indeed have been anaerobic in nature at some point and indeed it seems probable that anaerobic hydrocarbon degradation is the prevailing mechanism for deep subsurface petroleum biodegradation, marking an important step in the understanding of reservoir degradation conditions. ☐

Methods

Total hydrocarbon fractions from crude oils were obtained by silica gel chromatography using hexane as eluent and were analysed by gas chromatography and gas chromatography-mass spectrometry (GC-MS) to provide hydrocarbon distribution patterns that provided information on the extent of biodegradation that had been suffered by the oil, as described by the scale of 0–10 proposed by ref. 25.

Acid fractions of crude oil samples were obtained using the method described in ref. 30. Briefly, $\sim$150 mg of oil was loaded onto a strong anion exchange (SAX) quaternary amine solid phase extraction (SPE) column, and after removal of non-acid compounds, the crude carboxylic acid isolate was eluted with diethyl ether containing 2% (v/v) formic acid. This fraction was methylated with an ethereal solution of diazomethane, and cleaned up by eluting through a silica SPE column with a mixture of *n*-hexane and dichloromethane. 1-Adamantane carboxylic acid (Fluka) and 5β cholanic acid (Sigma) were used as extraction efficiency standards and the methyl ester of 1-phenyl-1-cyclohexane carboxylic acid was used as an internal standard. Reference standards were 2-naphthoic acid (Lancaster Synthesis), 5,6,7,8-tetrahydro-2-naphthoic acid and decahydro-2-naphthoic acid. 5,6,7,8-Tetrahydro-2-naphthoic acid and decahydro-2-naphthoic acid were not commercially available and were therefore synthesized by the catalytic hydrogenation of 2-naphthoic acid using molecular hydrogen and a palladium/graphite catalyst; the identities of these compounds were confirmed by nuclear magnetic resonance spectroscopy (NMR) and by comparison of the standards' mass spectra with those in the literature.

GS-MS analysis of carboxylic acid methyl esters was performed in full scan mode on a Hewlett Packard 6890 gas chromatograph linked to a HP 5973 MSD with some confirmatory analyses carried out using a Varian 1200 GC-MS-MS system. 2-Naphthoic acid, 5,6,7,8-tetrahydro-2-naphthoic acid and decahydro-2-naphthoic acids were quantified by comparison of their peak areas in the respective m/z (mass-to-charge ratio) 155, 131 and 164 mass chromatograms with the peak area of the internal standard in the $m/z = 159$ mass chromatogram. Response factors of the analyte compounds were assumed to be unity, while the response factors of the surrogate standards were calculated and corrected for. Detection limits for naphthoic and reduced naphthoic acids were 0.01 p.p.m.

Received 23 February; accepted 3 August 2004; doi:10.1038/nature02922.

---

1. Connan, J. in *Advances in Petroleum Geochemistry* (eds Brooks, J. & Welte, D. H.) Vol. 1, 299–335 (Academic, London, 1984).
2. Atlas, R. M. & Bartha, R. Hydrocarbon biodegradation and oil-spill remediation. *Adv. Microb. Ecol.* **12,** 287–338 (1992).
3. Palmer, S. E. in *Organic Geochemistry* (eds Macko, S. A. & Engel, M. H.) 511–534 (Plenum, New York, 1993).
4. Head, I. M., Jones, D. M. & Larter, S. R. Biological activity in the deep subsurface and the origin of heavy oil. *Nature* **426,** 344–352 (2003).
5. Magot, M., Ollivier, B. & Patel, B. K. C. Microbiology of petroleum reservoirs. *Antonie van Leeuwenhoek Int. J. Gen. Mol. Microbiol.* **77,** 103–116 (2000).
6. Boll, M., Fuchs, G. & Heider, J. Anaerobic oxidation of aromatic compounds and hydrocarbons. *Curr. Opin. Chem. Biol.* **6,** 604–611 (2002).
7. Widdel, F. & Rabus, R. Anaerobic biodegradation of saturated and aromatic hydrocarbons. *Curr. Opin. Biotechnol.* **12,** 259–276 (2001).
8. Röling, W. F. M., Head, I. M. & Larter, S. R. The microbiology of hydrocarbon degradation in subsurface petroleum reservoirs: perspectives and prospects. *Res. Microbiol.* **154,** 321–328 (2003).
9. Evans, C. R., Rogers, M. A. & Bailey, N. J. L. Evolution and alteration of petroleum in Western Canada. *Chem. Geol.* **8,** 147–170 (1971).
10. Horstad, I., Larter, S. R. & Mills, N. A quantitative model of biological petroleum degradation within the Brent Group reservoir in the Gullfaks field, Norwegian North Sea. *Org. Geochem.* **19,** 107–117 (1992).
11. Larter, S. R. *et al.* The controls on the composition of biodegraded oils in the deep subsurface. Part 1: biodegradation rates in petroleum reservoirs. *Org. Geochem.* **34,** 601–613 (2003).
12. Bastin, E. S. The presence of sulphate reducing bacteria in oil field waters. *Science* **63,** 21–24 (1926).
13. Kartsev, A. A., Tabasaranskii, Z. A., Subbota, M. I. & Mogilevski, G. A. *Geochemical Methods of Prospecting and Exploration for Petroleum and Natural Gas* (English translation edited by Witherspoon, P. A. & Romey, W. D.) (Univ. California Press, Berkeley, California, 1959).
14. Zengler, K., Richnow, H. H., Rosselló-Mora, R., Michaelis, W. & Widdel, F. Methane formation from long-chain alkanes by anaerobic microorganisms. *Nature* **401,** 266–269 (1999).
15. Rueter, P. *et al.* Anaerobic oxidation of hydrocarbons in crude oil by new types of sulfate-reducing bacteria. *Nature* **372,** 455–458 (1994).
16. Magot, M., Connan, J. & Crolet, J.-L. Les bactéries des gisements pétroliers. *La Recherche* **25** (268), 936–937 (1994).
17. Bennett, P. C., Siegel, D. E., Baedecker, M. J. & Hult, M. F. Crude oil in a shallow sand and gravel aquifer. I. Hydrogeology and inorganic geochemistry. *Appl. Geochem.* **8,** 529–549 (1993).
18. L'Haridon, S., Reysenbach, A.-L., Glénat, P., Prieur, D. & Jeanthon, C. Hot subterranean biosphere in a continental oil reservoir. *Nature* **377,** 223–224 (1995).
19. Elshahed, M. J., Gieg, L. M., McInerey, M. J. & Suflita, J. M Signature metabolites attesting to the *in situ* attenuation of alkylbenzenes in anaerobic environments. *Environ. Sci. Technol.* **35,** 682–689 (2001).
20. Rios-Hernandez, L. A., Gieg, L. M. & Suflita, J. M. Biodegradation of an alicyclic hydrocarbon by a sulfate-reducing enrichment from a gas condensate-contaminated aquifer. *Appl. Environ. Microbiol.* **69,** 434–443 (2003).
21. Gieg, L. M. & Suflita, J. M. Detection of anaerobic metabolites of saturated and aromatic hydrocarbons in petroleum-contaminated aquifers. *Environ. Sci. Technol.* **36,** 3755–3762 (2002).
22. Zhang, X., Sullivan, E. R. & Young, L. Y. Evidence for aromatic ring reduction in the biodegradation pathway of carboxylated naphthalene by a sulfate reducing consortium. *Biodegradation* **11,** 117–124 (2000).
23. Annweiler, E., Michaelis, W. & Meckenstock, R. U. Identical ring cleavage products during anaerobic degradation of naphthalene, 2-methylnaphthalene, and tetralin indicate a new metabolic pathway. *Appl. Environ. Microbiol.* **68,** 852–858 (2002).
24. Phelps, C. D., Battistelli, J. & Young, L. Y. Metabolic biomarkers for monitoring anaerobic naphthalene biodegradation *in situ*. *Environ. Microbiol.* **4,** 532–537 (2002).
25. Peters, K. E. & Moldowan, J. M. *The Biomarker Guide* (Prentice Hall, New York, 1993).
26. Aitken, C. M.. *Identification of Non-Hydrocarbon Metabolites of Deep Subsurface Anaerobic Petroleum Hydrocarbon Biodegradation* PhD thesis, Univ. Newcastle-upon-Tyne, UK (2004).
27. Watson, J. S.. *Hydrocarbon and Carboxylic Acid Compositions of Crude Oil Biodegraded in Marine Systems* PhD thesis, Univ. Newcastle-upon-Tyne, UK (1999).
28. Van der Linden, A. C. & Thijsse, G. J. E. The mechanisms of microbial oxidations of petroleum hydrocarbons. *Adv. Enzymol.* **27,** 469–546 (1965).
29. Seewald, J. S. Organic-inorganic interactions in petroleum–producing sedimentary basins. *Nature* **426,** 327–333 (2003).
30. Jones, D. M., Watson, J. S., Meredith, W., Chen, M. & Bennett, B. Determination of naphthenic acids in crude oils using non-aqueous ion exchange solid-phase extraction. *Anal. Chem.* **73,** 703–707 (2001).

Supplementary Information accompanies the paper on **www.nature.com/nature**.

Acknowledgements We thank I. Head, B. Bennett and H. Huang for comments and supporting data, and also the members of the BACCHUS biodegradation consortium for support, discussions and permission to publish. BACCHUS members include Norsk Hydro, Shell, Enterprise, Petrobras, TotalFinaElf, Exxon Mobil, JNOC, ConocoPhillips, BP Amoco and ChevronTexaco. We also thank H. Wilkes for discussions and the gift of succinate standards. We acknowledge support from the Natural Environment Research Council for research funds, including JREI awards and S.R.L. acknowledges additional support from NSERC and AIF.

Competing interests statement The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to D.M.J. (martin.jones@ncl.ac.uk).

Complex organic chemical balms of Pharaonic animal mummies

Stephen A. Buckley*, **Katherine A. Clark & Richard P. Evershed**

Organic Geochemistry Unit, Bristol Biogeochemistry Research Centre, School of Chemistry, Cantock's Close, Bristol BS8 1TS, UK

* Present address: Department of Archaeology, University of York, King's Manor, York YO1 7EP, UK

Millions of votive mummies of mammals, birds and reptiles were produced throughout ancient Egypt, with their popularity increasing during the reign of Amenhotep III (1400 BC) and thereafter. The scale of production has been taken to indicate that relatively little care and expense was involved in their preparation compared with human mummies[1–3]. The accepted view is that animals were merely wrapped in coarse linen bandages and/or dipped in 'resin' before death[2–4]. However, as with human mummification there was a range of qualities of treatments, and visual inspection of animal mummies suggests that the procedures used were often as complex as those used in humans (for example, evisceration and elaborate bandaging). Moreover, the ancient Egyptians treated animals with great respect, regarding them both as domestic pets and representatives of the gods; for example, the cat symbolized the goddess Bastet; the hawk, Horus; the ibis, Thoth, and so on. We report here the results of chemical investigations of tissues and wrappings from Pharaonic cat, hawk and ibis mummies using gas chromatography, gas chromatography–mass spectrometry, thermal desorption–gas chromatography–mass spectrometry and pyrolysis–gas chromatography–mass spectrometry[5,6]. The analyses reveal the presence of highly complex mixtures of

***n*-alkyl and cyclic biomarker components characteristic of fats, oils, beeswax, sugar gum, petroleum bitumen, and coniferous, *Pistacia* and possibly cedar resins. The mixture of balms is of comparable complexity to those used to mummify humans from the same period[6–8].**

One essential aspect of the mummification process was the treatment of the corpse and associated viscera with various organic embalming agents. Previous reports have shown that a variety of natural products possessing a range of preservative properties were applied during mummification, including beeswax[6–8], animal fats and plant oils[6–8], plant resins[6–9], petroleum bitumen[7–9] and essential oils[10,11]. Thus, a further criterion on which to assess the degree of care and sophistication employed in the mummification of animal mummies would be to determine the chemical composition of their balms. The specimens investigated herein (Fig. 1) are representatives of the latest mummy-making era of Pharaonic Egypt (XXIII dynasty (818–715 BC) to the XXX dynasty (380–343 BC)). As in our previous report on human mummies[7], we focused on diagnostic marker compounds that are resistant to degradation and can be related to specific natural products comprising the original embalming materials.

The embalming materials identified in the animal mummies (Table 1) are either sugar gum (that is, polysaccharide-based plant exudate, such as gum arabic) and/or lipid based. The fatty acid compositions of their extracts are indicative of both animal (low

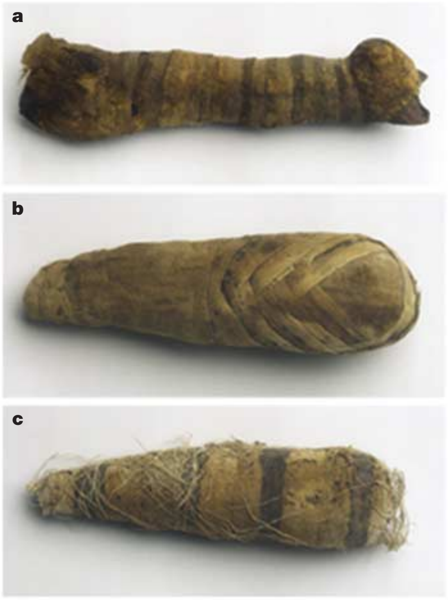

Figure 1 Photographs of animal mummies used in this investigation. **a**, Cat. **b**, Hawk. **c**, Ibis. Images were obtained after taking milligram to sub-milligram samples of balms and wrappings indicated in Table 1, showing that sampling for micro-scale analyses was essentially non-destructive.

Table 1 **Provenance and date of mummies, origin of balms and their chemical composition**

Mummy (museum number)	Date (yr BC) (dynasties)	Provenance	Sample description†	Inferred components of embalming 'resin'‡	Relative abundance (%)§
Hawk* (52.55.46)	818–664 (XXIII–XXV)	Tarkhan	'Resin' on wrapping underneath jaw/mandible	Fat/oil[a]	90
				Wax (beeswax?)[i,n]	10
			'Resin' on back, base of neck (head of spine)	Fat/oil[b]	90
				Wax (beeswax?)	10
			'Resin' on wrapping covering right breast	Fat/oil[c]	90
				Wax (beeswax?)	10
Hawk* (52.55.47)	818–664 (XXIII–XXV)	Tarkhan	'Resin'-soaked linen above right eye	Fat/oil[d]	30
				Wax[j,o]	70
Cat* (56.22.224)	664–343 (XXVI–XXX)	Beni Hassan	Blackened wrapping from base of mummy	Animal fat[e]	60
				Cedar resin (?)	4
				Pistacia resin	4
				Balsam/umbelliferae	1
				Beeswax[k,p]	30
				Gum resin (myrrh?)	1
			Detached 'resin'-soaked wrapping (location 1)	Animal fat[f]	61
				Cedar resin (?)	5
				Balsam/umbelliferae	1.5
				Beeswax[l,q]	31
				Gum resin (myrrh?)	1.5
				Petroleum bitumen	Trace‖
			Detached 'resin'-soaked wrapping (location 2)	Animal fat[g]	64
				Cedar resin (?)	2
				Pistacia resin	0.2
				Balsam/umbelliferae	0.5
				Beeswax[m,r]	33
				Gum resin (myrrh?)	0.5
			Red material in right ear	A sugar gum	95
				Plant oil[h]	5
				Beeswax	0.5
Ibis* (1969.112.42)	664–343 (XXVI–XXX)	Sakkara	'Resin'-soaked wrapping, covering right breast	A sugar gum	100
				Plant oil	Trace
				Wax	Trace
			'Resin'-soaked wrapping, covering left breast	A sugar gum	100
				Plant oil	Trace
				Wax	Trace

*Liverpool Museum.

†The term 'resin' denotes physical appearance and does not presuppose any chemical composition or biological origin.

‡Superscript letters (a–r) refer to the histograms in Fig. 2.

§Percentage of relative abundance on the basis of absolute concentrations in lipid extract, determined by gas chromatography on the basis of internal standards added at the extraction stage (thermal desorption was taken into account where appropriate). Compositions do not imply that they were the original formulations of the embalmers, owing to possible chemical changes over time.

‖Diagnostic steranes and hopanes present in trace concentrations. Concentration of bitumen not determined owing to chemical complexity.

abundance of $C_{16:0}$ compared with $C_{18:0}$) and plant (high abundance of $C_{16:0}$ compared with $C_{18:0}$) acyl lipid origins (see Fig. 2a–h). The ibis mummy treatment contains only trace amounts of fatty acids, being largely sugar based, although the high $C_{16:0}$ to $C_{18:0}$ ratios for the two samples seem to indicate a plant origin. In contrast, the three samples of wrappings taken from the mummified cat have $C_{16:0}$ to $C_{18:0}$ ratios of ~1.5, which taken together with the presence of cholesterol derivatives and triacylglycerols (with a high abundance of the $C_{18:0}$ fatty acyl moiety; Fig. 2) confirm an animal origin; a contribution from endogenous body fats cannot be ruled out. Red material that had been packed into the ears of the cat has a quite different $C_{16:0}$ to $C_{18:0}$ ratio (3.7) indicating a possible plant origin. The ratios of $C_{16:0}$ and $C_{18:0}$ in all the samples from the hawk mummies are very similar (~1.5), which suggests an animal fat origin. These samples all contained an unusually high abundance of 9,10-dihydroxyoctadecanoic acid, with the differing abundances of the *threo* and *erythro* isomers possibly reflecting differing sources or extents of stereomutation during the oxidation of the precursor oleic acid[12]. As in the previous study of human mummies, the results suggest that animal fats and plant oils were used as a less costly base on which to apply more exotic ingredients[7].

The presence of monosaccharides in the solvent extracts, and abundant sugar markers (levoglucosan, furan and pyran derivatives) in the thermal desorption–gas chromatography–mass spectrometry analyses, indicate the presence of a hydrolyzed plant sugar gum in the ibis mummy. Because both furanose and pyranose sugars were present, their origin cannot be the cellulose-based wrappings. Unfortunately, the low abundance of sugars in the extracts makes their precise origin difficult to assign; however, the identification of a sugar gum is consistent with a previous report of the use of a "gum" to secure mummy wrappings[13]. Analogous sugar gum markers were also detected in the red packing and wrappings of the cat mummy.

Conifer resin was identified in the three impregnated wrappings from the mummified cat. The normally abundant diterpenoids, particularly dehydroabietic acid, are highly oxidized, such that 7-oxodehydroabietic acid is most abundant with lesser amounts of 15-hydroxy-7-oxodehydroabietic acid (Fig. 3). Importantly, these diterpenoids are accompanied by sesquiterpenoids indicating that essential oil(s), such as cedar oil, was a component of the balm. Although tetramethylhexahydrobenzocycloheptadiene is characteristic of cedar resin and has been identified in a number of Egyptian vessels[10], the presence of this compound could not be unambiguously confirmed. However, the mass spectra of later eluting components of the neutral fraction exhibit $M^{+\bullet}$ 216 and 218, consistent with oxidation products of the aforementioned hydrocarbon. The precursor benzocycloheptadiene would be susceptible to autoxidation, and given the presence of the predominantly

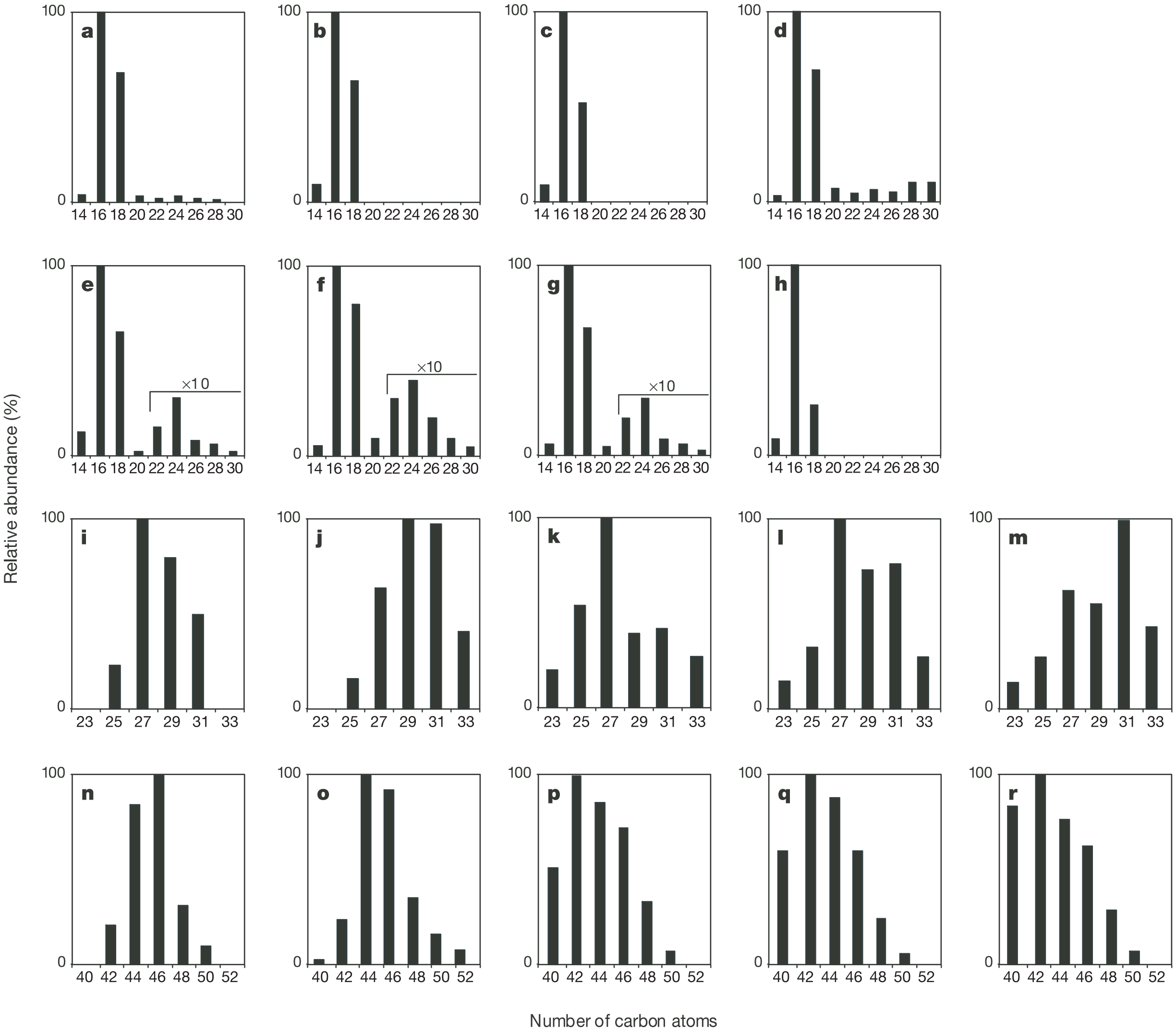

Figure 2 Histograms showing the distributions of alkyl lipids in balms of animal mummies. Fatty acids characteristic of plant oils and animal fats (**a**–**h**), and *n*-alkanes (**i**–**m**) and wax esters (**n**–**r**) present in balms containing beeswax as a significant component.

oxidized diterpenoids in the extract, then the oxidized forms of sesquiterpenoids would be expected to dominate.

The subject of mummification was written about extensively in the early part of the twentieth century[1,14], with the opinion that 'cedar oil' was in fact not true cedar, but a juniper. Unfortunately, our results do not confirm or refute this assertion, but do emphasize the problems of oxidation during either the preparation of balms or the deterioration of these materials over time, hindering confident assignments of this intriguing component. Hence, the report by classical author Diodorus Siculus on feline embalming[15], "… it is embalmed with cedria and the other substances which have the virtue of preserving bodies…" remains tantalizingly to be unambiguously confirmed.

However, triterpenoids were observed in two of the wrappings from the cat mummy (Table 1 and Fig. 3) including moronic acid as the dominant component, with lesser amounts of dammaranes, along with nor-α-amyrone (3-oxo-28-nor-urs-12-ene) and nor-β-amyrone (3-oxo-28-nor-olean-12-ene). The wrappings do not contain the euphane markers, isomasticadienonic acid and masticadienonic acid, identified in previous studies[7,8,16], but the presence of the diagnostic and relatively stable moronic acid clearly indicates that *Pistacia* resin[17] was used in embalming the cat.

The use of beeswax, characterized chemically by *n*-alkanes (C_{25} to C_{33}; Fig. 2i–m), wax esters (C_{40} to C_{50}; Fig. 2n–r) and hydroxy wax esters (C_{42} to C_{54}), is conclusively shown in the cat mummy. The solvent soluble extracts comprise 30–33% w/w beeswax (Fig. 4). The fourth sample from the wrappings of the cat mummy contains only traces of C_{25} to C_{31} *n*-alkanes, with no detectable wax esters, although the predominance of the C_{27} alkane indicates a probable beeswax origin. The sample taken from the hawk (museum number 52.55.46) also contains a significant amount of wax (~10% w/w), the wax ester distribution being similar to that of beeswax but with the C_{40} homologue being greatly reduced in abundance. The presence of *n*-alkanes (C_{25} to C_{31} with an odd-over-even predominance and

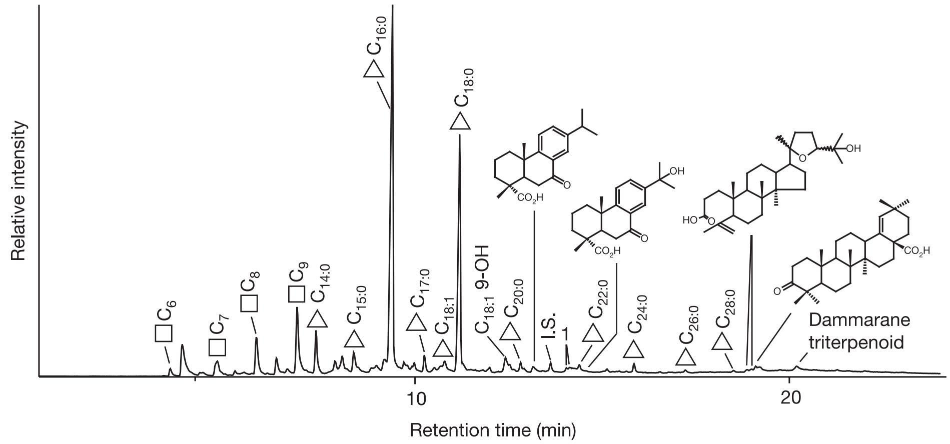

Figure 3 Reconstructed gas chromatography mass spectrometry (GC–MS) total ion chromatogram (TIC) of the trimethylsilylated acid fraction of blackened wrapping from the base of the mummy of the Late Pharaonic period cat. Peak identities ('x' indicates carbon chain length): open triangles, $C_{x:0}$ indicates saturated fatty acids; open squares, C_x indicates α,ω-dicarboxylic acids. $C_{18:1}$ 9-OH is 9-hydroxy-10-octadecenoic acid and 1 = $C_{18:0}$ 9,10-di-OH (*t* + *e*), which is 9,10-dihydroxyoctadecanoic acid (*threo* and *erythro* isomers). Also shown are the structures of two diterpenoid, and two triterpenoid, acids identified: 7-oxodehydroabietic acid; 15-hydroxy-7-oxodehydroabietic acid; 20,24-epoxy-25-hydroxy-3,4-seco-4(28)-dammaren-3-oic acid (two isomers); and moronic acid. I.S. is the internal standard (*n*-heneicosanoic acid).

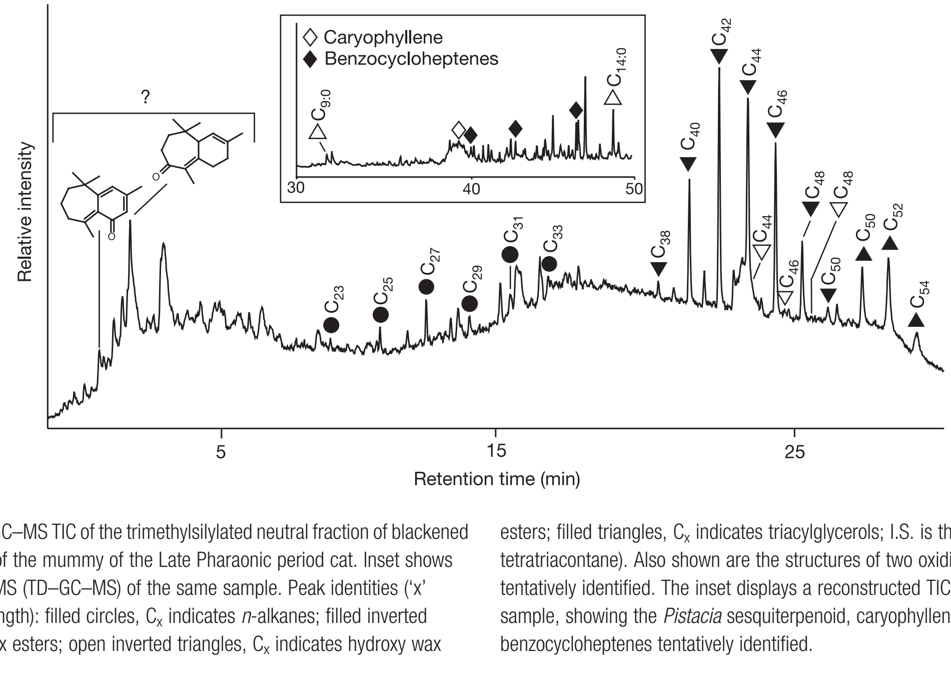

Figure 4 Reconstructed GC–MS TIC of the trimethylsilylated neutral fraction of blackened wrapping from the base of the mummy of the Late Pharaonic period cat. Inset shows thermal desorption–GC–MS (TD–GC–MS) of the same sample. Peak identities ('x' indicates carbon chain length): filled circles, C_x indicates *n*-alkanes; filled inverted triangles, C_x indicates wax esters; open inverted triangles, C_x indicates hydroxy wax esters; filled triangles, C_x indicates triacylglycerols; I.S. is the internal standard (*n*-tetratriacontane). Also shown are the structures of two oxidized benzocycloheptenes tentatively identified. The inset displays a reconstructed TIC of the TD–GC–MS of this sample, showing the *Pistacia* sesquiterpenoid, caryophyllene, and the benzocycloheptenes tentatively identified.

maximizing at C_{27}), the dominance of the $C_{16:0}$ acyl group in the wax esters and the saturated long-chain fatty acids in the $C_{22:0}$ to $C_{28:0}$ carbon number range, with $C_{24:0}$ predominating, all point to the presence of beeswax, albeit in a degraded state[7,8,18–20]. A similarly degraded beeswax was also seen in the other mummified hawk. The two samples from the ibis mummy also contain alkanes and wax esters indicative of a wax, but their low concentrations preclude a firm conclusion regarding its origin.

The gas chromatogram of the neutral fraction of the 'resin'-coated bandage taken from the cat mummy displayed a notable 'hump' (Fig. 4), which is indicative of a thermally mature petroleum product (that is, 'true' bitumen). The hydrocarbon fraction[6] contained characteristic bitumen-derived triterpanes and steranes, although their concentrations were about three orders of magnitude lower than those of other lipids. The source of this bitumen, determined using the ratios of the most characteristic bitumen biomarkers, suggests that it most closely resembles that from Abu Durba, Gulf of Suez, although a mixture with Dead Sea bitumen or another source is possible (Fig. 5)[21]. Gebel Zeit, another source thought to have been available to the Egyptians, has ratios markedly different to those calculated for the cat and can therefore be discounted as the source.

The identification of a range of natural sesqui-, di- and triterpenoids, wax esters, fatty acids and bitumen biomarkers demonstrate that commodities considered as exotic were used in animal mummification[1]. The complexity of the mixtures is analogous to those previously detected in human mummies[6–9,22–24]. The choice of natural products employed suggests that the ancient Egyptians had an appreciation of their preservative properties[7]. These findings provide further evidence for animal mummies being prepared using procedures as sophisticated as those employed in human mummification. □

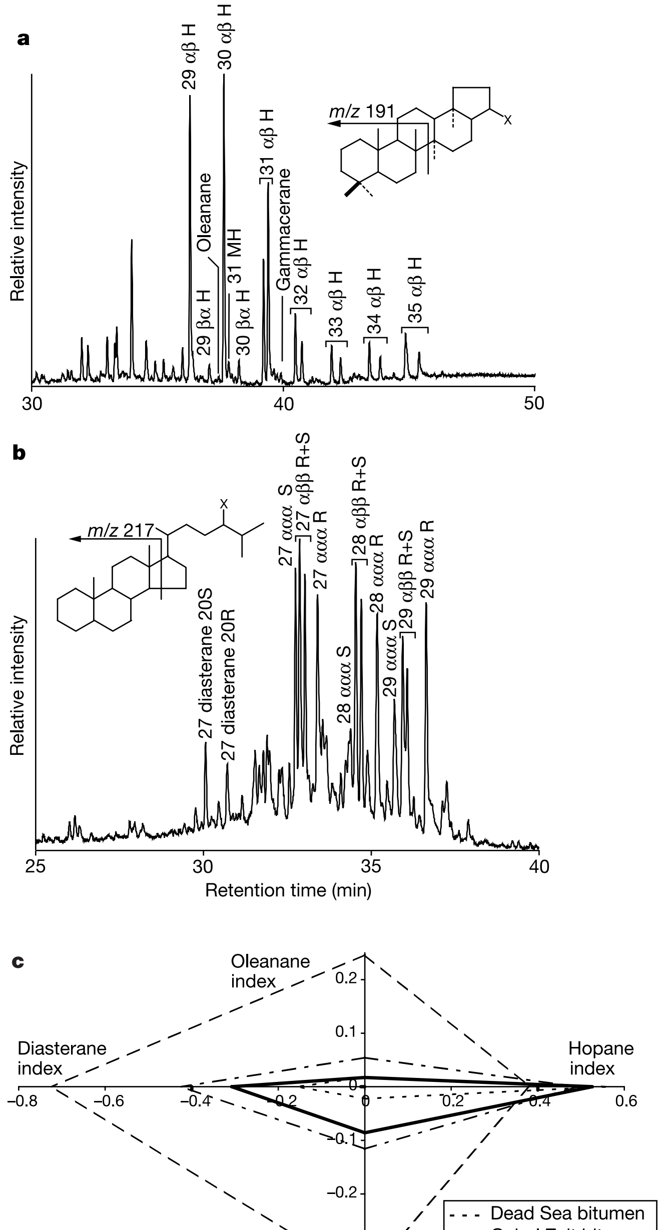

Figure 5 GC–MS selected ion monitoring mass chromatograms and star diagrams. **a**, **b**, Mass-to-charge ratio (*m/z*) 191 (**a**) and 217 (**b**) GC–MS selected ion monitoring mass chromatograms of saturated hydrocarbon fraction of 'resin'-coated bandages taken from the cat mummy. **c**, Star diagrams for reference petroleum bitumen and cat extract. Indices are calculated on the basis of peak heights as decimal fractions: oleanane index = oleanane/(oleanane + 17α,21β(H)-hopane); hopane C_{35} index = 17α,21β(H)-29-pentakishomohopanes/(17α,21β(H)-29-pentakishomohopanes + 17α,21β(H)-29-trishomohopanes); neonorhopane index = 18α(H)-30-neonorhopane/(18α(H)-30-neonorhopane + 17α(H),21β(H)-30-neonorhopane); diasterane index = 13β,17α-diacholestane 20S/(5α,14β,17β-cholestane 20S + 13β,17α-diacholestane 20S). Reference values taken from ref. 21.

Received 13 May; accepted 13 July 2004; doi:10.1038/nature02849.

---

1. Lucas, A. *Ancient Egyptian Materials and Industries*, 4th edn (Histories and Mysteries of Man, London, 1989).
2. Malek, J. *The Cat in Ancient Egypt* (British Museum, London, 1997).
3. Brier, B. *Egyptian Mummies* (Michael O' Mara Books, London, 1996).
4. Ikram, S. & Dodson, A. *The Mummy in Ancient Egypt* (Thames and Hudson, London, 1998).
5. Buckley, S. A., Stott, A. W. & Evershed, R. P. Studies of organic residues from ancient Egyptian mummies using high temperature gas chromatography mass spectrometry and sequential thermal desorption gas chromatography mass spectrometry and pyrolysis gas chromatography mass spectrometry. *Analyst* **124,** 443–452 (1999).
6. Connan, J. & Dessort, D. Du bitumen dans les baumes des momies egpytiennes (1295 av JC-300 ap JC): determination de son origine et evaluation de sa quantite. *C.R. Acad. Sci. Ser II* **312,** 1445–1452 (1991).
7. Buckley, S. A. & Evershed, R. P. Organic chemistry of embalming agents in Pharaonic and Graeco-Roman mummies. *Nature* **413,** 837–841 (2001).
8. Colombini, M. P., Modugno, F., Silvano, F. & Onor, M. Characterization of the balm of an Egyptian mummy from the 7th century B.C. *Stud. Conserv.* **45,** 19–29 (2000).
9. Maurer, J., Möhring, T., Rullkötter, J. & Nissenbaum, A. Plant lipids and fossil hydrocarbons in embalming material of Roman Period mummies from the Dakhleh Oasis, Western Desert, Egypt. *J. Archaeol. Sci.* **29,** 751–762 (2002).
10. Serpico, M. & White, R. in *Chemical Analysis of Coniferous Resins from Ancient Egypt using Gas Chromatography/Mass Spectrometry (GC/MS)* (ed. Eyre, C.) 1037–1048, (Proc. Seventh Intern. Congr. Egyptol., Leuven, 1998).
11. Koller, J., Baumer, U., Kaup, Y., Schmid, M. & Weser, U. Ancient materials - Analysis of a Pharaonic embalming tar. *Nature* **425,** 784 (2003).
12. Gunstone, F. D., Harwood, J. L. & Padley, F. B. *The Lipid Handbook* (Chapman and Hall, London, 1986).
13. Herodotus, *The Histories* (trans. De Sélincourt, A.) (Penguin, London, 1996).
14. Lucas, A. "Cedar"- tree products employed in mummification. *J. Egypt. Archaeol.* **XVII,** 13–21 (1931).
15. Diodorus, *The Library of History* (trans. Oldfather, C. H.) (Heinemann, London, 1935).
16. Mills, J. S. & White, R. The identity of the resins from the Late Bronze-Age shipwreck at Ulu-Burun (Kas). *Archaeometry* **31,** 37–44 (1989).
17. Serpico, M. & White, R. in *Ancient Egyptian Materials and Technology* (eds Nicholson, P. T. & Shaw, I.) 430–474 (Cambridge Univ. Press, Cambridge, 2000).
18. Evershed, R. P., Vaughan, S. J., Dudd, S. N. & Soles, J. S. Fuel for thought? Beeswax in lamps and conical cups from late Minoan Crete. *Antiquity* **71,** 979–985 (1997).
19. Evershed, R. P., Dudd, S. N., Anderson-Stojanovic, V. R. & Gebhard, E. R. New chemical evidence for the use of combed ware pottery vessels as beehives in ancient Greece. *J. Archaeol. Sci.* **30,** 1–12 (2003).
20. Regert, M., Colinart, S., Degrand, L. & Decavallas, O. Chemical alteration and use of beeswax through time: Accelerated ageing tests and analysis of archaeological samples from various environmental contexts. *Archaeometry* **43,** 549–569 (2001).
21. Harrell, J. A. & Lewan, M. D. Sources of mummy bitumen in ancient Egypt and Palestine. *Archaeometry* **44,** 285–293 (2002).
22. Connan, J. Use and trade of bitumen in antiquity and prehistory: molecular archaeology reveals secrets of past civilizations. *Phil. Trans. R. Soc. Lond. B* **354,** 33–50 (1999).
23. Rullkötter, J. & Nissenbaum, A. Dead-Sea asphalt in Egyptian mummies - molecular evidence. *Naturwissenschaften* **75,** 618–621 (1988).
24. Koller, J., Baumer, U., Kaup, Y., Etspüler, H. & Weser, U. Embalming was used in Old Kingdom. *Nature* **391,** 343–344 (1998).

Acknowledgements We thank J. Hayward of Liverpool Museum for making the samples available to us. Thanks also go to J. Carter, I. Bull, A. Gledhill and B. van Dongen for their technical assistance. The National Gallery Scientific Laboratory is thanked for the gift of cedar oil. The NERC provided financial support for mass spectrometry facilities and a studentship to K.A.C.

Competing interests statement The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to R.P.E. (r.p.evershed@bristol.ac.uk).

Early brain growth in *Homo erectus* and implications for cognitive ability

H. Coqueugniot[1], **J.-J. Hublin**[2], **F. Veillon**[3], **F. Houët**[1] & **T. Jacob**[4]

[1] *UMR 5199-PACEA, Laboratoire d'Anthropologie des Populations du Passé, Université Bordeaux 1, avenue des Facultés, 33405 Talence cedex, France*
[2] *Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, 04130 Leipzig, Germany*
[3] *Service de Radiologie I, Hôpital Hautepierre, avenue Molière, 67200 Strasbourg, France*
[4] *Department of Physical Anthropology, Gadjah Mada University, College of Medicine, Yogyakarta, Indonesia*

Humans differ from other primates in their significantly lengthened growth period. The persistence of a fetal pattern of brain growth after birth is another important feature of human development[1]. Here we present the results of an analysis of the 1.8-million-year-old Mojokerto child (Perning 1, Java), the only well preserved skull of a *Homo erectus* infant, by computed tomography. Comparison with a large series of extant humans and chimpanzees indicates that this individual was about 1 yr (0–1.5 yr) old at death and had an endocranial capacity at 72–84% of an average adult *H. erectus*. This pattern of relative brain growth resembles that of living apes, but differs from that seen in extant humans. It implies that major differences in the development of cognitive capabilities existed between *H. erectus* and anatomically modern humans.

Dental microstructure has been recently used[2] to determine when in the course of hominid evolution a modern human pattern of dental maturation appeared. Representatives of *H. erectus* have been shown to display a shorter period of dental development, suggesting that a modern human growth pattern evolved more recently. Another important aspect of human growth is 'secondary altriciality'. In most primates, brain growth slows down rapidly after birth[1] whereas hominids have to solve the evolutionary challenge of developing a large brain under substantial physiological, obstetrical and locomotor constraints[3]. An adaptive solution has been reached by giving birth to offspring with relatively small brains compared with adult brain size. Whereas *Macaca* newborns display an endocranial volume equivalent to 70% of adult size[1], the modern human brain represents only 25% of its adult size at birth and continues to grow at its fast fetal rate during the first year of life. At 1 yr of age the human brain is 50% of its adult size and at 10 yr 95% of the adult brain size is achieved. At birth, apes display an intermediate condition, with an endocranial volume approximately 40% of adult size in the common chimpanzee[4], with 80% of the adult volume being reached by the end of the first year.

Secondary altriciality has social consequences: modern human children require many years of parental support. It also influences the development of cognitive abilities. Most of human brain growth takes place in an 'enriched environment', while the individual is already interacting with the extra-maternal environment[5–7]. A prolonged interaction between peripheral somatic areas and developing related sensori-motor cortical areas could be one condition for the development of spoken language. When this important adaptation of the genus *Homo* appeared during the course of

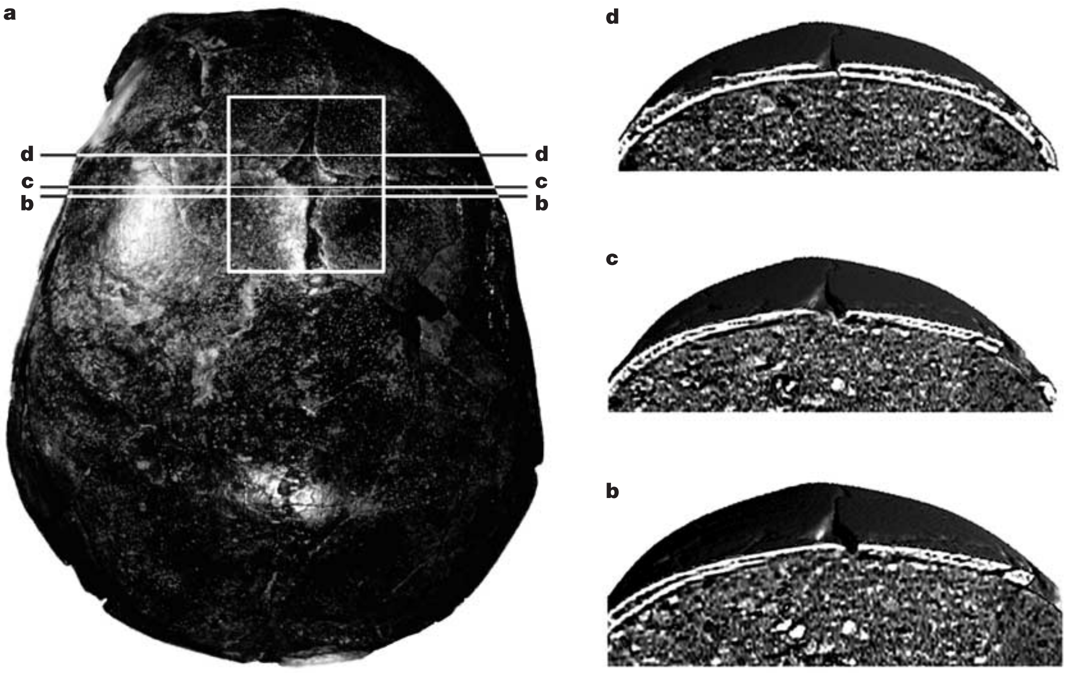

Figure 1 Superior view of the Mojokerto specimen (**a**) and three-dimensional reconstructions from axial CT scans of the anterior part of the skull (**b–d**). The location of the coronal cuts are indicated on **a**. The coronal cuts in **b** and **c**, located immediately behind the bregma, display the gap between the two parietals reduced to one compact table on both sides. The large depression located anteriorly to the bregma on the frontal visible on **d** results from damage of the outer table. Three-dimensional reconstructions of the skull were made with Voxel-man software (University of Hamburg)[28]. The photograph in **a** is from the Senckenberg Museum.

Copyright of Nature is the property of Nature Publishing Group and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.