

Hypsodonty, horses, and the spread of C₄ grasses during the middle Miocene in southern California

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ABSTRACT

Background: C₄ grasses were not abundant in North America during the middle Miocene (c. 15 Ma). They did not become abundant until around 7 Ma. One can analyse stable carbon ($\delta^{13}\text{C}$) and oxygen ($\delta^{18}\text{O}$) isotope values in the enamel of fossil horse teeth to determine the extent to which horses were eating C₄ grasses even during the period before those grasses became abundant.

Questions: In southern California, what proportion of a middle Miocene horse's diet was made up of C₄ grasses? Was the amount enough to influence the size and shape of horse teeth?

Organisms: Eighty-five specimens of five fossil horse species – *Acritohippus stylodontus*, *Archaeohippus mourningi*, *Merychippus californicus*, *Scaphohippus intermontanus*, and *Scaphohippus sumani* – from the middle Miocene (c. 16 Ma) of southern California (i.e. Barstow Formation, Cajon Valley Formation, and Temblor Formation).

Methods: To determine if C₄ grasses were present in middle Miocene horse diets, we analysed stable carbon ($\delta^{13}\text{C}$) and oxygen ($\delta^{18}\text{O}$) isotope values from the enamel of the fossils. If the result did indicate C₄ foraging at a locality, we modelled the percentage of C₄ grasses in equid diets using Stable Isotope Analysis in R (SIAR) v.4.2.2.

Results: Modelled percentage C₄ in equid diets was <20%. Each formation was statistically significantly different from the others in terms of $\delta^{13}\text{C}$ values. Barstow specimens had the highest values, those from Cajon Valley the lowest, and those from Temblor were intermediate. Those results indicate that horses ate C₄ grasses within the Barstow and possibly the Temblor Formation but not the Cajon Valley Formation. Within the Barstow sample, *Scaphohippus sumani* had statistically significantly lower $\delta^{13}\text{C}$ but statistically significantly higher $\delta^{18}\text{O}$ values than *Acritohippus stylodontus*, suggesting a higher proportion of C₃ grasses in the diet of *Scaphohippus sumani* versus a higher proportion of C₄ grasses for *Acritohippus stylodontus*. The latter species also had higher tooth crowns, consistent with a diet richer in C₄ grasses. There were no statistically significant differences between species at Cajon Valley for either $\delta^{13}\text{C}$ or $\delta^{18}\text{O}$. The $\delta^{13}\text{C}$ values for *Merychippus californicus* suggest that the habitats of the Temblor Formation had a low percentage (<6%) of C₄ plants.

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Conclusions: C_4 grasses lived in the mid-Miocene landscape in southern California up to 8 million years before the rapid increase in C_4 ecosystems that occurred worldwide about 7 to 5 Ma. Horses foraged these grasses, so hypotheses related to horse morphological evolution must take C_4 plants into account.

Keywords: C_3 plants, C_4 plants, Equidae, grasslands, hypsodonty, Miocene, stable isotope.

INTRODUCTION

The spread of grasslands in North America has been associated with diversification and morphological evolution in many mammalian clades, particularly in ungulate mammals (Simpson, 1951; Webb, 1977, 1983; Janis, 1993; Jacobs *et al.*, 1999; Janis *et al.*, 2000, 2002, 2004; Strömberg, 2006, 2011; Edwards *et al.*, 2010; McInerney *et al.*, 2011; Strömberg and McInerney, 2011). The initial spread of grasslands in the late Oligocene and early Miocene has been attributed solely to grass species that utilized the C_3 photosynthetic pathway, and that it was not until the late Miocene, between 7 and 5 million years ago (Ma), that grasses using C_4 photosynthesis spread globally in a period sometimes referred to as the rapid increase in C_4 ecosystems (RICE) (MacFadden and Cerling, 1996; Cerling *et al.*, 1997, 1998; Ehleringer *et al.*, 1997; Latorre *et al.*, 1997; Kohn and Cerling, 2002). Today, C_3 photosynthesis represents 85% of plant species, and is the typical pathway of most trees, shrubs, and cool-growing-season grasses (Ehleringer *et al.*, 1991), while C_4 photosynthesis is identified in less than 5% of plant species and is typical of warm-growing-season grasses and sedges (Ehleringer *et al.*, 1991; Ehleringer and Monson, 1993; Edwards *et al.*, 2010). Interestingly, while C_4 photosynthesis occurs in only 5% of extant plant species, this pathway represents about 25% of global plant abundance, and most herbivores eat a larger proportion of plants in C_4 -dominated ecosystems than they do in C_3 -dominated ecosystems (Heckathorn *et al.*, 1999; Edwards *et al.*, 2010). Thus, it is important to understand the evolution of this photosynthetic pathway and the influence it may have had on mammalian evolution.

Mammalian diversification during grassland expansion in North America resulted in the greatest diversity of mammalian herbivore genera during the Cenozoic (Van Valkenburgh and Janis, 1993). And, morphologically, adaptations to life in grassland habitats evolved, such as the evolution of hypsodonty and cursoriality (Stirton, 1947; Simpson, 1951; Janis *et al.*, 2000; Strömberg, 2006). These morphological adaptations were not confined to North America, as hypsodont herbivores began to thrive in Eurasia at this time as well (Raia *et al.*, 2011). The implication that C_3 photosynthesis was the only pathway involved in the initial spread of North American grasslands suggests that C_4 plants were neither present, nor could they have acted as a selective agent in mammal evolution at that time. However, recent studies utilizing various techniques have identified that C_4 plants were present on the landscape and consumed by ungulate species at least since the mid-Miocene (Fox and Koch, 2003, 2004; Cotton *et al.*, 2012, 2014; Feranec and Pagnac, 2013; Chen *et al.*, 2015). These studies generally show that C_4 abundance was less than 25% and that their occurrence was regional (Fox and Koch, 2003, 2004; Cotton *et al.*, 2012, 2014; Feranec and Pagnac, 2013; Chen *et al.*, 2015). However, consumption of C_4 plants by mammals during the mid-Miocene at the time of morphological evolution and diversification necessitates that this photosynthetic pathway be explored as a selective pressure.

C_4 plants have a number of characteristics that might serve as a basis for selection. For example, morphologically, C_4 plants characteristically contain bundle sheath cells (i.e. Kranz anatomy) that are more difficult to break down than C_3 plants which do not have this morphology, and possibly require stronger jaws and teeth, or additional chewing (Heckathorn

et al., 1999). Furthermore, C₄ plants contain a higher fibre content and are generally less nutritious than C₃ plants (Heckathorn *et al.*, 1999; Barbehenn *et al.*, 2004). Individuals would need to consume larger amounts of C₄ plants, requiring more chewing, than C₃ plants to obtain the same amount of nutrition (Heckathorn *et al.*, 1999).

One prominent morphological feature that spread during the early to middle Miocene in many mammalian herbivore taxa as an adaptation to feeding on or in grasslands is hypsodonty [i.e. high-crowned teeth (Stirton, 1947; Simpson, 1951; MacFadden, 1992; Janis *et al.*, 2000; Strömberg, 2006)]. In fact, hypsodonty has been identified as a ‘key adaptation’ for horses permitting diversification into the newly evolved grassland biome, and resulting in at least 16 equid genera by the late middle Miocene (Simpson, 1953; MacFadden, 1998, 2000; Carrasco *et al.*, 2007). Although resulting in a generally taller tooth, different horse clades show different characteristics of hypsodont teeth, with some having simple occlusal enamel patterning and a comparatively tall tooth versus other taxa with a more complex occlusal enamel pattern but a comparatively shorter tooth. If the ingestion of C₄ plants required more chewing, owing to the durability of the Kranz anatomy and/or the need to consume more forage because of its lower quality, more hypsodont teeth in animals consuming these plants would maintain or increase an individual’s longevity.

Here we investigate the effect of C₄ plants on mammal ecology and evolution during the mid-Miocene. We focus our investigation on equid species (i.e. horses) from southern California (Fig. 1). Equids represent an archetype of mammalian evolution to grassland environments, showing evolution of hypsodonty and cursoriality, for example, throughout the clade shortly after grassland evolution begins (Stirton, 1947; Simpson, 1951; MacFadden, 1992; Strömberg, 2006). Furthermore, southern California is geographically ideal for this study because the earliest known C₄ plant macrofossil in North America occurs in southern California’s Dove Spring Formation (Nambudiri *et al.*, 1978; Tidwell and Nambudiri, 1989), and there is a high abundance of fossil-bearing localities which yield a high diversity and abundance of specimens, particularly horses. In addition, previous analyses have shown the presence of C₄ plants in ungulates during the mid-Miocene (Feranec and Pagnac, 2013).

This study aims to address the following questions: (1) Are C₄ plants represented in horses from different mid-Miocene localities in southern California? (2) Do sympatric horse species partition resources within localities? (3) Does the ecological interpretation identified from stable carbon and oxygen isotope values match the ecology predicted from other techniques, particularly morphological analyses? (4) If C₄ is represented in the horse diets, what role might this pathway have played in selection of ungulate morphology, such as hypsodonty?

BACKGROUND

Geological setting

In this study, we analyse taxa from three disparate ecosystems from localities within three distinct formations: the Barstow Formation (14 localities), the Cajon Valley Formation (five localities), and the Temblor Formation (one locality).

Barstow Formation

The Barstow Formation is one of the most prominent Miocene sedimentary sequences in the central Mojave region of southern California. Approximately 1000 m of fluvial,

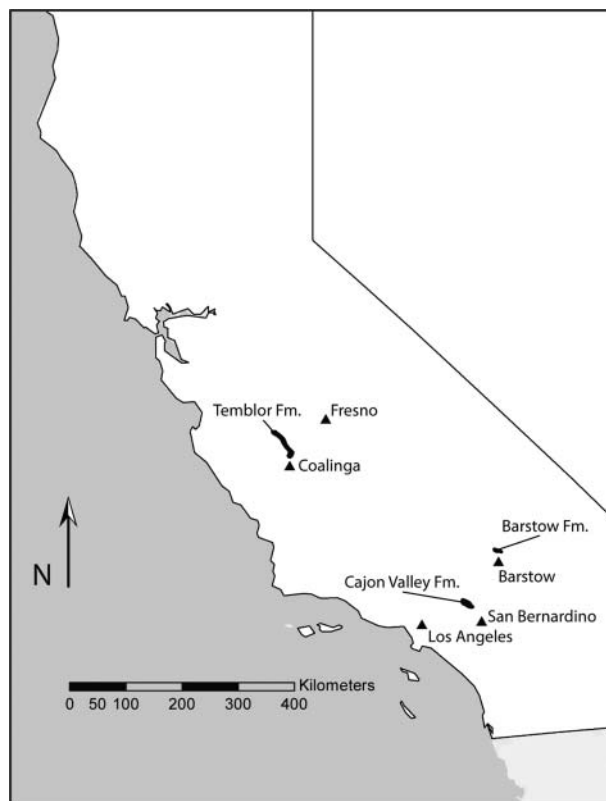


Fig. 1. Map of aerial extent of mid-Miocene formations from which the analysed equid specimens derive in this study.

lacustrine, and alluvial sediments comprise the predominant exposures within many uplifted areas of the region, including the Mud Hills, Yermo Hills, Alvord Mountains, and Calico Mountains (Woodburne *et al.*, 1990). Numerous ash-fall tuffs throughout its stratigraphic extent have provided precise geochronological calibration (Woodburne *et al.*, 1990; Woodburne, 1996), and detailed magnetostratigraphy has allowed interpretation of section completeness and rates of deposition (MacFadden *et al.*, 1990). K/Ar dates indicate ages from 19.3 to 13.4 Ma (Woodburne *et al.*, 1990; Woodburne, 1996). Extensive study of both the stratigraphy and fauna leave the Barstow Formation ideally suited for detailed analysis of palaeontological trends in a precise temporal framework.

The Barstow Formation has produced one of the most prolific and well-described mammalian faunas in the western United States (Pagnac, 2005, 2009; Woodburne, 2006). Recovered throughout the vertical extent, fossils are most abundant in the middle and upper portions. Abundant remains of camelids, equids, antilocaprids, canids, amphicyonids, and proboscideans have been recovered for over 100 years and comprise a significant portion of major vertebrate collections throughout the United States. Although this unit contains the exemplar fauna for the Barstovian NALMA, the biochronological character indicates a late Hemingfordian (He2) through late Barstovian (Ba2) age for the mammalian assemblage,

but the latest portion of the Barstovian is not retained (Pagnac, 2005, 2009; Woodburne, 2006). Specimens for this study were obtained from various localities in the middle to upper portions of the Barstow Formation.

Cajon Valley Formation

The Cajon Valley Formation, in specific reference to fluvatile sediments exposed in Cajon Pass, California, has had a confusing history of lithostratigraphic naming and interpretation. These sediments were originally considered part of the Punchbowl Formation, with more prominent outcrops exposed in the Devil's Punchbowl area some 40 km to the northwest near Valyermo (Noble, 1954a, 1954b; Dibblee, 1967; Woodburne and Golz, 1972). Thus, older literature treats both formations as a continuous stratigraphic unit offset by San Andreas slip by approximately 40–65 km (Dibblee, 1967).

Subsequent biochronological observations showed that the fauna of the Punchbowl Formation proper was early Clarendonian (Cl1–2) in age, while that of the yet unnamed Cajon Valley Formation was actually late Hemingfordian (He2) to early Barstovian (Ba1) (Tedford and Downs, 1965; Woodburne and Golz, 1972), significantly older than the fauna from the Punchbowl Formation proper. This led to recognition of the distinct lithostratigraphic and depositional nature of the two units. The Cajon Valley Formation was later proposed by Morton and Miller (2003) to describe a 730 m sequence of fault-isolated non-marine clastic rocks limited to the Cajon Pass region.

While some overlap in age is observed between the Cajon Valley and Barstow formations, lithologic and depositional differences abound. The Barstow Formation is characterized by relatively uninterrupted deposition of fluvial, lacustrine, and alluvial epiclastics over a period of 4 million years (Woodburne, 1991). In contrast, the Cajon Valley Formation accumulated episodically under high-energy pulses evidenced by coarse clastic material and emplacement of noticeable paleosols developed over approximately 4 million years (Woodburne and Golz, 1972).

The Cajon Valley Formation contains a mammalian fauna typical of the late Hemingfordian to early Barstovian. Mesodont and hypsodont equids abound, as do camelids and a modest mammalian microfauna (Woodburne and Golz, 1972; Wagner and Reynolds, 1983; Reynolds, 1991; Reynolds *et al.*, 2008). Some notable differences between the faunas of the Barstow and Cajon Valley formations have been documented in recent years, suggesting faunal variability in response to presumed environmental differences implied by lithologic character. An abundance of the dwarfed brachydont equid *Archaeohippus* in the Cajon Valley Formation and its near absence in the Barstow Formation (Pagnac and Reynolds, 2010), as well as the presence of chalicotheres in the Cajon Valley Formation contrasted by their complete absence in the Barstow Formation (Coombs and Reynolds, 2015) imply a more closed, vegetated environment in Cajon Pass conducive to smaller, browsing taxa. Specimens included in this study came from sites throughout the Cajon Valley Formation, but were generally associated with Ba1 faunas.

Temblor Formation

The Temblor Formation is exposed in outcrop in the southeastern foothills of the Diablo Range, north of the town of Coalinga, in the San Joaquin Basin, central California. This formation represents deposition during sea level rise, primarily eustatic in nature but compounded by regional subsidence of the local basin (Bartow, 1991; Bridges and Castle, 2003). A distinct transgressive event is recorded in five facies tracts – incised valley, estuarine,

tide- to wave-dominated shoreline, diatomite, and subtidal – leaving a 120 m thick remnant sequence of predominantly marine clastics (Bridges and Castle, 2003). The lowermost units record a series of fluvial events characterized by multiple fining sequences (Bridges and Castle, 2003).

The Temblor Formation is best known for abundant marine fossils, including desmostyliids, but the upper portion contains a significant terrestrial mammal assemblage. Faunas of note include the North Coalinga Local Fauna and the Sharktooth Hill Local Fauna, both early Barstovian (Ba1) in age (Tedford *et al.*, 2004). The mammalian fauna is typical of this time interval, exemplified by borophagine canids, ursids, mustelids, equids, rhinocerotids, camelids, palaeomerycids, and a microfauna of modest diversity (Tedford *et al.*, 2004; Prothero *et al.*, 2008). The intertongued marine beds allow correlation with benthic foraminiferal biostratigraphic zones, placing the mammalian fauna approximately at the Relizian–Luisian boundary, or 15.7 Ma (Barron and Issacs, 2001; Tedford *et al.*, 2004). All specimens from this study were derived from a single sample in the UCMP collections from V2124. This site is denoted as *Merychippus* zone 1 *sensu* Bode (1935), but more detailed stratigraphic information is unavailable.

EQUID TAXA

The medial Miocene represents an apex in equid diversity in North America with a minimum of 16 genera and 30 species currently recognized (MacFadden, 1998; Carrasco *et al.*, 2007). This noteworthy diversification of hypsodont taxa has been attributed to a number of causal factors, but there is no consensus on a satisfactory explanation.

Within hypsodont Equidae, cheek-tooth morphology takes two forms characterized by variable crown height and enamel complexity. The first, exemplified by the Hipparionini (e.g. *Neohipparion*, *Pseudhipparion*) exhibits moderately tall crowns and complex infolding of enamel plications on the perimeter of internal fossettes on the occlusal surfaces (MacFadden, 1998; Famoso and Davis, 2014). Taxa with this form presumably mitigate excessive wear by maximizing the surface area of the most durable tooth material, the enamel. The second form is found within the Equini, and entails much simpler enamel associated with a taller crown (MacFadden, 1998; Pagnac, 2006). Members of the Equini apparently moderate lifelong wear by producing more tooth material overall. All taxa included in this study – *Scaphohippus intermontanus*, *Scaphohippus sumani*, *Acritohippus styloodontus*, and *Merychippus californicus*) are members of the Equini exhibiting the latter dental morphology. Although complex enamel and relatively high crowns are characteristic of Hipparionini and Equini respectively, variation in both occurs within each tribe.

Scaphohippus sumani specimens from this study were derived from the Ba2 portion of the Barstow Formation. The cheek teeth of *S. sumani* are moderately hypsodont with an unworn crown height of 38–40 mm. Despite referral to the Equini, *S. sumani* exhibits moderately complex enamel with a maximum plication number of 6, suggesting a closer affinity to basal Hipparionini than to derived Equini (Pagnac, 2006).

Scaphohippus intermontanus specimens were obtained from the Ba2 portion of the Barstow Formation and the Ba1 portion of the Cajon Valley Formation. It has close dental similarity with *S. sumani*, but is differentiated by a slightly taller unworn crown of 40–50 mm. Additionally, *S. intermontanus* exhibits simpler enamel than that of *S. sumani* with a maximum plication number of 4, more closely adhering to the standard Equini condition (Pagnac, 2006).

Acritohippus styloidontus teeth were sampled from the Ba1 interval of the Barstow Formation. *Acritohippus styloidontus* exhibits notably different tooth morphology than that of *Scaphohippus*. The unworn crown height averages 35 mm, the lowest of the hypsodont equids from the Barstow Formation. Its enamel complexity is the simplest of all taxa in this study with a maximum plication number of only 3. The occlusal surface area of the cheek teeth of *A. styloidontus* is generally 25% larger than that of either species of *Scaphohippus* (Pagnac, 2006).

The only hypsodont equid present in abundance from the Temblor Formation is *Merychippus californicus*. The cheek teeth of *M. californicus* reach a moderate crown height of 38 mm, similar to that of *S. sumani*. However, *M. californicus* exhibits the most complex enamel of taxa from this study, with a maximum plication number of 8 (Merriam, 1915; Downs, 1956).

A single taxon of non-hypsodont Equidae from the subfamily Anchitheriinae was included in the study, *Archaeohippus mourningi*. It is a species of brachydont horse, a remnant taxon from an early radiation of low-crowned horses with lophate dentition common in the Oligocene (MacFadden, 1998). With the diversification of hypsodont forms in the Miocene, brachydont equids dwindle in both diversity and abundance. While typical Barstovian brachydont equids such as *Hypohippus* and *Megahippus* are sizeable, *Archaeohippus* exhibits extreme dwarfism. *Archaeohippus* is hypothesized to have occupied regions with more enclosed vegetation and underbrush cover (Pagnac and Reynolds, 2010). Specimens from the Barstow and Cajon Valley formations were included in this study.

Carbon isotope values in horses

In this study, we examine the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of tooth enamel of horses. The isotopic results in this study are expressed in standard δ -notation: $X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$, where $X = \delta^{13}\text{C}$ or $\delta^{18}\text{O}$ in parts per mil (‰) and $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{18}\text{O}/{}^{16}\text{O}$. The $\delta^{13}\text{C}$ values reported here are relative to the V-PDB standard, while the $\delta^{18}\text{O}$ values are reported relative to V-SMOW.

In mammals, the carbon isotope values of their food are reflected in their tissues (DeNiro and Epstein, 1978; Vogel, 1978; Tieszen *et al.*, 1979). Carbon isotope values are useful for diet studies because the different photosynthetic pathways used by plants [i.e. C₃, C₄, and crassulacean acid metabolism (CAM)] impart different $\delta^{13}\text{C}$ values to plant tissues, and the animals consuming those plants will reflect the isotope values ingested. Plants that utilize the C₄ photosynthetic pathway are relatively enriched in the heavy carbon isotope (^{13}C). Modern C₄ plants have a mean $\delta^{13}\text{C}$ value of $-12.5 \pm 1.1\text{‰}$ (O'Leary, 1988; Farquhar *et al.*, 1989; Ehleringer *et al.*, 1991; Ehleringer and Monson, 1993; Cerling *et al.*, 1997). C₃ plants, which include most trees, shrubs, and cool-growing-season grasses, are relatively enriched in the light carbon isotope (^{12}C). Modern C₃ plants have a mean $\delta^{13}\text{C}$ value of about -28.0‰ (Farquhar *et al.*, 1989; Ehleringer *et al.*, 1991; Ehleringer and Monson, 1993; Kohn, 2010). The CAM pathway is characteristic of succulent plants, such as cactus, and generally yield values that range between C₃ and C₄ plants (O'Leary, 1988; Ehleringer *et al.*, 1991; Ehleringer and Monson, 1993). We assume that the horse species in this study did not make CAM plants a significant part of their diet and so the isotopic effects of ingesting CAM plants are not considered in this study.

Mammalian herbivore tooth enamel is enriched by a consistent amount from their food, about $+13.8 \pm 0.2\text{‰}$ for horses (Cerling and Harris, 1999). Additionally, over time, both the CO₂ concentration and the $\delta^{13}\text{C}_{\text{CO}_2}$ value of the atmosphere has fluctuated, which affects the $\delta^{13}\text{C}$ value of plants and ultimately mammal tooth enamel (Friedli *et al.*, 1986; Marino and McElroy,

1991; Marino *et al.*, 1992; Tipple *et al.*, 2010). For example, as a result of the industrial revolution and fossil fuel burning, mean atmospheric $\delta^{13}\text{C}_{\text{CO}_2}$ values have become more negative by about 1.5‰ over the last few hundred years (Friedli *et al.*, 1986; Marino and McElroy, 1991; Marino *et al.*, 1992). Compared with the modern, Tipple *et al.* (2010) provide $\delta^{13}\text{C}_{\text{CO}_2}$ values over the Cenozoic. For the middle Miocene period represented in the southern California deposits analysed here, the $\delta^{13}\text{C}_{\text{CO}_2}$ values are generally over 2 parts per mil higher than modern (< 6‰). In fact, the time period represented by the Temblor Formation (i.e. 17 to 15 Ma) is shown to have the highest values for the Miocene, averaging -5.3‰ . $\delta^{13}\text{C}_{\text{CO}_2}$ was about -5.7‰ for the Barstow and Cajon Valley formations. Taking the discrimination of carbon isotopes from food to tooth enamel as well as the changes in the isotopic value of atmospheric CO_2 into account, suggests that horse species with an average C_3 diet in the Temblor should have tooth enamel $\delta^{13}\text{C}$ values around -11.5‰ , while an average C_3 diet for species within Barstow or Cajon Valley would be around -11.9‰ . If the species foraged in open, arid environments or closed canopy habitats, the $\delta^{13}\text{C}$ values could be higher or lower, respectively. Because the C_3 and C_4 baselines will affect how we interpret the presence and abundance of C_4 in diets, and by inference its presence on the landscape, we utilize a conservative approach focusing on arid ecosystems. Exact baseline values are presented and explained in the ‘Materials and methods’.

Oxygen isotope values in horses

Stable oxygen isotope values in mammal tooth enamel depend on the isotopic composition of ingested water, fractionation of isotopes between enamel and body water within the individual, and metabolism (Land *et al.*, 1980; Longinelli, 1984; Luz *et al.*, 1984; Luz and Kolodny, 1985; Koch *et al.*, 1989; Bryant and Froelich, 1995; Kohn, 1996; Kohn *et al.*, 1996). Herbivores ingest water either through drinking or from the plants they consume, and the isotope composition of water is affected by climatic factors, such as temperature, such that $\delta^{18}\text{O}$ values generally are more positive where and when it is warmer and more negative where and when it is colder (Dansgaard, 1964; Rozanski *et al.*, 1992; Fricke and O’Neil, 1996; Kohn and Welker, 2005).

It has been suggested that larger mammals that are obligate drinkers and have low metabolisms are those most likely to accurately reflect ingested $\delta^{18}\text{O}$ values (Longinelli, 1984; Luz *et al.*, 1984; Bryant and Froelich, 1995). All species in our study are large, *Archaeohippus mourningi* being the smallest (>45 kg), and based on modern analogues are suspected to be obligate drinkers. Thus, the $\delta^{18}\text{O}$ values we observed are likely primarily the result of climatic and/or environmental factors.

MATERIALS AND METHODS

We examined the tooth enamel of a total of 85 specimens from five species of equids from the middle Miocene of southern California: *Acritohippus stylodontus*, *Archaeohippus mourningi*, *Merychippus californicus*, *Scaphohippus intermontanus*, and *Scaphohippus sumani*. Because different teeth develop at different ages and our aim was to identify resource use of older, adult animals, we sampled primarily premolars and third molars, the teeth that are among the last ones to develop and mineralize (see www.evolutionary-ecology.com/data/3016Appendix.pdf) (Hillson, 2005). Sampling involved drilling about 5 mg of enamel powder from the tooth along a non-occlusal surface parallel to the growth axis and across its entire length using carbide drill bits and a variable speed dental drill. In

preparation for stable isotope analysis, the enamel powder samples were first treated with 30% hydrogen peroxide to remove organics for about 24 hours. Then, the hydrogen peroxide was decanted and the powder was washed with ultra-filtered water before being treated with 0.1 N acetic acid for about 24 hours to remove any diagenetic carbonates. The acetic acid was then decanted and the enamel powder was again washed with ultra-filtered water and air-dried. Our preparation procedure generally follows the methods of MacFadden and Cerling (1996) and Koch *et al.* (1997).

After treatment, the samples were analysed in the Geology Department at the University of California, Davis, the Department of Geosciences at Boise State University, Boise, and the Earth Sciences Department at the University of California, Santa Cruz. At UC Davis, an ISOCARB automated carbonate preparation system attached to a Micromass Optima gas source mass spectrometer was used, and samples were corrected to NBS-19 and UCD-SM92, an in-house marble standard here, with a precision of $\pm 0.1\%$. At Boise State, samples were analysed for stable carbon and oxygen isotope values using an automated extraction system (Gasbench II) attached to a ThermoFisher Delta V Plus continuous flow isotope ratio mass spectrometer. Raw data were standardized against NIST 18 and NIST 19 carbonates, whose analytical reproducibilities for both carbon and oxygen are 0.2–0.3%. At UC Santa Cruz, a ThermoFisher Kiel IV carbonate device interfaced to a ThermoScientific MAT-253 dual inlet isotope ratio mass spectrometer was used. During a run sequence, samples were corrected using the in-house Carrera Marble (CM) standard. NBS-18 and CM were used to correct for instrument-specific ionization effects, NBS-19 was run as a sample during runs for quality control and to monitor longer-term performance, and the precision was $\pm 0.1\%$. All isotope values are expressed relative to the Vienna PeeDee Belemnite (V-PDB) international standard.

In this study we performed two analyses. For the first, we compared the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ data within and among the analysed formations, observing whether the formations or species showed higher or lower isotope values. In this analysis, mean differences among the formations and/or species were compared using analysis of variance (ANOVA) and *post-hoc* Tukey-Kramer HSD tests. We used locality-averaged values to make comparisons among the sites. In one comparison, for $\delta^{18}\text{O}$ values between species within the Cajon Valley Formation, we used Welch's test because the data for each species revealed unequal variances. Tests for correlation between $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ were conducted for all data in the study as well as for all data within each locality. Statistical analyses for the above were conducted using JMP v.12 (SAS Institute Inc., 2015).

The second analysis was aimed at determining whether C₄ plants were present within each formation. We utilized a conservative approach, defining the 100% C₃ baseline and when C₄ plants must have been present. Foliar $\delta^{13}\text{C}$ values from modern, arid ecosystems with annual precipitation between 10 and 400 mm per year were averaged, yielding a mean of $-25.7 \pm 1.0\%$ (Kohn, 2010). These data exclude the unique values from the Atacama Desert, which we do not feel is an adequate/appropriate comparison to the ancient ecosystems analysed here due to its geological setting along the Andes and its extremely low rainfall. Using a foliar $\delta^{13}\text{C}$ value two standard deviations higher than the modern arid ecosystem mean, then adjusting for the difference in atmospheric CO₂ isotope values from the modern ($\delta^{13}\text{C}_{\text{CO}_2} = -8.0\%$) to the middle Miocene [Temblor Formation $\delta^{13}\text{C}_{\text{CO}_2} = -5.3\%$; Barstow and Cajon Valley formations $\delta^{13}\text{C}_{\text{CO}_2} = -5.7\%$ (Tippie *et al.*, 2010)], we calculate the 100% C₃ plant baseline at -21.0% for the Temblor Formation and -21.4% for the Barstow and Cajon Valley formations. Using the horse specific discrimination factor of $+13.8\%$

(Cerling and Harris, 1999), we consider tooth enamel $\delta^{13}\text{C}$ values more positive than -7.2‰ for Temblor specimens and more positive than -7.6‰ for Barstow and Cajon Valley specimens as necessarily including some proportion of C_4 plants in the diet.

If C_4 plants were determined to be present, the percentage of C_4 for each horse species was calculated. To calculate the percentage of C_4 for each species, we utilized SIAR (Stable Isotope Analysis in R) v.4.2.2 (R Core Development Team, 2015). SIAR is a Bayesian isotope mixing model capable of modelling the proportions of resource groups (e.g. C_3 plants, C_4 plants) in a consumer's diet (Parnell *et al.*, 2010). SIAR uses a Markov Chain Monte Carlo simulation to model the proportions of dietary sources in the diet of the consumer species on the basis of the stable isotope values of the different sources and the consumers. SIAR is also capable of incorporating the uncertainty of the trophic enrichment factor between the sources and the consumer into the model. Within the model for the C_3 source, we use the modern $\delta^{13}\text{C}$ values from arid ecosystems with precipitation between 10 and 400 mm per year, adjusted to account for atmospheric CO_2 $\delta^{13}\text{C}$ differences between the mid-Miocene and today [Temblor C_3 source = $-23.0 \pm 1.0\text{‰}$; Barstow and Cajon Valley C_3 source = $-23.4 \pm 1.0\text{‰}$ (Kohn, 2010; Tipple *et al.*, 2010)]. For the C_4 source, we use the modern $\delta^{13}\text{C}$ value for C_4 plants adjusted to account for the Miocene-to-modern atmospheric isotope $\delta^{13}\text{C}$ differences [$-9.8 \pm 1.1\text{‰}$ at Temblor; $-10.2 \pm 1.1\text{‰}$ at Barstow and Cajon Valley (Cerling *et al.*, 1997)]. We use a horse-specific trophic enrichment factor of $+13.8\text{‰} \pm 0.2$ (Cerling and Harris, 1999). The mean and median proportions as well as the 5% and 95% quantile ranges are used to compare the contribution of C_3 and C_4 plants for species within the formations containing C_4 plants.

RESULTS

Inter-locality comparison

For stable carbon isotopes, ANOVA showed significant differences among the three localities ($F_{2,82} = 58.53$, $P < 0.0001$), and *post-hoc* Tukey-Kramer HSD indicated that each locality was significantly different from the others at $P < 0.05$, with the Barstow Formation having the highest $\delta^{13}\text{C}$ values, the Cajon Valley Formation the lowest values, and the Temblor Formation intermediate values (Table 1, Fig. 2). Scrutinizing the individual $\delta^{13}\text{C}$ values obtained from each locality more closely, in order to determine the presence of C_4 plants, reveals that the highest $\delta^{13}\text{C}$ values are -6.2‰ within the Barstow Formation, -9.4‰ within the Cajon Valley Formation, and -7.2‰ within the Temblor Formation. For stable oxygen isotope values, ANOVA also showed significant differences among the localities ($F_{2,82} = 13.2$, $P < 0.0001$), and *post-hoc* Tukey-Kramer HSD indicated that the horses from the Temblor Formation had significantly higher $\delta^{18}\text{O}$ values than the specimens at the other two formations, which were not statistically significantly different from each other (at $P < 0.05$; Fig. 3). *Scaphohippus intermontanus* was the only species to occur within at least two formations, Barstow and Cajon Valley. Using *t*-tests we showed that the Barstow population had statistically significantly higher $\delta^{13}\text{C}$ values than the population from the Cajon Valley Formation ($t_{20} = 8.4$, $P < 0.0001$). A Levene's test on the $\delta^{18}\text{O}$ values showed unequal variances between the Barstow and Cajon Valley *S. intermontanus* populations ($F = 5.68$, $P > 0.03$). As a result, we used Welch's test (which does not assume equal variances), which showed no statistically significant difference in $\delta^{18}\text{O}$ between the samples ($F = 2.56$, $P = 0.13$). The correlation between $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values was significant only within the Temblor Formation ($r_{12} = 0.63$, $P = 0.017$).

Table 1. Isotope values for the specimens included in this study

Locality and species	<i>N</i>	Mean $\delta^{13}\text{C}$	SD $\delta^{13}\text{C}$	Mean $\delta^{18}\text{O}$	SD $\delta^{18}\text{O}$
Barstow Formation	53	-8.1	0.9	25.5	1.7
<i>Acritohippus stylodontus</i>	16	-7.6	0.2	25.3	0.4
<i>Scaphohippus intermontanus</i>	12	-7.7	0.2	24.6	0.5
<i>Scaphohippus sumani</i>	25	-8.4	0.2	26.2	0.3
Cajon Valley Formation	18	-10.9	1.0	24.8	1.5
<i>Archaeohippus mourningi</i>	8	-11.3	1.2	24.0	2.1
<i>Scaphohippus intermontanus</i>	10	-10.6	0.8	25.4	0.5
Temblor Formation	14	-9.0	1.3	27.6	1.3
<i>Merychippus californicus</i>	14	-9.0	1.3	27.6	1.3

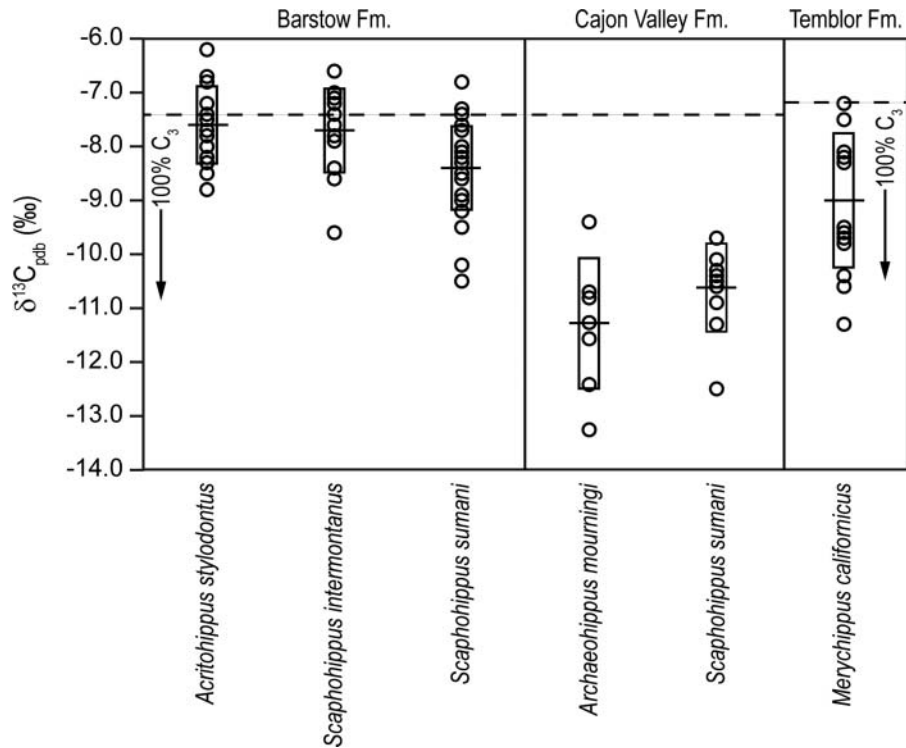


Fig. 2. Stable carbon isotope values from equid specimens analysed in this study. Each circle represents an individual and they are large enough to incorporate mass spectrometer precision. Solid line and box represent the mean and one standard deviation for analysed taxa. Dashed line represents the highest $\delta^{13}\text{C}$ value a 100% C₃ diet could result in equid tooth enamel based on the isotopic value of atmospheric CO₂ as well as the discrimination of isotopes from the diet to equid tooth enamel.

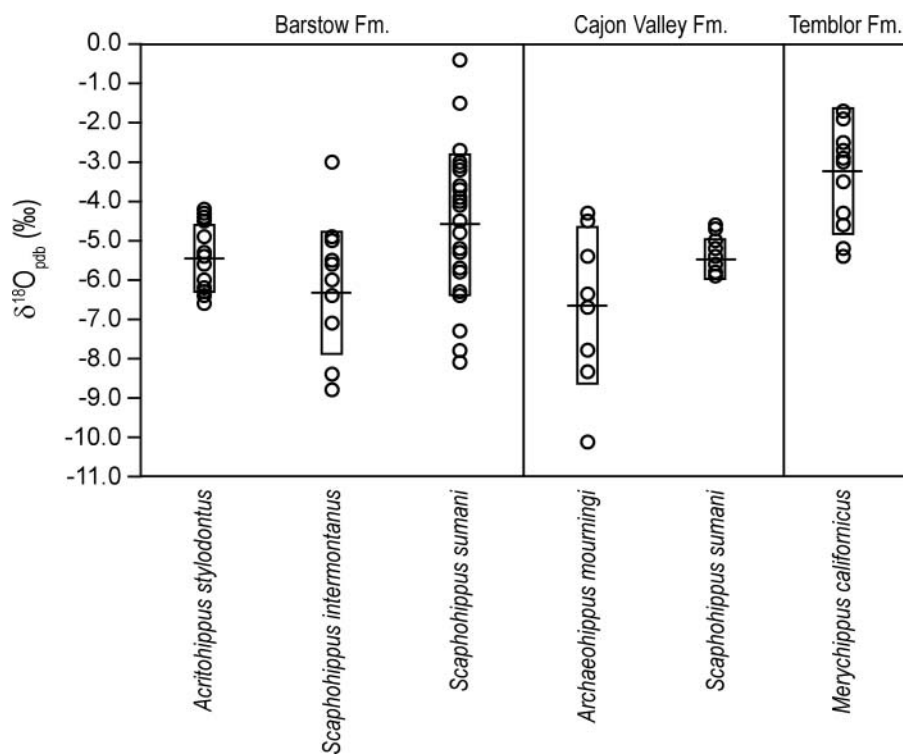


Fig. 3. Stable oxygen isotope values from equid specimens analysed in this study. Each circle represents an individual and they are large enough to incorporate mass spectrometer precision. Solid line and box represent the mean and one standard deviation for analysed taxa.

Intra-locality comparison

Within the Barstow Formation, ANOVA revealed significant differences ($F_{2,50} = 6.3$, $P < 0.004$) among the three horse species. *Post-hoc* Tukey-Kramer HSD tests indicated that *Scaphohippus sumani* had significantly lower $\delta^{13}\text{C}$ values than the other two species (at $P < 0.05$). *Acritohippus stylodontus* and *Scaphohippus intermontanus* were not statistically significantly different (Table 1, Fig. 2). ANOVA also showed significant differences in $\delta^{18}\text{O}$ values for the Barstow Formation specimens ($F_{2,50} = 4.2$, $P < 0.021$) with the *post-hoc* Tukey-Kramer HSD test showing that *Scaphohippus sumani* had the highest values and was significantly different from *Acritohippus stylodontus*, which displayed the lowest values (at $P < 0.05$; Fig. 3). *Scaphohippus intermontanus* was not statistically different from either *A. stylodontus* or *S. sumani*.

Within the Cajon Valley Formation, there were no statistically significant differences in $\delta^{13}\text{C}$ values between *Archaeohippus mourningi* and *Scaphohippus intermontanus* ($F_{1,16} = 1.8$, $P < 0.197$). For $\delta^{18}\text{O}$ values, Levene's test showed unequal variances between *A. mourningi* and *S. intermontanus* ($F = 10.1$, $P = 0.006$), while Welch's test showed no statistically significant difference between the species ($F = 3.27$, $P = 0.11$).

Modelling abundance of C₄ plants in mid-Miocene horse diets

Based on the $\delta^{13}\text{C}$ values obtained from each locality, it appears clear that C₄ plants were present in the diet of the Barstow horses (16 out of 53 individuals had values higher than -7.6‰), while no C₄ is apparent at Cajon Valley (all individuals $<7.6\text{‰}$). The values from the Temblor Formation present more of a problem. The cut-off value necessitating C₄ in the diet is -7.2‰ , and one of the 14 sampled individuals displayed that exact value – the remaining individuals all had lower $\delta^{13}\text{C}$ values. A strict interpretation of this value indicates only the presence of C₃ plants within the Temblor Formation. However, because of how isotopic values get incorporated into tooth enamel during development, a bulk $\delta^{13}\text{C}$ value of -7.2‰ would require that either this individual horse ate a diet exactly of -7.2‰ during the entire development of the sampled tooth, or that higher and lower $\delta^{13}\text{C}$ values were averaged over the months when this tooth formed (Passey and Cerling, 2002; Kohn, 2004). Although not examined here through serial sampling, serial sampling data on other horse species suggest the latter is a more accurate biological explanation (Cerling and Sharp, 1996; Fricke and O'Neil, 1996; Passey and Cerling, 2002; Kohn, 2004). But this then suggests inclusion of C₄ in this individual's diet. Therefore, even though the strict interpretation of data suggests only C₃ plants in the Temblor Formation, we decided it prudent to produce a model calculating percentage C₄ in horse diets at this locality as well as at Barstow where C₄ plants are known (this study; Feranec and Pagnac, 2013).

Based on the SIAR modelled results, at Barstow both *A. stylodentus* and *S. intermontanus* averaged about 15% C₄ in their diets, with a maximum of about 18% (Table 2; Fig. 4). These two species had a slightly higher percentage calculated for their diets compared with that of *S. sumani*, which had an average of about 9% C₄ in the diet and a maximum of about 11%. As expected, the calculated percentage C₄ in the diet of *M. californicus* from the Temblor Formation was low. If C₄ was present on the landscape, this species averaged only 3% and had a maximum of about 6% C₄ in its diet (Fig. 5).

DISCUSSION

Ecologically at Barstow, there appears to be resource use partitioning among the three sampled horse species. *Acritohippus stylodentus* and *Scaphohippus intermontanus* have statistically similar mean $\delta^{13}\text{C}$ values and the same modelled percentage of C₄ forage (up to

Table 2. Percentage C₄ in the diet of horse species within the Barstow Formation and Temblor Formation of southern California during the mid-Miocene based on the SIAR model (values shown are percentage C₄)

Locality and species	Mean C ₄	Median C ₄	5% quantile	95% quantile
Barstow Formation				
<i>Acritohippus stylodentus</i>	15.0	15.0	12.0	17.8
<i>Scaphohippus intermontanus</i>	14.4	14.4	10.7	17.9
<i>Scaphohippus sumani</i>	8.7	8.7	6.2	11.2
Temblor Formation				
<i>Merychippus californicus</i>	2.7	2.4	0.3	6.0

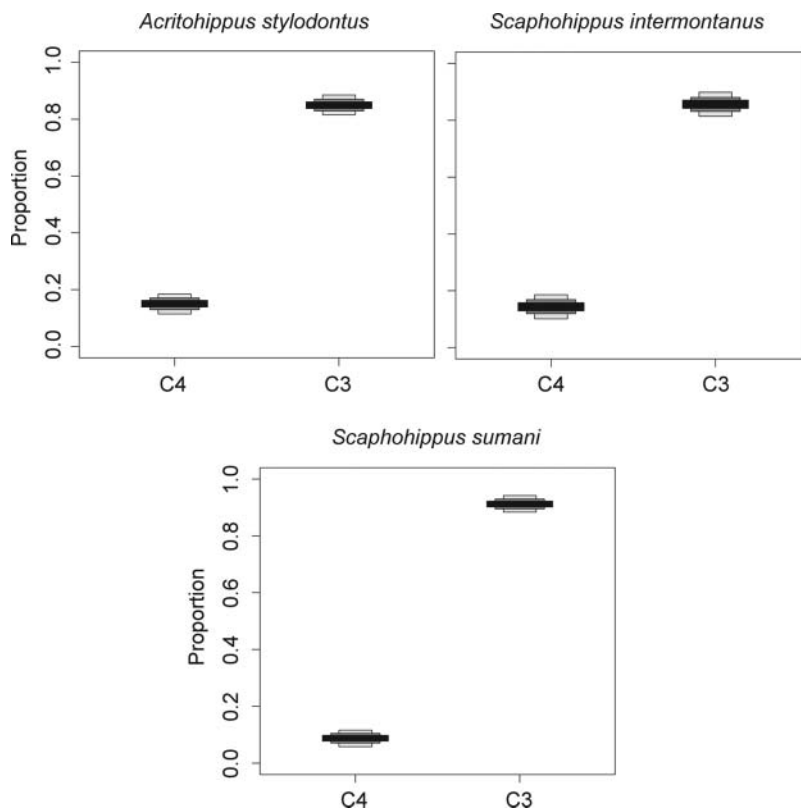


Fig. 4. SIAR Bayesian model results for the proportion of C₃ and C₄ plants included in the diets of equid species within the Barstow Formation. The model shows a low proportion of C₄ (<18%) in their diets from this locality. Quantiles (boxes) represent 25% (black), 75% (grey), and 95% (white) intervals.

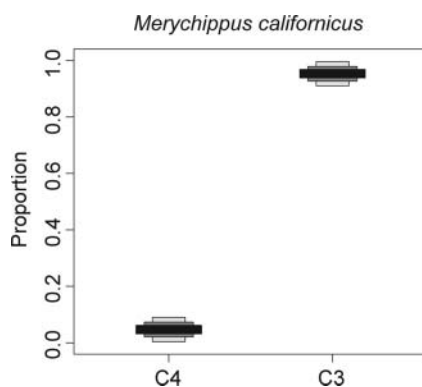


Fig. 5. SIAR Bayesian model results for the proportion of C₃ and C₄ plants included in the diet of *Merychippus californicus* within the Temblor Formation. The model shows a very low C₄ percentage (<6%) was possible at this locality. Quantiles (boxes) represent 25% (black), 75% (grey), and 95% (white) intervals.

18%). The higher $\delta^{13}\text{C}$ and modelled C₄ forage percentages suggest that these species lived and ate in more open grassland environments. This contrasts with *Scaphohippus sumani*, which shows lower $\delta^{13}\text{C}$ and thus lower modelled C₄ forage (up to 11%) than the other horse species. The higher $\delta^{18}\text{O}$ values observed in *S. sumani* compared with the other two species suggest that it either foraged more browse in open habitats or in a microhabitat that contained more C₃ grasses than the environments that *A. stylodontus* and *S. intermontanus* inhabited, rather than in more closed environments. We would expect individuals living in more closed environments to have lower isotopic values for both carbon and oxygen (Farquhar *et al.*, 1989; O'Leary *et al.*, 1992; Ehleringer and Monson, 1993; Koch, 1998; Bocherens, 2003; Drucker *et al.*, 2003; Cerling *et al.*, 2004; Drucker and Bocherens, 2004).

The diet implied from the isotope results at Barstow follows what would be suggested based on the characteristics of tooth morphology within each species. Although the occlusal enamel pattern is less complex, as mentioned above, *Acritohippus stylodontus* has taller teeth than either *Scaphohippus* species, and *S. intermontanus* has a slightly taller tooth, on average, than *S. sumani*. The differences in tooth morphology between *A. stylodontus* and *S. intermontanus* highlight that there is more than one way to be hypsodont in horses, and that these morphologies are capable of functioning for foraging on grasses and/or in grassland (i.e. grittier) environments. Compared with the tall but simple teeth in *A. stylodontus*, the complexity of the occlusal enamel pattern in *S. intermontanus* may help overcome its shorter teeth limitation. A shorter tooth would reduce an individual's longevity if foraging identically to a taller toothed individual. If the occlusal enamel complexity aids in chewing efficiency, then the wear rate should not reduce the tooth as quickly as a less complex occlusal enamel pattern, such as that observed for *A. stylodontus*. Less chewing would lead to less wear and longer tooth/individual longevity. In a recent series of papers, Famoso and colleagues (Famoso *et al.*, 2013, 2016; Famoso and Davis, 2014) explored the relationship between occlusal enamel complexity and factors such as diet, phylogeny, and climate change. These studies showed that increasing amounts of occlusal enamel are an adaptation to different diets and that the amount of occlusal enamel increased in ungulate species from the Miocene to the Pleistocene in North America, possibly in response to global cooling and aridity (Famoso *et al.*, 2013; Famoso and Davis, 2014). However, evolution of occlusal enamel complexity and hypsodonty were not correlated, implying the independent evolution of these features (Famoso *et al.*, 2016). The comparatively lower teeth in *S. sumani* follow its lower $\delta^{13}\text{C}$ values and suggested higher ingestion of C₃ plants.

At Cajon Valley, the lack of significant differences in either $\delta^{13}\text{C}$ or $\delta^{18}\text{O}$ values makes it difficult to suggest resource use differences between *Scaphohippus intermontanus* and *Archaeohippus mourningi*. The mean values observed between the species appear to follow the suspected ecology for each taxon, with the higher-crowned *S. intermontanus* showing a higher mean $\delta^{13}\text{C}$ value indicative of more open environments than the lower values displayed by the lower-crowned *A. mourningi*, which imply foraging in more closed habitats. Because *S. intermontanus* occurs at both Barstow and Cajon Valley, the isotopic data from these two populations reveal the habitat differences that were present between these two contemporaneous formations. Based on depositional environments, Pagnac and Reynolds (2010) suggested that sediment deposition at Cajon Valley was periodic and that soils were able to develop stable floras for thousands of years, while this was not the case at Barstow. Additionally, the abundance of *A. mourningi* and the presence of the Chalicothere, *Moropus* sp., is also indicative of more closed habitat within the Cajon Valley Formation (Coombs and Reynolds, 2015). The isotope results support this hypothesis, with the lower $\delta^{13}\text{C}$ values for

specimens of *S. intermontanus* from the Cajon Valley Formation indicative of an environment that is more closed, and the higher $\delta^{13}\text{C}$ values for this species at Barstow indicative of an environment that has more open habitats.

Specimens from the Temblor Formation are the only ones that show a significant correlation between the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values. Closer inspection of the $\delta^{13}\text{C}$ values (Fig. 2) shows two groupings of individuals, one with higher values similar to the values observed for horses at Barstow, and one with lower values similar to the values observed for horses at Cajon Valley. We suggest that these data are the result of preservation of specimens deriving from different environments. The Temblor horses with lower values could represent feeding either on browse in more closed habitats, or C_3 grasses in more open habitats. With *Scaphohippus sumani* at Barstow, we observe lower $\delta^{13}\text{C}$ values, but higher $\delta^{18}\text{O}$ values which we interpret as foraging C_3 grasses in more open environments. Because of the correlation of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values in the Temblor Formation, we suggest that the lower $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values for *Merychippus californicus* indicate the consumption of C_3 browse (e.g. trees and shrubs) as opposed to C_3 grasses. This would indicate that this species was a mixed-feeder eating grasses as well as browse.

The results support previous findings from the middle Miocene of southern California, in that Feranec and Pagnac (2013) previously showed the presence of C_4 plants in a number of ungulate taxa at Barstow. The SIAR modelled results presented here for Barstow, showing up to 18% C_4 in the diet of *Acritohippus styodontus* and *Scaphohippus intermontanus*, confirm that C_4 was present and abundant enough for taxa to incorporate the isotopic signal of this photosynthetic pathway into their tissues, at least at that locality. We consider the modelled percentage C_4 inclusion to be a conservative estimate of C_4 grasses likely present on the landscape at Barstow for two reasons. First, we used conservative estimates for the mid-Miocene C_3 and C_4 baseline values in the SIAR model. Second, horses are known to forage some percentage of browse in their diets (Koch, 1998; MacFadden *et al.*, 1999).

The lack of C_4 at Cajon Valley, and lack of – or extremely limited – C_4 abundance within the Temblor also supports our previous suggestion (Feranec and Pagnac, 2013) that local factors (e.g. local temperature, aridity) controlled the abundance of C_4 within particular geographic areas, in this case southern California (Pagani *et al.*, 1999; Keeley and Rundel, 2005; Osborne and Beerling, 2006; Tipple and Pagani, 2007; Osborne, 2008). This is not to say that local factors controlled the globally observed pattern, particularly for the RICE in the late Miocene (Cerling *et al.*, 1997; Ehleringer *et al.*, 1997; Latorre *et al.*, 1997; Kohn and Cerling, 2002). At least for southern California, the data presented here show that C_4 plants were present, but the ecosystems were still partially (e.g. Barstow) or wholly (e.g. Cajon Valley) C_3 -dominated. The abruptness and scale of the RICE, in our opinion, would be better explained by a more global mechanism, such as the previously suggested decrease in atmospheric CO_2 (Cerling *et al.*, 1997; Ehleringer *et al.*, 1997; Latorre *et al.*, 1997; Kohn and Cerling, 2002). We also suspect that more than one mechanism was likely responsible for the RICE (e.g. CO_2 decline and aridity), and that local factors played a role in refining the final abundance in a particular area. The pattern that we observe in southern California using stable isotope values from mammal tooth enamel is similar to patterns found elsewhere in North America in the mid- to late Miocene that utilized data provided by other techniques, such as palynology, phytolith analysis, and stable isotope analyses of pedogenic carbonates (Fox and Koch, 2003, 2004; McInerney *et al.*, 2011; Strömberg and McInerney, 2011; Cotton *et al.*, 2012, 2014; Chen *et al.*, 2015). C_4 grasses appear to have occurred in some measurable abundance on a regional basis, but the abundance is generally less than 25% until the late

Miocene (Fox and Koch, 2003, 2004; McInerney *et al.*, 2011; Strömberg and McInerney, 2011; Cotton *et al.*, 2012, 2014; Chen *et al.*, 2015).

Initial studies suggesting that the spread of grasslands in the Miocene of North America involved only C₃ plants obviated the influence of the photosynthetic pathway, particularly features of C₄ photosynthesis, on mammalian morphological evolution. In this view, mammalian adaptations, necessarily, would have evolved in response to the physiognomy of grasslands and to features displayed only by C₃ grassland species, not features displayed by C₄ species. Even if they were not dominant regionally, the presence of C₄ plants on the landscape and knowledge that ungulates were eating them in the mid-Miocene contemporaneously as mammalian morphologies (e.g. hypsodonty) appear to be adapting to this new environment, necessitates that C₄ plants be included in hypotheses of mammalian evolution at this time.

As mentioned in the Introduction, C₄ plants have a number of characteristics, such as Kranz anatomy, that might serve as the basis for selection in evolving morphological features like hypsodonty (Caswell *et al.*, 1973; Heckathorn *et al.*, 1999). The characteristics of C₄ plants may create a positive feedback loop promoting tooth wear. C₄ plants are less nutritious than C₃ plants, requiring ingestion and chewing of proportionally more foliage (Caswell *et al.*, 1973; Barbehenn *et al.*, 2004). Additionally, ingestion of silica, either through phytoliths or exogenous grit, has been shown to correlate positively with hypsodonty (Caswell *et al.*, 1973; McNaughton and Tarrants, 1983; McNaughton *et al.*, 1985; Hummel *et al.*, 2011; Hoffman *et al.*, 2015; Xia *et al.*, 2015). Silica not only has a physical effect increasing tooth wear, but also has been shown to reduce digestion in herbivores (Massey and Hartley, 2006; Massey *et al.*, 2007, 2008). The consumption of low-quality C₄ plants would require eating more and result in the consumption of more silica, which physically wears teeth and reduces nutrient uptake, requiring the need to forage and chew more, promoting additional tooth wear. While C₄ plants may have been involved in the evolution of hypsodonty *sensu lato*, the lack of statistically significant differences between species at Barstow does not currently suggest that this photosynthetic pathway had a selective effect on the different types of hypsodonty. That is, from the southern California data, we cannot distinguish whether ingestion of C₄ would influence selection for a taller but less complex tooth as in *Acritohippus styloidontus*, or a more complex but shorter tooth as in *Scaphohippus intermontanus*. A larger-scale analysis of the presence and abundance of C₄ and its correlation with the different forms of hypsodonty would be necessary to address this question accurately. What is clear from this study is that C₄ plants are present at certain localities in North America by the mid-Miocene, these plants are consumed by ungulates, and they need to become part of hypotheses related to the spread of grasslands and mammalian evolution at that time.

CONCLUSIONS

Analysis of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values from the tooth enamel of equid species from three mid-Miocene localities of southern California shows that C₄ plants were present at some localities, within the Barstow and possibly the Temblor formations, but not at others, such as the Cajon Valley Formation. Although C₄ plants were present, the maximum consumption of C₄ by any species was < 20%, indicating ecosystems were still C₃-dominated. At Barstow, isotopic values suggest resource use differences among the sympatric horse species and follow predictions based on characteristics of their teeth: more hypsodont species had higher $\delta^{13}\text{C}$ values. *Merychippus californicus*, the only sampled horse species from the

Temblor Formation, appears to have been a mixed-feeder having $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values indicative of both open habitat feeding, including the possibility of the consumption of C_4 plants, as well as values that indicate browsing in more closed habitats. Because C_4 plants are at least regionally present in North America in the middle Miocene, and these plants are consumed by ungulates, it is necessary that they become part of hypotheses related to the spread of grasslands and mammalian evolution at that time.

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