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PALEOECOLOGY OF NEBRASKA'S UNGULATES DURING THE EOCENE-
OLIGOCENE CLIMATE TRANSITION

by

Grant Stanley Boardman

A DISSERTATION

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PALEOECOLOGY OF NEBRASKA'S UNGULATES DURING THE EOCENE- OLIGOCENE CLIMATE TRANSITION

Grant Stanley Boardman, Ph.D.

University of Nebraska, 2013

Adviser: Ross Secord

The White River Group (WRG) preserves the Eocene-Oligocene climate transition (EOCT), an interval of global cooling and drying during the onset of Antarctic glaciation. In the Great Plains, a shift from forested conditions to drier woodland-savanna biomes is hypothesized to have occurred at this time. I test this hypothesis through the analyses of several paleoenvironmental proxies on the teeth of 12 WRG ungulate species: stable carbon and oxygen isotopes from tooth enamel, and mesowear and microwear texture. The EOCT shift toward more open habitats and lower vegetation density under drying climates should have resulted in an increase in mean carbon isotope values in vegetation and increase in abrasive ingesta in ungulates. These trends, in turn, should be reflected in the carbon isotope values of WRG ungulate teeth, as well as in their patterns of meso- and microwear. Data gathered for this study are grouped into two time-averaged faunas: one from the Chadronian North American land-mammal age (NALMA) (latest Eocene) and one from the Orellan NALMA (earliest Oligocene). Isotopic results suggest that both faunas inhabited drier, open canopied biomes, such as woodland-savanna or scrubland, and that wetter habitats became restricted in extent during the Orellan due to decreasing rainfall. Mesowear results suggest that the diets of several taxa living in open

habitats during the Chadronian were already abrasive enough to suggest mixed-feeding, and that all but one range-through taxon had static diets through this interval. Only the oreodont *Merycoidodon* sp. shows evidence for having had a more abrasive diet during the Orellan than it did during the Chadronian, suggesting that the expansion of open habitats led to greater utilization of grasses by this oreodont. Results from microwear texture analyses of extant taxa suggest that it is a useful tool in characterizing diets for WRG species. These results provide a better understanding of the effects of climate change on mammals in Nebraska during the EOCT.

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CHAPTER 1

INTRODUCTION

The Eocene-Oligocene climate transition (EOCT), is often cited as the most important interval of climate change during the Cenozoic because it heralds the switch from the warmer, equable, global greenhouse climates of the late Mesozoic and early Paleogene to the cooler, more seasonal, icehouse climates. Marine proxy data suggest significant cooling of mid- to high-latitude ocean temperatures (5-6°C) over a short interval during the earliest Oligocene, beginning at about 33.7 Ma (Zachos et al., 2008; Liu et al., 2009; Miller et al., 2009). This cooling coincided with the onset of continental glaciation in Antarctica and with changes in patterns of ocean circulation. Changes in the marine realm had a profound effect on invertebrates, causing major faunal turnover (Dockery and Louzet, 2003; Nesbitt, 2003; Pearson et al., 2008). The EOCT also had a significant, but heterogeneous effect on the continents, generally in the form of cooling. This effect coincides with major mammalian faunal turnover and immigration in places like Europe, but in North America the contemporaneous effect on mammalian faunal turnover seems comparatively minor (Prothero, 1999; Alroy et al., 2000; Hooker et al., 2009). Paleosols and non-mammalian faunas, in contrast, indicate fairly significant changes in aridity and vegetation structure (Hutchison, 1982, 1992; Evanoff et al., 1992; Retallack, 1992, 2007; Terry, 2001; Sheldon, 2009). In general these proxies suggest a change from semi-humid, forested conditions in the latest Eocene to progressively more arid and more open conditions in the earliest Oligocene. Though these changes may not have significantly affected mammalian turnover rates, I do expect to see changes in

aridity and vegetation structure reflected by the mammals in the form of changing habitats and diet.

This work compiles various proxy data from ungulates (hoofed-mammals) in an attempt to better characterize the ecological effects of the EOCT in the North American mid-continent. These proxies include: stable carbon and oxygen isotopes from tooth enamel, and mesowear and microwear analyses. A change to more open habitats should result in a mean increase in carbon isotope values in vegetation and an increase in abrasive ingesta, due to a more arid climate with lower vegetation density. These things should be reflected in the carbon isotope values, and in meso- and microwear texture measures of WRG ungulate teeth. Data gathered for this study are grouped into two time-averaged faunas: one from the Chadronian North American land-mammal age (NALMA) (latest Eocene) and one from the Orellan NALMA (early Oligocene).

Organization of chapters

The three research chapters that follow are intended to be stand-alone manuscripts to be submitted for peer review and eventual publication. Each research chapter addresses in a different manner the central themes of: (1) characterizing the paleohabitats and paleoecologies of ungulate species from the White River Group (WRG) of Nebraska, (2) and examining possible changes in ecosystem structure and ecology during the EOCT. For this reason, there is some reiteration of content, especially with respect to background about the WRG ungulates and the EOCT. Also, each chapter is formatted for the journal I intend to submit it to (or have submitted it to): Chapter 2 is published and uses the format of the Elsevier Journal *Paleogeography, Palaeoclimatology, Palaeoecology*; Chapters 3 and 4 are also currently formatted this way though after further consideration they may be

submitted to different journals. All figures, tables, and appendices are placed at the ends of the chapters.

Chapter 2: Stable isotope paleoecology of White River ungulates during the Eocene-Oligocene climate transition in northwestern Nebraska

In Chapter 2, I use stable carbon and oxygen isotopes from the carbonate portion of tooth enamel from WRG ungulates. Stable carbon isotopes have been employed to establish habitat partitioning in ancient herbivorous mammals and general biome occupation of faunas. Stable oxygen isotopes can also be used for establishing water dependence and climatic factors such as local humidity (Levin et al., 2006; Secord et al., 2008, 2010, 2012; Zanzazi and Kohn, 2008, Tütken and Vennemann, 2009). The primary goal of this chapter is to test the hypothesis that habitats became more open in response to increasing aridity in the Orellan by evaluating biomes, habitat partitioning, and water use among ungulates.

Chapter 3: Local feeding ecology of ungulates from northwestern Nebraska during the Eocene-Oligocene climate transition

The analysis of dental mesowear (Fortelius and Solounias, 2000), characterizes the shapes and relief of the cusps of fossil ungulate teeth as a proxy for the abrasiveness of diets. Ungulates with low-abrasion diets dominated by browse generally retain sharp cusps, whereas those with higher-abrasion diets including grasses have more rounded cusps. The evolution of hypsodont (high crowned) teeth is typically interpreted as an adaptation to compensate for excess wear incurred by increasingly abrasive diets. The acquisition of hypsodonty, however, has been shown to lag behind to onset of abrasive diets. In Chapter 3, I use new mesowear data and previously published hypsodonty data

from WRG ungulates to establish proxy diets and to test for a temporal shift to more abrasive diets in the Orellan, which would be expected with a change to more open habitats. The primary goals of this chapter are to establish the diets of particular taxa and test whether diets became more abrasive and/or teeth became more hypsodont, and therefore better adapted for the processing of abrasive diets, through this interval.

Chapter 4: Testing the utility of dental microwear texture as a proxy for diet in extant and fossil non-ruminant ungulates

Dental microwear texture analysis—a relatively new and highly automated method that examines three-dimensional wear surfaces—has been employed to reconstruct the diets of extant and extinct primates, carnivorans, and ruminant artiodactyls (Scott et al., 2005, 2009; Ungar et al. 2007, 2008, 2010, 2012; Scott, 2012). Scott (2012) and Ungar et al. (2012) demonstrated that this method is successful in differentiating diets in modern and fossil ruminant artiodactyls, but until now this method had not been applied rigorously to non-ruminant ungulates. In Chapter 4, I compile microwear texture data from extant perissodactyls and non-ruminant artiodactyls to establish a reference texture database for these groups. I then use this dataset to interpret diets for the WRG ungulates. The primary goals of this chapter are to demonstrate the utility of microwear texture analysis in characterizing the diets of extant and fossil non-ruminant ungulates, and to compare interpreted diets using texture analysis for the WRG ungulates to interpretations made on the basis of mesowear and other diet proxies.

References Cited

- Alroy, J., Loch, P.L., Zachos, J.C., 2000. Global climate change and North American mammalian evolution. *Paleobiology* 26, 259-288.
- Dockery, D. T., Lozouet, P., 2003. Molluscan faunas across the Eocene/Oligocene boundary in the North American Gulf Coastal Plain, with comparisons to those of the Eocene and Oligocene of France, in: Prothero, D.R., Ivany, L.C., Nesbitt, E.A. (Eds.), *From greenhouse to icehouse: the marine Eocene-Oligocene transition*. Columbia University Press, New York, pp. 303-340.
- Evanoff, E., Prothero, D.R., Lander, R.H., 1992. Eocene-Oligocene climatic change in North America: the White River Formation near Douglas, east-central Wyoming, in: Prothero, D.R., Berggren, W.A. (Eds.), *Eocene-Oligocene Climatic and Biotic Evolution*. Princeton University Press, Princeton, pp. 116-130.
- Fortelius, M., Solounias, N., 2000. Functional characterization of ungulate molars using the abrasion-attrition wear gradient: a new method for reconstructing paleodiets. *American Museum Novitates* 3301, 1-36.
- Hooker, J.J., Grimes, S.T., Matthey, D.P., Collinson, M.E., Shedlon, N.D., 2009. Refined correlation of the UK Late Eocene–Early Oligocene Solent Group and timing of its climate history. *Geological Society of America Special Paper* 452, 179-195.
- Hutchison, J.H., 1982. Turtle, crocodilian, and champsosaur diversity changes in the Cenozoic of the north-central region of western United States. *Palaeogeography, Palaeoclimatology, Palaeoecology* 37, 149-164.
- Hutchison, J.H., 1992. Western North America reptile and amphibian record across the Eocene/Oligocene boundary and its climatic implications, in: Prothero, D.R.,

- Berggren, W.A. (Eds.), Eocene-Oligocene Climatic and Biotic Evolution. Princeton University Press, Princeton, pp. 451-463.
- Levin, N.E., Cerling, T.E., Passey, B.H., Harris, J.M., Ehleringer, J.R., 2006. A stable isotope aridity index for terrestrial environments. PNAS 103, 11201-11205.
- Liu, Z.-H., Pagani, M., Zinniker, D., DeConto, R., Huber, M., Brinkhuis, H., Shah, S.R., Leckie, R.M., Pearson, A., 2009. Global Cooling During the Eocene-Oligocene Climate Transition. Science 323, 1187-1190.
- Miller, K., Wright, J.D., Katz, M.E., Wade, B.S., Browning, J.V., Cramer, B.S., Rosenthal, Y., 2009. Climate threshold at the Eocene-Oligocene transition: Antarctic ice sheet influence on ocean circulation. Geological Society of America Special Paper 452, 169-178.
- Nesbitt, E.A., 2003. Changes in shallow-marine faunas from the northeastern Pacific margin across the Eocene/Oligocene boundary, in: Prothero, D.R., Ivany, L.C., Nesbitt, E.A. (Eds.), From greenhouse to icehouse: the marine Eocene-Oligocene transition. Columbia University Press, New York, pp. 57-70.
- Pearson, P.N., McMillan, I.K., Wade, B.S., Jones, T.D., Coxall, H.K., Bown, P.R., H. Lear, C.H., 2008. Extinction and environmental change across the Eocene-Oligocene boundary in Tanzania. Geology 36, 179-182.
- Prothero, D.R., 1999. Does climatic change drive mammalian evolution? GSA Today 9, 1-5.
- Retallack, G.J., 1992. Paleosols and changes in climate and vegetation across the Eocene/Oligocene boundary, in: Prothero, D.R. and Berggren, W.A. (Eds.),

- Eocene-Oligocene Climatic and Biotic Evolution. Princeton University Press, Princeton, pp. 382-398.
- Retallack, G.J., 2007. Cenozoic paleoclimate on land in North America. *Journal of Geology* 115, 271-294.
- Scott, J.R., 2012. Dental microwear texture analysis of extant African Bovidae. *Mammalia* 6, 157-174.
- Scott, R.S., Ungar, P.S., Bergstrom, T.S., Brown, C.A., Grine, F.E., Teaford, M.F., Walker, A., 2005. Dental microwear texture analysis shows within-species diet variability in fossil hominins. *Nature* 436, 693-695.
- Scott, J.R., Godfrey, L.R., Jungers, W.L., Scott, R.S., Simons, E.L., Teaford, M.F., Ungar, P.S., Walker, A., 2009. Dental microwear texture analysis of two families of subfossil lemurs from Madagascar. *Journal of Human Evolution* 56, 405-416.
- Secord, R., Wing, S.L., Chew, A., 2008. Stable isotopes in early Eocene mammals as indicators of forest canopy structure and resource partitioning. *Paleobiology* 34, 282-300.
- Secord, R., Gingerich, P. D., Lohmann, K. C., MacLeod, K. G., 2010, Continental warming preceding the Palaeocene-Eocene thermal maximum. *Nature* 467, 955-958.
- Secord, R., Bloch, J. I., Chester, S. G. B., Boyer, D. M., Wood, A. R., Wing, S. L., Kraus, M. J., McInerney, F. A., Krigbaum, J., 2012, Evolution of the earliest horses driven by climate change in the Paleocene-Eocene Thermal Maximum. *Science* 335, 959-962.

- Sheldon, N.D., 2009. Nonmarine records of climatic change across the Eocene-Oligocene transition. *Geological Society of America Special Paper* 452, 241-248.
- Terry, D.O. Jr., 2001. Paleopedology of the Chadron Formation of northwestern Nebraska: implications for paleoclimatic change in the North American midcontinent across the Eocene-Oligocene boundary. *Palaeogeography, Palaeoclimatology, Palaeoecology* 168, 1-38.
- Tütken, T., Vennemann, T., 2009. Stable isotope ecology of Miocene large mammals from Sandelzhausen, southern Germany. *Paläontologische Zeitschrift* 83, 207-226.
- Ungar, P.S., Merceron, G., Scott, R.S., 2007. Dental microwear texture analysis of Varswater bovids and early Pliocene paleoenvironments of Langebaanweg, Western Cape Province, South Africa. *Journal of Mammalian Evolution* 14, 163-181.
- Ungar, P.S., Grine, F.E., Teaford, M.F., 2008. Dental microwear and diet of the Pliocene hominin *Paranthropus boisei*. *PLoS One* 3(4): e2044.
- Ungar, P.S., Scott, R.S., Grine, F.E., Teaford, M.F., 2010. Molar microwear textures and diets of *Australopithecus anamensis* and *Australopithecus afarensis*. *Transactions of the Royal Society B* 365, 3345-3354.
- Ungar, P.S., Scott, J.R., Curran, S.C., Dunsworth, H.M., Harcourt-Smith, W.E.H., Lehmann, T., Manthi, F.K., McNulty, K.P., 2012. Early Neogene environments in East Africa: Evidence from dental microwear of tragulids. *Palaeogeography, Palaeoclimatology, Palaeoecology* 342-343, 84-96.

- Zachos, J.C., Dickens, G.R., Zeebe, R.E., 2008. An early Cenozoic perspective on greenhouse gas warming and carbon-cycle dynamics. *Nature* 451, 279–283.
- Zanazzi, A., Kohn, M.J., 2008. Ecology and physiology of White River mammals based on stable isotope ratios of teeth. *Palaeogeography, Palaeoclimatology, Palaeoecology* 257, 22-37.

CHAPTER 2

Stable isotope paleoecology of White River ungulates during the Eocene-Oligocene climate transition in northwestern Nebraska

(Published as Boardman and Secord, 2013)

Abstract

This study analyzes carbon and oxygen stable isotopes from mammalian tooth enamel to evaluate ancient biomes, habitat partitioning, and water use among 12 ungulate taxa from the White River Group in Nebraska. The White River Group spans an interval of climate change from the upper Eocene to the lower Oligocene when global climate cooled in a heterogeneous fashion. This study tests whether changes in biome structure or mammalian ecology occurred during this interval. I found that mean $\delta^{13}\text{C}$ values for both Eocene and Oligocene faunas are consistent with drier, open biomes, that is, woodland-savanna or woody scrubland, rather than closed-canopy forest. A significant increase in mean faunal $\delta^{13}\text{C}$ values (+0.4‰) occurs in the lower Oligocene, primarily from the loss of individuals feeding in wetter-denser parts of the ecosystem. In the upper Eocene, the brontothere *Megacerops* sp. and the peccary *Perchoerus probus* have low mean $\delta^{13}\text{C}$ values consistent with feeding in wetter habitats (presumably riparian), whereas significantly higher mean values in the cursorial horse, *Meshippus* spp., and the clawed oreodont, *Agriochœrus antiquus*, are consistent with feeding in more open, drier habitats. The extinctions of *Megacerops* sp. and *Trigonias* sp., a rhinocerotid yielding relatively low $\delta^{13}\text{C}$ values, along with a decrease in the abundance of *P. probus* and other taxa, suggests a reduction in the extent of riparian habitats in the Oligocene. The rare tapir

Colodon sp. is the only taxon sampled with a mean $\delta^{13}\text{C}$ value low enough to indicate consistent feeding in a riparian habitat in the Oligocene. However, range-through taxa occupy the same relative positions in isotopic space in both the Eocene and Oligocene faunas suggesting stability in drier habitats. A significant increase also occurs in mean faunal $\delta^{18}\text{O}$ values (+1.0‰) in the Oligocene, primarily from the loss or reduction in abundance of water dependent species. Variance in $\delta^{18}\text{O}$ values increases significantly in the Oligocene, which is consistent with greater water stress in plants from drier conditions. Oxygen values increase in *A. antiquus* (+0.9‰), the most aridity-sensitive species in both faunas, as expected for drier conditions, but values in another aridity-sensitive taxon (*Mesohippus* spp.) are virtually unchanged. Together, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ enamel values suggest a shift to drier conditions in the early Oligocene although $\delta^{13}\text{C}$ values yielded more consistent results. The lack of a large negative shift in $\delta^{18}\text{O}$ values, expected with a decrease in mean annual temperature, suggests that any temperature changes in the early Oligocene were relatively small in the continental interior of North America.

Keywords: Paleoecology; stable isotopes; mammals; Eocene-Oligocene Climate Transition; Chadronian; Orellan.

1. Introduction

The transition from an equable, warmer climate in the Eocene to a cooler Oligocene climate is often seen as one of the most significant intervals of climate change in the Cenozoic. Marine studies suggest that mid- to high-latitude ocean temperatures

decreased globally by 5-6 °C over a geologically short interval of ~300 thousand years in the earliest Oligocene (Zachos et al., 2001, 2008; Liu et al., 2009; Miller et al., 2009).

Cooling coincided with large-scale continental glaciation in Antarctica and with the modification of ocean circulation patterns (Coxall et al., 2005; Katz et al., 2008; Lear et al., 2008; Zachos et al., 2008; Miller et al., 2009). In the marine realm, many invertebrate groups underwent major faunal turnover during this interval (e.g. Dockery and Lozouet, 2003; Nesbitt, 2003; Hansen et al., 2004; Pearson et al., 2008).

Studies of terrestrial sequences spanning the Eocene-Oligocene climate transition (EOCT) indicate that significant changes also occurred on the continents at mid-latitudes (Retallack, 1992; Collinson, 1992; Evanoff et al., 1992; Legendre and Hartenberger, 1992; Hooker, 1998; Myers, 2003; Hooker et al., 2004; Zanazzi et al., 2007), but that cooling was not uniformly distributed (Kohn et al., 2004; Grimes et al., 2005; Hooker et al., 2009). In Europe, for instance, cooling is associated with mammalian faunal turnover and immigration (Hooker et al., 2009), but in North America mammalian extinction rates were modest (Prothero and Heaton, 1996; Prothero, 1999; Alroy et al., 2000). A temperature drop as severe as 8.2 ± 3.1 °C was suggested for the mid-continent of North America (Zanazzi et al., 2007). However, most lines of evidence suggest increasing aridity with only minimal cooling (< 2 °C). Evidence for increased aridity includes changes in soil structure and root trace size (Retallack, 1992; Terry, 2001), geochemical trends suggesting a ~300 mm decrease in mean annual precipitation (Sheldon and Retallack, 2004; Retallack, 2007; Sheldon, 2009), turnover in the terrestrial gastropod fauna (Evanoff et al., 1992), and a rapid decline of the aquatic herpetofauna (Hutchison, 1982, 1992); all of these are consistent with a change to a more open biome in the early

Oligocene. Despite these corroborative proxies, several other studies indicate essentially no change in vegetation structure in Nebraska through this interval. Phytolith assemblages remain static (Strömberg 2004, 2005) and a study using mammalian isotopes from six ungulate taxa suggested that habitats remained stable through the EOCT (Zanazzi and Kohn, 2008).

In this study the stable carbon and oxygen isotope compositions of a large diversity of mammalian ungulates from faunas before and after the EOCT are used to test the hypothesis that vegetation structure became more open in the early Oligocene in response to greater aridity. To accomplish this, I infer biomes and habitats for the late Eocene and early Oligocene by comparing fossil compositions to those predicted for various environments using the isotopic compositions of modern floras. The isotopic compositions of seven extinct genera from the upper Eocene and lower Oligocene are analyzed here for the first time, greatly increasing the diversity of mammal species studied from this interval. This allows me to test for habitat partitioning among a wider range of species than in the previous study (Zanazzi and Kohn, 2008) and provides more accurate mean isotopic values for the faunas. As in the previous study, the fossils used here come from the White River Group (WRG) in northwestern Nebraska (Fig. 1), although our fossils were collected from different areas, and may be from different stratigraphic levels (levels were not published in the previous study).

A shift to a more open biome during the early Oligocene should have resulted in increased enamel carbon isotope values ($\delta^{13}\text{C}_\text{E}$) as some taxa shift to feeding in more open habitats and taxa occupying wetter-denser habitats are reduced in number or become extinct. It is further expected that a shift to more arid conditions will result in an

upward shift in enamel oxygen isotope values ($\delta^{18}\text{O}_\text{E}$) in aridity-sensitive species, relative to aridity-insensitive species, and that a marked decrease in mean annual temperature should result in a marked decrease in mean $\delta^{18}\text{O}_\text{E}$ faunal values.

2. Stable isotopes in mammals as paleoecological proxies

2.1. Carbon isotopes in mammalian tooth enamel

The carbon isotope composition of mammalian tooth enamel is a direct reflection of an animal's diet, with predictable metabolic enrichment (Lee-Thorp and van der Merwe, 1987). Carbon isotope values in medium to large ungulates are typically enriched by ~13 to 14‰ above the vegetation they consume (Cerling and Harris, 1999; Passey et al., 2005). Pre-Miocene biomes in the North American mid-continent were dominated by vegetation using the C_3 photosynthetic pathway (Calvin Cycle), with little or no influence from plants utilizing other photosynthetic pathways, such as CAM or C_4 (Cerling et al., 1993, 1997; Ehleringer and Monson, 1993; Tipple and Pagani, 2007; Secord et al., 2008; Zanzari and Kohn, 2008; Edwards et al., 2010). Thus, I assume that the primary influence on $\delta^{13}\text{C}_\text{E}$ values in the WRG faunas was from C_3 vegetation. C_3 plants include most trees, shrubs, and high-altitude or high-latitude grasses. Modern C_3 plants have a global mean $\delta^{13}\text{C}$ value of ~-27‰ and range from -21‰ to -37‰ (O'Leary, 1988, Cerling and Ehleringer, 2000).

The wide range of $\delta^{13}\text{C}$ values exhibited by C_3 vegetation is largely the result of environmental factors that vary among habitats, such as the availability of water and

nutrients, and the amount of solar radiation (O'Leary, 1988; Farquhar et al., 1989; van der Merwe and Medina, 1991; Ehleringer and Monson, 1993; Koch, 1998; Heaton, 1999). Leaf $\delta^{13}\text{C}$ values increase under conditions of water stress (Ehleringer et al., 1986; Stewart et al., 1995) and increasing amounts of solar radiation (Ehleringer et al., 1986). A third factor is the recycling of ^{13}C -depleted CO_2 under forest canopies, resulting in lower $\delta^{13}\text{C}$ leaf values (van der Merwe and Medina, 1991). Together these factors result in the most negative $\delta^{13}\text{C}$ values for C_3 plants occurring in closed canopy biomes, such as rainforests, and the most positive ones in dry open canopy biomes, such as woody scrublands (Farquhar et al., 1989; Stewart et al., 1995). The patterns seen in plant $\delta^{13}\text{C}$ values are reflected in herbivorous mammalian consumers, which provide a useful proxy for inferring ancient biomes, and habitat use within those biomes (Ambrose and DeNiro, 1986; Cerling and Harris, 1999; Cerling et al., 2004; Feranec, 2007).

2.2. *Oxygen isotopes in mammalian tooth enamel*

The oxygen isotope composition of mammalian tooth enamel can be used to infer drinking behavior and habitat preference for a taxon, as well as local climatic conditions such as humidity (Kohn, 1996; Kohn et al., 1996; Levin et al., 2006; Secord et al., 2008, 2010, 2012; Zanazzi and Kohn, 2008; Tütken and Vennemann, 2009). The factors influencing the $\delta^{18}\text{O}$ composition of mammalian bioapatites have been considered in detail elsewhere (Bryant and Froelich, 1995; Kohn, 1996), and will be described only briefly here. The oxygen isotopic composition of mammalian tissues is determined by the composition of a mammal's body water and temperature at the time of apatite formation.

Because most mammals maintain a fairly constant body temperature of $\sim 37^{\circ}\text{C}$, variation in $\delta^{18}\text{O}_{\text{E}}$ values among taxa is caused primarily by behavioral and physiological differences centering on whether or not the animal attains most of its water from drinking or from its food (Bryant and Froelich, 1995; Kohn, 1996).

Water-dependent taxa (i.e., frequent or obligate drinkers) should have $\delta^{18}\text{O}$ values more reflective of local drinking water (Bryant and Froelich, 1995; Kohn, 1996). These taxa, which are insensitive to ^{18}O -enrichment from the evaporation of leaf water or from physiological factors, have been called aridity-insensitive (EI taxa; Levin et al., 2006). In contrast, those that are less reliant on drinking and/or that get a significant part of their water from leaves are aridity-sensitive (ES taxa; Levin et al., 2006). In general, the highest $\delta^{18}\text{O}$ values in leaf water are expected to occur in open areas with high irradiance because of higher rates of evapotranspiration at the leaf surface, and the lowest values in more densely vegetated areas (Quade et al., 1995; Cerling et al., 2004; Feranec and MacFadden, 2006).

3. Materials and methods

The White River Group in northwestern Nebraska contains a rich record of superposed mammalian faunas during the latest Eocene and early Oligocene epochs. Fossils used here come from exposures of the Big Cottonwood Creek Member (BCCM) of the upper Chadron Formation and the Orella Member of the lower Brule Formation of the WRG in and around Toadstool Geologic Park, northwestern Nebraska (Fig. 1). Faunas from the former units were the original basis for the Chadronian and Orellan

North American Land Mammal “ages” (Wood et al., 1941; Woodburne 1987, 2004; Prothero and Emry, 2004). The boundaries for these informal “ages” are established on the first occurrences of mammal species alone (Fig. 2). Current durations for the “ages” are ~37.0 – 33.7 Ma for the Chadronian (late Eocene) and ~33.7 – 32.0 Ma for the Orellan (earliest Oligocene; Prothero and Emry, 2004).

A total of 114 samples from twelve genera (Table 1) of WRG ungulates were analyzed for this study (Appendix 1) – seven of them (*Colodon* sp., *Hyracodon nebraskensis*, *Perchoerus probus*, *Eotylopus reedi*, *Archaeotherium mortoni*, *Aepinacodon americanum*, and *Agriochoerus antiquus*) for the first time. The other five (except *Trigonias* sp.) were previously sampled by Zanazzi and Kohn (2008) from localities in the Toadstool Park area not represented in the University of Nebraska State Museum (UNSM) collections. I sampled mostly common taxa in order to get a good average representation of the environment, but I also sampled a few rare species in an attempt to include a wide range of habitats. Several taxa were sampled only from the Chadronian either because they were extinct by the Orellan (*Megacerops* sp., *Trigonias* sp., and *Eotylopus reedi*) or they became rare in the Orellan (*Aepinacodon americanum* and *Perchoerus probus*), in spite of a much larger Orellan collection. The tapir *Colodon* sp. is not present in the Chadronian collection at UNSM and is rare in the Orellan collection. The other six taxa (*Meshippus* spp., *Hyracodon nebraskensis*, *Subhyracodon* sp., *Merycoidodon* sp., *Agriochoerus antiquus*, and *Archaeotherium mortoni*) were sampled from both the Chadronian and Orellan. Table 1 includes a summary of suggested diets and habitats for these taxa at the generic level based on published studies. All specimens are curated at UNSM.

Most specimens used in this study were collected by parties from UNSM led by Schultz and Stout. Schultz and Stout (1955) established a series of loosely characterized, local “zones” for the White River Group (Fig. 2). Although the utility of the Schultz and Stout zones has been questioned (e.g., Prothero and Emry, 2004; Zanazzi et al., 2009), the stratigraphic distance of specimens collected by Schultz and Stout above or below marker beds (especially the upper purplish white [UPW] a dated volcanic ash) was often precisely recorded in field notes, allowing us to place many of these specimens into a well-resolved, sub-meter level, stratigraphic context. The UNSM collections can be placed in a strong geochronologic framework (Fig. 2) of paleomagnetic zones (Prothero and Swisher, 1992) and age estimates from volcanic ashes (Obradovich et al., 1995).

Of particular interest to my study is the placement of the Eocene-Oligocene (E-O) boundary, and the EOCT. On the basis of previous work, both the E-O boundary and the most rapid interval of cooling during the EOCT should occur in the upper part of polarity zone C13r, locally in “Orella A” of Schultz and Stout (Fig. 2). The Chadronian-Orellan boundary is approximately coincident with the E-O boundary, following the biostratigraphy of Zanazzi et al. (2009). The Chadronian-Orellan boundary had long been defined by the last appearance of brontotheres (Wood et al., 1941) but this definition has subsequently been challenged due to claimed diachrony of these last appearances among WRG exposures in Wyoming and Nebraska (Prothero and Whittlesey, 1998). The boundary was redefined by Prothero and Whittlesey (1998) based on the first appearances of the small ungulates *Hypertragulus calcaratus*, *Leptomeryx evansi*, and *Poebrotherium wilsoni*. Due to unresolved issues with sampling and possible minor diachrony of these

first appearances at Toadstool Park, Zanazzi et al. (2009) conservatively placed the Chadronian-Orellan boundary at 2 ± 5 m above the UPW, which I follow here (Fig. 2).

3.1. Sample treatment and statistical analysis

Isotopic sampling was done under a binocular microscope using a mounted, variable speed dental drill. Enamel powder (3-4 mg) was drilled from each tooth along a non-occlusal surface perpendicular to the growth axis using a 1 mm diamond burr. Care was taken to avoid decalcified or fractured enamel. Pretreatment followed Koch et al. (1997) except that samples were placed in a drying oven overnight at 60 °C to remove water, rather than being lyophilized. Samples were reacted with 2-3% reagent grade NaOCl for 24 hours to remove organic matter, rinsed five times with distilled water, and dried. Nonstructural carbonates were then removed by reacting samples in 1.0 M buffered acetic acid for 24 hours. This was again followed by rinsing and drying.

To avoid a potential bias from nursing, third molars, which form after weaning in extant eutherian mammals, were preferentially sampled. When third molars were not available, other late erupting teeth (e.g., fourth premolars, second molars) were sampled. The sampling of a single tooth position can also help to reduce variability from seasonal fluctuations in the $\delta^{18}\text{O}$ values of precipitation (e.g., Secord et al., 2010). This assumes that a species followed seasonal birthing cycles and that single teeth formed over only part of a year. These assumptions should be reasonable based on modern analogs of mammals in temperate climates (e.g., Hillson, 1986). Reducing seasonal variability in $\delta^{18}\text{O}$ values is desirable for comparing changes in $\delta^{18}\text{O}$ values between different time

intervals, but could cause isotopic separation among contemporaneous taxa biasing habitat interpretations. However, because of attenuation that takes place due to protracted mineralization (e.g., Passey et al., 2002) maximum seasonal differences in oxygen in serially sampled teeth are usually not more than a few per mil, and bulk sampling further attenuates seasonality. Moreover, I differentiate habitats primarily using differences in mean $\delta^{13}\text{C}_\text{E}$ values, and annual variability in carbon is relatively small in most serially sampled teeth from C_3 environments (Higgins and MacFadden, 2004; Nelson, 2005; Zanazzi and Kohn, 2008; Feranec et al., 2009; Kita et al., in prep.). Zanazzi and Kohn (2008) serially sampled teeth of *Subhyracodon* sp. from the Toadstool area and showed that seasonal variability in $\delta^{13}\text{C}_\text{E}$ values was very low. They concluded that variation in tooth position was of little concern for habitat interpretation in WRG mammals, with which I concur.

Isotopic measurements were done at the University of Michigan Stable Isotope Laboratory (UMSIL). Samples were reacted with phosphoric acid for 17 minutes at $77\pm 1.0^\circ\text{C}$ in a Finnigan MAT Kiel IV preparation device. Isotopic ratios of the resultant CO_2 were measured using a Finnigan MAT 253 triple collector isotope ratio mass spectrometer. Analytical precision at UMSIL is better than $\pm 0.1\text{‰}$ (1 standard deviation) for both $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values, based on international standards for carbonate (NBS-18, NBS-19) (Lora Wingate, lab manager – personal communication). Intra-lab enamel standards (LOX, from the African elephant, courtesy of D. L. Fox; MES-1, from fossil mammoth enamel) were used to monitor variance among batches. Mean values and variances were: $\delta^{18}\text{O}=31.89\pm 0.11\text{‰}$ and $\delta^{13}\text{C}=-5.65\pm 0.02\text{‰}$ (95% confidence, $n=16$) for

LOX and $\delta^{18}\text{O}=23.07\pm0.09\text{‰}$ and $\delta^{13}\text{C}=-9.63\pm0.02\text{‰}$ (95% confidence, n=16) for MES-1.

Isotopic results are expressed in standard δ -notation: $X = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 1000$, where X is the $\delta^{13}\text{C}$ or $\delta^{18}\text{O}$ value, and $R = {}^{13}\text{C} / {}^{12}\text{C}$ or ${}^{18}\text{O} / {}^{16}\text{O}$, respectively. The $\delta^{13}\text{C}$ values are reported relative to the Vienna PeeDee Belemnite (VPDB) standard and $\delta^{18}\text{O}$ values are reported here relative to the Vienna Standard Mean Ocean Water (VSMOW) standard. All errors on mean values are reported with 95% confidence of the mean (1.96*standard error) unless otherwise stated.

I performed a pairwise comparison of all genera in each fauna represented by two or more isotope values using Fisher's LSD test and Tukey's post hoc test. Tukey's test adjusts the level of significance by accounting for the number of pairs compared. In all cases, my use of "significance" refers to a probability of $\leq 5\%$ ($p \leq 0.05$) that the two compared mean values were drawn from the same population and are not significantly different. Welch's F-test was utilized for comparisons in which variances were significantly different; results for this test are noted in instances where its use was required. In cases where samples were not normally distributed the non-parametric Kruskal-Wallis test was used.

Mean values for faunal samples were also compared pair-wise to means for each of the predicted biomes to test for significant differences using the abovementioned tests. The same was done for taxa overlapping the ranges of predicted habitats. All uses of "significance" in text refer to statistical significance at $p \leq 0.05$ unless otherwise noted.

3.2. *Habitat modeling*

I follow the approach of Secord et al. (2008) for interpreting biomes and habitats in the White River Group (habitats and microhabitats, respectively, as used by Secord et al., 2008). In this study “biome” is used to characterize the general aspect of local vegetation structure, such as open or closed canopy, whereas “habitat” refers to areas within a biome, such as the understory in a closed canopy forest, or the riparian strip in a woodland-savanna biome. This approach uses plant $\delta^{13}\text{C}$ values from a variety of modern biomes. The modern biomes used here were chosen because they had similar sampling protocols, sampled a diversity of species, were reflective of intra-biome diversity, and they provided data from both wet and dry seasons that could be used to calculate a representative mean annual value. Mean $\delta^{13}\text{C}$ values were normalized to parameters for the latest Eocene/earliest Oligocene of Nebraska. Factors that were considered were: (1) the effects of latitude and altitude on $\delta^{13}\text{C}$ values in vegetation; (2) diet-enamel enrichment; and (3) changes in the composition of atmospheric CO_2 ($\delta^{13}\text{C}_\text{A}$) between the latest Eocene/earliest Oligocene and the present. I assume an average diet-to-enamel enrichment factor for WRG ungulates of 14.1‰ based on enrichment factors in extant ungulates calculated by Cerling and Harris (1999). Atmospheric carbon in the latest Eocene and earliest Oligocene was ~0.5‰ more positive than during the pre-industrial Holocene based on the dataset of Tipple et al. (2010). When considering the 1.5‰ decrease in $\delta^{13}\text{C}_\text{A}$ values caused by industrialization over the last two centuries (Friedli et al. 1986), the latest Eocene/earliest Oligocene $\delta^{13}\text{C}_\text{A}$ value was ~2.0‰ more positive than now. The total adjustment between normalized modern plant $\delta^{13}\text{C}$ values and WRG ungulate $\delta^{13}\text{C}_\text{E}$ values is +16.1‰. Model results indicate the following boundaries and

ranges for mean $\delta^{13}\text{C}_\text{E}$ values from Chadronian and Orellan ungulates: *closed canopy biomes (tropical rainforests and monsoon forests)*: $\leq -12.2\text{‰}$; *open canopy biomes (woodland-savannas, woody scrublands, seasonally dry forests, and C_3 grasslands)*: $< -12.2\text{‰}$ to -6‰ ; and *mixed C_3/C_4 biomes*: $> -6\text{‰}$. The upper boundary for C_3 vegetation represents very dry, water-stressed biomes.

3.3. Aridity Proxies

Levin et al. (2006) developed an “aridity index” for calculating differences in water deficit (WD) among faunas as a proxy for differences in environmental humidity. The aridity index uses the difference in mean $\delta^{18}\text{O}_\text{E}$ values between mammal species that were either aridity-sensitive (ES) or aridity-insensitive (EI). EI species are expected to track meteoric water values while ES species are sensitive to changes in humidity. The difference between mean values for EI and ES species is expected to increase with greater aridity as $\delta^{18}\text{O}$ values in ES species increase with greater aridity.

I chose *Archaeotherium mortoni* as the EI species since it was almost certainly an omnivore based on its brachydont and bunodont dentition (Joeckel, 1990; Table 1), and has a low mean $\delta^{18}\text{O}_\text{E}$ value relative to the rest of the fauna. I chose *Agriochoerus antiquus* as my ES species because it has the highest mean $\delta^{18}\text{O}_\text{E}$ value in the fauna, which is significantly elevated above many other taxa. Difference in Water Deficit (ΔWD), proportional to differences in humidity between two faunas, was determined using the following equation from Levin et al. (2006):

$$\Delta WD = \Delta \epsilon_{ES-EI} / 5.01 \times 10^{-3} \quad (1)$$

Where ΔWD is change in water deficit in mm/year between two faunas, $\Delta \epsilon_{ES-EI}$ is the difference in mean $\delta^{18}O_E$ values between ES and EI taxa between two faunas, and 5.01×10^{-3} is the slope (SE 1.98×10^{-3}) of the relationship between ES and EI species, calculated empirically from extant mammals. Ideally 10 specimens of *Archaeotherium mortoni* and 10 of *Agriochoerus antiquus* would be needed for both the Chadronian and Orellan faunas in order to determine fairly precise changes in water deficit (Levin et al., 2006). The recommended sample sizes could not be met for this study so uncertainty in estimates is fairly large.

I also utilized the general herbivore (#17) and the Dik-dik (#24) equations from Kohn (1996), to estimate changes in relative humidity using changes in mean faunal $\delta^{18}O_E$ values and changes in my aridity sensitive species (*Agriochoerus antiquus*), respectively.

4. Results

4.1. Isotopic changes across the EOCT

Significant increases of 0.4‰ (p=0.038, Fisher's LSD) and 1.0‰ (p=0.012, Welch's F-test) occur in faunal $\delta^{13}C_E$ and $\delta^{18}O_E$ values, respectively, from the Chadronian to the Orellan. These increases are caused primarily by Chadronian taxa with low $\delta^{13}C_E$ and/or low $\delta^{18}O_E$ values that are no longer present in the Orellan, or that were

not sampled in the Orellan because of decreased abundance, as discussed below. When all samples from only range-through taxa are considered, mean $\delta^{13}\text{C}_\text{E}$ and $\delta^{18}\text{O}_\text{E}$ values increase by 0.2‰ and 0.4‰, respectively, but neither change is significant ($p=0.328$, Fisher's LSD; $p=0.183$, Welch's F-test, respectively). Changes in individual range-through taxa are also not significant with 95% confidence but increases in $\delta^{13}\text{C}_\text{E}$ values in *Hyracodon nebraskensis* and $\delta^{18}\text{O}_\text{E}$ values in *Agriochoerus antiquus* are significant with 90% confidence (+0.8‰, $p=0.075$, Fisher's LSD; +0.9‰, $p=0.078$, Kruskal-Wallis; respectively). The *A. antiquus* sample from the Orellan is highly skewed by a single negative outlier (22.5‰, Appendix 1), however, and removal of this outlier results in a significant ($p=0.032$, Fisher's LSD) increase of 1.5‰ in *A. antiquus* $\delta^{18}\text{O}_\text{E}$ values. *Agriochoerus antiquus* is important since it has the highest $\delta^{18}\text{O}_\text{E}$ values in both faunas and is the strongest candidate for an aridity-sensitive taxon.

4.2. Chadronian and Orellan Biomes in northwestern Nebraska

Figure 3 shows that both Chadronian and Orellan faunal means plot in the range expected for dry, open canopied C_3 biomes with annual precipitation ranging from ~300-500 mm. Both means are significantly different from those predicted for “wet” biomes receiving >800 mm of precipitation, ranging from wet woodland-savanna to closed canopy rainforests but are indistinguishable from “dry” biomes such as dry woodland-savanna, woody scrublands, grasslands, and seasonally dry forests (Table 4A). A comparison of the Chadronian and Orellan faunas (Fig. 4, Tables 2 & 3) shows that mean $\delta^{13}\text{C}_\text{E}$ values for individual taxa in the Orellan fauna are all -9.3‰ or higher, with the

exception of the rare tapir, *Colodon* sp. (-12.2‰). This contrasts with the Chadronian fauna, in which five taxa (*Hyracodon nebraskensis*, *Subhyracodon* sp., *Trigonias* sp., *Megacerops* sp., and *Perchoerus probus*) have mean $\delta^{13}\text{C}_\text{E}$ values that plot in the range between -9.7‰ and -10.9‰. Thus, an isotopic “gap” is present in the Orellan fauna, previously filled by several taxa in Chadronian fauna. This gap represents relatively wetter-denser habitats. Notably, the taxa that fill this “gap” in the Chadronian become extinct or decrease in abundance in the Orellan.

4.3. Chadronian stable isotope paleoecology

Figure 4 shows mean $\delta^{13}\text{C}_\text{E}$ and $\delta^{18}\text{O}_\text{E}$ values for ungulates from the Chadronian. Isotopic values and descriptive statistics are reported in Table 2. The mean $\delta^{13}\text{C}_\text{E}$ and $\delta^{18}\text{O}_\text{E}$ values for all Chadronian samples are $-9.4 \pm 0.1\text{‰}$ and $23.2 \pm 0.2\text{‰}$ (n=67), respectively. *Meshippus* spp. (*M. bairdi* and *M. grandis* are combined here because they are statistically indistinguishable; $\delta^{13}\text{C}_\text{E}$ and $\delta^{18}\text{O}_\text{E}$, p=0.590 and p=0.712, respectively, Fisher’s LSD) has $\delta^{13}\text{C}_\text{E}$ values ($-8.4 \pm 0.3\text{‰}$, n=12) significantly higher than all other taxa except *Agriochoerus antiquus* ($-8.4 \pm 0.6\text{‰}$, n=3, p=0.981, Fisher’s LSD) and *Aepinacodon americanum* ($-8.9 \pm 1.0\text{‰}$, n=5, p=0.247, Fisher’s LSD). *Megacerops* sp. has $\delta^{13}\text{C}_\text{E}$ values ($-10.9 \pm 0.7\text{‰}$, n=7) significantly lower than all other taxa except *Subhyracodon* (-9.8‰ , n=2, p=0.204, Fisher’s LSD) and *Perchoerus probus* ($-10.3 \pm 1.0\text{‰}$, n=6, p=0.299, Fisher’s LSD). *Meshippus* has $\delta^{18}\text{O}_\text{E}$ values ($24.0 \pm 0.5\text{‰}$, n=12) significantly different from all other taxa except *Merycoidodon* sp. ($23.3 \pm 1.7\text{‰}$, n=8, p=0.135, Fisher’s LSD). *Agriochoerus* has significantly higher $\delta^{18}\text{O}_\text{E}$ values ($26.1 \pm 0.6\text{‰}$,

n=3) relative to all taxa except *Merycoidodon* sp. ($23.3 \pm 1.7\text{‰}$, n=8, p=0.098, Fisher's LSD) and *Hyracodon nebraskensis* ($25.2 \pm 1.1\text{‰}$, n=7, p=0.325, Fisher's LSD).

Archaeotherium mortoni has low $\delta^{18}\text{O}_\text{E}$ values ($21.9 \pm 1.4\text{‰}$, n=6), indistinguishable from all other taxa except *Mesohippus*, *Hyracodon*, and *Agriochoerus* (p=0.003, 0.003, and 0.005, respectively, Fisher's LSD).

Comparison of my isotopic results with those reported by Zanazzi and Kohn (2008) from the Toadstool area shows that most taxa are not significantly different (Table 5). However, $\delta^{13}\text{C}_\text{E}$ values for both *Mesohippus* sp. and *Merycoidodon* sp. are significantly more negative in the Zanazzi and Kohn dataset (Table 5).

4.4. Orellan stable isotope paleoecology

Mean $\delta^{13}\text{C}_\text{E}$ and $\delta^{18}\text{O}_\text{E}$ values for Orellan ungulates are shown in Figure 4. Table 3 reports isotopic values and descriptive statistics. The mean $\delta^{13}\text{C}_\text{E}$ and $\delta^{18}\text{O}_\text{E}$ values for all Orellan samples are $-8.9 \pm 0.2\text{‰}$ and $24.2 \pm 0.4\text{‰}$ (n=47), respectively. As with the Chadronian sample, *Mesohippus grandis* and *M. bairdi* are combined in *Mesohippus* spp. because they are statistically indistinguishable ($\delta^{13}\text{C}_\text{E}$ and $\delta^{18}\text{O}_\text{E}$, p=0.640 and p=0.989, respectively, Fisher's LSD). *Colodon* sp. has a significantly lower mean $\delta^{13}\text{C}_\text{E}$ (-12.2‰ , n=2) value compared to the other taxa sampled, but differs in mean $\delta^{18}\text{O}_\text{E}$ values only from *Agriochoerus antiquus*. The other six taxa examined have statistically indistinguishable $\delta^{13}\text{C}_\text{E}$ values. *Agriochoerus antiquus* ($27.0 \pm 1.3\text{‰}$, n=9) has $\delta^{18}\text{O}_\text{E}$ values significantly higher than all taxa except *Hyracodon nebraskensis* ($25.1 \pm 0.9\text{‰}$, n=10, p=0.108, Fisher's LSD).

Comparison of my isotopic results with those of Zanazzi and Kohn (2008) from the Toadstool area shows that taxa are not significantly different (Table 5).

4.5. Aridity Proxies

The aridity index (Levin et al., 2006) yields an estimated increase in water deficit of 90 ± 465 mm/yr (95% confidence) in the Orellan, based on mean $\delta^{18}\text{O}_\text{E}$ values for *Archaeotherium mortoni* (EI) and *Agriochoerus antiquus* (ES). Removing the negative outlier (mentioned above) from the Orellan dataset yields an increase of 204 ± 413 mm/yr. In both cases, propagated error is much greater than the estimated increase in water deficit, making this approach untenable. Taking a different approach, adapting the equation of Kohn for Dik-diks (1996, #24), an extant aridity-sensitive species (Levin et al., 2006), the 0.9‰ increase in $\delta^{18}\text{O}_\text{E}$ values in *A. antiquus* suggests a decrease in relative humidity of $\sim 7 \pm 9\%$ (95% confidence; $1.96 \times \text{SE}$), but uncertainty is larger than estimated change. Without the Orellan outlier a greater increase of $11 \pm 7\%$ with lower error is implied. However, these estimates do not take into consideration possible changes in the composition of precipitation unrelated to changes in humidity. Only a small, insignificant change (-0.1% , $p=0.833$, Fisher's LSD) occurs from the Chadronian to the Orellan in the $\delta^{18}\text{O}_\text{E}$ values of all presumed aridity-insensitive taxa (*Archaeotherium mortoni*, *Merycoidodon* sp., *Subhyracodon* sp.) averaged together, suggesting no change in meteoric water values. However, because of high uncertainty due to high $\delta^{18}\text{O}_\text{E}$ variability, a firm conclusion cannot be made using these aridity proxies.

5. Discussion

5.1. Biome and habitat interpretations in the White River Group

All taxa included in this study have $\delta^{13}\text{C}_\text{E}$ values consistent with a diet of C_3 vegetation, as expected from previous, pre-latest Miocene isotopic studies. However, some individuals appear to have been feeding in highly water stressed habitats and are approaching the upper range predicted for C_3 vegetation (C_3 - mixed C_3/C_4 boundary $\sim -6\text{‰}$). Both the Chadronian and Orellan faunas have mean values statistically indistinguishable from those predicted for open canopied biomes receiving ≤ 500 mm annual precipitation, such as “dry” woodland-savanna or woody scrubland, and significantly higher than “wet” open or closed canopied biomes receiving ≥ 800 mm of annual precipitation (Fig. 3, Table 4A). Although my mean faunal values are also consistent with C_3 grasslands (Table 4), Strömberg (2004, 2005) found only a small percentage of grass phytoliths in my study area, and it is unlikely that a treeless grassland biome was present. The brontothere *Megacerops* sp. and the peccary *Perchoerus probus* have relatively low mean $\delta^{13}\text{C}_\text{E}$ values, statistically indistinguishable from riparian habitat (Fig. 4, Table 4B). It seems unlikely that in a dry woodland-savanna or scrubland biome, any habitat other than riparian would have been extensive enough to support the common *Megacerops* sp., which probably had a large home range (see below). *Perchoerus probus*, with its much smaller size (~ 35 kg) and rarity, could conceivably have fed in poorly represented habitats not included in my model, such as areas around springs or ponds that should have similar $\delta^{13}\text{C}_\text{E}$ values to riparian habitat. Mean carbon values in the rhinoceroses *Trigonias* sp. (-9.8‰) and *Hyracodon nebraskensis* (-9.7‰)

are slightly below the Chadronian mean (-9.4‰) but are not low enough to be confidently assigned to a riparian habitat ($p=0.029$ and $p=0.047$, respectively, Fisher's LSD).

However, some individuals of these taxa, and of the entelodont *Archaeotherium mortoni*, have low enough values to suggest feeding in riparian areas.

The horse *Meshippus* spp. and the clawed oreodont *Agriochoerus antiquus* have the highest mean $\delta^{13}\text{C}_\text{E}$ values ($\sim -8.4\text{‰}$) of the Chadronian and Orellan, suggesting that they were feeding in open areas. Other species (*Subhyracodon* sp., *Eotylpus reedi*, *Merycoidodon* sp., and *Aepinacodon americanum*) have intermediate $\delta^{13}\text{C}_\text{E}$ values, implying that they were feeding on leaves and grasses in more densely vegetated areas than *Meshippus* spp. or *A. antiquus*. Significant differences in mean $\delta^{13}\text{C}_\text{E}$ values between my Chadronian samples of *Meshippus* spp. and *Merycoidodon* sp., and those of Zanazzi and Kohn (2008), suggest that these taxa (which could possibly include different species) were feeding in more densely vegetated areas in the populations sampled by Zanazzi and Kohn.

The tapir *Colodon* sp. has the lowest mean $\delta^{13}\text{C}_\text{E}$ value (-12.2‰) in the Orellan fauna, and is well separated from other taxa (Fig. 4). Its mean value is also lower than any in the Chadronian fauna, although single individuals of *Megacerops* sp. and *Perchoerus probus* are as low. The low values in *Colodon* undoubtedly represent feeding on leaves in or near water. These values are statistically indistinguishable from riparian habitat (Table 4B) although *Colodon* was rare in the WRG and could have fed on vegetation in more restricted habitats, such as around springs or ponds. This is especially interesting because it suggests that *Colodon* occupied a similar ecological niche to Neogene and modern tapirs, which also yield very low $\delta^{13}\text{C}_\text{E}$ values (DeSantis and

MacFadden, 2007; DeSantis, 2011). *Colodon sp.* appears to have been morphologically more comparable to extant *Tapirus* (Colbert, 2005) than to earlier forms. Early Eocene tapiromorphs, which were morphologically more generalized, inhabited a wider range of habitats, based on stable isotopes (Secord et al., 2008). These carbon data are the earliest evidence of tapirs occupying a position in the ecosystem similar to the one they occupy today.

5.2. Water dependence

The oreodont *Agriochoerus antiquus* has significantly higher $\delta^{18}\text{O}_\text{E}$ values than all other taxa, except *Hyracodon nebraskensis*, in both the Chadronian and Orellan faunas (Fig. 4; Tables 2b and 3b), and strongly suggests that it was getting a larger portion of water from leaves than other taxa, and/or that it had developed a drought-tolerant physiology allowing it to go without regular drinking. It is conceivable that some of the elevation in *A. antiquus* values was caused by sampling only third molars. This would mean that *A. antiquus* followed seasonal breeding cycles and that third molars formed when seasonal $\delta^{18}\text{O}_\text{E}$ values were highest. Although this is possible, it would also mean that the teeth sampled of the taxa to which *A. antiquus* is being compared formed during seasons when values were lower, and because *A. antiquus* maintains the same relative position in isotopic space (Fig. 4) in both faunas, that these conditions held in both the Chadronian and Orellan, in spite of other tooth positions being included in some other taxa. Thus, without additional supporting evidence, it is improbable that tooth position alone accounts for the elevated values exhibited in *A. antiquus*.

Hyracodon nebraskensis and *Mesohippus* spp. have the second and third highest mean $\delta^{18}\text{O}_\text{E}$ values in both the Chadronian and Orellan faunas, respectively, with higher variability in *H. nebraskensis* (Fig. 4, Tables 2b and 3b). As noted earlier, *Mesohippus* spp. was cursorial and was probably a browser or mixed feeder (Table 1). Cursoriality in modern mammals is usually associated with feeding in open areas as a defense against predators. Thus, I would expect that *Mesohippus* sp. was feeding on leaves in open areas, which is consistent with its high $\delta^{18}\text{O}_\text{E}$ and $\delta^{13}\text{C}_\text{E}$ values (Fig. 4). It is interesting, however, that *Mesohippus* sp. appears to be feeding in more closed areas in the Chadronian fauna analyzed by Zanazzi and Kohn (2008), as noted above. With regard to potential bias resulting from sampling third molars, Zanazzi and Kohn (2008) serially sampled five M3s each of *Mesohippus* sp. from the Chadronian and Orellan. Six of the individuals showed a decreasing trend in $\delta^{18}\text{O}_\text{E}$ values, while four individuals did not follow this pattern. In all cases except one, bulk sampling of these teeth would have yielded somewhat averaged annual values or seasonal lows (two teeth), rather than seasonal peaks. Thus the elevated $\delta^{18}\text{O}_\text{E}$ values in my *Mesohippus* spp. teeth cannot be reasonably attributed to sampling primarily third molars. Using *Mesohippus* spp. as a baseline for browsing/mixed-feeding in open areas, the significantly higher $\delta^{18}\text{O}_\text{E}$ values in *Agriochoerus antiquus* suggest that *A. antiquus* was less dependent on drinking water and had developed physiological adaptations for retaining water.

I interpret species with low $\delta^{18}\text{O}_\text{E}$ values to have been dependent on drinking water, following patterns seen in modern mammals (Bryant and Froelich, 1995; Levin et al., 2006). This includes the rhinocerotids, *Trigonias* sp. and *Subhyracodon* sp., the brontothere *Megacerops* sp., the entelodont *Archaeotherium mortoni*, the oromerycid

Eotylopus reedi, the peccary *Perchoerus probus*, and the anthracothere *Aepinacodon americanum* (Fig. 4, Tables 2b and 3b). *A. mortoni* and *P. probus* both have bunodont dentitions, suggesting an omnivorous diet, although Dewar (2008) suggested that *P. probus* was a hard-object browser (i.e., frugivorous “seed predator”) in his unpublished thesis. The low $\delta^{18}\text{O}_\text{E}$ values in these taxa are consistent with the expectation that non-folivores should have low $\delta^{18}\text{O}_\text{E}$ values relative to the rest of the fauna. The oreodont *Merycoidodon* sp. has intermediate values in the Chadronian, but lower relative values in the Orellan (Fig. 4), indicating that it was also water dependent. The significant increase in faunal mean $\delta^{18}\text{O}_\text{E}$ values (+1.0‰) occurs largely because of the extinction of water dependent taxa and from water dependent species that were sampled in the Chadronian but not in the Orellan due to decreased abundance. This suggests that conditions were less favorable for some water-dependent taxa in the Orellan.

5.3. Environmental response to the Eocene-Oligocene climatic transition

Isotopic results suggest reduction in the extent of wetter, denser habitats with increasing aridity during the Eocene-Oligocene climate transition. An isotopic “gap” in mean $\delta^{13}\text{C}_\text{E}$ values, between $\sim -9.5\text{‰}$ and -12‰ , opens in the Orellan, that was previously filled by mean values of five taxa in the Chadronian (*Hyracodon nebraskensis*, *Subhyracodon* sp., *Trigonias* sp., *Megacerops* sp., and *Perchoerus probus*) (Fig. 4, Tables 2 & 3). Low $\delta^{13}\text{C}_\text{E}$ values in these taxa suggest that they were feeding in wetter, denser parts of the ecosystem. As mentioned above, the low $\delta^{13}\text{C}_\text{E}$ values in *Megacerops* sp. and *P. probus* are consistent with feeding in wetter-denser habitats. The extinction of

Megacerops sp. and a marked decrease in the abundance of *P. probus*, based on the UNSM collections (12 specimens from the Chadronian collection versus 3 specimens from the Orellan collection, where the Orellan collection is roughly three times the size of the Chadronian), suggest reduction in the extent of the wettest parts of the ecosystem in the Orellan. The rare presence of the tapir *Colodon* sp. in the Orellan suggests such habitats were still present, at least transiently. However, with an estimated body mass of ~1900 kg (Mendoza et al., 2006), *Megacerops* sp. should have had a much larger individual home range than *Colodon* sp., which had a body mass of only ~150 kg. I estimate that their home ranges were 20 km² and 1.5 km², respectively, based on body size (Jetz et al., 2004). It is unlikely that *Megacerops* sp. became extinct due to competition with other mammals, since it was by far the largest mammal in the Chadronian, and no new large-bodied taxa appeared in the Orellan (Russell, 1973). Thus, reduction in the extent of its habitat is the most likely cause for its extinction. In regard to the other “gap” fillers, *Trigonias* sp. became extinct in the Orellan, while the mean $\delta^{13}\text{C}_\text{E}$ values for *H. nebraskensis* and *Subhyracodon* sp. increased, although not significantly.

Along with the reduction in taxa inhabiting wetter-denser habitats I also see an increase in mean faunal $\delta^{18}\text{O}_\text{E}$ values that reflects the loss or reduction of water dependent taxa in the Orellan, as noted above. The reduction of water dependent species and those inhabiting wetter habitats, along with an increase in $\delta^{18}\text{O}_\text{E}$ values in the most aridity-sensitive taxon (*Agriochoerus antiquus*), is consistent with increased aridity in the Orellan. However, a marked shift to more arid conditions would be expected to elevate $\delta^{18}\text{O}_\text{E}$ values in other taxa presumed to be aridity-sensitive (*Mesohippus* spp. and *H. nebraskensis*) but mean values in these taxa are virtually unchanged (Fig. 4). It is notable,

though, that *H. nebraskensis* has the highest $\delta^{18}\text{O}_\text{E}$ variability of any taxon in the Orellan (Table 3A), potentially masking large shifts in either direction. Another important factor is a significant increase in variance (SD increases by 46%; $p=0.002$, Welch's F-test) in $\delta^{18}\text{O}_\text{E}$ values (Tables 3A and 3B) in the Orellan. This is consistent with a shift to more arid conditions, which would cause increased water stress in plants, creating greater isotopic heterogeneity in the ecosystem. However, because of this high variability, larger samples are needed to adequately constrain changes in range-through taxa, and paleoenvironmental interpretations made from $\delta^{13}\text{C}_\text{E}$ values should be most reliable.

My findings are not consistent with the 7 to 8 °C decrease in mean annual temperature in the Orellan proposed by Zanzazi et al. (2007, 2009). Such a massive decrease in temperature should result in a large decrease in faunal $\delta^{18}\text{O}_\text{E}$ values, which is seen neither in my dataset nor theirs (Zanzazi et al., 2007, 2009). Their estimated drop in temperature is driven almost entirely by an increase in the $\delta^{18}\text{O}$ values of diagenetically reset bone carbonate, which they used as a proxy for soil carbonate. Groundwater $\delta^{18}\text{O}$ values may differ from surface water values, however, and could be influenced by soil water evaporation, by differential mixing of surface and ground water, and by tectonically influenced changes in regional groundwater flow (e.g., Evans and Welzenbach, 1998). A 7°C decrease should result in a decrease of ~4.1‰ in meteoric water values using the modern relationship between $\delta^{18}\text{O}$ values in precipitation and mean annual temperature at mid-latitudes (0.58‰/ °C; Dansgaard, 1964; Rozanski et al., 1993). The slope of this relationship could have been different in the Eocene-Oligocene if there had been a lower latitudinal temperature gradient (e.g., Fricke and Wing, 2004) but the change in $\delta^{18}\text{O}_\text{E}$ values would still have been substantial (e.g., a slope of 0.4‰/ °C,

results in a change of 2.8‰ in precipitation). A decrease of 4.1‰ in precipitation should result in a decrease of ~5.3‰ in mean faunal $\delta^{18}\text{O}_\text{E}$ values in the Orellan, using the $\delta^{18}\text{O}_\text{E}$ surface water slope of 0.76 from Kohn's (1996) general herbivore equation. Yet my results show an *increase* of 1.0‰ in mean faunal values. The large decrease expected in meteoric water values could theoretically have been offset by a ~45% decrease in relative humidity, using Kohn's general herbivore equation, but such a shift would represent a change equivalent to going from moist, sub-tropical conditions to those of a desert, and may be unprecedented. One other possibility is that the large negative shift in precipitation values was compensated for by a change in vapor source or a major reorganization of regional drainage resulting in a ~3-4‰ more positive water source in the Orellan. Changes in circulation through the EOCT are unresolved (e.g. Toggweiler and Bjornsson, 2000; Huber and Sloan, 2001; Huber et al., 2004; Hay et al., 2005; Sijp and England, 2005), and although this hypothesis cannot be falsified, it would require special circumstances and appears to be unlikely.

6. Conclusions

The goal of this study was to evaluate possible changes in biomes or habitat preference in WRG ungulates during the EOCT in response to climate change. Average carbon isotope values for both Chadronian and Orellan faunas suggest that they inhabited a fairly dry biome, such as woodland-savanna or woody scrubland. I find that range-through taxa retain approximately the same relative positions in isotopic space (Fig. 4) in both faunas, implying stability in the habitats occupied by these species. However, an

isotopic “gap” in mean $\delta^{13}\text{C}_\text{E}$ values, between $\sim -9.5\text{‰}$ and -12‰ , that was previously filled by five taxon means in the Chadronian opens up in the Orellan. The Chadronian “gap fillers” appear to have been taxa feeding in wetter parts of the ecosystem, suggesting a reduction in wetter habitats in the Orellan. This change to drier conditions does not appear to be the result of local conditions since two of these taxa (*Megacerops* sp. and *Trigonias* sp.) became extinct regionally in the Orellan. *Megacerops* sp. was by far the largest mammal alive in North America in the Chadronian and was abundantly represented. Thus, its extinction appears to represent an important change in ecosystem structure in the Orellan.

The significant increase of 1.0‰ seen in mean faunal $\delta^{18}\text{O}_\text{E}$ values in the Orellan is also consistent with drying, although the expected response of an upward shift in aridity-sensitive taxa yielded mixed results. 0.6‰ of this 1.0‰ increase resulted from taxa that were sampled in the Chadronian but not the Orellan due either to extinction or poor representation in the latter. This suggests that water dependent taxa did not fare as well as less water dependent taxa in the Orellan. The increase in $\delta^{18}\text{O}_\text{E}$ values in *Agriochœrus antiquus*, the most aridity-sensitive species in both of my faunas, is consistent with the expectation for drying, but significant change was not found in *Mesohippus* spp., which also appears to have been an aridity-sensitive taxon. A significant increase in variance in $\delta^{18}\text{O}_\text{E}$ values (Tables 3A and 3B) that occurs in the Orellan, is also consistent with an increase in aridity, but weakens the utility of using $\delta^{18}\text{O}_\text{E}$ values in range-through taxa to make paleoclimate interpretations. Paleoenvironmental interpretations made from $\delta^{13}\text{C}_\text{E}$ values, which have bearing on paleoclimate, should be more reliable because of lower overall variability.

Few studies have examined isotopic differences among a diversity of mammals in Pre-Miocene ecosystems dominated by C_3 vegetation. This study further demonstrates the utility of using stable isotopes in mammalian tooth enamel to identify ancient biomes and to recognize habitat differences and the effects of climate in Paleogene C_3 dominated ecosystems.

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References Cited

- Alroy, J., Loch, P.L., Zachos, J.C., 2000. Global climate change and North American mammalian evolution. *Paleobiology* 26, 259-288.
- Ambrose, S.H., DeNiro, M.J., 1986. The isotopic ecology of East African mammals. *Oecologia* 69, 395-406.
- Bryant, J.D., Froelich, P.N., 1995. A model of oxygen isotope fractionation in body water of large mammals. *Geochimica et Cosmochimica Acta* 59, 4523-4537.
- Cerling, T.E., Ehleringer, J.R., 2000. Welcome to the C₄ World, in: Gastaldo, R.A., DiMichele, W.A. (Eds.), *Phanerozoic Terrestrial Ecosystems*. Yale University Press, New Haven, pp. 273-286.
- Cerling, T.E., Harris, J.M., 1999. Carbon isotope fractionation between diet and bioapatite in ungulate mammals and implications for ecological and paleoecological studies. *Oecologia* 120, 347-363.
- Cerling, T.E., Wang, Y., Quade, J., 1993. Expansion of C₄ ecosystems as an indicator of global ecological change in the Late Miocene. *Nature* 361, 344-345.
- Cerling, T.E., Harris, J.M., MacFadden, B.J., Leakey, M.G., Quade, J., Eisenmann, V., Ehleringer, J.R., 1997. Global vegetation change through the Miocene-Pliocene boundary. *Nature* 389, 153-158.
- Cerling, T.E., Hart, J.A., Hart, T.B., 2004. Stable isotope ecology in the Ituri Forest. *Oecologia* 138, 5-12.
- Clark, J., Beerbower, J.R., Kietze, K.K., 1967. Oligocene sedimentation, stratigraphy, paleoecology, and paleoclimatology in the Big Badlands of South Dakota. *Fieldiana Geology Memoirs* Volume 5, 1-158.

- Codron, J., Codron, D., Lee-Thorp, J.A., Sponheimer, M., Bond, W.J., de Ruiter, D., Grant, R., 2005. Taxonomic, anatomical, and spatio-temporal variations in the stable carbon and nitrogen isotopic compositions of plants from an African savanna. *Journal of Archaeological Science* 32, 1757-1772.
- Colbert, M. W., 2005. The facial skeleton of the Early Oligocene *Colodon* (Perissodactyla, Tapiroidea). *Palaeontologia Electronica* 8: 12A, 1-27.
- Colbert, M.W., Schoch, R.M., 1998. Tapiroidea and other moromorphs, in: C.M. Janis, K.M. Scott, and L.L. Jacobs (Eds.), *Evolution of Tertiary mammals of North America, Volume 1: Terrestrial carnivores, ungulates, and ungulatelike mammals*, pp. 569-582.
- Collinson, M.E., 1992. Vegetational and floristic changes around the Eocene/Oligocene boundary in western and central Europe, in: Prothero, D.R., Berggren, W.A. (Eds.), *Eocene-Oligocene Climatic and Biotic Evolution*. Princeton University Press, Princeton, pp. 437-450.
- Coxall, H.K., Wilson, P.A., Pälike, H., Lear, C.H., Backman, J., 2005. Rapid stepwise onset of Antarctic glaciation and deeper calcite compensation in the Pacific Ocean. *Nature* 433, 53-57.
- Dansgaard, W., 1964. Stable isotopes in precipitation. *Tellus* 16, 436-468.
- DeSantis, L.G., 2011. Stable isotope ecology of extant tapirs from the Americas. *Biotropica* 43, 746-754.
- DeSantis, L.R.G., MacFadden, B., 2007. Identifying forested environments in Deep Time using fossil tapirs: evidence from evolutionary morphology and stable isotopes. *Courier Forschungsinstitut Senckenberg* 258, 147-157.

- Dewar, E.W., 2008. Dietary ecology and community paleoecology of early Tertiary mammals. Electronic Doctoral Dissertations for U Mass Amherst. Paper AAI3337037.
- Dockery, D. T., Lozouet, P., 2003. Molluscan faunas across the Eocene/Oligocene boundary in the North American Gulf Coastal Plain, with comparisons to those of the Eocene and Oligocene of France, in: Prothero, D.R., Ivany, L.C., Nesbitt, E.A. (Eds.), *From greenhouse to icehouse: the marine Eocene-Oligocene transition*. Columbia University Press, New York, pp. 303-340.
- Edwards, E.J., Osborne, C.P., Strömberg, C.A.E., Smith, S.A., C4 Grasses Consortium, 2010. The origins of C4 grasslands: Integrating evolutionary and ecosystem science. *Science* 328: 587-591.
- Ehleringer, J.R., Field, C.B., Lin, Z.-F., Kuo, C.-Y., 1986. Leaf carbon isotope and mineral composition in subtropical plants along an irradiance cline. *Oecologia* 70, 520-526.
- Ehleringer, J.R., Lin, Z.F., Field, C.B., Sun, G.C., Kuo, C.Y., 1987. Leaf carbon isotope ratios of plants from a subtropical monsoon forest. *Oecologia* 72, 109-114.
- Ehleringer, J.R., Monson, R.K., 1993. Evolutionary and ecological aspects of photosynthetic pathway variation. *Annual Review of Ecology and Systematics* 24, 411-439.
- Emry, R.J., Bjork, P.R., Russell, L.S., 1987. The Chadronian, Orellan, and Whitneyan land mammal ages, in: Woodburne, M.O. (Ed.), *Cenozoic mammals of North America: geochronology and biostratigraphy*. University of California Press, Berkeley, pp. 118-152.

- Evanoff, E., Prothero, D.R., Lander, R.H., 1992. Eocene-Oligocene climatic change in North America: the White River Formation near Douglas, east-central Wyoming, in: Prothero, D.R., Berggren, W.A. (Eds.), *Eocene-Oligocene Climatic and Biotic Evolution*. Princeton University Press, Princeton, pp. 116-130.
- Evans, J.E., Welzenbach, L.C., 1998. Episodes of carbonate deposition in a siliciclastic-dominated fluvial sequence, Eocene-Oligocene White River Group, South Dakota and Nebraska. *Geological Society of America Special Paper* 325, 93-116.
- Farquhar, G.D., Ehleringer, J.R., Hubrick, K.T., 1989. Carbon isotope fractionation and photosynthesis. *Annual Reviews of Plant Physiology and Molecular Biology* 44, 503-537.
- Feranec, R.S., 2007. Stable carbon isotope values reveal evidence of resource partitioning among ungulates from modern C₃-dominated ecosystems in North America. *Palaeogeography, Palaeoclimatology, Palaeoecology* 252, 575-585.
- Feranec, R.S., MacFadden, B.J., 2006. Isotopic discrimination of resource partitioning among ungulates in C₃-dominated communities from the Miocene of Florida and California. *Paleobiology* 32, 191-205.
- Feranec, R.S., Hadly, E.A., and Payton, A., 2009. Stable isotopes reveal seasonal competition for resources between late Pleistocene bison (*Bison*) and horse (*Equus*) from Rancho La Brea, southern California. *Palaeogeography, Palaeoclimatology, Palaeoecology* 271, 153-160.
- Fricke, H.C., Wing, S.L., 2004. Oxygen isotope and paleobotanical estimates of temperature and $\delta^{18}\text{O}$ -latitude gradients over North America during the early Eocene. *American Journal of Science* 304, 612-635.

- Friedli, H., Lotscher, H., Oeschger, Siegenthaler, U., Stauver, B., 1986. Ice core record of the $^{13}\text{C}/^{12}\text{C}$ ratio of atmospheric CO_2 in the past two centuries. *Nature* 324, 237-238.
- Gradstein, F.M., Ogg, J.G., Smith, A.G., 2004. A Geologic Time Scale 2004. Cambridge University Press, Cambridge (UK).
- Grandstaff, D.E., Terry, D.O. Jr., 2009. Rare earth element composition of Paleogene vertebrate fossils from Toadstool Geologic Park, Nebraska, USA. *Applied Geochemistry* 24, 733-745.
- Grimes, S.T., Hooker, J.J., Collinson, M.E., Matthey, D.P., 2005. Summer temperatures of late Eocene to early Oligocene freshwaters. *Geology* 33, 189-192.
- Hansen, T.A., Kelley, P.H., Haasl, D.M., 2004. Paleoecological patterns in molluscan extinctions and recoveries: comparison of Cretaceous-Paleogene and Eocene-Oligocene extinctions in North America. *Palaeogeography, Palaeoclimatology, Palaeoecology* 214, 233-242.
- Hay, W.W., Flögel, S., Söding, E., 2005. Is the initiation of glaciation on Antarctica related to a change in the structure of the ocean? *Global and Planetary Change* 45, 23-33.
- Heaton, T.H.E., 1999. Spatial, species, and temporal variations in the $^{13}\text{C}/^{12}\text{C}$ ratios of C_3 plants: implications for paleodiet studies. *Journal of Archaeological Science* 26, 637-649.
- Higgins, P., MacFadden, B., 2004. "Amount Effect" recorded in oxygen isotopes of Late Glacial horse (*Equus*) and bison (*Bison*) teeth from the Sonoran and Chihuahuan

- deserts, southwestern United States. *Palaeogeography, Palaeoclimatology, Palaeoecology* 206, 337–353.
- Hillson, S., 1986. *Teeth*. University of Cambridge Press, Cambridge.
- Hooker, J. J., 1998. Mammalian faunal change across the Paleocene-Eocene transition in Europe, in: Aubry, M.-P., Lucas, S. G., Berggren, W. A. (Eds.), *Late Paleocene-early Eocene Climatic and Biotic Events in the Marine and Terrestrial Records*. Columbia University Press, New York, pp. 419-441.
- Hooker, J.J., Collinson, M.E., Sille, N.P., 2004. Eocene-Oligocene mammalian faunal turnover in the Hampshire Basin, UK: calibration to the global timescale and the major cooling event. *Journal of the Geological Society of London* 161, 161-172.
- Hooker, J.J., Grimes, S.T., Matthey, D.P., Collinson, M.E., Shedlon, N.D., 2009. Refined correlation of the UK Late Eocene–Early Oligocene Solent Group and timing of its climate history. *Geological Society of America Special Paper* 452, 179-195.
- Huber, M., Sloan, L.C., 2001. Heat transport, deep waters and thermal gradients: Couple simulation of an Eocene “greenhouse” climate. *Geophysical Research Letters* 28, 3481-3484.
- Huber, M., Brinkhuis, H., Stickley, C.E., Doos, K., Sluijs, A., Warnaar, J., Schellenberg, S.A., Williams, G.L., 2004. Eocene circulation of the Southern Ocean: Was Antarctica kept warm by subtropical waters? *Paleoceanography* 19, PA4026.
- Hutchison, J.H., 1982. Turtle, crocodilian, and champsosaur diversity changes in the Cenozoic of the north-central region of western United States. *Palaeogeography, Palaeoclimatology, Palaeoecology* 37, 149-164.

- Hutchison, J.H., 1992. Western North America reptile and amphibian record across the Eocene/Oligocene boundary and its climatic implications, in: Prothero, D.R., Berggren, W.A. (Eds.), *Eocene-Oligocene Climatic and Biotic Evolution*. Princeton University Press, Princeton, pp. 451-463.
- Janis, C.M., 1982. Evolution of horns in ungulates: ecology and paleoecology. *Biological Reviews of the Cambridge Philosophical Society* 57, 261-318.
- Jessup, K.E., Barnes, P.W., Boutton, T.W., 2003. Vegetation dynamics in a *Quercus-Juniperus* savanna: An isotopic assessment. *Journal of Vegetation Science* 14, 841-852.
- Jetz, W., Carbone, C., Fulford, J., Brown, J.H., 2004. The scaling of animal space use. *Science* 306, 266-268.
- Joeckel, R.M., 1990. A functional interpretation of the masticatory system and paleoecology of entelodonts. *Paleobiology* 16, 459-482.
- Katz, M.E., Miller, K.G., Wright, J.D., Wade, B.S., Browning, J.V., Cramer, B.S., Rosenthal, Y., 2008. Stepwise transition from Eocene greenhouse to the Oligocene icehouse. *Nature Geoscience* 1, 329-334.
- Koch, P.L., 1998. Isotopic reconstruction of past continental environments. *Annual Review of Earth and Planetary Sciences*. 26, 573-613.
- Koch, P.L., Tuross, N., Fogel, M.L., 1997. The effects of sample treatment and diagenesis on the isotopic integrity of carbonate in biogenic hydroxylapatite. *Journal of Archaeological Science* 24, 417-429.
- Kohn, M.J., 1996. Predicting animal $\delta^{18}\text{O}$: Accounting for diet and physiological adaptation. *Geochimica et Cosmochimica Acta* 60, 4811-4829.

- Kohn, M.J., Schoeninger, M.J., Valley, J.W., 1996. Herbivore tooth oxygen isotope compositions: effects of diet and physiology. *Geochimica et Cosmochimica Acta* 60, 3889-3896.
- Kohn, M.J., Josef, J.A., Madden, R., Kay, R., Vucetich, G., Carlini, A.A., 2004. Climate stability across the Eocene-Oligocene transition, southern Argentina. *Geology* 32, 621-624.
- Kron, D.G., Manning, E., 1998. Anthracotheriidae, in: Janis, C.M., Scott, K.M., Jacobs, L.L. (Eds.), *Evolution of Tertiary Mammals of North America, Volume 1: Terrestrial carnivores, ungulates, and ungulatelike mammals*. Cambridge University Press, Cambridge (UK), pp. 381-388.
- LaGarry, H.E., 1998. Lithostratigraphic revision and redescription of the Brule Formation, White River Group, western Nebraska. *Geological Society of America Special Paper* 325, 63-91.
- Lear, C.H., Bailey, T.R., Pearson, P.N., Coxall, H.K., Rosenthal, Y., 2008. Cooling and ice growth across the Eocene-Oligocene transition. *Geology* 36, 251-254.
- Lee-Thorp, J.A., van der Merwe, N.J., 1987. Carbon isotope analysis of fossil bone apatite. *South African Journal of Science* 83, 712-715.
- Legendre, S., Hartenberger, J.-L., 1992. Evolution of mammalian faunas in Europe during the Eocene and Oligocene, in: Prothero, D. R., Berggren, W. A. (Eds.), *Eocene/Oligocene climatic and biotic evolution*. Princeton University Press, Princeton, pp. 516-528.
- Levin, N.E., Cerling, T.E., Passey, B.H., Harris, J.M., Ehleringer, J.R., 2006. A stable isotope aridity index for terrestrial environments. *PNAS* 103, 11201-11205.

- Liu, Z.-H., Pagani, M., Zinniker, D., DeConto, R., Huber, M., Brinkhuis, H., Shah, S.R., Leckie, R.M., Pearson, A., 2009. Global Cooling During the Eocene-Oligocene Climate Transition. *Science* 323, 1187-1190.
- MacFadden, B.J., 1987. Fossil horses from “Eohippus” (*Hyracotherium*) to *Equus*: scaling, Cope’s law, and the evolution of body size. *Paleobiology* 12, 355-369.
- Mead, A.J., Wall, W.P., 1998a. Paleoecological implications of the craniodental and premaxilla morphologies of two rhinocerotoids (perissodactyla) from Badlands National Park, South Dakota. *National Park Service Paleontological Research Volume 3*, 18-22.
- Mead, A.J., Wall, W.P., 1998b. Dietary implications of jaw biomechanics in the rhinocerotoids *Hyracodon* and *Subhyracodon* from Badlands National Park, South Dakota. *National Park Service Paleontological Research Volume 3*, 23-28.
- Mendoza, M., Janis, C.M., Palmqvist, P., 2006. Estimating body mass of extinct ungulates: a study on the use of multiple regression. *Journal of Zoology* 270, 90-101.
- Mihlbachler, M.C., Solounias, N., 2002. Body size, dental microwear, and brontothere diets through the Eocene. *Journal of Vertebrate Paleontology* 22 (Supplement), 88A.
- Mihlbachler, M.C., Rivals, F., Solounias, N., Semprebon, G.M., 2011. Dietary change and evolution of horses in North America. *Science* 311, 1178-1181.
- Miller, K., Wright, J.D., Katz, M.E., Wade, B.S., Browning, J.V., Cramer, B.S., Rosenthal, Y., 2009. Climate threshold at the Eocene-Oligocene transition:

- Antarctic ice sheet influence on ocean circulation. Geological Society of America Special Paper 452, 169-178.
- Myers, J.A., 2003. Terrestrial Eocene-Oligocene vegetation and climate in the Pacific Northwest, in: Prothero, D.R., Ivany, L.C., Nesbitt, E.A. (Eds.), From greenhouse to icehouse: the marine Eocene-Oligocene transition. Columbia University Press, New York, pp. 171-185.
- Nelson, S.V., 2005. Paleoseasonality inferred from equid teeth and intra-tooth isotopic variability. *Palaeogeography, Palaeoclimatology, Palaeoecology* 222, 122–144.
- Nesbitt, E.A., 2003. Changes in shallow-marine faunas from the northeastern Pacific margin across the Eocene/Oligocene boundary, in: Prothero, D.R., Ivany, L.C., Nesbitt, E.A. (Eds.), From greenhouse to icehouse: the marine Eocene-Oligocene transition. Columbia University Press, New York, pp. 57-70.
- Obradovich, J.D., Evanoff, E., Larson, E.E., 1995. Revised single-crystal laser-fusion $^{40}\text{Ar}/^{39}\text{Ar}$ ages of Chadronian tuffs in the White River Formation of Wyoming. Geological Society of America Abstracts with Programs 27, 77.
- O'Leary, M.H., 1988. Carbon isotopes in photosynthesis. *Bioscience* 38, 328-336.
- Passey, B.H., Cerling, T.E., Perkins, M.E., Voorhies, M.R., Harris, J.M., Tucker, S.T., 2002. Environmental change in the Great Plains: an isotopic record from fossil horses. *Journal of Geology* 110, 123-140.
- Passey, B.H., Robinson, T.F., Ayliffe, L.K., Cerling, T.E., Sponheimer, M., Dearing, M.D., Roeder, B.L., Ehleringer, J.R., 2005. Carbon isotope fractionation between diet, breath CO₂, and bioapatite in different mammals. *Journal of Archaeological Science* 32, 1459-1470.

Pearson, P.N., McMillan, I.K., Wade, B.S., Jones, T.D., Coxall, H.K., Bown, P.R., H.

Lear, C.H., 2008. Extinction and environmental change across the Eocene-

Oligocene boundary in Tanzania. *Geology* 36, 179-182.

Prothero, D.R., 1998b. Oromerycidae, in: Janis, C.M., Scott, K.M., Jacobs, L.L. (Eds.),

Evolution of Tertiary Mammals of North America, Volume 1: Terrestrial

carnivores, ungulates, and ungulatelike mammals. Cambridge University Press,

Cambridge (UK), pp. 426-430.

Prothero, D.R., 1999. Does climatic change drive mammalian evolution? *GSA Today* 9,

1-5

Prothero, D.R., Emry, R.J., 2004. The Chadronian, Orellan, and Whitneyan North

American Land Mammal Ages, in: Woodburne, M.O. (Ed.), Late Cretaceous and

Cenozoic Mammals of North America: biostratigraphy and geochronology.

Columbia University Press, New York, pp. 156-168.

Prothero, D.R., Heaton, T.H., 1996. Faunal stability during the early Oligocene climatic

crash. *Palaeogeography, Palaeoclimatology, Palaeoecology* 127, 239-256.

Prothero, D.R., Swisher, C.C.III, 1992. Magnetostratigraphy and geochronology of the

terrestrial Eocene-Oligocene transition in North America, in: Prothero, D.R. and

Berggren, W.A. (Eds.), Eocene-Oligocene Climatic and Biotic Evolution.

Princeton University Press, Princeton, pp. 46-73.

Prothero, D.R., Whittlesey, K.E., 1998. Magnetic stratigraphy and biostratigraphy of the

Orellan and Whitneyan land-mammal "ages" in the White River Group.

Geological Society of America Special Paper 325, 39-61.

- Quade, J., Cerling, T.E., Andrews, P., Alpagut, B., 1995. Paleodietary reconstruction of Miocene faunas from Pasalar, Turkey using stable carbon and oxygen isotopes of fossil tooth enamel. *Journal of Human Evolution* 28, 373-384.
- Radinsky, L., 1978. Evolution of brain size in carnivores and ungulates. *American Naturalist* 112, 815-831.
- Retallack, G.J., 1992. Paleosols and changes in climate and vegetation across the Eocene/Oligocene boundary, in: Prothero, D.R. and Berggren, W.A. (Eds.), *Eocene-Oligocene Climatic and Biotic Evolution*. Princeton University Press, Princeton, pp. 382-398.
- Retallack, G.J., 2007. Cenozoic paleoclimate on land in North America. *Journal of Geology* 115, 271-294.
- Russell, L.S., 1973. Geological evidence on the extinction of some large terrestrial vertebrates. *Canadian Journal of Earth Sciences* 10, 140-145
- Rose, K.D., 2006. *The Beginning of the Age of Mammals*. Johns Hopkins University Press, Baltimore.
- Rozanski, K., Aragus-Araguas, L., Gonfiantini, R., 1993. Isotopic patterns in modern global precipitation, in Swart, P.K., Lohmann, K.C., McKenzie, J. and Savin, S. (Eds.), *Climate change in continental climate records*. American Geophysical Union, *Geophysical Monograph* 78, 1-36.
- Sandquist, D.R., Cordell, S., 2007. Functional diversity of carbon-gain, water-use, and leaf-allocation traits in trees of a threatened lowland dry forest in Hawaii. *American Journal of Botany* 94, 1459-1469.

- Schultz, C.B., Stout, T.M., 1955. Classification of Oligocene sediments of Nebraska. Bulletin of the University of Nebraska State Museum 4, 17-52.
- Secord, R., Wing, S.L., Chew, A., 2008. Stable isotopes in early Eocene mammals as indicators of forest canopy structure and resource partitioning. *Paleobiology* 34, 282-300.
- Secord, R., Gingerich, P. D., Lohmann, K. C., MacLeod, K. G., 2010, Continental warming preceding the Palaeocene-Eocene thermal maximum. *Nature* 467, 955-958.
- Secord, R., Bloch, J. I., Chester, S. G. B., Boyer, D. M., Wood, A. R., Wing, S. L., Kraus, M. J., McInerney, F. A., Krigbaum, J., 2012, Evolution of the earliest horses driven by climate change in the Paleocene-Eocene Thermal Maximum. *Science* 335, 959-962.
- Sheldon, N.D., 2009. Nonmarine records of climatic change across the Eocene-Oligocene transition. *Geological Society of America Special Paper* 452, 241-248.
- Sheldon, N.D., Retallack, G.J., 2004. Regional paleoprecipitation records from the late Eocene and Oligocene of North America. *Journal of Geology* 112, 487-494.
- Sijp, W.P., England, M.H., 2005. On the role of the Drake Passage in controlling the stability of the ocean's thermohaline circulation. *Journal of Climate* 18, 1957-1966.
- Sjostrom, D.J., Hren, M.T., Horton, T.W., Waldbauer, J.R., Chamberlain, C.P., 2006. Stable isotopic evidence for a pre-late Miocene elevation gradient in the Great Plains-Rocky Mountain region, USA. *Geological Society of America Special Paper* 398, 309-319.

- Solounias, N., Semperebon, G., 2002. Advances in the reconstruction of ungulate ecomorphology with application to early fossil equids. *American Museum Novitates* 3366, 1-49.
- Stevens, M.S., Stevens, J.B., 1996. Merycoidodontidae and Miniochoerinae, in: Prothero, D.R. and Emry, R.J. (Eds.), *The Terrestrial Eocene-Oligocene Transition in North America*. Cambridge University Press, Cambridge (UK), pp. 498-573.
- Stewart, G.R., Turnbull, M.H., Schmidt, S., Erskine, P.D., 1995. ^{13}C natural abundance in plant communities along a rainfall gradient: a biological integrator of water availability. *Australian Journal of Plant Physiology* 22, 51-55.
- Strömberg, C.A.E., 2004. Using phytolith assemblages to reconstruct the origin and spread of grass-dominated habitats in the great plains of North America during the late Eocene to early Miocene. *Palaeogeography, Palaeoclimatology, Palaeoecology* 207, 239-275.
- Strömberg, C.A.E., 2005. Decoupled taxonomic radiation and ecological expansion of open-habitat grasses in the Cenozoic of North America. *Proceedings of the National Academy of Sciences* 102, 11980-11984.
- Terry, D.O. Jr., 1998. Lithostratigraphic revision and correlation of the lower part of the White River Group: South Dakota to Nebraska. *Geological Society of America Special Paper* 325, 15-37.
- Terry, D.O. Jr., 2001. Paleopedology of the Chadron Formation of northwestern Nebraska: implications for paleoclimatic change in the North American midcontinent across the Eocene-Oligocene boundary. *Palaeogeography, Palaeoclimatology, Palaeoecology* 168, 1-38.

- Terry, D.O. Jr., LaGarry, H.E., 1998. Big Cottonwood Creek Member: a new member of the Chadron Formation in northwestern Nebraska. *Geological Society of America Special Paper* 325, 117-141.
- Tipple, B.J., Pagani, M., 2007. The early origins of terrestrial C₄ photosynthesis. *Annual Review of Earth and Planetary Sciences* 35:435-461.
- Tipple, B.J., Meyers, S.R., Pagani, M., 2010. Carbon isotope ratio of Cenozoic CO₂: A comparative evaluation of available geochemical proxies. *Paleoceanography* 25, 1-11.
- Toggweiler, J.R., Bjornsson, H., 2000. Drake Passage and paleoclimate. *Journal of Quaternary Science* 15, 319-328.
- Tütken, T., Vennemann, T., 2009. Stable isotope ecology of Miocene large mammals from Sandelzhausen, southern Germany. *Paläontologische Zeitschrift* 83, 207-226.
- van der Merwe, N.J., Medina, E., 1991. The canopy effect, carbon isotope ratios and foodwebs in Amazonia. *Journal of Archaeological Science* 18, 249-259.
- Wall, W.P., Shikany, M.J., 1995. Comparison of feeding mechanisms in Oligocene Agriochoeridae and Merycoidodontidae from Badlands National Park. *National Park Service Paleontological Research Volume* 2, 27-33.
- Williams, D.G., Ehleringer, J.R., 1996. Carbon isotope discrimination in three semi-arid woodland species along a monsoon gradient. *Oecologia* 106, 455-460.
- Wood, H.E. II, Chaney, R.W., Clark, J., Colbert, E.H., Jepsen, G.L., Reeside, J.B. Jr., Stock, C., 1941. Nomenclature and correlation of the North American continental Tertiary. *Bulletin of the Geological Society of America* 52, 1-48.

- Woodburne, M. O., 1987, Cenozoic mammals of North America, geochronology and biostratigraphy, Berkeley, University of California Press, Berkeley.
- Woodburne, M. O., 2004, Late Cretaceous and Cenozoic mammals of North America: biostratigraphy and geochronology, New York, Columbia University Press, New York.
- Wright, D.B., 1998. Tayassuidae, in: Janis, C.M., Scott, K.M., Jacobs, L.L. (Eds.), Evolution of Tertiary Mammals of North America, Volume 1: Terrestrial carnivores, ungulates, and ungulatelike mammals. Cambridge University Press, Cambridge (UK), pp. 389-401.
- Zachos, J.C., Pagani, M., Sloan, L., Thomas, E., Billups, K., 2001. Trends, rhythms, and aberrations in global climate 65 Ma to Present. *Science* 292, 686-693.
- Zachos, J.C., Dickens, G.R., Zeebe, R.E., 2008. An early Cenozoic perspective on greenhouse gas warming and carbon-cycle dynamics. *Nature* 451, 279–283.
- Zanazzi, A., Kohn, M.J., McFadden, B.J., Terry, D.O. Jr., 2007. Large temperature drop across the Eocene-Oligocene transition in central North America. *Nature* 445, 639-642.
- Zanazzi, A., Kohn, M.J., 2008. Ecology and physiology of White River mammals based on stable isotope ratios of teeth. *Palaeogeography, Palaeoclimatology, Palaeoecology* 257, 22-37.
- Zanazzi, A., Kohn, M.J., Terry, D.O. Jr., 2009. Biostratigraphy and paleoclimatology of the Eocene-Oligocene boundary section at Toadstool Park, northwestern Nebraska, USA. *Geological Society of America Special Paper* 452, 197-214.

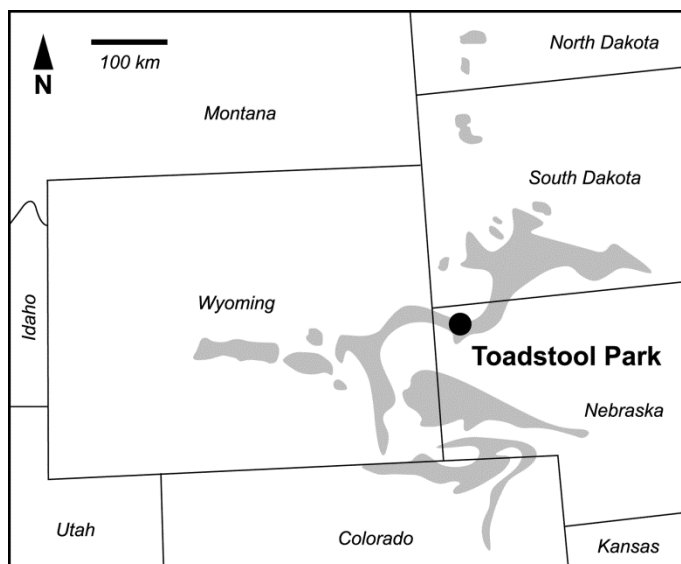


FIGURE 1. Location map of the study area in northwestern Nebraska superimposed on WRG outcrops (in gray). Modified from Emry et al. (1987).

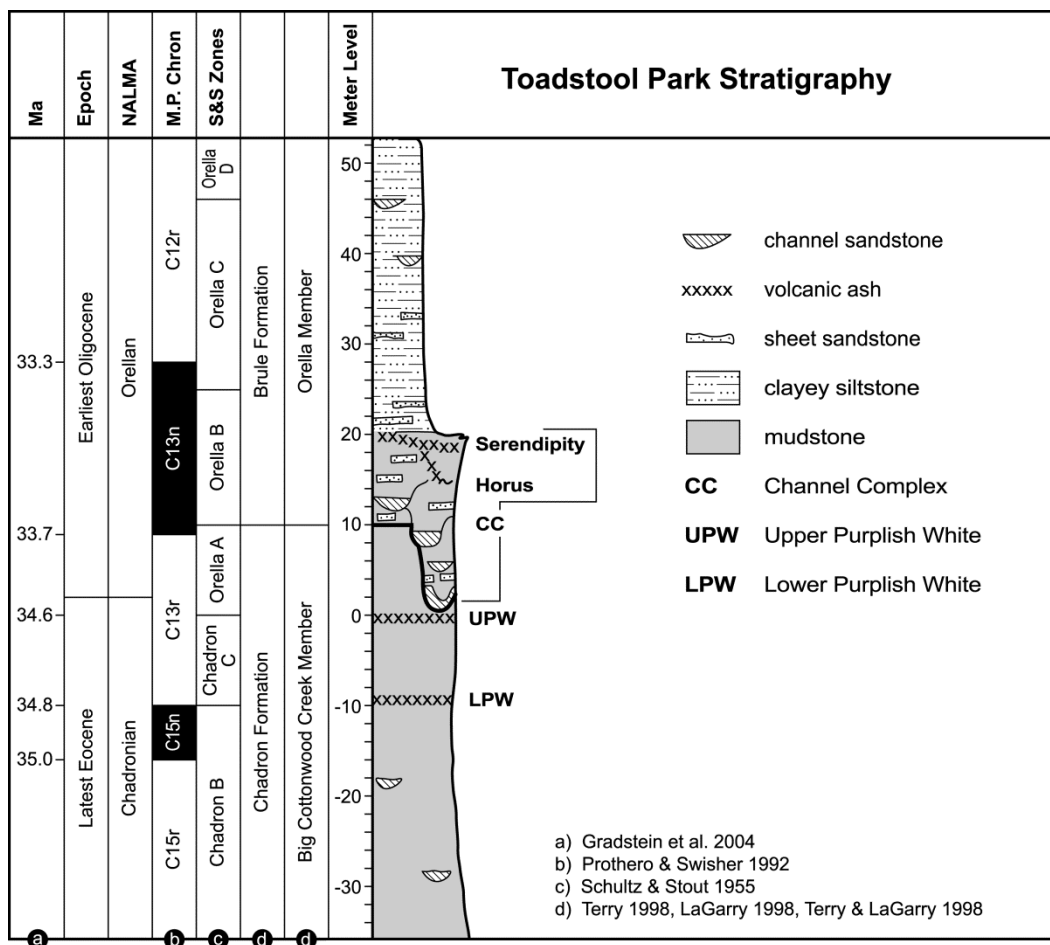


FIGURE 2. Chrono- and lithostratigraphy of the Toadstool Park stratigraphic section.

Volcanic ash dates from Zanazzi et al. (2009). Lithostratigraphy modified from Grandstaff and Terry (2009). Abbreviations: Ma, million years before present; M.P., magnetic polarity; NALMA, North American Land-Mammal “age”; S&S, Schultz and Stout zones.

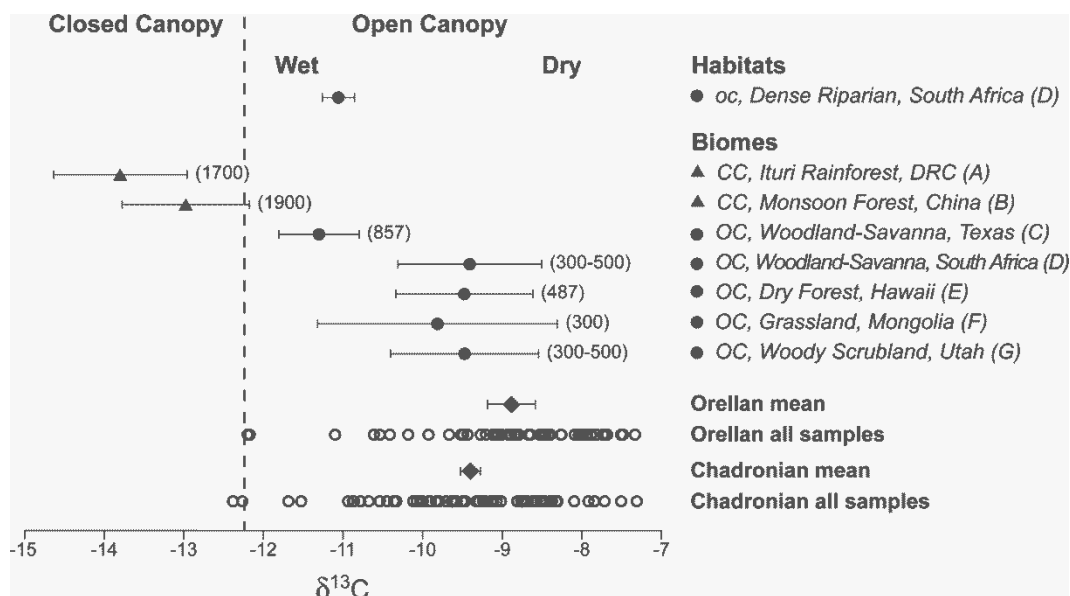


FIGURE 3. Predictive model for $\delta^{13}\text{C}_\text{E}$ values from different biomes and habitats in the late Eocene (Chadronian) and early Oligocene (Orellan). Predictions are based on $\delta^{13}\text{C}$ values from modern floras normalized to 42° N latitude, an altitude of 1.10 km (Sjostrom et al., 2006), and corrected for diet-enamel enrichment and change in atmospheric $\delta^{13}\text{C}$ values (see text). Solid triangles (closed canopy) and circles (open canopy) show mean $\delta^{13}\text{C}_\text{E}$ values expected for a diverse sampling of mammalian herbivores. Mean annual precipitation values (mm/year) for modern biomes (listed on right) used to make predictions are given in parentheses next to error bars. Solid diamonds show uncorrected mean values and open circles show individual values for the Chadronian and Orellan faunas. All error bars show 95% confidence of mean ($\pm 1.96 \cdot \text{SE}$). CC = closed canopy (black triangles), OC = open canopy (black circles). Data sources: A, Cerling et al., 2004; B, Ehleringer et al., 1987; C, Jessup et al., 2003; D, Codron et al., 2005; E, Sandquist and Cordell, 2007; F, Cerling and Harris, 1999; G, Williams and Ehleringer, 1996.

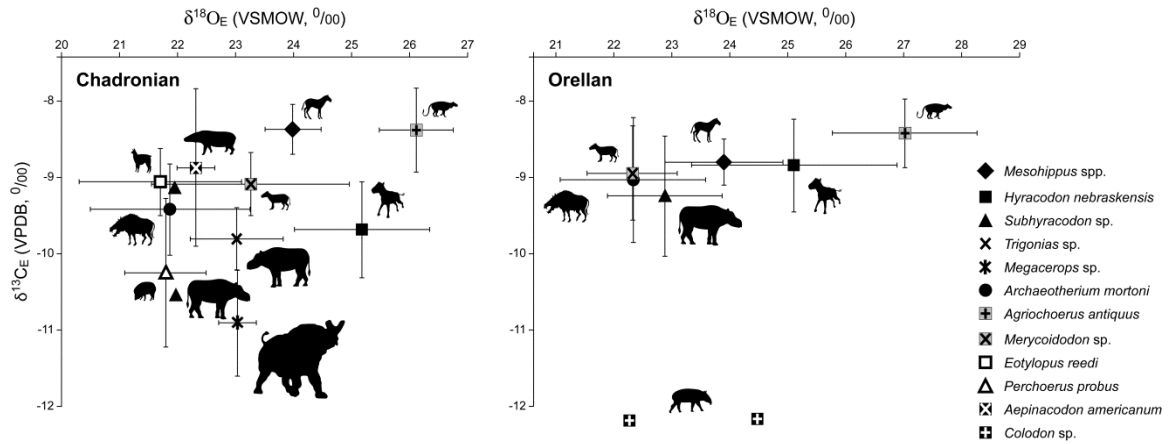


FIGURE 4. Scatter plot of $\delta^{13}\text{C}_\text{E}$ and $\delta^{18}\text{O}_\text{E}$ mean values for Chadronian and Orellan faunas. Error bars represent show 95% confidence of mean ($\pm 1.96 \cdot \text{SE}$) for taxa with >2 samples. Solid triangles without bars are individual samples.

Table 1. Summary of the latest hypothesized habitat preferences and diets of the studied White River ungulates.

Genus	Est. body mass	Dentition	Habitat	Diet
Perissodactyls				
<i>Mesohippus</i>	42 – 54 kg (1)	Brachy-lophodont; molariform premolars (1)	Woodlands (7, si)	Browser (1, mp; 20, mw), mixed-feeder/grazer (10, mc; 17, mc)
<i>Hyracodon</i>	160 kg (2)	Brachy-lophodont (3)	Open plains (3)	Browser (3mp; 10, mc)
<i>Subhyracodon</i>	500 kg (2)	Brachy-lophodont (3)	Open plains (7, si)	Mixed-feeder (3), browser (10)
<i>Trigonias</i>	500 kg (2)	Brachy-lophodont (3)	Open plains (7, si)	Mixed-feeder (3, 10)
<i>Colodon</i>	150 kg (2)	Bilophodont (15)	Forest (16)	Browser (10)
<i>Megacerops</i>	1900 kg (2)	Brachy-selenodont (4)	Forest (7, si)	Browser (9, mc), mixed-feeder (10, mc)
Artiodactyls				
<i>Archaeotherium</i>	180 kg (2)	Robust, apically worn premolars; bunodont molars (5)	Woodlands/Open plains (5)	High abrasion omnivore (5, mc/mp; 10, mw)
<i>Agriochoerus</i>	40-50 kg (18, 19)	Brachy-selenodont (6)	Forest/open plains (8)	Browser (11, mp), mixed-feeder (10, mc)
<i>Merycoidodon</i>	50 kg (2)	Brachy-selenodont (6)	Woodlands (7, si)	Browser/mixed feeder (11, mp), grazer (10, mc)
<i>Perchoerus</i>	35 kg (2)	Bunodont (14)	Riparian/open plains (8, sed)	Hard object browser (10, mc)
<i>Eotylopus</i>	20 kg (2)	Buno-selenodont (13)	Forest (13)	Mixed-feeder (10, mc)
<i>Aepinacodon</i>	280 kg (2)	Brachy-selenodont (12)	Semi-aquatic (12, sed)	Mixed-feeder (10, mc)

Habitat and diet interpretations based on: (mw = mesowear; mc = microwear; si = stable isotopes; mp = morphology; sed = sedimentology). References: (1) MacFadden (1987); (2) Mendoza et al. (2006); (3) Mead and Wall (1998a, b); (4) Rose (2006); (5) Joeckel (1990); (6) Stevens and Stevens (1996); (7) Zanazzi and Kohn (2008); (8) Clark et al. (1967); (9) Muhlbachler and Solounias (2002); (10) Dewar (2008); (11) Wall and Shikany (1995); (12) Kron and Manning (1998); (13) Prothero (1998), (14) Wright (1998), (15) Colbert and Schoch (1998); (16) DeSantis and MacFadden (2007); (17) Solounias and Semperebon (2002); (18) Radinsky (1978); (19) Janis (1982); (20) Muhlbachler et al. (2011).

Table 2. A) Descriptive statistics for Chadronian ungulate taxa examined in this study.

A. Chadronian Genus	n	Mean $\delta^{13}\text{C}_\text{E}$ (‰, VPDB)	S.D. (‰)	S.E. (‰)	Range (‰)	Mean $\delta^{18}\text{O}_\text{E}$ (‰, VSMOW)	S.D. (‰)	S.E. (‰)	Range (‰)
<i>Meshippus</i> spp.	12	-8.4	0.6	0.2	-9.5 to -7.3	24.0	0.9	0.2	22.4 to 25.0
<i>Hyracodon</i> <i>nebraskensis</i>	7	-9.7	0.7	0.3	-10.3 to -8.7	25.2	1.5	0.6	23.5 to 27.2
<i>Subhyracodon</i> sp.	2	-9.8	0.9	0.7	-10.5 to -9.1	22.0	0.0	0.0	22.0
<i>Trionias</i> sp.	7	-9.8	0.6	0.2	-10.8 to -9.2	23.0	1.1	0.4	21.2 to 24.9
<i>Megacerops</i> sp.	7	-10.9	0.9	0.4	-12.3 to -9.8	23.0	0.5	0.2	22.4 to 23.6
<i>Archaeotherium</i> <i>mortoni</i>	6	-9.4	0.8	0.3	-10.4 to -8.5	21.9	1.7	0.7	20.5 to 25.2
<i>Agriochoerus</i> <i>antiquus</i>	3	-8.4	0.5	0.3	-8.8 to -7.8	26.1	0.6	0.3	25.5 to 26.7
<i>Merycoidodon</i> sp.	8	-9.1	0.6	0.2	-9.7 to -8.1	23.3	2.5	0.9	19.2 to 26.4
<i>Aepinacodon</i> <i>americanum</i>	5	-8.9	1.2	0.5	-10.7 to -7.5	22.3	0.4	0.2	21.8 to 22.6
<i>Eotylpus reedi</i>	4	-9.1	0.5	0.2	-9.6 to -8.6	21.7	1.4	0.7	20.4 to 23.1
<i>Perchoerus probus</i>	6	-10.3	1.2	0.5	-12.3 to -9.1	21.8	0.9	0.4	20.5 to 22.6
Chadronian Fauna	67	-9.4	1.1	0.1	-12.3 to -7.3	23.2	1.7	0.2	19.2 to 26.7

B) Matrices of pairwise probabilities of mean differences in $\delta^{13}\text{C}_\text{E}$ (top) and $\delta^{18}\text{O}_\text{E}$ (bottom) values among Chadronian ungulate taxa. Values shown in bold indicate significance for $\alpha \leq 0.05$ using Fisher's least significant difference tests. Pairs that were also significant using Tukey's post hoc test are indicated by asterisks ($*\alpha \leq 0.05$).

B. Chadronian $\delta^{13}\text{C}_\text{E}$, $\delta^{18}\text{O}_\text{E}$	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.
1. <i>Mesohippus</i> spp.	1.00, 1.00										
2. <i>Hyracodon nebraskensis</i>	0.000* , 0.035*	1.00, 1.00									
3. <i>Subhyracodon</i> sp.	0.0097* , 0.007*	0.815, 0.020*	1.00, 1.00								
4. <i>Trigonias</i> sp.	0.000* , 0.044*	0.721, 0.008*	0.965, 0.227	1.00, 1.00							
5. <i>Megacerops</i> sp.	0.000* , 0.014*	0.019* , 0.003*	0.204, 0.015*	0.025* , 0.976	1.00, 1.00						
6. <i>Archaeotherium mortoni</i>	0.005* , 0.003*	0.534, 0.003*	0.551, 0.943	0.307, 0.169	0.010* , 0.111	1.00, 1.00					
7. <i>Agriochoerus antiquus</i>	0.981, 0.001*	0.022* , 0.325	0.106, 0.002*	0.005* , 0.002*	0.003* , 0.000*	0.068, 0.005*	1.00, 1.00				
8. <i>Merycoidodon</i> sp.	0.015* , 0.351	0.103, 0.093	0.191, 0.496	0.031* , 0.817	0.001* , 0.817	0.374, 0.260	0.098, 0.085	1.00, 1.00			
9. <i>Aepinacodon americanum</i>	0.247, 0.001*	0.167, 0.002*	0.361, 0.274	0.092, 0.193	0.008* , 0.016*	0.371, 0.588	0.523, 0.000*	0.662, 0.418	1.00, 1.00		
10. <i>Eotylopus reedi</i>	0.049* , 0.001*	0.153, 0.004*	0.227, 0.819	0.046* , 0.046*	0.006* , 0.043*	0.412, 0.878	0.111, 0.005*	0.924, 0.274	0.776, 0.383	1.00, 1.00	
11. <i>Perchoerus probus</i>	0.000* , 0.000*	0.317, 0.000*	0.678, 0.799	0.402, 0.047*	0.299, 0.007*	0.185, 0.925	0.041* , 0.000*	0.035* , 0.190	0.089, 0.247	0.101, 0.904	1.00, 1.00

Table 3. A) Descriptive statistics for Orellan ungulate taxa examined in this study.

A. Orellan Genus	n	Mean $\delta^{13}\text{C}_\text{E}$ (‰, VPDB)	S.D. (‰)	S.E. (‰)	Range (‰)	Mean $\delta^{18}\text{O}_\text{E}$ (‰, VSMOW)	S.D. (‰)	S.E. (‰)	Range (‰)
<i>Mesohippus</i> spp.	8	-8.8	0.5	0.2	-10.6 to -7.9	23.9	1.8	0.6	22.1 to 26.7
<i>Hyracodon</i> <i>nebraskensis</i>	10	-8.9	0.9	0.3	-10.6 to -7.9	25.1	2.9	0.9	20.7 to 28.3
<i>Subhyracodon</i> sp.	4	-9.3	0.8	0.4	-10.2 to -8.3	22.9	1.0	0.5	21.8 to 24.2
<i>Colodon</i> sp.	2	-12.2	0.0	0.0	-12.2	23.4	1.6	1.1	22.3 to 24.5
<i>Archaeotherium</i> <i>mortoni</i>	7	-9.0	1.1	0.4	-11.1 to -7.7	22.3	1.7	0.6	20.1 to 24.5
<i>Agriochoerus</i> <i>antiquus</i>	9	-8.4	0.7	0.2	-9.3 to -7.3	27.0	1.9	0.6	22.5 to 30.0
<i>Merycoidodon</i> sp.	7	-8.9	0.8	0.3	-10.4 to -7.8	22.3	1.1	0.4	21.0 to 23.4
Orellan Fauna	47	-8.9	1.1	0.2	-12.2 to -7.3	24.2	2.5	0.4	20.1 to 30.0

B) Matrices of pairwise probabilities of mean differences in $\delta^{13}\text{C}_\text{E}$ and $\delta^{18}\text{O}_\text{E}$ values among Orellan ungulate taxa. Values shown in bold indicate significance for $\alpha \leq 0.05$ using Fisher's least significant difference tests. Pairs that were also significant using Tukey's post hoc test are indicated by asterisks ($*\alpha \leq 0.05$).

B. Orellan $\delta^{13}\text{C}_\text{E}$, $\delta^{18}\text{O}_\text{E}$	1.	2.	3.	4.	5.	6.	7.
1. <i>Mesohippus</i> spp.	1.00, 1.00						
2. <i>Hyracodon nebraskensis</i>	0.896, 0.339	1.00, 1.00					
3. <i>Subhyracodon</i> sp.	0.233, 0.292	0.486, 0.161	1.00, 1.00				
4. <i>Colodon</i> sp.	0.001* , 0.681	0.001* , 0.433	0.008* , 0.656	1.00, 1.00			
5. <i>Archaeotherium mortoni</i>	0.580, 0.092	0.717, 0.036*	0.744, 0.569	0.006* , 0.461	1.00, 1.00		
6. <i>Agriochoerus antiquus</i>	0.217, 0.004*	0.303, 0.108	0.086, 0.002*	0.000* , 0.034*	0.196, 0.000*	1.00, 1.00	
7. <i>Merycoidodon</i> sp.	0.081, 0.052	0.829, 0.027	0.575, 0.410	0.001* , 0.289	0.869, 0.989	0.193, 0.000*	1.00, 1.00

Table 4.

A) Pairwise probabilities of difference in the $\delta^{13}\text{C}$ values between the whole Chadronian and Orellan faunal samples and possible vegetative biomes. Values in bold indicate significance for $\alpha \leq 0.05$ using Fisher's least significant difference tests. These tests clearly show that both faunas were living in "dry", open canopy habitats; based on phytolith assemblage data I can exclude grassland. B) Pairwise probabilities of difference in the $\delta^{13}\text{C}$ values between *Megacerops* sp., *Perchoerus probus*, *Colodon* sp. and riparian habitat.

A. Biomes	Chadronian Fauna	Orellan Fauna	
“Wet” Ituri Rainforest (DRC)	<0.001	<0.001	
“Wet” Monsoon Forest (China)	<0.001	<0.001	
“Wet” Woodland-Savanna (Texas)	<0.001	<0.001	
“Dry” Woody Scrubland (Utah)	0.916	0.219	
“Dry” C3 Grassland (Mongolia)	0.429	0.054	
“Dry” Woodland Savanna (South Africa)	0.871	0.506	
“Dry” Forest (Hawaii)	0.715	0.083	
B. Habitat\Taxon	<i>Megacerops</i> sp.	<i>Perchoerus probus</i>	<i>Colodon</i> sp.
Riparian (Open Canopy)	0.853	0.424	0.129

Table 5.

Statistics for Chadronian and Orellan taxa from the Toadstool Park area reported in Zanazzi and Kohn (2008). Differences (Δ) indicate whether the Zanazzi and Kohn (2008) means are more positive or more negative than means from my study. P-values shown in bold indicate significance for $\alpha \leq 0.05$ using Fisher's least significant difference tests.

Chadronian	n	$\delta^{13}\text{C}_\text{E}$	$\Delta\delta^{13}\text{C}_\text{E}$	p	$\delta^{18}\text{O}_\text{E}$	$\Delta\delta^{18}\text{O}_\text{E}$	p
<i>Mesohippus</i> sp.	7	-9.8	-1.4	<0.001	24.3	0.3	0.520
<i>Subhyracodon</i> sp.	2	-10.1	-0.3	0.822	23.0	1.1	0.143
<i>Megacerops</i> sp.	3	-11.1	-0.2	0.807	22.9	-0.1	0.780
<i>Merycoidodon</i> sp.	6	-10.1	-1.0	0.039	23.2	-0.1	0.948
Orellan							
<i>Mesohippus</i> sp.	8	-9.3	-0.5	0.128	23.2	-0.7	0.378
<i>Subhyracodon</i> sp.	2	-8.8	0.5	0.517	24.7	1.8	0.125
<i>Merycoidodon</i> sp.	7	-9.6	-0.6	0.213	23.3	1.0	0.247

APPENDIX 1.

Specimen #	Taxonomic ID	tooth	NALMA	Locality	^A S an S Zone	*Strat. Level (m)	d13C (VPDB)	d18O (VSMOW)
3-10-9-35 SP	<i>Archaeotherium mortoni</i>	left M/3	Chadronian	Sx-44	Chadron C	0.0	-10.1	21.9
359-38	<i>Archaeotherium mortoni</i>	right M/3	Chadronian	Dw-"C7"	Chadron	below 0	-9.6	21.9
76-38	<i>Archaeotherium mortoni</i>	right M/3	Chadronian	Sx-26	Chadron C	0.0	-8.8	21.0
4725-38	<i>Archaeotherium mortoni</i>	left M3/	Chadronian	Sx-44	Chadron C	0.0	-8.5	20.5
707-38	<i>Archaeotherium mortoni</i>	right M3/	Chadronian	Sx-34	Chadron B	-20.0	-10.4	20.7
no #	<i>Archaeotherium mortoni</i>	left M3/	Chadronian	Sx-29	Chadron	below 0	-9.2	25.2
no #; Talus	<i>Agriochoerus antiquus</i>	left M/3	Chadronian	Dw-104	Chadron C	0 to -12	-7.8	26.7
55-38	<i>Agriochoerus antiquus</i>	right M/3	Chadronian	Dw-0	Chadron C	0 to -6	-8.6	25.5
75-38	<i>Agriochoerus antiquus</i>	left M/3	Chadronian	Sx-26	Chadron C	-0.5	-8.8	26.1
603-77	<i>Merycoidodon sp.</i>	right M/3	Chadronian	Dw-104	Chadron C	-10.7	-9.5	26.4
1015-38	<i>Merycoidodon sp.</i>	left M/3	Chadronian	Sx-25	Chadron C	-6.1	-8.1	19.2
438-38	<i>Merycoidodon sp.</i>	right P/4	Chadronian	Sx-32	Chadron C	-3.0	-9.5	26.3
207-53	<i>Merycoidodon sp.</i>	left M/3	Chadronian	Dw-104	Chadron C	0.0	-9.6	23.8
609-38	<i>Merycoidodon sp.</i>	right M/3	Chadronian	Sx-29	Chadron C	-0.9	-9.2	23.6
1-16-7-36 SP	<i>Merycoidodon sp.</i>	left M/3	Chadronian	Sx-26	Chadron C	-1.2	-9.7	22.9
2597-63	<i>Merycoidodon sp.</i>	right M/3	Chadronian	Sx-35	Chadron C	0.0	-8.5	20.7
208-53	<i>Merycoidodon sp.</i>	left M/3	Chadronian	Dw-104	Chadron C	0.0	-8.7	23.1
132020	<i>Subhyracodon sp.</i>	right M3/	Chadronian	Sx-33	Chadron B	-30.0	-10.5	21.9
132026	<i>Subhyracodon sp.</i>	left M/2	Chadronian	Sx-34	Chadron C	-4.6	-9.1	21.9
516-38	<i>Trigonias sp.</i>	left P4/	Chadronian	Sx-26	Chadron B	-34.3	-9.2	23.1
2650-63	<i>Trigonias sp.</i>	right M/3	Chadronian	Sx-N Harrison	Chadron	below 0	-10.0	24.9
132022	<i>Trigonias sp.</i>	left M/2	Chadronian	Sx-29	Chadron B	-23.8	-10.1	23.2
132023	<i>Trigonias sp.</i>	left M3/	Chadronian	Sx-33	Chadron B	-30.0	-9.8	22.7
132024	<i>Trigonias sp.</i>	right M/3	Chadronian	Sx-33	Chadron B	-30.0	-9.3	22.9
132025	<i>Trigonias sp.</i>	right M/1	Chadronian	Sx-0	Chadron B	-15.2	-9.5	23.2
132015	<i>Trigonias sp.</i>	right P/4	Chadronian	Sx-31	Chadron C	-1.4	-10.8	21.2
11326	<i>Hyracodon nebraskensis</i>	right M/2	Chadronian	Sx-26	Chadron C	-0.5	-9.0	27.2
132000	<i>Hyracodon nebraskensis</i>	left M/1	Chadronian	Sx-0	Chadron B	-24.3	-9.9	24.5
132001	<i>Hyracodon nebraskensis</i>	left M/1	Chadronian	Dw-0	Chadron C	-4.0	-9.6	23.5
132002	<i>Hyracodon nebraskensis</i>	left M/1	Chadronian	Sx-33	Chadron B	-30.0	-10.9	24.6

132004	<i>Hyracodon nebraskensis</i>	left M/1	Chadronian	Dw-13	Chadron B	-13.6	-9.6	26.5
11246	<i>Hyracodon nebraskensis</i>	left M/3	Chadronian	Dw-?	Chadron B	-25.0	-8.7	23.8
132006	<i>Hyracodon nebraskensis</i>	right M/1	Chadronian	Sx-0	Chadron B	-24.4	-9.9	26.3
3222-91	<i>Megacerops</i> sp.	Mx/ frag	Chadronian	Sx-USDA	Chadron	below 0	-10.4	23.1
3008-91	<i>Megacerops</i> sp.	M/x frag	Chadronian	Sx-USDA	Chadron	below 0	-9.8	22.4
132-38	<i>Megacerops</i> sp.	M/x frag	Chadronian	Sx-"C4"	Chadron	below 0	-10.9	23.5
3287-91	<i>Megacerops</i> sp.	left M/3 frag	Chadronian	Sx-USDA	Chadron	below 0	-9.9	23.2
132013	<i>Megacerops</i> sp.	right M/3	Chadronian	Sx-33	Chadron B	-20.0	-11.7	22.9
132014	<i>Megacerops</i> sp.	left P/4	Chadronian	Sx-33	Chadron B	-20.0	-12.3	22.5
132018	<i>Megacerops</i> sp.	right M/1	Chadronian	Sx-33	Chadron B	-20.0	-11.5	23.6
131508	<i>Mesohippus grandis</i>	right M/3	Chadronian	Sx-18	Chadron C	0.0	-8.3	23.7
131528	<i>Mesohippus grandis</i>	right M/3	Chadronian	Sx-33	Chadron C	0.0	-8.4	22.4
131525	<i>Mesohippus grandis</i>	right M/3	Chadronian	Sx-33	Chadron C	-6.1	-7.7	24.9
131724	<i>Mesohippus grandis</i>	left M/3	Chadronian	Sx-26	Chadron B	-34.9	-9.5	23.9
131542	<i>Mesohippus grandis</i>	left M/2	Chadronian	Sx-26	Chadron B	-34.3	-8.5	23.9
131750	<i>Mesohippus grandis</i>	right M/2	Chadronian	Sx-8	Chadron C	0	-8.5	24.9
131725	<i>Mesohippus bairdii</i>	left M/3	Chadronian	Sx-25	Chadron C	-6.1	-8.4	23.9
131731	<i>Mesohippus bairdii</i>	right M/3	Chadronian	Sx-34	Chadron C	-4.6	-8.3	24.2
131768	<i>Mesohippus bairdii</i>	right M/3	Chadronian	Sx-29	Chadron C	-6.1	-8.7	24.7
131510	<i>Mesohippus bairdii</i>	left M3/	Chadronian	Sx-39	Chadron C	-2.4	-9.1	25.0
131577	<i>Mesohippus bairdii</i>	left M2/	Chadronian	Sx-18	Chadron C	0	-7.3	23.1
131728	<i>Mesohippus bairdii</i>	left M/3	Chadronian	Sx-33	Chadron B	-18.3	-7.9	24.1
50-38	<i>Aepinacodon americanum</i>	left M/3	Chadronian	Dw-"C3"	Chadron	below 0	-9.1	22.0
2657-63	<i>Aepinacodon americanum</i>	left M/3	Chadronian	Sx-34	Chadron	below 0	-10.7	21.8
127-54	<i>Aepinacodon americanum</i>	left M/2	Chadronian	Sx-33	Chadron B	below -12	-8.8	22.7
1-23-8-34 SP	<i>Aepinacodon americanum</i>	Ix and Px	Chadronian	Sx-0	Chadron	below 0	-7.5	22.6
2533-60	<i>Aepinacodon americanum</i>	Px	Chadronian	Sx-42	Chadron B	-23.2	-8.3	22.4
123701	<i>Pechoerus probus</i>	left M/3	Chadronian	Sx-26	Chadron B	-34.3	-12.3	22.2
No #	<i>Pechoerus probus</i>	left M/2	Chadronian	Dw-13	Chadron	below 0	-9.1	22.6
2148-73	<i>Pechoerus probus</i>	right M/3	Chadronian	Sx-29	Chadron C	-5.5	-9.6	20.9
53609	<i>Pechoerus probus</i>	right M/3	Chadronian	Sx-35	Chadron B	-24.7	-10.3	22.3
600-38	<i>Pechoerus probus</i>	left M/2	Chadronian	Sx-29	Chadron C	-9.9	-9.3	22.2
3081-011	<i>Pechoerus probus</i>	left M/2	Chadronian	Sx-35	Chadron B	-24.7	-10.9	20.5

316-66	<i>Eotylopus reedi</i>	left M/2	Chadronian	Dw-107	Chadron C	0 to -6	-9.6	20.4
?-82	<i>Eotylopus reedi</i>	right M/2	Chadronian	Creek"	Chadron	below 0	-9.2	22.7
1061-64	<i>Eotylopus reedi</i>	right M/3	Chadronian	Dw-107	Chadron	below 0	-8.8	20.5
628-46	<i>Eotylopus reedi</i>	left M/3	Chadronian	Sx-0	Chadron	below 0	-8.6	23.1
414-53	<i>Archaeotherium mortoni</i>	P/4	Orellan	Sx-13	Orella B	15.1	-7.7	20.7
434-54	<i>Archaeotherium mortoni</i>	right M/2	Orellan	Sx-36	Orella D	~47.0	-8.1	20.1
251-52	<i>Archaeotherium mortoni</i>	left P4/	Orellan	Sx-26	Orella C	27.1	-9.7	21.9
11-18-8-33	<i>Archaeotherium mortoni</i>	left M2/	Orellan	Sx-10	Orella C	25 to 46	-8.9	24.4
6-24-8-34 SP	<i>Archaeotherium mortoni</i>	frag M/?	Orellan	Sx-17	Orella C	25 to 46	-11.1	22.6
339-54	<i>Archaeotherium mortoni</i>	right M/3	Orellan	Sx-36	Orella A	2.1	-8.9	24.5
300-52	<i>Archaeotherium mortoni</i>	left P4/	Orellan	Sx-23	Orella D	50.5	-8.9	22.2
46048	<i>Agriochoerus antiquus</i>	left M3/	Orellan	Sx-18	Orella A	6.1	-7.7	26.9
52531 b	<i>Agriochoerus antiquus</i>	left M/3	Orellan	Sx-17	Orella A	2.7	-8.8	26.4
52530	<i>Agriochoerus antiquus</i>	left M/3	Orellan	Sx-17	Orella C	35.4	-9.3	28.9
84-15-8-35	<i>Agriochoerus antiquus</i>	right M/3	Orellan	Sx-11	Orella A	5.0	-7.3	27.3
46047	<i>Agriochoerus antiquus</i>	left M/3	Orellan	Sx-26	Orella A	6.1	-8.7	22.5
6-5-9-34 SP	<i>Agriochoerus antiquus</i>	left M3/	Orellan	Sx-9	Orella A	5.0	-8.0	27.1
46038	<i>Agriochoerus antiquus</i>	right M3/	Orellan	Sx-24	Orella C	35.4	-7.9	28.9
46050	<i>Agriochoerus antiquus</i>	right M3/	Orellan	Sx-11	Orella A	5.0	-8.9	27.7
17-22-8-34	<i>Agriochoerus antiquus</i>	right M3/	Orellan	Sx-12	Orella A	4.7	-9.2	27.3
37-28-8-34	<i>Merycoidodon sp.</i>	right M/3	Orellan	Sx-12	Orella A	4.7	-9.0	21.4
1545-38	<i>Merycoidodon sp.</i>	left M/3	Orellan	Sx-13	Orella B	15.1	-7.8	23.3
66-5-8-36 SP	<i>Merycoidodon sp.</i>	left M/3	Orellan	Sx-5	Orella C	25 to 46	-8.4	23.1
13-21-7-33	<i>Merycoidodon sp.</i>	left M/3	Orellan	Sx-4	Orella B	10 to 25	-10.4	22.8
3-24-7-33 SP	<i>Merycoidodon sp.</i>	left M/3	Orellan	Sx-4	Orella	above 0	-9.1	21.2
466-72	<i>Merycoidodon sp.</i>	left M/3	Orellan	Toadstool	Orella B	10 to 25	-9.4	21.0
2708-66	<i>Merycoidodon sp.</i>	right M/3	Orellan	Sx-1	Orella C	39.3	-8.5	23.4
132017	<i>Subhyracodon sp.</i>	left M3/	Orellan	Sx-39	Orella A	5.0	-9.1	21.8
132021	<i>Subhyracodon sp.</i>	left M2/	Orellan	Sx-26	Orella A	4.6	-8.3	22.7
132012	<i>Subhyracodon sp.</i>	right M/2	Orellan	Sx-19	Orella A	5.5	-9.5	22.8
132016	<i>Subhyracodon sp.</i>	right M3/	Orellan	Crawford 12	Orella B	23.5	-10.2	24.2
132003	<i>Hyracodon nebraskensis</i>	left M2/	Orellan	Sx-33	Orella B	17.4	-8.0	28.3
132005	<i>Hyracodon nebraskensis</i>	right M/3	Orellan	Sx-19	Orella C	35.7	-9.1	25.1

132007	<i>Hyracodon nebraskensis</i>	M/x? frag	Orellan	Sx-14	Orella A	5.0	-8.5	22.8
11359	<i>Hyracodon nebraskensis</i>	right M/1	Orellan	Sx-4	Orella B	23.2	-10.5	24.9
132008	<i>Hyracodon nebraskensis</i>	left M/2	Orellan	Sx-6	Orella C	25.6	-8.8	21.1
11257	<i>Hyracodon nebraskensis</i>	right M/1	Orellan	Sx-26	Orella A	8.8	-7.9	25.1
132009	<i>Hyracodon nebraskensis</i>	left M2/	Orellan	Sx-17	Orella B	21.0	-8.3	28.4
132011	<i>Hyracodon nebraskensis</i>	left M/3	Orellan	Sx-39	Orella A	6.0	-10.6	20.7
11357	<i>Hyracodon nebraskensis</i>	right M/1	Orellan	Sx-19	Orella C	29.0	-8.8	27.6
11216	<i>Hyracodon nebraskensis</i>	left M/1	Orellan	Sx-39	Orella A	8.5	-7.9	27.4
131734	<i>Mesohippus grandis</i>	right M/3	Orellan	Sx-19	Orella A	5	-9.5	22.9
131583	<i>Mesohippus grandis</i>	left M/3	Orellan	Sx-0	Orella A	7.9	-8.5	24.7
131779	<i>Mesohippus grandis</i>	left M/3	Orellan	Sx-14	Orella A	7.9	-8.4	22.4
131739	<i>Mesohippus grandis</i>	left M/3	Orellan	Sx-18	Orella A	2.3	-8.8	24.8
131774	<i>Mesohippus bairdii</i>	right M/2	Orellan	Sx-14	Orella A	3.9	-8.5	22.7
131749	<i>Mesohippus bairdii</i>	left M/3	Orellan	Sx-26	Orella A	8.8	-8.5	25.9
131741	<i>Mesohippus bairdii</i>	left M/3	Orellan	Sx-26	Orella A	8.8	-9.5	26.7
131769	<i>Mesohippus bairdii</i>	M/3	Orellan	Sx-13	Orella A	3.9	-8.6	22.1
2797-66	<i>Colodon sp.</i>	left M/2	Orellan	Sx-State Line	Orella	above 0	-12.2	22.3
45109	<i>Colodon sp.</i>	right M/3	Orellan	Sx-14	Orella	above 0	-12.2	24.5

^A**S and S Zone** from Schultz and Stout (1955) stratigraphy. Orella D, above 46m; Orella C, 25-46m; Orella B, 10-25m; Orella A, 0-10 m. Chadron C, 0 to ~-12m; Chadron B, below ~-12m.

* **Strat level** is in meters above (+) or below (-) the UPW; range of values reflect specimens of known zone but unknown meter level.

CHAPTER 3

Local feeding ecology of ungulates from northwestern Nebraska during the Eocene-Oligocene climate transition

Abstract

I evaluate the diets and potential increased dietary abrasion of eight ungulate species from the White River Group in Nebraska through assessments of dental mesowear and the hypsodonty index. The White River Group spans an important interval of heterogeneous global cooling and probable drying from the upper Eocene to the lower Oligocene, providing a testing ground for the effects of climate change on mammalian diet. In Nebraska, climate is interpreted as becoming more arid through this interval; if drying occurred, I would expect to find a shift to more abrasive diets in taxa occupying more open areas. I tested for increasing dietary abrasion in three range-through species (*Mesohippus bairdi*, *Hyracodon nebraskensis*, and *Merycoidodon* sp.), which on the basis of stable-isotope analyses, likely lived in the most open habitats. In the case of *M. bairdi* and *H. nebraskensis* dietary abrasion does not increase significantly, strongly suggesting that there was little change, if any, in the consumption of grass and grit by these mixed-feeding species. In *Merycoidodon* sp., however, dietary abrasion increases over time, indicating a shift from browsing to mixed-feeding. This shift suggests that *Merycoidodon* occupied a new ecological niche as grasses became more abundant, in effect avoiding more intensive competition with browsers. Analyses of mesowear and hypsodonty indicate that *Subhyracodon* sp. and *Aepinacodon americanum* were mixed-feeders, *Agriochoerus antiquus* and *Megacerops* sp. were browsers, and *Eotylopus reedi* was a

frugivore. An alternative interpretation to increasing grass biomass is that the amount of volcanic ash in the system increased during the Orellan. However, this hypothesis is inconsistent with a lack of abrasive wear in lower Oligocene *A. antiquus*, a browsing oreodont that appears to have lived in the most open habitats present, based on high carbon and oxygen isotope values. No species showed an increase in hypsodonty index values in the Orellan, an adaptation that might be expected for handling increased dietary abrasion. Overall mesowear results closely are consistent with other studies that suggest the expansion of dry, open habitats in the Orellan, with little effect on the diet of most open-habitat dwelling taxa.

Keywords: Mesowear; Hypsodonty Index; Mammal; Diet; Chadronian; Orellan.

1. Introduction

Studies of terrestrial sequences spanning the Eocene-Oligocene climate transition (EOCT) indicate that significant, but heterogeneous changes in climate occurred at mid-latitudes. Most proxy evidence from North America suggests increasing aridity with minimal cooling. This evidence includes changes in soil structure and root trace size (Retallack, 1992; Terry, 2001), geochemical trends suggesting significant decrease in mean annual precipitation but little change in mean annual temperatures (Sheldon and Retallack, 2004; Retallack, 2007; Sheldon, 2009), turnover in terrestrial gastropod and aquatic herpetofaunas (Hutchison, 1982, 1992; Evanoff et al., 1992), and enamel stable isotope data from ungulates (Boardman and Secord, 2013) suggesting reduction in the extent of wetter, more densely vegetated habitats and the expansion of sub-humid, woodland-savanna conditions in the early Oligocene. This period is also one of

increasing volcanism and volcanoclastic sedimentation in North America (LaGarry, 1998; Larson and Evanoff, 1998; Terry, 1998; Terry and LaGarry, 1998).

Mesowear (*sensu* Fortelius and Solounias, 2000) measures the relative abrasive nature of a diet by evaluating cusp relief and apical sharpness. This wear is cumulative over several months or even years, and as such correlates with diet and other ingesta. Grit, unintentionally ingested particulate matter, has been shown to affect the mesowear in captive ungulate populations. For instance, in giraffes free-ranging populations have mesowear consistent with their browsing diets but captive populations have mesowear consistent with grazing due to increased grit content in zoo foods (Clauss et al., 2007). This study illustrates the significant effect of grit on mesowear in circumstances when excessive grit is associated with food items regardless of plant type and proximity to the ground. Several other studies (e.g. Kaiser et al., 2011; Yamada, 2012) have shown that environmental grit has little effect on interpretation of mesowear in extant taxa living in open habitats because these animals are consuming abrasive foods in habitats that have abundant exogenous grit; suggesting mesowear detects diet composition and not necessarily habitat conditions. Mesowear has been used for several years to reconstruct the diets of ungulates and to test hypotheses regarding diet/habitat coevolution and the spread of grasslands in deep time (Kaiser et al., 2000; Kaiser and Solounias, 2003; Merceron et al., 2007; Croft and Weinstein, 2008; Semprebon and Rivals, 2010; Muhlbachler et al., 2011; Rivals, 2012; Fraser and Theodor, 2013). In this study I analyze mesowear to categorize the diet of the species examined.

The evolution of hypsodont (high-crowned) teeth in herbivorous mammals is generally accepted as an evolutionary adaptation for dealing with high rates of dental

wear associated with increasingly abrasive diets caused either by consuming more highly abrasive foods (phytolith rich grasses) or by consuming foods in more open habitats that may be covered in abrasive grit (e.g. Damuth and Janis, 2011; Jardine et al., 2012). This generality is supported by the correlation between crown height and dietary-abrasion/habitat-openness among extant ungulates (Janis, 1988; Damuth and Janis, 2011). It is difficult to untangle the importance of the two factors driving acquisition of hypsodonty in many lineages because processes favoring the expansion of grasses, cooling and drying climates with expanding open habitats, also favor the increased production of exogenous grit (Damuth and Janis, 2011). Acquisition of hypsodonty in some lineages may also potentially be driven by consumption of ash-laden food during extensive periods of increased volcanism, as postulated for South American notoungulates (Reguero et al., 2010). Given the period of time needed for these factors to drive selection for the evolution of increasing hypsodonty it should not be surprising that its acquisition in many lineages (e.g. oreodonts and equids) has been shown to lag behind the opening of habitats and spread of grasslands (e.g. Mhihlbachler and Solounias, 2006; Strömberg, 2005, 2006; Mhihlbachler et al., 2011) or to proceed it due to increased consumption of grit in the form of ash (as in notoungulates; Reguero et al., 2010; Croft and Weinstein, 2008) by several million years. Given the potential for lag or precociousness, tooth-crown height measured in a taxon at any given time represents its ability to deal with increasing abrasive wear, without necessarily reflecting the preferred diet of that taxon.

Herein, I characterize the abrasive nature of diets using mesowear and I evaluate whether or not there was a correlation between hypsodonty and dietary abrasion. Greater

hypsodonty might be expected with a shift to more abrasive diets. Eight non-bunodont ungulate species (Table 6) were studied. Mesowear in six of these species (*Hyracodon nebraskensis*, *Subhyracodon* sp., *Megacerops* sp., *Agriochoerus antiquus*, *Eotylopus reedi*, and *Aepinacodon americanum*) has not previously been examined. The mesowear patterns of three ubiquitous, range-through species (*Hyracodon nebraskensis*, *Mesohippus bairdi*, and *Merycoidodon* sp.) are of particular interest for testing whether or not aridification through this interval, presumably coupled with an increase in grass/grit abundance as habitats became more open, led to more abrasive diets. On the basis of stable isotopes from the same species (Boardman and Secord, 2013), these three range-through taxa did not move to more open habitats in the Orellan. Therefore, increasing dietary abrasion in these taxa is not expected *a priori* unless the abundance of ash adhering to leaves increased significantly, or unless these species switched to eating more grit-laden grass at ground level. I expect mesowear in all taxa to be affected if ash is present for extended periods of time. On the other hand, if individuals are consuming more grass an increase in abrasion in some species, but not others should reflect the increase in grass consumption.

2. Materials and methods

A total of 100 specimens representing 8 taxa were examined from two superposed, time-averaged faunas from the upper Eocene (Chadronian North American Land Mammal “age”) and lower Oligocene (Orellan North American Land Mammal “age”) White River Group (WRG) of northwestern Nebraska. All specimens are curated at the University of Nebraska State Museum (UNSM) in Lincoln, Nebraska.

From each fauna, all suitable specimens of the taxa of interest were sampled for mesowear. The optimal sample size of ten specimens recommended by Fortelius and Solounias (2000) could not be met for some of the taxa in this study. Results for taxa with sample sizes smaller than ten are considered suggestive but not robust. Overall sample sizes were small because only a very small percentage of specimens in the UNSM collections met quality standards as discussed below. From the Chadronian fauna, this includes *Mesohippus bairdi* (N = 10), *Megacerops* sp. (N = 10), *Merycoidodon* sp. (N = 12), *Hyracodon nebraskensis* (N = 4), *Aepinacodon americanum* (N = 3), and *Eotylpus reedi* (N = 7). From the Orellan, this includes *Mesohippus bairdi* (N = 10), *Merycoidodon* sp. (N = 11), *Hyracodon nebraskensis* (N = 14), *Agriochoerus antiquus* (N = 9), and *Subhyracodon* sp. (N = 10). M2/ is used to denote upper second molars, and M/2 to denote lower second molars.

2.1. Mesowear

Mesowear in the Chadronian and Orellan ungulate taxa was scored macroscopically according to cusp relief and cusp shape (Fig. 5) from the buccal side of M2/s, preferably the paracone, following the conventions of Fortelius and Solounias (2000). Teeth where both buccal cusps had damaged or broken apices were omitted from mesowear analysis, following the protocol of Fortelius and Solounias (2000). Also, to offset the potential effects of ontogeny on mesowear values in brachydont taxa noted by Rivals et al. (2007), all of the specimens were from the same ontogenetic age group. This age assessment was facilitated by sampling only second molars in specimens where the third molar had erupted and was in occlusion but was not yet worn. Isolated second molars were also scored where appropriate wear age could be confidently determined.

Cusp occlusal relief (OR) was scored as high (h) or low (l) following metric cutoffs from Fortelius and Solounias (2000). Cusp shapes were classified as: sharp (s), rounded (r), and blunt (b), following the standards in Fortelius and Solounias (2000). Mesowear from the lower dentition, which was scored from the lingual side of the M/2, was also considered in this study for taxa in which lower dentitions make up the bulk of the specimens in the UNSM collection (i.e. *Eotylpus reedi*). Mesowear from lower dentition was considered following the interpretational concerns of Franz-Odenaal and Kaiser (2003) because mesowear from lower molars have been shown to be less sharp relative to attendant upper molars in non-specialized feeders (i.e. mixed-feeders), but more or less the same in specialized feeders (i.e. browsers, grazers, and potentially frugivores). Each specimen was scored for cusp relief and cusp shape, then the percentages of high (%h), sharp (%s), and blunt (%b) were calculated for each taxon, as these three variables combined have shown the most utility in categorizing extant ungulates by diet (Fortelius and Solounias, 2000).

Among ungulate taxa with selenodont and lophodont dentition and predominantly transverse shearing mastication (i.e. perissodactyls and most artiodactyls), a diet low in abrasives (such as the browsing diet of the Sumatran rhinoceros, *Dicerorhinus sumatrensis*) maintains sharpened buccal cusp apices as the tooth wears. In contrast, a diet high in silica-rich grasses and/or abrasive particulates (such as the grazing diet of the American bison, *Bison bison*), or diets high in tough fruits which facilitate “tip crushing” (such as in the Bay duiker, *Cephalophus dorsalis*), result in more rounded or blunted buccal cusp apices.

2.2. Hypsodonty Index

Hypsodonty index (HI) values, measured as unworn lower third molar crown height divided by occlusal width (Janis, 1988), are reported for all Chadronian and Orellan ungulate taxa. Data for fossil taxa are derived from Jardine et al. (2012).

2.3. Dietary categorization of Chadronian and Orellan taxa

Four broad dietary categories are used in this study, following Fortelius and Solounias (2000) and Gagnon and Chew (2000). These are as follows: (1) frugivores, (2) browsers, (3) grazers, and (4) mixed-feeders. The diets of frugivores are composed predominantly (>70%) of fruits (including fruits, tubers, bulbs, concentrates, succulents, pods, and seeds) with little to no monocots (all grasses, reeds, and sedges). Browsers consume >70% dicots (including leaves, buds, shoots, twigs, flowers, and blossoms of trees, shrubs, forbs, semiaquatic plants, and non-grassy herbs). Grazers generally consume >70% monocots; and mixed-feeders fall in between browser and grazers in composition. Dietary assignments of each taxon from the Chadronian and Orellan assemblages were determined using discriminant function analysis (DFA) of hypsodonty index and mesowear variables, which have been shown accurately discriminate between ungulates based on diet (e.g. Fraser and Theodor, 2011). The extant dataset (Appendix 2) used includes 35 ungulates of known diet, representing the four broad categories described above, 28 of which have published hypsodonty index scores. DFA run with HI values correctly classifies diet for 71.4% of the 28 extant taxa defined *a priori*. The extant dataset was also run with mesowear variables, correctly identifying 80.0% of the 35 extant taxa defined *a priori*. All DFAs were performed using the MASS package in R (Venables and Ripley, 2002).

The observed mesowear parameters (%h, %s, and %b) for all extant and fossil taxa are also transformed to two dimensionless factors in a principal components analysis (PCA, Fig. 6 and Table 7) using PAST v.2.17 (Hammer et al., 2001) to detect dietary groupings and correlation of variables. This is done to connect the mesowear of a fossil species to that of an extant species of known diet to interpret potential specific dietary composition via nearest neighbor analogy.

3. Results

3.1. Mesowear and Hypsodonty Index

Mesowear variables, sample sizes, along with hypsodonty index values for all examined fossil taxa and interpretations based on mesowear and HI are included in Table 6. Raw data are included in Appendix 3. All fossil taxa in this study, except the sub-mesodont *Hyracodon nebraskensis* (HI=2), are brachydont (i.e. $HI \leq 1.5$) following the definitions of Janis (1988).

3.2. Dietary categorization of Chadronian and Orellan taxa

Table 6 shows proxy diet results from the DFA based on mesowear, and ability to handle increasing dietary abrasion, for each taxon examined from the Chadronian and Orellan assemblages.

3.2.1. Perissodactyls

The Chadronian brontothere, *Megacerops* sp. (100%h, 90%s, 0%b, HI=1, n=10) is classified as a browser in the DFA based on mesowear. The large Orellan rhinoceros, *Subhyracodon* sp. (100%h, 60%s, 0%b, HI=1, n=10) is classified as a mixed-feeder in the

DFA based on mesowear. Chadronian and Orellan *Hyracodon nebraskensis* (100%h, 50%h and 57%h, respectively, 0%b, HI=2, n=4 and n=12, respectively) were both classified as mixed-feeders in the DFA based on mesowear. Chadronian and Orellan *Mesohippus bairdi* have identical mesowear (100%h, 60%h, 0%b, HI=1, n=10 each) and are classified as mixed-feeders in the DFA based on mesowear. Results of the HI run of the DFA indicate low potential ability for handling increasing dietary abrasion in all perissodactyls examined.

3.2.2. Artiodactyls

The Orellan clawed oreodont, *Agriochoerus antiquus* (100%h, 100%h, 0%h, HI=1, n=9), is classified as a browser in the DFA based on mesowear. The Chadronian anthracothere, *Aepinacodon americanum* (100%h, 33.33%h, 0%b, HI=1, n=3), is classified as a mixed-feeder in the DFA based on mesowear. The oromerycid, *Eotylopus reedi* (100%h, 0%h, 0%b, HI=1, n=7), is classified as a frugivore in the Mesowear run. Chadronian *Merycoidodon* sp. (100%h, 66.7%h, 0%b, HI=1, n=12) is classified as a browser in the Mesowear run; Orellan *Merycoidodon* sp. (100%, 54.5%h, 0%b, HI=1, n=11), on the other hand, is classified as a mixed-feeder in the Mesowear run. Results of the HI run of the DFA indicate low potential ability for handling increasing dietary abrasion in all artiodactyls examined.

3.3. PCA Results

The factor loadings of the PCA represent the differentiation between and within extant feeding groups. Factor 1 (eigenvalues; 69.65%) differentiates frugivores, mixed-feeders, and browsers from grazers, and Factor 2 (eigenvalues; 23.04%) differentiates

browsers from mixed-feeders and frugivores. Browsers differ from mixed-feeders, frugivores, and grazers along factor 2 in having a higher percentage of sharp cusps, and grazers differ from the others along factor 1 in having a higher percentage of blunt cusps. In the PCA *Megacerops* sp., *Agriochoerus antiquus*, and Chadronian *Merycoidodon* sp. plot with the browsers, *Hyracodon nebraskensis*, *Subhyracodon* sp., *Mesohippus bairdi*, Orellan *Merycoidodon* sp., and *Aepinacodon americanum* plot with the mixed-feeders, and *Eotylopus reedi* plots with the frugivores.

4. Discussion

4.1. Interpretation of Diets

I present proxy interpretations of the diets of fossil ungulates based on mesowear (Table 6). Further refinement of diet compositions are interpreted based upon PCA results (Fig. 6) considering the diet composition of the nearest extant neighbors in PCA space.

4.1.1. Perissodactyls

Megacerops sp. is interpreted as a browser (Table 6), and its mesowear values are closest to the extant Black Rhino *Diceros bicornis*, White-tailed Deer *Odocoileus virginianus*, and the Okapi *Okapia johnstoni* suggesting a similar diet to these extant species. In this case, indicating a diet of leaves, shoots, and twigs of woody growth (Nowak, 1999). *Hyracodon nebraskensis* is categorized as a mixed-feeder, a diet which is more abrasive than expected given its low potential for handling increased dietary abrasion based on HI. The Orellan sample of *H. nebraskensis* is most similar in its wear values to the extant Wapiti *Cervus elaphus* and the Serow *Capricornis sumatraensis*.

Cervus elaphus has a seasonally variable diet, foraging primarily for grasses in the spring, and browse the remainder of the year (Nowak, 1999). The serow feeds on grass, shoots, and leaves (Nowak, 1999). Wear for the Chadronian sample of *H. nebraskensis* is slightly less abrasive, being closer to that of Thompson's Gazelle *Gazella thompsoni* and Muskox *Ovibos moschatus*, both of which have seasonal diets as well (Nowak, 1999). Therefore, I suggest that *H. nebraskensis* was likely a seasonal mixed-feeder, and that the slight increase in dietary abrasion in Orellan specimens is consistent with increasing aridity and expansion of water-stressed habitats (Retallack, 1992; Sheldon and Retallack, 2004; Sheldon, 2009; Boardman and Secord, 2013). This hypothesis can be further tested with analysis of microwear, with corroboration coming in the form of diet variability in such an analysis. *Mesohippus bairdi* and *Subhyracodon* sp. are both interpreted as mixed-feeders. As with *H. nebraskensis* their diets are more abrasive than expected given their low HI values (HI=1). *Subhyracodon* sp. and *M. bairdi* have mesowear values most similar to Thompson's Gazelle and the Muskox; therefore I suggest seasonal mixed-feeding for these taxa as well.

4.1.2. Artiodactyls

The clawed oreodont, *Agriochoerus antiquus*, is interpreted as a browser, with mesowear values closest to the Moose *Alces alces* and the Javan rhino *Rhinoceros sondaicus*. Both of these extant taxa feed on twigs, leaves, shoots, and fruit of woody plants (Nowak, 1999). I suggest a similar diet for *A. antiquus*. Here *Merycoidodon* sp. likely shifted to mixed-feeding in the Orellan, contrary to what was suggested by Mhielbachler and Solounias (2006) on the basis of mesowear. The Chadronian sample of *Merycoidodon* sp. has mesowear most similar to the mule deer *Odocoileus hemionus*,

which consumes leaves and twigs of woody plants, nuts, and lichen throughout the year, and has a large component of grass in the summer (Nowak, 1999). Orellan *Merycoidodon* sp. has mesowear most similar to the serow and Thompson's gazelle, suggesting a fairly significant increase in the amount of grass consumed. *Eotylpus reedi* has mesowear most similar to that exhibited by the frugivorous duikers (Fig. 6), suggesting a diet composed primarily of fruit (Gagnon and Chew, 2000). *Aepinacodon americanum* plots closest to the Impala *Aepyceros melampus*, which consumes equal parts grass and browse (Dunham, 1980; Nowak, 1999; Gagnon and Chew, 2000), suggesting that *A. americanum* was a mixed-feeder of similar diet composition.

4.2. Implications of dietary stasis across the EOCT

Overall the range-through taxa examined in this study (*Mesohippus bairdi* and *Hyracodon nebraskensis*) are interpreted to have had static diets, though *Merycoidodon* sp. appears to have switched to a mixed-feeding diet in the Orellan. These results echo those of Dewar (2008), and serve to indicate that, at least locally, the EOCT had little effect on the dietary strategy of most mammals, especially those living in previously established dry, open habitats despite increasing aridity and probable reduction of riparian habitat (Boardman and Secord, 2013). The apparent shift in wear and diet in *Merycoidodon* sp., does, however, suggest that some changes were occurring locally, perhaps in the form of increasing grass and grit abundance associated with the expansion of open habitats at the expense of wetter, more densely vegetated ones. Since *Merycoidodon* sp. does not appear to be moving to more open habitats in the Orellan (Boardman and Secord, 2013) I suggest that more abrasive mesowear is indicative of it consuming more abrasive foods (i.e. grasses/grit), filling an expanding niche space for

mixed-feeding. The results of this study tentatively refute the hypothesized role of volcanic ash as the chief agent of dental abrasion of these taxa. Even though nearly all Orellan taxa have mesowear indicative of abrasive diets none become more hypsodont. Also, *Agriochoerus antiquus* has the lowest abrasion mesowear (100% sharp cusps); if ash were a major factor I would expect it to have more abrasive mesowear.

5. Conclusions

The goal of this study was to reconstruct the diets and adaptation to increasing dietary abrasion of WRG ungulates during the EOCT, focusing on the potential increase in dietary abrasion associated with the expansion of dry, open habitats in the Orellan. All taxa examined had low hypsodonty index values, suggesting that none were particularly well adapted to increasing dietary abrasion, though mesowear results from several taxa (*Mesohippus bairdi*, *Hyracodon nebraskensis*, *Subhyracodon* sp., *Aepinacodon americanum*, and Orellan *Merycoidodon* sp.) suggest grass made up a large portion of their diets. The brontothere, *Megacerops* sp., and the clawed oreodont, *Agriochoerus antiquus*, are interpreted to be low abrasion browsers, both having mesowear well outside of expectations for any significant amount of grass in their diets. Mesowear results from two range-through taxa (*Hyracodon nebraskensis* and *Mesohippus bairdi*) suggest general dietary stasis during the EOCT in Nebraska, indicating that increasing aridity had little effect on their ecologies. However results from another range-through taxon, *Merycoidodon* sp., suggest it switched to a considerably more abrasive diet. This suggests more grass was available to it during the early Oligocene and that *Merycoidodon* sp. adapted to utilize this expanding resource perhaps to escape browsing competition from similar-sized ungulates.

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References

- Boardman, G.S., Secord, R., 2013. Stable isotope paleoecology of White River ungulates during the Eocene-Oligocene transition in northwestern Nebraska. *Palaeogeography, Palaeoclimatology, Palaeoecology* 375, 38-49.
- Clauss, M., Franz-Odenaal, T. A., Brasch, J., Castell, J. C., Kaiser, T., 2007. Tooth wear in captive giraffes (*Giraffa camelopardalis*): mesowear analysis classifies free-ranging specimens as browsers but captive ones as grazers. *Journal of Zoo and Wildlife Medicine* 38, 433-445.
- Croft, D.A., Weinstein, D., 2008. The first application of the mesowear method to endemic South American ungulates (Notoungulata). *Palaeogeography, Palaeoclimatology, Palaeoecology* 269, 103-114.
- Damuth, J., Janis, C.M., 2011. On the relationship between hypsodonty and feeding ecology in ungulate mammals, and its utility in paleoecology. *Biological Reviews* 86, 733-758.
- Dewar, E.W., 2008. Dietary ecology and community paleoecology of early Tertiary mammals. Electronic Doctoral Dissertations for U Mass Amherst. Paper AAI3337037.
- Dunham, K.M., 1980. The diet of Impala (*Aepyceros melampus*) in the Sengwa Wildlife Research Area. *Journal of Zoology* 192, 41-57.
- Evanoff, E., Prothero, D.R., Lander, R.H., 1992. Eocene-Oligocene climatic change in North America: the White River Formation near Douglas, east-central Wyoming,

- in: Prothero, D.R., Berggren, W.A. (Eds.), Eocene-Oligocene Climatic and Biotic Evolution. Princeton University Press, Princeton, pp. 116-130.
- Fortelius, M., Solounias, N., 2000. Functional characterization of ungulate molars using the abrasion-attrition wear gradient: a new method for reconstructing paleodiets. *American Museum Novitates* 3301, 1-36.
- Franz-Odenaal, T.A., Kaiser, T.M., 2003. Differential mesowear in the maxillary and mandibular cheek dentition of some ruminants (Artiodactyla). *Annales Zoologici Fennici* 40, 395-410.
- Fraser, D., Theodor, J.M., 2011. Comparing ungulate dietary proxies using discriminant function analysis. *Journal of Morphology* 272, 1513-1526.
- Fraser, D., Theodor, J.M., 2013. Ungulate diets reveal patterns of grassland evolution in North America. *Palaeogeography, Palaeoclimatology, Palaeoecology* 369, 409-421.
- Gagnon, M., Chew, A.E., 2000. Dietary preferences in Extant African Bovidae. *Journal of Mammalogy* 81, 490-511.
- Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4(1), 1-9.
- Hutchison, J.H., 1982. Turtle, crocodilian, and champsosaur diversity changes in the Cenozoic of the north-central region of western United States. *Palaeogeography, Palaeoclimatology, Palaeoecology* 37, 149-164.

- Hutchison, J.H., 1992. Western North America reptile and amphibian record across the Eocene/Oligocene boundary and its climatic implications, in: Prothero, D.R., Berggren, W.A. (Eds.), *Eocene-Oligocene Climatic and Biotic Evolution*. Princeton University Press, Princeton, pp. 451-463.
- Janis, C. M., 1988. An estimation of tooth volume and hypsodonty indices in ungulate mammals, and the correlation of these factors with dietary preference. In *Teeth revisited: proceedings of the VIIth international symposium on dental morphology*. Mémoires de Musée d'Histoire naturelle du Paris 53, 361-387.
- Jardine, P.E., Janis, C.M., Sahney, S., Benton, M.J., 2012. Grit not grass: concordant patterns of early origin of hypsodonty in Great Plains ungulates and Glires. *Palaeogeography, Palaeoclimatology, Palaeoecology* 365-366, 1-10.
- Kaiser, T.M., 2011. Feeding Ecology and Niche Partitioning of the Laetoli Ungulate Faunas, in: T. Harrison (ed.), *Paleontology and Geology of Laetoli: Human Evolution in Context. Volume 1: Geology, Geochronology, Paleoecology and Paleoenvironment, Vertebrate Paleobiology and Paleoanthropology*. Springer, pp. 329-354.
- Kaiser, T.M., Solounias, N., 2003. Extending the tooth mesowear method to extinct and extant equids. *Geodiversitas* 25, 321-345.
- Kaiser, T.M., Solounias, N., Fortelius, M., Bernor, R.L., Schrenk, F., 2000. Tooth mesowear analysis on *Hippotherium primigenium* from the Vallesian Dinotheriensande (Germany)—a blind test study. *Carolinea* 58, 103-114.

- Kaiser, T.M., Solounias, N., Schaller, G.B. 2011. Diet of Mongolian gazelles and Tibetan antelopes from steppe habitats using premaxillary shape, tooth mesowear and microwear analyses. *Mammalian Biology - Zeitschrift für Säugetierkunde* 76, 358-364.
- LaGarry, H.E., 1998. Lithostratigraphic revision and redescription of the Brule Formation, White River Group, western Nebraska. *Geological Society of America Special Paper* 325, 63-91.
- Larson, E. E., Evanoff, E., 1998. Tephrostratigraphy and source of the tuffs of the White River sequence. *Geological Society of America Special Papers* 325, 1-14.
- Louys, J., Meloro, C., Elton, S., Ditchfield, P., Bishop, L.C., 2011. Mesowear as a means of determining diets in African antelopes. *Journal of Archaeological Science* 38, 1485-1495.
- Merceron, G., Schulz, E., Kordos, L., Kaiser, T.M., 2007. Paleoenvironments of *Dryopithecus brancoi* at Rudabanya, Hungary: evidence from dental meso- and micro-wear analyses of large vegetarian mammals. *Journal of Human Evolution* 53, 331-349.
- Mihlbachler, M.C., Solounias, N., 2006. Coevolution of tooth crown height and diet in oreodonts (Merycoidodontidae, Artiodactyla) examined with phylogenetically independent contrasts. *Journal of Mammalian Evolution* 13, 11-36.
- Mihlbachler, M.C., Rivals, F., Solounias, N., Semprebon, G.M., 2011. Dietary change and evolution of horses in North America. *Science* 311, 1178-1181.

- Nowak, R.M., 1999. Walker's Mammals of the World, 6th edition, Volume 2. Johns Hopkins University Press, Baltimore, Maryland.
- Reguero, M.A., Candela, A.M., Cassini, G.H., 2010. Hypsodonty and body size in rodent-like notoungulates, in: Madden, R.H., Carlini, A.A., Vucetich, M.G., and Kay, R.F. (Eds.), The Paleontology of Gran Barranca: evolution and environmental change through the middle Cenozoic of Patagonia. Cambridge University Press, Cambridge, pp. 362-374.
- Retallack, G.J., 1992. Paleosols and changes in climate and vegetation across the Eocene/Oligocene boundary, in: Prothero, D.R. and Berggren, W.A. (Eds.), Eocene-Oligocene Climatic and Biotic Evolution. Princeton University Press, Princeton, pp. 382-398.
- Retallack, G.J., 2007. Cenozoic paleoclimate on land in North America. *Journal of Geology* 115, 271-294.
- Rivals, F., Muhlbachler, M.C., Solounias, N., 2007. Effect of ontogenetic-age distribution in fossil and modern samples on the interpretation of ungulate paleodiets using the mesowear method. *Journal of Vertebrate Paleontology* 27, 763-767.
- Rivals, F., 2012. Ungulate feeding ecology and middle Pleistocene paleoenvironments at Hundsheim and Deutsch-Altenburg 1 (eastern Austria). *Palaeogeography, Palaeoclimatology, Palaeoecology* 317-318, 27-31.
- Semprebon, G.M., Rivals, F., 2010. Trends in the paleodietary habits of fossil camels from the Tertiary and Quaternary of North America. *Palaeogeography, Palaeoclimatology, Palaeoecology* 295, 131-145.

- Sheldon, N.D., 2009. Nonmarine records of climatic change across the Eocene-Oligocene transition. Geological Society of America Special Paper 452, 241-248.
- Sheldon, N.D., Retallack, G.J., 2004. Regional paleoprecipitation records from the late Eocene and Oligocene of North America. Journal of Geology 112, 487-494.
- Stromberg, C.A.E., 2005. Decoupled taxonomic radiation and ecological expansion of open-habitat grasses in the Cenozoic of North America. PNAS 102, 11980-11984.
- Stromberg, C.A.E., 2006. Evolution of hypsodonty in equids: testing a hypothesis of adaptation. Paleobiology 32, 236-258.
- Terry, D.O. Jr., 1998. Lithostratigraphic revision and correlation of the lower part of the White River Group: South Dakota to Nebraska. Geological Society of America Special Paper 325, 15-37.
- Terry, D.O. Jr., 2001. Paleopedology of the Chadron Formation of northwestern Nebraska: implications for paleoclimatic change in the North American midcontinent across the Eocene-Oligocene boundary. Palaeogeography, Palaeoclimatology, Palaeoecology 168, 1-38.
- Terry, D.O. Jr., LaGarry, H.E., 1998. Big Cottonwood Creek Member: a new member of the Chadron Formation in northwestern Nebraska. Geological Society of America Special Paper 325, 117-141.
- Venables, W.N., Ripley, B.D., 2002. Modern Applied Statistics with S, Fourth Edition. Springer, New York, New York.
- Yamada, E., 2012. Mesowear Analysis of the Japanese Sika Deer (*Cervus nippon*) in Different Food Habits: Its Limitations and Applicability. Mammal Study 37, 93-103.

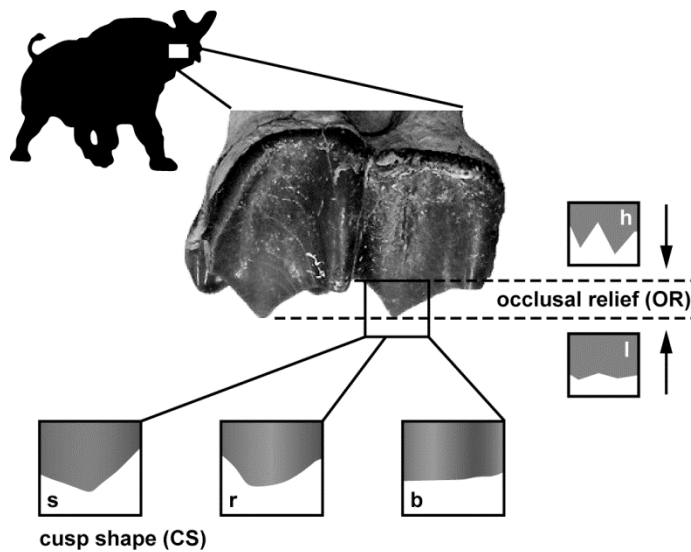


FIGURE 5. Mesowear variables of a brachydont cheek tooth (upper second molar; *Megacerops* sp.). Occlusal relief (OR) may be scored as “high” (h) or “low” (l); cusp shape (CS) is scored as “sharp” (s), “round” (r), or “blunt” (b). Figure modified from Kaiser (2011).

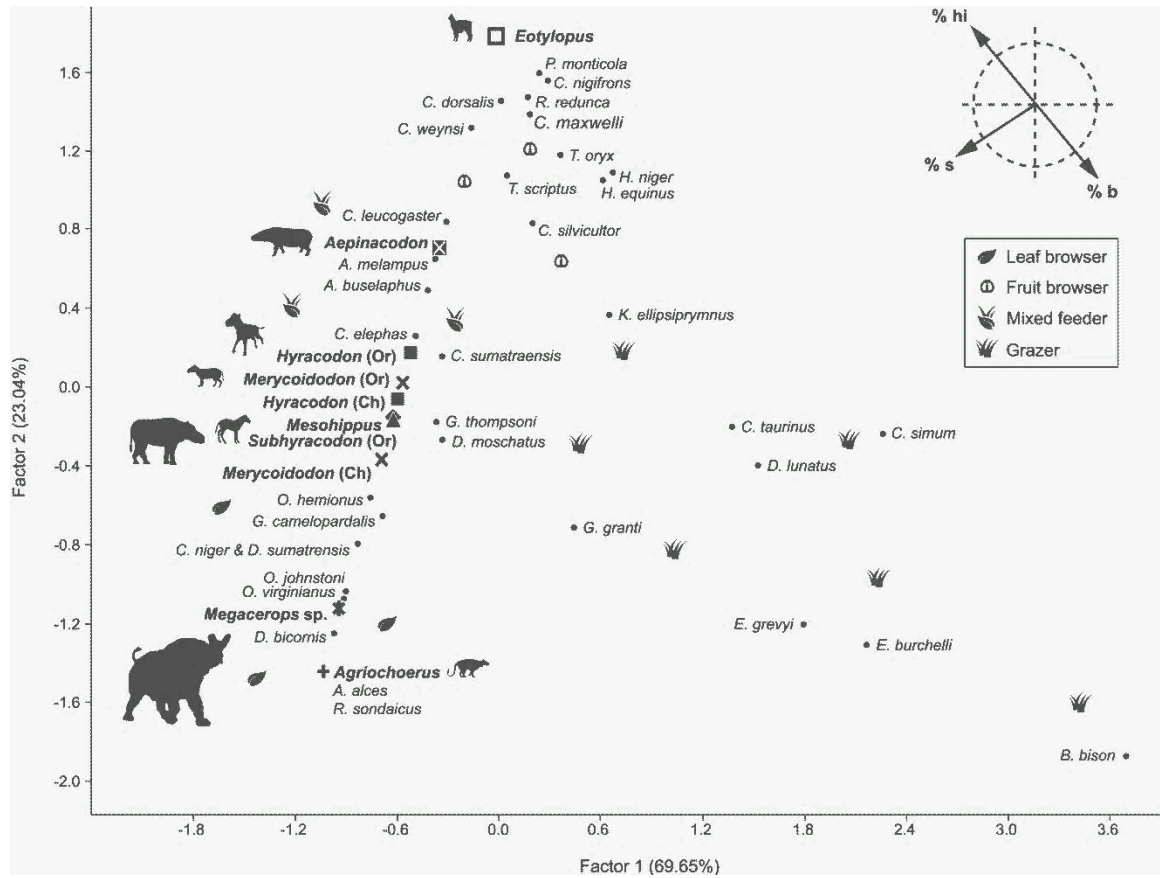


FIGURE 6. Principal components analysis based on extant species (see Appendix 2 for names), Or=Orellan, Ch=Chadronian. Each taxon was scored for cusp relief and cusp shape, percentages of high (%h), sharp (%s), and blunt (%b). Factor 1 (eigenvalues; 69.65%) differentiates frugivores, mixed-feeders, and browsers from grazers, and Factor 2 (eigenvalues; 23.04%) differentiates browsers from mixed-feeders and frugivores.

Table 6

Mesowear variables collected in this study and hypsodonty index (HI) values (Jardine et al., 2012), expected dietary abrasion based on DFA of HI values, and interpreted diet based on DFA from mesowear for sampled Chadronian and Orellan ungulate taxa from northwestern Nebraska. BR=browser, FR=frugivore, and MF=mixed-feeder, percentages of high cusp relief (%h), and sharp (%s) and blunt (%b) cusp shape.

Taxon	NALMA	n	Mesowear			HI	Expected Dietary	Diet from
			%h	%s	%b		Abrasion	Mesowear
Perissodactyls								
<i>Meshippus bairdi</i>	Chadronian	10	100	60.0	0	1	low	MF
	Orellan	10	100	60.0	0	1	low	MF
<i>Hyracodon nebraskensis</i>	Chadronian	4	100	50.0	0	2	low	MF
	Orellan	14	100	57.1	0	2	low	MF
<i>Subhyracodon</i> sp.	Orellan	10	100	60.0	0	1	low	MF
<i>Megacerops</i> sp.	Chadronian	10	100	90.0	0	1	low	BR
Artiodactyls								
<i>Agriochoerus antiquus</i>	Orellan	9	100	100	0	1	low	BR
<i>Merycoidodon</i> sp.	Chadronian	12	100	66.7	0	1	low	BR
	Orellan	11	100	54.5	0	1	low	MF
<i>Eotylopus reedi</i>	Chadronian	7	100	0.0	0	1	low	FR
<i>Aepinacodon americanum</i>	Chadronian	3	100	33.3	0	1	low	MF

Table 7. Statistics of the PCA: correlation between factors and variables are given as factor loadings according to the mesowear variable %h=percentage high occlusal relief, %s=percentage sharp cusps, %b=percentage blunt cusps. The eigenvalue and the percentage variation are given as characterization of the correlation matrix.

	Factor 1	Factor 2	Statistic
%h	-0.916	0.211	Factor loadings
%s	-0.685	-0.726	Factor loadings
%b	0.884	-0.346	Factor loadings
Eigenvalue	2.09	0.69	Correlation matrix
Variance (%)	69.65	23.04	Correlation matrix

Appendix 2. Mesowear variables and hypsodonty index values for extant taxa used in this study. Mesowear values for species in bold come from Louys et al. (2011), all other mesowear values come from Fortelius and Solounias (2000). HI values come from Janis (1988).

Species	DIET	%h	%s	%b	HI
<i>Cephalophus dorsalis</i>	FRUIT	92.3	7.7	0	1.15
<i>C. silvicultor</i>	FRUIT	70	20	0	2.23
<i>C. weynsi</i>	FRUIT	100	14.3	0	X
<i>C. niger</i>	FRUIT	100	80	0	X
<i>C. nigifrons</i>	FRUIT	77.8	0	0	X
<i>C. maxwelli</i>	FRUIT	81.3	6.3	0	X
<i>C. leucogaster</i>	FRUIT	100	29.2	0	X
<i>Philatomba monticola</i>	FRUIT	81.8	0	0	X
<i>Alces alces</i>	BROWSE	100	100	0	1.34
<i>Dicerorhinus sumatrensis</i>	BROWSE	100	80	0	1.67
<i>Diceros bicornis</i>	BROWSE	100	94.1	0	2.24
<i>Giraffa camelopardalis</i>	BROWSE	94	73.7	0	1.2
<i>Odocoileus hemionus</i>	BROWSE	100	72.7	0	1.59
<i>O. virginianus</i>	BROWSE	100	88.8	0	1.23
<i>Okapia johnstoni</i>	BROWSE	100	87.5	0	1.18
<i>Rhinoceros sondaicus</i>	BROWSE	100	100	0	1.72
<i>Tragelaphus scriptus</i>	BROWSE	100	51	0	2.54
<i>Aepyceros melampus</i>	MIXED	100	35.2	0	4.89
<i>Capricornis sumatraensis</i>	MIXED	100	45.4	4.5	3.93
<i>Cervus elephas</i>	MIXED	100	47.3	0	1.96
<i>Gazella granti</i>	MIXED	88	50	0	3.45
<i>G. thomsoni</i>	MIXED	88	55.4	1.3	3.77
<i>Ovibos moschatus</i>	MIXED	81	57.6	0	3.69
<i>Taurotragus oryx</i>	MIXED	100	50	0	2.91
<i>Alcelaphus buselaphus</i>	GRAZE	57	3.2	28	5.23
<i>Bison bison</i>	GRAZE	0	0	73.3	4.84
<i>Ceratotherium simum</i>	GRAZE	0	0	28	3.09
<i>Connochaetes taurinus</i>	GRAZE	55	15.3	28.8	4.94
<i>Damaliscus lunatus</i>	GRAZE	20	20	20	5.1
<i>Equus burchelli</i>	GRAZE	0	27	33.6	5.83
<i>E. grevyi</i>	GRAZE	0	34.4	24.1	5.8
<i>Redunca redunca</i>	GRAZE	91	6.4	2.5	X
<i>Hippotragus equinus</i>	GRAZE	85	3.8	0	4.28
<i>H. niger</i>	GRAZE	85	0	15	3.77
<i>Kobus ellipsiprymnus</i>	GRAZE	96	0	0	3.47

Appendix 3. Mesowear raw data for fossil taxa examined in this study.

Species	Specimen number	NALMA	Tooth	Occlusal Relief (OR)	Cusp Shape (CS)
<i>Mesohippus bairdi</i>	UNSM 131510	Chadronian	LM2/	High	Sharp
	UNSM 131529	Chadronian	RM2/	High	Round
	UNSM 131684	Chadronian	RM2/	High	Round
	UNSM 131721	Chadronian	LM2/	High	Round
	UNSM 48485	Chadronian	RM2/	High	Sharp
	UNSM 131519	Chadronian	RM2/	High	Round
	UNSM 465-54	Chadronian	RM2/	High	Sharp
	UNSM 2173-73	Chadronian	LM2/	High	Sharp
	UNSM 131705	Chadronian	RM2/	High	Sharp
	UNSM 131795	Chadronian	RM2/	High	Sharp
<i>Mesohippus bairdi</i>	UNSM 131659	Orellan	RM2/	High	Round
	UNSM 47-13-8-34SP	Orellan	LM2/	High	Sharp
	UNSM 131576	Orellan	RM2/	High	Sharp
	UNSM 131574	Orellan	RM2/	High	Sharp
	UNSM 131575	Orellan	LM2/	High	Round
	UNSM 131541	Orellan	RM2/	High	Round
	UNSM 131619	Orellan	LM2/	High	Sharp
	UNSM 131598	Orellan	RM2/	High	Round
	UNSM 131634	Orellan	RM2/	High	Sharp
	UNSM 131538	Orellan	RM2/	High	Sharp
<i>Hyracodon nebraskensis</i>	UNSM 11193	Chadronian	LM2/	High	Round
	UNSM 1502-59	Chadronian	RM2/	High	Sharp
	UNSM 11001	Chadronian	LM2/	High	Round
	UNSM 2536-63	Chadronian	RM2/	High	Sharp
<i>Hyracodon nebraskensis</i>	UNSM 11216	Orellan	LM2/	High	Sharp
	UNSM 11-8-33SP	Orellan	LM2/	High	Sharp
	UNSM 2282-73	Orellan	LM2/	High	Sharp
	UNSM 11191	Orellan	RM2/	High	Round
	UNSM 1500-65	Orellan	LM2/	High	Round
	UNSM 11012	Orellan	LM2/	High	Sharp
	UNSM 11007	Orellan	LM2/	High	Round

<i>Subhyracodon</i> sp.	UNSM 11071	Orellan	LM2/	High	Round
	UNSM 11065	Orellan	RM2/	High	Round
	UNSM 1085-50	Orellan	RM2/	High	Sharp
	UNSM 11181	Orellan	LM2/	High	Round
	UNSM 11198	Orellan	LM2/	High	Sharp
	UNSM 11205	Orellan	RM2/	High	Sharp
	UNSM 132003	Orellan	RM/2	High	Sharp
	UNSM 2-12-7-36 SP	Orellan	RM2/	High	Round
	UNSM 21-28-8-34 SP	Orellan	LM2/	High	Round
	UNSM 242-52	Orellan	LM2/	High	Sharp
	UNSM 395-53	Orellan	RM2/	High	Sharp
	UNSM 5016-67	Orellan	RM2/	High	Round
	UNSM 131-55	Orellan	LM2/	High	Sharp
	UNSM 132016	Orellan	RM2/	High	Sharp
	UNSM 2253-73	Orellan	LM2/	High	Round
	UNSM 219-52	Orellan	RM2/	High	Sharp
	UNSM 139-52	Orellan	RM2/	High	Sharp
<i>Megacerops</i> sp.	UNSM 3287-91	Chadronian	RM2/	High	Sharp
	Basement no #	Chadronian	LM2/	High	Sharp
	UNSM 1137	Chadronian	LM2/	High	Sharp
	UNSM 1131	Chadronian	LM2/	High	Sharp
	UNSM 1123	Chadronian	RM2/	High	Sharp
	UNSM 1135	Chadronian	RM2/	High	Round
	UNSM 2040-53	Chadronian	RM2/	High	Sharp
	Teaching collection	Chadronian	RM2/	High	Sharp
	Teaching collection	Chadronian	LM2/	High	Sharp
	UNSM 1539-59	Chadronian	RM2/	High	Sharp
<i>Agriochoerus antiquus</i>	UNSM 46047	Orellan	RM2/	High	Sharp
	UNSM 6-5-9-34	Orellan	LM2/	High	Sharp
	UNSM 46050	Orellan	RM2/	High	Sharp
	UNSM 46038	Orellan	RM2/	High	Sharp
	UNSM 46048	Orellan	LM2/	High	Sharp
	UNSM 46051	Orellan	LM2/	High	Sharp
	UNSM 46054	Orellan	LM2/	High	Sharp

<i>Merycoidodon</i> sp.	UNSM 46037	Orellan	LM2/	High	Sharp
	UNSM 7-8-33 SP	Orellan	LM2/	High	Sharp
	UNSM 3086-011	Chadronian	LM2/	High	Sharp
	UNSM 3102-011	Chadronian	LM2/	High	Sharp
	UNSM 2584-63	Chadronian	RM2/	High	Sharp
	3-3-9-34 SP Spc. A	Chadronian	RM2/	High	Sharp
	3-3-9-34 SP Spc. B	Chadronian	RM2/	High	Sharp
	UNSM 609-38	Chadronian	RM2/	High	Round
	UNSM 1005-38	Chadronian	RM2/	High	Sharp
	UNSM 407-54	Chadronian	LM2/	High	Round
	UNSM 25458-63 (1)	Chadronian	RM2/	High	Sharp
	UNSM 25458-63 (2)	Chadronian	LM2/	High	Round
	UNSM 28621	Chadronian	RM2/	High	Round
	UNSM 407-38	Chadronian	LM2/	High	Sharp
<i>Merycoidodon</i> sp.	UNSM 3012-011	Orellan	RM2/	High	Sharp
	UNSM 3022-011	Orellan	LM2/	High	Round
	UNSM 3023-011	Orellan	RM2/	High	Sharp
	UNSM 3052-011	Orellan	LM2/	High	Round
	UNSM 3053-011	Orellan	RM2/	High	Round
	UNSM 3105-011	Orellan	LM2/	High	Sharp
	UNSM 3111-011	Orellan	LM2/	High	Round
	UNSM 3112-011	Orellan	RM2/	High	Round
	UNSM 3119-011	Orellan	LM2/	High	Sharp
	UNSM 3120-011	Orellan	RM2/	High	Sharp
	UNSM 3121-011	Orellan	LM2/	High	Sharp
<i>Eotylopus reedi</i>	UNSM 316-66	Chadronian	LM/2	High	Round
	UNSM 1061-64	Chadronian	RM/2	High	Round
	UNSM -82	Chadronian	RM/2	High	Round
	UNSM 628-46	Chadronian	LM/2	High	Round
	UNSM 1136-64 (1163-64)	Chadronian	RM/2	High	Round
	UNSM 732-38	Chadronian	LM/2	High	Round
	UNSM 628-46*	Chadronian	RM/2	High	Round
<i>Aepinacodon americanum</i>	UNSM 5-10-8-33 SP	Chadronian	RM2/	High	Sharp
	UNSM 1507-62	Chadronian	RM2/	High	Round

UNSM 1-12-8-34 SP

Chadronian LM2/ High

Round

CHAPTER 4

Testing the utility of dental microwear texture as a proxy for diet in extant and fossil non-ruminant ungulates

Abstract

I test the utility dental microwear texture analysis on non-ruminant ungulates by examining eight extant but previously unstudied species. My results indicate that dental microwear texture analysis can accurately separate several dietary groups, namely browsers, frugivores, mixed-feeders, and grazers. This dataset of extant non-ruminants, while informative in itself, also provides a baseline against which microwear textures from extinct Eocene-Oligocene non-ruminants of the White River Group can be compared. Accordingly, diets for four perissodactyls, *Mesohippus bairdi* (n=7), *Hyracodon nebraskensis* (n=4), *Subhyracodon* sp. (n=4), and *Megacerops* sp. (n=6), and three artiodactyls, *Agriochoerus antiquus* (n=4), *Eotylpus reedi* (n=2), and *Perchoerus probus* (n=4) from the upper Eocene and lower Oligocene are interpreted. Dietary interpretations in this study are generally consistent with interpretations from previous studies based on mesowear and low-magnification, feature-based microwear. Microwear surfaces for *A. antiquus*, *P. probus*, and *E. reedi* have highly complex textures, and along with other texture values indicate primarily fruit consumption. Surface textures from *Megacerops* sp. and *H. nebraskensis* indicate a large browse component, whereas fairly anisotropic surfaces for *Subhyracodon* sp. and *Mesohippus bairdi* are consistent primarily with mixed-feeding. Microwear texture analysis is a useful tool for categorizing the diets of both extant and extinct non-ruminant ungulates.

Keywords: Microwear Texture; diet; non-ruminant; Eocene-Oligocene.

1. Introduction

Dental microwear, discrete features primarily caused by food contact with the occlusal surfaces of teeth, is accrued over short periods of time and then obliterated by new dental microwear. As such, microwear reflects the animal's last few meals (the "last supper" phenomenon of Solounias et al., 1994), or perhaps seasonal diets which may or may not be indicative of the animal's overall diet. Dental microwear texture analysis quantifies patterns of dental microwear from three dimensional enamel surfaces such that the diets of several different taxa of fossil mammals can be reconstructed (e.g. Scott et al., 2005; Ungar et al., 2008, 2010; Scott et al., 2009), ruminant artiodactyls (e.g. Ungar et al., 2007, 2012), and carnivorans (Stynder et al., 2012). For ruminant artiodactyls in particular this method is useful in separating extant taxa by diet (Scott, 2012), distinguishing between six dietary categories based on composition. Scott (2012) corroborated many generalities about differences in microwear texture variables correlated to diet as presented by Ungar et al. (2007). This method has not yet been applied to non-ruminant ungulates (i.e. perissodactyls, and artiodactyls outside of Ruminantia).

In this paper, microwear textures from a dataset of extant perissodactyls, the Plains Zebra *Equus burchelli*, Indian Rhinoceros *Rhinoceros unicornis*, Black Rhinoceros *Diceros bicornis*, and the Lowland Tapir *Tapirus terrestris* and artiodactyls outside of Ruminantia, the Llama *Lama glama*, Bactrian Camel *Camelus bactrianus*, Collared Peccary *Tayassu pecari*, and White-lipped Peccary *Pecari tajacu* are examined to test the

utility of this method in differentiating diet in non-ruminants. These results are then compared to seven fossil ungulate species from the upper Eocene and lower Oligocene White River Group (WRG) of northwestern Nebraska to characterize diet for the fossil taxa. The diet interpretations generated in this study for fossil taxa are compared with the interpreted diets from other studies using feature-based light microscopy microwear (Mihlbachler and Solounias, 2002; Solounias and Semprebon, 2002; Dewar, 2008) and mesowear (results from Chapter 3). I also compare my results with recent paleohabitat interpretations for these taxa made with stable isotopes (Boardman and Secord, 2013).

2. Materials

Specimens of extant taxa came from several institution collections, as follows: American Museum of Natural History, New York City, New York (AMNH); University of Kansas Natural History Museum, Lawrence, Kansas (KUM); National Museum of Natural History, Smithsonian Institution, Washington, D.C. (USNM); and University of Nebraska State Museum, Division of Zoology, Lincoln, Nebraska (ZM). Extant specimens were all collected in the wild (i.e. not from zoos) and are assumed to have eaten diets representative of the species in natural settings; information regarding the provenance of these specimens is included in Appendix 4. Fossil specimens used in this study are curated at the University of Nebraska State Museum (UNSM) in Lincoln, Nebraska. Fossil specimens come from exposures of the Big Cottonwood Creek Member (BCCM) of the upper Chadron Formation and the Orella Member of the lower Brule Formation of the WRG in and around Toadstool Geologic Park, northwestern Nebraska (Sioux and Dawes counties). Fossil specimens come from two superposed,

time-averaged assemblages the older being representative of the Chadronian North American Land Mammal “age” (NALMA, 37.0 – 33.7 Ma, Prothero and Emry, 2004) and the younger representing the Orellan NALMA (33.7 – 32.0 Ma, Prothero and Emry, 2004). All available upper and lower first and second molars from the extant and fossil taxa of interest were examined for suitability to this study.

All specimens were first examined for macroscopic damage (cracks, tool marks, etching, etc.) using a hand lens or light microscope; damaged specimens were not considered further. Non-damaged specimens were then cleaned using a gauze pad soaked in a 50/50 solution of acetone and ethanol. The pad was applied to the tooth and then covered with polyethylene film to provide a vapor atmosphere around the tooth. After five minutes, the film and pad were removed and any consolidant was gently removed with a cotton swab. Cleaned teeth were then molded using vacuum de-gassed Polytek Platsil 73-25, an addition-cure low-viscosity molding compound with standard 73-25 platinum curing agent. Molds were cured at ambient room temperature for 16 hours before removal from teeth. High-resolution casts were made with un-pigmented TAP Plastics 4 to 1 Super Hard epoxy. After mixing and pouring this material into the molds, they were then placed in a pressure pot at 400 PSI to cure into dense, bubble-free casts. Resultant optically clear casts were then mounted on the stage of the confocal imaging profiler and further inspected using the 100x objective. Postmortem microwear and preparation artifacts were noted following examples from El-Zaatari (2010). For instance, when potential wear features were seen to cover the wear facet and continue uninterrupted onto non-wear surfaces, I assumed this wear to be postmortem, and thus did not include those specimens in the analysis. Overall, casts on which more than 20%

of the scanned surfaces showed postmortem wear or preparation artifacts were excluded. I was able to obtain clean antemortem microwear data from 30 of the 40 extant specimens examined and 31 of the 100 fossil specimens examined that had been cast following the methods detailed above, illustrating the low-hit success for fossils with regards to microwear texture analysis noted elsewhere (e.g. Ungar et al., 2012).

3. Methods

Dental microwear texture analysis (DMTA), first introduced by Scott et al. (2005, 2006), is a method that utilizes scanning confocal microscopy and scale-sensitive fractal analysis to characterize three-dimensional dental microwear surfaces. Microwear texture data were collected from wear facet surfaces on the anterobuccal enamel band of the paracone of M1/ or M2/, or the posterobuccal enamel band on the protoconid of the M1/ or M2/ of high resolution resin casts made following the procedures noted above. The centers of these facets, or fields closest to it preserving antemortem microwear, were scanned using a Sensofar PL μ white-light confocal profiler (Solarius Development Inc., Sunnyvale, CA, USA). The 100x objective lens was used, surface elevations were sampled at lateral (x,y) intervals of 0.18 μm with vertical resolution of 0.005 μm . Four adjacent fields of 102 x 138 μm each were analyzed per specimen, with the total area of surface scanned per specimen being 204 x 276 μm , resulting in the generation of a point cloud of approximately 1.74 million points. Each point cloud was run through scale-sensitive fractal analysis using Toothfrax and Sfrax software (Surfract Corporation) following established microwear texture protocols as described in Scott et al. (2006) and Ungar et al. (2007).

I generated four texture variables for each specimen scanned: area-scale fractal complexity (*Asfc*), exact proportion length-scale anisotropy of relief (*epLsar*), textural fill volume (*Tfv*), and scale of maximum complexity (*Smc*). Complexity measures how surface roughness changes with scale of observation. As an example, a surface that is dominated by pits of various sizes or pits and scratches overlapping one another will result in high *Asfc* values. Anisotropy measures directionality of surface texture so that surfaces dominated by long, parallel scratches will have high *epLsar* values. Textural fill volume measures the differences between large- and small-volume features so that surfaces dominated by medium-sized pits or scratches (i.e. in the 2-10 μm range) will have high *Tfv* values. Scale of maximum complexity measures the point at which apparent surface roughness begins to trail off with decreasing observation scale so that surfaces dominated by large pits or large scratches will have high *Smc* values.

In this study I am analyzing microwear texture from extant non-ruminant ungulates (both artiodactyl and perissodactyl) with a range of tooth morphologies to compare to fossil taxa from related groups. Microwear texture analysis has been successfully used to characterize the relationship between diet and wear surface variables in some modern ungulate groups (Ungar et al., 2007; Merceron et al., 2010; Scott, 2012), and to evaluate the diet of fossil bovids (Ungar et al., 2007; Scott, in press) and tragulids (Ungar et al., 2012). These previous applications of texture analysis show significant differences in wear surface variables related to ungulate diet; strongly suggesting that taxa with grazing diets have less complex, more anisotropic surfaces, and lower textural fill volumes than browsing taxa do. These previous studies all included artiodactyl groups which share the basic ruminant selenodont tooth morphology.

The fossil non-ruminant ungulates (31 specimens representing 7 taxa) analyzed here were compared with one another and to a preliminary dataset of modern non-ruminant taxa (30 specimens representing 8 taxa of known diet) using a general linear model. A multivariate analysis of variance (MANOVA) was run on rank-transformed data with *Asfc*, *epLsar*, *Smc*, and *Tfv* as variables to determine whether the species (both extant and fossil) varied in overall microwear surface textures. I use this approach because microwear texture data are not typically normally distributed, and would thus be in violation of parametric analysis assumptions (Conover and Iman, 1981). ANOVAs were then run for each variable to assess sources of significant variation and multiple comparisons tests (Fisher's LSD) were conducted as necessary. All statistical tests performed on ranked data were done in PAST v.2.17 (Hammer et al., 2001).

The extant non-ruminant dataset includes species that fall into four of five broad dietary categories (Table 8), modified from Gagnon and Chew (2000). These are as follows: (1) obligate grazers, >90% monocots, (2) variable grazers, 60-90% monocots, varying seasonally, (3) mixed-feeders, which include their browser-grazer intermediates (30-70% monocots and dicots, <20% fruit) and generalists (>20% all food types), (4) browsers, >70% dicots, and (5) frugivores, >70% fruit. Monocots include all grasses, reeds, and sedges. Dicots include: leaves, buds, shoots, twigs, flowers, and blossoms of trees, shrubs, forbs, semiaquatic plants, and non-grassy herbs. Fruits include tubers, bulbs, concentrates, succulents, pods, and seeds.

Comparisons of Chadronian and Orellan ungulates to the extant non-ruminants were also made using discriminant function analysis (DFA) using the MASS package in R (Venables and Ripley, 2002). All ranked data from extant and fossil datasets were used

in the DFA. Discriminant function robustness was assessed by jack-knife cross-validation to see how well extant taxa with *a priori* diets would be classified when treated as unknowns. DFA was conducted to classify fossil specimens into a specific dietary category characterized by the preliminary extant non-ruminant sample. In this case generalized proxy diet is interpreted based on categorization of $\geq 70\%$ of specimens in a taxon to a particular dietary category, but due to small sample sizes, may not be considered fully robust. I also compare the proxy diet results for the fossil taxa studied here with interpretations from previous microwear and mesowear studies (Mihlbachler and Solounias, 2002; Solounias and Semprebon, 2002; Dewar, 2008; and my results from Chapter 3). A comparison is also made with habitat interpretations made by Boardman and Secord (2013) based on enamel stable carbon isotopes.

4. Results

Sample microwear texture images, box plots run in PAST v.2.17 (Hammer et al., 2001) from raw data, and descriptive statistics for extant and fossil non-ruminant ungulates are presented in Figs. 7 and 8, and Tables 8 and 9. Analytical statistics comparing fossil and extant specimens by taxon are presented in Table 10. Results for the DFA analysis are presented in Table 11. Raw data are reported in Appendix 4.

4.1. Extant and fossil specimens – general results

MANOVA results confirm that extant and fossil specimens vary in microwear textures. There is significant variation in all four texture variables. ANOVA and paired

comparison test results indicate that the most significant contributions to variation are seen in *Asfc* and *Smc*.

Asfc – Results for *Asfc* indicate extant, non-ruminant frugivores, *Pecari tajacu* (6.45, n=3) and *Tayassu pecari* (5.55, n=2), have significantly more complex surfaces than the extant grazer, *Equus burchelli* (1.74, n=4, p=0.005 and p=0.007, respectively, Fisher's LSD) and some extant browsers, namely *Diceros bicornis* (1.96, n=6, p=0.010 and p=0.013, respectively, Fisher's LSD) and *Tapirus terrestris* (1.87, n=5, p=0.006 and p=0.008, respectively, Fisher's LSD). *Tayassu pecari* also has more complex surfaces than the mixed-feeder *Lama glama* (2.04, n=3, p=0.032, Fisher's LSD). *Eotylopus reedi* (7.06, n=2) has significantly higher surface complexity than *Equus burchelli* (p=0.003, Fisher's LSD), *Diceros bicornis* (p=0.006, Fisher's LSD), *Tapirus terrestris* (p=0.004, Fisher's LSD), Chadronian *Hyracodon nebraskensis* (1.60, n=2, p=0.002, Fisher's LSD), Orellan *Hyracodon nebraskensis* (1.50, n=2, p=0.001, Fisher's LD), and Orellan *Subhyracodon* sp. (1.91, n=3, p=0.005, Fisher's LSD). *Perchoerus probus* (5.06, n=4) has significantly higher surface complexity than *Equus burchelli* (p=0.012, Fisher's LSD), *Diceros bicornis* (p=0.022, Fisher's LSD), *Tapirus terrestris* (p=0.014, Fisher's LSD), Chadronian *Hyracodon nebraskensis* (p=0.008, Fisher's LSD), and Orellan *Hyracodon nebraskensis* (p=0.002, Fisher's LSD). Chadronian *Hyracodon nebraskensis* has significantly less complex surfaces than *Pecari tajacu* (p=0.004, Fisher's LSD) and *Tayassu pecari* (p=0.004, Fisher's LSD). Orellan *Hyracodon nebraskensis* has significantly less complex surfaces than *Agriochoerus antiquus* (3.76, n=4, p=0.019, Fisher's LSD), *Pecari tajacu* (p=0.001, Fisher's LSD), and *Tayassu pecari* (p=0.001,

Fisher's LSD). *Subhyracodon* sp. has significantly lower surface complexity than *Pecari tajacu* ($p=0.009$, Fisher's LSD) and *Tayassu pecari* ($p=0.012$, Fisher's LSD).

EpLsar – Results for epLsar indicate *Equus burchelli* (0.0059, $n=4$) is statistically indistinguishable with respects to surface anisotropy from all extant and fossil taxa in this study except for *Eotylopus reedi* (0.0017, $n=2$, $p=0.042$, Fisher's LSD) and *Agriochoerus antiquus* (0.0016, $n=4$, $p=0.044$, Fisher's LSD).

Smc – Results for Smc indicate *Equus burchelli* (0.92, $n=4$) and *Diceros bicornis* (0.67, $n=6$) have surfaces with significantly larger surface features ($p<0.023$, Fisher's LSD) than extant mixed-feeders, *Camelus bactrianus* (0.20, $n=4$) and *Rhinoceros unicornis* (0.23, $n=3$), and frugivores, *Pecari tajacu* (0.19, $n=3$) and *Tayassu pecari* (0.21, $n=2$). *Equus burchelli* also has significantly larger surface features than Chadronian *Mesohippus bairdi* (0.26, $n=3$, $p=0.042$, Fisher's LSD), *Perchoerus probus* (0.23, $n=4$, $p=0.003$, Fisher's LSD), and Orellan *Subhyracodon* sp. (0.23, $n=4$, $p=0.003$, Fisher's LSD). *Diceros bicornis* has larger surface features than *Perchoerus probus* ($p=0.014$, Fisher's LSD) and *Subhyracodon* sp. ($p=0.016$, Fisher's LSD). *Megacerops* sp. has larger surface features than *Camelus bactrianus* ($p=0.024$, Fisher's LSD) and *Pecari tajacu* ($p=0.013$, Fisher's LSD).

Tfv – Results from Tfv indicate *Equus burchelli* (9251, $n=4$) is indistinguishable with respect to textural fill volume from all taxa in this study except for *Tayassu pecari* (15403, $n=2$, $p=0.029$, Fisher's LSD).

4.2. DFA analysis and comparison with preliminary extant non-ruminant baseline

Cross-validated results from DFA of the extant non-ruminant ungulate baseline presented here indicate correct classification of extant groups by diet ranging from 80 to 100% (Table 11A). Fossil proxy dietary classifications based on cross-validated extant discriminant functions indicate differences between species (Table 11B). 100% of *Eotylopus reedi* specimens (n=2) were categorized as frugivores. 75% of *Agriochoerus antiquus* specimens (n=3) were categorized as frugivores, 25% (n=1) as browser. 100% of *Megacerops* sp. specimens (n=6) were categorized as browsers. 75% of *Perchoerus probus* specimens (n=3) were categorized as frugivores, 25% (n=1) as mixed-feeder. 100% of Orellan and Chadronian *Hyracodon nebraskensis* specimens (n=2 each) were categorized as browsers. 75% of *Subhyracodon* sp. specimens (n=3) were categorized as mixed-feeders, 25% (n=1) as grazer. 100% of Chadronian *Mesohippus bairdi* specimens (n=3) were categorized as mixed-feeders, whereas 25% of Orellan *M. bairdi* specimens (n=1) were categorized as browser.

5. Discussion

The preliminary extant non-ruminant ungulate dataset presented here, though small, appears to provide an adequate baseline for preliminary comparison to fossil non-ruminants from the White River Group. In general the results from my extant taxa are similar in pattern and variability to that of extant bovids as presented by Scott (2012), clearly distinguishing between the *a priori* dietary groups (Fig. 8).

5.1. Microwear textures and extant non-ruminant diets

In the extant dataset, grazer wear surfaces (as represented by the obligate grazer *Equus burchelli*) have lower complexity and textural fill volumes than the other dietary groups, but higher anisotropy and scale of maximum complexity. *Equus burchelli* is a tall grass specialist (Grzimek, 1990a; Nowak, 1999) and has texture values similar to those of obligate grazing bovids (Scott, 2012). Frugivores *Pecari tajacu* and *Tayassu pecari* (Grzimek, 1990b; Nowak, 1999), as expected, have the most complex surfaces and highest textural fill volumes, and like their bovid analogs (Scott, 2012), also have low scale of maximum complexity and anisotropy values. In this study, browsers (*Diceros bicornis* and *Tapirus terrestris*) and mixed-feeders (*Rhinoceros unicornis*, *Lama glama*, and *Camelus bactrianus*), had surprisingly similar surface complexities and textural fill volumes, but browsers generally had lower anisotropy and significantly higher scale of maximum complexity values, similar to what is seen in extant bovids (Scott, 2012). Differences in microwear texture patterns between these dietary groups are caused by differences in the physical properties of the foods consumed, with similarities between groups notable when there is overlap in food type eaten. For instance, tough foods (i.e. monocots), require shearing/grinding, with lateral movement of the opposing occlusal surfaces relative to one another. This results in abrasives in grasses being dragged across the wear surface of the tooth, producing parallel scratches (Walker et al., 1978; Teaford and Runestad, 1992) which contributes to higher anisotropy values in grazers. Hard and/or brittle foods (i.e. dicots and fruits), on the other hand, require crushing, vertical contact of the opposing occlusal surfaces with the food item fracturing. This process results in the production of pits. Dicots and fruits have a large range of fracture properties, and as such, the pits produced can vary greatly in size, shape, and density

(Walker et al., 1978; Teafor and Runestad, 1992) leading to a broad range of surface complexity, textural fill volume, and scale of maximum complexity values based on the amount and type of dicots and/or fruit consumed.

5.2. *Fossil Diet Proxies*

Comparison of my proxy diet interpretations for fossil taxa based on microwear texture to those of low-magnification, feature-based microwear and mesowear studies requires some preface, as they differ fundamentally in approach to the characterization of wear surfaces and their correlation to diet. Whereas low-magnification feature-based microwear analysis requires the measurement and counting of individual discrete features, microwear texture analysis characterizes the entire surface in three dimensions and across a range of scales, taking advantage of surface variability at both higher and lower magnification. The two approaches to microwear also differ in the working space size; feature-based studies to which I compare my results generally look at a square frame (400 x 400 μm , on 35x magnification) that is much larger than the total working space for microwear texture analysis (204 x 276 μm , on 100x magnification). Though there is not a one-to-one correspondence between surface texture variables and discrete feature shape, volume, or density there is still the likelihood that these two approaches approximate similar diets in context. Both analyses examine wear as it is acquired over a very short period of time. The two different approaches, therefore, should be broadly comparable. Mesowear, a measure of tooth attrition versus abrasion in non-bunodont taxa (see Fortelius and Solounias, 2000), is cumulative over months or even years, and as such

potentially represents the “usual” diet of a species, providing a proximate background diet against which to compare the results of microwear analyses. Mesowear results for the non-bunodont taxa in this study are from Boardman and Secord (submitted).

The isotopic study of Boardman and Secord (2013) included all of the fossil taxa analyzed here, characterizing their general partitions in the biome in which they co-occurred. These taxa inhabited dry, open canopied biomes (most likely woodland-savanna or woody scrubland), based on $\delta^{13}\text{C}$ values, in which grasses are present (Strömberg, 2004, 2005), so I expected that many of the taxa, especially those in the more open habitats (e.g. *Mesohippus bairdi*, *Hyracodon nebraskensis*, *Agriochoerus antiquus*), would potentially utilize this food resource to some extent.

5.2.1. Artiodactyls

The small oromerycid, *Eotylopus reedi*, at ~20 kg, was expected to be reconstructed as a frugivore given its buno-selenodont dental morphology (Prothero, 1998) and the relationship between body size range and diet in modern ungulates (Bodmer, 1990). Among extant frugivorous ungulates average body-size (~25 kg) is significantly smaller than the average for browsers (~400 kg) and mixed-feeders (~700 kg) due in part to greater metabolic demand on small-bodied ungulates to consume high energy food like fruit (Bodmer, 1990). *E. reedi* is here reconstructed as a frugivore based on microwear texture, which agrees with the mesowear results of Boardman and Secord (submitted) but differs with the mixed-feeding interpretation of Dewar (2008) based on

low-magnification microwear features. Differing microwear results likely indicate periods in which *E. reedi* consumed foods other than fruit.

Agriochoerus antiquus, the clawed oreodont, is here reconstructed as a frugivore, which differs from previous interpretations of it as a mixed-feeder based on microwear features (Dewar, 2008). Mesowear results (results from Chapter 3) indicate it was a browser with a dietary composition similar to extant browsers such as the Moose (*Alces alces*) and the Javan Rhino (*Dicerorhinus sondaicus*). Both of these extant taxa eat the fruits of woody plants and more abrasive browse on occasion (Nowak, 1999), so variable microwear results in this taxon should not be surprising.

The tayassuid, *Perchoerus probus*, is a small (35 kg) peccary with bunodont dentition that is here reconstructed as a frugivore. Feature-based microwear results for *Perchoerus* from Dewar (2008) generally reflect the primarily frugivorous nature of modern peccaries (as he termed-it, hard object browser), as do the microwear texture results presented here. The modern peccaries (*Pecari tajacu* and *Tayassu pecari*) used in this study are seed predators (Barreto et al., 1997) and have surface textures very similar to *P. probus*, indicating that this fossil species likely had a diet analogous to its extant relatives.

5.2.2. *Perissodactyls*

Megacerops sp., the largest taxon in the fauna at ~1900 kg, is here reconstructed as a browser, which agrees with the light microscopy microwear results from Mihlbachler and Solounias (2002) but not those of Dewar (2008) which found it to be a mixed-feeder.

Mesowear results (results from Chapter 3) suggest that *Megacerops* sp. was a browser. I do not consider the mixed-feeding results of Dewar (2008) to be anomalous in light of the span of time microwear surveys. I expect that there would be periods of time in which *Megacerops* sp. consumed more abrasive materials, not unlike the extant “dirty” browsing Black Rhino (*Diceros bicornis*), to which its mesowear and microwear are very similar.

Microwear texture results for Chadronian and Orellan *Mesohippus bairdi* suggest that this species was a mixed-feeder, which is in general agreement with the results of feature-based low-magnification microwear studies (Solounias and Semprebon, 2002; Dewar, 2008) and mesowear results (results from Chapter 3). One specimen of Orellan *M. bairdi* was categorized as a browser in the DFA, suggesting support for my hypothesis of seasonal mixed-feeding as interpreted from mesowear (results from Chapter 3).

The microwear texture results for Chadronian and Orellan *Hyracodon nebraskensis* indicate that it was a browser, which is similar to the results of feature-based microwear studies (Dewar, 2008), but differs from the mixed-feeding diet attributed by mesowear (results from Chapter 3). This suggests that in both microwear studies, results may be reflective of seasons in which they ate less abrasive foods than expected, corroborating my hypothesis of seasonal mixed-feeding as interpreted from mesowear (results from Chapter 3). The larger rhinoceros, *Subhyracodon* sp., is here reconstructed as a mixed-feeder, differing from light microscopy microwear results from Dewar (2008), where this taxon is interpreted as a browser. These differences corroborate my hypothesis that this taxon was a seasonal mixed-feeder (results from Chapter 3).

6. Conclusions

This study provides a dataset of microwear textures from eight modern non-ruminant ungulates (a total of 30 specimens). Results indicate that dental microwear texture analysis can successfully distinguish among extant non-ruminant taxa with differing diets. These results are similar to those seen in modern bovids (Scott, 2012). The dataset of extant mammals generated here was used to interpret diet from the microwear textures of seven upper Eocene and lower Oligocene ungulate species, totaling 31 specimens. Microwear surfaces for *Agriochoerus antiquus*, *Perchoerus probus*, and *Eotylopus reedi* are highly complex; along with low anisotropy and scale maximum complexity and high textural fill volume values primarily indicate fruit consumption. Surface textures from *Megacerops* sp. and *H. nebraskensis* indicate a large browse component as surfaces are fairly complex with high scale of maximum complexity and textural fill volumes and low anisotropy. Fairly anisotropic surface values along with low scale of maximum complexity for *Subhyracodon* sp. and *Mesohippus bairdi* indicate mixed-feeding diets for these taxa. Results suggest that grass was an important food source for many WRG taxa during the latest Eocene-earliest Oligocene, which is consistent with results from stable isotopes indicating that these taxa inhabited dry, open woodland-savannas or woody scrublands in Nebraska during this interval.

Few studies have applied microwear texture analysis to Pre-Modern ungulates. This study further demonstrates the utility of using this technique in determining the diets of ungulates in deep time.

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References

- Barreto, G.R., Hernandez, O.E., Ojasti, J., 1997. Diets of peccaries (*Tayassu tajacu* and *T. pecari*) in a dry forest of Venezuela. *Journal of Zoology* 241, 279-284.
- Boardman, G.S., Secord, R., 2013. Stable isotope paleoecology of White River ungulates during the Eocene-Oligocene transition in northwestern Nebraska. *Palaeogeography, Palaeoclimatology, Palaeoecology* 375, 38-49.
- Bodmer, R.E., 1990. Ungulate frugivores and the browser-grazer continuum. *Oikos* 57, 319-325.
- Conover, W.J., Iman, R.L., 1981. Rank transformations as a bridge between parametric and nonparametric statistics. *The American Statistician* 35, 124-129.
- Dewar, E.W., 2008. Dietary ecology and community paleoecology of early Tertiary mammals. Electronic Doctoral Dissertations for U Mass Amherst. Paper AAI3337037.
- El-Zaatari, S., 2010. Occlusal microwear texture analysis and the diets of historic/prehistoric hunter-gatherers. *International Journal of Osteoarchaeology* 20, 67-87.
- Gagnon, M., Chew, A.E., 2000. Dietary preferences in Extant African Bovidae. *Journal of Mammalogy* 81, 490-511.
- Grzimek, B. (ed.), 1990a. Grzimek's Encyclopedia of Mammals, Volume 4. McGraw-Hill, New York.
- Grzimek, B. (ed.), 1990b. Grzimek's Encyclopedia of Mammals, Volume 5. McGraw-Hill, New York.

- Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4(1), 1-9.
- MacFadden, B.J., 1987. Fossil horses from “Eohippus” (*Hyracotherium*) to *Equus*: scaling, Cope’s law, and the evolution of body size. *Paleobiology* 12, 355-369.
- Merceron, G., Escarguel, G., Angibault, J.M., Verheyden-Tixier, H., 2010. Can dental microwear textures record inter-individual dietary variations? *PLoS One* 5, e9542.
- Mihlbachler, M. C., Solounias, N., 2002. Body size, dental microwear, and brontothere diets through the Eocene. *Journal of Vertebrate Paleontology* 22(3), 88A.
- Nowak, R.M., 1999. Walker’s Mammals of the World, 6th edition, Volume 2. Johns Hopkins University Press, Baltimore, Maryland.
- Prothero, D.R., Emry, R.J., 2004. The Chadronian, Orellan, and Whitneyan North American Land Mammal Ages, in: Woodburne, M.O. (Ed.), *Late Cretaceous and Cenozoic Mammals of North America: biostratigraphy and geochronology*. Columbia University Press, New York, pp. 156-168.
- Scott, J.R., 2012. Dental microwear texture analysis of extant African Bovidae. *Mammalia* 6, 157-174.
- Scott, J.R., submitted. Dental microwear texture analysis of fossil bovids from Hadar, Ethiopia: implications for the paleoenvironments of *Australopithecus afarensis*. *Journal of Vertebrate Paleontology*.
- Scott, J.R., Godfrey, L.R., Jungers, W.L., Scott, R.S., Simons, E.L., Teaford, M.F., Ungar, P.S., Walker, A., 2009. Dental microwear texture analysis of two families of subfossil lemurs from Madagascar. *Journal of Human Evolution* 56, 405-416.

- Scott, R.S., Ungar, P.S., Bergstrom, T.S., Brown, C.A., Grine, F.E., Teaford, M.F., Walker, A., 2005. Dental microwear texture analysis shows within-species diet variability in fossil hominins. *Nature* 436, 693-695.
- Scott, R.S., Ungar, P.S., Bergstrom, T.S., Brown, C.A., Childs, B.E., Teaford, M.F., Walker, A., 2006. Dental microwear texture analysis: technical considerations. *Journal of Human Evolution* 51, 339-349.
- Solounias, N., Semprebon, G., 2002. Advances in the reconstruction of ungulate ecomorphology with application to early fossil equids. *American Museum Novitates* 3366, 1-49.
- Solounias, N., Fortelius, M. & Freeman, P. (1994). Molar wear rates in ruminants: A new approach. *Annales Zoologici Fennici* 31, 219–227.
- Stynder, D.O., Ungar, P.S., Scott, J.R., Schubert, B.W., 2012. A dental microwear texture analysis of the Mio-Pliocene hyaenids from Langebaanweg, South Africa. *Acta Palaeontologica Polonica* 57(3), 485-496.
- Teaford, M.F., Runestad, J.A., 1992. Dental microwear and diet in Venezuelan primates. *American Journal of Physical Anthropology* 88, 347-364.
- Ungar, P.S., Merceron, G., Scott, R.S., 2007. Dental microwear texture analysis of Varswater bovids and early Pliocene paleoenvironments of Langebaanweg, Western Cape Province, South Africa. *Journal of Mammalian Evolution* 14, 163-181.
- Ungar, P.S., Grine, F.E., Teaford, M.F., 2008. Dental microwear and diet of the Plio-Pleistocene hominin *Paranthropus boisei*. *PLoS One* 3(4): e2044.

- Ungar, P.S., Scott, R.S., Grine, F.E., Teaford, M.F., 2010. Molar microwear textures and diets of *Australopithecus anamensis* and *Australopithecus afarensis*. Transactions of the Royal Society B 365, 3345-3354.
- Ungar, P.S., Scott, J.R., Curran, S.C., Dunsworth, H.M., Harcourt-Smith, W.E.H., Lehmann, T., Manthi, F.K., McNulty, K.P., 2012. Early Neogene environments in East Africa: Evidence from dental microwear of tragulids. Palaeogeography, Palaeoclimatology, Palaeoecology 342-343, 84-96.
- Venables, W.N., Ripley, B.D., 2002. Modern Applied Statistics with S, Fourth Edition. Springer, New York, New York.
- Walker, A., Hoeck, H.N., Perez, L., 1978. Microwear of mammalian teeth as an indicator of diet. Science 201, 908-910.
- Yakir, D., 1997. Oxygen-18 of leaf water: a crossroad for plant associated isotopic signals, in: Griffiths, H. (ed.), Stable isotopes: integration of biological, ecological, and geochemical processes. BIOS, Oxford, pp. 147–168.

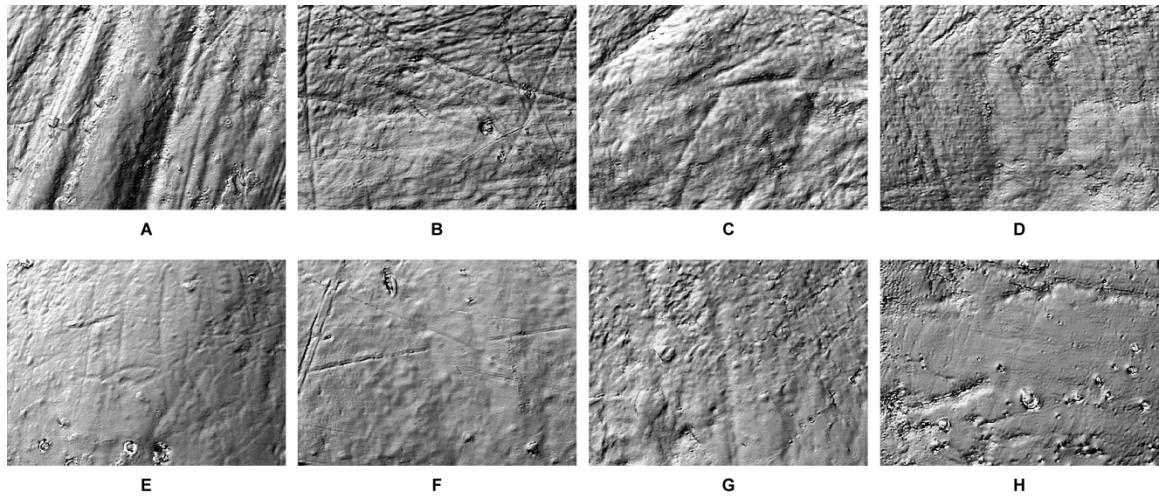


FIGURE 7. Photosimulation of microwear surfaces generated from points clouds for A) extant grazer *Equus burchelli* (KUM 7075), B) extant mixed-feeder *Lama glama* (USNM 172865), C) extant browser *Diceros bicornis* (USNM 162946), D) extant frugivore *Pecari tajacu* (AMNH 92341), E) *Mesohippus bairdi* (UNSM 131774), F) *Subhyracodon* sp. (UNSM 11654), G) *Megacerops* sp. (UNSM 328791), H) *Eotylopus reedi* (UNSM ?-38). Each represents a field of view 102 μm x 138 μm .

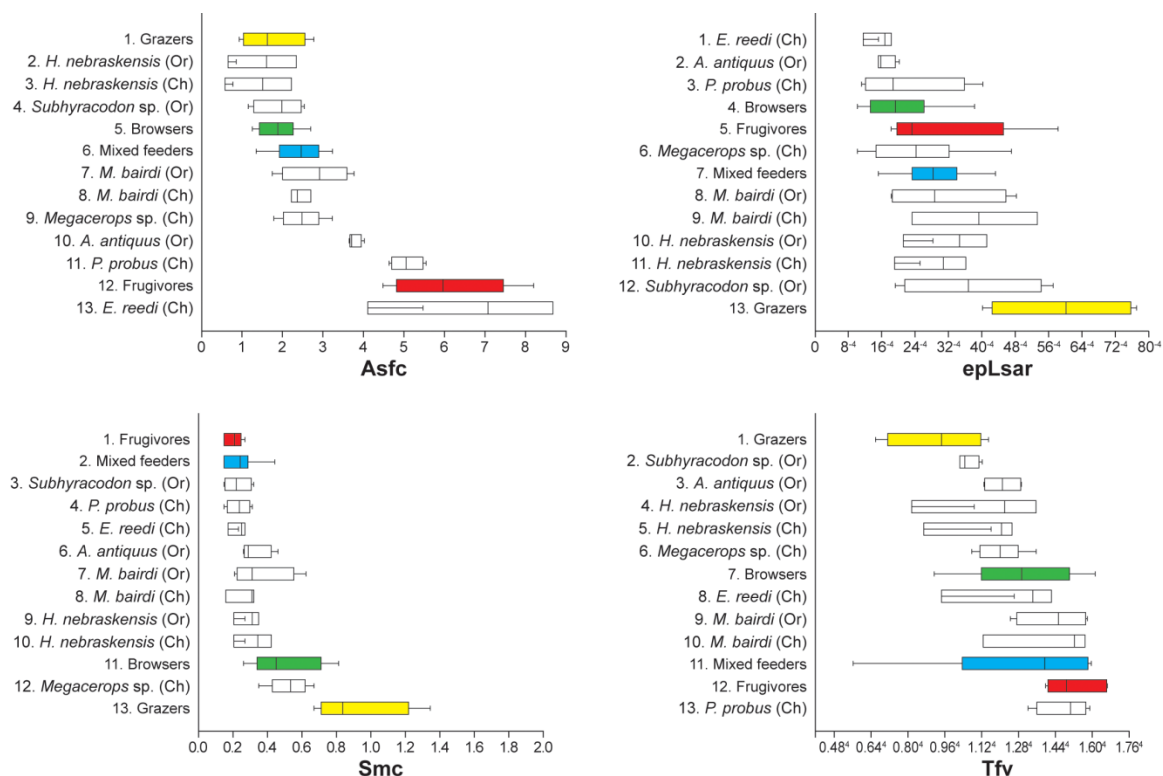


Fig. 8. Box-and-whiskers plots of microwear texture data for each variable by taxon (for the fossils) or diet category (for my extant baseline) arranged by median value. The hinges mark the first and third quartiles, the vertical lines between them are medians, each whisker represents a value 1.5 time the interquartile range, using the interpolated quartile method in PAST (Hammer et al., 2001).

Table 8

Descriptive microwear texture statistics for extant non-ruminant ungulates examined in this study.

Diet	Species		Asfc	epLsar	Smc	Tfv
Obligate Grazer	<i>Equus burchelli</i> Plains Zebra n=4	Mean	1.74	0.0059	0.92	9251.84
		Median	1.63	0.0060	0.84	9447.51
		SD	0.80	0.0018	0.29	2100.94
		Skewness	0.64	-0.1093	1.55	-0.46
Mixed-feeder	<i>Lama glama</i> Llama n=3	Mean	2.04	0.0022	0.31	14579.90
		Median	1.85	0.0024	0.34	15765.58
		SD	0.80	0.0007	0.15	2207.46
		Skewness	0.98	-1.1766	-0.94	-1.72
	<i>Rhinoceros unicornis</i> Indian Rhino n=3	Mean	2.74	0.0030	0.23	14994.71
		Median	2.71	0.0029	0.27	15203.93
		SD	0.47	0.0003	0.06	1090.08
		Skewness	0.36	1.5756	-1.73	-0.83
	<i>Camelus bactrianus</i> Bactrian Camel n=4	Mean	2.44	0.0031	0.20	9924.83
		Median	2.46	0.0030	0.18	9972.10
		SD	0.40	0.0010	0.06	3510.00
		Skewness	-0.34	0.1948	0.86	-0.08
		Mean	2.44	0.0031	0.20	9924.83
Browser	<i>Diceros bicornis</i> Black Rhino n=6	Mean	1.96	0.0020	0.67	13246.48
		Median	1.99	0.0018	0.70	13657.93
		SD	0.42	0.0010	0.13	2334.95
		Skewness	-0.03	1.0123	-1.62	-0.34
	<i>Tapirus terrestris</i> Lowland Tapir n=5	Mean	1.87	0.0022	0.33	12472.84
		Median	1.87	0.0022	0.34	11703.91
		SD	0.58	0.0011	0.08	2629.88
		Skewness	0.52	0.8602	0.77	-0.005
Frugivore	<i>Pecari tajacu</i> Collared Peccary n=3	Mean	6.45	0.0021	0.19	15169.60
		Median	6.68	0.0021	0.21	14895.55
		SD	1.87	0.0003	0.04	1333.74
		Skewness	-0.55	-0.9010	-1.57	0.89
	<i>Tayassu pecari</i> White-lipped Peccary n=2	Mean	5.55	0.0045	0.21	15403.65
		Median	5.55	0.0045	0.21	15403.65
		SD	0.57	0.0018	0.08	1768.16
		Skewness	-	-	-	-

Table 9

Descriptive microwear texture statistics for fossil non-ruminant ungulates from Nebraska.

Species	NALMA		Asfc	epLsar	Smc	Tfv
<i>Eotylopus reedi</i> n=2	Chadronian	Mean	7.06	0.0017	0.25	13417.70
		Median	7.06	0.0017	0.25	13417.70
		SD	2.27	0.0002	0.03	1138.24
		Skewness	-	-	-	-
<i>Agriochoerus antiquus</i> n=4	Orellan	Mean	3.76	0.0016	0.33	12114.78
		Median	3.69	0.0016	0.29	12102.82
		SD	0.17	0.0002	0.09	904.39
		Skewness	1.85	1.6185	1.64	0.01
<i>Megacerops</i> sp. n=6	Chadronian	Mean	2.47	0.0025	0.52	12021.53
		Median	2.48	0.0024	0.53	11989.46
		SD	0.53	0.0013	0.11	1023.96
		Skewness	0.14	1.0722	-0.42	0.40
<i>Perchoerus probus</i> n=4	Chadronian	Mean	5.06	0.0022	0.23	14795.81
		Median	5.04	0.0019	0.24	15045.01
		SD	0.40	0.0013	0.07	1145.33
		Skewness	0.19	1.3119	-0.21	-1.18
<i>Subhyracodon</i> sp. n=4	Orellan	Mean	1.91	0.0037	0.23	10603.74
		Median	1.98	0.0037	0.22	10466.72
		SD	0.61	0.0017	0.08	455.83
		Skewness	-0.50	0.1758	0.30	1.12
<i>Mesohippus bairdi</i> n=4	Orellan	Mean	2.83	0.0031	0.36	14328.26
		Median	2.92	0.0029	0.31	14525.25
		SD	0.84	0.0014	0.18	1599.02
		Skewness	-0.55	0.3706	1.44	-0.36
<i>Mesohippus bairdi</i> n=3	Chadronian	Mean	2.42	0.0038	0.26	14050.33
		Median	2.35	0.0039	0.31	15223.65
		SD	0.24	0.0015	0.09	2432.72
		Skewness	1.19	-0.2342	-1.71	-1.67
<i>Hyracodon nebraskensis</i> n=2	Orellan	Mean	1.50	0.0035	0.31	12225.91
		Median	1.50	0.0035	0.31	12225.91
		SD	1.03	0.0009	0.05	1875.74
		Skewness	-	-	-	-
<i>Hyracodon nebraskensis</i> n=2	Chadronian	Mean	1.60	0.0030	0.35	12074.09
		Median	1.60	0.0030	0.35	12074.09
		SD	1.05	0.0008	0.11	648.77
		Skewness	-	-	-	-

Table 10. Analytical statistics comparing fossil and extant species (ranked data).

A. MANOVA results

Between species	Test statistics	F	p
Wilk's λ	0.003	8.705	<0.001
Pillai Trace	2.449	4.341	<0.001

B. ANOVA results

Texture variable	F	df	p
Asfc	6.432	16, 44	<0.001
epLsar	2.578	16, 44	0.007
Smc	6.545	16, 44	<0.001
Tfv	3.349	16, 44	0.001

C. Paired comparisons (significant differences from Fisher's LSD only)

Variable	Species x Species	p
Asfc	<i>Equus burchelli</i> x <i>Pecari tajacu</i>	0.005
	<i>E. burchelli</i> x <i>Eotylopus reedi</i>	0.003
	<i>E. burchelli</i> x <i>Perchoerus probus</i>	0.012
	<i>E. burchelli</i> x <i>Tayassu pecari</i>	0.007
	<i>P. tajacu</i> x <i>Diceros bicornis</i>	0.010
	<i>E. reedi</i> x <i>D. bicornis</i>	0.006
	<i>Hyracodon nebraskensis</i> (Ch) x <i>Pecari tajacu</i>	0.004
	<i>H. nebraskensis</i> (Ch) x <i>E. reedi</i>	0.002
	<i>H. nebraskensis</i> (Or) x <i>Agriochoerus antiquus</i>	0.019
	<i>H. nebraskensis</i> (Or) x <i>P. tajacu</i>	0.001
	<i>H. nebraskensis</i> (Or) x <i>E. reedi</i>	0.001
	<i>P. probus</i> x <i>D. bicornis</i>	0.022
	<i>P. probus</i> x <i>H. nebraskensis</i> (Ch)	0.008
	<i>P. probus</i> x <i>H. nebraskensis</i> (Or)	0.002
	<i>Subhyracodon</i> sp. x <i>P. tajacu</i>	0.009
	<i>Subhyracodon</i> sp. x <i>E. reedi</i>	0.005
	<i>Tapirus terrestris</i> x <i>P. tajacu</i>	0.006
	<i>T. terrestris</i> x <i>E. reedi</i>	0.004
	<i>T. terrestris</i> x <i>P. probus</i>	0.014
	<i>T. pecari</i> x <i>D. bicornis</i>	0.013
	<i>T. pecari</i> x <i>H. nebraskensis</i> (Ch)	0.004
	<i>T. pecari</i> x <i>H. nebraskensis</i> (Or)	0.001
	<i>T. pecari</i> x <i>Lama glama</i>	0.032
	<i>T. pecari</i> x <i>Subhyracodon</i> sp.	0.012
	<i>T. pecari</i> x <i>T. terrestris</i>	0.008

epLsar	<i>E. burchelli</i> x <i>A. antiquus</i>	0.044
	<i>E. burchelli</i> x <i>E. reedi</i>	0.042
Smc	<i>E. burchelli</i> x <i>Camelus bactrianus</i>	0.001
	<i>E. burchelli</i> x <i>Pecari tajacu</i>	<0.000
	<i>E. burchelli</i> x <i>Rhinoceros unicornis</i>	0.004
	<i>E. burchelli</i> x <i>Mesohippus bairdi</i> (Ch)	0.042
	<i>E. burchelli</i> x <i>P. probus</i>	0.003
	<i>E. burchelli</i> x <i>Subhyracodon</i> sp.	0.003
	<i>E. burchelli</i> x <i>T. pecari</i>	0.003
	<i>D. bicornis</i> x <i>C. bactrianus</i>	0.004
	<i>D. bicornis</i> x <i>P. tajacu</i>	0.002
	<i>R. unicornis</i> x <i>D. bicornis</i>	0.022
	<i>Megacerops</i> sp. x <i>Camelus bactrianus</i>	0.024
	<i>Megacerops</i> sp. x <i>P. tajacu</i>	0.013
	<i>P. probus</i> x <i>D. bicornis</i>	0.014
	<i>Subhyracodon</i> sp. x <i>D. bicornis</i>	0.016
	<i>T. pecari</i> x <i>D. bicornis</i>	0.018
Tfv	<i>E. burchelli</i> x <i>T. pecari</i>	0.029

Table 11

Results of DFA for all dietary categories.

A. Cross-validation results: n, %. Numbers in bold indicate the number and percentage of cross-validations.				
	Grazers	Mixed-feeders	Browsers	Frugivores
Grazers	4, 100%			
Mixed-feeders		9, 90%	1, 10%	
Leaf browsers			11, 100%	
Fruit Browsers		1, 20%		4, 80%
B. Fossil classifications				
<i>Eotylopus reedi</i>				2, 100%
<i>Agriochoerus antiquus</i>			1, 25%	3, 75%
<i>Megacerops</i> sp.			6, 100%	
<i>Perchoerus probus</i>		1, 25%		3, 75%
<i>Hyracodon nebraskensis</i> (Ch)			2, 100%	
<i>Hyracodon nebraskensis</i> (Or)			2, 100%	
<i>Subhyracodon</i> sp. (Or)	1, 25%	3, 75%		
<i>Mesohippus bairdi</i> (Ch)		3, 100%		
<i>Mesohippus bairdi</i> (Or)		3, 75%	1, 25%	

Appendix 4. Raw data (median values from four scans) from extant and fossil taxa included in this study.

Species	Age	Locality	Specimen #	Median Raw Values			
				Asfc	epLsar	Smc	Tfv
<i>Equus burchelli</i>	Modern	South Africa	AMNH 82313	1.93	0.0071	0.84	10199.82
<i>Equus burchelli</i>	Modern	South Africa	AMNH 82314	2.77	0.0040	0.67	8695.19
<i>Equus burchelli</i>	Modern	Tanzania	AMNH 164127	1.33	0.0049	1.34	11501.24
<i>Equus burchelli</i>	Modern	Tanzania	KUM 7075	0.92	0.0077	0.83	6611.10
<i>Lama glama</i>	Modern	Argentina	USNM 92137	2.91	0.0028	0.15	12032.96
<i>Lama glama</i>	Modern	Peru	USNM 172862	1.35	0.0015	0.44	15765.58
<i>Lama glama</i>	Modern	Peru	USNM 172865	1.85	0.0024	0.34	15941.16
<i>Rhinoceros unicornis</i>	Modern	Nepal	AMNH 54455	2.71	0.0028	0.27	13815.18
<i>Rhinoceros unicornis</i>	Modern	Nepal	Cast 2616	2.30	0.0033	0.16	15203.93
<i>Rhinoceros unicornis</i>	Modern	Nepal	AMNH 54454	3.23	0.0029	0.27	15965.02
<i>Camelus bactrianus</i>	Modern	Mongolia	2008-1	2.35	0.0036	0.27	14108.46
<i>Camelus bactrianus</i>	Modern	Mongolia	2008-15b	2.88	0.0020	0.15	5646.66
<i>Camelus bactrianus</i>	Modern	Mongolia	2008-28	2.57	0.0025	0.21	10730.51
<i>Camelus bactrianus</i>	Modern	Mongolia	2008-33	1.94	0.0043	0.15	9213.68
<i>Diceros bicornis</i>	Modern	Kenya	USNM 162933	2.25	0.0036	0.42	14849.20
<i>Diceros bicornis</i>	Modern	Kenya	USNM 162946	1.59	0.0026	0.81	14389.32
<i>Diceros bicornis</i>	Modern	Tanzania	KUM 7074	2.49	0.0019	0.70	9952.64
<i>Diceros bicornis</i>	Modern	Tanzania	KUM 157345	1.43	0.0017	0.71	16135.84
<i>Diceros bicornis</i>	Modern	Tanzania	KUM 157348	2.21	0.0013	0.73	11225.36
<i>Diceros bicornis</i>	Modern	Kenya	ZM 5048	1.77	0.0010	0.65	12926.54
<i>Tapirus terrestris</i>	Modern	Venezuela	AMNH 77572	1.24	0.0015	0.34	15293.79
<i>Tapirus terrestris</i>	Modern	Venezuela	AMNH 77573	2.12	0.0010	0.26	11197.16
<i>Tapirus terrestris</i>	Modern	Venezuela	AMNH 77574	1.87	0.0023	0.27	15016.18
<i>Tapirus terrestris</i>	Modern	Brazil	AMNH 120996	2.70	0.0022	0.45	9153.18
<i>Tapirus terrestris</i>	Modern	Bolivia	AMNH 246974	1.42	0.0038	0.35	11703.91

<i>Pecari tajacu</i>	Modern	Brazil	AMNH 92341	4.47	0.0018	0.21	13994.18
<i>Pecari tajacu</i>	Modern	Brazil	AMNH 92839	8.19	0.0021	0.15	14895.55
<i>Pecari tajacu</i>	Modern	Brazil	AMNH 92842	6.68	0.0023	0.22	16619.09
<i>Tayassu pecari</i>	Modern	Brazil	AMNH 36654	5.95	0.0032	0.27	16653.93
<i>Tayassu pecari</i>	Modern	Brazil	AMNH 36656	5.14	0.0058	0.15	14153.38
<i>Eotylopus reedi</i>	Chadronian	Nebraska	UNSM 73238	5.46	0.0018	0.23	14222.56
<i>Eotylopus reedi</i>	Chadronian	Nebraska	UNSM ?-38	8.67	0.0015	0.27	12612.84
<i>Agriochoerus antiquus</i>	Orellan	Nebraska	UNSM 8415853SP	4.02	0.0015	0.27	12852.17
<i>Agriochoerus antiquus</i>	Orellan	Nebraska	UNSM 46047	3.71	0.0015	0.26	12942.25
<i>Agriochoerus antiquus</i>	Orellan	Nebraska	UNSM 52531a	3.68	0.0016	0.31	11311.23
<i>Agriochoerus antiquus</i>	Orellan	Nebraska	UNSM 46038	3.64	0.0020	0.46	11353.47
<i>Megacerops</i> sp.	Chadronian	Nebraska	UNSM 328791	1.78	0.0047	0.51	10777.56
<i>Megacerops</i> sp.	Chadronian	Nebraska	UNSM 1074	2.77	0.0010	0.60	12552.84
<i>Megacerops</i> sp.	Chadronian	Nebraska	UNSM 42254	2.73	0.0025	0.67	11253.45
<i>Megacerops</i> sp.	Chadronian	Nebraska	UNSM 272463	2.10	0.0016	0.56	11523.56
<i>Megacerops</i> sp.	Chadronian	Nebraska	UNSM 1134	3.22	0.0023	0.45	13566.40
<i>Megacerops</i> sp.	Chadronian	Nebraska	UNSM 1122	2.22	0.0027	0.35	12455.36
<i>Perchoerus probus</i>	Chadronian	Nebraska	UNSM 30811-011	4.86	0.0011	0.15	15892.57
<i>Perchoerus probus</i>	Chadronian	Nebraska	UNSM 53604	5.22	0.0022	0.25	14866.45
<i>Perchoerus probus</i>	Chadronian	Nebraska	UNSM 123701	4.62	0.0015	0.22	15223.56
<i>Perchoerus probus</i>	Chadronian	Nebraska	UNSM 76074	5.53	0.0040	0.31	13200.66
<i>Subhyracodon</i> sp.	Orellan	Nebraska	UNSM 11654	1.15	0.0057	0.27	10256.83
<i>Subhyracodon</i> sp.	Orellan	Nebraska	UNSM 212673	2.53	0.0028	0.15	10266.88
<i>Subhyracodon</i> sp.	Orellan	Nebraska	UNSM 13822394	1.72	0.0045	0.32	11224.69
<i>Subhyracodon</i> sp.	Orellan	Nebraska	UNSM 132012	2.23	0.0019	0.17	10666.56
<i>Mesohippus bairdi</i>	Orellan	Nebraska	UNSM 131774	2.73	0.0048	0.27	15499.93
<i>Mesohippus bairdi</i>	Orellan	Nebraska	UNSM 131769	1.75	0.0038	0.35	12455.65
<i>Mesohippus bairdi</i>	Orellan	Nebraska	UNSM 131601	3.10	0.0019	0.62	15806.88

<i>Mesohippus bairdi</i>	Orellan	Nebraska	UNSM 131585	3.75	0.0018	0.21	13550.57
<i>Mesohippus bairdi</i>	Chadronian	Nebraska	UNSM 3077-011	2.35	0.0023	0.32	11253.33
<i>Mesohippus bairdi</i>	Chadronian	Nebraska	UNSM 131519	2.69	0.0053	0.16	15674.02
<i>Mesohippus bairdi</i>	Chadronian	Nebraska	UNSM 202653	2.22	0.0039	0.31	15223.65
<i>Hyracodon nebraskensis</i>	Chadronian	Nebraska	UNSM 11001	2.34	0.0025	0.42	12532.84
<i>Hyracodon nebraskensis</i>	Chadronian	Nebraska	UNSM 3161011	0.86	0.0036	0.27	11615.34
<i>Hyracodon nebraskensis</i>	Orellan	Nebraska	UNSM 11266	0.77	0.0028	0.35	13552.26
<i>Hyracodon nebraskensis</i>	Orellan	Nebraska	UNSM 11383	2.22	0.0041	0.27	10899.56

CHAPTER 5

CONCLUSIONS

Stable-isotope analyses indicate important changes in ecosystem structure during the EOCT. Average carbon isotope values for both Chadronian and Orellan faunas suggest the presence of fairly dry biomes, in this case most likely woodland-savanna or woody scrubland. Many range-through taxa retain the same relative position in isotopic space in both faunas, indicating stability of the most open habitats through this interval. An isotopic “gap” in mean $\delta^{13}\text{C}$ values, between about -9.5‰ and -12.0 ‰, appears in the Orellan. This gap had been filled by five taxa that appear to have been feeding in wetter habitats in the Chadronian, suggesting the reduction of wetter habitats in the Orellan. Two of these “gap” fillers, *Megacerops* sp. and *Trigonias* sp., became extinct regionally in the Orellan, which suggests an important change in ecosystem structure in the form of open, water-stressed habitat expansion at the cost of wetter, more closed ones. An increase of 1.0‰ in mean faunal $\delta^{18}\text{O}_\text{E}$ values in the Orellan is also consistent with drying, although the expected response of an upward shift in aridity-sensitive taxa yielded mixed results. The increase in $\delta^{18}\text{O}_\text{E}$ values in *Agriochoerus antiquus*, the most aridity-sensitive species in both of the faunas studied, is consistent with the expectation for drying, but significant change was not found in *Mesohippus* spp., another taxon that appears to have been an aridity-sensitive. The significant increase in variance in $\delta^{18}\text{O}_\text{E}$ values that occurs during the Orellan is also consistent with an increase in aridity.

Dental wear results suggest that open habitat expansion had little effect on the feeding strategies of many WRG ungulates. All of the taxa examined in this study are characterized by low hypsodonty index values, suggesting that none of them were well

adapted for high levels of dietary abrasion. Mesowear results from several taxa (*Mesohippus bairdi*, *Hyracodon nebraskensis*, *Subhyracodon* sp., *Aepinacodon americanum*, and Orellan *Merycoidodon* sp.), however, suggest grasses made up a large portion of their diets, at least seasonally. The brontothere, *Megacerops* sp., and the clawed oreodont, *Agrichoerus antiquus*, are interpreted to have been low abrasion browsers, and both exhibit mesowear patterns well outside of what would be expected among mixed-feeders or frugivores. Mesowear results from two range-through taxa (*Hyracodon nebraskensis* and *Mesohippus bairdi*) suggest general dietary stasis during the EOCT in Nebraska, but results from *Merycoidodon* sp., suggest that it switched to a more abrasive diet in the early Oligocene. This suggests more grass was available to *Merycoidodon* sp. during the Orellan and that it adapted to utilize this expanding resource perhaps as a way of reducing competition with similar-sized ungulate browsers like *Agrichoerus antiquus*.

Results from microwear texture analysis indicate that the method is effective for characterizing the diets of non-ruminant ungulates. Dietary interpretations made in WRG ungulates using this dataset yielded results that were consistent with other dietary proxies. Expectations for dietary variability based on mesowear results were generally met, suggesting that many taxa were likely seasonal mixed-feeders as interpreted from mesowear.

The three studies presented in this dissertation provide ample evidence corroborating a transition to a more open biome structure in the early Oligocene of Nebraska, presumably due to climatic drying. These changes apparently had only a small effect on mammalian faunal turnover, however, even though *Megacerops* and *Trigonias*

appear to have become extinct as a result of shrinking habitat. Other faunal parameters, such as changes in the relative abundance of species and fauna evenness through the EOCT, have yet to be rigorously studied.