Vane, C.H.; **Kim, A.W.**; **Cave, M.R.**; Li, N.. 2014 Equal abundance of odd and even n-alkanes from cycad leaves: can the carbon preference index (CPI) faithfully record terrestrial organic matter input at low latitudes? *Encephalartos* (118).

Equal abundance of odd and even *n*-alkanes from cycad leaves: Can the carbon preference index (CPI) faithfully record terrestrial organic matter input at low latitudes?

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Abstract

Long chain *n*-alkanes from the leaves of thirteen extant cycad species within the Cycadacaea, Stangeriaceae, Zamiaceae families were measured by gas chromatography-mass spectrometry (GC-MS). Cycad *n*-alkane patterns ranged from nC_{10}-$nC_{37,}$ were unimodal in distribution, maximised at nC_{27}-nC_{33} and gave average chain length (ACL) values of 23.9 to 31.0. Low carbon preference indices (CPI) in the range of 0.88 to 2.70 were observed which is atypical of lipids from the leaves of terrestrial plants. Analysis of variance between the three families of true cycads showed that there were no significant differences between CPI values. The unusually low CPI values (<1.5) in nine of the thirteen cycad species analysed suggests that caution needs to be exercised in the use of *n*-alkanes distibutions as a chemical marker of terrestrial plant input in sediments from tropical and sub-tropical regions.

1. Introduction

Long chain *n*-alkanes from the cuticular waxes of higher plant leaves are characterised by a strong odd predominance, carbon preference index (CPI) values ranging from ~3 to 34 and average chain lengths (ACL) of 26 to 30 (Eglinton & Hamilton, 1967; Rommerskirchen *et al.*, 2006; Stojanovic *et al.*, 2003). In combination, the *n*-alkane distribution pattern and dominance of nC_{27}, nC_{29} and nC_{31} over lower molecular weight homologues nC_{15}, nC_{17} and nC_{19} have been widely applied as a proxy for terrestrial land plant input to lake, river, estuarine and coastal sediments (Jaffé *et al.*, 2001; Meyers, 2003; Wang *et al.*, 2003). Exceptions to the odd dominated *n*-alkane pattern from higher plants have been shown and on at least one occasion re-examined and corrected (Reddy *et al.*, 2000).

Investigations of the solvent soluble lipid content of Cycadophyta the ancient seed bearing plants found in tropical and subtropical regions reported that *n*-alkanes from the Zamiaceae family varied in a non-systematic manner with respect to genus (Osborne *et al.*, 1993). Epicuticular leaf waxes from the *Ceratozamia* and *Encephalartos* genera ranged from nC_{16} to nC_{33} with maxima at nC_{18} to nC_{19}. The same study reported that certain species *n*-alkane distributions were characterised by equal amounts of odd and even alkanes, as well as an absence of significant concentrations of branched chain isomers (Osborne *et al.*, 1993). The aim of this study was to examine the *n*-alkane distribution of Cycad foliage in order to improve our understanding of the utility of the *n*-alkane distributions and associated ratios in ancient sediments.

2. Methods

2.1 Samples and extraction

Fresh leaf material was obtained in October, 2006 from Fairy Lake Botanic Gardens, Shenzhen, China. Leaves were washed with 18 MΩ distilled water, freeze dried and ground using a freezer mill (SPEX CertiPrep 6850). Powdered leaves ~2.5 g were extracted in an accelerated solvent extraction system ASE 200 (Dionex) using 60 ml dichloromethane (DCM). Extracts were evaporated under a stream of dry nitrogen gas to a constant weight and reconstituted in 3 ml DCM.

2.2 Fractionation and Instrumental Analysis

Lipids were isolated by chromatography on columns containing 6 g Silica (deactivated) eluted with 40 ml *n*-hexane. The hydrocarbons were further fractionated into adduct and non-adduct fractions by urea adduction. Adduct fractions were analysed using a Varian CP3800 series gas chromatograph (GC) directly coupled with a Varian 1200L triple Quadrupole MS/MS system (GC/MS). Peak assignments were made by comparison with mass spectra and retention times of authentic standard compounds.

2. Results and discussion

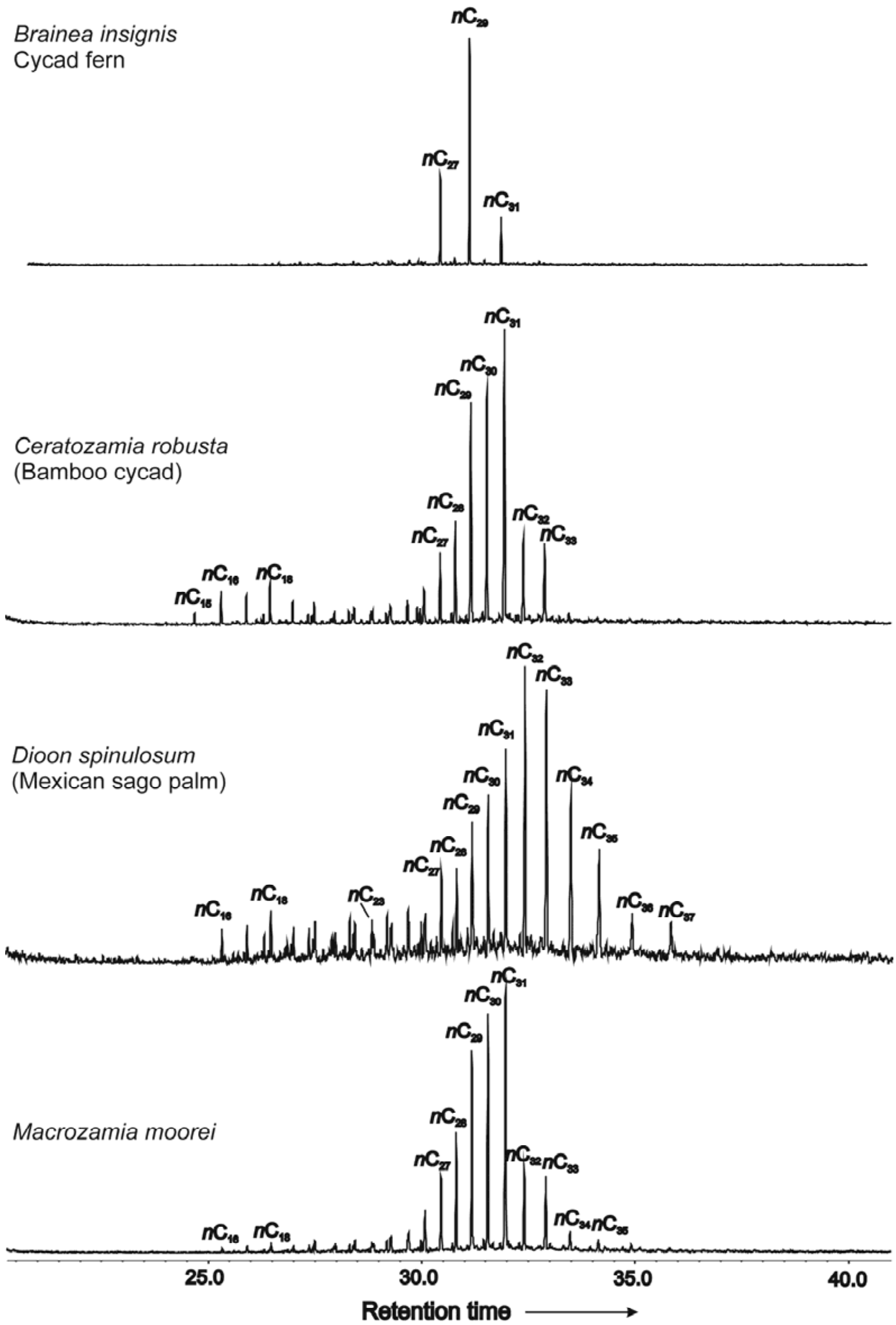

Figure 1. Gas chromatograms of the saturated hydrocarbon fraction extracted from cycad (*Ceratozmaia, Dioon, Macrozamia*) and tree fern (*Brainea*) leaves.

Alkanes ranging from nC_{10} to nC_{37} were identified in the leaves of 16 specimens from six plant families. For all specimens the major *n*-alkanes were C_{27}, C_{29}, C_{31} and C_{33} in contrast *n*-alkanes in the range C_{16} to C_{26} were mainly present at lower relative abundances (Fig. 1). Total amounts of *n*-alkanes ranged from 0.55 μg/g dry wt for *Cycas panzhihuaensis* to 6.8 μg/g dry wt for *Encephalartos gratus* (Table 1). The average chain length (ACL) ranged from 23.9 for *Cycas panzhihuaensis* to a maximum of 31.0 for *Encephalartos gratus,* confirming the notion that cycad leaves contain a broad range of *n*-alkanes (Table 1). By contrast long chain *n*-alkane distibutions for non-cycads, namely Equisetacea and Blechnaceae plant families, gave a limited distribution range dominated by C_{27}, C_{28}, C_{29} (Fig 1;Table 1). The carbon preference index (CPI) of the true cyacds (Cycadacaea, Stangeriaceae, Zamiaceae) ranged from as low as 0.88 in *Cycas panzhihuaensis* to 2.70 for *Zamia fischeri* Miq. The slight predominance of odd over even *n*-alkanes was confirmed by the mean CPI of the 12 cycad leaf specimens of 1.47. The cycad CPI values presented here are considerably lower than those usually reported for higher plant sourced *n*-alkanes which typically vary from about 5 to 30. An earlier study of *n*-alkane leaf waxes of 28 cycad species from Mexico, Florida and Australia reported that *Macrozamia moorei, Zamia fischeri*, *Zamia furfuracea* and *Ceratozamia robusta* yielded odd over even ratios of ~ 1.5, unimodal in distributions which were maximal at nC_{17} to nC_{21} and showed no significant quantities of branched chain isomers (Osborne *et al.*, 1993). In this current study we report broadly similar CPI values of 1.21 *Macrozamia moorei,* 1.30 *Ceratozamia robusta*, 1.32 *Zamia furfuracea,* but higher CPI values for the cycad *Zamia fischeri* at 2.70 (Table 1). Overall results differ from that of the earlier study of Osborne *et al* (1993) in that here the cycad *n*-alkanes were generally maximal at C_{29} to C_{31}. Furthermore, branched chain isomers were such a significant component of the solvent extracts that urea-adduction was required to remove these components in order to achieve baseline separation and facilitating quantification of individual *n*-alkane homologues. One plausible explanation for the difference in *n*-alkane distributions between this and previous studies of the same plant species could be that we analysed the whole leaf as compared to only extracting the surface epicuticular waxes of the leaves. Alternatively it has been speculated that the method of cultivation can modify epicuticular leaf wax *n*-alkane distributions, although this hypothesis remains untested (Osborne *et al.*, 1993).

Table 1. Summary of *n*-alkane data for cycad, horsetail and tree fern leaves

Family	**Genus and Species**	**Common Name**	**Conc. (μg/g)[a]**	**CPI[b]**	**ACL[c]**	**Odd Max**	**Even Max**
Equisetacea	*Equisetum arvenese*	Horsetail	2.21	4.90	28.4	29	30
Blechnaceae	*Brainea insignis* (Hook.) J.Sm	Cycad Fern	2.47	11.90	28.3	29	28
Cycadacaea	*Cycas miquelii* Warburg	Unknown	2.16	1.27	28.0	27	28
	Cycas panzhihuaensis L.Zhou & S.Y.Yang	Unknown	0.55	0.88	23.9	27	20
	Cycas revoluta Thunb	Sago Cycad	3.67	1.30	25.3	27	28
	Cycas thouarsii Lehm	Unknown	0.70	1.91	26.7	27	26
Oleandraceae	*Nephrolepis auriculata* (L.) Trim	Sword Fern	1.22	2.39	28.1	27	28
Stangeriaceae	*Stangeria eriopus* (Kunze) Baillon	Natal Grass Cycad	2.22	1.69	27.8	31	28
	Bowenia spectabilis Hook.ex J.D.Hook	Unknown	0.93	1.34	26.8	29	28
Zamiaceae	*Dioon spinulosum* Dyer	Mexican Sago Palm	1.52	1.00	29.4	33	32
	Encephalartos gratus Prain	Unknown	6.80	1.21	31.0	33	32
	Lepidozamia peroffskyana Regel	Pineapple Zamia	3.06	1.88	27.5	29	28
	Macrozamia moorei F.Muell	Unknown	3.79	1.21	29.1	31	30
	Zamia fischeri Miq.	Unknown	2.28	2.70	29.0	31	30
	Zamia furfuracea L.f.	Cardboard Palm	0.57	1.32	27.2	31	30
	Ceratozamia robusta Miq. Belize	Bamboo Cycad	3.67	1.30	28.1	31	30

[a]Total concentration of *n*-alkanes (n-C_{10} to n-C_{36}) in μg/g of dry leaf weight.
[b]CPI (carbon preference index)=(Σodd C from n-C_{10} to n-C_{36})/ (Σeven C from n-C_{10} to n-C_{36})
[c]ACL (average carbon chain length)=($\Sigma\%C_n \times n$)/ 100

The differences between the means of the CPI data (Table 1) for the three true cycad families Cycadacaea, Stangeriaceae, Zamiaceae were analysed using one-way analysis of variance (ANOVA) in the R statistical programming language (R Development Core Team (2008)). Figure 2 shows a comparative box plot of the CPI values for the three cycad families showing all three have similar median values and interquartile ranges with a possible outlier (*Zamia fischeri* Miq.) in the Zamiaceae family. The analysis of variance gave a non significant p-value of 0.8531 showing that there are no significant differences between the CPI values for the three families. As an additional check, a non-parametric equivalent method to the ANOVA, was also applied (Kruskal-Wallis) which confirmed no significant difference with a p-value of 0.5906.

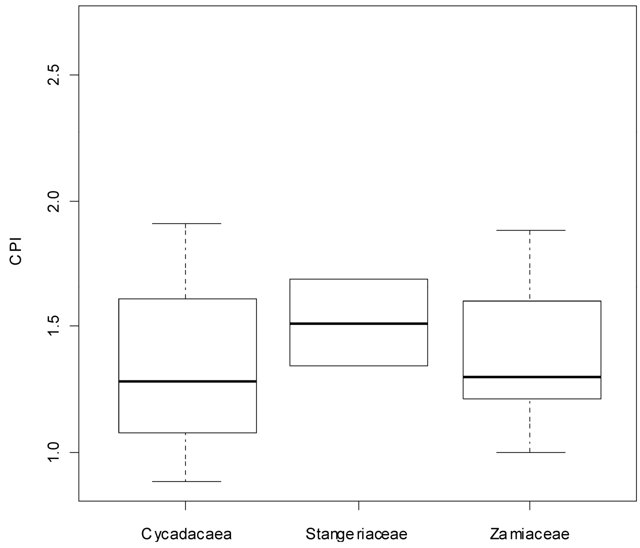

Figure 2. Boxplot of the CPI values for Cycadacaea (n=4), Stangeriaceae (n=2), Zamiaceae (n=7).

4 Conclusions

This study indicates that soils and sediments accumulating significant amounts of cycad foliage may have low CPI values and that the widely applied *n*-alkane based terrestrial source inference requires refinement particularly in tropical and subtropical soils where cycads grow naturally.

5. Acknowledgements

C.H. Vane is indebted to Nigel Birch at the British Consulate-General, Guangzhou, for logistical and financial assistance. This paper is published by permission of the Executive Director, British Geological Survey.

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