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Molecular organic matter in speleothems and its potential as an environmental proxy

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Abstract

Organic matter preserved in speleothems has considerable potential to record changes in the surrounding environment, particularly in the overlying vegetation. Here, we review three types of organic matter analysis relevant to speleothems: organic fluorescence, lipid biomarker analysis, and amino acid racemisation. Organic matter luminescence provides a useful non-destructive and rapid method for assessing dissolved organic matter quantity and quality, while biomarker analysis (amino acids and lipids) has the potential to provide a more detailed signal related to specific parts of the surrounding ecosystem such as the dominant vegetation regime and bacterial activity. Amino acid analysis has yet to prove demonstrably useful in stalagmites, due to the inability to characterise the sources of proteinaceous matter. However, the small but increasing body of work on lipid biomarker analysis in stalagmites has shown that a wide variety of recognisable biomarkers are preserved over long periods of time (>100 ka), can be recovered at temporal resolutions of <10 yr, and show meaningful changes through time. This approach is therefore of considerable potential value to Quaternary science.

1. Introduction

Speleothems (chemically precipitated cave deposits) occupy a valuable niche as proxies for palaeoenvironmental and climatic change. Caves provide sheltered environments that are more climatically stable than the Earth's surface (Wigley and Brown, 1976), whilst speleothems are generally chemically closed systems that alter little after lithification (Wigley and Brown, 1976). Therefore, the environmental records contained within speleothems are subject to far less geochemical and taphonomic disturbance than those in surface sedimentary deposits. Furthermore, a single speleothem can contain a multitude of proxies, including oxygen and carbon isotopes, trace elements and organic material, while the incremental nature of growth provides the opportunity for high-resolution dating. This means that, given sufficient material, a wide range of environmental and climatic parameters can be interpreted, including vegetation change, organic matter fluxes, variations in amount and seasonality of surface precipitation and temperature, and groundwater storage.

The vast majority of speleothem studies focus on inorganic proxies, in particular oxygen isotopes, which can be tied to other major climate records such as ice and ocean sediment cores (Fairchild et al., 2006). Until recently these studies have been generally unsuccessful at reconstructing climate, due to the complex forcing mechanisms affecting oxygen isotopes (McDermott, 2004). However,

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careful modern process studies mean that workers are now starting to provide quantified isotopic palaeoenvironmental data (e.g. Dorale et al., 1998; McDermott et al., 2001; Wang et al., 2001; Dykoski et al., 2005; Baker et al., 2007).

By and large, until the 1990s, organic material, although recognised as a significant minor component of speleothems (James et al., 1994), was mainly of interest as a potential contributor to the carbon isotope record, and as a source of speleothem colour (e.g. Caldwell et al., 1982). However, more recently there has been an increased focus on the actual organic matter itself. This has been brought about by the recognition of organic fluorescence in stalagmites (Baker et al., 1993; Shopov et al., 1994), and the recovery of usable pollen records (Bastin, 1978; Brook et al., 1990; Burney et al., 1994; Brook and Nickmann, 1996; McGarry and Caseldine, 2004). More recently, the field has been advanced by investigations into organic biomarkers, a proxy that is widely used in sediment and soil studies (e.g. González-Vila et al., 2003; Hanisch et al., 2003; Xie et al., 2003a; Zhang et al., 2004; Kim et al., 2007), and has been demonstrated to have potential in stalagmite work (Rousseau et al., 1995; Xie et al., 2003b, 2005; Blyth et al., 2006, 2007).

This paper reviews how organic matter in speleothems is currently used as an environmental proxy, and how improved analytical techniques can advance this further. It focuses on the organic matter trapped in the calcite during precipitation. 'Whole' contributions, such as pollen, are not considered here as they have been recently reviewed elsewhere (McGarry and Caseldine, 2004).

Organic matter in speleothems is important in environmental research because it can provide information on a number of different environmental variables including climate and ecosystem change. The latter is especially important, as organic matter in stalagmites, and particularly biomarkers, have the potential to record detailed changes in the vegetational and microbial ecosystems. Currently, records of such changes in the terrestrial environment are obtained from studies of soil and sediment cores and micro-fossil records (e.g. Ficken et al., 1998; Huang et al., 1999; Brincat et al., 2000). However, speleothems provide comparative environmental stability, with samples preserved for the Quaternary or longer (Richards and Dorale, 2003), and can be dated with relative ease, potentially back to several million years (Woodhead et al., 2006). Given this and the number of speleothem proxies available for multi-disciplinary work, molecular palaeoenvironmental information derived from stalagmites and flowstones is very valuable.

We therefore believe that a review is timely to gather together the small but increasing body of work on organic matter in speleothems, and identify how each proxy can contribute to palaeoclimatic studies. In presenting an overview of the subject, we can also identify where future work is needed to advance the field, and where additional novel approaches might be taken.

2. Sources of organic matter in speleothems

Organic matter of some type occurs in most carbonates, and is incorporated as a trace component either between or within the mineral crystals (Ramseyer et al., 1997). In speleothems (and other predominantly inorganically precipitated carbonates) the organics are found between crystals or within small pores (30–150 nm) in the crystal structure (Ramseyer et al., 1997), indicating that they derive from the water film present during calcite precipitation (Ramseyer et al., 1997). They can be incorporated into the growing formation either by adsorption onto the crystal or due to uptake as a fluid inclusion (Ramseyer et al., 1997).

Fig. 1 summarises the likely sources and transportation mechanisms of molecular organic matter in speleothems. There are four mechanisms by which organic material can be delivered to a speleothem: air, water, faunal transport, and autochthonous production.

Airborne material may consist of intact inclusions such as pollen or fungal spores or may include airborne volatile compounds or organic material adsorbed onto other particulate matter. There is currently no body of work investigating the distribution of airborne compounds in caves; however, work with pollen and fungal spores has demonstrated that the material is generally only found where the deposit has formed near an entrance or chimney, with studies demonstrating a negative correlation between

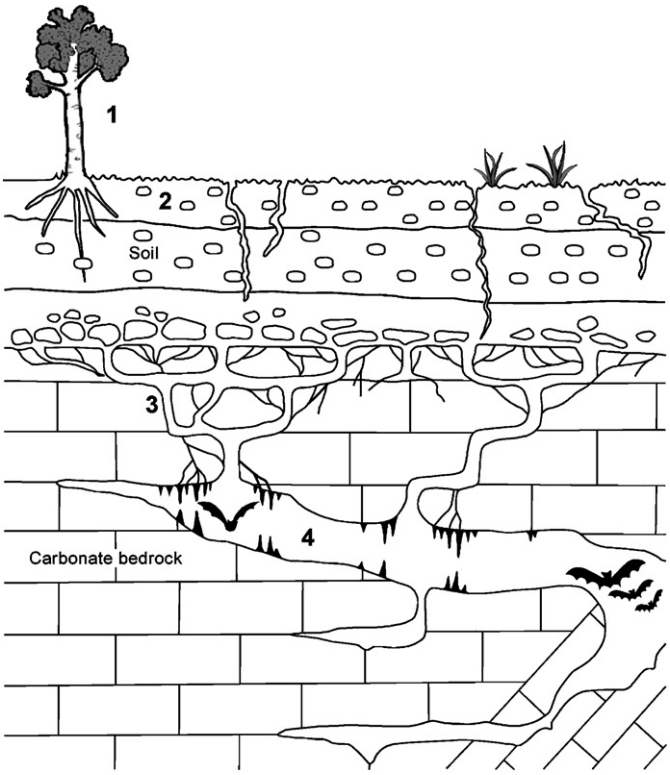

Fig. 1. The potential sources of molecular organic matter in stalagmites: (1) input from vegetation; (2) input from soil ecosystems; (3) bacterial input from limestone aquifer; and (4) input from cave fauna.

air-borne load and distance inside a cave (Camacho et al., 2000; McGarry and Caseldine, 2004). Since stalagmites formed near cave entrances can have fundamentally different mechanisms of formation compared to those forming in 'deep' cave environments (James et al., 1994), and since the vast majority of samples collected for palaeoenvironmental work are deposited in the dark zone some distance inside the relevant caves, airborne material is not considered in detail here. However, workers should always note the position of their stalagmite samples and the resulting potential for outside 'contamination'.

Material borne by animals and insects is more problematic. Although the material brought in from the surface environment generally consists of intact inclusions that can cling to coats (McGarry and Caseldine, 2004), it is likely that in caves with notable faunal populations, biomarkers may be derived from the animals themselves (Rousseau et al., 1995; Haile et al., 2007). This is especially the case where there is a significant amount of excreta deposited in the chamber (for example, bat guano) or where populations of small animals actually live on the speleothem surface. The latter scenario has gained credence from the discovery of fossilised mites within stalagmites (Polyak et al., 2001). As this mechanism for the deposition of biomarkers has yet to be investigated, it cannot be ruled out.

The majority of speleothems used in palaeoenvironmental research form in deep cave environments, where contact time with the groundwater is sufficient for supersaturation with respect to calcite to have occurred, and where the main transport mechanism for organic matter is the percolating meteoric water from which speleothems are precipitated. The majority of the organic matter so transported has been leached from the soil above, where it was produced by the decomposition of flora and fauna and by the metabolic processes of vegetation and the soil microbial community. Soil organic matter leaching is in turn controlled by factors such as partitioning between solid and solution phases, which is determined by hydrophobicity, solution pH, etc., and biological activity, which is controlled predominantly by climate (Lumsdon et al., 2005), with additional influences from human activity (such as vegetation clearances), and natural disturbances such as wild fires. Soil type will also have an effect, with particle size, the relative amounts of sand and clay, and the mineralogical composition also serving to affect the adsorption and so the availability of organic matter. Therefore, variations in soil type, soil solution and biological activity all have the potential to affect the transport of organic matter to the cave, and differential transportation maybe seen as the nature of the soil changes, either on a temporal or geographical scale.

The turnover of organic matter in soils varies (Lofts et al., 2001; Michalzik et al., 2003), broadly ranging from an 'old' fraction that turns over slowly ($\sim 10^2$–10^3 yr), to a 'new' fraction that decays very quickly ($\sim$1 yr); consequently, there will be a lag between the production of organic matter in the surface environment and its incorporation into speleothems (Genty et al., 1998). Studies of speleothems using bomb ^{14}C as a tracer suggest that, as would be expected, lags are variable, and, for the bulk organic matter found in speleothems, often in the range of 1–10 yr (Genty et al., 1998). The varying chemical structures of different organic compounds resulting in different amenabilities to transportation and potential for degradation means that these lag times may vary considerably, depending on the type of organic matter being studied. Therefore, any response of the signal to climate also depends on the type of organic matter under discussion, which soil organic matter fraction the material is derived from, and which fraction responds most to climate change.

It is possible that an additional proportion of the organic matter in the meteoric water is contributed by the limestone through which the water passes, particularly from biofilms on the internal rock surfaces. However, although there is evidence that limestone aquifers contain biofilms (Simon et al., 2001), and we have an improved understanding of the factors that promote the survival of groundwater microbial communities (organic content, grain size, pH, flow rate, etc. (John and Rose, 2005)), in the context of speleothem organic matter, this possibility has not been widely studied. In any case, there is a possibility that the limestone acts as a sink rather than a source, or as a combination of the two, depending on flow rates, degree of scouring, the relative hydrophobicity of the organic material (John and Rose, 2005), and particularly the thickness of limestone through which the material is transported, as significant filtering effects on organic matter have been observed (Baker et al., 1996).

The final source for molecular organic matter in speleothems is the autochthonous contribution made by the microbial communities within the cave. Biofilms are known to be present on speleothems (Northup and Lavoie, 2001; Cacchio et al., 2004), but work so far has focused on the role of microbes in the generation or destruction of cave minerals, including, but not confined to, calcite (Barton et al., 2001; Melim et al., 2001; Northup and Lavoie, 2001; Cacchio et al., 2004; Summers Engel et al., 2004). The possibility that these communities are also sources for organic matter preserved in speleothems has yet to be fully investigated. However, the microbial communities involved in the formation of stromatolitic stalagmites have been demonstrated to leave an amino acid record (James et al., 1994), and bacteria are known to be significant contributors to biomarker records in other contexts (Jones, 1969; Rohmer et al., 1980; Cardoso and Eglinton, 1983; Rütters et al., 2002; Wakeham et al., 2003; Weijers et al., 2006), both via the production of primary biomarkers and by the alteration of other organic material (Tremblay and Benner, 2006). So cave bacteria as a potentially major source for biomarkers in speleothems cannot be ruled out.

As shown above, the potential sources for organic matter in speleothems are numerous, complex, and at the current state of knowledge, ambiguous and under-researched. Understanding the behaviour of different organic compounds within the overlying soils and the cave system is a vital issue in stalagmite work. Initial information on the turnover of the organic matter at the surface may be gained from published studies of organic matter preservation in typical soil types (e.g. Bull et al., 2000). However, given the multitude of factors involved, and the fact that by necessity soil studies focus on the material recoverable, not the material removed from the soil (which may have been transported or degraded), there is an urgent need for holistic and contextualised research, which takes into account not only the behaviour of material in different soils, but also the amenability to transport through the limestone system and the potential impact of cave microbial communities on the transported signal.

Another concern that needs noting is the possibility that because they originate from soil and vegetation, or internal cave sources, speleothem organic matter signals could be recording very local environmental variations rather than regional climatic changes. In fact this can be argued of the majority of speleothem proxies, as they can rely on a local soil source or a sample specific hydrological routing for their delivery to the cave. The work of Charman et al. (2001) attempted to address this by comparing peaks in surface wetness seen in a peat humification record from NW Scotland, with those identified in a stalagmite organic fluorescence record from a hydrologically separate cave overlain by similar vegetation. The changes seen in these were then compared with other proxies including peat from elsewhere in Scotland and the GISP2 ice core. The results showed a replication of the main variations in the records. Similar changes were seen in the comparison of biomarker records derived from stalagmites with sea-surface temperature records (Xie et al., 2003b, 2005), indicating that the organic matter signal in these cases is being driven by regional or global climate changes rather than local hydrology. Nonetheless, recent studies into the reliability of stalagmite records (e.g. Fairchild et al., 2006) have shown the need for multi-proxy approaches, both to ensure that the changes being seen are regionally significant, and to clearly interpret the governing climatic mechanisms (Drysdale et al., 2006; Asrat et al., 2007).

The rest of this review focuses on the specific types of organic matter found in speleothems and how these can be used to interpret environmental change, and presents previously unpublished data to demonstrate the utility of these novel techniques.

3. Organic acid luminescence

3.1. Overview

The use of speleothem organic matter as a climate proxy has been spearheaded by studies of speleothem luminescence banding (Baker et al., 1993; Shopov et al., 1994). Organic acid luminescence relies upon the presence of a loosely held electron in the outer orbit of a molecule. The electron is excited to a higher energy level by the absorption of energy (e.g. a photon), and luminescence occurs when energy is lost as light as the electron returns to its original energy level (ground state). There are three measurable parameters, the luminescence intensity, the excitation wavelength, and the emission wavelength. The intensity reflects the amount of organic matter present, while the wavelength at which absorption (excitation) and emission occur is specific to the molecule, and therefore can be used to differentiate between different types of organic matter. Aromatic compounds such as humic acids provide a particularly good subject for study by luminescence due to their energy sharing electron structure (McGarry and Baker, 2000).

Luminescence spectrophotometry has been widely applied to speleothems (Baker et al., 1993, 1996, 1998, 1999; Baker and Bolton, 2000; for a review see McGarry and Baker, 2000), and stalagmite dripwaters (Baker et al., 1997; Baker and Genty, 1999; Baker, 2000; van Beynen et al., 2000). Stalagmite luminescence is dominated by excitation wavelengths between 300 and 420 nm and emission between 400 and 480 nm. Fluorescence in this region has been shown in modern soil, river and groundwater samples to be derived from 'humic' substances; relatively high molecular weight and aromatic dissolved organic matter (Newson et al., 2001; Baker and Spencer, 2004). Variations in the emission of this fluorescence to longer wavelengths reflect an increase in molecular weight or aromaticity in the signal (Bolton, 2004), which can be connected to the relative proportions of humic and fulvic acids present; humic acids having a higher molecular weight and greater degree of inter- and intramolecular bonding and aromaticity. The proportion of humic/fulvic acids is controlled by organic matter type, including vegetation regime (Baker and Genty, 1999), and climate, particularly rainfall, with increases in humic acids being related to higher soil moistures (Proctor et al., 2000).

The intensity of the organic luminescence has been shown to correlate strongly with dissolved organic carbon concentration in modern systems (e.g. Baker and Spencer, 2004). This also relates to climate, with temperature and rainfall affecting the rate of organic matter turnover and its release into the groundwater system. As a result, organic fluorescence intensity shows seasonal climate-induced fluctuations (Newson et al., 2001).

Both modern process studies and stalagmite records confirm that luminescence in stalagmites is derived from dissolved organic matter transported from overlying soils (White and Brennan, 1989), that there is a relationship between fluorescence wavelength and the organic matter composition (Baker et al., 1998; Perrette et al., 2005), and that seasonal climate driven variations can be observed (Tan et al., 2006).

3.2. Methods

Luminescence spectrophotometry utilises fibre-optic probes, which enable the non-destructive analysis of fluorescence organic matter trapped and preserved within the calcite. Typically, analyses have used 'off-the-shelf' techniques: either (1) mercury source microscopes to observe annual variations in luminescence that form annual laminae (Baker et al., 1993; Ribes et al., 2000), or (2) luminescence spectrophotometers with fibre-optic probe attachments, which can scan both excitation and emission wavelengths to obtain information on both the intensity and wavelength of the fluorescence (Baker et al., 1998). Occasionally, fixed wavelength laser systems have been utilised, either to measure fluorescence intensity variations at a fixed emission wavelength (Shopov et al., 1994) or at multiple emission wavelengths to obtain a 'fluorescence index' (Perrette et al., 2005).

Obtaining a satisfactory signal-to-noise ratio for the analysis of solid samples such as stalagmites requires a compromise between the need for a high fluorescence signal and any loss of optical and temporal resolution in achieving this. For example, Baker et al. (1998, 1999), Proctor et al. (2000) and Sundqvist et al. (2005) used 10 nm excitation and emission spectral bandwidths (SBWs) in order to gain sufficient fluorescence signal to analyse most stalagmite samples, limiting the reproducibility of the excitation and emission wavelengths of organic matter fluorescence centres to $\pm$3–5 nm. Fibre-optic spot size can also be limiting. Drysdale et al. (2006) employed a fibre-optic probe spot diameter of 4 mm, and were able to resolve a multi-centennial-scale period of reduced rainfall in a Holocene flowstone, while Baker et al. (1998) used a 1–2 mm spot: with a typical stalagmite growth rate of $\sim$100–250 μm yr^{-1}, this places the effective temporal resolution on a stalagmite at $\sim$5–20 yr. In contrast laser systems can achieve annual resolution but at the disadvantage of having a fixed excitation wavelength (Perrette et al., 2005).

Several recent technological developments have improved the utility of fluorescence analysis of dissolved organic matter in stalagmites. The addition of voltage control on the photomultiplier tube on recent off-the-shelf spectrophotometers has improved sensitivity and decreased detection limits. Meanwhile, scan speeds have improved by an order of magnitude in the last decade to $\sim$10^5 nm/min, leading to the generation of a fluorescence excitation and emission wavelength scan in 30–200 s, and increasing sample through-put. Lastly, the development of improved fibre-optic probes with smaller spot sizes has improved the temporal resolution that can be achieved.

We demonstrate these advances in Fig. 2, where high-resolution analyses of 5 stalagmites have been obtained using a Varian Cary Eclipse spectrophotometer with a focussed fibre-optic probe (beam size $\sim$0.1 mm). Stalagmite BA-99-4 from Ballynamintra Cave, Ireland has been subject to high-resolution ion-probe analysis and lamina counting (Fairchild et al., 2001): it has a mean growth rate of 15 μm yr^{-1} derived from lamina counting, exhibits trace element variations over the same scale and was deposited over the late Holocene. Stalagmite BFM-F1 from Brown's Folly Mine, England, was deposited over the last 150 years and has been previously analysed (Genty et al., 2001). Stalagmite growth rate based on maximum time of deposition is 230 $\pm$ 40 μm yr^{-1}, which is typical of the site as a whole. Stalagmite ETH-00-1 from Enda Abab Gerima Cave, Makden, Tigray, Ethiopia, is of unknown age but has clear visible laminae of 200–2000 μm scale; these are found in stalagmites throughout the country (Asrat et al., 2007; Baker et al., 2007) and are presumed to be annual. Mulwaree-2 is a stalagmite from the Mulwaree–Wollondilly Cave system, Wombeyan, Australia, that grew during marine isotope stage 5 and Onice-26 is an undated stalagmite sampled from Buca dell'Onice di Monte Girello, in the Alpi Apuane karst of central Italy. All stalagmite samples were moved at 100 μm steps for 2 mm, as well as at 1 mm steps for 10 mm, in order to investigate the variability of fluorescence intensities and wavelength variations at both spatial resolutions. The fluorescence index is the ratio of maximum emitted fluorescence intensities observed at $\sim$340 and 380 nm excitation, following Perrette et al. (2005).

For all samples, we observe variations in both fluorescence intensity and index at 100 μm steps, suggesting that significant data is lost when analysing at lower spatial resolutions. Fluorescence intensity variations are less noisy than those of the fluorescence index, while spatial variations become less noisy as sample growth rate increases: the sample with the slowest known growth rate (BA-99-4) possesses the noisiest fluorescence index variations, whereas the sample with the widest laminae (ETH-00-1, $\sim$1 mm) contains clear signal at the 100 μm level. This suggests that fluorescence intensity and index variations can be detected (using our apparatus) at $\sim$100 μm resolution, and that annual or even sub-annual resolution can be achieved for some samples.

3.3. Palaeoenvironmental applications

Speleothem luminescence provides a rapid and non-destructive approach for determining organic matter character and relative quantity. Organic matter character, in particular the aromacity of the constituent compounds, can be determined by variations in both fluorescence emission wavelength and fluorescence index, and can provide information on the overlying soil type at the time of speleothem formation. For example, Baker et al. (1998) presented variations in fluorescence emission wavelength, and inferred soil cover from caves in Yorkshire. The results showed that calcite fed by peat cover with blanket bog vegetation had the highest excitation and emission wavelengths, while that deposited under rankers and rendzinas covered by grassland and woodland showed the lowest fluorescence wavelengths. This is logical when

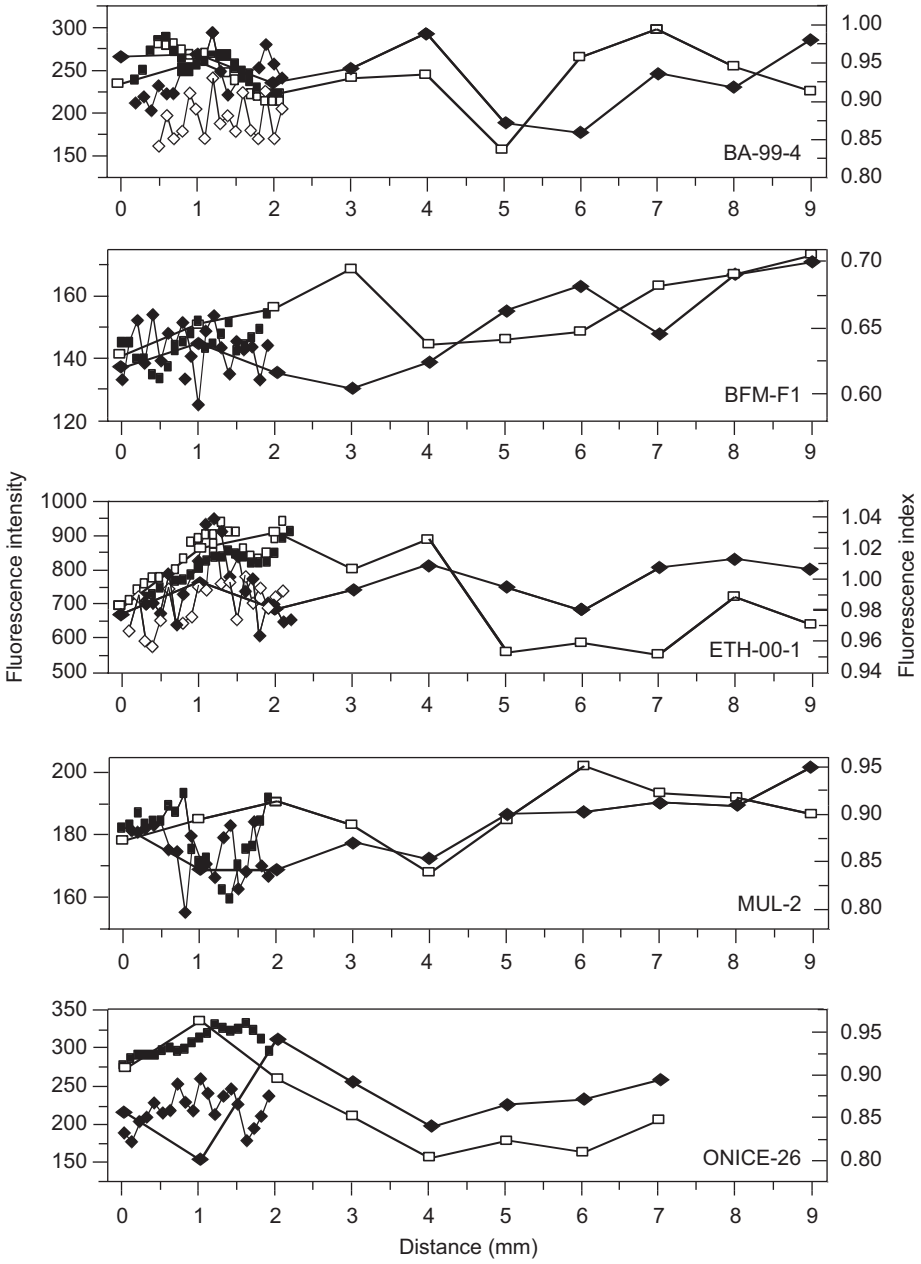

Fig. 2. Fluorescence time-series for five stalagmites. Fluorescence intensities are shown by squares, fluorescence index by diamonds. Replicate runs at ~2 mm offsets are shown by open squares and diamonds, respectively.

the relative degree of humification in the respective soil types is considered (Baker et al., 1998). A 70-yr time-series from a single stalagmite provided a correlation between the more subtle changes in fluorescence and the rainfall history of the area, albeit displaying a lag of around 10 yr (Baker et al., 1998), and for sites overlain by seasonally water saturated soils, the fluorescence index can provide a measure of relative wetness. In wet saturated soil years less degraded and more aromatic organic matter is transported, while in dry years the organic matter is more degraded and less aromatic. Proctor et al. (2000) demonstrated this effect in stalagmites by showing a correlation between fluorescence index and stalagmite annual lamina thickness for a stalagmite in NW Scotland deposited over the last 1000 years, both parameters being determined by the soil water table.

Fluorescence intensity variations in stalagmites have also been shown to occur seasonally, with annual fluxes of fluorescent material occurring in some regions in winter due to snowmelt, in other regions during monsoon seasons, and in others during soil wetting after a dry period. These can create annual lamination of the stalagmite, which can then be used to constrain dating and also for palaeoclimatic purposes, as the intensity of a fluorescence signal can relate to the amount of organic matter input. A review of the processes forming annual fluorescence

laminae can be found in Tan et al. (2006). However, long-term variations in the fluorescence intensity in speleothems have not been widely studied, due to the difficulty in quantifying fluorescence intensity against organic carbon concentration.

A further application is to combine variations in fluorescence index or wavelength with the relative fluorescence intensity as a measure of the relative contributions of 'fresh' soil derived water and 'old' stored groundwater. The underlying assumption is that there is little or no change in the soil organic matter character through time compared to the difference between soil and groundwater organic matter. Accordingly, speleothem fluorescence is interpreted to be from a soil source if there is a higher fluorescence intensity and wavelength, while groundwater sources are interpreted as having a lower intensity and fluorescence wavelength (Baker and Genty, 1999). Asrat et al. (2007) used this approach to determine the surface connectivity over the 500 yr period of deposition of an Ethiopian stalagmite, and applied this information to interpret the growth rate and ^{18}O and ^{13}C isotopic proxies contained within the sample.

3.4. Advantages, disadvantages, and research needs

The most significant advantage of fluorescent analysis of stalagmite samples is that it provides a non-invasive measure of dissolved organic matter quality and quantity, allowing the rapid collection of useful data without destructive analysis. The presence of fluorescence in itself suggests connectivity with the overlying soil and its associated biomarkers, and changes in fluorescence wavelength and/or intensity can help determine the best location for subsequent invasive biomarker analysis. It also implies a surface climate connection, which can help interpret inorganic stalagmite proxies such as ^{18}O and ^{13}C, which can contain a mixed surface and groundwater signal.

In terms of disadvantages, definite structural or concentration information cannot be recovered with this technique. Further work is also needed to bridge disciplines: organic matter fluorescence as a tool for organic matter characterisation is widely utilised in the freshwater, soil and engineering sciences, but this knowledge has yet to be fully transferred to the Quaternary science community. Finally, fluorescence intensity in stalagmites remains a semi-quantitative technique; future work needs to determine both fluorescence intensity and stalagmite TOC, in order to calibrate the luminescence intensity signal.

4. Lipid biomarkers

4.1. Overview

Lipid biomarkers are biologically derived fatty molecules such as fatty acids, alcohols and sterols. They are common across all environments, but different compounds may be specific to particular parts of the ecosystem (e.g. vegetation, bacteria, fungi, etc.); accordingly, by measuring the relative quantities of lipids present in an environmental record, it is possible to identify how the contributions of these different parts of the ecosystem have changed through time. The use of lipid biomarkers in climatic and environmental research has become well established over the last 30 yr (Brassell et al., 1986). The most common use has been in marine sedimentary environments (e.g. van Dongen et al., 2000; Hu et al., 2002; Boot et al., 2006), but terrestrial records (e.g. lake sediments, peat and soils) have also been studied (e.g. Meyers 1997, 2003; Ficken et al., 1998; Nott et al., 2000; Naafs et al., 2004) with success in characterising changes in vegetation and climate (e.g. Ficken et al., 1998; Marseille et al., 1999; Jansen et al., 2006; Jacob et al., 2007).

Due to the post-depositionally stable environment provided, the comparative ease with which they can be dated, and their direct connection with the overlying environment, stalagmites are an obvious potential archive for terrestrial biomarker records. The lipids preserved in speleothems are principally derived from the overlying soil and vegetation, having been transported from the surface by the percolating groundwater, although a significant proportion may be derived from the cave ecosystem (Xie et al., 2003b, 2005; Cacchio et al., 2004). This means that lipid records can potentially be used to interpret changes in vegetation type, as well as variations in the contribution of the soil fauna and cave fauna (both higher animals and microbes). Accordingly, several different compound groups can be targeted, depending on the desired research question, including both vegetation-specific compounds such as plant sterols and molecules derived from plant waxes, and bacterially specific groups such as branched fatty acids and 3-hydroxy acids.

However, the literature on biomarkers in cave deposits is currently limited. The preservation of lipids within speleothems was first demonstrated in the late 1980s when Cox et al. (1989) identified *n*-alkanes (straight chain hydrocarbons) within stromatolitic stalagmites. Stromatolitic stalagmites are not directly comparable to conventional speleothems, as they develop at least partially through microbial precipitation, and as they grow at cave entrances, they collect a greater amount of wind blown detritus (Cox et al., 1989; James et al., 1994). Lipids in speleothems deposited deeper within caves were first identified in the 1990s by Rousseau et al. (1992, 1995, 2005) who extracted plant and animal-derived sterols from a flowstone from the south of France. This work was followed by that of Xie et al. (2003b, 2005) who focussed on the relationship between major global climatic changes and a 10,000-yr lipid record in a Chinese subtropical stalagmite. Most recently, Blyth et al. (2007) have recovered a high-resolution biomarker record of the past 100-yr from an Ethiopian stalagmite and demonstrated the potential relationship between stalagmite biomarker records and vegetation change in the surrounding area.

4.2. Methods

Lipid biomarkers are extracted from environmental samples using organic solvents such as chloroform, dichloromethane, hexane and methanol, with the type of solvent selected and the precise extraction protocol (saponification, hydrolysis of the sample, liquid/liquid extraction, fractionation, derivatisation, etc.), depending on the type of environmental sample concerned, and the target compounds for analysis. Once the extract has been recovered and rendered amenable to study, the sample is most commonly analysed by gas chromatography–mass spectrometry (GC–MS), where a solvent containing extract is injected onto a gas chromatograph which resolves and separates the compounds into different peaks, and then passed into a mass spectrometer, which fragments the compounds into their component ions, allowing identification.

The three significant stalagmite biomarker studies published prior to 2006 (Rousseau et al., 1995; Xie et al, 2003b, 2005) all used conventional solvent extraction methods to recover the lipid signal: aliquots of crushed calcite powder were repeatedly mixed with organic solvents either by soxhlet extraction in the case of Xie et al. (2003b, 2005), or by a simple agitation technique in the case of Rousseau et al. (1995). However, because the solvent was only able to extract material from the surface of the powder and from within accessible pores, all three studies required initial calcite sub-samples of around 100 g (Rousseau et al., 1995; Xie et al., 2003b, 2005), giving a temporal resolution of approximately 1000 yr per subsample (Rousseau et al., 1995; Xie et al. 2003b, 2005). Given that other geochemical techniques applied to stalagmites are capable of yielding annual or sub-annual resolution, this was a major limitation on the approach.

The recent development of an optimised protocol for sample preparation (Blyth et al., 2006) has enabled a reduction in sample size of two orders of magnitude, making analysis of samples of <1–10 g of calcite feasible (Blyth et al., 2006, 2007), and allowing analyses at a temporal resolution of 10–100 yr. This method uses acid digestion of the whole calcite sample, rather than surface solvent extraction of powder, which has the advantage of both reducing contamination levels, and releasing compounds trapped within the calcite crystals (Blyth et al., 2006, 2007). The acid hydrolysis of the sample also facilitates the release of lipids bound within organic macromolecules, which cannot be released by direct solvent extraction (Mendoza et al., 1987a, b; Disnar et al., 2005; Pearson et al., 2005; Blyth et al., 2006). This serves to further increase the lipid yield, and widens the range of compounds that can be recovered, particularly those deriving from bacteria (for example, 3-hydroxy acids which are biomarkers for Gram-negative bacteria, are bound within lipopolysaccharides by ester and amide bonds, and cannot be recovered unless the latter are broken by acid hydrolysis (Mendoza et al., 1987b)). However, by recovering the organically bound and free lipid fractions together, there is a risk that environmental information may be lost, as the different organic fractions may be derived from different source pools, and may respond to climatic controls in different ways (Disnar et al., 2005). This issue is becoming increasingly important in the analysis of soils, with recent studies focusing on the optimisation and impact of sequential extraction techniques (Disnar et al., 2005), and stalagmite organic matter work would clearly benefit from similar investigations.

4.3. Palaeoenvironmental applications

The contrasting studies by Xie et al. (2003b) and Blyth et al. (2007) demonstrate the range of palaeoenvironmental applications lipid biomarker analysis in stalagmites can have, with the former looking at global climate change on a semi-geological scale, while the latter focuses on short-term anthropogenically induced local vegetation change.

The work of Xie et al. (2003b) used *n*-alkanes, *n*-alkanols, and *n*-alkan-2-ones, to link variations in the surrounding environment to global records of climate change during a time period of approximately 10,000–21,000 yr ago. In particular, attention was focussed on the ratio of high molecular weight (HMW: those with carbon chain lengths of C_{21} and above) and low molecular weight (LMW: carbon chains of C_{20} and below) compounds. HMW *n*-alkanols and *n*-alkanes are considered to be indicative of higher plants; the latter particularly are considered characteristic of leaf waxes, with certain carbon chain lengths being indicative of different vegetation types (Rieley et al., 1991; Meyers, 1997; Marseille et al., 1999; Pancost et al., 2002). LMW compounds are more commonly derived from lower organisms such as bacteria, fungi and algae, although they can also be the result of chemical degradation of other compounds (Bull et al., 2000). Similarly, *n*-alkan-2-ones are commonly found across soils and sediments and may have either a direct biological derivation or result from the degradation of *n*-alkanes and *n*-alkanoic acids.

In order to interpret the changes in the palaeoenvironmental signals through time, the ratio between the LMW compounds (representing microbial input either from the soil or in the cave), and the HMW lipids (broadly representing vegetational input) was examined, and the resulting record compared with sea-surface temperature alkenone records from the North Atlantic spanning the same time period. This showed that major cold events such as the Younger Dryas and Heinrich 1, were marked by an increase in LMW lipids. This was taken as a reflection of variation between vegetational and microbial input in the soil ecosystem with climatic changes. However, when looking at similar ratios of fatty acids in the sample, Xie et al. (2005) found that although the impact of Heinrich 1 was apparent, the Younger Dryas was not documented. This again demonstrates the need for a multi-proxy

approach, even within a single field of study such as lipid biomarkers.

Blyth et al. (2007) analysed a stalagmite from Mechara in south-east Ethiopia, which had grown over the past 100 yr. The area surrounding the cave had been subject to significant vegetation changes during that period, with the original wooded scrub being cleared to make way for a mixed agriculture of grain crops (tef, millet and maize), and cash crops (coffee, and the stimulant drug khat). This vegetation change appears to impact significantly on the lipid biomarker record in the stalagmite, with the chain length distribution of the HMW *n*-alkanes and the ratio of C_{27}/C_{31} *n*-alkanes (both previously suggested as markers for changes between arboreal and herbaceous vegetation (Marseille et al., 1999)) showing marked changes at the point in the stalagmite dated to the late 1930s (the time period when modern agriculture is reported as being introduced to the region). These changes coincide with variations in parameters relating to the amount of vegetation matter present (the Carbon Preference Index, which is a measure of the amount of fresh plant material in the *n*-alkane distribution; and the ratio of HMW/LMW *n*-alkanols and *n*-fatty acids, which, in line with the hypothesis put forward by Xie et al. (2003b), are suggested as representing the relative amounts of vegetation-derived compounds against those from microbial sources). In all cases the advent of the agricultural regime coincides with an increase in the amount of vegetation-derived material entering the system. Crucially, although several different lipid biomarker parameters appear to record the change in vegetation regime, the event is not clearly apparent in any of the traditional inorganic stalagmite proxies measured (carbon and oxygen isotopes, calcite fabric, and growth rate), which instead respond to principally to climatic parameters (Baker et al., 2007). If this pattern can be proven by repeated demonstration elsewhere, it offers the potential for lipid biomarker analysis in stalagmites to be utilised in decoupling anthropogenic and climatic impacts on palaeoenvironmental records.

4.4. Advantages, disadvantages, and research needs

One of the primary advantages of studying lipid records in stalagmites over terrestrial biomarker records from other contexts is the high degree of preservation over very long periods of time. Rousseau et al. (1995) looked specifically at the plant and animal sterols present within a flowstone floor deposited between 70,000 and 120,000 yr BP in Lazaret Cave near Nice, France. Although lipids in the wider environment change and degrade over time by oxidation and the loss of functional groups, comparison of the compounds found in 100,000-yr-old samples with those from an actively developing modern speleothem from the same area showed that there was no noticeable loss of signal in the older material. Quantification of the total lipid extracts from a range of stalagmites from different time-periods and geographical regions supports this (Table 1, Blyth, 2007): the amount of material recovered per gram of calcite is lower in samples from colder areas (NW Scotland and the Italian Alps), but is otherwise comparable, at 4–8 μg/g, regardless of the stalagmite age. This indicates that the chemically closed post-depositional environment provided by speleothems serves to 'freeze' the lipid signal intact. As stalagmites have a very great potential range of ages, from modern samples of a few tens or hundreds of years old, through to ancient material deposited over 4 million years ago (Woodhead et al., 2006), analysis of the lipid biomarkers preserved within them therefore has the potential to provide long-term records of environmental change and early human habitats at a level of temporal detail that is not currently available.

Table 1
The total measured lipid extract (μg/g calcite) from a range of stalagmites

Sample (with geographical location and age)	Average total measured lipids (μg/g calcite)
May Madken (Ethiopia, unknown)	8.80 ($n = 5$, sd = 1.27)
PDS 2 (Tuscany, modern)	5.05 ($n = 1$)
PDS 4 (Tuscany, modern)	8.57 ($n = 1$)
PDS 5 a (Tuscany, modern)	6.85 ($n = 1$)
PDS 5 c–f (Tuscany, 65–75 ka)	5.64 ($n = 4$, sd = 2.32)
Asfa 3 (Ethiopia, AD 1905–AD 2004)	5.82 ($n = 11$, sd = 3.00)
Tral-1 (N.W. Scotland, 2000 yr BP–present)	2.45 ($n = 7$, sd = 3.86)
Ern 79 (Italian Alps, modern)	1.73 ($n = 1$)
BR 36 (S.W. Victoria, Aus, unknown)	4.56 ($n = 1$)
M 7 (E. Victoria, Aus, unknown)	4.45 ($n = 1$)
Nul 1 (South Australia, >500 ka)	5.21 ($n = 1$)

N.B.: The total measured lipid extract is the sum of the *n*-alkanes, *n*-alkanols, *n*-alkanoic acids, sterols, straight chain di-alkanoic acids, 3-hydroxy acids, and *iso* and *anteiso* branched alkanoic acids measured in each sample.

Another advantage of lipid biomarker analysis is the ability to target specific compound groups and so study the relationship between changes in different sections of the ecosystem, principally plants and bacteria. The other proxies currently applied to stalagmites (for example carbon isotopes) can reflect changes in the source organic matter pool as a whole, but cannot separate them to this level of detail. In particular, the ability to recover specific bacterial markers is unique to lipid biomarker analysis in this context (DNA analysis has potential, but is largely untried and brings its own problems in terms of contamination and recovery). The use of biomarkers specific to bacteria (such as 3-hydroxy acids, branched fatty acids, and various hopanoids), as opposed to more general, broadly microbial, groups (such as LMW *n*-fatty acids) is currently highly under-utilised in stalagmite work, but with the development of an extraction method which enhances their recovery (Blyth et al., 2006), they offer considerable research potential.

However, lipid biomarker analysis in stalagmites also has a number of disadvantages, although many of these

relate to the current limited state of knowledge, and may be overcome by further research. From a technical perspective, the calcite sample size utilisable has been considerably improved by the optimised method of Blyth et al. (2006), but is still far from ideal when compared to the minimally invasive sampling methods that are available in other proxies. It is likely that advances in mass-spectrometry, allowing ever smaller sample sizes to be analysed, will improve the resolution available, while it is also entirely possible that the extraction method can be optimised still further, especially if workers target specific compound groups at the expense of a more general overview. However, the sample size required is dependent on the concentration of the lipids within the calcite, which will ultimately constrain the minimal amount of calcite that can be used. It may be necessary for workers requiring analysis of very small samples to take an entirely different analytical approach, for example Fourier transform infra-red sprectroscopy (FTIR) or secondary ion mass-spectrometry (SIMS).

A related issue is the current lack of a body of work demonstrating that the results from stalagmite analyses are truly replicable. Xie et al. (2003b) and Rousseau et al. (1992, 1995, 2005) did not state whether replicate samples were used in their analyses, while the size of stalagmite analysed by Blyth et al. (2007) prevented replicate subsamples from being taken. The only work currently existing on the repeatability of lipid signals from stalagmites is Blyth et al. (2006) which employed triplicate aliquots of a time-averaged homogenised stalagmite powder in the methodological investigations. However, this employed a minimum number of repeats, and in any case offers information only on the repeatability of the extraction yields relating to the method, not the repeatability of a genuine environmental signal. There is therefore a pressing need for work demonstrating that signals recovered from stalagmite time-series are robust within a single stalagmite sample, between stalagmite samples from a single cave, and between stalagmite samples from different caves but from the same region and subject to the same environmental regimes.

However, the most serious disadvantage affecting lipid biomarker analysis in this context is the current ambiguity of the source organic matter pools feeding the stalagmites, which at present limits the firmness with which environmental interpretations can be made. Although it is possible to distinguish bacterial input from vegetation input in certain compound groups (e.g. branched fatty acids and 3-hydroxy acids for bacteria, plant sterols and HMW *n*-alkanes with specific carbon number distributions for vegetation), many abundant groups (for example LMW *n*-fatty acids) have a mixed derivation. Furthermore, it is not yet possible to distinguish between different source populations of the same organism groups: this is a particular problem with bacteria, as the signal may derive from any or all of the soil OM pool, the limestone above the cave, or bacterial populations within the cave itself. Considerably more work is needed in this area before both the direct input of cave bacteria and their potential impact in terms of the alteration of transported organic matter can be understood.

An even more serious problem is identifying the different bulk organic matter pools from which the preserved lipids are derived, which requires a much deeper understanding of how the different compound groups are transported to the cave than is currently available. Due to their structural chemistry and the state in which they are released into the soil (free, bound within larger molecules, etc.), different lipid groups degrade at different rates and in different ways, and have varying hydrophobicities and amenability to adsorbing onto particulate matter. Therefore, different lipid groups released into the soil contemporaneously will not necessarily be transported to the stalagmite at the same rate. The issue is further complicated by variations in the potential effects on degradation and turnover time of variations in soil type and climatic conditions, along with the variations in transport speed caused by rock type and hydrological flow. This means that one model for lipid transportation will not necessarily be applicable to multiple cave sites, or even the same cave site over a prolonged time period. The work of Blyth et al. (2007) indicated a remarkably simple relationship between the lipid signal and the overlying vegetation regime, with no significant time lag being seen between the change to agriculture on the surface and the incorporation of the signal in the stalagmite. This was more remarkable due to there being a significant lag (~30 yr) in the incorporation of the atmospheric bomb ^{14}C spike in the stalagmite, and a significant storage component being apparent in the hydrological pathway (Baker et al., 2007). The working hypothesis proposed was that the lipids derived from the slow-turnover OM pool and transported via the dominant slower hydrological pathway had degraded to give a smoothed and therefore effectively invisible signal, which had then been over-written by less degraded material transported more quickly (Baker et al., 2007). This appears to be the most realistic hypothesis for the site given the evidence currently available, but to be supported with any firmness, more work needs to be done utilising samples from the same site known to be fed via different transport pathways. More generally, a much larger body of work is devoted to understanding the movement of lipids from the soil to incorporation within stalagmites in different geographical and environmental conditions needs to be developed.

5. Amino acids

5.1. Overview

A recent trend in the analysis of biomarkers in carbonates has been the study of amino acids (Lauritzen et al., 1994; Nyberg et al., 2001; O'Donnell et al., 2007; Penkman et al., 2007). There are 20 naturally occurring

amino acids which form the basic building blocks of proteins, and are also found in other biopolymers (e.g. peptidoglycans). All amino acids except glycine have a chiral centre and therefore can exist as two optical isomers, L and D, depending on the position of the functional groups. It is possible to measure both the concentration of amino acids present in a carbonate and also, by the ratio of D/L isomers, the extent of amino acid racemisation.

Proteins are exclusively synthesised from L amino acids (although peptidoglycan, which is a significant component of bacterial cell walls also contains D amino acids, see below). This artificial dominance in nature of the L-amino acid form is thermodynamically unstable, so a spontaneous reaction converting the L isomer into the D isomer occurs until an equilibrium of the two forms is reached; this is known as racemisation. The extent of racemisation is therefore a measure of the contribution from biologically synthesised D-amino acids and the further inter-conversion of L to D by abiotic and diagenetic means. Depending upon the nature and environment of the biopolymers and the temperature of burial, this process may take millions of years.

Amino acid racemisation has so far primarily been used to date biogenic carbonates (Hare and Abelson, 1968), specifically using the proteins involved in or entrapped by the process of biomineralisation. The extent of racemisation makes it possible to determine the relative age of fossil biominerals, and if the rate of inter-conversion between the two isomers can be determined, then it is possible to use racemisation to date the samples (Hare and Abelson, 1968). This approach, with the rate calibrated using other samples of known age, has been used to date bone (Bada, 1972), although with problematic results. Complications can occur due to variations in temperature, pH, and metal cations (Williams and Smith, 1977), all of which can affect the rate of racemisation, while groundwater contamination can contribute modern L isomers (Williams and Smith, 1977). The use of biominerals, such as shell, which are proposed to represent a closed system with respect to the original protein, has been more successful (Brooks et al., 1990; Parfitt et al., 2005; Penkman et al., 2007). There has been interest in studying racemisation in speleothems (Lauritzen et al., 1994), but due to the complexity of the system and the multiple potential sources of the amino acids (vegetation, bacteria, 'old' soil organic matter pools, 'young' soil organic matter pools, etc.), there are considerable problems with this approach.

5.2. Methodology

Amino acids are isolated from the intra-crystalline fraction of powdered speleothem, in order to avoid sampling surface compounds, which can be introduced during sample handling or variably leached away over time (Penkman et al., 2007). The intra-crystalline amino acids are operationally defined as the amino acids which persist after prolonged exposure of powdered carbonate to NaOCl (Berman et al., 1993; Sykes et al., 1995). Peptide bound amino acids are hydrolysed in 7 M HCl in a 110 °C oven, dried in a centrifugal evaporator and rehydrated for analysis by liquid chromatography (Penkman et al., 2007). In the only stalagmite amino acid study published to date, Lauritzen et al. (1994) sampled a flowstone at a resolution of approximately 1000 yr, and investigated one amino acid isomeric pair. Developments in high performance liquid chromatography (HPLC) mean that it is now possible to detect more amino acid pairs with much lower detection limits (Kaufman and Manley, 1998): in the data presented in Fig. 3 (discussed further below), 29 samples were taken, each consisting of 25–120 mg of calcite powder, and each representing a period of 1–60 yr. The amino acid analysis used reverse phase HPLC (following a modified method of Kaufman and Manley, 1998) and resolved 10 amino acid pairs, with a total concentration range of 100–300 pmol/mg calcite. This is an obvious improvement on the previously published capabilities of amino acid analysis in stalagmites.

5.3. Palaeoenvironmental applications

The principle use for amino acid analyses in other contexts has been exploitation of racemisation for dating purposes. Lauritzen et al. (1994) investigated the potential

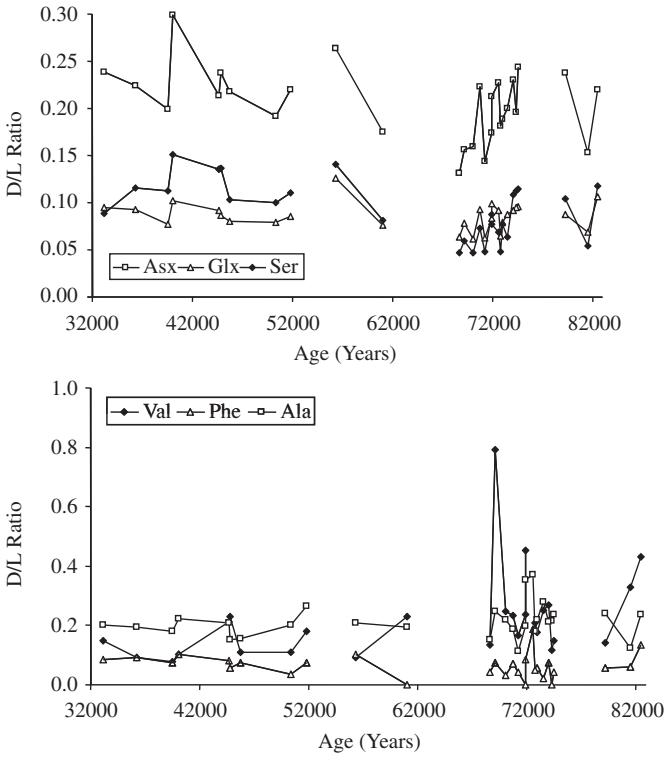

Fig. 3. D/L values versus time down core for the Grotte de Villars speleothem for six amino acids from the intra-crystalline fraction. Upper: Asx, aspartic acid/asparagine; Glx, glutamic acid/glutamine; Ser, serine. Lower: Val, valine; Phe, phenylalanine; and Ala, alanine. The sensitivity of the RP-HPLC technique enables the routine detection of multiple amino acids D/L values from small sample sizes, but no systematic increase in D/L with time is observed.

of this approach in speleothems, using the ratio of D-alloisoleucine to L-isoleucine (A/I) in the study of a Norwegian flowstone. It was found that the A/I value increased monotonically with age, suggesting that the method had potential as a chronological tool in this context. However, unlike bio-materials such as shell, speleothems are in no way a closed system for amino acids: as already discussed a significant proportion of organic matter in speleothems derived from the overlying soils, which contain amino acids from a wide range of sources including plants, fungi and bacteria. Organic matter turnover in soils preferentially decomposes L-amino acids (O'Dowd et al., 1997) while the contribution from the soil microbial community will contain significant amounts of D isomers from the peptidoglycan (predominantly D-Ala and D-Glx; Sowden et al., 1977; McCarthy et al., 1998: Willerslev et al., 2007), which will be further complicated by the additional contribution of amino acids (including D isomers) incorporated from bacteria either within the cave or along the flow paths. In short the amino acid signal in stalagmites may be of variable ages when entombed in the calcite (material from cave bacteria being fresher than material transported from the soil), and will invariably contain varying levels of D isomers. Therefore, although in the case of the Norwegian flowstone, the level of D-alloisoleucine incorporated into growing flowstone was low, suggesting that the organic matter was relatively fresh, these circumstances are likely to be site specific, and amino acid racemisation is likely to be of only very limited potential use as a chronological tool.

This is supported by the data presented in Fig. 3, which shows previously unpublished amino acid records from a 147 cm long stalagmite (Vil9) from Grotte de Villars, Dordogne, France; carbon and oxygen isotope data from this sample have been published in Genty et al. (2003) where they were observed to be coincident with Dansgaard–Oeschger events. It is immediately apparent from the graphs that these samples have highly variable amino acid records through time with no consistent trend. This contrasts not only with the results of Lauritzen et al. (1994), but also with $\delta^{13}C$ and $\delta^{18}O$ in the French sample, which clearly record stadial–interstadial climate change at the site (Genty et al., 2003). These results indicate that the amino acid record in stalagmites is very variable between sites, and has a far more complex derivation than simple time-dependent racemisation. Bulk composition is of little value in identifying source, and may be modified by selective partitioning along the flow path (Hedges et al., 1994; Aufdenkampe et al., 2001). However, amino acids contain information (isotopic composition, extent of racemisation) which may have the potential to help assess the environmental source of the organic matter.

5.4. Advantages, disadvantages, and research needs

The principal potential advantage of amino acid analysis in stalagmites is the relatively small sample size required, especially when compared to lipid biomarkers (25–120 mg of calcite, as opposed to 1–10 g). This would substantially improve the temporal resolution available in biomarker work, in some stalagmites potentially to an annual level. However, until a much greater understanding of the derivation of the amino acid in speleothems is developed, this will be of little practical use. Further work is needed to identify the different sources of the amino acid signal in speleothems, and to compare the records obtained to other speleothem proxies in order to identify climate driven responses.

6. Future development of the field

The most important progress in this field will be the development of a more advanced understanding of how organic matter is transported from the surrounding environment and preserved in speleothems. As discussed above, this will enable workers to have a much clearer understanding of the sources of the organic matter signal and their links to changes in the surrounding environment.

Beyond this however, progression may be made by expanding the range of information about the organic compounds that is recovered. The work on lipids and the unravelling of the different signals will be greatly accelerated by the application of compound specific isotope studies to the most interesting groups, as is now routinely applied in sedimentary biomarker studies (e.g. Cifuentes and Salata, 2001; Schwab and Spangenberg, 2007; Xu et al., 2007). This will be of particular use in investigating vegetation change, for instance between C3 and C4 plants, as it will provide an isotopic signal directly derived from the vegetation, and so circumvent issues of fractionation and disequilibrium that affect measurement of $\delta^{13}C$ in stalagmite calcite.

Outside of the compounds already being studied, the range of molecular material analysed should be increased. This could include plant DNA, which has the potential to offer a more detailed signal than lipid biomarkers, but is at present severely constrained by problems of extraction; or molecules such as low molecular weight sugars. These have yet to be firmly identified in stalagmites, but are found in soils and sediments (e.g. Teece and Fogel, 2007) and so would be expected to be present. A greater degree of information about human impacts on the environment could be obtained by the study of polycyclic aromatic hydrocarbons and related molecules derived from anthropogenic sources.

However, the most important of the available techniques is probably the extractive analysis of the humic fractions, including plant macromolecules, such as lignins. Macromolecular organic material forms the majority of organic matter in stalagmites (Ramseyer et al., 1997), and so offers the best opportunity for high-resolution analysis. Studies of lignin monomers have been successfully used in sediment research to recover detailed records of terrestrial ecosystem response to environmental change (e.g. Fisher et al., 2003;

Table 2
A summary of the molecular organic matter currently analysable in speleothems

Type of organic matter	Analytical method	Sample size required	Resolution	Advantages	Disadvantages
Fluorescence	Luminescence spectrophotometry	Non-destructive	Sub-annual	Non-destructive, high resolution, and rapid. Semi-quantitative bulk dissolved organic matter concentration information. Limited dissolved organic matter structural information.	Only limited information on dissolved organic matter character/structure.
Amino acids	High performance liquid chromatography (HPLC)	25–120 mg	>1 yr	Potential for high-resolution biomarkers. Can produce a signal at a smaller sample size than lipids, and modern technology allows very low detection limits.	Very little research published. Work needed to identify the different sources contributing to the stalagmite signal, and the mechanisms influencing variation between sites.
Lipid biomarkers	Gas chromatography–mass spectrometry (GC–MS)	0.5–10 g	>10 yr	Ability to distinguish clearly between vegetational, microbial and faunal inputs, so directly monitoring ecosystem change through time. Potential to identify different vegetational types (C3, C4 plants, etc.), especially if compound specific isotopes are used. Long records available in stalagmites due to closed chemical conditions post-deposition preventing compound degradation.	Still relatively large samples and coarse resolution compared to other techniques used in stalagmites. Further work needed to distinguish between surface and subterranean microbial signals. Work also needed to test local and regional variation and effect of hydrology.

Fuhrmann et al., 2003; Ishiwatari et al., 2006), and are the most promising biomarker for future stalagmite research.

7. Conclusion

Despite being a relatively recent innovation, the direct study of organic matter in speleothems has demonstrated great potential as a palaeoenvironmental proxy. Table 2 summarises the different approaches and their advantages and disadvantages.

Organic luminescence wavelength variations have been shown to relate to organic matter molecular weight and/or aromacity, which in some cases can broadly differentiate between vegetation regimes (Baker et al., 1998), or correlate with surface wetness and precipitation (Proctor et al., 2000). Where annual luminescent bands exist, these can also be used as a chronological tool to constrain other proxies, as well as providing a record of annual growth rate (Tan et al., 2006).

Biomarkers, although in their earliest stages of development as techniques relating to speleothems, have the potential to take this environmental information to a new level of detail. Lipids in particular are already proven to record ecosystem change in other environmental contexts, and the work so far clearly demonstrates that a record with differentiable signals is preserved within speleothems, and can be analysed at a resolution of down to 10 yr. Combined with annual laminations and the chemical stability of stalagmites, this will provide an exciting new proxy in the study of terrestrial ecosystem response to environmental change.

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