# Widespread Exploitation of the Honeybee by Early Neolithic Farmers

Mélanie Roffet-Salque[1], Martine Regert[2], Richard P. Evershed[1], Alan K. Outram[3], Lucy J. E. Cramp[1,4], Orestes Decavallas[5], Julie Dunne[1], Pascale Gerbault[6], Simona Mileto[1,7], Sigrid Mirabaud[8], Mirva Pääkkönen[9], Jessica Smyth[1,4], Lucija Šoberl[1,10], Helen L. Whelton[1], Alfonso Alday-Ruiz[11], Henrik Asplund[9], Marta Bartkowiak[12], Eva Bayer-Niemeier[13], Lotfi Belhouchet[14], Federico Bernardini[15,16], Mihael Budja[17], Gabriel Cooney[18], Miriam Cubas[19], Ed M. Danaher[20], Mariana Diniz[21], László Domboróczki[22], Cristina Fabbri[23], Jesus E. González-Urquijo[19], Jean Guilaine[24], Slimane Hachi[25], Barrie N. Hartwell[26], Daniela Hofmann[27], Isabel Hohle[28], Juan J. Ibáñez[29], Necmi Karul[30], Farid Kherbouche[25], Jacinta Kiely[31], Kostas Kotsakis[32], Friedriech Lueth[33], James P. Mallory[26], Claire Manen[24], Arkadiusz Marciniak[12], Brigitte Maurice-Chabard[34], Martin A. Mc Gonigle[35], Simone Mulazzani[36-37], Mehmet Özdoğan[38], Olga S. Perić[39], Slaviša R. Perić[39], Jörg Petrasch[40], Anne-Marie Pétrequin[41], Pierre Pétrequin[41], Ulrike Poensgen[42], C. Joshua Pollard[43], François Poplin[44], Giovanna Radi[23], Peter Stadler[45], Harald Stäuble[46], Nenad Tasić[47], Dushka Urem-Kotsou[48], Jasna B. Vuković[47], Fintan Walsh[49], Alasdair Whittle[50], Sabine Wolfram[51], Lydia Zapata-Peña[11‡], Jamel Zoughlami[52].

[1]Organic Geochemistry Unit, School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, UK. [2]CEPAM – Cultures et Environnements. Préhistoire, Antiquité, Moyen Âge. UMR 7264 Université Nice Sophia Antipolis – CNRS, 06300 Nice, France [3]Department of Archaeology, University of Exeter, Laver Building, North Park Road, Exeter, Devon, EX4 4QE, UK. [4]Department of Archaeology and Anthropology, University of Bristol, 43 Woodland Road, Bristol BS8 1UU, UK. [5]Université Michel de Montaigne Bordeaux 3, Bordeaux, France. [6]Research Department of Genetics, Evolution and Environment, University College London, London WC1E 6BT, UK. [7]Institut für Prähistorische Archäologie, Freie Universität Berlin, Altensteinstr. 15, Berlin 14195, Germany. [8]Département des restaurateurs, Institut National du Patrimoine, 150 avenue du Président Wilson, 93210 Saint Denis - La Plaine, France. [9]Department of Archaeology, University of Turku, 20014 Turun Yliopisto, Finland. [10]Present address: Laboratório HERCULES, Universidade de Évora, Palácio do Vimioso, Largo Marquês de Marialva 8, 7000-809 Évora, Portugal. [11]Department of Geography, Prehistory and Archaeology. University of Basque Country (EHU-UPV), Francisco Tomás y Valiente s/n, 01006 Vitoria-Gasteiz, Spain. [12]Institute of Prehistory, Adam Mickiewicz University, św. Marcin 78, 61-809 Poznań, Poland. [13]Museum Quintana – Archäologie in Künzing, Partnermuseum der Archäologischen Staatssammlung München, Osterhofener Str. 2, 94550 Künzing, Germany. [14]Musée archéologique de Sousse, Rue Marshall Tito, 4000 Sousse, Tunisia. [15]Centro Fermi, Museo Storico della Fisica e Centro di Studi e Ricerche Enrico Fermi, 00184 Rome, Italy. [16]Multidisciplinary Laboratory, The Abdus Salam International Centre for Theoretical Physics, 34151Trieste, Italy. [17]University of Ljubljana, Faculty of Arts, Department of Archaeology, Aškerčeva 2, P.O. 580, 1000 Ljubljana, Slovenia. [18]UCD School of Archaeology, University College Dublin, Dublin 4, Ireland. [19]International Institute for Prehistoric Research of Cantabria, University of Cantabria, Avd de los Castros s/n, 39005 Santander, Spain. [20]Townhouse 1, Hector Street Mills, Kilrush, Co. Clare, Ireland. [21]Departamento de História, Faculdade de Letras de Lisboa, Universidade de Lisboa, 1600-214 Lisboa, Portugal. [22]István Dobó Castle Museum, Vár út 1, 3300 Eger, Hungary. [23]Dipartimento Civiltà e Forme del Sapere, Università di Pisa, Via Galvani 1, 56126 Pisa, Italy. [24]CNRS - UMR 5608 - TRACES, Maison de la recherche, Université Toulouse Jean Jaurès, 5 Allée Antonio Machado, 31058 Toulouse cedex 9, France. [25]CNRPAH, Centre

National de Recherche Préhistorique, Anthropologique et Historique, Alger, Algeria. [26]School of Geography, Archaeology and Palaeoecology, Queen's University Belfast BT7 1NN, Northern Ireland, UK. [27]Universität Hamburg, Archäologisches Institut, Edmund-Siemers-Allee 1, Flügel West, 20146 Hamburg, Germany. [28]a.r.t.e.s. Graduate School for the Humanities Cologne, Graduiertenschule der Philosophischen Fakultät, Aachener Str. 217, 50931 Köln, Germany. [29]IMF-CSIC, Egipciacas 15, 08001, Barcelona, Spain. [30]Istanbul University, Faculty of Letters, Department of Prehistory, 34434 Laleli Istanbul, Turkey. [31]Eachtra Archaeological Projects, Lickybeg, Clashmore, Co. Waterford, Ireland. [32]School of History and Archaeology, Faculty of Philosophy, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece. [33]German Archaeological Institute, Podbielskiallee 69-71, 14 195 Berlin, Germany. [34]Musée Rolin, 3 rue des Bancs, 71400 Autun, France. [35]9 Chapel Close, Stranorlar, Co. Donegal, Ireland. [36]Aix-Marseille Université, CNRS, Ministère de la Culture et de la Communication, UMR 7269 LAMPEA, LabexMed, Aix-Marseille, France. [37]Dipartimento di Biologia Ambientale, Università degli Studi di Roma La Sapienza Rome 00185, Italy. [38]Mimarlık Fakültesi, Taşkışla Binası, Istanbul Teknik Universitesi, 34437 Istanbul, Turkey. [39]Institute of Archaeology Belgrade, Kneza Mihaila 35/4 11000 Belgrade, Serbia. [40]Eberhard-Karls-Universität Tübingen, Institut für Ur- und Frühgeschichte und Archäologie des Mittelalters - Abt. Jüngere Urgeschichte und Frühgeschichte - Schloß Hohentübingen, 72070 Tübingen, Germany. [41]Maison des Sciences de l'Homme et de l'Environnement C.N. Ledoux, CNRS & Université de Franche-Comté, 32 rue Mégevand, 25030 Besançon Cedex, France. [42]Kämpfenstr. 20, 78315 Radolfzell, Germany. [43]Archaeology, Faculty of Humanities, University of Southampton, Avenue Campus, Highfield, Southampton SO17 1BF, UK. [44]Muséum National d'Histoire Naturelle, 55 rue de Buffon, 75005 Paris, France. [45]University of Vienna, Vienna, Austria. [46]Landesamt für Archaeologie, Zur Wetterwarte 7, 01109 Dresden, Germany. [47]Department of Archaeology, Faculty of Philosophy, Belgrade University, 18-20 Čika Ljubina street, 11000 Belgrade, Serbia. [48]Departement of History and Ethnology, Democritus University of Thrace, Komotini, Greece. [49]Irish Archaeological Consultancy, Unit G1, Network Enterprise Park, Kilcoole, Co. Wicklow, Ireland. [50]Department of Archaeology and Conservation, Cardiff University, John Percival Building, Colum Drive, Cardiff CF10 3EU, UK. [51]State Museum of Archaeology Chemnitz, Stefan-Heym-Platz 1, 09111 Chemnitz, Germany. [52]Institut National du Patrimoine de Tunis - Musée archéologique de Carthage, Carthage, Tunisia.

‡ Deceased

**The pressures on Honeybee (*Apis mellifera*) populations, resulting from threats by modern pesticides, parasites, predators and diseases, have raised awareness of the economic importance and critical role this insect plays in agricultural societies across the globe. However, human's association with *A. mellifera* predates post-industrial revolution agriculture, as evidenced by the widespread presence of ancient Egyptian bee iconography dating to the Old Kingdom (*ca.* 2400 BC)[1]. There are also hints of Stone Age people harvesting bee products; for example, honey hunting is interpreted from rock art[2] in a prehistoric Holocene context and a beeswax find in a pre-agriculturalist site[3]. Significantly though, as to when and where the regular association of the *A. mellifera* with agriculturalists emerged is unknown[4]. One of the major products of *A. mellifera* is beeswax, which is composed of a complex suite of lipids including *n*-alkanes, *n*-alkanoic acids and fatty acyl wax esters. The composition is highly constant being determined genetically through the insect's biochemistry. Thus, the chemical 'fingerprint' of beeswax provides a secure basis for detecting this commodity in organic residues preserved at archaeological sites, which we now use to trace human's exploitation of *A. mellifera* temporally and spatially. Herein, we present secure identifications of beeswax in lipid residues preserved in pottery vessels of Neolithic Old World farmers. The geographical range of bee product exploitation is traced in Neolithic Europe, the Near East and North Africa providing the palaeoecological range of honeybees during Prehistory. Temporally, we demonstrate that bee products were exploited continuously, and probably extensively in some regions, at least from the 7$^{th}$ millennium cal BC, likely fulfilling a variety of technological and cultural functions. Thus, the close association of *A. mellifera* with Neolithic farming communities dates to the early onset of agriculture and may provide evidence for the beginnings of a domestication process.**

The honeybee holds a unique place in human culture. Notwithstanding its present day economic importance, it has been revered over the millennia for the sheer beauty and complexity of the social organisation within its colonies. For these reasons the honeybee is the most researched of the social insects, with its origin being regularly considered[5]. The last

114 Ice Age would have had a major impact on the honeybee with the ice sheets restricting
115 European populations to the northern Mediterranean hinterlands[6]. With the glacial retreat the
116 population would have subsequently expanded northwards. However, due to the lack of a
117 Holocene fossil record[7] the honeybee is ecologically invisible for most of the past 10,000
118 years.
119
120 Intriguingly, this is the period during which Neolithic agriculture emerged and spread out of
121 southeastern Anatolia and the Levant, with some human population movement into ecological
122 zones also conducive to the honeybee. Indeed, progressive woodland clearances by pioneer
123 prehistoric farmers may have opened up forests, favouring light-demanding shrubs, herbs and
124 fruit trees (e.g. *Rosaceae*)[8]. As to whether this would have exerted negative or positive
125 impacts on honeybee populations is unknown[8,9]. Given the latter, an opportunity exists to
126 investigate the presence and early exploitation of the honeybee by prehistoric farming
127 communities through the cultural materials recovered from Neolithic sites, namely their
128 recently invented pottery vessels, and in doing so to assess the palaeoecological range of the
129 honeybee in the Holocene.
130
131 Whilst the most obvious reason for exploiting the honeybee would be for honey, a rare source
132 of sweetener for prehistoric people, beeswax would likely have been an equally important
133 material for various technological, ritual, cosmetic and medicinal applications[10]. Indeed,
134 beeswax has been regularly detected in later archaeological and historic periods in lipid
135 extracts from the fabric of unglazed pottery vessels[11] where it is assumed to be a residue of
136 honey use in cooking, or from the use of vessels for processing wax combs[12-14], with beeswax
137 being absorbed through repeated contacts. Beeswax has also been detected as a fuel in lamps

and in larger vessels used as proto-beehives, *cf.* Classical Greece[15,16] and applied as a post-firing treatment to waterproof vessels[17].

The detection of beeswax in archaeological and historic contexts rests on its complex chemistry providing a unique and relatively recalcitrant chemical signature. Fresh beeswax comprises a complex mixture of aliphatic compounds consisting of series of homologues differing in chain-length by 2 methylene groups[18]. Medium-chain *n*-alkanes range from $C_{23}$ to $C_{31}$ (with $C_{27}$ dominating in *A. mellifera*), and *n*-alkanoic acids from $C_{20}$ to $C_{36}$. Monoesters comprise predominantly alkyl palmitates ($C_{38}$ to $C_{52}$), with characteristic hydroxy monoesters comprising long-chain alcohols ($C_{24}$ to $C_{38}$) esterified mainly to hydroxypalmitic acid, ranging between $C_{40}$ and $C_{54}$[18]. The hydrophobic nature of beeswax makes it relatively resistant to degradation. Hence, if protected from extensive microbial attack and/or exposure to high temperatures during anthropogenic manipulation, the aforementioned chemical characteristics can be used in assessing its presence[10,19] (Fig. 1-2).

Adopting this lipid biomarker approach we now explore the association of the honeybee with the spread of early Old World farmers based on lipid residue analyses of more than 6,400 pottery vessels (SI1-2). Combining our new findings with published occurrences of beeswax in prehistoric pottery allows the association between honeybees and early farmers to be mapped spatially and temporally through prehistory (Fig. 3-4).

The oldest evidence for beeswax comes from Neolithic sites in Anatolia dating from the 7$^{th}$ millennium cal BC, as these sites are the locations of the oldest pottery vessels in Europe and Eurasia. Most of the assemblages investigated comprised globular or bowl shape “cooking” vessels, an interpretation supported by the finding of ruminant and porcine animal fats in

significant numbers of vessels. No beeswax residues were detected during the intensive investigations of >380 vessels from the Levant, although only 34 residues were detected[20]. Moving into eastern Anatolia, the site of Çayönü Tepesi revealed two beeswax residues from 83 vessels from the 7$^{th}$ millennium including an exceptionally well-preserved residue containing all the biomarkers of beeswax (Fig. 1b-f). The free *n*-alkanols, dominated by $C_{30}$ and $C_{32}$ homologues, do not occur in fresh beeswax but are a feature of aged wax, due to hydrolysis of the wax esters. The high abundance of $C_{18:0}$ fatty acid suggests mixing with mammalian animal fat, the latter being common in other sherds in the assemblage[20]. The second sherd from this site contained a lower concentration of beeswax but all the biomarkers were clearly evident. These two residues establish the easterly limit of the beeswax detected in this investigation and provide the oldest unequivocal evidence of honeybee exploitation by early Neolithic farmers.

In central Anatolia extensive investigations of organic residues in 650 vessels, mainly from the site of Çatalhöyük, revealed abundant animal fat residues. Only one residue showed tentative evidence for beeswax based on wax esters, dominated by $C_{46}$ and $C_{48}$ homologues; however, the *n*-alkanols do not exhibit the familiar distribution. *n*-Alkanes were detectable but the distribution is skewed towards the higher homologues compared to that expected in fresh beeswax, although such distributional changes are frequently seen in historical and archaeological beeswax, assumed to arise by sublimation during ageing or heat treatment[10]. The tentative identification of this very early beeswax residue at Çatalhöyük is supported by the discovery of a striking depiction of honeycomb-like pattern painted on a wall at the site[21].

Analyses of ca. 570 cooking vessels from northwestern Anatolia revealed 72 lipid residues of which 4 were identified as containing beeswax, from Aşağı Pinar and Toptepe, dating to

188 5500-5000 cal BC. While the overall purity of the beeswax (2 were mixed with ruminant fat)
189 and lipid concentrations (20 to 220 μg $g^{-1}$) were quite variable, the distributions were
190 unmistakable. One of the beeswax finds from Toptepe is well-preserved, albeit with ageing
191 evident from the hydrolytically released free *n*-alkanols and slight distortions of the various
192 homologous series, through loss of lower homologues.
193
194 The most abundant evidence for honeybee exploitation by early farmers was seen in the rest
195 of the Balkan Peninsula. The full range of beeswax biomarkers was identified in sherds from
196 bowls, pans and sieves from the Late Neolithic sites of Paliambela, Greece (4900-4500 cal
197 BC), Măgura, Romania (Fig 2a; 5500-5200 cal BC) and Drenovac Turska Česma, Serbia
198 (5300-4700/4600 cal BC). A large number of beeswax residues were found in Neolithic
199 potsherds (11 residues out of 81 sherds analysed) from Attica, the Peloponnese and the
200 Cyclades (Aegean Islands), dating between 5800 and 3000 cal BC, firmly establishing the
201 long-tradition of bee exploitation in this region. Overall, the incidence of beeswax residues is
202 highest in the Balkan Peninsula with of the 1915 Neolithic sherds analysed, 473 yielded lipid
203 residues, of which 5.5% contained beeswax.
204
205 In Central Europe, pure beeswax was recovered from potsherds from *Linearbandkeramik*
206 (LBK) sites occupied by the earliest farmers of Austria and Germany (Oldest LBK) including
207 the sites of Brunn am Gebirge (5500-5400 cal BC) and Niederhummel (5360-5220 cal BC),
208 pushing back the date for bee exploitation in this region by ca. 1500 years[13] (Fig. 2b).
209 Beeswax was also detected in late 6$^{th}$ millennium LBK sites of Ludwinowo 7 and Wolica
210 Nowa, Poland[17]. In France, the exploitation of bee products is evident during the second half
211 of the 5$^{th}$ millennium at *Chasséen* sites (Font-Juvénal, Chassey-le-Camp and Bercy[10], France)
212 and 4$^{th}$ millennium at the Lake Village sites of Clairvaux-les-Lacs (3900 to 3700 BC) and

213 Chalain 3[22] and 4 (3200 to 3100 BC and 3040 to 2990 BC). High incidence of beeswax (ca.
214 15% of the detectable residues) was identified in 5th millennium sherds from two Slovenian
215 sites (Ajdovska jama and Moverna vas)[23].
216
217 Around 130 sherds have so far been analysed from the Iberian Peninsula. However, no
218 beeswax residues have yet been detected, although the overall preservation of organic
219 residues was poor. Further investigations will likely reveal examples of beeswax in Neolithic
220 pottery from this region.
221
222 The northerly limit of bee exploitation in northern Europe appears to be Denmark with two
223 beeswax finds in late Mesolithic and Neolithic contexts[24]. Around 5º to the south in southern
224 Britain beeswax is evident in 7 vessels amongst the ca. 670 Neolithic vessels analysed. These
225 findings clearly counter any arguments for a late introduction of the honeybee into the British
226 Isles[8,25]. Interestingly, however, investigations of nearly 1,200 Mesolithic and Neolithic
227 vessels from Ireland, Scotland and Fennoscandia[26,27] have failed to reveal any conclusive
228 evidence of beeswax (SI3). Given that organic residue preservation in these regions is
229 excellent, the lack of beeswax would seem to establish the ecological limit of *A. mellifera* at
230 that time. Similar arguments likely account for the absence of beeswax residues from >350
231 prehistoric pottery vessels from the Eurasian Steppe[28].
232
233 Finally, we report the first evidence for bee exploitation by Neolithic pastoralists in North
234 Africa. The analysis of 71 sherds from the Algerian site of Gueldaman revealed a single well-
235 preserved beeswax residue (5th millennium). The preservation is again exceptional with *n*-
236 alkanes, *n*-fatty acids and fatty acyl wax ester distributions providing an unequivocal
237 identification of beeswax. The presence of free long-chain *n*-alkanols and lack of hydroxy

fatty acid wax esters are indicative of diagenesis and/or use-related alteration. However, the overall distribution indicates the wax residue derives from *A. mellifera* (Fig. 2c).

In conclusion, the ca. 50 new finds of beeswax residues discussed above provide unequivocal evidence for the widespread exploitation of the honeybee by the early agriculturalists and pastoralists of the Near East, Europe and North Africa dating back nearly 9,000 years (Fig. 3). In all these regions the new data has either provided the first evidence of honeybee exploitation in a region, i.e. in North Africa, or pushed the chronology of human-honeybee association to substantially earlier dates, i.e. in Anatolia and Central Europe (Fig. 3b). The lack of evidence for beeswax use at Neolithic sites north of the 57$^{th}$ parallel North may suggest an ecological limit to the natural occurrence of honeybees. Indeed, harsh high-latitude conditions, even with temperatures warmer than today[29], would affect the foraging capabilities of honeybees[30]. Critically, in the absence of a Holocene fossil record for *A. mellifera*[7] these findings provide the first ancient biomolecule-based palaeoecological map of the distribution of an economically and culturally important animal (Fig. 4).

**Acknowledgements**

We thank the UK Natural Environment Research Council for partial funding of the mass spectrometry facilities at Bristol (contract no. R8/H10/63; www.lsmsf.co.uk) and English Heritage, European Research Council, Leverhulme Trust, Ministère de la Culture et de la Communication, Ministère de l'Enseignement Supérieur et de la Recherche (ACI Jeunes Chercheurs), Natural Environment Research Council, Région PACA, Royal Society and Wellcome Trust for funding.

**Author Contributions**

M.R.-S., M.R., R.P.E. and A.K.O. conceived and planned the project about beeswax in Prehistory. M.R.-S., M.R. and R.P.E. wrote the paper. M.R.-S., M.R., L.J.E.C., O.D., J.D., S.Mil., S.Mir., M.P., J.S., L.S., D.U.-K., H.L.W. and M.Bart. undertook planning of regional lipid residue analyses projects, sampling, analytical work and data analysis. P.G. created Figure 4 and SI3. All other authors either directed excavations or provided expertise in relation to pottery collections and essential insights into the study region and sites.

**Author information**

Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to M.R.-S. (melanie.salque@bristol.ac.uk), M.R. (martine.regert@cepam.cnrs.fr) or R.P.E. (r.p.evershed@bristol.ac.uk).

**Figure 1 | HT-GC-MS chromatograms of total lipid extract of a sherd from Çayönü Tepesi (6500-6000 cal BC) containing beeswax. a,** Partial total ion current chromatogram and **b-f,** mass chromatograms displaying ion masses of characteristic fragments from the main compound classes comprising the extract (*m/z* 85, 73, 103, 257 and 117, respectively) with the molecular structure of the most abundant component for each compound class. Blue squares, *n*-alkanes (AL); green circles, *n*-alkanoic acids (fatty acids, FA); red triangles, *n*-alkanols (OH); black stars, fatty acyl monoesters (WE); grey stars, hydroxyl fatty acyl monoesters (HWE); IS, internal standard (*n*-tetratriacontane); number n and n:i, acyl carbon number with zero or i degrees of unsaturations. Compounds on a grey background are interpreted as originating from mammalian animal fats.

**Figure 2 | Partial gas chromatograms of total lipid extracts from Neolithic sherds from each geographical region. a,** Mağura (5500-5200 cal BC); **b,** Niederhummel (5360-5220 cal BC) and **c,** Gueldaman ($5^{th}$ millennium BC). a-b are interpreted as mixture of animal fats and beeswax; c-d as pure beeswax. MAG, monoacylglycerols; DAG, diacylglycerols, TAG, triacylglycerols. Other peak attributions as in Figure 1.

**Figure 3 | Geographical distribution of prehistoric sites in the date range 7500 and 2000 cal BC yielding beeswax residues. a,** Locations of archaeological sites and **b,** Chronology of beeswax use in the Near East, the Balkan Peninsula, mainland Europe, Scandinavia, the UK and northern Africa. Neolithic finds in black, pre-Neolithic (hunter-gatherer contexts) in light grey and Bronze Age in dark grey. * Dental filling re-examined after Bernardini, F. *et al.* [PLoS ONE **7**, e44904 (2012)].

**Figure 4 | Regional distribution of beeswax residues in potsherd lipid extracts.** Interpolated map of Old World beeswax occurrences (proportion of beeswax residues per number of residues in pottery sherds, in %) during the Neolithic (inc. the Mesolithic sites available). Colours and colour key show the proportions of beeswax residues estimated by surface interpolation, where collection locations are represented by dots ($n$ = 154).

## References


1 Crane, E. *The world history of beekeeping and honey hunting*. (Duckworth publishers, 1999).

2 Dams, M. & Dams, L. R. Spanish art rock depicting honey gathering during the Mesolithic. *Nature* **268**, 228-230 (1977).

3 d'Errico, F. *et al.* Early evidence of San material culture represented by organic artifacts from Border Cave, South Africa. *Proceedings of the National Academy of Sciences* **109**, 13214-13219, doi:10.1073/pnas.1204213109 (2012).

4 Larson, G. & Fuller, D. Q. The evolution of animal domestication. *Annual Review of Ecology, Evolution, and Systematics* **45** (2014).

5 Wallberg, A. *et al.* A worldwide survey of genome sequence variation provides insight into the evolutionary history of the honeybee *Apis mellifera*. *Nature Genetics* **46**, 1081-1088 (2014).

6 Ruttner, F. *Biogeography and Taxonomy of Honeybees*. (Springer, 1987).

7 Buckland, P. I. & Buckland, P. C. in *Versions: BugsCEP v7.63; Bugsdata v8.01; BugsMCR v2.02; BugStats v1.22* (2006).

8 Limbrey, S. in *Archaeological aspects of woodland ecology* (eds M. Bell & S. Limbrey) 279-286 (British Archaeological Reports, 1982).

9 Wilson, E. O. *The insect societies*. (Belknap Press of Harvard University Press, 1971).

10 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).

11 Mayyas, A. S., Al-Qudah, M. A., Douglas, K. A. & Al-Ajlouny, F. K. Beeswax preserved in archaeological ceramics: function and use. *Annals of Faculty of Arts. Ain Shams University* **2** (accepted).

12 Copley, M. S. *et al.* Dairying in antiquity. III. Evidence from absorbed lipid residues dating to the British Neolithic. *Journal of Archaeological Science* **32**, 523-546 (2005).

13 Heron, C., Nemcek, N., Bonfield, K. M., Dixon, D. & Ottaway, B. S. The chemistry of Neolithic beeswax. *Naturwissenschaften* **81**, 266-269 (1994).

14 Decavallas, O. in *Cooking up the past: food and culinary practices in the Neolithic and Bronze Age Aegean* (eds C. Mee & J. Renard) 148-157 (Oxford Books Limited, 2007).

15 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).

16 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. *Journal of Archaeological Science* **30**, 1-12 (2003).

17 Salque, M. *et al.* Earliest evidence for cheese making in the sixth millennium BC in northern Europe. *Nature* **493**, 522-525 (2013).

18 Aichholz, R. & Lorbeer, E. Investigation of combwax of honeybees with high-temperature gas chromatography and high-temperature gas chromatography-chemical ionization mass spectrometry: I. High-temperature gas chromatography. *Journal of Chromatography A* **855**, 601-615 (1999).

19 Garnier, N., Cren-Olivé, C., Rolando, C. & Regert, M. Characterization of archaeological beeswax by Electron Ionization and Electrospray Ionization Mass Spectrometry. *Analytical Chemistry* **74**, 4868-4877 (2002).

20 Evershed, R. P. *et al.* Earliest date for milk use in the Near East and southeastern Europe linked to cattle herding. *Nature* **455**, 528-531 (2008).

21 Mellaart, J. Excavations at Çatal Höyük, 1962: Second Preliminary Report. *Anatolian Studies* **13**, 43-103 (1963).
22 Regert, M., Dudd, S. N., Van Bergen, P. F., Pétrequin, P. & Evershed, R. P. Investigations of solvent extractable lipids and insoluble polymeric components: organic residues in Neolithic ceramic vessels from Chalain (Jura, France). *British Archaeological Reports S* **939**, 78-90 (2001).
23 Šoberl, L. *et al.* Neolithic and Eneolithic activities inferred from organic residue analysis of pottery from Mala Triglavca, Moverna vas and Ajdovska jama, Slovenia. *Documenta Praehistorica* **XLI**, 149-179 (2014).
24 Heron, C., Craig, O. E., Forster, M., Stern, B. & Andersen, S. H. in *Shell middens in Atlantic Europe* (eds N. Milner, O. E. Craig, & G. N. Bailey) Ch. 7, 78-85 (Oxbow Books, 2007).
25 Carreck, N. Are honey bees (*Apis mellifera L.*) native to the British Isles? *Journal of Apicultural Research* **47**, 318-322 (2008).
26 Cramp, L. J. E. *et al.* Immediate replacement of fishing with dairying by the earliest farmers of the northest Atlantic coast. *Proceedings of the Royal Society B* **281**, 20132372 (2014).
27 Cramp, L. J. E. *et al.* Neolithic dairy farming at the extreme of agriculture in Northern Europe. *Proceedings of the Royal Society B* **281**, 20140819 (2014).
28 Outram, A. K. *et al.* The earliest horse harnessing and milking. *Science* **323**, 1332-1335 (2009).
29 Bartlein, P. J. *et al.* Pollen-based continental climate reconstructions at 6 and 21 ka: a global synthesis. *Clim Dyn* **37**, 775-802 (2011).
30 Abou-Shaara, H. F. The foraging behaviour of honey bees, *Apis mellifera*: a review. *Veterinarni Medicina* **59**, 1-10 (2014).

388 **Methods**

389 **Lipid residue analyses**

390 All solvents used were HPLC grade (Rathburn) and the reagents were analytical grade
391 (typically >98% of purity).

392

393 A sub-sample (1 to 3 g) from archaeological potsherds was cleaned with a modelling drill to
394 remove any exogenous lipids (from the soil and handling) and crushed with a solvent-washed
395 mortar and pestle. An internal standard (*n*-tetratriacontane, typically 20 μg) was added to the
396 powdered sherd to enable the quantification of lipid extract. Ground samples of sherds were
397 extracted with $CHCl_3$/MeOH [2:1 (vol/vol), 2x10 mL] using ultrasonication. Both
398 supernatants were combined and the solvent was removed under a gentle stream of nitrogen at
399 40 °C. Aliquots of the total lipid extract (TLE) were treated with 40 μL of *N*,*O*-
400 bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (Sigma
401 Aldrich) for 1 h at 70 °C and the BSTFA in excess evaporated under a gentle stream of
402 nitrogen. The trimethysilylated TLE was diluted in hexane (typically 50 to 150 μL) and
403 submitted to analysis by high temperature-gas chromatography (HT-GC) and HT-GC-mass
404 spectrometry (HT-GC-MS) to identify the major compounds present.

405

406 All TLEs were initially screened in a Hewlett-Packard 5890 Series II gas chromatograph
407 equipped with a fused-silica capillary column (15 m × 0.32 mm) coated with dimethyl
408 polysiloxane stationary phase (DB-1HT; film thickness, 0.1 μm; Agilent Technologies).
409 Derivatized extracts (1.0 μL) were injected on-column using a cool on-column inlet in track
410 oven mode. The temperature was held isothermally for 2 min at 50 °C and then increased at a
411 rate of $10°C \cdot min^{-1}$ and held at 350 °C for 10 min. The flame ionization detector (FID) was set
412 at a temperature of 350 °C. Helium was used as a carrier gas and maintained at a constant

flow of 4.5848 mL·min$^{-1}$. Data acquisition and processing were carried out using the HP Chemstation software (Rev. B.03.02 [341], Agilent Technologies).

HT-GC-MS analyses of trimethylsilylated aliquots were performed using a Thermo Scientific Trace 1300 gas chromatograph coupled with an ISQ single quadrupole mass spectrometer. Diluted samples were introduced using a PTV injector in split mode (split flow of 30 mL·min$^{-1}$, split ratio of 6.0) onto a 0.53 mm fused silica pre-column connected to a 15 m × 0.32 mm i.d. fused-silica capillary column coated with dimethyl polysiloxane stationary phase (Rxi-1HT; film thickness, 0.1 μm; Restek). The initial injection port temperature was 50 °C with an evaporation phase of 0.05 min, followed by a transfer phase from 50 °C to 380 °C at 0.2 °C·min$^{-1}$. The oven temperature was held isothermally for 2 min at 50 °C, increased at a rate of 10 °C·min$^{-1}$ to 280 °C, then at a rate of 25 °C·min$^{-1}$ to 380 °C and finally held at 380 °C for 5 min. Helium was used as a carrier gas and maintained at a constant flow 5 mL·min$^{-1}$. The MS was operated in the electron ionization (EI) mode (70 eV) with a GC interface temperature of 380 °C and a source temperature of 340 °C. The emission current was 50 μA and the MS set to acquire in the range of *m/z* 50-950 Daltons at 2 scans per second. Data acquisition and processing were carried out using the Thermo XCalibur software (version 3.0.63). Peaks were identified on the basis of their mass spectra, GC retention times, by comparison with the NIST mass spectral library (version 2.0) and by comparison with modern beeswax (from the Loire department, France).

**Construction of Figure 4**

The total number of archaeological sites investigated is 166, but only 154 of these fell within the geographical area of interest (longitude -10º to 42º and from latitude 25º to 62º, see SI1). To estimate the distribution of beeswax residues in continuous space from irregularly spaced

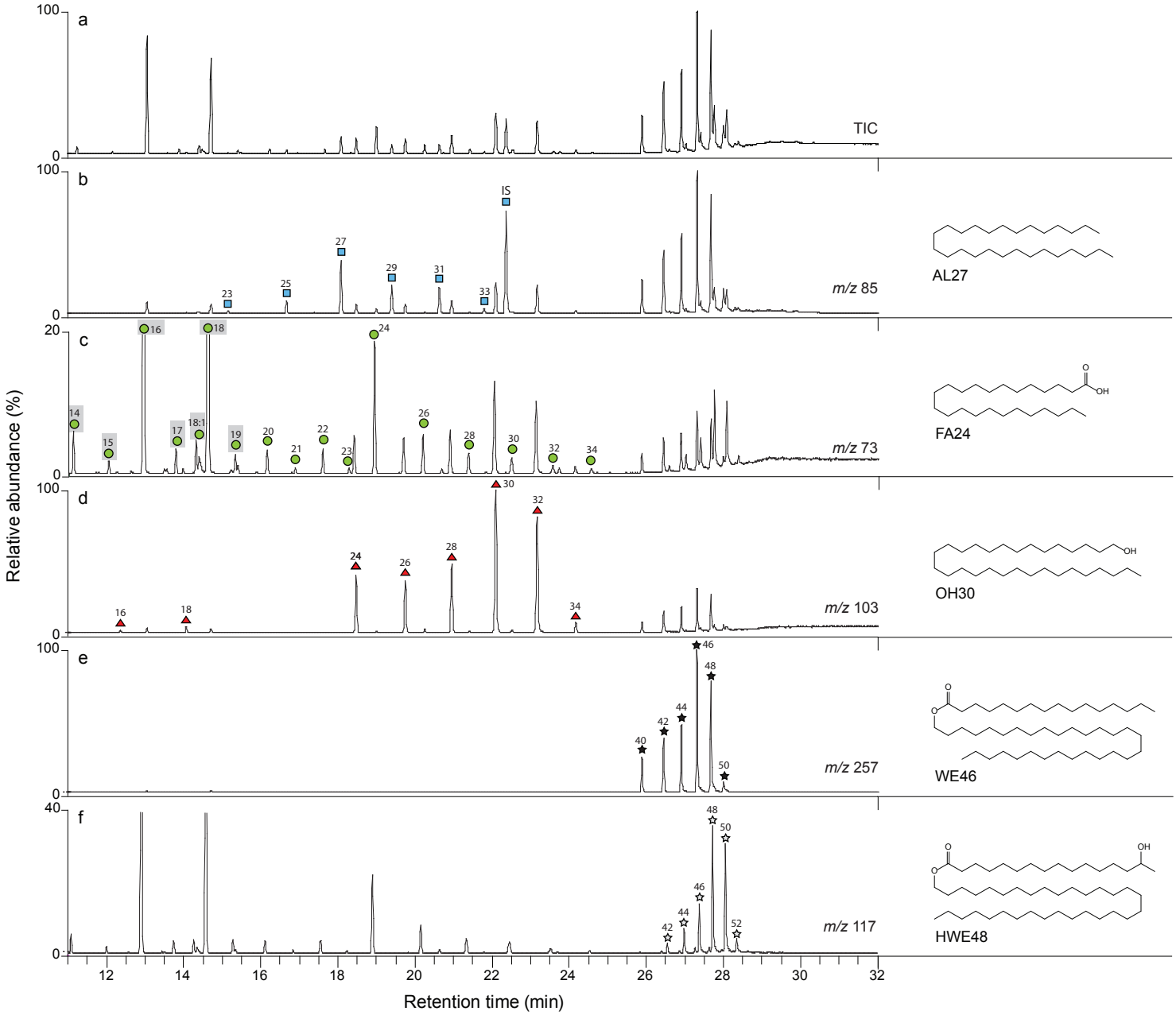
data, linear interpolation was performed in the triangles bounded by data points[31,32]. The output grid was made of 530 x 380 points evenly spaced over the range of latitude and longitude. No extrapolation was being used. Kriging was used to narrow the interpolation values to locations around data points (and not show interpolation values where there is no data). Kriging allows to obtain weights of the prediction locations based on the distance between data points, with lower variance where data points are and higher variance where there is no data. Interpolation, kriging and plotting were all performed in R version 2.15.1[33]. Interpolation was performed using the function 'interp' from the package 'akima' (CRAN repository, http://cran.r-project.org/web/packages/akima/akima.pdf). Kriging was performed using the function 'krige.conv' from the package 'geoR' (CRAN repository, http://cran.r-project.org/web/packages/geoR/geoR.pdf, further information on the package 'geoR' can be found at http://www.leg.ufpr.br/geoR). R code available upon request to p.gerbault@ucl.ac.uk.

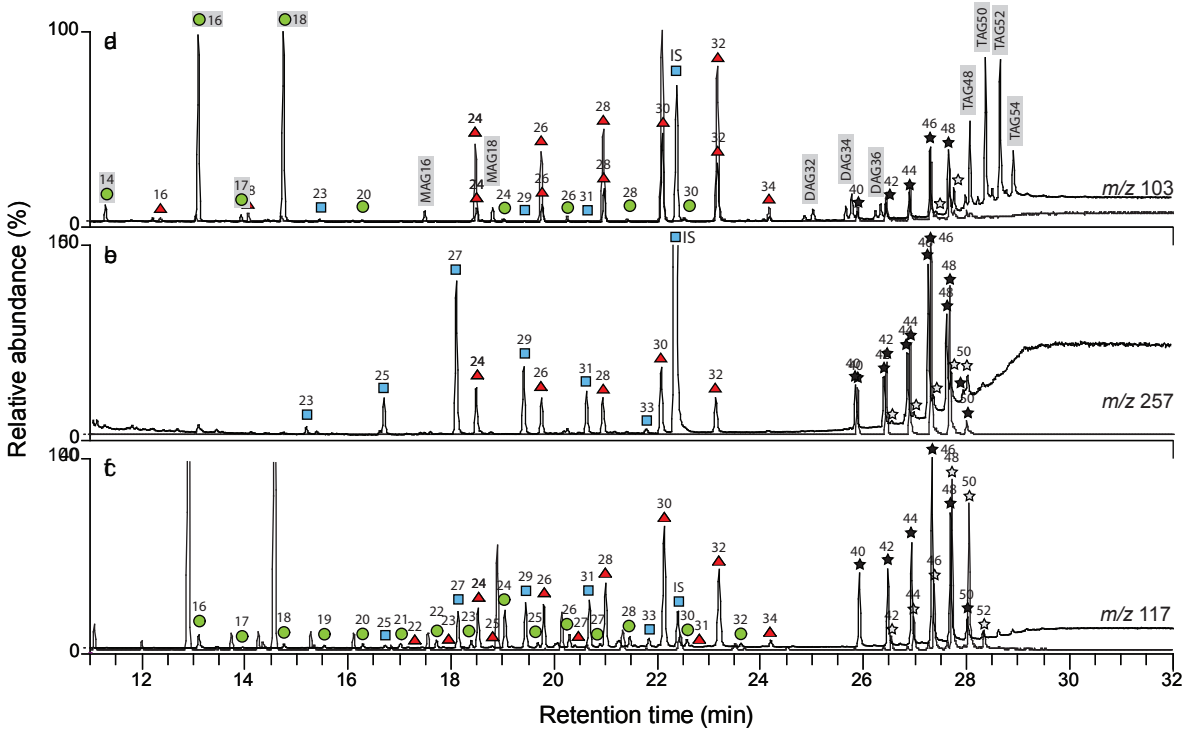
**References for Methods**

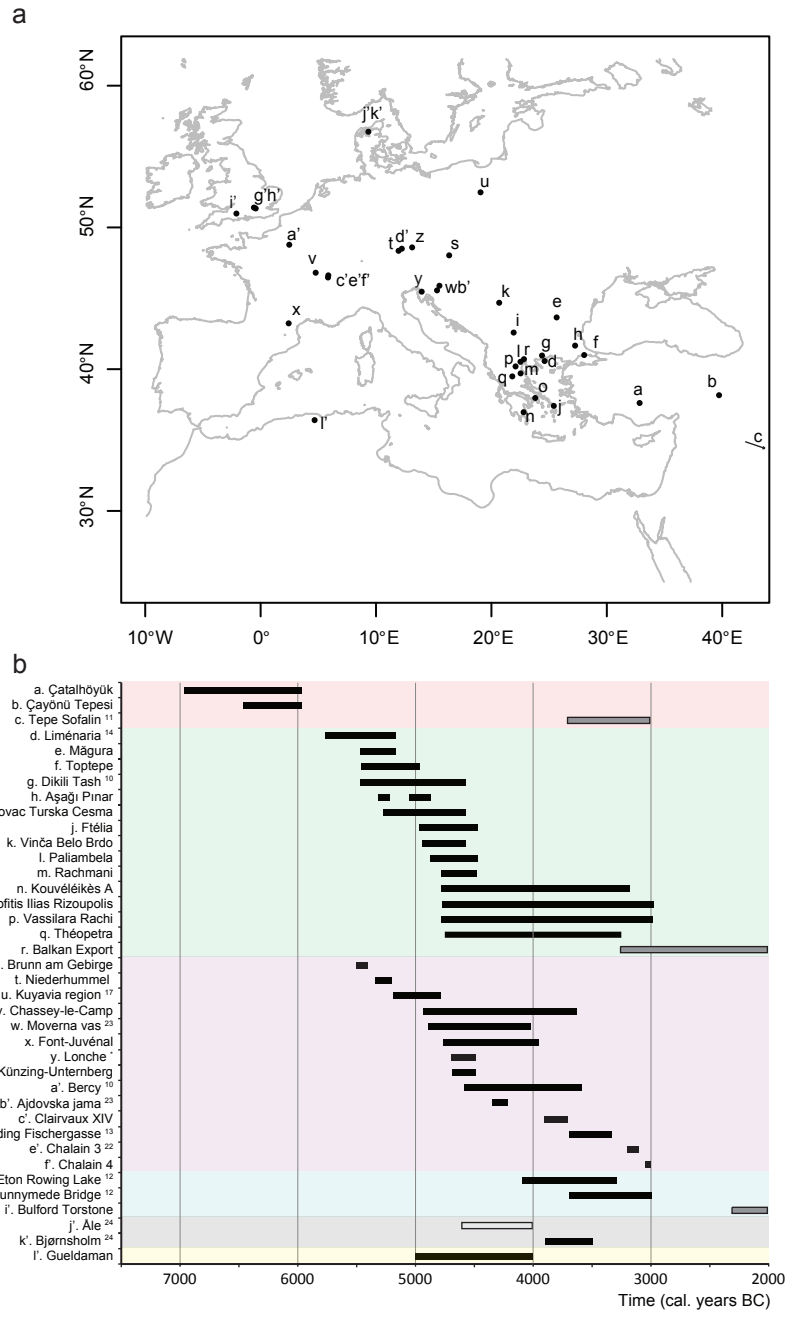

31 Akima, H. A method of bivariate interpolation and smooth surface fitting for irregularly distributed data points. *ACM transactions on mathematical software* **4**, 148-159 (1978).

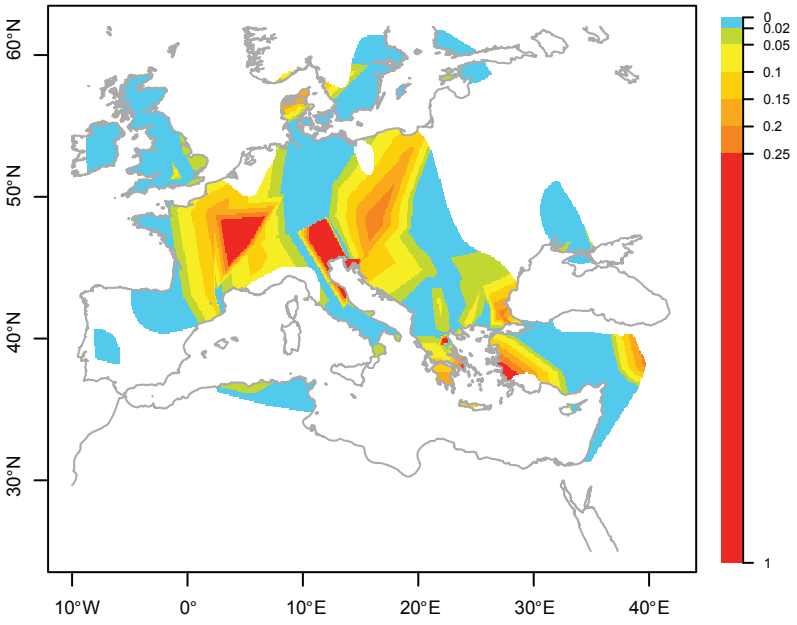
32 Akima, H. Algorithm 761: scattered-data surface fitting that has the accuracy of a cubic polynomial. *ACM transactions on mathematical software* **22**, 362-371 (1996).

33 The R Core Team. *R: a language and environment for statistical computing*. (R foundation for statistical computing, 2012).


a
TIC
b
IS
23
25
27
29
31
33
m/z 85
AL27
c
14
15
16
17
18:1
18
19
20
21
22
23
24
26
28
30
32
34
m/z 73
FA24
d
16
18
24
26
28
30
32
34
m/z 103
OH30
e
40
42
44
46
48
50
m/z 257
WE46
f
42
44
46
48
50
52
m/z 117
HWE48
Relative abundance (%)
Retention time (min)


a
b
c
m/z 103
m/z 257
m/z 117
IS
MAG16
MAG18
DAG32
DAG34
DAG36
TAG48
TAG50
TAG52
TAG54
Relative abundance (%)
Retention time (min)


a
60°N
50°N
40°N
30°N
10°W
0°
10°E
20°E
30°E
40°E
j'k'
u
i'
g'h'
a'
t
d'
z
s
v
c'e'f'
y
wb'
k
e
x
i
h
f
g
l
r
p
m
d
q
o
j
n
a
b
c
l'
b
a. Çatalhöyük
b. Çayönü Tepesi
c. Tepe Sofalin [11]
d. Liménaria [14]
e. Măgura
f. Toptepe
g. Dikili Tash [10]
h. Aşağı Pınar
i. Drenovac Turska Cesma
j. Ftélia
k. Vinča Belo Brdo
l. Paliambela
m. Rachmani
n. Kouvéléikès A
o. Profitis Ilias Rizoupolis
p. Vassilara Rachi
q. Théopetra
r. Balkan Export
s. Brunn am Gebirge
t. Niederhummel
u. Kuyavia region [17]
v. Chassey-le-Camp
w. Moverna vas [23]
x. Font-Juvénal
y. Lonche
z. Künzing-Unternberg
a'. Bercy [10]
b'. Ajdovska jama [23]
c'. Clairvaux XIV
d'. Ergolding Fischergasse [13]
e'. Chalain 3 [22]
f'. Chalain 4
g'. Eton Rowing Lake [12]
h'. Runnymede Bridge [12]
i'. Bulford Torstone
j'. Åle [24]
k'. Bjørnsholm [24]
l'. Gueldaman
7000
6000
5000
4000
3000
2000
Time (cal. years BC)


60°N
50°N
40°N
30°N
10°W
0°
10°E
20°E
30°E
40°E
0
0.02
0.05
0.1
0.15
0.2
0.25
1