greater Antilles would have to come up with an explanation not only for the distancing of the frequency of groups from the Amazon, but also for the chance approximation to the frequency of groups to that of the frequency found in Florida and in Mexico-Central America.

In contrast, a similar analysis of haplogroup C does not reveal any tendency. Puerto Rico is the only place of all the studies where group C1 is more frequent than group C2.

We decided, then, to study haplogroup C more closely. We took as a point of departure the extensive studies that have been conducted at the laboratory of Doug Wallace at Emory University in Atlanta. There, the mtDNA molecule has been completely analyzed for 14 nucleases of restriction in 338 Amerindians who belong to 21 New World tribes. These 338 Amerindians included 64 with mtDNA from haplogroup C. Upon doing the analysis of the 64 from haplogroup C, 25 distinct mtDNAs could be observed known as haplotypes. Of these 25 haplotypes, 21 are private, that it to say, they are encountered in only one of the 21 tribes; two haplotypes are semi-private, being encountered in only two Amazonian tribes; and two haplotypes are cosmopolitan. These last are AM32, which is found in seven tribes in North, Central, and South America, and AM43, which is found in four tribes in North, Central, and South America. The only difference between AM32 and AM43 is in the *Hae*III position 16,517, by which it is theorized that AM32 was the only haplotype from haplogroup C that

crossed the Bering Strait. A little later, the hypermutability of the *Hae*III 16,517 site permitted the generation of haplotype AM43 before more stable mutations arose. When the more stable mutations arose, the tribes were already formed, and social restrictions imposed upon intertribal marriage limited the haplotypes generated by the stable mutations to one, or a few, nearby tribes. Thus, the new haplotypes remained private or semi-private.

With this background in mind, our goal was to analyze the mtDNA of 79 Puerto Ricans who belonged to haplogroup C for the 17 sites of restriction in which Dr. Wallace's laboratory had found variability, as well as the 16,517 *Hae*III site, with the hope that some private haplotype would arise that would let us precisely locate the tribe's origin. We found only one additional variable site of restriction, in 7,013 *Rsa*I. We then proceeded to analyze the rest of the 96 mtDNAs of haplotype C that we had found for these two sites of restriction and we ended up with only three haplotypes. These were 104 (59.4%) AM79, 67 (38.3%) AM32, and four (2.3%) AM43. AM32 and AM43 are the haplotypes of the founders of the New World that are found as often in North as in Central and South America, which means we could not precisely locate their precedence. Not so for AM79. AM79 has only been found in two Amazon tribes, the Yanomamos and the Crajos. Until our study, they had not been found any other site in the world; therefore their presence in Puerto Rico brought us to fearfully conclude that we had erred about the majority of Puerto Rico's haplogroup C having an Amazonian origin. Where did AM32 and AM43 come from? We don't know. But the evidence leads me to suggest to you that they arrived in Puerto Rico long before AM79.

All mtDNA can be divided into two regions: a region codified where all its genes are found and that measures 15,447 nucleotides long and a region with genes some 1,122 nucleotides long. Within the region with genes, we have two hypervariable regions of between 300 and 400 nucleotides each, whose sequences can provide us with very valuable information if we analyze them using the method of median networks. In this method, the haplotypes that arise in the sequences are represented by circles of proportional size to the frequency within the population that they represent. The circles or haplotypes are interconnected, and the relationship between one haplotype and another is represented with cross-connecting lines below the connections among the circles. Each cross-connecting line represents a mutation between one haplotype and another.

We sequenced both hypervariable regions in 35 samples of haplogroup C, 21 of which belonged to AM79 and 14 to AM32. The differences are dramatic due to the production of haplotypes in the sequence and due to their distribution. The 21 samples of AM79 produce only four haplotypes, while the 14 AM32 produce seven. In AM79 we have a haplotype that represents 18 samples. From this haplotype arose the other three haplotypes of AM79, each one representing a single sample. In contrast, AM32 can be divided into two groups: one of them consists of eight samples represented in the six haplotypes, and the other consists of six samples represented by only one haplotype that is separated from the nearest haplotype by seven mutations.

The interpretation is the following. The simplicity of AM79 suggests a recent migration, or at least it did not occur far enough back in time to accumulate the mutations that produce a complete median network. Furthermore, the haplotype's location within the median network that represents the 18 samples connecting itself to the other three haplotypes of AM79 suggests that it is the founder haplotype of AM79. Moreover, its very high frequency suggests a rapid population expansion, like that which we could expect of an agrarian and ceramic culture that exploits the resources at its disposition more efficiently than archaic cultures.

AM32 is itself divided into two groups. One of them consists of one sole haplotype that represents six samples. This ought to correspond to a recent haplotype, but does not account for derivative haplotypes. Possibly it co-migrated with AM79 from South America, or perhaps it represents an even more recent migration. In contrast, the second group of AM32 presents a complete median network that includes six haplotypes representing only eight samples. The six haplotypes are differentiated one from another by one sole mutation; furthermore, there is none that occupies a clearly defined central position as is the case with AM79. This is a manifestation of an ancient group that could not achieve a population expansion over a long time period, which occasioned the accumulation of distinct haplotypes without the founder achieving such high frequencies. By the complexity of the group, this last group seems to belong to an ancient pre-ceramic group, almost as old as those of the haplogroup A that we will see as we continue.

Dr. Wallace's laboratory studied the complete mtDNA molecule for 120 Amerindians of haplogroup A in 21 tribes with 14 endonucleases of restriction. They generated 35 haplotypes, 30 of which were private and three semi-private. Only two were cosmopolitan: AM1 is found in nine tribes from North, Central, and South America, while AM9 is found in seven tribes in the same three large geographic regions. Again, the only difference found between AM1 and AM9 falls into the 16,517 *Hae*III position, hence the theory of there being only one founder group that crossed the Bering Strait and brought haplogroup C, could also apply for haplogroup A. We proceeded the same as we did for haplogroup C with the hope of discovering some private or semi-private haplotype that could help us to precisely locate the origin of those mtDNAs of haplogroup A. We analyzed 53 samples of haplogroup A for all the 21 sites of restriction for which Wallace's laboratory found variables in the mtDNAs of this haplogroup. Unfortunately, we only found two cosmopolitan haplotypes, AM1 and AM9, hence we could not precisely locate its origin.

We then proceeded to sequence the hypervariable regions within the region of the mtDNA that lacks genes. This was done in 42 samples of haplogroup A. The results produced two principal groups. The first group composes the most complete median network that we have had, which suggests that it represents the oldest migratory wave. It has two haplotypes that are very similar to each other; they are differentiated by one sole mutation that occupies central positions, like founder haplotypes. Both originate similar complex networks, which suggests that the two founder haplotypes belong to the same migratory wave. One of the founder haplotypes seems to have

spread out more than the other, which suggests that shortly after the migratory wave to Puerto Rico, one dominated the other. Associated with this large group are two haplotypes representing only one sample that is separated from its nearest haplotypes by three mutations in one case and by six in the other. These ought to represent post-Columbian migrations to Puerto Rico. Finally, separated by four mutations from the previous group, we have a second group with a level of complexity barely greater than that of the AM79 of haplogroup C. Nonetheless, the smaller size of the central haplotype of this group relative to its derivatives suggests that it did not enjoy the population expansion that AM79 enjoyed after its arrival in Puerto Rico; the smaller relative size also increases its estimated age. We could not conclude at present if this second group belonged to a younger ceramic migration or to a relatively recent archaic migration.

During this coming week, we hope to collect samples in the Dominican Republic with the intention of constructing median networks of the distinct indigenous haplogroups that we find here. As of this date, we can only say that we are lucky to have had a Dominican brother who passed through our laboratory and gave us a sample of his hair roots, which ended up belonging to haplogroup A. The sequence of bases of the hypervariable regions are closely linked but different than one of the hypothetical founders from the first migratory wave to Puerto Rico. That is to say that there is a relation with the haplotypes of Puerto Rico, but not as narrow as we had hoped. Of course, it is not possible to arrive at conclusions for an entire population on the basis of one sole sample.

Our expectations for the Dominican Republic and the hypotheses on which they are based are the following. The first is simple. Hispaniola is much bigger and with a larger quantity of natural exploitable resources than Puerto Rico. The second is that the archaic Indians appear to have originated in Yucatan or Florida, therefore they would have colonized Hispaniola before they colonized Puerto Rico. On the other hand, the ceramic culture arrived in Puerto Rico before Hispaniola. If it is true that the archaic Indians of the Caribbean are represented by haplogroup A and, within haplogroup C, also by haplotype AM79, then we hope for less frequency in the Dominican Republic of haplotype AM79 than in Puerto Rico and, on the other hand, a greater frequency of haplogroup A and the haplotype AM32.

Here is a "footnote" to Dr. Juan Carlos Martínez Cruzado's presentation, an informal E-mail message that he sent us on November 17. Thanks, Doctor, for permission to publish it! In the message, he talks about his analyses of the samples that we took in the three days after the conference and another quantity of samples taken by Lic. Arlene Alvarez, Director of the Regional Archaeological Museum of Altos de Chavón, assisted by Aldofo López, an independent scholar from Spain:

First of all, let me congratulate you for having graduated with honors as collectors of genetic material for posterity. The samples that Arlene sent are expanding the study very well. Thanks to you, we are getting a glimpse that there

will be much to say about the mitochrondrial DNA of the Dominican Republic at the next Archaeology Congress.

Up to this moment, we have tested for the types (haplogroups) A, B, C, and D on the 43 samples that we took while I was there. We have also tested for X (which is the only haplogroup that is absent in Puerto Rico) on 20 of them. Furthermore, we have tested the 32 samples that Arlene sent for A.

As an historian, Lynne is going to have a lot of work. It seems that the incidence of indigenous heredity in the Dominican Republic varies a lot with location. Something that we are beginning to see is that there is much more in the countryside than in the metropolis. But it is possible that in the countryside, also, there is substantial variability. The final results will give us an idea of which places the Taínos concentrated when they left the Spanish settlements. It's necessary to remember well the places where the samples were taken in order to be able to find the things like topography, vegetation, crops, and proximity to settled regions that these places have in common. Two things must always be kept in mind:

1) events of the last two or three centuries will have partially erased some of the genetic footprints left by the Taínos,
2) mitochrondrial DNA only detects the migration of women.

There is a general consensus in Puerto Rico that the barrios known as Indieras in Maricao have the most people of indigenous origin in Puerto Rico. Nonetheless, the incidence of indigenous mitochrondrial DNA in Indieras is no greater than that of any other place in Puerto Rico outside of its eastern third. Clearly, the genetic difference between Indieras and the greater part of the rest of Puerto Rico must be rooted in paternal inheritance. In the places that did not serve as refuges for the Taínos, the men were genetically neutralized but not the women. The difference in the refuges is that the men could also procreate for the following generations.

Until now, we have identified 15 indigenous samples in the Dominican Republic, 12 of which have been A and only 3 of which are C. The best place up to this moment has been Tubagua, which is where we first stopped along the route from Los Cocos to Santiago [the mountain road called *Ruta Turística*]. Of the 7 samples that we took there, 4 turned out to be indigenous: 2 A and 2 C. A place that could beat Tubagua is El Seibo. From there we have only tested 9 samples for A, and already 3 have given positive results. We still have to test for C. Another good place was Yásica, the second site where we stopped along the route from Los Cocos to Santiago. Of the 7 samples that we took there, 3 have had positive results (2 A and 1 C). The next best site was Monción. Of the 10 samples that we took there, 3 tested as indigenous, all A. It could be that San José de las Matas will end up better than Monción. There we have tested only for A so far, and 1 out of 7 was positive. Among the remainder of the indigenous samples, the only positive result we obtained was one from among the 10 samples we took at Los Cocos. It was A. The 3 samples that Lynne took in San Juan de la Maguana were blanks, as were the 6 samples that we took in Santo Domingo. We also did a test for A among the 16 samples from La Romana, and not one gave a positive result. This suggests that large coastal cities near Santo Domingo have little incidence. To me, it

nonetheless appears that Santiago de los Caballeros could have a much higher incidence. Dealing with a large city, it would be highly significant.

I will keep you updated.

AUTHOR

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Please cite this article as follows:

Martínez Cruzado, Juan C. (2002). The Use of Mitochondrial DNA to Discover Pre-Columbian Migrations to the Caribbean: Results for Puerto Rico and Expectations for the Dominican Republic. *KACIKE: The Journal of Caribbean Amerindian History and Anthropology* [On-line Journal], Special Issue, Lynne Guitar, Ed. Available at:
http://www.kacike.org/MartinezEnglish.pdf
[Date of access: Day, Month, Year].