

The Archaeologist and Ancient Bio-Molecules: Field Sampling Strategies to Enhance Recovery

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Recent developments in biotechnology should warrant the alteration of sampling strategies adopted by field archaeologists for the collection of human remains and other organic materials. New techniques include the ability to extract and amplify human and bacterial DNA using the polymerase chain reaction, and the identification of ancient proteins, lipids and carbohydrates from bones, tissues, artifacts and soils. This means preventing the loss of valuable information as a result of the failure to collect and to protect from contamination newly excavated material.

The burgeoning literature on molecular biology and archaeology of fossils (Brown and Brown 1992; Thomas 1993) has suggested the need for new attitudes towards the excavation and preservation of organic materials found in an archaeological context. Renfrew (1992) has questioned the value of the contribution of physical anthropology to our understanding of population movements in the past. Much of his criticism was directed at the validity of information derived from genetic studies of living populations for the understanding of the past. However, bio-molecules such as lipids, proteins, carbohydrates and particularly nucleic acids (DNA), do persist in the skeletal remains and soft tissues of ancient peoples, animals and plants. Increasingly sophisticated methods are now becoming available to enable the extraction of this information. Excavators should become familiar with the special techniques involved in the collection of specimens with a particular emphasis on the preservation and avoidance of contamination of ancient bio-molecules.

Bones and teeth are natural containers and may preserve some DNA internally, no matter how they are handled after excavation. Considerable data, however, may be lost due to the contamination of bones, and particularly soft tissues, as a result of poor recovery techniques.

We do not yet fully understand the circumstances under which organic matter such as ancient bio-molecules, cells or organisms survive and those conditions which are likely to lead to early degradation. Much work remains to be done in this area of molecular taphonomy. Only recently has it become known that cremated bones may well have a higher ancient DNA content than those from inhumations (Brown *et al.* 1995). In 1993, authors were still claiming (Ostinga *et al.* 1993) that acid bogs caused the complete destruction of any contained DNA. Subsequent experiments (Spigelman *et al.* 1995) have shown that this is incorrect and that inhibitors in the bog material (iron molecules, tannins etc.) can give falsely negative results. Thus we cannot afford to ignore any specimens, even those currently viewed as unpromising, given that future improvements in technique may well make the same a source of valuable information.

The recent development of the polymerase chain reaction (PCR) by Mullis and Faloona (1987), may be one of the major technical advances in the history of molecular genetics. The importance of this technique lies in its ability to amplify minute traces of DNA, either fragmented or intact, by a million times over a short period of time. Hagelberg and Sykes (1989) reported the recovery of 5 - 10 μ of DNA from 2g of

powdered ancient cortical bone. Their initial experiment suggested that the 'preservation of DNA in a bone depends less on the age of the specimen and more on the the burial condition of the skeleton.' (Hagelberg and Sykes 1989: 485). The bones they studied had ^{14}C dates ranging from the seventeenth-century English Civil War period to a 5450 BP cave burial at Wadi Mamud in the Judean Desert. The extracted DNA was used as a template for PCR, which is ideal for 'amplifying DNA in biological samples with little or degraded DNA' (*ibid.*). Successful results have been obtained with human bones aged more than 11,000 years (Brown and Brown 1992) and from insect remains aged 120 million years (Cano *et al.* 1991).

Hagelberg *et al.* (1991) noted that in skeletal remains the human DNA was severely degraded within eight years of burial, with almost all single stranded DNA fragments smaller than 300 nucleotides. However, similarly sized strands are preserved in specimens several thousand years old, suggesting that there is an initial phase of rapid DNA degradation which then slows or ceases. Once this equilibrium is reached, it may be disrupted during excavation if proper care is not taken to prevent contamination by modern micro-organisms or modern human DNA. The new micro-organisms may further degrade any surviving ancient DNA causing a potential loss of valuable, even unique, information.

Work at the Institute of Archaeology, London, has contributed to this knowledge. A joint project involving the Department of Medical Microbiology Division of Bacteriology at the University College London Medical School was the first to successfully extract bacterial DNA from ancient bones. This led to the isolation and amplification of a species specific sequence from *Mycobacteria tuberculosis* (TB) in bones showing morphological evidence of TB (Spigelman and Lemma 1993). Four bones with a date range from AD 600-1700 were found to contain this sequence (from Europe, Turkey and pre-European contact Borneo). Subsequently, the same techniques were used to extract ancient *M leprae* DNA from a bone (with a ^{14}C date of AD 600 +/- 50) excavated at the monastery of John the Baptist on the banks of the Jordan river by Joe Zias of the Israeli Antiquities Authority (Rafi *et al.* 1994a; 1994b).

Palaeofaeces may also be a source of ancient DNA. In a joint project involving the Institute of Archaeology, London and scientists from Thames Water plc, PCR has been utilised to amplify *Escherichia Coli* DNA from the gut contents of Lindow man, a bog body from Britain dated to 500 BC (Spigelman *et al.* 1995). This technological breakthrough may be used to provide clues for reconstructing human diet and to detect the prevalence of the many pathogens such as Cholera and *Yersinia* that do not leave morphologically diagnostic lesions on bones.

Modern archaeologists often express regret at the loss of material from past excavations where careful methods of excavation were not used (e.g. sieving and flotation). Present open area excavations are, however, destructive: once excavated, an area is contaminated and is essentially closed to further research, a direct contrast to the older 'Wheeler-Kenyon' excavation technique which ensured that much of the site may still be available for future research (Mazar 1990).

These problems can best be minimized by providing all excavations with the basic equipment needed for the collection of sterile uncontaminated samples. Quantities of material needed are small: one tooth or a few grammes of soil are sufficient. Refrigeration is desirable but in most cases not essential. A physical anthropologist is best qualified to decide which tissue is to be sampled and the method of collection. The

following guidelines (contained in appendices 1-3) developed during the 1993-4 excavations at Tel Beit Shean in Israel may help to improve the quality of the material received in the laboratory in the absence of a expert, preventing the destruction of skeletal or other material during sampling. These include:

- a) A summary of field collection methods (Appendix 1)
- b) A summary of the areas and materials in which ancient bio-molecules may be found (Appendix 2).
- c) A brief review summarising the relevance of such sampling processes for archaeologists (Appendix 3).

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Appendix 1

Steps to avoid specimen on-site contamination during excavation

- 1) Collect in sterile containers with sealable lids such as urine jars, or a stool collector where the spoon in the lid allows both collection and laboratory sampling using a no touch technique. The inside of the container should not be handled or breathed on, and the container should not be placed on the ground. The inner surface of the lid should be held facing down and replaced as soon as possible.
- 2) Sterile disposable gloves and masks should be worn when collecting the samples and the gloves (at least) changed for each sample to be collected.
- 3) Loose teeth are easiest to collect; if unavailable the whole mandible or another bone can be placed in a clean paper bag or aluminium foil (plastic wrapping should be avoided, as it causes sweating). Refrigeration is desirable but in most cases not essential. The bones or teeth can then be returned for further skeletal analysis after laboratory sampling. Bones should not be damaged in order to get a sample. Ribs, finger or toe bones are the least likely to contain residual DNA in significant amounts.
- 4) Try to stay down wind from the specimen so that droplet contamination caused by coughing, sneezing or even talking can be avoided until the specimen is sampled. Similarly avoid leaning over the specimen as this will promote contamination by sweat and skin cells particularly from the hair and scalp.
- 5) Speed is important: a specified period of time must be given to specimen collection avoiding distractions. Samples should be taken as soon as possible, certainly within one hour of exposure.
- 6) Direct sunlight, as well as being damaging to DNA, encourages bacterial growth; thus, exposure must be minimised.
- 7) Samples to be collected before any preservatives are applied or any washing or soaking is undertaken. Even tap water may be a source of contamination.
- 8) Ideally, if the specimen is to be used for population studies, samples should be taken from all excavators who may have been close to the specimen which must be taken for laboratory DNA analysis and comparison.
- 9) If possible, one member of the excavating team should be responsible for specimen collection and should receive instructions from a trained expert on sterile procedures, correct gloving, the no touch technique and the storage of collected materials.
- 10) Our ability to extract information is increasing rapidly, thus specimens should be collected for storage even if the information sought is not yet technically feasible. Sampling must also involve the use of proper controls; thus from a grave, DNA from the bacterial contents of the colon will require that the soil is sampled from the sacral area, or for lungs, the chest region. A control soil sample can be taken from the skull region and surrounding soil to ensure the bio molecule found is specific to the region sampled.
- 11) Some results may need verification in a second laboratory so a duplicate sterile sample should be taken if possible.

12) Refrigeration is the ideal storage method, but if not feasible a cool dark spot is sufficient

Appendix 2

Where ancient bio-molecules are to be found

Animal and human bones: burnt or cremated bone often has a higher content of ancient DNA, but most bone should contain some.

Plant remains such as charred and uncharred seeds.

Mummies.

Any remains skeletal or otherwise of any living organism (insect, fish, bacteria etc.).

Cesspits, latrines and palaeofaeces.

Fossils preserved in amber.

Bodies preserved in bogs or ice.

Soil around inhumations, the left side of the pelvis (the area where the colon disintegrates) in any burial.

Pathological specimens in medical museums.

Any site where bacterial action is reduced (eg. salt mines, near copper objects etc.)

Deep sea and lake sediments, palaeosols.

Appendix 3

How ancient bio-molecules may help archaeologists

1) Populations:

- a) Human: 1) Dispersal, colonisation, number and direction of migrations.
2) Invasions and assimilation or replacement by intrusive groups.
3) Family relationships.
4) Sex determination.
- b) Animal: 1) Dispersal, colonisation.
2) Domestication traits.
3) Family relationships.
4) Sex determination.

2) Plants:

- a) Species identification.
- b) Evolution and dispersal of domesticated plants.
- c) Reconstruction of past human food webs.

3) Disease:

- a) origin and spread of disease of microbes in humans and animals.
- b) Genetic changes in microbes and changes within specific chromosomes.
- c) Genetically determined human and animal diseases may be recognised in antiquity and monitored over time.

4) Climatic: Changes and variations discerned within plant bio-molecules.