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Molecular Palaeontological Evidence for Food-Web Relationships

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The presence of consumer organisms in ancient ecosystems has been indicated mainly by the occurrence of fossilized mineral skeletons. In comparison with primary producers and bacteria, non-photosynthetic eukaryotic organisms are minute contributors to organic carbon preserved in sediments. In principle, however, organic compounds produced by heterotrophs have the potential to enter the sedimentary record. The biomass of respiring heterotrophs is enriched in ^{13}C relative to its carbon source [1], and it has been shown in the modern environment that the carbon isotopic fractionation between photosynthetic primary producers and heterotrophs increases as a function of the trophic level of the consumer [2]. In ancient sediments, identification of organic compounds derived from specific heterotrophs and determination of their isotopic compositions are potential tools to reconstruct palaeo-food-web relationships. Here, we present unprecedented evidence of the presence in sediments of organic compounds derived from insect waxes. Analyses of the ^{13}C contents of these materials indicate that insects utilized the organic material of cyanobacterial mats as their main source of carbon and, thus, demonstrate that molecular palaeontology can reveal food-web relationships in ancient communities.

The lagoonal and sabkha sedimentary systems of the coast of Abu Dhabi (UAE) are the result of a rise in sea level which reached the region 8000 years ago and of a subsequent regression that began 4000–5000 years ago [3]. During both the transgression and the regression, microbial mats, an association of cyanobacteria and other bacteria, developed in the upper intertidal zone. Remains of these are found in sedimentary sequences in the Abu Dhabi sabkha [4, 5]. Hypersaline conditions of deposition and a desert hinterland minimized the number of organisms that could survive and contribute to the sediment.

The hydrocarbon biomarkers isolated from the transgressive microbial mat are dominated by C_{20} (Fig. 1, *I*), C_{21}, and C_{22} highly branched isoprenoids (*II* and *III* in Fig. 2) of unknown origin, a series of straight-chain n-alkanes (C_{20}–C_{38}) with a strong odd-over-even carbon-number predominance apparently indicating a higher plant contribution [9], and a series of C_{24}–C_{45} monomethylalkanes (MMA), dimethylalkanes (DMA), and trimethylalkanes (TMA, Fig. 2). Representative structures of these compounds are shown in Fig. 1 (*VI–XI*). The MMA are dominated by 3-, 5-, 9-, 11-, and 13-methylalkanes with odd-carbon straight chains (Fig. 2). Three series of DMA were unambiguously identified, partly by comparison with synthesized standards (Fig. 1, *VIII*, *IX*, *XI*). The most abundant homologous series is the 3,9-DMA series (Fig. 1, *IX*), but smaller amounts of 3,7-, 3,11-, and 3,13-DMA isomers are also present. The less abundant DMA series is comprised of complex mixtures of 5,X-DMA (with X=9, 11, 13, 15) and 9,13- and 11,5 -DMA (Fig. 1, *XI*), respectively. Low amounts of 3,X,Y-TMA, including 3,7,11-TMA (Fig. 1, *X*), were also encountered.

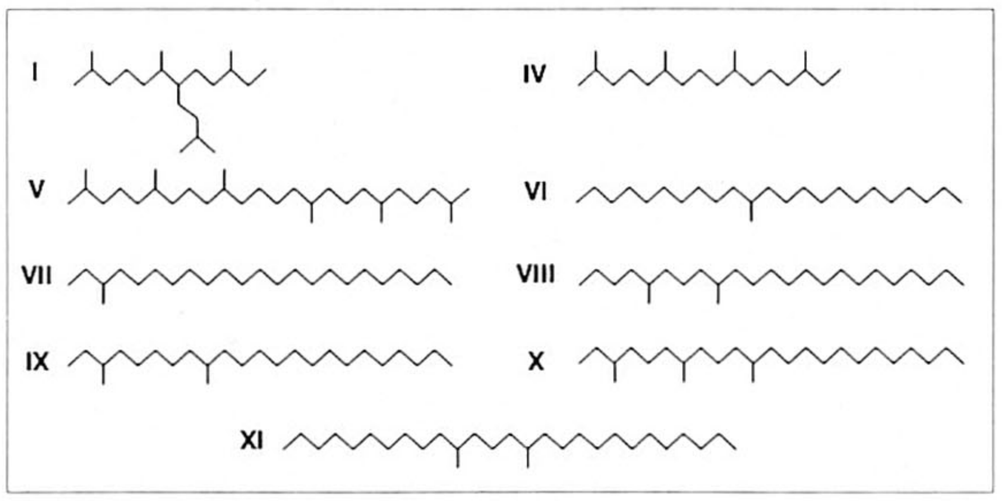

Fig. 1. Molecular structures of roman-numbered compounds in Fig. 2. *I* 2,6,10-trimethyl-7-(3-methylbutyl)dodecane, *IV* 2,6,10,14-tetramethylhexadecane (phytane), *V* 2,6,10,15,19,23-hexamethyltetracosane (squalane), *VI* 11-methyltricosane, *VII* 3-methyltricosane, *VIII* 5,9-dimethyltricosane, *IX* 3,9-dimethyltricosane, *X* 3,7,11-trimethyltricosane, *XI* 11,15-dimethylheptacosane. Compounds *VIII*, *IX*, and *XI* were unambiguously identified by synthesis of authentic standards. 5,9-Dimethyltricosane (*VIII*) was prepared by a Grignard reaction of the magnesium salt of 1-bromo-4-methyloctane and hexadecan-2-one followed by dehydration (5% H_2SO_4 in tetrahydrofuran, 20 °C, 24 h) and hydrogenation (PtO_2, 10% AcOH in EtOAc). 3,9-Dimethyltricosane (*IX*) and 11,5-dimethylheptacosane (*XI*) were similarly prepared from 1-bromo-6-methyloctane and hexadecan-2-one and 1-bromo-4-methyltetradecane and tetradecan-2-one, respectively. Hexadecan-2-one and tetradecan-2-one were prepared, respectively, by oxidation of hexadecan-2-ol and tetradecan-2-ol with pyridinium chlorochromate in CH_2Cl_2 [6]. The appropriate bromides were prepared by a copper halide-catalyzed monosubstitution in 1,5-dibromopentane and 1,3-dibromopropane (2x) by the magnesium salts of 2-bromobutane, 2-bromohexane, and 2-bromododecane, respectively [7]. 2-Bromododecane was prepared by reaction of dodecan-2-ol with tertiary phosphine dibromide in dimethylformamide [8]

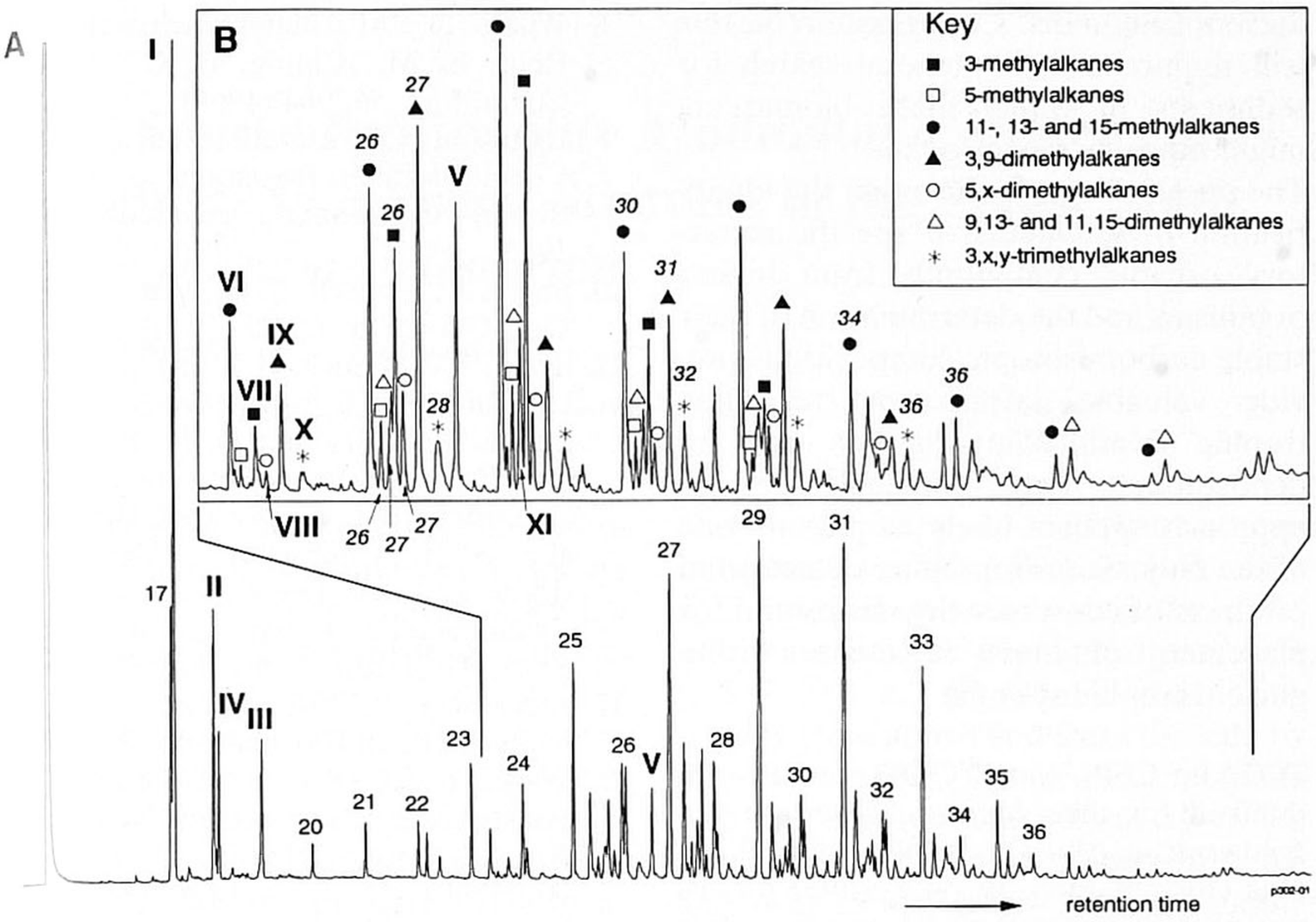

Fig. 2. A) Gas chromatogram of the saturated hydrocarbon fraction of a transgressive microbial mat (aged ca. 5500 B.P.) of Abu Dhabi sabkha. *Arabic numbers* correspond to the number of carbon atoms of n-alkanes. B) Part of the 5-Å molecular-sieved hydrocarbon fraction of this sample. *Italic numbers* refer to total number of carbon atoms of the MMA, DMA, and TMA indicated. *Roman numbers* correspond to the structures given in Fig. 1. The freeze-dried sample was powdered in a rotary mill and Soxhlet-extracted with MeOH/CH_2Cl_2 (1:7.5, v/v) for 24 h. The hydrocarbon fraction was obtained by alumina column chromatography using hexane/CH_2Cl_2 (9:1/v:v) as an eluent, followed by argentiferous thin-layer chromatography on silica gel with hexane as a developer. GC conditions: Carlo-Erba 5300, equipped with an on-column injector, and a fused silica capilary column (25 m × 0.32 mm) coated with CP-Sil 5 (film thickness 0.12 µm). He was used as a carrier gas, temperature programmed from 50 to 150 °C at 10 °C min^{-1} and from 150 to 320 °C (30 min hold time) at 4 °C min^{-1}. Identifications were made through analyses with gas chromatography-mass spectrometry (GC-MS). MS conditions: VG-70S instrument, ionization voltage 70 eV, mass range m/z 40–800, cycle time 1.8 s

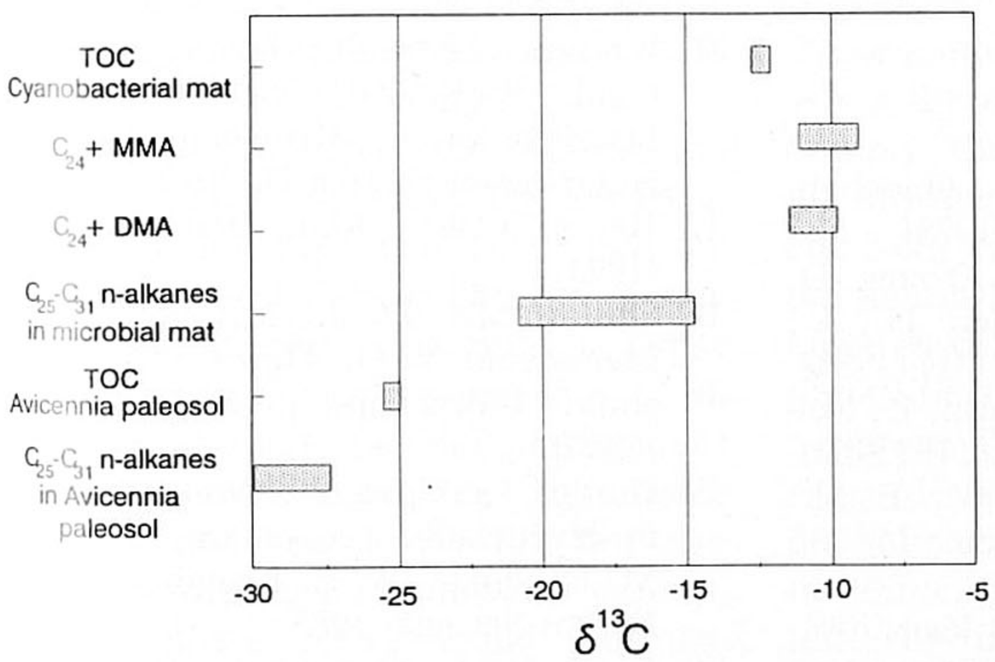

Fig. 3. Carbon isotopic values versus PDB ($\delta^{13}C$) obtained from total organic carbon (TOC) and individual compounds of the hydrocarbon fraction of a transgressive microbial mat (ca. 5500 B.P.) and a mangrove paleosol of the Abu Dhabi sabkha. $\delta^{13}C=10^3[(R_x-R_s)/R_s]$ in ‰, where $R=^{13}C/^{12}C$, x designates sample and s designates PDB standard with $R_s=0.0112372$. For isotopic analyses of total organic carbon ($\delta^{13}C_{org}$), minerals were removed by dissolution in HCl and HF and resulting kerogens were combusted in a sealed quartz tube at 850 °C for more than 4 h. CO_2 produced during combustion was cryogenically distilled and collected for mass spectrometric analyses on a Finnigan Delta E mass spectrometer. Contents of ^{13}C in individual saturated hydrocarbons were determined using isotope ratio monitoring GCMS [15]. The column used was a Hewlett-Packard Ultra 1 (length 50 m, 0.32 mm i.d.) with a film thickness of 0.52 µm. The oven temperatures were programmed from 50 to 150 °C at 10 °C min^{-1} and from 150 to 320 °C (30 min hold) at 3 °C min^{-1}

MMA, DMA, and TMA with 24 to 45 carbon atoms and with methyl groups located specifically at odd-numbered carbons have not been identified in cyanobacterial and bacterial products or in modern microbial mats [10]. Shorter-chained MMA and DMA (C_{15}–C_{21}) with methyl groups at both odd- and even-numbered carbon positions are produced by cyanobacteria [5, 10]. These compounds are, however, absent in the fossil mats examined here. This is consistent with prior reports of their biodegradability [4, 11]. In contrast, the long-chain (C_{24}–C_{45}) branched hydrocarbons found in the fossil mats are prominent constituents of cuticular waxes of insects [12] and have not been reported in other organisms. Long-chain MMA and DMA have been tentatively identified in Pre-Devonian sediments [13] that formed before insects first appeared [14], but the structures proposed and their distribution are dissimilar to those found here and in insect waxes. Hence, an origin from insect waxes is suggested for the branched alkanes in the ancient mats.

Stable carbon isotopic data are summarized in Fig. 3. The longer-chained MMA and DMA are slightly enriched in ^{13}C (average δ values of –9.8 and –10.5 ‰, respectively) relative to the total organic carbon of the microbial mat ($\delta^{13}C_{org}$ = –12.3 ‰) and strongly enriched relative to higher plant products. The enrichment of ^{13}C observed in the mat is typical of microbial assemblages growing in hot, hypersaline waters with low contents of dissolved CO_2 [16]. The enrichment of ^{13}C in the MMA and DMA relative to the mat carbon is significant. It indicates that the MMA and DMA are not algal or cyanobacterial products (if they were, depletion in ^{13}C relative to total mat carbon would be expected [17]). Insects are, however, commonly enriched in ^{13}C by 2 ‰ relative to their carbon source [1]. Moreover, there is one report that cuticular hydrocarbons from insects are enriched in ^{13}C relative to the insect's carbon source by 1 ‰ [18]. The ^{13}C contents of the MMA and DMA (Fig. 3) are thus consistent with an origin from cuticular waxes of insects grazing on the microbial mats.

Values of $\delta^{13}C$ for unbranched long-chain n-alkanes (C_{25}–C_{31}) in the mats average –18.7 ‰ but spread between –14.8 and –21.1 ‰. This indicates that these alkanes derive partly from the Avi-

cennia mangrove (mangrove paleosol: $\delta^{13}C_{n\text{-alkanes}} \approx -28.7$ ‰) which grew lower in the intertidal zone [4] and partly from a more ^{13}C-depleted precursor.

Cyanobacteria and bacteria are not known precursors of long-chain linear alkanes [11] and the isotopic values of the linear alkanes of the mat must therefore result from the mixing of mangrove- and insect-derived compounds.

In the terrestrial environment, insects are by far the most abundant primary consumers [14]. They are abundant in intertropical coastal hypersaline environments. Insect burrows were observed in the upper intertidal and supratidal modern sediments of Abu Dhabi as well as in equivalent sedimentary environments [19, 20]. In Solar lake (Sinai), and in the Gavish sabkha (Sinai), insects are primary consumers of the microbial assemblages and extensively burrow in intertidal sediments including microbial mats [19]. Ants and insect larvae were also cited as important burrowers and grazers of microbial mats in Andros Island (Bahamas) [21]. The transgressive microbial mats of Abu Dhabi studied here were also intensively burrowed. Although the excavators of these burrows are not precisely known, insects are the most likely candidates.

Molecular, isotopic, and sedimentological evidence thus combine to indicate that cuticular waxes of insects grazing the microbial mats were preserved in these Holocene sediments. Insects have not previously been cited as contributors to sedimentary organic matter. Their soft bodies and unmineralized exoskeletons generally result in a poor fossil record [14]. However, their cuticular waxes contain compounds particularly refractory to biologically mediated oxidation. In terms of sedimentary total organic carbon, contributions of insects may be negligible, but the preservation potential of the molecular constituents of their cuticles is high and it is therefore plausible that they could contribute to the hydrocarbon fraction of some sediments. Considering their abundance in ancient biospheres, insects may have been a significant source of hydrocarbons in some ancient sediments. Confirmation on this will require a more general search for sediments in which these biomarkers might have been overlooked.

The observations confirm that the identification in sediments of specific refractory organic compounds from higher organisms and the determination of their stable carbon isotopic compositions provide valuable information regarding trophic relationships at the time of deposition. The molecular-isotopic approach appears likely to provide one of the only means for secure detection of products of consumer organisms and for placement of those organisms within ancient trophic systems.

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