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Mitochrondial DNA in the Dominican Republic

Dr. Fernando Luna Calderón

A brief introduction by the editor: In the past, studies of the Taínos relied upon history, archaeology, and anthropology. Today, a new method of research is helping to shed light on ancient questions about them and their ancestors—genetics. The Human Genome Project, an international effort aimed at identifying and mapping the entire sequence of the more than 30,000 human genes and 3 billion chemical base pairs that make up human DNA, was completed ahead of its 2003 schedule. Today, scientists like Dr. Fernando Luna Calderón are using the new knowledge and methods learned from the Human Genome Project to trace human migrations, to identify human origins. Specifically, Luna Calderón proposes to clearly identify who the ancestors of the Taínos and other indigenous Caribbean peoples were, and their routes of migration and settlement. These are brand new studies, the results of which have not yet been realized. In this paper, Luna Calderón explains the biological base of the research and the methods he is using to obtain DNA samples and perform the required analyses on them.

The physical-chemical bases of inheritance

The method of selection of animal and vegetable lineages began back in the Neolithic with the aim of attaining more productive individuals. In a simple form, those agriculturalists and shepherds understood that characteristics were transmitted from generation to generation. This fact was affirmed by the monk Gregory Mendel, who, using worms, demonstrated that factors remained as mysterious entities, although he did not know their exact nature; thus they were transmitted from generation to generation. One hundred years later, those mysterious entities have been almost completely clarified and the theme of inheritance is perhaps one of the best known in biology. Since 1910 those "factors" have received the name of genes.

Chromosomes and genes

Living beings are the product of repeated divisions of an egg and zygote, which differentiate themselves from the

sister cells in order to realize multiple functions. The zygote is nothing more than an initial male and female cell. These cells are known in scientific jargon by the names ovum and spermatozoid. These gametes carry a gene for each given character; that is, each zygote possesses two genes.

If you observe a cell's cycle, after it has split off from its progenitor cell, there is no change in the nucleus or in the cytoplasm. But at the moment of cell division, the nucleus disappears, and the material in its interior distributes itself into filament-like structures that coil up and can be seen if one uses special colors. These coils are called chromosomes, and their number is constant in each living cell.

The chromosomes are divided longitudinally, forming two long arms of steps called chromatics, united in their middle parts by a short "trunk." At the moment of division, the chromosomes emigrate toward the center of the cell. There they separate and form a new cell. From this a transverse partition is formed that gives birth to the two sister cells. The chromatics also distend themselves and form new muscles. The process of cell division involves a longitudinal division of the chromosomes to form chromatics that are distributed separately toward the sister cells. A little before the cell division, a chromatic appears with a new chromatic, constituting a new chromosome.

There is a relationship between genes and chromosomes. Thus each new gene possesses a homolog in each cell; the chromosome is identical in form and size to the cell's other chromosomes. In the same way, the homologous genes proceed from their opposites in every pair of identical chromosomes that proceed from the mother and father. It is not a random process. The chromosomes are the cellular structures in which genes are situated. The combination of human genes is constituted of dozens of thousands and they are found distributed in 46 chromosomatically duplicated structures.

The molecule of inheritance

Phenotype and genotype are the products of biochemical reactions that take place in each living organism. These reactions, to make themselves faster, count on the presence of some molecules called enzymes, whose mission is to accelerate the organic processes, increasing their velocity. For the process, they use their geometric forms that permit them to adhere themselves easily to the molecules that intervene.

The structures of the genes should be stable. That is, they are not easily susceptible to random changes. Their molecules ought to have the capacity of duplicating themselves with fidelity, in order that the information is transmitted without change generation after generation. The structures of the said molecules ought to be healthy, which guarantees that their information transmits itself in the simplest form possible in the zygotic structures capable of directly influencing the physiological process. Life appeared on earth some three million years ago and needed a molecule capable of perpetuating itself and reproducing itself. That molecule is DNA (Deoxyribonucleic acid).

The structure of DNA

DNA is a molecule made up of albumins, called nucleotides. The

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nucleotide has various parts: Ortho-phosphate group, attached to a ring of deoxyribose, which has a nitrogenous base. The Ortho-phosphate group and the deoxyribose have a peculiar structure. That is, four distinct groups, a hexagonal ring united to a pentagonal ring, in which case it is called purine base (Adenine-Guanine) or better by one single hexagonal ring in which case it would be pyramidine base (Timine-Citosine).

The nucleotides locate themselves one by one in two chains in the manner of the shell of a snail—a form that is called double helix. The steps of the staircase are made up of nitrogenous bases from both chains, with very weak electric charges, which are called hydrogen bridges.

The nitrogenous bases allow only one thing to face Adenine, and normally that is Timine. Meanwhile only Citosine faces the Guanine. The base sequence of the chain has but one possible path. In effect, the stability of the molecule is important, and because of this the hydrogen connections come into being. In the duplication process of DNA, it produces an unpairing of the two chains for the synthesis of a new complementary chain. This breaking process creates an enzyme because such a separation would be difficult if the connections were covalent. This type of structure, as described, permits the duplication of the DNA molecule, which is produced through the simple separation process of the two chains, each one of which acts as a mold for the synthesis of the complementary chain.

The DNA's information

A gene is essentially a fragment of DNA with an average longitude of a thousand base pairs with the corresponding Ortho-phosphate and deoxyribose molecules. The enzymes are molecules of natural proteins formed by small entities called aminoacids. Twenty aminoacids are known that form in the normal protein path. The translator gene uses an alphabet of four that serve to write twenty (20) different words by which the twenty aminoacids are known.

If the DNA upon duplication were perfect, all the molecules of DNA would be equal. The first should have synthesized itself randomly three million years ago. Evidently, that did not happen, proven by the diversity of living beings that populate the universe. In other words, the duplication of DNA information is incompatible with the evolution of living beings. Evolution has been possible thanks to errors and to the random duplication of DNA; this is what has produced genetic mutations.

Translation of the information

In the translation, the first problem is the DNA of the nucleus. While the synthesis of proteins is produced outside of it, in the cellular cytoplasm, the existence of a molecule capable of transporting the genetic message from the nucleus to the cytoplasm is necessary. This molecule is the messenger RNA—mRNA (Ribonucleic acid)—with a structure similar to that of DNA.

The Vikings of the 11th century made contact with aboriginal groups from the American continent. They touched the eastern coast of Greenland and arrived at a territory called Vinland. There they established various settlements, leaving ample archaeological evidence. Christopher Columbus, seeking The

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Indies of Spices and Gold, discovered the New World in 1492. Thus he made contact with a people with a marginal culture characterized by a variety of subsistence patterns: fishing, hunting, gathering, and intensive agriculture. In the 18th century, the majority of intellectuals were convinced that the first Americans came from Northeastern Asia.

The Count of Bufón, Luis Leclerc, recognized the seeming morphology between Amerindian and Asiatic groups. Also Blumenbach, in the same century, presented a classification of the human races in his book, *Native Variability of Human Genetics*. In the 1940s and 1950s, it was taught that the ancestors of the Amerindians were a product of the mixture of the two racial ingredients Caucasian and Mongoloid (Birdsell 1951).

Genetics supports the evidence of these theories, the seeming morphology, the craneometric affinities, and the cultural similarities. In the genetic evidence we find various genetic and blood markers, which are: protein series and DNA haplotypes. Genetic trees were constructed based on 120 alleles of world populations. This permitted an exhaustive study of the genetic markers, like the Greek gene and the Gamma globuline halotype. In a morphologic similarity, Amerindians and Asiatics share: scarcity of hair (beardless), hair that is black and straight, the Mongolic birthmark, epicanthic fold, shovel teeth, and three-root molars. In the cranium there is a similarity by the tendency toward brachycephalization among the Indians that is similar to the Asiatics.

Among the cultural similarities are the following: the exploitation of the environment with techniques adapted to concrete problems, the belief in spirits, the presence of shamans, the type of living quarters, the stick calendar, etc. The environment was exploited in similar ways by both groups. There is no doubt, apart from the mitochrondrial DNA studies, that Amerindian groups descended from Asiatic groups.

Mitochrondrial DNA in the Taíno groups of Hispaniola

Since long ago, some researchers maintained the theory that these Arawak-language-based groups were extinct as a result of maltreatment, illnesses, suicides, and the rupture of their familiar unity. Nonetheless, studies done by archaeologists and genetic researchers on the present population indicate that these groups were decimated, but not annihilated. Toward that aim, we worked with materials that proceeded from the Aboriginal Cemetery of La Caleta, Santo Domingo, upon which we made bone cuttings in order to study their mitochrondrial DNA.

In total, we analyzed twenty-seven (27) samples and had them examined at a laboratory in the Biology Department of the University of Barcelona, Spain, by doctors Bertrand Petite and Charles Lalueza Fox. The HVRI was amplified with the frequency results subjected to controls that permitted only two CID of the principal ancestral mitochrondrial DNA to appear in the work.

Of this sample, 75% belong to haplogroup C and 25% to haplogroup D. This data is comparable to those encountered on the South American continent, and its sequence demonstrates a reduced diversity of mitochrondrial DNA.

The Taínos are culturally marginal groups that made contact with European groups under the command of Christopher Columbus in 1492. That

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Columbian expedition had the support of the Catholic Kings Ferdinand and Isabella and its mission continued the project of Mina that was begun on the coasts of Africa.

It is very possible that, upon the arrival of Columbus in the New World, there existed about 300,000 aborigines. Nonetheless, in 1525, the majority of that population was dead, a result of maltreatment, illnesses, famines, etc., which has been called "ecocide." Around 1502, the first slaves were brought in to help the aboriginal laborers who were almost annihilated.

On the other hand, it seems that the Carib Indians of the Lesser Antilles disappeared as a different human group. Some 16 sequences were found that were clearly of African origin. We need new studies to seek genetic affinities of these groups in relation to the other persons in the Americas.

The chronicles talk about two aboriginal groups: Taínos and Caribs. The Taínos inhabited the Greater Antilles and the Caribs the Lesser Antilles. The Taínos were organized into *cacicazgos* and had an economy of subsistence based on fishing, hunting, and gathering. They developed a high level of ceremonialism directed by a *behique* (shaman) or cacique, who, by means of a ritual called the *cohoba* ritual, put themselves in contact with their gods, announcing good harvest, wars, or illnesses.

These agricultural societies practiced a type of dance called *areito*, by means of which, through song and dance," they transmitted their culture from generation to generation. There were *areitos* for war, agricultural, marriage, death, dirges, etc.

The predecessor groups, according to archaeological studies, proceded from Trinidad-Tobago (Banwari Trace) and Belize in Central America. The archaeological excavations done by the teams from the Museum of Dominican Man and in other areas of the Caribbean indicate that the Taínos moved into the islands beginning about 5,000 B.C. from the lower part of the Orinoco River Valley, while groups from Trinidad and Central America migrated around A.D. 1,000. In Cuba, these groups received the name of Guanahatabeyes and are characterized for being fishers, hunters, and gatherers.

According to these studies, it seems that the Antillean population is lineal from the southeast toward the northeast, following the configuration of the chain of the Antillean islands. It is important to know if the Caribs came from South America or not; this is something that lends itself to verification by the scientists who study mitochrondrial DNA. Mitochrondrial DNA is being used extensively for the construction of history about all the populations of the past and present. This is done with great determination under analysis and restrictions that consist of verifying the genetic differences.

At Berkeley, a group of scientists, among whom Wes Brown and Mark Stoneking are prominent, have applied this technique to samples of present existing humans who come fom various parts of the world. Mitochrondrial DNA is inherited through the maternal line. This material is taken from the mitochrondria, a cellular organ that converts the nutritive substance into energy that is utilizable by the cell.

Different from nuclear DNA, which forms a chain of long fibers, some of which are a double helix covered with proteins, the mitochrondrial DNA presents itself in small rings grouped in filaments. While the nuclear DNA is enclosed and

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has some 100,000 genes with all the information necessary to produce a living human, mitochrondrial DNA contains only 37. In America, many studies of the mitochrondrial DNA of Amerindians show that it corresponds to four lineages called A, B. C, and D, and a residual lineage called X by Bandelt in 1995. This ancestral lineage is found in some European populations.

Greenbelg postulated in 1986 that there were three Amerindian migrations: Nadene and Eskimo-Aleut from Asia, crossing the Bering Strait, populated the Americas. The first sequence was made in 1991 and showed a high quantity of mitochrondrial DNA in a simple tribe, subjecting it to a scenario much more complex than that hoped for since the model of the three migrations.

Our proposal in this study is to use the mitochrondrial DNA left in the bones by the Taínos of Hispaniola and from other islands in order to seek the genetic affinities of these groups in relation to the present Amerindians, and thus to reconstruct the origins of the groups that live in the Caribbean. We intend to analyze the genetic composition in order to give a clear image of all that is relative to this migration.

The materials and the method for this study consist in the extraction and amplification of the 27 bone cuttings taken from the Cemetery of La Caleta in Santo Domingo, Dominican Republic. This cemetery, located on the southern coast of the country, contains burials in fetal position accompanied by Boca Chican ceramics, and its dating runs from about 670 through 1680, give or take 100 years. All of these dates are before the arrival of Columbus.

Strict methods are taken so that the samples are not contaminated with a positive air pressure in the principal laboratory. Sterile gloves, small filters, and frequent bleaches were some of the methods adopted during the process. The external surface of the bones was cleaned with a sterile razor and part of the scrapings were made into powder in a coffee grinder. The material was incubated half a night at 37 degrees centigrade with 8.5 ml. of water, 1 ml. 5% SDS, 0.5 ml. 1 m tris/HCL PH 8.0, and 50 uL of 1 mg/ml with Protinase K, obtaining it three times.

In order to ascertain the correct environment for the attribution of the first four lineages of Amerindian mitochrondrial DNA, small fragments from each lineage were taken. They were amplified in frequencies previously identified for haplogroup A, B, C, and D. All were cloned and sequenced. New research could show in a clear and precise way the manner in which Asiatic and Amerindian groups migrated thousands of years ago.

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Dr. Fernando Luna Calderón, Dominican, is a human biologist and paleontologist who perfected his skills at the Smithsonian Institution in Washington, D.C. He studied medicine for five years at the Universidad Autónoma de Santo Domingo and also studied clinical psychology at the Universidad Mundial Dominicana and CDEP. He was director of the Physical Anthropology Department of the Museum of Dominican Man from 1973 until 2000. At the same institution, he was subdirector in 1983-86 and 1996-2000, occupying the position of director from May through August of 2000. Since 2000, he has been director of the Museum of Natural Science. Luna Calderón has conducted hundreds of archaeological excavations across national territory. Furthermore, he has excavated very important aboriginal cemeteries in Puerto Rico, Martinique, Venezuela, Ecuador, and Cuba. He has published the *Atlas of Bone Pathology* and many other books in collaboration with important figures of Dominican, Venezuelan, Italian, and Spanish archaeology, as well as scientific articles in national and foreign magazines.

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