Assessment of Indigeneity in Fossil Organic Matter: Amino Acids and Stable Isotopes [and Discussion]
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Assessment of indigeneity in fossil organic matter: amino acids and stable isotopes

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SUMMARY

Whereas the presence of amino acids in fossil materials is clearly related to biosynthesis, an indigenous relationship with the fossil may be suspect. At present, attempts to establish the indigeneity of amino acids in fossils are based on distribution and stereochemistry. However, fossil systems are not closed and racemization may be retarded in organic materials within a fossil matrix. The advent of new technologies has resulted in alternative methods for evaluating the authenticity of fossil organic matter. A comparison of the stable carbon isotope compositions of the D- and L-enantiomers of individual amino acids facilitates the evaluation of indigeneity. Here we report the application of this method for determining the origin(s) of amino acids in fossils and extraterrestrial samples. Further, nitrogen isotope compositions of fossil materials should reflect trophic order, with increasing enrichment in $\delta^{15}N$ with progression up the food chain. Establishment of the trophic position of a fossil would further reinforce the appraisal of indigeneity based on the isotopic composition of its residual organic matter. Results of initial studies on Cretaceous age vertebrates which are consistent with the suggested feeding morphologies of these organisms are also presented.

1. INTRODUCTION

The occurrence of amino acids in fossils and sediments is clearly indicative of biosynthesis. However, the fact that geological materials rarely exist as closed systems from the time of burial to exhumation poses a significant challenge with respect to the isolation of amino acids that are derived from the proteinaceous material that was originally present in an organism. The problem of indigeneity becomes compounded with the passage of time, as earlier exchange of organic compounds between a fossil and its surrounding matrix becomes obscured.

For Quaternary age fossils, the establishment of indigeneity has relied on the distribution, relative abundance and stereochemistry of amino acids. For example, well preserved, Quaternary age fossil shells are expected to have relatively high concentrations of aspartic and glutamic acid and, with increasing time, lesser amounts of unstable amino acids, for example serine and threonine, and increasing concentrations of amino acids that form via the decomposition of protein amino acids, for example alanine and α-aminobutyric acid (Miller & Hare, 1980). In addition, the observation that amino acids will undergo racemization with the passage of time (e.g. Hare & Mitterer 1966) implies that the occurrence of non-racemic (L-enantiomer predominating) amino acids in order fossil materials is likely to reflect recent contamination from the surrounding environment (Williams & Smith 1977).

The above criteria for indigeneity have proven to be ambiguous at best. This is because it has not been possible to distinguish amino acids introduced into fossils via exchange from the surrounding environment. More importantly, the extent of amino acid racemization in fossils may appear abnormally low if the amino acids that are isolated for analysis are derived from well preserved, higher molecular mass proteinaceous material (Kimber & Griffin 1987) or a non-proteinaceous humic component that formed naturally in the shell via the condensation of indigenous amino acids and sugars (Hoering 1980).

The recent development of new methodologies and instrumentation has made it possible to determine the stable carbon and nitrogen isotope compositions of individual D- and L-enantiomers of amino acids in fossil systems (Serban *et al.* 1988; Silfer *et al.* 1991). As amino acids appear to retain their stable carbon and nitrogen isotopic integrity during racemization (Engel & Macko 1986), it may be possible to compare the isotopic compositions of the D- and L-enantiomers of individual amino acids in fossil systems to establish indigeneity. Here we report several applications of this new molecular approach for establishing the indigeneity of amino acids in fossils and in extraterrestrial materials.

In addition, an alternative approach for establishing the authenticity of organic matter in fossils is presented that relies on the observation that amino acid constituents of organisms have distinct stable isotopic compositions and that bulk isotope compositions reflect their trophic levels in food chains (Deniro & Epstein

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1978, 1981; Fry & Sherr 1984). The stable nitrogen and carbon isotope compositions of a high molecular mass (HMM) material isolated from Cretaceous age reptiles have been determined. The fact that the stable isotope data fit a trophic level pattern that is consistent with the morphology of these organisms supports the possible preservation of an indigenous signal in these ancient organic constituents of fossil bone and teeth.

2. STABLE ISOTOPE COMPOSITION OF AMINO ACID ENANTIOMERS IN FOSSILS

As previously discussed, the utility of amino acid distributions for establishing indigeneity is hampered by the ever present uncertainty of exchange with the surrounding sediment at various times. In addition, whereas the presence of significant concentrations of unstable amino acids as serine and threonine in fossils is commonly attributed to modern contamination, the occurrences of serine and threonine in HMM proteinaceous fraction represent unusually well-preserved Cretaceous age ammonite shells have been taken as evidence that the fractions represent original material (Weiner & Lowenstam 1980; Buchardt & Weiner 1981).

It is commonly assumed that most protein amino acids in fossil systems should be entirely racemic after several hundred thousand to several million years. However, there have been reports of the occurrences of indigenous, non-racemic amino acids in mass HMM fractions of well-preserved, older fossil shells ranging in age from Miocene (Hoering 1980) to the Late Cretaceous (Buchardt & Weiner 1981). The preservation of non-racemic amino acids may be attributed to retardation of the racemization reaction via the physical or chemical incorporation of amino acids into humic materials (Engel & Hare 1982). Simulation experiments in which humic-like materials are formed via the reaction of amino acids and sugars in aqueous solutions clearly indicate that the racemization rates for amino acids incorporated into the insoluble humic materials may be greatly diminished relative to the free amino acids remaining in solution in solution (figure 1).

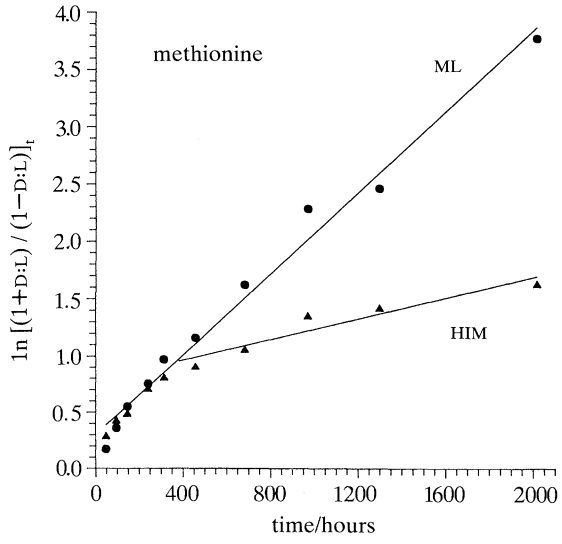

Figure 1. Racemization of methionine as a function of heating time, in the presence of glucose, valine and phenylalanine, at 100 °C. Abbreviations: ML = mother liquor; HIM = acid hydrolysate of insoluble melanoidin (Rafalaska *et al.* 1991).

Alternatively, the occurrence of non-racemic amino acids in order fossil materials may result from the actual preservation of a percentage of the original protein structure (Buchardt & Weiner 1981). In fact, Buchardt & Weiner (1981) have proposed that, for a series of late Cretaceous ammonites, lower racemization rates may indicate better preservation of the original protein material and, contrary to conventional wisdom, higher racemization rates are indicative of enhanced diagenetic effects.

Given the above uncertainties that result from the fact that the 20 common protein amino acids are ubiquitous to all organisms, present and past, new criteria that are independent of amino acid distribution and stereochemistry are required to establish the indigeneity of these compounds in fossil materials. It has been suggested that an alternative approach for establishing the indigeneity of amino acids in fossils would be to compare the stable carbon and nitrogen isotope compositions of the respective D- and L-enantiomers of individual amino acids (Dungworth 1976; Pillinger 1982; Engel & Macko 1984). Assuming that a fossil system has remained closed, the D-enantiomer that results from racemization should retain a stable carbon and nitrogen isotopic composition that is identical to that of the L-enantiomer from which it formed (Engel & Macko 1986). Whereas it is conceivable that a contaminating amino acid introduced to the system via, for example, migrating fluids, might have a carbon or nitrogen isotopic composition that is identical to the indigenous component, it is highly improbable that the contaminant would have both the same carbon and nitrogen isotopic compositions as the original amino acid in a fossil.

The isolation of individual D- and L-enantiomers of an amino acid from a fossil for stable isotope analysis by conventional high performance liquid chromatography (HPLC) separation techniques and the subsequent analysis by isotope ratio mass spectrometry (IRMS) presents a formidable challenge. A large enough fossil sample (in the case of fossil shell, about 18 g) must be processed to recover sufficient quantities of each enantiomer (about 1 mg) for combustion and isotopic analysis by IRMS. Also, given the potential for fractionation during the requisite cation-exchange (Macko *et al.* 1987) and reversed-phase (Macko *et al.* 1990) chromatographic steps of this type of isolation procedure, it is essential that all recoveries of the target compounds be quantitative.

We have recently succeeded in isolating the D- and L-enantiomers of glutamic acid from the total hydrolysate of a fossil mollusc shell (*Mercenaria*). The *Mercenaria* shell (ILC-B) is a well-preserved reference specimen from Mark Clark Pit, Charleston, South Carolina. The sample has been assigned an approximate age of 100000 to 250000 years before present

Table 1. *Elemental abundances and isotopic compositions of* D*-Glu and* L*-Glu isolated from the total hydrolysate of the fossil Mercenaria (ILC-B) shell (Serban et al. 1988)*

amino acid	millimoles carbon	millimoles nitrogen	C:N[a]	$\delta^{13}C$[b] (‰)	$\delta^{15}N$[b] (‰)
D-Glu	0.020806	0.004087	5.091	−20.74	+7.92
L-Glu	0.025540	0.005110	4.998	−16.28	+10.21

[a] Elemental abundances of carbon and nitrogen were determined by measuring the volume of gases (CO_2, N_2) that evolved during combustion of the samples for stable isotopic analyses.
[b] $\delta^{15}N$ reference = air (0 ‰); 2 standard deviation (s.d.) = 0.2 ‰. $\delta^{13}C$ reference = NBS−22 (−29.8 ‰) relative to PDB carbonate (0 ‰); 1 s.d. = 0.1 ‰.

(B.P.) (Wehmiller 1984). Details of the methodology that was employed for the isolation and stable isotope analysis of D-glutamic acid and L-glutamic acid are reported in Serban *et al.* (1988). As shown in table 1, the C:N values for the glutamic acid enantiomers isolated from the shell are virtually identical to the C:N value for pure glutamic acid (5.0). The D:L value for glutamic acid in the total acid hydrolysate of this shell was 0.39. This is in good agreement with the average D:L value for glutamic acid in *Mercenaria* ILC-B previously reported for an interlaboratory study (0.43; Wehmiller 1984). The stable carbon isotope values for D- and L-glutamic acid fall within the range of $\delta^{13}C$ values that have been reported for marine humic materials (Hoering 1974) and are similar to $\delta^{13}C$ values reported by Hoering (1980) for insoluble organic matter isolated from a modern *Mercenaria* shell (−16.20‰) and a Miocene age *Mercenaria* shell (−19.58‰). Also, the $\delta^{15}N$ and $\delta^{13}C$ values for D- and L-glutamic acid are similar to values observed for HMM, proteinaceous material isolated from a Pliocene age fossil pelecypod (*Mitra heilprini*) from the Caloosahatchee Fm., Florida ($\delta^{15}N$ = +8.6‰, $\delta^{13}C$ = −20.1 ‰). What is surprising, however, is the fact that the D-enantiomer of glutamic acid was depleted in ^{13}C and ^{15}N by several per mil relative to the L-enantiomer (table 1).

Whereas the disparity in isotopic composition between the D- and L-enantiomers in this apparently well-preserved shell might be construed as evidence for input from the surrounding environment, i.e. contamination, it is at present speculated that the difference may at least in part reflect isotopic fractionation resulting from the partial hydrolysis of the original protein (Serban *et al.* 1988). The racemisation of amino acids in the HMM proteinaceous material is very slow. In this shell the amino acid enantiomers of the HMM material is nonracemic in all cases (table 2), in contrast to values suggesting a racemic mixture in the total hydrolysate. It is therefore possible that a high percentage of the D-glutamic acid in the fossil shell forms subsequent to the hydrolytic release of L-glutamic acid as a component of lower molecular weight (LMM) peptides. In support of this latter hypothesis is our recent observation of stable carbon and nitrogen isotopic fractionation during the partial hydrolysis of the peptide glycylglycine (Silfer *et al.* 1990). It was observed that the free glycine released during partial hydrolysis of this dipeptide was depleted in ^{13}C and ^{15}N relative to the residual dipeptide (figures 2 and 3, respectively). Further support for the hypothesis of Serban *et al.* (1988) has been presented by Bada *et al.* (1989) in heating experiments done on collagen. The possible occurrence of hydrolytic effects will have to be reconciled before the application of this isotope method for establishing absolute indigeneity. It should be noted, however, that in a recent study of the isotopic composition of the D- and L-enantiomers of glutamic and aspartic acid, which were isolated from the total hydrolysate of shells from late Pleistocene/Holocene land snails from the Negev Desert, the D- and L-enantiomers of the respective amino acids were found to have virtually identical stable isotope compositions (M. H. Engel, S. A. Macko & G. A. Goodfriend, unpublished results). Apparently hydrolytic isotope

Table 2. *Amino acid* D*:*L *values for fossil Mercenaria shells (Serban et al. 1988)*

sample	analytical method	Ala	Val	Ile	Leu	Pro	Asp	Glu	Phe	Orn	Lys
dialysed shell (ILC-B)	GC	0.135 ±0.006	0.076 ±0.007	0.079 ±0.003	0.096 ±0.003	ND[b]	0.434 ±0.004	0.156 ±0.010	0.164 ±0.003	0.328 ±0.009	0.156 ±0.008
	HPLC	—	—	0.03	—	—	—	—	—	—	—
ILC-B powder	GC	0.670 ±0.017	0.425 ±0.044	0.499 ±0.029	0.468 ±0.007	0.489 ±0.096	0.700 ±0.063	0.385 ±0.004	0.567 ±0.007	0.806 ±0.025	0.515 ±0.053
	HPLC	—	—	0.50	—	—	—	—	—	—	—
ILC-b powder[c]	GC	0.725 ±0.034	0.449 ±0.068	0.540 ±0.081	0.497 ±0.049	0.595 0.105	0.705 0.028	0.432 0.017	0.583 0.059	NR[d]	NR
	HPLC	—	—	0.525 ±0.055	—	—	—	—	—	—	—

[a] The D- and L-amino acid enantiomers were derivatized (*N*-trifluoroacetyl isopropyl esters) for GC analysis by using a Hewlett-Packard 5890 GC (splitless injection) equipped with an NPD detector and a 50 m × 0.25 mm i.d. fused silica column coated with Chirasil-Val (Applied Science, State College, Pa). Details of the derivatization procedure and chromatographic conditions have been previously reported by Frank *et al.* (1978). Amino acid D:L values are an average of at least three chromatographic determinations.
[b] ND = not determined.
[c] The mean values for the ILC-B powder are the results of an interlaboratory study reported by Wehmiller (1984*b*).
[d] NR = not reported.

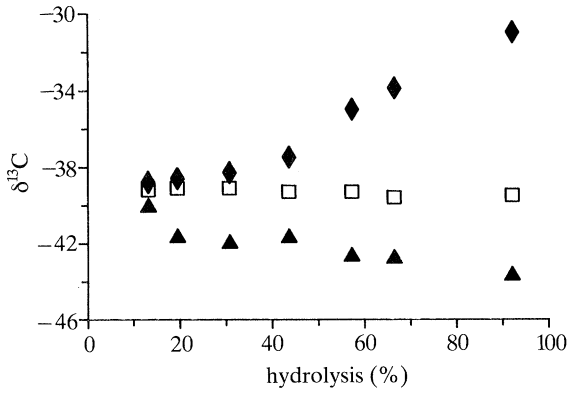

Figure 2. Stable carbon isotope compositions of products from the hydrolysis of glycylglycine (diamonds) to free glycine (triangles) at 160 °C. Squares are the total solution organic material in solution (Silfer *et al.* 1991).

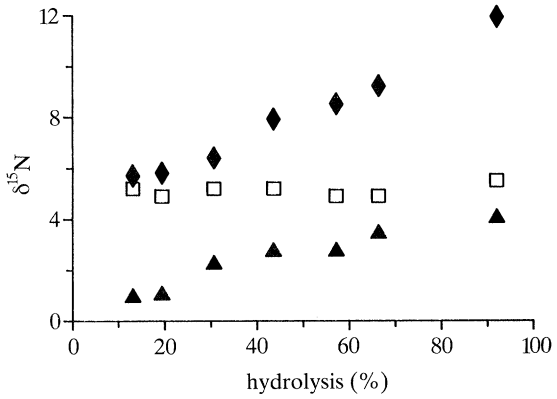

Figure 3. Stable nitrogen isotope compositions of products from the hydrolysis of glycylglycine (diamonds) to free glycine (triangles) at 160 °C. Squares are the total organic material in solution (Silfer *et al.* 1991).

fractionation effects may be diminished during accelerated racemization of amino acids in well-preserved fossil materials in warm, arid environments.

Clearly there is much to be learned from the stable isotope composition of individual organic compounds in fossil systems with respect to biosynthetic pathways, environments of deposition and preservation. However, the limitations of the analytical approach described above are self evident. The sample size that is required and the time and expense for the chromatographic separation of individual components for conventional IRMS analysis are prohibitive. As will be discussed below, recent developments of procedures for the direct stable carbon isotope analysis of nanomole concentrations of amino acid enantiomers by combined gas chromatography–isotope ratio mass spectrometry (GC–IRMS) circumvents many of the aforementioned problems.

3. GC–IRMS ANALYSIS OF AMINO ACIDS IN THE MURCHISON METEORITE

Despite the unique nature of the data with regard to the source and history of organic materials (see, for example, Gilmour *et al.* (1984); Macko *et al.* (1989)), only a limited number of additional reports utilizing molecular or stable isotope characterizations using conventional separations have appeared. The recent development of GC–IRMS systems has permitted a more routine direct stable carbon isotope analysis of individual compounds (Freedman *et al.* 1988; Hayes *et al.* 1989). Volatile organic compounds (i.e. hydrocarbons) in crude oils and rock extracts are able to be routinely assessed for their stable carbon isotope compositions using GC–IRMS (e.g. Franchi *et al.* 1989; Freeman *et al.* 1990; Kennicutt & Brooks 1990). The application of this method to amino acid analysis, however, is complicated by the fact that amino acids are non-volatile, multifunctional molecules that require derivatization before analysis. Whereas the derivatization process introduces additional carbon atoms and an apparent, yet reproducible fractionation during esterification and acylation, we have succeeded in developing a procedure for determining the stable carbon isotope composition of amino acid enantiomers by GC–IRMS. Derivatization of amino acid standards of known stable carbon isotope composition in conjunction with natural samples permits computation of the original, underivatized amino acid $\delta^{13}C$ values via a correction for the carbon introduced and isotopic fractionation during the derivatization process (Silfer *et al.* 1991). Here we present an application of this method for determining the indigeneity of amino acids in the Murchison meteorite.

Engel & Nagy (1982) previously reported that several amino acid constituents of the Murchison meteorite were not racemic. The amino acid distribution in this Murchison stone was similar to that reported for other Murchison stones (Engel & Nagy 1983; Shock & Schulte 1990). Thus the conventional criteria of amino acid abundance and distribution were not deemed sufficient to confirm whether the occurrence of non-racemic amino acids (L-enantiomer predominating) was indigenous or a terrestrial overprint (i.e. contamination).

It has previously been reported that the bulk amino acid extract of the Murchison meteorite is substantially enriched in ^{13}C relative to terrestrial organic matter (Chang *et al.* 1978; Epstein *et al.* 1987). It was therefore hypothesized that if the D- and L-enantiomers of amino acids in the Murchison meteorite were derived entirely from an extraterrestrial, prebiotic synthetic pathway, they should reflect the ^{13}C enrichment that has been observed for the bulk extracts.

Recent analyses of an interior sample of a second Murchison stone (Engel *et al.* 1990) revealed a bulk isotopic composition and amino acid distribution similar to that previously reported by Engel & Nagy (1982). As in the previous study, it was observed that amino acids in the extracts of this stone were partially racemized but not entirely racemic. The stable carbon isotope composition of individual free amino acids isolated from this stone were determined by GC–IRMS (table 3). It is readily apparent that the individual components are indeed enriched in ^{13}C relative to terrestrial organic compounds that, owing to fractionation during photosynthesis, are depleted in ^{13}C. Alanine was not racemic (D:L = 0.85). Whereas L-

Table 3. *Stable carbon isotope compositions and concentration of individual amino acids isolated from the Murchison meteorite (Engel et al. 1990)*

amino acid	concentration (nmol g^{-1})	$\delta^{13}C$[a] (‰)
α-aminoisobutyric acid	16.0	+5
L-glutamic acid	4.6[b]	+6
isovaline	7.5[c]	+17
glycine	28.1	+22
D-alanine	[b]	+30
L-alanine	12.9[b]	+27

[a] The $\delta^{13}C$ values are corrected for carbon introduced during derivatization. $\delta 1^{13}C(‰) = [(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{PDB} - 1] \times 10^3$.

[b] Concentrations reported for L-glutamic acid and L-alanine represent the total contribution of both enantiomers.

[c] This value includes a minor contribution of valine that co-eluted with isovaline during HPLC analysis.

alanine is slightly depleted in ^{13}C relative to D-alanine, mass balance considerations indicate that if the excess L-enantiomers was contributed from a terrestrial source, it would have had to have a stable carbon isotope composition of about +10 ‰. Thus, a terrestrial overprint appears to be unlikely. Having recently completed the interfacing of a GC with a more sensitive IRMS, experiments are how underway to determine the stable isotope composition of the D- and L-enantiomers of other amino acids in various extracts of the Murchison meteorite. Clearly the application of this method holds great promise for evaluating the origin and indigeneity of organic constituents in terrestrial and extraterrestrial materials.

4. FOOD WEBS: AN ALTERNATIVE INDIGENEITY CRITERIA FOR ORGANIC MATTER IN FOSSILS USING STABLE ISOTOPES

Numerous studies utilizing stable isotope analyses of tissues from living organisms have documented the capability for the estimation of diet of the organism and of trophic relations among organisms in a food web in both freshwater and marine ecosystems (reviewed by Fry & Sherr (1984)). A similarity exists between an organism and its diet as a result of biosynthetic incorporation of the carbon and the nitrogen of the diet into the tissues of the consumer. The isotope enrichments observed will in the first case be affected by the isotopic compositions of the primary production the food chain consumes (Harrigan *et al.* 1989). Small fractionations of about ±1‰ have been reported for carbon, whereas there has generally been observed about a 3‰ enrichment in nitrogen with each trophic level (figure 4). Long food chains, as exist in the High Arctic, have been observed to have organisms with the greatest enrichment in nitrogen isotopes as a result of this effect (Harrigan-Ostrom *et al.* 1989*a*, *b*). The exact cause of the nitrogen enrichment has not been well documented but has been suggested to be associated with protein utilization by the consumer and the observed enrichment proteins have (about 3‰, also) relative to the whole organism (Wada 1980; Wada *et al.* 1987; Macko 1988). Individual amino acids within a protein also have a wide distribution of isotope compositions (Macko *et al.* 1987) which are the result of isotopic fractionations associated with metabolic effects during the biosynthesis of the amino acid, such as in the transfer of nitrogen during transamination (Macko *et al.* 1986). The primary structure of a protein will then influence the isotopic composition of the whole protein. Some proteins which have more of an anomalous structure (i.e. collagen) will be more heavily biased toward the principal amino acids as a result.

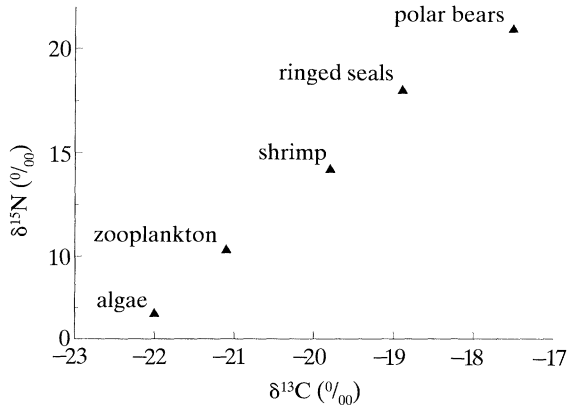

Figure 4. Example of enrichment seen in food chains with increasing trophic position in a study of organisms from Resolute Bay, North West Territories, Canada (Harrigan-Ostrom *et al.* 1991).

Of increasing interest to palaeontology and anthropology is the use of stable isotopes toward the understanding of fossil organisms, many of which are extinct and any information regarding their relationship to the overall community is regarded as precious. Modern animal food chains, and attendant isotope distributions and fractionations, are used in establishing the feeding relations among a fossil assemblage. The basis for this approach lies in the limiting fact that indigenous organic material must be preserved in the fossil in order to make such an evaluation. Evaluation of the authenticity of that remnant can be attempted by using the methods described above. Alternatively, the isotope composition of the high HMM fraction, could also be used if some facets of the trophic order were known about a fossil assemblage, such as was studied by Harrigan-Ostrom *et al.* (1990). In fact, in some cases, such a bulk parameter may indicate the indigeneity of the overall fossil material. Because often a large fraction of such HMM isolates cannot be further evaluated by standard separation or hydrolysis techniques, with the amino acids remaining locked in a refractory matrix, the bulk measurements can be used to evaluated even those non-hydrolysable portions.

Through the isolation and isotope characterization of HMM materials isolated from bones and teeth of the late Cretaceous fossils, and comparison with associated sediments from which the bones were removed, a strong trophic structure seems to have been preserved. Interestingly, none of the amino acids analysed for stereochemistry in this HMM material was racemic, in

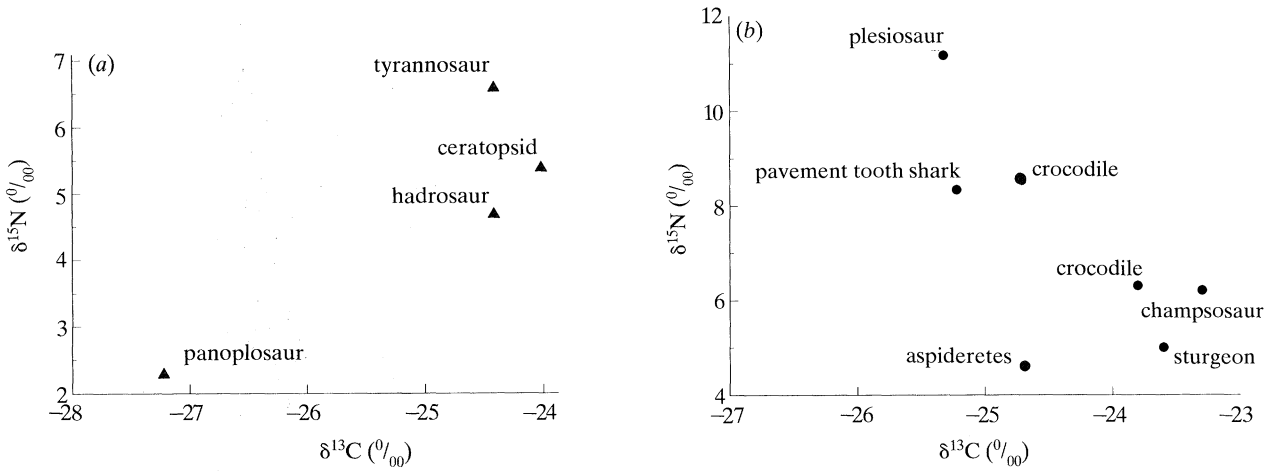

Figure 5. Stable carbon and nitrogen isotope compositions of teeth and bone high molecular mass organic matter from late Cretaceous fossils. (*a*) Terrestrial food web; (*b*) aquatic food web (Harrigan-Ostrom *et al.* 1990).

Table 4. *Amino acid enantiomer ratios of late Cretaceous fossils and sediment from the Judith River Formation, Alberta, Canada (Harrigan-Ostrom et al. 1990)*

	D:L alanine	D:L apartic acid	D:L glutamic acid
tyrannosaur bone	0.01	0.07	0.08
sediment	0.19	0.12	0.25
ceratopsid bone	0.15	0.09	0.23
hadrosaur bone	0.08	0.05	0.12
tyrannosaur tooth	0.21	0.05	0.04
ceratopsid tooth	0.11	0.02	0.03
hadrosaur tooth	0.23	0.10	0.07
crocodile tooth	0.15	0.04	0.03

line with the above previous observations (table 4). A clear enrichment in nitrogen isotope signatures of the fossil organic materials is observed with advancement up the food chain (figure 4). Carnivores are more enriched than the omnivores which are more enriched than the herbivores. Further, with the suggestion that the materials then appear to be related to the original isotope signals of the living, particular feeding strategies of the animals may be able to be discerned. Animals that feed on a specific plant types, or exhibit special feeding strategies may be proposed. The fact that such a particular relation is exhibited in the set of fossils indicates the results are more than coincidence. Obviously, the best evaluations of authenticity will draw on as many of the methods as possible in assessment.

5. CONCLUSIONS

The assessment of indigeneity in fossil organic materials has previously depended on the observations of predictable rates of decomposition for amino acids and consistent rates of racemization. Reported are instances of findings that appear to be incongruous with such assessments. Evidence is presented which suggests alternative strategies for the evaluation of the indigeneity of organic materials isolated from fossils and meteorites. Principally, these methods call for amino acid comparisons among similar materials, isotopic evaluation of bulk HMM isolates, and the isotopic analysis of the enantiomers of the amino acids. With such capability of assessment, the confidence in the interpretation of the fossil or in the origin of the organic components is strengthened.

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Discussion

J. L. Bada (*Scripps Institution of Oceanography, University of California at San Diego, U.S.A.*). The D:L ratios (D:L Ala, D:L Asp, D:L Glu) Dr Macko gives for the dinosaur material are just the kind of ratios one finds in bacterial cell walls. Perhaps he is seeing the remains of bacteria that ate the collagen in the original dinosaur bones?

S. Macko. Certainly the possibility exists that we could be looking at bacterial remains if we consider that the D-amino acids are principally in Asp, Glu and Ala. As far as isotopes go, however, we do not know what bacterial compositions will be if they are grown on a mixed amino acid substrate like dinosaur protein. The facts remain, however, that the high molecular mass materials are non-racemic and the isotopes of that material show a clear enrichment with trophic level consistent with our knowledge of feeding morphology. For some types of research, this may be the information needed, and not that the organic was 'authentic' fossil remains.

B. Halstead (*Department of Geology, Imperial College, London, U.K.*). One of the basic controls in assessing levels of contamination, or the more preferable term used by the authors, indigeneity, is to analyse the surrounding sediment as well as the fossil. When this is done, with shales for example, there is a zone of amino acid enrichment surrounding the fossil, out of which it has been clearly leached (Armstrong & Halstead Tarlo 1966). The degree of racemization acts as a useful measure of degree of suspicion of the analyses. The amino acid compositional profiles obtained from some fossils suggest to me that they are the result of contemporary bacterial contamination (Armstrong *et al.* 1983).

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