APABE Supplementary Guidance Note 1

Sampling human remains for ancient DNA analysis
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1. Introduction

This Guidance is intended to assist clergy, curators, university academic staff, public bodies such as English Heritage and anyone else with human remains in their care when they receive a request for DNA analysis. It should be read in conjunction with other published Guidance, in particular the Church of England/English Heritage *Guidance for best practice for treatment of human remains excavated from Christian burial grounds in England* (2005), the Association of Diocesan and Cathedral Archaeologists *Guidance Note Notes 1 (2004) and 2 (2010)*, and the *Human Tissue Act (2004)*. In addition, APABE is able to offer advice, free of charge, on the quality of individual proposals.

Most human DNA research has been performed on living populations. Nevertheless, evidence has been adduced to explore trends in human evolution, the dispersion of human groups and reasons for the current world distribution of modern *Homo sapiens*. DNA comparison has been further developed for determination of paternity, scenes of crime evidence and the identification of the dead, whether through accidental death, suicide, homicide, act of war or genocide.

The ability to extract human (and other) DNA from ancient skeletons has opened up new fields of study. Much of this work is exemplary and generally the field holds great promise as techniques are further refined. However, there are some cases where the motives or interpretations of those who promote individual research projects, and indeed the value of the projects themselves, ought to be questioned.

2. A History of ancient DNA studies

2.1 The science

DNA is the molecule that contains the genetic information needed for a living organism to develop and function. When an organism dies the DNA molecules start to degrade, breaking up into shorter strands. These fragments are sections (’sequences’) of the original DNA and can be used in ancient DNA comparisons and identifications. Analysis of ancient DNA (aDNA) commenced in the 1980s with the demonstration that it could be detected in mummified material and the skins of extinct animals. The study of aDNA is problematic: even if the genetic material survives, the quantity is often too small for making valid comparisons.

The introduction of polymerase chain reaction (PCR) in the late 1980s enabled small traces of DNA sequences to be amplified into quantities sufficient for study. The chief drawback of the PCR method is that it will amplify all DNA present in a bone or other tissue sample, irrespective of source. New technological developments (some that do not rely on having the DNA amplified) will advance aDNA studies. These new methods, known as “new generation sequencing methods” in the scientific literature, are particularly suitable for aDNA; they target relatively short stretches of DNA and can generate several gigabytes of genetic data.

Regardless of the techniques used for DNA work, there is a risk of cross-contamination with extraneous DNA at every stage of examination. Scrupulous

laboratory measures have to be taken to prevent, detect and remove any contamination of the sample with modern DNA. Ideally results should be replicated in another laboratory observing the same anti-contamination precautions.

Under ideal conditions, DNA in human remains can survive for millennia, but in reality much aDNA work is unsuccessful, due to poor DNA survival or other factors. Whether aDNA survives in a particular burial cannot be predicted with accuracy. Factors that favour aDNA survival include a cool, dry burial environment, and relatively recent date (centuries rather than millennia). Acidic soils and free-draining soils may destroy not only DNA but also the skeletal remains themselves.

DNA analysis involves removing a small sample (generally less than one gram) of tissue from a bone or tooth or other surviving parts of the body. Those responsible for authorising the sampling of remains should consider whether the knowledge potentially generated by the proposed work justifies this intervention. The research proposal should identify an important issue or question that cannot be answered using non-destructive analysis. The likelihood of achieving the stated aims of the research should also be considered, and if necessary appropriate expert advice should be sought.

Scientific and ethical considerations require that permitted sampling should be minimally destructive and that the scientific value and visual integrity of the bone should be preserved as far as possible. Sampling should be undertaken in an anatomically uninformative location; in the case of a bone that has already been sampled the material should where possible be taken as an extension of the existing sample.

2.2 Range of applications: population studies, disease and evolution

The field of ancient human genetics generates substantial scientific and public interest, and has grown considerably over the past three decades. Ancient DNA analysis of human bone has a range of applications in archaeology.

- Studies of ancient populations can reveal the presence or absence of a particular genetic trait. It can shed light on the origins of that trait, changes in its frequency over time and the underlying causes of those changes, which may be genetic drift, selection within a population or population replacement. One such genetic trait is the persistence of lactase in adulthood, which allows the consumption of raw milk; this is believed to have evolved with the emergence of sedentary agriculture from earlier more mobile hunter-gatherer life-styles. Research has shown that the frequency of this trait in the modern Swedish population is substantially higher than in the Middle Neolithic hunter-gatherer population from the same region. The difference in frequency could not have arisen from genetic drift and is due to either selection or replacement of hunter-gatherer populations by sedentary agriculturalists (Malmström et al 2010).

- aDNA can shed light on the origins of populations that appear to have left no descendants; it can reveal patterns of continuity and discontinuity in a regional population. For example, analysis of maternally inherited mitochondrial DNA (mtDNA) in Neolithic skeletons from central Europe has shown that the first

Neolithic farmers did not have a strong genetic influence on modern European female lineages (Haak et al 2008).

- Individual relationships and patterns of kinship can be established within burial assemblages, providing insights into the social organization of ancient populations. A direct child-parent relationship was detected in a 4,600-year-old grave from Eulau, Germany, providing the oldest molecular genetic evidence for a nuclear family (Haak et al 2008). Sex determination of skeletons can provide revealing insights into practices such infanticide.

- Ancient human bones have been analysed for the DNA of disease-causing organisms that the deceased may have been incubating at the time of death. Detection of pathogenic DNA can be used to diagnose disease in human remains, providing valuable information on the antiquity, geographic origins, spread and evolution of infectious diseases. This approach has been successful in instances of infections causing bony changes, such as in leprosy and tuberculosis. A recent study of Iron Age skeletons with spinal lesions recovered from a cemetery in South Siberia found that four cases were due to infection with *Mycobacterium bovis* rather than *Mycobacterium tuberculosis*, providing useful information on the history of tuberculosis (Taylor et al. 2007). Not all infectious diseases leave traces on human bone, particularly if death occurs rapidly. There is potential to screen for a wide range of diseases in human skeletal remains including syphilis, bubonic plague, malaria, typhus, influenza and smallpox. In future it may also be possible to screen archaeological bone for inherited diseases caused by specific genetic mutations.

- Ancient DNA analysis can also make a contribution to the understanding of recent human evolution, providing information on dates of evolutionary divergence between modern humans and extinct relatives. A large part of the Neanderthal genome has now been sequenced using DNA extracted from fossils, revealing a generic contribution of around 1-4% in non-African modern humans (Green et al 2010).

3. Personal identification: Case studies and controversies

3.1 Introduction

Much analysis of modern DNA is concerned with issues of personal identification and relationships. Often the aim is to provide legal evidence, such as in the Coroner's court for identifying the deceased, the High Courts for forensic use of DNA traces in serious crime cases, the Divorce Courts in cases of disputed paternity, and War Crimes Tribunals in cases of suspected genocide.

In modern forensic DNA work, personal identification can normally be established with some certainty by matching multiple sequences of nuclear (chromosomal) DNA between the deceased and a living close relative. This approach, and the high degree of certainty associated with it, is not normally feasible with aDNA due to its degraded nature. Nevertheless, questions of identity and genetic relationships of identified historic individuals can potentially be addressed using aDNA extracted from their

remains. Comparison of aDNA extracted from ancient bones with the DNA of known or putative close relatives (living or deceased) can be undertaken either to confirm the identity of the deceased or to support the claims of individuals who claim a relationship to the deceased. In such cases, a descendant on a 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. However, even in such cases, results may be equivocal for what they can say about the personal identification of the deceased - for example, breaks in biological descent lines commonly occurred in the past due to adoption or illegitimacy.

3.2 Famous individuals

Many exhumations were undertaken by antiquaries but few of these were for the specific purpose of identifying the remains. However, where the human remains from these excavations have been preserved until the present day, attempts at retrospective identification have often been made. Thus, authentication of such remains may be attempted by aDNA or other analysis. This has been the case with remains attributed to Dante (d1321), Petrarch (d1327), Jeanne d'Arc (d1443), Sir John Talbot (d1453), Cardinal John Morton (d1499), Oliver Cromwell (d1658), Emanuel Swedenborg (d1772), Mozart (d1791) and Thomas Paine (d1809).

Since the last decade of the 20th century there has been a steady demand to conduct exhumations for the purposes of biochemical analysis and identity confirmation. Different arguments in favour of exhumation have been adduced but the results have been variable. A list of some of these is given in Appendix 1. Most of these examples are taken from continental Europe or the Americas; regulation of exhumation in the UK has been rather more stringent.

4. The legal and ethical framework

4.1 Introduction

The treatment of human remains involves making decisions that take into account, *via* appropriate consultation, the views of individuals and groups with legitimate interests in those remains, within the relevant legal parameters. In England these interests include those of the dead themselves, their surviving family and descendants, the Church and other bodies responsible for the care of the dead, representatives of religions and faith systems, the scientific community (including archaeologists), and the general public, particularly those with direct links to the place of burial.

4.2 The secular legal framework

In most circumstances secular legislation provides a framework for regulating the disturbance and removal of human remains and any proposals to undertake DNA testing will be carefully considered on their merits where applications for exhumation licences are made. The exception is remains under 100 years old, to which the *Human Tissue Act* (2004) applies, requiring a specific consent. It is stressed that this guidance note is not concerned with cases under 100 years old. Under the 2004 Act it is an offence to possess human tissue, including hair, nails and gametes (i.e. cells connected with sexual reproduction), with the intention of analysing the DNA without

the consent of the person from whom the tissue came or of those close to them if they have died. Medical diagnosis and treatment, criminal investigations, etc. are excluded.

The secular legal system has traditionally recognised that human remains and the archaeological evidence for the rites that accompanied their burial are important sources of scientific information and of legitimate interest to the research community. This includes archaeologists, osteologists, medical and forensic scientists, historians and others, experts who can also mediate the evidence for the wider general public.

Access to the remains for research will be determined by the organisation entrusted with their care, for example English Heritage or other national organisation, the landowner, a museum, local authority or archaeological organisation. These should consult the relevant guidance, including this document. APABE itself can give non-binding advice, or facilitate contacts with suitably qualified persons.

4.3 The position of the Church of England

The Church of England considers that human remains should be treated with respect and reverence. The phrase 'laid to rest' used in the Anglican Funeral Service, being common parlance for burial, implies that remains should not normally be disturbed. The law of the Church of England, which applies to many thousands of burial grounds in England (mostly churchyards), is protective. It encompasses a principle that remains entrusted to the safe custody of the Church should lie undisturbed, unless authority is granted for a good and proper reason; if they are disturbed they should eventually be re-interred.

The safe custody of the Church does not mean that human remains may never be disturbed. Church law recognises that the living, including church congregations, also have rights that may come into conflict with this principle. Human remains (whether corpse or cremation) under the protection of the consistory court of a Church of England diocese cannot be disturbed without lawful permission in the form of a faculty.

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 of legitimate academic and public interest. Analysis of human remains, including (within reasonable limits) destructive analysis, which includes the taking of DNA samples, is therefore acceptable provided that research aims are clearly and adequately justified and that permission is given by the relevant authorities and the living close family of the individual involved, if known.

Proposals to remove and/or destroy parts of skeletons should be submitted to rigorous scrutiny. This is particularly so in cases where the identity of the individual is known and sensitivities are consequently heightened.

Guidelines have been developed through judicial decisions as to what circumstances may lead to the granting of a faculty for exhumation or disturbance. Although, as noted above, burial is not necessarily final, the principal guideline is that human remains are not to be disturbed on a whim; consistory courts require the submission of a cogent and persuasive case.

Spurious, trivial or poorly researched applications are therefore likely to be refused. There was an important judgement in the case at Bosham Holy Trinity, heard in the consistory court of Chichester Diocese in November 2005. An application that sought to identify a burial in the church as that of King Harold II was rejected due to the poor quality of its research content and the small likelihood of obtaining a meaningful result.

Another landmark ruling related to an attempt to confirm the identification of the body of Bartholomew Gosnold in Jamestown, U.S.A. Exhumation for the purpose of cross-matching the DNA with that from a burial, thought to be his sister Elizabeth, from the church of All Saints in Shelley, Suffolk, was allowed by the consistory court of the Diocese of St Edmundsbury & Ipswich in March 2006. Despite the existence of valid questions which might potentially have been answered, no agreement could be reached regarding the significance of the results.

4.4 The ethics of exhumation for the purposes of DNA sampling

Secular ethics encompass both knowledge-based ethics and those associated with the need for respectful treatment of human remains. Frequently, these considerations coincide, but in some instances they may be in conflict. Decisions should be seen to be made in the public interest and in an accountable way.

As we have seen in the previous sections, DNA is increasingly one of the main tools for scientists using the latest medical and forensic techniques to investigate the behaviour, diseases, cause of death and lineage of historic figures, as well as the often less controversial study of anonymous ancient populations. Targeting known individuals raises serious ethical questions, typified in the debate caused by analysing DNA from President Thomas Jefferson to determine whether he had fathered a child with his slave Sally Hemmings. In this case, as so often, the results were inconclusive, the context of political and social agenda distracting, and the facts of the case easily submerged by sensationalist reporting. The question that must always be asked, in expectation of cogent justification, is whether the project is in the public interest, and whether that interest is sufficient to override the rights of an individual, family or community to privacy.

4.5 Conclusion

The field of ancient genetics generates both scientific and public interest. It is a developing field of great promise, but it can also excite unrealistic expectations. Those wishing to undertake aDNA analysis involving human remains, whether by exhuming burials from churchyards or accessing material in museum or other collections, should submit a research design for the proposed work to those responsible for the care of the remains. The research design (see EH/CofE 2005, Annexe E6, especially paragraph 190) should include details of the project, its justification, the methods to be used, and the applicant's experience in undertaking such work. In arriving at a decision, those entrusted with the care of the human remains have to weigh up the likelihood of a successful outcome, and whether the knowledge thereby generated would fully justify the intervention. This should be

achieved by careful evaluation of the research design, in the light of expert advice from APABE and other relevant sources.

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APPENDIX 1

Case studies of DNA analysis for personal identification of historic individuals

1993 At San Vincente, Bolivia, permission was given to exhume remains ascribed to 'Butch' Cassidy and 'The Sundance Kid' who had vanished in 1908.
No match was found with living relatives.

1996 Zachary Taylor, US President, 1849-50, was believed by some to have met his sudden death by poisoning.
Exhumation was unable to substantiate this.
(There are frequent requests to exhume the body of President Abraham Lincoln (and use aDNA analysis to examine the theory that he suffered from Marfan's Syndrome.
These have always been refused).

1999 Prince Edward's 'Ardent' company planned to make a TV programme based on a further exhumation of the skeletal remains in Westminster Abbey attributed to the 'Princes in the Tower'. The chief aim was to apply scientific tests not invented at the time of the first exhumation in 1933 (e.g. radiocarbon dating, aDNA). Because the Abbey is a 'Royal Peculiar', the permission of the sovereign was first required (just as George V had acquiesced in 1933).
HM Queen refused this request.

2000 DNA analysis from a heart burial, said to be that of Louis XVII of France, was compared with DNA extracted from the preserved hair of Queen Marie Antoinette, the putative mother. The aim was to assess the case of the Pretender Naundorf, who claimed to be the King, having escaped the French Revolution in which his parents were executed.
A positive match was obtained, destroying the case.

2002 There is a tradition that King Richard II of England did not die in 1399 but escaped to Scotland and finally was buried at Stirling. The aim of the investigation was to exhume the body in Stirling and compare the DNA with that of Richard's father, Edward the Black Prince, buried in Canterbury Cathedral.
This project never progressed further, probably because of the difficulty in obtaining the requisite permissions from Canterbury.
(Similar claims have been made that Edward II was not assassinated in 1327 and the requisite exhumations proposed.
No action has occurred.)

2003 It was claimed that King Harold II was buried in Bosham church, Sussex, not at Battle Abbey, Waltham Abbey or at other establishments that purport to be his last resting place. Exhumation was sought to compare bone DNA with two alleged descendants but it was shown that these two did not have matching DNA.
The request was rejected (see above).

2004 A body believed to be that of Christopher Columbus in Seville Cathedral was exhumed.
Identification via *extracted DNA failed*.

2004 There were attempts to identify two of the crew of the Confederate submarine *LT Huntley* which sunk in 1864, *via* comparison of DNA in bones with that of exhumed relatives.
Success was claimed in one case, not in the other.

2005 A tomb in the churchyard at Sevenoaks, Kent, contains the body of Henry Locock (d.1907) claimed by some to be the "love child" of Princess Louise, daughter of Queen Victoria. Locock's grandson sought a faculty to exhume the body and test its DNA for possible relationship to the Royal family. A consistory court at Rochester turned down his request and the grandson appealed to the Court of Arches of Canterbury which began its hearing in September 2004. Part of the evidence considered was the emerging Church of England/English Heritage Guidance (CoE/EH 2005).
The Appeal was rejected.

2005 A skull believed to be that of Wolfgang Amadeus Mozart had been in a private collection for over 200 years. It was proposed to authenticate it by extracting DNA and comparing it with DNA from his grandmother and his niece (both exhumed for the purpose).
There was no match between the DNA from the preserved skull and that from the putative relatives nor between the DNA of the supposed grandmother and the other female relative.

2005 A grave in Frombok Cathedral, Poland, opened for this purpose, was provisionally identified as that of the astronomer Niclaus Copernicus (d 1543). DNA was extracted from the skeleton within and was compared with an authentic sample of the astronomer's hair preserved in a book at the University of Uppsala.
A 97% confidence level in the identification was claimed, but its significance has never been explained.

2006 Mary, Duchess of Burgundy (d1482) was buried in Our Lady's Church, Bruges, Belgium. Remains disclosed during works of renovation have been DNA-tested, and several skeletons are possible candidates.
At the present time there is no known relative with whom to compare mtDNA.

2006 Various members of the Medici family were exhumed from the church of San Lorenzo, Florence.
Their family relationships were confirmed by aDNA from their bones. Further chemical analysis has failed to find any evidence of poisoning.

2006 The skeleton of Elizabeth Tilney was exhumed from Shelley parish church, Suffolk for DNA comparison with the Jamestown, Virginia, body attributed to Bartholomew Gosnold, her brother and founder of Jamestown.
No match was found.

2007 Permission was granted to compare aDNA from three female skeletons, excavated at Mechelen, Belgium – any one of which could be that of Margaret of York, Duchess of Burgundy (d.1503) – with that from a reserved specimen of the hair of her brother, King Edward IV of England, supplied by the Ashmolean Museum.

The investigation foundered owing to failure to demonstrate that Edward's hair still contained extractable DNA.

2008 A vault in Jacob's Cemetery, Weimar, was thought to hold the body of Friedrich Schiller (d. 1805). Since his death, some 86 skulls have been retrieved from the vault, each one potentially that of Schiller. Two skulls regarded as particularly strong candidates were chemically analysed to examine stable isotopes; facial reconstructions were performed. aDNA was extracted and compared with that of five specially exhumed close relatives of Friedrich Schiller.
No DNA match with either of the two putative Schiller skulls was found.