Journal of Archaeological Science (2003) **30,** 1–12
doi:10.1006/jasc.2001.0827, available online at http://www.idealibrary.com on IDEAL®

New Chemical Evidence for the Use of Combed Ware Pottery Vessels as Beehives in Ancient Greece

Richard P. Evershed and Stephanie N. Dudd

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

Virginia R. Anderson-Stojanovic

Department of Classics and Fine Art, Wilson College, 1015 Philadelphia Avenue, Chambersberg, PA 17201-1285, U.S.A.

Elizabeth R. Gebhard

University of Illinois at Chicago, Chicago, IL, U.S.A.

(*Received 13 September 2000, revised manuscript accepted 17 February 2001*)

Coarseware vessels from excavations at Isthmia, Greece, in contexts dating from the Hellenistic and Roman periods, resemble ceramic beehives used by the ancient Greeks and still in recent use on the Cycladic islands and Crete. Chemical investigations of absorbed residues were performed with the aim of obtaining direct evidence for the use of these vessels as beehives. High-temperature gas chromatography (HT-GC) and HT-GC/mass spectrometry (HT-GC/MS) were used to screen lipid extracts for the presence of compounds characteristic of beeswax. Samples of beeswax taken from a 19C ethnographic beehive was used as reference material. Potsherds from 10 pithoi recovered from the same Isthmia excavation served as controls. A significant proportion of the sherds from the putative beehive vessels contained compounds, i.e. *n*-alkanes, wax esters, fatty acids and long-chain alcohols congruent with those seen in the reference beeswax. $\delta^{13}C$ values were determined for the individual components of the lipid extracts and reference beeswax by means of compound specific GC-combustion-isotope ratio mass spectrometry (GC-C-IRMS). On the basis of the molecular structures, carbon number distributions and $\delta^{13}C$ values 16 of the 40 sherds studied were shown to contain residues of beeswax. None of the pithoi contained beeswax residues although two yielded residues consistent with degraded triacylglycerols.

Keywords: BEESWAX, BEEHIVES, ARCHAEOLOGY, ISTHMIA, GREECE, LONG-CHAIN ALCOHOLS, n-ALKANES, WAX ESTERS, COMPOUND-SPECIFIC $\delta^{13}C$ VALUES, GAS CHROMATOGRAPHY, MASS SPECTROMETRY, ISOTOPE RATIO MASS SPECTROMETRY.

Introduction

Excavations at various sites in Greece have revealed a number of finds of similar curious red ceramic vessels and lids which it has been argued were beehives (Jones, Graham & Sackett, 1973 and references therein). The vessels are of two general types. One type is fairly long (0·6–0·65 m) and tapers to a rounded, closed end (referred to hereafter as type 1), while the other type (referred hereafter as type 2) shown in Figure 1a is characterized by its straight sides, flattened bottom, and opening at one end, somewhat resembling a large flowerpot. The vessels are wider at the mouth than at the base and manufactured from a coarse gritty fabric. The unusual feature, common to both types of vessel, are the grooves that cover half of the internal surface. It is thought that the grooves were made by drawing a comb across the clay before it was fired. Examples of this grooving or combing can be seen on the potsherds in Figure 1b & c. Although there is some variation in the dimensions of the type 2 vessels, typical sizes are 0·29–0·33 m high, with interior diameters varying from 0·34–0·4 m. A small opening, either rectangular or round, is present at the bottom of the wall, or in the base close to the wall. The round holes vary from 0·026–0·030 m in diameter, while the rectangular slits vary from 0·025–0·040 m by 0·15–0·10 m. While complete vessels have

0305–4403/03/$-see front matter

Figure 1. (a) The near complete upright (type 2) combed ware vessel recovered from excavations at Isthmia, and (b) characteristic combing of the interior surface a sherd that forms the basis of the classification of this ware type.

been found, it is more common to find potsherds that can be linked to this vessel type by the combing on one surface (Figure 1b & c).

The excellent summary by Jones, Graham & Sackett (1973) provides compelling evidence for the use of Type 1 vessels, which they refer to as combed ware, as beehives. Only the most salient points raised in their article are presented below. Their conclusion, based on the shape and interior grooving, is that these vessels were beehives, although it should be emphasized that direct evidence for this is lacking. They argue that such vessels would have provided an enclosed space of about the right size for a swarm of bees to colonize. The roughening, or combing, of the internal wall of the vessels would provide an ideal surface for the honeycomb to cling to. Evidence that such vessels functioned as beehives, comes from ethnographic examples found in several countries around the Mediterranean e.g. Crete and Malta (Crane, 1983), where similar, albeit somewhat larger, pottery beehives are still used today. Moreover, the horizontally stacked pottery beehives that appear in Egyptian paintings dated at 3000–1000 years BC bear a close resemblance to the type 1 vessels described above. Thus, while type 1 vessels would have been lain horizontally, the type 2 vessels would probably have stood upright with the comb hanging down from either the lid or wooden bars. The use of wooden bars would have been consistent with

the development from the horizontal type of hive towards top-bar hives and the modern movable frame arrangement (Infantidis, 1983; Crane, 1983).

Although no pictorial evidence exists showing the ancient Greeks engaged in apiculture, there is some literary evidence. Aristotle certainly studied bees and from his comments in Historia Animalium (summarized in Jones, Graham & Sackett, 1973) it is apparent that the ancient Greeks kept bees. There is some confusion as to whether his comments suggest primitive hives, such as the pottery hives, or a more modern version, in which the combs are removable, as is widely used today. However, the fact that Pliny's ex-consul needed to have a special transparent hive to observe bees, and Aristotle's conviction that there was not enough known about bees, both seem to indicate that a removable comb version was probably not available to them or their sources of information.

Jones, Graham & Sackett, (1973) draw attention to the fact that "combed" vessels have been found primarily at rural sites, but are not unknown in the centre of ancient Athens (Sparkes & Talcott, 1970; Thompson, Thompson & Rotroff, 1987; Ludorf, 1999). Interestingly, their absence at one country house, and presence in abundance at another, indicates that they were not an absolutely essential part of every household, and that when they were used, their large numbers suggest they were connected with the productive economy of the community. This is exactly the situation that would be expected to be encountered with an activity such as beekeeping. While such vessels have also been found in areas that would be in keeping with the idea that they are beehives, it has been argued that these pots were not beehives, since some sherds have been recovered in an area identified as a military encampment. It is has been proposed that these vessels are more likely to have been used as water carriers, since soldiers are unlikely to have engaged in apiculture. However, the wide mouth, narrow bottom and lack of handles, would have made it impossible to carry the vessels with water inside. It has also been argued that the Greeks had a more advanced type of hive, or that they would have built them out of wood. Crane also states that the volume is too small for them to have been used as hives (Crane, pers. comm.).

In view of the questions that remain concerning the function of these vessels, there is clearly a need for direct evidence in order to establish an association with beekeeping. The identification of beeswax residues in the ancient vessels, or sherds derived from such vessels, would provide very compelling evidence for their use as beehives. In an effort to provide such evidence Bu'Lock (in Jones, Graham & Sackett, 1973) examined yellow waxy residues obtained by solvent extraction of a number of sherds from this type of vessel recovered from excavations at Vari, Greece. Although GC was performed and the results consistent with the presence of beeswax, they were rather inconclusive, due to the lack of unambigous peak identifications, which would have required mass spectral data or co-injection of reference compounds. The technological developments that have occurred in the fields of GC and MS since Bu'Locks early attempt have lead to considerable advances being made in the study of organic residues from archaeological ceramics (e.g. Evershed *et al.*, 1997, 1999). This paper reports the results of a investigation of organic residues from combed vessels in an attempt to obtain direct evidence for the use of combed vessels as beehives based on the detection of beeswax absorbed into the fabric of the vessels.

Materials and methods

Archaeological samples

The vessels which are the subject of this investigation were either horizontal or upright hives. Samples submitted to analysis were wall fragments and thus can only tell us that they were beehives but not whether they were the upright or horizontal type (the entire profile is required to identify the type). A nearly complete example of the type 2 beehive (Figure 1a) along with some 50 fragments from other hives, was recovered in excavations in a Hellenistic settlement on the ridge (Rachi) south of the Sanctuary of Poseidon at the Isthmus of Corinth. The restored top-bar hive from the Rachi settlement, discovered in 1955 (Broneer, 1958; Kardara, 1961) was inscribed with the name Orestada and has been discussed at length by Crane (1993) where it has been suggested that it could not have been a beehive because of its small size. Furthermore, the existence of the flyhole at its base was also considered open to doubt. The additional fragments have been identified since that time from work in 1955 and from the more recent excavation campaign in 1989 (Anderson-Stojanovic, 1996). Their inclusion in destruction debris dated to ca. 200 BC provides a terminus *ante quem* for their period of use. Two additional nearly complete upright hives and 30 fragments come from excavations in the Sanctuary of Poseidon and its environs (Gebhard & Hemans, 1998). So far as we know, these pieces from Isthmia are the only known ancient examples of the top-bar-hive that have been identified. Owing to the lack of evidence elsewhere in the ancient world for the upright hive, and the absence of clear indications of its existence in the ancient sources, there is clearly a need for direct association of these vessels with beekeeping.

Table 1 provides details of the samples studied. Samples were exported under license from Greece to the University of Bristol for analysis. All the samples were water washed and stored in brown paper bags, at room temperature, until required for analysis. The reference ethnographic beeswax (sampled from cylindrical pottery vessels known to have been used as beehives on Crete in the 19th century AD) was stored in sealable plastic bags. Although 100 years old the composition of the ethnographic beeswax was largely

Table 1. Details of potsherds from pithoi and combed ware vessels subject of this investigation

Sample no.	Object	Munsell	Date	Area	Feature	Context	Lot	Lipid content (μg g^{-1})	Lipid compos.	Lipid type
28	Pithos	2·5YR 6/8 grey core	3 BC	Rachi	House XI	Constr.	89–487	tr[a]	TAG[b]	Oil
29	Pithos	2·5YR 6/6 grey core	3 BC	Rachi	House XI	Constr.	89–487	83	FA[c], TAG	Oil
30	Pithos	5YR 7/6	3 BC	Rachi	House XI	Constr.	89–487	tr	—	—
31	Pithos	2·5YR 6/8	3 BC	Rachi	House XI	Constr.	89–487	32	FA, TAG	Oil
32	Pithos	2·5YR 6/8	3 BC	Rachi	House X	Destr.	89–497	tr	—	—
33	Pithos	5YR 7/6	3 BC	Rachi	House X	Destr.	89–497	tr	—	—
34	Pithos	2·5YR 6/8	3 BC	Rachi	House X	Destr.	89–497	tr	—	—
35	Pithos	5YR 7/6	3 BC	Rachi	House VII	Destr.	89–506	tr	—	—
36	Pithos	2·5YR 7/6	3 BC	Rachi	House VII	Destr.	89–506	tr	—	—
37	Pithos	7·5YR 8/4	3 BC	Rachi	North Bldg	Fill	89–180	tr	—	—
38	Beehive	7·5YR 7/4	AD 13	Fortress	South Gate	—	58-DFO-008	tr	—	—
39	Beehive	10YR 7/1	AD 13–14	Fortress	South Gate	—	56-DFO-016	tr	—	—
40	Beehive	7·5YR 7/4	AD 1–2	Fortress	Tower 14	—	67-T14-041	tr	—	—
41	Beehive	5YR 6/4	AD 5–6	Fortress	Tower 14	—	67-T14-041	tr	—	—
42	Beehive	5YR 7/6	Mixed	Fortress	NE Gate	—	67-RMN-010	tr	—	—
43	Beehive	7·5YR 7/4	AD 6–7	Fortress	NE Gate	—	67-RMN-071	14	AL[d], OH[e], We[f]	Beeswax
44	Beehive	7·5YR 7/4	Mixed	Fortress	NE Gate	—	67-RMM-040.1	tr	—	—
45	Beehive	7·5YR 7/4	Mixed	Fortress	NE Gate	—	67-RMN-036	tr	—	—
46	Beehive	7·5YR 7/2	AD 13–14	Fortress	NE Gate	—	67-RMN-024	27	U[g]	—
47	Beehive	5YR 7/6	4 BC–AD 3	Fortress	NW Precinct	—	67-NWP-014	262	AL, OH, WE	Beeswax
48	Beehive	7·5YR 7/4	3 BC	Rachi	House VIII	Fill	89–522	tr	U	—
49	Beehive	5YR 6/4	3 BC	Rachi	House XI	Destr.	89–492	73	AL, OH	Beeswax
50	Beehive	7·5YR 7/4	3 BC	Rachi	House XI	Destr.	89–486	49	—	—
51	Beehive	7·5YR 7/4	3 BC	Rachi	House XI	Destr.	89–486	116	AL	Beeswax
52	Beehive	7·5YR 7/4	3 BC	Rachi	Street 1	Destr.	89–115	149	AL	Beeswax
53	Beehive	7·5YR 6/4	3 BC	Rachi	House I, II	Destr.	89–101	tr	—	—
54	Beehive	7·5YR 7/4	3 BC	Rachi	House I, II	Wash	89–101	29	U	—
55	Beehive	5YR 7/6	3 BC	Rachi	House I, II	Wash	89–101	354	U	—
56	Beehive	7·5YR 7/4	3 BC	Rachi	House II	Wash	89–104	24	U	—
57	Beehive	NA	3 BC	Rachi	House II	Destr.	89–106	113	AL, OH, WE	Beeswax
58	Beehive	2·5YR 6/8	Roman	Stadium	Floor	Destr.	1219	tr	AL	Beeswax
59	Beehive	2·5YR 6/8	Roman	Sanctuary	Stoa	Surf.	1237	tr	—	—
60	Beehive	7·5YR 7/4	—	Sanctuary	NW Dump	—	408	16	AL, OH, WE	Beeswax
61	Beehive	2·5YR 6/8	—	Sanctuary	—	Fill	1315	tr	—	—
62	Beehive	NA	Byzan	Sanctuary	NE	—	1514	34	AL, OH, WE	Beeswax
63	Beehive	NA	Roman	Sanctuary	NE	—	1529	82	AL, OH, WE	Beeswax
64	Beehive	5YR 7/4	Roman	Sanctuary	NE	—	1536	64	AL, OH, WE	Beeswax
65	Beehive	NA	Roman	Sanctuary	NE Cave	—	1564	18	AL	Beeswax
66	Beehive	5YR 7/6		Sanctuary	NE	—	1831	tr	—	—
67	Beehive	2·5YR 6/6	Byzan	Stadium	—	Sand	2020	245	AL, OH, WE	Beeswax
68	Beehive	7·5YR 7/4	Byzan	Stadium	—	Fill	2026	21	AL	Beeswax
69	Beehive	7·5YR 7/4	Roman	Stadium	—	Fill	2033	tr	—	—
70	Beehive	5YR 7/6	Roman	Stadium	—	Fill	2041	tr	—	—
71	Beehive	2·5YR 6/8	Roman	Stadium	Water channel	—	2042	tr	—	—
72	Beehive	7·5YR 8/4	Roman	Stadium	Water channel	—	2053	tr	—	—
73	Beehive	7·5YR 7/4	Roman	Theatre	North end	—	2090	tr	—	—
74	Beehive	NA	Roman	Theatre	Northeast	Surf.	2127	66	AL, OH, WE	Beeswax
75	Beehive	7·5YR 7/4	Roman	Stadium	North tunnel	—	2234	151	U	—
76	Beehive	7·5YR 7/4	Roman	Sanctuary	SE	—	89–911	168	U	—
77	Beehive	7·5YR 7/4	3 BC	Rachi	House XVII	Destr	153	tr	—	—
78	Beehive	7·5YR 8/2	3 BC	Rachi	House I	Destr.	89–114	10	AL	Beeswax

[a]tr = trace <5 μg g^{-1}; [b]FA = fatty acids; [c]TAG = triacylglycerols; [d]AL = *n*-alkanes; [e]OL = long-chain 1° alcohols; [f]WE = wax esters; [g]U = unknown.

unchanged from that of fresh beeswax [cf. Figure 3 with Figure 4 in Charters *et al.* (1995)].

Preparation of samples

Solvents and chemicals. The solvents and reagents used were of the highest grade available, in order to avoid possible contamination. AnalaR and HPLC grade solvents were used as supplied, and water was doubly distilled.

Extraction of lipid from pottery samples. The extraction procedure used was based on the method used by Evershed, Heron & Goad (1990) and Charters *et al.*

(1993) although the surface cleaning step was avoided since the beeswax would not have been expected to have penetrated the pottery so deeply as lipids from food preparation cooking or the storage or processing of fats and oils. A small piece of the potsherd was taken and ground into a fine powder, in a degreased pestle and mortar. The sample (2 *g*) was accurately weighed into a vial and 20 µl of the internal standard solution [1 mg ml^{-1} solution of *n*-tetratricontane ($C_{34}H_{70}$) in cyclohexane] added. The lipid was then extracted by ultrasonication with 10 ml of chloroform/methanol (2/1 v/v) solution, for 2 × 15 min (with a 30 min pause between, to allow for cooling). The samples were then decanted into glass centrifuge tubes and spun at 3000 rpm for 15 min, to remove suspended pottery. The supernatant was decanted into a round bottomed flask and most of the solvent removed on a rotary evaporator, before transferring the residual solution into a vial. The solvent was then removed under a stream of nitrogen with gentle warming. This gave the total lipid extract, which was stored in a refrigerator at 4°C until required for analysis. An analytical blank was also prepared by the same method with each batch of pottery samples.

Dissolution of pure beeswax. A small amount of the ethnographic (taken from a AD 1800–1900 Cretan beehive) beeswaxes were weighed out (5–10 mg) into a vial and dissolved in 2 ml of chloroform/methanol (2/1 v/v) by ultrasonication for 15 min. Aliquots of this solution were taken as required for GC, GC/MS, saponification, etc.

Derivatization of total lipid extracts

The total lipid extract was re-dissolved in solvent (chloroform/methanol 2/1 v/v), and a small portion (usually a 1/5 aliquot) transferred to a sample vial, where the solvent was removed under a stream of nitrogen. *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA; 20 µl) containing 1% v/v trimethylchlorosilane (TMCS) was then added and the resulting solution heated to 65°C for 20 min. The BSTFA was removed under a stream of nitrogen and the sample re-dissolved in an appropriate volume of cyclohexane for analysis by GC, GC/MS, or GC-C-IRMS.

Saponification of beeswax

A known amount of total lipid extract (up to 150 µg in the case of the reference beeswax) was dissolved in an appropriate amount of chloroform/methanol (2/1 v/v), and added to 2 ml of a 10% NaOH in MeOH solution, in a screw-capped vial. This mixture was then heated at 70°C in a water bath for 30 min and then left to cool. After acidification (1 ml 2 M HCl), and dilution (3 ml of water), the lipid was extracted three times by adding diethyl ether (3 ml), shaking, and then removing the uppermost (organic) layer. The combined organic layers were then transferred to a round bottomed flask and reduced almost to dryness using a rotary evaporator. The residual solution was transferred to a vial and the remaining solvent evaporated under a stream of nitrogen. The resulting saponified lipid extract was trimethylsilyated by the same method as a total lipid extract and analysed by GC and GC-MS.

Fractionation of saponified beeswax and organic residues

Separation of fatty acids from the lipid extract. Since baseline resolution of components is required for GC-C-IRMS, the saponified beeswax was separated into its three main compound classes namely: *n*-alkanes, fatty acids, and long-chain alcohols. Fatty acids were separated from the other components by solid phase extraction using aminopropyl Bond Elute columns [covalently bonded silica gel (particle size–40 µm)]. The column was conditioned with hexane, then the sample (dissolved in 6 ml of dichloromethane/isopropanol, 2/1 v/v) was introduced onto the column. This was eluted with a further 6 ml of dichloromethane/isopropanol (2/1 v/v) and then 12 ml of 2% acetic acid in diethyl ether. The first fraction contained the neutral components and the later fraction the fatty acids. After separation the solvent was evaporated from each fraction under a stream of nitrogen. The separated lipid fractions were stored in a refrigerator until required for analysis.

Separation of alkanes and long-chain alcohols. A small scale flash chromatography column was prepared, using silica gel (60–80 mesh, dried in an oven overnight) and conditioned using hexane. The sample was then added to the top of the column and eluted with 6 ml of hexane (containing the alkanes), followed by 6 ml of diethyl ether (containing the alcohols). The solvent was removed from both fractions by gentle heating under a stream of nitrogen, and the samples stored in a refrigerator until required for analysis.

Instrumentation

Gas chromatography. GC analyses were carried out using an HP 5890A instrument connected to an Opus V PC, with HP chemstation software. Samples were introduced by on-column injection, both manually and by autosampler. The column used was a polyimide clad 15 m × 0·32 mm i.d., bonded phase wall coated open tubular (WCOT) fused silica capillary column, coated with DB-1 stationary phase (0·1 µm film thickness, immobilized dimethyl polysiloxane). Helium was the carrier gas, with a column head pressure of 15 psi with the column effluent being monitored by a flame ionization detection. The temperature program used involved an isothermal hold at 50°C for 2 min after injection, followed by temperature programming to 350°C at 10°C min^{-1}, followed by a further isothermal hold at 350°C for 10 min.

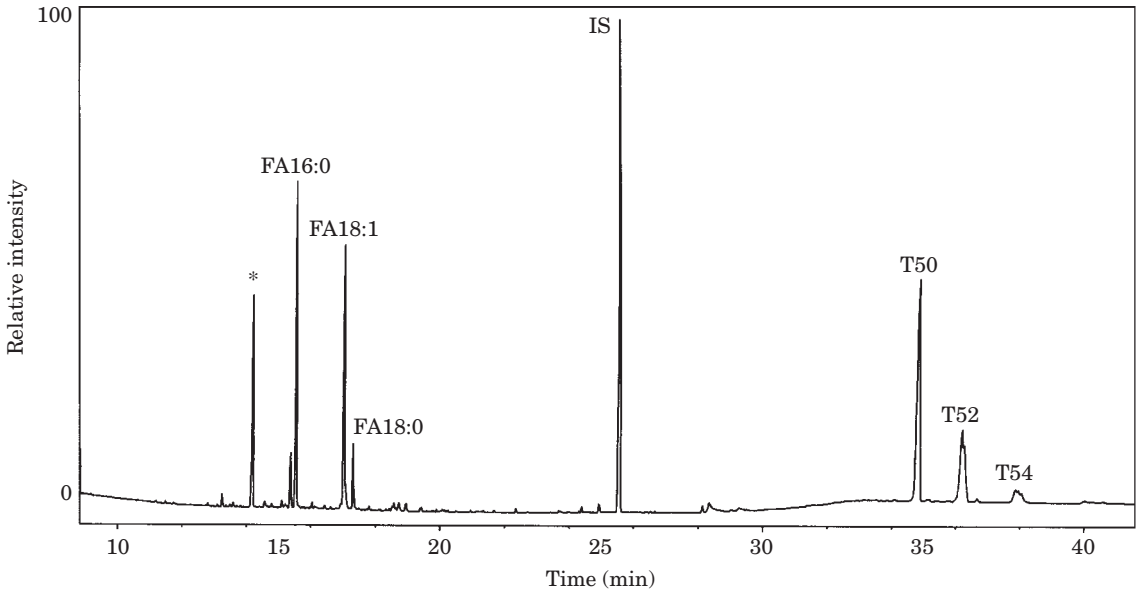

Figure 2. Partial high temperature GC profile of the trimethylsilylated total lipid extract of a sherd derived from pithos sherd 31.

Gas chromatography/mass spectrometry. GC/MS analyses were carried out using a Carlo Erba HRGC 5160 Mega series GC instrument, coupled to a Finnigan 4500 quadrupole MS. On-column injection was used with a 25 m × 0·32 mm i.d. DB-1 WCOT capillary column (0·25 μm film thickness). The temperature program was the same as used above for the stand alone GC. The MS operating details were, electron multiplier potential 2 KV, filament current 0·35 mA, electron energy 35 eV, and the spectra were recorded every 1 s, over the range *m/z* 50 to 800. The identification of individual compounds was based upon the GC elution orders, interpretation of the spectra, and comparison with reference spectra.

Gas chromatography-combustion-isotope ratio mass spectrometry. GC-C-IRMS analyses (Matthews & Hayes, 1978; Evershed *et al.*, 1994) were carried out using a Varian 3500 GC attached to a Finnigan MAT Delta-S isotope ratio mass spectrometer via a Finnigan MAT combustion interface. Electron ionization was used, with an 18 cm effective radius for the magnetic sector with extended geometry. The column used was a 50 m × 0·32 mm i.d. WCOT fused silica capillary column, with a 0·25 μm film of CP-Sil 5CB. The temperature program used involved an isothermal hold at 50°C for 2 min, followed by temperature programming to 150°C at 10°C min^{-1}. The temperature was then further raised to 300°C at 4°C min^{-1}, and held isothermally for 30 min. The minimum peak detection voltage was set at 0·3 V. The $\delta^{13}C$ values of trimethylsilylated components were corrected for the contribution from the added carbon by the method of Jones *et al.* (1991).

Results and discussions

This investigation of the lipid extracts of sherds from the combed ware vessels used GC, GC-MS and GC-C-IRMS to derive quantitative, structural, distributional and compound specific isotopic information. All extracts were submitted to GC analysis to screen for the presence of lipid. Only those samples that yielded appreciable material were submitted to GC/MS and GC-C-IRMS. Samples of ethnographic beeswax were analysed in parallel with the archaeological samples for comparative purposes in an effort to confirm the presence or absence of beeswax in the ancient vessels. The storage pithoi were chosen as controls since they would not have been used as beehives.

GC and GC/MS analyses of total lipid extracts

Extracts of 41 sherds from combed vessels and 10 sherds from pithos (controls) were submitted to HT-GC analysis as the first phase in the investigation. Of the 51 vessels studied 26 contained >5 μg g^{-1} of lipid detectable by GC (Table 1). Close inspection of the GC chromatograms from the pithos showed none of them to contain lipid distributions that could in any way be related to beeswax. Eight out of the 10 pithos contained only trace amounts of lipid which were not studied further. The remaining two pithos yielded 83 and 32 μg g^{-1} of lipid dominated by $C_{16:0}$, $C_{18:1}$ and $C_{18:0}$ fatty acids and C_{48}, C_{50} and C_{52} triacylglycerols of unknown origin.

A rather different picture was presented by the extracts of the combed ware vessels. At least 16 of these sherds contained components which suggested an origin in beeswax. For example, sample 64 yielded

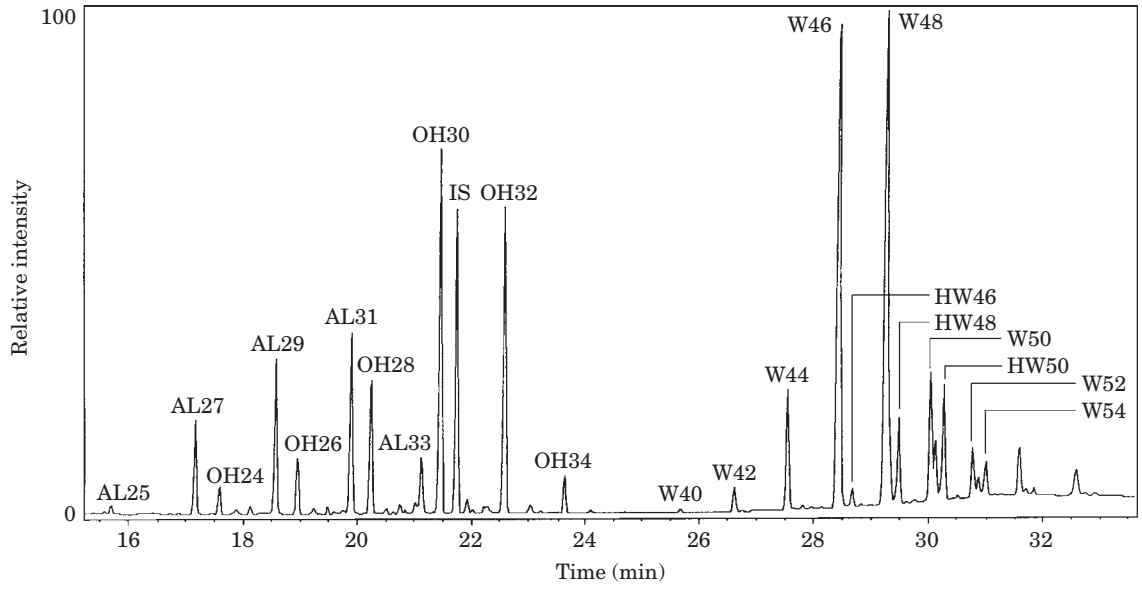

Figure 3. Partial high temperature GC profile of the trimethylsilylated total lipid extract of combed ware sherd 64 derived from an upright (type 2) putative beehive.

82 μg g^{-1} of lipid which produced the GC profile shown in Figure 3. The complex mixture of components was shown by GC/MS to comprise four significant homologous series of components. Eluting at the shortest retention times was a series of C_{23} to C_{33} carbon number *n*-alkanes displaying a unimodal distribution possessing a strong odd-over-even predominance. Eluting somewhat later was a series of C_{24}–C_{34} long-chain alcohols in which *n*-tricontanol (C_{30}) and *n*-dotricontanol (C_{32}) were the major components. Eluting at longer retention times were a series of C_{40}–C_{54} carbon number palmitic acid wax esters. Interestingly, the carbon number range of the fatty alcohol moieties in the long-chain wax esters closely mirrors that seen for the earlier eluting long-chain 1° alcohols. Eluting at somewhat longer retention times than the wax esters is a series of hydroxy palmitic acid wax esters in which the C_{48} and C_{50} homologues were the most abundant components. An almost exactly analogous distributions of components was seen in the lipid extracts of potsherds 62 and 63 although their overall lipid content was somewhat lower (33 and 64 μg g^{-1}). Sherd 67 yielded a somewhat similar distribution of components although the *n*-alkanes were much less in evidence, with the distribution maximizing at C_{31}.

Although the distributions of the various components shown in the GC profile for the extract of sample 64 in Figure 3 is not exactly analogous to that of the ethnographic beeswax, shown in Figure 4 and the histograms presented in Figures 5 and 6, there is sufficient evidence, to suggest that the lipid extracts of the sherds from combed ware vessels of this type do indeed represent residues of beeswax (see also Tulloch, 1970, 1971; Tulloch & Hoffman, 1972; Brüschweiler, Felber & Schwager, 1989; Mills & White, 1994; Charters *et al.*, 1995; Evershed *et al.*, 1997). A recent report (Regart *et al.*, 2001) discusses variations of this nature in the composition of ancient beeswax in relation to its deterioration with time. Thus, the data obtained from these sherds provide very compelling evidence to support the arguments in favour of the interpretation of the function of these vessels as beehives.

Particularly characteristic is the distribution of *n*-alkanes seen in the lipid extracts of sherds 62 and 63, since it exactly mirrors that seen in fresh and ethnographic beeswax, and indeed the latter is shown in Figures 3 and 4. Also of significance is the series of wax esters seen at longer retention time in the GC profile shown in Figure 3. Although the distribution does not exactly match that seen in the beeswax, and overall these components are present at much lower abundance in the archaeological samples, the carbon number range and the dominance of palmitic acid and hydroxy palmitic acid esters is a very characteristic feature of beeswax.

A number of the other sherds examined and found to contain reasonable amounts of lipid bore a less strong resemblance to beeswax but still retained sufficient characteristics to suggest that they had indeed originated from beeswax. For instance, sherds 43, 47, 57, 58, 65, 68 and 78 yielded lipid extracts that comprised almost entirely *n*-alkanes (14, 262, 113, 10, 18, 21 and 10 μg g^{-1} respectively) with an exactly analogous carbon number range to that seen in the ethnographic beeswax. The *n*-alkane distributions of these sherds together with those of the others subject of this investigation are presented as histograms in Figure 5. The extract of vessel 43 was also dominated by *n*-alkanes although the distribution resembled that seen

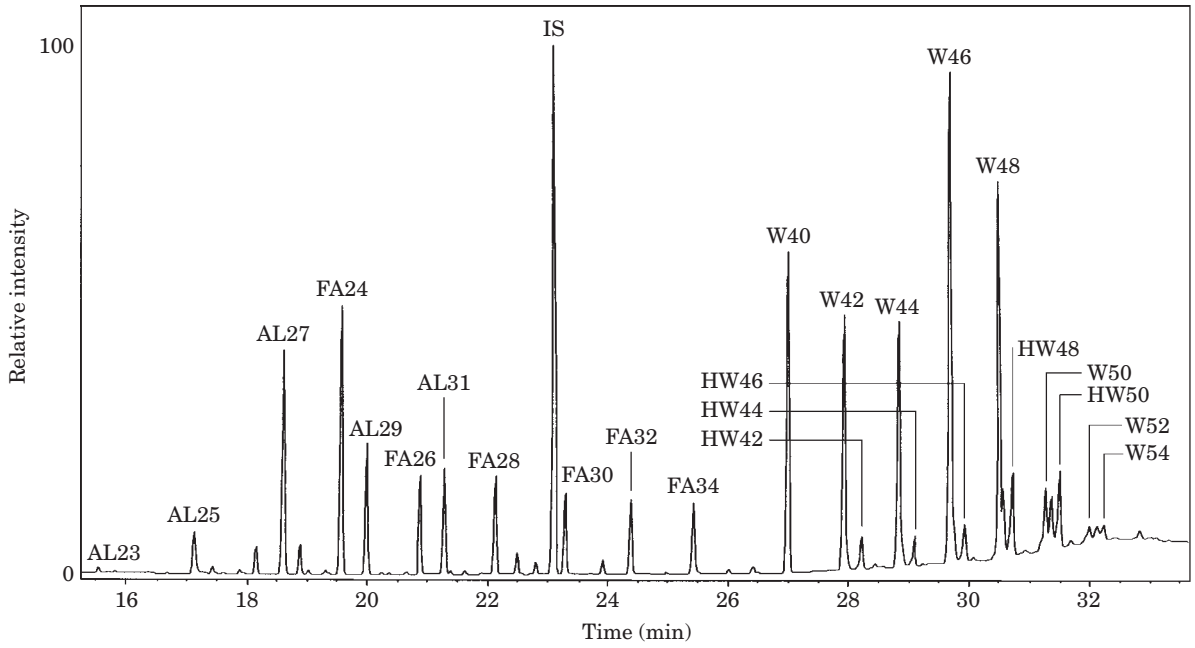

Figure 4. Partial high temperature GC profile of the trimethylsilylated total lipid extract of a AD 1800–1900 sample of beeswax samples from an ethnographic beehive from Crete.

in vessel 64 where the distribution maximized at C_{31}. The sherds from vessels 60 and 74 also yielded lipid extracts containing the characteristic mixture of *n*-alkanes described above (Figure 5) although these two sherds also exhibited appreciable amounts of free *n*-alkanols in the C_{24}–C_{34} carbon number range. Hence, these extracts somewhat resembled those of sherds 62, 63, 64 and 67, but lacked the intact wax esters seen both in these sherds and fresh beeswax. The *n*-alkanol and wax esters distributions obtained from the analysis of the extracts of the sherds subject of this investigation are presented in Figures 6 and 7.

Compound specific stable carbon isotope ($\delta^{13}C$) analyses

Compound specific $\delta^{13}C$ measurements were performed on the *n*-alkane and *n*-alkanol components of the sherd lipid extracts and reference beeswax in order to provide further molecular information on which to base interpretations of the use of the combed vessels. The $\delta^{13}C$ values of the *n*-alkanes from extracts of the ancient vessels are presented in Table 2 together with those of the reference ethnographic beeswax. The values for the extracts of the ancient vessels lie in the range $-24{\cdot}5$ to $-26{\cdot}8$‰ while those of the reference beeswax cover the range $-25{\cdot}1$ to $-26{\cdot}7$‰. The close agreement between the values obtained from the ancient samples and the reference beeswax is entirely consistent with an origin for the *n*-alkanes in beeswax. Interestingly, the $\delta^{13}C$ values for the beeswax *n*-alkanes are significantly less depleted in ^{13}C compared to values typically recorded for higher plant epicuticular wax components (Rieley *et al.*, 1991; Lockheart, van Bergen & Evershed, 1997). The significance of these values will be discussed in further detail below.

Saponification was performed on those samples that had been shown to contain wax esters. The resulting *n*-alkanols were purified by 'flash' adsorption column chromatography, trimethylsilylated and the $\delta^{13}C$ values obtained by GC-C-IRMS. Stable isotope values were recorded for fewer vessels for the *n*-alkanols than for the *n*-alkanes. Notwithstanding this the data presented in Table 3 shows excellent agreement to exists between the ancient vessels and the reference ethnographic beeswax, thus providing further evidence for the origin of the ancient lipids being beeswax.

Variations in the chemical composition of ancient and modern beeswax

It is becoming increasingly apparent that the composition of beeswax found in association with pottery vessels can be altered during its use and burial. Heron *et al.* (1995) noted a complete loss of the *n*-alkanes from a sample of beeswax recovered from a Neolithic ceramic jar as a result of heating. While in our own work on Late Minoan lamps and conical cups from Crete we noted not only a loss of the *n*-alkanes, but also alterations in the distributions of wax esters (Evershed *et al.*, 1997). Whilst the loss of the *n*-alkanes could be accounted for by the heat generated by the lamps during their use, as in the case of the Neolithic jar, an explanation for the changes in the distribution of the wax esters is less obvious but are probably

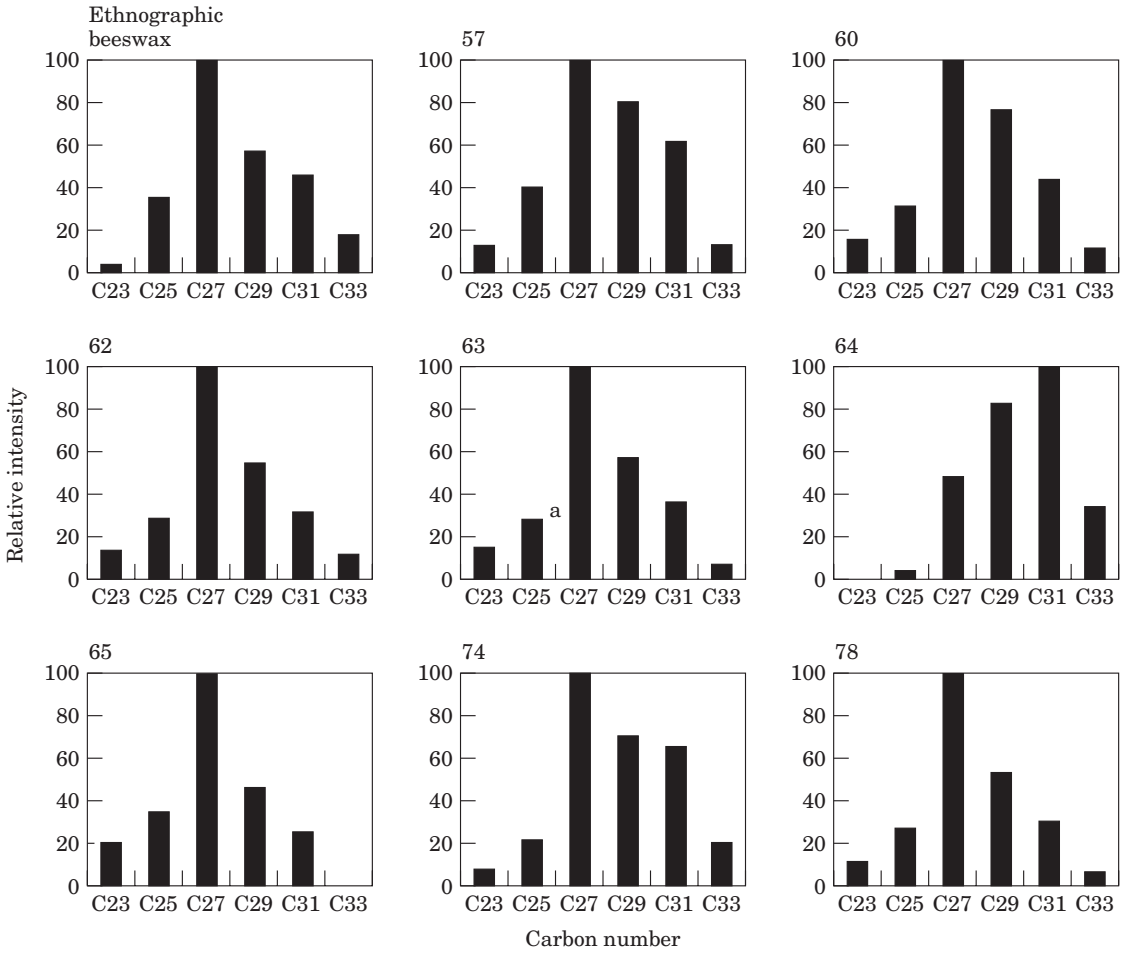

Figure 5. Distributions of *n*-alkanes isolated from solvent extracts of the reference ethnographic beeswax and combed ware putative beehive sherds. Distributions were determined from the areas under the peaks in the GC chromatograms of the fractionated total lipid extracts. The numbers associated with each histogram correspond to the sample numbers given in Table 1. See "Materials and Methods" for further details.

driven by diagenetic processes. Evidence for hydrolysis of the wax esters comes from the presence of free *n*-alkanols in the total lipid extracts of the ancient beehives, although interestingly, the free fatty acids, palmitic and hydroxypalmitic, are not seen, presumably since they have been lost from the sherds by microbial action and groundwater leaching. Likewise, free fatty acids were absent from the total lipid extracts of the lamps and conical cups from Crete.

While beeswax has been detected previously in ceramic vessels in most cases evidence exists for use of the vessels in the processing of beeswax or honey rather than in its production (Charters *et al*., 1995; Heron *et al*., 1995; Evershed *et al*., 1997, 1999). For example, the presence of extensive sooting on the exterior surfaces of a medieval jar and an inturned-rimmed bowl from the U.K. indicated heat had been introduced during the mixing of beeswax and animal fat in the vessels (Charters *et al*., 1995). Significantly, no trace of evidence of heating over a fire exists on any of the combed ware vessels recovered from Isthmia, a parameter again fully consistent with the use of the vessels as beehives.

While the lack of beeswax residues in more than half of the vessels might appear to raise some concern, this most likely relates to differences in the burial histories of individual sherds rather than differences in function; the loss of lipid is known to be very rapid from potsherds deposited in exposed contexts where weathering effects are most extreme. The length of time and intensity of vessel use prior to discard will also affect the amount of beeswax preserved, although it would be impossible to deconvolute this from diagenetic effects. Likewise, while the differences in the composition of the ancient beeswax residues compared with the reference beeswax might also appear to be inconsistent with its origin, however, this again is to be entirely anticipated as a result of differing burial contexts producing differing degrees of diagenetic alteration in the absorbed lipid during the *ca*. 2000 year period of burial, and does not in any way affect the conclusions of this investigation.

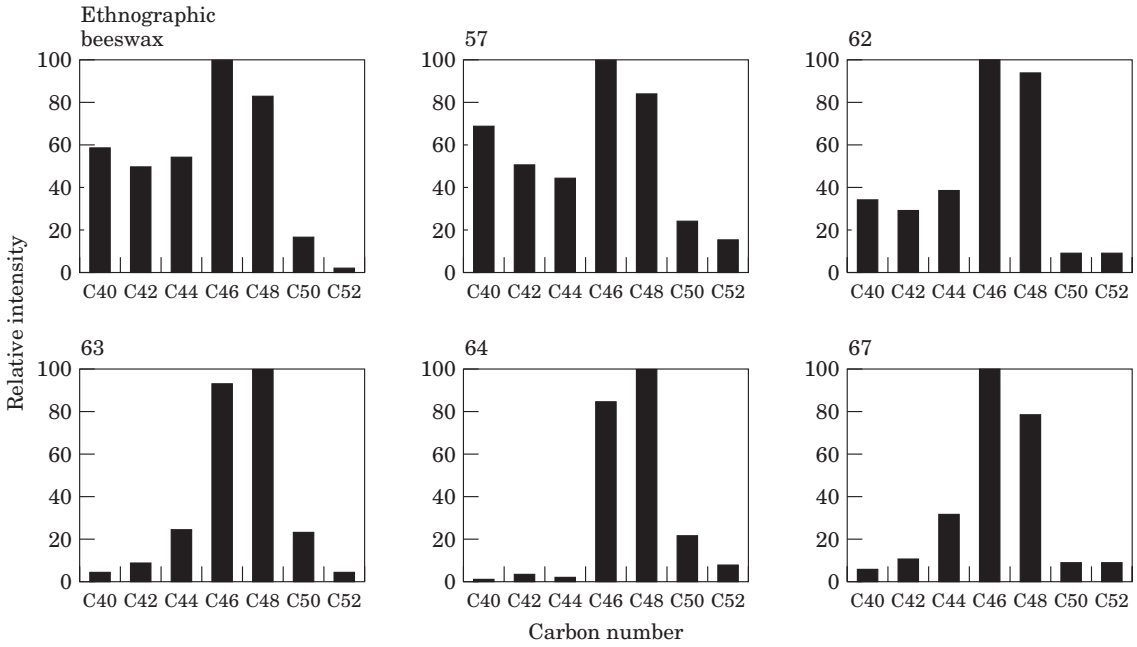

Figure 6. Distributions of wax esters isolated from solvent extracts of the reference ethnographic beeswax and combed ware putative beehive sherds. The numbers associated with each histogram correspond to the sample numbers given in Table 1. See "Materials and Methods" for further details.

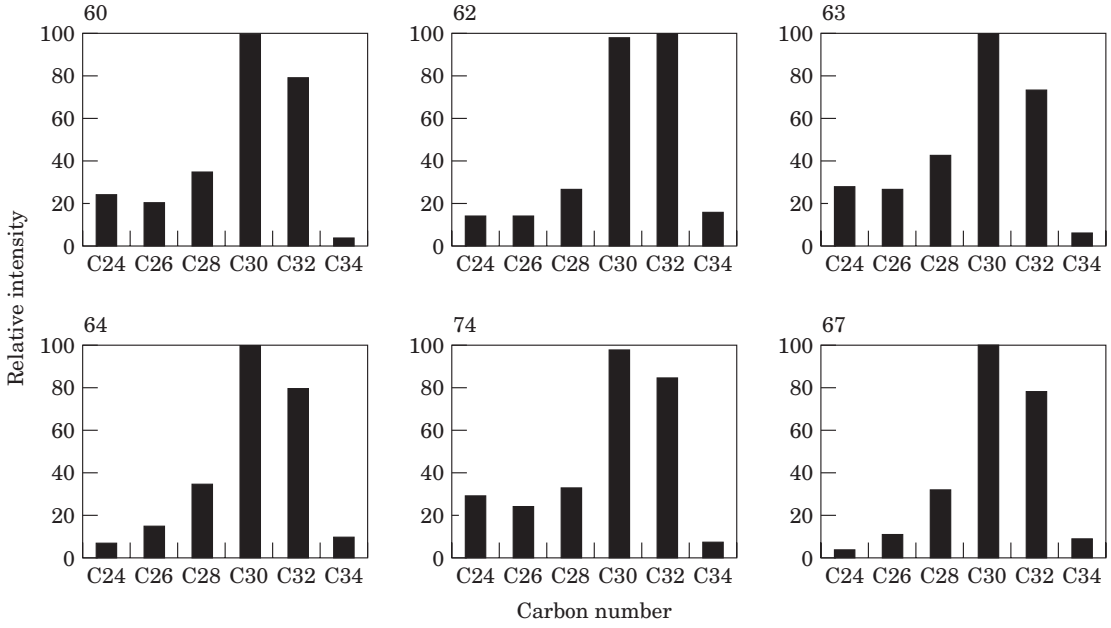

Figure 7. Distributions of *n*-alkanols isolated from solvent extracts of the reference ethnographic beeswax and combed ware putative beehive sherds. The numbers associated with each histogram correspond to the sample numbers given in Table 1. See "Materials and Methods" for further details.

Conclusions

The molecular data, i.e. structures, distributions and stable carbon isotope values, presented above for the total lipid extracts of combed ware pottery from Isthmia confirm that a significant proportion of the ombed ware vessels had been intimately associated with beeswax during their use. This investigation provides another example of way in which compound specific $\delta^{13}C$ values provide an essential adjunct to the structures and distributions of indicator compounds, particularly where familiar "fingerprint" distributions

Table 2. $\delta^{13}C$ values for individual alkanes from sherd extracts and reference beeswax

Combed ware vessel	$\delta^{13}C$ values (‰)					
	n-C_{23}	*n*-C_{25}	*n*-C_{27}	*n*-C_{29}	*n*-C_{31}	*n*-C_{33}
43	−26·2	−26·2	−25·5	−25·9	−26·2	−26·0
47	−25·2	−25·3	−24·6	−25·5	−25·5	−26·2
57	−26·8	−25·9	−25·1	−25·9	−26·6	−26·3
58	−25·5	−26·0	−25·1	−25·9	−25·6	−26·8
62	−25·6	−24·8	−25·8	−25·7	−25·3	−26·4
63	−25·4	−24·8	−24·5	−24·9	−24·9	−25·5
64	nd	nd	−25·4	−25·6	−25·7	−25·8
65	−26·8	−25·4	−24·5	−25·1	nd	nd
68	−26·9	−25·3	−25·0	−25·4	−25·5	−25·7
78	−26·6	−26·5	−26·2	−26·8	−26·8	−26·8
Reference	−25·1	−25·8	−26·1	−26·4	−26·6	−26·7

nd=not determined.

Table 3. $\delta^{13}C$ values for long-chain alcohols from sherd extracts and reference beeswax

Combed ware vessel	$\delta^{13}C$ values* (‰)				
	n-C_{24}	*n*-C_{26}	*n*-C_{28}	*n*-C_{30}	*n*-C_{32}
62	−25·6	−24·5	−25·2	−25·5	−25·7
63	−25·4	−25·1	−25·2	−25·0	−25·1
64	nd	nd	−25·3	−25·6	−25·5
Reference beeswax	−26·3	−26·0	−26·2	−26·2	−25·2

*$\delta^{13}C$ values corrected for trimethylsilylating reagent (Jones *et al.*, 1991).
nd=not determined.

have been altered through diagenesis. The specific chemical characteristics of the total lipid extracts of the ancient vessels are assigned as beeswax include:

(i) The presence of *n*-alkanes in several of vessels with distributions maximizing at C_{27} is highly diagnostic of an association of the ancient vessels with beeswax (see Figure 5).
(ii) The presence of long-chain palmitic acid wax esters in the C_{40}–C_{52} carbon number range with distributions resembling that of the reference beeswax (see Figure 6).
(iii) The presence of long-chain hydroxypalmitic acid wax esters is highly diagnostic of beeswax (cf. minor components eluting together with the more abundant wax esters in the chromatograms shown in Figures 3 & 4).
(iv) The presence of free *n*-alkanols in the C_{24}–C_{34} chain length range with distributions closely resembling that seen for the wax esters of beeswax. Thus, the free *n*-alkanols are assumed to derive through hydrolysis of the palmitic and ω-hydroxypalmitic acid wax esters during vessel use and burial (Figure 7).
(v) The characteristic $\delta^{13}C$ values recorded for the homologous *n*-alkane components of the ancient vessels lie in the range −24·5 to −26·9‰ compare very closely with those of the reference beeswax (−25·1 to −26·7‰) thus removing any possibility that these residues might derive from plant waxes rather than beeswax (Table 2).
(vi) As for (v) above the range of $\delta^{13}C$ values recorded for the *n*-alkanols derived from the wax esters (−24·5 to −25·7‰) of the ancient vessels compare closely with those of the reference beeswax (−25·2 to −26·3‰; Table 3) and so remove any possibility that these residues derive from plant waxes rather than beeswax.
(vii) None of the pithoi (control group) contained any of the chemical components present in the combed ware vessel. Two of the pithoi contained residues consistent in composition with degraded olive oil.

Hence, our chemical data confirm unambiguously the presence of beeswax in the combed ware vessels, and the support the compelling arguments presented by Jones, Graham & Sackett (1973) for the use of combed ware vessels a beehives. Most significantly, our data appear to refute the assertion of Crane (pers. comm.) that the dimensions of the upright (type 2) combed ware vessel are too small for them to have served as beehives. It appears inconceivable that the combed ware vessels served any function other than as beehives.

Acknowledgements

We are grateful for comments of J. Ellis Jones who read a draft of this article. Mr J. F. Carter and Mr A. R. Gledhill are thanked for their assistance with organic and stable isotope mass spectrometric analyses. The NERC are thanked for mass spectrometry facilities (Grants: GR3/2951, GR3/3758, GR9/02572, FG6/36101) and a research grant (GR3/10153).

References

Anderson-Stojanovic, V. R. (1996). The University of Chicago Excavations in the Rachi Settlement at Isthmia, 1989. *Hesperia* **65,** 57–98.

Broneer, O. (1958). Excavation at Isthmia, Third campaign, 1955, 1956. *Hesperia* **27,** 1–37.

Brüschweiler, V. H., Felber, H. & Schwager, F. (1989). Bienenwachs–zusammensetzung und beurteilung der reinheit durch gaschromatogrhische analyse. *Fat Science and Technology* **91,** 73–79.

Charters, S., Evershed, R. P., Goad, L. J., Heron, C. & Blinkhorn, P. W. (1993). Quantification and distribution of lipid in archaeological ceramics: implications for sampling potsherds for organic residue analyses. *Archaeometry* **35,** 211–223.

Charters, S., Evershed, R. P., Blinkhorn, P. W. & Denham, V. (1995). Evidence for the mixing of fat and wax in archaeological ceramics. *Archaeometry* **37**(1), 13–28.

Crane, E. (1983). *The Archaeology of Beekeeping*. London: Duckworth.

Evershed, R. P., Heron, C. & Goad, L. J. (1990). Analysis of organic residues of archaeological origin by high temperature gas chromatography/mass spectrometry. *Analyst* **115,** 1339–1342.

Evershed, R. P., Arnot, K. I., Collister, J., Eglinton, G. & Charters, S. (1994). Application of isotope ratio monitoring gas chromatography/mass spectrometry to the analysis of organic residues of archaeological interest. *Analyst* **119,** 909–914.

Evershed, R. P., Vaughan, S. J., Dudd, S. N. & Soles, J. S. (1997). Fuel for thought? Beeswax in lamps and conical cups from late Minoan Crete. *Antiquity* **71,** 979–985.

Evershed, R. P., Dudd, S. N., Charters, S., Mottram, H., Stott, A. W., Raven, A., van Bergen, P. F. & Bland, H. A. (1999). Lipids as carriers of anthropogenic signals from Prehistory. *Philosophical Transactions of the Royal Society* **354,** 19–31.

Gebhard, E. R. & Hemans, F. P. (1998). University of Chicago Excavations at Isthmia, 1989: III. *Hesperia* **67,** 405–456.

Heron, C., Nemcek, N., Bonfield, K. M., Dixon, D. & Ottaway, B. (1994). The chemistry of Neolithic beeswax. *Naturwissenschaften* **81,** 266–268.

Ifantidis, M. D. (1983). The movable nest hive: a possible forerunner to the moveable comb hive. *Bee World* **64,** 79–87.

Jones, J. E., Graham, A. J. & Sackett, L. H. (1973). Excursus on the combed ware: evidence for beekeeping. *The Annual of the British School at Athens* **68,** 397–414.

Jones, D. M., Carter, J. F., Eglinton, G., Jumeau, E. J. & Fenwick, C. S. (1991). Determination of $\delta^{13}C$ values sedimentary straight chain and cyclic alcohols by gas chromatography/isotope ratio mass spectrometry. *Biological Mass Spectrometry* **20,** 641–646.

Kardara, C. (1961). Dyeing and weaving works at Isthmia. *American School of Archaeology* **65,** 261–266.

Kolattukudy, P. E. (Ed.) (1976). *Chemistry and Biochemistry of Natural Waxes*. Amsterdam: Elsevier.

Lockheart, M. J., van Bergen, P. F. & Evershed, R. P. (1997). Variation in the stable carbon isotope compositions of individual lipids from the leaves of modern angiosperms: implications for the study of higher land plant-derived sedimentary organic matter. *Organic Geochemistry* **26,** 137–153.

Ludorf, G. (1998/99). ''Leitformen der attischen Gebrauchskeramik: Der Bienenkorb,'' Boreas (Munstersche Beitrage zur Archaeologie) 21/22, pp. 41–130.

Matthews, D. E. & Hayes, J. M. (1978). Isotope ratio monitoring gas chromatography-mass spectrometry. *Analytical Chemistry* **50,** 1465–1473.

Mills, J. S. & White, R. (1994). *Organic Chemistry of Museum Objects*. London: Butterworths.

Regert, M., Colinart, S., Degrand, L. & Decawallas, O. (2001). Chemical alteration and use of beeswax through time: accelerated ageing tests and analysis of archaeologcial samples from various environmental contexts. *Archaeometry* **43,** 549–569.

Rieley, G., Collier, R. J., Jones, D. M. & Eglinton, G. (1991). The biogeochemistry of Ellesmere Lake, UK-I: source correlation of leaf wax inputs to the sedimentary lipid record. *Organic Geochemistry* **17,** 901–912.

Sparkes, B. A. & Talcott, L. (1970). *Back and Plain pottery of the 6th, 5th and 4th Centuries (The Athenian Agora XII)*. Princeton.

Thompson, H., Thompson, D. B. & Rotroff, S. I. (1987). *Hellenistic pottery and terracottas*. Princeton.

Tulloch, A. P. (1970). The composition of beeswax and other waxes secreted by insects. *Lipids* **5,** 247.

Tulloch, A. P. (1971). Beeswax: structure of the esters and their component hydroxy acids and diols. *Chemistry and Physics of Lipids* **6,** 235.

Tulloch, A. P. & Hoffman, L. L. (1972). Analysis of whole beeswax by gas liquid chromatography. *Journal of the American Oil Chemists Society* **49,** 696.