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AUSTRALIAN CENTRE FOR ANCIENT DNA
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ANCIENT DNA ANALYSIS OF HUMAN REMAINS

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PROJECT OUTLINE

ANCIENT DNA ANALYSIS OF HUMAN SPECIMENS

As part of the Genographic Project, an ambitious, multi-center, 5-year project to trace the Journey of Man, the National Geographic Society (NGS) is funding the genetic analysis of ancient human specimens from around the world (<https://genographic.nationalgeographic.com/genographic/index.html>).

Ancient DNA provides a means to trace the migration patterns and relationships of human groups through time, offering a powerful additional source of information to standard physical anthropological and archaeological research. Ancient DNA provides a genetic capacity to travel through time, and examine the genetic diversity, relationships and origin of archaeological groups. Furthermore, within the archaeological context it can help shed light on kinship, demography, and the social organization of past populations.

ANCIENT DNA

Studies of ancient human DNA are famously difficult, being both expensive and highly-sensitive to modern human DNA contamination. As part of the Genographic Project, NGS has formed a partnership with The Australian Centre for Ancient DNA (ACAD), an internationally-leading group in the analysis of ancient human specimens. Directed by Prof Alan Cooper, one of the leading researchers in the field, ACAD has developed a range of powerful new techniques that allow high resolution analysis of ancient human DNA while also monitoring and detecting modern contamination. The new methods change considerably what is possible and are now available for use by all NGS-funded research expeditions, and for other archaeological and anthropological studies.

WHAT WILL BE DONE?

A concise analysis of the hypervariable D-loop region of the mitochondrial genome (mt genome) as well as the complete mt genome sequencing of selected haplogroups will be the main focus of this study. This has two reasons:

- a) Due to the higher copy number (~1.000-10.000:1 per cell) of mitochondrial DNA (mtDNA) the chances to be detected after degradation and diagenesis of the specimen are much higher than for nuclear DNA.
- b) Numerous and extensive studies on global modern human mtDNA diversity have revealed reliable scaffold of the mtDNA phylogeny and at the same provide a huge dataset for comparison with extant populations in a global as well as a small-scale perspective.

If DNA is preserved in excellent conditions, we will be able to target regions of the nuclear genome. This allows us to conduct sex typing and analysis of autosomal short tandem repeats (STRs) as known from the genetic fingerprint in forensic medicine. In addition, in male individuals we will type the Y haplogroup (the paternal counterpart to the mtgenome) to track back paternal lineages through time.

WHAT IS REQUIRED?

Ancient DNA analyses can be performed on small (0.1-0.5g) samples of bone, teeth, hair or fecal remains, and can be accomplished without any discernible damage to the specimen (especially tooth roots, which leave the form and placement of the crown unaltered). The key problem is that the samples must remain uncontaminated with modern human DNA, which requires the use of gloves (and protective clothes) when samples are collected. Specimens should not be washed, as human DNA in water will penetrate and contaminate the specimen. It is important to note that in many situations, the biochemical preservation may not be good enough to allow DNA survival, and ACAD will work with researchers to evaluate the potential of material before analysis. ACAD is available to coordinate with researchers to advise and assist with sampling protocols and analysis.

WHAT IS THE TIMELINE AND THE FUNDING?

Support from the National Geographic Society for genetic analysis within the Genographic Project is available until the end of 2011, and requires only the cost of sample transport to ACAD on the part of individual researchers. The amount of time required for the analyses will vary according to the amount of material, and specific question, but will generally be between 3-9 months.

CONTACT DETAILS

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The ACAD website (www.adelaide.edu.au/acad) includes further details, e.g. about protocols for sending samples to Australia, and should be used in addition to direct contact with ACAD members.

SAMPLING GUIDE

SAMPLING PROTOCOL FOR ANCIENT HUMAN REMAINS

BACKGROUND

The ancient DNA (aDNA) component of The Genographic Project (TGP) will take place in the Australian Centre for Ancient DNA (ACAD), University of Adelaide, using tooth, bone and hair samples from human specimens around the world. We would like to encourage all archaeologists/anthropologists to seek possible ancient samples for us during their activities, as this will expand both the geographic and temporal coverage and significance of the aDNA work. We are happy to provide advice and assistance in the logistical, practical, (and political) aspects of locating and collecting samples. We will attempt to type both mt and nuclear (Y & autosomal) DNA single nucleotide polymorphisms (SNPs) in the ancient samples to complement datasets generated by the other TGP centres and beyond. Although Y and autosomal loci are likely to be difficult in less well-preserved samples, we try to apply the best techniques available at the time. A research & development program into new genomic and microarray approaches is also underway in order to increase the amounts of SNP data per specimen.

The ACAD facility is a dedicated, positive air-pressure complex in a building isolated from any molecular research, and features field-leading anti-contamination and detection methods. Pre-existing contamination within samples can be minimized if intact specimens (*eg* whole bones) can be sampled at ACAD using the specialist equipment (*eg* surface removal with a shot-blaster), but archaeologists and anthropologists are likely to come across opportunities where it is necessary to take the samples quickly in the field (freshly excavated). The latter is actually the situation we would prefer. For such cases, the following guide covers the steps involved in identifying and collecting samples that are most likely to contain DNA.

The dominant problem with genetic studies of ancient humans is contamination with modern human DNA, which becomes extreme in geographic regions where the authentic aDNA is genetically similar to members of the archaeological or museum team (*eg* Europe). Both bones and teeth can easily be contaminated by handling (*eg* during collection or curation), and the DNA can be absorbed deeply inside the specimen over time. This problem is vastly exaggerated if the specimen has been washed, as this will encourage complete penetration. In most environmental conditions teeth are the most resilient sample material, are also easiest to collect, and offer (limited) protection from handling contamination. Dense cortical bone is an alternative sample, and involves the removal of a small chunk (approx. 2cm x 2cm) via a thin cutting disc (preferably with diamond blade) or even a hacksaw. Where possible, a good approach to combat the risk of contamination is to analyze two physically remote samples (*eg* a tooth and a limb bone) from the same specimen. (NB This

may still be confounded if the whole specimen has been washed using contaminated water). Hair also provides a good source of ancient DNA, however, its limitations are the availability in the archaeological record (mostly restricted to mummies) and its restriction to mtDNA only. This should therefore be taken into consideration before samples are taken.

SAMPLING IN THE FIELD.

Avoid direct or close contact with the specimen as your sweat (and breath, hair etc) contains DNA, which can be absorbed into the bone or teeth. At least two layers of gloves (and a breathing mask where possible) should be used when collecting a sample. The most suitable sample DNA-containing material will nearly always be characterized by excellent biochemical preservation (*eg* high collagen/protein content) and show minimal diagenetic/mineral alteration. Good signs include fresh-looking, dense heavy bones with smooth and intact surfaces, to more obvious evidence such as strong, jagged, flexible edges at broken ends (as opposed to a crumbly chalky white interior). Beware that a chalky (protein-leached) interior matrix can often be hidden by a thin, smooth surface layer (*esp.* under articulation faces), but low bone density and any cracks should reveal the underlying diagenetic alteration. Such damage often varies considerably within a single specimen, and it is generally helpful to search for the most promising area (keeping in mind to avoid important morphological characters if necessary). A good sign of protein (and therefore potentially DNA preservation) is a burning smell when cutting bone or teeth. This should smell like a nightmare trip to the dentist (*i.e.* a cross between burning hair and bacterial agar plates), rather than the acrid inorganic smell of rock-cutting. Black scorch marks indicate a lack of protein, and are a bad sign.

Sampling bone: Where the sample must be taken in the field, a hacksaw or drill with cutting disk should be used to obtain an 8-10mm square (3/8 inch) section of dense cortical bone from diaphyses of long bones (normally limb). Femur or tibia is best, humerus is an alternative (see figure next page). Try to minimize heat build-up during sampling, *eg* use cutting disks as opposed to a drill bit. Denser bone is more likely to preserve DNA, especially in adverse environments, so thick cross sections of limb bones are much preferable to porous bone such as phalanges and ribs. Where possible, whole bones sent to Adelaide will allow us to take the least obtrusive and cleanest samples under controlled conditions (positive air pressure, still air labs etc). If the skeletons and *esp.* the skulls are disassembled we also recommend taking the petrous bone as it also contains dense compact bone.

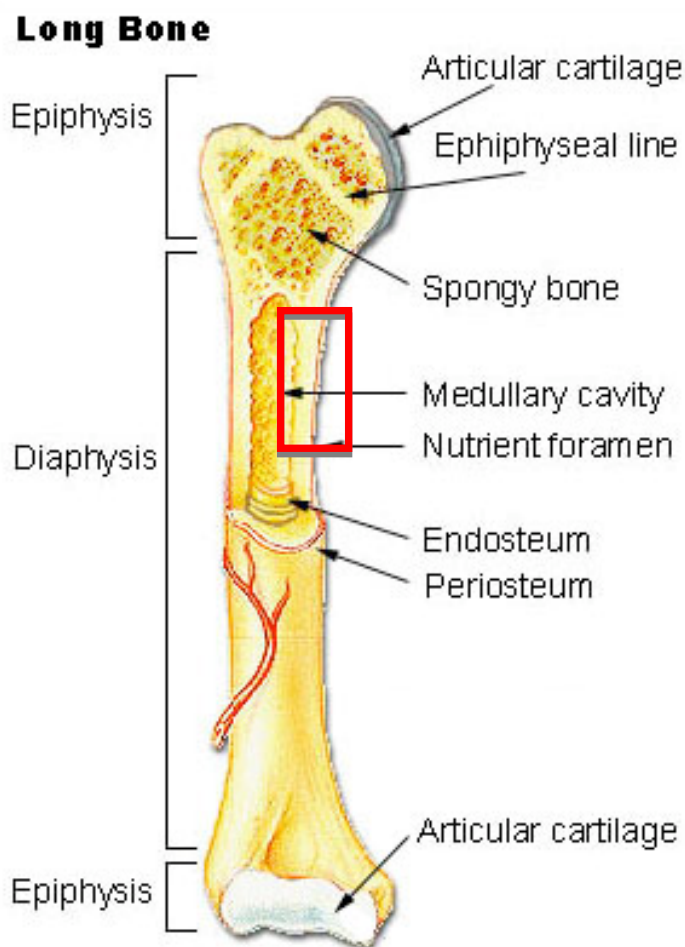


Figure. Preferred sampling area in long bones (red rectangle).

Sampling teeth: The best sample is a single-rooted tooth, as it comes out of the socket more easily. Teeth should be ‘gently’ extracted using (gloved) fingers and repeated small movements as opposed to single big heaves. Canines can be taken quickly and easily, molars can sometimes be difficult and require some experience to extract from the alveolars but provide more material to analyze. Avoid metal tools as these often lead to spalling or breakage. Leave dental calculus in place where possible.

Once removed, samples should be stored in sterile plastic ziplock bags or equivalent tubes and labelled on the outside. In addition to locality and specimen number, please note whether the material has been handled or washed, the age of museum collection and any other details. As a general guideline, samples should be kept at roughly the same conditions as they had been found. Samples from temperate climates can be kept at room temperature, or refrigerated and permafrost samples should be kept frozen. But repeated humidity changes (freeze and thaw cycles) should be minimized/avoided, as condensing water and its crystallization will promote diagenesis.

ANALYSIS IN ADELAIDE:

After arrival and unpacking in the lab, the surface of all samples will be UV-irradiated from all sides. Bone sections and tooth samples will be surface-sterilized using a shot-blaster or the surface will be removed mechanically using disposable cutting discs. Alternatively, samples are immersed in commercial bleach and rinsed with EtOH (NB Both approaches will not help if the contamination is deep inside the sample), and then sectioned and ground to fine powder using a Mikrodismembrator (tungsten carbide balls and stainless steel containers).

Alternative method for teeth samples, in case the sample should be returned roughly intact (e.g. for permanent display in a museum):

Drilling teeth: The tip of the tooth root is cut off with a thin diamond cutting disk, and the interior of the tooth root and dental cavity excavated with a dental drill (0.5mm) at low speed (100rpm). The resulting powder forms the sample for DNA extraction. Once the cavity is excavated, the root tip can be reattached with glue. At this point, there should be no sign (short of an X-ray) that the tooth has been removed or sampled, and crown wear patterns and tooth dimensions will be unaltered.

ANCIENT DNA ANALYSIS:

We will use cutting-edge molecular methods to test the authenticity of the results, but the critical issue is whether the sample is free of contamination before we receive it. We use PCR to amplify various mitochondrial (and nuclear if preservation allows) loci and clone the resulting products to look for contamination or sequence damage. Samples will be divided and DNA extracted and separately analysed from both subsets. An additional subset of the samples will also be independently tested in another aDNA laboratory (on case to case basis) for further quality assurance. (This critical element is often over-looked in aDNA studies). While we are working on new methods to promote the retrieval of short ancient fragments of DNA in favor of larger contaminating modern DNA, this issue will continue to dominate studies of ancient human specimens.

Any assistance you can provide in locating or sampling ancient specimens will be of great help to our work, and the overall success of the project as a whole.

Many thanks!!

QUICK SAMPLING GUIDE

1. Putting on gloves is not as easy as you think: Put on the first one by touching only its cuff, try to avoid touching its fingers. Take the 2nd one out of the box with the gloved hand and try not to touch its fingers with your (bare/ungloved) hand.
2. Put on a 2nd pair on top of these following the same procedure and change the outer one regularly between samples. Important: Avoid scratching your nose, rubbing your eyes etc.
3. Wear a medical face mask, or if possible/available wear a fresh full body suit or at least some hair cover/net.
4. Choose appropriate samples (see illustrations below 2 and 3).
5. Take a minimum of 2 samples per individual/specimen.
6. Put samples into sterile ziplock bags or tubes and label outside (!) and store as cool as possible. Take notes/details in your field book (transfer later into an electronic version).
7. Clean sampling area and tools used with commercial bleach and/or a detergent solution (commercial soap).
8. Change gloves between individuals.

SAMPLES CONSIDERED AS APPROPRIATE FOR ADNA ANALYSIS



Physical appearance, preservation:

Fresh-looking, „ivory gloss“, intact, smooth surfaces,
Compacta/cortical bone is dense and compact

SAMPLES CONSIDERED AS NOT APPROPRIATE FOR ADNA ANALYSIS



Physical appearance, preservation:

Broken, dull surfaces, evidence of mineral alterations
and/or microbial attack, compacta bone is white and „biscuit-like“ or very brittle