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Stable isotope patterns found in early Eocene equid tooth rows of North America: Implications for reproductive behavior and paleoclimate

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ABSTRACT

Reproductive behaviors of early Eocene equids were likely different from their modern-day counterparts as a result of their small body size and warmer global temperatures. To better understand the paleoenvironment and ecology of these early horses, teeth of *Protorohippus montanum* jaws from a single stratigraphic locality in early Eocene sedimentary deposits of Wyoming were sampled for oxygen and carbon isotope analysis. These enamel data along with an inferred body mass of ~7.5 kg suggests that adult teeth formed over a matter of months, with the final tooth erupting at ~1.3 years of age. Thus, average isotopic values from each tooth likely represent environmental conditions from a single season. Results indicate two isotopic patterns. In the case of isotopic "pattern A," the second forming molars (m/2s) have significantly lower isotopic ratios compared to other teeth within the jaw. This suggests the first forming molars (m/1s) formed during a cooling fall season, followed by formation of the m/2s during the cooler winter. In the case of isotopic "pattern B," results suggest that m/1s formed during a warming spring season, while the m/2s formed during the following warmer summer season. Isotopic ratios of m/1s may represent an individual's birth season (or *in utero* season, depending on timing of initial formation), implying that early equids experienced at least two birth seasons per year. These results suggest that temperatures and reduced seasonality of the early Eocene played a strong role in early equid birth cycles. Lastly, the mean and variance in carbon and oxygen isotope ratios was not significantly different across tooth positions of all jaws when all individuals were grouped together, regardless of their pattern assignment. Such results indicate that isotopic data from fourth premolars, first molars, second molars, and third molars can be combined for purposes of temporal reconstructions of paleoclimate, thus increasing the potential sample sizes for these types of studies.

1. Introduction

When equids first appeared at the beginning of the Eocene, they were only about 15% the body mass of extant equids, suggesting their biology and ecology may have considerably evolved since then (MacFadden, 1986; Tacutu et al., 2013). In addition to the body size disparity, warmer climatic conditions of the early Eocene may have also contributed to ecological and life history differences. For example, reproduction characteristics such as litter size and birth seasons in modern mammals are strongly correlated with body size, photoperiod, and climate, making it likely that early Eocene equids reproduced differently compared to their more recent descendents (Millar, 1977; Blueweiss et al., 1978; Maiorana, 1990; McNab, 1990; Guerin and Wang, 1994; Nagy et al., 2000; Bradshaw and Holzapfel, 2007; Bronson, 2009). Unfortunately, gestational characteristics of extinct animals are often difficult to reconstruct from the fossil record because such behaviors are not typically associated with anatomical characters. Furthermore, when such anatomical characters do exist, they are not always well-preserved. For example, in some mammals, the uterus may preserve placental scars, each scar representing potential offspring (Davis and Emlen, 1948).

Oxygen and carbon isotope ratios of tooth enamel have been used extensively to infer aspects of mammalian paleoecology and reconstruct paleoclimate. For example, stable isotope ratios of different teeth along mammal tooth rows (i.e., jaws) have been shown to preserve information about an animal's reproductive behavior (Bryant et al., 1996a, 1996b; Fricke and O'Neil, 1996; Balasse, 2002). As tooth enamel forms, it records the isotope ratio of body water and metabolic byproducts of digestion during the interval over which it grew. The oxygen and carbon isotope ratios of body water and metabolic byproducts are controlled by oxygen isotope ratios of local water supplies and the carbon isotope ratio of local food sources which, in turn, can vary seasonally due to changes in climatic variables like temperature and precipitation (Dansgaard, 1964; Longinelli and Nuti, 1973; Longinelli, 1984; Luz et al., 1984; Kohn, 1996). As each tooth forms in succession during

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early ontogeny, its isotope ratio is thus reflective of environmental variables like temperature and precipitation during the interval over which it formed. By comparing the stable isotope ratios of early forming teeth to late forming teeth, the timing of birth cycles and the magnitude of seasonal environmental changes can be captured (Bryant et al., 1996a, 1996b; Fricke and O'Neil, 1996; Fricke et al., 1998; Balasse et al., 2003).

Inter-tooth isotopic variability is also important in the context of stratigraphic studies of paleoclimate where stable isotope records obtained from mammalian fossil enamel are used to reconstruct environmental changes through time (Koch et al., 1992, 1995; Secord et al., 2008, 2010, 2012). For instance, early forming teeth are thought to be isotopically variable, and thus less reliable for paleoclimate reconstructions, due to the influences of mother's milk prior to weaning. Mother's milk has already undergone physiologically-induced isotopic fractionation, introducing an additional step in tracing an isotopic signal from the environment (Bryant and Froelich, 1995; Bryant et al., 1996a; Fricke and O'Neil, 1996; Camin et al., 2008). However, isotopic variability between early- and late-forming teeth has rarely been investigated explicitly in fossil taxa. Because early equids are some of the most commonly preserved animals of early Eocene sedimentary deposits, their teeth have been used in several studies for temperature and carbon cycle reconstruction (Koch et al., 1995; Secord et al., 2008, 2012). The early Eocene is a particularly interesting period because of generally warm greenhouse temperatures interspersed with numerous short-term extreme global warming events known as hyperthermals (Bowen et al., 2006; Gingerich, 2006; Zachos et al., 2008; Abels et al., 2012). However, despite the prevalence of early equids throughout early Eocene stratigraphic records and their common use in stratigraphic studies of paleoclimate, no systematic study of isotope variability across their tooth rows has been conducted. Therefore, it is not clear whether some tooth positions are better suited than others for these types of stratigraphic paleoclimate reconstructions.

In this study we use stable isotope ratios of enamel from a sample of North American early Eocene equid tooth rows from a single locality within the Bighorn Basin of Wyoming in order to (1) better understand reproductive cycles of early horses in comparison to more recent, larger-bodied, and better studied equids, and (2) determine which teeth of an early Eocene horse's tooth row have the least amount of isotopic variability and thus are best suited for stratigraphic studies of paleoclimate.

2. Background

2.1. *Protorohippus*

The early equid specimens used in this study are referred to the species *Protorohippus montanum*. Traditionally these specimens have been referred to the genus *Hyracotherium* (= *Eohippus*), which was long considered to include the earliest equids from North America and Europe (Owen, 1841; Hooker, 1994; Froehlich, 2002; Wood, 2009). However, it is now thought that the type species, *Hyracotherium leporinum*, was probably more closely related to palaeotheres than to equids (Hooker, 1994; Froehlich, 1999, 2002; Rose, 2006), and the genus *Hyracotherium* has recently been split into six new genera including *Protorohippus* (Froehlich, 1999, 2002; note that these results are not universally accepted, e.g., Rose, 2006; Wood, 2009).

Early Eocene equids were characterized by an elongate snout, primitive molars, and long limbs with some cursorial adaptations (Radinsky, 1969; Rose, 2006). Based on evidence of low male/female ratios in fossil assemblages indicated by canine and cranial size bimodality (e.g., males having larger canines), some early equid species were thought to have been sexually dimorphic and polygynous, similar to modern day horses (Gingerich, 1981; Wood, 2009). Early North American equids have been estimated to weigh between 4 and 35 kg—the smallest being about the size of a house cat (Gingerich, 1981; MacFadden, 1986; Rose, 2006). Early equids had low-crowned (brachydont) teeth designed for compressive chewing of fruits, seeds, tender leaves, and perhaps even herbaceous dicots, woody shrubs, and specialized ferns (Gingerich, 1981; Rensberger et al., 1984; Janis, 1990; MacFadden, 1992, 2000).

2.2. Stable isotope ratios and temporal records of tooth enamel formation

Tooth enamel is composed of hydroxyapatite, or "bioapatite," with a general mineral formula of $Ca_5(PO_4, CO_3)_3(OH, CO_3)$. Studies have shown that, unlike bone and dentine, enamel bioapatite is rarely recrystallized once it precipitates due to its low organic collagen content and larger crystals, and thus is more resistant to post-burial diagenetic processes (Lee-Thorp and van der Merwe, 1991; Fricke and O'Neil, 1996; Koch et al., 1997; Kohn and Cerling, 2002; Fricke, 2007). Studies of modern herbivorous mammals indicate that the primary source of oxygen in enamel bioapatite is ingested water (e.g., from surface waters of streams or ponds, water in vegetation, and water on vegetation), and the primary source of carbon is consumed vegetation (Longinelli, 1984; Ambrose and Norr, 1993). Stable carbon and oxygen isotope ratios of these sources typically vary on seasonal to geological timescales due to environmental and ecological changes. Because mammalian bioapatite precipitates in isotopic equilibrium with body water, it can provide a faithful record of the oxygen isotope ratio of ingested water and the carbon isotope ratio of consumed food, and thus can be used to reconstruct environmental and ecological changes over various time scales.

The oxygen isotope ratio ($\delta^{18}O$[1]) of ingested surficial water can be estimated from the oxygen isotope ratio of bioapatite using established physical models for mammals of various sizes (Bryant and Froelich, 1995; Kohn, 1996; Hoppe et al., 2004a; Podlesak et al., 2008). In turn, $\delta^{18}O$ of meteoric water is linked to local atmospheric temperatures (Dansgaard, 1964; Longinelli, 1984; Bryant and Froelich, 1995; Kohn and Cerling, 2002; Fricke, 2007). $\delta^{18}O$ of atmospheric water vapor changes when more ^{18}O versus ^{16}O is preferentially removed from cooling air masses in the form of precipitation. As an air mass continues to cool, less ^{18}O is available to rain out, contributing to progressively lower $\delta^{18}O$ values of local meteoric waters. This means that higher $\delta^{18}O$ values of meteoric water generally indicate warmer temperatures, and lower $\delta^{18}O$ values generally indicate cooler temperatures (Dansgaard, 1964; Rozanski et al., 1993), and thus likewise for $\delta^{18}O$ of tooth enamel. During especially warm and arid conditions, $\delta^{18}O$ of local water sources (e.g., ponds and leaf water) can be higher due to evaporative isotopic-enrichment (Sharp, 2007). This study restricts bioapatite $\delta^{18}O$ interpretations to *relative* changes in temperature, as *absolute* estimates of temperature require many additional assumptions.

Carbon isotope ratios ($\delta^{13}C$[1]) of mammalian bioapatite ultimately reflect the carbon isotope ratios of consumed plant material, which in turn vary in response to photosynthetic pathways and climatic conditions (O'Leary, 1988; Farquhar et al., 1989; Kohn and Cerling, 2002). C3 photosynthesizing plants, which were common in the early Eocene and probably formed the basis of early equid diets (Wang et al., 1994; Koch et al., 1995), have $\delta^{13}C$ values ranging from −36‰ to −21‰, averaging ~ −26‰ (Cerling and Quade, 1993; Kohn and Cerling, 2002; Sharp, 2007). Changes in temperature and/or aridity can cause variations in $\delta^{13}C$ within C3 plants. During periods of higher temperatures, aridity, and sunlight, plants will close their stomata to conserve water. This directly decreases their influx of CO_2 and results in relatively high plant tissue $\delta^{13}C$ because there is less discrimination between ^{13}C and ^{12}C during tissue growth. Thus $\delta^{13}C$ of plants and mammalian tooth enamel can fluctuate seasonally (O'Leary, 1988; Farquhar et al., 1989; O'Leary et al., 1992). C3 plants of the early Eocene exhibited a similar

[1] Stable isotope ratios may be expressed using "delta" notation, where $\delta = (R_{sample}/R_{standard} - 1) \times 1000$, reported in per mil (‰), with R representing the abundance of the heavy to light isotope (^{18}O–^{16}O for $\delta^{18}O$, and ^{13}C–^{12}C for $\delta^{13}C$).