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Widespread exploitation of the honeybee by early Neolithic farmers

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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, the association of humans with *A. mellifera* predates post-industrial revolution agriculture, as evidenced by the widespread presence of ancient Egyptian bee iconography dating to the Old Kingdom (approximately 2400 BC)¹. There are also indications of Stone Age people harvesting bee products; for example, honey hunting is interpreted from rock art² in a prehistoric Holocene context and a beeswax find in a pre-agriculturalist site³. However, when and where the regular association of *A. mellifera* with agriculturalists emerged is unknown⁴. 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 as it is determined genetically through the insect's biochemistry. Thus, the chemical 'fingerprint' of beeswax provides a reliable basis for detecting this commodity in organic residues preserved at archaeological sites, which we now use to trace the exploitation by humans of *A. mellifera* temporally and spatially. Here 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 seventh millennium cal BC, likely fulfilling a variety of technological and cultural functions. 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.

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101 The honeybee holds a unique place in human culture. Notwithstanding its present-day economic
102 importance, it has been revered over the millennia for the sheer beauty and complexity of the social
103 organization within its colonies. For these reasons the honeybee is the most researched of the social
104 insects, with its origin being regularly considered⁵. The last Ice Age would have had a major effect on
105 the honeybee with the ice sheets restricting European populations to the northern Mediterranean
106 hinterlands⁶. With the glacial retreat, the population would have subsequently expanded northwards.
107 However, due to the lack of a Holocene fossil record⁷, the honeybee is ecologically invisible for most
108 of the past 10,000 years.

109 Intriguingly, this is the period during which Neolithic agriculture emerged and spread out of
110 southeastern Anatolia and the Levant, with some human population movement into ecological zones
111 also conducive to the honeybee. Indeed, progressive woodland clearances by pioneer prehistoric
112 farmers may have opened up forests, favouring light-demanding shrubs, herbs and fruit trees (for
113 example, *Rosaceae*)⁸. Whether this would have exerted negative or positive effects on honeybee
114 populations is unknown^{8,9}. Given the latter, an opportunity exists to investigate the presence and early
115 exploitation of the honeybee by prehistoric farming communities through the cultural materials
116 recovered from Neolithic sites, namely their recently invented pottery vessels, and in doing so, to assess
117 the palaeoecological range of the honeybee in the Holocene.

118 Although the most obvious reason for exploiting the honeybee would be for honey, a rare source of
119 sweetener for prehistoric people, beeswax would likely have been an equally important material for
120 various technological, ritual, cosmetic and medicinal applications¹⁰. Indeed, beeswax has been regularly
121 detected in later archaeological and historic periods in lipid extracts from the fabric of unglazed pottery
122 vessels¹¹ where it is assumed to be a residue of honey use in cooking, or from the use of vessels for
123 processing wax combs¹²⁻¹⁴, with beeswax being absorbed through repeated contacts. Beeswax has also
124 been detected as a fuel in lamps and in larger vessels used as proto-beehives, for example Roman Greece
125 (second century BC to fourth century AD)^{15,16} and applied as a post-firing treatment to waterproof
126 vessels¹⁷.

127 The detection of beeswax in archaeological and historic contexts rests on its complex chemistry
128 providing a unique and relatively recalcitrant chemical signature. Fresh beeswax comprises a complex
129 mixture of aliphatic compounds consisting of series of homologues differing in chain-length by two
130 methylene groups¹⁸. Medium-chain *n*-alkanes range from C₂₃ to C₃₁ (with C₂₇ dominating in *A.*
131 *mellifera*), and *n*-alkanoic acids from C₂₀ to C₃₆. Monoesters comprise predominantly alkyl palmitates
132 (C₃₈ to C₅₂), with characteristic hydroxyl monoesters comprising long-chain alcohols (C₂₄ to C₃₈)
133 esterified mainly to hydroxypalmitic acid, ranging between C₄₀ and C₅₄ (ref. 18). The hydrophobic
134 nature of beeswax makes it relatively resistant to degradation. Hence, if protected from extensive
135 microbial attack and/or exposure to high temperatures during anthropogenic manipulation, the
136 aforementioned chemical characteristics can be used in assessing its presence^{10,19} (Figs 1 and 2).

137 Adopting this lipid biomarker approach, we now explore the association of the honeybee with the spread
138 of early Old World farmers based on lipid residue analyses of more than 6,400 pottery vessels
139 (Supplementary Information sections 1 and 2). Combining our new findings with published occurrences
140 of beeswax in prehistoric pottery allows the association between honeybees and early farmers to be
141 mapped spatially and temporally through prehistory (Figs 3 and 4).

142 The oldest evidence for beeswax comes from Neolithic sites in Anatolia dating from the seventh
143 millennium cal BC, as these sites are the locations of the oldest pottery vessels in Europe and Eurasia.
144 Most of the assemblages investigated comprised globular or bowl shape ‘cooking’ vessels, an
145 interpretation supported by the finding of ruminant and porcine animal fats in significant numbers of
146 vessels. No beeswax residues were detected during the intensive investigations of > 380 vessels from
147 the Levant, although only 34 residues were detected²⁰. Moving into eastern Anatolia, the site of Çayönü
148 Tepesi revealed two beeswax residues from 83 vessels from the seventh millennium including an
149 exceptionally well-preserved residue containing all the biomarkers of beeswax (Fig. 1b–f). The free *n*-
150 alkanols, dominated by C₃₀ and C₃₂ homologues, do not occur in fresh beeswax but are a feature of aged
151 wax, due to hydrolysis of the wax esters. The high abundance of C_{18:0} fatty acid suggests mixing with
152 mammalian animal fat, the latter being common in other sherds in the assemblage²⁰. The second sherd
153 from this site contained a lower concentration of beeswax but all the biomarkers were clearly evident.
154 These two residues establish the easterly limit of the beeswax detected in this investigation and provide
155 the oldest unequivocal evidence, to our knowledge, of honeybee exploitation by early Neolithic farmers.
156 In central Anatolia, extensive investigations of organic residues in 650 vessels, mainly from the site of
157 Çatalhöyük, revealed abundant animal fat residues. Only one residue showed tentative evidence for
158 beeswax based on wax esters, dominated by C₄₆ and C₄₈ homologues; however, the *n*-alkanols do not
159 exhibit the familiar distribution. *n*-Alkanes were detectable but the distribution is skewed towards the
160 higher homologues compared to that expected in fresh beeswax, although such distributional changes
161 are frequently seen in historical and archaeological beeswax, assumed to arise by sublimation during
162 ageing or heat treatment¹⁰. The tentative identification of this very early beeswax residue at Çatalhöyük
163 is supported by the discovery of a striking depiction of a honeycomb-like pattern painted on a wall at
164 the site²¹.

165 Analyses of approximately 570 cooking vessels from northwestern Anatolia revealed 72 lipid residues
166 of which 4 were identified as containing beeswax, from Aşağı Pinar and Toptepe, dating to 5500–5000
167 cal BC. Although the overall purity of the beeswax (two were mixed with ruminant fat) and lipid
168 concentrations (20 to 220 µg per gram of sherd) were quite variable, the distributions were
169 unmistakable. One of the beeswax finds from Toptepe is well preserved, albeit with ageing evident from
170 the hydrolytically released free *n*-alkanols and slight distortions of the various homologous series,
171 through loss of lower homologues.

172 The most abundant evidence for honeybee exploitation by early farmers was seen in the rest of the
173 Balkan Peninsula. The full range of beeswax biomarkers was identified in sherds from bowls, pans and

174 sieves from the Late Neolithic sites of Paliambela, Greece (4900–4500 cal BC), Măgura, Romania (Fig.
175 2a; 5500–5200 cal BC) and Drenovac Turska Česma, Serbia (5300–4700/4600 cal BC). A large number
176 of beeswax residues were found in Neolithic potsherds (11 residues out of 81 sherds analysed) from
177 Attica, the Peloponnese and the Cyclades (Aegean Islands), dating between 5800 and 3000 cal BC,
178 firmly establishing the long tradition of bee exploitation in this region. Overall, the incidence of
179 beeswax residues is highest in the Balkan Peninsula, where of the 1,915 Neolithic sherds analysed, 473
180 yielded lipid residues, of which 5.5% contained beeswax.

181 In Central Europe, pure beeswax was recovered from potsherds from *Linearbandkeramik* (LBK) sites
182 occupied by the earliest farmers of Austria and Germany (oldest LBK) including the sites of Brunn am
183 Gebirge (5500–5400 cal BC) and Niederhummel (5360–5220 cal BC), pushing back the date for bee
184 exploitation in this region by approximately 1,500 years¹³ (Fig. 2b). Beeswax was also detected in late
185 sixth millennium LBK sites of Ludwinowo 7 and Wolica Nowa, Poland¹⁷. In France, the exploitation
186 of bee products is evident during the second half of the fifth millennium at *Chasséen* sites (Font-Juvénal,
187 Chassey-le-Camp and Bercy¹⁰) and fourth millennium at the Lake Village sites of Clairvaux-les-Lacs
188 (3900 to 3700 BC) and Chalain 3 (ref. 22) and 4 (3200 to 3100 BC and 3040 to 2990 BC). High
189 incidence of beeswax (approximately 15% of the detectable residues) was identified in fifth millennium
190 sherds from two Slovenian sites (Ajdovska jama and Moverna vas)²³.

191 Around 130 sherds have so far been analysed from the Iberian Peninsula. However, no beeswax residues
192 have yet been detected, although the overall preservation of organic residues was poor. Further
193 investigations will likely reveal examples of beeswax in Neolithic pottery from this region.

194 The northerly limit of bee exploitation in northern Europe appears to be Denmark with two beeswax
195 finds in late Mesolithic and Neolithic contexts²⁴. Around 5° to the south in southern Britain, beeswax
196 is evident in 7 vessels amongst the approximately 670 Neolithic vessels analysed. These findings clearly
197 counter any arguments for a late introduction of the honeybee into the British Isles^{8,25}. Interestingly,
198 however, investigations of nearly 1,200 Mesolithic and Neolithic vessels from Ireland, Scotland and
199 Fennoscandia^{26,27} have failed to reveal any conclusive evidence of beeswax (Supplementary
200 Information section 3). Given that organic residue preservation in these regions is excellent, the lack of
201 beeswax would seem to establish the ecological limit of *A. mellifera* at that time. Similar arguments are
202 likely to account for the absence of beeswax residues from >350 prehistoric pottery vessels from the
203 Eurasian Steppe²⁸.

204 Finally, we report the first evidence for bee exploitation by Neolithic pastoralists in North Africa. The
205 analysis of 71 sherds from the Algerian site of Gueldaman revealed a single well-preserved beeswax
206 residue (fifth millennium BC). The preservation is again exceptional with *n*-alkanes, *n*-fatty acids and
207 fatty acyl wax ester distributions providing an unequivocal identification of beeswax. The presence of
208 free long chain *n*-alkanols and lack of hydroxy fatty acid wax esters are indicative of diagenesis and/or
209 use-related alteration. However, the overall distribution indicates the wax residue derives from *A.*
210 *mellifera* (Fig. 2c).

211 In conclusion, the approximately 50 new finds of beeswax residues considered above provide evidence
212 for the widespread exploitation of the honeybee by the early agriculturalists and pastoralists of the Near
213 East, Europe and North Africa dating back nearly 9,000 years (Fig. 3). In all these regions the new data
214 have either provided the first evidence of honeybee exploitation in a region, as in North Africa, or
215 pushed the chronology of human–honeybee association to substantially earlier dates, as in Anatolia and
216 Central Europe (Fig. 3b). The lack of evidence for beeswax use at Neolithic sites north of the 57th
217 parallel North may suggest an ecological limit to the natural occurrence of honeybees. Indeed, harsh
218 high-latitude conditions, even with temperatures warmer than today²⁹, would affect the foraging
219 capabilities of honeybees³⁰. Critically, in the absence of a Holocene fossil record for *A. mellifera*⁷ these
220 findings provide the first ancient biomolecule-based palaeoecological map of the distribution of an
221 economically and culturally important animal (Fig. 4).

222

223 **Online Content** Methods, along with any additional Extended Data display items and Source Data, are available
224 in the online version of the paper; references unique to these sections appear only in the online paper.

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285 **Methods**

286

287 **Lipid residue analyses.** All solvents used were HPLC grade (Rathburn) and the reagents were
288 analytical grade (typically > 98% of purity).

289 A sub-sample (1 to 3 g) from archaeological potsherds was cleaned with a modelling drill to remove
290 any exogenous lipids (from the soil and handling) and crushed with a solvent-washed mortar and pestle.
291 An internal standard (*n*-tetratriacontane, typically 20 μ g) was added to the powdered sherd to enable
292 the quantification of lipid extract. Ground samples of sherds were extracted with $\text{CHCl}_3/\text{MeOH}$ (2:1
293 (vol/vol), 2 \times 10 ml) using ultrasonication. Both supernatants were combined and the solvent was
294 removed under a gentle stream of nitrogen at 40 $^\circ\text{C}$. Aliquots of the total lipid extract (TLE) were treated
295 with 40 μ l of *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane
296 (Sigma Aldrich) for 1 h at 70 $^\circ\text{C}$ and the BSTFA in excess evaporated under a gentle stream of nitrogen.
297 The trimethylsilylated TLE was diluted in hexane (typically 50 to 150 μ l) and submitted to analysis by
298 high-temperature gas chromatography (HTGC) and high temperature gas chromatography-mass
299 spectrometry (HTGC/MS) to identify the major compounds present.

300 All TLEs were initially screened in a Hewlett-Packard 5890 Series II gas chromatograph equipped with
301 a fused-silica capillary column (15 m \times 0.32 mm) coated with dimethyl polysiloxane stationary phase
302 (DB-1HT; film thickness, 0.1 μ m; Agilent Technologies). Derivatized extracts (1.0 μ l) were injected
303 on-column using a cool on-column inlet in track oven mode. The temperature was held isothermally for
304 2 min at 5 $^\circ\text{C}$ and then increased at a rate of 10 $^\circ\text{C min}^{-1}$ and held at 350 $^\circ\text{C}$ for 10 min. The flame
305 ionization detector (FID) was set at a temperature of 350 $^\circ\text{C}$. Helium was used as a carrier gas and
306 maintained at a constant flow of 4.6 ml min^{-1} . Data acquisition and processing were carried out using
307 the HP Chemstation software (Rev. B.03.02 (341), Agilent Technologies).

308 HTGC/MS analyses of trimethylsilylated aliquots were performed using a Thermo Scientific Trace
309 1300 gas chromatograph coupled with an ISQ single quadrupole mass spectrometer. Diluted samples
310 were introduced using a PTV injector in split mode (split flow of 30 ml min^{-1} , split ratio of 6.0) onto a
311 0.53 mm fused silica pre-column connected to a 15 m \times 0.32 mm i.d. fused-silica capillary column
312 coated with dimethyl polysiloxane stationary phase (Rxi-1HT; film thickness, 0.1 μ m; Restek). The
313 initial injection port temperature was 50 $^\circ\text{C}$ with an evaporation phase of 0.05 min, followed by a
314 transfer phase from 50 $^\circ\text{C}$ to 380 $^\circ\text{C}$ at 0.2 $^\circ\text{C min}^{-1}$. The oven temperature was held isothermally for
315 2 min at 50 $^\circ\text{C}$, increased at a rate of 10 $^\circ\text{C min}^{-1}$ to 280 $^\circ\text{C}$, then at a rate of 25 $^\circ\text{C min}^{-1}$ to 380 $^\circ\text{C}$ and
316 finally held at 380 $^\circ\text{C}$ for 5 min. Helium was used as a carrier gas and maintained at a constant flow 5
317 ml min^{-1} . The mass spectrometer was operated in the electron ionization (EI) mode (70 eV) with a GC
318 interface temperature of 380 $^\circ\text{C}$ and a source temperature of 340 $^\circ\text{C}$. The emission current was 50 μ A
319 and the mass spectrometry set to acquire in the range of m/z 50–950 Daltons at two scans per second.
320 Data acquisition and processing were carried out using the Thermo XCalibur software (version 3.0.63).
321 Peaks were identified on the basis of their mass spectra, gas chromatography (GC) retention times, by

322 comparison with the NIST mass spectral library (version 2.0) and by comparison with modern beeswax
323 (from the Loire department, France).

324

325 **Construction of Fig. 4.** The total number of archaeological sites investigated is 166, but only 154 of
326 these fell within the geographical area of interest (longitude – 10° to 42° and from latitude 25° to 62°,
327 see Supplementary Information section 1). To estimate the distribution of beeswax residues in
328 continuous space from irregularly spaced data, linear interpolation was performed in the triangles
329 bounded by data points^{32,33}. The output grid was made of 530 × 380 points evenly spaced over the range
330 of latitude and longitude. No extrapolation was being used. Kriging was used to narrow the interpolation
331 values to locations around data points (and not show interpolation values where there is no data).
332 Kriging allows to obtain weights of the prediction locations based on the distance between data points,
333 with lower variance where data points are and higher variance where there is no data. Interpolation,
334 kriging and plotting were all performed in R version 2.15.1 (ref. 34). Interpolation was performed using
335 the function ‘interp’ from the package ‘akima’ (CRAN repository, [http://cran.r-](http://cran.r-project.org/web/packages/akima/akima.pdf)
336 [project.org/web/packages/akima/akima.pdf](http://cran.r-project.org/web/packages/akima/akima.pdf)). Kriging was performed using the function 'krige.conv'
337 from the package ‘geoR’ (CRAN repository, <http://cran.r-project.org/web/packages/geoR/geoR.pdf>,
338 further information on the package ‘geoR’ can be found at <http://www.leg.ufpr.br/geoR>). R code
339 available upon request to P.G.

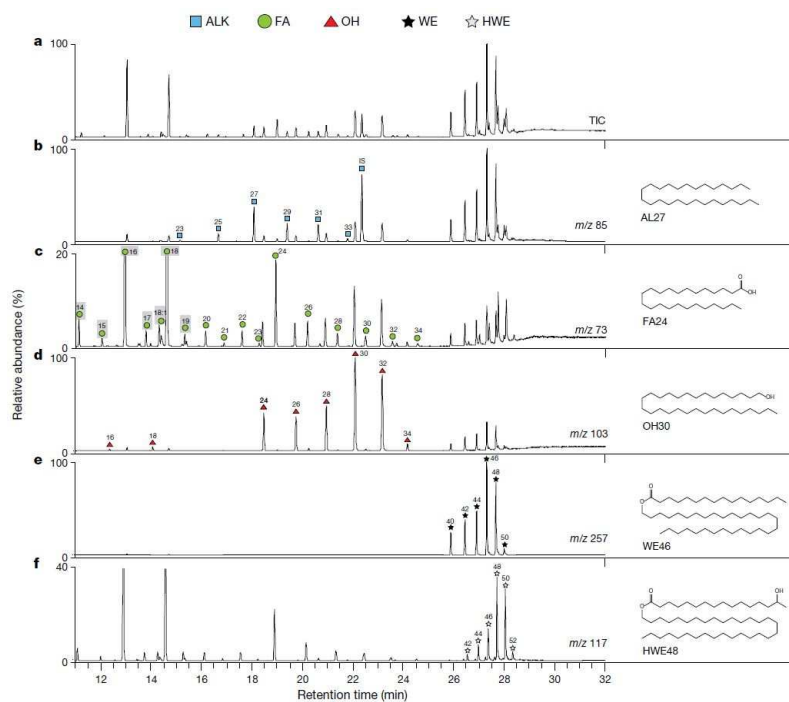
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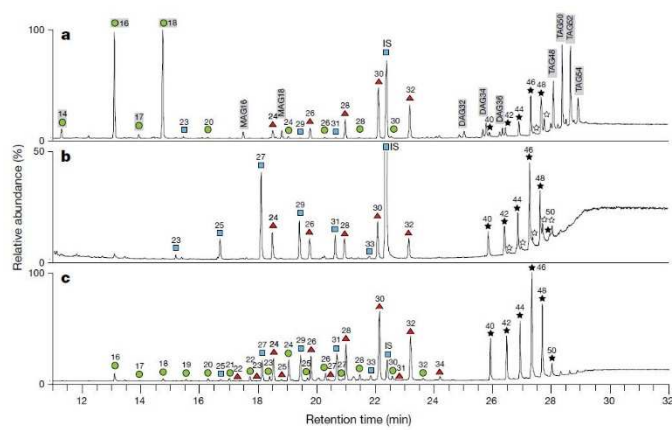
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346 Computing, 2012).

347 **Figure 1**



348

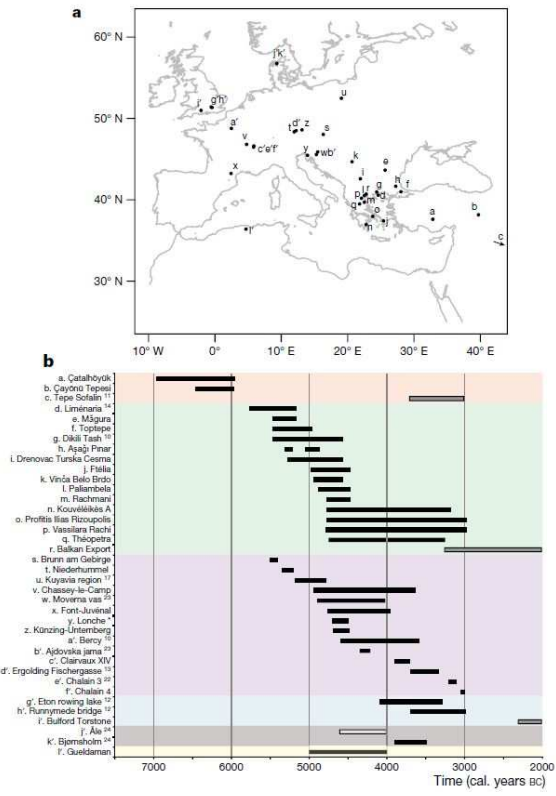
349 **Figure 2**



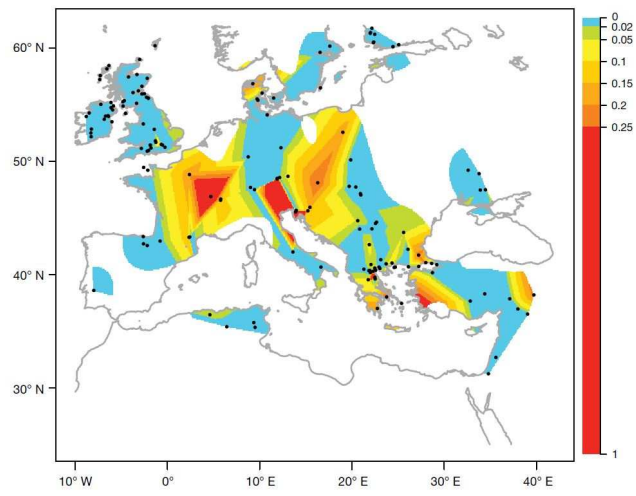
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352 **Figure 3**



353
354 **Figure 4**



355
356

357 **Figure captions**

358

359 **Figure 1 | High-temperature gas chromatography/mass spectrometry chromatograms of total lipid extract**
360 **of a sherd from Çayönü Tepesi (6500–6000 cal BC) containing beeswax. a–f**, Partial total ion current
361 chromatogram (**a**) and mass chromatograms (**b–f**) displaying ion masses of characteristic fragments from the main
362 compound classes comprising the extract (m/z 85, 73, 103, 257 and 117, respectively) with the molecular structure
363 of the most abundant component for each compound class. Squares, *n*-alkanes (ALK); circles, *n*-alkanoic acids
364 (fatty acids, FA); triangles, *n*-alkanols (OH); black asterisks, fatty acyl monoesters (WE); grey asterisks, hydroxyl
365 fatty acyl monoesters (HWE); IS, internal standard (*n*-tetratriacontane); number *n* and *n:i*, acyl carbon number
366 with zero or *i* degrees of unsaturations. Compounds shown with a grey background are interpreted as originating
367 from mammalian animal fats.

368 **Figure 2 | Partial gas chromatograms of total lipid extracts from Neolithic sherds from each geographical**
369 **region. a**, Mağura (5500–5200 cal BC). **b**, Niederhummel (5360–5220 cal BC). **c**, Gueldaman (fifth millennium
370 BC). **a** is interpreted as mixture of animal fats and beeswax; **b** and **c** as pure beeswax. MAG, monoacylglycerols;
371 DAG, diacylglycerols, TAG, triacylglycerols. Other peak attributions as in Fig. 1.

372 **Figure 3 | Geographical distribution of prehistoric sites in the date range 7500 and 2000 cal BC yielding**
373 **beeswax residues. a**, Locations of archaeological sites. **b**, Chronology of beeswax use in the Near East, the Balkan
374 Peninsula, mainland Europe, Scandinavia, the UK and northern Africa. Neolithic finds in black, pre-Neolithic
375 (hunter-gatherer contexts) in light grey and Bronze Age in dark grey. * Dental filling re-examined after ref. 31.

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

380

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386

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389 J.S., L.S., H.L.W., M.Bart. and D.U.-K. undertook planning of regional lipid residue analyses projects, sampling,
390 analytical work and data analysis. P.G. created Figure 4 and Supplementary Information section 3. All other
391 authors either directed excavations or provided expertise in relation to pottery collections and essential insights
392 into the study region and sites.

393

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395 declare no competing financial interests. Readers are welcome to comment on the online version of the paper. R
396 code is available upon request to P.G. (p.gerbault@ucl.ac.uk). Correspondence and requests for materials should
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