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δ^{13}C and δ^{18}O analysis of Cenozoic Camelidae tooth enamel, Great Plains, U.S.A.

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Abstract

Neogene fossil Camelidae and Equidae tooth enamel from the Great Plains, U.S., preserve similar δ^{13}C values. There is no stable isotope evidence for distinct habitats or niche partitioning between these two mammals that might elucidate the time lag between C4 biome expansion and equid morphological adaptations and dietary records that indicate a C4 dominated diet in the Neogene. The stable isotope composition (δ^{13}C) of paleosol carbonates from the Great Plains indicates C4 grass comprised approximately 20% of plant biomass during the Miocene, increased to about 40% by the early Pliocene, and reached modern abundance by the early Pleistocene (~80%). Equids evolved high-crowned teeth (hypodonty), an adaptation for open habitats and/or grazing by ca. 18 Ma, and δ^{13}C values of equid tooth enamel indicate that equids maintained C3-dominated diets during the Miocene until 6.6 Ma. At this point several species began to consume a mixed diet of C3 and C4 vegetation while others maintained their C3-dominated diets. Camels and horses coexisted in the Great Plains during the Neogene, indicating that niche partitioning must have occurred in some way that is not discernible through analysis of stable carbon and oxygen isotopes.

Keywords: Neogene, carbon isotopes, oxygen isotopes, palaeoecology, Camelidae, Equinae, soil carbonates, climate
Introduction

The Neogene (~24 Ma to present) is marked by considerable worldwide environmental change, including a global expansion of C4 biomass beginning around 7-8 Ma (Cerling et al., 1997a; Fox et al., 2004) and continuing to the present day. The widespread expansion of C4-dominated grasslands in the Neogene has been linked to decreasing global temperature and locally increasing aridity and seasonality (Cerling et al., 1998; Fox et al., 2004). The increase of C4 plant abundance in numerous ecosystems in the late Miocene is attributed to a decrease of atmospheric $p$CO$_2$ (Cerling et al., 1997a) due to increased continental chemical weathering in the tectonically active Himalayan region (Raymo and Ruddiman, 1992). The increase of C4 plants due to decreasing $p$CO$_2$ is questioned as there is little or no evidence for a sharp, permanent drop in atmospheric $p$CO$_2$ in the late Miocene (Figure 1) (Pagani et al., 1999; Pearson and Palmer, 2000; Zachos et al., 2001). Isotope records from coeval paleosols from different continents show variable C3 and C4 plant dominance between locations, indicating that the expansion of C4 grasses may be best explained by local climate variations rather than global factors (Fox, 2003).

The Great Plains are the largest continuous grasslands in North America, extending from the Rocky Mountains to the coniferous and deciduous forests of the Appalachian Plateau, and from the Gulf of Mexico to the Canadian parkland. In this paper, I use the isotopic record ($\delta^{13}$C and $\delta^{18}$O) from fossil camelid teeth as a proxy for diet to interpret the abundance of C3 and C4 biomass in the Great Plains ecosystem. Fossil camel tooth enamel was analyzed to see if their isotopic signatures are similar or different from the isotopic record of horses in order to investigate possible
Figure 1: a) $\delta^{18}O$ and b) $pCO_2$ records of biomarkers from marine algae at eight Deep Sea Drilling Project and Ocean Drilling Project sites in SW Pacific, Atlantic, and Indian Oceans indicate that the expansion of C4 plants is not due to a sudden decrease in $pCO_2$ in the Miocene (Adapted from Pagani et al., 1999).
resource partitioning behaviors. Analysis of δ13C isotopes in tooth enamel of seven different Camelidae genera from the Barstovian, Clarendonian and Hemiphillian (NALMA) (Lundelius, 1987) of Nebraska exhibit the same characteristics as the Equidae δ13C record, showing a simultaneous shift from consuming primarily C3 grasses to consuming both C3 and C4 grasses in the late Miocene. The similarity between the horse and camel isotope signatures indicates that there is no evidence for niche partitioning as horses and camels shared the resources equally without changing their niche.

Methods

Sample collection and preparation

Nebraska is at the modern transition between southern C4-dominated and northern C3-dominated grasslands and contains an abundant record of Neogene camels (Teeri and Stowe, 1976; Tieszen et al., 1997; Passey et al., 2002). Slight temperature and/or pCO2 fluctuations might be amplified in the δ13C values recorded in camel tooth enamel as the C3/C4 transition moved north and south of the present Nebraska (Passey et al., 2002).

For sample pretreatment and preparation I followed the methods of Koch et al., 1997. Approximately 10-50 mg of powdered hydroxylapatite (HA) was obtained from each specimen (7 different camel genera from the Barstovian, Clarendonian and Hemiphillian) from the University of Nebraska State Museum by drilling along a longitudinal section of the buccal margin of each tooth using a hand-held Braessler variable speed rotary drill with a carbide burr bit. Rather than serial sampling of individual teeth, this method was used because bulk sampling exhibits an accurate value
for mean food intake during enamel mineralization (Feranec and MacFadden, 2000). For analysis of $\delta^{13}C$, aliquots of HA powder were treated with 2-3% sodium hypochlorite (NaOCl) for 24 hours to oxidize and remove organic matter, after which the powder was rinsed five times with ultrapure water. The samples were then treated with 1 M acetic acid (CH$_3$COOH) buffered with 1 M calcium acetate, Ca(C$_2$H$_3$O$_2$)$_2$, for 24 hours to remove labile CO$_2$ and any calcite or dolomite contamination. The samples were rinsed with ultrapure water five more times and then vacuum freeze-dried for 12 hours to remove any remaining water. The isotopic compositions of the CO$_2$ in the samples were then analyzed using a Kiel II automatic carbonate preparation device coupled to a Finnigan MAT-252 Isotope Ratio Mass Spectrometer (IRMS) at the University of Minnesota. $\delta^{13}C$ data are reported in per mil deviations from the international VPBD carbonate standard (0 ‰).

**Background**

*C3 and C4 plants and their photosynthetic pathways*

Modern plants use three photosynthetic pathways to get energy: the C3, the C4, and the *crassulacean acid metabolism* (CAM) pathways. The C3 pathway is the least complicated and has been used by earliest plants in the Devonian, when CO$_2$ was the most abundant gas in the atmosphere, up to the present day. It is believed that the C4 and CAM photosynthetic pathways evolved more recently in response to the decrease of atmospheric CO$_2$ levels in the Cenozoic (Ehleringer et al., 1991). This paper is concerned with C3 and C4 plants, as CAM plants only account for a small fraction of terrestrial primary productivity (Passey et al., 2002).
C3 and C4 plants use different photosynthetic pathways that fractionate carbon isotopes differently during photosynthesis. In plant physiology and geochemistry applications, the isotopic $\delta^{13}C/\delta^{12}C$ ratio is conventionally expressed as a $\delta^{13}C$ (‰) value where:

$$\delta^{13}C_{\text{sample}} = \left( \frac{\left[^{13}C/^{12}C\right]_{\text{sample}}}{\left[^{13}C/^{12}C\right]_{\text{standard}}} - 1 \right) \times 1000 \quad (1)$$

C3 plants utilize the Calvin cycle photosynthetic pathway and have a mean $\delta^{13}C$ value of about -26.7 ‰ (Cerling et al., 1997a) relative to the Vienna PeeDee Belemnite (VPDB) standard, which has a $\delta^{13}C$ of 0 ‰ (equation 1) (Figure 2). C3 plants use the enzyme ribulose-1, 5-bisphosphate carboxylase oxygenase (rubisco) to fix carbon into 3-carbon compounds (hence the term C3) and may lose up to 50% of this recently fixed carbon through photorespiration because of high light, temperature, and oxygen concentration. Modern C3 plants make up more than 85% of Earth’s terrestrial plant species and include trees, shrubs, and cool growing season grasses (Jacobs, 1999; MacFadden and Higgins, 2004).

Modern C4 plants dominate the Great Plains and are mostly warm growing season grasses that utilize the Hatch-Slack photosynthetic pathway and have a mean $\delta^{13}C$ value of about –12.5 ‰ (VPDB) (Figure 3) (Cerling et al., 1997a; Fox, 2003). Carbon is first incorporated into 4-carbon compounds in C4 grasses (hence the term C4). Relative to C3 plants, C4 plants are more efficient under low atmospheric $pCO_2$ (Ehleringer et al., 1997) because C4 plants internally concentrate CO$_2$ before carbon is fixed through the Calvin cycle (Pagani et al., 1999). C4 plants use the enzyme phosphoenolpyruvate (PEP) carboxylase, rather than rubisco, during initial carbon
Figure 2: $\delta^{13}C$ values for (a) modern grasses, (b) modern mammalian tooth enamel, and (c) fossil tooth enamel > 8 Ma. The modern and fossil samples are taken from Europe, Asia, Africa, North America and South America and include bovids, equids, giraffids, notungulates, proboscideans and rhinocerids. In (c) the $\delta^{13}C$ axis has been shifted by 1.5‰ due to anthropogenic effects on the $\delta^{13}C$ in the atmosphere as a result of fossil-fuel burning (Marino et al. 1991) (Adapted from Cerling et al., 1997).
Figure 3: Modern C4 grass productivity as a percentage of total grass productivity in the Great Plains, U.S. C4 grasses dominate non-agricultural biomass south of about 43° N (Adapted from Tieszen et al., 1997).
fixation, resulting in lower photorespiratory carbon losses in C4 plants because of the inability of PEP carboxylase to fix oxygen. Diffusion of oxygen in air increases with increasing temperature because the O\textsubscript{2} molecules move more quickly. However, because photorespiration in C4 plants is very low, the increased O\textsubscript{2} levels at higher temperatures do not affect them.

The C4 pathway is strongly regulated by light and allows for more efficient carbon fixation at higher temperatures and low CO\textsubscript{2} because C4 plants internally concentrates CO\textsubscript{2} in the bundle sheath and are able to limit water loss by closing their stomata for longer. CO\textsubscript{2} in the bundle sheath cells is much greater (> 2000 ppmv) than atmospheric CO\textsubscript{2}, reducing the loss of C to photorespiration and increasing the overall efficiency of photosynthesis (Jacobs, 1999). CO\textsubscript{2} used during C3 photosynthesis passes through stomata into internal spaces within the leaf where it then diffuses into wet mesophyll cells and becomes available for photosynthesis. In sunny, more arid environments, C3 plants are unable to keep their stomata open for long periods of time because of the threat of desiccation, thereby limiting their fractionation opportunities. C4 plants are preferred in warm, sunny environments because of their ability to concentrate CO\textsubscript{2} in the bundle sheath, but cannot compete with C3 plants in moist, colder, and darker environments because their photosynthesis takes more energy. C3 plants can keep their stomata open longer in these wet environments without experiencing desiccation, thereby eliminating the advantage experienced in drier climes by C4 plants.

\[ \delta^{13}C \text{ as recorded in plants} \]
C3 and C4 plants accurately record the atmospheric CO$_2$ and can act as proxies for paleoclimate studies. While C4 plants keep a more precise record of pCO$_2$, their use for paleoclimate studies is limited because these plants have only become common in the late Miocene to early Pliocene (Cerling and Harris, 1999; Sage, 2001). C3 plants, on the other hand, have been widespread since the Devonian, but display a more variable carbon isotopic discrimination (Figure 2a) (Cerling et al., 1998; Arens et al., 2000). Isotopic fractionation during carbon assimilation in C3 vascular plants can be described by the equation:

\[
\delta^{13}C_p = \delta^{13}C_a - a - (b - a) C_p/C_a
\]  

(2)

Where $\delta^{13}C_p$ is the carbon isotopic composition of C3 plant tissue and $\delta^{13}C_a$ is the carbon isotopic composition of atmospheric CO$_2$; $a$ is the isotopic discrimination due to the diffusion of $^{13}$CO$_2$ versus $^{12}$CO$_2$ through air; $b$ is the isotopic discrimination imparted during carboxylation by rubisco, the primary carbon-fixation enzyme in C3 plants; and $C_p/C_a$ is the ratio of intercellular to atmospheric pCO$_2$ expressed in parts per million by volume (ppmv) (Farquhar et al., 1989; Arens et al., 2000).

The $\delta^{13}$C of modern C3 plants averages about –26.7 ‰ compared to C4 plants, which average about –12.5 ‰ (Deines, 1980; Cerling et al., 1997a; Fox, 2003). Due to the burning of fossil fuels and the subsequent release of CO$_2$ into the atmosphere however, the $^{13}$C has changed by about 1.5 ‰ since the Industrial Revolution, so it is likely that pre-Industrial Revolution plants are also enriched in $^{13}$C by about 1.5 ‰ relative to modern plant biomass (Marino and McElroy, 1991). Due to these
anthropogenic effects, the mean δ\textsuperscript{13}C of ancient C3 and C4 plants probably has values of approximately -25 ‰ and -11 ‰, respectively (Figure 2) (Cerling et al., 1998). C3 plants have highly variable δ\textsuperscript{13}C compositions depending on their location. CO\textsubscript{2} in closed canopied environments may be significantly more negative due to respired CO\textsubscript{2}. Leaves obtained near the ground in these closed canopies can be several per mil more negative than C3 plants or leaves from higher elevations in the same forests (O'Leary, 1981). Water-stressed (xeric) ecosystems are enriched in \textsuperscript{13}C (-22 ‰) while closed canopy systems are depleted in \textsuperscript{13}C (-35 ‰) (van der Merwe and Medina, 1989; Ehleringer and Monson, 1993). The δ\textsuperscript{13}C recorded in plants is primarily controlled by the availability of CO\textsubscript{2}: when the CO\textsubscript{2} level is high, plant cells have the opportunity to discriminate between \textsuperscript{12}C and \textsuperscript{13}C, thereby creating a greater \textsuperscript{13}C to \textsuperscript{12}C ratio. CO\textsubscript{2} is the limiting factor to growth in low CO\textsubscript{2} conditions and the plant cells use all available CO\textsubscript{2}, regardless of its isotopic signature (O'Leary, 1981).

*Paleosol isotope record in the Great Plains, U.S.A.*

Stable isotope records of paleosols is a valuable paleoclimate proxy because carbonate paleosols directly record the proportions of C3 and C4 plants that were present in an ecosystem at a certain time (Fox, 2003). Stable isotope composition of 274 paleosol carbonates from the Great Plains, U.S.A. (south of ~43°N), indicates a uniform proportion of C4 biomass (12-34%) throughout the Miocene, an increasing proportion between 6.4 and 4.0 Ma, and present levels (80%) by 2.5 Ma (Figure 4; 5) (Fox et al., 2003; Tieszen et al. 1997). The expansion of C4 grasses throughout the Neogene has been attributed to a drop in atmospheric CO\textsubscript{2} (Cerling et al., 1997a), although there is a
Figure 4: 24 carbonate paleosols localities in South-Central Great Plains, U.S., where 274 samples were taken and analyzed for δ13C isotope composition (Figure 5; Appendix 1) (Adapted from Fox et al., 2003).
Figure 5: δ13C values recorded from 274 paleosol carbonates from the Great Plains, U.S. Throughout the Miocene there was about a 20% abundance of C4 grasses, that increased to about 40% in the late Pliocene and reached modern abundance by the Pleistocene (Fox et al., 2003). A δ13C value of -7 ‰ is used as the cutoff between pure C3 and C3/C4 mixed grasses (Cerling et al., 1997b; Marino and McElroy, 1991). See Figure 4 for sample localities (Adapted from Fox et al., 2003).
disparity between the timing of C4 expansion in the Great Plains and the appearance of low atmospheric CO$_2$ (Figure 1) (Latorre et al., 1997; Cerling and Harris, 1999; Pagani et al., 1999; Zachos et al., 2001; Sage and Anonymous, 2004).

Isotopic analysis of mammalian tooth enamel

Bones and teeth are made of a highly substituted form of the mineral hydroxylapatite, $\text{Ca}_5[\text{PO}_4,\text{CO}_3]_3[\text{OH,F}]$, which contains about 3-4\% weight of structural carbonate (Legeros, 1981). Teeth themselves are composed of 3 types of mineralized tissue: enamel, dentin, and cementum, and consist primarily of carbonated hydroxylapatite (Hoppe et al., 2004b). In order to get the most reliable and best results, most stable isotope studies focus on the isotopes that are most resistant to diagenetic alteration (oxygen and carbon) in the best-preserved tissues (bone, dentin, cementum, enamel). CO$_3$ in teeth is believed to have diagenetic resistance and is therefore ideal for isotope analysis.

Although abundant, bone samples are not ideal for isotope analysis because of their high porosity and organic content that makes them vulnerable to recrystallization and isotopic change. Dentin has a much lower porosity than bone, which reduces the potential for its diagenetic alteration. Tooth enamel is the hardest and least porous tissue, making it the least likely material to undergo alteration (Kohn and Cerling, 2002). While the $\delta^{13}\text{C}$ in bone CO$_3$ may be changed by diagenesis (Nelson et al., 1986; Kohn and Cerling, 2002), there is no evidence for the alteration of the isotopic signature in enamel.
CO₂ for samples throughout the Miocene (Cerling et al., 1997b; Feranec and MacFadden, 2000; Kohn and Cerling, 2002; Koch et al., 2004).

Hydroxylapatite from fossil horse and camel teeth is useful because 1) camel and horse fossils are abundant across a broad geographic and temporal range, 2) extant camels and horses provide a very close analogue to many fossil species, and 3) the teeth mineralize incrementally over a period of years and offer a temporal record of isotopic variation that can yield information about seasonal changes in behavior and/or local climatic conditions (Sharp and Cerling, 1998; Feranec and MacFadden, 2000; Hoppe et al., 2004a).

δ¹³C as recorded in mammalian tooth enamel

Mammalian tooth enamel is used as a proxy for C4 biomass in the Cenozoic for numerous reasons: 1) mammalian fossils are abundant in the geological record, 2) tooth enamel is resistant to diagenesis and preserves dietary preferences, 3) the isotopic signature recorded in enamel of C3 biomass is easily distinguished from C4 biomass, and 4) mammalian herbivores are selective feeders, so their teeth record any ecological signal provided by their diet (Cerling et al., 1998).

Analysis of tooth enamel of extant large mammals show an enrichment of ¹³C by ~ 14.3 ‰ relative to their diet (Figures 2, 6). C3 plants have δ¹³C values ranging from about –22 ‰ to –35 ‰ which would indicate that the transition or “cut-off” from a C3 to C4 diet would occur at a δ¹³C value for tooth enamel of about –8 ‰ (Cerling et al., 1997b; Fox, 2003). If the shift of δ¹³C ~ 1.5 ‰ in the atmosphere and plants caused by anthropogenic effects is taken into account, then the transition for a pure C3 diet may be
Figure 6: δ13C values of C3 plants (left side = 0% C4) and C4 grasses (right side = 100% C4) on the bottom. The top shaded region represents the enrichment of tooth enamel carbonate relative to plant foods of about 14 ‰ (Figure 2; Cerling et al., 1997) with C3 feeders on the left and C4 grazers on the right (Adapted from MacFadden, 2002).
even less negative; - 7 ‰ (Marino and McElroy, 1991). The -8 ‰ δ13C is an extremely conservative cut-off due to the fact that modern C3 plants with δ13C ≥ -22 ‰ are rare (Kohn and Cerling, 2002), which may make the cutoff for C3 and C4 plants even more negative. It is important to take into account that δ13C values in plants can vary by up to 10 ‰ depending on which part of the plant tissue is used to make the measurement (O'Leary, 1981). Preferential eating by mammals of certain plant tissues from borderline C3/C4 plants could result in an artificially bolstered δ13C signature that might lead to incorrect conclusions about whether an animal is eating a C3 or a C4 plant.

δ18O isotopes

Enamel δ18O is an indirect recorder of temperature, in the sense that mean annual temperature (MAT) and surface water δ18O are positively correlated for middle to high latitudes (Rozanski et al., 1993). Increased temperature and aridity are associated with enriched δ18O signatures while decreased temperature and increased humidity are associated with depleted δ18O values (Feranec and MacFadden, 2000; Passey et al., 2002). Mammal tooth δ18O enamel records are dependent upon the isotopic composition of the ingested water (Bryant and Froelich, 1995; Feranec and MacFadden, 2000), though the physiological fractionations can be complex. In addition to the biological complexity, diagenetic alteration of carbonate δ18O is possible though it has been shown that single fossil teeth can preserve seasonal δ18O signals (Fricke et al., 1998). δ18O records from mammals can exhibit differences of several per mil because of different physiological or behavioral reasons such as panting versus sweating, stem feeding versus leaf feeding,
dry-food feeding versus succulent-food feeding, as well as other factors that may affect the isotopic signature in mammalian body water (Kohn, 1996; Passey et al., 2002).

\[ \delta^{13}C \]

**δ\textsuperscript{13}C record of Neogene horse teeth**

Stable carbon isotope studies of horse tooth enamel show a distinct biotic shift around 7 Ma from primarily C3 to C4 plants (Figure 7) (Janis, 1997; Passey et al., 2002) that corresponds to a similar isotopic change in carbonate paleosols during the same time (Fox, 2003). In the early Miocene horses ate primarily C3 plants with some evidence of a mixed C3/C4 diet. The first known horses with mixed and C4-dominated diets become evident at 6.6 Ma (Wang et al., 1994; Cerling et al., 1997b). If -8 ‰ δ\textsuperscript{13}C is used for the C3-C4 cutoff, then there are some horses potentially eating C4 as early as 15 Ma. The -8 ‰ δ\textsuperscript{13}C cutoff is very conservative as it is likely that these early values at 15 Ma are reflecting temperature, light, or water stressed C3 plants. Around ~7 Ma however, isotopic evidence indicates that horse diets began to shift to a more mixed C3/C4 diet with a strong directional swing towards a C4 dominated diet (Figure 7) (Passey et al., 2002).

δ\textsuperscript{13}C data from some fossil horses and camels show a strong and distinct shift to a C4-dominated diet in the Miocene while paleosol records show a uniform and consistent C4 biomass during the same time period. This contrast between the paleosol and horse and camel record may indicate that the isotopic paleo-dietary study of parallel clades (horses and camels) may not reveal complex ecological interactions within communities, such as niche partitioning.
Figure 7: The red and blue points represent the δ13C values recorded in the fossil tooth enamel of horses (Passey et al., 2002), while the green points represent the δ13C values recorded in fossil tooth enamel from camels (Appendix 1). Throughout the Miocene, horses and camels predominantly ate C3 grasses until about 6.6 Ma when some horses and camels begin to eat C4 as well as C3 grasses. The black triangles at Thomson Quarry represent several species of horse and camel, while the samples collect at Ashfall represents several different species of camels and rhinos. It is clear that some of the animals found at Ashfall and Thomson were eating C4 grasses at the same time that the majority of horses and camels were eating C3 grasses (Adapted from Passey et al., 2002).
Equid morphological studies: hypsodonty

Tooth morphology is often used to infer the diet of fossil taxa because mammalian tooth shape is believed to reflect feeding ecology (Janis, 1997). Horses, as well as several other ungulate lineages, developed high-crowned teeth (hypsodonty) in the middle Miocene in what is believed to be an adaptational response to the expansion of grasslands during this time (MacFadden, 2005). Hypsodonty is defined as the ratio of maximum height to maximum antero-posterior length for unworn molars and is believed to be a morphological adaptation to open, grass-dominated habitats where the longer teeth are meant to deal with silica-rich grasses or windblown dust that became part of the horse’s diets (Wang et al., 1994; MacFadden, 2000). On the hypsodonty index (HI) Hypsodont taxa have HI > 3.5, Mesodont are 1.5 < HI < 3.5, and Brachydont are HI < 1.5. Brachydont teeth are generally believed to be for diets that consist of primarily browsing, while hypsodonty is advantageous for a primarily grazing habitat. Hypsodonty evolved in equids around 18 Ma (MacFadden, 1998; Stromberg, 2006), more than 4 Ma after C3 grasses were established in the Great Plains, U.S.A. (Figure 8). Browser species richness declined as grazer species increased during the middle and late Miocene, from ~16-7 Ma. Overall species richness of both browsers and grazers declined after ~7 Ma (Figure 9). If HI > 2.5 is used to define a truly hypsodont horse (Janis, 2004a), then the offset between grassland expansion and hypsodonty evolution in fossil equids in the Great Plains is extended to about 10 Ma. The offset between the adaptation for a grazing diet, hypsodonty, occurs more than 10 Ma years prior to the expansion of the primary grazing material, C4 grasses, in the Central Great Plains. Whether hypsodonty evolved in
Figure 8: a) Horses develop hyssodonty, an adaptation for open habitat grazing and C4 consumption, around 18 Ma.  
b) Consumption of C4 plants arose independently in several derived clades at approximately the same time, indicating that there were no horses who were specialized C4 consumers (Adapted from Stromberg, 2006).
Figure 9: Ungulate tooth crown height shows evidence for ecological changes in Neogene samples. Around 18 Ma ungulates began to develop hysodonty, an adaptation for open habitats and grazing. Browser species richness declined as grazer species increased during the middle and late Miocene, from ~16-7 Ma. Overall species richness of both browsers and grazers declines after ~7 Ma (Adapted from Janis et al., 2002).
response to open habitats or coevolved with the expansion of grasslands is argued, but no strong conclusions can be asserted until further study is performed (Stromberg, 2006).

**Results**

The mean $\delta^{13}C$ values for the 35 camel samples from the Barstovian through the Hemphillian from Nebraska (Figure 10) show a discrete increase to more positive per mil values. Mean $\delta^{13}C$ values for Barstovian samples are $-10.83 \%e$ while Clarendonian samples have a mean of $-8.82 \%e$, and Hemphillian samples have a mean $\delta^{13}C$ value of $-8.76 \%e$ (VPBD) (Appendix 1, Figure 7). $\delta^{13}C$ values of camels are relatively consistent but indicate a slight increase in C4 diet throughout the Miocene and are similar to the $\delta^{13}C$ of horse tooth enamel over the same period (Janis et al. 2002).

The mean $\delta^{18}O$ values for the Barstovian samples are $-3.80 \%e$ while the Clarendonian samples exhibit a $\delta^{18}O$ mean value of $-3.81 \%e$. The Hemphillian samples have a mean $\delta^{18}O$ value of $-3.42 \%e$ (Appendix 1, Figure 11). These samples do not differ significantly and further analysis and sampling is essential to study the implications of the oxygen isotopic signatures.

**Discussion**

Carbon isotopes are sometimes used to show isotopic discrimination of resource partitioning (Feranec and MacFadden, 2000) and can help to reveal possible niche partitioning behavior among ungulates (MacFadden and Higgins, 2004). While an animal’s “habitat” refers to where the animal lives, the same animal’s “niche” refers to the way in which that animal uses and interacts with the habitat. By analyzing two
Figure 10: Mapped localities of sampled fossil camel teeth from Nebraska, U.S. 35 tooth samples from seven different camilidae genera were sampled from the Barstovian, Clarendonian and Hemphillian (North American Land Mammal Ages) and analyzed for their δ13C and δ18O content (Figure 7; 11; Appendix 1).
Figure 11: δ18O values of 35 fossil camel teeth from Nebraska. The mean δ18O values for the Barstovian samples are – 3.80 ‰ while the Clarendonian samples exhibit a δ18O mean value of - 3.81 ‰. The Hemphillian samples have a mean δ18O value of -3.42 ‰ (Figure 10; Appendix 1).
animals that coexist in the same environment and use the same resources one can illuminate how the two animals share the space. There are three possible outcomes when two different populations share the same space and resources; 1) the two populations share the resources equally and do not change the niche; 2) niche partitioning occurs as one or both of the populations alters the niche to reduce overlap interactions (see (McNaughton, 1976); 3) one population fails to compete and is driven to extinction. According to Gause’s Law of competitive exclusion, only 2) or 3) is possible; no two species that compete for the exact same resources can stably coexist as one of the competitors will inevitably have a slight advantage over the other and the second competitor will be driven to extinction or will be forced to occupy a different ecological niche. Similar-sized organisms, such as camelids and equids, which are most likely feeding in a community with limited resources, should have to partition resources in order to decrease competition and to ensure survival (Hutchinson, 1958). From the fossil record we know that option 3) did not occur because there is a great abundance of coeval equid and camelid fossil material present in the Great Plains. Analysis of camel tooth enamel enables the comparison of the camel data alongside the equid isotopic data of Janis et al. 2002, and may give clues into the possible niche partitioning behaviors of the two mammals.

The δ¹³C ratios from the camel teeth exhibit the same characteristics as the horse teeth, showing a simultaneous shift from consuming primarily C3 grasses to consuming C3 and C4 grasses in the late Miocene. Contrary to Gause’s Law and the niche theory set out by Hutchinson (1958), late Miocene horses and camels appear to have successfully shared the resources of their environment equally, with neither of the animals exhibiting a
distinct evolutionary advantage. Coeval camels and horses may have been feeding on different plant foods, or may have been foraging at different times (c.f. McNaughton, 1976). The lack of significant differences between the δ¹³C records of Camelids and Equids suggests that the two families were competing for the same resources in an environment where there were ample resources available. Camelids and Equids may have been partitioning resources temporally, although serial sampling of fossil teeth for δ¹³C values has shown no temporal difference in feeding among coeval C4-grazers (Feranec and MacFadden, 2000). Niche partitioning must have occurred in some way that was not discernible through analysis of stable carbon and oxygen isotopes. C4 biome expansion in the Neogene may have been due to an increased competition for browse, forcing the development of open habitats dominated by C4 plants (c.f. Feranec and MacFadden, 2000). Further sampling of Blancan camels could help to explore similarities and differences between the camels and horses as the expansion of C4 grasses developed thoroughly after 5 Ma.

Present camel samples have limited usefulness as they do not cover some of the crucial C3 to C4 dietary transition of the horses from 6.6 to 5 Ma. Camelid samples from the Coffee Ranch fauna would be ideal as there are some camel fossils there that demonstrate different behavior relative to the horses during the same time (Passey et al., 2002). The paleosol δ¹³C record indicates a consistent C4 biomass concentration in the Great Plains during the Miocene, while the δ¹³C record from some horse and camel teeth shows evidence for an increasing C4 biomass diet during this same time (Figure 7). The contrast between the paleosol data and the horse and camel data indicates that something ecological may have happened in the mammal community. Seasonal changes in primary
production may have occurred on a temporal scale that was not recorded in the soil, but may have been important to mammals and the biological fractionation of isotopes in enamel.

Conclusions

My data show that there are no significant differences in the $\delta^{13}C$ values of camels and horses during the Neogene. These two genera are isotopically similar, giving no evidence for niche partitioning and suggesting that they were either competing for the same resources where there were abundant resources available, or they were partitioning food resources in ways that are not apparent through analysis of carbon isotope values.

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Appendix 1: 35 Camelidae teeth were sampled from the University of Nebraska State Museum, and the enamel was analyzed for its \( \delta^{13}C \) and \( \delta^{18}O \) signature. The samples represent 7 different Camelidae genera from the Barstovian, Clarendonian, and Hemphillian (NALMA). See Figure 7 and Figure 11 for graphical representation of the \( \delta^{13}C \) and \( \delta^{18}O \) results.

<table>
<thead>
<tr>
<th>Species</th>
<th>Carbon Site</th>
<th>County</th>
<th>Locality</th>
<th>Loc. Name</th>
<th>Age (Ma)</th>
<th>NALMA</th>
<th>NALMA mod</th>
<th>Tooth</th>
<th>( \delta^{13}C )</th>
<th>( \delta^{18}O )</th>
</tr>
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