



Chemotaxonomic classification of fossil leaves from the Miocene Clarkia lake deposit, Idaho, USA based on *n*-alkyl lipid distributions and principal component analyses

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Abstract

Fossil leaves which show excellent preservation of lipids and other organic compounds are preserved in abundance at the Miocene Clarkia lake deposit in northwestern Idaho, USA. Eighty three fossils from nine genera which are commonly found at the site, namely *Quercus*, *Platanus*, *Magnolia*, *Pseudofagus*, *Fagus*, *Cocculus*, *Taxodium*, *Metasequoia* and *Sequoia* were investigated to assess whether their chemical compositions can be used in chemotaxonomic comparisons. Chromatographically separated *n*-alkane and *n*-alkanol sub-fractions were examined by GC and GC/MS to establish whether characteristic distributions were evident between fossils and distinct from the enclosing sediment. Chemotaxonomic comparisons were performed between the individual fossil specimens and six sediments using the *n*-alkane and *n*-alkanol profiles and principal component analysis (PCA). Characteristic distributions were observed for certain genera, e.g. *Platanus* and *Quercus*, which were reproducible between multiple specimens of the same genus. Concentrations of the lipids in fossils were markedly greater than in the six sediments in all of the fossils except the conifers examined. These findings demonstrate that *n*-alkyl lipid distributions from fossil leaves have chemotaxonomic utility, with potential for comparisons with modern taxa. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Plant fossil chemotaxonomy; Clarkia lake deposit; PCA; Molecular preservation; *n*-Alkyl lipid distributions; Palaeo-environmental change

1. Introduction

The chemical compositions of the epicuticular waxes from modern leaves have been used to examine chemotaxonomic relationships between different taxa. In particular, the distribution profiles of *n*-alkyl lipids derived from these waxes have been used in comparative studies of modern plant species (Eglinton et al., 1962; Dyson and Herbin, 1968; Osborne et al., 1989; Maffei, 1994;

Mimura et al., 1998). Alkyl lipid classes such as *n*-alkanes, *n*-alkanols and fatty acids are hydrophobic in nature. Consequently they are expected to remain in close association with a buried leaf macrofossil rather than migrating into the enclosing material, assuming that the conditions within a sediment favour lipid preservation, e.g. anoxia. Since these compounds are the most abundant components of epicuticular waxes of higher plants we anticipate that they will still be detectable above the sedimentary background even if some degradation has occurred during senescence, prior to deposition, or during burial.

The abundance of well preserved fossil leaves in the Miocene (17–20 Ma) lake sediments at Clarkia, Idaho, provides the opportunity to perform chemical analyses on many individual fossil specimens. Initial studies of

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the Clarkia site investigated and characterised the lipid composition of the sediment at site P-33, as well as analysing a number of fossil leaves from the locality (Logan, 1992; Logan and Eglinton, 1994; Logan et al., 1995). These studies detected biomarkers of plant, bacterial and algal origins. Inputs from bacterial sources included three isomers ($\Delta^{17(21)}$, $\Delta^{13(18)}$, Δ^{21}) of the bacterial lipid diploptene (hop-22(29)-ene), C_{27} – C_{32} hopanes (mainly 17β , 21β configuration), 3β -methylhopenes and hopanoid acids. β -amyrin, phytosterols and a suite of des-A and aromatic triterpenoids, resulting from the degradation of higher plant triterpenols, were also detected. Algal inputs were reflected in the presence of 24-ethyl sterenes, sterols and series of diols (α,ω ; $\alpha,\omega-1$; 1,3). Dinoflagellate inputs were represented by abundant dinosterol and dinostanol. All of the above compounds were either not sufficiently characteristic of a particular organism or too degraded/transformed to present the opportunity to be used in chemotaxonomy, with the exception of 3β -methylhopenes which are recognised as biomarkers for methanogenic bacteria (Summons and Jahnke, 1992). However, the most dominant compounds detected in the Clarkia sediments and higher plant fossils were homologous series of *n*-alkanes, *n*-alkanols and fatty acids. Their hydrophobic nature and association with identifiable fossil specimens makes them suitable candidates for chemotaxonomic analyses where distributional differences between genera could be compared. Fossil specimens in the study by Logan and co-workers (1995) were analysed as total lipid extracts, and while overall differences between fossil genera were apparent it was difficult to discern the subtle differences in distributions of *n*-alkyl lipids. Two subsequent studies of Clarkia fossil leaves achieved further separation of the total lipid extracts into chromatographic subfractions (Huang et al., 1995, 1996). On the basis of these encouraging early results, this comprehensive study was undertaken of fossil leaves and sediments from the Clarkia P-33 site where a number of specimens of each genus were examined. The aims were to establish whether characteristic distributions of *n*-alkyl lipids were preserved between a number of different fossil taxa. This research extends our use of plant and animal macro-fossil and microfossil specimens as a means of deriving new information on taphonomic and diagenetic processes.

2. Samples and site description

2.1. Sampling of fossils

Fossils were collected during two visits to the Clarkia locality (September 1994 and April 1996). Several sites have been excavated in the region, although most of the fossils examined in this study originate from the renowned P-33 site where the best preservation is evident

(descriptions of the local geology are provided in Smiley and Rember, 1979). Large blocks of fossil-bearing sediment were cut from the deposit mainly at stratigraphic level 2b (Fig. 1) approximately 2.5 m from the base of the 7.6 m sedimentary sequence. The P-33 sediments are estimated to be between 15.3 and 22 million years old based on K/Ar dating of the Priests Rapids basalts which intrude into the deposit, however, comparisons of the taxa preserved at Clarkia with similar Miocene sites in the region have narrowed the age range of the deposit to between 17 and 20 million years (Smiley and Rember, 1985a,b). Several fossils were sampled from Unit 5 higher up (ca. 6.5 m from the base) in the sedimentary sequence from the same site. A few specimens were also collected for comparative purposes from site P-35 at Oviatt Creek, a younger Miocene deposit (~12.8 Ma) located 32 km south of the Clarkia basin. Sediment blocks were either split at the site to obtain fossils, or returned to the University of Idaho, Moscow, USA or University of Bristol, UK for processing.

2.2. Sampling of sediments

Samples of fossil-bearing sediments were also taken intact from the Clarkia P-33 site for chemical comparisons with the fossils. Sediment was sampled from five horizons (Fig. 1) of the exposure at the P-33 site, 2b, 2d, 4 (ash), and from below, 5a, and above, 5c, a thin layer of ash in Unit 5. A block of sediment from the geologically younger P-35 Oviatt Creek site was also removed for analysis. Fossils of nine abundant genera were analysed in this study. They are listed in Table 1, and comprised six angiosperm (dicotyledons) and three gymnosperm (conifers) genera.

2.3. Identification of fossils

The majority of the Clarkia plant fossils are comparable to other fossil floras in the Columbia Plateau region of the Pacific Northwest, in particular the Mascall and Latah floras, although some taxa resemble species represented in older Oligocene floras (Smiley and Rember, 1985a and references therein). Some genera discovered at the site have not previously been identified at other sites, in particular, one new taxon *Pseudofagus idahoensis* has been classified as a novel extinct genus and species unique to Clarkia (Smiley and Huggins, 1981). In addition to *P. idahoensis*, two other taxa at the site have been identified to the 'species' level in this paper, i.e. *Magnolia latahensis* and *Platanus dissecta*, these classifications have been assigned based on species present in the flora of deposits of similar age in the Pacific Northwest region (J. Smiley, personal communication). Other taxa examined have only been identified to the genus level and may represent more than one closely related species. The majority of *Quercus* specimens

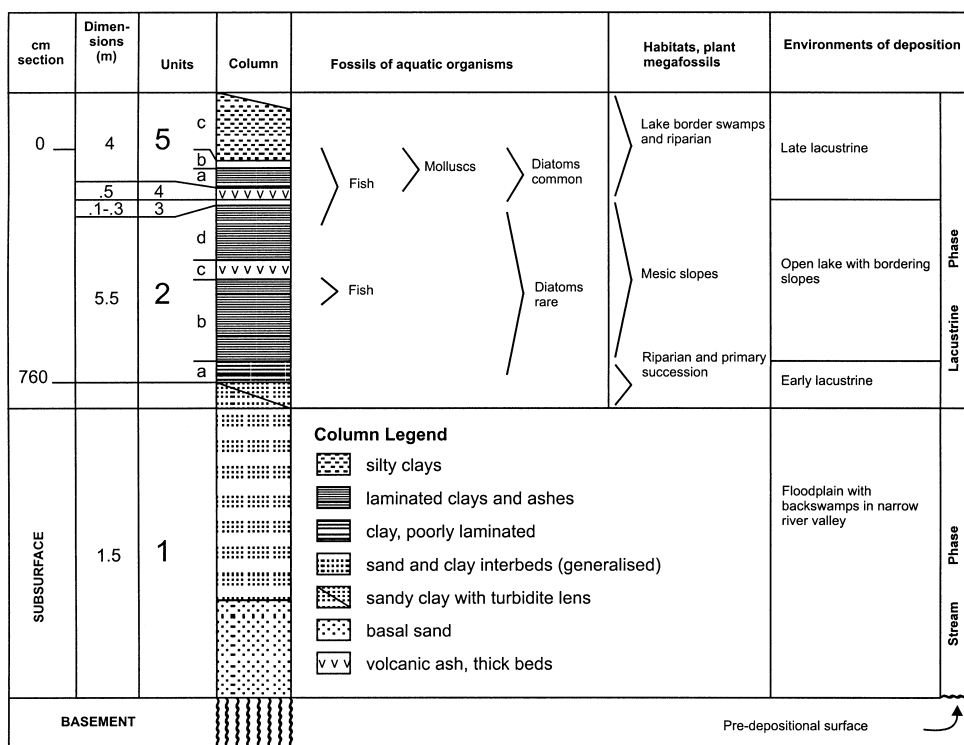


Fig. 1. Stratigraphic profile of the Clarkia P-33 deposit. The majority of fossil leaves were sampled from within the 2b sediments. Adapted with permission from Smiley and Rember 1985b.

Table 1
Fossil species examined from the Clarkia fossil sites

Genus and species	Common name	Family	Number of samples analysed
Angiosperms			
<i>Quercus</i> sp.	Oak	Fagaceae	17 red, 2 evergreen
<i>Platanus dissecta</i>	Plane	Platanaceae	12
<i>Pseudofagus idahoensis</i>	n/a (extinct)	Fagaceae	19
<i>Fagus</i> sp.	Beech	Fagaceae	2
<i>Cocculus</i> sp.	Cocculus	Menispermaceae	5
<i>Magnolia latahensis</i>	Magnolia	Magnoliaceae	16
Gymnosperms			
<i>Taxodium</i> sp.	Bald or swamp cypress	Taxodiaceae	6
<i>Metasequoia</i> sp.	Dawn redwood	Taxodiaceae	1
<i>Sequoia</i> sp.	Redwood	Taxodiaceae	2

were identified as belonging to the red subgenus *erythrobalanus* (Metcalf and Chalk, 1950; Phillips, 1978). This subclassification was performed by examination of the leaf margins for characteristic features, specifically *aristae* (*Q. Leng*, personal communication). There appeared to be at least two closely related red oak species represented in the assemblages at both sites P-33 and P-35. In addition, two of the oak specimens were of a completely different species, these were thought to be

swamp-dwelling evergreen or “live” oaks (which are sometimes classified as belonging to the white *leucobalanus* subgenus). Modern red oaks and the majority of modern white oaks are deciduous trees.

2.4. Description of fossils

A total of 83 fossils and six sediments were examined in the course of this study. The majority of fossils were

black in colour although several were orange or green/brown. The upper leaf surfaces of some of the fossils still had a waxy appearance (e.g. *Magnolia* specimens) and several showed evidence of morphological damage (e.g. by insect mining or fungi). Descriptions of the appearance and condition of fossils are presented in Table 2.

3. Experimental

3.1. Sample preparation

Sediment blocks were split along bedding planes to reveal fossils. Fossil leaves were sampled if they appeared to be one of the nine genera listed in Table 1, of a reasonable size and in good morphological condition, or unusual in some respect. After selection, leaves were identified, logged and photographed. Removal of specimens from the enclosing sediments was achieved by clean careful lifting and scraping using a scalpel. The fossil fragments were brushed onto a piece of aluminium foil and transferred to a vial. This process was found to be the most effective in minimising the amount of sediment removed along with the fossil as in many cases the fossil remains were structurally distinct from the sediment. However, a number of the fossil *Cocculus* and *Pseudofagus* specimens were more intimately associated with the sediment so were more likely to exhibit lipid distributions containing an appreciable sedimentary component. In order to prevent degradation or evaporative losses of lipids within the fossil material, solvent (dichloromethane [DCM]/methanol, 2:1 v/v) was added to the vial in sufficient quantity (ca. 100–300 μ l) to cover the sample. Fossil samples were stored at -20°C .

3.2. Extraction of samples

Aliquots (typically 50 μ l) of a mixture comprising five standards were added to fossils prior to extraction (cf. Lockheart et al., 1997). The solvent covering the samples was removed under nitrogen. Fossil specimens were extracted by sonication in DCM/methanol (2:1 v/v; 5 x 1 ml) for 15 min per extraction, and the supernatant was transferred into a vial to obtain a total lipid extract. The extraction solvent was evaporated under nitrogen and then redissolved in a small volume of DCM/isopropanol (2:1 v/v, ca. 1 ml).

Sediment samples were prepared for extraction by freeze-drying as described by Huang et al. (1996). When the sediment blocks were cut into pieces any fragments containing leaf macrofossils were removed. Total lipid extracts were isolated from homogenised sediment samples (ca. 60 g) by refluxing for 24 h with ca. 200 ml

DCM/methanol, 2:1 (v/v), in defatted cellulose thimbles using a Soxhlet apparatus. Copper turnings (2–3 g) were added to the solvent reservoir in order to desulphurise the total lipid extract. An aliquot of each sediment total lipid extract (ca. 1/10 by volume) was taken for further separation.

3.3. Chromatographic separation

Chromatographic separation of sediment extracts was performed as described in previous studies (Huang et al., 1995, 1996). Chromatography of fossil extracts followed a similar procedure but was scaled down to allow for the small size of samples. Briefly, the total lipid extracts of fossils and sediments were separated into ‘acid’ and ‘neutral’ subfractions using aminopropyl solid phase extraction cartridges (Bond Elut, Varian). Silica gel ‘flash’ column chromatography was employed to further separate the ‘neutral’ fraction. In the case of fossils a small flash column was utilised, i.e. a glass column with capacity ca. 3 ml and internal diameter 5 mm which was packed with silica gel (0.4 g; Fluka 60, 0.035–0.070 mm particle size, 220–440 mesh). The following solvents were passed through the column to yield five fractions: (i) hexane (3.1 ml) (ii) hexane/DCM (9:1 v/v, 1.5 ml), (iii) DCM (2.0 ml), (iv) DCM/methanol (1:1 v/v, 3.1 ml), and (v) methanol (2.3 ml). Elemental sulphur was present in some fossil samples and eluted in the hexane fractions. This was removed by the addition of a few copper turnings.

3.4. Derivatisation of alcohol fractions

Alcohols were converted to trimethylsilyl ethers prior to gas chromatographic analysis using an excess of *N,O*-bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane (BSTFA + 1% TMCS, Sigma Chemical Co.). Samples were heated with the reagent in capped vials at 60°C for 1 h. Excess reagent was removed under a stream of nitrogen.

3.5. Gas chromatography (GC)

The hydrocarbon and derivatised alcohol fractions dissolved in dichloromethane (100 μ l) were analysed using a Hewlett Packard 5890 Series II GC fitted with an on-column injector port and flame ionisation detector. Each sample was injected (1 μ l) onto a CP-SIL 5CB capillary column (Chrompack; 50 m x 0.32 mm, 0.12 μ m film thickness) with hydrogen as carrier gas at a head pressure of 10 psi. The GC oven was temperature programmed as follows: isothermal at 40°C for 1 min, then rising to 200°C at $10^{\circ}\text{C min}^{-1}$, and finally to 300°C at $3^{\circ}\text{C min}^{-1}$, with an isothermal period of 15 min. Data was acquired and processed on a PC using Chemstation software (Hewlett Packard).

Table 2
Description of fossils from the Clarkia (P33) and Oviatt Creek (P35) sites

Sample code	Site and horizon	Colour	Description
<i>Quercus (evergreen)</i>			
FL12	P33 2b	Brown/black	Small toothed "live oak"
FL23	P33 2b	Black	Small size, small toothed "live oak"
<i>Quercus (red)</i>			
FL34	P35	Black/brown	Thick, lobed
FL35	P35	Black	Large toothed
FL36	P35	Black/red	Large toothed, half oxidised, same species as FL35
FQR3	P33 2b	Black/brown	
FQR6	P33 2b	Black/brown	Similar to FQR3
FQR9	P33 2b	Red/brown	Same species but larger than FQR3 and 6
FQR15	P33 2b	Black	Thick residue
FQR16	P33 2b	Black	Easily lifted from sediment
FQR17	P33 2b	Brown/black	
FQR22	P33 2b	Black	Fragment, similar to FMR17
FQR23	P33 2b	Black	Fragment
FQR24	P33 2b	Brown/black	Thick similar to FQR3, 6, 9
FQR25	P33 2b	Black/brown	Thick
FQR28	P33 2b	Brown/black	
FQR29	P33 2b	Black	
FQR30	P33 2b	Black	
FQR37	P33 2b	Black/brown	
<i>Platanus dissecta</i>			
FX10	P33 2b	Brown/black	Medium sized specimen
FX11	P33 2b	Black	Complete and large fossil
FP26	P33 2b	Brown/ black	Fragment showed good venation and detail at margins
FP56	P33 2b	Black/brown	Very large
FP57	P33 2b	Black	Evidence of fungus/insect holes?
FPR12	P33 2b	Black	Large, half oxidised, thick
FPR13	P33 2b	Black	Very large
FPR14	P33 2b	Black	Small leaf but formed a thick layer on the sediment
FPR26	P33 2b	Black	Thick fossil difficult to remove from sediment below
FPR27	P33 2b	Black	Large
FQR35	P33 2b	Brown/black	Initially misidentified as a different genus (<i>Quercus</i> sp.)
FPR38	P33 2b	Black	Medium sized
<i>Pseudofagus idahoensis</i>			
FX9	P33 2b	Red	Large, good condition
PF46	P33 2b	Red/brown	Large, good condition
PF52	P33 2b	Red/brown	Large, good condition
PF53	P33 2b	Brown	Very large
PF4	P33 2b	Black	
PF7	P33 2b	Black	Large, immature?
PF9	P33 2b	Dark brown	Small leaf
PF19	P33 2b	Black	
PF20	P33 2b	Black	Good venation
PF27	P33 2b	Brown	Obscured partially by a legume leaf (not sampled)
PF51	P33 2b	Red/brown	Medium size with good venation
PF59	P33 2b	Red	Good condition.
PFR5	P33 2b	Brown/black	Large
PFR10	P33 2b	Black/brown	Thick layer on sediment
PFR11	P33 2b	Red/orange	Large, very well preserved
PFR21	P33 2b	Black	Large
PFR31	P33 2b	Red/brown	Very large and broad
PFR33	P33 2b	Brown/black	
PFR36	P33 2b	Brown/black	Well preserved, base of leaf brown

(Table continued on next page)

Table 2 (continued)

Sample code	Site and horizon	Colour	Description
<i>Fagus sp.</i>			
FFR19	P33 2b	Brown/orange	Good venation
FFR20	P33 2b	Brown/black	
<i>Cocculus sp.</i>			
FC25	P33 2b	Brown/black	
FC31	P33 2b	Black	Bedded in a clay layer
FC37	P35	Black	Remains dried out cf. other specimens
FC45	P33 2b	Black	Thick, fine detail well preserved
FC58	P33 2b	Red/brown	Complete leaf
<i>Magnolia latahensis</i>			
FX3	P33 2b	Red	
FX4	P33 2b	Green/brown	
FX6	P33 2b	Brown	Excellent condition
FM1	P35	Black	Very thick, possibly <i>Persea</i> sp.
FM11	P33 2b	Black	
FM42	P33 2b	Red/brown	Veins light in colour
FM50	P33 2b	Green	Very good condition
FM54	P33 2b	Red/brown	Approx. half size of FM50 mottled appearance
FM55	P33 2b	Red/orange	Curled up from the sediment after sunlight exposure
FM64	P35	Black	Mouldy white appearance, insect holes?
FMR1	P33 2b	Red/brown	Nearly complete
FMR2	P33 2b	Red/brown	Thinner compression than FMR1 with good morphology
FMR4	P33 2b	Red/black	Excellent preservation, red colour on white clay
FMR7	P33 2b	Brown/black	Very good condition, complete fossil with red ends
FMR8	P33 2b	Black	Thinner than FMR7 with good morphology
FMR32	P33 2b	Black	Half leaf fragment
<i>Taxodium sp.</i>			
FT3	P33 2b	Black	2 shoots combined
FT61	P33 2b	Brown/black	1 long shoot
FT62	P33 2b	Black	Part of shoot
FT63a	P35	Red brown	1 shoot
FT63b	P35	Black/brown	2 shoots with thick stems
FT76	P33 5c	Brown/black	1 shoot, poor condition, pyrite encrusted
<i>Metasequoia sp.</i>			
FT77	P33 5c	Black	1 shoot, excellent structural preservation
<i>Sequoia sp.</i>			
FT80	P35	Black	1 shoot
FT81	P35	Brown/red	1 shoot
<i>Dryophyllum sp.</i>			
FQR18	P33 2b	Black	Toothed margins, initially identified as different <i>Quercus</i> sp.

3.6. Gas chromatography–mass spectrometry (GC/MS)

Compound identification was carried out by GC/MS using a Carlo Erba 5160 Mega series GC coupled to a Finnigan 4500 quadrupole mass spectrometer operating in positive electron ionisation mode (70 eV), scanning from m/z 50 to 600, with a cycle time of 1 s. Column type and oven conditions were identical to those used in GC analyses, except that helium was used as the GC/MS carrier gas.

3.7. Treatment of data

The large amount and complexity of the fossil data set provided the opportunity to perform principal component analysis (PCA) on the relative abundance distributions of *n*-alkanes and *n*-alkanols. The data was processed using Systat for Windows v. 5.02 (Systat Inc.). A description of the principles and application of this technique to organic geochemical data can be found in Ficken (1994) and Stankiewicz et al. (1997).

4. Results

In the following sections reference is made to individual fossil specimens by unique codes (e.g. FMR1). Descriptions of the fossils these codes correspond to are presented in Table 2.

4.1. Aliphatic lipid composition of *Clarkia* fossil leaves and sediments

4.1.1. *n*-Alkanes in fossils

The hexane fractions contained the hydrocarbon components of the fossils and were, without exception, dominated by a series of *n*-alkanes with chain lengths ranging between C₂₁ and C₃₅ (Figs. 2 and 3). For most samples a high odd/even predominance of *n*-alkane chain lengths was evident. Figs. 2 and 3 depict, by fossil type, histograms of the mean concentrations for each chain length of *n*-alkane where the mean value is indicated by the wide bar height and standard deviations are shown by the I-bars; the numbers of specimens of each fossil genus analysed (*n*) are also given. For genera

where samples were examined from both P-33 and P-35 sites a mean histogram for each site is presented (i.e. *Cocculus*, *Magnolia*, *Quercus* and *Taxodium*). Distributions for specimens of red and evergreen *Quercus* are presented separately in Fig. 2.

Table 3 details some typical features of the fossil *n*-alkane distributions as well as giving ranges within each species of: (i) carbon preference index, CPI; (ii) average chain length, ACL, and (iii) total *n*-alkane concentration. In most cases the individual fossil specimens of a particular species/genus displayed a similar *n*-alkane profile to the calculated mean distribution pattern of that genus. Individual histograms and tabulated *n*-alkane concentrations for each specimen are reported in Appendices 5 and 6 of Lockheart (1997).

4.1.2. Sedimentary *n*-alkanes

The concentrations of *n*-alkanes in sediments from the different stratigraphic horizons at sites P-33 and P-35 are depicted as histograms in Fig. 4. The sedimentary *n*-alkane distributions were remarkably consistent between the five P-33 samples analysed; C₂₉ dominated

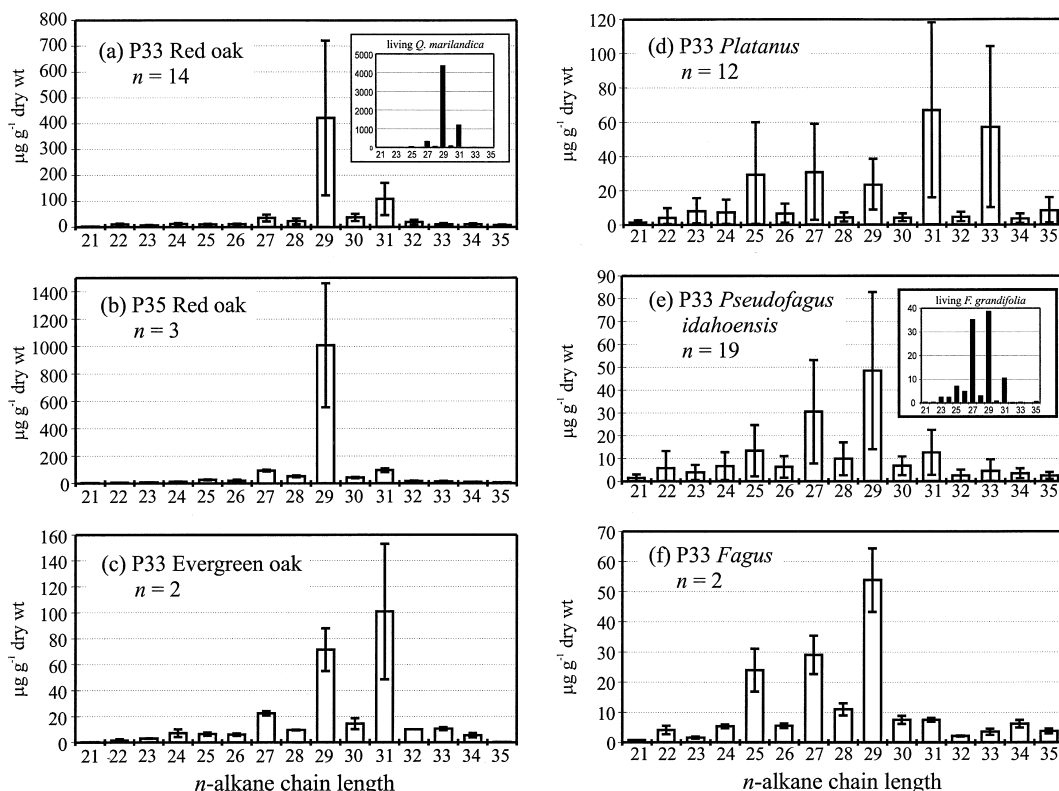


Fig. 2. Mean distributions of *n*-alkanes from four fossil genera. (a) P-33 red oaks comprised at least two similar species (inset histogram depicts profile of living *Q. marilandica* for comparison), (b) P-35 red oaks were also of two species which resembled P-33 specimens. (c) Evergreen oaks from P-33 showed different morphology from the red oaks. (e) *Pseudofagus idahoensis* is a genus and species unique to *Clarkia*. The inset is of living *Fagus grandifolia* which morphologically resembles this extinct species. Wide bars are mean concentrations with standard deviations indicated by I-bars (*n* = number of fossils analysed).

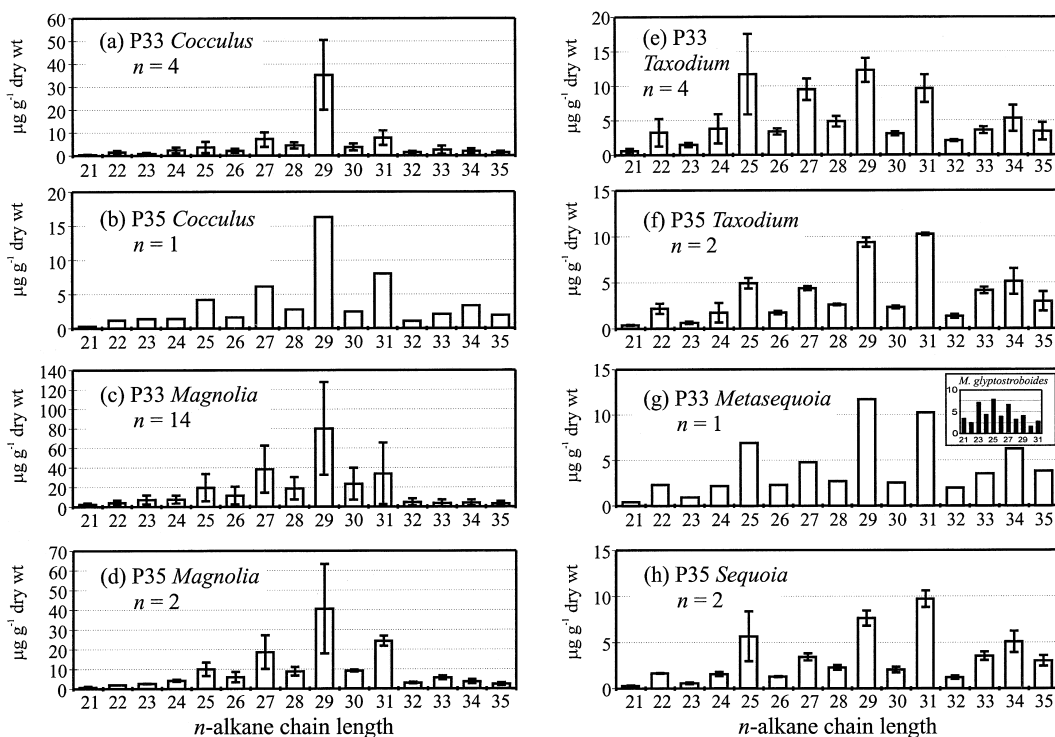


Fig. 3. Mean distributions of *n*-alkanes in fossil *Cocculus* (a-b), *Magnolia* (c-d) and three genera of the coniferous family Taxodiaceae (e-h) collected from sites P-33 and P-35. The inset histogram in (g) shows the *n*-alkane distribution of modern *Metasequoia glyptostroboides*; note that concentrations of *n*-alkanes in this species are lower than most of the sediments in Fig. 4. Wide bars are mean concentrations with standard deviations indicated by I-bars (n = number of fossils analysed).

distributions, with CPI ranging between 3.5 and 5.0 (Table 3). Concentrations of *n*-alkanes in the five clay, fossil bearing, layers from the two sites were lower than in most of the fossils analysed, especially the red oak fossils from P-33 and P-35 (Fig. 2 cf. 4; Table 3). The *n*-alkane profile of Oviatt Creek sediment (P-35) resembled the *Clarkia* sediments except that C_{31} was the dominant component and the concentrations of *n*-alkanes were approximately half those measured in *Clarkia* sediments.

4.1.3. *n*-Alkanols in fossils

Aliphatic compounds also tended to dominate the alcohol fractions from fossils. *n*-Alkanol homologues between 22 and 34 carbons in length were the major constituents with a strong even/odd predominance in all cases. Histograms, and a summary of the prominent features of the fossil *n*-alkanol distributions are shown in Figs. 5 and 6 and Table 4, respectively. The *n*-alkanol concentrations were often greater than the *n*-alkanes in the same fossil specimen, with the exception of red oak fossils where *n*-alkanes showed exceptionally high concentrations in some cases (Tables 3 and 4).

4.1.4. Sedimentary *n*-alkanols

Sediment *n*-alkanol profiles (Fig. 4) showed marked differences between different horizons of the deposit. This was in contrast with the *n*-alkanes which showed a consistent distribution pattern between all layers. The *n*-alkanols in the Unit 4 ash layer were present at approximately one seventieth of the concentration of those in the clay layers. The sediments from horizon 2b of the P-33 deposit (the level at which most of the fossils analysed were uncovered) possessed a broad distribution with C_{30} *n*-alkanol in the greatest concentration. Oviatt Creek sediment (P-35) closely resembled the P-33 2b sample in *n*-alkanol composition but concentrations were approximately half those detected in the *Clarkia* P-33 sample.

4.2. Chemosystematic analyses of *n*-alkyl lipids

Figs. 2–6 show that it is possible to distinguish between related genera in some cases on the basis of their *n*-alkyl lipid distributions. The main features of these distributions are summarised in Tables 3 and 4, but in order to fully appreciate the extent of variation

Table 3
Characteristics of leaf *n*-alkane distributions in Clarkia leaf fossils and sediments

Fossil genus/species	Site/horizon and number of specimens	Mean carbon preference index (CPI) ^a	Average chain length (ACL) ^b	Total <i>n</i> -alkane concentration $\mu\text{g/g}_{\text{dw}}$ mean $\Sigma\text{C}_{21}\text{--C}_{35}$	Features of <i>n</i> -alkane distribution (e.g. relative abundances and type of distribution, 'narrow' = 2 or 3 dominant components, 'broad' = most of $\text{C}_{21}\text{--C}_{33}$ components are abundant)
<i>Quercus</i> sp.	P-33 2b evergreen oak (2)	4.1±0.7	29.5±0.3	270	Narrow, unimodal, C_{29} max. with C_{31} moderate in many cases (25–50% C_{29} concentration). Chain lengths $> \text{C}_{29}$ usually more abundant than those $< \text{C}_{29}$. Evergreen oaks gave different distribution (i.e. FL12 and FL23). Red oaks from P33 and P35 had very similar distributions.
	red oak (14)	4.9±2.0	29.1±0.2	710	
<i>Platanus dissecta</i>	P-35 red oak (3)	9.4±4.7	28.9±	1400	Broad, bimodal, maxima at C_{25} or C_{27} and C_{31} or C_{33} in most samples. A few showed narrower distributions with $\text{C}_{29}\text{--C}_{33}$ dominant.
	P-33 2b (12)	7.3±3.3	29.6±0.7	250	
<i>Pseudofagus idahoensis</i>	P-33 2b (19)	3.3±1.0	28.1±0.3	150	Broad, unimodal, C_{29} max., with C_{27} always high, chain lengths $< \text{C}_{29}$ more dominant than those $> \text{C}_{29}$.
<i>Fagus</i> sp.	P-33 2b (2)	3.0±0.2	28.0±0.2	160	Broad, unimodal, similar to <i>Pseudofagus idahoensis</i> with less C_{31} and more C_{25} .
<i>Cocculus</i> sp.	P-33 2b (4)	3.9±0.6	28.8±0.1	74	Narrow, unimodal, C_{29} max with C_{27} and C_{31} in similar abundances (ca. 25–33% of C_{29}).
	P-35(1)	3.0	28.9	54	
<i>Magnolia latahensis</i>	P-33 2b (14)	2.7±0.9	28.3±0.4	260	Medium-broad, unimodal, C_{29} max. with $\text{C}_{30} > \text{C}_{31}$ sometimes. Weaker samples showed relatively high C_{27} and C_{25} homologues. CPI lower than most other species.
	P-35(2)	2.8±0.7	28.8±0.2	140	
<i>Taxodium</i> sp.	P-33 2b (3) 5c (1)	2.1±0.4	28.5±0.4	78	Broad, quite variable, sometimes bimodal. C_{29} high in most samples but C_{25} sometimes major.
	P-35 (2)	2.2±0.3	29.4±0.1	54	
<i>Metasequoia</i> sp.	P-33 5c (1)	2.1	29.2	62	Broad, C_{29} max., with C_{31} and C_{25} also high.
<i>Sequoia</i> sp.	P-35 (2)	2.3±0.1	29.5±0.2	49	Broad, chain lengths between C_{25} and C_{31} abundant. C_{25} dominant in FT80.
Sediments	P-33 2b, 2d, 4 ash, 5a, 5c	P33 3.5 to 5.0 P-35 5.3	28.2 to 29.4	P-33 samples 60 to 74 1.8 (4 ash) 37 (P-35)	Sediments show broad distributions between C_{21} and C_{35} ; C_{max} is C_{29} for P-33 samples and C_{31} for P-35. Ash layer is low in concentration

^a CPI calculated from *n*-alkane concentrations according to $(2\Sigma_{\text{odd}} \text{C}_{21}\text{--C}_{35})/(\Sigma_{\text{even}} \text{C}_{20}\text{--C}_{34} + \Sigma_{\text{even}} \text{C}_{22}\text{--C}_{36})$

^b ACL calculated according to $(\Sigma c_i \times i)/\Sigma c_i$ where c_i is the concentration of the *n*-alkane containing *i* carbons (chain lengths $i = 21$ to 35).

CPI, ACL and total *n*-alkanes are mean values over all the samples examined with ± 1 s.d. indicated; < denotes an s.d. of less than 0.05.

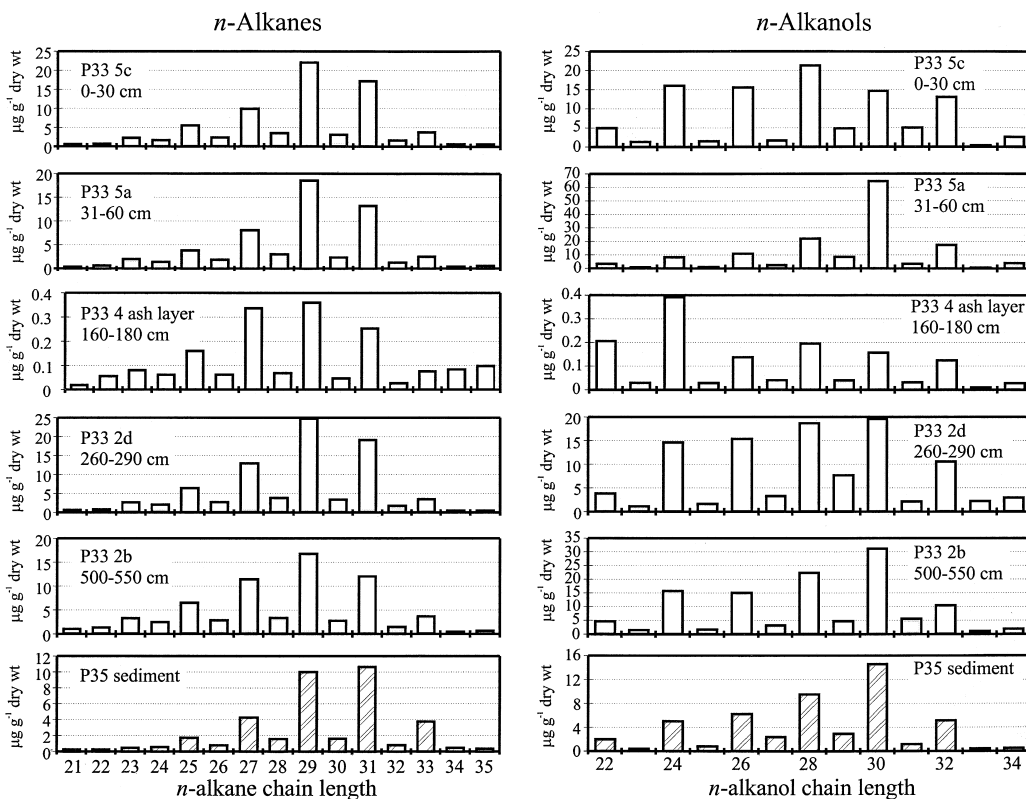


Fig. 4. Distributions of *n*-alkanes and *n*-alkanols in sediments at five different horizons of site P-33. Distribution profiles for sediment from the younger P-35 Oviatt Creek site is also shown (cross hatched). The majority of leaf fossils analysed were sampled from the 2b horizon.

within and between genera it is preferable to examine the individual distributions. Space constraints preclude the presentation of histograms for every sample [but see Appendices 5 and 6 of Lockheart (1987)]. A more robust means of exploring the relationships between the different samples is to apply principal component analysis (PCA) to the data set.

4.2.1. Principal component analyses of *n*-alkane and *n*-alkanol distributions

PCA was used to compare the relative abundance distributions of *n*-alkanes and *n*-alkanols for the fossils and sediments. The concentrations of *n*-alkyl lipid homologues obtained from 83 fossil and 6 sediment samples were subjected to two separate PCA analyses using:

- (i) *n*-alkanes using relative abundances of C_{21} – C_{35} *n*-alkane homologues (15 variables)
- (ii) *n*-alkanols using relative abundances of C_{22} – C_{32} even chain length *n*-alkanol homologues (6 variables).

Data were pre-treated by normalising to the most abundant compound in each distribution in order to

eliminate possible bias toward genera possessing high concentrations of lipid, e.g. *Quercus*. The odd chain length *n*-alkanols were not included in the analysis since they were minor components, with higher associated quantification errors. Only the first three principal component axes (PC1, PC2 and PC3) were determined in the two analyses since these accounted for more than 75% of the total variance within the respective data sets.

4.2.2. PCA of *n*-alkanes

The first three principal component axes accounted for 75.3% of the variance in the *n*-alkane data set, i.e. PC1 46.2%, PC2 17.9%, PC3 11.2%. Fig. 7(a–c) depicts the loadings for PC1, 2 and 3, respectively, and cross-plots of PC1 vs PC2 and PC1 vs PC3 component scores for the set of samples are presented in Fig. 7d and e. The loadings plot for PC1 (Fig. 7a) shows that this component reflects the abundance of C_{29} *n*-alkane vs other homologues. *Quercus* and *Cocculus* samples had the most negative scores of the sample set (Fig. 7d and e; y axis) as a result of their *n*-alkane distributions being dominated by the C_{29} compound (Figs. 2 and 3). *Platanus* fossils also showed negative scores due to the moderate abundance of the C_{29} homologue in their

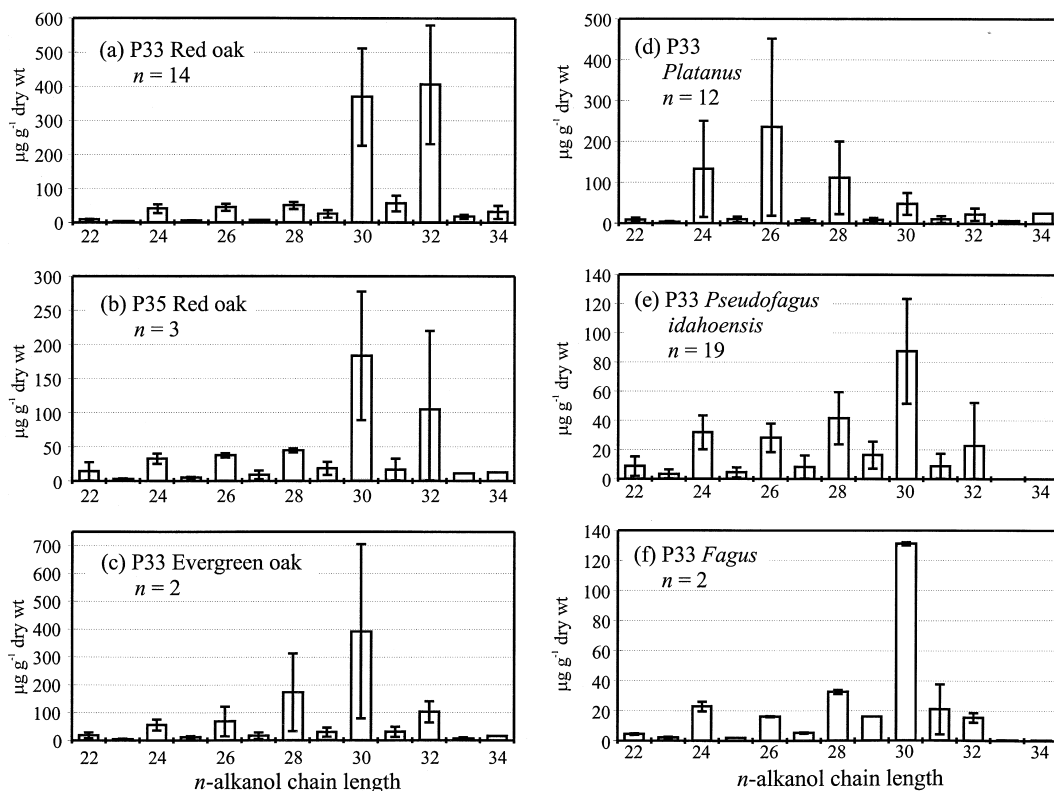


Fig. 5. Mean distributions of n -alkanols in four *Clarkia* fossil genera. (a) and (b) show red oak fossils from sites P-33 and P-35 and (c) shows evergreen oaks from P-33. (d) *Platanus dissecta* showed a distinct distribution with a maximum at C_{26} n -alkanol whereas (e) *Pseudofagus* and (f) *Fagus* specimens maximised at C_{30} . Wide bars are mean concentrations with standard deviations indicated by I-bars (n = number of fossils analysed).

distributions. The loadings plot for PC2 (Fig. 7b) shows a mid chain length (C_{26} – C_{30} ; positive scores) vs odd long chain (C_{31} , C_{33} , C_{35} ; negative scores) component relationship. The majority of *Platanus* fossils scored highly against this component axis (Fig. 7d; x axis), as they were the only fossils of the nine genera analysed that displayed a mid and long-chain length bimodal distribution of n -alkanes with a high CPI (Fig. 2). The loadings plot for PC3 (Fig. 7c) shows a component relationship of C_{21} , C_{22} , C_{34} and C_{35} n -alkanes versus most other chain lengths. The conifers *Taxodium*, *Metasequoia* and *Sequoia* were separated from other genera against this component (Fig. 7e; x axis) as they displayed very broad distributions of n -alkanes, with relatively greater abundances of short and long chain length compounds compared with the other genera examined (Fig. 3).

The crossplots of scores against two component axes (Fig. 7d and e) revealed groupings for *Quercus*, *Platanus* and *Cocculus*. *Quercus* specimens formed a tight group on the PC1 vs PC2 plot but with five of the nineteen samples analysed scattered elsewhere on the plot, speci-

fically, evergreen oaks FL12 and FL23 and three P-33 red oaks FQR9, 22 and 23 (Table 2). The similarity in the distributions of *Cocculus* and *Quercus* can be seen. *Platanus* samples were well separated from the other genera by PC2, and the three samples of this genus that showed unusual distributions, FPR14, 26 and 38 plotted in different regions from the majority (Fig. 7d, FPR14 and FPR38 are depicted in histograms 'E' and 'H', respectively in Fig. 8). *Magnolia* and *Pseudofagus* specimens were, on the whole, not very well separated against any component axis due to the moderate variation in the distributions of examples of these two genera. However, there was a group of six *Magnolia* fossils that plotted apart from the other specimens against PC2 (circled in Fig. 7d). These six examples had a subtly different n -alkane distribution from the other *Magnolia* specimens where abundances of $C_{30} > C_{31}$ (compare histogram 'A' for FM54 with 'B' for FM50 in Fig. 8). The similarity of the P-33 sediments 5a, 5c and 2d is emphasised by the close proximity in which they plot on Fig. 7d. P-33 2b, P-35 and ash layer 4 plot apart from the others (due to subtle differences in their distributions;

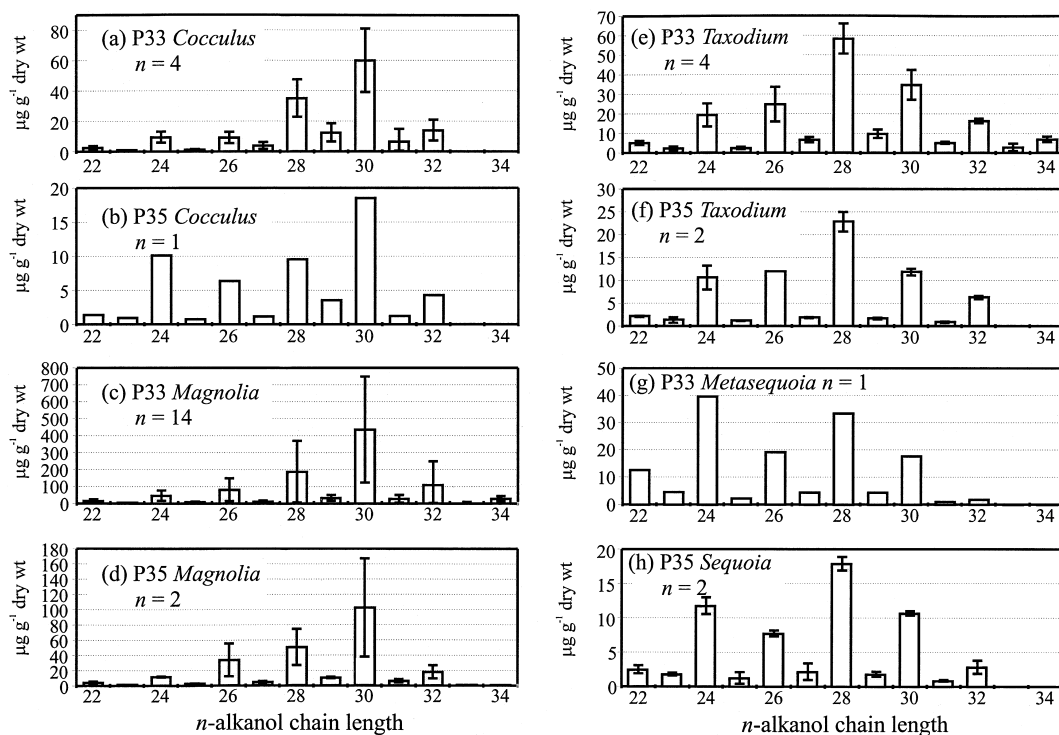


Fig. 6. Mean distributions of *n*-alkanols in fossil *Cocculus* (a-b), *Magnolia* (c-d) and three genera of the coniferous family Taxodiaceae (e-h), from sites P-33 and P-35. Wide bars are mean concentrations with standard deviations indicated by I-bars (*n* = number of fossils analysed).

Fig. 4). However, only the ash layer 4 plots apart from the others on the basis of scores against PC3 (Fig. 7e).

4.2.3. PCA of *n*-alkanols

The first three principal component axes accounted for 79.7% of the variance in the *n*-alkanol data set, i.e. PC1 46.2%, PC2 20.1%, and PC3 13.4% (Fig. 9). The loadings plot for PC1 (Fig. 9a) clearly shows that it represents the variance of C₃₀ and C₃₂ *n*-alkanols (negative loadings) vs shorter chain length *n*-alkanols (positive loadings). Consequently, many of the red oak fossils scored negatively against this component since the C₃₀ and C₃₂ homologues were typically dominant in this genus (Fig. 5a and b). The loadings plot for PC2 (Fig. 7b) shows that this component reflects the dominance of the C₂₆ *n*-alkanol (negative loading) over all other homologues (positive loadings). Most of the *Platanus* fossils displayed distributions that maximised at C₂₆ (Fig. 5d), and as a result fossils of this genus tended to show negative scores against PC2 (Fig. 9d). There were three exceptions, samples FPR14, 26 and 38, that had scores in a different range from the rest of the *Platanus* specimens. These were the same three samples that separated from the majority of the other *Platanus*

specimens, when PCA was applied to the *n*-alkanes (Fig. 7e). The third component PC3 (Fig. 9c) is more complicated and shows negative loadings of C₂₂, C₂₈ and C₃₀ *n*-alkanols vs positive loadings of C₂₄, C₂₆ and C₃₂ homologues. The pattern in the distributions that this component represents is difficult to see clearly by examination of the individual histograms (Lockheart, 1997; Appendix 6e–h). However, the majority of specimens of *Quercus* were distinguishable from samples of other genera on the basis of their scores against this component (Fig. 9e).

The crossplot of PC1 vs PC2 scores of fossil and sediment distributions was particularly effective in showing the distinctions that exist between the distributions of different fossil genera. However, the oak fossil specimens were more scattered on these plots for the *n*-alkanols than for those of the *n*-alkanes (Fig. 7 cf. Fig. 9). FL12, FL35 and FQR9 were the furthest away from the main group. Many of the groupings that were apparent in the *n*-alkane analysis were also evident in the *n*-alkanol analysis. For instance, the six *Magnolia* fossils that had slightly different *n*-alkane distributions (C₃₀ > C₃₁) and formed a distinct group in Fig. 7d, were also grouped apart from

Table 4
Characteristics of *n*-alkanol distributions in *Clarkia* leaf fossils and sediments

Fossil genus/species	Site/horizon and number of specimens	Mean carbon preference index (CPI) ^a	Average chain length (ACL) ^b	Total <i>n</i> -alkanol concentration μg/g _{dwt} mean ΣC ₂₂ –C ₃₄	Features of <i>n</i> -alkanol distribution (e.g. relative abundances and type of distribution, ‘narrow’ = 2 or 3 dominant components, ‘broad’ = most of C ₂₂ to C ₃₀ components are abundant)
<i>Quercus</i> sp.	P33 2b evergreen oak (2)	8.2±2.0	29.1±0.1	900	Narrow, unimodal distributions in general. P33 red oaks had C ₃₀ or C ₃₂ max., often both were abundant. Chain lengths < C ₃₀ were present at ca. 10% abundance of C _{max} and components > C ₃₂ were minor. P35 red oaks FL34, FL36 and P33 evergreen oak FL23 were very similar with C ₃₀ max. and other homologues < 50% C ₃₀ . FL12 resembled P33 red oaks.
	red oak (14)	9.4±2.1	30.3±0.4	1000	
	P35 red oak (3)	10.4±4.3	28.4±0.3	320	
<i>Platanus dissecta</i>	P33 2b (12)	16.0±7	27.0±1.1	590	Broad, unimodal, nine fossils showed distributions with C ₂₆ (or C ₂₄) max. with declining concentrations of longer homologues. Three fossils showed different distribution (as was true for their <i>n</i> -alkanes also), i.e. FPR14 and FPR26 were bimodal, C ₃₀ max., with abundant C ₂₈ , FPR26 had C ₃₀ max.
<i>Pseudofagus idahoensis</i>	P33 2b (19)	6.8±2.2	28.2±0.5	260	Broad, bimodal, C ₃₀ max., with C ₂₄ often higher in concentration than C ₂₆ , chain lengths < C ₃₀ much more dominant than those > C ₃₀ .
<i>Fagus</i> sp.	P33 2b (2)	5.8±2.1	28.9±0.1	270	Broad, unimodal, similar to <i>Pseudofagus idahoensis</i> with more C ₃₀ .
<i>Cocculus</i> sp.	P33 2b (4)	6.7±1.8	28.6±0.4	150	Narrow-medium, unimodal, C ₃₀ max. with C ₂₈ ca. 50–75% abundance of C ₃₀). C ₂₆ and C ₂₄ featured, often with C ₂₄ slightly > C ₂₆ . P-35 specimen (FC37) similar to P-33 samples but showed C ₂₄ > C ₂₈ .
	P35 (1)	6.8	27.5	57	
<i>Magnolia latahensis</i>	P33 2b (14)	12.3±5.4	28.6±1.0	880	Two distinct unimodal distributions: (a) stronger samples – C ₃₀ max. with C ₂₈ and C ₂₆ at 25–50% C _{max} and other homologues minor, (b) weaker samples – C ₃₀ max. with C ₂₈ and C ₃₂ other major homologues variable. The two P-35 samples resembled P-33 samples, i.e. FM64 type (a), FM1 type (b).
	P35 (2)	10.1±4.7	28.7±	240	
<i>Taxodium</i> sp.	P33 2b (3) 5c (1)	6.1±1.3	28.2±0.2	190	Broad, unimodal. generally symmetrical about max. of C ₂₈ . Two samples showed C ₂₄ slightly > C ₂₆ .
	P35 (2)	10.1±1.7	27.5±0.2	72	
<i>Metasequoia</i> sp.	P33 5c (1)	7.7	26.2	140	Broad, bimodal, C ₂₄ max. with C ₂₈ also high.
<i>Sequoia</i> sp.	P35 (2)	7.6±2.0	27.1±0.1	60	Broad, like <i>Metasequoia</i> sp. but C ₂₈ > C ₂₄ .
Sediments	P-33 2b, 2d, 4 ash 5a, 5c	4.8 (2d) to 8.3 (5a)	26.4 to 29.1	P-33: 100 to 140 1.4 (4 ash) 50 (P-35)	Broad distributions between C ₂₂ and C ₃₄ ; concentrations are more variable. C ₃₀ is max. for all except 4 ash

^a CPI was calculated from *n*-alkanol concentrations according to $(2\Sigma_{\text{even}} C_{22}\text{--}C_{34})/(\Sigma_{\text{odd}} C_{21}\text{--}C_{33} + \Sigma_{\text{odd}} C_{23}\text{--}C_{35})$.

^b ACL was calculated according to $(\Sigma c_i \times i)/\Sigma c_i$ where c_i is the concentration of the *n*-alkanol containing i carbons (chain lengths $i = 22\text{--}34$). CPI, ACL and total *n*-alkanols are mean values over all the samples examined with ±1 s.d. indicated; < denotes an s.d. of less than 0.05.

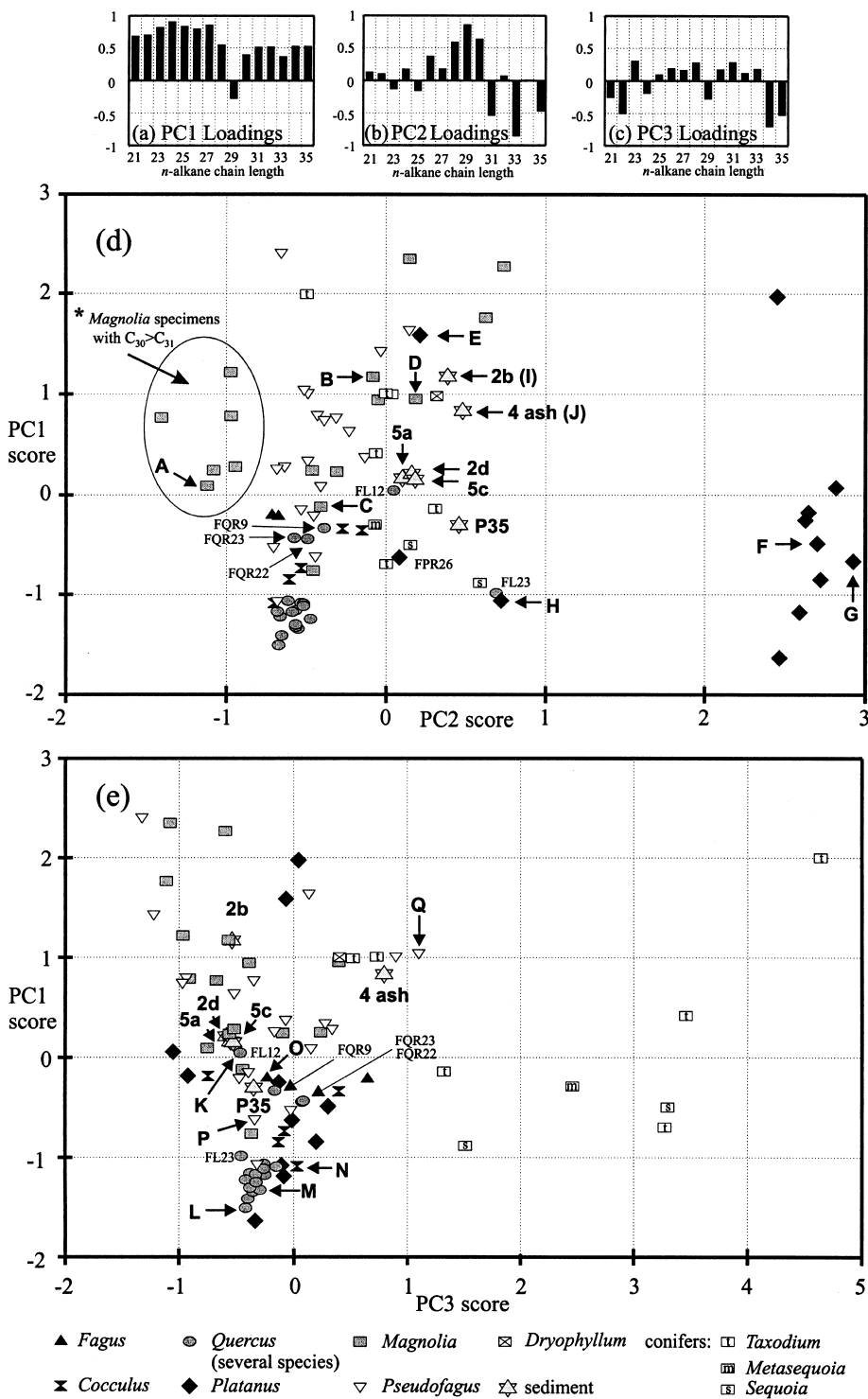


Fig. 7. Plots of (d) PC1 vs PC2, and (e) PC1 vs PC3, for scores from PCA of relative abundance distributions of *n*-alkanes. *Quercus* fossils formed a tight grouping against all three components. Evergreen oaks FL12 and FL23, and red oaks FQR9, 22 and 23 plotted apart from this main group. *Platanus* fossils and *Magnolia* specimens with $C_{30} > C_{31}$ *n*-alkane (circled) were well separated according to PC2. Component loadings for PC1, 2 and 3 are depicted as histograms above the scores plots (a)–(c). Specimens A–Q are described in the text and depicted in Fig. 8. N.B. The ellipse around these six samples is for clarity only, and does not have any statistical significance.

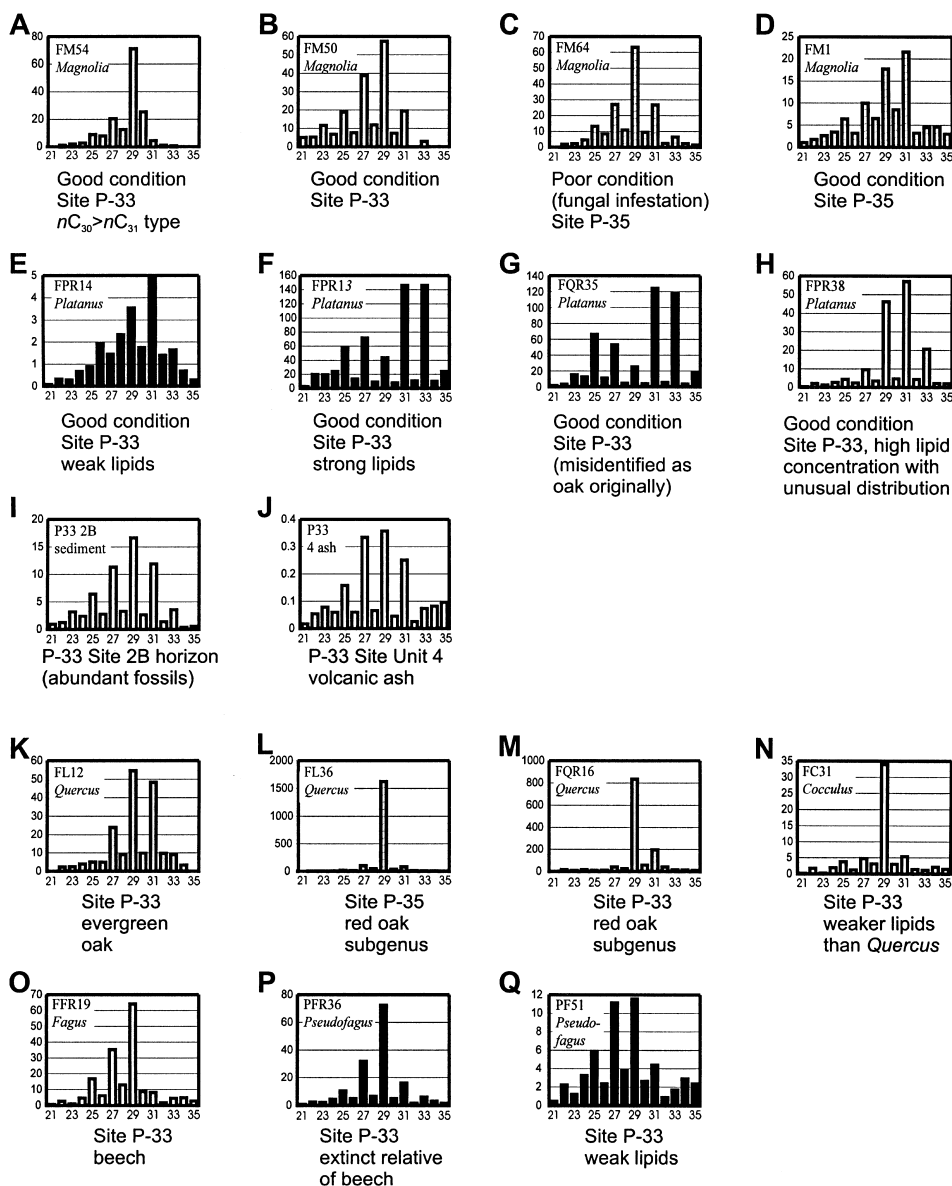


Fig. 8. *n*-Alkane distributions for selected specimens (labelled on Fig. 7) x axis = chain length, y axis = $\mu\text{g g dry wt}^{-1}$ alkane. Specimens were selected to represent a range of different genera, sites, lipid concentrations and physical conditions. Note that: (1) lipid preservation is independent of morphological condition (B cf. C); (2) specimens from sites P-35 and P-33 can show similar distributions (L cf. M; C cf. B); (3) Fagaceae genera can show similar (O cf. P) or very different (M cf. P) distributions; (4) red oaks were distinct from evergreen oaks (M cf. K); (5) in one case genera of different families showed similar distributions, e.g. N cf. M- *Cocculus* sp. cf. *Quercus* sp. (red).

the other *Magnolia* specimens by PC3 for the *n*-alkanol analysis (circled on Fig. 9e). The sediment samples displayed a greater degree of variation in the *n*-alkanol analysis than in the *n*-alkane analysis. In particular, the sediment from the 5a layer did not plot closely to the 5c and 2d samples as was seen in the *n*-alkane analysis.

5. Discussion

5.1. Overview of chemotaxonomy using fossil and modern leaf lipid compositions

The lipid profiles depicted in Figs. 2–6 show that plant *n*-alkanes and *n*-alkanols are preserved in individual

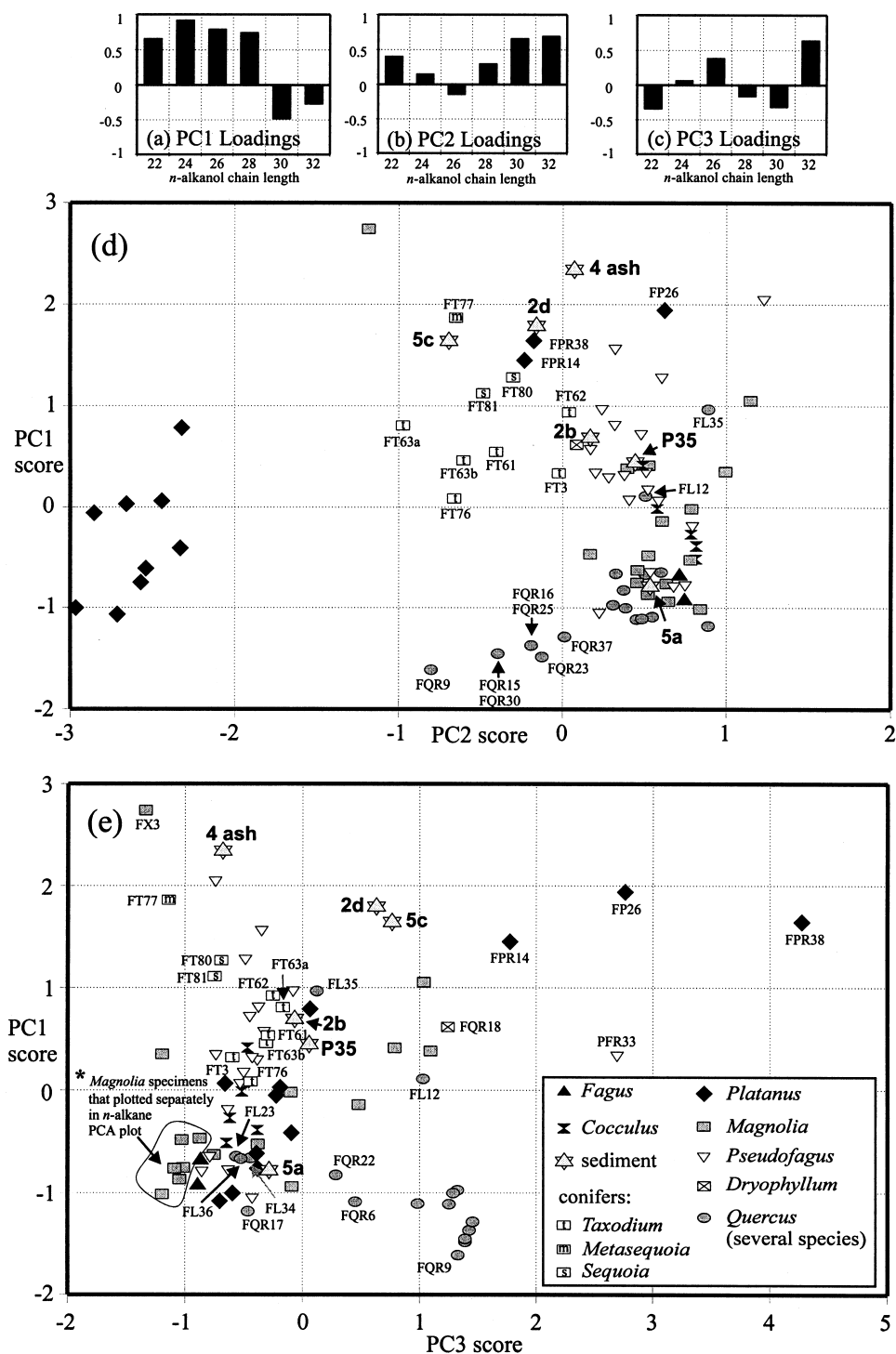


Fig. 9. Plots of (d) PC1 vs PC2 and (e) PC1 vs PC3, for scores from PCA of relative abundance distributions of *n*-alkanols. *Platanus* fossils were clearly separated from other fossil genera by their PC2 scores. Conifer specimens were partially separated from the main group, against PC2 and showed trends within the grouping, i.e. specimens of the same genus and site plotted close to one another. *Quercus* fossils were more dispersed, and there were some similarities with the trends observed in the PCA scores plots for *n*-alkanols. *Magnolia* fossils also showed similar groupings as in Fig. 7. Component loadings for PC1, 2 and 3 are depicted as histograms above the scores plots (a–c). *N.B. The loop around these six samples is for clarity only, and does not have any statistical significance.

specimens of different fossil genera after ca. 17–20 million years of burial. Distinctive distribution patterns were observed between some genera and, in certain cases, significantly higher concentrations than the enclosing sediment, were also apparent although others showed a degree of mixing with sedimentary lipids. As a result, the potential exists for exploiting these lipid distributions for chemotaxonomic purposes and palaeoenvironmental reconstruction.

The possibility of using the distributions of long chain *n*-alkyl lipids of epicuticular waxes (for reviews see Tulloch, 1976; Baker, 1982; Walton, 1990) as a chemosystematic tool to distinguish between different modern plant genera was first explored by Eglinton (1962) in their investigation of the Sempervivoideae. These early attempts have been repeated in many subsequent investigations (e.g. Dyson and Herbin, 1968; Osborne et al., 1989; Maffei, 1994; Mimura et al., 1998; Skorupa et al., 1998). Several of these studies have shown that different species of the same genus can show similar *n*-alkyl lipid distributions and that it is sometimes possible to distinguish between taxa of different genera belonging to the same family (especially if they differ morphologically). However, Herbin and Robins (1969) noted that the distribution patterns of leaf lipids are not always adequate to differentiate between taxa even at the genus level, especially when examining closely related genera of the same family. Their observation will also apply to the fossils examined from *Clarkia*, i.e. not all of the fossil genera will be expected to be chemically distinct in lipid composition.

This study examined a range of genera thus permitting comparisons to be made between members of the same family and between taxa which are not closely related. For instance, two fossil genera of the family Fagaceae which share a similar morphology have been examined, i.e. *Fagus* and *Pseudofagus* (Smiley and Huggins, 1981) as well as a member of the Fagaceae family which does not share similar morphological characteristics, i.e. *Quercus* sp. Also analysed were specimens of *Taxodium* sp., *Metasequoia* sp. and *Sequoia* sp.; three coniferous genera of the family Taxodiaceae, which showed morphological similarity.

The possibility of diagenetic alteration of the original leaf lipids in the sediments might be expected to complicate the chemosystematic study of fossils. The fact that lipid concentrations were higher in most fossils than the enclosing sediments and that several genera showed very distinctive distribution patterns (especially *Quercus* and *Platanus*) is compelling evidence that chemosystematic analyses are possible for certain taxa from this locality. However, in specimens where *n*-alkyl lipid levels were low in the leaf, contributions of sedimentary lipids may obscure the plant signal.

The results of this study provide the opportunity to make chemosystematic comparisons of fossils using

distributions, concentrations and principal component analyses on a number of levels, such as between: (i) fossils of different genera; (ii) specimens of the same genus; (iii) subdivisions of a single genus, e.g. red oaks cf. evergreen oak fossils; and (iv) several genera belonging to the same family, e.g. Taxodiaceae and Fagaceae families.

5.2. Comparisons between *Clarkia* sediments and fossils

5.2.1. *n*-alkyl lipid distribution patterns

Many of the nine fossil genera examined in this study showed distinct distributions of *n*-alkyl lipids from the enclosing sediments (Fig. 4) and other genera (fossil *n*-alkanes Figs. 2 and 3, fossil *n*-alkanols Figs. 5 and 6). In some cases the distributions were very distinctive, e.g. the bimodal *n*-alkane distribution of *Platanus* (Fig. 2d) or *n*-alkane and *n*-alkanol distributions of red *Quercus* specimens (Figs 2a and b and 5a and b). *Pseudofagus* fossils shared clear similarities with the lipid distribution of the 2b sediment from which they originated. This could arise from the lipids in the original leaf being obscured by sedimentary components. However, since the mean ($n = 19$) total *n*-alkane concentrations in *Pseudofagus* were found to be approximately double (150 $\mu\text{g/g}$) that of the most concentrated sediment (range 60–74 $\mu\text{g/g}$; Table 3) this resemblance to the sediment appears coincidental. In fact, a number of living Asian *Fagus* species (*F. grandifolia*, *F. japonica*, *F. orientalis*) which resemble *Pseudofagus idahoensis* display an analogous pattern of *n*-alkanes to the fossil species (see inset Fig. 2e for distribution of modern *F. grandifolia*; Lockheart, 1997).

A sedimentary lipid contribution will always be evident in any fossil although this will be negligible in the most lipid-rich specimens. The background of *n*-alkanols contributed to fossils from the sediments will vary according to where the fossil was sampled in the stratigraphic column. In contrast to the *n*-alkanes, the distribution of *n*-alkanols was shown to vary throughout the deposit (Fig. 4) probably as a result of input or productivity changes in the lake through time.

5.2.2. Comparisons between genera on the basis of *n*-alkyl lipid concentration

The concentrations of *n*-alkyl lipids in the fossils were in most cases higher than those detected in the sediments (with the exception of the coniferous genera *Taxodium*, *Metasequoia* and *Sequoia*). However, it is not unexpected that the concentrations of lipids in sediments expressed in $\mu\text{g/g}$ dry weight are lower than the fossils since the organic carbon content of the sediment matrix is lower due to inorganic diluents. It would have been preferable to express lipid concentration relative to total organic carbon, however, the paucity of fossil tissue precludes this measurement being undertaken routinely.

In situations where different fossil genera shared similar distribution patterns of lipids, e.g. *n*-alkanes of *Quercus* (red oaks; Fig. 2a and b) and *Cocculus* (Fig. 3a) the concentrations were sometimes sufficiently different to distinguish between the two genera. For instance, the concentrations of *n*-alkanes were considerably higher in *Quercus* than *Cocculus* fossils (mean total *n*-alkane concentrations for P-33 specimens, 710 and 74 $\mu\text{g}/\text{g}_{\text{dw}}$, respectively; Table 3) despite their similar distributions. However, these two genera were readily separable on the basis of their *n*-alkanol distributions (and *n*-alkanol concentrations), i.e. red *Quercus* sp. were dominated by C_{30} and C_{32} whereas *Cocculus* showed a unimodal trend which maximised at C_{30} (Fig. 5a and b cf. Fig. 6a). In certain cases, e.g. Taxodiaceae genera, the *n*-alkanol homologues were present in significantly higher concentration than the *n*-alkanes; the latter may have been present at lower concentrations on the original leaf.

Most of the other genera examined in this study showed similar ranges of concentrations and, hence, for these taxa concentration would not be a useful parameter for comparisons (Tables 3 and 4).

With the exception of a few of the *Quercus* fossils the concentrations of *n*-alkanols were higher than the *n*-alkanes from the same specimen. *n*-Alkanols also tend to be higher in concentration than *n*-alkanes in modern leaves although this is dependent on species (Gülz et al., 1989; Rieley et al., 1991; Gülz and Müller, 1992). It is likely that some of the *n*-alkanols present in the fossils derive from the hydrolysis of wax esters that were originally present in the epicuticular waxes (Rieley et al., 1991). The fact that these compounds were not detected when the DCM fraction from several of the fossils was analysed by high temperature-GC reinforces this assertion (Lockheart and Evershed, unpublished results). *n*-Alkanols will also have been contributed from algal remains and debris from other lacustrine organisms in the encroaching sediments (Meyers and Ishiwatari, 1993). However, for the majority of fossils, i.e. those from the 2b horizon, the concentrations of the *n*-alkanols were appreciably higher than those of the enclosing sediments (total *n*-alkanols: 2b sediment 120 $\mu\text{g}/\text{g}_{\text{dw}}$, fossils 140 to 1000 $\mu\text{g}/\text{g}_{\text{dw}}$ Table 4). Hence, the contributions from exogenous sources have minimal impact on the preserved lipid profiles.

5.2.3. Principal component analyses

PCA treatment of the relative abundance distributions of *n*-alkyl lipids from the fossils emphasised the differences between genera, and individual specimens. The technique was most effective when applied to specimens of *Quercus* and *Platanus* due to their characteristic distribution patterns. However, the technique was not universally successful in separating some genera of different families, for instance *Magnolia* (Magnoliaceae) and *Pseudofagus* (Fagaceae) showed a certain degree of similarity in their distributions and accordingly are

intermingled on the PCA scores plots (especially *n*-alkanes; Fig. 7d and e). The Taxodiaceae family members displayed a wide range of scores on the *n*-alkane plots, probably due to the low levels of lipid originally present in leaves of these specimens (based on concentrations in modern *M. glyptostroboides*, see inset in Fig. 3g) and the correspondingly greater influence of sedimentary lipids. Although differences in epicuticular composition between members of the same family cannot be discounted (Borges de Castillo et al., 1967), when PCA was applied to the *n*-alkanol distributions of these coniferous taxa the specimens tended to group according to genus and site (Fig. 9d and e).

The similarity between the distributions of the sedimentary *n*-alkanes is borne out by the proximity in which they cluster on the scores plots (Fig. 7d and e). Conversely, the lack of uniformity in the *n*-alkanol distributions through the stratigraphic column can be seen by greater scattering of data points on the scores plots in Fig. 9. The changes in concentrations of the shorter chain length *n*-alkanols (C_{24} and C_{26} ; Walton, 1990) relative to their longer chain counterparts (C_{28} and above; Collister et al., 1994) through the deposit may reflect variations in the size and types of communities of contributing organisms within the lake and the vegetation catchment area due to the changing environment. Further differences in lipid distributions may result from compounds contributed by nearby plants but for which fossil remains are rarely preserved as a consequence of taphonomic bias, e.g. grasses and ferns.

5.3. Comparisons between specimens of the same genus: distributions

The variability in concentration was sometimes quite large between specimens of the same genus for *Quercus* sp., *Magnolia latahensis* and *Pseudofagus idahoensis*, as can be seen in the large standard deviations indicated by I-bars on Figs. 2–6. However, the distribution patterns were, in most cases, consistent between examples of these fossil species (Appendices 5 and 6, Lockheart, 1997). Hence, the large standard deviations that were apparent in the mean histograms for some of the species mainly reflect changes in overall concentration of lipids (affecting all homologues in proportion) rather than variability of the distribution patterns themselves (PCA provides a better measure of this as it was performed on relative abundances).

In a few cases where concentrations of lipids in the fossils were low the characteristic distribution patterns were lost; this was often accompanied by a reduced CPI, e.g. FM1 *Magnolia*, FPR14 *Platanus* and PF51 *Pseudofagus* (labelled D, E and Q, respectively, in Fig. 8). Furthermore, these 'weak' samples tended to show prominent even carbon number *n*-alkanes at short and long chain lengths. Interestingly, several other fossil specimens

showed considerable deviation from the typical distribution despite exhibiting high lipid concentration. For example, two of the *Platanus* samples analysed (FPR38 and FPR26) showed quite different distributions from most with C_{29}/C_{31} dominant rather than the more usual C_{31}/C_{33} . This can be seen by comparing *Platanus* histograms F (FPR13) and G (FQR35) with H (FPR38) in Fig. 8. The *n*-alkanol distributions of the FPR38 and FPR26 specimens were also found to be distinct from the other taxa examined which suggests that they might in fact be specimens of a different genus (and family?) altogether which resembles *Platanus* in appearance.

In those specimens where deviations from the mean distribution were significant, the identification of the fossil in question was verified using photographs, or remnants of the fossils. In this way, two of the ‘oak’ fossils were found to have been misclassified, i.e. one ‘oak’ was found to be a fragment of a large *Platanus dissecta* leaf (FQR35 labelled G in Fig. 8), while the identity of a second fossil (FQR17) was revised to a *Dryophyllum* sp. fossil (Q. Leng, personal communication).

5.3.1. Comparisons between specimens of the same genus: PCA

As mentioned above, certain fossils for which the absolute concentration of lipids were low possessed distributions which deviated from the mean distributions (as depicted in Figs. 2–6). Such specimens were also separated from other specimens of the same type by PCA in Figs. 7 and 9, e.g. FM1, FPR14 and PF51. Histograms of these are depicted in Fig. 8 (labelled D, E, and Q) alongside specimens of the same genus which exhibited higher lipid concentrations and more typical distributions (D cf. A–C; E cf. F, G; and Q cf. P).

5.3.2. Distributional variations between *Magnolia* specimens

The 14 specimens of *Magnolia* that were collected from the P-33 site showed two subtly different *n*-alkane distribution patterns (Fig. 8 histogram A cf. B and C), yet morphologically the fossils seemed to represent a single species. Six of the specimens from the P-33 site (FX4, FX6, FM42, FM54, FMR4, FMR32) showed an unusual feature in that the C_{30} *n*-alkane was higher in concentration than the *n*- C_{31} homologue (Fig. 8 histogram A; Lockheart, 1997, Appendix 6c). The *n*-alkanol distributions for this group of six fossils also showed slight differences from the other *Magnolia* samples (Lockheart, 1997; Appendix 6g). As a consequence, these specimens formed distinct groups on the PCA scores plots for the *n*-alkane and *n*-alkanol analyses, respectively (circled in Figs. 7d and 9e). The other eight *Magnolia* fossils had the more usual odd predominance over the whole *n*-alkane distribution (e.g. Fig. 8 histograms B and C). The cause of these differences is uncertain but

they may reflect: (i) local variations in the conditions for preservation within the sediment; (ii) the presence of more than one species of *Magnolia* in the fossil assemblage; (iii) phenotypic diversity between trees of the same species which may lead to small variations in the distribution of the original leaf waxes, or (iv) the canopy position from which the leaf originated. Sun and shade leaves from the same tree can show quite different *n*-alkane distributions and concentrations, e.g. in *Quercus pyrenaica* and *Quercus robur* (Lockheart, 1997).

5.4. Distinctions between subdivisions of the genus *Quercus*

The majority of oak fossils were remarkably consistent in composition of *n*-alkyl lipids, especially the *n*-alkanes. The Clarkia P-33 samples were dominated by one or more species that have affinities with modern deciduous red oak species (subgenus erythrobalanus), such as aristate margins. These species are associated with floodplain-slope type vegetation (Smiley and Rember, 1985a). Two examples of ‘evergreen’ fossil oak species were identified (FL12 and FL23) which are usually associated with swamp-type vegetation (Smiley and Rember, 1985a and b) and these, as expected, displayed different *n*-alkane distributions compared with the more common deciduous red oaks. The *n*-alkanol distributions of these two specimens also differed from the P-33 red oaks but, coincidentally, resembled the distributions from three samples of a potentially different species of red oak from the younger P-35 site (FL34, FL35, FL36). It is noteworthy that the red oak lipid profiles are quite different from some living species of white oak (subgenus leucobalanus) (e.g. *Quercus robur*, Prasad and Gülz, 1990, Rieley et al., 1991). Furthermore, several living species of red oak have been shown to have very similar distributions to the Clarkia fossils. The distribution of modern *Q. marilandica* is inset in Fig. 2a and is similar to that of the fossils (along with *Q. rubra* and *Q. negra*; Lockheart, 1997). A detailed analysis of a large number of living examples of the Fagaceae family has been performed and this data will be compared with Clarkia fossils in a forthcoming paper.

5.5. Comparisons between several genera of the same family

5.5.1. Taxodiaceae family

The nine fossil samples representing three different genera of this family provided further opportunity to examine the chemotaxonomic relationships between related taxa. Slight morphological differences in the form of the stems and leaves exist between the three genera facilitating their identification in the fossil assemblage at Clarkia (see Fig. 10 for photographs). The *n*-alkane concentrations in all three of these genera

were low (Table 3 and Appendix 5a, Lockheart 1997; C_{\max} between 8.8 and 21 $\mu\text{g}/\text{g}_{\text{dw}}$) and in the same range as sedimentary *n*-alkanes. While C_{29} or C_{31} *n*-alkanes formed the dominant homologues in the conifer specimens the C_{22} , C_{24} and C_{34} *n*-alkanes were also prominent in their distribution profiles. The fossil bearing sediments had concentrations of the dominant *n*-alkane (either *n*- C_{29} or *n*- C_{31}) of 17 $\mu\text{g}/\text{g}_{\text{dw}}$ (C_{29} , P-33 2b), 22 $\mu\text{g}/\text{g}_{\text{dw}}$ (C_{29} , P-33 5c) and 11 $\mu\text{g}/\text{g}_{\text{dw}}$ (C_{31} , P-35). Hence, lipids derived from the enclosing sediments may make a significant contribution to the fossil distributions, in this instance. The low concentrations of *n*-alkanes in the fossil Taxodiaceae were consistent with the levels detected in leaves of a modern *Metasequoia glyptostroboides* (dawn redwood) sampled from Westonbirt Arboretum, Gloucestershire, UK (*n*-alkane distribution depicted in inset of Fig. 3g). This modern species had a broad distribution that maximised at C_{25} . Closer examination of the fossil distribution for the sole *Metasequoia* fossil FT77 from P-33 horizon 5c (Fig. 3g) reveals that it may indeed show a distribution similar to modern *M. glyptostroboides* (Fig. 3g inset) overlaid with the P33-5c sedimentary profile (Fig. 4). It is possible that the other two related genera (*Taxodium* and *Sequoia*) will share similar distributions to the modern *Metasequoia* although

other members of the Taxodiaceae (*Cryptomeria* and *Cunninghamia*) have been reported with differing distributions (Borges de Castillo et al., 1967). Hence, the lipid distributions of fossils of these two taxa may also take the form of a composite of a leaf lipid profile (maximising at C_{25} *n*-alkane) above a sedimentary background (maximising at C_{29} or C_{31}). The specimens of Taxodiaceae were well separated from other genera according to PC3 which reflected the prominent long and short chain homologues (Fig. 7c and e). Variation in the extent of contributions from exogenous (sediment-derived) *n*-alkanes may account for the lack of clear trends in PCA scores between Taxodiaceae specimens (e.g. Fig. 7e).

Approximately 40% of all the fossil remains identified in the upper 5a and 5c layers of the Clarkia P-33 sediments were *Taxodium* with ca. 5% *Metasequoia* and *Sequoia* leaves (Smiley and Rember, 1985a), the lipid profile of these layers is similar to that of the less conifer-rich Unit 2 sediment (Fig. 4). A *Taxodium* rich deposit in the Weissenloster Basin, Germany also showed a distribution pattern of *n*-alkanes which was dominated by C_{29} (maximum) and C_{31} *n*-alkane (Otto et al., 1994).

In contrast, the *n*-alkanols in the Taxodiaceae fossils were present at higher concentration than in the enclosing

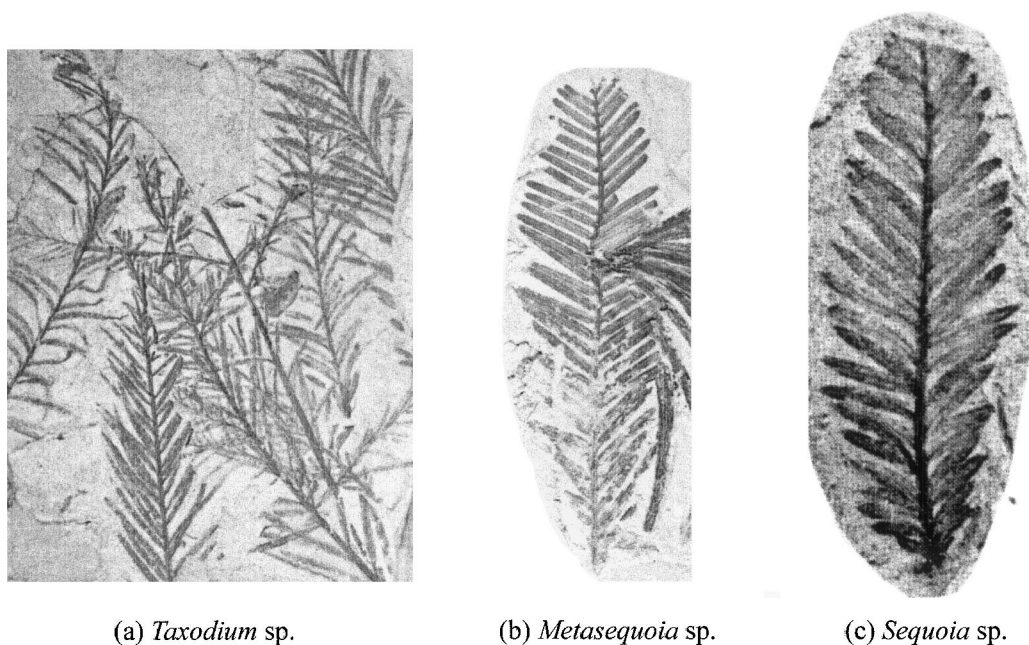
(a) *Taxodium* sp.(b) *Metasequoia* sp.(c) *Sequoia* sp.

Fig. 10. Examples of genera of the family Taxodiaceae commonly found in Clarkia (P-33) and Oviatt Creek (P-35) sediments: (a) *Taxodium* (like modern swamp cypress of SE USA), (b) *Metasequoia* (relative of modern dawn redwood of China), and (c) *Sequoia* (resembles modern coast redwood of Oregon). Subtle differences in the morphology of the branchlets enable their taxonomic identification. *Taxodium* and *Metasequoia* differ in that *Taxodium* has a more acute angle between leaves and the shoot, and the leaves are slightly offset rather than paired, as is the case for *Metasequoia*. *Sequoia* leaves are arranged in two offset rows along a more robust shoot. Photographs are enlarged to emphasise detail. Photographs are reproduced with permission from the Clarkia guidebook and roadlog (Smiley and Rember, 1979).

sediments. As a result, the distributions were more consistent and showed clear differences from the sediments. The plot of PC1 vs PC2 scores from PCA of *n*-alkanols emphasises the subtle differences between the samples of different genera and from different sites (Fig. 9d). The *n*-alkanol distributions of the three *Taxodium* fossils from P-33 2b sediments (FT3, 61 and 62) resembled those of the two fossils from site P-35 (FT63a, b) although they showed lower carbon preference indices (Lockheart, 1997, Appendices 5b and 6h). The distribution of *n*-alkanols extracted from the single example of *Taxodium* from the P-33 5c strata (FT76) was more similar to the *Taxodium* specimens from P-35 than the three from P-33 2b. *Metasequoia* (FT77) and *Sequoia* (FT80 and 81) were distinguishable from the six *Taxodium* fossils on the basis of the prominence of the C₂₄ *n*-alkanol (Fig. 6g and h).

5.5.2. *Fagaceae* family

The three members of this family examined provide the opportunity to compare the lipid profiles between two genera that are morphologically similar, i.e. *Fagus* and *Pseudofagus*, and one that is not, i.e. *Quercus*. Fossil *Fagus* leaves were generally very similar to *Pseudofagus* although they possessed fewer secondary veins and the leaves were smaller in size (the fruit of *Pseudofagus* also differs quite markedly from modern *Fagus* sp., Smiley and Huggins, 1981). The *n*-alkyl lipid profiles of these two genera were very similar to each other and also extant *F. grandifolia*, *japonica* and *orientalis* which closely resemble both fossil genera. *Quercus* specimens are not only different in appearance but also in lipid composition to the fossil *Fagus* and *Pseudofagus* specimens. This difference is also apparent between living examples of these genera (compare inset histograms in Fig. 2a and e).

The clear analogies between fossils and living members of the *Fagaceae* will have great value in assessing the prevailing climatic in Miocene *Clarkia*. This may be achieved by considering the climatic preferences of extant species that have the closest chemical and morphological affinities with the fossils.

5.6. Preservation of lipids

5.6.1. Preservation inferred from distributions and concentration of lipids

Most of the fossil specimens of the same genus resembled one another in the distribution of *n*-alkyl lipids. The fact that the concentration of lipid in most fossils was higher than in the enclosing sediments provides further evidence that the lipids endogenous to the leaf remained intimately associated with the macro-fossil throughout the period of burial.

It is not unreasonable to assume that the low concentrations and atypical distributions in a few of the fos-

sils (discussed earlier) arise from unfavourable pre or post depositional conditions. The rapid burial of the *Clarkia* fossils under mud/silt introduced to the lake during heavy regional rainstorms has been suggested as a contributory factor to the excellent state of preservation of many of the fossils (Smiley and Rember, 1985b). This proposed rapid incorporation into the sediments coupled with anoxic conditions would also favour the preservation of organic materials such as lipids because of the limited activity of fungi and bacteria. However, if a leaf remained uncovered at the surface of the sediments for an extended period of time, greater opportunity for degradation would arise, since many of the soluble components including phytotoxic tannins would leach out into water within a few hours (Spicer, 1991). It is possible that the burial of the specimens which showed poor chemical preservation was delayed in some way, resulting in more extensive pre and post depositional degradation.

5.6.2. Preservation in relation to morphological appearance

Three specimens mentioned previously (*Magnolia* FM1, *Platanus* FPR14 and *Pseudofagus* PF51) that showed low concentrations of lipid exhibited no morphological indications to suggest that a greater degree of degradation of lipids had occurred (e.g. colour, evidence of fungal infestation or insect damage). Furthermore, when the sample set was considered as a whole, there did not appear to be an obvious correlation between the physical appearance of the various fossils and the qualitative or quantitative survival of lipids.

Pre-depositional damage to leaves, deduced from the physical appearance of fossils, was apparently of little consequence as far as the preservation of lipids was concerned. For instance, one *Magnolia* fossil (FM64, Fig. 8 histogram C) was morphologically quite different from other specimens, it appeared to be in poor physical condition; it was light grey in colour and covered in darker speckles, perhaps evidence of fungal infestation (see Williams, 1985 for description of epiphytic fungi on *Clarkia* fossils). Yet, this fossil displayed a similar *n*-alkane distribution to the most exceptional fossil sampled, a green *Magnolia* fossil (FM50, Fig. 8 histogram B). Likewise, one of the *Platanus* fossils (FP57) displayed typical distributions and concentrations of *n*-alkyl lipids despite being in poor physical condition with numerous holes, presumably the result of pre-depositional insect mining.

6. Conclusions

Lipid profiles of individual fossil leaves and sediments from two Miocene lake deposits in northern Idaho, *Clarkia* P-33 and Oviatt Creek P-35, were studied to assess the chemotaxonomic potential of *n*-alkyl lipids

from intact macrofossils. The following conclusions can be drawn from the results.

1. It was clear from comparisons of distributions and concentrations of *n*-alkyl lipids between genera that lipids associated with the epicuticular waxes of the leaf were preserved and in most cases distinct above a weaker background signal of sedimentary *n*-alkyl lipids. These distribution patterns often differed between genera belonging to the same or different families. Principal component analyses of the relative abundance distributions of *n*-alkane and *n*-alkanol homologues emphasised these distinctions between genera and also revealed subtle variations within examples of a single fossil genera (e.g. *Magnolia*). In certain cases, differences in *n*-alkyl lipid distributions were sufficient to distinguish between fossil genera (e.g. *n*-alkanes: *Platanus*, *n*-alkanols: *Quercus*).
2. A few samples showed atypical distribution patterns, these were usually characterised by reduced concentrations of alkyl lipids which showed an increased sedimentary signal and lower carbon preference indices, these specimens were suggested to have been degraded. The extent of post-depositional lipid preservation appeared to be unrelated to the gross morphological appearance of the leaf. Furthermore, pre-depositional damage to leaves through fungal infestation or insect mining was not reflected in the distributions or concentrations of preserved *n*-alkyl lipids.
3. Subtle differences in distributions were evident between specimens of *Magnolia*. Specifically, six of the fourteen P-33 samples showed an unusual feature in that the C₃₀ *n*-alkane was higher in concentration than the C₃₁ homologue. The cause of this difference remains unresolved but possibilities proposed included: (i) differences in the extent of lipid preservation, (ii) more than a single species being represented in the assemblage, (iii) phenotypic variation between trees that contributed leaves to the sediments, and (iv) differences in lipid compositions due to local environmental conditions, e.g. the intensity of sunlight that a leaf received in the canopy.
4. Three genera of the family Taxodiaceae; *Sequoia*, *Metasequoia* and *Taxodium*, were examined and showed low concentrations of *n*-alkanes and distributions that appeared similar to the *n*-alkane distributions of the embedding sediment. However, in some cases a weak distribution maximising at *n*-C₂₅ derived from endogenous leaf wax components (which resembled that of a modern *Metasequoia glyptostroboides*) was detectable above the underlying sedimentary *n*-alkane pattern (which maximised at *n*-C₂₉ or *n*-C₃₁). *n*-Alkanol

concentrations were higher in specimens of these three genera and closer similarities in distribution patterns were evident between specimens than were observed in the *n*-alkanes. Specimens of the same genus or originating from the same site grouped together upon PCA treatment of the *n*-alkanol distribution data.

5. Differences in the distributions and concentrations of *n*-alkyl lipids between specimens from two subdivisions of the *Quercus* genus were also seen. Deciduous red oak fossil specimens showed very distinct *n*-alkane lipid profiles compared with the two examples of evergreen oak fossils. The fossil red oak profiles also resembled those of the *n*-alkanes extracted from the epicuticular waxes of several living red oak species.

The results from this study demonstrate that in certain cases it is possible to use the distributions of simple lipid classes preserved in plant macrofossils to perform chemotaxonomic comparisons between genera preserved in the fossil record. Furthermore, the potential exists to use these characteristic distribution profiles of fossil lipids in comparisons with epicuticular waxes of modern flora in order to establish taxonomic relationships. Results from studies of this nature will be presented in a future paper.

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