Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Molecular Preservation [and Discussion]

Geoffrey Eglinton, Graham A. Logan, R. P. Ambler, J. J. Boon and W. R. K. Perizonius

Phil. Trans. R. Soc. Lond. B 1991 **333**, 315-328
doi: 10.1098/rstb.1991.0081

Email alerting service Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click **here**

To subscribe to *Phil. Trans. R. Soc. Lond. B* go to: **http://rstb.royalsocietypublishing.org/subscriptions**

This journal is © 1991 The Royal Society

Downloaded from rstb.royalsocietypublishing.org

Molecular preservation

GEOFFREY EGLINTON[1] AND GRAHAM A. LOGAN[1,2]

[1] *Organic Geochemistry Unit, University of Bristol, School of Chemistry, Cantock's Close, Bristol BS8 1TS, U.K.*
[2] *Department of Geology, University of Bristol, Wills Memorial Building, Queens Road, Bristol BS8 1RJ, U.K.*

SUMMARY

The differing patterns of molecular abundances in organisms are fundamental to the understanding of the biomolecular palaeontological record. All organisms contain DNA, RNA, protein, polysaccharides and lipid components, together with glycolipids, lipopolysaccharides and other complex molecules. Certain biopolymers, however, are restricted in their distributions; for example, lignin, cutin and sporopollenin are found only in terrestrial plants. The detailed chemical structures, namely the bond types present and their precise intramolecular environments, determine resistance to degradation. Observations of biomolecular preservation are compared with predictions based on chemical structure and on conditions encountered during decay.

1. INTRODUCTION

Hamlet: How long will a man lie i' the earth ere he rot?
Gravedigger: Faith, if he be not rotten before he die – as we have many pocky corses now-a-days, that will scarce hold the laying in, – he will last you some eight year or nine year: a tanner will last you nine year.
Hamlet: Why he more than another?
Gravedigger: Why, sir, his hide is so tanned with his trade that he will keep out water a great while; and your water is a sore decayer of your whoreson dead body.

W. Shakespeare, *Hamlet V, i*, '*A Churchyard*'

This paper explores the relations between the molecular composition of living organisms and the nature of fossil organic matter through consideration of the mechanisms of decay and preservation. Briefly, we review what is preserved, where, when and how.

All living organisms contain the essential biopolymers: nucleic acids, proteins and carbohydrates. They also contain a wide variety of other organic compounds made up of carbon, hydrogen, nitrogen, oxygen, sulphur, phosphorus and other elements, of which the fat-soluble lipids are important components. Biomolecular palaeontology is the study of the molecular record of ancient life recorded in fossils and sediments. Studies have shown that the fossil record contains large quantities of molecular debris derived from the biomolecular input. Molecules of obvious biosynthetic origin occur in sediments of Archean age (Jackson *et al.* 1986; Summons *et al.* 1988), some as early as *ca.* 3000 Ma before present (BP). The preservation potential of biopolymers and other individual biomolecules varies considerably with molecular structure, depositional environment and diagenetic history. Indeed, biomolecular palaeontology has much to reveal concerning the processes which result in the preservation or decay of the original biomolecules. There is also the possibility of using fossil biomolecular structures for assessment of palaeoenvironments and for the study of evolution itself. The field is highly dependent upon techniques of analytical chemistry and here two separate areas of work emerge: the study of soluble components and the study of the insoluble debris.

2. MOLECULAR COMPOSITION OF BIOMASS

Table 1 lists the main groups of biomolecule in terms of the susceptibility to attack of the linkages holding the molecules together and the molecular groupings which may be attacked. The table is, of necessity, much simplified in relation to the wide variety of situations in which fossilization may occur, but an attempt is made to assign an overall preservation potential to the different compound classes. The potential for preservation is, of course, highly dependent upon the physical state of the organic material. This applies at the gross level – in terms of entrapment in mineral matrices or tissue membranes – and at the molecular level itself.

(a) *Nucleic acids*

DNA in its simplest form is a single chain macromolecule (single strand) made up of deoxyribose sugar units linked at the 5′ and 3′ positions by phosphate ester groupings. Each deoxyribose unit carries a purine or pyrimidine base attached at the 1′ position as a C–N bond. Ribonucleic acid (RNA) has the same basic structure with ribose replacing deoxyribose. The consequence of this structure in terms of preservation versus destruction is that the macromolecular nature of the chain is entirely dependent on the phosphate ester link (see figure 1).

Phil. Trans. R. Soc. Lond. B (1991) **333**, 315–328
Printed in Great Britain

The Royal Society is collaborating with JSTOR to digitize, preserve, and extend access to *Philosophical Transactions: Biological Sciences.* www.jstor.org

Downloaded from rstb.royalsocietypublishing.org

Table 1. *Fossilization of biomolecules: susceptibility to modification and destruction against preservation potential*

Compound class[a]	Susceptible linkages	Susceptible groups	Preservation potential[b]
nucleic acids (DNA, RNA)	ester (phosphate) C–N link (sugar-base)	heterocyclic rings amino	*
proteins	peptide	side-chain functionalities chiral centre	*
carbohydrates	acetal	hydroxyl amide	*
lipids including (glycolipids, lipo-polysaccharides, resins)	ester, ether, amide	hydroxyl carboxy ester	**
cutin[c], suberin[c],	ester, ether	hydroxyl, carboxy	***
cutan[c], etc.	?	?	****
sporopollenin[c], algaenan, etc.	?	?	****
lignin[c]	ether	methoxyl aromatic rings	****

[a] A more detailed list of biomolecules in relation to their preservation potential is given by Tegelaar (1990).
[b] * to **** represent a very approximate scale of resistance to degradation during decay of an organism.
[c] Restricted to vascular plants.

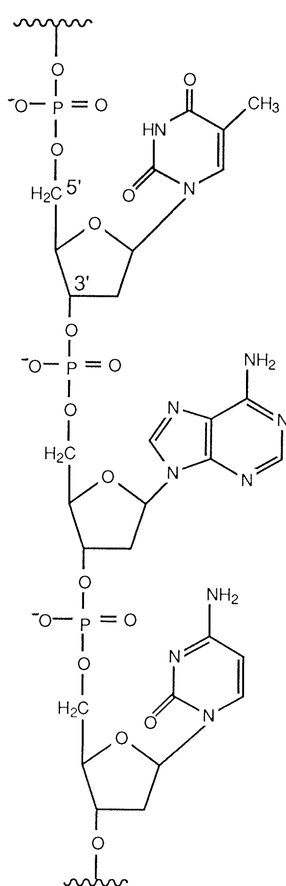

Figure 1. A three-base fragment of a DNA chain. The phosphodiester bonds link the deoxyribose sugar units at the 3′ and 5′ positions: hydrolytic cleavage of one of these bonds results in breakage of the DNA chain at that point. The bases (from top to bottom: thymine, adenine and cytosine) are attached to the sugar units at carbon 1 as a CN link, cleavage of which results in loss of the bases and hence the ability to pair with the appropriate base in the complementary chain. This ability is also lost when the bases are themselves altered, through oxidation for example.

The development of the polymerase chain reaction (PCR) has allowed the amplification of extremely low concentrations of DNA and indeed, it is theoretically possible to amplify single fragments of DNA for sequencing. A number of papers have now appeared concerning fossil DNA (see Pääbo *et al.* 1989; Golenberg, this symposium; Sidow *et al.*, this symposium). Pääbo (1989) in his paper on archaeological and sub-fossil dry soft tissues in the age range to 13000 BP, shows that where there was microscopic preservation he was able to detect up to 140 base pair (b.p.) double-strand sequences, although extensive damage could be observed in the form of DNA–DNA cross links, marked reductions in the content of cytosine and thymine bases, and the presence of fragmented and saturated pyrimidines. Alkali sensitivity was enhanced with about 1 site per 20 being present. However, much work is needed to understand properly the processes underlying the preservation and destruction of DNA and RNA molecules. It is apparent that initial rapid desiccation of tissues limits endogenous hydrolytic damage, such as the cleaving of phosphate links to produce shorter strands. Such dry tissues remain subject to oxidative damage, resulting in loss and alteration of pyrimidine bases and of sugar moieties. Pääbo has suggested that oxidative attack is rapid at first but then plateaus off, possibly owing to limited access to some DNA portions, hidden within the mass of biomolecular debris. The initial oxidative attack may be ascribed to OH radicals that are known to attack DNA bases, for example thymine is converted to thymineglycol. Oxygen and hydrogen peroxide are not thought to attack DNA, but cupric ion is reported to release hydroxyl radicals from these reagents (Aruoma & Halliwell, 1991) and hence it may be that chelation of copper ion and other metal ions, by humic acids or precipitation of copper sulphide in the presence of H_2S, could minimize oxidation in the early stages of

Downloaded from rstb.royalsocietypublishing.org

fossilization. This may explain less extensive attack under reducing conditions. Binding of the DNA to mineral surfaces may also lead to enhanced preservation (Romanowski *et al.* 1991).

(*b*) *Proteins*

Proteins are linear polymers of α-amino acid residues linked by the peptide bond (CONH). Organisms almost universally have the same 20 or so different α-amino acids making up their proteins, with the α-CH carbon having the S-(L) absolute configuration. The molecular integrity of protein molecules can be lost during fossilization through hydrolysis of the peptide links, resulting in progressively shorter chains of α-amino acid units. Such shorter units, provided that hydrolysis is non-random, may still carry sufficient information for recognition of the protein by immunological techniques. Other processes of degradation include attack on side chain functionalities, such as amino, carboxyl, hydroxy, sulphydryl and other groupings (see Ambler & Daniel, this symposium). All of these processes result in loss or partial loss of identity of protein molecules, though again, selective attack, for example on only the amino groups, could still allow possible recognition by appropriate analytical procedures. Finally, the key chiral α-carbons of the amino acid units are subject to epimerization resulting in gradual racemization of the protein molecule. This process of racemization (as measured for the amino acids themselves; for example, those liberated by hydrolysis of the debris), has received much attention as a dating and stratigraphic tool; it is discussed elsewhere in this symposium. There is clear evidence that the rate of epimerization of these C–H groups is highly dependent not only on the environmental condition but also the nature of the amino acids themselves and the intramolecular situation, i.e. protein sequence.

The tertiary structure of proteins is likely to play a major part in determining the resistance to modification during fossilization and diagenesis. Some proteins, such as myoglobin, are tightly folded on themselves through the operation of hydrogen bonding, cross linking (e.g. via disulphide bonds), dipolar interactions and involvement with water molecules. Hence, without unfolding of the molecules through the action of polar denaturing agents or appropriate enzymes, attack on interior amino acid residues may prove impossible. Likewise, tight packing through hydrogen bonding and cross linking are common features of fibrous structural proteins such as those of muscle tissue (collagen) and hair (keratin). Such structural proteins are often assumed to survive better, though little research has specifically addressed this area. Thus, collagen (figure 2), the quaternary structure of which involves a triple helix of three extended protein chains, may be expected to be especially resistant when enclosed within, and protected by, minerals. Such assemblages occur in bones and teeth. A particular example here concerns osteocalcin, an extremely acidic protein that bonds strongly to the hydroxy apatite mineral matrix of teeth and

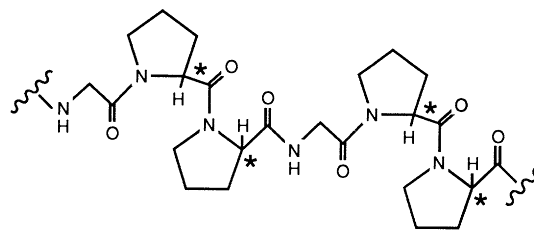

Figure 2. A portion of a single strand of the protein collagen. The six α-amino acid residue sequence from left to right is: glycine-proline-proline-glycine-proline-proline. Hydrolysis of any of the five peptide bonds (CONH) shown cleaves the chain at that point. Of the two types of α-amino acid present, only the proline units have a chiral α-CH capable of epimerization(*) and hence racemization. Three collagen strands self-assemble to form the tightly bonded triple helix that provides the elastic properties of this biopolymer.

bones, where intimate association with the mineral phase seems to protect it in part from diagenetic modification (Huq *et al.* 1985). Dehydration can lead to the preservation of protein, but protection from atmospheric air and moisture would appear to be important conditions during desiccation (Sensabaugh *et al.* 1971). Certainly, evidence of the survival of protein fragments is provided by immunological techniques (see, for example, Lowenstein (1981); Lowenstein *et al.* (1981); Lowenstein & Ryder (1985); Muyzer *et al.* (1988); Collins *et al.* (1991), whereas electrophoretic techniques were used by Robins & Brew (1990) to detect fossil proteins in the carbonate tests of 300000 years BP foraminifera.

As with the nucleic acids, both dehydration and immobilization of the protein molecules are important factors in limiting mineralization, especially in relation to microbial enzymic attack. Higher rates of degradation occur in the presence of water. Part of the reason for this lies in reactions that occur between proteins and carbohydrates, which result in melanin type polymers. Even theoretically 'anhydrous' shell glycoprotein, which is *ca.* 30% carbohydrate, could self-hydrolyse and condense (Collins *et al.* 1991).

(*c*) *Carbohydrates*

The simplest carbohydrates are monosaccharides, typically C_5 or C_6 compounds, which are water-soluble and of very low survival potential. On the other hand, many polysaccharides, formed by the condensation of the hydroxyl of one molecule with the carbonyl group of another as ketal or acetal links are long chain and branched three-dimensional structures which are insoluble in water. Again, as with the proteins, tertiary and quaternary structures probably play an important part in controlling susceptibility to mineralization. Thus cellulose (figure 3), a linear chain structural polysaccharide, forms hydrogen bonded bundles twisted together to form rope-like structures that are themselves grouped together as fibres. However, the exterior surfaces of such closely packed molecular assemblages must still exhibit many exposed hydroxyl groups and readily hydrolysed acetal links, susceptible

Downloaded from rstb.royalsocietypublishing.org

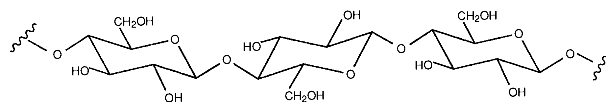

Figure 3. Portion of cellulose molecule. The D-glucose units are linked $-\beta$-1,4 to form a polyacetal chain, which is readily hydrolysed and hence depolymerized under acidic conditions. The long (*ca.* 10^3 units) chains self-assemble to form strong fibres through multiple intermolecular hydrogen bonds formed by the numerous free hydroxyl groups.

to attack by extracellular enzymes, such as those exuded by wood-rotting fungi. There is growing evidence that some portions of polysaccharides are preserved in fossilized tissues, as evidenced by pyrolysis studies and solid state ^{13}C nuclear magnetic resonance (NMR) (see for example, Wilson *et al.* (1987); Hatcher *et al.* (1989)).

(*d*) *Lignin*

Lignin (figure 4) is an important biopolymer present in vascular plant tissues. It is a heterogeneous and ill-defined polymer that varies somewhat in structure, depending on the plant species involved. Major monomer components comprise hydroxy propyl benzene (C_9) units, variously oxygenated in the alkyl group and the benzene ring. These monomers form three-dimensional structures through a variety of ether links and carbon–carbon bonds, the whole being rather resistant to alteration during early diagenesis. However, demethylation of the methoxy groups substituent upon the benzene rings is an established process (Bates *et al.* 1991). Information is limited as to the changes in structure of lignin with burial, the most used techniques for analysis being pyrolysis and controlled oxidation. None the less, lignin, as one of the more resistant biopolymers, appears to be concentrated relative to others during diagenesis (Meyers *et al.* 1980; Benner *et al.* 1984; Boon *et al.* 1989; Stout *et al.* 1988). Lignin moieties have been obtained from Carboniferous age fossil plant debris (Logan & Thomas, 1987).

(*e*) *Lipids*

Lipids are defined as that group of compounds that are soluble in organic solvents. They are of medium molecular mass and amenable to purification by fractionation and eventual characterization as individual compounds. Hence fossil lipids have provided among the best evidence so far of molecular preservation, fully detailed in terms of specific structures. Biolipids are synthesized by all organisms, typically to serve as cell membrane components and energy stores. Lipids have a wide variety of carbon skeletons made up of linear and branched chains and cyclic structures. These carbon skeletons are fully saturated, apart from occasional double bonds or substituent oxygenated functions. This aliphatic nature of most lipids results in low water solubility and hence high preservation potential, although some alteration, for example hydrogenation of double bonds, aromatization of rings,

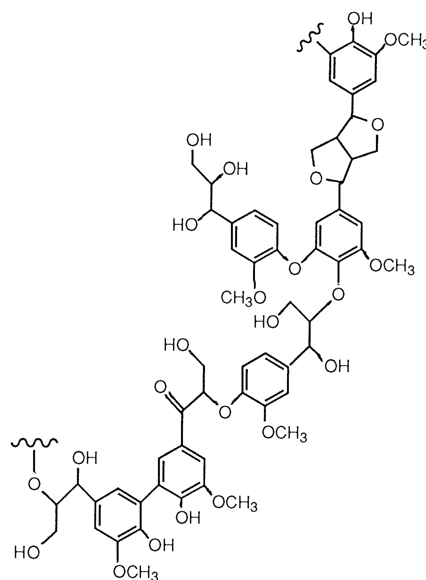

Figure 4. Stylized structure of a portion of the three-dimensional network of a softwood lignin. The various linkages (ethers, carbon–carbon) shown are drawn in a fairly arbitrary arrangement between C_9 aromatic alcohol units carrying one or more phenolic hydroxyl or methoxyl groupings on the benzene ring.

or loss of functional groups may take place during diagenesis. The realization that the structure of the original precursor biolipid may often be inferred from the structure of the resulting geolipids has led to the concept of the lipid 'biomarker'. Recognition and quantification of biomarkers may be useful in assessing palaeoenvironment conditions and processes of diagenesis (Brassell 1985; Brassell & Eglinton 1986; Brassell *et al.* 1983).

Many lipid components in organisms are linked into larger molecules via amide, ester, ether or other linkages, for example as storage lipids in the form of triglyceride esters. Once hydrolysed, the free fatty acids are more water soluble and more readily utilized by microbial enzymes and by chemical reactions requiring aqueous conditions. Here, much discussion and controversy has centred around the origin of adipocire, the mysterious waxy material sometimes found in quantity in long buried human corpses: in effect, are human body fats the source of the adipocire? The calcium salts of the typical glyceride fatty acids are apparently

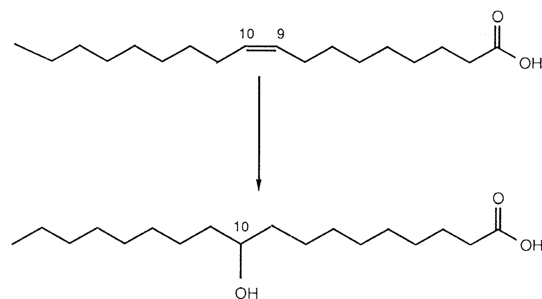

Figure 5. Microbially induced hydration of oleic acid (*cis*-$\Delta^{9,10}C_{18}$ monoacid) to 10-hydroxyoctadecanoic acid.

Downloaded from rstb.royalsocietypublishing.org

BIOLOGICAL SCIENCES THE ROYAL SOCIETY PHILOSOPHICAL TRANSACTIONS OF

major components of adipocire but there is little recent work.

The topic was described in detail by Werner Bergmann (1963) in his pioneering chapter 'The Geochemistry of Lipids' in Breger's *Organic Geochemistry*. Bergmann quotes Sir Thomas Browne's contribution of 1658:

> In an Hydropicall [dropsical] body ten years buried in a Church-yard, we met with a fat concretion, where the nitre of the Earth, and the salts and lixivious liquor of the body, had coagulated large lumps of fat, into the consistence of the hardest castle-soap (Castile soap); whereof part remaineth with us.
>
> (T. Browne, 1658 *Hydriotaphia*, *Urne-Buriall*, *or*, *A Discourse of the Sepulchrall Urnes lately found in Norfolk*, *London*. Printed for Hen. Browne at the Sign of the Gun in Ivy-Lane).

Bergmann went on to quote the findings of De Fourcroy (1790) concerning the re-organization of the Cemetery of the Innocents during the years 1786–1787. This whole topic deserves to be 'disinterred' and new information is available on the lipids found in burials retrieved from bogs. Evershed & Connolly (1988) and Evershed (1990) have demonstrated that the water-logged peat environment, which is high in humic acids and tannin-like materials, results in the preservation of some tissues, for example muscle, through modification of the collagen fibres and through toxic effects on microorganisms. However, this acidic environment does bring about rapid hydrolysis of ester links, including phosphate links in DNA and phospholipids, in triacylglycerides and steryl esters, although the conditions do seem to result in preservation of glycosoaminoglycans and glycopeptides. The aqueous environment does not solubilize the lipid components, so their concentrations remain high in the original tissues, for example cholesterol in human muscle. Where aerobic microorganisms can access materials, such as exposed skin tissues, then microbially induced hydration of unsaturated fatty acids, for example, oleic acid to 10-hydroxyoctadecanoic acid (figure 5) occurs, and likewise reduction of cholesterol to 5α- and 5β-H cholestanols. There is some evidence that shorter chain lipids, particularly those less than C_{20} which tend to be more soluble in water, are preferentially lost as a consequence of microbial activity (Fukushima *et al.* 1987) and that unsaturated fatty acids are degraded faster than saturated fatty acids (Parkes & Taylor 1983).

(*f*) *Lipid biopolymers*

Several biopolymers of lipid-like composition are listed in table 1. Cutin and suberin are biopolymers comprised mainly of fatty acid units (e.g. C_{16}, C_{18} chain length) interlinked three dimensionally through ester and ether bonds, which form the protective cuticular materials of the stems and roots of many higher plants (Holloway 1982). Sporopollenin is largely of unknown structure but is the highly resistant polymeric coating of spores and pollens (Guilford *et al.* 1988). Recently identified biopolymers, based largely on saturated carbon chains, include the cutans, algaenans, etc.; the precise structures are not fully characterized as yet but much information can be found in the article by de Leeuw *et al.* (this symposium).

Finally, unsaturated biolipids themselves can generate polymers which are largely resistant to normal diagenetic processes. Thus the hydrocarbons in plant resins polymerize to form fossil amber (Brackman *et al.* 1984; Mills *et al.* 1984), also discussed in de Leeuw's *et al.* (this symposium).

3. FOSSIL ORGANIC MATTER

The biochemical content of living organisms may be preserved within individual fossils or distributed in the sediment matrix. There is, of course, scope for research with all principal classes of biomolecule. However, to date, lipids have received the most detailed study, mainly from organic geochemists and petroleum geochemists. Free lipids are the most abundant component of the solvent-soluble extracts and mixtures of geolipids can be fraction ated and individual components identified using standard analytical practices, such as gas chromatography (GC), liquid chromatography (LC), gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–mass spectrometry (LC–MS).

Complex mixtures of organic compounds can be extracted from fossil organic matter by hydrolytic and other chemical degradation procedures. Estimates of the contents of carbohydrate, protein and other biopolymeric debris have been made using this approach, through firm inferences as to the precise molecular nature of the debris may not be drawn from such studies. Non-destructive spectroscopic techniques such as solid state NMR should be particularly valuable in this respect (Bates & Hatcher 1989).

Most fossil organic matter is insoluble and is termed 'kerogen'. Its composition is complex and heteropolymeric and is highly dependent on the original source material and the subsequent thermal history of the sediment or fossil. In most cases the kerogen would appear to contain the debris of resistant biopolymers, together with condensation, polymerization and alteration products of other biochemical components such as amino acids, sugars and lipids (Durand 1980; Tissot & Ungerer 1990). Recent studies, based on pyrolysis and chemical degradation techniques, have led to the realization that the complex heteropolymeric structures of the kerogen debris contain some well preserved, normally labile, components. For example, epimerization of chiral centres in biolipid moieties in the debris has apparently been much retarded as compared with free lipids, whereas highly functionalized lipids are evidently bound more or less intact via their functional groups. Careful work by Michaelis *et al.* (1989) has revealed that the sites of bonding of lipid biomarker molecules, such as hopanoids, steroids and acyclic isoprenoids, can be demonstrated by using specific ether cleavage reagents that place deuterium atoms at the site of bonding to the organic matrix. For example, extended hopanoids are found to be bonded at several carbon atoms in the side chain, whereas steroids are

Downloaded from rstb.royalsocietypublishing.org

Table 2. *Factors limiting molecular damage during fossilization*

(a) Nature and composition of organisms

(i) *Gross morphology and tissue composition*

These factors undoubtedly play a major part in controlling decay reactions. Hard parts, sclerotized cuticles, etc. restrict the movement of reactants. Soft tissues, with their very high water content, are readily decomposed. Biopolymers, other biochemicals and organic debris are entrapped and hence somewhat protected during formation of the crystalline matrix of shells, bones and teeth. However, trapped species include water and other reactants, thereby favouring some reactions; diffusion may still occur along crystal boundaries and fractures.

(ii) *Macromolecular structure of biopolymer assemblages*

Most cell organelles, such as chloroplasts, ribosomes and nuclei, contain biopolymers, such as proteins, carbohydrates, DNA, RNA etc. as quaternary structures, in which the individual biopolymer molecules are stacked and tightly packed into compact assemblages more resistant to attack.

(iii) *Content of reactants*

Organisms contain considerable quantities of natural antioxidants (and other controllers of radical/ionic reactions) which may continue to play a part in controlling the fossilization process. However, initial decay processes brought about by autolytic enzymes and by resident microbes are often extremely rapid and effective.

(b) Physico-chemical conditions and microbial and enzymatic processes

(i) *Movement*

Restriction of gross physical movement, including transport of organisms and debris, limits physical damage and exposure to reactants and decomposing organisms. Thus disturbance, modification and destruction through action of worms and other higher organisms are greatly reduced or eliminated in dysoxic and anoxic environments.

(ii) *Water*

Complete dehydration is not commonly achievable in natural environments. A water medium assists many reactions and biochemical and microbial activity, and in solid matrices adsorbed and interstitial water molecules may still take part in hydrolysis reactions. The absence of a water medium may facilitate gaseous diffusion of reactants.

(iii) *pH, Eh, temperature, light*

Environmental conditions, such as pH, Eh, especially in terms of microniches, exert major constraints on fossilization process, but largely on an interactive basis with the microbial population. Chemical reactions are greatly accelerated at higher temperatures but most microbial communities constantly adapt to changing circumstances.

(iv) *Ions*

Major role as microbial nutrients, electron acceptors and catalysts of hydrolysis. Chelation of metal ions is an important consideration.

(v) *Microbiota*

Microbial activity is generally the dominant aspect of decay processes, interdependent with other factors (above). Thus, electron acceptors and donors are crucial for microbial activity. Availability may be controlled by physico-chemical factors (pH, Eh, etc.), the presence of chelating agents such as humic acids, and by removal through precipitation as insoluble species (e.g. sulphides).

(vi) *Organic matter*

Plays a major part in the preservation of the remaining organic components through 'sacrificial' decomposition. Quantity and bioavailability are both important. Adsorbtion of organic matter at surfaces may stimulate or inhibit microbial decay.

(c) Factors relating to longer term survival of biomolecular information

(i) *Compaction and lithification*

Increasing compaction by overburden expels pore waters, eventually leading to greatly limited movement of ions and molecules in compacted, extremely fine grained sediments. However, reactions may be enhanced through increased contact of organic debris with clay mineral surfaces. Microbial activity will be limited as porosity and permeability tend towards low values and must cease where diagenetic cementation becomes complete.

(ii) *Radiation*

Potassium and uranium ions in clays emit radiation which causes molecular damage.

(iii) *Radicals*

Radical trapping by a variety of organic compounds inhibits attack. Radicals may be generated by radiation damage and exposure to light and other processes.

(iv) *Temperature*

Raised temperatures consequent upon deep burial effect well established major thermolytic changes in the molecular debris, eventually resulting in the generation of petroleum and natural gas. However, even the evolved hydrocarbons (*n*-alkanes, steranes, triterpanes, etc.) carry clear 'biomarker' signals of their biological origin, as employed in oil exploration.

attached at the A-ring, as would be expected for the original hydroxyl groups. This approach has been used on a contemporary humic acid fraction and on organic matter in sediments of Precambrian age, revealing the release of lipid moieties of clear microbial origin. A wider application of this analytical technique should produce valuable results.

4. PHYSICO-CHEMICAL AND BIOCHEMICAL BASIS FOR PRESERVATION VERSUS DESTRUCTION OF BIOMOLECULES

The molecular aspects of fossilization processes – 'molecular taphonomy' – have received little detailed attention until recently (Logan *et al.* 1991). Table 2 presents a brief listing of factors important to the eventual preservation of organic matter through limitation of damage to molecules following the death of the organism and its subsequent fossilization. It provides a framework for the debate that is now needed. The three main topics listed in table 2 are further discussed below under related headings.

The first two relate to the all important, although extremely brief, time interval of very early diagenesis which immediately follows death of an organism. Rapid burial in sediment and debris greatly enhances preservation, as any physical barrier reduces the availability of electron acceptors and nutrients re-

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

Table 3. *Examples of exceptional biomolecular preservation*

Type or site(s) (age)	Preserved materials	Conditions	Molecular preservation	Comments	References
bogs; Florida, The Netherlands, U.K. bogs (10^3–10^4 years)	human brains, skin	waterlogged, acidic, anoxic, high humic acids	DNA, protein (not bone), lipids	well-preserved morphology of tissues; 'tanning' processes	Evershed (1990); Evershed & Connolly (1988); Pääbo *et al.* (1988)
permafrost: Siberia, Alaska, Utah ice caves (10^4 years)	mammoth muscle, skin, hair, bones, etc.	freeze-dried in soil (desiccated), presumably < 0 °C, high humic environment?	DNA, protein, lipids	extremely well-preserved morphology	Prager *et al.* (1980); Lowenstein *et al.* (1981)
hypersaline marine bottom water: Mediterranean, Gulf of Mexico (10^3 years)	marine vascular plant debris	permanently cold (< 5 °C), anoxic, hypersaline, stratified water	lipids, carotenoid and chlorophyll pigments	well-preserved morphology	Klinkhammer & Lambert (1989)
marine upwelling sediments: Peru Margin ODP 112, Cap Blanc, Oman, etc. (10^3–10^6 years)	marine planktonic debris	high organic input, cold (< 5 °C), anoxic, high sulphide formation or methanogenesis	lipids, carotenoid and chlorophyll pigments	extensive sulphur incorporation in high sulphide sediments	Farrimond *et al.* (1990); Mossman *et al.* (1990); Repeta (1990)
marine shell deposits: U.S.A., New Zealand (10^6 years)	carbonate shells	mild alkaline, crystalline brachiopod shells, in clay matrix	glycoprotein (immunoresponse)	rapid hydrolysis and melanoidin formation	Muyzer *et al.* (1988); Collins *et al.* (1991)
tree resin (ambers): Baltic, Indonesia	cross-linked hydrocarbon copolymers, flies, etc.	oxidative cross linking in air	hydrocarbon skeletal structures including some unsaturation	solid resin prevents microbial attack and hydrolysis but allows oxidation	Mills *et al.* (1984); van Aarssen *et al.* (1990)
lacustrine mud rock: Clarkia (Idaho) (10^7 years)	complete leaves and fish	waterlogged anoxic clay, non-permeable matrix?	DNA, lipids flavanoids, carotenoid and chlorophyll derivatives	high sedimentation rate of fine clays; anoxic, stratified, humics?	Riesenberg & Soltis (1987); Golenberg *et al.* (1990)
lacustrine oil shale: Messel (Germany) (10^7 years)	plants, animals; matrix of unicellular algal cell walls	stratified, anoxic water column	biopolymers, lipids, chlorophyll derivatives including porphyrins	sub-tropical eutrophic, stratified high microbial methanogenesis	Goth *et al.* (1988); Robinson *et al.* (1989); Hayes *et al.* (1987)

quired for microbial decomposition. Even so, gas production from the decay process may physically break up the decaying organisms. Observations of decay and the evidence from the fossil record point to the norm in an aquatic environment being extremely rapid partial destruction of the biological debris within days to years. This period is then followed by a virtually exponential fall off of rate of decay thereafter, over years to millions of years, when a relatively closed, stable, sedimentary environment has been established. These kinetic considerations must apply to the biomolecular structures as well as to the gross organic debris if, for example, preservation of DNA fragments into the Miocene is to be explained. Restriction of the movement of gases and solutions must occur if biological debris is to escape complete mineralization to CO_2 and water, etc. during the period of extremely rapid early decomposition. The system must also remain 'closed'.

(*a*) *Composition of organic matter*

The composition of the organic matter undergoing fossilization in part determines the preservation potential, as different types of molecule will degrade at different rates and the particular local environments of these molecules within cells may provide different degrees of protection. This topic is addressed under the first heading in table 3 and also in § 5.

Some information is available on the comparative rates of degradation of biomolecules (Parkes *et al.* 1990; Capone & Kiene 1988; cf. the Berner (1980) G. model

Downloaded from rstb.royalsocietypublishing.org

of available organic carbon with different reactivities). In general, proteins and carbohydrates degrade more quickly than either lipids or lignin (Meyers *et al.* 1980; Benner *et al.* 1984; Hedges *et al.* 1985). Some lipid components, such as fatty acids, are much more rapidly degraded than, for example, the sterols and hydrocarbons (Cranwell 1981). These differences in bioavailability relate to the functionality of the molecules and the ease with which they can be metabolized by a decay community. The physical location of the biomolecules is also an important factor: thus cellulose is intimately mixed with lignin in plant organelles, resulting in great difficulty of enzymatic attack on either biomolecule (J. J. Boon, personal communication). Lipids sited within cuticular cutin are reported to survive degradation better than those of cellular and epicuticular wax origin during leaf decay (Wannigama *et al.* 1981). Interestingly, even some components of dissolved organic matter appear to be resistant to complete microbial degradation (Gibson *et al.* 1989; Parkes *et al.* 1990; Christensen & Blackburn 1982).

(*b*) *Microbial activity*

The role of biology, mainly microbiology, is of fundamental importance in the degradation of organic matter and in determining the physico-chemical environment in a sediment. The availability of appropriate nutrients, both electron acceptors and donors, together with the relative absence of toxic metal ions (Kelly *et al.* 1979) and the presence of the appropriate ranges in pH and ionic concentrations determine the decomposer community (Nedwell 1984). Consortia of microorganisms are present in almost all environments and quickly colonize and exploit detrital material but eventually come up against limiting physico-chemical conditions (Eglinton 1985; Capone & Kiene 1988). However, biological mediation of the taphonomic process is almost universal, as the ranges of physico-chemical parameters that may be tolerated by organisms are extremely wide. Microorganisms can metabolize approximately 99% of organic matter deposited during sedimentation (Jorgensen 1983). Indeed, preservation of readily degradable biopolymers requires exceptional conditions. Microbial activity is extremely pervasive so we must look for situations where activity is inhibited or at least restricted. However, most environments are limited in terms of energy sources – suitable reactant ions and organic compounds – rather than physico-chemical conditions.

Environmental situations where biomolecules can be included and microbial activity excluded are very rare. They include the incorporation of molecules into biominerals such as intracrystalline molecules in growing shells and entrapment of organisms in free resin which then forms a resistant polymeric mass (e.g. amber) around the organism. Desiccation and freezing of organic material can severely reduce the activity of microbes but, again, chemical reactions such as oxidation and enzymic activity generally will continue to degrade the organic matter.

Microbial activity will be restricted in environments that exhibit extremes in salinity, pH or temperature. Such physico-chemical conditions limit the microbial community available to break down organic matter. For example, in dense brine pools the extremely low bacterial diversity limits the extent of attack on biological debris (Klinkhammer & Lambert 1989). Similarly, other situations such as acidic moorland pools and peats, and high temperature thermal springs, limit the diversity of the decomposer populations. However, reduced diversity in itself does not necessarily result in reduced decomposition as very limited populations can mineralize recalcitrant compounds such as xenobiotics: thus, the rumen is specialized but efficient. It is difficult to make direct comparisons as the inputs to extreme systems are themselves very different from other environments. Another example is provided by environments high in humic acids and other phenolic geopolymers that slow anaerobic activity and thereby increase the preservation potential of biomolecules (Harvey *et al.* 1986).

Decaying organic matter may experience a series of 'zonations' representing a dominance by different groups of microorganisms and the associated chemical conditions. During decay a succession of specialized microorganisms progressively utilizes different reactants while processing substrate molecules derived from the decaying organic matter. There is a general sequence of electron acceptors ranging from O_2 in aerobic situations to CO_2 in fully anaerobic conditions (Nedwell 1984). Oxygen utilization is often limited to the top few m or cm of bottom sediment, with anaerobic conditions beneath (Revsbech *et al.* 1980; Reimers *et al.* 1986). Indeed, the absence of oxygen is claimed not to inhibit decomposition of organic matter (Calvert *et al.* 1991) in comparison with aerobic conditions. In shallow ocean waters and shelf sediments extensive sulphate reduction can be the dominant degradation mechanism (Jorgensen 1982). Even in deeper marine sediments and freshwater environments, where sulphate reduction is limited by low sulphate concentrations, anaerobic microbial degradation can continue via methanogenesis.

Recent studies as part of the Ocean Drilling Program have shown that microbial activity, and hence decomposition and modification of organic material, continues to remarkable depths in marine sediments, in excess of 500 m below the sea floor (Cragg *et al.* 1991) and that microbial activity will actually increase with depth if nutrients and electron acceptors are supplied by brine incursion from below (Parkes *et al.* 1990). Bacteria also produce their own biomass and biomarkers during decomposition and effectively add to and dilute depositional signatures.

(*c*) *Longer term survival of biomolecular information: early to late diagenesis*

Considerable progress has been made in characterizing the reactions of early diagenesis taking place in marine sediments, again largely as a result of the availability of long sediment cores from the deep ocean obtained by the Deep Sea Drilling Project, the Ocean

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Drilling Project and similar enterprises. Reactions identified to date include condensation and carbon–carbon bond formation. For example, polyunsaturated hydrocarbons undergo polymerization. Sulphur is definitely involved in early diagenesis, resulting in both cross linking via S and S–S groups and the formation of sulphur-containing organic biomarkers (Sinninghe Damste 1988; Hutchison 1990). However, the reactive sulphur species is yet to be established. Defunctionalization reactions such as the conversion of sterols to sterenes are also well established and there is good evidence for hydrolytic bond breaking (Mackenzie *et al.* 1982). Isomerization reactions include the epimerization of amino acid units in proteins and the formation of diasterenes from sterenes.

The aqueous environment of an unconsolidated sediment will favour hydrolysis reactions, involving the cleavage of ester, peptide, phosphate and other susceptible linkages of the biochemical macromolecules (polysaccharides, proteins, nucleic acids, etc.). Binding of a molecule into a macromolecular structure may help preserve it relative to unbound organic matter (Lee *et al.* 1977; Nishimura & Koyama 1977), for example, as a result of exclusion of water by the hydrophobic macromolecular debris.

Many of the diagenetic reactions mentioned above have been observed to proceed slowly over time spans of 10^5–10^7 years at the relatively low temperatures (e.g. 5–30 °C) prevailing in the deep sea sedimentary columns. The molecular changes can be followed by sampling at appropriate intervals down the core.

Further work is needed to clarify the precise nature of both early and late stage diagenetic reactions, in terms of reactants and intermediates involved, ions and free radicals. Sedimentary organic matter has been shown to contain a number of powerful antioxidants and radical trapping reagents, such as the tocopherols, which derive directly from the decaying organisms. These must limit radical reactions during diagenesis, mopping up radicals generated by radiation damage, thermolysis and other processes.

Pressure is a factor that may be important later on in diagenesis but has received little study. Lithostatic pressure helps in the compaction of the incipient fossil and its immediate environment, thereby limiting access to water, microorganisms and chemical species. Diffusion can be extremely slow in a highly compacted sedimentary situation, being further restricted as permeability and porosity are reduced through the formation of crystalline mineral cements within the pore spaces. Compression of organic debris may have a twofold effect: facilitation of intermolecular reaction, including cross-linking, through enforced proximity and, by contrast, steric inhibition of attack. Supramolecular behaviour must indeed be an important aspect of the reactivity of biomolecular debris in fossil organic matter.

5. COMPARATIVE ASPECTS OF BIOMOLECULAR PRESERVATION

Taphonomic studies may now be extended to the molecular level, whereby the environments of fossilization are considered in terms of the differential preservation of individual classes of biomolecule. Thus, in a peat bog environment, where humic acid concentrations are high and the waterlogged mass of plant debris is anoxic, animal skin and muscle tissue proteins are often well preserved, whereas bones are dissolved, some lipids are preserved and others hydrolysed and modified. Studies are needed for all important classes of biomolecule on a comparative basis in a range of environments. The interpretational framework will require chemical, biochemical and microbiological input and will need to take account of where the biomolecules reside within the decaying system, for example, whether they are localized in storage or structural organelles. Most taphonomic research to date has largely been concerned with the formation of macrofossils, mainly in regard to gross morphology, with some attention to cellular aspects. However, the underlying microbiology and chemistry are very important.

Organic geochemical studies of fossil organic matter are normally conducted with portions of unseparated sediment or entire pieces of rock. Hence, the originating biology and the fossil molecular content have been linked without the benefit of being able to locate the compounds within specific fossils or their tissues. The direct correlation of biological input with fossil product needs to be addressed: an approach we might term 'actualistic molecular taphonomy'. We need to know the relative susceptibilities of different classes of organic matter to degradation under specific conditions and the ways in which the molecular alteration processes change with respect to both space and time as organisms undergo decay and fossilization.

The main questions to be addressed in relation to the fate of biological compounds are: (i) what factors retard or stop their breakdown in the very short term following death of the organism and (ii) What factors ensure long-term survival through millions of years of burial?

(*c*) *Exceptional biomolecular preservation: some type situations*

It is valuable at this stage in the development of biomolecular palaeontology to select from the literature fossil locations that appear to represent exceptional biomolecular preservation (see table 3), according to the following three criteria. First, the locations differ markedly in physico-chemical respects, such as acidity, temperature and hypersalinity. Second, there is good documentation of exceptional preservation of organic matter in terms of morphology and amount. Third, there is evidence from organic geochemical and biochemical studies of a good preservation at the molecular level. However, it should be noted that almost all molecular palaeontological work has been done by using a single compound type or group of related compounds. Few integrated studies have been carried out to discover how different compounds have behaved within the same organism from the same environment. Some attempt has been made to categorize the key conditions relating to the

Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

preservation and also suspected causative factors. Table 3 is illustrative rather than exhaustive. We need now to locate further deposits typifying particular aspects of preservation and environmental conditions. Certainly, a number of other locations in the literature might be placed under categories listed in table 3. Allison & Briggs (1991) have provided a review of the major localities where non-mineralized tissues are preserved in the fossil record.

There is a perception (for example Butterfield (1990); Tegelaar *et al.* (1989)) that organic remains are more common than is usually appreciated, but the vast majority are presumably diagenetically altered, where preservation of unaltered biomolecules would still be very exceptional. This view needs to be supported with lists of sites and biomolecular analyses of the fossils preserved at those sites. At this stage, it would seem best to concentrate on the study of contemporary depositional environments and on sites of relatively young geological age, as conditions during very early diagenesis, or produced during early diagenesis, are critical in controlling preservation and mineralization. Thus, in table 3, most sites listed are of Quaternary age. However, one site of Miocene age is the fossil assemblage at Clarkia, Idaho, for which preservation of DNA remnants has been claimed, as discussed elsewhere in this symposium. In the following section, we illustrate the biomolecular palaeontological approach to this type locality by a specific study of the lipid content of a plant leaf fossil. Future research will need to encompass detailed studies of biopolymers and other molecular categories within samples from this and other localities if biomolecular palaeontology is to be put on to a proper comparative basis.

(*b*) *The plant fossil assemblage in the lacustrine mud rock of Miocene Age at Clarkia, Idaho, U.S.A.*

This Miocene lake deposit has been renowned for its plant fossils for some years. The site is also remarkable for the extent of inter- and multidisciplinary studies conducted on it, largely as a result of the work of Dr Jack Smiley of the University of Idaho at Moscow. However, the report by Golenberg *et al.* (1990), of the finding of magnolia DNA in a compression fossil of a magnolia leaf at this site was an event of major significance for biomolecular palaeontology. The earlier work on organic geochemistry of these sediments and fossils has been reported in several papers (e.g. Nicklas & Giannasi 1985). This deposit had suffered no appreciable thermal stress, having been buried by, at the most, a few hundred metres of sediment over the last 20 Ma and hence, its organic matter must be 'immature', in the terminology of the organic geochemist. However, with limited analytical information, Giannasi & Nicklas (1981) claimed to find a range of saturated cyclic hydrocarbons, such as cholestane, ergostane, oleanane and gammacerane, in association with the fossil tissue. These compounds are geolipids which are normally found in 'mature' sediments and petroleums and hence are completely out of place in these highly immature sediments. Indeed, in the present work they were absent from our extracts and almost certainly derived from contamination in the earlier studies. Other claims for the Clarkia sediments and fossils included flavanone pigments, carotenoids and chlorophyll derivatives. Analytical evidence for the flavanones appears satisfactory and the finding of these compounds seems appropriate. However, the Clarkia literature contained no report of the lipid distributions that might be expected for a well-preserved leaf. We have begun a programme of work on the lipid content of the Clarkia leaf fossils and the matrix of enclosing sediment.

Figure 6 shows a GC trace for the total lipid extract of a beech (*Betula*) leaf fossil, after derivatization (trimethylsilylether derivatives). The fossil tissue had been removed from its enclosing sediment by using a scalpel and the lipids extracted by using methanol and dichloromethane in a modified Bligh and Dyer extraction procedure. The GC–MS study revealed homologous series of *n*-alkanes and *n*-alkanols with high Carbon Number Preference Indices and carbon number maxima typical of modern leaf wax lipids. The distribution of components is very similar to those of a modern leaf wax, indicating excellent preservation of the lipids with very little biodegradation or diagenesis. Functionalized steroids and pentacyclic triterpenoids are also present in the extract. In principle, it should be possible to relate the distribution patterns of such lipid components to those of modern counterpart species. However, it is evidence from our comparative studies that the lipid profiles for individual fossil leaves closely resemble those of the surrounding matrix sediments. The implications are that at least some of the leaf lipids become disseminated in the sediment matrix. Thus, it may prove difficult to discern the provenance of the lipids in such a rich fossil assemblage. The way forward may be twofold: firstly, to carry out such analyses at the microscopic level, so that the compounds may be profiled (e.g. by pyrolysis–mass spectrometry) from within the fossil leaf tissue through into the matrix. Secondly, because most of the organic matter is insoluble in organic solvents, and hence may be expected to be static within the fossil during its fossilization, then characterization of the insoluble organic debris in the leaf fossil tissue compared with that distributed in the matrix of the sediment should be highly informative. Preliminary results conducted by using ruthenium tetroxide as oxidant have shown that the amounts of carboxylic acid oxidation products generated from the fossil tissue are about 20 times greater than those from the surrounding sediment matrix (G. A. Logan & G. Eglinton, unpublished results). Such comparative studies should assist greatly in understanding the processes of preservation and destruction through which the incipient fossil passes during emplacement and fossilization.

6. CONCLUSIONS

The study of organic matter in the fossil record has revealed valuable information not obtainable from other sources, but knowledge of this record at the molecular level is still very limited. However, advances in analytical instrumentation and in techniques such as

Downloaded from rstb.royalsocietypublishing.org

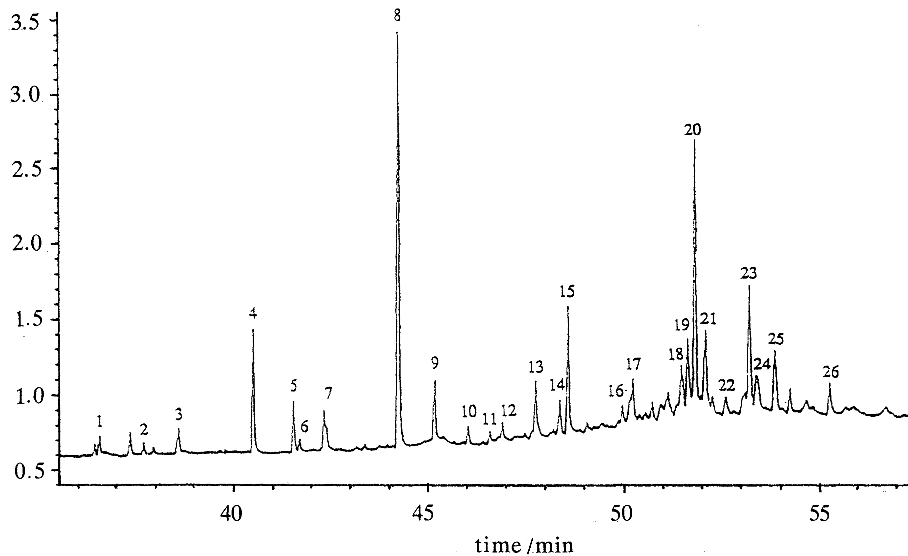

Figure 6. GC trace of total lipid extract of *Betula* fossil from Clarkia Lake deposit (Miocene), Idaho, U.S.A. Extracted by sonication in DCM, 1:1 DCM: MeOH, MeOH, evaporated under nitrogen then derivatized with BSTFA. Run on Carlo Erba 5300, 50m, Chrompack CP-Sil-5CB column, programed at 50 °C (2 min), 150 °C (10 °C min^{-1}), 310 °C (4 °C min^{-1}), isothermal 20 min Peak annotation: 1, C_{25} *n*-alkane; 2, C_{22} *n*-alcohol; 3, C_{26} *n*-alkane + unknown; 4, C_{27} *n*-alkane; 5, C_{24} *n*-alcohol; 6, perylene; 7, C_{28} *n*-alkane + unknown; 8, C_{29} *n*-alkane; 9, C_{26} *n*-alcohol; 10, C_{30} *n*-alkane; 11, hop-17(21)-ene; 12, C_{27} *n*-alcohol; 13, C_{31} *n*-alkane; 14, 5α-cholestan-3β-ol; 15, C_{28} *n*-alcohol; 16, C_{28} *n*-acid; 17, C_{29} *n*-alcohol + olean-12-ene-3-one; 18, β-amyrin; 19, 24-ethyl-5α-cholestan-3β-ol; 20, C_{30}, *n*-alcohol; 21, 4α,23,24-trimethyl-5α-cholest-22-en-3β-ol; 22, arborenone; 23, 4α,23,24-trimethyl-5α-cholestan-3β-ol; 24, friedelin; 25, $C_{30}H_{49}OTMS$ ($M^{+\cdot}$ = 498); 26, C_{32} *n*-alcohol.

PCR now allow very low concentrations of organic compounds to be studied and even the insoluble organic debris is beginning to yield molecular information. The interpretation of the new molecular data will require the development of a parallel understanding of molecular taphonomy, i.e. the processes of decay at the molecular level and their consequences in terms of biasing the eventual record. Experiments following decay under different environmental conditions are needed. The complexity of the problem, the variety of decay processes and the range of decomposer communities of microorganisms are so great that there will be a need to limit variables through careful laboratory experiments as well as studies of contemporary environments. To understand palaeoenvironments we need to develop rational models for the sedimentary conditions giving rise to exceptional preservation of biological debris. Table 2 is an attempt to summarize the factors limiting molecular damage during fossilization. The treatment is necessarily simplistic and incomplete but at least represents a start.

It is apparent that immobilization of the molecular debris and the limitation or prevention of access by reactive species, including compounds required for microbial metabolism, is crucial to preservation at the molecular level. Location of the molecular structures within protective material, whether it be other organic matter or mineral matrix, is perhaps the single most important factor, as illustrated by the enhanced preservation obtained within certain tissues and within the mineral matrices of bones and shells. Compression of the debris and the presence of tightly hydrogen bonded and covalently cross-linked portions of the molecular structures must sterically inhibit attack on them. Such considerations may explain the existence of a few replicable lengths of DNA sequence even where almost all the original DNA has been destroyed. Presumably largely intact fragments lie deeply buried under much degraded and altered debris, much as a weathered crust may protect an interior portion of an exposed sedimentary rock. Furthermore, we can speculate that carefully chosen biochemical techniques may prove effective in repairing damaged fossil DNA or RNA fragments in the laboratory, just as repair enzyme systems do in living cells. PCR could then be used to amplify sufficient of the repaired fragment to allow sequencing of small sections of the fossil molecule.

We thank Dr Derek Briggs, Dr Mathew Collins and Dr John Parkes for helpful and detailed criticisms of the manuscript, and Dr Jack Smiley for advice and assistance in obtaining Clarkia fossils. G.A.L. thanks the Natural Environment Research Council for a studentship GT4/88/GS11. NERC is also thanked for the funding of GC and GC–MS analytical facilities (GR3/6619).

REFERENCES

Allison, P. A. & Briggs, D. E. G. 1991 Taphonomy of non-mineralized tissues. In *Taphonomy – releasing the data locked in the fossil record* (ed. P. A. Allison & D. E. G. Briggs). New York: Plenum Press.

Aruoma, O. I. & Halliwell, B. 1991 DNA damage and free radicals. *Chem. Brit.* **27**, 149–151.

Bates, A. L. & Hatcher, P. G. 1989 Solid-state 13C NMR studies of a large fossil gymnosperm from the Yaallourn Open Cut, Latrobe Valley, Australia. *Org. Geochem.* **14**, 609–617.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

Bates, A. L., Hatcher, P. G., Lerch, H. E., Blaine Cecil, C., Neuzil, S. G. & Supardi 1991 Studies of a peatified angiosperm log cross section from Indonesia by nuclear magnetic resonance spectroscopy and analytical pyrolysis. *Org. Geochem.* **17**, 37–45.

Benner, R., Maccubbin, A. E. & Hodson, R. E. 1984 Anaerobic biodegradation of the lignin and polysaccharide components of lignocellulose & synthetic lignin by sediment microflora. *Appl. envir. Microbiol* **47**, P998–1004.

Bergmann, W. 1963 Geochemistry of lipids. In *Organic geochemistry* (ed. I. A. Breger), pp. 503–542. Oxford: Pergamon.

Berner, R. A. 1980 In *Biogeochemistry of organic matter at the sediment water interface*, pp. 35–44. CNRS Int. Colloq.

Boon, J. J., Stout, S. A., Genuit, W. & Spackman, W. 1989 Molecular palaeobotany of *Nyssa* endocarps. *Acta bot. neerl.* **38**, 391–404.

Brackman, W., Spaargaren, K., van Dogen, J. P. C. M., Couperus, P. A. & Bakker, F. 1984 Origin and structure of the fossil resin from an Indonesian Miocene coal. *Geochim. cosmochim. Acta* **48**, 2483–2487.

Brassell, S. C., Eglinton, G. & Maxwell, J. R. 1983 The geochemistry of terpenoids and steroids. *Biochem. Soc. Trans.* **11**, 575–586.

Brassell, S. C. & Eglinton, G. 1986 Molecular geochemical indicators in sediments. In *Organic marine geochemistry* (ed. Mary L. Sohn), pp. 10–32. American Chemical Society.

Brassell, S. C. 1985 Molecular changes in sediment lipids as indicators of systematic early diagenesis. *Phil. Trans. R. Soc. Lond.* A**315**, 57–75.

Butterfield, N. J. 1990 Organic preservation of non-mineralizing organisms and the taphonomy of the Burgess Shale. *Palaeobiology* **16**, 272–286.

Calvert, S. E., Karlin, R. E., Toolin, L. J., Donahue, D. J., Southon, J. R. & Vogel, J. S. 1991 Low organic carbon accumulation rates in Black Sea sediments. *Nature, Lond.* **350**, 692–695.

Capone, D. G. & Kiene, R. P. 1988 Comparison of microbial dynamics in marine and freshwater sediments: contrasts in anaerobic carbon catabolism. *Limn. Oceanogr.* **33**, 725–749.

Christensen, D. & Blackburn, T. H. 1982 Turnover of ^{14}C labelled acetate in marine sediments. *Mar. Biol.* **71**, 113–119.

Collins, M. J., Muyzer, G., Curry, G. B., Sanberg, P. & Westbroek, P. 1991 Macromolecules in brachiopod shells: characterization and diagenesis. *Lethaia* **24** (4). (In the press.)

Cragg, B. A., Harvey, M. J., Fry, J. C., Herbert, R. A. & Parkes, R. J. 1991 Bacterial biomass and activity in the deep sediment layers of the Japan Sea, Hole 798B ODP Leg 128. *Proc. ODP.* (In the press.)

Cranwell, P. A. 1981 Diagenesis of free and bound lipids in terrestrial detritus deposited in a lacustrine sediment. *Org. Geochem.* **3**, 79–89.

Damste, J. S. 1988 *Organically-bound sulphur in the geosphere: a molecular approach.* Ph.D. thesis, University of Delft.

Durand, B. (ed.) 1980 *Kerogen: insoluble organic matter from sedimentary rocks.* (519 pages.) Paris: Editions Technip.

Eglinton, G. 1985 The alchemy of life: probing the molecular record. In *Science and uncertainty* (ed. S. Nash), pp. 192–219. Northwood, Middlesex: Science Reviews Ltd.

Evershed, R. P. 1990 Lipids from samples of skin from seven Dutch Bog Bodies: preliminary report. *Archaeometry* **32**, 139–153.

Evershed, R. P. & Connolly, R. C. 1988 Lipid preservation in Lindow Man. *Naturwissenschaften* 75, 143–145.

Farrimond, P., Poynter, J. P. & Eglinton, G. 1990 Molecular composition of sedimentary lipids off the Peru Margin, Leg 112. *Proc. Ocean Drill. Prog. sci. Res.*, Site 679–688, **112**, 547–554.

Fukushima, K., Yamamoto, S., Uzaki, M., Morinaga, S. & Ishiwatari, R. 1987 Characterization of the microbial degradation products of a submerged plant with particular reference to the production of the kerogen-like material. *Chem. Geol.* **64**, 169–179.

Giannasi, D. E. & Nicklas, K. J. 1981 Comparative paleobiochemistry of some fossil and extant Fagaceae. *Am. J. Bot.* **68**, 762–770.

Gibson, G. R., Parkes, R. J. & Herbert, R. A. 1989 Biological availability and turnover rate of acetate in marine and estuarine sediments in relation to dissimilatory sulphate reduction. *FEMS Microbiol. Ecol.* **62**, 303–306.

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

Goth, K., de Leeuw, J. W., Putlmann, W. & Tegelaar, E. W. 1988 Origin of Messel oil shale kerogen. *Nature, Lond.* **336**, 759–761.

Guilford, W. J., Schneider, D. M., Labovitz, J. & Opella, S. J. 1988 High resolution solid state 13C NMR spectroscopy of sporopollenins from different plant taxa. *Pl. Physiol.* **86**, 134–136.

Harvey, H. R., Fallon, R. D. & Patton, J. S. 1986 The effect of organic matter and oxygen on the degradation of bacterial membrane lipids in marine sediments. *Geochim. cosmochim. Acta* **50**, 795–804.

Hatcher, P. G., Breger, I. A., Szeverenyi, N. & Maciel, G. E. 1989 Nuclear Magnetic Resonance studies of ancient buried wood: II. Observations on the origin of coal from lignite to bituminous coal. *Org. Geochem.* **14**, 9–18.

Hayes, J. M., Takiguiku, R., Ocampo, R., Callot, H. J. & Albrecht, P. 1987 Isotopic composition and probable origins of organic molecules in the Eocene Messel shale. *Nature, Lond.* **329**, 48–51.

Hedges, J. I., Cowie, G. L., Ertel, J. R., Barbour, R. J. & Hatcher, P. G. 1985 Degradation of carbohydrates and lignins in buried woods. *Geochim. cosmochim Acta* **49**, 701–711.

Holloway, P. J. 1982 The chemical constitution of plant cutins. In *The plant cuticle*, Linnean Society Symposium Series, 10 (ed. D. F. Cutler, K. L. Alvin & C. E. Price), pp. 45–48. London: Academic Press.

Huq, L. N., Rambaud, S. M., Teh, L. C., Davies, A. D., McCulloch, B., Trotter, M. M. & Chapman, G. E. 1985 Immunochemical detection and characterization of osteocalcin from Moa bone. *Biochem. biophys. Res. Commun.* **129**, 714–720.

Hutchison, J. 1990 *Structural studies of sulphur-rich sedimentary organic matter.* Ph.D. thesis, University of Bristol.

Jackson, M. J., Powell, T. G., Simmons, R. E. & Sweet, I. P. 1986 Hydrocarbon shows and petroleum source rocks in sediments as old as 1.7×10^9 years. *Nature, Lond.* **322**, 727–729.

Jorgensen, B. B. 1983 Processes at the sediment-water interface. In *The major biogeochemical cycles and their interactions* (ed B. Bolin & R. B. Cook), pp. 477–515. Chichester: Scope, John Wiley & Sons.

Jorgensen, B. B. 1982 Mineralization of organic matter in the seabed – the role of sulphate reduction. *Nature, Lond.* **296**, 643–645.

Kelly, D. P., Norris, P. R. & Brierley, C. L. 1979 Microbiological methods for the extraction and recovery of metals. In *Microbial technology: current state, future prospects* (ed. A. T. Bull, D. C. Ellwood & C. Ratledge), pp. 263–308. Cambridge University Press.

Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Klinkhammer, G. P. & Lambert, C. E. 1989. Preservation of organics during salinity excursions. *Nature, Lond.* **339**, 271–274.

Lee, C., Gagosian, R. B. & Farrington, J. W. 1977 Sterol diagenesis in Recent sediments from Buzzards Bay, Massachusetts. *Geochim. cosmochim. Acta* **41**, 985–992.

Logan, G. A., Collins, M. J. & Eglinton, G. 1991 Preservation of biomolecules. In *Taphonomy – releasing the data locked in the fossil record* (ed. P. A. Allison & D. E. G. Briggs). New York: Plenum Press.

Logan, K. J. & Thomas, B. A. 1987 The distribution of lignin derivatives in fossil plants. *New Phytol.* **105**, 157–173.

Lowenstein, J. M. 1981 Immunological reactions from fossil material. *Phil. Trans. R. Soc. Lond.* B **292**, 143–149.

Lowenstein, J. M., Sarich, V. M. & Richardson, B. J. 1981 Albumin systematics of the extinct mammoth and Tasmanian wolf. *Nature, Lond.* **291**, 409–411.

Lowenstein, J. M. & Ryder, D. A. 1985 Immunological systematics of the quagga Equidae. *Experientia* **41**, 1192–1193.

Mackenzie, A. S., Brassell, S. G., Eglinton, G. & Maxwell, J. R. 1982 Chemical fossils: the geological fate of steroids. *Science, Wash.* **217**, 491–504.

Meyers, P. A., Leenheer, M. J., Erstfeld, K. M. & Bourbonniere, R. A. 1980 Changes in spruce composition following burial in lake sediments for 10,000 years. *Nature, Lond.* **287**, 534–535.

Michaelis, W., Richnow, H. H. & Jenisch, A. 1989 *Science and total environment*, pp. 41–50. Amsterdam: Elsevier.

Mills, J. S., White, R. & Gough, L. 1984 The chemical composition of Baltic Amber. *Chem. Geol.* **47**, 15–39.

Mossman, J. R., Aplin, A. C., Curtis, C. D. & Coleman, M. L. 1990 Sulphur geochemistry at site 680 & 686 on the Peru margin. *Proc. Ocean Drill. Prog. sci. Res.*, Site 679–688, **112**, 455–464.

Muyzer, G., Westbroek, P. & Wehmiller, J. F. 1988 Phylogenetic implications and diagenetic stability of macromolecules from Pleistocene and Recent shells of *Merceneria mercenaria* Mollusca, Bivalvia. *Hist. Biol.* **1**, 135–144.

Nedwell, D. B. 1984 The input and mineralization of organic carbon in anaerobic sediments. In *Advances in microbial ecology* vol. 7 (ed. K. C. Marshall), pp. 93–131. New York & London: Plenum Press.

Nicklas, K. J. & Giannasi, D. E. 1985 The paleobiochemistry of fossil angiosperm floras. Part II. diagenesis of organic compounds with particular reference to steroids. In *Late Cenozoic history of the Pacific Northwest* (ed. C. J. Smiley), pp. 175–183.

Nishimura, M. & Koyama, T. 1977 The occurrence of stanols in various living organisms and the behaviour of sterols in contemporary sediments. *Geochim. cosmochim. Acta* **41**, 379–385.

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

Pääbo, S. 1989 Ancient DNA: extraction, characterization, molecular cloning and enzymatic amplification. *Proc. Natn. Acad. Sci. U.S.A.* **86**, 1939–1948.

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.

Parkes, R. J. & Taylor, J. 1983 The relationship between fatty acid distributions and bacterial respiratory types in contemporary marine sediments. *Estuar. coast. Shelf Sci.* **16**, 173–198.

Parkes, R. J., Cragg, B. A., Fry, J. C., Herbert, R. A. & Wimpenny, J. W. T. 1990 Bacterial biomass and activity in deep sediments from the Peru Margin. *Phil. Trans. R. Soc. Lond.* A **331**, 139–153.

Prager, E. M., Lowenstein, J. M. & Sarich, V. M. 1980 Mammoth albumin. *Science, Wash.* **209**, 287–289.

Reimers, C. E., Fischer, K. M., Merewether, R., Smith, K. L. & Johnke, R. A. 1986 Oxygen microprofiles measured *in situ* in deep ocean sediments. *Nature, Lond.* **320**, 741–744.

Repeta, D. J. 1990 Carotenoid diagenesis in Pleistocene to Miocene sediments from the Peru Margin. *Proc. Ocean Drill. Prog. sci. Res.* Site 679–688, **112**, 567–572.

Revsbech, N. P., Sorensen, J. & Blackburn, T. H. 1980 Distribution of oxygen in marine sediments measured with microelectrodes. *Limnol. Oceanogr.* **25** (3), 403–411.

Riesenberg, L. H. & Soltis, D. E. 1987 Flavonoids of fossil Miocene Platanus and its extant relatives. *Biochem. Sys. Ecol.* **15**, 109–112.

Robbins, L. L. & Brew, K. 1990 Proteins from the organic matrix of core-top and fossil planktonic foraminifera. *Geochim. cosmochim. Acta* **54**, 2285–2292.

Robinson, N., Eglinton, G., Cranwell, P. A. & Zeng, Y. B. 1989 Messel oil shale (Western Germany): assessment of depositional palaeoenvironment from the content of biological marker compounds. *Chem. Geol.* **76**, 153–173.

Romanowski, G., Lorenz, M. G. & Wackernagel, W. 1991 *Appl. Envir. Microbiol.* **57**, 1057–1061.

Sensabaugh, G. F., Wilson, A. C. & Kirk, P. L. 1971 Protein stability in preserved biological remains. *Int. J. Biochem.* **2**, 545–557.

Sinninghe Damsté, J. S. 1988 Organically-bound sulphur in the geosphere: a molecular approach Ph.D. thesis, Tech. University of Delft.

Stout, A. A., Boon, J. J. & Spackman, W. 1988 Molecular aspects of the peatification and early coalification of angiosperm and gynosperm woods. *Geochim. cosmochim. Acta* **52**, 405–414.

Summons, R. E., Brassell, S. C., Eglinton, G., Evans, E., Horodyski, R. J., Robinson, N. & Ward, D. M. 1988 Distinctive hydrocarbon biomarkers from fossiliferous sediment of the Late Proterozoic Walcott Member, Chur Group, Grand Canyon, Arizona. *Geochim. cosmochim. Acta* **52**, 2625–2637.

Tegelaar, E. W. 1990 Resistant biomolecules in morphologically characterized constituents of kerogen: a key to the relationship between biomass and fossil fuels. Ph.D. thesis. Tech University of Delft.

Tegelaar, E. W., De Leeuw, J. W., Derenne, S. & Largeau, C. 1989 A reappraisal of kerogen formation. *Geochim. cosmochim. Acta* **53**, 3103–3106.

Tissot, B. & Ungerer, Ph. 1990 Advances in organic geochemistry, a narrow path between fundamental interests and industrial needs. *Org. Geochem.* **16**, 21–45.

van Aarssen, B. G. K., Cox, H. C., Hoogendoorn, P. & de Leeuw, J. W. 1990 A cadinene biopolymer in fossil and extant dammar resins as a source for cadinanes and bicadinanes in crude oils from South East Asia. *Geochim. cosmochim. Acta* **54**, 3021–3031.

Wannigama, G. P., Volkman, J. K., Gillan, F. T., Nichols, P. D. & Johns, R. B. 1981 A comparison of lipid components of the fresh and dead leaves and pneumatophores of the mangrove *Avicennia marina*. *Phytochemistry* **20**, 659–666.

Wilson, M. A., Verheyen, T. V., Vassallo, A. M., Hill, R. S. & Perry, G. J. 1987 Selective loss of carbohydrates from plant remains during coalification. *Org. Geochem.* **11**, 265–271.

Discussion

R. P. Ambler (*Institute of Cell and Molecular Biology, University of Edinburgh, U.K.*). What is the dynamic state of the chemical

Downloaded from rstb.royalsocietypublishing.org

environment in which the Clarkia leaves (for instance) have been preserved (water flow, Cu^{2+}/Fe^{3+} flow, O_2 potential, etc.)?

G. Eglinton. The sediments in the unfractured blocks appear to be saturated with water that must be anoxic in view of the reduced state of iron salts present (dark grey colour that changes to an orange–yellow colour on exposure to air). No information is available about the copper ion concentration.

J. J. Boon (*FOM-AMOLF, Amsterdam, The Netherlands*). Professor Eglinton's methodology in scraping his samples is rather crude. It is no longer necessary to do this because there are now mass spectrometry methods that can characterize organics on the microgram scale. I suggest, therefore, that he can be more 'microscopic' in his chemical approach. Extractions can even be done on a microlitre scale and need very small samples.

G. Eglinton. We regard our methodology as being appropriate for the present exploratory stage of examination of these fossil leaf tissues, during which our aim is to characterize both major and minor organic compounds by GC–MS techniques. In addition, milligram quantities of tissue and sediment scrapings enable us to survey portions of the fossils and adjacent regions for the distribution of extractable compounds. We are aware of the power of pyrolysis-MS techniques, such as those employed by Dr Boon at the FOM and look forward to using them in our planned joint exploration of the Clarkia fossils.

J. J. Boon. Enzymes such as cellulase do not work on dried tobacco leaves. Only after treatments with proteases do the polysaccharidases work. So there are layers covering the cell wall and perhaps the cytosolic components which help the preservation process.

G. Eglinton. I believe that supramolecular behaviour will emerge as an important factor in biomolecular palaeontological studies, for example, where portions of molecular structure are shielded from chemical, enzymatic or direct bacterial attack by other molecular structures and debris.

W. R. K. Perizonius (*Department of Anthropo-Osteology, University of Utrecht, The Netherlands*). I stress another type of preservation that, although it has nothing to do with molecular taphonomy, in my opinion is just as important, namely that of the existing scientific collections of ancient human, animal and plant remains. To survive hundreds or even millions of years does not mean that present-day bureaucracy can be survived as well. I do not know about the situation in the U.K. but I do know about the so called 'reburial issue': actions by American Indians as well as Australian Aborigines have already forced the reburial of important archaeological collections which they see as the remains of their ancestors. Biomolecular palaeontology may offer new arguments against these financial and emotional pressures.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES