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METHOD ARTICLE

APPROACHES FOR ANCESTRY STUDIES IN ARCHAEOGENOMICS

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ABSTRACT. *Archaeology involves the study of intriguing findings, and since the last century the field has benefited from scientific developments originating in the most diverse disciplines. This work aims to present a scientific development that can greatly contribute to archaeology: genomics. A discussion about the concept of ancestry, one of the possible products of the archaeogenomic approach, is also presented here along with a brief explanation of the main techniques for ancestry estimation. The availability of such a development means that archaeologists now have methods at their disposal that could not have been dreamed of just a few decades ago.*

KEYWORDS. *Ancestry, Archaeology, Genomics.*

RESUMO. *A Arqueologia envolve o estudo de descobertas intrigantes e, desde o século passado, este meio é favorecido por desenvolvimentos científicos originados nas mais diversas disciplinas. Este trabalho tem como objetivo apresentar um desenvolvimento científico que pode contribuir bastante para a Arqueologia: a Genômica. Uma discussão sobre o conceito de ancestralidade, um dos possíveis produtos da abordagem arqueogenômica, também é apresentada aqui, juntamente com uma breve explicação sobre as principais técnicas de estimação de ancestralidade. A existência de tal desenvolvimento significa que os arqueólogos têm agora à sua disposição variados métodos que não poderiam ter sido sonhados há apenas algumas décadas atrás.*

PALAVRAS-CHAVE. *Ancestralidade, Arqueologia, Genômica.*

INTRODUCTION

Archaeology is a field that involves the study of intriguing contexts and findings, be they prehistoric or historic, in a constant effort to obtain as much information as possible about them. In many cases, more precisely in archaeological contexts characterized by the presence of human burials, basic information about the ancient individuals—such as their respective ancestry—still remains uncertain, and any conclusion regarding this aspect belongs to the realms of conjecture.

In order to better address the scientific problems that commonly arise in this field, it is necessary that the archaeologist knows how to identify and choose or propose the most appropriate method for that specific context. Fortunately, since the middle of the last century, the archaeological métier has also been favored by a variety of scientific and technological developments originating in the most diverse scientific disciplines, e.g., chemistry, biology and engineering (Walker 2005; Shillito 2013).

Obviously, it is not necessary for the archaeologist to know how to apply all these techniques from other scientific areas, however, it is undeniable that the existence of such a methodological variety certainly encourages, at least, a multidisciplinary training. In addition, their applications are only half the effort, since it is also necessary for a certain level of knowledge that allows

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for the appropriate analyses and interpretations of the resulting data, since they are not narrative or descriptive interpretations in themselves (Santos 2016).

This work aims, then, to present a relatively recent scientific development that has already contributed greatly to archaeological discussions, which is genetics or genomics—or archaeogenetics/archaeogenomics when applied to archaeological findings.

In the sections below, in addition to presenting this scientific development, the discussion that led to the use of archaeogenomic methods will also be outlined along with a brief explanation of the techniques that can most contribute to the resolution of specific but common scientific problems in archaeology, such as ancestry estimation of ancient human individuals or remains.

Finally, emphasis will be given here to methods that involve microscopic data analysis, rather than macroscopic information, since it is already known that specific morphological—macroscopic—characters are not necessarily associated with specific genetic or genomic ancestry[1] (Moreno-Mayar *et al.* 2018a).

ARCHAEOGENOMICS

Archaeogenetics, or archaeogenomics, is a term coined and introduced by Colin Renfrew that can be defined as a discipline that studies the human past through the use of Molecular Genetics techniques (Renfrew 2001; Renfrew *et al.* 2005), i.e., with the extraction of genetic material from archaeological human bone remains, information that allows an approximation to the life history of that individual.

From such analysis it is then possible to estimate the ancestry of a given individual or even the putative kinship relationships among different individuals, in addition to the identification of microscopic pathogens (such as viruses and bacteria) that may have caused their respective deaths (Hummel 2007; Herrmann *et al.* 2012).

Over the last decades, the extraction and analysis of genetic material from archaeological bones, the so-called "ancient DNA" (aDNA), has become a method of common use in Europe and North America to solve scientific problems concerning paleomigrations (Santos 2008; Santos *et al.* 2022, 2023), paleopathology, and human evolution itself, as well as to complement the analysis of morphometric characteristics from bone remains—usually leading to even more precise conclusions (Hummel 2003, 2007; Hermann *et al.* 2012; Petraglia *et al.* 2012). Nationally, however, such an approach has generally been appropriated by sciences other than archaeology (Freitas 2001, 2002, 2006).

As previously stated, only one of the possible products of an archaeogenomic approach, which is the estimation of ancestry, will be presented here. A brief discussion about the concept of "ancestry" will follow below.

THE CONCEPT OF ANCESTRY

Over the last few decades, studies involving the estimation of ancestry have been common practice in the field of archaeology—especially when the objects under study are human remains (Santos 2008, Santos *et al.* 2023; Dirkmaat *et al.* 2008; Dirkmaat & Cabo 2012; Solari *et al.* 2016; Silva *et al.* 2016). It is a concept and variable arising from the forensic sciences, where it has a prominent role of characterizing (or even identifying)[2]—in the form of a biological profile—human remains found in contexts of this nature (Birx 2010; Iscan & Steyn 2013).

The concept of ancestry is hardly defined in the works that involve it—it is treated almost as an axiom, when there seems to be no need for a definition, perhaps because it is considered obvious, or quite the opposite: because it is problematic (Birx 2010). However, the authors who employ the concept offer some clues about how they seem to think "ancestry" and the possibilities of information that its application can provide.

Adams (2007: 43) suggests that "[g]enerally, forensic anthropologists tend to classify individuals into three main groups: Caucasoid, or white/European; Negroid, or black/African; and Mongoloid or Native American/

[1] For example, on the relationship between a putative Australasian ancestry and a distinct cranial morphology observed in ancient Native American individuals—originally suggested by Bernardo and Neves (2009) for a couple individuals unearthed in *Serra da Capivara* —, Moreno-Mayar and colleagues (2018a, p 362) state that: "[a]lthough we detected the Australasian signal in one of the [...] individuals identified as a Paleoamerican, it is absent in other Paleoamericans [...]. This indicates that the Paleoamerican cranial form is not associated with the Australasian genetic signal, as previously suggested [...]."

[2] Together with the estimates of biological sex, age and height forming the so-called "Big Four"—which would be the main and primordial information of the biological profile of an analyzed individual (Adams 2007: 31).

Table 1. Stereotypical description of craniofacial traits of "The Three Main Human Races" from Krogman (1955). Source: Iscan and Steyn (2013: 197).

Dimensions	Caucasoid			Negroid	Mongoloid
	Nordic	Alpine	Mediterranean		
Skull length	Long	Short	Long	Long	Long
Skull breadth	Narrow	Broad	Narrow	Narrow	Broad
Skull height	High	High	Moderately high	Low	Middle
Sagittal contour	Rounded	Arched	Rounded	Flat	Arched
Face breadth	Narrow	Wide	Narrow	Narrow	Very
Face height	High	High	Moderately high	Low	High
Orbit	Angular	Rounded	Angular	Rectangular	Rounded
Nasal opening	Narrow	Moderately wide	Narrow	Wide	Narrow
Lower nasal margin	Sharp	Sharp	Sharp	Guttered	Sharp
Nasal profile	Straight	Straight	Straight	Downward slant	Straight
Palate shape	Narrow	Moderately wide	Narrow	Wide	Moderately wide
General impression of skull	Massive, rugged, elongated, ovoid	Large, moderately rugged, rounded	Small, smooth, elongated, pentagonoid to ovoid	Massive, smooth, elongated, constricted, oval	Large, smooth, rounder

Asian" and goes on to bluntly affirm that "[t]he best area to estimate race/ancestry is from the skull, especially the bones of the face". The three groups cited by Adams refer to the proposition originally made by Krogman in 1955 of the "Three Main Human Races"[3] (Krogman 1955; Iscan & Steyn 2013). In his work, Krogman proposed the distinction of these three "races"—where the Caucasoid was still subdivided into Nordic, Alpine and Mediterranean—through observation and analysis of craniofacial morphological characteristics (Table 1) (Krogman 1962).

Similarly, Iscan and Steyn (2013) argue that it is possible to identify at least three kinds of ancestry—African, Asian and European—based on morphometric analyses performed on bones of the human skeleton in general. Hefner (2009), in turn, uses the craniofacial morphological characters present in multiple American populations to segregate five distinct ancestries: American black, American white, Amerindian, Asian, and Hispanic. Finally, something similar is enforced by forensic anthropologists, as presented by Sauer (1992), who divide Mongoloid ancestry into two: Amerindian and Asian—in addition to black and white (Iscan & Steyn 2013).

After this brief but comprehensive presentation of how the concept is employed in the forensic sciences

[3] Some years later, Krogman himself withdrew from the use of the concept of race, changing "Races" to "Stocks" (Krogman 1962: 190).

—and a significant portion of the fields of anthropology and archaeology—, it is possible to propose that, in general, ancestry can be defined as: the estimation of the geographical, biological and/or ethnic origins (as well as the "cultural affiliation") of an individual based on their bone morphometric characteristics.

The application of this forensic approach in archaeology is naturally logical, then, for two reasons: (1) they share the same research object—human remains—even though the contexts might be different and (2) a similar curiosity: the "origin" of the analyzed individual, albeit for different purposes—individual identification for the forensic sciences (Adams 2007; Iscan & Steyn 2013), and for paleomigration studies in archaeology (Santos 2008; Santos *et al.* 2023), for example.

In human genealogy studies, a composite term appears. According to the International Society of Genetic Genealogy (ISOGG), "biogeographic ancestry" is defined as "the estimation of one's biological, ethnic and/or geographical origins based on DNA analysis".[4] There are a number of works in the biological, medical, and/or forensic sciences that employ such a definition (Shriver & Kittles, 2004; Halder *et al.* 2008; Bouakaze *et al.* 2009). The concept of "biogeographic ancestry" proposed by the ISOGG brings a new perspective of analysis: genetics. In this case, the estimation of ancestry as performed in the forensic sciences does not seem to be satisfactory to reach a more precise conclusion about the origin of a given individual.

Adams himself criticizes the possibilities of answers that the forensic sciences offer:

> "Clearly, these groups do not encompass the diversity of the modern world, and the skeletons of some people do not fit comfortably into these broad classifications. Another consideration is that admixture is a possibility. Admixture refers to a situation where a person has parents that fall into different racial groups. For example, if someone has a Caucasoid mother and a Negroid father, he or she would likely have some skeletal features typical of both groups" (Adams 2007: 43).

The definition of the "biogeographic ancestry" concept presented by ISOGG, in turn, sounds rather pretentious. How to define ethnicity based on genetic analysis? (and this question also extends to the forensic science analyses). According to Nagel (1994, p. 153), ethnicity is based on two "building blocks": "identity and culture." The question is then rephrased: how to approach these two aspects of ethnicity based on genetic analysis?

Given the limitations of both definitions mentioned above, it is necessary to consider an alternative to the concept of ancestry. Weiner (2010) argues that, in order to obtain the greatest possible amount of information from the archaeological record, and to provide interpretations with the least degree of uncertainty, the archaeologist should seek to carry out an investigation that encompasses both perspectives of this record: the macroscopic and microscopic, thus exploring all possible sources of information. Agreeing with this argument, it is admitted that this approach should be used, therefore, for ancestry studies—in order to carry out the most comprehensive characterization possible. In this sense, it is proposed in this work to define the concept of ancestry as the estimation of an individual's geographical origin from his biological characteristics—thus, both perspectives are included, morphometric and genomic analyses; and, therefore, ethnic and/or cultural concerns in such application are excluded.

In archaeogenomics, the concept of ancestry appears in the form of two other concepts that are used interchangeably (Santos 2008, 2022): haplotype and haplogroup. Haplotype is defined as the genetic profile of an individual (Goodwin *et al.* 2011). Haplogroup, on the other hand, can be defined as the simple grouping of individuals who share the same haplotype, or similar haplotypes, and is generally associated with a specific geographic region (Hummel 2007).

As an example of this last statement, here are some of the denominations chosen by the consortium that carried out the *1000 Genomes Project* (1kGP)—the largest genomic sequencing project for global human populations: "Chinese Dai in Xishuangbanna, China" (CDX) in East Asia, "British in England and Scotland" (GBR) in Europe, "Gambian in Western Divisions in the Gambia" (GWD) in Africa, "Colombians from Medellin, Colombia" (CLM) in the Americas, and "Punjabi from Lahore, Pakistan" (PJL) in South Asia (1000 Genomes Project Consortium *et al.* 2015). Genomically, haplotypes are defined by single nucleotide polymorphisms (SNPs)[5] present in the genetic material that each individual carries, usually inherited from

[4] "Biogeographical ancestry" in ISOGG Wiki (2020): <https://isogg.org/wiki/Biogeographical_ancestry>.

[5] Or variants, genetic mutations that affect a single nitrogenous base (adenine, cytosine, guanine or thymine) (Carracedo 2005).

Figure 1. A SNP sample (in red). Source: The authors (2020).

their ancestors. When the same combinations of SNPs (haplotype) occur frequently within a given population, there is a haplogroup, associated with the geography occupied by that population (Byrnes *et al.* 2012). And this is the way by which the ancestry of an individual is measured.

This estimation of ancestry through the classification of haplotypes in haplogroups is constantly used successfully in archaeogenomics studies carried out in the most diverse regions of the globe (Posth *et al.* 2019; Kampuansai *et al.* 2020).

Finally, it is clearly noticeable here the use, even if minimally, of some fundamentals of classification and typology, albeit with very specific terms: haplogroups and haplotypes represent the ideas of "groups" and "types" originally brought to the archaeological métier by professionals today considered as culture-historical archaeologists (Spaulding 1953; Ford & Steward 1954); while the SNPs are the variables or attributes that define these types and groups.

However, it is important to emphasize, differently from certain culture-historical claims, that it is not possible to work on this typology and/or classification within a "space-time framework" (Brainerd 1951: 303), since a given ancestry is not limited by time, as it is constantly updated through the centuries[6] (Semino *et al.* 2000; Carracedo 2005; Kayser *et al.* 2005; Goodwin *et al.* 2007; Hummel 2007).

METHODS FOR ANCESTRY ESTIMATION

Currently, to obtain genomic data, the samples under study are subjected to Next Generation Sequencing (NGS) techniques, which allow for the obtention of millions of short sequences—fractions of the genome—even in ancient/archaeological remains. These millions of "short reads" are the raw data that result from a sequencing procedure, provided as text files in the FASTQ format.

This allows for a given individual's whole genome to be completely "covered" by the short reads between tens and hundreds of times, thus decreasing the probability of errors in the final sequence of each sample —something especially important for studies of ancient DNA, since such a molecule undergoes constant decay over time, after the death of an organism (taphonomy) (Hummel 2007; Linderholm 2015; Mutzenberg *et al.* 2015; Santos 2016; Santos & Sullasi 2016; Sullasi *et al.* 2017, 2018; Santos *et al.* 2020).

In the NGS Era, the technologies developed by *Illumina*® have dominated the genomic sequencing market, and the fact that the required monetary resources involved in the process of whole-genome[7] sequencing have dropped has been attributed to them (Linderholm 2015).

However, it is not exactly the whole genome of an individual that is analyzed. Since the genomic difference between two human individuals is only 0.1% —i.e., when comparing two human genomes, only one position in every 1,000 nitrogenous bases (adenine, A; cytosine, C; guanine, G; and thymine, T) is different between them (Figure 1)—in genomic analyses only the SNPs are considered, since analyzing 99.9% of similar data would be a waste of time and resources (monetary and computational), especially given the human genome size: more than 3 billion nitrogenous bases. Thus, a difference of 0.1% still means more than 3 million SNPs (Naidoo *et al.* 2011).

To get to the SNPs, it is necessary to map (compare) the raw sequencing data (the data from the FASTQ file) of each ancient individual with a human reference genome, available as a text file in the FASTA format. It is a process, made possible by the application of compu-

[6] It is correct to affirm that this "update" has taken place since the "biological appearance" of these ancestry, even in prehistoric periods, due to the random mutations that have occurred and still occur in the human genome over time (Santos 2008; Cassidy *et al.* 2016; Martiniano *et al.* 2017).

[7] The DNA sequence present both in the *Mitochondria* and in the chromosomes (Linderholm 2015).

Table 2. List of 1kGP populations and their respective numbers of individuals (*n*). Source: The authors (2020) with data from *1000 Genomes Project Consortium* and colleagues (2015).

Population Description (Population Code)	Region	*n*
Han Chinese in Beijing, China (CHB)	EAS	103
Japanese in Tokyo, Japan (JPT)	EAS	104
Southern Han Chinese (CHS)	EAS	105
Chinese Dai in Xishuangbanna, China (CDX)	EAS	93
Kinh in Ho Chi Minh City, Vietnam (KHV)	EAS	99
Utah Residents (CEPH) with Northern and Western European Ancestry (CEU)	EUR	99
Toscani in Italia (TSI)	EUR	107
Finnish in Finland (FIN)	EUR	99
British in England and Scotland (GBR)	EUR	91
Iberian Population in Spain (IBS)	EUR	107
Yoruba in Ibadan, Nigeria (YRI)	AFR	108
Luhya in Webuye, Kenya (LWK)	AFR	99
Gambian in Western Divisions in the Gambia (GWD)	AFR	113
Mende in Sierra Leone (MSL)	AFR	85
Esan in Nigeria (ESN)	AFR	99
Americans of African Ancestry in SW USA (ASW)	AFR	61
African Caribbeans in Barbados (ACB)	AFR	96
Mexican Ancestry from Los Angeles USA (MXL)	AMR	64
Puerto Ricans from Puerto Rico (PUR)	AMR	104
Colombians from Medellin, Colombia (CLM)	AMR	94
Peruvians from Lima, Peru (PEL)	AMR	85
Gujarati Indian from Houston, Texas (GIH)	SAS	103
Punjabi from Lahore, Pakistan (PJL)	SAS	96

tational algorithms and/or programs, which is universal and mandatory for any and all genomic work. Thus, the mapping phase involves standard procedures that allow very little room for customization.[8]

The SNPs from ancient individuals can then be compared with the same data from modern individuals, which serve as a reference for the ancestry analysis. Although this procedure sounds odd—ancestry estimation of ancient individuals with the use of extant data—it is a standard procedure in genomics studies (Flegontov *et al.* 2019), and it is so because there is not a significant number of ancient individuals sequenced around the world that would enable reliable conclusions from old samples just yet. In other words, the procedure of using extant samples as a reference for ancient ones is still what allows the best approximation for the study of ancient ancestry.

Examples of modern samples that can be used are the 2,504 individuals that were originally published under the 1000 Genomes Project (1kGP), belonging to 26 populations from 5 different geographic regions

[8] For a detailed description of the procedures, see *Session2_ReadAlignment_VariantCalling* at <https://github.com/Saguiomics/AAAGs_2018>. This describes a protocol to obtain the SNPs through mapping raw sequencing data, of any human sample, to a reference genome.

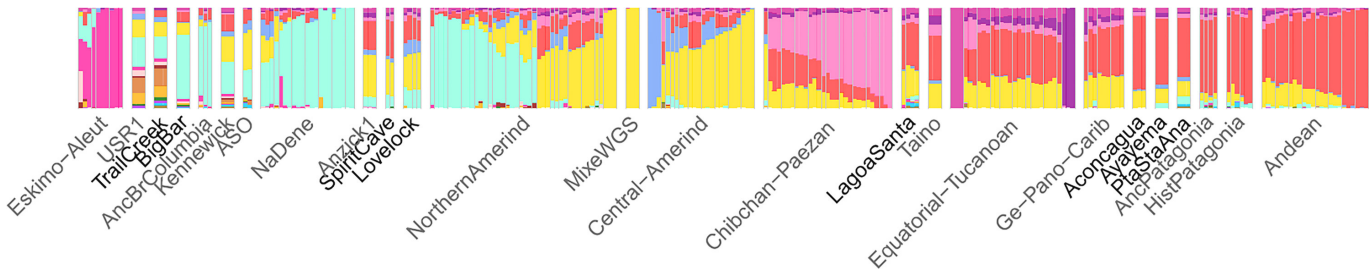

Figure 2. Sample bar graph produced from the results (percentages) of an ADMIXTURE® analysis (in this example, k = 16) on genomic data from dozens of ancient and modern human individuals (vertical bars).
Source: Adapted from Moreno-Mayar and his colleagues (2018a: 3).

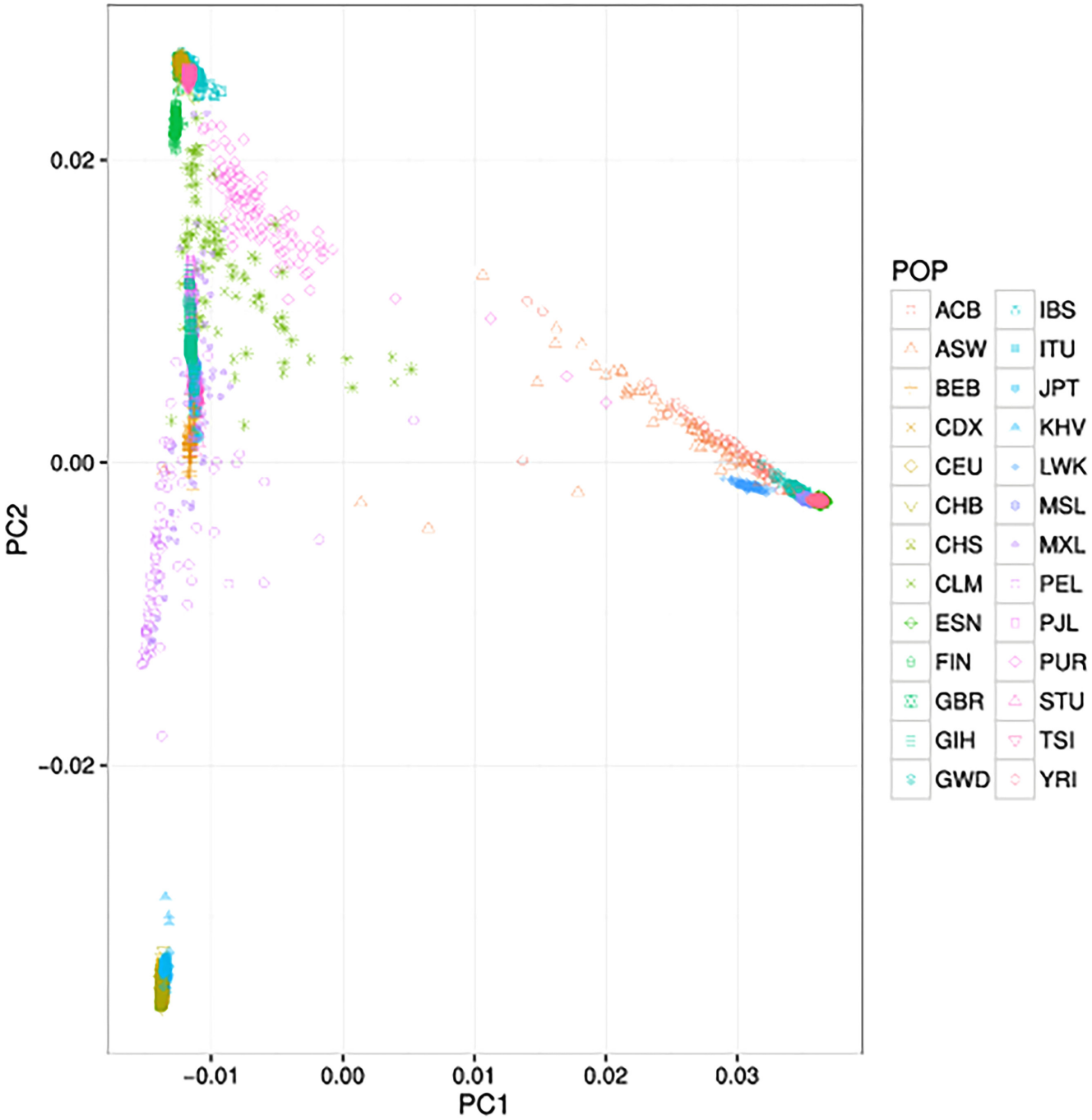

Figure 3. Sample scatter plot produced with the two principal components resulting from a PCA analysis performed on the 1kGP samples genomic data (colored dots). Source: <https://apol1.blogspot.com/2016/10/1000-genomes-project-phase-3-principal.html>.

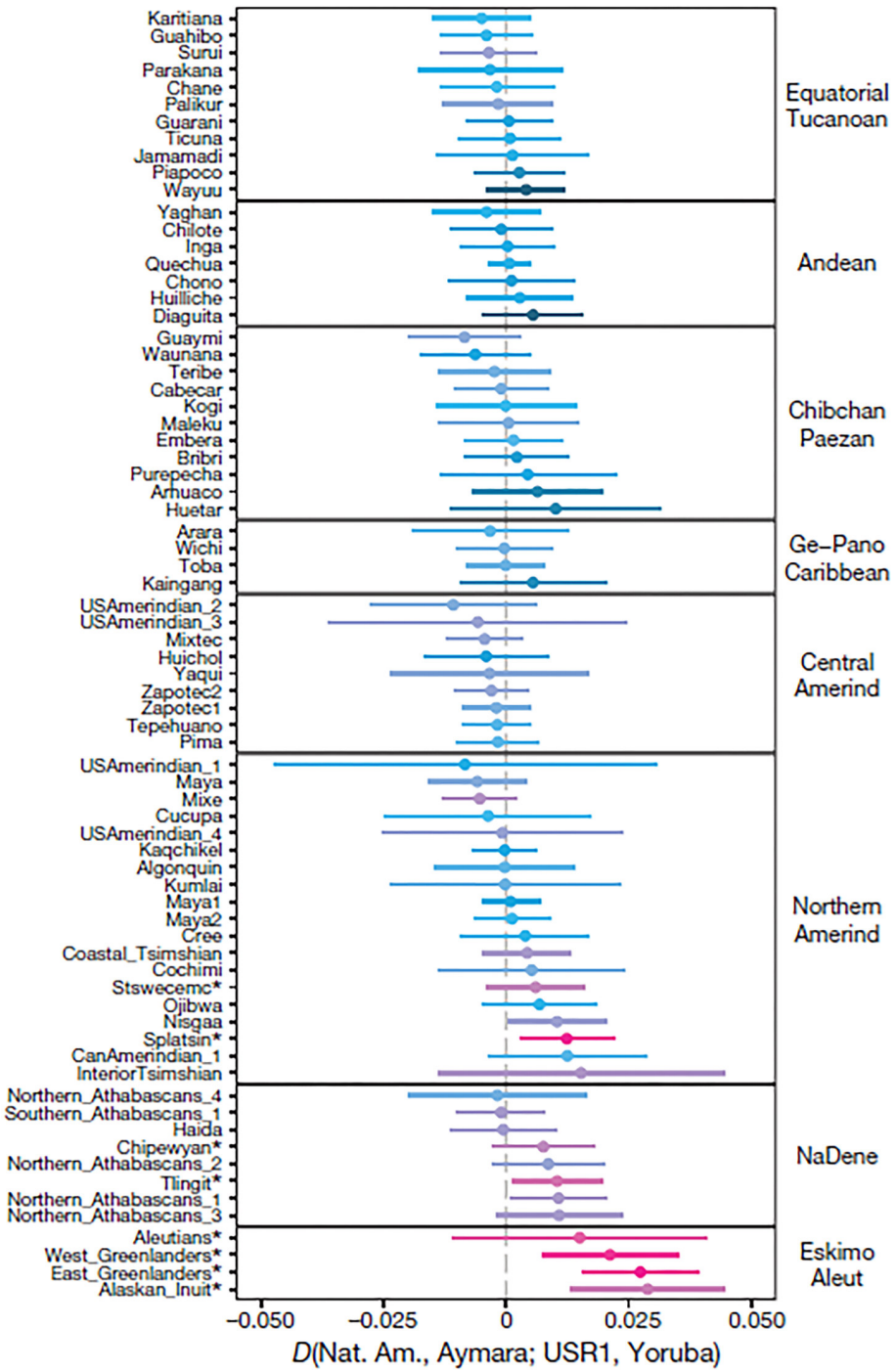

Figure 4. Sample graph produced from the application of f_3-Statistics on genomic data of an ancient human individual (center, USR1) and from dozens of modern populations (sides). Source: Adapted from Moreno-Mayar and his colleagues (2018b: 2).

of the planet (Table 2): East Asia (EAS), Europe (EUR), Africa (AFR), Americas (AMR) and South Asia (SAS) (1000 Genomes Project Consortium *et al.* 2015).

With a dataset composed of SNPs from ancient and modern samples, some ancestry analyses can then be performed, however, only those that allow for visual interpretations will be presented here: (1) ADMIXTURE®, (2) Principal Component Analysis (PCA), and (3) f_3-Statistics.

ADMIXTURE® is a software and tool that aims to statistically estimate the ancestry of one or more individuals from the frequency of occurrence of their respective SNPs.

The tool requires the input of an arbitrary number (k) referring to the number of ancestries that one seeks to observe in the individuals. After that, based on the SNPs frequencies, the software statistically defines which SNPs make up the k-ancestry.

The result is a list of individuals and the respective percentages of each of the k-ancestry in the dataset (Alexander *et al.* 2009). From these percentages, a bar graph can then be produced in order to interpret the possible results (Figure 2). Since the number of ancestries existing in a given human genome is unknown, a range of values for k—Flegontov and his colleagues (2019), for example, employed between 5 and 20—is generally used, and only the result for k that has the lowest cross-validation error (CV-error), among all k values after 100 iterations, is presented (Flegontov *et al.* 2019).

The software does not include any information regarding existing and/or previously defined ancestry, so that is why individuals from modern populations are used as references for the analysis of the ancient ones.

The second technique is the PCA, which generates the maximum dispersion of a given dataset based on all its respective variables (Abdi & Williams 2010). To obtain better visualization and further interpretations, a scatter plot can then be produced with its results.

In this case, what is sought to be observed is the genetic distance of all the individuals analyzed, taking into account all the millions or thousands of SNPs identified in the dataset—each SNP being a variable under analysis. Thus, based on the respective positions of individuals in the midst of dispersion, it is possible to observe those who have similar genetic affinities or ancestry (Figure 3).

As in the first technique presented here, it is recommended to also include individuals from modern populations in the PCA analysis—in order to have some references. A PCA analysis can be performed using the *Plink*® software, specifically created with the goal of analyzing genomic data (Chang *et al.* 2015).

Finally, the last ancestry estimation technique to be presented here is the f_3-Statistics, which aims to statistically estimate, also by SNP frequency, how genetically close an individual is to different populations. This analysis, unlike the above-presented ones, is carried out on an individual-by-individual basis (Figure 4) (Moreno-Mayar *et al.* 2018b).

CONCLUSIONS

It is notable here how much genomics can contribute to archaeological contexts characterized by the presence of human inhumations, and how rich the corpus of microscopic information that this type of trace can contain.

Properly answering the basic questions that involve the archaeological *métier* is the first step to providing more conclusive interpretations about the formation of a given context. The theoretical and methodological contributions presented here are also suggested to make this first step possible.

Fortunately, as previously stated, in the last few years there has been a noticeable drop in the costs of genomic procedures in general, and this scenario has gradually facilitated access to these technologies. The trend is, therefore, that the advent of even more technological innovations in this field will also allow for a greater expansion of archaeogenomics studies, including the study of issues that are not even considered today.

Lastly, it is important to note that the occurrence of these innovations, accompanied by the development of existing techniques, means that archaeologists have at their disposal, or at least will have, a wide portfolio of methods that could not have been dreamed of just a few decades ago.

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