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Organic residue analysis of Egyptian votive mummies and their research potential

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Vast numbers of votive mummies were produced in Egypt during the Late Pharaonic, Ptolemaic, and Roman periods. Although millions remain *in situ*, many were removed and have ultimately entered museum collections around the world. There they have often languished as uncomfortable reminders of antiquarian practices with little information available to enhance their value as artefacts worthy of conservation or display. A multi-disciplinary research project, based at the University of Manchester, is currently redressing these issues. One recent aspect of this work has been the characterization of natural products employed in the mummification of votive bundles. Using gas chromatography–mass spectrometry and the well-established biomarker approach, analysis of 24 samples from 17 mummy bundles has demonstrated the presence of oils/fats, natural waxes, petroleum products, resinous exudates, and essential oils. These results confirm the range of organic materials employed in embalming and augment our understanding of the treatment of votives. In this first systematic initiative of its kind, initial findings point to possible trends in body treatment practices in relation to chronology, geography, and changes in ideology which will be investigated as the study progresses. Detailed knowledge of the substances used on individual bundles has also served to enhance their value as display items and aid in their conservation.

Keywords: Organic residue analysis, ATR–FTIR, GC–MS, Mummification practices, Votive animal mummies, Ancient Egypt

Introduction

Since 2000, multi-disciplinary research into the practice of non-human mummification in ancient Egypt has been conducted at the Kay Nanette Hinkley (KNH) Centre for Biomedical Egyptology, University of Manchester. As a continuation of this early work, the Ancient Egyptian Animal Bio Bank (AEABB) was established in 2010. This project, designed to evaluate the nature and condition of mummified animal remains curated outside Egypt and provide a repository for the data obtained, forms the largest cross-collection study of its kind (McKnight *et al.*, 2011). To date, 800 individual mummies, principally votives, have been investigated using non-invasive imaging techniques, with photography employed to record exterior appearance and radiographic techniques (X-radiography and computed tomography) used to provide insights into the contents without jeopardizing the integrity of the bundle (McKnight, 2010; McKnight & Atherton, 2014). This approach has revealed that approximately two-thirds of votive bundles contain skeletal remains while the remaining third were constructed from non-skeletal material. A broad list of species have been identified along with inclusions, either intentionally placed to add support and provide form to the bundle, or accidentally incorporated during the mummification and wrapping process (McKnight *et al.*, in press).

During the course of this research, it became evident that the current state of preservation of a number of votive mummies allowed small samples to be collected without further damage to the bundle. Much of this material was detached debris or loose portions of packing materials and bundle wrappings. Visual and microscopic examination indicated that organic residues comprised a significant component of these samples as they included fragments of a resinous nature, balm-impregnated textiles, residue-coated feathers, and packing materials (e.g. reeds) and mixed debris associated with the bundles (Fig. 1). As

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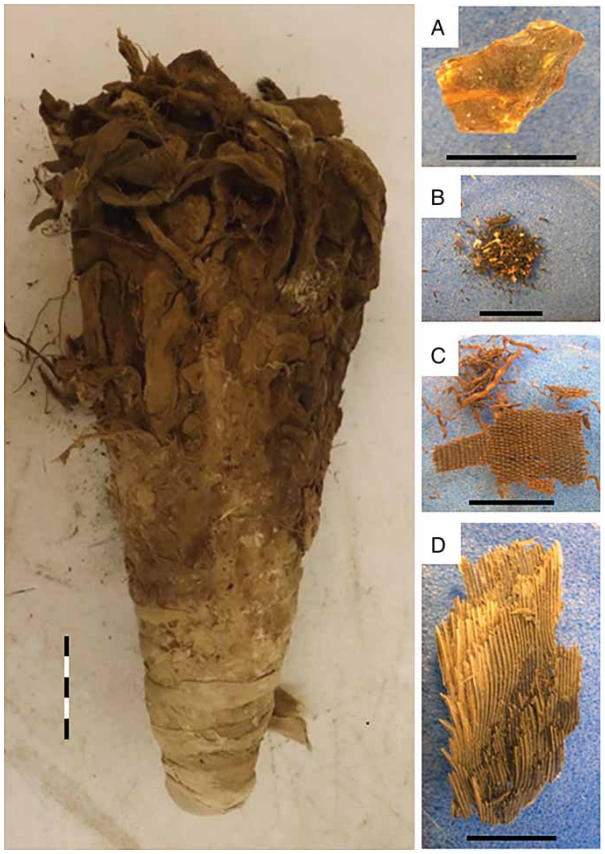

Figure 1 Votive ibis bundle, AEABB401, showing poorly attached portions suitable for analysis (main image) and materials detached from mummy bundles (A–D). (A) Resin fragment from foot area, ibis, AEABB162; (B) debris from base of ibis mummy, AEABB471; (C) residue-impregnated textile loose from left side, bird of prey, AEABB004; (D) residue-coated wing feather, left side, *Accipiter* sp., AEABB384. Scale bars = 1 cm.

ancient texts provide little information about the organic substances used as part of non-human mummification processes (Dunand & Lichtenberg, 2006, pp. 108–11) and detailed data from the chemical analysis of only four votive bundles is currently in the public domain (Buckley *et al*., 2004), a decision was taken to investigate this aspect of their treatment.

The aim of this study was to add to the limited corpus of knowledge regarding the substances used in the preparation of votive mummies and highlight the level of information retained in the small samples available for analysis. To this end, materials were selected from 17 votive mummy bundles for initial assessment by attenuated total reflection Fourier transform infrared spectroscopy (ATR–FTIR) followed by analysis of those indicating the presence of organic residues by gas chromatography–mass spectrometry (GC–MS). The rationale behind this methodology was to establish a protocol that would meet the requirements of different institutions (Fig. 2). Museums, as custodians of historically and culturally important artefacts, are often justifiably wary of scientists seeking to apply destructive techniques. Permission to apply such methods, even to small detached samples, may be refused so a demonstration of the level of information obtainable using a non-destructive technique (ATR–FTIR) was deemed desirable. Moreover, as visual assessments may be incorrect this first-pass evaluation would avoid unnecessary sample treatment.

The focus of the research was, however, the application of GC–MS and the well-established biomarker approach (Evershed, 2008). This minimally destructive technique enables suites of compounds to be characterized, permitting identification of a wide range of organic materials of archaeological interest (Colombini & Modugno, 2009). Consideration of the value added by such data in conjunction with minimum sample dimensions and the impact of sample processing on textile or other substrates would then enable discussions to be initiated and informed decisions to be made concerning future sample collection strategies. Designed to complement the non-invasive programme of research already being undertaken at the KNH Centre, University of Manchester, it is hoped that such information relating to embalming materials will form part of the growing database which can be interrogated for research purposes, facilitate the work of conservators and serve to enhance the role of votive mummies within museum collections.

Archaeological context

The ancient Egyptians are the only civilization known to have intentionally preserved the remains of other animals after death through artificial mummification. The non-human mummy bundles produced have been categorized into four groups: cult animals (e.g. the Apis bull), pets/companion animals, victual (food) mummies, and votives (Ikram, 2005; McKnight, 2010). These last account for the vast majority of these animal mummies, yet their intended purpose is the least well understood. Deposited in their millions in dedicated catacombs often associated with temple complexes, they have been discovered in all areas of Egypt but appear to be a late phenomenon extending from the 25th Dynasty (Kushite Empire) to the end of the Roman Period (Ikram, 2005).

In Egyptian thought, gods, humans, and other animals were perceived as closely inter-related entities with transmigration possible in certain circumstances (Bleiberg, 2013). This intimate association extended into the mortuary sphere as surviving texts and tomb inscriptions, and the mummification of pets and cult animals throughout the Pharaonic period demonstrate that the afterlife was accessible to all, providing the correct rituals were performed (Dunand & Lichtenberg, 2006, pp. 108–09; Barbash, 2013).

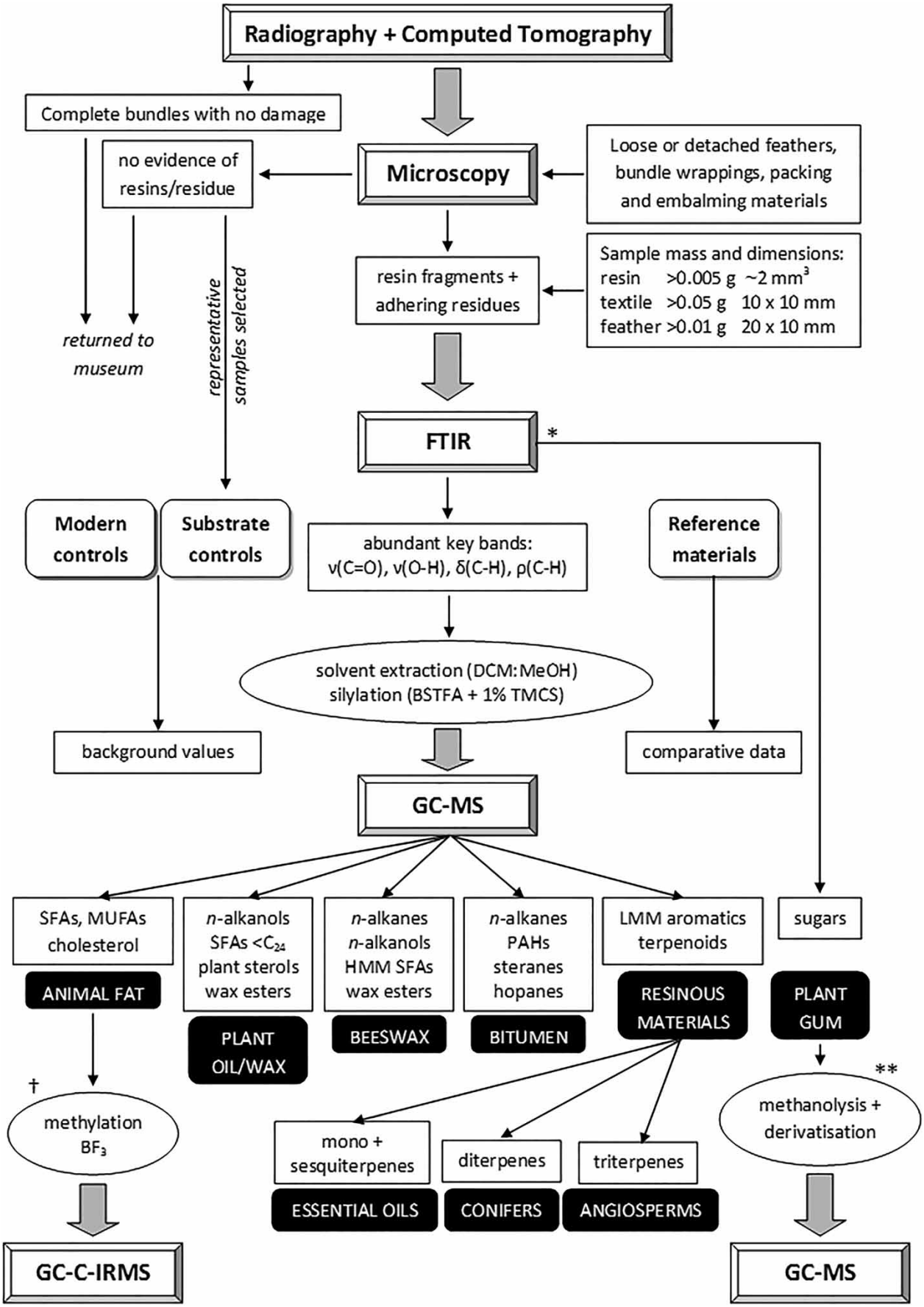

Figure 2 Protocol developed to facilitate the selection of samples from Egyptian votive animal mummies and maximize the structural and molecular information obtained from these sensitive artefacts without causing further damage. The scheme includes ongoing work to evaluate the saccharide content of these samples, based on the FTIR method of Ménager *et al*. (2014)* and GC–MS method of Bleton *et al*. (1996). When in sufficient abundance, oil/fat components will be analysed in future investigations using GC-C-IRMS (after Evershed, 2009).†**

During the Late Period, influenced by the upheaval resulting from a series of external conquests and exposure to new ritual practices, this practice appears to have evolved to include the concept of votive mummies (Ikram, 2005). Sanctified through their association with Egyptian deities and their temple enclosures, it is thought that these creatures were embalmed in order to act as an oracular device through whose agency requests for advice, comfort, or protection could be delivered direct to the god honoured by the offering (McKnight, 2010; Bleiberg, 2013).

Preservation of the likeness of the individual was a key element in this rite of passage. Without a recognizable image, the *ka* (spirit) would not be able to reunite with the body, the *ba* (essence) of the god, another sacred incarnation, and the appeals borne by votive animals would not reach their destination (El Mahdy, 1989, pp. 11–13; Smith, 2009, pp. 4–6). The process of mummification was, therefore, invested with enormous significance with the natural products employed key to its success in both physical and ritual terms (Baumann, 1960; Dunand & Lichtenberg, 2006, pp. 108–11). As with other aspects of the treatment of the body, however, very little detail has been passed down from antiquity regarding the organic substances selected (Elliot Smith & Dawson, 1991, pp. 45–67). This is particularly true with regards to votive animal mummies. Chemical investigation of residues associated with votive bundles has also rarely been undertaken despite the 'unparalleled opportunities' offered by the vast numbers extant (Aufderheide, 2003, p. 404).

Organic residue analysis has a well-established history in archaeological research (Evershed, 2008). Using instrumental techniques, principally GC–MS, a wide variety of materials can be characterized with minimal destruction of the artefact (Colombini & Modugno, 2009). These include the natural substances used as part of Egyptian embalming processes which range from animal fats and plant oils to beeswax and plant exudates (Lucas & Harris, 1962, pp. 303–37; Serpico, 2000; Serpico & White, 2000a). The latter comprise natural gums, resins, and gum-resins whose identification can provide insights not only into ritual actions but also aspects of technology and trade or tribute relations (Baumann, 1960). The majority of this research has focussed on human mummification practices from their earliest manifestations (Jones *et al.*, 2014), through the Pharaonic and Intermediate periods (e.g. Vieillescazes & Coen, 1993; Koller *et al.*, 1998; Colombini *et al.*, 2000; Hamm *et al.*, 2004) to their continuance during Ptolemaic and Roman rule (e.g. Buckley & Evershed, 2001; Maurer *et al.*, 2002; Corcoran & Svoboda, 2010).

Recently, victual mummies, deposited in human tombs as food offerings for the deceased, have also been investigated (Clark *et al.*, 2013; Ikram 2012). Only two publications, however, provide data regarding the chemical analysis of votive mummies, Buckley *et al.*'s (2004) seminal study of four bundles curated in Liverpool Museum and recent research conducted by the Brooklyn Museum, New York, on 20 examples from their own collection (Bruno, 2013). The results of these studies have shown that a similar range of substances to those used in human mummification were employed on some votive bundles but by no means all.

Nonetheless, the small sample pool investigated to date leaves many questions unanswered. These include concepts such as the differential treatment of species, variations in recipes used by different temple complexes, and changes made in response to the fluid political, economic, and ideological conditions in the Late Pharaonic, Ptolemaic, and Roman periods. In addition, the lack of detailed information regarding the nature and treatment of votives often prevents curators from utilizing these intriguing artefacts as informative and attractive exhibits. Thus, although significant numbers form part of museum collections around the world, they are rarely displayed. These issues can only be addressed by a systematic multi-disciplinary programme of research which incorporates samples from a broad range of votive mummies, preferably those with provenance attached. In museum collections, such provenanced bundles are, however, relatively rare as a result of nineteenth century practices, including the bulk transportation of votives and the actions of private collectors (Zivie & Lichtenberg, 2005). Thus, part of the research being undertaken by the AEABB is aimed at providing evidence of a chronological sequence based on wrapping techniques and accelerator mass spectrometry (AMS) dating (McKnight & Atherton-Woolham, 2015). It is hoped that the sampling protocol, findings, and discussion presented in this paper will facilitate the broader, collaborative research agenda.

Experimental

Sample selection

Samples were collected from 17 votive animal mummies (Table 1 and Supplementary Table S1). These were chosen to provide data relating to the treatment of a range of species, with eight bundles specifically targeted since information regarding their chronological and/or geographical origins were available.

Sampling was conducted using pre-cleaned tweezers, with permission and under the guidance of museum curators and conservators. The materials selected were securely associated with each mummy bundle (contained within the wrappings or still

Table 1 Summary of analytical data for votive mummies sampled. For further details regarding the bundles, see Supplementary Table S1

Species *provenance*	AEABB number	Sample description and context	Institution details	Key findings
Dog/Jackal *Ptolemaic?*	001	Degraded linen and mixed debris — loose in tissue paper	Grantham Museum, Grantham, UK	Minimal/no organic residue (FTIR) Below level of significance (GC–MS)
Cat	144	Degraded linen and mixed debris — loose in box	Nottingham Castle Museum, Nottingham, UK	Fat/oil, cholesterol present
Cat	191	Linen and mixed debris from head region — sweepings	Bristol Museum & Art Gallery, Bristol, UK	Fat/oil, cholesterol present Wax (beeswax?)
Cat	595	Residue-coated linen from neck region — loose in tissue paper	Old Speech Room Gallery, Harrow School, Harrow, UK	Fat/oil, cholesterol present
Bird of prey	004	Residue-coated linen (x4) portions — loose in bag and on bundle	Kirklees Museum Batley, UK	Fat/oil, cholesterol present Pinaceae resin *Pistacia* spp. Resin
Falco sp.	027	Dark-stained linen — debris within tissue paper	Garstang Museum, University of Liverpool, Liverpool, UK	Fat/oil Beeswax Pinaceae resin *Pistacia* spp. Resin
Hawk	146	Sticky, shiny black residue from rear aspect — area of damage	Nottingham Castle Museum, Nottingham, UK	Bitumen (modern)
Bird of prey *Late Period*	153	Stained tail feathers — exposed distal end	Royal Albert Memorial Museum, Exeter, UK	Minimal/no organic residue (FTIR)
Accipter sp.	384	Residue-coated feathers (3×) — left shoulder/wing	Museum of Fine Arts, Boston, USA	Fat/oil Natural wax Pinaceae resin Triterpenes
Bird of prey *Kafr Ammar*	475	Stained, residue-coated tail feathers (2×) — exposed layers	New Walk Museum, Leicester, UK	Wax (beeswax?) *Pistacia* spp. resin
Ibis *Roman*	162	Resinous fragments (2×) + charred (?) mass from foot area	Oriental Museum, Durham University, Durham, UK	*Pistacia* spp. resin (resinous fragments) No organic extract (charred mass)
Ibis *Ptolemaic Saqqara*	165	Reed fragments & mixed debris — removed from packaging	Oriental Museum, Durham University, Durham, UK	Fat/oil, cholesterol present Bitumen Conifer essential oil Terpenes — traces
Ibis *Ptolemaic/ Roman Memphis*	464	Degraded linen and mixed debris from head end — loose in wrappings	Buckinghamshire Museum, Aylesbury, UK	Minimal/no organic residue (FTIR)
Ibis *Ptolemaic/ Roman Memphis*	465	Degraded linen & mixed debris — loose under abdomen	Buckinghamshire Museum, Aylesbury, UK	Minimal/no organic residue (FTIR)
Ibis?	471	Mixed debris from base — loose after photography	Elgin Museum, Elgin, Scotland, UK	Fat/oil Bitumen Conifer essential oil Pinaceae resin
Bird? *Ptolemaic/ Roman*	401	Solid mass of mark material with feather and textile impressions	Museum of Fine Arts, Boston, USA	Fat/oil, cholesterol present Conifer essential oil *Pistacia* spp. resin?
Cat	150 control	Fragment of plain linen, no residue apparent — from bag of debris	Derby Museum & Art Gallery, Derby, UK	Degraded cellulose (FTIR) Below level of significance (GC–MS)

loosely attached). Each sample was placed into a separate glass jar with a Teflon-coated lid to minimize contamination and stored in environmentally monitored conditions at the KNH Centre, University of Manchester. Details of the source, location, and nature of each sample were recorded and the materials selected were photographed.

At this stage of the project, only damaged bundles were sampled as their condition permitted direct access to the mummy without causing further deterioration. The benefits of this approach were deemed to outweigh issues regarding how representative a single sample might be of the totality of materials employed in each bundle, although multiple samples were collected where possible. In addition, the greater potential of environmental contamination on samples of this nature is acknowledged. Although this may be of significance with regards to the detection of certain

ubiquitous compounds (e.g. commonly occurring carboxylic acids), biomarkers characteristic of natural products such as plant resins are far less likely to derive from contamination. It is the latter that have the greater potential to address the questions posed above.

Sample evaluation using ATR–FTIR

A preliminary assessment was undertaken using the non-destructive technique ATR–FTIR. Measurements were performed using a PerkinElmer Frontier spectrometer (4000–650 cm^{-1}, 16 scans, 4 cm^{-1} spectral resolution) without sample pre-treatment. The presence of organic matter was ascertained through evaluation of diagnostic vibrational group frequencies as reported in the literature (Shearer, 1989; Derrick *et al.*, 1999, pp. 82–108; Colombini *et al.*, 2009; Ménager *et al.*, 2014) and in comparison with controls.

Sample preparation for organic residue analysis

Organic residue analyses and interpretations were conducted using established protocols designed to minimize contamination and maximize recovery of extractable organic compounds (Stern *et al.*, 2003; Brettell *et al.*, 2014). Each sample of interest was sub-divided where possible. In order to establish the minimum amount of each type of material (e.g. resin fragments or mixed debris) from which meaningful results could be obtained, a range of masses was evaluated. The dimensions of the various substrates (e.g. balm-impregnated textiles or residue-coated feathers) were also considered due to their differing densities and as balances of sufficient accuracy are unlikely to be available in many contexts. This approach established that 5–10 mg (c. 2 mm^3) was more than sufficient for characterization when a specific material such as a resin fragment was available. Where the residues were impregnated within or adhering to substrates of differing density, textile fragments c. 10 mm^2 (50–100 mg) and feather portions c. 20 × 10 mm (10–50 mg) were required. When the sample consisted of mixed detritus with a considerable contribution from inorganic components and/or degraded textiles, <200 mg was found to be desirable although some indication as to the substance(s) present was obtained from as little as 50 mg.

These sub-samples were solvent extracted in dichloromethane: methanol (DCM:MeOH, 2:1, *v*/*v*, 3 × 2 ml) aided by ultrasonication and centrifuged to facilitate separation of the soluble and insoluble fractions. The solvent extracts were combined and excess solvent evaporated under a stream of nitrogen. To promote chromatographic separation, trimethylsilyl derivatives were produced using ~0.5 ml of *N*,*O*-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane. Excess reagent was removed by evaporation at room temperature and the derivatized samples re-diluted in DCM (~0.1 ml) for analysis by GC–MS. All solvents were high-performance liquid chromatography (HPLC) grade.

GC–MS analysis

Analysis was carried out using an Agilent 7890A GC system, fitted with a 30 m × 0.25 mm, 0.25 μm DB-5MS Ultra-Inert 5% phenyl methyl siloxane phase fused silica column (Agilent), connected to a 5975C inert XL triple axis mass selective detector. The splitless injector and interface were maintained at 300 and 280°C, respectively, and the carrier gas, helium, at constant flow (1.5 ml/min). The temperature of the oven was programmed from 50 °C (isothermal for 2 minutes) to 350°C (isothermal for 10 minutes) at a gradient of 10°C/minute. Electron impact (EI) spectra were obtained at 70 eV with full scan from m/z 50 to 800 amu.

Control and reference materials

As feathers from avian mummies formed the substrate for a number of the residues, modern examples from two bird of prey species (*Accipiter nisus* and *Falco tinnunculus*) were analysed as controls. One sample of pale brown, apparently residue-free, linen associated with a cat bundle (AEABB150) was also selected (Table 1 and Supplementary Table S2). These samples were initially evaluated by ATR–FTIR and then underwent the same processes as the archaeological materials in order to ascertain the nature of any intrinsic lipids present in the organic solvent-soluble fraction. In addition, a range of botanically and geographically certified reference materials were analysed to confirm the nature of the plant exudates identified. These comprised of Pinaceae and *Pistacia* spp. resins and conifer essential oils (Supplementary Table S3).

Results

Results from the analysis of 24 samples from 17 votive animal mummies are summarized in Table 1. The analytical protocol is illustrated schematically in Fig. 2.

Preliminary assessment using ATR–FTIR

An initial evaluation was undertaken using ATR-FTIR, with spectral contributions of textile and feather substrates established through the analysis of controls (Fig. 3A). Eight samples contained little or no organic matter (other than the substrate, where present). The remainder showed bands characteristic of aliphatic hydrocarbons (strong C–H stretching (ν), bending (δ), and rocking (ρ) modes at ~2950–2850, ~1460/1380, and 750–720 cm^{-1}, respectively) with oxygen-containing functional groups and/or admixed with sterols (e.g. a broad ν(O–H) band,

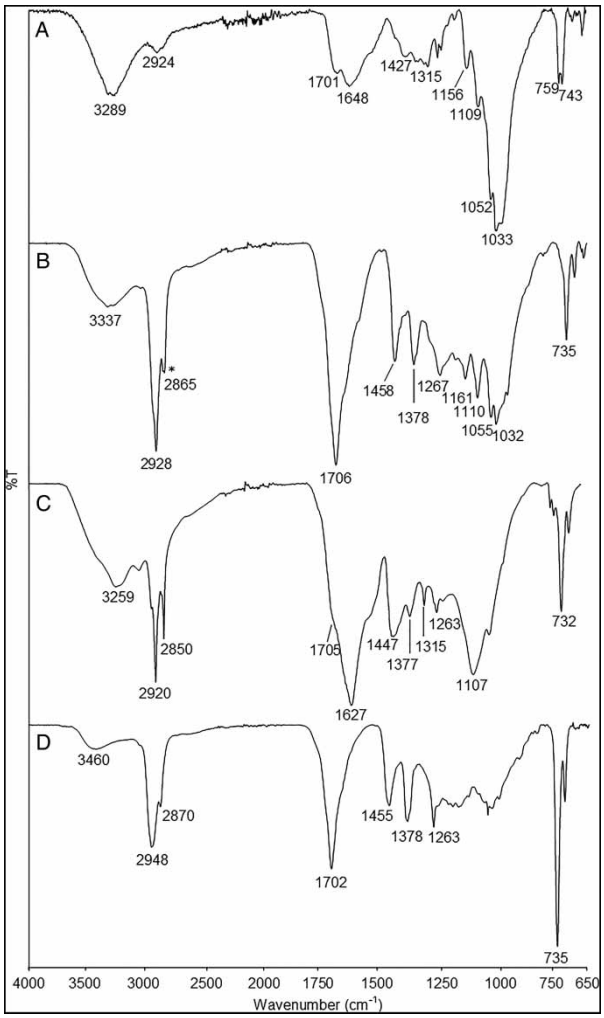

Figure 3 ATR–FTIR spectra (A) plain archaeological textile control, votive cat bundle AEABB150, showing bands characteristic of degraded cellulose; (B) residue-coated textile, bird of prey bundle AEABB004, with bands indicating a mixed of lipid components, possibly including an animal fat; (C) debris sample, votive ibis AEABB471, suggesting the presence of a diterpenoid resin; (D) resin fragment, votive ibis AEABB162, with bands diagnostic of a triterpenoid resin.

3500–3300 cm^{-1}; a strong ν(C=O) band between 1760 and 1665 cm^{-1}, which falls ~1708 cm^{-1} in unsaturated carboxylic acids; and ν(C–O) bands in the fingerprint region, ~1320–1000 cm^{-1}; Fig. 3B) and/or terpenic components (e.g. strong ν(C=O) bands, ~1720–1690 cm^{-1}; a shifted broad ν(O–H) band ~3450–3300 cm^{-1}; ν(C–O) bands between 1280 and 1230 cm^{-1}, generally ~1240 cm^{-1}; CH_2 and CH_3 group bending (δ) at 1460–1450/1377–1385 cm^{-1} and rocking (ρ) modes ~730–740 cm^{-1}) (Fig. 3C and D). The ν(C–H) band at 2865 cm^{-1} marked in Fig. 3B may indicate an animal fat since this is reportedly not present in vegetable oils (Shearer, 1989, p. 214). The strong bands at 1627 and 1315 cm^{-1} in Fig. 3C probably denote the presence of calcium oxalates which are common components of degraded organic materials (Sutherland *et al.*, 2013).

Analysis by GC–MS

Results of GC–MS analysis of the samples for which ATR–FTIR indicated the presence of organic matter, other than the substrate, are presented as total ion current (TIC) and extracted ion (XIC) chromatograms of the trimethylsilylated solvent extracts. Assignments are based on the molecular mass, established fragmentation patterns and relative retention times of the detected compounds and in comparison with the mass spectral literature.

Contaminants and controls

The majority of the samples contained low levels of phthalate plasticisers and related compounds. These modern contaminants probably derive from plastic packaging that had come in contact with the mummy bundles at some point during their curational history, although a proportion may be analytical artefacts (i.e. from piercing of the vial septum). Four of the samples, including the archaeological textile control, which showed little evidence of additional organic matter using ATR–FTIR, were also evaluated by GC–MS and the near-absence of extractable organic residues confirmed.

The two modern feather controls were likewise assessed as, although the degradation pathways of mammalian body fats are well attested (Evershed *et al.*, 2002; Forbes *et al.*, 2004), avian species have received less attention. Their solvent extracts contained low levels of saturated carboxylic (fatty) acids (SFAs: $C_{16:0}$, $C_{18:0}$) and cholesterol together with 5α-cholestanol, its aerobic microbial reduction (biohydrogenation) product. These ubiquitous components of degraded body tissues provided a baseline from which to consider the relevant archaeological samples. They also indicated that, although recognizable, lipids associated with the feathers of deceased birds are generally only present in trace amounts. Nonetheless, as *in vivo* epidermis or uropygial (preen) gland secretions consist of wax esters, triacylglycerols and cholesterol (Rajchard, 2010) and vary according to species, season, health, and nutrition (Jacob, 1976), careful consideration is required in each case.

The modern feathers also permitted the impact of solvent extraction on the integrity of this substrate to be considered. Both remained intact and appeared unaltered by the process. Likewise, little change was noted in the textile control and other substrates from which the archaeological residues were obtained. The integrity and structural details of both the fabric and feathers were maintained (Supplementary Fig. S1). This is of considerable importance as such materials can, if required, be returned intact to the contributing museums once the organic residues have been extracted.

Oils and fats

Almost all of the comminuted debris and adhering residue samples that provided positive results contained a limited range of *n*-alkanes along with SFAs (no unsaturated moieties such as monounsaturated fatty acids (MUFAs) were detected). These saturated compounds are ubiquitous end products of the degradation of plant and animal tissues and so could derive from either source or a combination of both. Some mammalian contribution is, however, indicated by the presence of cholesterol and its derivatives, diagnostic markers for animal fats (Buckley & Evershed, 2001; Forbes *et al*., 2002; Fig. 4A), in all but three of these samples. As those that provided negative results comprised feathers (AEABB384) and a textile fragment (AEABB027) from the outer layers of their respective bundles, the most parsimonious explanation is that the lipids identified in the remainder derived from the decomposition of the wrapped remains. Nonetheless, an applied animal fat or an admixture with a plant oil cannot be ruled out. In future studies, if sufficient fatty matter is recovered, gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) could be undertaken. This technique can distinguish between plant oils and animal fats derived from ruminant and non-ruminant sources based on the $\delta^{13}C$ values of key lipid components (Meier-Augenstein, 1999; Evershed, 2009). To be effective in the context of votive mummies, however, reference data would need to be obtained from comparative arid environment species as dietary ratios are affected by environmental variables and research to date has focussed on old world domesticates (Evershed, 2009).

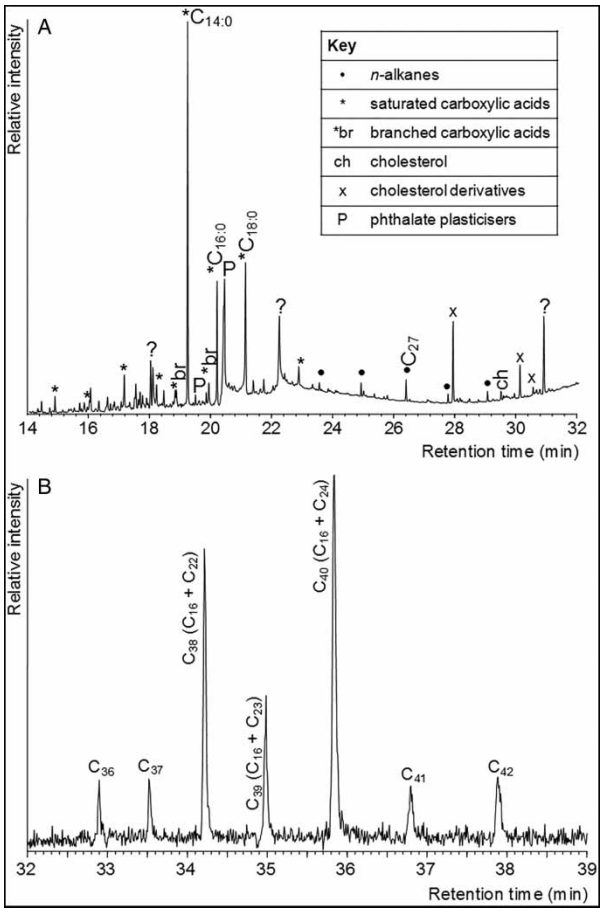

Figure 4 GC–MS data from two votive cat mummies. (A) Partial TIC chromatogram of solvent extract of degraded linen and debris from AEABB144 with components indicative of animal fat and/or plant oil; (B) XIC (*m*/*z* 257) of loose material from AEABB191 showing homologous series of palmitic wax esters ($C_{36–42}$).

Natural waxes

Samples from four of the mummy bundles, a cat (AEABB191) and three birds of prey (AEABB027, AEABB384, and AEABB475), provided evidence of the presence of a natural wax. Characterized by high molecular mass (HMM) *n*-alkanes with an odd-over-even carbon number predominance (C_{23}–C_{33}) and long-chain monoesters of palmitic acid ($C_{34–46}$) (Fig. 4B), these compounds may derive from the epicuticular waxes of higher plants (Rieley *et al*., 1991) including the grasses and reeds used for packing materials in the bundles. Beeswax is an alternative source as this plant-derived product of the genus *Apis* contains a similar range of components and the *n*-alkane maximum in three of the samples falls at C_{27} (except AEABB384), a distribution diagnostic of this substance (Regert *et al*., 2001).

Previously reported in both human and non-human mummies (Buckley & Evershed, 2001; Buckley *et al*., 2004), beeswax is, however, generally typified by wax esters greater than 40 carbons in length (rather than the range observed here) and their degradation products (Heron *et al*., 1994; Regert *et al*., 2001). The latter include HMM *n*-alkanols, traces of which were detected in AEABB191 and AEABB027 although these may also be present in leaf waxes whose natural composition is highly variable (Eglington & Hamilton, 1967). Inconsistencies in the composition of beeswax have likewise been recorded as a result of heating and/or taphonomic factors (Evershed *et al*., 1997; Regert *et al*., 2001) making definitive determination problematic. The additional presence of long-chain SFAs ($C_{22:0}$–$C_{30:0}$) with a maximum at $C_{24:0}$ together with ω-hydroxy- and oxocarboxylic acids in sample AEABB027 does, however, support a diagnosis of beeswax in this instance (Garnier *et al*., 2002).

Fossil hydrocarbons

The outer surface of one of the mummy bundles sampled, a hawk (AEABB146), had an unusual dark sticky appearance that suggested a modern treatment may have been applied. The glossy black droplets analysed contained a homologous series of *n*-alkanes (Fig. 5A) in conjunction with low levels of hopanes (Fig. 5B), pentacyclic terpenes derived from bacteria,

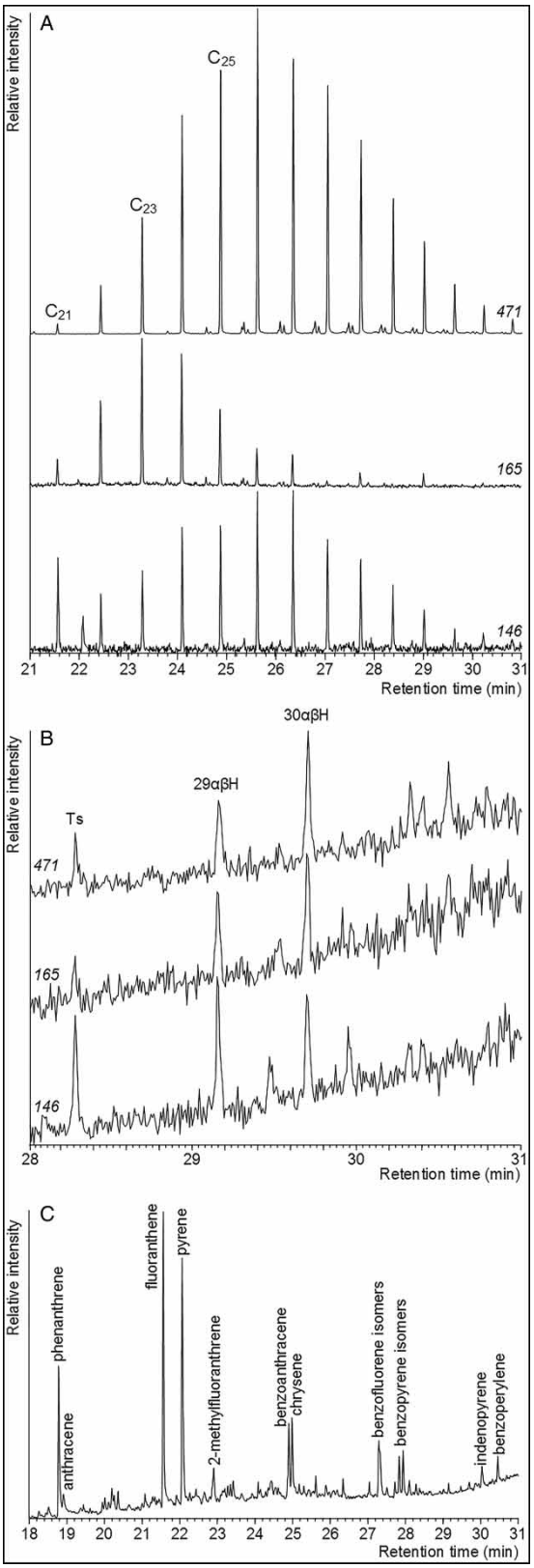

Figure 5 GC–MS data indicating fossil hydrocarbon material in samples from a votive hawk (AEABB146) and two ibis (AEABB165, AEABB471) mummy bundles. See text for identifiers. (A) XIC (*m*/*z* 71) showing characteristic homologous series of *n*-alkanes; (B) XIC (*m*/*z* 191) indicating traces of hopanes; (C) TIC chromatogram showing polyaromatic hydrocarbons in the sample from the hawk (AEABB146), confirming the sticky black coating is a modern application.

which are petroleum biomarkers. Key compounds such as pristane and phytane were not observed but the *n*-alkane pattern suggested that this substance could derive from a waxy oil in the early stages of maturity, possibly a type III kerogen or coal (Hunt, 1996, p. 402; Killops & Killops, 2005, p. 381). The latter is more likely as the major components in the TIC are aromatic intermediate and naphthenic hydrocarbons (Fig. 5C). These moieties are commonly found in coal-sourced crude oils (Hunt, 1996, pp. 329–31, 404–05; Killops & Killops, 2005, pp. 150–53) with diagnostic ratios (e.g. anthracene/phenanthrene) also consistent with the combustion of a bituminous coal (Yunker *et al.*, 2002; Dong *et al.*, 2012). The absence of expected biomarkers such as steranes and T_m (17α(H)-22,29,30-trisnorhopane) may, therefore, be due to heating prior to application as T_m is less thermally stable than T_s (18α(H)-22,29,30-trisnorneohopane). Samples from two ibis bundles (AEABB165, AEABB471) also contained trace amounts of hopanes, with 29αβ-norhopane (29αβH) and 30αβ-hopane (30αβH) most clearly identifiable, together with a classic array of *n*-alkanes ($C_{21–33}$) denoting a fossil hydrocarbon input. The low abundance of the hopanes and absence of steranes and polyaromatic hydrocarbons (PAHs) prevented any attempt at geochemical fingerprinting.

Resinous plant exudates

Many of the samples contained biomarkers characteristic of resinous plant exudates. These terpenic compounds form part of the resin fraction and principally derive from conifers (Pinaceae, Cupressaceae, and Araucariaceae) and certain angiosperms (Anacardiaceae, Burseraceae, Dipterocarpaceae, Leguminosae, and Styraceae) (Langenheim, 2003, pp. 24–41). The lower molecular mass (LMM) mono- and sesquiterpenes (10 carbons and 15 carbons, respectively) are of widespread, highly variable occurrence and are prone to losses over archaeological time due to their volatility (Serpico, 2000). Fortunately, the HMM di- and triterpenes (20 carbons and 30 carbons, respectively) are more limited in their botanical origins due to the different biosynthetic pathways employed by different botanical families. These water-insoluble components also survive well in the archaeological record as they are relatively immobile and resistant to decay (Pollard & Heron, 2008, pp. 235–69). Thus, coniferous resins are principally characterized by diterpenoids with three main skeletal types (abietane, pimarane, or labdane), depending on family. In contrast, angiosperm exudates are generally exemplified by triterpenoids (Langenheim, 2003, pp. 36–38). The detection of resinous substances in the archaeological record focuses on these marker compounds which

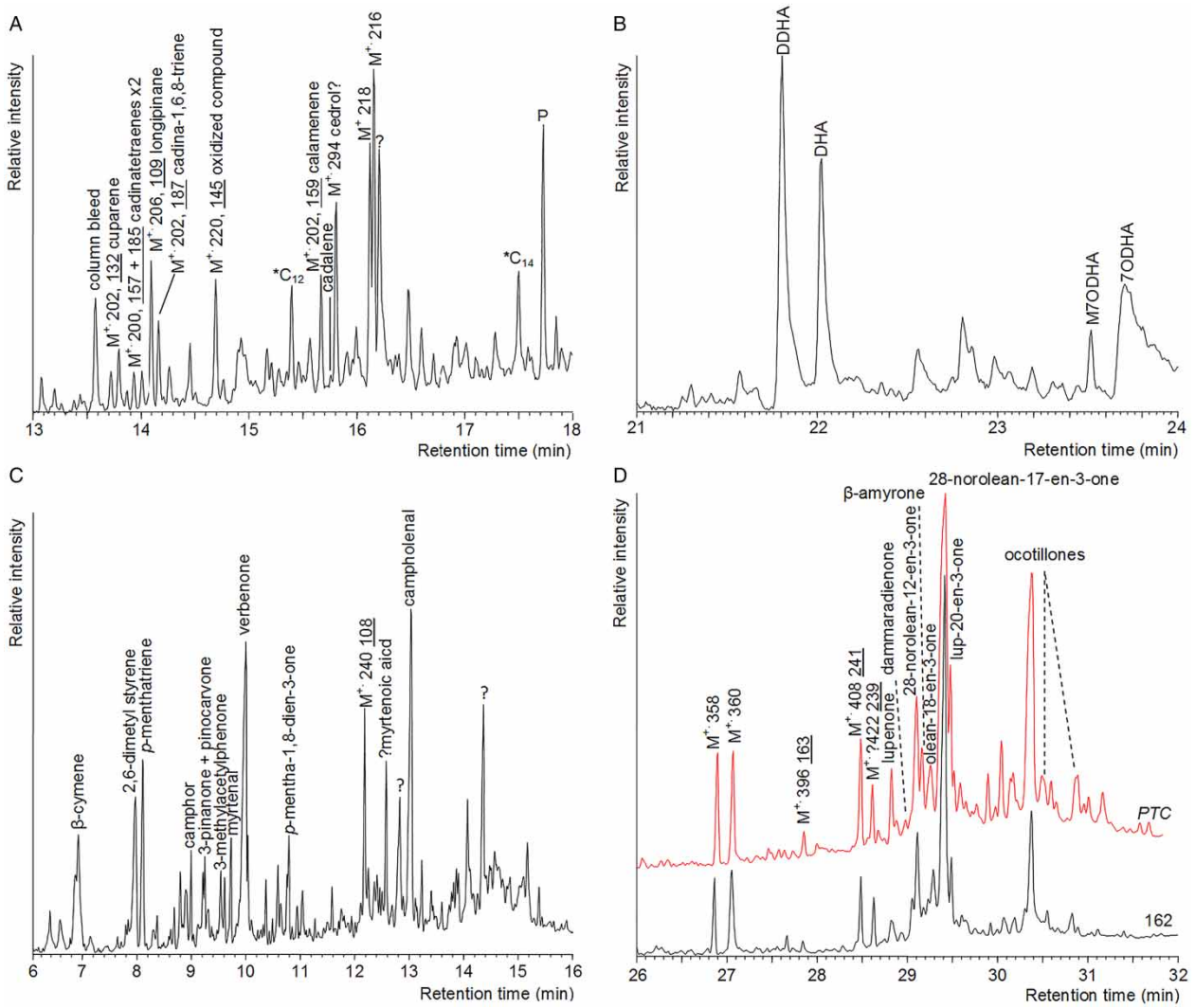

Figure 6 GC–MS data indicating resinous plant exudates in samples from an *Accipiter* sp. (AEABB384) and two votive ibis (AEABB471, AEABB162) mummy bundles. See 4a for key. Mass spectral base peaks are underlined. (A) Partial TIC chromatogram showing traces of sesquiterpenes suggestive of a conifer essential oil in debris from ibis AEABB471; (B) partial TIC chromatogram showing diterpenoids indicative of a Pinaceae resin in residue coating a feather from *Accipiter* sp. mummy, AEABB384; (C) partial TIC chromatogram showing mono- and sesquiterpenes; and (D) triterpenes from resinous fragments associated with the ibis AEABB162 indicative of a *Pistacia* spp. resin; data in (D) are compared with a modern reference sample from *P. terebinthus*, Cyprus (PTC).

permit identification of source at least to the level of genus or sub-family (Evershed, 2008).

The ibis bundles that showed evidence of bituminous substances (AEABB165, AEABB471) also contained an array of sesquiterpenes, as did AEABB401, an animal (bird?) mummy curated in the Museum of Fine Arts, Boston (Fig. 6A). Due to their low abundance and similar fragmentation patterns definitive identification of these compounds proved problematic. Those most clearly characterized are cuparene and calamenene (cadina-1,3,5-triene) which have been reported in the resin, wood and wood extracts of the Cupressaceae (Enzell & Erdtman, 1958; Kamatou *et al*., 2010) and Pinaceae (Colombini *et al*., 2000; Koller *et al*., 2003). Tentative classification of other moieties including LMM compounds containing aromatic ring structures such as the two potential benzocycloheptenes described by Buckley *et al*. (2004) support the presence of a conifer product, probably an essential oil (Koller *et al*., 2003).

In addition, traces of dehydroabietic acid (DHA) and 7-oxo-dehydroabietic acid (7ODHA) were observed in AEABB471. These oxidized and dehydrogenated abietane-skeleton diterpenes are biomarkers for conifer resins derived from the sub-family Pinaceae which includes pines, cedars, and larches (Colombini & Modugno, 2009). The diterpenoids produced by these genera are very similar although an abundance of abietic and pimaric acids is more characteristic of *Pinus* spp. exudates (Mills & White, 1977). These primary resin acids are, however, rarely found in archaeological materials due to natural processes such as oxidation, with aged Pinaceae resins characterized by increased levels of DHA, didehydroabietic acid

(DDHA), 7ODHA, and neutral abietadienes (Colombini *et al*., 2000, 2005). Thermal degradation also leads to dehydrogenation, decarboxylation, and a higher degree of aromatization of the cyclic compounds present so a mixture of DHA acid and various neutral compounds is again produced (Egenberg *et al*., 2002) with retene the final stable product of Pinaceae resin tars (Robinson *et al*., 1987) and significant levels of methyl dehydroabietate (MDHA) indicative of the pyrolysis of resinous Pinaceae woods (Hjulström *et al*., 2006). These markers of extensive heating were not observed in this instance. The presence of an aged conifer exudate that may have been subjected to low-temperature pre-application processing is, therefore, suggested.

In addition, highly degraded triterpenic compounds were observed in AEABB165 and AEABB401. Based on their key fragment ions, the majority appeared to have oleana(e)ne skeletons with 28-norolean-17-en-3-one most abundant. This neutral compound is formed from oleanonic acid (Pastorova *et al*., 1998) which is present in the exudates of certain angiosperms such as *Pistacia* spp. and *Liquidambar* spp. (Assimopoulou & Papageorgiou, 2005a; Hovaneissian *et al*., 2008). A degradation pathway from the more widely occurring 3β-oleanolic and/or 3α-*epi*oleanolic acids also seems feasible but has not been confirmed. In addition, lupa(e)ne derivatives and ocotillones (oxidized dammaranes) were detected in AEABB401. These defunctionalized and oxidized species have been identified in degraded *Pistacia* spp. resins (van der Doelen *et al*., 1998; Stern *et al*., 2003). As these aromatic secretions, better known as mastic or terebinth, have been widely reported in Ancient Egyptian contexts (e.g. Colombini *et al*., 2000; Serpico & White, 2000b, 2001; Clark *et al*., 2013), they are the most likely source here although the absence of diagnostic precursor resin acids precludes a definitive assignment.

Likewise, samples from four of the birds of prey (AEABB004, AEABB027, AEABB384, AEABB475) provided evidence of di- and triterpenic compounds. Based on their characteristic fragmentation patterns as detailed in the literature (Budzikiewicz *et al*., 1963; Assimopoulou & Papageorgiou, 2005a), these comprised oxidized abietane-skeleton diterpenes (MDHA, DDHA, DHA, methyl 7-oxodehydroabietate (M7ODHA) and 7ODHA) in AEABB004, AEABB027, and AEABB384 (Fig. 6B), 28-norolean-17-enes in all four bundles, dammarane derivatives in AEABB004 and ocotillones, oxidized dammaranes with furan derivative side chains in AEABB004, AEABB027, and AEABB475. This combination is indicative of the presence of a Pinaceae resin and a highly degraded triterpenoid exudate, with the range of compounds observed closely resembling those reported in aged *Pistacia* spp. resins (Stern *et al*., 2003). These findings are supported by possible traces of pimaric acid in AEABB004 and moronic acid in AEABB027.

Greater clarity was obtained by characterization of the fragments of resinous appearance from the foot area of the mummified ibis, AEABB162. These materials contained a near-identical suite of terpenoids which comprised mono- and sesquiterpenes together with both pentacyclic and tetracyclic terpenic compounds (Fig. 6C). Although no triterpenoid acids were detected, a range of biosynthetic transformation and environmental oxidation products were identified. As detailed above, the majority have oleana(e)ne skeletons with lupa(e)ne and dammarane derivatives, including ocotillones (oxidized dammaranes), as minor contributors. This combination is again consistent with aged *Pistacia* spp. Exudates (Stern *et al*., 2003; Assimopoulou & Papageorgiou, 2005a, 2005b). These observations were supported by comparison with modern *Pistacia* spp. resins. Indeed, the solvent extracts of exudates obtained from two closely related species, *Pistacia terebinthus* and *Pistacia khinjuk*, showed a remarkable correspondence with those from the archaeological materials (Fig. 6D). In contrast, reference samples from *Pistacia lentiscus*, which has been shown to be taxonomically distinct (Golan-Goldhirsh *et al*., 2004), were generally dominated by the classic biomarkers: moronic, oleanonic, *iso*masticadienonic, and masticadienonic acids.

Sugars, detected as silyated derivatives characterized by 204 and 217 fragment ions (Mogoşanu *et al*., 2011), were also noted in the sample obtained from the Hawk bundle (AEABB027). These could relate to apparent use of beeswax in the preparation of this mummy although, as reported for two votives analysed by Buckley *et al*. (2004), they could also derive from a natural plant gum or gum-resin. Further work investigating the presence of saccharides is currently being undertaken using a water extraction (after Bleton *et al*., 1996; Ménager *et al*., 2014). These results will be published in due course if they add to our understanding of the substances used in the treatment of these votive bundles.

Discussion

The majority of the adhering and comminuted organic residues analysed were brown-black amorphous masses of indeterminate origin. This visual appearance is due to natural taphonomic processes such as oxidation but has resulted in misconceptions about the nature of the substances employed. Bitumen is the classic example as it was originally thought to be the major component used in embalming (Baumann, 1960; Lucas & Harris, 1962, p. 271) although

frankincense has also often been incorrectly cited due to mistranslation of the term *sntr* (Loret, 1949; Serpico & White, 2000b; Stern *et al*., 2003). The visual appearance of the shiny black matter coating on the mummified hawk (AEABB146), therefore, raised some concerns. This abundant sticky layer varied considerably from the usual desiccated exterior of votive bundles. Radiography indicated that the mummy itself was genuine based on body position and wrapping phases but supported the hypothesis that this outer layer was a more recent addition. GC–MS analysis demonstrated the petroleum-based nature of this substance, which still incorporated a range of highly volatile PAHs, and confirmed that it represented a modern consolidation of the bundle. The remainder of the organic components identified in the other votives, with the exception of phthalate plasticizers, are consistent with substances of ancient origin.

In common with Buckley *et al*. (2004) and Bruno (2013), our results show that the majority of the bundles contained a fat/oil. This contribution probably derives from the decomposition of intrinsic body tissues although an admixture with an applied fat/oil cannot be ruled out. One interesting observation is that a fat/oil was the only substance identified from the votive cat mummies, with the exception of a natural wax, possibly beeswax, in AEABB191, despite these bundles being some of the most elaborately wrapped. The various bird mummies, however, showed evidence of having been treated with a mixture of plant products comprising resinous exudates and/or essential oils. In addition, traces of natural waxes (plant and/or beeswax) and fossil hydrocarbons (bitumen) were noted. This contrasts with the findings of Buckley *et al*. (2004) which showed that only the Ptolemaic/Roman period cat mummy from Beni Hasan had been prepared in this manner while the Late Pharaonic avian species assessed had received a less lavish treatment.

Nonetheless, the palette of substances identified here are similar both to those recorded in their seminal study of four bundles curated in Liverpool Museum (Buckley *et al*., 2004) and during analysis of votive mummies from the Brooklyn Museum collection (Bruno, 2013). This corpus of research demonstrates that essential conifer oils (which may represent the 'cedar oil' described by classical authors) (Koller *et al*., 2003), and Pinaceae resins, are most frequently observed alongside traces of triterpenoids, generally consistent with *Pistacia* spp. resins. To date, all of those afforded this more extensive treatment and whose provenance is reported pertain to sites in Lower and Middle Egypt and date from the Ptolemaic/Roman periods. Were these regions better connected with European markets, more progressive, or simply wealthier than the south? This pattern may, of course, be the result of sample availability or taphonomy but it provides an intriguing hypothesis that requires testing through the targeted analysis of provenanced bundles.

What is clear is that chemical analysis of votive bundles has confirmed the dominant role played by plant products in the embalming process as implied in ancient texts (Oldfather, 1933, pp. 311–13; Rackham, 1968, pp. 421–23; Rawlinson, 1992, pp. 160–61; Smith, 2009, pp. 215–348). From a practical perspective, the widespread use of resinous exudates supports the view that their antimicrobial properties were fully appreciated by the Egyptians (Koller *et al*., 2005) although without prior desiccation and/or evisceration soft-tissue preservation would have been temporary due to the internal bacterial load. In the votives analysed this may have been counteracted through the use of heated resins as the terpenic components identified indicate considerable, possibly thermal, degradation, although this could also be an artefact of deposition in an arid environment. Nonetheless, it has been suggested that the application of hot resins mixed with beeswax and/or bitumen, all of which were identified here, might have inhibited the action of intrinsic bacteria and, once cooled, have formed an insect and moisture repellent coating (Aufderheide, 2003, pp. 253–54).

Of the range of substances identified, the plant exudates (alongside bitumen if present in sufficient abundance, Nissenbaum & Buckley, 2012), also hold the greatest potential for investigating broader questions relating to fluctuating trade and tribute relations as they would have had to have been imported into Egypt (Loret, 1949; Lucas & Harris, 1962, pp. 316–24; Weser *et al*., 1998). Indeed, those identified in the treatment of votives were also used in human mummification (e.g. Colombini *et al*., 2000; Serpico, 2000; Maurer *et al*., 2002; Koller *et al*., 2005; Corcoran & Svoboda, 2010) where chronological variation in the substances selected has previously been noted (Buckley & Evershed, 2001). The latter study provided evidence for an increase in the use of conifer resins in the Ptolemaic and Roman periods due, perhaps, to fashionable novelty, ease of extraction and relative abundance (Howes, 1949, pp. 86, 105–10) or the influence of Roman eschatology in which *Pinus* spp., in particular, appear to have been closely associated with immortality or mourning (Alcock, 1980; Brettell *et al*., 2015). Thus, illumination of the material aspects of votive mummification may also enable aspects of symbolic action to be explored and the impact of new ideologies on this most conservative of social arenas, death and burial, to be considered.

The validity of such patterns, perceived through analysis of a limited number of samples and in

comparison with previous studies, can be tested by research on individual species, more geographically provenanced bundles, and those whose date of manufacture can be established (through documentary evidence, AMS dating, or wrapping styles). The systematic evaluation of this abundant resource using a combination of radiography, microscopy, and organic residue analysis should then, as discussed above, enable a wide variety of questions to be addressed encompassing economic, socio-cultural, and ritual spheres of action. Additional work investigating the presence of saccharides (after Bleton *et al*., 1996; Ménager *et al*., 2014), potentially indicative of the uses of gums or gum-resins, is already in progress with the incorporation of GC-C-IRMS planned as appropriate. Thus, the protocol detailed here, which aims at minimal disruption of the mummy bundle while obtaining the maximum level of information, highlights the value of this largely untapped reservoir of evidence. The results of future research in this area, compiled in the form of a comprehensive database, will provide a valuable resource for others seeking to understand the material, technical, and ritual aspects of votive mummification and serve to enhance the value of these artefacts to the museums in which they are curated.

Conclusion

The analysis of organic residues from a wide range of archaeological contexts is a well-established technique but one that has rarely been applied to non-human mummies. This study adds considerably to the number of votives bundles investigated using gas chromatography–mass spectrometry and establishes a protocol aimed at promoting continued work in this field. Our findings, in comparison with those of other researchers, confirm that a similar range of substances was used in the embalming of both humans and other animals during the Late Pharonic, Ptolemaic, and Roman Periods in Egypt. This reinforces our current understanding of Egyptian thought regarding the divine attributes of all species and enhances our knowledge of this mortuary rite. Moreover, detailed information regarding the substances employed in the preparation of individual mummy bundles will aid in their conservation.

In combination with the non-invasive programme of research and database established at the KNH Centre, University of Manchester, the systematic application of organic residue analysis to votive bundles has the potential to permit ever more intricate hypotheses about life and death in Late Period Egypt to be addressed. Regeneration of interest in these abundant and yet mysterious artefacts should also assist in their re-contextualization both on the world stage and in terms of their social history, so that they may be informatively displayed in museum collections. Thus, by telling as much of their story as possible without causing additional damage to the mummy bundle, it is hoped that these sanctified creatures will reclaim their significance and, in effect, gain the immortality promised in antiquity.

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