Analysis of Ancient Bone DNA: Techniques and Applications [and Dicussion]
Author(s): Erika Hagelberg, Lynne S. Bell, Tim Allen, Alan Boyde, Sheila J. Jones, J. B. Clegg, S. Hummel, T. A. Brown, R. P. Ambler
Source: *Philosophical Transactions: Biological Sciences*, Vol. 333, No. 1268, Molecules Through Time: Fossil Molecules and Biochemical Systematics (Sep. 30, 1991), pp. 399-407
Published by: The Royal Society
Stable URL: http://www.jstor.org/stable/55428
Accessed: 27/05/2010 06:20

Analysis of ancient bone DNA: techniques and applications

ERIKA HAGELBERG[1], LYNNE S. BELL[2], TIM ALLEN[3], ALAN BOYDE[2], SHEILA J. JONES[2] AND J. B. CLEGG[1]

[1] *MRC Molecular Haematology Unit, Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU, U.K.*
[2] *Hard Tissue Research Unit, Department of Anatomy and Developmental Anatomy, University College London, London WC1E 6BT, U.K.*
[3] *Oxford Archaeological Unit, 46 Hythe Bridge Street, Oxford OX1 2EP, U.K.*

SUMMARY

The analysis of DNA from ancient bone has numerous applications in archaeology and molecular evolution. Significant amounts of genetic information can be recovered from ancient bone: mitochondrial DNA sequences of 800 base pairs have been amplified from a 750-year-old human femur by using the polymerase chain reaction. DNA recovery varies considerably between bone samples and is not dependent on the age of the specimen. We present the results of a study on a small number of bones from a mediaeval and a 17th-century cemetery in Abingdon showing the relation between gross preservation, microscopic preservation and DNA recovery.

1. INTRODUCTION

(*a*) *The polymerase chain reaction and bone DNA*

Developments in molecular biology have provided new tools for analysing ancient DNA from archaeological remains and museum specimens (Higuchi *et al.* 1984; Pääbo 1985; Pääbo *et al.* 1988; Thomas *et al.* 1989; Hagelberg *et al.* 1989; Golenberg *et al.* 1990). In particular, with the polymerase chain reaction (PCR) (Saiki *et al.* 1985), significant phylogenetic information has now been recovered from a number of ancient tissues preserved frozen, in dry environments, or in water-logged deposits. PCR is a technique for the enzymic synthesis of a DNA segment, whereby the exact sequence to be amplified is specified by two primers, short molecules of single-stranded DNA designed to match opposite ends of the two complementary strands of the target DNA, bounding the fragment to be replicated. Repeated cycles of denaturation, annealing of the primers to the target DNA and extension of the segment between the primers by a DNA polymerase, result in the exponential accumulation of the target DNA fragment that can then be sequenced by conventional techniques. In recent studies, PCR has been shown to be an essential tool for the analysis of ancient DNA, as it can be used for degraded and chemically modified DNA samples (Pääbo *et al.* 1989). The oldest tissue from which phylogenetically useful sequences have been recovered is a compression fossil of a *Magnolia* leaf from a Clarkian lake bed dating from 17–20 Ma, as described by Golenberg *et al.* (1990, and this symposium).

Hagelberg *et al.* (1989) described the amplification of mitochondrial DNA (mtDNA) sequences from human bones several hundred years old. This has important implications for archaeology, anthropology and palaeontology as bones are preserved under a wider range of environmental conditions than those which allow preservation of soft tissue remains. Other studies have now been published describing the amplification of mtDNA fragments from ancient human skeletal material (Horai *et al.* 1989; Hänni *et al.* 1990).

Reports of the analysis of bone DNA have met with some scepticism, as the results could be artefacts caused by amplification of trace amounts of modern DNA from people handling the material. However, Hagelberg & Clegg (1991) confirmed that authentic DNA can indeed be extracted and amplified from ancient bone, as analysis of DNA from a 16th-century pig bone revealed an unambiguous pig sequence. Additional evidence for the applicability of these techniques was provided by work done in the course of a murder investigation, in which bone DNA typing was used to confirm the identity of the victim (Hagelberg *et al.* 1991). In this context, note that in a comprehensive review of forensic anthropology, Iscan (1988) stated that bone DNA typing was impossible unless some soft tissue remained on the skeleton.

Despite these developments, DNA typing from ancient bones is far from being a routine technique because little is known about the factors affecting DNA preservation and recovery. The preservation of DNA in bone is highly variable (E. Hagelberg, unpublished observations) and the relation between DNA content and parameters such as protein content is poorly understood. No work has yet been done to show how environmental conditions, such as depth of burial or

Figure 1. Plan of the Civil War cemetery in Abingdon. Four bone samples from the mass grave (3115, 3117, 3118 and 3123) and one from an adjacent single grave (3071) were used in this study. The unusual north–south orientation of the graves was part of the Puritan reaction against Anglican church ritual during the rule of Parliament.

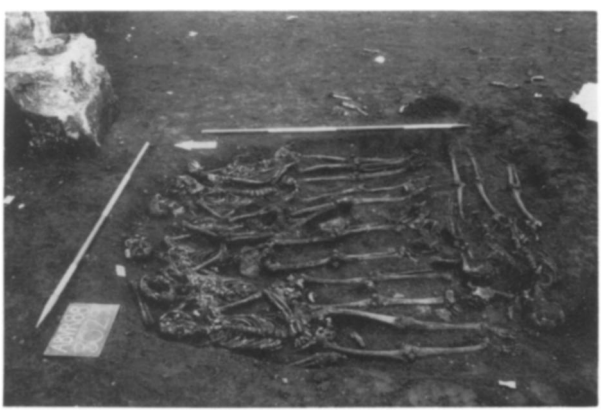

Figure 2. Mass burial of nine men, one with a musket ball between the ribs, from the Civil War cemetery in Abingdon.

soil pH, may affect DNA survival. The histological preservation of bone has also been shown to be variable, and skeletal material may be profoundly altered and reorganized by the effects of diagenesis (Hackett 1981; Bell 1990; Bell *et al.* 1991).

The purpose of this study was to investigate whether a relation exists between gross state of preservation of bone, as defined in archaeological terms, histological preservation, and the ability to extract DNA and amplify mtDNA sequences from the tissue. MtDNA is used frequently in ancient DNA studies because it is relatively small and well characterized (the human mtDNA genome consists of 16569 base pairs and its sequence was published by Anderson *et al.* 1981), evolves on average five to ten times faster than nuclear DNA and is inherited through the maternal line. Mammalian cells have approximately 10^3–10^4 copies of mtDNA so it is fairly likely that some mtDNA could persist in degraded samples.

We describe here the preliminary results of DNA analysis of a small number of samples from skeletons recovered in the course of archaeological excavations in Abingdon, Oxfordshire, U.K.

(*b*) *The Abingdon cemeteries*

Excavations by the Oxford Archaeological Unit were done within the former precinct of Abingdon Abbey before redevelopment, and involved both the mediaeval town cemetery and a cemetery of the English Civil War period (Allen 1989, 1990*a*, *b*). In total, nearly 1000 skeletons have been recovered from the mediaeval cemetery and over 280 from the Civil War cemetery. The mediaeval cemetery was used from *ca.* A.D. 1100 to A.D. 1540 and has more than twenty successive layers of graves that overlay and cut into one another. In contrast, the Civil War cemetery was in use only between A.D. 1644 and 1663 and consisted of a single layer of burials that did not intercut.

Interestingly, the Civil War graves are all oriented north–south, in contrast to the mediaeval graves which all lie east–west. During the English Civil War and the rule of Oliver Cromwell much of the Anglican ritual was considered Papist, hence the unconventional orientation of the graves. The start of the cemetery can be dated from the capture of Abingdon by the Parliamentarians in May 1645, the end by the passing of the Test Act in 1663 when Anglican burial rites became compulsory again. The cemetery was not predominantly military, as the burials include old people, children and women. As shown in figure 1, most of the burials contained only one skeleton, with the exception of several group burials of a woman and child or two children, and one mass grave of nine individuals, one with a musket ball between his ribs (figure 2). The burial register for 1644–45 contains an entry for the burial of nine prisoners from the town gaol, which probably corresponds to the grave of the nine men, especially as the gaol at that time was in the Abbey gateway only 50 m away.

In addition, a small mediaeval graveyard approximately 1 km northwest of the Abbey was excavated between 1977 and 1978 by the Abingdon Archaeological and Historical Society (Harman & Wilson 1981). There were at least 19 adult and 3 child burials, all in individual graves, and no evidence of intercutting, although there were at least two successive layers of graves.

In this study we examined five human femur samples from skeletons excavated between 1988 and 1989 from the Civil War Cemetery and one femur from the small mediaeval graveyard.

2. MATERIALS AND METHODS

Mid-shaft sections of the femora were cut longitudinally and DNA extracted from one of the fragments, and the remaining portion was used for the

microscopic study. The bone fragments were processed for DNA analysis as described in Hagelberg & Clegg (1991). Care was taken at all stages to avoid contamination by modern DNA: disposable sterile containers and pipette tips were used throughout, as well as sterile reagents and solutions dedicated solely for work on ancient DNA. Blank control extractions (containing no bone) were done in parallel with the bone extractions to monitor contamination from laboratory reagents and equipment.

DNA amplifications were done by the method recommended by Perkin-Elmer Cetus in 25 μl reactions containing 2 units *Thermus aquaticus (Taq)* DNA polymerase and 160 μg ml^{-1} bovine serum albumin. The addition of bovine serum albumin in PCR reactions is useful to overcome the effect of a powerful PCR inhibitor of unknown origin present in many ancient DNA extracts (Pääbo *et al.* 1988; Hagelberg *et al.* 1989). Two microlitres of bone DNA extract were used in each amplification reaction. A blank reaction containing no DNA was set up in each experiment to check for contamination of the PCR reagents. The amplifications consisted of 35 cycles of denaturation at 95 °C (1 min), annealing at 55 °C (1 min), and extension at 72 °C (1 min) and were done in a Perkin-Elmer Cetus DNA Thermal Cycler. After amplification, 10 μl of the PCR reactions were subject to electrophoresis on agarose minigels and stained with ethidium bromide to visualize the DNA fragments under ultraviolet light.

The mtDNA primers used in this work are the previously described primers A and B which specify a 121 base pair (b.p.) fragment in a small non-coding region (region V) (Wrischnik *et al.* 1987), primers D18, D6 and H408 of the hypervariable region (D-loop) of human mtDNA (Higuchi *et al.* 1988; Vigilant *et al.* 1989), and the highly conserved mtDNA primers (Kocher *et al.* 1989) specifying a 375 b.p. fragment of the cytochrome *b* gene (primers L14841, H15149).

For the microscopic study, thick mid-shaft sections were taken from the femora using a water-cooled diamond saw and allowed to air dry. The sections were then placed in distilled methylmethacrylate monomer which was polymerized at 37 °C. After polymerization, the embedded specimens were cut transversely, polished using graded abrasives, and finally polished with fine diamond abrasive (1 μm) on a rotary lap. Each block face was then coated with carbon *in vacuo*.

The specimens were examined by using a Cambridge Stereoscan S4-10 scanning electron microscope (SEM) operated in backscattered electron (BSE) mode working at 20 kV beam voltage. A four-segment solid-state BSE detector was used for compositional imaging, summing the signal from all four segments. The images were dominated by differences in the mean atomic number of the volume probed by the scanning beam and so provided a sensitive indicator of mean density and micromorphology.

3. RESULTS

We examined five human femur samples from the Civil War cemetery in Abingdon and one human femur from the small mediaeval cemetery. Of the five bone samples from the Civil War cemetery, four were from the mass grave and one from a single grave adjacent to the mass grave (figure 1), as follows: from mass burial, skeletons numbers 3115, 3117, 3118 and 3123 (males aged *ca.* 17–24 years); single burial, skeleton number 3071 (adult male). The femur sample from the mediaeval cemetery was from a male aged 20–25 (radiocarbon date Har-3474 750±80 before present (1950) = A.D. 1200).

(*a*) *DNA amplification*

DNA was extracted from 1.2 g of powdered bone, and little difference in the quantity of DNA was observed between all 17th-century bone samples after electrophoresis on an agarose gel and staining with ethidium bromide. Segments of mtDNA of 121 b.p. (region V) and 229 b.p. (D-loop) could be amplified from all DNA samples except bone DNA from skeleton 3123: DNA extracted from this sample could not be amplified, although the DNA extracted from the other three skeletons in the same grave could be amplified readily. To obtain an idea of the extent of damage of the bone DNAs, samples 3115, 3117, 3118 (mass grave), 3071 (single burial) and the mediaeval bone DNA was subjected to PCR with four pairs of primers specifying DNA fragments of varying length, ranging

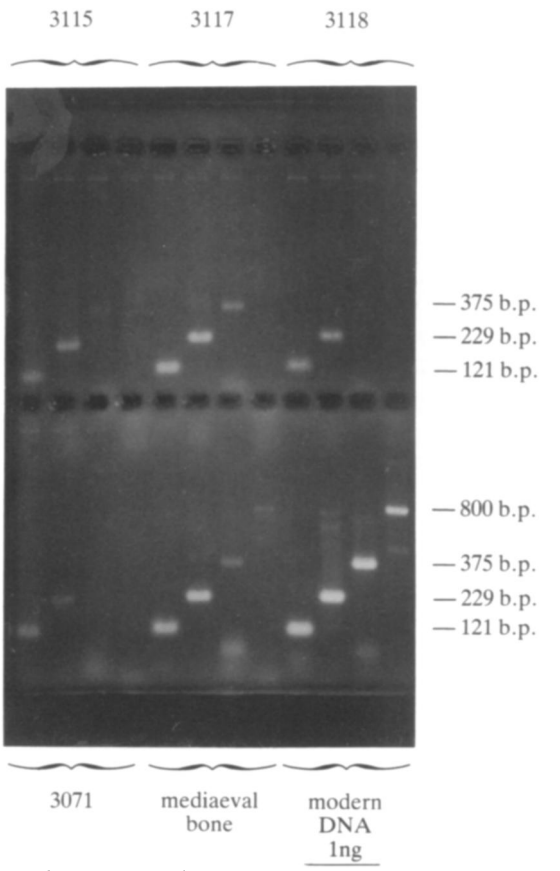

Figure 3. PCR amplifications of mtDNA from bone DNAs and control modern DNA, as follows: DNA samples from skeletons 3115, 3117, 3118, 3071 (Civil War cemetery); bone DNA from mediaeval skeleton; control modern DNA (1 ng). The amplified products were 121 b.p. (primers A and B); 229 b.p. (primers D18 and D6); 375 b.p. (primers L14841, H15149); 800 b.p. (primers D18 and H408).

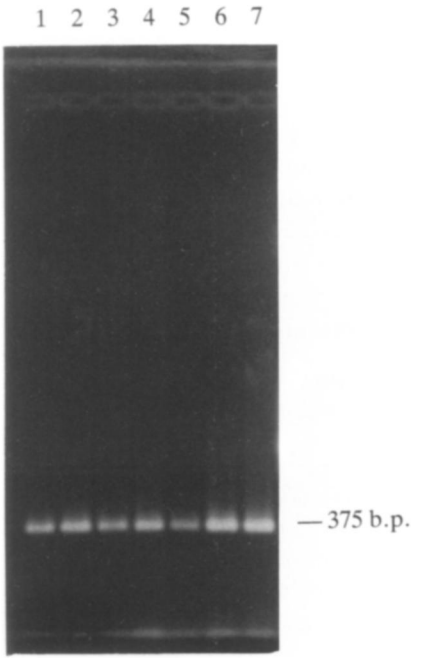

Figure 4. Effect of ancient bone DNA extracts on amplification of modern DNA. All the reactions contained 1 ng of modern DNA but reactions 1–6 contained 1 μl DNA from the following bone samples: (1) 3115; (2) 3117; (3) 3118; (4) 3123; (5) 3071; (6) mediaeval bone. Reaction (7) contained modern DNA only. Note the slight inhibitory effect of several of the bone samples, particularly 3115, 3118, and 3071. The amplified product was the 375 b.p. fragment of the mtDNA cytochrome *b* gene.

from 121 to 800 b.p. The results (figure 3) suggested that DNA sample 3071 (single burial) was highly degraded, as only slight amplification of the 229 b.p. fragment could be obtained, whereas the three samples 3115, 3117 and 3118 from the mass burial were better preserved as the 375 b.p. fragment could be amplified (though not 800 b.p.). The most striking DNA preservation was observed with the mediaeval bone DNA, with clear amplification of the 800 b.p. fragment.

From the point of view of DNA preservation the mediaeval bone was better preserved than the 17th-century samples, assuming that failure to amplify reflects the extent of damage to the DNA. Samples may amplify poorly because of DNA damage, i.e. the DNA might be broken into small segments, but failure to amplify efficiently could also be a result of inhibition of the *Taq* polymerase by some unknown substance in the ancient DNA. We tested whether the ancient samples inhibited PCR by adding aliquots of the ancient DNA to reactions containing modern DNA (1 ng). The results (figure 4) show that the 17th-century samples inhibited slightly the amplification of modern DNA, with inhibition being stronger in 3115, 3118 and 3071, although there was little difference between them, and what inhibition there was could not account for the total failure to amplify sample 3123 described above.

(*b*) *Microscopic examination*

The microscopic examination revealed that the 17th-century bones had undergone similar, and sometimes quite extensive, diagenetic alteration, whereas the spatially distinct mediaeval specimen had extremely good micromorphology. The 17th-century specimens exhibited diagenetic changes similar to those described in Bell (1990), believed to be caused by invasive bacterial activity. No fungal invasion was evident in any of the specimens examined.

Sample 3115 (figures 5–7) had fairly good microscopic preservation. The medullary third and central third of the cortex showed large areas of bone with an intact system of osteocyte lacunae, although diagenetic foci were present among large fields of intact bone. Diagenetic alteration was only extensive at the subperioseal aspect of this sample. In contrast, samples 3117 (figures 8–10), 3118 (figures 11–13) and 3071 (figure 17) showed a similar degree of poor preservation. The distribution of diagenetic alteration concentrated on the medullary and subperiosteal thirds of the cortex, where little recognizable bone could be identified. The middle third of the cortices had undergone diagenetic changes within every single osteonal system in the plane of each section, although more bone was recognizable and intact osteocyte lacunae could be seen (figures 9, 12 and 17). Sample 3123 (figures 14–16) had extremely poor preservation; its entire cortex from medullary aspect through to the periosteal aspect having been almost entirely remodelled *post mortem*. Closer inspection of locations between diagenetic focal lesions revealed occasional intact osteocyte lacunae and very small areas of bone with unaltered morphology and density.

The bone from the small mediaeval graveyard showed by far the best preservation. This sample (figure 18) had nearly perfect micromorphology, with its osteonal and osteocytic network intact. Only a very small area of the circumferencial lamellae showed localized demineralization, but otherwise the state of preservation of this specimen was excellent.

4. DISCUSSION

We have already shown in several studies (Hagelberg *et al.* 1989; Hagelberg & Clegg 1991; Hagelberg *et al.* 1991) that genetic information can be recovered from archaeological and forensic bones, but little is known about the factors affecting DNA preservation. To extract DNA from extremely ancient and valuable skeletal remains we will need to know the conditions under which DNA is preserved and which are the best samples to test. However, preservation may be defined in a variety of different ways; for an archaeologist, who is primarily concerned with gross morphology, the quality of preservation as assessed in the field depends on the weight, robustness and completeness of the bones. If the bones are dense, not brittle, and the surfaces are undamaged, the skeleton is said to be well preserved; if the bones are damp, the surfaces are pitted or rub off when touched and the bone is spongy, the preservation is poor.

The site of the Abingdon Abbey precinct, where the large mediaeval town cemetery and the Civil War cemetery were situated, sits upon gravel terrace deposits, overlaid by an orange sandy silt of post-

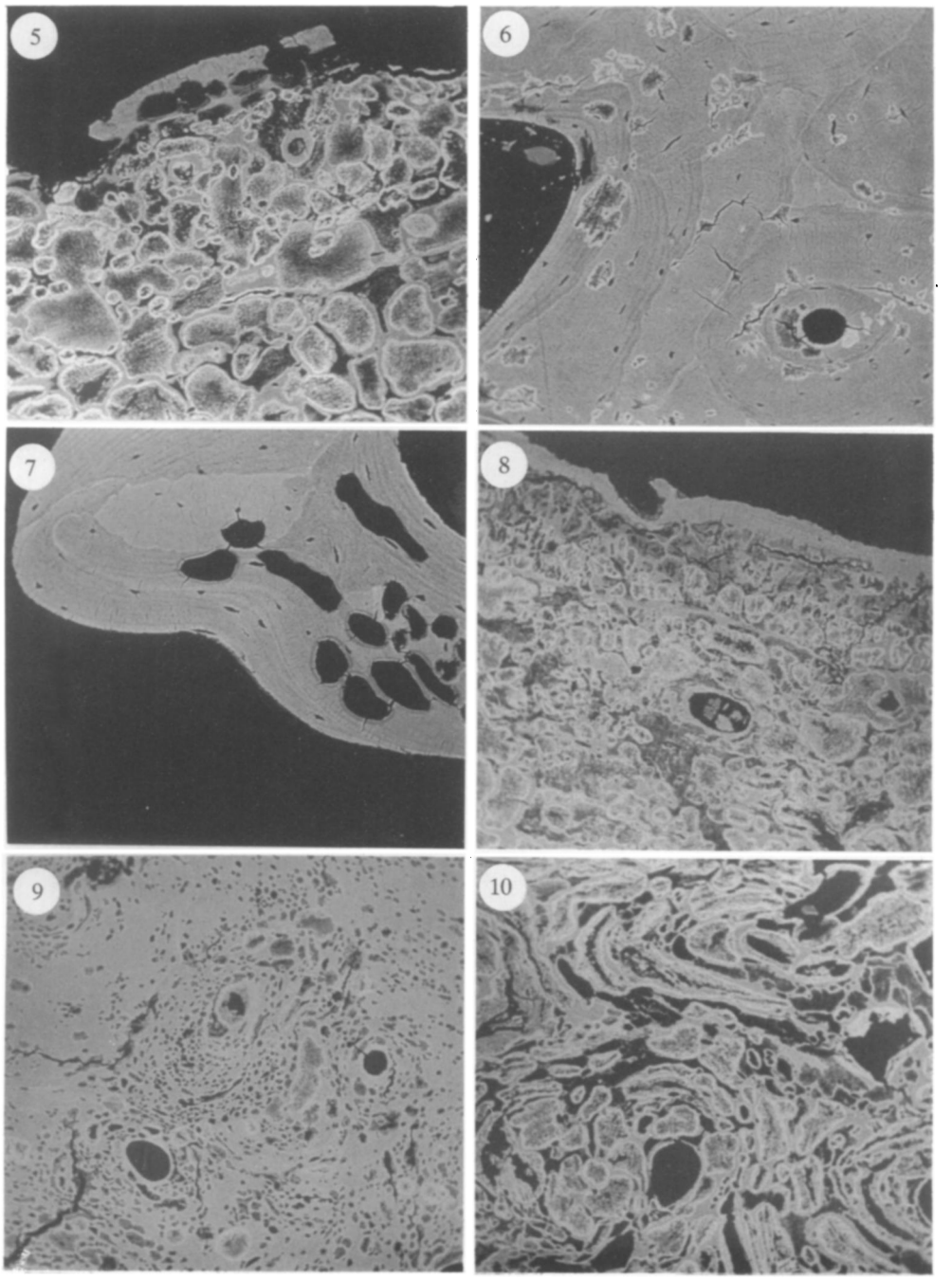

Figure 5. Specimen 3115. Transverse section (TS). Extensive diagenetic changes to bone concentrated at subperiosteal aspect. A small amount of circumferential lamellae is still intact. Field width (FW) 320 μm.

Figure 6. Specimen 3115. TS. Midcortical region exhibiting osteonal systems with localized areas of diagenetic alteration, usually centred on osteocyte lacunae. FW 320 μm.

Figure 7. Specimen 3115. TS. A single trabeculum with recognizable areas of bone, combined with focal areas of diagenetic demineralization. FW 320 μm.

Figure 8. Specimen 3117. TS. Extensive diagenetic remodelling at subperiosteal aspect. Several circumferential lamellae remain intact. FW 330 μm.

Figure 9. Specimen 3117. TS. Midcortical region showing a moderate amount of diagenetic remodelling. FW 330 μm.

Figure 10. Specimen 3117. TS. Region close to medullary aspect affected by extensive diagenesis. FW 330 μm.

glacial origin. Gravel deposits vary in their pH values depending on geographical location, but calcareous gravels are alkaline and are favourable to the gross preservation of bone (Brothwell 1981). The soils of Abingdon are alkaline–neutral, depending on the degree of mixing of the gravel into the overlying soils by human agencies such as ploughing. The large mediaeval town cemetery was in use for more than 400 years and consisted of more than 20 layers of graves overlaying and intercutting each other. In consequence, the soil around the skeletons was very mixed and reworked, resulting in a friable loam with varying

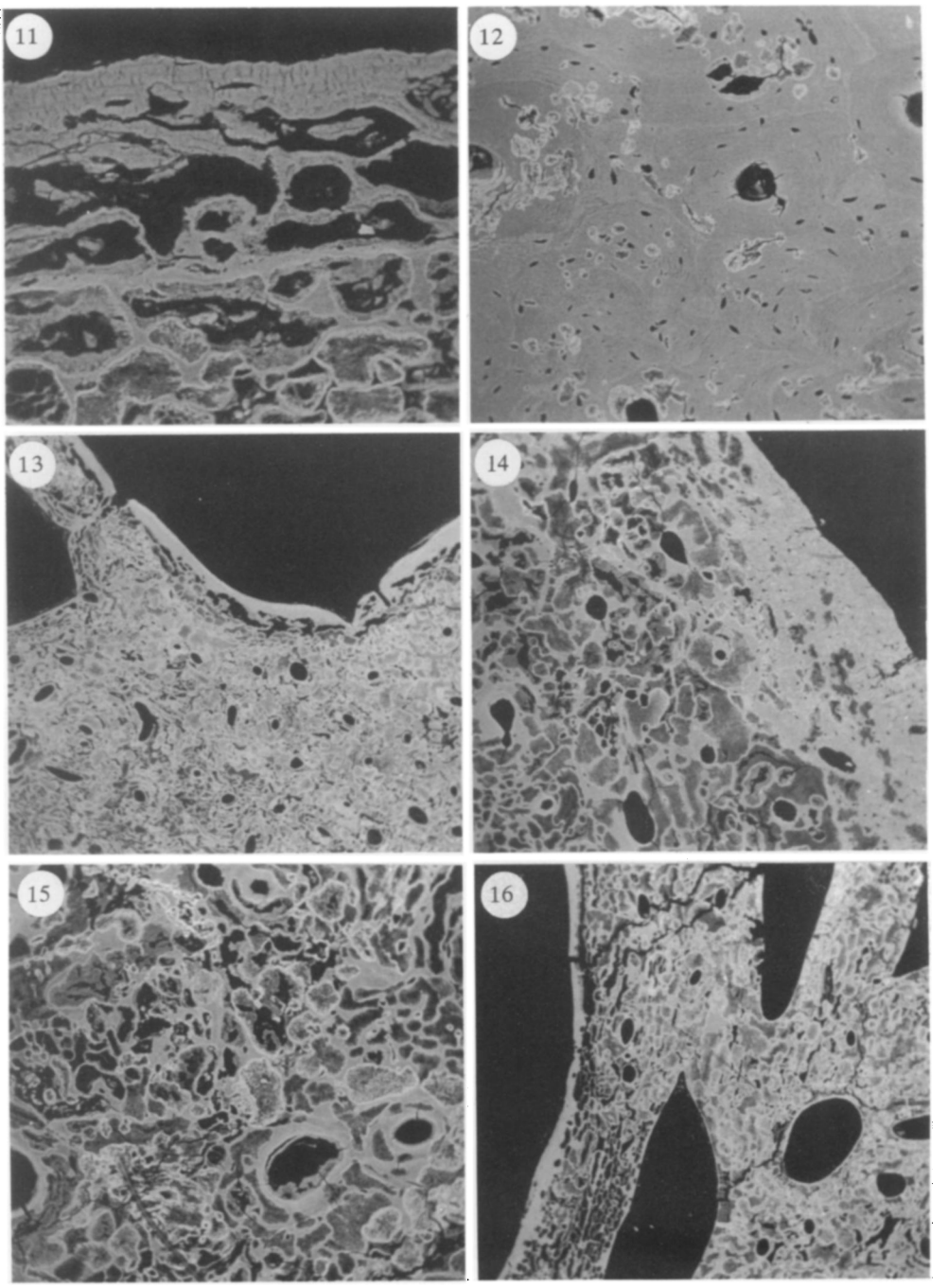

Figure 11. Specimen 3118. TS. Extensive diagenetic remodelling increasing and decreasing overall density. Some circumferential lamellae are intact with two osteocyte lacunae visible. FW 150 μm.

Figure 12. Specimen 3118. TS. Small area of midcortical bone with osteonal systems and interstitial lamellae altered by focal diagenetic lesions, many incorporating osteocyte lacunae. FW 365 μm.

Figure 13. Specimen 3118. TS. Medullary aspect and adjoining trabecular bone affected extensively by diagenetic remodelling. The endosteal rim remains unaltered. FW 3640 μm.

Figure 14. Specimen 3123. TS. Subperiosteal region showing extensive diagenetic remodelling, although some bone is intact at the most external aspect. FW 670 μm.

Figure 15. Specimen 3123. TS. Midcortical region extensively affected by diagenesis. Haversian canals are still evident. FW 330 μm.

Figure 16. Specimen 3123. TS. Medullary aspect of bone showing the extensive remodelling common throughout this specimen. FW 1330 μm.

percentages of gravel. No mediaeval skeletons from this cemetery have yet been examined for DNA survival (the only mediaeval bone was from the small graveyard 1 km from the town cemetery) but the mediaeval bodies, although buried much longer than those of the Civil War, were very well preserved from an archaeo-

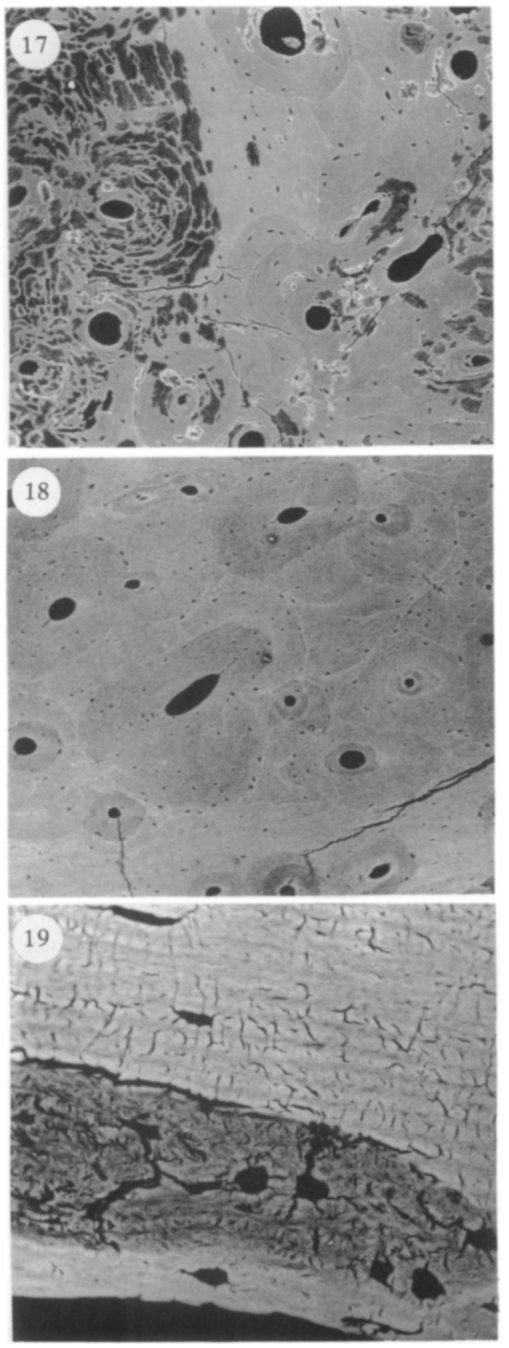

Figure 17. Specimen 3071. TS. Border between subperiosteal altered bone (left) and relatively unaltered bone (right). FW 670 μm.

Figure 18. Mediaeval specimen. TS. Typical field of excellent bone preservation. Osteonal systems with varying relative densities reflecting their maturation, with interstitial lamellae, cement lines more highly mineralized, and a network of osteocyte lacunae. FW 1260 μm.

Figure 19. Mediaeval specimen. TS. Small area of localized diagenetic demineralization situated just under the subperiosteal surface of the bone. Note enlarged osteocyte lacunae within this demineralized zone, and the evident canalicular network throughout the adjacent bone. FW 170 μm.

logical point of view. The constant reworking of the soil may have influenced gross preservation by aiding drainage and circulation of calcareous material.

In contrast, the Civil War (17th-century) cemetery was in use for less than 20 years and consisted of a single layer of discrete burials. The graves were cut into Saxon and mediaeval garden soils 0.8 m deep, below which was the orange sandy sealing gravel, although in places there were ditches and pits of Roman and earlier date filled with silty loam. Some of the graves were deep and cut into the gravel substrate, resulting in calcareous soil and good bone preservation; others were surrounded by the silt fills of earlier features or were shallow and did not reach gravel, and preservation was poor. The mass burial and the single burial 3071 were both shallow, being *ca.* 1 m and 0.9 m below the contemporary surface, and as such were in soft soil and did not cut into the gravel. The Civil War skeletons were quite orange or brown in colour on excavation, whereas the mediaeval ones were almost white, but colour was not a clear guide to gross preservation as some of the Civil War skeletons were orange but dense and well preserved.

From the point of view of tissue micromorphology, the changes to skeletal material are described in quite different terms. Here the geochemical term diagenesis is used to describe the alterations to skeletal and dental tissues after death. It includes all processes that can affect degradation and remineralization, both in and out of the ground, but excludes the effects of high temperature and pressure (Lapedes 1978; Pate & Brown 1985). This geochemical term is used commonly in archaeology to describe *post mortem* changes to bone and teeth, and has been shown histologically to be highly variable in its distribution and morphology (Clement 1963; Poole & Tratman 1978; Hackett 1981; Garland 1989; Bell 1990; Bell *et al.* 1991). Many of the changes are thought to be caused by the separate or joint action of bacteria and fungi (Wedl 1864; Hackett 1981), although other microorganisms are implicated and may be environment specific (Ascenzi & Silvestrini 1984; Bell *et al.* 1991).

In this study we show a clear relation between DNA recovery and the relative microscopic preservation of the bone. We had already assumed that DNA preservation was not directly related to the age of a specimen, as the mediaeval (13th-century) bone had consistently given better results in DNA amplification than the 17th-century bones (21 skeletons from the Civil War cemetery have been examined for DNA recovery (E. Hagelberg, unpublished observations)). The mediaeval bone showed by far the best microscopic preservation, with nearly perfect micromorphology; it also showed very good gross preservation and was very dense and light in colour. The factors contributing to this excellent preservation are not understood; the depth of burial of the skeletons in the small mediaeval graveyard was difficult to measure owing to topsoil stripping caused by extensive building works, but was probably only 0.5 to 1 m in depth from the contemporary ground level (Harman & Wilson 1981).

Relatively uniform preservation might have been expected within the mass grave from the Civil War, as all the skeletons were of young men of similar age, buried at the same time. However, skeleton 3123 was extremely badly preserved, with almost complete *post*

mortem remodelling; the sample taken from this skeleton has not yielded amplifiable DNA so far. The skeleton was buried slightly deeper than the other skeletons at the foot of the grave, partly covered by two other bodies, but this alone cannot explain its poor preservation compared with the three other samples from the mass grave, 3115, 3117 and 3118. These showed similar preservation to 3071 (the sample from the single grave adjacent to the mass grave).

These results show that significant variability in preservation can occur within an archaeological site, whether described as gross preservation, microstructure, or DNA recovery. In population surveys of cemeteries it would be necessary to take several samples from the skeletons to be studied, particularly if a skeleton exhibited poor gross preservation, to maximize the chance of recovering some well-preserved tissue. Our study suggests that even in poorly preserved bones there might be regions of bone with unchanged morphology, particularly in the inner third of the cortex, between the diagenetically remodelled endosteal and periosteal layers. Histological screening of skeletal samples would optimize DNA recovery.

These results are encouraging for future research in bone DNA typing, although it remains to be seen to what extent DNA can be amplified from very ancient bone. Although many technical problems need to be solved, not least how to avoid or at least monitor contamination from modern DNA (Hagelberg & Clegg 1991) and the occurrence of PCR artefacts such as 'jumping PCR' (Pääbo *et al.* 1990), PCR and sequencing of bone DNA will become essential tools in anthropology and palaeontology.

This work was supported by a research grant to E.H. under the NERC Special Topic in Biomolecular Palaeontology. The Abingdon Vineyard project was funded by the Vale of the White Horse District Council. The microscopic examination of the bone samples was supported by grants from the MRC, SERC and the Central Research Fund of the University of London.

REFERENCES

Allen, T. 1989 Abingdon Vineyard redevelopment. *South Midl. Archaeol.* **19**, 44–47.

Allen, T. 1990*a* Abingdon Vineyard redevelopment. *South Midl. Archaeol.* **20**, 73–74.

Allen, T. 1990*b* Abingdon. *Curr. Archaeol.* **121**, 24–27.

Anderson, S., Bankier, A. T., Barrell, B. G., de Bruijn, M. H. L., Coulson, A. R., Drouin, J., Eperon, I. C., Nierlich, D. P., Roe, B. A., Sanger, F., Schreier, P. H., Smith, A. J. H., Staden, R. & Young, I. G. 1981 Sequence organisation of the human mitochondrial genome. *Nature, Lond.* **290**, 457–465.

Ascenzi, A. & Silvestrini, G. 1984 Bone-boring marine micro-organisms: an experimental investigation. *J. hum. Evol.* **7**, 409–420.

Bell, L. S. 1990 Palaeopathology and diagenesis: an SEM evaluation of structural changes using backscattered electron imaging. *J. Archaeol. Sci.* **17**, 85–102.

Bell, L. S., Boyde, A. & Jones, S. J. 1991 Diagenetic alteration to teeth *in situ* illustrated by backscattered electron imaging. *Scanning* **13**. (In the press.)

Brothwell, D. R. 1981 *Digging up bones: the excavation, treatment and study of human skeletal remains*, 3rd edn. Oxford University Press.

Clement, A. J. 1963 Variations in the microstructure and biochemistry of human teeth. In *Dental anthropology*. ed. D. R. Brothwell). Pergamon Press.

Garland, A. N. 1989 Microscopical analysis of fossil bone. *Appl. Geochem.* **4**, 215–229.

Golenberg, E. M., Giannasi, D. E., Clegg, M. T., Smiley, C. J., Durbin, M., Henderson, D. & Zurawski, G. 1990 Chloroplast DNA sequence from a Miocene *Magnolia* species. *Nature, Lond.* **344**, 656–658.

Hackett, C. J. 1981 Microscopical focal destruction (tunnels) in excavated human bones. *Med. Sci. Law* **21**, 243–265.

Hagelberg, E. & Clegg, J. B. 1991 Isolation and characterization of DNA from archaeological bone. *Proc. R. Soc. Lond.* B **244**, 45–50.

Hagelberg, E., Gray, I. C. & Jeffreys, A. C. 1991 Identification of the skeletal remains of a murder victim by DNA analysis. *Nature, Lond.* **352**, 427–429.

Hagelberg, E., Sykes, B. & Hedges, R. 1989 Ancient bone DNA amplified. *Nature, Lond.* **342**, 485.

Hänni, C., Laudet, V., Sakka, M., Begue, A. & Stehelin, D. 1990 Amplification of mitochondrial DNA fragments from ancient human teeth and bones. *C.r. Acad. Sci., Paris.* **310**, Series III, 365–370.

Harman, M. & Wilson, B. 1981 A mediaeval graveyard beside Faringdon Road, Abingdon, *Oxoniensia* **46**, 56–61.

Higuchi, R., Bowman, B., Freiberger, M., Ryder, O. A. & Wilson, A. C. 1984 DNA sequences from the quagga, an extinct member of the horse family. *Nature, Lond.* **312**, 282–284.

Higuchi, R., von Beroldingen, C. H., Sensabaugh, G. F. & Erlich, H. A. 1988 DNA typing from single hairs. *Nature, Lond.* **332**, 543–546.

Horai, S., Hayasaka, K., Murayama, K., Wate, N., Koike, H. & Nakai, N. 1989 DNA amplification from ancient human skeletal remains and their sequence analysis. *Proc. Japan Acad.* **65**, 229–233.

Iscan, M. Y. 1988 Rise of forensic anthropology. *Yearb. phys. Anthrop.* **31**, 203–230.

Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Pääbo, S., Villablanca, F. X. & Wilson, A. C. 1989 Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc. natn. Acad. Sci. U.S.A.* **86**, 6196–6200

Lapedes, D. N. (ed.) 1978 *McGraw-Hill encyclopedia of the geological sciences* pp. 153–155. New York: McGraw-Hill.

Maniatis, T., Frisch, E. F. & Sambrook, J. 1982 *Molecular cloning: a laboratory manual.* Cold Spring Harbor University press.

Pääbo, S. 1985 Molecular cloning of ancient Egyptian mummy DNA. *Nature, Lond.* **314**, 644–645.

Pääbo, S. 1989 Ancient DNA: extraction, characterization, molecular cloning, and enzymatic amplification. *Proc. natn. Acad. Sci. U.S.A.* **83**, 1939–1943.

Pääbo, S., Gifford, J. A. & Wilson, A. C. 1988 Mitochondrial DNA sequences from a 7,000-year-old-brain. *Nucl. Acids Res.* **16**, 9775–9787.

Pääbo, S., Higuchi, R. G. & Wilson, A. C. 1989 Ancient DNA and the polymerase chain reaction. *J. biol. chem.* **264**, 9709–9712.

Pääbo, S., Irwin, D. M. & Wilson, A. C. 1990 DNA damage promotes jumping between templates during enzymatic amplification. *J. biol. chem.* **265**, 4718–4721.

Pate, F. D. & Brown, K. A. 1985 The stability of bone strontium in the geochemical environment. *J. hum. Evol.* **14**, 483–491.

Poole, D. F. G. & Tratman, E. K. 1978 Post-mortem changes on human teeth from late upper Palaeolithic–Mesolithic occupants of an English limestone cave. *Arch. Oral Biol.* **23**, 1115–1120.

Saiki, R. K., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A. & Arnheim, N. 1985 Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science, Wash.* **230**, 1350–1354.

Thomas, R. H., Schaffner, W., Wilson, A. C. & Pääbo, S. 1989 DNA phylogeny of the extinct marsupial wolf. *Nature, Lond.* **340**, 465–467.

Vigilant, L., Pennington, R., Harpending, H., Kocher, T. D. & Wilson, A. C. 1989 Mitochondrial DNA sequences in single hairs from a southern African population. *Proc. natn. Acad. Sci. U.S.A.* **86**, 9350–9354.

Wedl, C. 1864 Ueber einen im Zahnbein und Knochen keimenden Pilz. *Mineral. Biol. Erdkunde* **50**, 171–193.

Wrischnik, L. A., Higuchi, R. G., Stoneking, M., Erlich, H. A., Arnheim, N. & Wilson, A. C. 1987 Length mutations in human mitochondrial DNA: direct sequencing of enzymatically amplified DNA. *Nucl. Acids Res.* **15**, 529–542.

Discussion

S. Hummel (*Institut für Anthropologie der Universität Göttingen, Göttingen, F.R.G.*). How does Dr Hagelberg distinguish between evolutionary changes in DNA sequences and those changes produced by diagenetic processes? Are there any safe tools for diagnosis?

E. Hagelberg. Direct sequencing of PCR products should distinguish between these possibilities.

S. Hummel. In the case of highly repeated sequences, direct sequencing the PCR products will solve the problem. However, in the case of amplifying sequences that are less often repeated in the genome, or present only as single copies, the problem that a 'wrong' target might be the 'parent' of the overwhelming reaction should be kept in mind, I think, especially as ancient DNA might only reveal very few intact (not nicked) targets.

T. A. Brown (*Department of Biochemistry and Applied Molecular Biology, UMIST, Manchester, U.K.*). Is the amount of human DNA present in human bones, and its length, dependent on the extent of microbial contamination present?

E. Hagelberg. Yes, the heavily contaminated bones have less human DNA. The best bones with respect to human DNA amplification are undamaged ones.

R. P. Ambler (*Institute of Cell and Molecular Biology, Division of Biological Sciences, University of Edinburgh, U.K.*). In the case of bone DNA that is destroyed by bacteria and fungi, does the decaying bone environment attract or support characteristic microorganisms (e.g. characterized by 16*S* rRNA)?

E. Hagelberg. Yes, work of this kind is being done at present in several laboratories, although I myself have not worked in this area.