

Evolution, the Key to Understand Life. Recent Research from Latin America

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RESUMEN: *La visión del mundo en el oscurantismo (Edad Media) no podría ser más monótona. La versión establecida era que el mundo había sido creado por una entidad divina y las opiniones divergentes se consideraban una herejía y enfrentaban la fuerte oposición de la Iglesia. Todo cambió con la publicación, en 1859, de la obra precursora de Charles Darwin, El origen de las especies. La naturaleza no fue creada para servirnos; la especie humana, más bien, fue sólo una más en una multitud de otras entidades biológicas. A esta revolución darwiniana le siguieron otras en el siglo subsiguiente y el siguiente medio siglo, las cuales implicaron: a) la síntesis evolutiva moderna, b) la dilucidación de la estructura del material genético; c) las técnicas de manipulación del ADN y d) la bioinformática y la nanotecnología. El medio siglo de investigaciones de nuestro grupo continuó con estos cambios, entre los más recientes se incluyen algunos de los ejemplos que se describen a continuación: 1. comparaciones entre los genomas arcaicos y modernos, 2. enfoques genómicos para la historia amerindia y 3. interacciones entre los genes y la cultura con rasgos específicos. La técnica molecular y la bioinformática nos permiten tener una vista detallada de nuestro pasado así como una mejor comprensión de nuestro presente y de las condiciones que predicen mejor nuestro futuro.*

PALABRAS CLAVE: *Evolución, Darwin, ADN, genética amerindia, biología y cultura.*

ABSTRACT: *The world view in the Dark (Middle Ages) could not be more monotonous. The world would have been created by a divine entity in a fixed version, and divergent opinions would be considered a heresy and subjected to strong opposition by the church. Everything changed with the publication in 1859 of Charles Darwin's seminal work The Origin of Species. Nature was not created to serve us; instead, the human species was just one in a multitude of other biological entities. To this Darwinian revolution others followed in the ensuing one and a half century, involving: a) The Modern synthesis, b) elucidation of the structure of the genetic material, c) techniques of DNA manipulation, and d) bioinformatics and nanotechnology. The half-a-century investigations of our group followed these changes, and examples of some of the most recent will be described, involving: 1. comparisons between archaic and modern genomes, 2. genomic approaches to Amerindian history, and 3. gene-culture interactions involving specific traits.*

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Molecular and bioinformatic techniques are allowing us to have a detailed view of our past, better understanding of our present, and conditions to better predict our future.

KEYWORDS: *Evolution, Darwin, DNA, genomes, Amerindian genetics, biology and culture.*

WORLD VIEWS

We are curious creatures. Since we crossed the human threshold questions were raised about our past, present and future, and self-consciousness seems to be exclusive of our species. Along time, as we progressed in the sociocultural way, explanation theories were developed about ourselves, the others, and the world in general. These world views can be classified in three categories: *a) magic; b) metaphysical; and c) scientific* [Gottschall, 2003].

The magic view of the world was established at mankind's dawn, from a pre-logic mentality that did not distinguish between wishes and realities. There was no need for a relationship between past and present events and the day-to-day was characterized by unexplained facts that could only be understood creating a mythology as vast as the world itself.

Around the seventh century before Christ there is a marked change in attempts to explain the world through a set of rational knowledge and not empirical or revealed evidences. This relationship between the knowledge of being (ontology) and things (cosmology) characterized the metaphysical view of the world.

On the other hand, around the middle of the 17th century, the present scientific model started to be delineated. The basis of the scientific view of the world is the principle of cause and effect. Through a detailed analysis of a portion of reality we search how one phenomenon resulted in another. This perspective is basically materialist, with no need for supernatural explanations [Gottschall, 2003].

THE DARWINIAN REVOLUTION

The world view in the Dark (Middle) Ages could not be more monotonous. The world would have been created by a divine entity in a fixed version, and therefore present conditions would be exactly those that existed at the beginning. The act of creation, which occurred in seven days, would have been relatively recent. God's words, reproduced in the bible, could not

be questioned. Divergent opinions would be considered a heresy and subjected to strong opposition by the church.

Everything changed with the publication in 1859 of Charles Darwin's (1809-1882) seminal work *The Origin of Species*. Nature was not created to serve us; instead, the human species was just one in a multitude of other biological entities. The book a masterpiece of scholarship, summarizing 17 years of observations and ideas that were elaborated since the formulation of the theory that attributed a key role in the evolutionary process to natural selection. Five other editions were published between 1860 and 1887 under the author's supervision, updating the knowledge generated since its publication.

The book compared the organic variability found in the wild with that present in domesticated species; considered the concept of natural as compared it to that of artificial selection (performed by mankind); the role of hybridity as a factor inducing but also preventing novelty; geographic distribution factors; and affinities of the organized beings in terms of morphology and embryology.

Table 1.
Eleven books that established the foundations of the synthetic theory of evolution, together with the lifetime of their authors

Author	Lifetime	Title of the book	Year
Ronald Fisher	1890-1962	<i>The Genetical Theory of Natural Selection</i>	1930
Sewall Wright	1889-1988	<i>Evolution in Mendelian Populations</i>	1931
Edmund E. Ford	1901-1988	<i>Mendelism and Evolution</i>	1931
John B.S. Haldane	1892-1964	<i>The Causes of Evolution</i>	1932
Theodosius Dobzhansky	1900-1975	<i>Genetics and the Origin of Species</i>	1937
Julian S. Huxley	1887-?	<i>Evolution. The Modern Synthesis</i>	1942
Ernst Mayr	1904-2005	<i>Systematics and the Origin of Species</i>	1942
George G. Simpson	1902-1984	<i>Tempo and Mode in Evolution</i>	1944
Michael J.D. White	1910-1983	<i>Animal Cytology and Evolution</i>	1945
Bernhard Rensch	1900-1990	<i>Neuere Probleme der Abstammungslehre</i>	1947
G. Ledyard Stebbins	1906-2000	<i>Variation and Evolution in Plants</i>	1950

Source: Provine [1971]; Mayr and Provine [1980]; Mayr [1982] and Freire-Maia [1988].

OTHER REVOLUTIONS

One of the weaknesses of Darwin's theory, recognized by him, was the ignorance, at the time, of the laws that determined the biological inheritance of living forms. This occurred despite the fact that these laws had been clearly delineated by Gregor Mendel (1822-1884) in his fundamental publication of 1866, seven years after the first edition of the *Origin of Species*. It would take almost one century for the successful marriage between genetics and evolution. It occurred through the so-called *Synthetic Theory of Evolution*, developed between 1930 and 1950. Eleven books that established the foundations of the theory are listed in Table 1, together with the names of their authors.

Fisher, Wright and Haldane established the mathematical-statistical bases, and Dobzhansky's book is considered the main reference to the fusion between these bases and empirical studies. Dobzhansky apparently used Darwin's book as model (as suggested by the title of his book), but contrary to Darwin, who spent 17 years between the formulation of his theory and the publication of the book that documented it, Dobzhansky wrote his classic in just four months [Provine, 1986]. The extension of the theory to zoology and systematics was done by Ford, Mayr, and Rensch; to paleontology by Simpson; to cytogenetics by White, and to botany by Stebbins. The theory's denomination as synthetic was given by Huxley, who included in it the embryology approach and his extraordinary ability to develop general principles.

The genetic molecular era started in 1953, with the brilliant model developed by James D. Watson (1928-present) and Francis H. C. Crick (1916-2004) of the DNA structure. They have been helped in an important way by Rosalind E. Franklin (1921-1958) and Maurice H. F. Wilkins (b. 1916). The DNA structure was revealed, but we needed to know how it functioned, and it was Crick again who conceived the need for an intermediary in the DNA protein process, messenger RNA; and that together with Sidney Brenner identified the nature of the genetic code.

Four techniques were basic for the generalized study of DNA. The first was related to the use of the restriction endonucleases which allow DNA cleavage in specific regions. Its application was initially proposed by Danna and Nathans [1971]. Starting from this point Stanley N. Cohen and three colleagues [1973] developed the cloning technique (introduction and multiplication of a region cut from the DNA of an organism in another). Manual DNA sequencing was established by Frederick Sanger (b. 1918) and two colleagues in 1977 [Sanger *et al.*, 1977]. But the democratization of the DNA study was made

possible through the development in 1985 of the polymerase chain reaction (PCR), invented by Kary Mullis and colleagues from the Cetus Corporation, Berkeley, California, USA. Techniques for the automatization of DNA studies, in turn, made possible the creation of a new area of science, *genomics*, and bioinformatic techniques allowed the generalized use through the internet of a fabulous amount of data.

ARCHAICS AND MODERNS

In the remaining part of this paper I will give some examples derived from our research team that indicate the potential of genomics to identify important human evolutionary questions and clarify aspects of our history.

Modern humans evolved from earlier species of *Homo* that originated in Africa some 2.0-2.5 million years ago afterwards migrating into Eurasia and other continents at different epochs. Among premodern *Homo* none reached the notoriety or was more discussed than Neanderthals (*Homo neanderthalensis* or *Homo sapiens neanderthalensis*); and there are reasons for this. One of the fossils found in the Feldhofer cave of the type locality the Neander ("New Man" in Greek) Valley was the first fossil of an extinct archaic hominin recognized as such. Moreover, fossils of this morphological type were found widely distributed in Europe and the Near East, with relatively recent evidence of its presence in Central Asia and Siberia. Its temporal distribution is also curious – typical morphological Neanderthal traits start to appear in Europe about 400 thousand years ago, and eventually abruptly disappear between 30 and 28 thousand years before present (YBP) [Dodge, 2012]. In the popular media and arts they were characterized as bloody and violent. This view was contested by a North American physical anthropologist, Carleton S. Coon (1904-1981) who suggested in 1939 through a drawing that a Neanderthal in modern dress could not be distinguished from a common *Homo sapiens* [Trinkaus and Shipman, 1994].

In parallel with these developments, ancient DNA techniques became more precise, and Green *et al.* [2010] provided a description of a whole draft sequence of the Neanderthal genome, while Reich *et al.* [2010] furnished the same type of data from a finger found in the Denisova cave in Siberia of a hominin who should have shared a closer common ancestor with Neanderthals than with modern humans. The paleogenomics of archaic hominins are opening new avenues for the understanding of our evolutionary past [Lalueza Fox and Gilbert, 2011].

Paixão-Côrtes *et al.* [2012] took advantage of the availability of the complete genomes of the chimpanzee, *Homo sapiens*, *Homo neanderthalensis* and the Denisova specimen. They started from a total of 10 447 non-synonymous substitutions in which the derived allele, as compared to the chimpanzee, was fixed or nearly fixed in humans, verifying that 381 of these genes determined radical amino acid changes. Of these, 40 participate in a single independent functional cluster, 77 to several of them, influencing multiple phenotypic traits, and 21 are olfactory receptor genes. Interestingly, generally these changes are present in all three hominin genomes, including those related to neurogenesis and cognition. The conclusion is that the selective sweep that gave rise to *Homo sapiens* could have started before the modern/archaic human divergence.

THE HUMAN DIASPORA

Presently there is consensus that all modern human populations had their origin in Africa (to the racists horror!) and that the out of Africa expansion should have occurred between 50 to 100 thousand YBP. Asia's colonization would have started about 60 thousand YBP, and from there humans reached Europe 35 thousand YBP. Dates for Oceania are 50 thousand YBP and for America 20 thousand YBP. These are, of course, approximate numbers and rely on the source of data from which they were obtained (archeological, paleoanthropological, genetic, linguistic). See Crawford [2007] for a recent review.

Why did these prehistoric migrations occur, and generally, why do human individuals or populations migrate? Two sets of factors can be envisaged, environmental, or inherent to individuals or groups. Along the times, changes in the ocean levels offered the opportunity of colonization of areas that were previously submersed. Examples are the Bering region (Beringia) which connected eastern Siberia with Alaska in North America; and in the Pacific the Sahul region, which linked Australia to Tasmania and New Guinea in the past.

Hunters and gatherers need larger territories than agriculturalists for their subsistence, favoring mobility; war between tribes or states is another factor, while at the individual level persons who are against the status quo are more prone to migrate.

It is important to stress that models involving prehistoric migration waves based on genomic data are theoretical simplifications. Both, intercontinental or intracontinental migrations, of short or long extension, did

not involve deliberation; what happened were contingencies like those indicated above, which conditioned mobility.

GENOME APPROACHES TO AMERINDIAN HISTORY

America's "discovery" by the Europeans in the 15th century posed a question: who were the strange people who inhabited the land? Paul III's (1468-1549) papal bull solemnly recognized their human status in 1537. But from where did they come? The absence of fossils of high antiquity in the Americas would eliminate the autochthonous origin hypothesis, and for more than a century scholars considered the questions: *a*) from where did they come?; *b*) how long ago?; and *c*) how many migratory waves would have occurred?

Reich *et al.* [2012] approached this question with a degree of genomic detail previously unimagined as possible. They tested 364470 single nucleotide polymorphisms (SNPs) in 493 subjects of 52 Native American populations; 245 from 17 Siberian groups; and 1613 from 57 other, non-Amerindian, populations.

Previous to this paper the general consensus was that all present-day Native Americans would have derive from a single migration wave [Salzano, 2007]. The evidence now assembled indicates that the great majority of Native American populations —from Canada to Chile's southern region— derive their ancestry from a homogeneous "First American" ancestral population that probably crossed the Bering Strait about 18 thousand YBP. Two additional streams of Asian gene flow, however, occurred more recently, one involving the Eskimo-Aleut people and another the Na-Dene-speaking Chippewyan of North America.

These results represent a return to a hypothesis proposed by three North American researchers: J. H. Greenberg, a linguist, C. G. Turner, a physical anthropologist, and S. L. Zegura, a geneticist [Greenberg *et al.*, 1986]. Other findings indicated: *a*) a southward expansion facilitated by the coast, with sequential population splits and little gene flow after divergence, especially in South America; and *b*) that the Panamanian Chibchan speakers would have ancestry from both North and South America. Achilli *et al.* [2013] also proposed further streams of gene flow in northern North America.

BIOLOGY AND CULTURE. I. LACTASE PERSISTENCE IN ADULTHOOD

It is traditionally considered that a fundamental distinction between our species and those of other animals is the complexity of our culture. But the term is of difficult definition. We can characterize it as a set of beliefs, values, behavior and traditions associated with a given population; as well as the information that is capable of affecting the acquired individual behavior through teaching, imitation, and other forms of social learning [Laland *et al.*, 2010].

Concepts on the relationship between biology and culture suffered a curious evolution. In the beginning of the 20th century there was a consensus that biology (genes) could markedly influence behavior and cultural processes. Afterwards, however, due to the concepts of many scholars, including, for instance, Franz Boas (1858-1942), there was an extreme change, and the contrary opinion that biology had nothing to do with culture prevailed. Presently the dominant view is that they interact in several ways, and that both sets of variables should be considered when dealing with complex phenomena [Salzano, 2012].

Lactase persistence during adulthood is a paradigmatic trait which revealed the importance of a cultural variable (cattle raising and milk consumption) in a specific genetic condition. Lactase persistence is due to mutations in the 70 kilobases *LCT* gene enhancer region. In addition, extensive interethnic polymorphism is also found within the gene itself.

Friedrich *et al.* [2012] studied 316 individuals from four Brazilian Amerindian populations for 12 polymorphisms (common variants) in both the enhancer region and the *LCT* gene. For the enhancer region the only allele associated with lactase persistence observed was -13910*T, whose frequency varied from 0.5% in the Xavante to 7.6% in the Guarani-Ñandeva. With the exception of the Xavante, its presence should be due to non-Amerindian (European) admixture. This region, therefore, is characterized by the absence of variability. On the other hand, a completely different picture is shown by the coding *LCT*, which is highly polymorphic, with 15 haplotypes (specific gene arrangements) presenting a heterogeneous distribution. Therefore different evolutionary factors can be at play even in relatively close DNA portions.

BIOLOGY AND CULTURE. II. GENE-CULTURE COEVOLUTION IN MESOAMERICA

Human cultural practices have drastically modified environmental conditions and behaviors, leading to rapid and substantial genomic changes often associated to positive selection and adaptation. Agriculture and animal husbandry can be regarded as key factors on this regard, leading to what has been called niche construction. This approach emphasizes the ability of organisms to modify their environment in such a way that natural selection factors are changed, and they therefore can act as coagents of their own evolution.

The 230Cys allele of the ATP-binding cassette transporter A1 (*ABCA1*) gene, exclusively present in Amerindian and Mestizo persons of this ethnic group was studied in 1,905 individuals from 50 Meso and South American populations. In addition, 20 other autosome SNPs of a putative neutral region surrounding the locus was investigated in 126 of these individuals by Hünemeier *et al.* [2012]. The estimated 230Cys age (8,268 YBP) was compatible with its origin in the American continent.

*ABCA1*230Cys* leads to low high density lipoprotein-C (HDL-C) levels in their carriers due to a reduction in the intercellular flux of this substance. This effect would favor the permanence of cholesterol within the cells, and energy storage. Adipose tissue would favor several biological functions, including the capacity to deal with fluctuations in energy supply, that would occur in starvation periods (characteristic of prehistoric conditions), regulation of reproductive functions, and energy supply to the immune system. In modern times, however this mutation which was initially favored by selection, would become harmful due to food abundance and availability. This variant would be, therefore, another example of the “trifly” genotype postulated by the North American geneticist James V. Neel (1915-2000) to explain the present high prevalence of diabetes mellitus in our populations.

One of the most interesting findings of Hünemeier *et al.* [2012] was the 94% correlation between the ages of maize fossil polens found in archeological sites of Mesoamerica and the 230Cys allele frequencies in extant populations situated nearby (Table 2). The interpretation of this finding is as follows: maize, the most important food plant of the Americas, was domesticated from teosinte (*Zea mays parviglumis*) between 6.3 and 10.0 thousand YBP. This event conditioned an important change in the ways of living of the people in populations where the plant was cultivated. Gradually hunter and gathering was abandoned as subsistence resource and substituted by agriculture. This change led to vulnerability due to crop losses determined by environmental factors (for instance, excess of rains, droughts) or different types of plagues, resulting in periods of generalized famine. It would

be in these circumstances that *ABCA1*230Cys* would be favored, since it would determine more resistance to such situations. Changes to modern conditions would have been too recent to revert the trend.

Table 2.
Comparison between the ages of maize fossil polens found in archeological sites of Mesoamerica and the *ABCA1*230Cys* frequencies observed in nearby extant populations¹

Archeological sites	Years before present	Geographic region	Nearby Amerindian population	Sample size	<i>ABCA1*230Cys</i> (%)
Guilá Naquitz	9212	Oaxaca	Zapoteco	125	24
San Andrés	7122	San Andrés	Maya	110	20
Zoalpilco	5835	Mexico State	Nahuatl	267	17
Zipacate	5318	Guatemala	Kaqchikel-Quiche	17	15
Laguna Pompal	4818	Veracruz	Totonaco	113	13
Gatun Lake	4468	Panama	Guaymi	35	15
Lago Cote	3096	Costa Rica	Cabecar	24	10

¹ Spearman's correlation coefficient: 0.94.

Source: Hünemeier *et al.* [2012].

South America presents a larger diversity of environments, persons or cultures than Mesoamerica. It is possible that in this region the factors responsible for the high prevalences of this allele would be diverse. For instance, cholesterol has an important role in the infectious processes, like the entry and replication of Dengue virus type 2 and flaviviral infection, and it was demonstrated that *ABCA1* gene deletions confer complete resistance against cerebral malaria in mice [Combes *et al.*, 2005]. Additional work in this region would be welcome.

JUST ANOTHER SPECIES?

It is therefore clear that although Darwin displaced us from the center of the universe, we are a special type of creature, due to the unprecedent op-

portunities provided by culture. With a certain degree of exaggeration Alter [2007] asserted that "When *Homo sapiens* became human they also became cyborgs, since the reflexivity of culture bends biology to such an extent that biology itself becomes an artifact of culture"! This interconnection is evident in the research examples reviewed here.

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