 DOI: 10.24425/academiaPAS.2023.147023

History Inscribed in Bones

Anna Juras, PhD, ME
is an Assistant Professor at the Institute of Human Biology and Evolution, Faculty of Biology, Adam Mickiewicz University in Poznań. She is a biotechnologist and anthropologist by training, and her research concerns the study of ancient human DNA. She is interested in the origin of historical and prehistoric populations in Central Europe and their migrations from the Neolithic to the Middle Ages.
anna.juras@amu.edu.pl

Researchers hard at work in the ancient DNA laboratory at the Faculty of Biology, Adam Mickiewicz University

Obtaining genetic material from ancient human remains is challenging mainly due to the risk of sample contamination.

Anna Juras
Maciej Chyleński

Institute of Human Biology and Evolution
Department of Biology
Adam Mickiewicz University in Poznań

Deoxyribonucleic acid, or DNA, is found in the cells of all living organisms. It is a polymer composed of four types of subunits called nucleotides – thymine (T), cytosine (C), guanine (G) and adenine (A) – by means of which the information stored in DNA is encoded. DNA is also present in skeletal remains; then it is then called ancient (aDNA). Unlike genetic material from living organisms, aDNA is highly fragmented and exhibits characteristic changes in its structure, which occur as a result of deposition and postdeposition processes occurring after the organism died (*post-mortem*). In the first phase, DNA becomes fragmented under the influence of endogenous nucleases, which are enzymes that cleave nucleic acids (including DNA) and are released as a result of cell death. Next, DNA becomes degraded by microorganisms (mainly bacteria). Subsequently, over time, DNA comes to be further degraded by environmental factors, including water, temperature, oxygen, and UV radiation. For example, the influence of water can lead to hydrolytic depurination (which cleaves DNA at the purine sites, i.e. G and A) or to nucleotide deamination. This latter phenomenon typically involves the conversion of C into uracil, i.e. a nucleotide that is later interpreted as T. Therefore, as a result of these changes, the ends of aDNA fragments contain T instead of C, and in the complementary strand, A appears in place of G. These mutations occur gradually and are typical of aDNA, primarily affecting the last 10 nucleotides of each DNA fragment.

The time elapsed between deposition and the retrieval of a given sample for molecular analysis is undoubtedly a limiting factor, although it is primarily the environment in which the bone materials were deposited that ultimately determines how well aDNA is preserved. Interestingly, after just around 150 years, the average length of surviving aDNA fragments is only about 40 to 80 base pairs. All these factors, coupled with the fact that bone material is susceptible to contamination by modern, exogenous DNA, make working with ancient genetic material extremely challenging.

Contamination of materials

Contamination with modern DNA posed a formidable obstacle to aDNA research until recently, due to the lack of good detection tools. The most serious concern is contamination of human remains with DNA from the researchers working with the material, including archaeologists, physical anthropologists, museum professionals, and laboratory staff. Direct sources of contamination may include the hair, skin fragments, sweat particles, and breath of individuals handling the samples. This means that from the very first stages of working with bone materials, including sample collection, must be carried out with certain precautions – including the use of coveralls, masks, disposable gloves, and sterile tools, such as bone-cutting instruments. Collected samples should also be stored in a frozen state to halt further degradation of the aDNA. Contamination with modern DNA is particularly worrying because the methods of amplifying the genetic material (necessary for analysis and interpretation) may preferentially amplify the modern genetic material by mistake.

> Ancient DNA (aDNA) can easily become contaminated by products of already amplified modern DNA.

The greatest risk, however, is that aDNA may become contaminated by products of already amplified modern DNA. The concentrations of DNA resulting from the use of the polymerase chain reaction (PCR) are incomparably greater than the amounts of aDNA to be found in the materials under study. It is estimated that just one drop of aerosol released by opening up a tube containing amplified DNA can contain more of it than an entire aDNA isolate. Therefore, the work of isolating ancient genetic material needs to be carried out in special "clean" laboratories, which

Maciej Chyleński, PhD

an archaeologist and geneticist, employed at the Institute of Human Biology and Evolution, Faculty of Biology, Adam Mickiewicz University in Poznań. He specializes in the study of ancient DNA, and his research interests mainly focus on the reconstruction of the kinship structure of prehistoric populations and its relationship to migration processes. He is also involved in bioinformatic analyses of ancient nuclear genomes obtained by high-throughput sequencing.

maciej.ch@amu.edu.pl

must be physically separated from modern labs where amplified DNA is worked with. This should involve having the two types of laboratories situated in separate buildings (or at least separate wings of a building) and installing a separate, filtered air circulation system to generate positive air pressure in the clean room. Additionally, protective clothing must be required, including full coveralls, masks, eye protection, and double gloves. The aDNA laboratory itself should have a designated area, called an anteroom or sluice room, where staff can change into protective clothing. When work is underway, all surfaces, equipment, and tools should be regularly decontaminated using DNA-degrading agents and UVC radiation-emitting lamps. Having a sterile laboratory and adhering to strict working procedures allows the risk of ancient bone samples becoming contaminated with contemporary human DNA to be kept to a minimum and also avoids possible cross-contamination between the bone samples under study.

> With the rapid development of high-throughput sequencing technology, the amount of data on ancient DNA is surging.

Another type of contamination encountered during aDNA research is referred to as environmental contamination, and it is directly related to the degrading activity of microorganisms and their ubiquitous DNA. Bone samples are typically heavily contaminated with genetic material of bacterial origin. The fraction of such material often constitutes more than 90 percent of all the DNA isolated from them. Procedures used in clean laboratories allow for partial elimination of environmental contaminants present on the surface of bone materials, but completely eliminating them is not possible.

Verifying authenticity

The objective of all these careful measures taken when working with ancient DNA is to obtain isolates of the highest possible quality for aDNA research. This means striving to obtain isolates with the smallest ratio of environmental to endogenous DNA and minimizing the risk of contamination with modern DNA. In the case of human remains, distinguishing human aDNA fragments from bacterial genetic material does not pose significant methodological difficulties, but it still requires significant effort and financial resources. It is generally accepted that bone materials containing at least 1 percent endogenous human DNA are suitable for analysis. This means that for such samples, 99 percent of the data generated is treated as representing environmental contamination.

Verifying the authenticity of aDNA sequences and, therefore, determining the level of potential contamination in the form of contemporary human DNA may be somewhat more problematic. Nevertheless, we now have methods and appropriate statistical tools that allow us to confirm that we have obtained authentic endogenous DNA. High-throughput sequencing provides us with sequences of entire

Collection of bone materials for aDNA research

Teeth and the petrous parts of the temporal bone are the materials from which ancient DNA is most commonly isolated

DNA fragments. Such sequences carry additional information that is useful for detecting contamination. Due to the large amounts of data, this information can be quantified and analyzed statistically. In this case, we rely on features typical of aDNA, such as post-mortem mutations, including changes from C to T and G to A. A sufficiently high fraction of sequences exhibiting such mutations indicates the presence of aDNA. Additionally, we analyze the length distribution of DNA fragments, which, in the case of ancient genetic material, should show a predominance of short and very short fragments. Furthermore, we can estimate the degree of contamination by examining sequences mapped to haploid parts of the studied genomes, which should appear in one identical and unaltered variant in each individual. For humans, these genome segments include the mitochondrial DNA and, in the case of male individuals, the X and Y chromosomes. Any discrepancies in the sequences in these parts of the genome may indicate their origin from more than one person, pointing to the contamination of the DNA sample.

Advances and discoveries

The study of aDNA (referred to as archaeogenetics, paleogenetics or biomolecular archaeology) is one of the most rapidly advances branches of bioarchaeology. Thanks to the rapid development of high-throughput sequencing technology, the amount of available data in ancient DNA research is surging. Consequently, in the last decade, we have progressed from studying fragments of individual mitochondrial genomes to studying complete nuclear genomes of hundreds of individuals. The increasing amount of data generated also makes it possible to unambiguously determine the authenticity of the results obtained – something that was not possible with older methodologies, based on direct amplification of only selected DNA fragments. The growing volume of data and declining costs of sequencing are allowing analyses to be performed with increasingly better resolution.

Research on human aDNA primarily focuses on historical and prehistoric populations, mainly in terms of their origins, migrations, and reconstructing their biological relatedness. However, all research findings are the outcome of the ongoing struggle against contamination with both environmental and human DNA – a struggle we may not always be aware of when casually reading the results of such analyses. For example, in our project concerning the study of Bronze Age populations inhabiting the present-day territories of Poland and Ukraine 4200–3200 years ago, out of 175 examined DNA isolates, 82 exhibited high levels of environmental DNA contamination, excluding them from further analysis. Two further samples had to be excluded from the study due to contamination with contemporary human DNA. Ultimately, the ancient nuclear genomes of only 91 individuals were suitable for further analysis. Nevertheless, the results demonstrated that a significant migration occurred around 3800 years ago at the transition between the Early and Middle Bronze Age, substantially altering the European gene pool. ■

Further reading:

Chyleński M., Makarowicz P., Juras A. et al, Patrilocality and hunter-gatherer-related ancestry of populations in East-Central Europe during the Middle Bronze Age, *Nature Communications* 14/2023, https://doi.org/10.1038/s41467-023-40072-9