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Moose teeth as monitors of environmental isotopic parameters

J.L. Bada[1], R.O. Peterson[2], A. Schimmelmann[1], and R.E.M. Hedges[3]

[1] Amino Acid Dating Laboratory (A-012B) Scripps Institution of Oceanography, University of California at San Diego, La Jolla, CA 92093, USA
[2] School of Forestry and Wood Products, Michigan Technological University, Houghton, MI 49931, USA
[3] Radiocarbon Accelerator Unit, Research Laboratory for Archaeology and the History of Art, Oxford University, 6 Keble Road, Oxford OX1 3QJ, UK

Summary. The radiocarbon activities of amino acids isolated from crown first molar dentin of moose born between 1948 and 1984 on Isle Royale National Park, USA closely follows the bomb radiocarbon signal generated from atmospheric nuclear weapons testing. This demonstrates that these amino acids are metabolically inert and have recorded the isotopic parameters of the environment during the first year or two of the animal's life. The moose teeth amino acids provide both carbon and nitrogen isotopic chronologies for Isle Royale for a period of nearly four decades. The carbon isotopic record reflects both anthropogenic alterations of the global atmospheric carbon cycle (the "Suess" effect), and changes in forest ecology and moose feeding habitat. The nitrogen isotopic chronology is more variable than the carbon record and is the result of environmental and biological factors which are poorly understood.

Key words: Teeth – Bomb radiocarbon – Isotope chronologies

The stable isotopic ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) have been increasingly utilized during the last decade in investigations of natural biogeochemical cycles. The extent of fractionation (e.g., the separation of isotopes by biological, chemical and/or physical processes) can provide information about food chain dynamics, dietary sources and habitat characteristics (for example, see Rundel et al. 1989 and Owens 1987 and references therein). Detailed historical isotopic chronologies, however, are not easily obtained and when available, their interpretation is not straightforward. Climatic variability, ecosystem changes, and anthropogenic alterations each affect isotopic ratios. For terrestrial environments, the best carbon isotopic time records have been obtained from tree rings (Leavitt and Long 1983, 1988; Peng et al. 1983; Stuiver et al. 1984). Problems exist in the interpretation of these records because the factors which may cause variations in the tree ring carbon isotopic ratios are complex and poorly understood (Francey and Farquhar 1982; Leavitt and Long 1988). Nitrogen isotopic chronologies have never been previously obtained.

Mammalian teeth should be excellent recorders of environmental isotopic parameters. In most mammals, teeth are formed during the first few years of life, and the proteins encapsulated in these calcified tissues are metabolically inert. This is demonstrated by the fact that the amino acids obtained from enamel and dentin of known age mammals show increasing racemization (e.g., the conversion of the natural L-amino acids into the uncommon D isomers) with age (Bada 1984). Thus, the isotopic ratios of carbon and nitrogen in amino acids obtained from tooth proteins should reflect the environmental isotopic values during the first few years of the animal's life.

We report here an investigation of the carbon and nitrogen isotopic ratios in dentin amino acids isolated from moose teeth from Isle Royale National Park, USA (48° N, 89° W). These animals were selected because of the large number of specimens available, and the isolated habitat and well studied ecosystem of Isle Royale. To demonstrate that the amino acids obtained from the moose teeth are formed in the first few years of life and that they are metabolically inert, we have utilized the bomb radiocarbon signal generated from atmospheric nuclear weapons testing in the late 1950s and early 1960s (Bada et al. 1987).

Methods

The moose of Isle Royale National Park have been the focus of long-term research since 1958 and several hundred skeletons have been collected (Peterson 1977; Allen 1979). For our study, thirteen animals were selected which were born between 1948 and 1984. The year of birth was estimated from the year of death, and the animal's age determined from counts of cementum annulations (Wolfe 1969). This aging technique tends to slightly overestimate (+0.5 year) the true age for animals with ages in the range 2–11 years (Gasaway et al. 1978). The estimated ages of older animals likely have greater uncertainties.

Sample processing was carried out at the Mt. Soledad Radiocarbon Laboratory at UCSD in order to avoid radiocarbon contamination. The amino acids were isolated from the crown of the first molar of the lower jaw. This tooth was selected because calcification of the crown begins in

Offprint requests to: J.L. Bada

utero and is completed during the first year of life (Hillson 1986). After grinding off the enamel, a chunk of crown dentin weighing between 0.3 and 0.5 grams was removed from the lingual (tongue) side of the tooth. Dentin samples were hydrolyzed in 6M HCl for 24 h at 100° C and the amino acids then isolated by cation exchange chromatography as described elsewhere (Bada 1985). All reagents used in these processing steps were specially prepared in order to minimize contamination problems (e.g., water and HCl were doubly distilled).

The radiocarbon activities of the isolated amino acids were determined by accelerator mass spectrometry (AMS) at the Radiocarbon Accelerator Unit, Oxford University (Batten et al. 1986). A portion of the carbon dioxide generated during preparation of the AMS targets was also saved for $\delta^{13}C$ analysis.

Before combustion of the samples for carbon and nitrogen stable isotopic analyses, any NH_3 which may have remained in the amino acid extracts from desalting (e.g., in the cation exchange procedure) was removed by raising the pH to 11 (with addition of 0.1 M KOH) and evaporation in vacuo. The samples were then acidified to pH 1–2 with HCl in order to remove any carbonate derived from the alkaline treatment, transferred into quartz ampules (O.D. 9 mm, length 15 cm), and then again evaporated to dryness in vacuo. The samples were then combusted (Stump and Frazer 1973) to obtain carbon dioxide and nitrogen and the elemental C/N ratios were determined manometrically. The isotopic compositions were determined by mass spectrometry at the UCLA stable isotope facility; the precisions are $\pm 0.1‰$ for $\delta^{13}C_{PDB}$ and $\pm 0.2‰$ for $\delta^{15}N_{AIR}$.

Results and discussion

The results of the various measurements are summarized in Table 1. The C/N ratios of the amino acid extracts obtained from the moose dentin samples are very similar and have an average value of 3.36 ± 0.05. This value is consistent with the C/N ratio measured in dentin amino acids (Masters 1987), and illustrates the purity and homogeneity of our amino acid extracts.

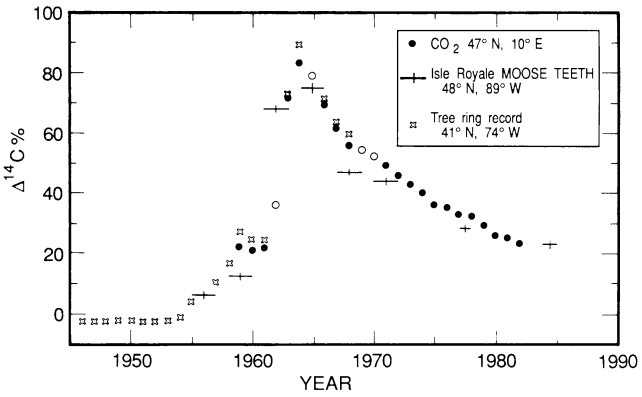

Fig. 1. Northern hemispheric radiocarbon activity of atmospheric CO_2 (Levin et al. 1985), tree rings (Cain 1975), and amino acids isolated from Isle Royale moose first molar crown dentin. The CO_2 and tree ring records overlap between 1960–1970; the years when the values are indistinguishable are indicated by the open circles

The atmospheric detonation of thermonuclear weapons during the period from the late 1950s to the early 1960s nearly doubled the radiocarbon activity of tropospheric carbon dioxide by 1964 (Levin et al. 1985). Although this bomb radiocarbon signal has steadily decreased since the ratification of the limited atmospheric test ban treaty in October 1963, the current radiocarbon activity of atmospheric carbon still exceeds pre-bomb levels by about 15–20%. As can be seen in Fig. 1, the radiocarbon activity of amino acids isolated from crown first molar dentin of moose closely tracks the atmospheric carbon dioxide and tree ring bomb radiocarbon signal. This demonstrates that the amino acids isolated from moose first molar crown dentin are indeed synthesized during the first year or so of the animal's life, and that they are then isolated from active metabolic processes.

Table 1. Carbon and nitrogen elemental ratios, radiocarbon, and stable carbon and nitrogen isotopic ratios in Isle Royale moose first molar crown dentin amino acids

Year of death	Estimated age (yrs)	Interval represented by dentine sample[a]	C/N	$\Delta^{14}C\%$[b]	$\delta^{13}C‰$[c]	$\delta^{15}N‰$
1959	11	1948–50	3.45		−22.2	+4.6
1960	8	1952–53	3.25		−22.7	+8.8
1969	14	1955–57	3.34	6.6±1.2	−22.2, −22.3	+5.4
1973	15	1958–60	3.47	12.7±0.9	−22.8, −22.6	+3.9
1977	16	1961–63	3.27	68.4±1.0	−22.9, −23.0	+5.9
1980	16	1964–66	3.40	75.1±0.9	−23.0, −23.2	+3.7
1985	18	1967–69	3.31	46.6±0.9	−23.4, −23.6	+6.2
1986	16	1970–72	3.42	43.6±0.9	−23.6, −23.8	+3.6
1986	14	1972–73	3.39		−23.8	+4.9
1986	12	1974–76	3.30		−23.2	+4.2
1985	8	1977–78	3.40	28.5±1.0	−23.6, −24.1	+5.0
1986	6	1980–81	3.33		−23.7	+4.3
1986	2	1984–85	3.39	23.7±1.4	−23.3, −22.8	+4.2

[a] For 1st molars with unworn crowns, the dentine sample is estimated to be from the first year of life. For those with worn crowns, the dentine sample represents accumulation during the first 2 years of life

[b] $\Delta^{14}C\%$ is calculated relative to the 1950 activity = 0%

[c] The values on the right were obtained using the CO_2 produced during preparation of AMS targets

The moose teeth radiocarbon results indicate that the moose ages estimated from cementum annulations are reasonably accurate, with the possible exception of the oldest ages. For example the greatest deviation from the bomb radiocarbon signal is for the animal estimated to be 18 years old. Its true age is more likely 15–16 years. The results in Figure 1 suggest that the bomb signal can be used to assess the year of birth, and thus biological age, of animals whose age cannot be readily established using other techniques. A comparison of the radiocarbon activity in first molar crown dentin amino acids with the atmospheric carbon dioxide bomb radiocarbon record offers a way of directly evaluating an animal's year of birth. For animals born before the episode of atmospheric nuclear testing this technique would obviously only provide a minimum age estimate. Problems could arise in deciding which side of the bomb signal represents the actual birth year; for example, atmospheric CO_2 radiocarbon activities were roughly equal in 1962 and 1975 (see Fig. 1). This could be resolved by ^{14}C analysis of amino acids isolated from crown dentin of teeth formed later than the first molar, such as the fourth premolar. A comparison of the ^{14}C activities in the first molar and later formed teeth would reveal whether they were formed in the rapidly changing interval prior to 1964 or in the period of gradually decreasing radiocarbon activity which followed the atmospheric test ban treaty.

The stable carbon and nitrogen isotopic chronologies for the period 1948 to 1985 obtained from the Isle Royale moose teeth amino acids extracts are shown in Fig. 2. Also included is the $\delta^{13}C$ record for atmospheric carbon dioxide (Keeling et al., in press) over the last few decades; this shows the magnitude of the "Suess" effect (Suess 1955; Keeling 1979), which results from the dilution of natural atmospheric carbon dioxide by ^{13}C-depleted CO_2 released from fossil fuel combustion. During the interval between 1948 and 1973 the carbon isotopic signal recorded in the moose teeth amino acids shows a fairly systematic enrichment in the light isotope of about 1.5‰. This is about twice as large as the enrichment observed in atmospheric carbon dioxide; thus, only about half of the light carbon isotopic enrichment observed in the moose teeth can be attributed to the "Suess" effect.

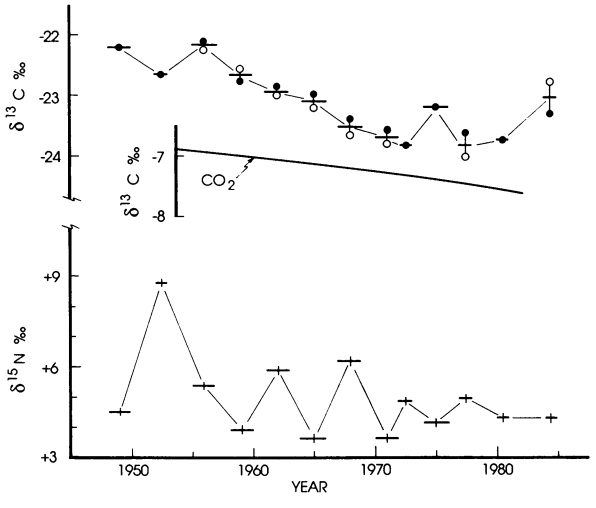

Fig. 2. $\delta^{13}C$ and $\delta^{15}N$ chronologies recorded in Isle Royale moose teeth between 1948 and 1985. The open circles are measurements carried out using the CO_2 produced during production of the AMS targets. Also shown is the $\delta^{13}C$ trend for northern hemispheric CO_2

Although the factors responsible for the excess light carbon isotopic enrichment observed in moose teeth are possibly many, one candidate is the shift in moose winter feeding habitat which took place during the sample period. Major fires burned about 20% of Isle Royale in 1936 and 1948. These burns provided optimum winter habitats for moose throughout the 1940s and 1950s. In winter surveys during the late 1940s, Krefting (1951) found few moose outside the burn areas, and Cole (1953, 1956) continued to find numerous moose in the deciduous regrowth in the burns throughout the 1950s. After 1960, annual aerial surveys revealed very little use of the old burn regions except when snow cover was unusually low. By the early 1960s, fewer than 10% of the moose were found in the burns and by the early 1970s the old burns supported almost no moose (Peterson 1977). By this time, moose existed in winter in older forests dominated by balsam fir (*Abies balsamera*). During the period from the late 1940s to the early 1970s, we infer a major change in the moose winter food intake from a predominantly deciduous diet of aspen (*Populus termuloides*) and white birch (*Betula papyrifera*) to one dominated by balsam fir. This shift could have resulted in the moose sampling in a fir dominated forest, the "canopy" effect, wherein leaves near ground level in a dense forest are depleted in light carbon in comparison to those at treetop and in well exposed vegetation. In a dense forest, the CO_2 near ground level is enriched in light carbon relative to that of the surrounding open air by as much as 5–6‰ (Medina and Minchen 1980; Schleser and Jayasekara 1985; van der Merwe and Medina 1989). This arises from the production of isotopically light CO_2 from the oxidation of plant derived soil organic material. The "canopy" effect is largely due to the assumulation of the isotopically light CO_2 near ground level (van der Merwe and Medina 1989), although the reduced light intensity at the forest floor is also a contributor (Francy and Farquhar 1982). The "canopy" effect would be minimal in a well ventilated, exposed deciduous regrowth habitat, but would be increasingly apparent as the moose shifted to the dense fir forest based diet. A similar explanation has been suggested to explain variations in the carbon isotopic ratios of East African animals from forested vs. open habitats (Ambrose and DeNiro 1986). There also exist differences in the isotopic ratios of plants which arise, however, from developmental stage, physiological capability and other environmental factors (Tieszen and Boutton 1989), and these may also generate fluctuations in carbon isotopic chronologies. Whether the carbon isotopic signal in moose teeth indeed reflects changes in forest ecology and moose winter feeding strategies will require further study of the isotopic composition of Isle Royale moose dietary components.

Since the late 1970s, the carbon isotopic signal in moose teeth tends to show an enrichment in the heavy isotope, which is a reversal of the trend seen in the earlier decades. The moose born during this period were larger and had a better nutritional status than those born a decade earlier (Peterson 1988), which is mainly due to reduced moose density and recovery of food supplies. It is unclear whether this parameter would affect carbon isotopic fractionation. Since the record after 1975 is admittedly sparse, additional measurements need to be carried out in order to verify the observed trend.

The nitrogen isotopic ratios recorded in the moose teeth (see Fig. 2) show much larger variability than those observed in the carbon isotopic chronology. During the sampled time interval, the nitrogen isotopic ratios change by more than 5‰, compared to a 1.5‰ change observed for carbon isotopes. We feel that the variations in the nitrogen isotopic record are real and not an artifact of the sampling and processing procedure. This conclusion is based on the results we have obtained on some other animals (n = 3) in which we determined the isotopic composition of the dentin amino acids isolated from both the right and left first molars of the same lower jaw (e.g., 3 pairs, 6 teeth total). These analyses demonstrated that the $\delta^{15}N$ and $\delta^{13}C$ values were reproducible to within $\pm 0.6‰$ and $\pm 0.1‰$, respectively.

The Isle Royale nitrogen isotopic chronology shows no consistent pattern other than it is less variable during the later part of the record than during the first part. The factors that affect nitrogen isotopic environmental and biogenic signals over time are so poorly understood that it would be premature to attempt an interpretation of the Isle Royale moose teeth record, especially since as far as we can ascertain this is the first nitrogen isotopic chronology which has been obtained. Changes in feeding habitat, nutritional status and forest ecology are each probably important as well as are climatic changes which may affect plant nitrogen fixation. The large variability we have observed in the nitrogen isotopic ratios of moose teeth over a short time interval of only 40 years, however, is somewhat surprising considering the restricted habitat of Isle Royale. Although factors such as rainfall variability (Heaton et al. 1986) and water stress (Schoeninger and DeNiro 1984; Ambrose and DeNiro 1986) have been shown to affect nitrogen isotopic ratios, our results suggest that additional environmental and biological factors generate significant changes in nitrogen isotopic fractionation.

Conclusions

We have shown that moose teeth act as natural recorders of carbon and nitrogen environmental isotopic ratios. Amino acids isolated from the crown of the first molar are metabolically inert and preserve the radiocarbon activities and stable carbon and nitrogen isotopic ratios present in the animal's habitat during the first year or so of life. Using teeth from animals with known birth years, it is possible to obtain an isotope chronology for a particular region. Applying this technique to various animal species from diverse geographical areas will be useful in evaluating both the regional and global biogeochemical variability of carbon and nitrogen isotopic fractionation. In cases where known age animals are not available, the bomb radiocarbon signal recorded in molar crown dentin amino acids offers a way of evaluating the year of birth.

Provided diagenetic processes have not significantly scrambled the original isotopic signals, this technique for assessing isotopic chronologies could possibly be extended to well dated fossil teeth thus providing long term carbon and nitrogen isotopic records.

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