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THE ROYAL SOCIETY

Molecular taphonomy of animal and plant cuticles: selective preservation and diagenesis

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The nature of organic material and the environment in which it is deposited exert a major influence on the extent to which biomacromolecules are preserved in the fossil record. The role of these factors is explored with a particular focus on the cuticle of arthropods and leaves. Preservation of the original chemistry of arthropod cuticles is favoured by their thickness and degree of sclerotization, and the presence of biominerals. Decay and burial in terrestrial as opposed to marine, and anoxic rather than oxygenated conditions, likewise appear to enhance preservation. The most important factor in the long-term preservation of the chemistry of both animal and plant cuticles, however, is diagenetic alteration to an aliphatic composition. This occurs even in amber, which encapsulates the fossil, eliminating almost all external factors. Some plants contain an original decay-resistant macromolecular aliphatic component but this is not the case in arthropods. It appears that the aliphatic components of many plant as well as animal fossils may be the result of diagenetic polymerization. Selective preservation as a result of decay resistance may explain the initial survival of organic materials in sediments, but in many cases longer-term preservation relies on chemical changes. Selective preservation is only a partial explanation for the origin of kerogen.

Keywords: cuticle; aliphatic; molecular taphonomy; selective preservation; polymerization; kerogen

1. INTRODUCTION

Organisms contain a range of biopolymers that may be preserved in the sedimentary record: nucleic acids (DNA, RNA), proteins, polysaccharides and lipids, as well as the biomacromolecules that are an integral part of structural tissues in plants (e.g. algaenan, lignin and sporopollenin) (Briggs & Eglinton 1994). DNA is highly susceptible to both hydrolysis and oxidation (Lindahl 1993) and this is reflected in the rarity of reliable traces in fossils more than about 100 000 years old. Proteins also decay rapidly, and are preserved on a time-scale similar to that of DNA (see Bada *et al.*, this issue; Collins *et al.*, this issue). Polysaccharides, such as cellulose and chitin, are readily biodegraded. Where they form structural tissues, however, through cross-linking with other molecules, recognizable traces may last for millions of years. Lipids, the most widely studied fossil biomolecules, can be extracted using organic solvents, and are widely employed in investigations of archaeological (Evershed, this issue) and more ancient materials. The lipid in the living organism is readily altered by diagenesis, but it commonly yields a lipid product diagnostic of the precursor, a so-called biomarker. Biomarkers carry a diversity of evidence of ancient environments (light, temperature, oxygen levels) and the source of hydrocarbons (reviews in Engel & Macko 1993). Lipids usually are investigated as dispersed chemical fossils in sedimentary organic matter rather than as part of identifiable fossil remains. The organic (as opposed to biomineralized) remains of plants and animals studied by palaeontologists are commonly decay-resistant macromolecular structures such as animal cuticles, algal cell walls, plant cuticles, spore and pollen walls, propagules, and the woody tissues of plants (van Bergen *et al.* 1995). The preservation and diagenetic alteration of these morphological remains and their molecular composition are intrinsically linked.

The factors that promote the preservation of molecules, or at least limit their degradation, have yet to be investigated in detail. Recent experimental evidence (Stankiewicz *et al.* 1998*c*) indicates that decay normally occurs in days or months. The transfer of organic macromolecules from the living biota to the fossil record is controlled by (i) the nature and composition of the organism, (ii) the depositional context, i.e. conditions prevalent at the time of death and burial (both physico-chemical, and microbial), and (iii) controls on the longer-term survival of the molecules such as enclosing lithology and degree of metamorphism (see Eglinton & Logan 1991, table 2).

Some macromolecules are more decay-resistant, and have a higher preservation potential, than others. DNA decays very rapidly; the detection of sequence information in ancient material has only been possible with the development of the polymerase chain reaction (PCR). This technique has revolutionized archaeology by allowing a range of questions in prehistory to be addressed with materials up to 100 000 years old. At the other end of the spectrum lies decay-resistant macromolecular material like algaenan and sporopollenin which has a very high preservation potential. In this case analysis with pyrolysis–gas chromatography/mass spectrometry (py–GC/MS) has permitted major advances. This technique can generate diagnostic spectra from as little as 100 μg of otherwise intractable materials. Py–GC/MS has

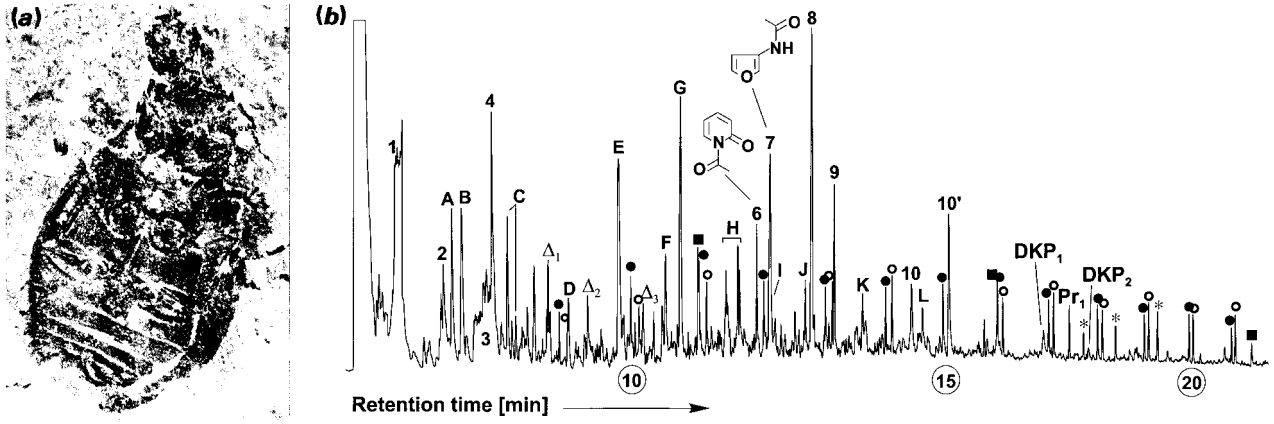

Figure 1. (*a*) Ventral view of a beetle (Coleoptera: Curculionoidea) from level 12 of the Oligocene (24.7 Ma) of Enspel, Germany. (*b*) Total ion chromatogram (pyrolysis at 610 °C for 10 s) of cuticle of specimen illustrated in (*a*). Specimens were solvent extracted in CH_2Cl_2 prior to analysis to eliminate contaminants. Key to peaks: numbers indicate major pyrolysis products derived from chitin: 1, acetic acid; 2, pyridine; 3, acetamide; 4, methylpyridine; 5, 2-pyridinemethanol; 6, *N*-acetyl-2-pyridone; 7, 3-acetamidofuran; 8, 3-acetamido-5-methylfuran; 9, 3-acetamido-4-pyrone; 10, 10′, oxazoline derivatives; solid squares, other important components directly related to chitin polymer. Letters indicate products derived from amino acids A, pyrrole; B, toluene; C, methylpyrroles; D, dimethylpyrrole; E, phenol; F, 2-methylphenol; G, 4-methyl phenol; H, dimethyl and ethyl phenols; I, vinylphenol; J, indole; K, methylindole; L, dimethylindole; DKP_1, 2,5-diketopiperazine of pro-ala; DKP_2, 2,5-diketopiperazine of pro-val. Δ_{1-3}, cyclic ketones derived from carbohydrate moieties; solid circles, *n*-alk-1-enes; open circles, *n*-alkanes; * , contamination. Circled numbers indicate the carbon number in the aliphatic chains. Chemical structures are given for some of the important pyrolysis products. (For further details see Stankiewicz *et al.* (1997*c*).)

been used successfully to analyse the molecular structure of both fossil and modern biological tissues (e.g. de Leeuw *et al.* 1991; van Bergen *et al.* 1995). Recent experiments (Stankiewicz *et al.* 1998*a*) have shown that the results obtained from the two major categories of pyrolysis device, filament and Curie-point, both combined with GC/MS, are directly comparable. Pyrolysis is selective and only crudely quantitative, and should be used in association with other techniques (e.g. colorimetry) to determine the amounts of different macromolecular components present (e.g. Bierstedt *et al.* 1998). Scanning electron microscopy allows the morphological changes induced by decay to be correlated with chemical changes.

The relative preservation potential of different macromolecules was assessed by Tegelaar *et al.* (1989*a*,*b*). The identity of chemical species *per se*, however, provides only a crude guide to their preservation potential, which also depends on how they are incorporated into the tissues of an organism. Where cellulose is mixed with lignin in the walls of fruits and seeds it is much more decay resistant than in isolation (van Bergen *et al.* 1994*b*–*d*, 1995). Where the protein collagen is sclerotized, as in polychaete jaws, it breaks down very slowly compared to the non-sclerotized collagen in the cuticle of the same worm (Briggs & Kear 1993). The preservation potential of macromolecules is increased where they are incorporated into structural tissues. Biomineralization may also protect organic molecules in fossils.

All macromolecular material can be degraded and recycled under appropriate conditions. Scavengers must be inhibited to allow the preservation of organic remains. Invertebrates may be eliminated by the absence of oxygen, leaving a carcase undisturbed, but anaerobic conditions do not prevent decay. Indeed most decay in marine settings is the result of sulphate-reducing anaerobes. Organic remains survive only where chemical (e.g. hydrolysis, oxidation) and biological (enzymatic and microbial activity) degradation is prevented, or where the micro-organisms present cannot use the molecular material as an energy source. Molecules may be protected by encapsulation (Eglinton & Logan 1991; Knicker & Hatcher 1997), within shells and other biomineralized material, or within organic matter including resins, but even these settings do not ensure long-term protection from chemical alteration.

This paper reviews recent research on the preservation of animal and plant cuticles, and discusses the wider implications of the results for our understanding of molecular taphonomy. The main focus, therefore, is on decay-resistant macromolecules, either individually, or in complexes (lignin–cellulose, chitin–protein). Three major taphonomic factors are considered: the nature of the cuticle, the depositional context (trapped in resins, buried in sediments), and longer-term diagenetic changes.

2. CONTROLS ON MOLECULAR PRESERVATION

(a) *The nature of the cuticle*

The structure of arthropod cuticle is an important factor in controlling the fossilization of its molecular components. Thick sclerotized cuticles of beetles from the Oligocene (25 Ma ago) of Enspel, Germany (Stankiewicz *et al.* 1997*c*) preserve the oldest traces of chitin so far discovered (figure 1). Some of the protein also survives, and is still clearly evident (in scanning electron microscope images) as the cuticle matrix that encloses the chitinous fibres. Chitin remnants are absent, however, in the thinner more delicate cuticles of flies from the same deposit. Biominerals may also promote the preservation of the organic components. The biomineralized cuticle of the freshwater crayfish *Astacus* from the Pliocene of Willershausen (Briggs *et al.* 1998*b*) yielded the major

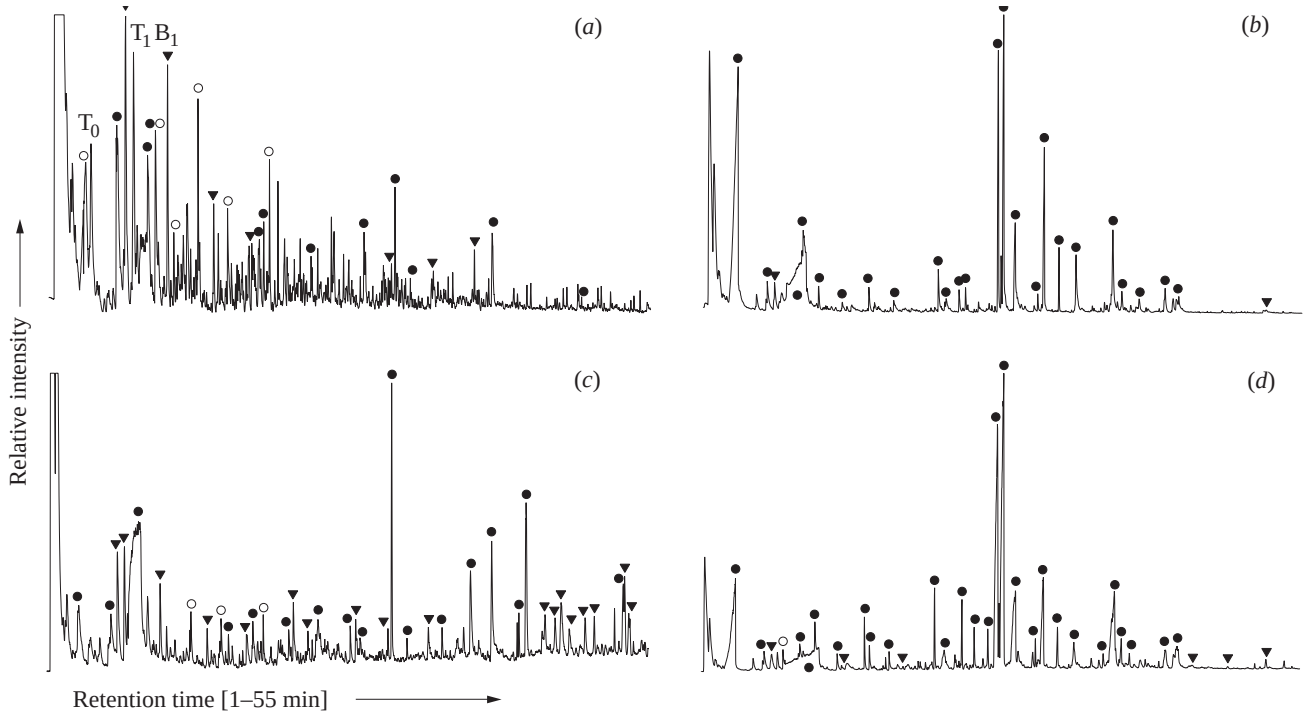

Figure 2. Total ion chromatograms (pyrolysis at 610 °C for 10 s) of cuticle of crayfish (Decapoda, Astacidae). Specimens were solvent extracted in CH_2Cl_2 prior to analysis to eliminate contaminants. (*a*,*b*) *Astacus* sp., exuviae, Pliocene, Willershausen, Germany; (*a*) before and (*b*) after decalcification. (*c*,*d*) *Pacifastacus leniusculus*, Recent, freshly killed; (*c*) before and (*d*) after decalcification. Key to peaks: solid circles, pyrolysis products derived from chitin; open circles, pyrolysis products of non-chitinous polysaccharides; filled triangles, pyrolysis products of proteins; T_0 and T_1, thiophene and methylthiophene; B_1, methylbenzene (toluene). (For further details see Briggs *et al.* (1998*b*).)

pyrolysis products of chitin (as well as carbohydrates, thiophenes and minor amino-acid-derived products) (figure 2*a*). Proteins were depleted significantly compared with their abundance in the cuticle of living *Pacifastacus*, which was used for comparison (figure 2*c*). Following decalcification, the cuticle of both fossil *Astacus* and living *Pacifastacus* yielded a remarkably similar and much simpler trace, essentially the same as that obtained for 'standard' commercially available chitin (figure 2*b*,*d*). Some of the organic components of the cuticle, particularly the proteins, were lost during the acid treatment because they were presumably cross-linked to the $CaCO_3$. The biomineral may have been instrumental in promoting the preservation of this protein fraction. The cuticle of an orthopteran insect from the same facies as *Astacus*, however, preserved protein markers even though it lacked a biomineralized component. In this case the protein was presumably bonded to other components of the cuticle.

Plant tissues are not biomineralized, and therefore might be expected to have a poor fossil record. Unlike arthropod cuticle, however, some plant tissues contain decay-resistant aliphatic macromolecules (Nip *et al.* 1986*a*,*b*; Tegelaar *et al.* 1989*b*; Largeau *et al.* 1990; see de Leeuw & Largeau (1993) for a review). Cutan is the decay-resistant aliphatic component of plant cuticles; cutin is much more readily decayed. Cutan was originally discovered in the cuticle of *Agave americana* (Nip 1986*a*,*b*). Subsequent pyrolysis of 13 flowering plants, two conifers and *Ginkgo* demonstrated that many cuticles contain both cutin and cutan components (so-called mixed cuticles) (Tegelaar *et al.* 1991). Cutan was also demonstrated in some 18 different fossil plants (where cutin does not survive), and their preservation was attributed to its presence.

The cuticle of *Agave americana* was reinvestigated recently by Mösle *et al.* (1997). The cuticle was released with hydrogen pyroxide in aqueous acetic acid. Lipids were extracted; the polysaccharide and lignin were disposed of by acetyl bromide in acetic acid, after which saponification removed the cutin (Mösle *et al.* 1997). The residue yielded a characteristic pyrolysate which was dominantly aliphatic. This aliphatic signature, which was evident even when just the lipids had been removed, indicates the presence of cutan. Mösle *et al.* (1997) investigated how leaves of *Ginkgo biloba* are affected by the same treatment. Following lipid extraction, the pyrolysate of *Ginkgo* revealed a range of products (e.g. polysaccharides, phenols from lignin, fatty acids from cutin) including low abundance aliphatic components (alkene–alkane doublets from C_8 to C_{33}). Saponification, however, resulted in complete dissolution of the cuticle; no non-saponifiable residue like that in *Agave* remained. Thus the cuticle of *Ginkgo* does not contain cutan. The original report of highly resistant material in *Ginkgo* cuticle (Nip *et al.* 1986*a*) was based on the pyrolysis of untreated material. The aliphatic components do not impart any significant decay resistance to the cuticle of *Ginkgo*, as they are present in a saponifiable macromolecule. This result casts doubt on the widespread occurrence of cutan in the cuticles of living plants. The presence of cutan in other plant cuticles (Tegelaar *et al.* 1991) has yet to be tested. An alternative explanation for the fossilization of *Ginkgo* leaves, and possibly other plant cuticles, may be required (Mösle *et al.* 1997).

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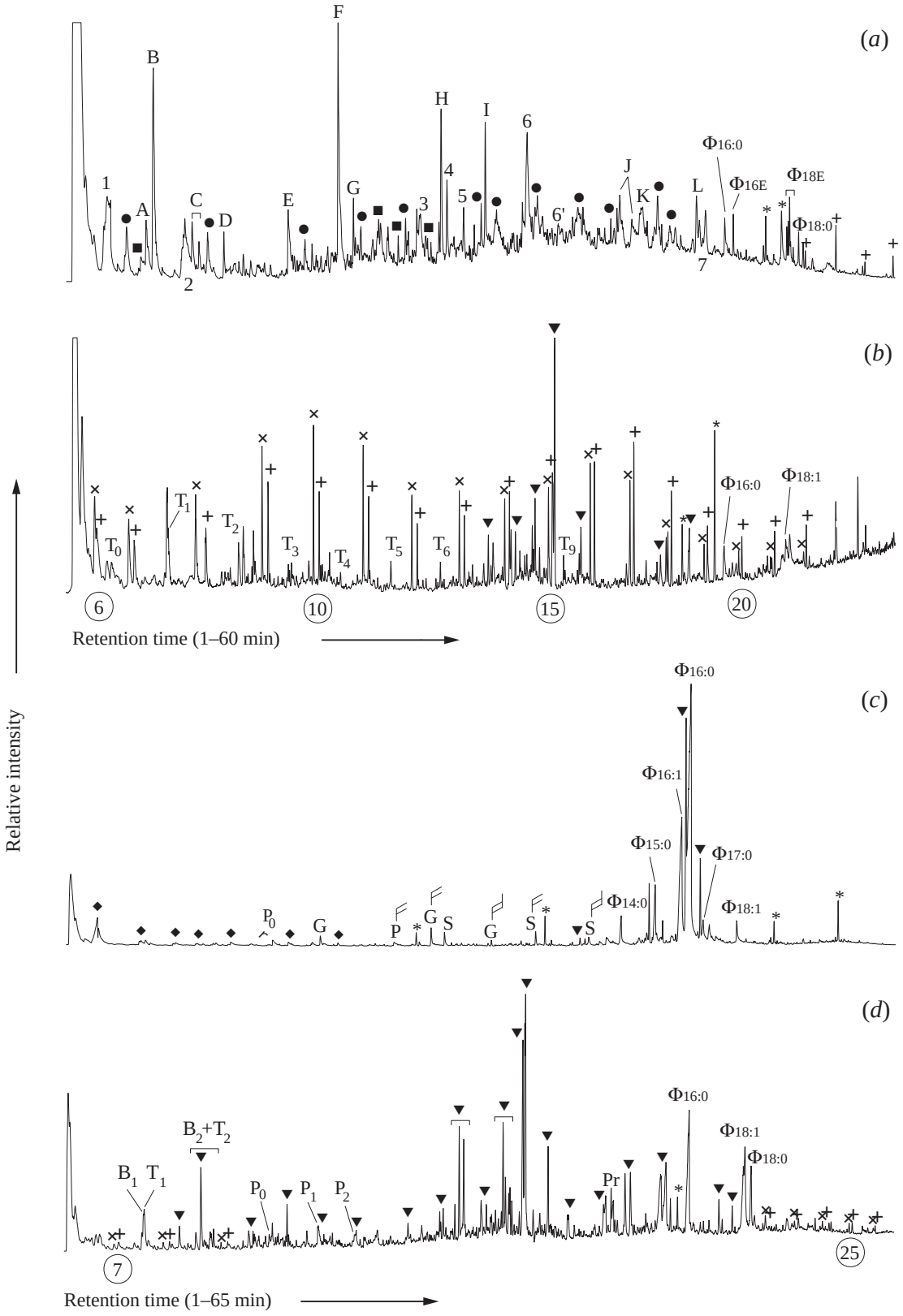

Figure 3. Total ion chromatograms (pyrolysis at 610 °C for 10 s) of tissue from (*a*) abdomen of modern stingless bee, *Plebeia frontalis*, and (*b*) stingless bee, *Proplebia dominicana*, from 25–30 Ma Dominican amber; leaf tissue of (*c*) modern *Hymenaea verrucosa* from Madagascar, and (*d*) *Hymenaea* from 25–30 Ma Dominican amber. Specimens were solvent extracted in CH_2Cl_2 prior to analysis to eliminate contaminants. (For further details see Stankiewicz *et al*. (1997*c*).) Key to peaks: (*a*,*b*): numbers indicate major pyrolysis products derived from chitin: 1, acetic acid; 2, acetamide; 3, 3-acetamidofuran; 4, 3-acetamido-5-methylfuran; 5,3-acetamido-4-pyrone; 6, 6′ and 6″-oxazoline derivatives; 7, 1,6-anhydro-2-acetamido-2-deoxyglucose; filled squares, other important components directly related to chitin polymer. Letters indicate products derived from amino acids: A, pyrrole; B, toluene; C, methylpyrroles; D, ethenylbenzene; E, phenol; F, 4-methyl phenol; G, ethylcyanobenzene; H, indole; I, methylindole;

(b) *A special case: trapped in resin*

The remarkable morphological and chemical preservation of organisms in amber is well-known (Poinar & Hess 1985; Grimaldi *et al.* 1994; Poinar *et al.* 1996*a*,*b*). The resin traps and encapsulates insects, leaves and other organisms, in a medium with well-known bacteriocidal properties. Amber inclusions are an important source of information on the taphonomic processes that affect organic remains, because the resin is thought to eliminate most external agents. Amber appeared the most promising source of ancient DNA. Sequences were obtained from a stingless bee (Cano *et al.* 1992), a weevil (Cano *et al.* 1993), and a termite (DeSalle *et al.* 1992), as well as from a leaf of the resin-producing plant *Hymenaea* (Poinar *et al.* 1993). However, these results were thrown into doubt by rates of DNA degradation that indicate that the molecule is unlikely to survive for more than 10 000 years (Lindahl 1993). Attempts to replicate the original reports of DNA in amber have been unsuccessful. A team at the Natural History Museum in London spent two years (funded by the NERC Ancient Biomolecules Initiative) working on DNA in amber and younger resins (Austin *et al.* 1997). Inclusions in Oligocene Dominican amber and in younger Quaternary resins from East Africa were analysed but none yielded any authentic ancient DNA (Austin *et al.* 1997; see also Walden & Robertson 1997).

Amber provides an opportunity to explore the fate of more decay-resistant parts of organisms which have been protected from normal decay. Until recently no one had considered the macromolecular composition of the inclusions; only amino-acid racemization had been investigated (Bada *et al.* 1994; Wang *et al.* 1995) in spite of seven reports of DNA from insects and plants (see Stankiewicz *et al.* 1998*d*). Insect cuticle is a complex of chitin and proteins that can be extremely tough and durable, not least because of its protective function. Py–GC/MS of insects in east African resins no more than 20 000 years old showed traces of chitin and proteins, albeit partly degraded (Stankiewicz *et al.* 1998*d*). Stingless bees and beetles in 25–30 million-year-old Dominican amber, however, preserved no markers characteristic of either chitin or proteins (figure 3*a*,*b*). The cuticle had been transformed into material that yields a py–GC/MS trace dominated by long, straight chain hydrocarbons (Stankiewicz *et al.* 1998*d*). Alteration of the cuticle through time had removed all trace of its original chitin–protein composition.

Leaves of the resin-producing plant *Hymenaea*, a tropical legume, were similarly analysed (Stankiewicz *et al.* 1998*d*). Pyrolysis of modern *Hymenaea* leaves yielded a pyrolysate dominated by cutin markers, with less abundant markers of lignin and polysaccharide (figure 3*c*). Pyrolysis of *Hymenaea* from sub-fossil resin showed that the cutin polymer was partly degraded, but lignocellulose was well preserved. The pyrolysate of *Hymenaea* from Dominican amber, however, lacked lignin markers and was dominated by resin markers (figure 3*d*). Like that of the sub-fossil resin, it included a series of *n*-alk-1-enes and *n*-alkanes indicating the presence of aliphatic polymer. This last was not evident in the pyrolysate of a modern *Hymenaea* leaf, so it must be a product of diagenesis. Where resistant macromolecules like lignin in *Hymenaea*, and chitin in insects, are not preserved in amber inclusions, there seems little likelihood of traces of the much more fragile DNA molecule remaining.

Among earlier reports of DNA in ancient materials, one of the most celebrated was that from leaves in the Miocene Clarkia deposit of Idaho (Golenberg *et al.* 1990; Golenberg 1991). Here too, attempts to replicate the original results in other laboratories have failed; Sidow *et al.* (1991) detected only bacterial DNA. Investigations of the other macromolecules in the leaf tissues showed that the chemical preservation of the Clarkia fossils reflects the thermal immaturity of the sediment (Logan & Eglinton 1994). Cellulose, hemicellulose, cutin and protein have been lost, but lignin and an aliphatic biopolymer were detected using pyrolysis (Logan *et al.* 1993). The lipids from the leaf waxes of Clarkia plants differ between species suggesting that they reflect the gross characteristics of the original leaf waxes. However, a comparison of these epicuticular wax lipids with those of modern leaf tissues showed evidence of decay: wax esters, aldehydes, certain sterols, and triterpenoids are absent (Logan *et al.* 1995). The extensive degradation is incompatible with the preservation of DNA in these Clarkia fossils.

Although authentic DNA has yet to be extracted from fossils more than *ca.* 100 000 years old, its analysis is becoming widespread in addressing archaeological questions. Methods need to be developed that allow samples to be screened, so that PCR amplification is attempted on only the most promising material. Reports of ancient DNA from both amber and the Clarkia sediments remain to be authenticated by replication in different laboratories, and in both cases more decay-resistant macromolecules in fossils have been altered. Thus, associated macromolecules could provide a measure of the quality of preservation of a fossil

Figure 3. (*Cont.*) J, 2,5-diketopiperazine of Pro-Ala; K, 2,5-diketopiperazine of Pro-Gly; L, 2,5-diketopiperazine of Pro-Pro; filled circles, other important components related to proteins. Filled triangles, pyrolysis products directly related to resin polymer; ×, *n*-alk-1-enes; +, *n*-alkanes; *, contaminants; T_n, alkylthiophenes, B_n, alkylbenzenes, where *n* indicates the extent of alkyl substitution (0, none; 1, methyl; 2, ethyl or dimethyl, etc.). $\phi_{x:y}$, fatty acids, where *x* indicates a carbon number and *y* refers to degree of unsaturation (0, fully saturated; 1, monounsaturated; 2, diunsaturated). Circled numbers indicate the carbon number in the aliphatic chains. (*c*,*d*): filled triangles, pyrolysis products directly related to resin polymer; filled diamonds, polysaccharide pyrolysis products; empty diamonds, hemicellulose marker; ×, *n*-alk-1-enes; +, *n*-alkanes. P_n, alkylphenols; B_n, alkylbenzenes; T_n, alkylthiophenes, where *n* indicates the extent of alkyl substitution (0, none; 1, methyl; 2, ethyl or dimethyl, etc.). G, 2-methoxyphenol; S, 2,6-dimethoxyphenol; Pr, prist-1-ene; *, contaminants. $\phi_{x:y}$, fatty acids, where *x* indicates a carbon number and *y* refers to number of unsaturation (0, fully saturated; 1, monounsaturated; 2, diunsaturated). Circled numbers indicate the carbon number in the aliphatic chains. Side chains of the G and S components are attached at carbon position 4 of the aromatic ring. (For further details see Stankiewicz *et al.* (1998*d*).)

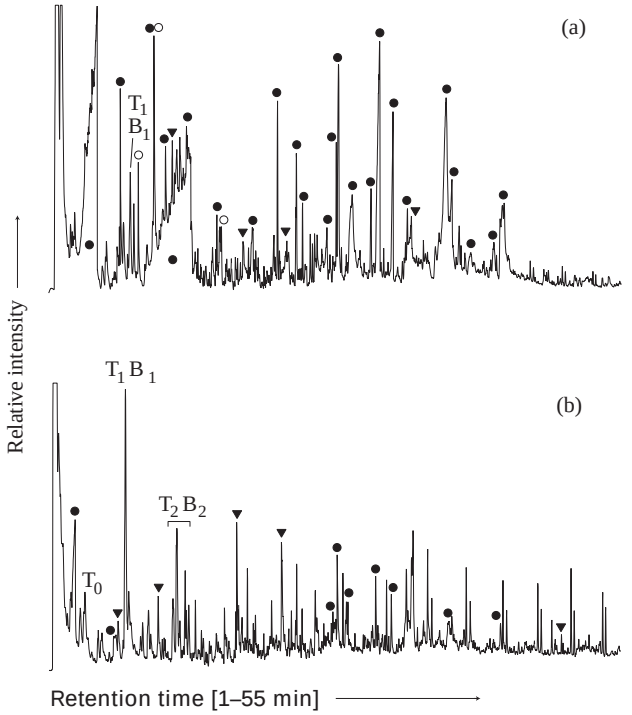

Figure 4. Total ion chromatograms (pyrolysis at 610 °C for 10 s) of cuticle of insects from Pliocene lake sediments, Willershausen, Germany: (*a*) beetle (Coleoptera: Curculionoidea) from anoxic sediment near the lake centre, (*b*) orthopteran from oxic sediment near the lake margin. Specimens were solvent extracted in CH_2Cl_2 prior to analysis to eliminate contaminants. Key to peaks: solid circles, pyrolysis products derived from chitin; open circles, pyrolysis products of non-chitinous polysaccharides; filled triangles, pyrolysis products of proteins; T_0, T_1 and T_2, thiophene, methylthiophene and dimethylthiophenes; B_1 and B_2, methylbenzene and dimethylbenzenes. (For further details see Briggs *et al.* (1998*b*).)

that might indicate whether DNA is likely to be preserved.

(c) *The sedimentary context*

Analyses of the cuticles of insects and leaves trapped in amber show that, even in this protected environment, diagenesis results in profound alteration within a few tens of millions of years. Clearly investigations of the influence of depositional environment on diagenetic changes must concentrate on examples from the Quaternary and Tertiary. Analyses of fossils from marine and terrestrial settings, and observations on rates of decay, suggest that chitin degrades more rapidly in marine than in fresh water settings (Stankiewicz *et al.* 1998*b*). Sedimentary context is the major control on the preservation of animal and plant cuticles, but to date there have been few systematic investigations of its impact on the preservation of macromolecular remains of fossils.

An investigation of the chitin content of beetles from a range of Quaternary deposits showed that the main control on preservation was environment, not age (Stankiewicz *et al.* 1998*b*). Beetles from some peat horizons preserved relatively low concentrations of chitin compared to those from some much older clastic sediments; other examples from peat preserved levels approaching those in modern beetles. Silty lithologies can preserve levels of chitin greater even than those in the asphalts of La Brea (Stankiewicz *et al.* 1997*b*, 1998*b*). The controls on preservation are complex, but contrasts in pH may be important; more acidic environments may enhance chitin degradation through acidic hydrolysis.

An investigation of shrimps and insects preserved in Pliocene lake sediments at Willershausen, Germany (Briggs *et al.* 1998*b*), demonstrated that both the depositional setting and the nature of the organism influenced molecular preservation. All the fossil insects analysed from the lake sediments yielded pyrolysis products characteristic of chitin, but the degree of preservation varied. A sample from dark, finely laminated anoxic marl from the lake centre yielded the most complete suite of chitin derivatives (figure 4*a*); specimens from lighter coloured sediments deposited in oxygenated conditions nearer the lake shore displayed a reduced range (figure 4*b*). Furthermore, a colorimetric assay revealed almost 38% chitin in the specimen from the deeper facies compared to less than 6% in specimens from shallower water sediments. Depositional setting has also been shown to influence the preservation of plant macromolecules. Ligno-cellulose in the seed coats of water plants, for example, is better preserved in coarse-grained sediment than it is in finer-grained lithologies (van Bergen *et al.* 1994*a*). This apparently counter-intuitive observation (given the detrimental effects of porosity on preservation) emphasizes that the influence of sedimentary environment on molecular preservation needs further investigation.

4. SELECTIVE PRESERVATION AND LONGER-TERM DIAGENETIC CHANGES

The most decay-resistant molecules occur in algal cell walls (algaenan), plant cuticles (cutan), spore and pollen walls (sporopollenin), propagules (lignin-cellulose), periderm, secondary xylem and resins (van Bergen *et al.* 1995). The discovery of some of these components in sedimentary organic matter prompted a new model for kerogen, attributing its origin to selective preservation of chemically resistant biomacromolecules (Nip *et al.* 1986*a*,*b*; Tegelaar *et al.* 1989*a*; Largeau *et al.* 1990; Derenne *et al.* 1991; de Leeuw *et al.* 1991). This model has enjoyed a vogue in the last ten years in place of that of Tissot & Welte (1984), which involved random repolymerization and recondensation of lipids, sugars, amino acids and other moieties. Tegelaar *et al.* (1989*a*, table 1) listed macromolecules from different taxa and arranged them in order of their resistance to decay. This approach predicted not only which organisms contribute to kerogen, but also which organic tissues were most likely to survive decay and supply identifiable fossil remains to the sedimentary record.

The most resistant biomacromolecules are derived from algae and vascular plants; proteins and chitin are shown to have a very low preservation potential (Tegelaar *et al.* 1989*a*). Predictably, therefore, fossil animals have received little attention from organic geochemists. Paradoxically, however, many fossil arthropods are preserved as the organic remains of non-biomineralized skeletons (Bartram *et al.* 1987; Briggs *et al.* 1998*a*), as are graptolites (Briggs *et al.*

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1995). Thus animal remains do contribute, even if only in a small way, to sedimentary organic matter. Moreover, a substantial proportion of sediment nitrogen may have an animal origin (Stankiewicz & van Bergen 1998).

Arthropod cuticle consists of chitin linked by a catechol moiety to protein. The cuticle may be strengthened by cross-linking (tanning and sclerotization), particularly in insects, and of course by biomineralization. Laboratory and field-based experiments have shown that chitin decays more slowly than the protein constituents of the cuticle (Baas *et al.* 1995; Stankiewicz *et al.* 1998*c*). Thus it would appear that selective preservation of chitin must account for the extensive fossil record of non-biomineralized arthropods. This prediction is borne out by studies of fossil arthropods in Quaternary and Tertiary sequences (using py–GC/MS and quantitative colorimetric assay). The proportion of chitin remaining in insect cuticles from various Quaternary deposits, including the Pleistocene asphalt deposits of California, ranged up to 37% dry weight (Bierstedt *et al.* 1998; Stankiewicz *et al.* 1997*b*, 1998*b*), whereas the protein constituents had largely degraded.

The decay resistant properties of chitin must contribute to the preservation of fossil arthropods, not least because there can be no fossil unless some tissue remains! However, this selective preservation is only part of the story. Not all the insects preserved in Oligocene Lake Enspel show traces of chitin (Stankiewicz *et al.* 1997*c*). The flies, which have a thinner cuticle than the beetles, yielded a pyrolysate dominated by aliphatic components (*n*-alk-1-enes and *n*-alkanes). Arthropod fossils from older deposits, ranging back as far as Silurian, also failed to show any trace of chitin (Stankiewicz *et al.* 1997*a*). The remains are mainly aliphatic, their pyrolysates dominated by series of *n*-alk-1-enes and *n*-alkanes. In some cases the fossil cuticles are aromatic in composition, dominated by C_0–C_3 alkylated benzenes and alkylated indenes (Stankiewicz *et al.* 1997*a*). The chemical composition of these fossils cannot be explained solely by selective preservation. The components of the fossil material are not simply the remains of the degraded cuticle of living arthropods.

The cuticle of living arthropods lacks macromolecular aliphatic components, but they are present in the tissues of living plants. Both fossil arthropod and plant cuticles, however, may be aliphatic. Is selective preservation the major factor in the fossilization of plants, whereas some other process must be invoked to explain the preservation of arthropods?

A number of fossil localities yield the organic remains of plants and animals in association, allowing their preservation to be compared in the same environmental context. Fossil cuticles of arthropods (scorpion, eurypterid) and plants (cordaite, pteridosperm) from the Upper Carboniferous of Lone Star Lake, Kansas and Joggins, Nova Scotia were analysed using py–GC/MS (Stankiewicz *et al.* 1998*e*). The fossil arthropods yielded no trace of chitin, but an aliphatic signature made up of an homologous series of alkanes and alkenes, with associated phenolic and aromatic constituents (figure 5*a*). The pyrolysate appeared to be specific to the type of organism. The aliphatic trace derived from scorpions from both Joggins and Lone Star was very similar, the abundance of *n*-alk-1-enes and *n*-alkanes decreasing sharply after carbon number 17 (a similar trace was obtained from a Silurian scorpion from Ontario: Stankiewicz *et al.* 1997*a*). The eurypterid from Joggins, on the other hand, yielded more long-chain aliphatic components as well as abundant phenols. The pyrolysates from these fossils are completely different to that of a modern scorpion cuticle, which, following solvent extraction, yields products characteristic of chitin cross-linked with proteins. Thus the preservation of the fossil arthropods cannot be attributed simply to selective preservation but must have involved diagenesis. The diagenetic process, however, allowed a signature characteristic of the particular organism to be retained.

Although the fossilization of arthropods cannot be explained by selective preservation, this was the favoured hypothesis for plants (Tegelaar *et al.* 1989*a*, 1991). The most useful living analogue for the ancient cordaite and pteridosperm material analysed from Kansas and Nova Scotia was *Araucaria araucana*, the monkey puzzle tree. The pyrolysate of *Araucaria* cuticle is dominated by fatty acids, thermal breakdown products of cutin; no long, straight-chain aliphatic hydrocarbon components were released. The pyrolysates of the fossil plants, on the other hand, were dominated by homologous series of alkane–alkene doublets: C_6 to C_{30} *n*-alk-1-enes and *n*-alkanes, most abundant from C_{10} to C_{14}, with minor phenolic and other benzenoid components (figure 5*b*). The pyrolysates of the fossils showed no sign of products derived from cutin or lignocellulose. Nonetheless, the pyrolysates of the cordaites from both localities were similar but differed from that of the pteridosperm. Thus the plants, like the arthropods, appear to retain a signature that is to some degree taxon specific. Furthermore, the plants differed from the arthropods in displaying a higher proportion of C_{20} to C_{30} hydrocarbons. Where previous analyses of fossil plants have yielded aliphatic dominated pyrolysates (Nip *et al.* 1986*a*; van Bergen *et al.* 1994*b*), they have been interpreted as the result of selective preservation, reflecting the decay resistance of cutan. In this case, however, the cuticle of the living *Araucaria*, a close analogue of the fossil plants, does not contain any cutan, nor is cutan present in the closely related seed plant *Ginkgo* (Mösle *et al.* 1997). Thus the composition of the fossil leaves, like that of the arthropods, must be the result of diagenetic alteration (Stankiewicz *et al.* 1998*e*).

Not only the chemical composition, but also the preserved morphology of the arthropods and plants indicate that they have undergone diagenesis (Stankiewicz *et al.* 1998*e*). Although the external morphology is well preserved, the ultrastructure (viewed with the transmission electron microscope) has been lost, leaving a thinner largely amorphous layer in place of the original cuticle. The fact that both arthropods and plants yield different characteristic pyrolysates eliminates the possibility that the organic material in these deposits was altered by replacement with randomly polymerized materials (Tissot & Welte 1984), or the transfer of chemical constituents between the remains of plants and animals (a possibility raised by Baas *et al.* (1995)). Diagenesis must have been specimen specific. In the case of the arthropods it may have involved polymerization of the cuticle together with epicuticular lipids or internal tissues (Stankiewicz *et al.* 1997*a*). Polymerization of the plant cuticle may have incorporated the cross-esterified fatty acids

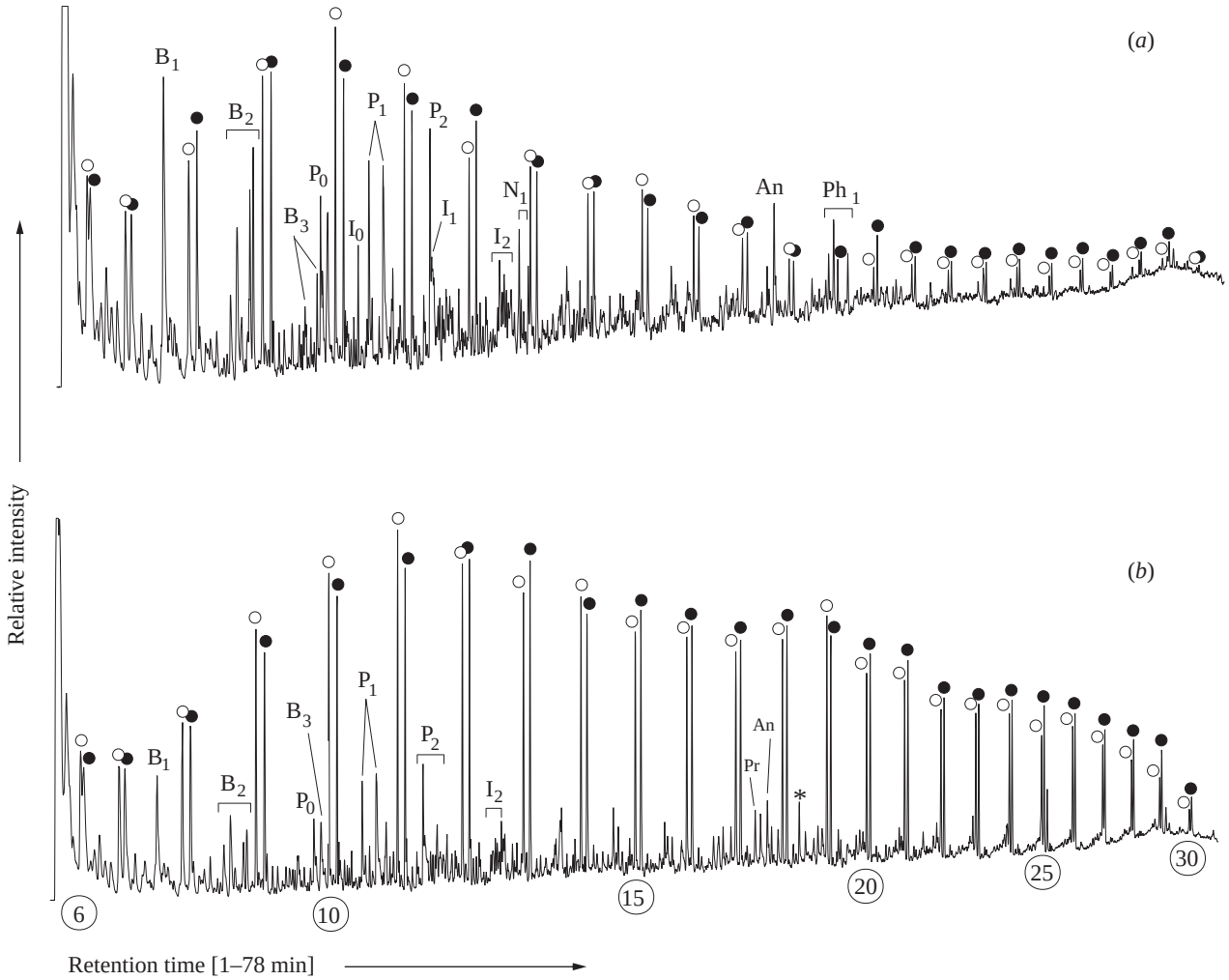

Figure 5. Total ion chromatograms (pyrolysis at 610 °C for 10 s) of arthropod and plant cuticle from Carboniferous of Lone Star Lake, Kansas, USA: (*a*) scorpion; (*b*) cordaite. Specimens were solvent extracted in CH_2Cl_2 prior to analysis to eliminate contaminants. Key to peaks: numbers in circles below pyrograms indicate carbon number for alkene–alkane pairs. Open circles, *n*-alk-1-enes; closed circles, *n*-alkanes; B_n, alkylbenzenes; P_n, alkylphenols; N_n, alkylnaphthalenes; Pr, prist-1-ene; I_n, alkylindenes; An, anthracene; Ph_1, methylphenanthrenes, where *n* indicates the extent of alkyl substitution (i.e. 1, methyl; 2, dimethyl or ethyl, etc.). (For further details see Stankiewicz *et al.* (1998*e*).)

within it (Stankiewicz *et al.* 1998*e*). A similar process must be invoked to explain the aliphatic nature of fossil graptolites, the periderm of which is thought to have been originally proteinaceous (Briggs *et al.* 1995).

Diagenetic change clearly relies on time. Fossil arthropods in deposits of Mesozoic or older age have been diagenetically altered; only samples from a small number of Tertiary or younger localities preserve traces of chitin or proteins. Both insect and plant cuticles in 25–30 Ma amber have been altered to an aliphatic composition whereas in 20 000-year-old sub-fossil resins the original components are at least partly intact (Stankiewicz *et al.* 1998*d*). The effects of time, however, are tempered by other factors, namely chemical alteration proceeds at different rates in different organisms and settings. Even where some Tertiary arthropods preserve traces of their original composition (Stankiewicz *et al.* 1997*c*), others, even from the same beds, display a predominantly aliphatic signature. Likewise lignin may survive relatively unaltered in Tertiary seed coats (van Bergen *et al.* 1995), whereas contemporaneous samples may yield quite different chemical signatures.

5. CONCLUSIONS

It is clear that the nature and composition of an organism, and the depositional setting, have a profound influence on the degree to which its molecular composition is preserved. The detailed mechanisms that control the preservation of macromolecules in the fossil record still require systematic investigation. Further research will rely on analysis of identifiable fossil remains so that the source of macromolecules can be explicitly determined. Some of the controls are amenable to laboratory experiment. In addition, however, all require investigation in the field; sampling from measured sections will allow the preservation of the material to be directly related to depositional setting.

Recent investigations have shown that selective preservation is inadequate to explain the survival and incorporation of all plant and animal remains into the fossil record. Fossil arthropod and least some fossil plant remains are the product of polymerization. The occurrence of polymerized insect remains at Enspel, together with others that preserve traces of chitin and protein,

shows that this process can occur in 25 Ma; it has yet to be demonstrated on a shorter time-scale. Cutan occurs in *Agave americana*, but its occurrence in other plants has yet to be confirmed; in many plant fossils similar polymeric material is likely to be the result of diagenetic alteration. A similar origin is possible for other biomacromolecules such as sporopollenin (see de Leeuw & Largeau 1993) in fossil material. Selective preservation may be important to ensure that macromolecular material survives the normal processes of rapid decay, but ultimately fossilization involves long-term diagenetic alteration, a process with important implications for the interpretation of fossil assemblages and for the origin of kerogen.

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Discussion

W. G. Chaloner (*Royal Holloway, University of London, UK*). In view of the extensive occurrence of aliphatic residues in both plant and animal fossil material, is it not possible that these components may migrate from one source to another through the matrix?

D. E. G. Briggs. We originally considered that the aliphatic composition of the cuticles of fossil arthropods, which cannot be explained by selective preservation, might be a result of diagenetic substitution of chitin by more resistant organic matter from a plant source (Baas *et al.* 1995; Van Bergen *et al.* 1995). Our recent research, however, has made this view untenable for a number of reasons: (i) aliphatic polymers are characteristically highly insoluble and immobile; (ii) beetle cuticles in Oligocene lake sediments from Lake Enspel, Germany, retain a chitin–protein signature even though the enclosing rock matrix is strongly aliphatic (Stankiewicz *et al.* 1997*c*); (iii) a significant aliphatic component is found in the cuticles of insects from Oligocene Dominican amber, where encapsulation in resin clearly rules out an external source (Stankiewicz *et al.* 1998*d*); (iv) arthropod and plant cuticles from the same Carboniferous fossil localities reveal distinct differences in the distribution and relative abundance of long-chain alkenes–alkanes, as well as a higher content of phenolics in the arthropod cuticle. These differences are incompatible with the transfer of material from plant to arthropod fossil (Stankiewicz *et al.* 1998*e*). We now consider that the aliphatic composition of fossil arthropod cuticles is a product of the diagenetic transformation of components of the arthropod itself.

R. P. Ambler (*University of Edinburgh, UK*). Have any pyrogram markers been recognized that are characteristic of aerobic or anaerobic taphonomy for materials like animal cuticle?

D. E. G. Briggs. I am not aware that this has been done for resistant macromolecules such as those preserved in ancient plant and animal cuticles. It would involve quantitation of peaks in pyrograms of many replicate samples from well-characterized environments followed by statistical analysis of the results to identify any distinct groupings. In practice, once incorporated into sediment, cuticles will undergo degradation and diagenesis under anoxic conditions. Microbial degradation under oxic or

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anoxic conditions does, however, have an important impact on the lipid biomarkers incorporated into sediments (see Teece *et al*. 1998).

S. Macko (*University of Virginia, USA*). Obviously, pyrolysis can only show that intact portions of a molecule have survived in a fossil. For chitin identification chitinase activity is an alternate approach to test for larger molecule survival. How far back would you suggest chitinase activity would exist?

D. E. G. Briggs. Enzymatic methods have been used to identify traces of chitin in fossils as old as Cretaceous (Brumioul & Voss-Foucart 1977). However, given the problems of sensitivity and specificity, we advocate using a combination of py–GC/MS and a more quantitative method such as colorimetry (Bierstedt *et al*. 1998). This should be supported by microscopy to confirm that structural and chemical preservation are consistent. To date, the oldest traces of chitin we have discovered using this approach are in 25 million-year-old beetles from Lake Enspel, Germany (Stankiewicz *et al*. 1997*c*).

J. Bada (*University of California—San Diego, USA*). We have found excellent preservation of amino acids in amber-entombed bees and other insects. You are looking at only aromatic amino acids, which are minor components of these tissues. So I suggest you should use caution in saying that proteins are not preserved in insects in amber.

D. E. G. Briggs. Our analyses of a modern bee reveal not only ethylcyanobenzene and indole (products of aromatic amino acids), but also diketopiperazines (indicative of at least dipeptide chains which include aliphatic amino acids and proline). Diketopiperazines are also evident in a bee from *ca*. 2000-year-old Kenyan ancient resin. However, none of these pyrolysis products, nor others characteristic of chitin, proteins, or even peptide fragments, are evident in Dominican amber insects. They revealed resin markers, some fatty acids, alkylthiophenes (in the bee) and alkylbenzenes (in the beetle), but dominantly alkene–alkane doublets. Thus, our study (Stankiewicz *et al*. 1998*d*) revealed no evidence for intact proteins in amber insects, although it does not eliminate the possibility that very low concentrations of single amino acids are present.

J. Hubbard (*King's College, London, UK*). Is there not a simple analytical method whereby TEM images can be traversed for chemical signatures (as used for medical purposes)?

D. E. G. Briggs. Yes, laser pyrolysis provides a possible method but, as far as I am aware, it has yet to be applied to ancient cuticles.

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