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CARBON AND NITROGEN ISOTOPIC EVIDENCE FOR TERTIARY GRASSLAND DISTRIBUTIONS AND THE EVOLUTION OF HYPSODONTY IN NORTH AMERICAN GREAT PLAINS HORSES (32 MA TO RECENT)

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Shawn Gilbert Clouthier

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ABSTRACT

CARBON AND NITROGEN ISOTOPIC EVIDENCE FOR TERTIARY GRASSLAND DISTRIBUTIONS AND THE EVOLUTION OF HYPSODONTY IN NORTH AMERICAN GREAT PLAINS HORSES (32 MA TO RECENT)

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Isotopic analysis of indigenous organic matter from a geographically and temporally constrained series of fossil horse teeth is presented. Carbon and nitrogen isotope and amino acid analyses of modern and ancient horses serve to assess natural isotopic variation in skeletal remains and verify authenticity of ancient organic matter. Carbon isotope values ($\delta^{13}C$) show the relative amounts of C_3 and C_4 plants in ancient horse diets which is used to assess Tertiary grassland distributions and the advent of high-crowned horse teeth. Organic $\delta^{13}C$ data reveal that C_4 grasses comprised 20-30% of the diet of Great Plains horses in the middle Miocene (15.5 Ma to 12 Ma). This is in contrast to inorganic $\delta^{13}C$ studies of horse enamel carbonate which showed a C_4 proliferation 7 Ma to 5 Ma related to decreased global CO_2 levels. Since horses with C_4 dominate diets were localized to arid regions after 7 Ma and never inhabited the Great Plains from 32 Ma to 3.3 Ma, the $\delta^{13}C$ data does not support a late Miocene drop in global CO_2. The origin of C_4 grass in the mid-Miocene postdates the evolution of hypsodonty in equids by at least 2 Ma. Consequently, a C_4 radiation cannot be the primary cause for the origin of high-crowned horse teeth. The expansion of C_3 grasses and abrasive grazing substrates are likely causes for the evolution of horse hypsodonty.

Dedicated to my grandmothers, Betty Clouthier and Mildred Romanack;

I will forever cherish our abridged time together.

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With Love,

Shawn

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KEY TO ABBREVIATIONS AND SYMBOLS

AMNH= American Museum of Natural History, New York.
MSU= Michigan State University, East Lansing.
SMU= Southern Methodist University, Dallas.
UA= University of Arizona, Tucson.
UCR= University of California, Riverside.
UF= University of Florida, Gainsville.
UMNH= Utah Museum of Natural History, Salt Lake City.
UNSM= University of Nebraska State Museum, Lincoln.
F:AM= Frick Collection, American Museum.

HPLC= High Performance (Pressure) Liquid Chromatography.
GC= Gas Chromatograph.
MS= Mass Spectrometer.
MSD= Mass Selective Detector.
FID= Flame Ionization Detector

HMW= High Molecular Weight.
TFA= Trifluoroacetic Acid.
RIA= Radioimmunoassay.
ELISA= Enzyme Linked Immunosorbent Assay.
PCR= Polymerase Chain Reaction.

C_3= Plant photosynthetic pathway (Calvin-Benson) utilizing three carbon intermediates.
C_4= Plant photosynthetic pathway (Hatch-Slack) utilizing four carbon intermediate molecules.
CAM= Plant photosynthetic pathway (Crassulacean Acid Metabolism) which alternates between the C_3 and C_4 cycles during daylight and night conditions.

RM1= Mammalian upper molar terminology (e.g. Right First Molar).
LdP4= Mammalian upper deciduous molar terminology (e.g. Left Fourth Deciduous Premolar).
RMax= Right Maxilla Bone.
Ma= Megannum, temporal unit meaning millions of years ago.
NALMA= North American Land Mammal Age.

INTRODUCTION

The horse family (Equidae) spans 58 million years and is perhaps the most well-known fossil assemblage in the world. Sequential modification in skeletal morphologies have long been cited as evidence for a gradual, stepwise evolutionary pattern for equids (Huxley, 1870; Kowalevsky, 1874; Stirton, 1947). Among other evolutionary trends, the gradual development of hypsodont dentition (high-crowned teeth) became dogmatic in paleontology. Today a purely anagenetic view of horse evolution such as this is considered simplistic. The current phylogeny of the family Equidae, including the origin and prevalence of hypsodonty, is depicted in Figure 1.

The most common hypothesis for the morphological transition from low-crowned (brachyodont) to high-crowned (hypsodont) teeth among the Equidae is as an adaptation in response to the widespread radiation of siliceous savanna grasslands in the Miocene (Stirton, 1947; Simpson, 1951; MacFadden et al., 1991). Grasses with a high silica content are more abrasive than the fleshy leaves upon which brachyodont horses browsed. Consequently, higher tooth crowns may have evolved as the dietary percentage of silica-bearing grasses increased. Alternatively, equid hypsodonty may have arisen, concurrent with the transition to a grazing lifestyle, in response to the incorporation of grit characteristic of sandy savanna substrates (Stirton, 1947; Simpson, 1951). Evolutionary change in the grinding surface of horse molars

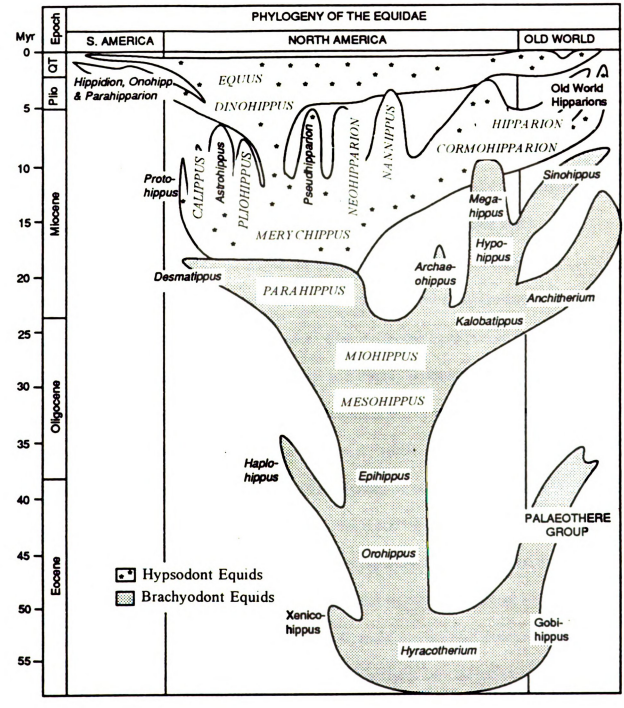


Figure 1 Phylogeny of the family Equidae throughout its 58 million year evolutionary history. The capitalized genera signifies those used in this study (modified from MacFadden, 1992).

over their phylogeny is represented in Figure 2. Both hypotheses concerning the origin of hypsodonty in horses have been championed and criticized with no real evidence upon which to substantiate either position. Recent advances in the field of isotope geochemistry have made it possible to test these and other interesting paleobiological questions. This study, for the first time, uses stable carbon and nitrogen isotopes of the organic remnant from a time series of fossils to determine if the spread of savanna grasslands influenced the diet of ancient horses and consequently contributed to the advent of hypsodonty among the Equidae.

Geochemical analyses provide information about origins and transformations of organic matter in the natural environment. Stable isotopes have proved particularly effective for evaluating dietary components and trophic positions of organisms owing to the close association between the isotope composition of an organism and its diet (DeNiro and Epstein, 1978b; 1981; Harrigan et al., 1989). This relationship exists for modern and ancient organisms (Vogel, 1978; Tieszen et al., 1979a; Cormie and Schwarcz, 1994). Provided unique geochemical signals exist, stable isotopes can be used to investigate the paleoecologies of extinct organisms. The transition to a savanna-based diet would provide a unique geochemical signal resulting from the difference in carbon isotope values ($\delta^{13}C$) of C_3 and C_4 vegatation (mean $\delta^{13}C$ = -27‰ and -13‰, for C_3 and C_4 plants, respectively). Savannas exist in regions of seasonal contrasts usually related to water stress and are among the first ecosystems to utilize the evolutionarily more recent C_4 photosynthetic pathway. Given these conditions, the importance of savanna grasslands in ancient horse diets should be recorded in the $\delta^{13}C$

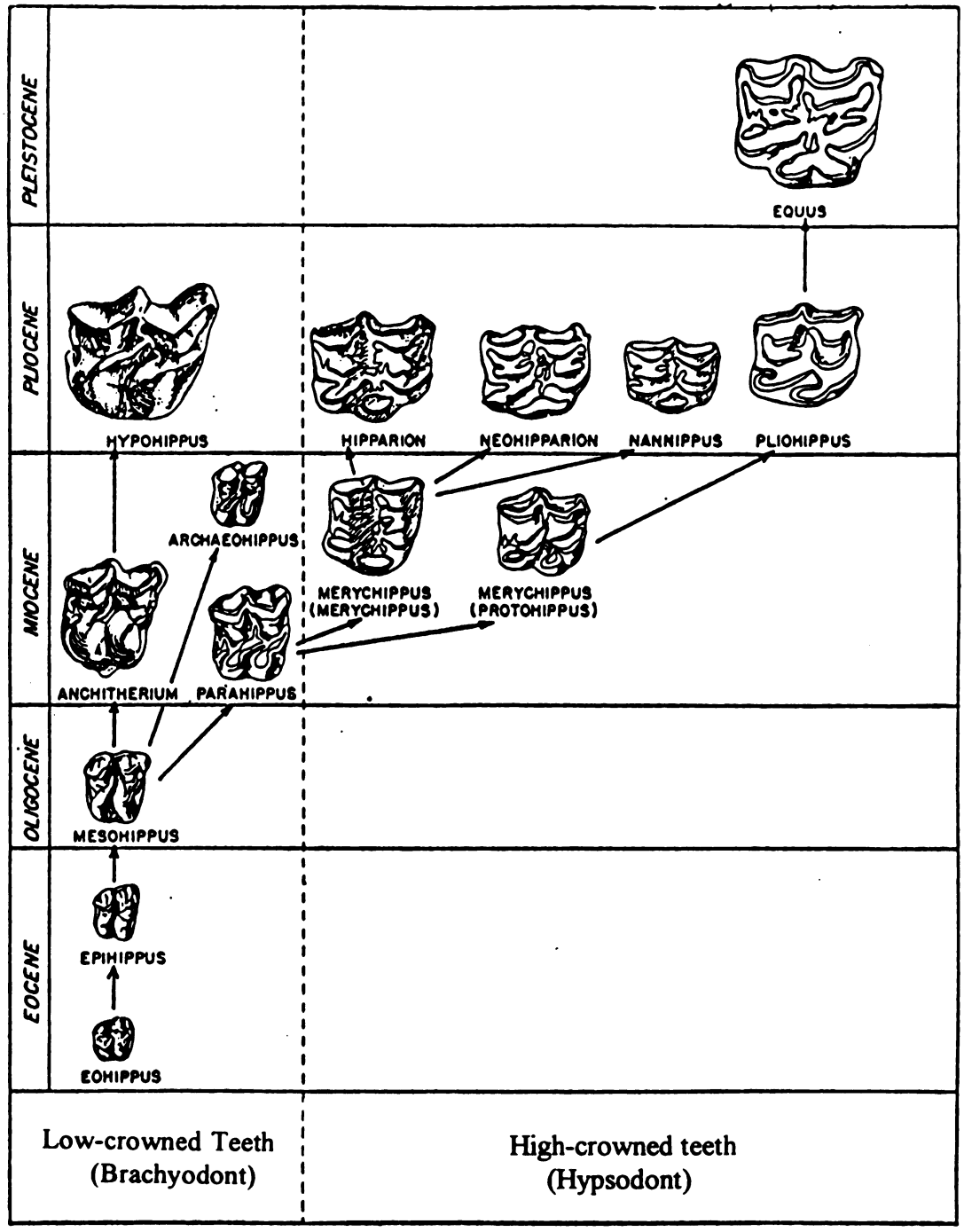


Figure 2 Horse dental evolution showing the transition from the relatively undifferentiated brachyodont condition to the more complex enamel foldings of the hypsodont equids (modified from Simpson, 1951).

values of fossil horse teeth. This information can then be used to evaluate the traditional paleontological interpretations concerning the evolution of hypsodonty in the family Equidae of North America.

Before attempting paleoecological reconstructions, indigeneity of the fossil organic matter must be established. When working with ancient organic material, the possibility of postmortem and/or modern contamination must be addressed. Numerous attempts to establish an objective indigeneity criterion have been proposed (DeNiro, 1985; Macko and Engel, 1991; Ostrom et al., 1994). Abundances and distributions of amino acids and isotope values of the ancient organic material isolated from tooth and bone serve as indicators of indigenity. Diagenetic effects such as age and depositional environment may alter elemental compositions and isotope values of fossils (DeNiro, 1985). Therefore, this study evaluates the effects of diagenesis by comparing the amino acid signatures and isotope values of ancient and modern terrestrial herbivores. This thesis reports HPLC results concerning the reproducibility and recovery of amino acids subsequent to ion exchange chromatographic purification. In addition, gas chromatographic results detailing the distribution and relative abundance of amino acids recovered from equid fossils are used to assess indigeneity. Comparisons of ancient and modern isotope values designed to investigate indigeneity require knowledge of the isotope variability of the species being studied. Very little isotope data currently exists for modern and fossil horses and for large herbivores in general. Thus, another aim of this research is to enhance the current data on the natural isotopic variation in large terrestrial herbivores. This knowledge will provide a

framework which will facilitate the evaluation of postmortem alteration effects. Distinguishing diagenetic alteration from natural isotopic variation is best accomplished using a well-known spatially and temporally constrained series of fossils. The family Equidae has a remarkably complete fossil record. For this reason, and for the intruiging paleoecological questions concerning the origin of hypsodonty, a time series a horse teeth and bones (32 Ma to Recent) was selected for this isotope study.

Stable Isotopes

Applications of stable isotope mass spectrometry in the field of vertebrate paleontology are of recent origin. Nonetheless, ratios of naturally occurring isotopes, specifically carbon (^{12}C and ^{13}C) and nitrogen (^{14}N and ^{15}N), enhance investigations on prehistoric vertebrates including assessments of ancient biochemistries (Ostrom and Fry, 1993), interpretations of paleo-community structures (Bocherens et al., 1994; Ostrom et al, 1993) and paleoclimate analyses using vertebrate remains (Thackeray and Lee-Thorp, 1992). By convention, isotope values are given by the following equation:

$$\delta^{x}Y = \frac{(R_{Sample})}{(R_{Standard} - 1)} * 10^{3}$$

where Y is the element of interest, x is the trace isotope and R is $^{13}C/^{12}C$, $^{15}N/^{14}N$, or

the ratio of any trace to abundant isotope of a particular element. The units for this equation are permil (‰). Isotope values are reported relative to international standards, Pee Dee Belemnite (Belemintella americana) for carbon and atmospheric N_2 for nitrogen.

Carbon Isotopes- Dietary Sources

Carbon isotopes have proved useful in elucidating sources of dietary carbon in a wide variety of modern as well as fossil animals (DeNiro and Epstein, 1978b; Tieszen et al., 1979b; Lee-Thorp and Van der Merwe, 1987; Bocherens et al., 1990). These studies have shown that the $\delta^{13}C$ values of consumers tissues are similar to the food consumed. Laboratory experiments demonstrate the $\delta^{13}C$ values of the organic fraction of small mammalian tissues differ from the diet by about 1‰ to 3‰ (DeNiro and Epstein, 1978b). Large terrestrial herbivores (e.g. elephants) commonly exhibit up to a 6‰ fractionation between the $\delta^{13}C$ values of skeletal tissues and dietary constituents (Vogel, 1978; Vogel, 1990a). Fractionation in horses likely falls between these two size extremes. Variation in carbon isotope values are a result of kinetic fractionation during metabolic processes (Silfer et al., 1992). Kinetic fractionations also account for the large differences in $\delta^{13}C$ values of C_3 and C_4 vegetation. Knowlege of the carbon isotopic signatures of C_3 and C_4 plants becomes important when attempting to reconstruct horse paleodiets.

The two priniciple plant photosynthetic pathways (C_3 and C_4, respectively) have

$\delta^{13}C$ values which form a nearly continuous, non-overlapping sequence. Terrestrial C_3 plant $\delta^{13}C$ values range from -24‰ to -34‰ and average -27‰ (Smith and Epstein, 1971; Galimov, 1985). Assuming a 4‰ fractionation between bone and diet, horses with pure C_3 diets would have $\delta^{13}C$ values between -20‰ and -30‰. Plants which utilize the C_4 biochemistry are enriched in ^{13}C relative to C_3 plants. The $\delta^{13}C$ values of C_4 plants range from -5‰ to -19‰ with an average $\delta^{13}C$ value of about -13‰ (Smith and Epstein, 1971; Tieszen, 1978; Galimov, 1985; Keegan, 1989). Assuming the same 4‰ fractionation, horses consuming only C_4 vegetation would have $\delta^{13}C$ values between -1‰ and -15‰. A third plant biochemical pathway exists, the CAM cycle, which in many cases is isotopically indistinguishable from C_4 photosynthesis. However, CAM photosynthesis is only found in succulents and cacti which are not likely to represent an abundant food source for horses on the Great Plains. Thus, carbon isotope analyses allow for discrimination between C_3 and C_4 based diets of extinct horse species.

Modern savanna grasslands (e.g. the Serengeti plains of Africa) are characterized by C_4 grasses which have adapted to avoid the problem of photorespiration by utilizing an intermediate four-carbon molecule such as malate during photosynthesis. This adaptation allows C_4 plants to sequester carbon dioxide inside their bundle shealth cells which provides a selective advantage over their less sophisticated C_3 relatives in arid climates where conditions regularly exceed 28°C (82°F). Other conditions which favor the C_4 metabolic pathway, aside from low CO_2 availability and high temperatures, are high light levels, high salinity and low moisture

levels. In summer months these conditions are regularily attained in modern savannas. More than half the species of known grasses utilize the C_4 pathway (Downton, 1975). These are the so-called warm season (tropical) grasses as they are uncommon in temperate regions where daily minimum summer temperatures drops below 10°C (50°F) (Teeri and Stowe, 1976). Other C_4 plants include maize, sorghum, and sugarcane. Only a single C_4 tree is known, the euphorbia tree of Hawaii (James A. Teeri, personal communication). In contrast, all other deciduous and coniferous trees and shrubs utilize the C_3 pathway. Wheat, soy, oats, clover, rice, nuts, most fruits and vegetables, and the cool season (temperate) grasses are all examples of C_3 plants.

Determining the abundance of C_4 plants in the diet through isotope studies of organic materials is dependent upon an understanding of the initial $\delta^{13}C$ values of the individual dietary components (in this case, C_3 and C_4 vegetation). In addition, the turnover rate of carbon and the observed isotopic discrimination between the herbivore and its diet must be evaluated. Isotope values of C_3 and C_4 plants are well characterized. The average carbon isotope values of extant C_3 and C_4 vegetation (-27‰ and -13‰, respectively) can be used to predict the percentage of C_4 plants in the diets of ancient horses provided carbon turnover rates are understood. Turnover rates of many herbivore carbon reservoirs such as milk, fat, muscle and hair are rapid (days to months, in most cases) (Teiszen et al., 1983; Boutton et al., 1988). Collagen, having an extremely long turnover rate, may record yearly or longer accumulations of carbon in large herbivores with slower metabolisms (Teiszen et al., 1983). This knowledge of turnover rates in large herbivores is crucial since climate variaitions may

alter plant distributions causing seasonal differences in herbivore tissues rather than actual dietary changes. Previous attempts have been made to assess the percent contribution of C_4 plants using mass balance models (Vogel, 1978; Vogel et al., 1986; Nordt et al., 1994; Cormie and Schwarcz, 1994). This thesis reports percent C_4 contributions in ancient horse diets computed as follows:

$$\% C_4 = \frac{\delta^{13}C_D - \delta^{13}C_{C3}}{\delta^{13}C_{C4} - \delta^{13}C_{C3}} * 10^2$$

The $\delta^{13}C_{C3}$ and $\delta^{13}C_{C4}$ terms are the mean values for modern C_3 and C_4 plants. The $\delta^{13}C_D$ is the isotope value of the horses diet which is determined from the $\delta^{13}C$ value of the ancient horse in question taking into account the observed discrimination between horses and their diet. To insure accuracy when determining the percent contribution of C_4 plants in the diet of horses, all of these variables must be well characterized.

Nitrogen Isotopes- Trophic Effects

Nitrogen isotope values ($\delta^{15}N$) are useful ecological indicators. The $\delta^{15}N$ values of skeletal tissues from consumers are ~3‰ enriched in $\delta^{15}N$ over the diet

(DeNiro and Epstein, 1981; Macko et al, 1987). This observation makes it possible to use nitrogen isotopes as trophic level indicators in modern and ancient ecosystems. The usefulness of nitrogen isotopes in paleontological studies has recently been demonstrated by the ability to delineate trophic level structure in Cretaceous communities (Ostrom et al., 1993) and paleophysiologies in Pleistocene bears (Bocherens et al., 1994).

Ancient trophic reconstructions using nitrogen isotopes rely on a knowledge of source and cycling of nitrogen in the modern environment. Most terrestrial plants which obtain nitrogen from the soil have $\delta^{15}N$ values between 3‰ and 6‰, but may be as high as 9‰ (Delwiche and Steyn, 1970; DeNiro and Hastorf, 1985). Nitrogen fixing plants such as legumes (e.g. soy, peas, clover) typically have $\delta^{15}N$ values closer to that of atmospheric nitrogen (0.0‰). Low $\delta^{15}N$ values for terrestrial herbivores may indicate the influence of legumes or other nitrogen fixing plants in the diet. Terrestrial herbivores with pure non-leguminous diets are expected to have $\delta^{15}N$ values between 6‰ and 9‰. Terrestrial carnivores should range from 9‰ to 12‰.

The nitrogen cycle in marine communities is quite different from that of the terrestrial realm. Marine plants are up to 4‰ enriched in ^{15}N relative to terrestrial plants (Ambrose and DeNiro, 1989). Anamolously high values from terrestrial regions are often observed in modern and ancient communities (DeNiro and Hastorf, 1985; Vogel et al., 1990a). These apparent discrepancies have been interpreted as climatic effects (Heaton et al., 1986), diagenetic alteration (DeNiro and Hastorf, 1985), or marine influences (Ambrose and DeNiro, 1989). Since it is unlikely fossil equids

consumed marine plants, this explanation is untenable for this research. However, climatic effects and diagenesis are not so easily dismissed. Aridity potentially controls $\delta^{15}N$ values owing to differential nitrogen metabolism by animals during water stress (Heaton et al., 1986; Vogel et al., 1990b). Soil nitrogen contamination and extensive groundwater throughput represent possible modes of diagenetic isotope alteration. Whether these diagenetic agents would serve to enrich or deplete isotope values of the original organic nitrogen is unclear. The role that biomineralization reactions have in influencing the preservation potential of organic matter in fossils is only beginning to be studied (Masters, 1987). Preliminary research investigating fractionation associated with diagenetic reactions exists (Silfer et al., 1992; Qian et al., 1993), but little is currently known about this very important topic. For these reasons, a more in-depth consideration of how diagenesis is thought to proceed and how indigenous fossil molecules are identified is presented.

Diagenesis and Indigeneity

Underlying stable isotopic analyses of fossils from similar depositional environments and of similar age is the notion that the original isotopic signature persists unchanged indefinitely or is shifted by a consistent, detectable amount (Peggy H. Ostrom, personal communication). However, this assumption is invalid when dealing with fossils of different ages from different depositional settings owing to the exceptionally labile nature of most biological molecules. Post-depositional change

differentially alters the retention capabilities of organic molecules owing to newly established affinities with the mineral phase (Masters, 1987). Therefore, it is important to investigate diagenetic pathways of change if indigenous organic components from fossils are to be accurately identified.

This thesis addresses the concerns of diagenesis using carbon and nitrogen isotopes and amino acid analyses. Carbon and nitrogen isotope values of organic matter from ancient horses that are consistent with traditional paleontological interpretations and with trends observed in modern horses indicate a lack of diagenetic alteration. Alternatively, the extent to which $\delta^{13}C$ and $\delta^{15}N$ values of the horse fossils deviate from trends that characterize modern horses may be useful for assessing degrees of diagenetic alteration once natural isotopic variability is taken into account. Published isotope values of terrestrial herbivores with similar diets to horses serve as proxies used to define the natural isotope variation within horses.

Amino acid analyses are used to verify indigeneity of ancient materials as well. Fossil amino acid patterns that are consistent with modern amino acid signatures (e.g. presence of hydroxyproline and high concentrations of glycine and proline) likely retain an indigenous organic fraction. Elevated concentrations of the acidic amino acids (aspartic acid and glutamic acid) are consistent with the observation that acidic macromolecules with a high affinity for the mineral phase are preferentially retained during diagenesis (Hare, 1980). Fossils which are found to have little or no glycine, proline and/or hydroxproline and have high concentrations of aspartic acid and glutamic acid are likely to have experienced severe diagenetic alteration. These fossils

are poor candidates for paleoecological investigations.

Previous Studies

Abelson (1955) detected the presence of amino acids in fossil hydrolysates. Shortly thereafter methods were devised to determine whether or not amino acids isolated from fossils were authentic. One of the original methods for verification of the authenticity of organic material isolated from fossils consists of assessing amino acid enantiomer ratios. In solution, amino acids (except for glycine) exist in one of two stereoisomers, the L-optical isomer and the D-optical isomer (Corrigan, 1969). In living organisms the vast majority of organic molecules are formed from L-configuration amino acids. Upon death, a racemization reaction proceeds toward equilibrium of the D and L enantiomers. Previously, ancient materials which were found to contain a significant L-amino acid component were assumed to represent non-indigenous components. However, using this technique fails to recognize indigenous material within fossils which have undergone alteration. Polymerization and polycondensation reactions proceed under the conditions of early diagenesis (50-100°C) and result in indigenous HMW material with low D/L ratios (indicative of contamination) (Serban et al., 1987; Ostrom, 1990). Consequently, amino acid racemization data has to be dealt with carefully. The best approach for detecting indigenous organic components within ancient remains is one which employs several lines of evidence.

Current Research

An assessment of indigeneity of organic components within fossils is presently based on the distribution and relative abundance of amino acids and various immunological/biochemical assays in addition to amino acid racemization studies (Armstrong et al., 1983; Macko and Engel, 1991; Lowenstein, 1988). Amino acid hydrolysates from ancient materials which have been desalted on an ion exchange resin can be detected by HPLC using a fluorometer with post-column introduction of O-phenylaldehyde (OPA). This facilitates determination of the distribution and relative abundances of the amino acids present. Another technique used to identify amino acids in ancient materials involves the use of a GC. In this technique a liquid sample is combusted to a gaseous state then separated under helium flow prior to introduction to a detector (such as a FID or a MSD). Provided the amino acids are derivitized beforehand, a GC can be used to verify molecular identities. Derivitization is required in order to make the amino acids more volatile during the GC combustion step. Derivitization is complicated when dealing with fossil organic materials. Hydrolysis of ancient organic matter results in low molecular weight (1,000-6,000mw) peptides and salts which interfere with the derivitization step. This situation is avoided by performing a column purification of the amino acid extract prior to derivitization. Of considerable concern is the possible loss of amino acids during the desalting step.

Immunological approaches such as RIA and ELISA involve raising antisera against specific fossil proteins. These methods have been very successful for verifying fossil organic matter authenticity and elucidating relationships among extinct taxa (e.g. the Tasmanian wolf and the Quagga)(Lowenstein et al., 1981; Lowenstein and Ryder, 1985). In addition, DNA-DNA hybridization experiments and amplification and sequencing of intact mitochondrial or genomic DNA within ancient materials by PCR are becoming more popular indigeneity assays (Marshall, 1988; Pääbo et al., 1989).

The use of C/N ratios of extracted "collagen" for verifying sample indigeneity (with C/N= 2.9 to 3.6 being considered indigenous) has been proposed (DeNiro and Epstein, 1981). However, the C/N ratio of polymerized, authentic fossil "collagen" is frequently high. Thus, the C/N criterion may well exclude some indigenous polymerized samples and might even include altered samples which coincidentally fall within the acceptable 2.9 to 3.6 range. Therefore, the use of C/N ratios for assessing indigeneity of organic matter from fossils is of limited use at present.

A recent approach for paleomolecular indigeneity involves analyzing the isotopic signature of individual amino acid enantiomers (Silfer et al., 1991; Ostrom et al., 1993). This approach, in association with the other indicators, could provide a more objective criterion for assessing the indigeneity of fossil organic matter. Although this study does not report isotope values on individual enantiomers, amino acid distribution, abundance and racemization data is utilized in conjunction with carbon and nitrogen isotope values of the HMW material isolated from fossils to characterize ancient organic matter for indigeneity purposes. Once indigenous organic

matter has been identified, interpretations concerning the paleoecology of extinct organisms can be formulated.

Tertiary Paleoecology

Perrisodactyl (rhinocerotids, tapiroids, titanotherids, and equids) diversity throughout the Tertiary has recently been correlated with climatic events and vegetational changes (Janis, 1989). Interestingly, C_4 grasses are first known in abundance from the time when equid diversity sharply declines. Hindgut fermenters (such as horses) might have been inherently less capable of processing the expanding, nutritionally poor C_4 grasses compared to ruminant artiodactyls. Researchers have hypothesized the drop in equid diversity may relate to a C_4 diversification (Wang et al., 1994). This hypothesis is more parsimonious than the once dominant view that equids were ecologically out-competed by more efficient grazing artiodactyls. The demise of the horse family was most likely in response to Tertiary vegetational changes which fortuitously favored ruminant artiodactyls (Janis, 1989). High-crowned teeth confer an adaptive advantage to individuals who regularily consume materials harder than the apatite of enamel and dentine (e.g. plant opal, soil grit particles)(Jones and Handreck, 1967; Walker et al., 1978). Thus, the expansion of abrasive grasslands may well explain the advent of hypsodonty in horses without invoking interspecific competition. This view does not distinguish between siliceous components of C_3 and C_4 grasses or the influence that gritty substrates may have played in the evolution of

hypsodonty. The best way to decipher this relationship is to assess fossil grass originations and distributions using paleontological and stable isotopic evidence.

Variables used to determine modern grass distributions can be used to reconstruct paleotemperature and climatic conditions prevalent during the time when C_4 grasses first evolved. This information provides clues to assess whether or not there is a correlation between the radiation of abrasive grasses and the evolution of high-crowned teeth in horses. The use of stable carbon and nitrogen isotopes provides a more objective way to verify the existence of the postulated grassland-hypsodonty relationship in light of all the available evidence.

The Miocene World

Paleopedological (fossil soil) evidence suggests a transition from mostly woodlands to shrublands (with some locally widespread grasslands) occurred during the mid to late Oligocene (Retallack, 1983a; 1983b). In the early Miocene, vegetation of the continental interior was comprised of a forest/grassland mosaic as evidenced by the low endemism of grasses and the presence of a large browsing fauna (Axelrod, 1985). Paleoclimatological studies suggest the middle Miocene (18 Ma to 15 Ma) was a tropical, high-moisture environment, the warmest interval of the Neogene (Zubakov and Borzenkova, 1990). Concommitant with the warming trend, the middle Miocene experienced the first occurrence of high levels of seasonality in both temperature and precipitation which profoundly affected floral distributions (Janis, 1989). The Great

Plains experienced annual precipitation double present levels during the mid-Miocene (Axelrod, 1985).

By the late Miocene, the Great Plains had become much drier (Axelrod, 1985). A temperature-constrained herpetofauna (mainly the occurrence of the tortoise, Geochelone, and abundant crocodiles) indicate that the late Miocene was still very warm even in extreme northern latitudes (Holman, 1971). These hot, arid conditions were favorable to drought-adapted floras such as the C_4 savanna grasses. Additionally, the rise of a scrubland-dwelling caenophidean snake fauna at the expense of the arboreal boid fauna supports a mid to late Miocene savanna radiation (Holman, 1976).

The middle to late Miocene in North America was a time of increasing diversity of the hypsodont equids (Janis, 1989; MacFadden, 1992). Consequently, the C_4 biochemistry which likely arose contemporaneously with the expansion of warm season grasslands, might have influenced the development of high-crowned teeth in horses. Alternatively, the possibility exists that the C_4 biochemistry is much more ancient and merely proliferated with the expanse of grasslands during the Miocene. Whichever is true, an assumed correlation between the diversification of siliceous grasses and hysodonty in horses remains.

The Pliocene Epoch

By the end of the Miocene an extensive ice cap had developed in Antarctica (Woodruff et al., 1981). Subsequent to the Miocene, continued global cooling and

increased aridity altered grassland distibutions once again presumably causing the drastic decline in equid diversity throughout the Pliocene (Janis, 1989). The Miocene-Pliocene extinction event that greatly reduced equid diversity left the world with two horse genera, only one of which (Equus) exists today. Axelrod (1985) envisioned the Pliocene as an epoch of gradual drying, but argued for a significant woodland habitat on the Great Plains. An essentially modern North American snake fauna by Upper Pliocene times indicates a progression toward recent landscape organization (Holman, 1979). Large grassland and forest-dwelling herpetofaunas from the Upper Pliocene also indicate a gradual modernization of the landscape with tall-grass prairies pervasive in the west (Great Plains) and short-grass plains and deciduous forest prevalent in the Central Lowlands region of North America (Rogers, 1976). This scenario pre-supposes the development of a moisture gradient from west to east. A continental rain-shadow developed subsequent to the uplift of the Rocky Mountains in the middle Miocene which was responsible, in part, for the evolution of the Pliocene landscape structure. The Plio-Pleistocene was a time of varied climate fluctuations which most assuredly influenced the distribution of the C_3 and C_4 grasses (Ralph E. Taggart, personal communication).

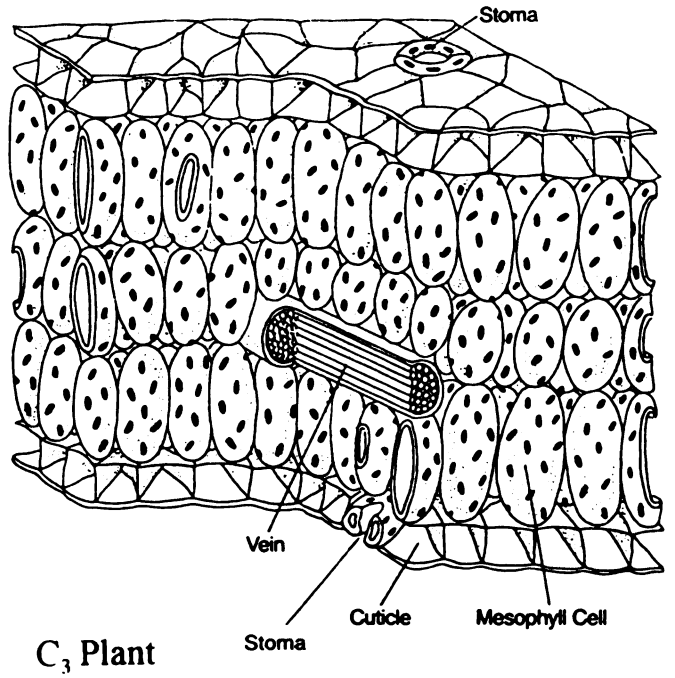
Grasses and Hypsodonty

If C_4 grasses had an influence on the evolution of hypsodonty, they must have existed in the early-middle Miocene (circa 18 Ma) when horse tooth crown heights

began to increase. The C_4 photosynthetic pathway may have existed as far back as the Cretaceous (Brown and Smith, 1972). The fossil record, however, has yielded only one definitive C_4 grass of Miocene age (from Nebraska, approximately 7 Ma to 5 Ma) (Thomasson et al, 1986). A C_4 grass from California originally assigned to the Pliocene (Nambudiri et al., 1978) is likely late Miocene (7 Ma to 5 Ma) based upon mammalian faunal correlations (MacFadden, 1984). This paucity of fossil evidence might suggest that no relationship exists between the evolution of C_4 grasses and the evolution of hypsodonty in horses. However, many fossil plants have a notoriously poor fossil record and the identification of C_4 photosynthesis in fossil plants is difficult even under the best circumstances. Carbon isotope analyses do not require morphological preservation to determine photosynthetic pathways of extinct plants. In fact, $\delta^{13}C$ analyses do not even require the plant itself. All that is required is tissue from the herbivore. Therefore, $\delta^{13}C$ analyses of equid remains should prove useful for determining when C_4 grasses were first incorporated into horse diets.

One reason C_4 grasses are thought to be responsible for equid hypsodonty despite the lack of ample fossil evidence is the greater abrasiveness of most C_4 grasses compared to C_3 grasses. Silica inclusions may have evolved as an anti-predation strategy of the diversifying C_4 grasses in response to herbivore grazing. Silica bodies are often found along the ribs of C_4 grasses, but rarely are they observed in C_3 grasses (Kaufman et al., 1985). Silica bodies are located over the vascular bundle cells of C_4 grasses where they alternate with cork cells (Kaufman et al., 1985). Plants which exhibit "Kranz anatomy" (a specialized C_4 leaf structure) have low mesophyll:bundle-

shealth area (Thomasson et al., 1986)(Figure 3). Consequently, many C_4 grasses possess more hypoderm, a lignified, fibrous material surrounding the vascular bundle-shealth cells within the stem (Elias, 1942). This larger amount of lignin, together with cellulose and siliceous components make C_4 grasses less digestable, less nutritious and necessitate herbivores to process much more grass. This extra processing is inefficient for an herbivore with access to a more nutritous food source (e.g. C_3 vegetation). However, some C_3 grasses contain opal phytoliths within the leaf epidermis which may deter herbivory in much the same way that silica inclusions function in C_4 grasses (Jones, 1964). The identification of opal phytoliths and silica inclusions within anatomical features of plants represents the first empirical evidence suggesting the evolution of a more abrasive grass structure might have led to the evolution of high-crowned teeth in horses. The use of stable isotopes promise a fuller insight into the relative importance of C_3 and C_4 plants in horse diets and into the influence these vegetations had on the development of hypsodont teeth in horses.



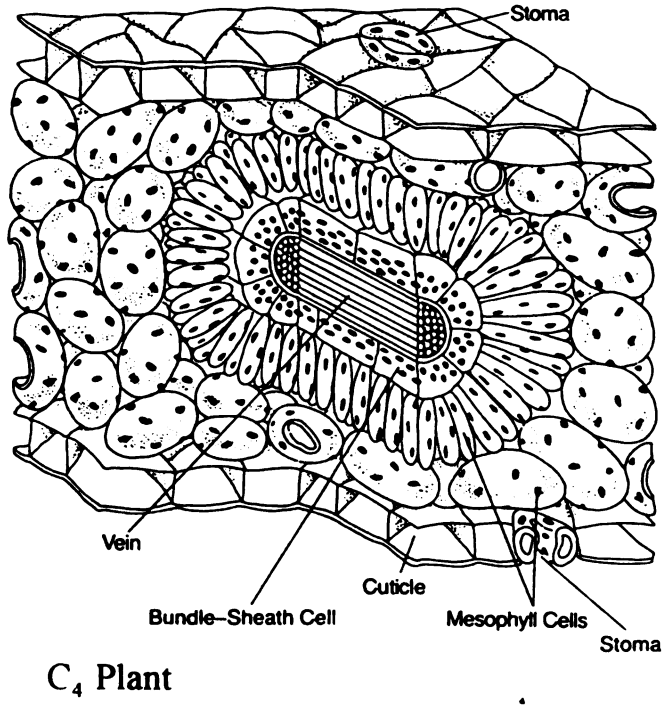


Figure 3 Arrangement of the various cell layers in C_3 and C_4 plants. The vascular bundle-shealth cells of C_4 plants, absent in C_3 plants, are surrounded by silica bodies and lignin fibers making them less digestible (modified from Wessells and Hopson, 1988).

MATERIALS AND METHODS

Sample Procurement

Modern horse skeletal and dental specimens were obtained from MSU Veterinary Medical Center (courtesy, Peter Ocello) and the AMNH (courtesy, J. Daniel Bryant) (Figure 4; Table 1). Modern wapiti (Cervus canadensis) material was also obtained through MSU (courtesy, Dr. William Cooper) (Table 1). Fossil horse material was supplied by the AMNH (courtesy, Dr. Richard Tedford), the UNSM (courtesy, Dr. Michael Voorhies) and the UA (courtesy, Dr. Everett Lindsay) (Table 2). Specimen selection was confined to individuals 32 Ma and younger owing to the size constraints of geologically older specimens. At present, horse teeth from the Oligocene and earlier are too small to obtain enough HMW organic matter for isotopic analysis without combining samples.

Specimen Documentation

Isolation of organic matter for isotopic analysis requires that a portion of the specimen be destroyed. For modern specimens this does not usually present a problem. When dealing with an exhaustible, exceptionally valuable entity such as a

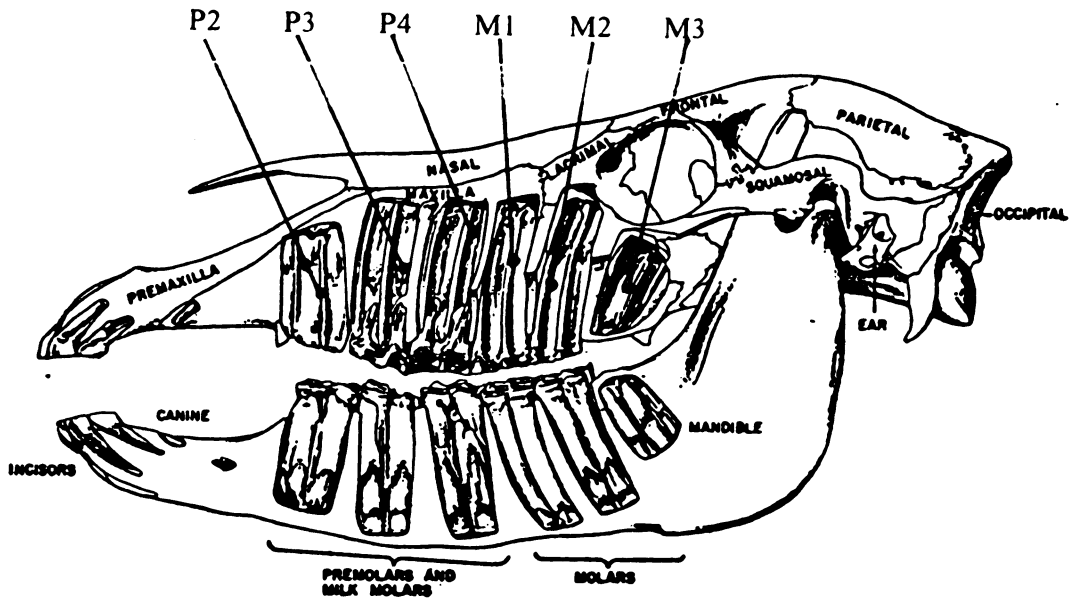


Figure 4 Modern horse skull and teeth shown in cross-section to illustrate the pronounced hypsodont condition. Premolar and molar tooth position nomenclature is given (modified from Simpson, 1951).

Table 1 Taxonomy and relevant information for modern horses and wapiti

Table 1 Taxonomy and relevant information for modern horses and wapiti.

Sample Number	Genus species	Sampled Element	Geographic Locality
MSU: Cer. 1-3	Cervus canadensis	RM1	Hanna Co., Wyoming
MSU: Cer. 4-6	Cervus canadensis	RdP4	Hanna Co., Wyoming
MSU: Cer. 7-9	Cervus canadensis	R Dentary Bone	Hanna Co., Wyoming
MSU: Cer. 10	Cervus canadensis	Hair	Hanna Co., Wyoming
MSU: Cer.11	Cervus canadensis	Plant Debris inTeeth	Hanna Co., Wyoming
MSU: Cer.12	Cervus canadensis	Connective Tissue	Hanna Co., Wyoming
MSU: Cer. 13	Cervus canadensis	Muscle (Masseter)	Hanna Co., Wyoming
MSU: Bot. 1	?	Meadow Hay	Macomb Co., Michigan
MSU: Eq. 1a	Equus caballus	RM1 (Labial)	Clinton Co., Michigan
MSU: Eq. 1b	Equus caballus	RM1 (Labial)	Clinton Co., Michigan
MSU: Eq.1c	Equus caballus	RM1 (Labial)	Clinton Co., Michigan
MSU: Eq.1d	Equus caballus	RM1 (Labial)	Clinton Co., Michigan
MSU: Eq.1e	Equus caballus	RM1 (Labial)	Clinton Co., Michigan
MSU: Eq.1f	Equus caballus	RM1 (Labial)	Clinton Co., Michigan
MSU: Eq.1g	Equus caballus	RM1 (Labial)	Clinton Co., Michigan
MSU: Eq.1h	Equus caballus	RM1 (Labial)	Clinton Co., Michigan
MSU: Eq.1i	Equus caballus	RM1 (Labial)	Clinton Co., Michigan
MSU: Eq.1j	Equus caballus	RM1 (Lingual)	Clinton Co., Michigan
MSU: Eq.1k	Equus caballus	RM1 (Labial)	Clinton Co., Michigan
MSU: Eq.1l	Equus caballus	RM1 (Lingual)	Clinton Co., Michigan
MSU: Eq. 2	Equus caballus	RM2	Clinton Co., Michigan
MSU: Eq. 3	Equus caballus	RM3	Clinton Co., Michigan
MSU: Eq. 4	Equus caballus	RP2	Clinton Co., Michigan
MSU: Eq. 5	Equus caballus	RP3	Clinton Co., Michigan
MSU: Eq. 6	Equus caballus	RP4	Clinton Co., Michigan
MSU: Eq. 7	Equus caballus	R Maxilla Bone	Clinton Co., Michigan
MSU: Eq. 8	Equus caballus	RM1 (6N HCl)	Clinton Co., Michigan
MSU: Eq. 9	Equus caballus	RM1 (3N HCl)	Clinton Co., Michigan
F.M.M. 1219	Equus sp.	R Max (6N HCl)	? Co., Nebraska

Table 2 Taxonomy and relevant information for ancient horses.

Specimen Number	Genus species	Age (Ma)	Formation	Skeletal Element(s)
UALP 7810	Equus sp.	3	Rexroad Fauna	RM1 or RM2?
F: AM 129114	Equus simplicidens	4	Keim	RM1 or RM2?
F: AM 129444	"Dinohippus" leidyanus	5.5	"Hemphill Beds"	RM3
UNSM 35-56B	"Dinohippus" leidyanus	6	Uptegrove Fauna	RM3 (Labial)?
UNSM 316-56	Nannippus lenticularis	6	Uptegrove Fauna	LM (Labial)?
F: AM 129443	Neohipparion eurystyle	6.5	"Hemphill Beds"	RM3
AMNH 17599A	"Dinohippus" leidyanus	7	Snake Creek	RP2
UNSM 2010-70B	Calippus sp. nov.	7	Cambridge Fauna	LM3
UNSM 5157-73	Hipparion cf. forcei	7	Cambridge Fauna	LM?
F: AM 129442	Cormohipparion occidentale	8.5	"Hemphill Beds"	RM3
F: AM 129445	Cormohipparion occidentale	9.5	Ash Hollow	LP2
F: AM 129446	Cormohipparion occidentale	9.5	Ash Hollow	LM3
F: AM 129454	Cormohipparion occidentale	10	Ash Hollow	RP2
F: AM 129441	Cormohipparion occidentale	10.5	"Clarendon Beds"	L Max., LdP4-LM2
F: AM 129447	Pliohippus pernix	12	Valentine	LM3
F: AM 129448	Pliohippus pernix	12	Valentine	LM3
F: AM 129450	Pliohippus pernix	12	Valentine	RM3
F: AM 114068	Pliohippus pernix	12	Valentine	RP4
F: AM 129316	Merychippus insignis	15	Olcott	L Max., LP1-LP3
F: AM 129453	Merychippus sp. (isonesus)	15.5	Olcott	LP4-M1
F: AM 110575	"Merychippus" primus	17	Sheep Creek	L Max., LP4-LM3
AMNH 20513	"Merychippus" primus	17	Sheep Creek	LP4
F: AM 129451	"Merychippus" tertius	17.5	Sheep Creek	LM2
F: AM 128784	"Parahippus" sp.	18.5	Running Water	R max., RM2
F: AM 129455	Mesohippus sp.	30.5	Brule	Part. L acetabulum
F: AM 74067	Miohippus obliquidens	32	Chadron	Part. R acetabulum

fossil it is of utmost concern. Consequently, all fossil material utilized in this thesis represents either highly abundant fossils (e.g. horse teeth) or remains which are unlikely to be useful in morphological/taphonomical studies. In the future digital, three-dimensional computer imaging will make possible the ability to preserve the morphometric dimensions of fossils in virtual reality. Unfortunately, this technology is not currently available. Therefore, all specimens were measured, sketched and photographed (from two separate angles) to preserve the morphologies. A portion of every fossil was saved in its original state for comparative purposes to future studies. Illustrations of two representative fossil horse specimens obtained from the AMNH are included (Figure 5, Figure 6).

Locality and Age Description

Documentation concerning stratigraphy and chronometry, depositonal environment and paleontological information was obtained from Cenozoic Mammals of North America (Woodburne, 1987) or from a series of bulletins of the AMNH (Galusha, 1975; Skinner et al., 1977; Skinner and Johnson, 1984) (Table 3). Voorhies (1990) provided additional biostratigraphic and radiometric information relevant to the horses analyzed in this study. Without exception, the geologic age assigned to each fossil was determined by the loaning institution (Table 3). In most instances there is good agreement between the age calibrations of the separate institutions. For one locality (Burge Quarry) there is considerable dispute concerning its proper NALMA

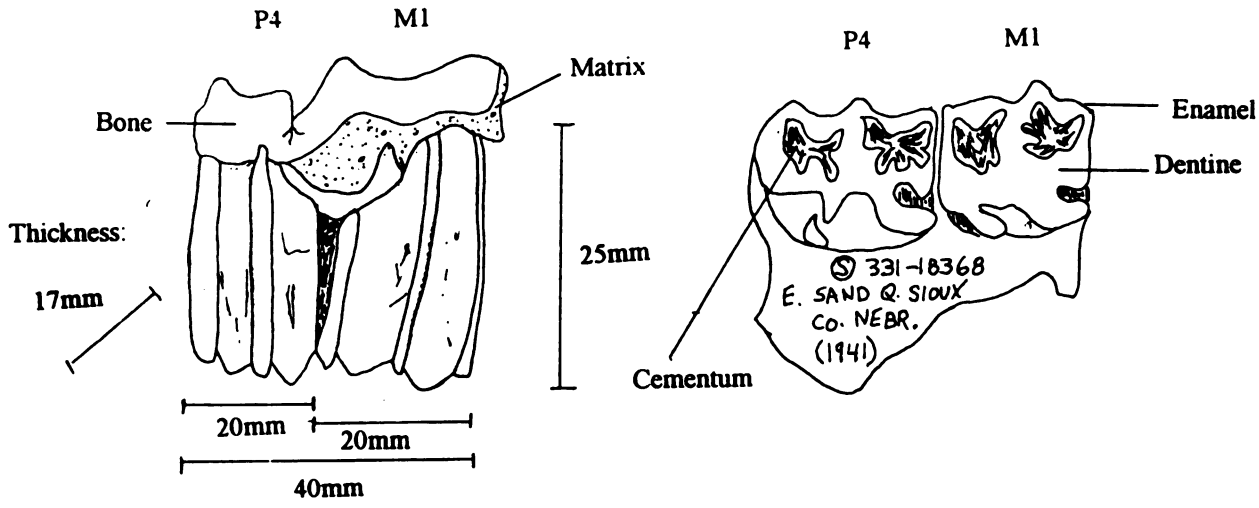


Figure 5 Labial view (left illustration) and occlusal surface (right) of LP4-LM1 of a 15.5 Ma merychippine horse, Merychippus sp. (cf. isonesus) (F:AM 129453).

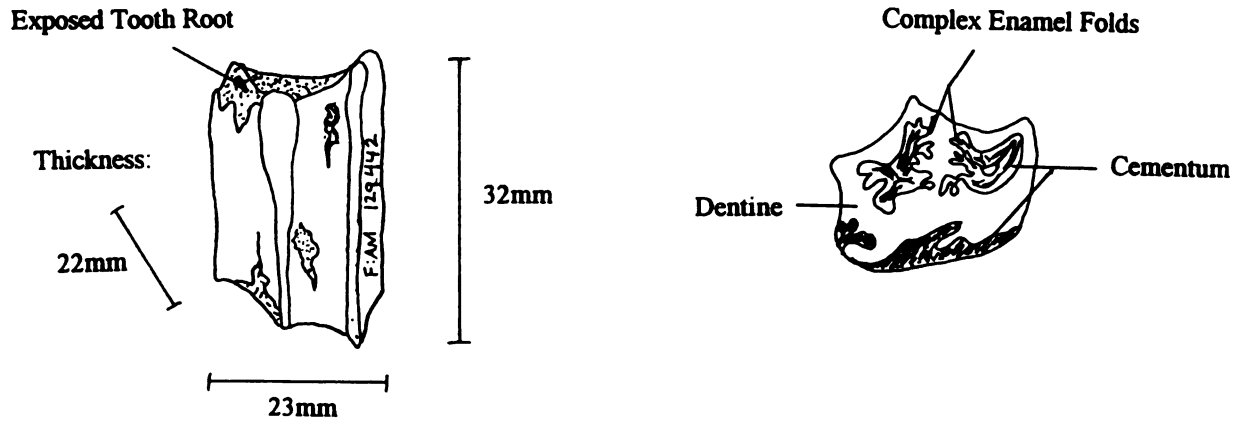


Figure 6 Labial view (left illustration) and occlusal surface (right) of RM3 of Cormohipparion occidentale, 8.5 Ma, from Ellis County, Oklahoma (F:AM 129442).

placement. The loaning institutions (AMNH) designation of Latest Barstovian (12 Ma) has been preserved in this case in lieu of Early Clarendonian (11 Ma) as some paleontologists advocate. Aside from Burge quarry, all other age estimates can be considered accurate to within ± 0.5 Ma (J. D. Bryant, personal communication).

Preservational States

The state of preservation was defined for each fossil and used to determine which samples would be analyzed (Table 3). Preservation was defined herein to include the gross morphological condition of the fossil (e.g. exposed tooth roots, extensively worn crown enamel, root casts from plants present, etc.), the amount of preserving agent (e.g. consolidants) and the amount of encasing matrix around the fossil. Fossils which were found to be mostly or wholly intact, predominantly free from perserving agents and easy to remove from their matrix were selected for isotopic analysis. With the exception of one specimen (F:AM 129441), all horses analyzed in this study had attained adulthood. Maturity was evaluated by surveying the wear patterns on the tooth enamel.

Table 3 Locality, age, and preservation information of horse fossils.

Specimen Number	Age (Ma)	NALMA	Quarry	Depositional Environment	Preservational State
UALP 7810	3	L. Blancan	Safford-Duncan	Gila Conglomerate, Clay, Sand	Fragmentary Upper Molar
F: AM 129114	4	L. Blancan	Magill Ranch	?	1/4 Transverse Section
F: AM 129444	5.5	L. Hemphillian	Denver Quarry	Stream Channel Deposits	Exposed Tooth Roots, Matrix
UNSM 35-56B	6	L. Hemphillian	Uptegrove Quarry	UNSM Locality Cn 101	Molar Fragment
UNSM 316-56	6	L. Hemphillian	Uptegrove Quarry	UNSM Locality Cn 101	Labial Portion of Upper Molar
F: AM 129443	6.5	L. E. Hemphillian	Box T Quarry	Stream Channel with Basalts	Plant Root Traces, Heavy Pres.
AMNH 17599A	7	E. Hemphillian	Aphelops Draw Q. 1	Channel Fill	Plant Root Traces, Light Pres.
UNSM 2010-70B	7	M. Hemphillian	Amebeldon Quarry	UNSM Locality Ft 40	Unworn Lingual Molar Fragment
UNSM 5157-73	7	M. Hemphillian	Amebeldon Quarry	UNSM Locality Ft 40	Labial Portion of Upper Molar
F: AM 129442	8.5	E. E. Hemphillian	Port of Entry Pit	Stream Channel	No Preservative, Matrix Encrusted
F: AM 129445	9.5	L. Clarendonian	Xmas Quarry	Point bar, Well-sorted Quartz Sand	Light Preservative, Little Matrix
F: AM 129446	9.5	L. Clarendonian	Xmas Quarry	Point bar, Well-sorted Quartz Sand	Light Preservative Coating
F: AM 129454	10	L. Clarendonian	Clayton Quarry	Gray Sand with Sand Clasts	Light Preservative Coating
F: AM 129441	10.5	E. Clarendonian	MacAdams Quarry	?	Matrix Encased Fossil
F: AM 129447	12	L. L. Barstovian	Burge Quarry	X-bedded Sands/ Clay Lenses	Thick Preservative,Thick Matrix
F: AM 129448	12	L. L. Barstovian	Burge Quarry	X-bedded Sands/ Clay Lenses	Thick Preservative,Thick Matrix
F: AM 129450	12	L. L. Barstovian	Burge Quarry	X-bedded Sands/ Clay Lenses	Light Preservative,Thick Matrix
F: AM 114068	12	L. L. Barstovian	Burge Quarry	X-bedded Sands/ Clay Lenses	Very Light Preservative
F: AM 129316	15	E. Barstovian	Echo Quarry	Stream Cut	Matrix Packed in Tooth Roots
F: AM 129453	15.5	E. Barstovian	East Sand Quarry	Channel-trench Fill	Thick Preservative Coating
F: AM 110575	17	L. Hemingfordian	Thomson Quarry	Channel Deposit	Light Preservative Coating
AMNH 20513	17	L. Hemingfordian	Thomson Quarry	Channel Deposit	Little Matrix Around Tooth
F: AM 129451	18	L. Hemingfordian	Long Quarry	Massive Pinkish Deposits	Light Preservative Coating
F: AM 128784	18.5	E. Hemingfordian	Cottonwood Creek Q.	?	Thickest Preservative Coating
F: AM 129455	30.5	E. Orellan	Bartlett Ranch	Channel/ Flood Plain Facies	Quartz Recrystallization, No Pres.
F: AM 74067	32	L. Chadronian	Brecht Ranch	Channel/ Flood Plain Facies	Light Preservative Coating

Preparatory Methods

Tooth and Bone Cleaning and Preparation

All samples were thoroughly cleaned with dental instruments to remove any exogenous contamination (e.g. matrix) within tooth or bone cavities. On occassion small arthropod exoskeletons were removed from the pulp cavity of a few of the modern horse teeth. The outer enamel surface from every sample was removed using a dremel tool or a chisel to remove external contamination and any preservative when present. If this procedure did not visibly remove the preserving agent the fossil was not analyzed further. Throughout the cleaning procedure gloves were worn and only ashed glassware and dental tools were used to prevent contamination. The complex inner enamel folds of the hypsodont horse teeth made dentine-specific isolation virtually impossible (Figure 7). Consequently, all interior tooth layers were extracted together. The inner enamel, dentine and cementum complex, free from preservative, was then cleaned rigorously with dental probes to remove any remaining sediments or impurities from within the pulp cavity. The cleaned inner complex material from either the tooth or bone was then sectioned with a dremel saw. These fragments were pulverized in a steel mill and subsequently blended to a fine grain size in a Waring blendor or, alternatively, ground in a glass mortar. Tooth and bone powders were stored at room temperature prior to demineralization.

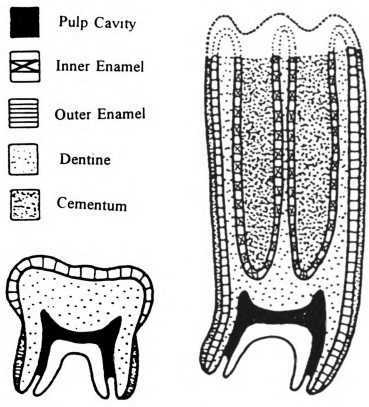


Figure 7 Cross-sectional view showing the relationship of the layers of a molar tooth from an extant hypsodont horse, Equus (right), and a low-crowned molar from an extinct horse, Parahippus (left). Note the complex inner enamel foldings of the hypsodont horse (modified from MacFadden, 1992).

Hydrochloric Acid Demineralization Technique

Granulized samples were demineralized in cold (4°C) hydrochloric acid (either 12.5%, 25%, or 50% HCl for modern samples; 50% HCl for all fossil material). Hydrochloric acid dilutions (1N, 3N, and 6N) were accomplished using 12N HCl (Optima, Sigma) in varying volumes of distilled, deionized water. Modern samples were treated with different acid normalities to determine the relationship between acid strength and demineralization efficiency. Samples were allowed to demineralize for twenty-four hours under these conditions. Some modern samples did not acheive a full demineralization after twenty-four hours. In these cases the supernatant was decanted from the undemineralized pellet and fresh, chilled, dilute HCl was added to the beaker. If the sample still was not fully demineralized after another hour at 4°C it was ground in a glass mortar over ice to facilitate demineralization.

Dialysis and Lyophilization Procedure

Immediately following demineralization, the HCl-soluble fraction from each sample was placed into dialysis. The supernatant (containing the HCl-soluble organic fraction) was pipetted from any residual undemineralized pellet. Typically there was a small percentage (>5% dry weight) of non-calcitic material which did not demineralize. The supernatant was then placed into a newly hydrated dialysis bag (Spectra/por 1, 6,000-8,000 molecular weight cutoff) secured with labeled clips. The

dialysis bags (usually 3-4 together) were then placed into a 4.0L beaker of distilled water at 4°C for 14 days. Dialysis water was changed three times on the first day of dialysis, twice per day for the first week and once per day for the final week in dialysis. This procedure purified the HMW organic extract by removing anions, cations and other low molecular weight material. The HMW organic isolates were removed from dialysis and placed into individual 250ml ball flasks. The inner surface of the dialysis tubing was rinsed with ~5ml of quartz distilled water to minimize sample loss. This fraction was added to the ball flask. Samples were lyophilized for twenty-four to forty-eight hours depending upon the initial volume of the sample.

Homogenation Technique

The lyophilized material from each sample was homogenized with a Wig-L-Bug amalgamator (Henry Schein Dental Supply Company). Compared to other techniques (e.g. mortar and pestel), the Wig-L-Bug provided the best homogenation efficiency and the least sample loss. The HMW homogenates were stored in a freezer at -5°C prior to isotopic analysis.

Stable Isotope Analyses

Isotope analyses were performed using a modification of the Dumas combustion followed by cryogenic gas line separation (Macko et al, 1987).

Alternatively, an automated technique (Carlo Erba analyzer) involving separation on a GC column and subsequent transfer to the MS under helium flow was utilized. The next several sections deal with the methodology involved in the preparation of samples for the manual mass spectrometry method.

Quartz Tube Evacuation and Combustion

Samples for manual isotope analysis were prepared by placing between 17mg and 24mg of HMW fossil powder in a 6mm quartz tube. Approximately two inches of copper oxide and one inch of copper metal shavings (Cu) were added to the sample prior to sealing the quartz tube under vaccuum. The sealed tubes were shaken to thoroughly mix the combustion reagents with the sample powder. The tubes were then combusted in a furnace at 850°C, then 650°C, and finally 550°C for one hour each. Following the combustion, the tubes were allowed to return to room temperature over night so as to quantitatively convert the nitrous oxides to N_2 gas. This protocol converts the solid tissue sample to CO_2, N_2 and H_2O vapor which can be cryogenically purified then isotopically analyzed.

Cryogenic Gas Line Separations

Sealed, combusted quartz tubes were introduced to a cracking apparatus connected to an evacuated gas line. A liquid nitrogen dewer (-195°C) in contact with

the gas line served to trap the carbon dioxide and water vapor once the sample gases were introduced. The sample N_2 was allowed to equilibrate for five minutes after cracking. The nitrogen gas was collected on a molecular sieve containing zeolite. In a similar manner, carbon dioxide gas was collected using an isopropyl-liquid nitrogen slush (-65°C to -70°C) into pyrex tubes which were sealed before being removed from the gas line. Prior to collection of the CO_2 gas, percent carbon determinations were made using a calibrated manometer. The purified CO_2 and N_2 gases were then analyzed on a VG PRISM dual inlet mass spectrometer.

Dual Inlet Mass Spectrometry

Samples of N_2 gas were introduced into the dual inlet of the mass spectrometer by heating the molecular sieve to approximately 300°C with a heating cord. Carbon dioxide samples were cracked into the spectrometer. All samples were analyzed in comparison to laboratory gas standards which were previously calibrated against NBS standards (e.g. PDB and N_2). Precision for $\delta^{13}C$ and $\delta^{15}N$ replicate analyses on the MS was ± 0.1‰.

Carlo Erba Method

In addition to isotope analyses approximately ten $\delta^{13}C$ values were determined using a Carlo Erba elemental analyzer interfaced to the VG PRISM stable isotope ratio

mass spectrometer. This required no more than 1mg of sample. The Carlo Erba combusts the sample carbon and nitrogen to CO_2 and N_2 using a modification of the Dumas method. Water resulting from this combustion is trapped using magnesium perchlorate. The purified sample gases are separated via a GC then sent on to the MS for analysis. Instrument precision for the Carlo Erba is typically 0.2‰.

Amino Acid Analyses

An amino acid recovery experiment designed to quantify amino acid loss during column purification was performed. Solutions of known concentration of ten amino acids were placed over a cation exchange resin column. Standard solutions of identical amino acid concentration were made for each sample. After column purification, sample eluents were run by HPLC to assess amino acid recoveries. Amino acid analyses were subsequently performed on fossil organic matter hydrolysates by gas chromatography. Three representative fossil samples (4.0 Ma, 5.5 Ma and 10.0 Ma) were chosen for amino acid analysis. The 4.0 Ma sample was chosen because its appearance was consistent with being diagenetically altered (exposed inner surfaces and abberant coloration indicating possible diagenesis). The 5.5 Ma sample was selected because it appeared to be the least altered (intact structure, no visible signs of alteration) of the entire time series. The 10.0 Ma sample was neither visibly well preserved nor significantly altered, but was intact. Calculations of the mole percent of each amino acid were made. The HMW material

extracted from modern bovine bone (Ostrom, 1990) was used as the standard for comparisons to ancient equid amino acid patterns.

Organic Matter Hydrolysis

Five to ten milligram aliquots of HMW organic matter isolated from individual fossil horse teeth were each hydolyzed in 1ml of 6N HCl at 100°C for a period of twenty-four hours. Hydolyzates were dried under filtered air, then rehydrated with 500µls double distilled water to concentrate the amino acids before proceeding with the desalting procedure. Modern amino acid standards did not require concentration in this manner and were placed directly over the resin column.

Column Chromatographic Purifications

To assess percent recoveries and reproducibilities, amino acid standards of known composition and concentration were placed over an affinity ion exchange chromatography column. Fossil samples were desalted by the same procedure used for the amino acid standards. Glass burets (25ml) were packed with approximately 10ml of ion exchange resin (BioRad, Amberlite 50W-X8 100 mesh size) freshly hydrated in pH 2 quartz distilled water. The column was then equilibrated to a slightly acidic pH by washing with 3 to 4 bed volumes of quartz distilled water. Protonation of the column in this manner facilitates the ion exchange reaction between the resin and the

sample amino acids. Next, the sample amino acid solution was added to the top of the column. Two bed volumes of quartz distilled water were placed over the column immediately at flow rates between 0.2ml/min. and 0.7ml/min. Slower flow rates increase the ion exchange capabilities of the resin. Multiple bed volumes of quartz distilled water were added to the column to remove all the salt contaminants. Amino acids were eluted from the column by the addition of 2 to 3 bed volumes of 3N ammonium hydroxide (NH_4OH). The eluent was rotoevaporated at 40-45°C to a volume of approximately 1ml, dried down under filtered N_2 gas and rehydrated to approximately 500µl in pH 2 water. A 200µl aliquot of the resulting solution was dried and used for derivitization.

Derivitization Procedure

Desalted hydrolyzates were derivitized to their TFA esters (Engel and Hare, 1985). The derivitization involved esterification with an acidified isopropyl alcohol followed by acylation using TFA (Engel and Hare, 1985). To each vial 500µl of acid alcohol (1:4 acetyl chloride:isopropanol) was added. The samples were allowed to reflux for one hour at 100-110°C in teflon sealed vials, quenched in an ice bath and dried under filtered N_2 gas at room temperature. To each sample 100µl of methylene chloride (CH_2Cl_2) was added and the drying step was repeated. Samples were esterified with 500µl of TFA and 500 µl CH_2Cl_2 for 10 minutes at 100-110°C. This reaction was quenched in an ice bath then dried under N_2 gas. The derivitized amino

acids were resuspended in a known volume (typically 1µl) of CH_2Cl_2 and injected into the GC for analysis.

Enantiomer Assessment

The relative abundance of amino acids in a sample were estimated using a GC (HP5790A) interfaced with a MSD (HP5971). Individual derivitized amino acid solutions were injected into the GC inlet where they were combusted (at 280°C) to the vapor phase. Separation of amino acids was achieved by maintaining a constant column temperature of 90°C for seven minutes then changing to a final temperature of 200°C at a rate of 1.6°C per minute. The mobile phase, helium gas, transported the sample gas down the capillary column (internal diameter 0.25mm). The coating on the 50m long Chirasil-Val (Applied Science) column acts as the stationary phase and was composed of N-propionyl-L-valine terbutylamide coupled to a co-polymer of carboxyalkylmethylsioxene and dimethylsiloxene. Amino acids were identified both by retention time and by comparison of the sample ion fragmentation patterns to an amino acid standard mass spectral library. Ion fragmentation patterns were analyzed over the range from 50-500 mass to charge (m/z) units at 1.7 cycles per second.

RESULTS AND DISCUSSION

Yield and Recovery Experiments

Demineralization and Lyophilization

Fossil material ground to a uniform powder in a shorter time period and demineralized more efficiently than did modern materials. Lyophilized material consisted of a light brown powder and occassionally a fluffy white component. These may represent organic fractions in different conformations. Some of the powderized material probably was silicified crystals which would not demineralize.

High Molecular Weight Organic Matter Yields

Percent yields of HMW organic matter obtained from all modern and fossil samples were determined. The results from four separate demineralization techniques used for modern and ancient materials (1N, 3N, 6N HCl and 6N-2Hr.) are reported (Table 4, Table 5, Table 6). Duplicate sample numbers denote separate elements from the same individual. Modern horse and wapiti tissues treated in 1N HCl yield from 0.3% to 10.5% HMW material and have an average value of 3.0 ± 1.9% (Table 4).

Table 4 High molecular weight organic matter yields obtained from modern horses and wapiti.

Sample/Element	Genus species	Demin. Mass (mg)	Organic Mass (mg)	Percent Yield (%)
MSU: Cer 1 RM1	Cervus canadensis	1540	19	1.2
MSU: Cer 1 RM1	Cervus canadensis	1560	12	0.8
MSU: Cer 1 RM1	Cervus canadensis	1550	26	1.7
MSU: Cer 2 dP4	Cervus canadensis	1070	29	2.7
MSU: Cer 2 dP4	Cervus canadensis	1080	32	3.0
MSU: Cer 2 dP4	Cervus canadensis	1080	23	2.1
MSU: Cer 3 Dent.	Cervus canadensis	1220	128	10.5
MSU: Cer 3 Dent.	Cervus canadensis	1380	41	3.0
MSU: Cer 3 Dent.	Cervus canadensis	1090	32	2.9
MSU: Eq 1a RM1	Equus caballus	1960	71	3.6
MSU: Eq 1d RM1	Equus caballus	1150	48	4.2
MSU: Eq 1f RM1	Equus caballus	870	66	7.6
MSU: Eq 1j RM1	Equus caballus	2260	48	2.1
MSU: Eq 1k RM1	Equus caballus	2410	44	1.8
MSU: Eq 1l RM1	Equus caballus	2180	28	1.3
MSU: Eq 2 RM2	Equus caballus	2200	78	3.5
MSU: Eq 3 RM3	Equus caballus	5040	16.8	0.3
MSU: Eq 4 RP2	Equus caballus	2520	83	3.3
MSU: Eq 5 RP3	Equus caballus	2500	87	3.5
MSU: Eq 6 RP4	Equus caballus	2560	41	1.6
MSU: Eq 7 R Max.	Equus caballus	2000	64.5	3.2
MSU: Eq 8 RM1	**Equus caballus**	**2000**	**185.2**	**9.3**
MSU: Eq 9 RM1	*Equus caballus*	*2500*	*159.9*	*6.4*
F.M.M. 1219 R Max.	**Equus sp.**	**2480**	**305.3**	**12.3**
		Mean (1N HCl)	3.0	1.9
		Mean (3N HCl)	*6.4*	*NA*
		Mean (6N HCl)	**10.8**	**NA**

Table 5 High molecular weight organic matter yields obtained from ancient horse tooth and bone.

Specimen	Taxonomic Designation	Age (Ma)	Demin. Mass (mg)	Organic Mass (mg)	Percent Yield (%)
UALP 7810	Equus sp.	3	4500	218.2	4.8
F: AM 129114	Equus simplicidens	4	4990	232.3	4.7
F: AM 129444	"Dinohippus" leidyanus	5.5	2520	156.7	6.2
UNSM 35-56B	"Dinohippus" leidyanus	6	3030	248	8.2
UNSM 316-56	Nannippus lenticularis	6	2000	28.6	1.4
F: AM 129443	Neohipparion eurystyles	6.5	2990	235.1	7.9
UNSM 5157-73	Hipparion sp. (cf. forcei)	7	3030	114.6	3.8
AMNH 17599A	"Dinohippus" leidyanus	7	4520	245.4	5.4
UNSM 2010-70B	Calippus sp. nov.	7	2500	168.4	6.7
F: AM 129442	Cormohipparion occidentale	8.5	3040	166	5.5
F: AM 129445	Cormohipparion occidentale	9.5	3490	200	5.7
F: AM 129446	Cormohipparion occidentale	9.5	2520	76	3.0
F: AM 129454	Cormohipparion occidentale	10	3030	191	6.3
F: AM 129441 Max.	Cormohipparion occidentale	10.5	3010	330.2	11.0
F: AM 129441 LM1	Cormohipparion occidentale	10.5	1500	121	8.1
F: AM 129441LdP4	Cormohipparion occidentale	10.5	1980	68.9	3.5
F: AM 129441LM2	Cormohipparion occidentale	10.5	2250	124.6	5.5
F: AM 129447	Pliohippus pernix	12	3990	694	17.4
F: AM 129450	Pliohippus pernix	12	3010	148	4.9
F: AM 114068	Pliohippus pernix	12	1520	146.1	9.6
F: AM 129316 LP3	Merychippus insignis	15	2520	148.7	5.9
F: AM 129316 Max.	Merychippus insignis	15	2660	125.4	4.7
F: AM 129453	Merychippus sp.(cf. isonesus)	15.5	4500	337.8	7.5
F: AM 110575LP4	"Merychippus" primus	17	1340	16.6	1.2
F: AM 110575 Max.	"Merychippus" primus	17	2500	244.1	9.8
AMNH 20513	"Merychippus" primus	17	3000	32.5	1.1
F: AM 129451	"Merychippus" tertius	18	2540	46.5	1.8
F: AM 128784	"Parahippus" sp.	18.5	2010	325	16.2
F: AM 129455 Acet.	Mesohippus sp.	30.5	2620	67.5	2.6
F: AM 74067 Acet.	Miohippus obliquidens	32	1250	46.9	3.8

Table 6 High molecular weight organic matter yields obtained from a two hour demineralization experiment.

Specimen	Genus species	Age (Ma)	Demin. Mass (mg)	Organic Mass (mg)	Percent Yield (%)
F: AM 129450-1	Pliohippus pernix	12	1500	52.6	3.5
F: AM 129450-2	Pliohippus pernix	12	1500	54.6	3.6
F: AM 129450-3	Pliohippus pernix	12	1500	61.2	4.1
			Mean (6N HCl)	**2 Hr. Demin. Expt.**	**3.8 +/- 0.3%**

These same tissues yield 6.4% and 10.8% HMW material when treated with 3N HCl and 6N HCl, repectively. Modern organic matter yields of this magnitude are consistent with the small fraction of organic matter present in mammalian dentine. In most cases, bones yield a larger percentage of HMW organic matter compared to teeth (e.g. F:AM 129441 and F:AM 110575, Table 5). The HMW organic material retrieved from horse fossils demineralized twenty-four hours in 6N HCl range from 1.1% to 17.4% dry weight yield and average 6.1 ± 3.7% (Table 5). This yield is similar to the dry weight percent yields obtained from prehistoric remains by other researchers (DeNiro and Epstein, 1981; Nelson et al., 1986). Fossil HMW material obtained from the two hour demineralization experiment gave a mean value of 3.8 ± 0.3% (Table 6). Variations in the yield among fossils could relate to either differential diagenetic alteration or to variable contributions from aluminosilicate minerals that were not removed during demineralization. The mean yield of HMW organics from modern materials isolated after demineralization in 1N HCl (3.0 ± 1.9%) is similar to that obtained from fossils treated with 6N HCl (6.1 ± 3.7%). Consequently, modern and fossil yields are comparable despite the difference in demineralization technique.

Percent Carbon Yields

The mean percent organic carbon yields for the fossils analyzed in this study was 2.2 ± 1.0% (Table 7). Despite being small, this value is similar to the published

Table 7 Percent organic carbon yields from select modern and fossil samples.

Specimen	Genus species	Age (Ma)	Percent Carbon (%)
F.M.M. 1219	Equus sp.	0	28.5
UALP 7810	Equus sp.	3	3.7
F: AM 129114	Equus simplicidens	4	5.3
UNSM 35-56B	"Dinohippus" leidyanus	6	3.5
UNSM 316-56	Nannippus lenticularis	6	1.8
UNSM 316-56	Nannippus lenticularis	6	2.3
UNSM 5157-73	Hipparion sp. (cf. forcei)	7	1.9
AMNH 17599A	"Dinohippus" leidyanus	7	2.3
UNSM 2010-70B	Calippus sp. nov.	7	0.7
F: AM 129445	Cormohipparion occidentale	9.5	1.5
F: AM 129446	Cormohipparion occidentale	9.5	1.3
F: AM 129454	Cormohipparion occidentale	10	1.4
F: AM 129454	Cormohipparion occidentale	10	3.5
F: AM 129441 Max.	Cormohipparion occidentale	10.5	2.5
F: AM 129441 Max.	Cormohipparion occidentale	10.5	3.0
F: AM 129441 LM1	Cormohipparion occidentale	10.5	2.0
F: AM 129441LdP4	Cormohipparion occidentale	10.5	2.7
F: AM 129441LM2	Cormohipparion occidentale	10.5	3.4
F: AM 129447	Pliohippus pernix	12	1.2
F: AM 129448	Pliohippus pernix	12	1.8
F: AM 129450	Pliohippus pernix	12	1.7
F: AM 129450	Pliohippus pernix	12	1.7
F: AM 129450-A	Pliohippus pernix	12	1.6
F: AM 129450-B	Pliohippus pernix	12	1.7
F: AM 129450-C	Pliohippus pernix	12	1.2
F: AM 129316 LP3	Merychippus insignis	15	1.4
F: AM 129316 Max.	Merychippus insignis	15	1.1
F: AM 129453	Merychippus sp.(cf. isonesus)	15.5	2.8
F: AM 110575 Max.	"Merychippus" primus	17	1.7
AMNH 20513	"Merychippus" primus	17	1.8
F: AM 129451	"Merychippus" tertius	18	1.7
F: AM 128784 RM2	"Parahippus" sp.	18.5	3.7
F: AM 128784 Max.	"Parahippus" sp.	18.5	1.8
F: AM 129455 Acet.	Mesohippus sp.	30.5	1.9
F: AM 74067 Acet.	Miohippus obliquidens	32	3.7
		Mean	**2.2% +/- 1.0 %**

percent carbon yields from ancient materials of DeNiro and Werner (1988) (0.3% to 10.7%) and Ostrom et al. (1990) (2.0% to 9.0%).

Amino Acid Recovery Experiment

The results from three representative amino acids with varying initial concentrations (aspartic acid, glutamic acid and serine) are reported (Table 8). Percent recoveries are reported as mean percent recovered of each amino acid (Table 9). The data in Table 8 and Table 9 demonstrate that column purification does not result in appreciable amino acid loss. Aspartic acid and serine experienced no appreciable loss irrespective of initial sample concentration. As much as 12.6% of the original glutamic acid may be lost during the purification process when the initial concentration is 0.1nmol/μl or higher. A loss of this magnitude is not problematic for interpreting overall amino acid abundances and distributions. For lower concentrations, glutamic acid loss is of less concern (e.g. 98.9% mean recovery when C_i = 0.01nmol/μl). The increase in loss of glutamic acid at higher concentrations relates to a saturation of the theoretical plates within the column and might be avoided by using a larger resin bed. As such, amino acid loss during purification is minimal. Consequently, all fossil samples analyzed for their amino acid content were first purified by ion exchange chromatography to remove salts, minerals and HMW material remaining after hydrolysis which might inhibit the derivitization step.

Table 8 Mean peak areas for three amino acids, before column purification and after column purification, with varying initial concentrations.

Amino Acid Standard II **0.2nmol/20ml**
(Pre-purification)

Amino Acid	Mean Peak Area (mV-sec)	σ
ASP	8.80E+06	1.13E+06
GLU	9.09E+06	3.49E+05
SER	2.06E+07	1.26E+06

Amino Acid Standard II **0.2nmol/20ml**
(Post-purification)

Amino Acid	Mean Peak Area (mV-sec)	σ
ASP	1.05E+07	2.42E+05
GLU	1.02E+07	1.96E+05
SER	2.79E+07	2.16E+05

Amino Acid Standard I **2.0nmol/20ml**
(Pre-purification)

Amino Acid	Mean Peak Area (mV-sec)	σ
ASP	1.51E+07	2.70E+05
GLU	1.91E+07	2.31E+06
SER	3.58E+07	6.79E+05

Amino Acid Standard I **2.0nmol/20ml**
(Post-purification)

Amino Acid	Mean Peak Area (mV-sec)	σ
ASP	1.54E+07	5.60E+05
GLU	1.67E+07	3.92E+06
SER	3.70E+07	5.12E+06

Table 9 Mean percent recoveries of aspartic acid, glutamic acid and serine from ancient horse organic matter following column purification.

Amino Acid	Concentration	Minimum % Recovery	Mean % Recovery
ASP	2.0nmol/20ml	96.1%	100.0%
ASP	0.2nmol/20ml	97.5%	100.0%
GLU	2.0nmol/20ml	59.8%	87.4%
GLU	0.2nmol/20ml	87.8%	98.8%
SER	2.0nmol/20ml	87.4%	100.0%
SER	0.2nmol/20ml	100.0%	100.0%

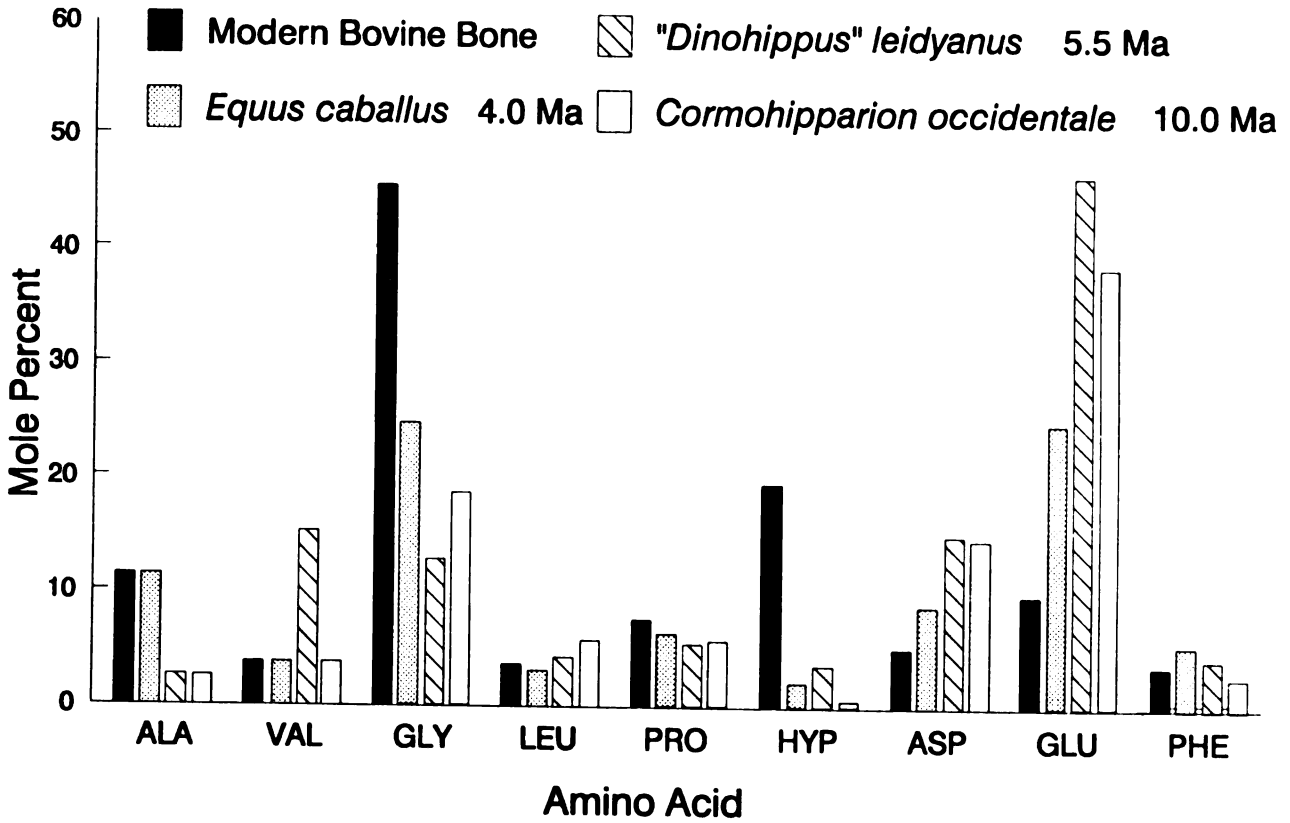
Amino Acids in Fossil Horse Tooth and Bone

The relative abundance and distribution of amino acids in three fossils of different ages were determined (Table 10). The presence of hydroxyproline and high concentrations of glycine and proline (verified both by retention time and mass fragmentation patterns) in each sample suggest an indigenous component is retained in these fossils (Figure 8, Figure 9, Figure 10). Modern tooth and bone collagen is predominantly composed of glycine, proline and hydroxyproline (Hare, 1980). High concentrations of aspartic acid and glutamic acid are consistent with the suggestion that acidic macromolecules are preferentially preserved during diagenesis owing to their high selectivity for the mineral phase (Masters, 1987; Robbins and Brew, 1990). The lack of large concentrations of serine and threonine (common contaminating amino acids) also suggest indigenous organic matter was recovered from each of these three fossils. The presence of several D/L pairs in the chromatogram of the 4.0 Ma fossil could be construed as evidence for contamination of this specimen (Figure 8). However, this is not the case given the resemblance of the hydroxyproline, glycine and proline amino acids to the modern bovine bone pattern.

Implications for Indigeneity Assessment of Fossils

The amino acid patterns of bones and teeth change as a function of time. However, a direct relationship between age of the fossil and amino acid loss

Table 10 Amino acid distributions and abundances for three fossils, 4.0 Ma, 5.5 Ma and 10.0 Ma (F:AM 129114, F:AM 129444 and F:AM 129454, respectively) in relation to the amino acid pattern of modern cow bone (modern amino acid data is reprinted from Ostrom, 1990).



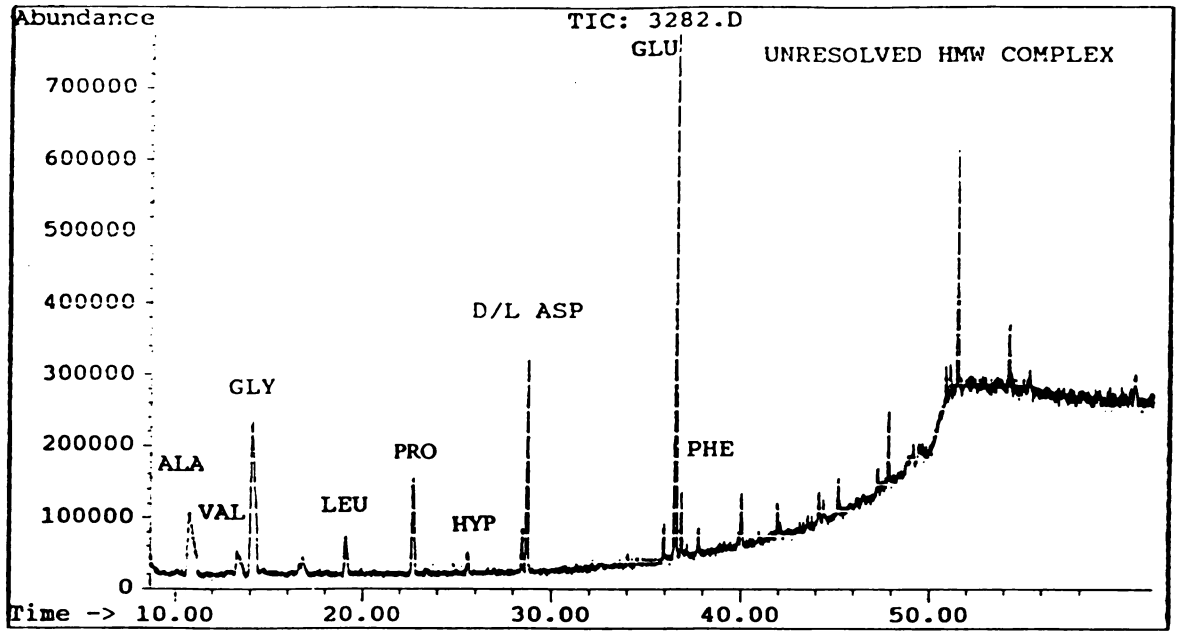
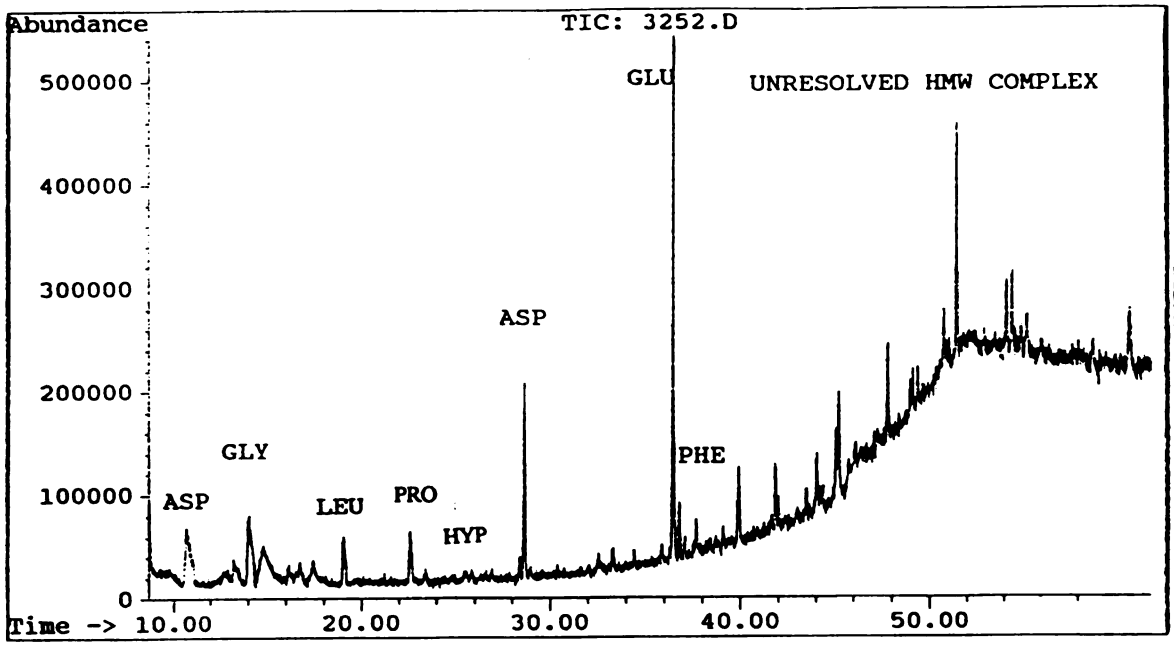


Figure 8 Amino acid chromatogram for a 4.0 Ma fossil tooth (F:AM 129114). Note the presence of hydroxyproline and D/L aspartic acid peaks.



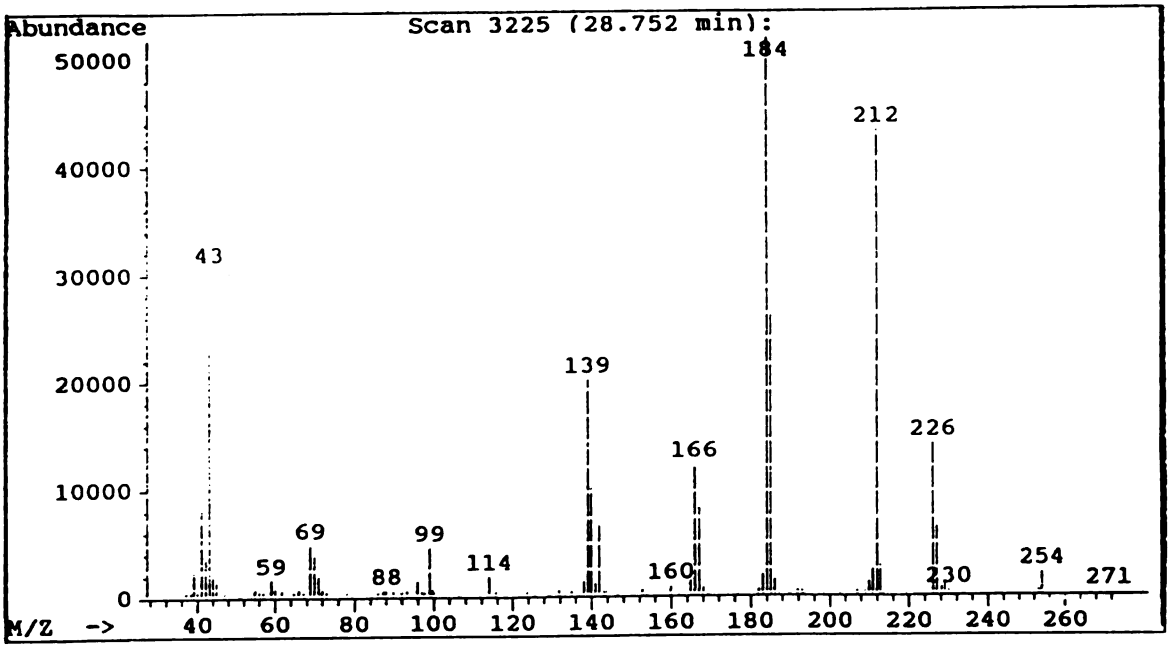


Figure 9 Amino acid chromatogram for a 5.5 Ma fossil tooth (F:AM 129444). The mass fragmentation pattern for hydroxyproline is given below the chromatogram.

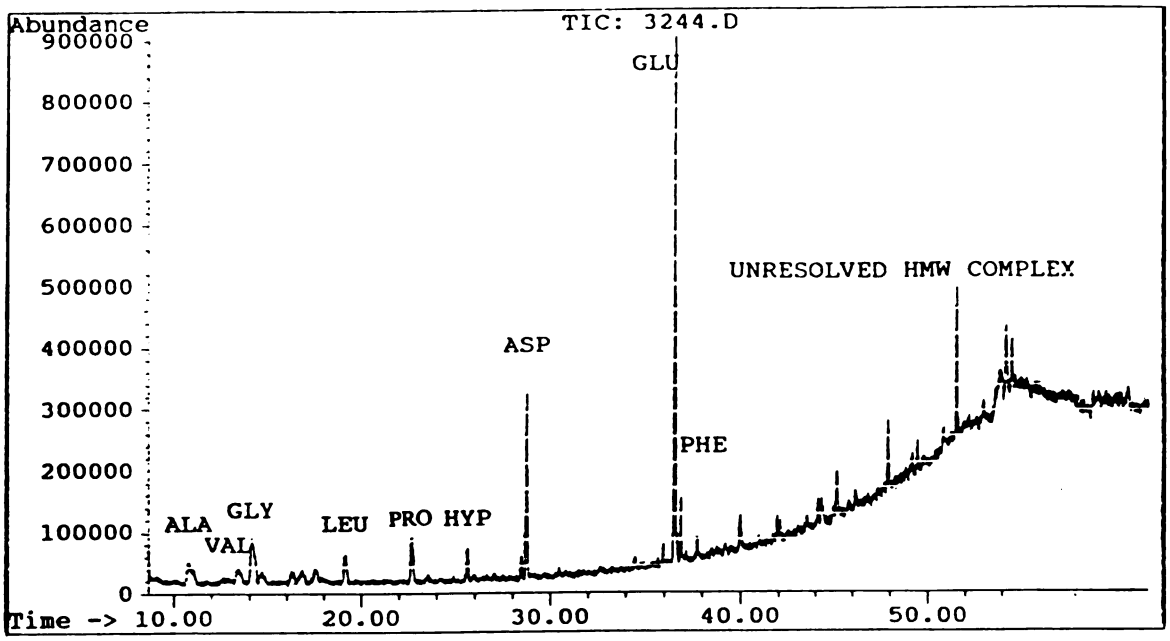


Figure 10 Amino acid chromatogram for a 10.0 Ma fossil tooth (F:AM 129454). Note the large abundance of the glycine and proline peaks and the presence of a hydroxyproline peak.

does not exist. Complexification of acidic amino acids with organic molecules may stabilize organic matter in fossils over time. Further research directed toward assessing how organic matter survives diagenesis is needed in order to better understand indigeneity of ancient materials.

For this study, amino acid analyses supply several indications that an indigenous organic component has been isolated from these fossils including: 1) The distribution and abundance of amino acids is similar to the amino acid pattern of a modern terrestrial herbivore (a cow); 2) The presence of a distinct hydroxyproline peak which is unique to tooth and bone; 3) The lack of large concentrations of serine and threonine (common soil contaminants). These indications suggest an indigenous component was obtained for fossils which were intact and which appeared to be moderately to well preserved. Even the poorly preserved 4.0 Ma fossil had an indigenous organic signal. Unquestionably, the organic fraction in these fossils has undergone alteration, but most of them should retain an indigenous fraction judging by this amino acid data, particularly if the fossil is intact. In order to more conclusively demonstrate the indigeneity of organic material isolated from this time series of fossil horses, an evaluation of the carbon and nitrogen isotope values was undertaken.

Isotope Studies of Modern and Ancient Herbivores

Isotope values of modern equids were determined in order to better understand the natural isotopic variation in horse hard parts. This understanding provides

comparative data which may facilitate an indigeneity assessment of fossil organic matter. Carbon isotope values of the modern terrestrial herbivore tissues measured range from -20.1‰ to -24.2‰ (Table 11). The nitrogen isotope range for these same specimens is 2.3‰ to 6.8‰ (Table 11).

Procedural Reproducibility

Procedural reproducibility on modern samples was evaluated through replicate demineralizations and dialyses of five aliquots of the labial half of a single horse molar (RM1). Of the five replicate demineralizations, three $\delta^{13}C$ and five $\delta^{15}N$ determinations were made. The procedural reproducibility for modern horse $\delta^{13}C$ and $\delta^{15}N$ measurements was 0.2‰. Reproducibility for the fossil $\delta^{13}C$ analyses was typically 0.1‰ to 0.3‰. Reproducibility for fossil $\delta^{15}N$ values was 0.4‰.

Testing For Procedural Errors

The three samples which compose the two hour demineralization experiment (F:AM 129450-1, F:AM 129450-2, and F:AM 129450-3) have mean $\delta^{13}C$ and $\delta^{15}N$ values of -23.6 ± 0.1‰ and 4.2 ± 0.5‰, respectively (Table 6). Although the $\delta^{13}C$ values are not significantly affected by a reduction in demineralization acid exposure time, the $\delta^{15}N$ values are on average 3.9‰ depleted in ^{15}N. This depletion is accounted for by a larger incorporation of ^{14}N-rich low molecular weight material

Table 11 The $\delta^{13}C$ and $\delta^{15}N$ values of modern wapiti and horses.

Sample Number	Genus species	Element Sampled	δ13C	δ15N
MSU: Cer. 1	Cervus canadensis	RM1	ND	5.0
MSU: Cer. 2	Cervus canadensis	RM1	-22.9	5.7
MSU: Cer. 3	Cervus canadensis	RM1	-22.9	5.4
MSU: Cer. 4	Cervus canadensis	RdP4	-23.2	5.8
MSU: Cer. 5	Cervus canadensis	RdP4	-22.8	6.1
MSU: Cer. 6	Cervus canadensis	RdP4	-23.0	5.1
MSU: Cer. 7	Cervus canadensis	R Dentary Bone	ND	5.1
MSU: Cer. 8	Cervus canadensis	R Dentary Bone	-22.6	4.9
MSU: Cer. 9	Cervus canadensis	R Dentary Bone	-23.0	4.0
MSU: Cer. 10	Cervus canadensis	Hair	-24.0	ND
MSU: Cer. 11	Cervus canadensis	Plant Debris inTeeth	ND	6.3
MSU: Cer.12	Cervus canadensis	Connective Tissue	-24.2	5.3
MSU: Cer. 13	Cervus canadensis	Muscle (Masseter)	ND	5.0
MSU: Bot. 1	?	MI Meadow Hay	-28.1	ND
MSU: Eq. 1a	Equus caballus	RM1.1A (labial)	-21.1	6.1
MSU: Eq. 1b	Equus caballus	RM1.1B (labial)	-21.1	5.9
MSU: Eq. 1c	Equus caballus	RM1.1C (labial)	-20.9	6.0
MSU: Eq. 1d	Equus caballus	RM1.2 (labial)	-20.8	5.8
MSU: Eq. 1e	Equus caballus	RM1.3 (labial)	-20.3	5.9
MSU: Eq. 1f	Equus caballus	RM1.4A (labial)	-20.6	6.1
MSU: Eq. 1g	Equus caballus	RM1.4B (labial)	ND	5.7
MSU: Eq. 1h	Equus caballus	RM1.4C (labial)	ND	5.9
MSU: Eq. 1j	Equus caballus	RM1.5 (lingual)	-21.4	5.7
MSU: Eq. 2	Equus caballus	RM2	-20.5	6.3
MSU: Eq. 3	Equus caballus	RM3	-20.8	6.0
MSU: Eq. 4	Equus caballus	RP2	-20.1	5.8
MSU: Eq. 5	Equus caballus	RP3	-20.2	5.9
MSU: Eq. 6	Equus caballus	RP4	-20.3	5.6
MSU: Eq. 7	Equus caballus	R Maxilla Bone	-20.4	5.6
MSU: Eq. 8	Equus caballus	RM1 (6N HCl)	ND	6.0
MSU: Eq. 9	Equus caballus	RM1 (3N HCl)	-20.7	6.8
F.M.M. 1219	Equus sp.	R Maxilla Bone	-21.8	2.3

remaining in response to the poor demineralization efficiency. Hence, samples which are not fully demineralized will not provide reliable nitrogen isotope data. Correspondingly, these three samples are not used when interpreting horse paleocology.

Isotope Variation Within Modern Materials

Separate tissues from a single organism are often found to be isotopically distinct from one another (Teiszen et al., 1983). For example, DeNiro and Epstein (1978b) found the $\delta^{13}C$ values of soft anatomies of mice to be approximately -21.0‰ while bones from the same mouse strain average approximately -18.0‰. Differences such as these results from fractionation during metabolism. Natural isotopic variation within a species must be understood before accurate paleoecological assessments can be made.

Intraspecific Variance in a Juvenile Wapiti

In an effort to understand natural isotope variation among separate tissues from an individual, various skeletal and soft tissues from a single juvenile wapiti were measured. The various wapiti tissues range from -22.8‰ to -24.2‰ in $\delta^{13}C$ and from 4.0‰ to 6.1‰ in $\delta^{15}N$ (Figure 11). Comparisons of $\delta^{13}C$ and $\delta^{15}N$ values among tissues from an individual wapiti shows that variation is typically larger for nitrogen

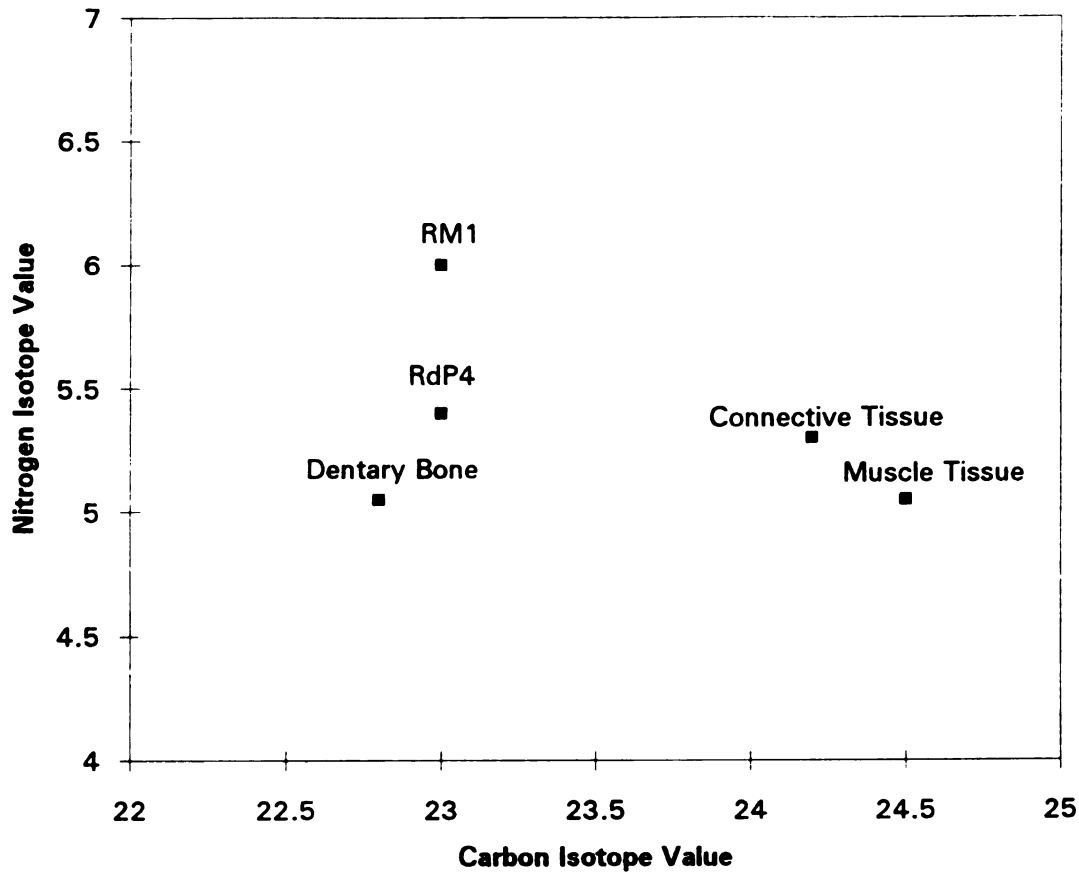


Figure 11 Natural variation in the $\delta^{13}C$ and $\delta^{15}N$ values of a juvenile wapiti.

isotopes than for carbon isotopes. For example, the mean $\delta^{13}C$ value for modern wapiti bone is -22.8 ± 0.1‰ whereas the mean $\delta^{15}N$ value for modern wapiti bone is 4.7 ± 0.6‰. Natural isotope variation is small in comparison to isotope effects from alternate sources such as diagenesis or dietary changes (e.g. transition from a C_3 to a C_4-based diet) (DeNiro and Hastorf, 1985; Vogel, 1978). The mean $\delta^{13}C$ value for the modern juvenile wapiti skeletal materials (teeth and bones) is -22.9 ± 0.2‰ while the corresponding mean $\delta^{15}N$ value is 5.2 ± 0.6‰ (Figure 11). The large variation in nitrogen isotope values could be due to changes in the juvenile's diet that occurred during development. The transition from breast milk to the herbivorous adult diet can result in natural isotope differences between tissues emplaced at different times. Enrichment in the $\delta^{15}N$ of milk teeth of 2‰ over bone collagen occurs in juveniles and in species whose teeth stop growing early in life (Bocherens et al., 1994). A lack of similar variation in $\delta^{13}C$ and other lines of evidence (e.g. grass found within teeth) indicate this juvenile was weaned from its milk diet. Nonetheless, the potential confounding variable that juvenile specimens introduce may make them poor indicators of natural isotopic variation. Consequently, the natural variation in the carbon and nitrogen isotope values of modern horse skeletal materials was also investigated.

Interspecific Variance Between Wapiti and Horses

The carbon isotope values of modern horse teeth and bones range from -20.1‰ to -21.8‰ (Table 11). The mean $\delta^{13}C$ value for all modern horse skeletal

material is -20.7 ± 0.4‰ versus -22.9 ± 0.2‰ for wapiti materials. Natural variation in $\delta^{13}C$ values of skeletal remains of horses and wapiti are similar whereas the $\delta^{13}C$ values themselves are different (t-test, t = -12.2, df = 16.2, α = 0.05). A $\delta^{13}C$ difference of 2.2‰ exists between modern wapiti skeletal material and modern horse skeletal material. This likely reflects the different feeding strategies of wapiti and horses, wapiti consuming a considerably larger amount of browse (C_3 plants). A "canopy" effect might also explain the 2.2‰ depletion given preliminary results which indicate a 5‰ to 6‰ depleted carbon pool on dense forest floors (Van der Merwe and Medina, 1989). Alternatively, this isotope difference may be attributed to the age of the wapiti since a milk diet would serve to deplete the its $\delta^{13}C$ value (Boutton et al., 1988).

Currently, little information is available concerning the magnitude of depletion associated with milk-based diets in undomesticated mammals. Studies have shown that lipids are regularly 7-8‰ more negative than other biological fractions (DeNiro and Epstein, 1977). Whole wapiti milk is assuredly not as depleted as a pure lipid fraction. Tyrrell et al. (1984) found dairy cow milk to be 1‰ depleted in $\delta^{13}C$ and Boutton et al. (1988) found 1.8‰ to 2.2‰ depletions in bovine $\delta^{13}C$ milk values compared to the diet. These depletions are consistent with the observation that milk fat is typically less than 10% in most mammals (Iverson, 1992). The presence of a substantial amount of plant debris within the selenodont lophs of this juvenile's cheek teeth indicates a weaned individual. If the individual was recently weaned a milk signature might persist for some time. This does not appear to be the case, however,

since the mean $\delta^{13}C$ for this individual is similar to the $\delta^{13}C$ values published for other mature artiodactyls (deer, mainly) (DeNiro and Weiner, 1988; Cormie and Schwarcz, 1994). The wapiti had not reached full maturity since it had not yet lost its deciduous premolars. Therefore, the juvenile wapiti appears to be in transition to adulthood and it's adult diet.

The $\delta^{15}N$ values of modern horse teeth and bones range between 5.6‰ and 6.3‰ (Table 11). The mean $\delta^{15}N$ values for the modern horse skeletal material analyzed is 5.9 ± 0.3‰ compared to 5.2 ± 0.6‰ for the wapiti. A statistical difference in the mean horse and wapiti $\delta^{15}N$ values exists (t-test, t = -4.0, df = 11.7, α = 0.05). This result may indicate fundamental differences in the diets of wapiti and horses in terms of the amount of legumes consumed.

Variation Between Different Horses

Carbon isotope values for modern horses are similar between separate individuals and within the same individual (Figure 12). A difference in $\delta^{13}C$ of 1.4‰ was observed for bone between a horse from Michigan and another from Nebraska. The difference in $\delta^{13}C$ for these two horses is similar to the 1.5‰ variance Vogel (1978) found between individual ungulates with like diets. The $\delta^{13}C$ value for bone from the modern Michigan horses is -20.4‰ while the Nebraska horse bone is -21.8‰. These values are also in good agreement with the organic $\delta^{13}C$ values of

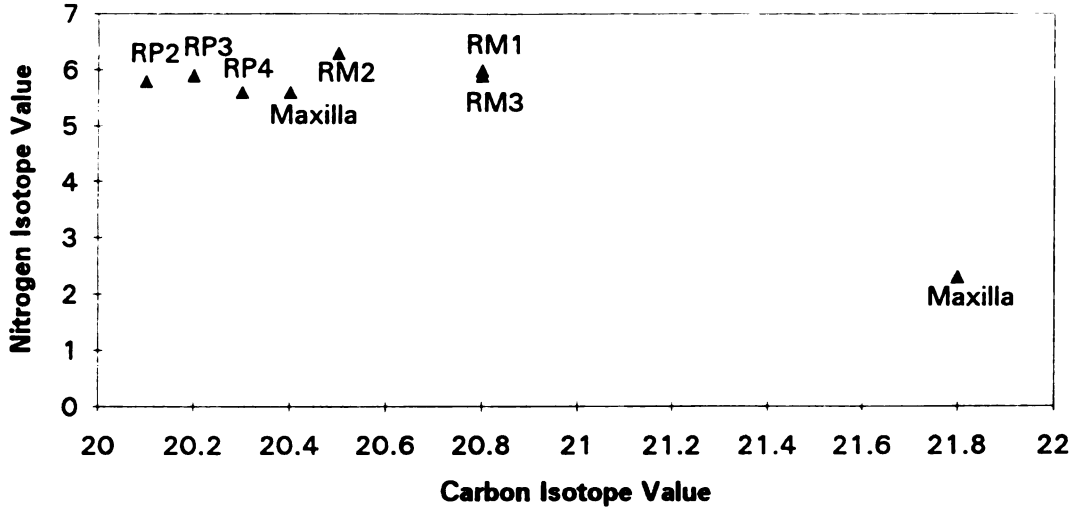


Figure 12 The relationship between the $\delta^{13}C$ and $\delta^{15}N$ values of skeletal tissues (premolar and molar teeth and maxilla bone) from a modern horse.

modern horse bone (-21.2‰) published elsewhere (Schoeninger and DeNiro, 1984). No such comparison was possible for recent horse teeth in that this study is the first to characterize modern equid dentine isotopically.

Modern horse nitrogen isotope values were also found to be similar within an individual (Figure 12). Nitrogen isotope variation between individuals can be large even for individuals with similar diets (Bada et al., 1990). The nitrogen isotope value of the modern horse from Nebraska (F.M.M. 1219) differed substantially from the Michigan horse. The abberant 2.3‰ nitrogen isotope value of the Nebraska horse may reflect consumption of plants with altered nitrogen values due to the influence of fertilizers which have $\delta^{15}N$ values of about 0.0‰ (Schoeninger and DeNiro, 1984). Alternatively, this horse may have eaten a large amount of legumes such as clover which have $\delta^{15}N$ values near 0.0‰ (Delwiche et al., 1979; Schoeninger and DeNiro, 1984). A 60% incorporation of total nitrogen with a $\delta^{15}N$ signature of 0.0‰ will shift a natural bone value of 5.6‰ to 2.3‰.

The mean $\delta^{15}N$ value for modern horse skeletal materials is 5.9 ± 0.3‰ excluding the 2.3‰ value which may record fertilizer influences. The mean nitrogen isotope value of modern horse is similar to the modern mean $\delta^{15}N$ value for terrestrial herbivores (5.3 ± 1.9‰) determined by Schoeninger and DeNiro (1984). However, the mean $\delta^{15}N$ value of the modern horse is slightly more depleted than the expected value for a terrestrial herbivore consuming a non-leguminous diet. Consequently, the modern horses analyzed in this thesis likely ate a percentage of clover or some other leguminous, herbaceous ground cover. Noteworthy is the fact that the $\delta^{15}N$ values

reported herein are consistent with expectations based on modern horse trophic level position as well as nitrogen isotope values of horses reported in the literature (DeNiro and Epstein, 1981; Schoeninger and DeNiro, 1984).

Variability in Horse Tooth and Bone

The mean $\delta^{13}C$ value for all modern equid teeth is -20.6 ± 0.4‰. Replicate analyses of RM1 yielded a mean $\delta^{13}C$ value of -20.9 ± 0.4‰ (n=7). All other adult molars and premolars had $\delta^{13}C$ values slightly enriched over the RM1 mean (Table 10). There is no statistical difference in $\delta^{13}C$ values within molars (t-test, t = -1.2, df = 3.0, a = 0.05), within premolars (t-test, t = 3.0, df = 1.0, α = 0.05) or between tooth and bone (t-test, t = 0.7, df = 2.3, α = 0.05). The mean $\delta^{13}C$ values for modern horse bone and teeth are -21.0 ± 0.6‰ and -20.6 ± 0.4‰, respectively. Undoubtedly, natural isotopic differences exist within tooth types and between tooth and bone, but these differences are smaller than the reproducibility of the technique. No differences were observed in the $\delta^{15}N$ values within molars (t-test, t = -1.4, df = 1.2 a = 0.05), within premolars (t-test, t = 5.0, df = 1, α = 0.05) and between molars and premolars (t-test, t = -1.4, df = 4.1, α = 0.05). In addition, there was no difference between tooth and bone of the same individual (t-test, t = -0.6, df = 2.1, α = 0.05). Determination of the $\delta^{13}C$ and $\delta^{15}N$ values of horse dentine are significant as a means to begin distinguishing natural isotopic variation from the potential effects of diagenesis or dietary shifts recorded in fossil teeth.

Isotope Variation in Ancient Horses

Carbon isotope values of the fossil Equidae ranges from -18.4‰ to -26.7‰ (Table 12). Nitrogen isotope values fall between 0.5‰ and 14.0‰ (Table 12). Consistent with the modern modern data, no significant carbon or nitrogen isotope differences were observed between fossil horse premolars and molars (carbon t-test, $t = -0.6$, $df = 2.9$, $\alpha = 0.05$; nitrogen t-test, $t = -1.2$, $df = 29.9$, $\alpha = 0.05$) or between fossil horse bone and teeth (carbon t-test, $t = -0.8$, $df = 3.2$, $\alpha = 0.05$; nitrogen t-test, $t = -0.7$, $df = 4.3$, $\alpha = 0.05$). Within an individual, $\delta^{13}C$ and $\delta^{15}N$ values of bone frequently deviate from tooth isotope values (e.g. F:AM 129316, F:AM 110575)(Table 12). These differences cannot be ascribed to tooth formation prior to weaning as with the juvenile wapiti resulting in a milk signature for two reasons. First, all but one of the ancient horses were fully mature (as evidenced by their lack of deciduous teeth and the wear patterns present on their adult teeth). Secondly, the hypsodont teeth of horses continue to grow throughout life (Bocherens et al., 1994). Therefore, any milk signature would soon be replaced by the adult diet signature. The intraspecific isotope differences between tooth and bone might relate to differential susceptabilities to diagenesis of these two skeletal elements. This can best be evaluated through a comparison of the isotope values of modern and ancient skeletal elements from various horses.

Table 12 The $\delta^{13}C$ and $\delta^{15}N$ values of ancient horse teeth and bones. Italicized isotope values are presummed to be diagenetically altered or had ion beams to low to achieve an accurate isotope value.

Specimen	Genus species	Age (Ma)	δ13C	δ15N
UALP 7810	Equus sp.	3	-23.3	5.9
F: AM 129114-1	Equus simplicidens	4	-25.2	1.3
F: AM 129114-2	Equus simplicidens	4	-24.9	0.5
F: AM 129444-1	"Dinohippus" leidyanus	5.5	-23.4	5.7
F: AM 129444-2	"Dinohippus" leidyanus	5.5	-22.9	ND
UNSM 35-56B	"Dinohippus" leidyanus	6	-22.4	4.2
UNSM 316-56-1	Nannippus lenticularis	6	-22.0	5.4
UNSM 316-56-2	Nannippus lenticularis	6	*-23.3*	ND
F: AM 129443	Neohipparion eurystyles	6.5	-22.9	5.3
UNSM 5157-73	Hipparion sp. (cf. forcei)	7	-24.5	6.8
AMNH 17599A-1	"Dinohippus" leidyanus	7	-24.5	5.6
AMNH 17599A-2	"Dinohippus" leidyanus	7	-25.5	ND
UNSM 2010-70B	Calippus sp. nov.	7	-24.2	5.6
F: AM 129442	Cormohipparion occidentale	8.5	-22.7	7.2
F: AM 129445	Cormohipparion occidentale	9.5	-23.6	6.0
F: AM 129446	Cormohipparion occidentale	9.5	-24.3	6.1
F: AM 129454-1	Cormohipparion occidentale	10	-23.6	6.2
F: AM 129454-2	Cormohipparion occidentale	10	-23.5	ND
F: AM 129441Max.	Cormohipparion occidentale	10.5	-22.6	4.3
F: AM 129441Max.	Cormohipparion occidentale	10.5	ND	4.2
F: AM 129441LdP4	Cormohipparion occidentale	10.5	-23.1	5.8
F: AM 129441LM1	Cormohipparion occidentale	10.5	-21.0	ND
F: AM 129441LM2	Cormohipparion occidentale	10.5	-21.3	ND
F: AM 129447	Pliohippus pernix	12	-19.3	7.4
F: AM 129448	Pliohippus pernix	12	-23.0	ND
F: AM 129450-A	Pliohippus pernix	12	-22.9	8.5
F: AM 129450-B	Pliohippus pernix	12	-22.8	6.6
F: AM 129450-C	Pliohippus pernix	12	-22.7	8.6
F: AM 129450-D	Pliohippus pernix	12	-23.3	7.1
F: AM 129450-1	Pliohippus pernix	12	-23.4	4.1
F: AM 129450-2	Pliohippus pernix	12	-23.2	4.5
F: AM 129450-3	Pliohippus pernix	12	-24.2	3.9
F: AM 114068	Pliohippus pernix	12	-25.7	ND
F: AM 129316 LP3	Merychippus insignis	15	-21.1	1.6
F: AM 129316 Max.	Merychippus insignis	15	*-25.0*	*14.0*
F: AM 129453	Merychippus sp. (cf. isonesus)	15.5	-21.1	6.9
F: AM 110575 LP4	"Merychippus" primus	17	-25.5	8.5
F: AM 110575 Max.	"Merychippus" primus	17	*-18.4*	*10.0*
AMNH 20513	"Merychippus" primus	17	*-22.6*	ND
F: AM 129451	"Merychippus" tertius	17.5	-26.2	8.3
F: AM 128784 RM2	"Parahippus" sp.	18.5	-27.5	9.8
F: AM 128784 Max.	"Parahippus" sp.	18.5	-26.7	ND
F: AM 129455-1	Mesohippus sp.	30.5	-25.4	6.2
F: AM 129455-2	Mesohippus sp.	30.5	-25.3	ND
F: AM 74067	Miohippus obliquidens	32	-26.6	ND

Comparisons of Modern and Ancient Isotope Values

With an understanding of natural isotopic variability in modern and ancient horses, inferences about the indigeneity of organic matter extracted from fossil bones and teeth are possible. Isotope fluctuations not attributable to natural variation may be due to diagenetic alteration of the isotope signal or to actual changes in the paleodiet of the horse in question. Evidence for indigeneity of ancient organic matter includes: 1) Similarity in the distribution and relative abundance of amino acids of the fossil compared to the modern pattern 2) Percent yields of organic matter obtained from the fossil which are consistent with the range of yields from other studies 3) Similarity in the $\delta^{13}C$ and $\delta^{15}N$ values of the fossil compared to a modern analog. Fossils which conform to these criteria likely retain an indigenous organic matter fraction. Fossils which do not conform to these criteria have undergone some amount of diagenetic alteration and consequently are not good candidates for reconstructing equid paleoecologies. Amino acid and percent yield data used to assess indigeneity of fossil horse organic matter has previously been discussed. Stable isotope data is particularly important as an independent method for verifying indigeneity.

Modern and fossil isotope values of the organic matter from terrestrial herbivores were compiled from the literature for comparative purposes (Figure 13). Values of herbivores from arid and non-arid regions are reported separately since $\delta^{15}N$ values may change as a function of aridity (Heaton et al., 1986; Ambrose and DeNiro, 1989). In general, the isotope values of the fossil horses fall within the range of

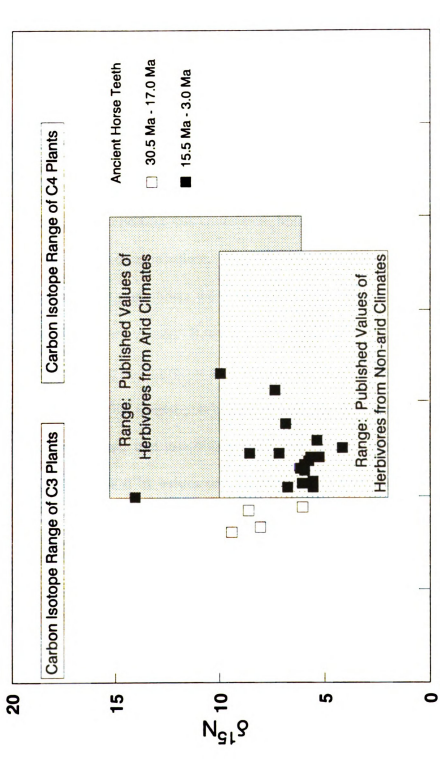


Figure 13 Ranges of $\delta^{13}C$ and $\delta^{15}N$ values of organic matter from a majority of the published modern terrestrial herbivores including the ancient horses analyzed in this study. Herbivores from arid and non-arid regions have different ranges because $\delta^{15}N$ values are often different in arid locations as compared to areas with higher moisture levels.

modern values of herbivores from arid and non-arid regions. Depleted $\delta^{13}C$ values of four ancient horses (F:AM110575, F:AM 128784, F:AM 129451, F:AM 129455) likely reflect a higher dietary contribution of C_3 plants. These horses are 17.0 Ma and older and likely existed before the widespread evolution of C_4 plants. Two horse bones (F:AM 129316, F:AM110575) have $\delta^{13}C$ and $\delta^{15}N$ values which are not consistent with those of modern herbivores (Figure 13). These specimens may exhibit diagenetic alteration. Supporting this contention, bones and teeth from the same individual may have different isotope values. Three ancient bones (for which $\delta^{15}N$ values of tooth and bone from the same horse were determined) have $\delta^{15}N$ values which differ by 1.5‰ or more from tooth values. Bone appears less likely to retain an indigenous organic fraction owing to its greater porosity compared to teeth. The evaluation of these bones as diagenetically altered precludes their use as reliable paleoecological indicators. The possibility exists that low $\delta^{15}N$ bone values record a dietary contribution of legumes. Similarly, high $\delta^{15}N$ values could document a sick organism in nitrogen stress or an individual from an especially arid locality (Heaton et al., 1986; Ambrose and DeNiro, 1989). Owing to the extensive isotopic alteration present in these bone samples, it is unlikely that any causal factor other than diagenesis is indicated.

Evaluation of Diagenetic Effects

An initial aim of this research was to attempt to quantify diagenesis in the fossils which were deemed to be altered in some regard. A time series of fossils from

similar depositional environments should have provided insight into diagenetic processes. However, quantification of diagenesis was not possible because some fossils from the same deposits exhibited very different degrees of alteration. For example, a maxilla bone (F:AM 110575 LMax.) from Thompson Quarry was 7.1‰ enriched in $\delta^{13}C$ and 1.5‰ enriched in $\delta^{15}N$ relative to a tooth (F:AM 110575 LP4) from the same location. Additional effort must be placed on obtaining samples with precise stratigraphic control within a quarry to best investigate diagenetic effects. Geochemical characterization of the associated sediment would also aid in understanding diagenesis. Provided precise depositional and stratigraphic information is attainable, isotope studies may be useful for correlating the extent of postmortem processing with one or more environmental parameters. This would enhance our understanding or organic matter preservation in fossils. Conceivably, altered fossils may be shown to be useful for paleoecological reconstructions if diagenesis can be accurately modeled and predicted. As this was not possible for this study, the diagenetically altered samples were not used for evaluating equid paleoecology.

Interpretations of Tertiary Paleoecology of the Horse Family

Percent C_4 in Horse Diets

The first step towards an understanding of Tertiary paleoecology and the evolution of hypsodonty in equids is to assess the abundance of siliceous grasses in

the diets of various horses. This assessment relies on two assumptions: 1) Knowledge of the $\delta^{13}C$ values of the dietary constituents; 2) The degree to which these endmember values are fractionated by the horse. Mean $\delta^{13}C$ values for modern C_3 and C_4 plants are well characterized (-27‰ and -13‰, respectively). A modern C_3 plant eaten by horses was measured to determine if grasses common to horse diets were similar to the $\delta^{13}C$ mean of C_3 plants. Meadow hay from New Baltimore, Michigan was found to have a $\delta^{13}C$ value of -28.1‰. Therefore, mean $\delta^{13}C$ values should be useful when modeling the amount of C_4 vegetation in horse diets. Metabolic fractionation is not well constrained in horses, so several representative values for other mammals were used to model equid fractionation (DeNiro and Epstein, 1978b; Vogel, 1978; Schoeninger and DeNiro, 1984). The component of C_4 plants in the diet of all fossil and modern horses assuming four separate theoretical equid $\delta^{13}C$ fractionations equal to 1‰, 3‰, 4‰ and 5‰ were calculated using mixing equations (Table 13). Carbon isotope fractionations of about 1‰ to 2‰ are characteristic of small mammals (DeNiro and Epstein, 1981) and are probably too small for herbivores as large as horses. The largest terrestrial herbivores may have $\delta^{13}C$ fractionations of 5.1‰ to 5.3‰ (Ambrose and DeNiro, 1989) and possibly as high as 6.1‰ (Vogel, 1978). Thus, carbon isotope fractionation in horses is likely on the order of 3‰ to 4‰. The relative abundance and distribution of C_4 plants in modern environments in relation to the percent C_4 values determined for modern horses can be used to model the appropriate fractionation associated with horses. The percentage of C_4 plants

Table 13 Percent C_4 vegetation in the diet of modern and ancient terrestrial herbivores assuming four separate fractionations. Based upon modern C_4 distributions in North America, horse metabolic fractionation is on the order of 3‰ to 4‰.

δ13C	Sample Number	Genus species	Age (Ma)	% C4 ϵ = 1	% C4 ϵ = 3	% C4 ϵ = 4	% C4 ϵ = 5
-22.93	MSU: Cer 1	Cervus canadensis	0	21.9	7.6	0.5	0.0
-22.94	MSU: Cer 1	Cervus canadensis	0	21.9	7.6	0.4	0.0
-23.18	MSU: Cer 2	Cervus canadensis	0	20.1	5.9	0.0	0.0
-22.79	MSU: Cer 2	Cervus canadensis	0	22.9	8.6	1.5	0.0
-22.99	MSU: Cer 2	Cervus canadensis	0	21.5	7.2	0.1	0.0
-22.59	MSU: Cer 3	Cervus canadensis	0	24.4	10.1	2.9	0.0
-22.98	MSU: Cer 3	Cervus canadensis	0	21.6	7.3	0.1	0.0
-23.98	MSU: Cer 4	Cervus canadensis	0	14.4	0.1	0.0	0.0
-24.20	MSU: Cer 6	Cervus canadensis	0	12.9	0.0	0.0	0.0
-24.50	MSU: Cer 7	Cervus canadensis	0	10.7	0.0	0.0	0.0
-20.89	MSU: Eq 1c	Equus caballus	0	36.5	22.2	15.1	7.9
-20.77	MSU: Eq 1d	Equus caballus	0	37.4	23.1	15.9	8.8
-20.31	MSU: Eq 1e	Equus caballus	0	40.6	26.4	19.2	12.1
-20.60	MSU: Eq 1f	Equus caballus	0	38.6	24.3	17.1	10.0
-21.39	MSU: Eq 1j	Equus caballus	0	32.9	18.6	11.5	4.4
-21.07	MSU: Eq 1k	Equus caballus	0	35.2	20.9	13.8	6.6
-21.06	MSU: Eq 1l	Equus caballus	0	35.3	21.0	13.9	6.7
-20.52	MSU: Eq 2	Equus caballus	0	39.1	24.9	17.7	10.6
-20.83	MSU: Eq 3	Equus caballus	0	36.9	22.6	15.5	8.4
-20.06	MSU: Eq 4	Equus caballus	0	42.4	28.1	21.0	13.9
-20.20	MSU: Eq 5	Equus caballus	0	41.4	27.1	20.0	12.9
-20.31	MSU: Eq 6	Equus caballus	0	40.6	26.4	19.2	12.1
-20.37	MSU: Eq 7	Equus caballus	0	40.2	25.9	18.8	11.6
-20.70	MSU: Eq 9	Equus caballus	0	37.9	23.6	16.4	9.3
-21.80	F.M.M. 1219 Max.	Equus sp.	0	30.0	15.7	8.6	1.4
-23.30	UALP 7810	Equus sp.	3	19.3	5.0	0.0	0.0
-25.20	F: AM 129114-1	Equus simplicidens	4	5.7	0.0	0.0	0.0
-24.90	F: AM 129114-2	Equus simplicidens	4	7.9	0.0	0.0	0.0
-23.40	F: AM 129444	"Dinohippus" leidyanus	5.5	18.6	4.3	0.0	0.0
-22.40	35-56B	"Dinohippus" leidyanus	6	25.7	11.4	4.3	0.0
-21.98	316-56-1	Nannippus lenticularis	6	28.7	14.4	7.3	0.1
-23.30	316-56-2	Nannippus lenticularis	6	19.3	5.0	0.0	0.0
-22.87	F: AM 129443	Neohipparion eurystyles	6.5	22.4	8.1	0.9	0.0
-24.50	5157-73	Hipparion sp. (cf. forcei)	7	10.7	0.0	0.0	0.0
-24.45	AMNH 17599A-1	"Dinohippus" leidyanus	7	11.1	0.0	0.0	0.0
-25.51	AMNH 17599A-2	"Dinohippus" leidyanus	7	3.5	0.0	0.0	0.0
-24.16	2010-70B	Calippus sp. nov.	7	13.1	0.0	0.0	0.0

Table 13 (cont'd)

δ13C	Sample Number	Genus species	Age (Ma)	% C4 ϵ = 1	% C4 ϵ = 3	% C4 ϵ = 4	% C4 ϵ = 5
-22.67	F: AM 129442	C. occidentale	8.5	23.8	9.5	2.4	0.0
-23.60	F: AM 129445	C. occidentale	9.5	17.1	2.9	0.0	0.0
-24.26	F: AM 129446	C. occidentale	9.5	12.4	0.0	0.0	0.0
-23.58	F: AM 129454-1	C. occidentale	10	17.3	3.0	0.0	0.0
-23.50	F: AM 129454-2	C. occidentale	10	17.9	3.6	0.0	0.0
-22.60	F: AM 129441Max	C. occidentale	10.5	24.3	10.0	2.9	0.0
-23.10	F: AM 129441LdP4	C. occidentale	10.5	20.7	6.4	0.0	0.0
-20.96	F: AM 129441LM1	C. occidentale	10.5	36.0	21.7	14.6	7.4
-21.30	F: AM 129441LM2	C. occidentale	10.5	33.6	19.3	12.1	5.0
-19.30	F: AM 129447	Pliohippus pernix	12	47.9	33.6	26.4	19.3
-23.00	F: AM 129448	Pliohippus pernix	12	21.4	7.1	0.0	0.0
-22.75	F: AM 129450-B	Pliohippus pernix	12	23.2	8.9	1.8	0.0
-25.70	F: AM 114068	Pliohippus pernix	12	2.1	0.0	0.0	0.0
-21.10	F: AM 129316 LP3	Merychippus insignis	15	35.0	20.7	13.6	6.4
-25.00	F: AM 129316 Max.	Merychippus insignis	15	7.1	0.0	0.0	0.0
-21.07	F: AM 129453	Merychippus sp.	15.5	35.2	20.9	13.8	6.6
-25.50	F: AM 110575 LP4	"Merychippus" primus	17	3.6	0.0	0.0	0.0
-18.40	F: AM 110575 Max.	"Merychippus" primus	17	54.3	40.0	32.9	25.7
-22.60	AMNH 20513	"Merychippus" primus	17	24.3	10.0	2.9	0.0
-26.18	F: AM 129451	"Merychippus" tertius	17.5	0.0	0.0	0.0	0.0
-27.45	F: AM 128784	"Parahippus" sp.	18.5	0.0	0.0	0.0	0.0
-26.70	F: AM 128784	"Parahippus" sp.	18.5	0.0	0.0	0.0	0.0
-25.40	F: AM 129455	Mesohippus sp.	30.5	4.3	0.0	0.0	0.0
-25.33	F: AM 129455	Mesohippus sp.	30.5	4.8	0.0	0.0	0.0
-26.60	F: AM 74067	Miohippus obliquidens	32	0.0	0.0	0.0	0.0

in various regions throughout North America is known (Figure 14). The modern day percent distributions of C_4 plants in the Midwest and Nebraska most closely parallels the 3‰ and 4‰ fractionation models (Table 13, Figure 14). Having established a way to assess percent C_4 vegetation in horse diets, an evaluation of the paleoecological significance of the data can then be made.

Temporal and Spatial Distibution of Isotope Data

Despite being constrained to the Great Plains, some of the horses which compose this time series are from distant geographical regions (e.g. Nebraska and Texas). An investigation of $\delta^{13}C$ values in association with age and locality information aids in interpreting the stable isotope data (Figure 15). A striking feature of viewing data in pictoral format is the perspective it provides concerning the distribution of the data points. The relative proximities of the horses (both geographically and temporally) is another salient feature of viewing data pictorally. Without this map, the fact that 85% of this time series is constrained to western Nebraska might easily go unrecognized. The distribution of data points in space and time are fundamental concerns of the interpretive aspect of science. Approaching the isotope data with a visual perspective facilitates interpretations and enhances paleoecological assessments.

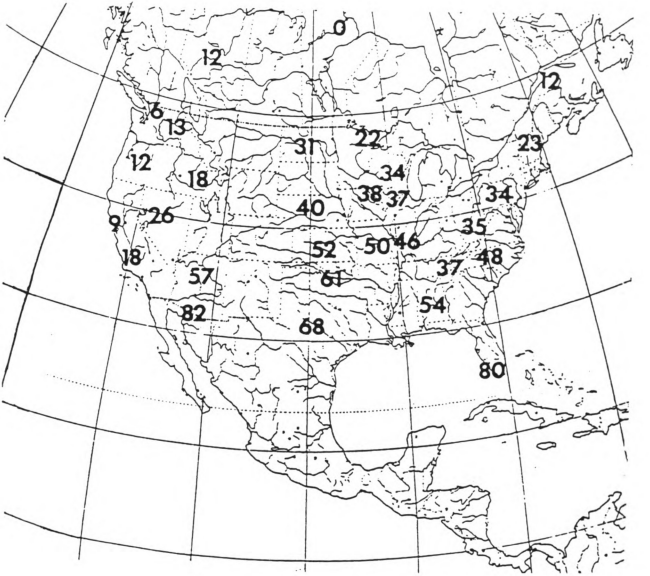


Figure 14 The percent distributions of modern C_4 plants in various regions of North America (modified from Teeri and Stowe, 1976).

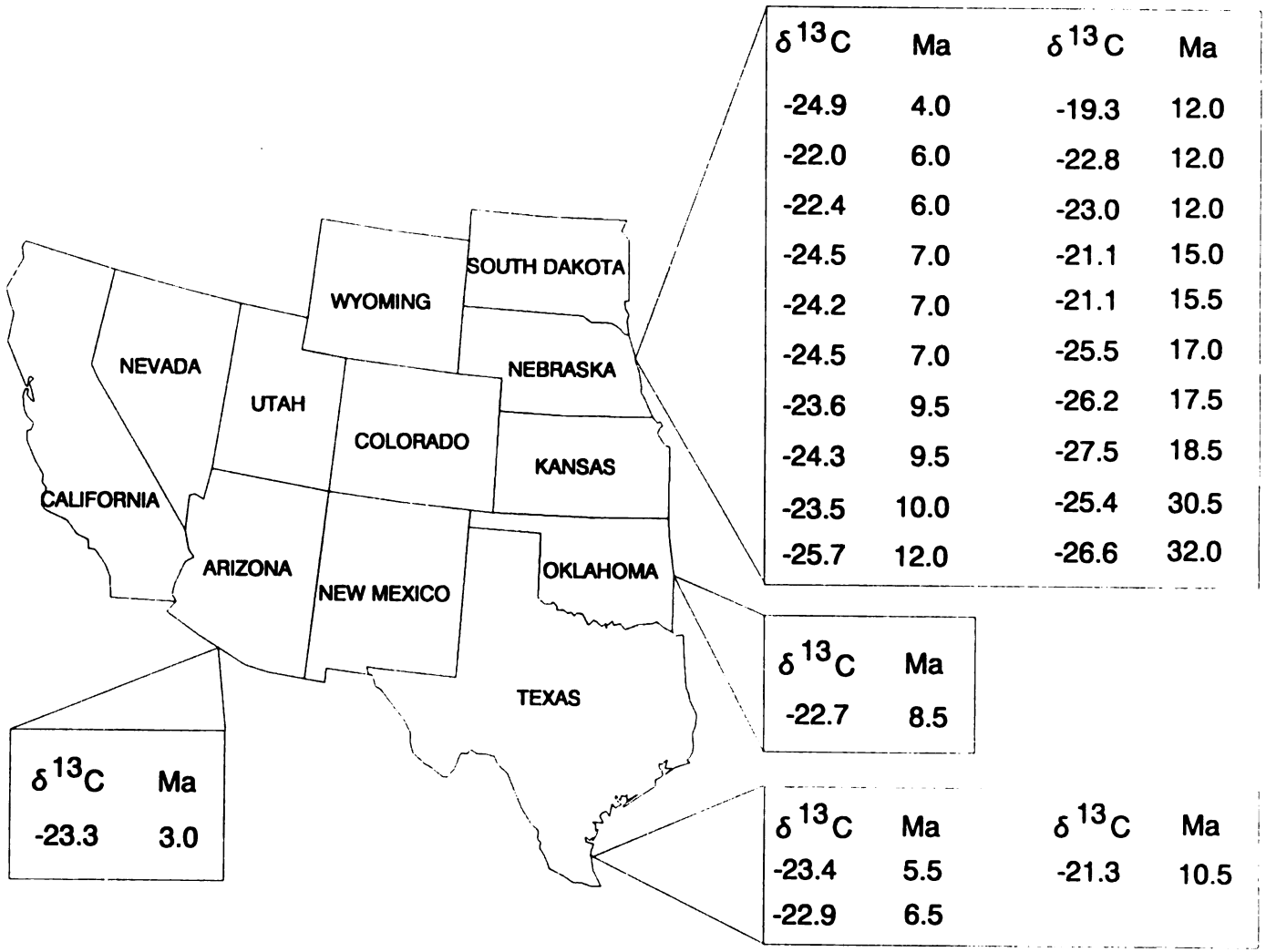
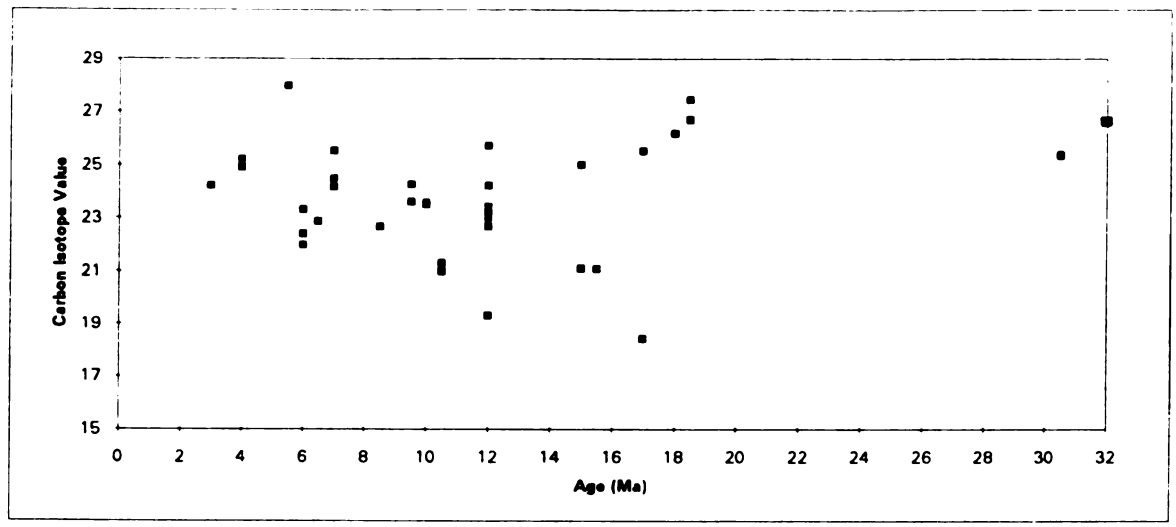


Figure 15 Carbon isotope values of ancient North American horses (32.0 Ma to 3.0 Ma) in association with age and geographical information.

Isotope Changes Over Time

If horse diets changed from C_3-based to C_4-based any time in the last 32.0 Ma this signature should be recorded in the $\delta^{13}C$ values of this time series. Linear regression of the $\delta^{13}C$ values ($r^2 = 0.01$) versus time reveals no correlation between the isotopic signature and age of the fossils (Figure 16). Similarly, no trend exists between $\delta^{15}N$ values and time ($r^2 = 0.15$; Figure 16). The lack of an obvious trend in $\delta^{13}C$ and $\delta^{15}N$ versus time is not at all surprising given the wide array of species and Great Plains depositional localities.

Rather than looking for linear temporal trends, a better way to interpret the data is to divide it into separate populations based on traditional paleobotanical assessments. Before about 17 Ma savanna grasslands composed only a small fraction of the Tertiary landscape (Axelrod, 1985). The mean $\delta^{13}C$ value of horses from this study that are 17 Ma or older is -26.2 ± 0.7‰ which is consistent with a pure C_3 diet given the range and mean $\delta^{13}C$ values for modern C_3 plants. The oldest C_4 grass known is from the latest Miocene (7 to 5 Ma) (Thomasson et al., 1986). Therefore, 7 Ma is another convenient place to subdivide the time series. Prior to 7 Ma, but subsequent to 17 Ma, the mean $\delta^{13}C$ value of horses is -22.8 ± 1.8‰. Although not a large component (~25%) of horse diets, the percent C_4 mixing equations used in this thesis suggests that C_4 plants were present by somewhere between 15.5 Ma and 12 Ma on the North American Great Plains (Table 13). After 7 Ma, when C_4 grasses are first documented in the fossil record, the mean $\delta^{13}C$ value of horses is -23.7 ± 1.0‰. If



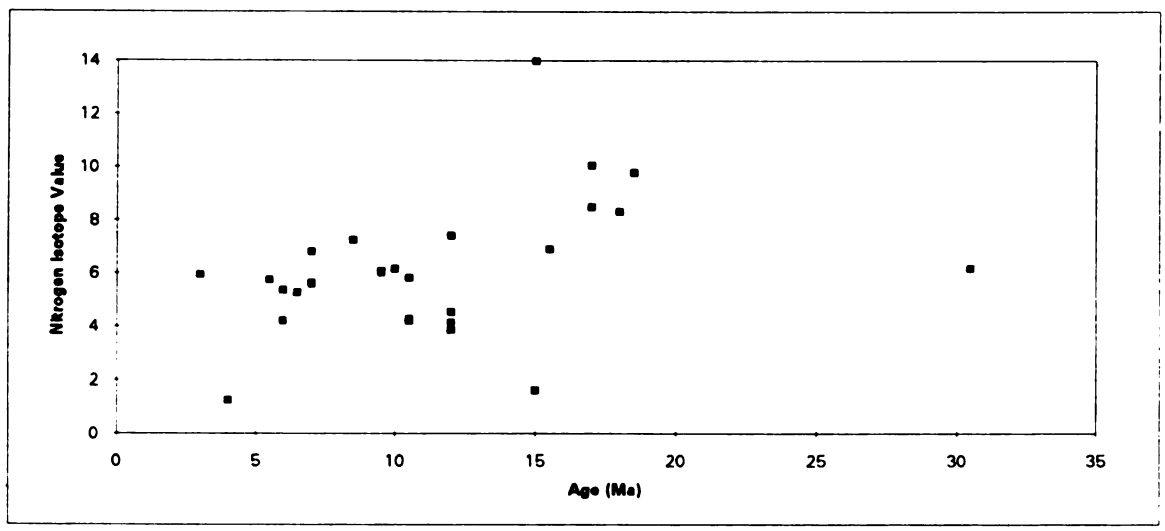


Figure 16 The relationship between $\delta^{13}C$ and $\delta^{15}N$ values of ancient horse organic matter and geologic age of the specimens.

anything, there appears to be a decrease in the amount of C_4 vegetation in the diet of these Great Plains horses after 7 Ma. These findings are not in agreement with some lines of evidence concerning the distribution and prevalence of C_4 grasses during the Tertiary (Cerling, 1992; Cerling et al., 1993; Wang et al., 1994). However, the appearance of C_4 vegetation by 15.5 Ma to 12 Ma on the Great Plains closely agrees with age estimates determined for the first occurrence of C_4 vegetation in South America (MacFadden et al, 1994) and Kenya (Morgan et al., 1994).

Comparisons with Other Isotope Studies

Savanna-type grasslands of limited extent first appear in East Africa about 9 Ma to 8 Ma based on paleosol isotope data (Cerling, 1992). Cerling et al. (1993), analyzing $\delta^{13}C$ values of carbonates and palaeosols and mammalian tooth enamel, provided evidence for a C_4 expansion presumably at the expense of C_3 vegetation at approximately 7 Ma to 5 Ma in North America and Pakistan. A C_4 proliferation did not occur between 7 Ma and 5 Ma on the Great Plains based upon carbon isotope data from the organic material preserved in fossil horse teeth (Table 13). Even if horse diets were to record a C_3/C_4 shift there is no reason to assume all equids would change uniformly. A shift in $\delta^{13}C$ values of all horses between 7 Ma and 5 Ma would only be expected if C_4 grasses rapidly radiated blanketing the Great Plains in a short period of time providing a new food resource for horses, a situation which has little evidence currently.

Researchers have suggested that a global drop in atmospheric CO_2 levels may account for the apparently rapid appearance of C_4 dominated habitats in North America and Pakistan (Cerling et al., 1993). A subsequent study of the enamel carbonate of prehistoric herbivores from Pakistan and Kenya was not consistent with a decrease in global CO_2 levels (Morgan et al., 1994). In that study, Morgan et al. (1994) provided evidence for the existence of C_4 grasses by 9.4 Ma in Pakistan and 15.3 Ma in Kenya. These ages are too old to be ascribed to a late Miocene global drop in atmospheric carbon dioxide levels. Another recent study of the inorganic tooth enamel carbonate from a subset of North American horses reports a change in carbon isotope values at approximately 7 Ma to 5 Ma (Wang et al., 1994) (Figure 17). This evidence in association with enamel carbonate isotope data from South American ungulates led MacFadden et al. (1994) to postulate the appearance of C_4 grasses 10 million years earlier in the Southern Hemisphere. Obviously, the late Miocene global CO_2 issue has raised considerable interest. Consequently, a re-examination of some of the $\delta^{13}C$ data upon which the CO_2 hypothesis rests might facilitate a better understanding of this controversial topic.

Reinterpreting Previous Studies

A spatial evaluation of prehistoric horses studied by Wang et al. (1994) reveals an extensive geographical distribution which is not as apparent when the data is viewed in tabular form. Specimens are located in the southeast, along the western

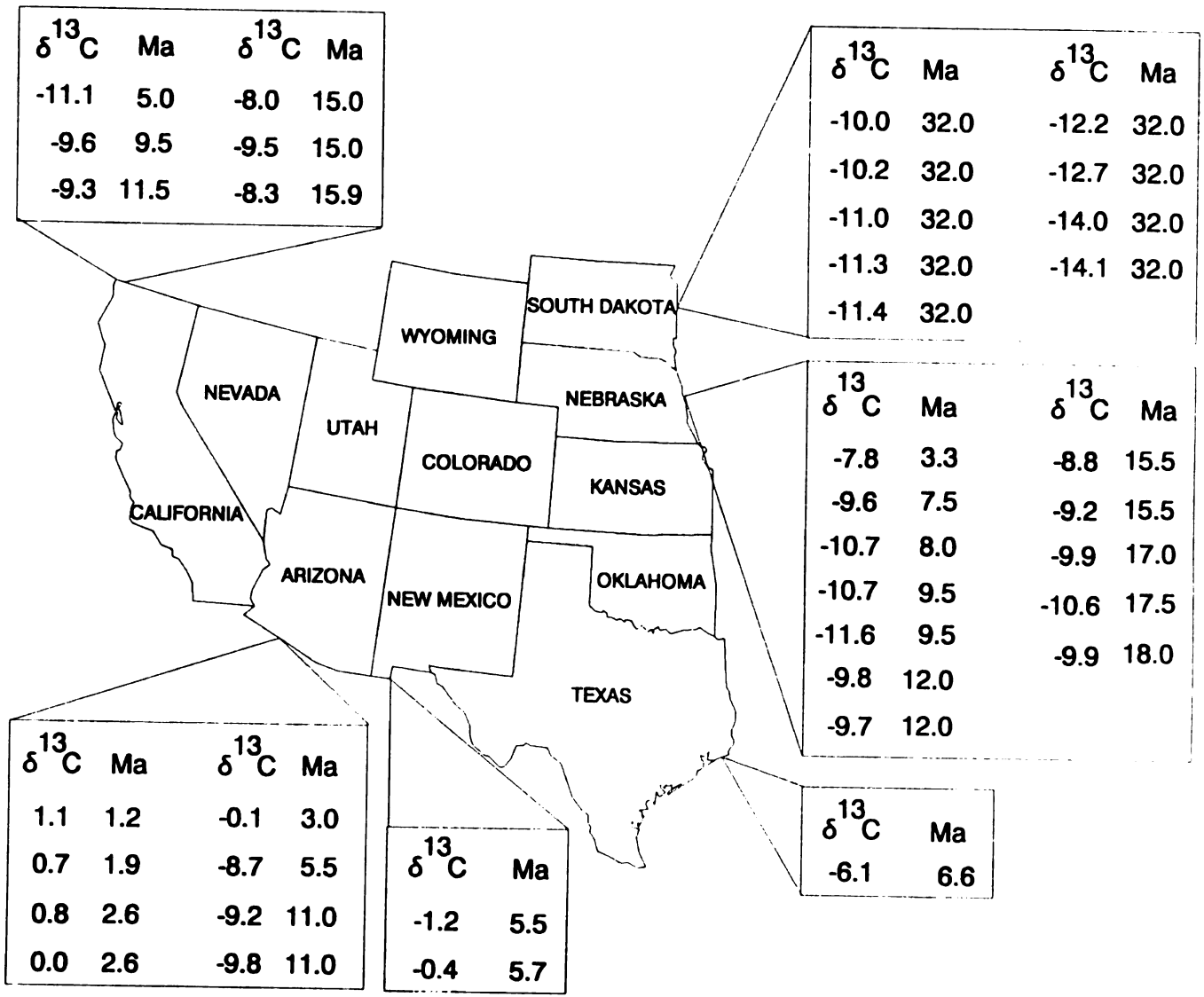


Figure 17 Geographical location, age and $\delta^{13}C$ values of ancient horse enamel carbonate (modified from Wang et al., 1994).

coast and on the Great Plains of North America. Today these regions are highly disparate in terms of climate with extensive desert and scrubland in the southwest and a more variable climate regime in the central Great Plains region. These localities differ not only in minimum, maximum and mean temperature, but also precipitation, soil moisture content (owing to differences in soil water retentions), light intensity, degree of shading, seasonality, latitude, altitude and a whole host of other climate variables. These conditions are the same envionmental parameters which influence the distribution of C_3 and C_4 plants in modern ecosystems (Teeri and Stowe, 1976). Variable climatic conditions similar to today inevitably existed during the latest Miocene and Pliocene. The presence of fossil reptiles and amphibians (Egelhoff and Norden Bridge Faunas) in regions well outside modern ranges and a dramatic reptile turnover indicate variable climates throughout the late Tertiary (Holman, 1973; Holman 1982). Indeed, paleoclimates may have been as variable as in modern times. Consequently, it seems very unlikely a single parameter such as an atmospheric CO_2 decrease would control Tertiary vegetational changes. In addition, a global drop in carbon dioxide levels would serve to cool global temperatures offsetting any inherint advantage C_4 plants would enjoy under low pCO_2 conditions. Moreover, the isotope data of Wang et al. (1994) does not support the global CO_2 hypothesis upon closer inspection.

Enamel carbonate $\delta^{13}C$ values below -12‰ are indicative of C_3 dominated diets. Carbonate $\delta^{13}C$ values between -1‰ to +1‰ indicate pure C_4 diets. Consequently, equal portions of C_3 and C_4 plants in a horses diet will result in

carbonate $\delta^{13}C$ values near -5‰ to -6‰. The only horses of Wang et al. (1994) which show a clear diet shift between 7 Ma and 5 Ma are from the arid soutnwestern region of North America (Ocate, New Mexico not Ocote, Mexico as published; SMU-uncat). If all specimens younger than 7 Ma are considered, only five more individuals from Arizona (UA 12/526, UA 52/1539, UA 7352, UA 7354/6552, UA 25-0/718A) show the diet shift. Noteworthy, the 7 Ma to 5 Ma shift does not begin from a pure C_3 diet immediately prior to 7 Ma.

Both inorganic and organic $\delta^{13}C$ values reveal a small percentage (20-30%) of C_4 vegetation in horse diets as early as the middle Miocene (~15.5 Ma to 12.0 Ma). Due to limitations imposed by the mass balance model assumptions (e.g. mean $\delta^{13}C_{C3}$ $\delta^{13}C_{C4}$, etc.), only percent contributions of C_4 vegetation larger than 7% are considered significant. Grasslands containing C_4 plants may well have existed prior to 15.5 Ma, but would have constituted less than 1/10 of the vegetation in horse diets. Contributions of less than 10% C_4 grasses in the diet of ancient horses are not likely to have had a major influence on tooth morphology.

None of the Great Plains horses in the 7 Ma to 5 Ma time range possess C_4 dominanted diets. The Nebraska horses (AMNH 17599A and F:AM 128963) from 7.5 Ma to 3.3 Ma clearly have C_3-based diets. Furthermore, only one Texas horse (SMU 70533) and one Florida horse (UF uncat. 3A) exhibit transitional diets. Consequently, isotope data of Wang et al. (1994) does not demonstrate a diet shift between 7 Ma and 5 Ma and thus does not support a decrease in global CO_2 levels in the late Miocene as proposed. Rather, the isotope data only indicates an abundance of

C_4 vegetation in the southwest region of North America after 7 Ma, a condition likely attributable to elevated temperature and/or aridity of this localized region. The absence of an abrupt C_3/C_4 transition in the Great Plains horses of Wang et al. (1994) is consistent with the findings of this thesis. This suggests that distributions of C_4 plants were far more provincial than previously thought.

Three horses measured by Wang et al. (1994) (AMNH 17599A, F:AM 128784 and F:AM 114068) were also analyzed in this thesis. The $\delta^{13}C$ values of both the enamel carbonate and organic matter from these horses predict similar C_3-based diets. This result supports the view that both isotope methods can provide reliable paleoecological data.

Paleoecological Ramifications of Equid Isotope Data

All the available evidence regarding the rise of the C_4 grassland biome during the Tertiary points to a provincial history. Plants utilizing the C_4 biochemistry have evolved independently in thirteen families (James A. Teeri, personal communication). A phylogeny such as this is best explained by adaptation to local envionmental conditions, not global changes. The C_4 pathway may have evolved first in the Southern Hemisphere if tropical climate conditions began earlier in the southern latitudes. However, the first appearance of C_4 vegetation seems to be nearly contemporaneous for both North America (this study), South America (MacFadden et al, 1994) and regions within Africa (Morgan et al., 1994). This suggests that

conditions favorable to the C_4 biochemistry (e.g. aridity, warmer temperatures, etc.) were expanding during the middle Miocene. A multitude of herpetological, paleopedological and paleobotanical evidence agrees with this contention (Holman, 1971; Retallack, 1983a; 1983b). Despite the ongoing large scale climate trends, local environmental conditions have been far more important in determining the abundance and distribution of C_4 grasses throughout their evolutionary history. Were CO_2 the only controlling variable, C_4 vegetation would be far more pervasive between 7 Ma and 5 Ma than the fossil record indicates. In fact, sound evidence in support of a widespread C_4 vegetation radiation at the end of the Miocene has yet to be demonstrated. Recent research indicates that C_4 grasses were minor constituents in Greece throughout the last 11 Ma (Quade et al., 1994). This is consistent with a provincial distribution for C_4 grasses throughout their history.

Savanna grasses began with patchy seasonal distributions in local favorable environments. As these environments expanded so did the territory of the C_4 grasses. Only in restricted high temperature and aridity regions did the C_4 grasses proliferate at the expense of the C_3 grasses (Tieszen, 1979b). Whereas the radiation of the first grasses (C_3) can rightfully be called an explosion, C_4 grasses diversified rather unimpressively. This interpretation is consistent both with the meager fossil record of C_4 grasses as well as the stable isotope record contained within fossil teeth and soils (Thomasson et al., 1986; Morgan et al., 1994; Quade et al., 1994).

Siliceous C_4 vegetation was present during the middle Miocene as traditionally supposed (Simpson, 1951). But, the first occurrence of C_4 plants does not precisely

coincide with the advent of hypsodont dentition in North American horses. Merychippine high-crowned horses are known from the early Miocene (~18 Ma) at least 2 Ma to 3 Ma before C_4 vegetation is first documented. Hence, C_4 grasses cannot be the main cause for the evolution of hypsodonty in horses. The cause might then be the transition from a browsing to a grazing diet consisting of C_3 grasses. The incorporation of grit particles most likely influenced the evolution of horse hypsodonty as well.

The most primitive hypsodont horse, "Merychippus" gunteri, arose between 17.7 Ma and 16.2 Ma (MacFadden et al., 1991). "Merychippus" gunteri is thought to be ancestral to "Merychippus" primus (MacFadden and Hulbert, 1988). These horses are often assumed to be the first equid grazers. If so, they must have been consuming abrasive C_3 grasses off gritty substrates. Grazing ungulates were not uncommon in the middle Miocene. For example, Teleoceras (an extinct hypsodont rhinoceros) has been discovered with siliceous grass anthoecia contained within the teeth presumably indicative of a grazing diet. Some authors justifiably caution against the assumption that all hypsodont ungulates were grazers (Janis, 1988; Hulbert, 1993). Digestive strategy (e.g. hindgut versus foregut fermentation), substrate quality and plant abrasiveness all influence whether or not high-crowned teeth evolve. Possibly the most important prerequisite for hypsodonty is preadaptation of enamel microstructure (Pfretzschner, 1993). The parahippine ancestor which gave rise to the merychippine linneage had an enamel microstructure capable of undergoing the transition to the hypsodont condition (Pfretzschner, 1993). Not all ungulates possess this fortuitous

enamel microstructure. Presumably, hypsodonty will evolve in response to an abrasive grazing diet provided the precursor enamel structure exists. All members of the Equinae possess this prerequisite enamel microstructure (Pfretzschner, 1993). Therefore, it is not unreasonable to assume a grazing diet led to equid hypsodonty in light of the isotope data generated by this thesis as well as recent research on tooth wear patterns in horses (Hulbert, 1982) and other mammals (Walker et al., 1978). A diet of predominantly siliceous C_3 grasses (with associated grit) thus appears to have been the major causal factor in the evolution of equid hypsodonty.

The presence of C_4 vegetation on the Great Plains after about 15.5 Ma to 12.0 Ma served only to perpetuate the runaway selection toward higher crown heights as equids continued to graze on the diversifying savanna grasses. In some local favorable environments C_4 grasses may have dominated year round. However, C_4 grasses did not achieve modern-day savanna distributions until the Pleistocene (Cerling, 1992). Organic and inorganic carbon isotope data suggest that C_4 plants never comprised more than 40% of the vegetational biomass consumed by Great Plains horses throughout the Tertiary (32 Ma to 3.3 Ma). This realization is surprising given the dominance of C_4 grasses in some modern environments.

Several reasons might explain a maximum of 40% C_4 vegetation in horse paleodiets. First, evidence exists that demonstrates some herbivores have an aversion to C_4 plants (Caswell et al., 1973; Vogel et al., 1986). Some biochemical intermediates (e.g. malate, aspartate, etc.) are present in C_4 plants that do not exist in C_3 plants. However, these are generally short-lived intermediates which do not

accummulate to any extent in plant tissues (Ralph E. Taggart, personal communication). There are few if any well documented cases where C_4 plants sequester secondary metabolites to deter herbivory. Horses prefer to eat clover over crab grass given a choice (personal observation), but this is not selective herbivory so much as dietary preference of the horse. To an extent, herbivore preference will account for the amount and kinds of grasses consumed (Tieszen et al., 1979a). However, resource partitioning among megaherbivores and grass abundances also determine which food type is consumed.

Selective herbivory in horses might evolve if C_4 vegetation was of poorer nutrional quality than C_3 grasses. Quite a bit of evidence suggests that C_4 plants are indeed nutritionally a less valuable food resources to a wide range of taxa (Bryant et al., 1989; Maschinski and Whitham, 1989). Relative to C_3 plants, C_4 have a lower nitrogen content indicating the different nutritional values of these two plant types (James A. Teeri, personal communication). Grasses utilizing the C_4 pathway produce four to five times less photosynthetic products and quickly transport their synthesized starches and sugars to their stem and roots thus reducing the availability of high-energy metabolites (Steve Stephenson, personal communication). As compared to C_3 grass, a larger percentage of C_4 grass high in silica, lignin and cellulose remains unprocessed following digestion of hindgut fermenters like horses (Janis, 1989). These observations suggest that C_4 grasses are an energetically and nutritionally poor food source. Yet, African zebras live quite well on C_4-based diets today even in regions where C_3 grasses are available (Vogel, 1978). Thus, there does not seem to be much

evidence in favor of C_4 avoidance among the Equidae.

Another possibility why most fossil horses do not exhibit pure C_4 diets relates to seasonal influences of available vegetation. Modern Nebraskan grasslands contain about 40% C_4 species (Teeri and Stowe, 1976). The relative abundance of C_3 and C_4 grasses is seasonally controlled. Vogel et al. (1986) found that C_4 species dominate all of southern Africa except in the winter rainfall areas. If horses grazed on essentially pure C_3 diets during the wet season and ate 100% C_4 grasses during the drier summer months, $\delta^{13}C$ values would be intermediate depending on how long the wet and dry seasons lasted. In other words, C_4 vegetation may never have been available more than 40% of the year on the Great Plains through the Mio-Pliocene. Migration in horses would bring them into contact with new types of vegetation. Unless migrating horses traveled through the harshest climates, an increase in the available abundance of C_4 vegetation would not occur. In all likelihood, the contribution of C_4 in the diet of fossil horses roughly records the percentage available in the horses home range. Consequently, it is fair to assume that C_4 grasses were seasonally controlled, locally important, but far less widespread on the Great Plains during the Mio-Pliocene than has recently been hypothesized.

CONCLUSIONS

Based upon stable isotopic evidence from the organic remnant of horse tooth and bone the rise of the savanna grassland biome on the Great Plains of North America began between 15.5 Ma and 12.0 Ma. These ages are consistent with traditional interpretations, but disparate with recent isotope studies of inorganic equid enamel carbonate which purportedly provided evidence for largescale savanna grassland proliferation between 7 Ma and 5 Ma attributed to a global CO_2 drop at the end of the Miocene. Mass balance calculations demonstrate that C_4 vegetation comprised fewer than 40% of the biomass consumed by Great Plains horses between 32 Ma and 3.3 Ma. In contrast, horses from the arid southwest region of North America had diets predominantly (50-90%) composed of C_4 vegetation in the late Miocene and Pliocene. A provincial grassland distribution pattern such as this indicates that global atmospheric CO_2 fluctuations in the late Miocene are insufficient for explaining the expansion of C_4 grasslands. Other factors which control the distribution of modern C_4 vegetation such as regional temperature, moisture availability and latitudinal effects controlled the distribution and abundance of savanna grasses throughout their phylogeny.

Savanna vegetation did not become an important dietary constituent of Great Plains horses until the middle Miocene (15.5 Ma to 12.0 Ma), approximately 2 Ma

after the evolution of horse hypsodonty. Therefore, C_4 vegetation cannot be the primary causal agent of high-crowned teeth in horses. Although not the original cause, siliceous C_4 grasses likely contributed to the evolution of hypsodonty among the Equidae. The incorporation of C_3 grasses containing opal phytoliths and the expansion of abrasive grazing substrates are the most likely causes for the increase in equid tooth crown heights. The precise role that abrasive C_3 grasses and gritty prairie soils played in the evolution of horse hypsodonty requires further investigation. Undeniably, the combination of C_3 grasses, C_4 grasses and grit particles worked in concert to produce the remarkable hypsodont teeth which characterize members of the family Equidae for the last eighteen million years.

APPENDIX

APPENDIX 1

Vertebrate taxonomy to the specific level of the ancient and modern ungulates analyzed in this study including the original references when known (modified from Carroll, 1988).

Class Mammalia (Linnaeus, 1758)

Superorder Ungulata (Novacek, 1986)

Order Artiodactyla

Family Cervidae

Cervus canadensis

Order Perissodactyla (Owen, 1848)

Family Equidae (Gray, 1821)

Calippus sp.nov.
Cormohipparion occidentale
"Dinohippus" leidyanus
Equus caballus
Equus simplicidens
Hipparion sp.(cf.forcei)
Nannippus lenticularis
Neohipparion eurystyle
Mesohippus sp.
Merychippus insignis
"Merychippus" primus
"Merychippus" tertius
"Merychippus" sp.(cf.isonesus)
Miohippus obliquidens
"Parahippus" sp.
Pliohippus pernix

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