Ancient DNA: a direct window into the human past

ADN antigo: uma janela para o nosso passado

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Abstract The origin and history of our species have for long captured our imagination and guided philosophical and scientific inquiry alike. Traditionally, the study of the human past was a central theme of various disciplines across the humanities, in particular history, archaeology, anthropology, or linguistics. These disciplines mostly focus on understanding the roots and cultural evolution of contemporary human populations, including the plethora of religions, ethnicities, behaviours, and languages that characterize the diversity of human life on the planet.

Resumo A origem e a história da nossa espécie há muito capturam a imaginação humana e orientam as nossas linhas de investigação filosófica e científica. Tradicionalmente, o estudo do passado foi objeto central das ciências sociais e humanas, em particular a história, a arqueologia, a antropologia ou a linguística. Estas disciplinas focam-se essencialmente na compreensão da origem e evolução cultural de populações contemporâneas, incluindo a grande variedade de religiões, etnias, comportamentos e idiomas que compõem a diversidade humana no planeta.

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Recently, a technological revolution in molecular biology, which made it possible to obtain genetic material from biological remains and cultural artefacts unearthed at archaeological sites and stored in museum collections, has provided unparalleled information on the biological history of past human populations. Over the last decade, ancient DNA emerged as a crucial tool for understanding human origins, population movement, or environmental adaptation. The exponential growth in ancient DNA studies makes cooperative, interdisciplinary research efforts imperative to enable appropriate integration of knowledge across complementary scientific disciplines. These interdisciplinary approaches are particularly relevant for studying the recent human past, where the higher availability of historical, archaeological, anthropological, and linguistic data can successfully allow for meaningful interpretations of the genetic data.

Keywords: Ancient DNA; human origins; demography; interdisciplinary approaches.

Uma revolução tecnológica recente possibilitou a obtenção de material genético a partir de amostras biológicas e artefactos culturais obtidos em escavações arqueológicas ou depositados em coleções de museu, permitiu obter informações sem precedentes sobre a história biológica das populações humanas passadas. Na última década, o ADN antigo emergiu como ferramenta crucial para entender as origens da espécie humana, movimentos populacionais passados ou episódios de adaptação. No entanto, é imperativo que o crescimento exponencial dos estudos de ADN antigo seja acompanhado pelo desenvolvimento de projetos verdadeiramente colaborativos e interdisciplinares que integrem o conhecimento de disciplinas tradicionais. Tais abordagens interdisciplinares serão particularmente decisivas para estudar o passado recente devido à maior concentração de dados históricos, arqueológicos, antropológicos e linguísticos que podem finalmente permitir interpretações adequadas dos dados genéticos.

Palavras-chave: ADN antigo; origens humanas; demografia; perspetivas interdisciplinares.

Studying the human past

The scientific disciplines studying the human past (i.e., archaeology, anthropology, linguistics, history, and evolutionary biology) rely on distinct (albeit complementary) types of data. The availability of suitable data that can be subject to investigation by these different sciences sets clear boundaries on their scope and application and somehow splits the study of our remote origins and evolution as a species from analysis of very recent episodes in our past. For example, documented historical data can be extremely useful for understanding recent events but offers no information about the periods of human history preceding the emergence of written records (i.e., *prehistory*), or insights about the past of human populations for which such records simply do not exist. Similarly, the

study of language evolution contains limitations in resolution when analysing the deeper human past, despite the likely remote origins of human oral communication (Miyagawa et al., 2014). In contrast, archaeological, anthropological, and genetic data contain valuable information to disentangle both the remote and recent history of human populations. Important methodological developments over the last decades led to significant breakthroughs despite the limitations imposed e.g., by the sparsity of the fossil record or the difficulties associated with archaeological age-modelling (Carleton et al., 2018). The exponential growth in data generation fostered the development of novel theoretical models for explaining human origins and the events that shaped contemporary human diversity. Notwithstanding the great potential of these data, the distinct limitations of the different sciences make it a necessity to develop truly integrated and interdisciplinary approaches that bridge the existing gaps across the disciplines to construct a holistic view of the human past (Williams and Teixeira, 2020).

Evolutionary biology and population genetics

In 1859, Charles Darwin published *On the Origin of Species by Means of Natural Selection, or The Preservation of Favoured Races in the Struggle for Life*, a book that is commonly regarded as having set the foundations of evolutionary biology (Darwin, 1859). This work details Darwin's perspectives on how differential reproduction and the survival of individuals carrying favourable traits, which were transmitted to the next generation (although no explanation was offered at the time on the mechanistic features of this process), could explain the diversity of life on Earth. The *Origin* conveniently avoided the topic of human evolution, which Darwin addressed later in a separate book, *The Descent of Man, and Selection in Relation to Sex* (Darwin, 1871). Arguably, and despite ensuing political and religious pressure, Darwin's work decisively marked the beginning of the biological study of the origin and evolution of the human species, which at the time were (in essence) dominated by philosophical and religious perspectives.

The critical contributions of Charles Darwin were accompanied by a truly revolutionary work by Austrian friar and mathematician Gregor Mendel, who is accurately regarded as "the father of genetics". Mendel formally established the so-called laws of inheritance (Mendel, 1866), allowing for probabilistic predictions on the transmission of physical traits (i.e., phenotypes) across generations that are encoded by genetic configurations (i.e., genotypes) on each parental lineage (Wood and Robinson, 2012).

Crucially, while it can be considered that Darwin's work laid the foundations for the development of evolutionary biology, the formal and mechanistic framework that finally established it as a scien-

tific discipline was only developed upon the incorporation of Mendel's laws and the emergence of population genetics.

The development of theoretical population genetics revealed how the interplay of different evolutionary forces (i.e., mutation, recombination, migration/gene flow, genetic drift, and natural selection) acting through time can shape the distribution of genetic variation in species and populations and, ultimately, the diversity of life on Earth (Nei, 1975; Hartl and Clark, 1989). Accordingly, the past biological history of a population can be evaluated, from the perspective of a population geneticist, by examining the abundance and frequency of genetic variants segregating in that population and building mathematical models that enable us to predict (even if evaluating *the past*) the demographic and adaptive processes that led to the observed genetic variation (Haldane, 1930). Population genetics is thus the study of how the different evolutionary forces interplay to shape genetic diversity in a well-defined probabilistic framework (Wright, 1931).

Evolution is a continuous process of (imperfect) transmission of genetic information from one generation to the next, leading to a gradual accumulation of genetic mutations along the different branches of an evolutionary species tree. In the case of humans, significant advances allowed for an overall understanding of the specific trajectory (or trajectories, as we shall see below) of our evolutionary branch since the pioneering works of Darwin, Mendel, and the establishment of evolutionary biology and population genetics as scientific disciplines (Williams and Teixeira, 2020).

Models of human evolution

Initially, the study of our origins mostly focused on the morphological analysis of the various fossil specimens that document the evolution of hominids over the last few million years. This approach eventually led to the emergence of two competing models for explaining the origin of anatomically modern humans (AMH): *Multiregional Evolution* (Wolpoff et al., 1984) and *Out of Africa* (Stringer and Andrews, 1988). Briefly, the main divergence between these two models pertains to the last two million years of human evolution and the relative contributions of different fossil groups to the origin of contemporary human populations.

Multiregional Evolution posits that different Pleistocene hominin groups, including *Homo erectus* or *H. neanderthalensis* (i.e., the Neanderthals), represent intraspecific morphological diversity within the human species that resulted from the relative isolation of these geographically separated fossil groups over time. However, *multiregionalism* states that this isolation was never complete and that a continuous network of gene flow across geographies facilitated the spread of advantageous traits, following the pioneering work of Franz Weidenreich (Wolpoff and Lee, 2014). Despite explicitly pro-

posing a global contribution of different hominin groups to the evolution of AMH, multiregional evolution does not offer any specific predictions on the magnitude of the contributions of each group.

In contrast, the competing *Out of Africa* model envisions a much more recent origin for AMH. According to this model, the ancestors of contemporary humans first evolved in Africa around 250,000 years ago and spread across the world through a massive migratory movement that replaced the different hominin groups documented in the fossil record (e.g., *H. erectus*, Neanderthals) (Stringer and Andrews, 1988).

Population genetics and human evolution

During the second half of the last century, the exponential availability of genetic data for worldwide human populations enabled the application of population genetics theory to disentangle major demographic and adaptive chapters in our history (Race and Sanger, 1950; Cavalli-Sforza, 1963; Cavalli-Sforza and Bodmer, 1971; Menozzi et al., 1978; Cavalli-Sforza et al., 1994; Jobling et al., 2004; Nielsen et al., 2017). Importantly, this opened the possibility to directly contrast genetic results to complementary archaeological, anthropological, linguistic, and historical data, and to thus provide a complementary source of information regarding AMH origin (Williams and Teixeira, 2020; Bergström et al., 2021).

The analysis of worldwide mitochondrial DNA (mtDNA), which is transmitted from the maternal side and thus recapitulates the evolutionary history of female lineages in a population, revealed that contemporary human diversity derives from a common African ancestral lineage from around 200,000 years ago, providing strong support to the *Out of Africa* model of human origins (Cann et al., 1987). However, it should be noted that mtDNA contains no information on most genealogical ancestors in a population, as it solely tracks the evolution of a single maternal lineage. In fact, the proportion of ancestral genetic information captured by mtDNA (or the Y-chromosome, which tracks the paternal lineages) decreases exponentially as we go further back in time, as the number of ancestors increase by a factor of two per generation. Notwithstanding these limitations, these results were interpreted at the time as providing definitive evidence for the recent African origin of humanity, seemingly excluding contributions from non-African fossil specimens, such as *H. erectus* or Neanderthals (Stringer and Andrews, 1988).

However, the limitations of the so-called uniparental markers (i.e., mtDNA and Y-chromosome) make it necessary to investigate *recombinant* genetic markers that are inherited both by the maternal and paternal lineages, and which provide information for the entirety of the genealogical ancestors of a population (Tavaré, 1984). The analysis of these recombinant markers, which comprise most of the human genome, provided further support to the *Out of Africa* model and brought

new light to the amazing migration that took our ancestors from Africa to peopling the entire world ~60-50,000 years ago (for a comprehensive review see Bergström et al., 2021). Moreover, a great number of genetic studies over the years helped unveil several examples of genetic adaptation of human populations to fluctuating selective pressures as humans migrated into new environments, including disease, diet, UV-light exposure or altitude (see Key et al., 2014; and Rees et al., 2020 for reviews on the subject).

The ancient DNA revolution

A recent, amazing technical accomplishment in molecular biology enabled the retrieval and analysis of genetic information from organic material unearthed at archaeological sites or stored in museum collections (Higuchi et al., 1984; Pääbo, 1989; Hofreiter et al., 2001a; Pääbo et al., 2004; Orlando et al., 2021). Crucially, the ancient DNA revolution was mainly driven by an attempt to decipher the evolutionary history of modern humans, whereby some of the most significant developments in the field are deeply intertwined with the search for our own origins (Orlando et al., 2021). These amazing developments led to the attribution of the 2022 Nobel Prize in Physiology or Medicine to ancient DNA pioneer Svante Pääbo, "*for his discoveries concerning the genomes of extinct hominins and human evolution*".

The analysis of DNA obtained from archaeological or museum specimens (of bone, skin, hair, or seeds), as well as environmental (soil) and material artifacts (e.g., pendants), presents significant technical challenges. First, the amount of endogenous (i.e., *truly ancient*) DNA present in the samples is often very low when compared to standard (i.e., modern) samples obtained from e.g., buccal swabs or blood. Second, the low levels of endogenous DNA make contamination from modern DNA (sourced from the environment or the researcher(s)) a significant problem. Finally, ancient DNA biomolecules often undergo transformations associated with post-mortem damage (Hofreiter et al., 2001b; Hansen et al., 2001; Briggs et al., 2007; Brotherton et al., 2007; Ho et al., 2007; Dabney et al., 2013), such as the fragmentation of the DNA molecule and the chemical deamination of cytosine into uracil, leading to the erroneous incorporation of thymine in the DNA sequence during amplification (Briggs et al., 2007). These transformations of the endogenous DNA present in a sample are mostly affected by the environmental conditions of preservation (usually, colder and drier environments are preferable to warmer and more humid ones) and the age of the sample.

Importantly, ancient DNA researchers have capitalised on these typical DNA damage patterns to efficiently authenticate truly ancient DNA molecules (Skoglund et al., 2014), as these chemical transformations do not occur in modern DNA sequences that may be present as a result of contamination. In any case, it

should be noted that the misincorporation of spurious DNA nucleotides in the sequence during amplification has the potential to interfere with downstream population genetic analyses (Axelsson et al., 2008). Accordingly, the computational processing of ancient DNA data is crucial to ensure the authenticity of the data, eliminate biases caused by molecular damage and laboratory treatments, and make it possible to generate a 'clean' dataset suitable for downstream population genetic inference. This typically involves the implementation of different steps (such as demultiplexing, adapter removal (Schubert et al., 2016), trimming, and collapsing of overlapping read pairs) using publicly available bioinformatic pipelines (Schubert et al., 2014; Peltzer et al., 2016). The filtered DNA sequencing reads are then mapped to available reference genomes (of the species of interest), and the authenticity of the ancient DNA molecules is typically obtained by analysing their relative size and damage profiles (i.e., the excess of cytosine to thymine (C->T) transitions at the end of the DNA reads).

Despite these limitations, the implementation of ancient DNA approaches significantly impacted our understanding of what makes us human by revealing the genetic makeup of the Neanderthals (Krings et al., 1997; Serre et al., 2004; Green et al., 2006; 2008; 2010; Noonan et al., 2006; Briggs et al., 2009) and identifying a completely new hominin species from DNA extracted from a finger phalanx uncovered at Denisova Cave, in Siberia (Reich et al., 2010). The Denisovans had not been previously recognized in the fossil record and thus represent the first-ever discovery of a new species solely based on genetic information.

The analysis of the Neanderthal and Denisovan genomes revealed both hominin groups are part of the global human family and direct ancestors of contemporary human populations (Green et al., 2010; Reich et al., 2010; 2011). These observations confirm some of the expectations of *Multiregional Evolution* that a non-negligible proportion of global human genetic ancestry is derived from now-extinct hominin relatives living across the world (Wolpoff et al., 1984). Specifically, Neanderthal DNA represents approximately 2% of the genetic ancestry of populations living outside of Africa as a result of distinct admixture events after the *Out of Africa* migration (Villanea and Schraiber, 2019; Mafessoni, 2019). More intriguingly, Denisovan DNA is mostly found in human populations presently living in Island Southeast Asia (ISEA), New Guinea and Australia (Reich et al., 2011), which has fostered intense debates about the true identity of this group and whether they could be represented by fossil specimens in Asia and ISEA (e.g., *H. erectus*, *H. floresiensis* and *H. luzonensis*), for which no genetic data currently exists (Teixeira and Cooper, 2019; Teixeira et al. 2021).

In addition to provide crucial information for refining our understanding on the biological origin of modern hu-

mans, ancient DNA studies have also provided unprecedented insights on more recent events in human prehistory (Liu et al., 2021). For example, these studies have successfully demonstrated that the contemporary genetic diversity of European populations can be traced back to three distinct ancestral population groups (Lazaridis et al., 2014) of Palaeolithic hunter-gatherers (Skoglund et al., 2012; Sánchez-Quinto et al. 2012), Neolithic farmers, and Bronze Age pastoralists. The latter has been associated with the Yamnaya culture and the likely spread of proto-Indo-European languages in the continent (Allentoft et al., 2015; Haak et al., 2015). Ancient DNA has also been used to further our understanding of the prehistory of Indigenous populations, in particular in the Americas (e.g., Scheib et al., 2018; Moreno-Mayar et al., 2018; Posth et al., 2018; Sikora et al., 2019; Nägele et al., 2020; Roca-Rada et al., 2020; 2021; Villa-Islas et al., 2023) and Oceania (Tobler et al., 2016), with growing efforts by the scientific community to decrease the bias towards European-centric studies coupled with the need for the integration of strong ethical considerations and community-driven research.

Intriguingly, with very few exceptions, ancient DNA studies continuously revealed significant and dynamic population movements, as well as extensive changes in ancestry in human populations across time. Beyond estimating previously hidden details of the demographic history of modern humans, ancient DNA data presents a unique opportunity to identify targets of natural selection using time-series data. So far, most studies have focused on examples of adaptive introgression of genetic variants inherited from Neanderthals and Denisovans – for a comprehensive review see Dannemann and Racimo (2018). However, a few studies have already leveraged large-scale ancient DNA repositories from human populations to uncover episodes of human adaptation to the environment (Mathieson et al., 2015; Souilmi et al., 2022). Interestingly, the most recent of these studies proposes population admixture during the Holocene has masked genomic signatures of hard selective sweeps in European populations, suggesting this type of adaptation might be more common than previously thought, and demonstrating the power of the time-series sampling characteristic of ancient DNA (Souilmi et al., 2022).

In addition, the availability of ancient genomes has also helped the reconstruction of one of the major transformative cultural and behavioural changes in the history of the human species – the domestication of animals and plants during the Mesolithic-Neolithic transition around 10,000 years ago (Frantz et al., 2020). As extensively documented in the archaeological record, and first occurring in the Fertile Crescent, humans gradually began to abandon their nomadic lives of hunting and gathering to settle and establish the world's first villages and urban centres (Zeder et al., 2006; Vigne, 2011), leading to extensive debates regarding the spread of

people and animals along with their culture. Accordingly, evidence gathered from ancient human and animal genomes showed the significant cultural changes associated with the Neolithization process were (at least to a certain extent) accompanied by changes in the genetic ancestry of human populations in Europe (e.g., Haak et al, 2005; 2010; Burger et al., 2007; Bramanti et al., 2009; Brotherton et al., 2013; Günther et al., 2015; Szécsényi-Nagy et al., 2015; Lazaridis et al., 2016; Rivollat et al., 2020). Moreover, ancient genomics provided new insights into the complex demographic dynamics behind the origin and geographical spread of the most emblematic domesticated species, including dogs, cattle, pigs or horses (e.g., Skoglund et al., 2015; Park et al., 2015; Frantz et al., 2016; 2019; Botigué et al., 2017; Gaunitz et al., 2018; Verdugo et al., 2019; Sinding et al., 2020; Librado et al., 2021).

While the contribution of ancient genomics to our understanding of the demographic and adaptive prehistory of humanity, as well as the domestication of animals and plants, has been significant, a growing amount of research has been devoted to important historical periods. In this context, significant attention has been devoted to major documented pandemic events, including the reconstruction of the genomes of the pathogenic strains responsible for some of the worst epidemics in human history, such as the genetic profiling of *Yersinia pestis*, the bacterial strain responsible for the Black Death pandemic (Bos et al., 2016; Spyrou et al., 2016; 2019).

Finally, and of great interest to the readership of this special edition on *Early Medieval Bioanthropology*, a recent study successfully identified the oldest known case of a genetic syndrome to date, from a 1,000-year-old individual who lived in the Medieval village of Castro de Avelãs, in the northern Portugal (Roca-Rada et al., 2022). Specifically, the researchers identified an extra copy of the X chromosome in this individual by analysing ancient DNA obtained from the petrous bone. In a truly integrated approach, the team combined the genetic evidence with archaeological and bioanthropological data to confirm the clinical diagnosis. This study also presents a new statistical method that opens the possibility of investigating the incidence of other genetic syndromes through time (e.g., Down Syndrome), confirming the power of interdisciplinary approaches to the study of the human past.

"With great power comes great responsibility"

The aforementioned studies represent but a subset of the exciting work in the field of ancient genomics of the past decade. Importantly, they show us the potential for ancient DNA to inform and/or challenge current theories regarding the origin of contemporary humans, and their cultural practices or languages, but also highlight the necessity for interdisciplinary dialogues that extend beyond

traditional disciplinary focus. In this regard, this author believes progress has been rather slow, with multidisciplinary (but not interdisciplinary!) approaches often failing to accurately integrate the perspectives and hypotheses of the different disciplines.

In any case, the recent explosion in the availability of ancient DNA data followed extraordinary developments in DNA sequencing technologies and a significant increase in computational power. Undeniably, ancient DNA has great power to uncover hidden events in the human past, whereby the growing trend in the number of ancient DNA publications observed in the last decade is likely to further accelerate in the foreseeable future. Importantly, the continuous improvement in ancient DNA analysis will pave the way for the generation of a large number of high-quality ancient genomes, in particular for the recent past, where limitations associated with DNA preservation are less common. This novel ancient genomic data will provide a unique opportunity to employ time-serial sampling and population genetics modelling to precisely infer the changes in the frequency of genetic variants and detailed demographic and adaptive episodes in human history.

However, this will necessarily bring a significant responsibility for geneticists to build complete models of human history by fully incorporating data from complementary scientific disciplines. Importantly, particular attention and care should be devoted to the implementation of appropriate ethical guidelines, that ensure 1) regulations are followed in places where the remains were obtained; 2) a detailed plan is prepared before beginning any study; 3) damage to the remains is minimized (while acknowledging that these analyses are inherently invasive and destructive); 4) data is made available following publication to ensure scientific reproducibility; and 5) the perspectives and sensitivities of associated stakeholders are respected and discussed *a priori* (Alpaslan-Roodenberg et al., 2021).

The study of the recent past arguably harbours the strongest potential for the development of truly interdisciplinary efforts due to the abundance of historical, linguistic, anthropological, and archaeological data available. To fulfill this potential, it is necessary to promote further engagement between geneticists, archaeologists, anthropologists, historians, and linguists, and to support the development of truly holistic models of human history by establishing a continuous dialogue across the different disciplines.

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