pca

european journal of
postclassicalarchaeologies

volume 10/2020

SAP Società Archeologica s.r.l.

Mantova 2020

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"Post-Classical Archaeologies" is indexed in Scopus. It was approved on 2015-05-13 according to ERIH PLUS criteria for inclusion and indexed in Carhus+2018. Classified A by ANVUR (Agenzia Nazionale di Valutazione del sistema Universitario e della Ricerca).

DESIGN

Paolo Vedovetto

PUBLISHER

SAP Società Archeologica s.r.l.
Strada Fienili 39/a, 46020 Quingentole, Mantua, Italy
www.archeologica.it

Authorised by Mantua court no. 4/2011 of April 8, 2011

For subscription and all other information visit the web site www.postclassical.it.

ISSN 2039-7895

pca
european journal of
postclassicalarchaeologies

volume 10/2020

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Pere Gelabert*

Past diseases: present questions and future perspectives from an archaeogenetic approach

The assessment of the health condition of past individuals is a central topic in the study of ancient populations: both from an archaeological and genetic point of view. The genetic study of ancient human individuals and human-related environments has already been successfully used to understand the relationship of ancient populations with health and disease. The present article aims to present which are the current explored applications of archeogenetics in this direction. The article is divided in three sections that group the major research lines: the direct study of pathogenic data from ancient human remains, the study of human evolution linked to disease and the study of human related environments. Additionally, the article aims to discuss the potential scope and limitations of this method.
Keywords: ancient DNA, pandemics, plague, malaria, metagenomics, health condition

La valutazione dello stato di salute degli individui del passato è un argomento centrale per lo studio delle popolazioni antiche, sia dal punto di vista archeologico che genetico. Lo studio genetico degli individui antichi e degli ambienti a loro legati è stato già applicato per comprendere la relazione tra le popolazioni antiche e la salute. Il presente articolo vuole presentare le attuali applicazioni dell'archeogenetica per tali fini. L'articolo è diviso in tre sezioni che raggruppano le principali linee di ricerca: lo studio diretto di dati patogeni dai resti umani antichi, lo studio dell'evoluzione umana collegata alle malattie e lo studio degli ambienti relativi all'uomo. Inoltre, l'articolo vuole discutere potenzialità e limiti di questo metodo.
Parole chiave: *DNA antico, pandemie, peste, malaria, metagenomica, stato di salute*

1. Introduction: health and paleogenomics

Health can be defined as physical, social and mental well being (WHO 2005). Two of these three categories can not be easily assessed through the archaeological and genetic study of ancient remains. The physical condition, in contrast, can be partially inferred from the study of archaeological human remains. In consequence, the study of health in an-

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cient populations based on biological material has been always focused on the study of the physical condition of the individual.

The health status of the individual is a complex reality, therefore is relevant to understand that the study of specific signals and markers provide very relevant information about specific stress conditions but may not be enough for assessing the global health condition of the individual (see Reitsema *et al.* 2014). When extrapolating health status observations from a sample study to a population level, more precautions must be taken into account. In Wood *et al.* 1992 the cautions that sciences based on the study of skeletal assemblages must control are divided in two categories: factors that can be addressed by a more accurate methodology like: problems with the sex and age estimation of skeletons, inadequate sample size and non representative sample, differential bone preservation conditions and differential diagnosis of the disease causing the lesion. The second category groups the relevant limitations that can only be addressed by changing the research strategy. In this category we include all the bias sources that relate to the non-correctly addressed heterogeneity of the population.

The study of ancient Desoxyribonucleic Acid (aDNA) of ancient biological remains is a relatively new approach in comparison to the large trajectory of bioarcheological research. In 1985, short sequences of DNA from Egyptian mummies were cloned with bacteria (Pääbo *et al.* 1985). Shortly after, the first DNA sequences extracted from skeletal tissue were sequenced and published (Hagelberg *et al.* 1989). Since then, multiple publications have reported the analysis of DNA from a constantly growing number of individuals from multiple historic periods. The field has evolved rapidly and currently millions of gigabases (GB) of ancient genomic data are publicly available (see Skoglund *et al.* 2018). These studies have allowed: the direct reconstruction of the genetic and social structures of ancient societies (e.g: Amorim *et al.* 2018; Mittnik *et al.* 2019; Schroeder *et al.* 2019), the unraveling of the migrating routes of extinct populations (e.g.: Haak *et al.* 2015; Lazaridis *et al.* 2016; Hofmanová *et al.* 2016; Mathiesson *et al.* 2018; Moreno-Mayar *et al.* 2018; Olalde *et al.* 2019; Narasimhan *et al.* 2019), the evolution of the *Homo* genus while enabling genetic comparison of our genome with extinct taxa such as Neanderthals or Denisova (e.g.: Meyer *et al.* 2012; Prüfer *et al.* 2014; Slon *et al.* 2018) and the study of past pandemics (e.g.: Bos *et al.* 2011; Schuenemann *et al.* 2013; Bos *et al.* 2014). The field of paleogenomics is gradually shifting to more complex questions that rely on the capacity of integrating diverse orientations. The novel trends aim to combine archaeological, anthropological, genomic and climatic data to un-

ravel past environments or the dynamics of ancient human populations in context with the environment and culture.

In the particular case of classical and post-classical times (8th century BC to 7th century AD), only few publications have included the analysis of individuals from these historical periods, the most relevant ones are: a time-scale genetic study of more than 100 individuals from the city of Rome, covering all the classical times, which revealed that the genetic diversity during Imperial Rome was way higher than the present-day Rome population (Antonio *et al.* 2019). This finding suggests that the city of Rome was more cosmopolitan in Imperial times than nowadays. Also relevant is a whole cemetery study of two Longobard sites from the 6th century CE in Northern Italy and Hungary. The genetic and archaeological analyses of the site graves unraveled complex social structures in these communities. These findings were achieved through the combined study of isotopes, grave goods and DNA (Amorim *et al.* 2018). One of the most relevant findings from the cited publication was the discovery, in the Longobard cemetery of Collegno (Italy), of individuals with low class profiles, according to grave gods with Italian genetic ancestry while the upper class graves belonged to Longobard genetic-ancestry individuals, which suggests a leading-class replacement in Northern-Italy in this period. Classical samples are also found in studies including: classical and post-classical Iberia (Olalde *et al.* 2019), Roman Lebanon (Haber *et al.* 2019), Roman Britain (Schiffels *et al.* 2016) or Egyptian mummies from the Ptolemaic Kingdom (Schuemman *et al.* 2017). All these publications have been able to characterize the individuals from these periods, giving information about the migrating movements and population changes of the last millenium.

Ancient DNA analysis, together with isotopic analyses and radiocarbon dating involve invasive and destructive sampling. This can make the study of ancient DNA polemic and ethically inadequate, as it can be incompatible with the bioarchaeological study of ancient remains. Although several bones have been used for aDNA sampling, the petrous bone, and especially the inner ear, clearly shows the highest content of DNA in the whole skeleton (Pinhasi *et al.* 2015). Therefore, the petrous bone has become the primary target of all the paleogenomic studies, as it guarantees the maximum amount of endogenous DNA in the whole skeleton, which is crucial in aDNA research. However, the removal of the complete petrous bone for sampling has an important impact on the capacity to demarcate landmarks used in craniometric diversity comparison, which represents a relevant problem. In order to solve this issue, research on less invasive sampling techniques is one of the priorities of the

field. One of the most relevant novel techniques in this direction is the Cranial Base Drilling Method (CBDM). This method allows the sampling of the petrous portion of complete skulls without altering the morphology of it, just performing a little incision on the cranial base, from where the inner ear is targeted (Sirak *et al.* 2017). Other alternatives to the sampling of the petrous bones are: the sampling of the tooth cementum (Hansen *et al.* 2017) or the destruction of the ear ossicles, that yield to significant endogenous DNA recovery and do not jeopardize future craniometric studies (Sirak *et al.* 2020). These findings show the interest on finding minimally invasive sampling techniques more compatible with the bioarchaeological research.

2. Archaeogenetics and disease

Ancient DNA can be obtained from a wide range of samples; from environmental material (Willerslev *et al.* 2003) to human manufactured objects (Schablitsky *et al.* 2019), and its study has multiple applications. When referring to the assessment of health from archeological samples these applications can be grouped in three different categories: a) Analysis of the human genetic variation linked with susceptibility, predisposition and resistance to disease, b) The direct identification and study of ancient pathogens in ancient biological remains and c) The study of the human-related environment and how it influences the health status of the individual.

2.1. Human genetic adaptation to disease

Modern humans evolved in Africa and left the continent at least 200,000 years BP (Hershkovitz *et al.* 2018). During the last millennia humans have spread and colonized all the continents and ecosystems. It is widely accepted that the present day human genetic diversity is the result of local adaptations of the different human populations that settled diverse ecosystems (Balaresque *et al.* 2007). Infectious diseases have been accompanying humans since the arose of our species, it is estimated that these pathologies have been the principal cause of death among all human history (Dye 2014), representing the most significant selective pressure that have acted upon our genomic variation (Fumigalli *et al.* 2017).

Farming originated in the Middle-East 11,000 years ago and started expanding through Europe 8,5000 years ago (Skoglund *et al.* 2012). The

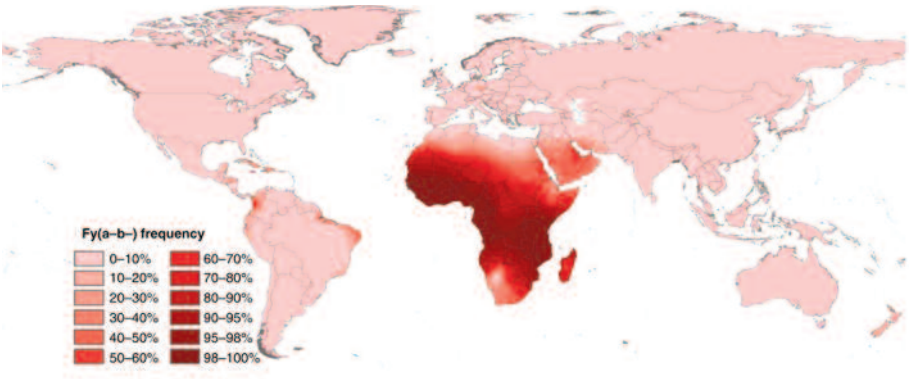

Fig. 1. Global distribution of the Duffy negative phenotype. Global prevalence of the phenotype (Howes *et al.* 2011).

technological revolution led to massive migrating movements that replaced most of the European populations by farmers with Anatolian ancestry (Hofmanová *et al.* 2016; Lazaridis *et al.* 2016), which expanded into all the continent during the next 5,000 years (Skoglund *et al.* 2012). The arise of farming was contemporary in place and in time with multiple domestication processes of animals (Zeder 2008). The Neolithic onset was succeeded by the arose of sedentary and highly inhabited human communities. The increase in population densities in these sedentary sites linked with the close contact of humans with livestock conduit to the spread of zoonotic diseases (Pearce-Duvet 2006). Zoonotic diseases are infectious diseases that have an animal reservoir, which represent most of the documented infectious agents acting upon human cells (Woolhouse *et al.* 2005). As different climatic conditions favour the propagation of different pathogens it is observed that different human populations exhibit particular adaptations to several infectious diseases as a result of a long coexistence. These adaptations are observed as prevalent genotypes in specific populations. Normally, the favored genotypes are found in genes that codify for proteins that interact with an specific pathogen. The most well known examples of such adaptation are probably sickle cell anemia (Gouagna *et al.* 2010), Beta thalassemia (Waterhall *et al.* 1997) and Duffy negative phenotype (Miller 1976) (fig 1). All of these three genetic variants confer a major resistant phenotype against malaria. High frequencies of these variants are observed in present-day African populations exposed for many centuries to the disease.

ing of human genetic adaptations, allowing a direct observation and tracking of genetic variation origin and spread in past human populations (Malaspinas *et al.* 2012; Gamba *et al.* 2014; Marinack *et al.* 2017; Nielsen *et al.* 2017; Mathiesson *et al.* 2015). Particular applications of this direction have revealed that notorious infectious diseases such as malaria have not represented an important selective pressure in ancient European populations (Gelabert *et al.* 2017). Similar approaches have also allowed the identification of genetic variants that have fluctuated in different periods revealing the presence of human adaptation to diet such as the capacity of tolerating the lactose or the efficiency in the metabolism of fatty acids (Mathiesson *et al.* 2015; Ye *et al.* 2017; Buckley *et al.* 2017; Sverrisdóttir *et al.* 2017), adaptations in the immune system (e.g.: Olalde 2014; Hofmanová 2016; Lindo *et al.* 2016; Broushaki *et al.* 2016) as well as adaptations to specific climatic conditions (Günther *et al.* 2018).

2.2. Past pandemics: direct study through archaeogenetics

The study of past pandemics has gained certain popularity in recent decades, and especially the direct study of pathogens recovered from biological and archeological remains, usually extracted from human bone or teeth. The main limiting factor in the study of past pathogens is the preservation of these in the skeletal tissue as not all pathogens can be recovered from bones. Some pathogens have very low infectious loads and some are too acute to be preserved in bone. Examples of these pathologies without skeletal affectation or low pathogenic load are: cholera or viral infections such as flu.

Multiple pandemics have affected human populations during history (fig. 2), however its impact and etiological causing agent can not be always known from the macroscopic study of ancient human remains or historical texts. The research on past infectious diseases and pandemics, before the arose of aDNA, has been conducted through the paleopathological study of ancient skeletal assemblages combining macroscopic, histological and radiological diagnosis of skeletal lesions that can be linked to disease (Buikstra, Roberts 2012). Since the early 1990s, the usage of molecular techniques has enabled the direct sequencing of pathogenic species in ancient skeletal assemblages (Spigelman *et al.* 1993; Salo *et al.* 1994; Arriaza *et al.* 1995; Drancourt *et al.* 1998). These first results were obtained with the usage of Polymerases Chain Reaction (PCR) techniques. PCR techniques imply the recovery of only known and short fragments of DNA. The limited output has two main consequences: A) the recovered sequences can be enough for diagnosis

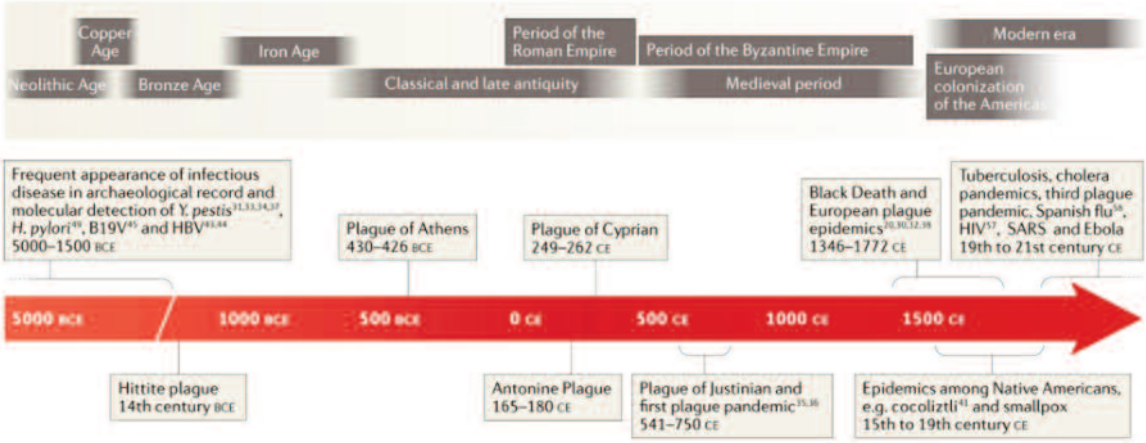

Fig. 2. Major pathogenic outbreaks that have affected humanity in the past (Spyrou *et al.* 2019).

but not for molecular phylogenetic studies, B) PCR results are difficult to be reproduced, which means the existence of false positive identifications (Gilbert *et al.* 2004; Shapiro *et al.* 2006). Since the arose of Next Generation Sequencing (NGS) techniques, the amount of genetic data sequenced has experimented a big boost, allowing the sequencing of complete pathogenic genomes (Bos *et al.* 2011) and the study of the molecular particularities of the ancient DNA which allow the identification and discrimination of modern contamination (Sawyer *et al.* 2012). The implementation of sensible bioinformatic pipelines (Key *et al.* 2017) have resulted in a drastic reduction of the false positives of pathogenic analyses with NGS data.

Pathogenic DNA can be targeted and extracted from diverse sources. To maximize the chances of recovering the pathogen it is necessary to target the specific tissues that can more probably preserve it. While petrous bone is normally the choice for endogenous ancient DNA, its low irrigation make it less indicated when looking for pathogens, as it was demonstrated with Bronze Age plague victims (Margaryan *et al.* 2018). *Y. pestis* can be targeted in the inner cavity of the teeth as it is preserved in the pulp chamber (Drancourt *et al.* 1998; Schuenemann *et al.* 2011). Other pathogenic species recovered from teeth are: *Plasmodium falciparum* (Marinack *et al.* 2016), Hepatitis B virus (Mühelmann *et al.* 2018; Krause-Kyora *et al.* 2018), *Mycobacterium leprae* (Schuenemann *et al.* 2013; Schuenemann *et al.* 2018), *Salmonella enterica* (Vå-

gene *et al.* 2018; Key *et al.* 2020), and *Borrelia recurrentis* (Guelli *et al.* 2018). Mycobacterium tuberculosis (Bos *et al.* 2014), *Treponema pallidum* (Schuenemann *et al.* 2018) and *Mycobacterium leprae* (Schuenemann *et al.* 2013; Schuenemann *et al.* 2018) have been isolated from bone. Other pathogens have been recovered from less conventional sources such ass: *Plasmodium falciparum* and *vivax* from antique microscope slide collections (Gelabert *et al.* 2016; de-Dios *et al.* 2019; van-Dorp *et al.* 2019), *Vibrio cholera* from medical collections (Devault *et al.* 2014) and others.

In the following section two relevant diseases will be presented: malaria and the plague. Both diseases have had relevant implications in human history and these diseases are the two more relevant that have been recovered and studied from classical individuals.

2.2.1. Malaria

Malaria is an infectious disease caused by the infection of diverse protist species of the *Plasmodium* genus (fig 3). To date there are six described species capable of infecting human cells. *P. falciparum* is known to be the most lethal one, while *P. vivax* is the one with the broader distribution. Currently malaria is widely prevalent in Africa. It is estimated that up to 455,000 people died from paludism in 2016 (World malaria report 2017, WHO). Recent discoveries have pointed out that the relationship between *P. falciparum* and humans occurred recently, and probably linked with the farming expansion. Farming led to a dramatic boost of both human populations and densities. The disparity between human and great apes population size turned into a selective pressure for Anopheles to feed on humans (Carter *et al.* 2003), which motivated the selection of human feeding genotypes. Therefore, *P. falciparum* would have also been forced to select those genotypes that conferred a major success both for human and mosquito infection. This process promoted an

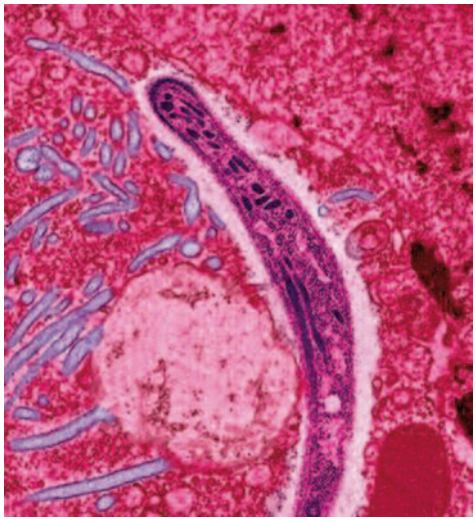

Fig. 3. *Plasmodium falciparum* sporozoite migrating through the cytoplasm of a mosquito cell.

important population growth of *P. falciparum* that genetically appears as a bottleneck dated in 5,000 years BP (Otto *et al.* 2018).

P. falciparum would have spread from Africa to all tropical and subtropical climates within the last 6,000 years (Carter *et al.* 2003; Otto *et al.* 2018). The expansion towards Europe seems to be much more recent, probably occurred in historical times. The spread of *P. vivax* is suggested to be quite comparable, *P. vivax* has also an African origin and after a complex path would have colonized Europe in recent times. The proposed estimations are around 10,000 years BP (Culleton *et al.* 2011). These estimations also link the arrival of *P. vivax* in Europe with the onset of farming (Liu *et al.* 2014). Nevertheless there are other proposed dates that set this up to 265,000 years BP (Escalante *et al.* 2005; Mu *et al.* 2005).

Malaria was present in Europe in classical times (Sallares *et al.* 2004). Genetic studies based on ancient samples have revealed that *P. falciparum* malaria was probably introduced in Europe from India. This introduction could have been following the military campaigns of Alexander the Great in India in the 4th century BCE (Gelabert *et al.* 2016; de-Dios *et al.* 2019), while *P. vivax* European malaria is likely to have an African origin (Gelabert *et al.* 2016; van-Dorp *et al.* 2019).

Related to the evidence of malaria in the Mediterranean in classical times, Hippocrates of Cos the Greek medical doctor of the Pericles era, considered as one of the fathers of medical science, described episodes of fevers in Classical Greece concordant with malaria symptoms. This writing would represent the oldest written record of malaria infection. This record, however, does not allow us to know the extent of malaria presence at this time in the region, even though it is possible that was vastly ubiquitous (Sallares 2002). Before the arose of NGS, only one PCR result from a 5th century CE Italian child from Lugano in Umbria was available (Sallares *et al.* 2001). Recent intents have certified the presence of *P. falciparum* in two individuals from Velia and Vagnari, two Italian coastal populations from the 1st-2nd century CE by

Fig. 4. Individual from which malaria was recovered in ancient Italy (Marinack *et al.* 2016).

the sequencing of the *P. falciparum* mitochondrial genome (Marciniak *et al.* 2016) (fig. 4).

2.2.2. The Plague

Y. Pestis infection is the cause of bubonic plague. This bacteria originated and evolved from *Y. pseudotuberculosis*, much less pathogenic than *Y. pestis* (Achtman *et al.* 1999). Nowadays it is still present in rodent reservoirs and it is endemic in 17 countries (WHO 2017). Three main *Y. pestis* epidemics have affected Europe in historical times. The oldest documented one is the Plague of Justinian, that lasted from the 6th to the 8th century AD (Russell *et al.* 1968). Probably the most famous one is the pandemic that devastated Europe in the 14th century. Named the Black Death, this epidemic could have killed up to 40% of the European population and was present in Europe until the 18th century (Zietz *et al.* 2004; Benedictow *et al.* 2004). The most recent plague pandemic occurred between the 18th and 19th centuries (Cohn *et al.* 2008; Stenseth *et al.* 2008). Based on literary records, possibly earlier *Y. pestis* outbreaks occurred in Europe prior to the Justinian plague, such as the Plague of Athens (5th century BC) and Antonine plague (2nd century AD). The lack of concluding DNA evidence do not allows neither the confirmation of such events nor the identification of the pathogen linked with the historical records (Drancourt *et al.* 2002). The study of plague, in general, is complicated by the presence of false positives, as other bacterial species such as such as *Y. pseudotuberculosis*, found on the soil can be confused with *Y. pestis* (Gilbert *et al.* 2004), and is especially challenging when working with low coverage samples (Rasmussen *et al.* 2015; Andrades *et al.* 2017; Keller *et al.* 2019).

The Justinian plague (541 CE) is the name that received the first documented *Y. pestis* outbreak that originated in Northern Africa, according to written records, in the mid-6th century CE (Harper 2017; Sarris 2013) and was present in Europe until the 8th century CE. Molecular data, nevertheless, do not support this hypothesis. To date, the aDNA of *Y. pestis* has been found in samples from human remains dated to this pandemic from Germany (Feldman *et al.* 2016; Wagner *et al.* 2014; Keller *et al.* 2019), Britain, Spain and France (Keller *et al.* 2019). The comparison of these samples with first pandemic ones from China and Kyrgyzstan (Cui 2013; Eroshenko 2017) as well as a 2nd-3rd century CE sample from Tien Shan mountains (De Barros Damgaard 2018) has revealed that the First plague Pandemic likely has an Asian origin. This plague could have accessed the Mediterranean via the Indian Ocean and the Red Sea since India was well connected by marine traffic with the

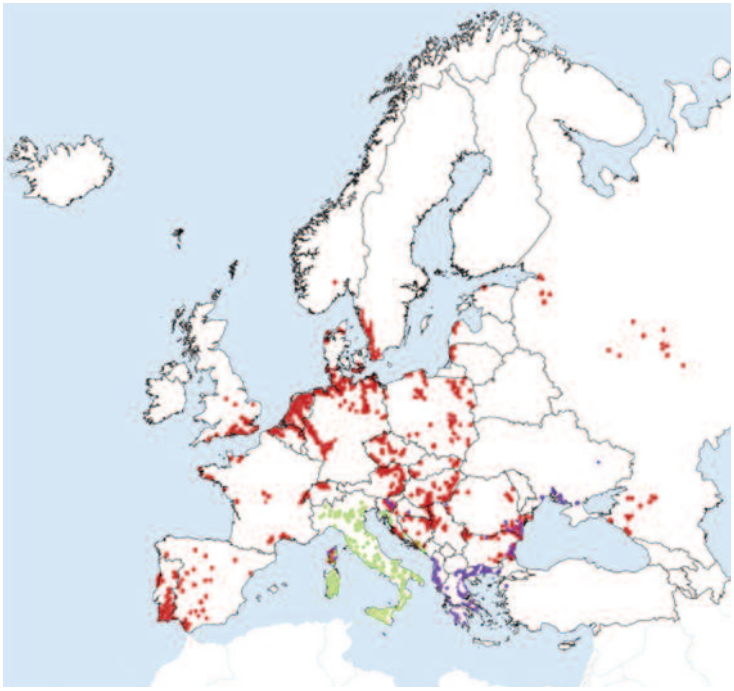

Fig. 5. Distribution of the three relevant anopheles species present in Europe: *A. atraparvus* (red), *A. labranchiae* (green), *A. sacharovi* (purple) (from Piperaki *et al.* 2016).

early Byzantine Empire (Harper 2017). The comparison of the European strains has pointed the presence of local reservoirs at that time, as not all the genomes from the 6th century are related. For example, two different *Y. pestis* strains were identified in the same site in the South of France (Keller *et al.* 2019). The study of Justinian plague strains has also revealed interesting facts related to cultural practices. The individual from which the British *Y. pestis* genome was recovered was found in a single burial, indicating that the plague did not change the burial practices in this location, although the presence of mass burials has traditionally been linked with pathogenic outbreaks (Keller *et al.* 2019).

The understanding of the causes of the dramatic impact of pathogenic outbreaks is crucial for the prevention of future pandemics. As an example of this, it is relevant to mention the polemic surrounding possible future *Plasmodium* outbreaks in the Mediterranean linked with changes related to global warming (Sainz-Elipe *et al.* 2010). Even that global warming will favor the malaria-related mosquito, which are still present in the Mediterranean (fig. 5), it would seem that the European medical atten-

Fig. 6. Sources from which ancient DNA has been obtained: a) crops, seeds and plants, b) fecal samples, c) ceramic objects, d) ancient insects, d) dental calculus, e) ancient medical preparations, f) ancient books and parchment, g) ancient shell, h) ancient capillary, i) ancient bone and teeth.

tion standards as well as the capacity of the European governments to conduct effective surveillance on the vector would prevent future malaria outbreaks in Europe (Piperaki *et al.* 2016).

2.3. Human health indicators evaluated with environmental samples

Paleogenomics are not restricted to the analysis of human bone and teeth samples. The growing attention of paleogenomics linked with the new technical improvements after the development of NGS techniques has promoted the number of studies that have targeted aDNA from multiple and alternative sources (fig. 6). Up to date, aDNA has been obtained and analyzed from: ancient shells, ceramic objects, skin and hair tissues, environmental samples such as lake cores, plants, ancient seeds, laboratory preparations, books, coprolites, dental calculus and animals (see Green *et al.* 2017). Some of these sources potentially contain DNA that can be used to infer health conditions of ancient human populations or study the connection between human populations and ancient environments.

Commensal bacterial, fungal and viral species are found in all the human tissues exposed to the environment such as; mouth, ear, skin, digestive track or reproductive organs. The commensal microorganisms play critical roles in host immunity, metabolic pathways or stress response (Koskella *et al.* 2017). The diverse environments that host all these species are known as microbiomes. Microbiomes vary within people, populations and time (Davenport *et al.* 2017). The composition of these tissues has been observed to be related to the genetics of the individual but also with habits such as diet or medical drug treatments. In recent years, the usage of NGS techniques together with refined bioinformatic pipelines has enabled the study of multiple human microbiomes (fig. 7).

The human oral microbiome is a complex ecosystem that plays important roles related to human health. Its alterations can also cause dis-

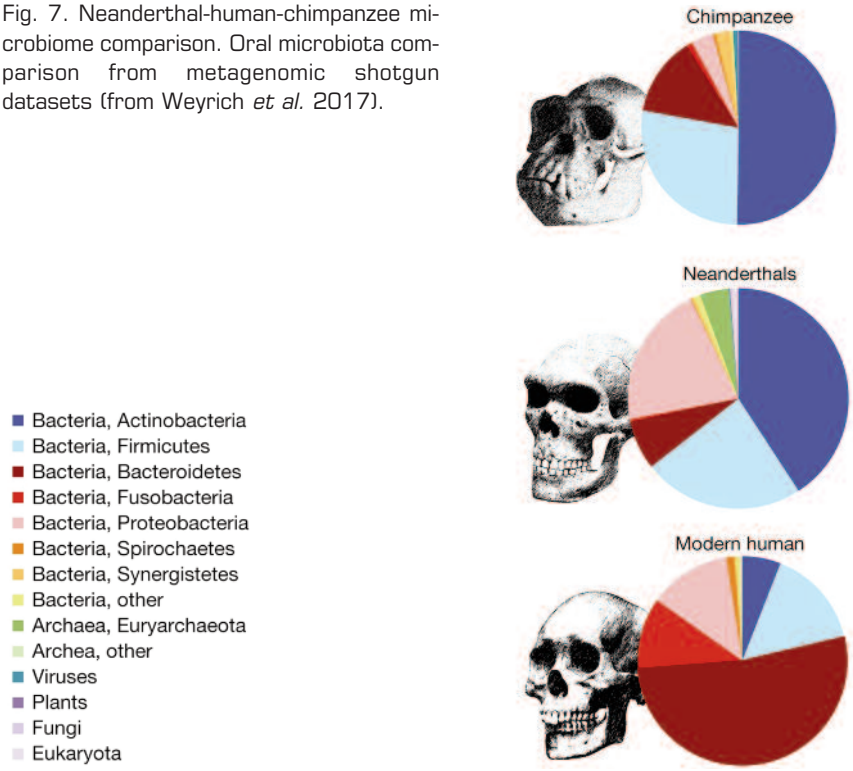

Fig. 7. Neanderthal-human-chimpanzee microbiome comparison. Oral microbiota comparison from metagenomic shotgun datasets (from Weyrich *et al.* 2017).

ease such as cancer, depression or metabolic disorders (Gill *et al.* 2006, Turnbaugh *et al.* 2007; He *et al.* 2015). The study of the oral microbiome can also provide information about past diets that can be obtained from dental calculus (e.g. Weyrich *et al.* 2015; Weyrich *et al.* 2017). Both ancient genomes and proteins can be obtained from dental calculus. The study of past dental calculus has revealed insights on the diet of the ones that suffered the great Irish Famine (1845-1849), the reconstructed diets from different individuals provided information about sex specific patterns (Geber *et al.* 2019). Interestingly, the metagenomic data can be preserved for thousands of years as the reconstruction of a Neanderthal dental calculus evidenced. This study showed that the Neanderthal microbiome composition was more similar to the one of a present day chimpanzee rather than to the microbiomes of modern humans, pointing the great connection between diet and microbiome (Weyrich *et al.* 2017) (fig. 7). In a shorter scale it has also been observed, by the comparative study of dental calculus, that oral microbiomes correlate with the diet in different periods, and a major shift is observed in modern times, while populations from the Neolithic to the Middle Ages have sim-

Fig. 8. Eighth-century coprolite used for ancient DNA analysis (Tito *et al.* 2012).

ilar microbiome profiles (Adler *et al.* 2013). A very recent discovery has also revealed the potential of human-used objects to assess the oral microbiome composition as it is the case of a Stone Age chewing gum (Jansen *et al.* 2019).

Coprolites, which can be normally found in mummified bodies (Tito *et al.* 2012) are another source of intestinal microbiomes. Alternatively to mummified bodies, coprolites can be also naturally preserved in caves, as the ones studied in a Clovis Culture cave in Oregon, dated to 14,000 years BP. This source allowed the sequencing and analysis of the mitochondrial DNA of Clovis settlers, that showed the typical Paleoamerican mtDNA haplogroups (Jenkins *et al.* 2012). Coprolites can preserve parasites and commensal species of the gut microbiome, as well as the food present people ingested right before dead (Poinar *et al.* 2001; Tito *et al.* 2012) (fig. 8). Several studies have already shown the potential of this method, especially with very well preserved human remains such as the body of the Iceman from the Alps (Maixner *et al.* 2015). Nevertheless, The approach is not free from limitations, as metagenomic samples from the intestinal tract can be easily recovered contaminated by modern bacterial and fungal species, as it has already been revealed (Tito *et al.* 2012). An especial mention must be arisen when referring to the capacity of discrimination between modern and ancient bacterial

DNA. The technique that is currently used is basically identifying the species found in the sample and assessing the contamination based on the presence of deamination signals. This approach however has a strong limitation which is that is not able to detect and study ancient bacterial species that are not found anymore, as we do not have its included in the reference panels.

The environment has a strong effect in the gene expression, although it is not easy to explore this connection in ancient samples. Epigenetics are described as the mechanisms that affect the expression of genes during the life of the individual, which means that these signals do not alter the genetic sequence but affect how genetics are expressed in the individual. There exist several modifications that the environment can cause on the DNA sequences, however only DNA methylation can be easily assessed from ancient remains (Llamas *et al.* 2012; Smith *et al.* 2015). These modifications are known to be associated with multiple biological traits; many epigenetically regulated genes are relevant during the embryogenesis and human development, and alterations on its can be the cause of disease or abnormal development (Okano *et al.* 1999, Gokham *et al.* 2020), mostly through the silencing or enhancing of differential gene expression (Jones *et al.* 2012). There are multiple factors that are responsible for these differential expressions such as: the composition of the diet, exposure to heavy metals or exposure to chemicals or even changes in the climate that result in temperature fluctuations (Cao-Lei *et al.* 2014). It has been shown that the exposure of humans to catastrophes can result in alterations in the methylation patterns as for example the Dutch famine of 1944-1945 (Veenendaal *et al.* 2013) or the Ice storm that devastated Quebec in 1998 (Cao-Lei *et al.* 2014).

The listed examples evidence that the contribution of archaeogenetics to the assessment of health is not just limited to the study of pathogens in ancient human skeletons, which is probably the most well-known application, but also to the environmental and genetic factors that are the source and the consequences of the interaction of the human populations with disease. In the next section the most relevant conclusions and the possible future directions of the field are presented.

3. Conclusions and new perspectives on archaeogenetics related to health status

The study of ancient genomes and metagenomes for assessing the health conditions of past populations is gaining popularity in the field of archaeogenetics, as it can be observed by the increasing number of pub-

lications that target health-related questions. Researchers have made big efforts in recovering diverse diseases from ancient human remains that have allowed the sequencing of multiple pathogens and commensal species from a wide variety of environments.

The macroscopic study of disease has already shown how human populations have suffered, adapted and coped with past major health problems issues. In the future, a closer connection between archaeology, pathogen recovery and environmental data can yield to the understanding of the differential causes of mortality and morbidity in past populations, as well as to a much accurate estimation of the consequences of past diseases in human history. This data complemented with the genetic scans on the human genome can also help to understand the evolutionary fingerprint of disease in the human genome and populations.

For a more accurate understanding of the health conditions of past populations and individuals it is crucial to be conscious of aDNA in the recovery and discrimination of results, which means that a negative result can never be understood as a definitive statement. And more relevant it is strictly necessary to contextualize the genetic and molecular findings with historical and archeological research to avoid biases due to an erroneous study design, an inadequate sampling or a weak understanding of past conditions context. If these limitations are considered and the synergy between multidisciplinary researchers grows, with the available techniques and information, it is possible to arise a very precise understanding of the living conditions of past cultures as well as a very accurate estimation of past mortalities, morbidities and selection forces.

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