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Science and the Dead

A guideline for the destructive sampling of archaeological human remains for scientific analysis

Advisory Panel on the
Archaeology of Burials in England

Contents

Executive summary

Scientific analyses involving destruction of parts of bones and teeth from human remains are becoming increasingly widely used in archaeology. The more important techniques include radiocarbon dating; carbon and nitrogen stable isotope studies for shedding light on ancient diets; strontium and oxygen isotopic analyses to investigate geographical origins of people; DNA analysis for looking at genetic questions and for studying infectious diseases; and the cutting of histological sections to study changes due to age, disease and other factors. Institutions responsible for curating archaeological human remains, principally museums, archaeological field units and university departments, are increasingly receiving requests from researchers to sample remains in their care. Clergy and others responsible for historic churchyards and other burial grounds are also receiving an increasing number of requests from those wishing to exhume remains for research purposes. The aim of this document is to provide a framework which will help those organisations in responding to such applications. The remit of the document is English remains over 100 years old.

Following an introductory preamble, the legal framework pertaining to destructive sampling is set out, and some pertinent ethical considerations are discussed. There then follows a series of practical recommendations aimed at helping organisations in decision-making regarding requests for destructive sampling and, in cases where destructive sampling is permitted, ways in which it can be undertaken in order to minimise damage to remains. The science behind radiocarbon, isotopic, DNA and histological analyses is described, as are the likely nature of the bone or tooth samples required for each technique. Several case studies are given for illustrative purposes.

The main recommendations are as follows:

- In general, the benefit of generating new knowledge by the application of techniques that require destructive sampling needs to be weighed against the imperative of preservation of skeletal collections intact and, in the case of church burial grounds, the presumption of the church against disturbance of remains.
- When faced with a request for destructive sampling the following need to be assessed:
  - the likelihood of obtaining useful knowledge and the value of that knowledge;
  - whether that knowledge could be obtained by non-destructive analyses;
  - the experience and competence of those who intend to undertake the work;
  - the effects of the destructive analyses on the future research potential of the remains.
- Expert casework advice should be sought, if needed, from the Advisory Panel on the Archaeology of Burials in England (APABE) or other sources.
- For burials of known identity, permission should be sought from surviving family members, if known.
- If sampling is approved, it should be minimally destructive commensurate with the purposes of the research, and removal of any material should be properly documented.
- Decisions concerning destructive sampling should be made in the public interest and in an accountable manner.

1 Preamble

The scientific study of ancient human skeletons has traditionally relied upon measurement and visual examination of the remains. In recent years, scientific techniques involving destructive analysis of samples taken from bones or teeth have become increasingly important. The earliest of these laboratory analyses to find regular use in archaeology was radiocarbon dating, which started to become commonplace from the 1960s. More recently, analysis of stable isotopes from bones and teeth has provided new insights into the diets and the geographic origins of populations and individuals in the past. Study of ancient DNA has helped us investigate genetic relationships and has also enabled the study of the evolution of pathogenic bacteria from their DNA preserved in the bones of those who died from infectious disease. Microscopic study of cut sections of bone has helped the diagnosis of disease, and may provide a way of investigating activity patterns in past populations.

These newer techniques have not replaced traditional methods, but rather are used alongside them. The potential increase in knowledge that such techniques offer is clearly a positive development, but it has resulted in an increase in requests to museums and other institutions that curate human remains for work that involves destruction of samples from bones and teeth. In addition, clergy and others responsible for churchyards and other historic burial grounds are increasingly facing requests for exhumation of specific burials for research purposes. In these cases, the proposed work often involves 'forensic' study of a burial of a known historical figure, for example to confirm their identity, to assess their genetic relationship with living putative descendants, or to answer questions about their life or manner of death. Frequently, these requests involve work using destructive analyses of remains.

Some work on human remains, for example detailed case studies to illustrate a particular disease or work on remains of identified historic personages, may target a single skeleton. However, the focus of the scientific study of human remains as an academic discipline has largely shifted from works focusing on a single or a few 'interesting' skeletons towards problem-orientated studies with an emphasis on the identification of patterning in data at the population level. For research into archaeological questions and for methodological research directed at testing and improving scientific techniques, large numbers (more than 100) of skeletons are often needed. In addition, technological advances have begun to facilitate the processing of large numbers of samples for DNA and other analyses. Requests for sampling large numbers of skeletons from museum and other collections for destructive analysis have grown in recent years and are likely to continue to do so. Increasingly, the different techniques are used in conjunction (e.g. DNA and stable isotopic techniques for personal identification). Hence, there has also been an increase in requests for the application of multiple destructive techniques.

Some large organisations may have suitably trained staff to advise on the best response to a request for destructive sampling, and to formulate general policy, but for many this is not the case. The aim of this guidance is to provide non-specialists with responsibility for human remains with relevant scientific and other information on destructive analyses to aid them in their deliberations when they are faced with requests from researchers to obtain samples for destructive analysis from remains in their care. It is also hoped that the document may prove useful to museums and other organisations which curate human remains, such as archaeological fieldwork units and university departments, when formulating their own policies in this area. In addition, the reader is reminded that the Advisory Panel on the Archaeology of Burials in England (APABE) is available to provide specific casework advice on such matters (www.britarch.ac.uk/apabe).

Some of the discussion in this document may also be relevant to destructive sampling of other classes of archaeological remains, particularly faunal remains.

In keeping with APABE's remit, the scope of this document is restricted to remains over 100 years old (herein termed archaeological) from burial sites in England. The focus is on skeletal remains, as these are normally the only parts preserved in archaeological burials in England. Destructive analysis refers to removal of a bone, tooth, or parts thereof (herein termed sampling) for analyses which will result in the complete or partial destruction of the tissue removed. This guideline does not attempt to be comprehensive, but focuses on the more frequent types of analysis that require destructive sampling: radiocarbon dating, stable isotope work, DNA analyses and histological studies. Of course, those with responsibilities for human remains may be faced with requests for destructive sampling for purposes besides those discussed in this document. These include lead concentration and isotopic analyses for assessing human lead burden and sources of lead ingestion in the past; trace element analyses for investigating diet; and analysis of mycolic acids to look at infectious diseases, such as tuberculosis. Casework advice on destructive sampling for these or other techniques not covered in this guideline can be obtained from APABE.

The structure of this guideline is as follows: After a brief outline of the legal and ethical framework for destructive sampling of human remains, there are

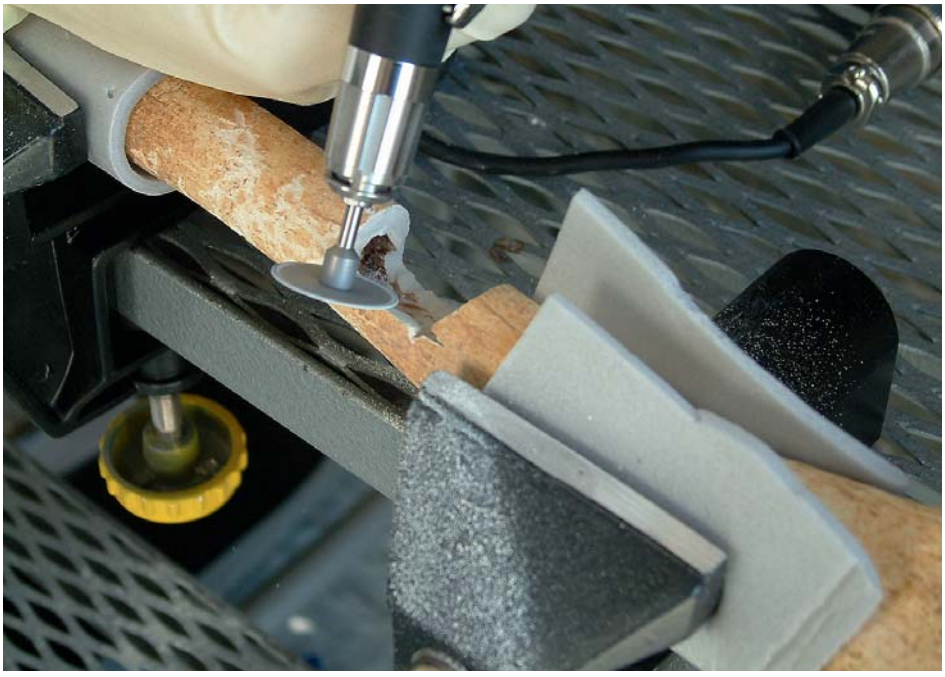


Fig 1 A sample being cut from a femur for radiocarbon dating. The bone is held in two small vices, padding being used to prevent damage to the specimen. The small rotary electric saw has been used to remove the sample.

sections devoted to the different techniques. These give a brief outline of the science, details of the sorts of things that can be learnt, and the nature of the samples that may be required.

This document draws upon the *Guidance for the Care of Human Remains in Museums* (http://webarchive.nationalarchives.gov.uk/+/http://www.culture.gov.uk/reference_library/publications/3720.aspx), published by the Department of Culture, Media and Sport. For Christian burials, it also draws upon the English Heritage/Church of England document 'Guidance for Best Practice for Treatment of Human Remains Excavated from Christian Burial Grounds in England' (http://www.english-heritage.org.uk/publications/human-remains-excavated-from-christian-burial-grounds-in-england/) particularly Section E6, and on Guidance Notes 1 and 2 from the Association of Diocesan & Cathedral Archaeologists (http://www.britarch.ac.uk/adca/projects-issues.html).

2 Overview of destructive sampling

2.1 Legal framework

In England it is unlawful to disturb buried human remains without lawful authority. Secular burial law is generally aimed at regulating the way in which human remains or grave markers are cleared from burial grounds. Permission to excavate archaeological burials is administered via the Ministry of Justice. The secular legal system recognises the public benefit of scientific work on human remains. Destructive sampling of collections of human remains excavated from archaeological sites and curated in museums or other institutions is not normally subject to legal constraint. It is generally the curating institution which grants (or withholds) permission for destructive sampling of remains in its care. However, in cases where permission for exhumation(s) is sought from the Ministry of Justice for the specific purpose of scientific research involving destructive sampling, the Ministry will evaluate carefully the proposals for destructive sampling when the application for the exhumation licence is considered. Different constraints apply to remains less than 100 years old, which are subject to the Human Tissue Act (2004). Such remains lie beyond the scope of this guideline, but in brief, under this Act it is an offence to hold human tissue less than 100 years old without a Human Tissue Authority Licence. In addition, consent of the person from whom the tissue came, or if they have died, from those close to them, for analysis of DNA is required. Analyses for the purposes of medical diagnosis or treatment and for criminal investigations are excluded.

In burial grounds under Church of England jurisdiction (mostly churchyards), ecclesiastical law, as well as secular statutes, applies. Human remains cannot be disturbed without ecclesiastical permission, usually issued in the form of a Faculty. The Church of England considers that human remains should be treated with reverence and respect, and ecclesiastical law is protective. It draws upon the principle that remains entrusted to the Church should normally lie undisturbed. This does not, however, mean that human remains should never be disturbed. Church law recognises that the living, including church congregations, have rights which may come into conflict with this principle. The Church also recognises that human remains, and the archaeological evidence for the rites that accompanied their burial, are important sources of scientific information and that this information is of legitimate academic and public interest. Analysis of human remains, including destructive analyses, is therefore potentially acceptable provided that the research aims are adequately justified and permission has been granted by the relevant authorities. Under the Church Faculty system, as well as authorising exhumation of burials, the Consistory Court also regulates their treatment once exhumed, and therefore has the authority to grant or withhold permission for destructive sampling. Proposals to remove and/or destroy parts of skeletons are subject to rigorous scrutiny. This is particularly so when the personal identity of the individual is known and sensitivities are consequently heightened.

2.2 Ethical considerations

A number of ethical considerations need to be borne in mind when considering applications for destructive sampling. Some of these fall under the general rubric of knowledge-based ethics. Analysis of human remains offers important insights into the human past. Their study may also help to test and improve existing methods of skeletal analysis, and to develop new techniques that are useful both for archaeology and forensic science. Most would consider that the accrual of knowledge is a significant benefit for humanity. A museum or other institution holding archaeological remains for research purposes may be considered to have stewardship of that material. That is, they hold it in trust for the benefit of the wider community and for the benefit of future generations. There is, therefore, a moral imperative towards the preservation of collections in ways which safeguard the information they contain. When it comes to destructive sampling there is a tension between the imperative to generate new knowledge and the imperative towards preserving collections intact. This dilemma lies at the heart of decisions concerning destructive sampling. In addition to the above considerations, requests to the clergy for exhumations of skeletal remains from churchyards for research purposes will also need to be weighed against the

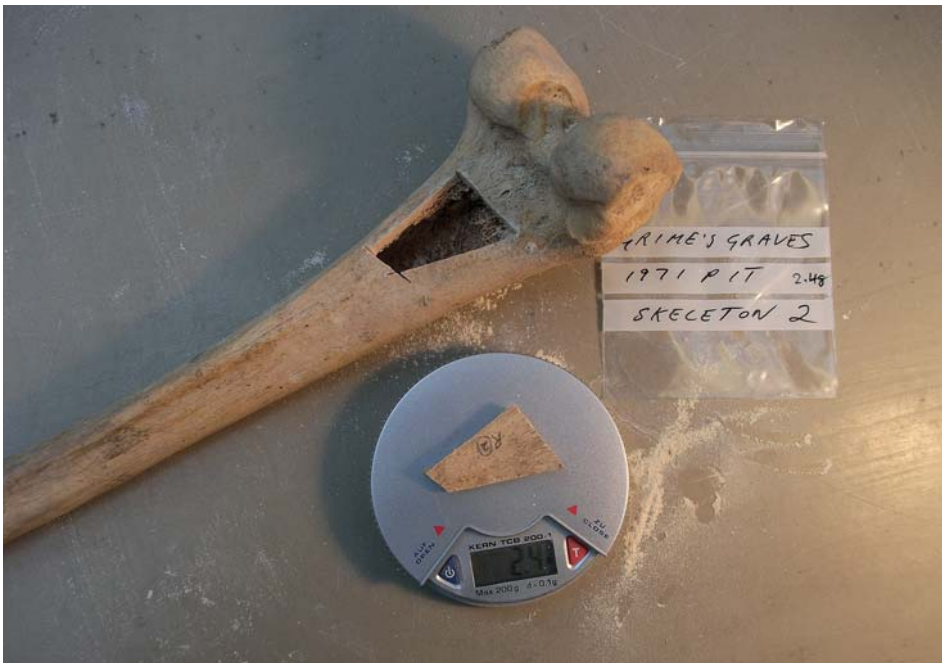


Fig 2 Four intersecting cuts from an electric rotary saw, like that illustrated in Fig 1, have been used to remove a quadrangle of bone from a femur for radiocarbon dating.

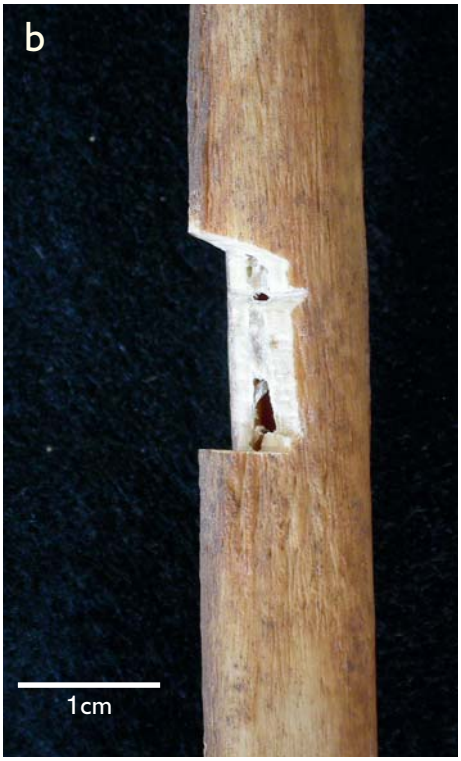


Fig 3 (a, b) A fibula (one of the lower leg bones). A sample for carbon and nitrogen stable isotope analysis has been removed from the shaft of the bone. Despite the bone's slender shaft, removal of the sample has been achieved without cutting completely through the bone, preserving it intact for measurement. The sample was removed using a small hand saw with a round-section 360° cutting blade. The lower part of this bone is thickened due to disease. The sample was taken from the upper part to avoid damaging this area.

Church's presumption against disturbance of remains.

Targeting the remains of historic personages or other known individuals may raise additional concerns. On the one hand, study of known-identity burials may have important scientific benefits – for example, known age and sex skeletons are essential for testing methods of estimating age and determining sex from skeletal remains. However, in other cases, results may be of a sensitive nature that could offend or embarrass living descendants, and they may raise complex social or political sensitivities. For example, work on DNA was conducted using remains from the American President Thomas Jefferson to attempt to address the question of whether he had fathered a child by his female slave, Sally Hemmings. The results were inconclusive. In this case, as in some others, associated social and political agendas, and sensationalist reporting in the media, overshadowed the scientific aspects of the work. In cases such as this, the question that must be addressed is whether the project is in the wider public interest and whether this is sufficient to override any wishes that an individual, family or community may have to privacy.

2.3 General considerations regarding destructive sampling

From the above discussion can be drawn a set of general points to be considered when assessing applications for destructive analysis, either from remains curated in museums or other institutions, or in the form of requests that include exhumation of selected remains from churches or churchyards.

- Are the questions to be addressed by the work of general archaeological, historical or other significance?
- If so, can they be addressed adequately using non-destructive techniques? Only if this is not the case should destructive analyses be contemplated.
- Are the researchers sufficiently competent and experienced to conduct the work proposed?
- Any programme of destructive analysis should be carried out within a coherent research programme and should stand a realistic chance of advancing knowledge.
- If the feasibility of a technique is questionable, then thought should be given to conducting a pilot study on a small number of samples, before permission is given for a fuller programme entailing destruction of larger numbers of samples.
- For work involving the exhumation of identified individuals, permission should be sought from surviving family members, if known.
- Only the quantity of material needed to address the research questions should be removed, and the number, location on the skeleton, and size of samples that the researchers intend to remove should be made explicit. It should also be remembered that as methods are refined the general trend has been towards requirement for smaller samples, and this trend is likely to continue.
- The likely effect of sampling on future research potential of the remains is a key issue. To this end, the location in the skeleton from which a sample is to be taken should be carefully considered:
  - Sampling from anatomical landmarks (points from which measurements are taken) or from areas important for sex or age determination should be avoided.
  - Unless the study specifically requires it, sampling from diseased bone should be avoided.

- Teeth are particularly rich in biological information and should not be sampled unless the required data could not be acquired from sampling bone. If a tooth is to be sampled, then its antimere (the corresponding tooth from the opposite side of the jaw) should preferably be present.
- Samples should preferably be taken from bones or teeth that are already incomplete, damaged or fragmentary.
- In the past, chemical consolidants may have been applied to archaeological bone to try and strengthen it. This may cause problems for some scientific analyses, so sampling such areas is best avoided
- If appropriate, thought should be given to the visual impact of sampling – for example on the suitability of the specimen for future museum display.
- All sampling should be fully documented, so that future researchers can see what has been taken.
- Any unused samples should be returned as they may obviate the need for future sampling.
- The skeletal element that is to be sampled should be fully recorded and measured prior to sampling. In some cases it may be appropriate to produce a cast of the parts to be destroyed or to conduct a surface laser scan or CT scan so that a virtual replica can be produced. Unless exhumation is for the specific purpose of research involving destructive analysis, sampling should not normally be permitted on site during excavation.
- Publications and, if appropriate, the raw data arising from the scientific analyses should be lodged with the organisation which granted access to the remains.
- Casework advice should be sought from APABE or other sources as necessary to aid decision-making
- Decisions concerning permissions for destructive sampling should be made in the public interest. Those making decisions should be willing to be held accountable for their judgements.

Fig 4 (a, b) A disc of bone has been removed for trace element analysis from this femur using a 15mm diameter plug-cutting drill. The shaft of a limb bone takes the form of a cylinder of bone surrounding a cavity which, in life, contains the marrow. The plug-cutter has removed a disc from one wall of the cylinder. The bone is broken towards its lower end. Further damage to this area was avoided during sampling as the two sides of the break fit back together to form a complete specimen for measurement.

3 Radiocarbon dating

3.1 The science

Isotopes are atoms of a chemical element with different masses. Some are radioactive and steadily decay, transmuting into other elements. Others are stable – they are non-radioactive and do not change in abundance over time. Carbon has three naturally occurring isotopes: ^{12}C, ^{13}C and ^{14}C. These three isotopes do not occur equally, with carbon in the atmosphere and biosphere consisting of 99% ^{12}C, 1% ^{13}C and about one part in a million million of ^{14}C. ^{12}C and ^{13}C are stable isotopes. ^{14}C is radioactive with a half-life of 5730 ± 40 years. From this it derives its name *radiocarbon*.

Radiocarbon is formed in the upper atmosphere by the interaction of neutrons, produced by cosmic rays, with nitrogen atoms. Once radiocarbon has been produced it rapidly forms carbon dioxide and mixes through the atmosphere, dissolves in the ocean, and enters the terrestrial food chain through photo-synthesis. Consequently, the ^{14}C content of a living terrestrial organism is in equilibrium with that of the contemporary atmosphere.

When a plant, human or animal dies it no longer takes in ^{14}C and thus over time the proportion of radiocarbon falls at a rate that is determined by the law of radioactive decay. By measuring the proportion of ^{14}C that remains, it is possible to estimate the time since the organism died.

Unfortunately, as the production of radiocarbon in the atmosphere is not constant, a year in the radiocarbon age timescale does not have an equivalent interval in the calendar timescale and for this reason calibration is required. Progress in the extent and resolution of the data available for calibration means that the current internationally agreed calibration curve extends to 50,000 years before present. This provides a common standard and means that all calibrated dates are comparable.

Radiocarbon is present in such low abundance it puts a statistical limit on the precision of a radiocarbon determination. A fundamental aim during measurement is therefore to measure the isotope ratio as accurately and precisely as possible. The two main methods of measuring ^{14}C are decay counting methods (using liquid scintillation and gas proportional counters)

and accelerator mass spectrometry (AMS) where the radiocarbon atoms are directly detected. Since the mid-1980s the introduction of accelerators for the direct detection of radiocarbon has allowed a whole range of much smaller samples to be measured.

Bones and teeth are some of the most complex materials commonly used in radiocarbon dating. Bone, and dentine in teeth, are composed of a mineral part (hydroxyapatite, a form of calcium phosphate) and an organic part, chiefly composed of the protein collagen. It is normally carbon from the organic part that is used for radiocarbon dating. Following the death of an individual, degradation of a bone's molecular structure and the incorporation of exogenous molecules as a result of chemical and environmental processes can influence subsequent radiocarbon measurements. Research into effective pretreatment methods continues, with the aim of reducing the contaminants present in the sample from the environment and to minimise the addition of further contaminants. With human bone, attempts to improve on the widely used simple extraction of protein (collagen) have included molecular-size selection using ultrafiltration and the selection of individual amino acids.

Bones that have undergone thorough burning at high temperatures (i.e. cremation) no longer contain organic carbon and so until recently have not been suitable for radiocarbon dating. However, in the last decade the successful dating of the mineral part of burnt bone has opened up the possibility of dating cremated deposits.

Humans have a markedly variable and mixed diet and as such frequently derive carbon from more than one reservoir. The measurement of carbon and nitrogen stable isotope ratios (see Section 4) can be used to determine the potential for diet-induced radiocarbon offsets if an individual has taken up carbon from a reservoir not in equilibrium with the terrestrial biosphere, for example, marine or carbonate-rich freshwater resources. For technical reasons, this issue affects radiocarbon measurements on unburnt bone, but not those on cremated bone. In practice, dietary effects have not been found to be significant for interpreting radiocarbon dates on human bone from England before the Viking period.

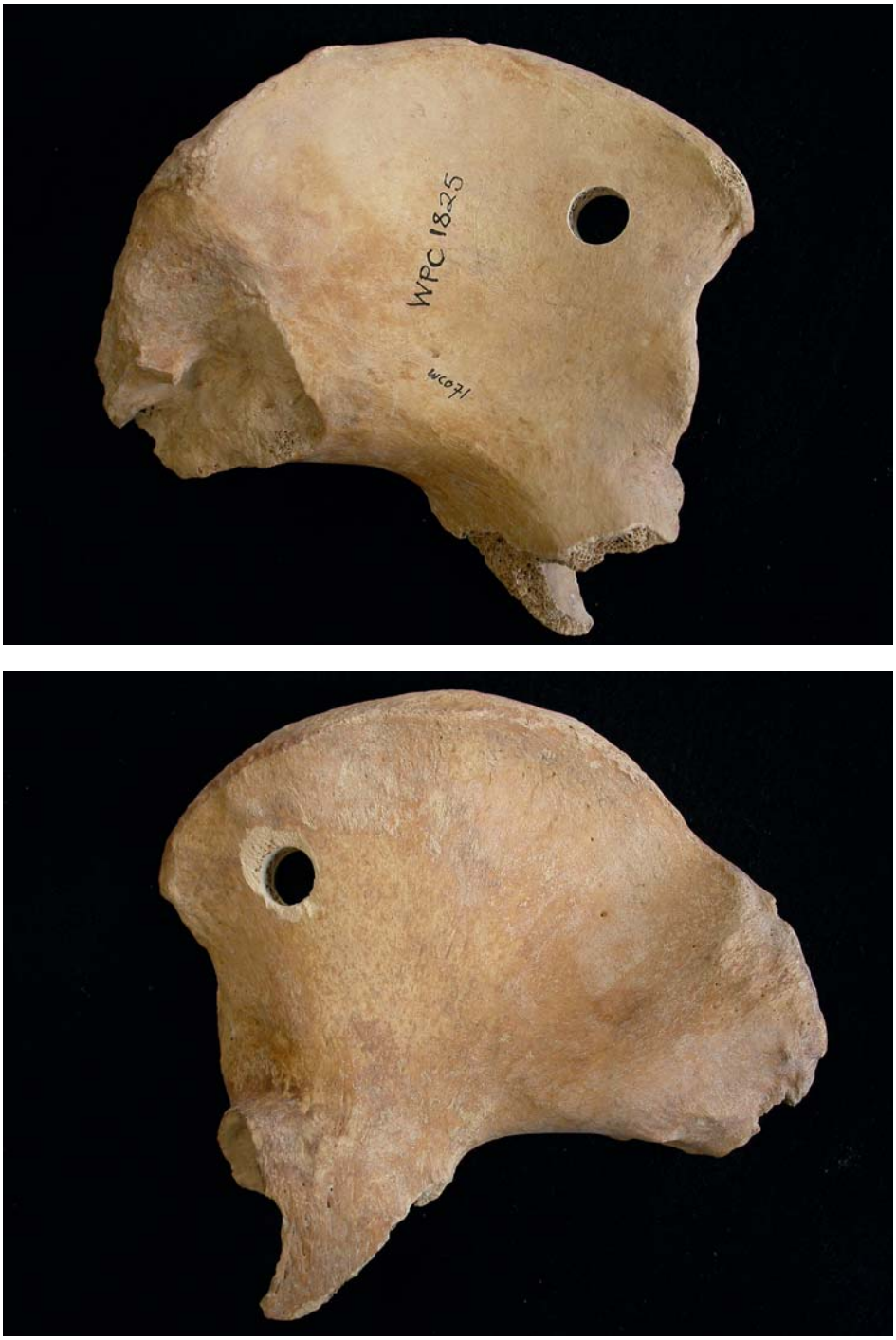


Fig 5 (a, b) Part of a left hip bone. A 10mm-diameter disc of bone has been removed by a plug-cutting drill for the purposes of a histological study. Unlike limb bones, such as the femur in Fig 4, hip bones lack a marrow cavity, so it has been necessary to drill right through the bone to remove the sample. The side from which the drill entered the bone (a) shows a hole with clean edges, but on the other side (b) there has been some splintering of bone around the hole. This was caused as the drill broke through. It is difficult to prevent this sort of damage entirely because the irregular shape of a bone makes it impossible adequately to support the reverse side as it is drilled, but it can be reduced if the pressure on the drill is minimised as the hole nears completion.

3.2 What can we learn from radiocarbon dating?

Chronology provides a fundamental structure for understanding the past, with timing unravelling the sequence of past events and the tempo of change. Increasingly refined chronological frameworks from burial grounds are enhancing understanding and appreciation of the value of such burials, particularly when combined with other investigations such as stable isotope analysis.

The ability to chronologically divide the human population of cemeteries such as St Mary Spital, London (10,516 mainly medieval burials) using radiocarbon dating and archaeological phasing means that it is possible to track developments in demographic change and variations in health of the population that lived and died in this part of London. The radiocarbon dating programme also identified cases of pre-Columbian syphilis and mass burial pits predating the great Black Death outbreak of AD 1348.

Dating of the human maxilla from Kent's Cavern, Torquay, one of the most important Palaeolithic sites in the country, sheds light on the origins of the earliest anatomically modern humans in Europe. Dating of a selection of the 50 or more

Fig 6 A right femur from which multiple samples have been removed for various purposes over the years. The midshaft and the subtrochanteric areas (indicated by the two dotted lines) have been avoided as these are important landmarks for measurements and other osteological observations. This burial also preserved a left femur, and in this case a decision was taken to keep that bone fully intact and to repeatedly sample this bone. When several samples are removed from one bone, care should be taken that the structural integrity of the bone is not unduly compromised. In this case the femur was strong and well-preserved, so it was thought that the removal of samples would not lead to significant risk of inadvertent breakage during handling.

bodies once present in Aveline's Hole, Burrington Combe, Somerset, confirmed the site as one of the largest early Mesolithic burial sites in Europe. The results suggest use of the cave for burial over, at the most, a century or two, in the mid to late ninth millennium cal BC.

When considering individual radiocarbon dates, it must be remembered that the bandwidth of calibrated radiocarbon dates is not only a function of the errors quoted on radiocarbon ages and on the calibration data, but also on the shape of the calibration curve. Thus for some time periods the bandwidth is relatively large, for example *c* 750–400 cal BC for a person who actually died in 500 BC, i.e. where the actual ages fall on a 'plateau'. It can also be relatively precise: cal AD 1410–1470 for a person who died in AD 1425, where it falls on a 'steep' section of the calibration curve.

In the last decade the use of a Bayesian statistical approach has proved to be the most effective method available for producing estimates of chronology. In archaeological terms this means that we analyse new data that we have collected about a problem (the 'standardised likelihoods' – radiocarbon dates) in the context of our existing experience and knowledge about that problem (our 'prior beliefs' – for example, the stratigraphic relationship between graves). This allows us to arrive at a new understanding of chronology which incorporates both our existing understanding of the problem and our new data ('posterior belief'). Bayesian modelling of radiocarbon dates from southern English long barrows has provided chronologies at a generational scale for these early Neolithic mortuary structures.

3.3 Sampling for radiocarbon dating

The most common human remains submitted for dating are unburnt bones from which typically 1g of bone is needed for AMS dating and about 200g for liquid scintillation and gas proportional counters. Samples from the larger dense bones of the body (femur, tibia, humerus or mandible) are preferred. Sampling of complete unburnt bones for AMS dating is usually undertaken with a mechanical drilling kit.

The preservation of unburnt bone can be greatly influenced by the burial environment, resulting in chemical and physical degradation. Over 90% of the collagen content can be lost in some environments, which restricts the potential for radiocarbon and stable isotope analysis. A rapid technique, determining the %N content of whole bone, which requires very little material (< 5mg bone), has been shown to be very successful in predicting whether a bone is suitable for dating. This prescreening method reduces the amount of destructive sampling, in addition to saving time and money spent on unsuccessful dating.

For teeth, the preferred samples are incisors, canines and molars, and attempts should be made where possible to leave enamel in good condition for other analyses (e.g. strontium and oxygen isotopes – see Section 5). For cremated bone, a 2g sample that needs to be fully calcined (i.e. completely white or grey) not just charred is required. In exceptional circumstances other material suitable for dating may also be preserved, e.g. hair, skin and other soft tissue. For large human bone assemblages, the use of Bayesian simulation models to identify the minimum number of samples needed to provide meaningful answers has proved especially valuable.

4 Stable isotopes and ancient diets

4.1 The science

Carbon and nitrogen each have two stable isotopes. Ratios of the two stable isotopes in each of these elements are different in different classes of foods. These differences are passed on to the tissues of the consumer. Hence carbon and nitrogen stable isotopes can be used to study ancient diets.

Carbon stable isotope ratios differ in plants using different photosynthetic pathways to manufacture carbohydrates from atmospheric carbon dioxide. Most temperate zone vegetation uses the so-called C3 pathway. Some plants from warmer regions use the C4 pathway. In addition, both carbon and nitrogen stable isotope ratios differ in marine and terrestrial foods. In north-west Europe there are no indigenous C4 foods, so most stable isotope work has concentrated on studying marine contributions to diets. For nitrogen isotopes, there is a small trophic-level effect, so ratios change as one ascends a food chain. In principle, this means that it may be possible to say something about the relative importance of meat versus plant foods, but in practice interpretation in these terms is often difficult because a number of non-dietary factors also seem to exert (fairly minor) influences on nitrogen isotope ratios in bone. Because breastfeeding infants are exclusively consuming a product of the mother's body, they are one trophic level higher. Nitrogen isotope ratios have been used to study the duration of breastfeeding in past societies.

Intact collagen preserves lifetime carbon and nitrogen stable isotope ratios,

and this is the part of the bone usually analysed in dietary studies. In living people, collagen in bone is continually renewed. During infancy this process is rapid, but by adulthood it slows down, so that analysing adult bone collagen gives a measure of diet averaged over years or decades. Collagen in dentine from the teeth is not renewed like that in bone, so analysing this material gives indications of diet whilst the dentine was forming as the tooth developed during childhood. All the nitrogen in collagen, and most of the carbon, comes from dietary protein, so results tell us mainly about the protein part of the diet. Some workers also advocate analysing carbon stable isotopes from bone mineral (there is no nitrogen in the mineral part of bone). This has the advantage that results appear to reflect whole diet rather than being biased towards protein, but a drawback is that bone mineral is very vulnerable to alteration of its chemical and isotopic composition in the soil. Methods have been developed to wash out extraneous carbon which has contaminated the bone mineral from the soil, but their effectiveness is unclear.

The great majority of dietary work involving stable isotopes uses carbon and nitrogen, but some other elements are also beginning to attract attention. Hydrogen stable isotope ratios in bone collagen may prove a useful indicator of the meat: vegetable ratio of the diet. Depending on local geology, sulphur stable isotopes may be different in terrestrial and freshwater foods, so sulphur isotope ratios in bone collagen may also have dietary potential.

In the laboratory, tests are done to identify whether a sample contains intact collagen. Usually, bone and tooth samples from burials on English archaeological sites contain sufficient intact collagen for successful stable isotope determinations, so stable isotope work normally produces usable results, but in cases where collagen survival is thought to be doubtful, measurement of nitrogen content, as described in Section 3.3, can be used as a pre-screening technique. Dietary information cannot be obtained from isotopic analysis of cremated bone. To analyse stable isotopes in collagen, the collagen is extracted from the bone or dentine in the laboratory and then purified. This material is then burnt and the resulting gases analysed using an isotope-ratio mass spectrometer. This measures the relative abundance of the different isotopes present.

4.2 What can we learn about diet from stable isotope analysis?

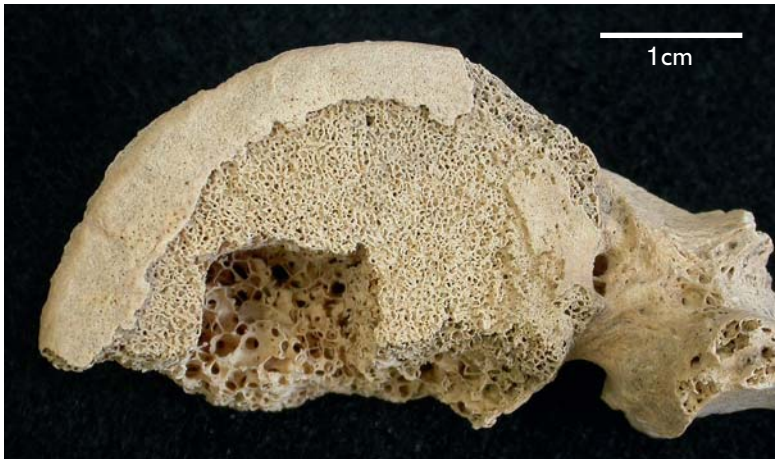
To study diet, carbon and nitrogen stable isotopes are usually used together. Most work attempts to address questions of broad archaeological or historical interest, so multiple skeletons rather than single burials are normally the focus. Currently, most studies use anything from about 30 to more than 100 skeletons, often from several archaeological sites, depending upon the questions to be investigated.

A number of studies have looked at the way in which diet changed with the advent of farming in the Neolithic period. Results show that prior to the Neolithic, coastal groups in Britain relied heavily on sea foods, but these resources were abandoned with the introduction of farming. In Britain, this change in diet seems to have occurred abruptly rather than gradually, unlike in some other parts of Europe where marine foods continued to be exploited in significant quantities into the Neolithic. Other work has focused on how a person's social position influenced their diet, and how diet varied with geographical location. At Roman Dorchester, isotopic work indicated that the wealthier members of society consumed more marine foods. Stable isotope work on skeletons from communities near the coast and from a large inland town showed that in late medieval times (11th–16th century AD) the inhabitants consumed more marine foods than did individuals from a small inland rural community. This is consistent with other evidence in suggesting that there was a developed sea fishing industry in the late medieval period, with trade in salted fish to inland commercial centres, but that fish may not have been very important in rural diets. By contrast, analysis of skeletal material from the early medieval period (5th–7th century AD) showed that little seafood was consumed, even by coastal communities.

Breastfeeding practices have received increasing archaeological attention, as they are important determinants of family size and of maternal and infant health in

Fig 7 A fourth lumbar vertebra. A slice about 0.5cm in width has been removed as part of a histological study of osteoporosis. Although the study produced valuable data, the extent of the destruction involved has compromised subsequent work. For example, bone density measurement (another way of studying osteoporosis) involving this vertebra is not now possible. Measurement studies are also compromised. For example, this individual had to be omitted from a study investigating how the shape of vertebrae influenced the occurrence of stress fractures of the lumbar spine in this population.

Fig 8 This already incomplete vertebra has been sampled for DNA. A cube about 1 cm^3 has been cut from the spongy bone using a scalpel. Two of the other vertebrae from this individual showed abscessing, apparently due to tuberculosis, and aims of the DNA work were to try to confirm this diagnosis and to attempt to determine whether the disease was bovine tuberculosis contracted from cattle or whether it was the form of the disease transmitted person to person. To have sampled the diseased vertebrae would have destroyed significant parts of the bony lesions, so a decision was taken to sample from an undiseased vertebra even though this may have reduced the chances of detecting pathogen DNA. In fact, the DNA amplifications were successful. They confirmed the diagnosis and indicated that the person had the form of the disease contracted from other people rather than from infected cattle or dairy products.



premodern communities. This can be investigated with nitrogen isotope analysis, either using bone samples from infants and children of different ages, or using adult skeletons, sampling from parts of teeth that were developing at different times during childhood. At a British medieval site, nitrogen isotope data suggested that breastfeeding was continued until children were about 18 months old. This prolonged period of breastfeeding seems to have had beneficial results: infant mortality in that community appeared low by premodern standards.

4.3 Sampling for carbon and nitrogen stable isotope work

Typically, less than 0.5g of bone is needed for carbon and nitrogen stable isotope determinations. For adults this is normally taken from a long-bone shaft. Collagen in these locations is renewed more slowly than in other bones such as ribs, and so provides a good overall indication of long-term diet. When bone samples are used to assess age of weaning in infants and children, typically bones with more rapid turnover (those that are rich in spongy bone, e.g. ribs) are sampled so that the delay with which the weaning signal is manifest in bone is minimised. Archaeological bones are often fragmentary. If an appropriately sized bone fragment is present then this is normally used for analysis. Otherwise a small saw is used to cut a piece of bone of suitable size. Care is taken not to cut completely through an intact or minimally damaged bone. One way of doing this is to make two closely placed parallel saw cuts which pass less than halfway through the bone; the saw can then be twisted to break the slice of bone free.

Increasingly, breastfeeding studies are using micro-sampling of dentine from teeth from adult skeletons. Recent technical developments mean that many samples of tissue which developed at different ages may be obtained from a single tooth, but doing this involves cutting the tooth vertically, partially destroying it.

As mentioned in Section 3.1, carbon and nitrogen stable isotope determinations are routinely conducted on bone samples submitted for radiocarbon dating. This is because it is important to detect individuals who consumed significant amounts of marine foods, as incorporation of marine carbon into bone collagen tends to make radiocarbon dates too old, and a correction is needed for this. Although carbon stable isotope measurements made on AMS machines and used to correct for fractionation as part of radiocarbon dating cannot be used in dietary studies, the carbon and nitrogen stable isotope measurements routinely reported along with radiocarbon ages on dating certificates can be. This is because they are measured on an isotope-ratio mass spectrometer. When both radiocarbon dating and dietary studies are envisaged, with careful planning destruction of material can be kept to a minimum.

5 Strontium and oxygen stable isotope ratios and geographical origins of people in the past

5.1 The science

Strontium isotope ratios vary in different types of rock. There are therefore systematic differences in plants and animals in areas with different geology, and these are passed on to the tissues of consumers. Oxygen isotopes vary in rainwater in different regions according to factors which include climate, altitude and distance from the coast. Oxygen isotope ratios vary in different living organisms, and hence in different foods, but this does not matter very much for human studies as the isotopic composition of drinking water is the prime determinant of the oxygen isotopic composition of human tissues (an exception is suckling infants – during breastfeeding oxygen isotope ratios are altered).

Unlike most carbon and nitrogen stable isotope work, strontium and oxygen isotope analyses use the mineral part of skeletal tissues and not collagen. As mentioned above, the mineral part of bone and dentine appears very vulnerable to changes in composition during burial, but dental enamel appears highly resistant to alteration. Therefore, most strontium and oxygen work on human remains uses dental enamel. Unlike bone, dental tissues are not continually renewed, so the isotopic composition of dental enamel reflects the locale in which the person lived as a child when the enamel was forming. A local baseline for oxygen or strontium values in the location in which the individual was buried (and by implication lived immediately prior to death) can be established from geological or rainfall maps or, better still, from analysis of local archaeological remains of domestic animals not likely to have roamed far. If the isotopic composition of dental enamel differs from this baseline, then the person likely spent at least part of their childhood elsewhere.

Oxygen isotope ratios in waters in Britain overlap with those in other locations, for example, in continental Europe, particularly north-western areas and parts of the Mediterranean basin. Strontium isotopes will be similar in regions of similar geology regardless of geographic separation. Oxygen isotopes are generally most useful for distinguishing among individuals on a fairly large spatial scale; strontium isotopes may, depending on geology, enable smaller spatial distinctions to be made. In practice, most workers use both strontium and oxygen isotopes in conjunction in order to narrow down the number of possible locations where a person may have spent their childhood. Nevertheless, isotopic determinations are most suitable for excluding certain locations as places of childhood residence, and would not without other evidence allow a location to be identified unambiguously.

5.2 What can we learn about mobility from stable isotope studies?

In archaeology, applications of strontium and oxygen stable isotope analysis may entail studies of one or a few burials. These normally involve those that are unusual in some way, for example, regarding where or how the deceased were buried, or they may have been buried with grave goods suggesting a non-local origin. However, increasingly, studies involving greater numbers of burials are being undertaken in order to address broader questions.

For example, an unusual prehistoric mass grave containing seven individuals (three adult males, a teenager and three children) was found recently near Stonehenge. The remains date to the later third millennium cal BC. Oxygen and strontium stable isotope determinations from the teeth of the adults showed that they had originated from outside the local area. Wales was one possibility compatible with the isotope results. An initial suggestion was the Preseli Hills, the likely origin of the bluestones used in the building of Stonehenge. This could show a fascinating connection, but this was only one possibility for the place of origin of these people; others included western England, Brittany and Portugal.

At the Roman Fort at Catterick, North Yorkshire, burials dating from the 2nd–3rd centuries AD showed greater isotopic diversity than burials from the 4th century. This seemed consistent with the idea that in the Roman army an early policy of more diverse recruitment was later supplanted by greater recruitment from the local population.

Turning to a later period, in the early medieval (5th–6th century AD) cemetery at West Heslerton, North Yorkshire, isotopic analyses suggested that about one-sixth of the burials were of first-generation migrants, perhaps originating from Scandinavia.

At Whithorn cathedral priory, Scotland, oxygen and strontium analysis of burials of high-ranking late medieval (11th–14th century AD) clergy suggested that they originated outside the local area, unlike lower status and lay individuals who seemed to have spent their childhoods locally. That these important positions were not held by locals was consistent with documentary sources which suggested that outside political interests held sway in the appointment of clerics to senior positions at Whithorn. Appointees were often senior men from important Scottish monasteries or clerks in the households of the king or nobility.

5.3 Sampling for strontium and oxygen isotope analyses

Strontium and oxygen isotopic analyses normally require dental enamel, not bone. About 50mg of material is needed, and sampling typically partially or wholly destroys the enamel crown of a tooth. Some studies may require analysis of enamel from more than one tooth from each individual sampled – different teeth develop at different times during infancy and childhood so analysing more than one tooth enables more detailed work to be done on childhood residence. Because of the effect of breastfeeding on infant oxygen isotope ratios, the parts of tooth crowns that formed in infancy are best avoided in work studying mobility, but these parts can be sampled as another way of looking at breastfeeding practices. Cremation normally destroys tooth crowns so isotopic studies cannot be performed.

6 DNA

6.1 The science

DNA is the molecule that contains the genetic information necessary for living organisms to develop, function and reproduce. It is present in two locations in human cells. Most DNA is located in the chromosomes in the nucleus of the cell. Some also lies in the mitochondria, the cell's energy-generating units. Most chromosomal or nuclear DNA is inherited from both parents; mitochondrial DNA is inherited solely from the mother. Mitochondrial DNA is present in multiple copies – each cell contains about 8,000 identical copies of mitochondrial DNA but only one set of chromosomes.

When an organism dies, the long DNA molecules rapidly degrade leaving only small amounts of DNA composed of short fragments. In the 1980s it became clear that these fragments of DNA could be detected and analysed in ancient human and other biological remains. Because there is so little of it, ancient DNA (aDNA) normally needs to be amplified into sufficient quantities before it can be studied. The usual way of doing this is with a technique called polymerase chain reaction (PCR), which is a way of generating many copies of a selected region of a DNA fragment. A particular DNA sequence of interest in mitochondrial DNA is more likely to survive the ravages of time than a sequence in nuclear DNA simply because there were more copies to start with. Therefore, much aDNA work uses mitochondrial DNA. In human remains, both human DNA and DNA from infecting micro-organisms that were present at time of death have been successfully studied.

Much aDNA work fails to produce results. This may be due to poor survival of DNA in the remains in question or for other reasons. Success rates in published aDNA studies vary widely, and doubtless many of the less successful works fail to reach the publication stage. Predicting in advance whether aDNA work is likely to be successful is very difficult. Factors that favour aDNA survival include a cool, dry burial environment and relatively recent date (i.e. an age in centuries rather than millennia). Acidic and free-draining soils may destroy not only the DNA but even the skeletal remains themselves. Cremated bone is not generally suitable for DNA studies.

6.2 What can we learn from aDNA?

Generally speaking, aDNA work can shed light on genetic relationships between people or populations, and can help us to determine the sex of a person when it is not possible from the bones. It can also help to identify and study infectious diseases in the past.

With regard to broad genetic questions about the history of human populations, recent work on DNA from Neanderthal skeletal remains suggests that up to about 4% of DNA in modern Europeans may come from Neanderthals, implying that a small amount of inter-breeding did occur between Neanderthals and early modern humans, but this is controversial. Study of Mesolithic and Neolithic human DNA suggests that some of the first Neolithic farming groups in Europe share affinities with modern south-west Asian populations, and genetic discontinuities have been found between Mesolithic hunter-gatherer and Neolithic farming groups. These results seem consistent with other evidence in suggesting the spread of farming to Europe from a centre in the Near East was not just

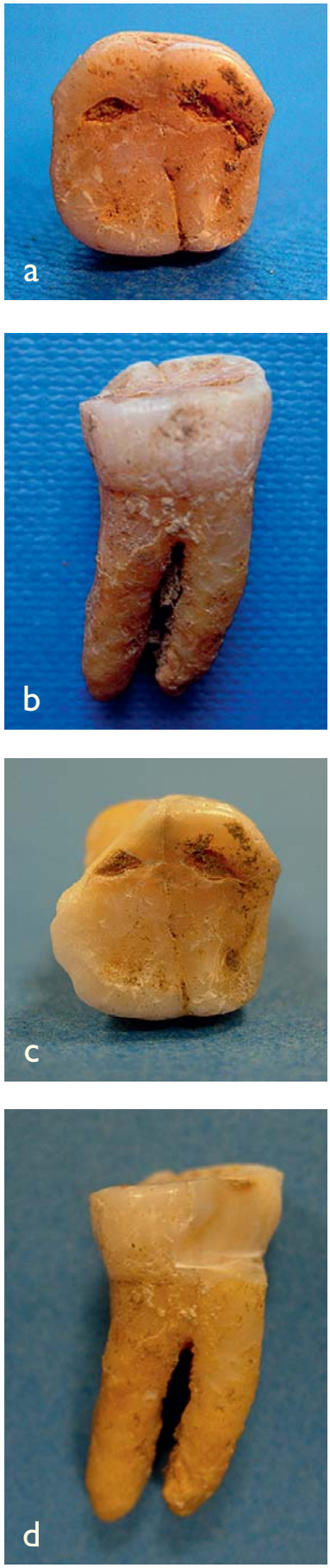


Fig 9 A molar tooth before **(a, b)** and after **(c, d)** removal of part of the crown for isotopic analysis. Although some measurement and other studies of crown morphology will now not be possible on this specimen, much of the crown has been preserved intact.

a transmission of ideas but involved at least some migration of people.

Individual relationships and patterns of kinship can sometimes be discerned, providing insights into the social organisation of early populations. A direct child-parent relationship was suggested in a 4,600-year-old grave containing two adults and two children from Germany, providing the oldest evidence of a nuclear family.

Much analysis of modern DNA is concerned with issues of personal identification and relationships in a legal setting. In modern forensic cases, personal identification can normally be established with some certainty. Forensic work generally relies on matching multiple sequences of nuclear DNA with a close relative. However, this approach, and the high degree of certainty associated with it, is not usually feasible with aDNA due to its degraded nature. Nevertheless, questions of identity and genetic relationships of identified historic individuals can potentially be investigated using aDNA extracted from their remains. Comparisons of DNA extracted from ancient bones with the DNA of known or putative close relatives (living or dead) may be used to confirm the identity of the deceased, or to refute or support individuals who claim a biological relationship to the deceased. However, attempts often fail to provide conclusive results. This may reflect problems with the amplification of the aDNA due to poor DNA survival or for other reasons. In addition, DNA from a relative via the direct male or female line from the deceased or his/her siblings is normally needed to establish good evidence for descent, and hence for the personal identity of the buried individual. It is normally difficult to trace such lines over many generations, and breaks in descent lines often occurred in the past due to adoption or illegitimacy.

In most cases, we can determine the sex of adults by looking at the bones, but if the diagnostic parts are missing or damaged this may not be possible. In addition, sex identification is difficult in infants or children. Therefore, here are potential uses of aDNA. Among Romano-British populations, infanticide (killing of newborn children) seems to have been commonly practiced to limit family size. Some have postulated people in Roman times preferred male children, so more female infants may have been killed. However, aDNA analysis of infant bones from Roman sites in Britain provided no support for this, boys and girls being present in similar numbers.

Study of the DNA of pathogenic bacteria present in human remains can aid the study of disease in earlier times. For example, some Iron Age skeletons recovered from a cemetery in Siberia showed spinal abscesses that seemed to be due to tuberculosis. Study of pathogen DNA from the burials confirmed this and, furthermore, indicated that the disease was the type transmitted from animals (probably cattle) rather than caught from other people. Conversely, work on remains from a medieval English village found that the skeletal tuberculosis cases were exclusively of the type transmitted person-to-person. Recent work has confirmed that the Black Death in medieval Europe was caused by the plague bacillus *Yersinia pestis*, which some had doubted. It also revealed that it was caused by a variant of the bacterium which apparently no longer exists. This may help explain apparent differences in virulence between ancient and modern *Y. pestis* infections.

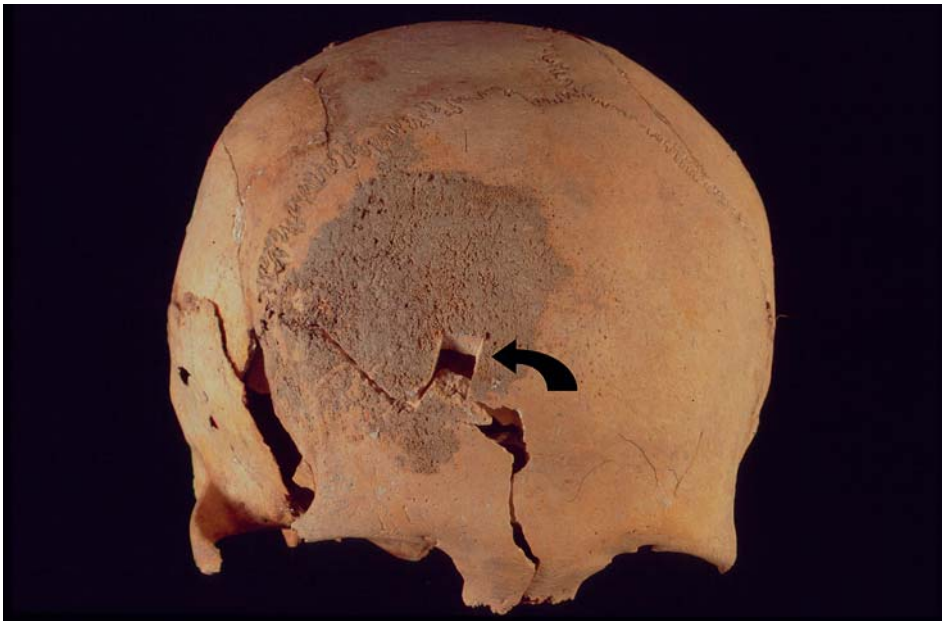


Fig 10 A cranium showing an area of pitted bone on its surface. Within this diseased area, a cube of bone (arrowed) has been removed for histological analysis using a small hand saw. The sample was taken from an area that was already rather damaged. The nature of the bony changes suggested that this was a case of prostate cancer that had spread to the skeleton, and the histological examination of the bone sample supported this.

6.3 Sampling for aDNA

DNA analysis generally involves removing a small sample (varying from milligrams up to about one gram) from a bone or tooth. DNA is present in all cells (with a few exceptions such as red blood cells). The skeleton, the bone and the dentine of the teeth each contain cells and hence are potential sources of DNA. DNA is not present in tooth enamel as it lacks a cell structure. Even if a body preserves some soft tissue, DNA generally survives better in the skeleton. In general, human DNA seems to survive best in dentine, so teeth generally offer better prospects than bone. Samples are generally drilled from dentine from the tooth interior. With care, much of the tooth can be preserved intact. Removing bone for DNA work usually involves cutting away a small cube with a scalpel or, for harder bone, using a small saw.

For work on DNA of infecting bacteria, the best places to sample from are usually the diseased parts of the skeleton (if there are any) as this is where concentration of pathogens was often greatest. For infections that are disseminated via the bloodstream, skeletal sites without bone lesions may also give positive results. Taking a DNA sample from a bone lesion may mainly or entirely destroy the diseased area, severely compromising any future work.

7 Bone histology

7.1 The science

Histology is the microscopic study of cut sections of tissue. Under the microscope, most bone is not amorphous, but has a regular internal structure. In the dense bone (called cortical bone) that makes up most of the limb bones and forms the outer layer of other bones, channels transmit tiny blood vessels which nourish the bone, and layers of bone are arranged concentrically around these vessels, forming microscopic structures called osteons. Various factors may cause minor or major alterations in the microscopic structure of bone. These changes may be noted as present or absent or else the frequency or size of various features may be quantified (often using computer image analysis software), a process called histomorphometry.

There are some differences in the microstructure of human and animal bones, so it may be possible to determine whether small bone fragments are human using histology. This can also be done on cremated bone fragments, although allowance has to be made for shrinkage of osteons on burning.

As a person grows older, various alterations occur in bone microstructure, including aspects of the size and character of the osteons. Quantitative study of these changes has been suggested as a way of estimating age at death in adults. However, like other techniques for age determination in adult skeletons, it does not seem very reliable, and debate continues over what features should be counted and where in the skeleton samples should be taken.

Another thing that may influence size and density of osteons is the mechanical forces placed on a bone during life. This has led some people to argue that this is a way of investigating activity patterns in past peoples. The usual technique is to compare microstructure in a load-bearing bone, such as a femur or other limb bone, with that in a rib from the same individual to control for the effects of age and other physiological factors. Again there is debate over what aspects of microstructure best reflect mechanical loading in life. Age determination and activity studies target cortical bone.

As well as causing grossly visible changes in the bones, skeletal disease may also affect bone microstructure. This means that examination of histological sections can help us diagnose disease. For diseases that cause distinct lesions on bone, what is needed is generally a section cut from the lesion itself. Some diseases, such as osteoporosis and rickets, affect the metabolism of the skeleton as a whole. In such cases, the sampling site is not normally constrained by the location of lesions.

The three types of hard tissue that make up teeth – dentine, enamel and cementum – are deposited in layer structures, the former two during childhood, the latter throughout life. Irregularities in the incremental layers in enamel correspond to disturbances in enamel formation, which in turn may relate to episodes of disease or poor nutrition during the time in childhood when the enamel was laid down. Studies of the frequency and timing of these features enable the study of patterns of childhood disease and malnutrition. Most of the enamel layers outcrop on the surface of the tooth, so irregularities in them can be observed without damage to the specimen. Large defects can be seen with the naked eye, and smaller ones can be studied microscopically on the tooth surface, at least in unworn teeth. However, layers deposited near the beginning of tooth formation are covered by later ones, so for the study of these a vertical section of the tooth needs to be cut.

Because it is laid down throughout life, counting incremental layers in cementum (the material that coats the roots of the teeth and helps anchor them into the jaw) has been suggested as a method for estimating age at death in adults. The method involves cutting a vertical section and counting the lines under the microscope. Results so far have been mixed, but more work on skeletons of known age at death (e.g. those with gravestones or coffin plates) is needed to evaluate the technique.

Soil-dwelling micro-organisms attack bone collagen during burial. As well as reducing the collagen content, this results in dissolution and redeposition of bone mineral at the microscopic level. This results in increased porosity and progressive obliteration of microarchitectural features. There is no correlation between surface appearance of bone and destruction of the histological structure. Outwardly well-preserved bones can show highly degraded microstructural features and vice versa. It has been shown that survival of DNA tends to be poorer in bones with advanced microstructural deterioration but the relationship is not strong enough to predict DNA survival in individual cases.

7.2 What can we learn from the histological study of bones and teeth?

Studies of disease may concentrate on a single or a few skeletons suspected of having a particular disease. Sometimes, sampling of more individuals may be required in disease studies. For example, many skeletons of different ages at death might be sampled to investigate the extent of age-deterioration in structural features of bone in osteoporosis. Reasonable numbers of skeletons (over about 30) are needed for studies of patterning in disturbances in enamel development and for investigating differences in activity patterns within or between communities. Work on testing methods of age determination usually requires large numbers (about 100 or more).

Histology is becoming an increasingly important aid to the diagnosis of disease in ancient bones. It is of more use in some types of disease than others – it is particularly useful in the study of disease caused by imbalances in bone metabolism, such as osteoporosis and vitamin deficiency conditions. Poor mineralisation of bone is a characteristic feature of vitamin D deficiency, which manifests as rickets in children and osteomalacia in adults. Vitamin D is made in the skin on exposure to natural light, so the prime cause of vitamin D deficiency was lack of exposure of the skin to sunlight, either because natural light was attenuated by industrial pollution or because some people failed to expose their skin for occupational, cultural or other reasons. In cases where diagnosis is unclear, obtaining a histological section helps identify whether vitamin D deficiency was present. For example, five adult skeletons from a 19th-century burial site in Birmingham showed clear signs of osteomalacia. A further two had ambiguous changes, but a diagnosis of osteomalacia was confirmed using bone histology.

Osteoporosis causes loss of bone density in the elderly, especially women, and renders them vulnerable to fracture, particularly hip fracture. Study of bone density showed that women from a medieval village lost similar amounts of bone density after the menopause as modern women. However, the medieval women did not show the hip fractures that are a prominent and life-threatening feature of the disease today. There are a number of possible reasons for the lack of hip fractures in the medieval group. One, which was suggested by a study of cut-sections of vertebrae, was that structural features key to the strength of the spongy trabecular bone of the hip and spine persisted into old age, aiding maintenance of structural integrity even in the face of loss of bone density, helping to protect the women against fracture.

Study of microstructural deterioration of bone during burial can help us understand how bone degrades in the soil. At a churchyard in a deserted English village, medieval bones showed severe microstructural degradation, but this was much less marked in later burials (17th–19th century). Studies like this have the potential to inform us of the rates of decay of bone under different soil conditions.

7.3 Taking histological samples

Samples, generally less than 1cm wide, are taken by sawing free a slice of bone (usually a half section so as not to cut completely through the bone), or by removing a plug of bone with a small drill. The sample then needs to be prepared, a process that normally involves embedding it in a resin and grinding and polishing the surface to be examined. If samples of diseased bone are required then this may significantly compromise future studies of the lesions.

Work aimed at investigating activity patterns normally requires more than one bone to be sampled per skeleton. In some cases advanced microstructural deterioration can make it impossible to study many microstructural features, and it is difficult to predict in advance the preservation of bone at the histological level (the outward appearance of the bone is no guide to this).

8 Case Studies

8.1 The case of the Arctic explorer

In the Old Royal Naval College at Greenwich there is a monument to the mid-19th-century expedition to the Canadian Arctic commanded by Sir John Franklin. The purpose of the expedition was to discover the North-West Passage, but disaster overtook it and all members perished. In the late 19th century a skeleton of an officer from the expedition was recovered from the Arctic and interred beneath the monument. Studies done in the 19th century by the great Victorian biologist Thomas Henry Huxley led to the belief that the skeleton was of Henry LeVesconte, a lieutenant aboard one of Franklin's ships.

In 2009, a plan was implemented for a programme of building works which included the removal of the Franklin Monument to a more prominent position in the chapel at the Old Royal Naval College. This provided an opportunity for the first modern scientific examination of the skeleton. The purposes of the scientific work were two-fold. First, to try and confirm or refute the personal identification made in the 19th century and, second, to attempt to shed light on reasons for the loss of the expedition. This latter has been debated over the last 150 years, and various theories have been put forward. These include that the men may have suffered lead poisoning from the tinned food that the expedition was provided with, or that they may have been weakened by tuberculosis.

The memorial was not on land subject to Church of England jurisdiction, so permission to disturb the remains was sought from the Ministry of Justice. This was forthcoming, with the proviso that the remains be reinterred beneath the monument when the building works were complete.

The authorities at Greenwich contacted the closest living relative of Henry LeVesconte, and obtained his agreement to the removal of the remains and to their scientific study which, it was explained, might potentially involve destruction of bone or tooth samples. The remains were removed to a laboratory for scientific analysis.

Henry LeVesconte was known from historic documents to have spent his childhood in Devon. In an effort to try and check this identification, a tooth was removed from the skeleton, and strontium and oxygen isotope analysis undertaken. The strontium values were not very

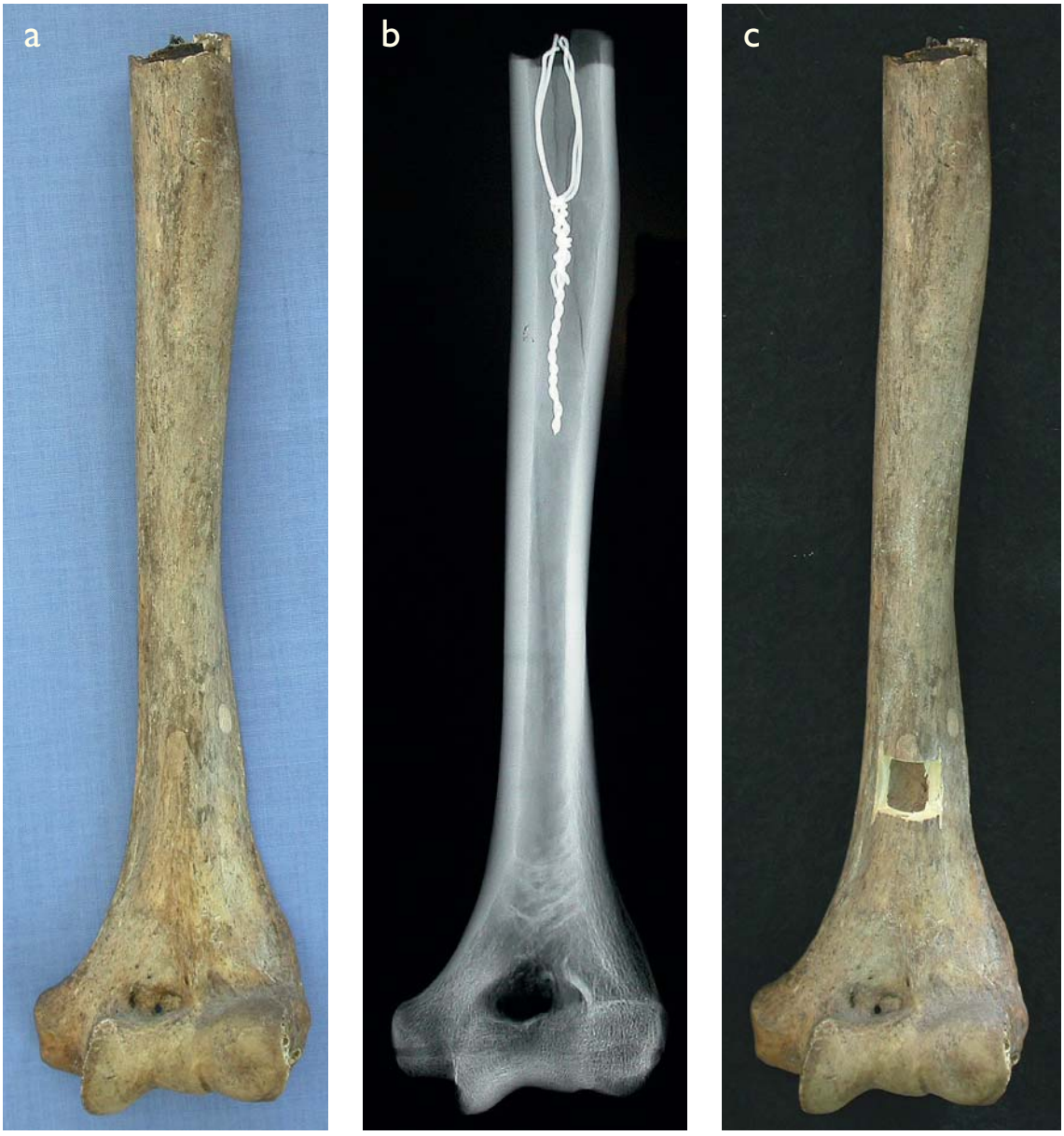


Fig 11 (a) Part of a humerus (upper arm bone). Attempts were made in the 19th century to repair a break in this specimen by inserting a gutta percha dowel reinforced with wire (which can be seen just protruding from the top of the bone). The other limb bones in this skeleton were intact, so this one was selected for sampling for radiocarbon dating. An X-ray **(b)** was taken prior to sampling so that the dowel could be visualised. The wire shows clearly, and within the bone the end of the dowel, projecting just beyond the wire, is faintly visible. This enabled the 19th-century repair to be avoided when the sample was removed **(c)**.

diagnostic – they matched those found in many regions of Britain (although they did allow areas on chalk geology to be excluded) – but the oxygen isotope ratio was atypical of south-west Britain and more consistent with an origin in eastern Scotland or eastern England. This meant that the original identification of the skeleton as LeVesconte was unlikely to be correct. Several officers on the expedition are known to have grown up in regions consistent with the isotopic results. Portraits of some of these men exist, and facial reconstruction from the skeleton permitted tentative identification of the remains as of one of the ship's assistant surgeons, who came from Fife in Scotland. The researchers informed the LeVesconte relative of the results, and initiated contact with living relatives of the expedition's assistant surgeon to inform them of the findings and to attempt to reconstruct family history with a view to assessing the viability of confirming the identity of the burial using DNA analysis. However, it transpired that confirming the identification using DNA was unlikely to be feasible because genealogical research showed that the individual in question fathered no recorded children and no continuous male or female line could be traced from his siblings.

The skeleton showed no obvious signs of tuberculosis, but the disease does not always affect the bones. A bone sample from a vertebra was analysed for bacterial DNA from the microorganisms that cause the disease, and this proved negative. This could mean that the DNA failed to survive or that the individual did not have tuberculosis. Microscopic study of a cut section of cortical bone indicated that the microstructure of the bone survived very well. Although not conclusive, this suggests that bacterial DNA probably ought to have survived if the individual had had tuberculosis, so the negative results may more likely mean that he did not have the disease.

The part of the tooth that was not used for the isotope work and a sample of bone were used for analysis of lead. Work on this is still ongoing, but it is hoped that the content and distribution of lead within the tissues at a microscopic level may help to shed more light on the lead-poisoning theory.

The remains were reburied beneath the Franklin Memorial in 2009, and a special memorial service was held. It was attended by descendants of the explorers, and representatives from Canada, including the Canadian High Commissioner, as well as by some of the researchers who had been involved in the work on the skeleton.

8.2 Parity and weaning in 18th- and 19th-century London

The human osteological series from Christ Church Spitalfields, London, includes many skeletons of adults and juveniles who could be identified from the inscriptions on their coffin plates. The series is held temporarily at the Natural History Museum in London under a Faculty and with permission from the friends of Christ Church Spitalfields. It has been widely used for osteo-archaeological, forensic and clinical research. In particular, the coffin plate series of identified skeletons has been used to test and develop techniques for estimation of age at death, sex determination, and to evaluate other ideas and assumptions concerning the interpretation of human skeletons.

Nitrogen stable isotope determinations are routinely used as a measure of trophic level, and can be used to track the weaning process. Studies of adult skeletons at some archaeological sites have revealed that adult males and females had different values, suggesting a possible difference in male and female diet. An alternative explanation is that nitrogen stable isotope ratios in female skeletons were lower due to a succession of pregnancies (nitrogen metabolism may be altered during pregnancy). The series from Christ Church Spitalfields provided an opportunity to test this idea since parity status has been researched using historical records. If the number of pregnancies has an effect on nitrogen stable isotope ratios, it is predicted that adult females who had not had children would have the same values as adult males, females who had experienced many pregnancies (four or more) would have lower values, and females who had experienced one to three pregnancies would show intermediate values.

An application was submitted for sampling rib bone from skeletons of adult females of known parity, a control sample of adult males and a series of infants and juveniles in order to (1) investigate the possible effect of parity on isotope determinations and (2) reconstruct weaning behaviour in 18th- and 19th-century London. The application specified that samples would be removed alongside an existing break and that skeletons with no damaged ribs would not be sampled. All bones were photographed by the curator and images were attached to the destructive testing application. The application was considered and approved by the curator, Head of Collections, and Head of Department. The analyses were undertaken blind – the researcher who conducted the isotope determinations did not know the sex, age or parity status of individual skeletons until after the analyses were complete.

The study revealed no significant difference in isotope determinations between adult males and adult females with no documented pregnancies, few pregnancies or many pregnancies. The study did not support the suggestion that parity status could account for male and female differences in nitrogen isotope ratios in archaeological populations. Results do not prove that parity does not affect nitrogen stable isotope ratios since other factors such as sample size, possible under-recording of pregnancies (which were estimated from baptism registers) and bone turnover during the interval between last pregnancy and death could influence the outcome of this study. The weaning study provided useful information on the diversity of nursing behaviour in 18th- and 19th-century London. It also revealed that nitrogen stable isotope ratio elevation associated with breastfeeding can be detected in the ribs of infants by the age of 5–6 weeks, which is useful information for the interpretation of other archaeological series.

8.3 The lost bones of Harold II, the last Saxon king of England

King Harold II was killed in 1066 at the Battle of Hastings. His burial place has never been conclusively identified. The Bayeux Tapestry shows Harold praying at Bosham Church prior to his journey to Normandy in 1064. The Holy Trinity Church, Bosham, has been suggested as a possible last resting place for his remains. In 1954, during works renewing the floor of the church, a medieval stone coffin was encountered, and was reported to contain the bones of an adult male. Could this burial be Harold? A project initiated in 2003 aimed to excavate the grave and take a bone sample for DNA analysis to try and shed further light on this possibility. A television production company undertook to meet the costs of the work. Because the burial lay in a church still in use for worship, it was on land subject to the legal effects of consecration. Therefore, the granting of a Church of England Faculty was required if the work was to proceed. The Consistory Court of the Diocese of Chichester met to consider the case.

The Consistory Court was mindful of the presumption in ecclesiastical law

against disturbance of human remains. However, it also recognised the legitimate historic interest in establishing the burial place of King Harold, the only monarch since Edward the Confessor for whom this is not known. It was therefore open in principle to allowing the work, provided that the applicants could make a compelling case for it. To help the Court arrive at a decision they took advice from appropriate experts.

A report from a historian considered the likelihood, based on historic evidence, that Harold was interred at Bosham, and considered other existing theories – that he was buried at Waltham Abbey or his body was disposed of at sea. The report indicated that although it was impossible to disentangle fact from literary or politically inspired artifice, the most compelling case could be made for Waltham Abbey, a foundation that Harold himself had endowed. Neither written sources, nor the Bayeux Tapestry, supported Bosham as a burial place.

With regard to the scientific analysis, the applicants proposed removing a sample of bone from the femur of the burial for their DNA work (the 1954 excavation had shown that the burial lacked a skull so there were no teeth to sample). For the purposes of identification, the DNA would need to be compared with that of paternal ancestors or descendants. The applicants proposed comparison with present-day members of the Godwin family, Harold's living relatives. Y chromosomal DNA would potentially be useful if a direct patrilineal descent could be traced genealogically from Harold or a sibling. This seemed highly unlikely over so many centuries, but nevertheless, three people came forward, each claiming a direct patrilineal link with Harold. However, testing of their DNA showed they had three different Y chromosome sequences. Because a comparator who could demonstrate an unambiguous direct patrilineal link with Harold was lacking, exhuming the remains and sampling them for DNA seemed futile.

Taking into account the advice they had received on the historical and scientific aspects of the case, the Consistory Court concluded that, although the question of King Harold's burial place was of potential interest, the applicant's proposed programme of archaeological and scientific work was not likely to advance knowledge in that respect. It was felt that the case made by the applicants was insufficient to override the presumption of non-disturbance of human remains. Hence, the application was refused.

9 Procedures and terms of access for human remains: the example of St Peter's Church Barton-upon-Humber

St Peter's Church Barton-upon-Humber is no longer used for worship, and is under the care of English Heritage. Part of the church is used for the storage of over 2,700 burials excavated during archaeological investigations in the church and churchyard. The church is still consecrated. Placement of remains here satisfies a wish expressed by the Church that human remains should be returned to consecrated ground after excavation, and at the same time allows the remains to continue to be accessed by researchers. They are an internationally important collection, and are much in demand for research purposes. To illustrate some of the procedures for managing access to this collection, including for the purposes of destructive sampling, the pro forma which applicants need to complete is reproduced below, as are the procedures for considering applications for access, and the terms under which access may be granted.

9.1 Pro forma for access to remains

Pro formas help ensure that the correct information is gathered prior to considering an application, and that different applications are treated fairly and openly. Set out below is the pro forma which applicants need to complete to request access to the human remains stored at St Peter's Church. Note that part B of the form refers to requests for destructive sampling.

9.2 Procedures for considering requests for access to remains

A Barton Human Remains Research Committee (BHRRC) was set up to administer access to the remains at St Peter's Church. The BHRRC comprises representatives of the English Heritage and Parochial Council of St Mary's Church, Barton-upon-Humber, as well as external experts in human remains. The aim was to assemble a committee with a mixture of expertise and experience in Church, curatorial, archaeological and scientific matters. The BHRRC considers requests for access to human remains using the flow-chart set out below. Where the proposed work involves destructive analyses, it considers the proposals against the criteria set out in Section 2.3 of the current document.

9.3 Standard terms of access to the human remains from St Peter's Church Barton-upon-Humber

Procedures for consideration of applications

Access to the skeletal material kept at Barton is normally restricted to suitably qualified individuals conducting research in a relevant discipline, although requests for access for other reasons will be considered.

Postgraduate students may be granted access provided a supervisor's letter of recommendation is submitted. Under-graduate applications will be considered only in exceptional circumstances.

Applications will be considered by the Barton Human Remains Research Committee (BHRRC) and applicants will be informed of their decision

The BHRRC reserves the right to seek external advice as necessary

General terms of access

Applicants are reminded that it is a legal and ethical obligation that human remains be at all times treated with respect

Human skeletal remains are fragile. Applicants should handle remains with care at all times.

Any material removed from boxes for study on- or off-site should be returned to its correct bag and box after study.

The BHRRC should be informed of any problems associated with the curation of the collection, e.g. damage to or deterioration of specimens.

The BHRRC should be provided with a copy of the dissertation, thesis or published articles based on the study of material in the collection.

English Heritage and the BHRRC should receive acknowledgement in any published articles based on study of remains in the collection.

Whilst working on the remains at Barton, all reasonable requests from the English Heritage staff at Barton Church should be complied with.

The BHRRC reserves the right to confer additional terms of access in individual cases as it sees fit.

The BHRRC reserves the right to terminate access if the access conditions are violated.

Additional conditions covering loans of material

Researchers must not remove any remains from site without written permission from the BHRRC

In cases where permission has been granted by the BHRRC to remove remains

APPLICATION FOR RESEARCH ACCESS TO THE ENGLISH HERITAGE HUMAN SKELETAL COLLECTION AT BARTON-UPON-HUMBER

PART A: to be completed by all applicants

Applicant

Name: Academic affiliation:

Email: Telephone:

Postal address:

Academic status of applicant

Masters student/PhD student/University employee (please specify job title)/Other (please specify):

Name of supervisor

Email: Telephone:

Postal address:

FOR STUDENT APPLICATIONS A SUPERVISOR'S LETTER OF RECOMMENDATION SHOULD BE SUBMITTED ALONG WITH THE COMPLETED FORMS

Aims and purpose of research. Please explain briefly the nature of your research and why the skeletal material you are requesting access to is needed for it. Include the overall rationale for your research and how the Barton collection contributes to this. Please also specify whether you require access to the entire collection or a subset of it (eg juvenile skeletons etc). *Please summarise the above in no more than 500 words.*

Data to be recorded and methods to be used

Dates when access will be required

Is loan of material requested? YES/NO

If yes please give details of material required; loan period requested; where material will be kept whilst on loan

Does the work involve destructive analysis YES/NO

If yes, please fill in PART B of this form

Is publication intended? YES/NO If yes please give details

I have read and accept the procedures and terms of access

Signature of applicant (scanned signature acceptable)

Countersignature by supervisor (students only; scanned signature acceptable)

PART B: to be completed by applicants conducting destructive analyses

Please detail what destructive techniques are to be used

What specific research questions will the analyses address?

What is the likelihood of useful information being obtained?

Please indicate:

a what skeletal elements are to be sampled and at what location on the bone

b how many specimens will be sampled

c how much tissue will be taken from each specimen

d how samples will be removed.

Please also specify the context numbers that you intend to sample from, if known at this stage.

Please email completed forms to Simon Mays: simon.mays@english-heritage.org.uk

from site, a loan agreement form must be completed and countersigned by the EH curator/registrar at the time remains are taken.

In cases where BHRRC grants permission for loan of material, a date by which material should be returned will be specified.

Loans will normally be for a period of less than 6 months. Requests for loans for periods of more than 6 months will only be considered in exceptional circumstances.

Researchers should be able to provide safe and secure transportation for remains borrowed, and details should be agreed with the curator.

Researchers should ensure that any remains borrowed are kept in a secure store under conditions which ensure the physical integrity of the remains and which comply with standards set out in *Guidance for the Care of Human Remains in Museums* (DCMS, 2005) and *Guidance for Best Practice for Treatment of Human Remains Excavated from Christian Burial Grounds in England* (English Heritage, 2005). These and any further conditions of loan will be incorporated into a loan agreement signed by both parties.

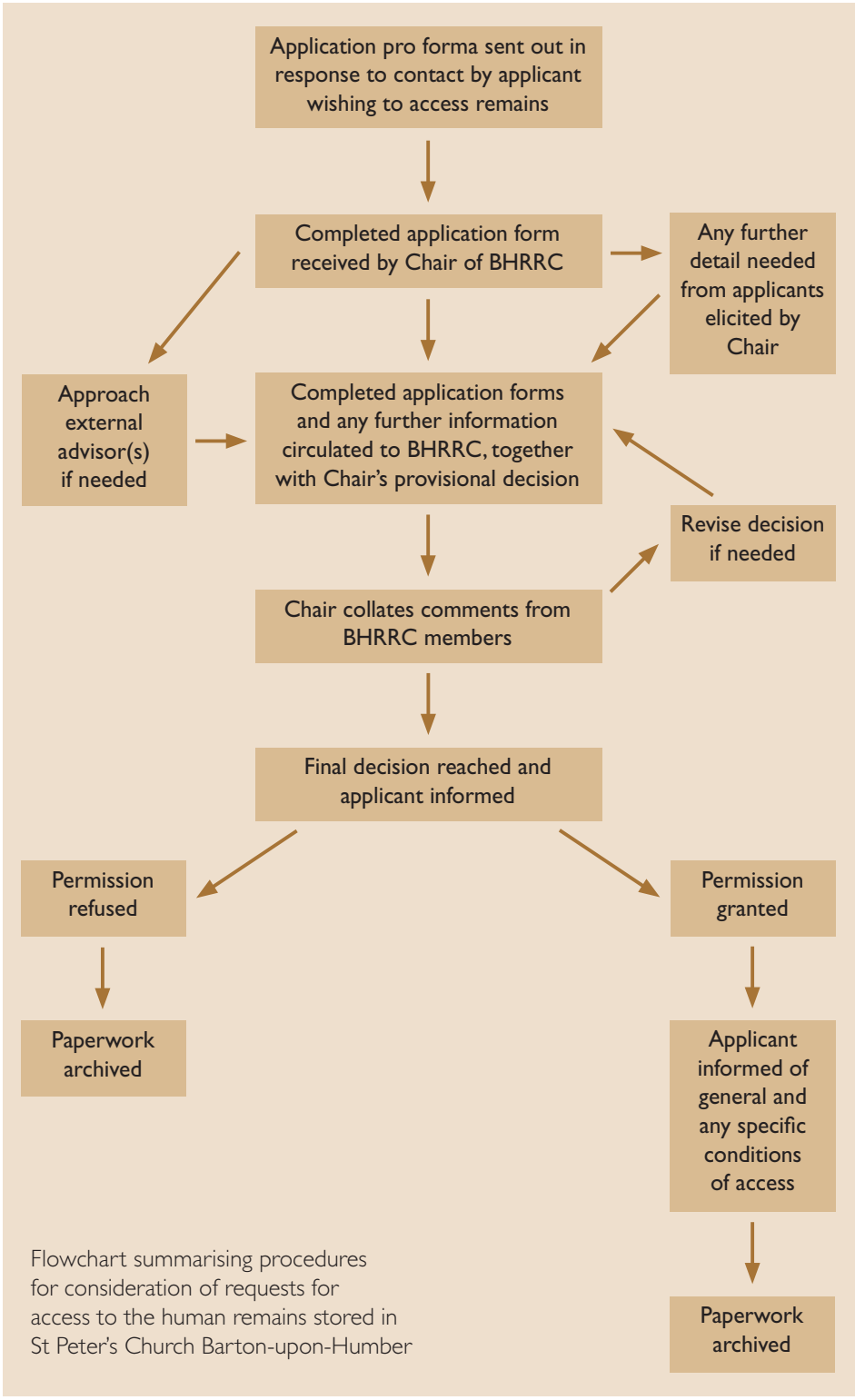
Additional conditions covering destructive analyses

No samples should be removed for destructive analysis without written permission from the BHRRC.

For permission for destructive analysis to be given, the BHRRC needs, minimally, to satisfy itself that the research questions could not be adequately addressed using non-destructive techniques; that the analyses have a realistic prospect of producing useful knowledge; and that the sampling strategy is designed to keep damage to the collection to a minimum.

When samples are removed for destructive analysis, a list of the samples taken should be presented to the BHRCC. In addition, a note should be placed in the box from whence each specimen was taken giving brief details of the sample removed; the analysis that will be performed on it; the date the sample was removed; and the name and affiliation of the researcher who took the sample.

Any unused samples removed for destructive analysis should be returned by the researcher to their correct bag and box.



Flowchart summarising procedures for consideration of requests for access to the human remains stored in St Peter's Church Barton-upon-Humber

10 Further reading

The following books are introductions to the scientific study of human remains aimed at the non-specialist. They contain sections on the techniques described in this guideline.

Mays S 2010. *The Archaeology of Human Bones*, 2nd edition. Routledge, London.

Roberts C A 2009. *Human Remains in Archaeology: A Handbook*. Practical Archaeology Handbooks No. 19. Council for British Archaeology, York.

To follow up the case studies:

'The case of the Arctic explorer'. Mays S, Ogden A, Montgomery J, Vincent S, Batterby W, Taylor G M 2010. New light on the personal identification of a member of Sir John Franklin's last expedition to the Arctic, 1845. *Journal of Archaeological Science* 38: 1571–1582.

'Parity and weaning in 18th- and 19th-century London'. Nitsch E K, Humphrey L T, Hedges R E M 2011. The effect of parity status on delta(15)N: looking for the 'pregnancy effect' in 18th and 19th century London. *Journal of Archaeological Science* 37: 3191–3199. And: Nitsch E K, Humphrey L T, Hedges R E M 2011. Using stable isotope analysis to examine the effects of economic change on breastfeeding practices in Spitalfields, London, UK. *American Journal of Physical Anthropology* 146: 619–628.

'The lost bones of Harold II, the last Saxon king of England'. The judgement of the Consistory Court of the Diocese of Chichester regarding the Bosham case is set out in http://www.diochi.org.uk/downloads/Consistory%20Court/Judgements/031210%20Bosham%20Holy%20Trinity%20%20Judgment%20dated%2010%20December%202003.PDF

11 Where to get advice

The Advisory Panel on the Archaeology of Burials in England (APABE) was set up in 2010 under the auspices of the Ministry of Justice, English Heritage and the Church of England. APABE gives free casework advice to professionals involved in archaeological projects in England dealing with human remains. Its members cover a wide range of expertise, and its remit encompasses advice on ethical and legal matters as well as scientific advice. APABE can be contacted via its website: www.britarch.ac.uk/apabe

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Front cover: left: an archaeological excavation at a post-medieval cemetery; centre: sampling a bone for radiocarbon dating; right: a bone from which multiple samples have been taken.

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