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Palaeogeography, Palaeoclimatology, Palaeoecology 249 (2007) 271–282
PALAEO
www.elsevier.com/locate/palaeo

Carbon and nitrogen isotopic analysis of Pleistocene mammals from the Saltville Quarry (Virginia, USA): Implications for trophic relationships

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Received 1 June 2006; received in revised form 29 January 2007; accepted 1 February 2007

Abstract

The late Rancholabrean (>10,000 years ago) Saltville Quarry in Virginia, USA preserves a community of basal herbivores, including several families of giant mammals, representing a range of potential feeding strategies (browsers versus grazers) and digestive mechanisms (ruminants versus non-ruminants). In this study, carbon and nitrogen isotopic analysis of well-preserved bone collagen from a variety of body elements was used to determine the trophic relationships among these herbivores. A range of $\delta^{15}N$ values was observed with ruminants and non-ruminants clustering into two distinct groups. Analysis of a young mammoth revealed a relatively high $\delta^{15}N$ composition, which is consistent with a milk-dominated diet and supports the previously noted "juvenile effect" observed in the isotopic analyses of sub-adult mammals. In addition, the isotopic evidence supports the view that a giant ground sloth was primarily herbivorous, as opposed to competing hypotheses for an omnivorous or carnivorous nature. The measured ^{13}C abundances across the range of mammals, and of fossil organic matter, indicate a lack of C-4 grasses in the regional environment and a reliance on C-3 vegetation by all herbivores included in this study. These findings may record a regional geographic transition between C-3 and C-4 dominated grasslands. However, the overlapping isotopic signatures may also be considered as a potential indicator of extreme competition amongst the basal herbivores in this ecosystem, which has broader implications for the subsequent mass extinction of mega-herbivore clades at the end of the Pleistocene epoch.

Keywords: Stable isotopes; Trophic analysis; Paleoecology; Saltville; Pleistocene

1. Introduction

The study of paleoecology is important insofar as it provides information on how ancient animals lived and interacted with their environment. Examining diet can be an extremely useful tool in this endeavor as it provides insight into the food chain. Previous paleodietary studies have considered the concept of the niche in Pleistocene North America, defining particular functions and positions of mammals within their respective habitats (MacFadden and Cerling, 1996; Koch et al., 1998; Feranec and MacFadden, 2000; Coltrain et al., 2004; Kohn et al., 2005). Determination of the proportion of herbivore browsers, grazers, and intermediate feeders

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doi:10.1016/j.palaeo.2007.02.002

(mixed feeders) within a community yields insight into niche splitting. The partitioning of resources allows for an increase in animal diversity due to a decrease in competition for food.

On the North American east coast during the Rancholabrean North American Land Mammal Age (ca. 0.3 to 0.01 Ma) of the Pleistocene epoch there were several families that included morphologically large species. These included the Proboscidea (mammoths, mastodons), Xenarthra (ground sloths), Artiodactyla (bovids, deer, camelids), and Perissodactyla (horses and tapirs), all of which appeared to occupy the same mega-herbivore niche. Considering that present day North America lacks such diversity of mega-herbivores, the question arises as to just how these giant herbivores co-existed. It has been suggested that one or more species of giant ground sloth were not strict herbivores, but rather omnivores, opportunistic scavengers, insectivores, or possibly even carnivores (Martin, 1975; Hansen, 1978; Naples, 1989; Cork, 1994; Farina, 1996; Farina and Blanco, 1996). *Mammuthus columbi*, one of the largest herbivores of the Cenozoic, is a suspected grazer based on hypsodonty and carbon isotopic data indicative of a primarily grassy diet (Koch et al., 1998; Feranec and MacFadden, 2000; Sanchez et al., 2003; Feranec, 2004), while *Mammut americanum* is suspected to be a browser or a more general opportunistic herbivore (Laub et al., 1994; Koch et al., 1998; Gobetz and Bozarth, 2001; Sanchez et al., 2003; Coltrain et al., 2004). This suggested partitioning of plant resources may have relieved some of the stress on the total community food source and allowed each large herbivore to occupy an individual ecospace.

Stable isotopic analyses of a representative North American Rancholabrean herbivore community may

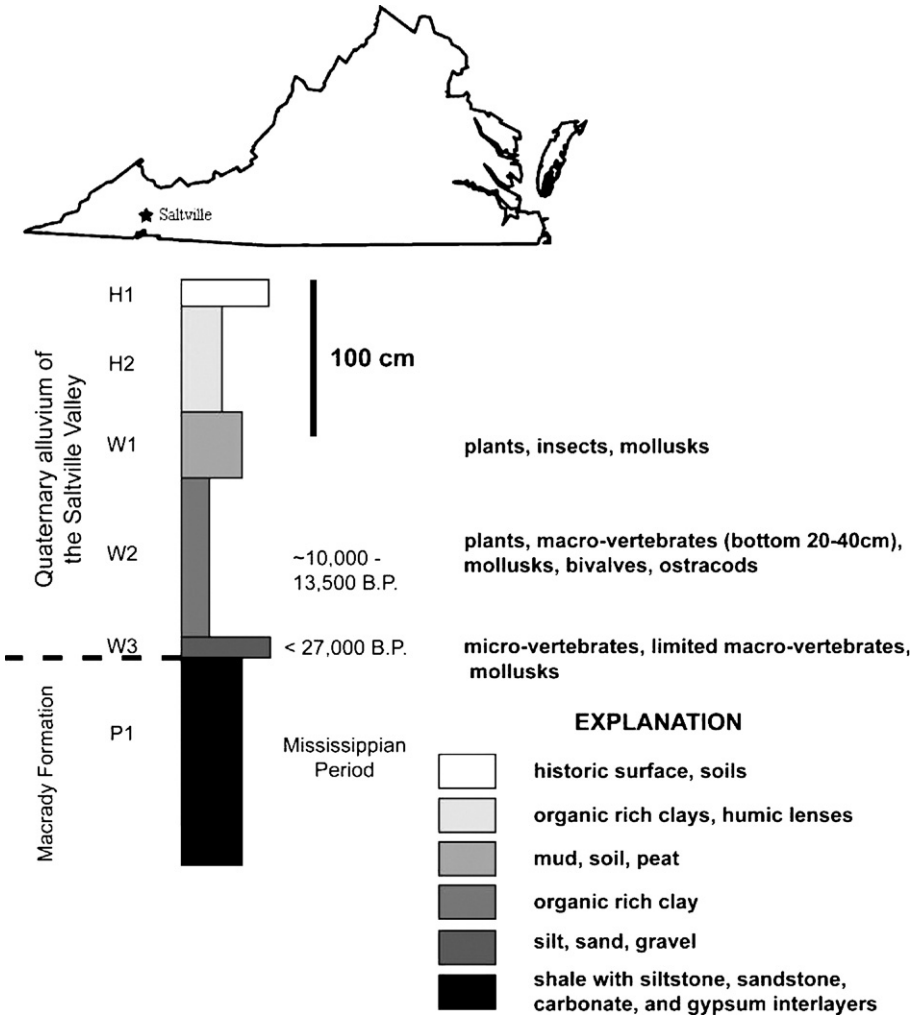

Fig. 1. Map of Virginia, USA showing the location of Saltville, simplified stratigraphic column of the Saltville Quarry sedimentology after McDonald (1984).

provide the quantitative evidence necessary to determine niche partitioning within this extinct ecosystem. Samples from the Saltville Quarry in Virginia, USA constitute a collection of large herbivores from the late Rancholabrean. This study initially aimed to delineate competitive niches amongst the herbivores, and potentially add new data to the ongoing controversy over omnivory and carnivory within the ground sloths.

2. Samples and geologic setting

The fossil bearing strata of the Saltville Valley are located in the southwestern tip of Virginia (Fig. 1). Sediments consist of loosely packed Quaternary alluvium composing eight different stratigraphic units ranging in age from upper Holocene to ~27,000 ka (McDonald, 1984). The units are primarily fluvial and lacustrine in origin and suggest periodic transitions between moist swampy areas and drier fluvial systems as evidenced by cycles of peaty muds and silty–sandy clays. The vegetation varied from sedge meadows and alder swamps to pine and spruce forests (Ray et al., 1967; McDonald, 1984). The majority of large mammal remains are found in units ranging in age from ~12 to 20 ka. Animal remains include *Megalonyx jeffersonii*, *Equus* sp., *Mammut americanum*, *Mammuthus primigenius*, *Rangifer* sp., *Bison* sp., *Bootherium* sp., *Symbos cavifrons* (junior synonym for *Bootherium*), *Odocoileus*, *Alces*, and unidentified large carnivores (McDonald, 1984). Specific samples for this study were obtained from the Smithsonian Museum of Natural History (NMNH) and the Virginia Museum of Natural History (VMNH). Table 1 lists all sampled species and body elements.

In addition to these animal remains, the organic fraction of two sediment samples from the Saltville site was analyzed for carbon isotope composition in an effort to estimate the dominant flora available for consumption. Sediment sample VMNH-A was collected directly from one of the primary sampling sites in the quarry. Sample VMNH-B was obtained from sediment removed from a mammoth tooth upon preparation of the sample.

3. Stable isotopic reconstruction of trophic levels

Due to our inability to observe extinct animals directly in their natural habitat, preserved chemical signatures in bones and teeth are often used to analyze

Table 1
Sample list

Genus/species	Common name	Collection[a]	Museum designation	Body element
Bootherium	Musk ox A	VMNH	95-51W-19S	Rib
Bootherium	Musk ox B	VMNH	95-54W-25S-1	Rib
Bootherium	Musk ox C	VMNH	95-54W-25S-2	Rib
Bootherium	Musk ox D	VMNH	92-63W-23S-8	Ball joint
Bootherium	Musk ox E	VMNH	10N5E	Scapula (?)
Bootherium	Musk ox F	VMNH	2349	Mandible
Bootherium	Musk ox G	VMNH	92-61N-20S-2	Rib
Bootherium	Musk ox G	VMNH	92-61N-20S-1	Rib
Bootherium	Musk ox H	VMNH	92-58W-21S-1	Rib
Bootherium	Musk ox I	VMNH	59W-25S-SV2	Metacarpal
Bootherium	Musk ox J	VMNH	93-26S-55W-7	Cranial element
Bootherium	Musk ox K	NMNH	None listed	Scapula
Bootherium	Musk ox L	NMNH	23264	Cranium
Bootherium (“*Symbos cavifrons*”)	Musk ox N	NMNH	23705	Cervical
Mammut	Mastodon A	VMNH	55W-27S (#038)	Ankle element
Mammut americanum	Mastodon B	NMNH	8071	Patella
Mammut americanum	Mastodon C	NMNH	215076	Longbone
Mammuthus (juvenile)	Mammoth A	VMNH	92-28S-63W-1	Mandible
Mammuthus columbi	Mammoth B	NMNH	None listed	Longbone
Mammuthus (“*Elaphus primigenius*”)	Mammoth C	NMNH	None listed	Thoracic Centrum
Megalonyx jeffersonii	Megalonyx	NMNH	23737	Femur
Rangifer tarandus	Caribou	NMNH	23700	Antler
Cervalces	Deer A	NMNH	23704	Calcaneum
Cervalces	Deer B	NMNH	23750	Antler
Equus	Horse	NMNH	23703	Femur
Unidentified	Ovibovine	NMNH	None listed	Pelvis

[a] NMNH (National Museum of National History); VMNH (Virginia Museum of Natural History).

feeding habits and subsequently determine trophic levels within a fauna. Specifically, nitrogen and carbon isotopes from bone collagen can provide distinctions between different trophic levels within a food chain (DeNiro and Epstein, 1978; Schoeninger and DeNiro, 1984).

During the processes of excretion, animals preferentially retain ^{15}N over ^{14}N (Sutoh et al., 1987). Therefore, the nitrogen isotopic composition of an animal mimics its diet but is offset to higher ^{15}N due to the enrichment of ^{14}N in urine and other related waste products. Nitrogen isotopic compositions are shown to step ~3.0–4.2‰ with each trophic level in a food chain (DeNiro and Epstein, 1981; Minagawa and Wada, 1984; Schoeninger and DeNiro, 1984; Post, 2002; Bocherens and Drucker, 2003) where isotope abundances are measured in the standard permil notation according to the following formula:

$$\delta^{15}N = [(R_{sample} - R_{standard})/R_{standard}] * 1000$$

where $R = {}^{15}N / {}^{14}N$ and the accepted standard is atmospheric N_2.

Carbon isotopes behave similarly in the sense that ^{13}C is more readily retained than ^{12}C with each step in trophic level. However, the isotopic difference is only ~1‰ between steps and it is therefore more difficult to detect subtle differences in feeding strategies (DeNiro and Epstein, 1978; Rau et al., 1983; Post, 2002; Bocherens and Drucker, 2003). The $\delta^{13}C$ values are determined by the same formula for nitrogen where $R = {}^{13}C / {}^{12}C$ and the accepted standard is V-PDB.

Carbon isotopes have the added benefit of providing information regarding different types of plant consumption. The C-3 and C-4 photosynthetic pathways of plants each produce characteristic isotopic signatures. The C-3 plants, which consist of dicots, most trees, shrubs, herbaceous plants, and some grasses yield $\delta^{13}C$ values of $\sim -26.5 \pm 3$‰ (Smith and Epstein, 1971; O'Leary, 1988; Heaton, 1999). The C-4 plants, which consist of a specific subset of grasses and sedges, exhibit a $\delta^{13}C$ of $\sim -12.5 \pm 3$‰ (Smith and Epstein, 1971; O'Leary, 1988). The carbon isotopic difference between plants and herbivore bone collagen is ~2–5‰ (van der Merwe, 1982; Balasse et al., 1999; Roth and Hobson, 2000). This results in a $\delta^{13}C$ of ~−21.5‰ for browsers (herbivores that consume primarily trees, shrubs, and herbs that are virtually all C-3). Grazers (herbivores that consume primarily grasses) will exhibit a $\delta^{13}C$ value that is indicative of the dominant type of grass in the local area. The presence of C-4 variety grasses will result in a $\delta^{13}C$ of herbivore bone collagen of ~−7.5‰. An extremely low abundance or complete absence of C-4 grasses will result in similar $\delta^{13}C$ signatures between browsers and grazers. While there is a certain amount of natural variation in these values, the C-3 and C-4 signatures are nonetheless distinct from one another. Therefore, the carbon isotopic data are valuable for distinguishing both the presence of C-4 grasses and the different herbivore niches within a food web that contains this specific subset of plants.

The sediment samples provide insight to the predominant plant types available to the herbivores at the Saltville site. In the absence of diagenetic or heat-induced alteration, the isotopic signature of insoluble organic matter (i.e. kerogen) is believed to be an accurate reflection of original plant material incorporated into the sediment (Hayes et al., 1983). Therefore, an ecosystem dominated by either C-3 or C-4 plants should become apparent in isotopic analyses of autochthonous sediments.

4. Methods

4.1. Sample preparation

Animal bones collected for this study were drilled using a rotary tool and diamond tip powdering bit or a plug bit. Approximately 150–300 mg of bone was obtained. In this study, no effort was made to separate the compact and cancellous portions of the bone.

Specimens were processed according to the procedures of Stafford et al. (1988). Specimens were first sonicated in ultra-pure Milli-Q water to remove any clinging sediment material or any labile salts. They were then dried overnight. This was followed by a demineralization soak in 0.6 M HCl (~1 mL acid:20 mg bone) at 4 °C with fresh acid added every 24 h until reaction ceased. The solid bone residue was then rinsed in ultra-pure water and dried overnight. The dried bone material was soaked in 5 mL of 0.03 M HCl at 90 °C for 24 h. The acidic solution was separated from the solid bone residue and freeze-dried to isolate a crude collagen extract.

This extract was then soaked in 10 mL of 6 M HCl at 100 °C for 24 h. It was centrifuged and the supernatant containing the denatured protein as well as any humic contaminants was poured off and saved. A 5 cc plastic syringe was loaded with 2–3 cc of Serdolit® PAD-I resin (0.1–0.2 mm particle size). The resin was conditioned using three bed volumes of 6 M HCl. The supernatant was then allowed to pass through the resin at ~200 μL/min. The eluant was saved. The column was then rinsed with two bed volumes of 6 M HCL; both

Table 2
Data

Genus/species	Common name	% yield [a]	Collagen %N [b]	Collagen %C [b]	$\delta^{15}N$ [c]	$\delta^{13}C$ [c]	C:N
Bootherium	Musk ox A	18.8	8.73	24.22	5.81	−19.99	3.24
Bootherium	Musk ox B	18.1	7.90	21.27	2.29	−20.48	3.14
Bootherium	Musk ox C	9.8	7.22	22.43	2.20	−20.95	3.62
Bootherium	Musk ox D	9.8	9.19	24.87	2.35	−20.40	3.16
Bootherium	Musk ox E	24.8	10.23	27.71	2.66	−20.61	3.16
Bootherium	Musk ox F	11.9	8.75	23.98	2.36	−20.94	3.20
Bootherium	Musk ox G	7.6	9.17	26.66	5.58	−20.36	3.39
Bootherium	Musk ox G	8.9	8.53	24.06	5.55	−20.55	3.29
Bootherium	Musk ox H	22.4	10.40	27.93	5.68	−19.91	3.13
Bootherium	Musk ox I	28.8	10.00	26.35	2.49	−20.67	3.07
Bootherium	Musk ox J	13.3	8.68	23.55	2.33	−20.64	3.17
Bootherium	Musk ox K	13.7	9.02	24.17	2.50	−20.71	3.13
Bootherium	Musk ox L	12.6	9.22	24.73	2.19	−20.81	3.13
Bootherium ("S*ymbos cavifrons*")	Musk ox N	30.4	11.47	30.06	1.99	−20.90	3.06
Mammut	Mastodon A	25.9	8.99	24.00	3.04	−21.15	3.11
Mammut americanum	Mastodon B	9.9	9.65	30.20	3.60	−22.40	3.65
Mammut americanum	Mastodon C	11.3	8.71	25.48	2.52	−22.08	3.41
Mammuthus (juvenile)	Mammoth A	21.2	10.16	26.64	5.60	−20.59	3.06
Mammuthus columbi	Mammoth B	12.3	9.43	25.42	5.60	−21.35	3.15
Mammuthus ("*Elaphus primigenius*")	Mammoth C	6.4	5.25	19.29	3.96	−22.00	3.53
Megalonyx jeffersonii	Megalonyx	19.2	11.17	29.52	4.65	−20.66	3.08
Rangifer tarandus	Caribou	13.1	9.06	25.54	0.79	−20.28	3.29
Cervalces	Deer A	14.9	10.37	28.76	1.53	−21.11	3.24
Cervalces	Deer B	12.1	9.58	25.60	1.39	−20.28	3.12
Equus	Horse	23.8	11.39	30.02	3.87	−22.71	3.08
Unidentified	Ovibovine	10.1	9.73	28.13	2.37	−21.79	3.37

[a] % yield indicates the weight percent of total collagen extracted from the sample.
[b] Collagen %N and collagen %C indicate the weight percent of nitrogen and carbon in the collagen final product.
[c] Isotopic values are in permil units.

rinses were saved with the eluant. The resulting solution was diluted up to 50 mL with ultra-pure water and freeze-dried.

The resin for this process is thoroughly washed before use. It is first rinsed by gravity filtration with 10 bed volumes of 50% acetone followed by 10 bed volumes of ultra-pure water. It is then soaked in alternating baths of 3 M NaOH and 3 M HCl at 80 °C for 30 min per soak. The resin is soaked three times in each bath and then rinsed copiously with ultra-pure water. It is transferred into a Soxhlet apparatus and extracted for 24 h in 100% acetone. It is rinsed excessively with water and then extracted again using 100% methanol. The resin is given a final water rinse and stored in 1 M HCl until use.

All isolated bone collagen was weighed (400–500 μg) and folded into tin cups. The samples were introduced into a Eurovector Elemental Analyzer where CO_2 and N_2 gas were produced by combustion of the collagen at 1020 °C. The gases, moved along in a continuous flow of helium, were separated by a GC column, and introduced into a Micromass continuous flow gas source mass spectrometer for carbon and nitrogen isotopic and abundance analyses. All analyses were completed at the University of Maryland Stable Isotope Geochemistry Laboratory.

The sediment samples were powdered and decalcified with 3 M HCl overnight (~1 mL acid:30 mg sediment powder). These were then rinsed with ultra-pure Milli-Q water and dried. Aliquots of the decalcified sediments were weighed (4–8 mg) and folded into tin cups prior to EA combustion analysis of %C and $\delta^{13}C$ compositions.

Reproducibility of $\delta^{13}C$, $\delta^{15}N$, and C:N values, as well as machine stability were monitored using NIST-912a urea standard. The standard exhibited a maximum error of 0.30‰ (2σ) for $\delta^{15}N$, and 0.39‰ (2σ) for $\delta^{13}C$ during individual runs of samples (refer to Table 2 for errors associated with individual data points). A modern bovine bone was also used as an in-house standard to monitor reproducibility and accuracy of all values obtained.

4.2. Examination of diagenesis

Although the bone fragments are geologically young, they were preserved in porous terrestrial sediments and

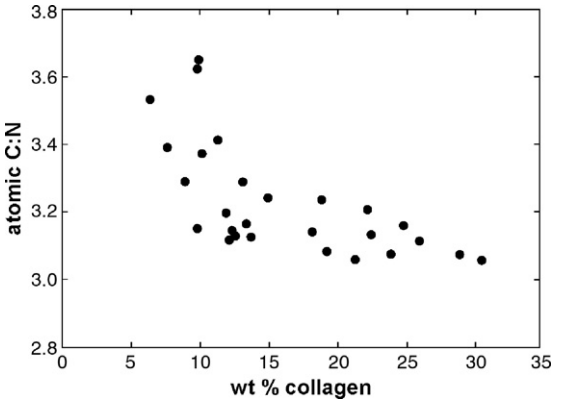

Fig. 2. C:N ratios plotted against the collagen yield as a weight percent of the whole bone sample.

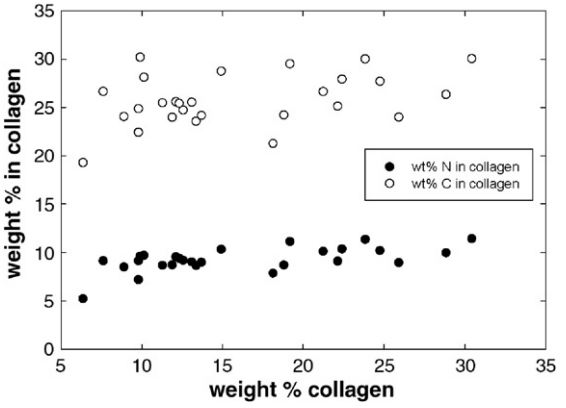

Fig. 3. Carbon and nitrogen yields as % of collagen extract plotted against the collagen yield as a weight percent of the whole bone sample.

should be examined for potential diagenetic alteration before the collagen isotopic data are accepted as primary. Research on modern and fossil samples has noted that unaltered bone collagen has an atomic C:N ratio of 2.8–3.6 (DeNiro, 1985; Ambrose, 1990; Bocherens et al., 1996; Bocherens et al., 1997; Drucker et al., 2001; Drucker et al., 2003). The percent total carbon yields from collagen range from ~30 to 45%, and percent total nitrogen yields range from ~11 to 16% (Ambrose, 1990; Bocherens et al., 1996; Bocherens et al., 1997; Drucker et al., 2003). These values are unique to bone collagen due to the unique pattern of amino acid residues in this complex helical protein. The relatively high amounts of proline and hydroxyproline amino acids in collagen contribute to its characteristic C:N ratio. Additionally, previous fossil studies have achieved a collagen yield of ~1–21% of the whole bone weight (Ambrose, 1990; Bocherens et al., 1991; Bocherens et al., 1994a; Coltrain et al., 2004). In this study, fossil specimens were monitored for their C:N ratio, %C and %N in collagen extract, as well as overall yield of collagen and compared to modern bone data.

5. Results

5.1. Preservation

Virtually all of the samples exhibited atomic C:N ratios and percent yields of collagen within the acceptable range for unaltered collagen (Table 2, Fig. 2). The C:N ratios ranged from 3.06 to 3.65; the percent yield of collagen extracted ranged from 6.4 to 30.5%. The yields of nitrogen and carbon in the collagen ranged from 5.3 to 11.5% and 19.3 to 30.1%, respectively. Eight samples, which had elevated C:N ratios above 3.65, were excluded from further consideration.

A trend of increasing C:N ratios with decreasing collagen yield is apparent in the data (Fig. 2). The upper limit of acceptable C:N ratios was determined by comparison of the weight percent C and N in the collagen with both C:N and overall weight percent yield of collagen. The individual weight percent yields of both carbon and nitrogen are not well correlated with the overall yield of collagen (Fig. 3). The low R^2 values of 0.16 and 0.35 for weight percent C and weight percent N, respectively, suggest that little of the variation in the elemental yields is governed by the amount of collagen extracted from the bone. Comparison of weight percent C and weight percent N with C:N ratios suggests no significant correlation (Fig. 4) as indicated by R^2 values of 0.042 and 0.33, respectively. A low yield of carbon and nitrogen may suggest that some of the original collagen has been removed from the bone. However, the C:N ratio is consistent with samples yielding higher amounts of these two elements, thus we argue that the

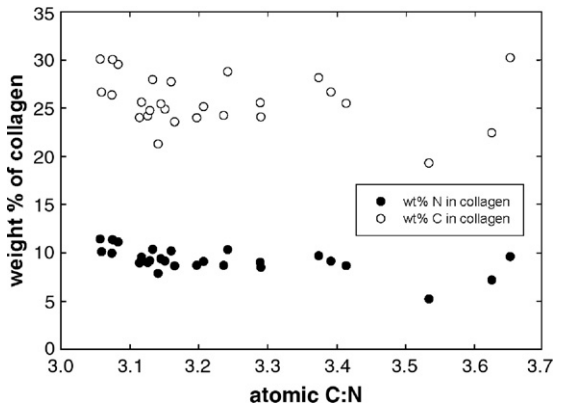

Fig. 4. Carbon and nitrogen yields as % of collagen extract plotted against C:N ratios.

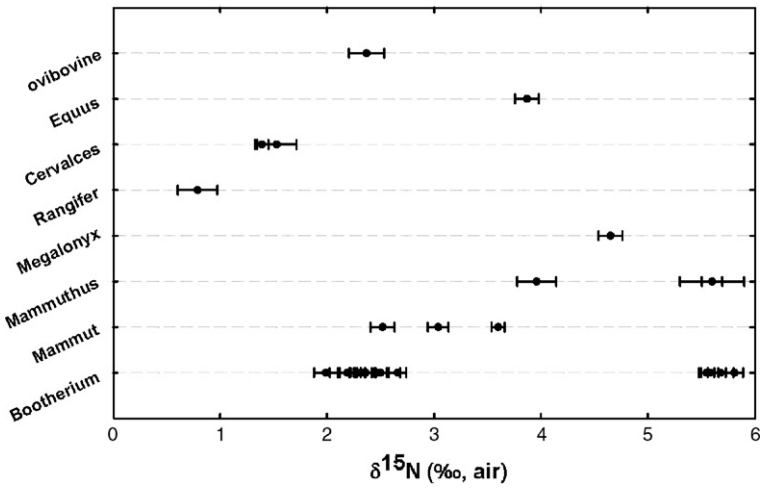

Fig. 5. Nitrogen isotopic data grouped according to genus.

remaining collagen is intact and well preserved. Therefore, the upper limit of 3.65 for acceptable C:N values was determined by considering samples that maintained the trend of independence between the yield percents and C:N ratios.

The collagen yield of a few samples is higher than the range of previously published literature values range of 1–21%. This is likely due to the difference in methods of processing. The literature range of 1–21% was achieved using a NaOH soak to remove humic and fulvic contaminants, as opposed to the PAD resin used in this study for the same purpose. While the NaOH method has been proven to produce quality data, it also runs the risk of hydrolyzing collagen protein and hence prematurely removing it from the bulk sample early in the extraction chemistry, thereby resulting in a slightly lower yield. This study does not dispute the validity and quality of the NaOH method. Rather, we chose to use the resin due to very limited sample sizes and the subsequent necessity to avoid premature collagen removal and achieve higher yields. Modern bone processed in this lab using the resin method has produced collagen yields ranging 20–30%, values that rival the maximum yields achieved during fossil bone processing. This suggests that the Saltville sample set includes a few exceptionally well-preserved specimens while the remainder exhibits a slightly lower, yet adequate, quality of preservation.

The percent nitrogen and carbon contained in the final collagen product appear lower than the previously published yields for carbon and nitrogen from extracted collagen. However, it should be noted that the

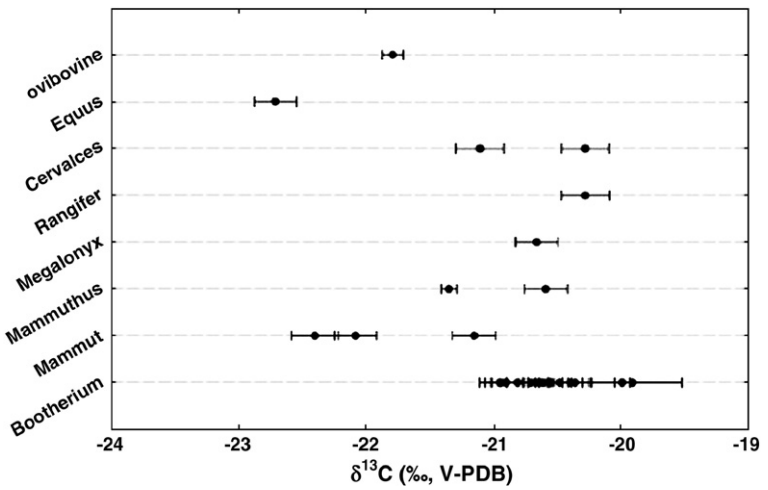

Fig. 6. Carbon isotopic data grouped according to genus.

gelatinous collagen final product exhibited a remarkable tendency to hydrate quickly after freeze-drying. Therefore, during the weighing process before introduction to the mass spectrometer, an unknown portion of the sample weight was contributed by secondary water. This affects the apparent percent yields of carbon and nitrogen, but does not alter the C:N ratio. Therefore, after determination of the upper acceptable limits of C:N ratios, these C:N ratios were used to determine the diagenetic state of the final collagen product.

5.2. Carbon and nitrogen isotopes

The nitrogen isotopic data yield a range of ~5‰ with various trends exhibited amongst the different animals (Fig. 5). The *Rangifer* specimen exhibited the lowest value with a δ^{15}N of +0.79‰. The highest values were included in the *Bootherium* specimens which showed a range in δ^{15}N values of +1.99‰ to +5.81‰. Observations to note include the apparent separation into two distinct groups of the *Bootherium* as well as the consistency of the *Megalonyx* data with that of the other herbivores in the sample group. The mammoths show a range of values (+3.96‰ to +5.60‰) that rival the highest δ^{15}N exhibited by the *Bootherium*. However, this apparent similarity in ranges may be due to the presence of a mammoth juvenile (see Discussion).

The carbon isotopic data yielded a range of ~3‰ with considerable overlap between animals (Fig. 6). *Equus* showed the lowest δ^{13}C value of −22.71‰. The most enriched values belonged to the *Bootherium*, which showed a range of −22.54‰ to −19.91‰. The apparent similarity between the *Megalonyx* and the rest of the sample group is once again visible. Finally, the sediment samples yielded δ^{13}C values of −25.58‰ and −26.45‰. This mimics the collagen data in that neither set of data has values above −19‰.

6. Discussion

The range in bone collagen nitrogen isotopic data from this study suggests two potential factors that could cause such a distribution: 1) natural variation in the nitrogen isotopic composition of plants in the Saltville area during the late Pleistocene, or 2) the presence of both ruminants and non-ruminants in this fauna. The former can be considered a potential factor based on observations of various terrestrial flora and associated herbivore fauna exhibiting δ^{15}N ranges of at least 5‰ or much greater (Schoeninger and DeNiro, 1984; DeNiro, 1985; Ambrose and DeNiro, 1989; Ambrose, 1991; Bocherens et al., 1994a; Bocherens et al., 1996; Handley et al., 1999; Drucker et al., 2001; Coltrain et al., 2004). The natural variation in soil δ^{15}N values and the fractionation during different methods of nitrogen incorporation into plant tissues (i.e. nitrogen fixing versus non-nitrogen fixing plants) are the primary factors contributing to the observed δ^{15}N range in plant tissues (Shearer and Kohl, 1989; Virginia et al., 1989; Gebauer, 1991; Nadelhoffer and Fry, 1994). If these are the primary factors influencing the nitrogen isotopic composition of the herbivores in this study, then both the observed overlap and range of δ^{15}N values are expected given that the animals would all be consuming plants from a similar soil and nutrient base while still potentially being slightly selective over plant choice. Particularly notable is the depleted δ^{15}N value of the *Rangifer* in this study which suggests selectivity for lichen, a food which is commonly ^{15}N depleted (Virginia and Delwiche, 1982). This pattern of *Rangifer* exhibiting the most depleted δ^{15}N values of all herbivores within an ecosystem has been previously recorded in both modern and Pleistocene faunas (Bocherens et al., 1994b; Fizet et al., 1995; Iacumin et al., 2000; Drucker et al., 2001).

The presence of both ruminants and non-ruminants presents the alternate possibility that digestive physiology is responsible for the observed range in nitrogen isotopic values in this study. This idea can be explored by first examining the mammoths and the mastodons. The mammoth specimens plot relatively high in the range of δ^{15}N values for this ecosystem with one adult in particular showing a value as high as +5.60‰. The mammoths also show a statistically higher average value than the mastodons ($df=1$, $\alpha=0.15$). While a larger data set including additional proboscideans is required to support this finding, it is in agreement with previous research (Bocherens et al., 1996; Coltrain et al., 2004). This pattern is commonly attributed to the presence or absence of a ruminant digestive strategy and the subsequent variations in isotope fractionation that may take place during digestion. Ruminants (or foregut fermenters) generally seek protein-rich diets and digest their stomach microbial symbiotes, whereas non-ruminants have a lower protein diet and generally exhibit a lower degree of microbial symbiosis (Stevens and Hume, 1995).

Examination of ecosystems including proboscideans, as well as other basal herbivores, reveals differing patterns amongst ruminants and non-ruminants in terms of their nitrogen isotopic compositions. Coltrain et al. (2004) found higher δ^{15}N values for known ruminants in a Pleistocene ecosystem whereas Bocherens et al.

(1996) found relatively lower values for ruminants. Despite this disagreement, the ruminants and non-ruminants in both studies tended to cluster with their respective groups. Based on modern analogues and sister taxa, the ruminants from Saltville include the *Cervalces*, *Rangifer*, *Bootherium*, and unidentified ovibovine specimen. With the exception of one subset of musk oxen, these data represent the lower end of the δ^{15}N range observed in this study (average δ^{15}N = +2.89±1.59‰ 1σ, n=18). The suspected non-ruminants include the *Equus*, *Mammut*, *Mammuthus*, and *Megalonyx* specimens. These specimens all fall in the upper end of the δ^{15}N range (average δ^{15}N=+4.11± 1.12‰ 1σ, n=8). A simple *t*-test suggests that these two populations exhibit significantly different means (df=18, p=0.038). Exclusion of the enriched group of musk oxen specimens supports this analysis even more strongly (df=8, p=0.0011). A directional Mann–Whitney test suggests that the ruminants exhibit an average value that is significantly lower than the non-ruminants (p=0.0091). While it appears that the ruminants and non-ruminants tend to cluster within two distinct populations, it should be noted that several of the taxa are represented by only one specimen. Until a greater number of specimens are analyzed, this nitrogen isotope study cannot conclusively determine the relative position of ruminants versus non-ruminants. Multiple specimens for each genus would be an ideal statistical scenario, but the unfortunate reality is that the fossil record is often meager. Despite these statistical weaknesses, it does appear that digestive strategy plays at least some role in the determination of nitrogen isotopic ratios in herbivore bone collagen.

The single mammoth juvenile included in the study exhibited a relatively high δ^{15}N value (+5.60±0.10‰, 2σ). Modern and fossil systems indicate that juveniles often plot one trophic level higher than adults of the same species due to the apparent nitrogen isotopic enrichment in mammalian milk (Fogel et al., 1989; Bocherens et al., 1994a,b; Balasse et al., 1999). This, however, does not explain the relatively high nitrogen isotopic signatures observed in at least one other mammoth and several of the musk oxen. The mammoth adult that exhibits a similar nitrogen isotopic signature to the mammoth juvenile was not identifiable at the species level. The potential taxonomic difference between this adult and the juvenile may explain the overlap in isotopic data. The musk oxen with higher δ^{15}N values were all sampled from rib fragments which were not age indicative. Further comparisons with known musk ox juveniles are necessary to determine if the higher δ^{15}N values observed in some of the musk oxen from this study are due to the aforementioned "juvenile effect".

The *Megalonyx* specimen presented a unique opportunity to examine the debate concerning the potential carnivorous nature of the giant ground sloths. The data show a relatively high δ^{15}N value (+4.65±0.11‰, 2σ) for this particular sloth. Based on sister taxa and modern sloths, the giant ground sloths of the Pleistocene are generally considered to be non-ruminants. This study supports this view due to the similarity in δ^{15}N values between the *Megalonyx* and other non-ruminants. However, previous research has also suggested that the giant ground sloths may have been omnivorous based on the biomechanical structure of their forearms allowing for precise butchering of captured prey; furthermore they may have evolved highly developed brains allowing for the necessary sensory aptitude for hunting (Aramayo, 1988; Dozo, 1989; Farina, 1996). Our ability to obtain only one quality sloth specimen from the Saltville locality prevents a statistical comparison between the *Megalonyx* and the other herbivores, yet the fact that the *Megalonyx* δ^{15}N and δ^{13}C values plot well within the range of the rest of the herbivores (Figs. 5 and 6) strongly suggests a herbivorous diet. The possibility of a small meat component in the *Megalonyx* diet remains possible, but it is highly unlikely that this animal partook of a strict meat diet. This is in agreement with previous findings for a Pleistocene *Megalonyx* from Alberta (Bocherens et al., 1994b) and the Pleistocene sloth *Paramylodon harlani* (Coltrain et al., 2004), which exhibited carbon and nitrogen isotopic values similar to those of strict herbivores from the same ecosystem.

While it is expected that the nitrogen isotopic data would exhibit an overlap among the different herbivores, it is equally expected that the carbon isotopic data should show some degree of partitioning between C-3 and C-4 plant consumers. However, the data show a noticeable lack of difference in δ^{13}C values (Fig. 6). All of the data plot within the range indicating a primarily C-3 diet for these herbivores, thus suggesting a general absence of C-4 grasses in this area. This unexpected result is difficult to explain in light of previous research showing a definite presence of C-4 grass consumption in similar east coast ecosystems (MacFadden and Cerling, 1996; Koch et al., 1998; Feranec and MacFadden, 2000; Kohn et al., 2005). However, some studies have shown a similar lack of carbon isotopic discrimination to that observed here with the herbivores exhibiting primarily a C-3 signature (Bocherens et al., 1996; Coltrain et al., 2004). The differences could possibly be explained by location. The C-4 plants tend to be adapted for warmer

and drier climate regimes, and the studies showing a pronounced influence of C-4 plants in animal diets are geographically south of the Saltville locality. The region including the Saltville Quarry consisted of primarily cool mixed forests including pines, spruce, oak, and sedge, while regions further south exhibited slightly more open temperate forests (Ray et al., 1967; Webb, 1981; Williams et al., 2000; Williams et al., 2001; Webb et al., 2004). Grass pollen counts are relatively low in this region with relative abundances generally less than 10% (Ray et al., 1967; Williams et al., 2000). The two sediment samples analyzed as part of this study support the idea that C-4 plants were scarce as both samples show an isotopic signature indicative of primarily C-3 plants. This apparent predominance of C-3 type plants at the Saltville locality may explain the lack of any strict C-4 grass diets apparent in the carbon isotopic compositions of the animals. It is likely that the herbivores commonly considered quintessential grazers, such as *Equus*, did in fact consume grasses and herbaceous vegetation. They were simply not reliant on C-4 grasses. This study then suggests that the transition between C-4 and C-3 dominated grasslands would necessarily have occurred south of the Saltville, Virginia fossil locality during the time period 12–20 ka.

An alternate explanation for the overlapping carbon isotopic values of these herbivores is a compaction of niches among C-3 browsers and formerly C-4 grazers due to climatic or environmental stresses. Climate transitions often induce changes in floral distribution and/or abundance, which may in turn lead to increased competition for a shrinking or shifting food base. Specifically, a shift in the distribution of open grasslands could result in competition between browsers and those grazers formerly reliant on C-4 grasses. While it is not possible to support or further develop this idea with our limited isotopic data, the coincidence of extreme climate changes and mass extinction at the end of the Pleistocene should be considered in a larger analysis of trophic structure, niche compaction, and collapse. A causal link between the extinction of the late Pleistocene North American terrestrial fauna and climate-induced vegetation change may be considered later with additional data from sites spanning different geographic localities from the latest Pleistocene.

7. Conclusions

The herbivore fauna from the Saltville Quarry shows a range of nitrogen isotopic values likely indicating some natural variation among plant species in this region, as well as potential differences in the digestive strategies of the studied species. The data follow previously published trends where mammoths show a slightly higher nitrogen isotopic signature than mastodons, and a mammoth juvenile also exhibited a relatively high nitrogen isotope value likely due to the enrichment of mammalian milk. This isotopic signature has been previously observed, but only in limited fossil occurrences. Despite the sample size of only one juvenile in this study, the results still suggest that such a signature may be present in various other fossil fauna and should therefore be considered in the analyses of any sample sets including sub-adults. This study also contributes further insights into the digestive physiology of the giant ground sloths. This is a group of animals that has no modern analogue and has therefore been subject to numerous hypotheses concerning its potential consumption of meat. This contribution to the previously existing data set on extinct sloths supports the idea of an herbivorous diet of a non-ruminant nature. The question of sloth digestive physiology is not yet conclusively answered, but more studies of this type can certainly provide valuable quantitative evidence to the debate.

Finally, the lack of enriched carbon isotopic values among the herbivores at Saltville suggests either a complete lack of C-4 plants at this site or a compaction of niches due to a reduced food source. Rather than a total absence of C-4 plants, it is more likely that C-4 plants were extremely limited in this region and were therefore unable to provide a complete food base for one or more species. Those species generally reliant on grazing would have consumed the C-3 varieties within the grasses and herbaceous plant groups. This apparent C-4 limitation may have been present throughout the Pleistocene, or it may be a result of the severe climate changes associated with the late Pleistocene deglaciations. A time series palynological study of the Saltville area would greatly aid in this interpretation and should be considered for future work. Meanwhile, this study presents an additional suggestion that the observed carbon isotopic pattern represents an intriguing compaction of niches at the base of this food chain which could have implications for the extinction of this mega-herbivore fauna. Future analyses of other late Pleistocene fauna including carnivores are warranted to further examine this issue.

Acknowledgements

We thank Nick Fraser and the Virginia Museum of Natural History as well as Bob Purdy and the Smithsonian National Museum of Natural History for providing sample material. We also thank the editors of

this journal for their consideration and H. Bocherens and M.J. Kohn for their many helpful comments and very constructive criticisms. Funding for this project was supplied by NSF grant EAR040709-8545.

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