Ecology and physiology of White River mammals based on stable isotope ratios of teeth

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Abstract

To characterize the ecology and physiology of common late Eocene–early Oligocene White River mammals, we analyzed the carbon and oxygen isotope composition of tooth enamel carbonate for six of the most abundant taxa: the perissodactyls *Brontops* (brontothere), *Mesohippus* (equid) and *Subhyracodon* (rhino); and the artiodactyls *Merycoidodon* (oreodont), *Leptomeryx* (leptomerycid) and *Poebrotherium* (camelid).

$\delta^{13}C$ values of middle and rear molars (M2s and M3s) and premolars range from $-13.1‰$ to $-7.7‰$ (V-PDB), consistent with pure C3 diets. In the late Eocene, *Mesohippus*, *Merycoidodon*, and *Leptomeryx* show indistinguishable average $\delta^{13}C$ values ($\sim -10‰$). In contrast, *Brontops* and *Subhyracodon* exhibit lower ($-11.2‰$) and higher ($-8.7‰$) values, respectively. Early Oligocene values for *Mesohippus* and *Merycoidodon* remain indistinguishable from each other and lower than the value of *Leptomeryx*, *Poebrotherium*, and *Subhyracodon* ($\sim -8.5‰$). These results likely indicate niche separation in terms of habitat preference between the investigated sympatric taxa. More specifically, assuming a $\delta^{13}C$ value of atmospheric CO_2 of $-5.5‰$, our data suggest a preference of *Brontops* for mesic forested areas, of *Mesohippus* and *Merycoidodon* for woodlands, and of *Subhyracodon* and *Poebrotherium* for more open habitats (e.g., grasslands). The higher Oligocene versus Eocene average $\delta^{13}C$ exhibited by *Leptomeryx* possibly reflects a preference of the new Oligocene species *L. evansi* for more open and/or xeric habitats relative to the Eocene species *L. speciosus*.

Late Eocene and early Oligocene average $\delta^{18}O$ of *Mesohippus*, *Merycoidodon*, *Leptomeryx*, and *Subhyracodon* are similar ($\sim 25‰$, V-SMOW), possibly indicating comparable water dependency for these taxa. In contrast, the higher $\delta^{18}O$ of *Poebrotherium* (26.6‰) suggests lower water dependency whereas the lower $\delta^{18}O$ of *Brontops* (23.0‰) may result from a high water dependency or, more likely, from its preference for humid habitats. Because hind-gut fermentation in perissodactyls requires high water intake, whereas fore-gut fermentation does not, our results might indicate the presence of fore-gut fermentation in early Oligocene camelids but its absence or incomplete development in late Eocene–early Oligocene oreodonts and leptomerycids.
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1. Introduction

The Eocene–Oligocene White River Group of the northern Great Plains is a sequence of volcaniclastic and siliciclastic sediments deposited within fluvial, lacustrine, and eolian environments. These sediments, whose

outcrops are usually scenic badlands that extend from southwestern North Dakota to northeastern Colorado, represent one of the most productive fossil mammal terrains in the world. Research on White River fossils has provided crucial information on late Paleogene paleoclimates and on mammalian evolution (e.g., Prothero and Berggren, 1992). Within the impressive fossil mammal record of the White River Group, seven ungulate genera are particularly common: the perissodactyls *Mesohippus*, *Subhyracodon*, *Trigonias* and *Brontops*; and the artiodactyls *Merycoidodon*, *Leptomeryx,* and *Poebrotherium*. The main purpose of this paper is to investigate various aspects of their paleoecology and physiology from their stable carbon and oxygen isotope compositions. Our data may be used to evaluate and refine previous hypotheses derived from studies that employed different methods such as functional morphology, modern analogues, and sediment association.

The stable isotope analyses are used to address the following specific questions:

(1) Did White River mammals feed exclusively on C3 vegetation? Is there any evidence of C4 grasses? Although C4 grasses did not become abundant in global ecosystems until the late Miocene (~7 Ma; Cerling et al., 1997), a recent study proposed the presence of a small to moderate (12–34%) component of C4 grasses in the Great Plains prior to that time (Fox and Koch, 2003). Enamel carbon isotope ratios are employed to investigate whether a C4 isotopic signal is present in any of the investigated taxa.

(2) In what kind of habitat(s) did White River mammals live? Is there isotopic evidence for niche partitioning among these taxa? Although most previous carbon isotope investigations have focused on the quantification of C3 browse versus C4 graze in the diet of ancient mammalian herbivores, a smaller but consistent body of research (e.g., Quade et al., 1995; Bocherens et al., 1995, 1997; Bocherens and Sen, 1998; Cerling et al., 2004; MacFadden and Higgins, 2004) has also employed carbon isotope ratios to explore niche partitioning in pure C3 ecosystems. Enamel $\delta^{13}C$ is therefore employed to investigate whether these White River mammals preferred particular habitats and partitioned available food resources.

(3) How did the investigated taxa respond to the major, abrupt environmental and climatic change that occurred in North America across the Eocene–Oligocene transition? Paleosols (Retallack, 1983,

1992), leaf morphology (Wolfe, 1992, 1994; Gregory and McIntosh, 1996; Chase et al., 1998), and stable isotope (Zanazzi et al., 2007) investigations all indicate a large and abrupt cooling event in the Great Plains ~33.5 Ma. Paleosol studies, along with the faunal trends investigated by Hutchison (1982), Hutchison (1992), and Evanoff et al. (1992), also indicate a trend towards drier conditions, with a decrease in mean annual rainfall from more than ~1000 mm/yr in the late Eocene to ~700 mm/yr in the early Oligocene (Retallack, 1992). This climate shift could have induced a major change in the vegetation and ecosystems structure. Specifically, Retallack (1983, 1992) suggests that the dense, humid forests characteristic of the late Eocene were replaced by arid woodlands and wooded grasslands in the early Oligocene. By analyzing the carbon isotope composition of Eocene and Oligocene teeth we investigated whether the investigated taxa changed habitat and/or diet in response to this major climatic event.

(4) Is there isotopic evidence for a difference in the digestive physiology between the fossil artiodactyls and the perissodactyls? Theoretical models (Kohn, 1996) and modern observations (Kohn et al., 1996) indicate that drought-tolerant animals exhibit higher $^{18}O/^{16}O$ ratios relative to more water-dependent ones. Modern perissodactyls are hind-gut fermenters and are all very water dependent. In contrast, modern artiodactyls, most of which are fore-gut fermenters, show a wide range of water dependency and include very drought-tolerant animals. Because hind-gut fermentation in perissodactyls is thought to have developed in the early Eocene (Janis, 1976), an inter-taxa comparison of enamel oxygen isotope ratios may help resolve existing uncertainties regarding the physiology of the digestive system in late Eocene–early Oligocene artiodactyls.

2. Background

2.1. White River mammals

Mesohippus (Order Perissodactyla, Family Equidae) was an ancestral horse about 60 cm tall with an estimated body mass of ~50 kg (MacFadden, 1987). It first appeared in the middle Eocene (early Duchesnean NALMA, ~40 Ma) and became extinct in the late Oligocene (Arikareean NALMA, 28 Ma; MacFadden, 1998). *Mesohippus* had brachydont (i.e., low-crowned),

lophodont (i.e., with the cusps forming continuous ridges) cheek teeth with three molariform premolars well adapted to browse fruits, seeds, and leaves. It had longer legs relative to its Eocene ancestors and was the first horse with tridactyl (i.e., three-toed) feet. In paleoenvironmental reconstructions of the late Eocene Chadron formation of South Dakota, *Mesohippus* is seen as having lived preferentially in riparian forests (Clark et al., 1967). In the reconstructions of the early Oligocene Brule formation, *Mesohippus* is still seen predominantly in riparian forests but also in swamp areas and, to a lesser extent, in open plains far from any streams (Clark et al., 1967; Retallack, 1983).

Subhyracodon (Order Perissodactyla, Family Rhinocerotidae) is the most common late Eocene–Oligocene rhinocerotid. *Subhyracodon* (in some early works called *Caenopus*) had a sub-cursorial habitus with tridactyl feet and brachydont lophodont molars. In the late Eocene, *Subhyracodon* is thought to have predominantly inhabited savannas (Clark et al., 1967). According to Clark et al. (1967) and Retallack (1983), the specimens found in Oligocene sediments indicate that *Subhyracodon* preferred riparian forests and open plains. Mead and Wall (1998) described *Subhyracodon* as a selective mixed feeder inhabiting wooded habitats proximal to the riparian strip. This hornless rhino first appeared in the late Eocene (early Chadronian NALMA, ∼ 37 Ma) and survived until the late Oligocene (Arikareean NALMA, ∼ 28 Ma; Prothero, 1998) to give rise to later North American rhinos. Its close relative *Trigonias* is described as a cow-sized, hornless rhino with a saddle-shaped head. In contrast to *Subhyracodon*, *Trigonias* had canines and incisors in the upper jaw and retained a functional fifth metacarpal. *Trigonias*, which became extinct in the early Oligocene (Orellan NALMA, ∼ 32 Ma; Prothero, 1998), has been found exclusively in river channel deposits. For this reason, and because of its characteristic short and heavy limbs, *Trigonias* is believed to have been a semi-aquatic rhino (Clark et al., 1967).

Larger than any other coexisting mammal, the elephant-sized (2.5 m at the shoulder), tridactyl *Brontops* (Order Perissodactyla, Family Brontotheriidae) belongs to the group informally known as titanotheres (meaning "thunder beasts") or brontotheres. The most striking feature of *Brontops* is the two hornlike prominences that arise anterior to the orbit. The exact phylogenetic relationship of brontotheres to other perissodactyls has not been firmly established. *Brontops* had brachydont, selenodont (i.e., crescent-shaped) cheek teeth specialized to browse relatively soft, leafy vegetation in dense forests (Mihlbachler and Solounias, 2002). *Brontops* first appeared in the late Eocene (early Chadronian NALMA,

∼ 37 Ma) and became extinct, like all the brontotheres, in the latest Eocene (Chadronian–Orellan NALMA boundary, ∼ 33.5 Ma; Mader, 1998).

Merycoidodon (Order Artiodactyla, Family Merycoidodontidae) is the most common fossil in the White River Group. It belongs to the superfamily Oreodontoidea whose members are commonly called oreodonts. Entirely restricted to North America, oreodonts had a post-cranial morphology similar to suids but cervid-like brachydont–selenodont molars indicative of a folivorous (i.e., leaf-based) diet (Janis, 1982). The digestive physiology of the oreodonts is still a matter of speculation. According to Janis (1982), oreodonts had some sort of enlarged fore-stomach but lacked the derived system of regurgitation and cud chewing seen in extant ruminants. *Merycoidodon*, whose estimated body weight is ∼ 50 kg, had five toes on the front legs and four on the back. It first appeared in the late middle Eocene (Duchesnean NALMA, ∼ 38 Ma) and became extinct in the late Oligocene (Arikareean NALMA, ∼ 28 Ma; Lander, 1998). Lander (1998) suggested that *Merycoidodon* was a mixed browser-grazer inhabiting gallery forests. Slightly in contrast with this interpretation, Clark et al. (1967) indicated a preference of late Eocene *Merycoidodon* for savanna or savanna-forest habitats. Likewise, Wall and Shikany (1995) indicated that Oligocene *Merycoidodon* had specializations characteristic of grazers adapted to savanna grasslands. Clark et al. (1967) and Retallack (1983) suggested, on the other hand, that early Oligocene *Merycoidodon* preferred riparian forests rather than open plains or swamps.

The second most common fossil artiodactyl of the White River sediments is the hornless, tetradactyl (i.e., four-toed) *Leptomeryx* (Infraorder Tragulina, Family Leptomerycidae) which is thought to be the earliest and most primitive ruminant (Benner et al., 2002). Along with the very similar *Hypertragulus*, the small, rabbit-sized *Leptomeryx*, which possessed typical selenodont molars, is closely related to the living fossil chevrotains, or "mouse deer". *Leptomeryx* first appeared in the middle Eocene (late Uintan NALMA, ∼ 41 Ma) and became extinct in the early Miocene (early Hemingfordian NALMA, ∼ 18 Ma; Webb, 1998). Whereas several species existed in the Eocene, only *Leptomeryx evansi*, which appeared around the Eocene–Oligocene transition, is present in Oligocene sediments (Heaton and Emry, 1996). According to Wall and Collins (1998), *Leptomeryx* possessed all the traits of a browser. Clark et al. (1967) and Retallack (1983) indicated that *L. evansi* showed a definite preference for open plain habitats (savanna woodlands and shrub steppe).

Poebrotherium (Order Artiodactyla, Family Camelidae) was an ancestral camelid believed to be a member of

Table 1
Summary of the ecological and physiological characteristics of White River mammals derived from this study and previous investigations

Previous studies						This study		
Genus	Estimated body mass	Dentition	Habitat	Diet	Digestive system	Diet	Habitat	Oxygen isotope composition and implications
Mesohippus	42.2–53.8 kg (1)	Brachydont–lophodont; molariform premolars (1)	Riparian forests, swamps, open plains (4, 5)	Browser (1)	Hind-gut fermentation (2)	C3 plants	Woodlands	Medium $\delta^{18}O$→Water dependent→hind-gut fermentation?
Subhyracodon	250 kg (2)	Brachydont–lophodont (6)	Riparian forests, open plains (4, 5, 6)	Browser/mixed feeder (4, 6)	Hind-gut fermentation (2)	C3 plants	Open plains	Medium $\delta^{18}O$→Water dependent→hind-gut fermentation?
Trigonias	250 kg (2)	Brachydont–lophodont (6)	Close to rivers (semi-aquatic?) (4)	Browser/mixed feeder (4)	Hind-gut fermentation (2)	C3 plants	Open plains (no aquatic)	Medium $\delta^{18}O$→Water dependent→hind-gut fermentation?
Brontops	1900 kg (3)	Brachydont–selenodont (7)	Dense forests (7)	Browser (7)	Hind-gut fermentation (2)	C3 plants	Forests	Low $\delta^{18}O$ (and low $\delta^{13}C$) →Humid environment?
Merycoidodon	50 kg (2)	Brachydont–selenodont (2)	Riparian forests-open plains (4, 8, 9)	Browser/mixed feeder (2, 8)	? (2)	C3 plants	Woodlands	Medium $\delta^{18}O$→Water dependent→hind-gut fermentation?
Leptomeryx	2.5 kg (2)	Selenodont (11)	Open plains (4, 5)	Browser (11)	Fore-gut fermentation? (12)	C3 plants	Woodlands (*L. speciosus*), open plains (*L. evansi*)	Medium $\delta^{18}O$→Water dependent?→hind-gut fermentation? Nocturnal feeder?
Poebrotherium	8 kg (2)	Brachydont–selenodont (10)	Mostly open plains (2, 4, 10)	Browser/mixed feeder (2, 10)	Fore-gut fermentation (2, 13)	C3 plants	Open plains	High $\delta^{18}O$→Drought tolerant→fore-gut fermentation?

References: (1) MacFadden (1987); (2) Janis (1982); (3) Mendoza et al. (2006); (4) Clark et al. (1967); (5) Retallack (1983); (6) Mead and Wall (1998); (7) Mihlbachler and Solounias (2002); (8) Lander (1998); (9) Wall and Shikany (1995); (10) Wall and Hauptman (2001); (11) Wall and Collins (1998); (12) Benner et al. (2002); (13) Langer (1974).

the central stock from which modern camels and llamas have evolved. Unlike other contemporary ungulates, *Poebrotherium* had already evolved a relatively long slender neck and limbs and had a long, tubular snout. Because of these characteristics, Janis (1982) suggested that this camel was a highly selective browser, feeding on low-level herbaceous plants in an open-country habitat. In conformity with this interpretation, Clark et al. (1967) suggest a preference of the late Eocene *Poebrotherium* for savanna habitats. In contrast, in the Oligocene, *Poebrotherium*, although uncommon, occurred in different habitats without showing any particular preference (Clark et al., 1967). Similar conclusions were drawn by Wall and Hauptman (2001) who suggested that *Poebrotherium* was a browser or mixed feeder with broad dietary preferences ranging over a variety of habitats. *Poebrotherium* was the earliest two-toed artiodactyl, had a body weight of approximately 20 kg (Janis, 1982), and brachydont–selenodont dentition. It first appeared in the late Eocene (Chadronian NALMA, ~37 Ma) and became extinct in the late Oligocene (Arikareean NALMA, ~28 Ma; Honey et al., 1998). A

summary of the existing knowledge regarding the ecology and physiology of these mammals (along with the major findings of this study) is presented in Table 1.

2.2. Carbon isotopes in teeth: diet and habitat

Because land plants are the ultimate source of carbon in mammalian herbivores, the main factor affecting the carbon isotope composition of tooth enamel is the carbon isotope composition of the ingested plants. In turn, the carbon isotope composition of land plants is mainly a function of the photosynthetic pathway used to fix atmospheric CO_2. The C3 pathway (or Calvin cycle) is the most common, occurring in all trees, almost all shrubs and herbs, and many grasses. C3 plants have a mean $\delta^{13}C$ value of -27 ± 3‰[2] (all means reported $\pm1\sigma$) (Farquhar

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[2] All isotope composition are expressed with the conventional δ notation in which the ratio of heavy to light isotopes in a sample is expressed as the permil (‰) difference between the ratio in the sample (R_{sa}) and the ratio in a standard (R_{st}), normalized to R_{st}: $\delta = ((R_{sa}/R_{st})-1) * 1000$.

et al., 1989). C4 (or Hatch–Slack) photosynthesis, on the other hand, occurs in warm climate grasses, some sedges and herbs, and a few shrubs. C4 plants have a mean $\delta^{13}C$ of -13 ± 2‰ (O'Leary, 1988). CAM (Crassulacean acid metabolism) plants, mainly succulents, form a third photosynthetic group and show a wide range of $\delta^{13}C$ values that can overlap with those seen in C3 and C4 plants. However, CAM plants represent a minor fraction of total terrestrial plant biomass and are not considered important in Eocene–Oligocene ecosystems. For this reason, they will not be considered further here. The distinct, non overlapping carbon isotope composition of C3 versus C4 plants forms one basis for the use of enamel $\delta^{13}C$ to reconstruct the diet of extinct mammalian herbivores.

In addition to allowing quantification of C3 versus C4 plant consumption in post 7 Ma ecosystems, enamel $\delta^{13}C$ can be employed to identify different habitats and/or water stress in pure C3 ecosystems. The basis of this approach is the wide range in $\delta^{13}C$ values exhibited by C3 plants under different environmental conditions. Plants under water stress conditions close their stomata and show less discrimination against ^{13}C. High $\delta^{13}C$ values (up to ~ -22‰) are therefore characteristic of open, xeric habitats (Farquhar et al., 1989). Conversely, very negative $\delta^{13}C$ values (up to ~ -35‰) are found in closed canopy forests because of recycling of ^{13}C-depleted CO_2 and low irradiance (van der Merwe and Medina, 1991).

Plant carbon ingested by herbivores is incorporated into the mineralized tissues of the animal. In mammals, these tissues have a mineralogy similar to hydroxyapatite $[Ca_5(PO_4)_3(OH)]$ with substitutions of CO_3 (normally 2–4 wt.%; Driessens and Verbeeck, 1990) for PO_4 and OH. The CO_3 component of tooth enamel therefore assumes a $\delta^{13}C$ value that reflects the carbon isotope composition of the ingested plants. However, both metabolism and biomineralization fractionate ingested carbon so that bioapatites are enriched in ^{13}C relative to the bulk diet. In this paper we will follow the result of the most recent study which suggests an enrichment factor of 13.3 ± 0.3‰ for non-ruminants and of 14.6 ± 0.3‰ for ruminants (Passey et al., 2005). Therefore, assuming an atmospheric CO_2 $\delta^{13}C$ value of -5.5‰ (calculated from late Eocene–early Oligocene foraminifera compositions), enamel $\delta^{13}C$ values for pure C3 and C4 diets range from ~ -6 to -18‰ and from ~ -1 to $+4$‰, respectively. Intermediate values would indicate a mixed diet. In addition, in pure C3 ecosystems, low (from ~ -15 to -21‰) enamel $\delta^{13}C$ values will be indicative of mesic, closed-canopy forests, intermediate (from ~ -13 to -8‰) of woodlands, and high ($> \sim -8$‰) of xeric grasslands (Cerling and Harris, 1999).

2.3. Oxygen isotopes in teeth: digestive physiology and habitat

The oxygen isotope composition of tooth enamel has recently been widely used for paleoclimate investigations in continental areas (see reviews of Koch, 1998; Kohn and Cerling, 2002). The basis for these reconstructions is the linear dependence of the oxygen isotope composition of mammalian apatite on local rainwater composition which, in turn, is a proxy for average surface temperature. However, in addition to rainwater $\delta^{18}O$, several other physiological, environmental, and behavioral factors affect enamel $\delta^{18}O$ (Kohn, 1996). The dependence on these additional factors complicates the use of this paleoclimate proxy but may allow, on the other hand, the reconstruction of important aspects of the paleoecology and physiology of extinct taxa.

One important factor affecting enamel $\delta^{18}O$ is the water dependency of the animal. Modern observations (Kohn et al., 1996) and theoretical models (Kohn, 1996) indicate that enamel $\delta^{18}O$ in obligate drinkers shows a first-order dependence on rainwater $\delta^{18}O$. In contrast, drought-tolerant animals usually deviate to higher values because they obtain proportionally more water from leaves which are more affected by evaporative enrichment relative to surface water. Therefore, sympatric water-dependent and drought-tolerant animals may exhibit different $\delta^{18}O$ values (Kohn, 1996). This difference is expected to be more pronounced in arid versus humid ecosystems because leaf water enrichment in ^{18}O is inversely proportional to relative humidity.

A theoretical model proposed by Bryant and Froelich (1995) indicates that the rate of water turnover is proportional to the body mass of an animal. However, Kohn (1996) and Kohn et al. (1996) showed that this model fails to predict the composition of several modern taxa. For instance, gazelle and oryx are large but drought-tolerant and exhibit high $\delta^{18}O$ values relative to more water-dependent animals such as zebras and hippos. A more applicable generalization regarding water balance may concern differences in digestive physiology (Kohn and Fremdt, 2007). In this context, the different anatomy of the digestive system in perissodactyls and artiodactyls may result in important, inter-taxa differences in enamel $\delta^{18}O$ values of sympatric animals.

Most artiodactyls are fore-gut fermenters or ruminants. Fore-gut fermenters possess a complex, multi-chambered stomach. In the members of the infraorder Pecora (i.e., cervids and bovids), fermentation occurs in the first two chambers, the rumen and the reticulum which both absorb the main products of fermentation, short-chain fatty acids. In the reticulum, a softened mass

called the "cud" is also formed. When the animal is at rest, this softened mass is regurgitated, allowing the animal to "chew its cud", or "ruminate". After the action of potent salivary enzymes, the food is swallowed a second time and enters the third chamber, the omasum, where muscular walls knead it further. The fourth, and final, chamber, the abomasum, is the true stomach and is where protein digestion is completed (Feldhamer et al., 2004). The members of the suborder Tylopoda (i.e., camels and llamas) posses a slightly different, three-chambered stomach whose compartments are however analogous to the rumen, reticulum, and omasum of the Pecora (Kay and Maloiy, 1989). Unlike ruminant artiodactyls, perissodactyls, which are hind-gut fermenters, do not regurgitate their food. Hind-gut fermenters masticate food as they eat, initiating digestion with salivary enzymes. Digestion continues within the simple stomach, and food then moves rapidly into the small intestine and into the cecum where fermentation of ingested cellulose by microorganisms occurs (Feldhamer et al., 2004).

Each of these two kinds of digestive system in herbivores has its own advantages and disadvantages. Of relevance for this study, fore-gut fermenters are able to recycle urea and can store large amount of water in the rumen. This allows some of these mammals to survive with very little water, whereas perissodactyls must drink often to balance the urea in their urine (McNab, 2002). Modern perissodactyls are indeed very water-dependent whereas modern artiodactyls show a wide range of water dependencies, including extremely drought-tolerant animals. Whereas no doubts seem to exist about the presence of hind-gut fermentation in late Eocene-early Oligocene perissodactyls, the physiology of the digestive system of the investigated fossil artiodactyls (particularly of the oreodonts) is still uncertain. An inter-taxa comparison of the $\delta^{18}O$ values of these sympatric fossil taxa potentially allows the identification of water dependent versus drought tolerant animals and may therefore provide valuable information about the evolution of the digestive systems in herbivores.

In addition to physiological information, enamel $\delta^{18}O$ can be used to investigate the preference of a taxon for a certain habitat. Taxa inhabiting preferentially closed-canopy forests tend to show low $\delta^{18}O$ values because the high humidity of these environments decreases leaf water enrichment in ^{18}O due to evapotranspiration (Quade et al., 1995). In addition, data on extant taxa have shown that aquatic animals tend to exhibit lower average $\delta^{18}O$ values than sympatric
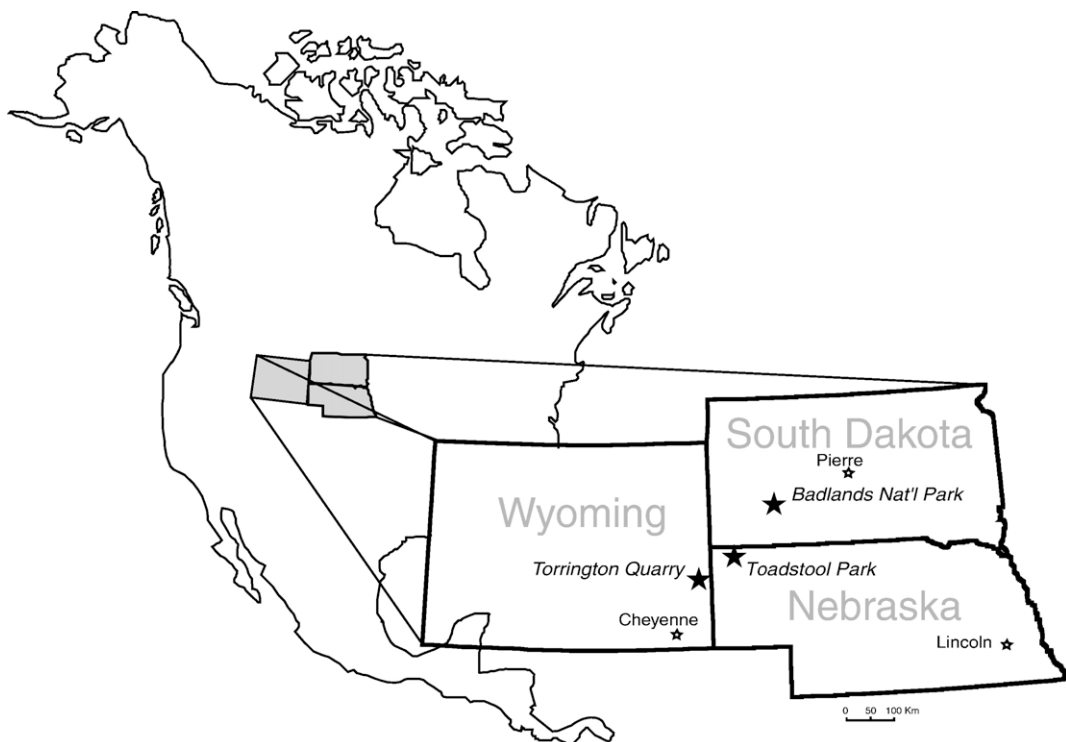

Fig. 1. Location map showing the sampling sites: Toadstool Park (northwestern Nebraska), Badlands National Park (southwestern South Dakota), and Torrington Quarry (eastern Wyoming).

Table 2
Descriptive statistics and results of parametric and nonparametric tests for Eocene $\delta^{13}C$ and $\delta^{18}O$

Genus	*n* teeth	Mean $\delta^{13}C$ (‰)	S.D. (‰)	Range (‰)	Mean $\delta^{18}O$ (‰)	S.D. (‰)	Range (‰)
Mesohippus	7 (4 M3s, 3 M2s)	−9.8	0.6	−10.8 to −8.5	24.2	1.6	21.3 to 27.1
Subhyracodon	6 (4 M3s, 2 Prem.)	−8.7	1.0	−11.3 to −7.7	25.0	1.3	21.5 to 27.3
Trigonias	1 (M3)	−8.1	0.1	−8.2 to −8.0	26.9	0.6	25.5 to 27.5
Brontops	3 (2 M2s, 1 Dec. Prem.)	−11.2	1.4	−12.9 to −8.5	23.0	1.0	20.7 to 25.5
Merycoidodon	10 (4 M3s, 3 M2s, 3 Prem.)	−10.0	0.9	−12.1 to −8.9	24.3	2.6	20.6 to 28.5
Leptomeryx	24 (15 M3s, 9 M2s)	−9.8	1.1	−12.5 to −8.3	24.6	1.3	21.6 to 27.5

Results of parametric and nonparametric pair-wise inter-taxa comparisons (S=significant, NS=non significant). The upper right portion and lower left portion of this table show results for oxygen and carbon isotope comparisons, respectively.

$\delta^{18}O$ / $\delta^{13}C$	*Mesohippus*	*Subhyracodon*	*Brontops*	*Merycoidodon*	*Leptomeryx*
Mesohippus		S (0.009, 0.011)	S (<0.001,<0.001)	NS (0.791, 0.893)	NS (0.372, 0.296)
Subhyracodon	S (<0.001,<0.001)		S (<0.001,<0.001)	NS (0.056, 0.217)	NS (0.155, 0.091)
Brontops	S (<0.001,<0.001)	S (<0.001,<0.001)		S (0.001, 0.024)	S (<0.001,<0.001)
Merycoidodon	NS (0.298, 0.761)	S (<0.001,<0.001)	S (<0.001,<0.001)		NS (0.695, 0.744)
Leptomeryx	NS (0.743, 0.228)	S (<0.001,<0.001)	S (<0.001,<0.001)	NS (0.156, 0.161)	

P-values are reported in parentheses (the first is for *t*-tests, the second for Mann–Whitney tests).

terrestrial ones (Bocherens et al., 1996; Clementz and Koch, 2001). For instance, hippos, which live in the water for most of the day, show the lowest $\delta^{18}O$ among the large herbivores of east Africa (Koch et al., 1991; Bocherens et al., 1996). Therefore, a comparison of the $\delta^{18}O$ of the two rhinocerotid *Subhyracodon* and *Trigonias* may indicate whether one of these two rhinos had a stronger preference for an aquatic versus a terrestrial habitat.

3. Materials and methods

3.1. Study sites and specimens sampled

The teeth analyzed in this study are from Toadstool Park (northwestern Nebraska), Badlands National Park (western South Dakota), and Torrington Quarry (eastern Wyoming) (Fig. 1). The specimens from Toadstool Park were provided by the Florida Museum of Natural History or collected in the field in August 2005. The specimens from Badlands National Park and Torrington Quarry are housed in collections at the American Museum of Natural History and Denver Museum of Nature and Science.

The stratigraphy of the White River Group in South Dakota and Nebraska has been recently revised (LaGarry, 1998; Terry, 1998; Terry and LaGarry, 1998). In northwestern Nebraska, the White River Group rests

unconformably on the Cretaceous Pierre Shale and is overlain by the Arikaree Group. It is subdivided into the Chamberlain Pass, Chadron, and Brule Formations. The Chadron Formation is now further subdivided into Peanut Peak and Big Cottonwood Creek Members and corresponds temporally to the upper part of the Chadronian NALMA (late Eocene, 37 to 33.8 Ma; Prothero, 1996). The Brule Formation is subdivided into the Orella and Whitney Members and "brown siltstone". The Orella Member corresponds temporally to the Orellan NALMA (early Oligocene, 33.8–32 Ma; Prothero, 1996). The late Eocene samples from Toadstool are from the Big Cottonwood Creek Member of the Chadron Formation. This sequence is composed primarily of volcaniclastic overbank silty claystones interbedded with channel sandstones, lacustrine limestones, and volcanic ashes (Terry and LaGarry, 1998). The early Oligocene samples are from the volcaniclastic clayey siltstones, interbedded with sheet sandstones of the Orella Member of the Brule Formation.

The samples from Badlands National Park of western South Dakota were entirely collected from the lower member of the Brule Formation. This early Oligocene unit was named Scenic Member by Bump (1956). In this location, the Big Cottonwood Creek Member is not present; the Scenic Member conformably overlies the Peanut Peak Member of the Chadron Formation and

Table 3
Descriptive results of parametric and nonparametric tests for Oligocene $\delta^{13}C$ and $\delta^{18}O$

Genus	n teeth	Mean $\delta^{13}C$ (‰)	S.D. (‰)	Range (‰)	Mean $\delta^{18}O$ (‰)	S.D. (‰)	Range (‰)
Mesohippus	22 (15 M3s, 1 M2, 6 Prem.)	−9.9	1.2	−13.1 to −8.2	24.5	2.0	20.4 to 28.8
Subhyracodon	2 (1 M3, 1 Prem.)	−8.7	0.5	−9.6 to −7.7	24.8	1.4	21.1 to 27.2
Merycoidodon	5 (3 M3s, 2 M2s, 1 Prem)	−9.6	0.9	−11.1 to −7.9	23.9	1.9	20.4 to 26.8
Leptomeryx	13 (7 M3s, 6 M2s)	−8.5	0.5	−9.6 to −7.8	23.7	0.9	21.9 to 25.3
Poebrotherium	7 (2 M3s?, 5 M2s)	−8.6	0.7	−10.5 to −7.9	26.6	0.9	24.8 to 28.9

Results of parametric and nonparametric pair-wise inter-taxa comparisons (S=significant, NS=non significant). The upper right portion and lower left portion of this table show results for oxygen and carbon isotope comparisons, respectively.

$\delta^{18}O$ / $\delta^{13}C$	*Mesohippus*	*Subhyracodon*	*Merycoidodon*	*Leptomeryx*	*Poebrotherium*
Mesohippus		NS (0.392, 0.329)	NS (0.143, 0.219)	NS (0.157, 0.172)	S (<0.001,<0.001)
Subhyracodon	S (<0.001,<0.001)		**S (?)** (0.03, 0.054)	S (0.011, 0.009)	S (<0.001,<0.001)
Merycoidodon	NS (0.236, 0.461)	S (<0.001,<0.001)		NS (0.781, 0.503)	S (<0.001,<0.001)
Leptomeryx	S (<0.001,<0.001)	NS (0.308, 0.428)	S (<0.001,<0.001)		S (<0.001,<0.001)
Poebrotherium	S (<0.001,<0.001)	NS (0.439, 0.057)	S (<0.001,<0.001)	NS (0.821, 0.877)	

P-values are reported in parentheses (the first is for *t*-tests, the second for Mann–Whitney tests).

intertongues with the overlying Poleslide Member. The Scenic Member consists of pedogenically modified volcaniclastic mudstones and siltstones interbedded with channel sandstones (Clark et al., 1967; Retallack, 1983).

In Wyoming, the White River Group is labeled as a formation and contains the Chadron and Brule Members separated by a widespread, thick, white tuff bed (5 tuff; Evanoff et al., 1992) which has a $^{40}Ar/^{39}Ar$ age of 33.59± 0.02 Ma (Obradovich et al., 1995). The late Eocene *Subhyracodon* and *Trigonias* samples from Wyoming were collected from the exposures of the Chadron Member southwest of Torrington. This unit correlates with the Chamberlain Pass Formation of northwestern Nebraska and is composed of brilliantly colored sands and/or conglomerates (Schultz and Stout, 1955).

A total of 101 teeth were analyzed in this study, 51 for the late Eocene and 50 for the early Oligocene. Part of the oxygen isotope data of these teeth have been previously published in a study aimed at reconstructing the climate change across the Eocene–Oligocene transition (Zanazzi et al., 2007). For our analyses, enamel was selected because of all biogenic tissues it is the least susceptible to diagenetic isotopic alteration (see review of Kohn and Cerling, 2002). Preference was also given to middle and rear molars (M2s and M3s) and premolars because the first molars (M1s) mineralize wholly or partially before weaning and could be isotopically biased as a result of milk consumption.

3.2. Sample preparation and isotope analyses

The teeth analyzed in this study were sampled by removing 2–3 mm wide vertical strips of enamel with a slow speed diamond-wafering saw. The enamel strips were sub-sampled perpendicular to the growth axis at intervals of 1.25 mm and the outer layer of enamel and adhering dentin were removed with a dental drill. Because of differences in tooth size and crown height,
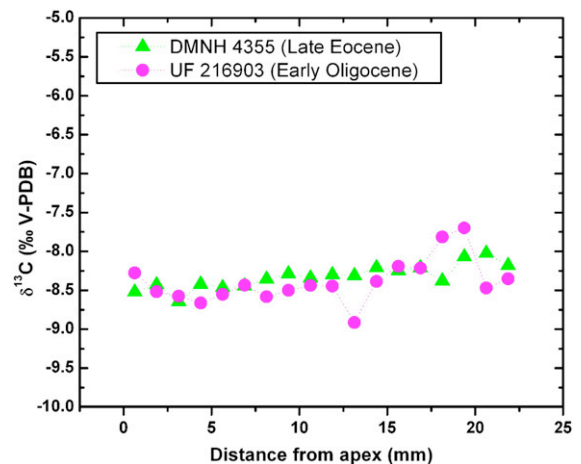

Fig. 2. $\delta^{13}C$ vs. distance along tooth for Eocene and Oligocene *Subhyracodon* teeth. The small intra-tooth variability in $\delta^{13}C$ suggests a small range in the $\delta^{13}C$ of the diet over seasonal time scales.

Leptomeryx yielded only one analyzable sub-sample, *Subhyracodon* and *Brontops* yielded on average sixteen, *Mesohippus*, *Merycoidodon*, and *Poebrotherium* five. For the analyses of the carbonate component of enamel, powdered samples were treated with H_2O_2 to remove organic contaminants and with an acetic acid-calcium acetate buffer to remove diagenetic carbonates (Koch et al., 1997). The samples were then dissolved in phosphoric acid and analyzed by an ISOCARB automated carbonate system attached to a VG OPTIMA isotope ratio mass spectrometer housed at the Department of Geological Sciences, University of South Carolina. Precision of these analyses is typically better than ±0.2‰ (1σ). Results are reported relative to the V-PDB ($\delta^{13}C$) and V-SMOW ($\delta^{18}O$) standards. Average composition of NBS 120c was found to be −6.1‰ ($\delta^{13}C$) and 28.7‰ ($\delta^{18}O$).

4. Results

A different number of M2s, M3s, and premolars have been analyzed for each taxon and time period (Tables 2 and 3). Because different teeth in a jaw grow at different times of the year, a correct interpretation of the data requires a complete understanding of the seasonal variations in enamel δ-values. With respect to $\delta^{13}C$, the small intra-jaw and intra-tooth variability exhibited by most of the samples suggests a fairly constant carbon isotope composition of the ingested plants throughout the year (Fig. 2). This finding suggests pooling data to include M2s, M3s, and premolars. In contrast, intra-tooth and intra-jaw $\delta^{18}O$ values show a higher variability reflecting different time of enamel mineral-

ization coupled with seasonal variation in rainwater composition (Bryant et al., 1996; Fricke and O'Neil, 1996; Kohn et al., 1998). Analyses of intra-tooth $\delta^{18}O$ profiles reveal that analogous teeth (e.g., M3s) from different individuals of the same taxon exhibit different patterns of variability (Fig. 3). Consequently, we believe that pooling the $\delta^{18}O$ values of the different teeth (i.e., M2s, M3s, premolars) for each taxon provides an accurate estimate of Eocene and Oligocene mean $\delta^{18}O$.

Tables 2 and 3 report descriptive statistics and results of pair-wise comparison tests for the isotope compositions of the investigated taxa. Fig. 4 shows box and whiskers plots for Eocene and Oligocene $\delta^{13}C$ and $\delta^{18}O$ values (*Trigonias* has been excluded from the figures because of limited sample size). Both parametric (two-tailed *t*-tests, ANOVA) and nonparametric (two tailed Mann–Whitney tests, Kruskal–Wallis) statistical tests were performed on the data. Statistical significance is based on $P<0.05$.

4.1. $\delta^{13}C$ values

Kruskal–Wallis and ANOVA tests suggest statistically significant inter-taxa differences among both Eocene and Oligocene samples ($P<0.001$). With respect to the Eocene samples, further analysis indicates that *Mesohippus* (−9.8±0.6‰), *Merycoidodon* (−10.0±0.9‰), and *Leptomeryx* (−9.8±1.1‰) show indistinguishable average $\delta^{13}C$ values. In contrast, *Subhyracodon* (−8.7±1.0‰) and *Brontops* (−11.2±1.4‰) mean $\delta^{13}C$ are significantly higher and lower, respectively, than the values of the other three taxa (Fig. 4A, Table 2). Eocene samples of *Subhyracodon* are from two different
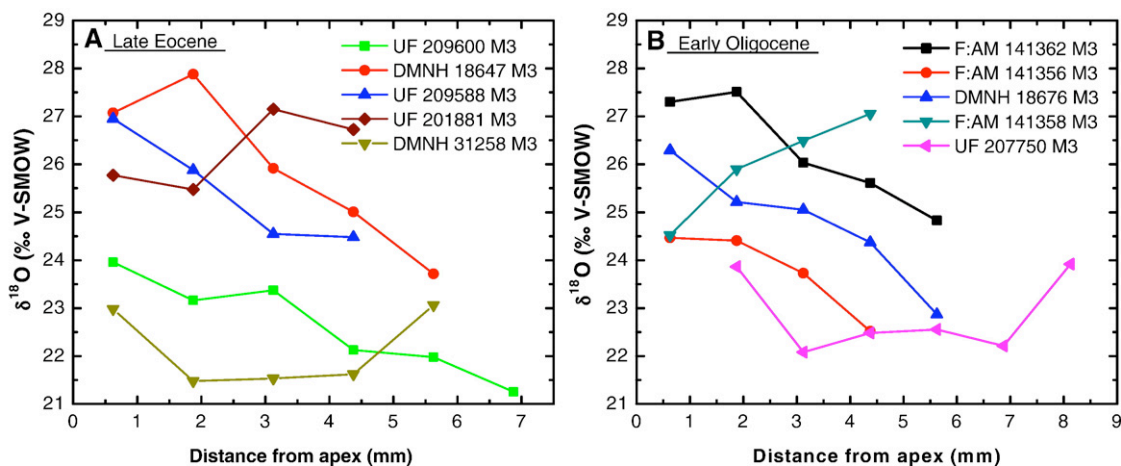

Fig. 3. $\delta^{18}O$ vs. distance along tooth for Eocene (A) and Oligocene (B) *Mesohippus* rear molars (M3s). The different patterns of variability reflect different time of enamel mineralization coupled with seasonal variations in rainwater composition.
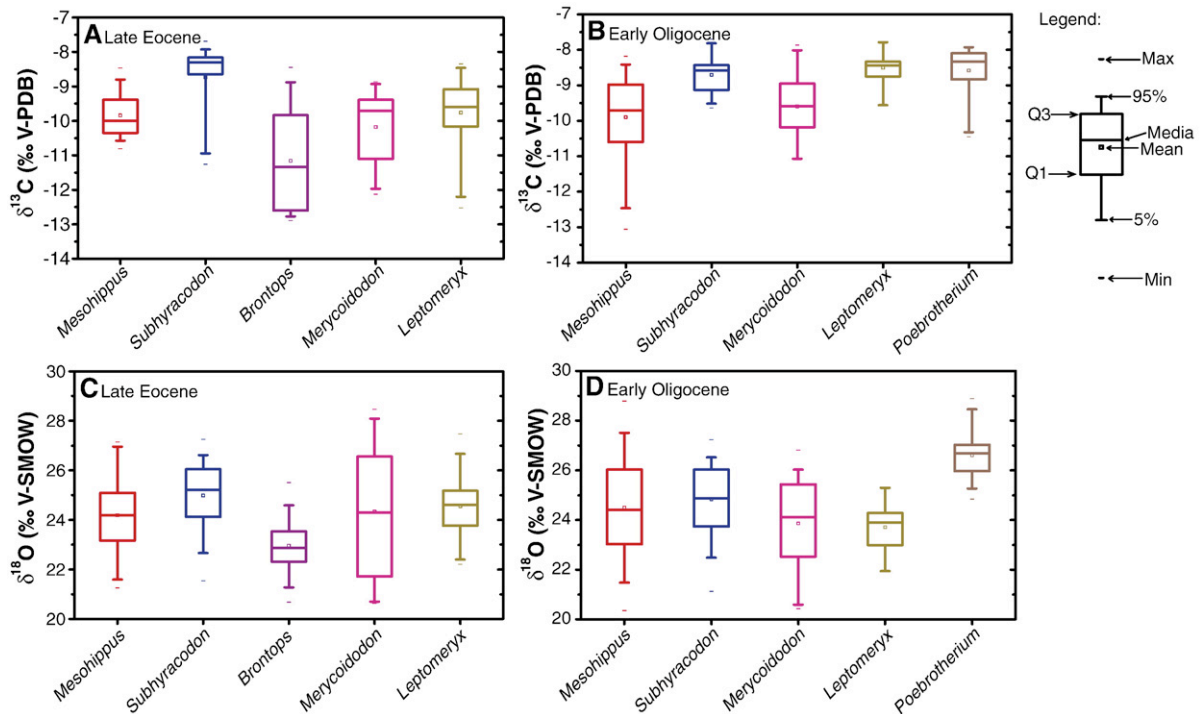
Fig. 4. Top: box and whiskers plots for Eocene (A) and Oligocene (B) $\delta^{13}C$ values. Bottom: box and whiskers plots for Eocene (C) and Oligocene (D) $\delta^{18}O$ values. The lower and upper sides of the boxes indicate the lower and upper quartile, respectively. Inside the boxes, the solid lines indicate the median and the hollow squares the mean. The length of the whiskers indicates the 5 to 95-percentile range. Outside the boxes, the segments indicate the minimum and maximum values.

localities, Toadstool Park and Torrington Quarry. Statistical analysis indicates that average $\delta^{13}C$ value for the Toadstool samples (-10.3 ± 0.8‰) is significantly lower than the average value of the Torrington samples (-8.2 ± 0.2‰) [$P<0.001$; Mann–Whitney and t-test, $n=22$ (Toadstool), $n=67$ (Torrington)]. The mean $\delta^{13}C$ of the *Trigonias* tooth from Torrington (-8.1 ± 0.1‰) is slightly higher than the average value obtained for *Subhyracodon* in this locality. The difference between the two values is, however, only marginally significant or insignificant [$P=0.030$, Mann–Whitney; $P=0.126$, t-test; $n=67$ (Subhyracodon), $n=16$ (Trigonias)].

Analysis of Oligocene samples indicates that average $\delta^{13}C$ values for *Mesohippus* (-9.9 ± 1.2‰) and *Merycoidodon* (-9.6 ± 0.9‰) are indistinguishable. Average $\delta^{13}C$ of *Subhyracodon* (-8.7 ± 0.5‰), *Poebrotherium* (-8.6 ± 0.7‰), and *Leptomeryx* (8.5 ± 0.5‰) are indistinguishable from each other and higher than the values of *Mesohippus* and *Merycoidodon* (Fig. 4B, Table 3). Oligocene samples of *Mesohippus* include eight teeth from Toadstool and fourteen teeth from the Badlands. Mann–Whitney and t-tests indicate that the samples from Toadstool show a significantly higher mean $\delta^{13}C$ value (-9.4 ± 0.8‰) relative to the

samples from the Badlands (-10.2 ± 1.3‰; $P<0.001$, Mann–Whitney and t-tests, $n=37$ (Toadstool), $n=58$ (Badlands)].

Analysis of the taxon-specific change in mean $\delta^{13}C$ across the Eocene–Oligocene transition indicate that *Mesohippus* [$P=0.466$, Mann–Whitney; $P=0.801$, t-test; $n=29$ (Eocene), $n=91$ (Oligocene)], and *Merycoidodon* [$P=0.071$, Mann–Whitney; $P=0.053$, t-test; $n=42$ (Eocene), $n=26$ (Oligocene)] Eocene versus Oligocene values are indistinguishable. The difference in Eocene versus Oligocene mean $\delta^{13}C$ values for *Subhyracodon* is insignificant or marginally significant [$P=0.012$, Mann–Whitney; $P=0.862$, t-test; $n=89$ (Eocene), $n=30$ (Oligocene)]. *Leptomeryx* is the only taxon showing a clear difference in Eocene versus Oligocene average $\delta^{13}C$ [$P<0.001$, Mann–Whitney and t-tests; $n=24$ (Eocene), $n=13$ (Oligocene)].

4.2. $\delta^{18}O$ values

Kruskal–Wallis and ANOVA tests indicate significant inter-taxa difference among both Eocene and Oligocene samples ($P<0.001$). With respect to the Eocene samples, *Mesohippus* (24.2 ± 1.6‰), *Merycoidodon* (24.3 ± 2.6‰),

and *Leptomeryx* ($24.6 \pm 1.3‰$) show indistinguishable average $\delta^{18}O$ values. *Subhyracodon* average $\delta^{18}O$ ($25.0 \pm 1.3‰$) is higher than the value of *Mesohippus* but indistinguishable from the values of the artiodactyls. *Subhyracodon* average $\delta^{18}O$ is also lower than the mean value obtained for the tooth of *Trigonias* ($26.9 \pm 2.0‰$) [$P < 0.001$, Mann–Whitney and *t*-tests; $n = 89$ (*Subhyracodon*), $n = 16$ (*Trigonias*)]. Average $\delta^{18}O$ of *Brontops* ($23.0 \pm 1.0‰$) is significantly lower than the values of all the other taxa (Fig. 4C, Table 2). Discarding the M2s and the premolars and considering only the M3s would cause *Merycoidodon* to have the lowest mean $\delta^{18}O$ ($21.9 \pm 1.0‰$) but would not change the values of the other taxa significantly.

Similarly to the Eocene samples, average $\delta^{18}O$ of Oligocene *Mesohippus* ($24.5 \pm 2.0‰$), *Merycoidodon* ($23.9 \pm 2.0‰$), and *Leptomeryx* ($23.7 \pm 0.9‰$) are indistinguishable. *Subhyracodon* mean $\delta^{18}O$ ($24.8 \pm 1.4‰$) is higher than the value of *Leptomeryx* but not different from the values of *Mesohippus* and *Merycoidodon*. *Poebrotherium* mean $\delta^{18}O$ ($26.6 \pm 0.9‰$) is significantly higher than the values of all the other taxa (Fig. 4D, Table 3). Again, considering only the M3s would cause *Merycoidodon* to have the lowest $\delta^{18}O$ ($22.0 \pm 1.2‰$) but would leave the average values of the other taxa virtually unchanged.

None of the taxa shows a significantly different Eocene versus Oligocene mean $\delta^{18}O$, with the exception of *Leptomeryx*. The difference in this case is, however, only marginally significant [$P = 0.05$, Mann–Whitney; $P = 0.044$, *t*-test; $n = 24$ (Eocene), $n = 13$ (Oligocene)].

5. Discussion

The interpretation of $\delta^{13}C$ values in terms of dietary reconstructions is fairly straightforward: all the investigated ungulate genera were feeding predominantly on C3 vegetation. None of the investigated enamel samples has a $\delta^{13}C$ higher than $-6‰$ which can be considered a "cutoff" value for the identification of an important component of C4 grasses in the diet of Eocene–Oligocene mammalian herbivores. However, because fossil grasses are known from the early Eocene (Crepet and Feldman, 1991), a small fraction of C4 biomass might have been present in the early Tertiary Great Plains and might have been incorporated in the diet of the investigated taxa. As a result, the inter-generic differences in $\delta^{13}C$ may reflect habitat preference or subtle differences in the abundance of C3 versus C4 plants in the diet.

If we assume a small C4 component in Eocene and Oligocene ecosystems, a percentage of C3 versus C4 plant biomass in the diet of each taxon can be calculated. These calculations assume a constant, Eocene–Oligocene $\delta^{13}C$ of C3 and C4 plants of $-24.5‰$ and $-10.5‰$, respectively. Results indicate that all the taxa consumed more than 80% C3 plants. *Brontops* had the highest average C3 intake (100%), *Subhyracodon* the lowest (85%). Consumption of C3 vegetation for *Mesohippus*, *Merycoidodon*, *Leptomeryx*, and *Poebrotherium* was $\sim 90\%$. This assumption implies that the geographical differences in average $\delta^{13}C$ (i.e., Toadstool Park versus Badlands and Torrington Quarry) exhibited by *Mesohippus* and *Subhyracodon* may partially reflect regional variations in the abundance of C3 versus C4 vegetation in the ancient ecosystems. Similarly, the higher Oligocene versus Eocene average $\delta^{13}C$ exhibited by *Leptomeryx* can be interpreted to reflect a stronger preference for C4 plants of the new Oligocene species *Leptomeryx evansi* relative to the Eocene species *Leptomeryx speciosus*.

A different interpretation of the $\delta^{13}C$ values assumes the presence of only C3 plants in Eocene–Oligocene ecosystems. Under this scenario, the inter-taxa variability in $\delta^{13}C$ values likely reflects differences in habitat preference. Hence, using a $\delta^{13}C$ of atmospheric CO_2 calculated from foraminifera compositions ($-5.5‰$), the average Eocene $\delta^{13}C$ value exhibited by *Mesohippus*, *Merycoidodon* and *Leptomeryx* ($\sim -10‰$) is consistent with a preference of these taxa for woodlands. Conversely, the lower mean value exhibited by *Brontops* ($\sim -11‰$) suggests a preference for closer forests and the higher mean value of *Subhyracodon* ($\sim -9‰$) is indicative of drier and/or more open habitats (e.g., grasslands). With respect to the Oligocene samples, the average $\delta^{13}C$ exhibited by *Mesohippus* and *Merycoidodon* is still indicative of a preference for woodlands. However, the very high range in $\delta^{13}C$ ($\sim 5‰$) exhibited by *Mesohippus* suggests, as indicated by Clark et al. (1967) and Retallack (1983), the presence of this taxon in different habitats including open plains. Oligocene *Subhyracodon*, *Poebrotherium*, and *Leptomeryx* show higher mean $\delta^{13}C$ relative to the sympatric *Mesohippus* and *Merycoidodon* indicating a stronger preference for more open and/or xeric environments. This finding is consistent with the cursorial features of these taxa which suggest that they were well adapted to running on dry grounds in open-country conditions.

The pure C3 assumption implies that geographical differences in $\delta^{13}C$ reflect regional variations in vegetation openness and/or water stress conditions rather than variation in C3 versus C4 plant abundance. Also, the increase in $\delta^{13}C$ across the Eocene–Oligocene transition exhibited by *Leptomeryx* would reflect a stronger preference of *Leptomeryx evansi* (relative to

L. speciosus) for more open and/or xeric environments rather than for C4 plants. In contrast to *Leptomeryx*, the indistinguishable Eocene versus Oligocene $\delta^{13}C$ value exhibited by the other taxa implies that their habitat did not change significantly as a result of the climate change of the Eocene–Oligocene transition. Quite surprisingly, neither interpretation supports the presence of closed-canopy conditions in both Eocene and Oligocene samples. In fact, the lowest $\delta^{13}C$ value observed in White River mammals is −13.1‰, obtained for the *Mesohippus* premolar F:AM 141355. Subtracting the enamel-diet enrichment factor (13.3‰) would yield a composition for the plants of −26.4‰. This value, although lower than an average Eocene–Oligocene C3 value (∼−25‰), is still within the range of "normal" C3 ecosystems and does not clearly indicate closed-canopy conditions where values lower than ∼−28‰ would be expected.

Based only on the carbon isotope data obtained, it is not possible to determine without uncertainty whether the inter-taxa differences in $\delta^{13}C$ reflect different diet versus habitat preference or a combination of both. However, a few observations can be made in favor of a habitat hypothesis. First, sedimentological and paleosol studies (Clark et al., 1967; Retallack, 1983) indicate the presence of a mosaic of habitats in the Eocene–Oligocene Great Plains that included both open, xeric plains and forested, mesic environments. Under these conditions, C3 plants would exhibit a wide range of carbon isotope composition that could well explain the observed inter-taxa variability. Second, because C4 plants are less nutritious than C3 plants (Demment and Van Soest, 1985; Ehleringer and Monson, 1993), it is reasonable to assume that animals would try to incorporate as much C3 vegetation as possible. Therefore, even if a small component of C4 plants had been present in the Eocene–Oligocene Great Plains, most animals would have likely selected C3 plants, largely reducing the C4 isotopic signal in tooth enamel. Finally, our results in terms of habitat preference are generally consistent with the data obtained in most previous morphological and sedimentological studies (Clark et al., 1967; Retallack, 1983; Lander, 1998; Wall and Hauptman, 2001).

Concerning the oxygen isotope data, the similar or indistinguishable average $\delta^{18}O$ exhibited by *Mesohippus*, *Subhyracodon*, *Merycoidodon*, and *Leptomeryx* in both Eocene and Oligocene samples suggests similar water dependency for these taxa. Because the body size of *Leptomeryx* was more than an order of magnitude smaller than that of *Mesohippus*, *Subhyracodon* and *Merycoidodon*, this finding further indicates the unreliability of body-mass-based models in predicting enamel $\delta^{18}O$. In addition, because hind-gut fermentation in perissodactyls is thought to have originated in the early Eocene and requires, in contrast to fore-gut fermentation, large amounts of water intake, these results suggest that the artiodactyls *Merycoidodon* and *Leptomeryx* were either very water dependent fore-gut fermenters or that fore-gut fermentation had not yet developed in these late Eocene–early Oligocene artiodactyls. It is important to note, however, that a complex interaction of many physiological and ecological factors affect enamel $\delta^{18}O$. As indicated by Kohn (1996), important factors include mechanisms of heat loss (panting versus sweating, rate of transcutaneous water vapor loss) and dietary preferences (C3 versus C4 plants, leaves versus stems). These factors cannot be known for extinct taxa such as *Leptomeryx* and *Merycoidodon*. Our results, therefore, must be interpreted with caution.

Results for *Leptomeryx*, however, can be interpreted in light of the ecological and physiological characteristics of the extant lesser mouse-deer (*Tragulus javanicus*). This species, which is considered a descendant of the Leptomerycidae, is the world's smallest ruminant and the most primitive ungulate still in existence. As indicated by Nolan et al. (1995), *T. javanicus* is a very drought-tolerant animal. Its daily water intake (258 ml/day; 182 ml/kg $M^{0.82}$) is at the lower end of the range found for other ruminants in the humid tropics. Assuming for *Leptomeryx* a similar water intake as in *T. javanicus*, the relatively low $\delta^{18}O$ of *Leptomeryx* might seem in contrast with its assumed drinking habits. A possible explanation for our results is related to the fact that mouse-deer are nocturnal. Hence, by feeding at night when relative humidity is higher than during the day and plant and local water $\delta^{18}O$ are most similar, *Leptomeryx* might have assumed a composition similar to that of the diurnal, more water-dependent *Mesohippus* and *Subhyracodon*.

In contrast with the similar or indistinguishable $\delta^{18}O$ of *Mesohippus*, *Subhyracodon*, *Merycoidodon*, and *Leptomeryx*, the higher $\delta^{18}O$ of *Poebrotherium* possibly indicates lower water dependency for this taxon. A low water dependency in *Poebrotherium* may suggest that the behavioral and physiological adaptations that allow modern camels to cope with several days of water deprivation were already present in this early Oligocene camelid. More specifically, *Poebrotherium* could have possessed the large fore-gut that serves as a water reservoir in extant ruminants (Macfarlane et al., 1963; Hoppe et al., 1976). On the other hand, the low $\delta^{18}O$ of *Brontops* may indicate a high water dependency. However, since *Brontops* also shows a low $\delta^{13}C$ value, indicative of a dense forest habitat, its low $\delta^{18}O$ more likely results from the high humidity of this habitat which

leads to decreased $\delta^{18}O$ in leaf water and consequently in herbivores feeding on such plant material (Quade et al., 1995; Bocherens and Sen, 1998). It is noteworthy that *Brontops*, the only one among the investigated taxa showing a strong preference for a humid forested habitat, is also the only one that went extinct across the Eocene–Oligocene transition. With respect to aquatic versus terrestrial habitat preference in Eocene rhinocerotids, the higher $\delta^{18}O$ of *Trigonias* relative to *Subhyracodon* does not support previous hypotheses that *Trigonias* was semi-aquatic. Our data, however, do not rule out the possibility that *Trigonias* spent part of its daily life in water. Moreover, the sample size for *Trigonias* is too small for a statistically significant comparison. Further analyses of *Trigonias* teeth are therefore required to test the hypothesis of its semi-aquatic lifestyle.

In sum, our carbon data are consistent with a pure C3 diet for all of the investigated taxa. Stable carbon isotopes analyses cannot distinguish between C3 browsers and C3 grazer or mixer feeders, so resource partitioning in terms of browse versus graze cannot be investigated with this technique. However, White River mammals exhibit significant inter-taxa differences in average $\delta^{13}C$ suggesting niche partitioning in terms of habitat preference among *Brontops* (inhabiting forested areas), *Mesohippus–Merycoidodon–Leptomeryx* (inhabiting preferentially more open woodlands), and *Subhyracodon–Poebrotherium* (inhabiting open plains and/or xeric areas). Further niche separation could have been practiced by means not discernible through isotope analysis (e.g., selection of different plant taxa in the same habitat, differential preference for certain parts of the plants, different activity time).

With respect to oxygen isotopes, our data suggest similar water dependency for *Mesohippus*, *Merycoidodon*, *Subhyracodon*, and *Leptomeryx*. In contrast, data for *Poebrotherium* suggest a lower water dependency relative to the other taxa. Our results possibly indicate that fore-gut fermentation had not yet developed in late Eocene–early Oligocene oreodonts and leptomerycids. Janis and Constable (1993) suggested that, because hind-gut fermenters require larger jaw muscle and greater occlusal surface than fore-gut fermenters, digestive physiology in extinct ungulates can be inferred from studies of craniodental design. Perhaps future studies applying this technique will help resolve the uncertainties about the digestive physiology of these extinct clades.

6. Conclusions

The main goal of this study was to investigate various aspects of the paleoecology and digestive physiology of common White River mammals by analyzing the carbon and oxygen isotope composition of their teeth. The carbon isotope data presented here indicate diets consisting predominantly of C3 vegetation for all the investigated taxa, although the possibility of the consumption of small amounts of C4 plants is not excluded. If the diet were constituted by 100% C3 plants, as seems most likely, the inter-taxa differences in average $\delta^{13}C$ suggest niche partitioning in terms of habitat preference for both Eocene and Oligocene taxa. Specifically, *Brontops* likely inhabited preferentially mesic forests, *Merycoidodon* woodlands, *Subhyracodon*, and *Poebrotherium* open plains and/or xeric areas. Data for *Mesohippus* indicate its presence in different habitats; its average composition suggests however a preference for woodlands. According to our data, all the taxa except *Leptomeryx* were not affected in terms of their habitat and/or diet by the climate change of the Eocene–Oligocene transition. Data for *Leptomeryx* possibly indicate a habitat change from woodlands in the late Eocene to grasslands in the early Oligocene.

Interpretation of oxygen isotope data is complex because of the interaction of ecological and physiological factors that determine enamel $\delta^{18}O$. However, our data are consistent with low water dependency in *Poebrotherium* and higher in *Mesohippus*, *Merycoidodon*, *Subhyracodon*, and *Leptomeryx*. The low water dependency inferred for *Poebrotherium* possibly indicates the presence of an enlarged fore-gut in this early Oligocene camelid. In contrast, the high water dependency inferred for *Merycoidodon* and *Leptomeryx* perhaps indicates the absence or incomplete development of fore-gut fermentation in late Eocene–early Oligocene oreodonts and leptomerycids.

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