PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Introduction to Molecular information and prehistory. A themed issue

Phil. Trans. R. Soc. Lond. B 1999 **354**, 3-5
doi: 10.1098/rstb.1999.0355

THE ROYAL SOCIETY

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Introduction

1. INTRODUCTION: THE INTERFACE BETWEEN PREHISTORIC STUDIES AND MOLECULAR SCIENCE

An important shift has taken place at the interface between archaeology and the life and earth sciences. Since its inception, archaeology has drawn upon the concepts and techniques of both disciplines, first because the material components of human life are largely carbon-based organic structures, and second because much archaeological evidence is extracted from sedimentary sequences. Much of what is exploited was at the cutting edge of 19th century research, for example the understanding of stratigraphy and the foundations of plant geography and ecology. During recent decades, the concerns of both earth and life sciences have moved towards issues addressed at the molecular and atomic level. Over the last few years, archaeology has moved in a similar direction as a result of two things. The first is a series of advances in analytical method that allow molecular analyses to reach back further in time, and with greater precision, to archaeological remains. The polymerase chain reaction has opened up enormous possibilites for ancient DNA analysis, and amino-acid racemization and isotope techniques have also had great impact (Evershed *et al.*, this issue; Macko *et al.*, this issue). The second is a rephrasing of archaeological questions to dovetail with the possibilities of ancient and modern molecular evidence. This has been most clear in relation to those concerning lineage and migration, which have found a very profitable interface with molecular genetics. There are various other archaeological questions, ranging from the exchange of goods through to the usage of pottery and consumption of food that increasingly have a molecular dimension. In the past decade, archaeology has grown from interfacing with the more established earth and life sciences to interfacing at their cutting edge at the molecular and atomic levels. In the conference from which this issue derived, we aimed to bring together speakers at the forefront of this new interface.

One major development in this field has been the Ancient Biomolecule Initiative (ABI), a scientific thematic programme funded by the NERC for five years, and reaching completion earlier this year. This had as its principal aim the furtherance of knowledge of the fate of biomolecules in archaeological and fossil materials. Project teams characterized the decay processes and decay products of a range of biomolecules, notably DNA, protein, carbohydrates, lipids and decay-resistant macromolecules. They applied this new information to taphonomic, palaeontological, palaeoenvironmental and archaeological problems.

Those projects relating directly to archaeology were generally based on DNA studies and issues relating to the last 10 000 years. These include human migrations, domestication of cattle, camelids and horses, and of the crops, wheat and sorghum. In biomolecular taphonomy–palaeontology, the topics spanned millions of years and included insects in amber, fossil bacteria in salt crystals, proteins in bones and teeth, insect and plant cuticles, as well as fossilization, bacterial necromass, bacterial biomarkers (hopanoids) in lake sediments, and the differential persistence of lipid biomarkers in the geological record. The various ABI project teams have presented full papers in a special issue of *Ancient Biomolecules*, published in 1998, and ABI researchers have also presented papers at the conference recorded here, alongside international scholars conducting related research.

2. REPHRASING THE QUESTIONS

The rephrasing of archaeological questions has been most clear in the context of questions about lineage and migration, that apply to the origins of the human species itself (cf. Stone & Stoneking, this issue), and to subsequent beginnings and migrations, particularly of farming communities (cf. Merriwether, this issue; Sykes, this issue), and of their plant and animal foods (Brown, this issue; MacHugh *et al.*, this issue). There has also been a shift in approaching questions about economic resources beyond their origins and spread. Connan's paper in this issue illustrates how a complex natural organic material, such as bitumen can yield precise information on exchange and provenance. Approaches are also changing to the contents of an 'empty' pot. It is one of those occasional good fortunes of archaeology that one of the major durable remains, pottery, is also relatively porous. Its pores often retain a range of diagnostic molecules, absorbed from the organic materials which the pot had contained during its original usage for storage or food preparation. Trapped within the minute pores of the pot, even some easily degradable lipids seem to be protected from degradation. Furthermore, the spatial distribution of the molecules in the clay matrix around a pot's interior can reveal a great deal about the precise use of a container (Evershed *et al.*, this issue).

An important series of questions, both of interpretation and archaeological heritage management, revolve around biomolecular survival. The number of the unsuccessful ancient DNA analyses of bone and other archaeological materials illustrate the limits to our understanding of when, and where, biomolecules survive. What is clear is that, although there is sometimes a reasonable relationship between the preservation of an organic tissue and its contained biomolecules, structurally intact tissue is no guarantee of contained intact biomolecules. The processes affecting biomolecular persistence are explored by Briggs, Collins *et al.* and Bada *et al.*, in this issue.

3. HOW PERSISTENT ARE ANCIENT BIOMOLECULES?

A feature central to the above developments has been the increased sensitivity with which we can detect and characterize ancient biomolecules, sometimes in very small quantities indeed. Our abilities to do so depend, not only on our analytical methods, but also on the scope for those molecules' persistence in the ground. The complex biomolecules that are continuously rebuilt and repaired within living systems invariably have some tendency to break down after the death of that system. In the short term, measured in days and years, the agents of breakdown are autolytic enzymes and other biological systems feeding on their remains. In the longer term, measured in thousands or millions of years, the chemical processes of hydrolysis and oxidation are the principal drivers. The relationship between physical, biological and chemical processes of breakdown is itself quite complex, the outcome of certain processes setting the boundary conditions for other processes. To take the example of bones; whether the bones were initially scavenged, buried or burnt could first transform the biological decay, in turn triggering varied longer-term trajectories of chemical breakdown. The logic of this variation is gradually being revealed, for example by the work of Bada *et al.* (this issue) and Collins *et al.* (this issue).

It is clear that persistence varies from setting to setting. It is not only a function of the stability of the original molecule, however, but also the impact of diagenetic changes. Alteration of molecules may, in some circumstances, enhance their preservation potential. Investigating the influence of environment on the chemistry of fossilization and biomolecular preservation requires an interdisciplinary approach and documentation of the geochemistry and occurrence of different categories of organic remains in various ancient environmental settings is fundamental (Briggs, this issue).

Archaeologists have tended to be most familiar with the initial, biological breakdown processes, which have most conspicuously acted upon specimens that are hundreds or thousands of years old. For that reason, much emphasis in collection has been placed upon sediments in which either oxygen or water have been excluded, in other words the extremes of wet (waterlogged, air-free) and dry. What has been underestimated is the importance of factors affecting chemical, as well as biological, breakdown. Whilst waterlogging may arrest decay sufficiently for morphologically intact biota to survive, the abundance of water may greatly accelerate hydrolysis of the component molecules. The long chain molecules in those intact biota may be considerably shortened. The rates of many chemical processes are highly dependant upon temperature, and archaeological recovery may now need to show far greater awareness of ambient temperatures, and their impact on biomolecular preservation. In hot environments, they may be of strategic value in seeking out cave sites and high altitude sites for optimal biomolecular recovery. In summary, factors affecting both biological and chemical transformation will need to be fed into decisions about archaeological recovery. Persistence of gross biological form, through the arrest of normal decay, is not a direct indication of the persistence of its component biomolecules.

Points in the earth's crust in which biological and chemical processes are truly arrested are proving to be rare indeed. Certain species of bacteria are able to grow and reproduce several kilometres below the sea floor (Parkes *et al.* 1994), and other species persist within the heart of rock salt formations (Gemmell *et al.* 1998). Biomolecular persistence is rarely a simple consequence of an inactive matrix. Breakdown due to molecular cleavage is always occurring at some rate. However, it does not seem to be complete, even moving from the above extreme environments to the most biologically active soils. Here, the level of biomolecular breakdown may run as high as 99.9%, but even that leaves residues that are discernible and can be characterized by current analytical methods, especially as they accumulate to form quite substantial components of sediments.

4. CHARACTERIZATION OF BREAKDOWN PATHWAYS

The growing ability of modern analytical methods to target specific fossil tissues, even specific cells, in turn contributes to a much greater understanding of the pathways by which those molecules breakdown to form the fossil record. As indicated above, understanding of organic breakdown within archaeology has tended to focus upon general redox potential, pH and hydrological conditions, and how they effect the common categories of decay organisms. Within the earth sciences, there has been a greater emphasis on chemical change, but a high level of generalization about the biological pathways from which breakdown was initiated. In recent years, the ability to analyse small quantities has brought an understanding of chemical and biological process together. As a consequence, the contribution of particular biological taxa to very old organic sediments can be established (Briggs, this issue), and chemical transformation can be placed in the context of biological microstructure in the more recent archaeological specimens (Collins *et al.*, this issue).

5. THE BREAKDOWN OF DNA

One of the most tantalizing molecules, from the point of view of breakdown, is DNA. This in part has to do with the great interest shown in the progressive back-dating of ancient DNA records over the last decade, with

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

claims of detection in specimens up to 100 000 000 years old (Austin *et al*. 1998). Lindahl (1997) put forward a series of kinetic arguments against such long persistence, which have largely been supported by the work of Austin *et al*. (1998) which showed that the earliest claims were not reproducible. There is certainly a consensus that, in cold climates at least, preservation of DNA of 50 000 years of age is in both theory and practice acceptable, and accounts for DNA from, amongst other things, extinct woolly mammoth and Neanderthal teeth. The case is however not closed for much earlier DNA, particularly in water-free micro-sites (cf. Bada *et al*., this issue).

Whatever the case as regards the persistence of pre-Quaternary DNA, there is now a much greater realism about the potential and status of ancient DNA analysis within the context of wider genetic studies. It is widely agreed that the place of ancient DNA analyses is to address highly specific questions generated from a much broader analysis of modern variation. Those questions will often relate to the precise dates and contexts only available through archaeology and also to recently extinct varieties and species with a direct bearing on surviving species.

6. PRESERVING MOLECULAR INFORMATION

Much of this issue is concerned with the kinds of molecular information that are available to assist our understanding of prehistory. That new understanding needs also to feed back into how our material heritage is curated and preserved. At a very simple level, the kind of work described by Evershed *et al*. (this issue) has clear implications for the desirable treatment of excavated pottery fragments, and particularly for the practices of 'cleaning' and storage. One of the most important organic materials in archaeology is skeletal material, such as tooth, bone and antler. Their preservation has often been thought of as an issue primarily of the matrix pH, in relation to the solubility of the mineral component. The research described by Collins *et al*. (this issue) opens up a whole range of other issues about the preservation of molecular information within them.

Another major organic material of archaeological importance is 'peat' and other anoxic accumulations of partially decomposed organic material, generally referred to as waterlogged material. While we understand in very general terms that the low levels of oxygen are central to preservation in these conditions, our specific understanding is limited, and that affects our ability to predict and control preservation *in situ*. Briggs (this issue) argues that we cannot reduce survival within peat to any single variable, and certainly not age alone. The local environment of objects in peat will often constitute the main control, with pH and sediment type interacting with biomolecular degradation in complex ways.

7. BIOMOLECULAR ARCHAEOLOGY

As mentioned at the outset, the molecular approach to prehistory is in its infancy. However even in the ten years since the polymerase chain reaction gave the ancient DNA field a significant boost, it has gone through a series of transformations. Many of the earlier contributions can be seen as rather particular 'showcase' examples, featuring the earliest or the most spectacular find. This was followed, in the early days of the ABI, by excessive optimism about how ancient biomolecules on their own might answer many of archaeology's key questions. Where that optimism paid off, this phase did yield some very impressive case studies, such as the research into American colonization discussed by Merriwether (this issue), and the Pacific work discussed by Hagelberg *et al.* (this issue). This has in turn been followed by a more sanguine approach, with a better and more cautious understanding of biomolecular persistence, and a selective and targeted use of information about ancient molecules in the context of a sound understanding of the present, such as is illustrated by Sykes' paper in this issue. However, the work has only just begun, and the results described in these papers are only the beginnings of what will undoubtedly remain a fruitful interplay between the cutting edge of a range of research disciplines. Palaeoclimatology, molecular archaeology and human prehistory have a synergy which cries out for intensive development.

REFERENCES

Austin, J., Smith, A. B., Fortey, R. A. & Thomas, R. H. 1998 Ancient DNA from amber inclusions: a review of the evidence. *Ancient Biomolecules* (NERC ABI Special Issue). (In the press.)

Gemmell, R. T., McGenity, T. J. & Grant, W. D. 1998 Use of molecular techniques to investigate possible long-term dormancy of halobacteria in ancient halite deposits. *Ancient Biomolecules* (NERC ABI Special Issue). (In the press.)

Lindahl, T. 1997 Facts and artifacts of ancient DNA. *Cell* **90**, 1–3.

Parkes, R. J., Cragg, B. A., Bale, S. J., Getliff, J. M., Goodman, K., Rochelle, P. A., Fry, J. C., Weightman, A. J. & Harvey, S. M. 1994 A deep bacterial biosphere in Pacific Ocean sediments. *Nature* **371**, 410–413.

Martin K. Jones,
Derek E. G. Briggs,
Geoffrey Eglinton,
Erika Hagelberg