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Diet and habitat of unique individuals of *Dinohippus mexicanus* and *Neohipparion eurystyle* (Equidae) from the late Hemphillian (Hh3) of Guanajuato and Jalisco, central Mexico: stable isotope studies

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ABSTRACT

Stable carbon and oxygen isotopes were determined in molar enamel from fossil Pliocene equids from Rancho El Ocote in the San Miguel Allende basin, Guanajuato, and from Santa María, Tecolotlán basin, Jalisco. At each locality, the source was one molar from an individual *Dinohippus mexicanus* and one molar from an individual *Neohipparion eurystyle*. Results indicated that the *N. eurystyle* individuals from both localities had been C_3/C_4 mixed feeders, and had lived in open-zone vegetation (δ^{13}C: -3.1‰ to -1.3‰; δ^{18}O: -4.9‰ to -6.4‰). On the other hand, the *D. mexicanus* from Rancho El Ocote had fed upon C_4 plants and lived in open zones (δ^{13}C: -1.3‰; δ^{18}O: -4.9‰), whereas the *D. mexicanus* from Santa María was a C_3/C_4 mixed feeder with considerable consumption of C_3 plants (δ^{13}C: -7.7‰; δ^{18}O: -6.4‰). These results could be contrasted to suggestions from previous isotopic work that *D. mexicanus* in Mexico predominantly fed on C_4 plants and further samples analyses are warranted. This study contributes to the understanding of the Pliocene equid taxa from central Mexico and emphasizes the presence of different diets, ranging from exclusive C_4 to mixed C_3/C_4 plants.

Key words: *Dinohippus mexicanus*; *Neohipparion eurystyle*; stable isotopes; late Hemphillian, Mexico.

RESUMEN

En este trabajo se analizó un molar de Dinohippus mexicanus y uno más de Neohipparion eurystyle, ambos recolectados de la localidad de Rancho el Ocote, en la Cuenca de San Miguel de Allende; de la misma forma también se analizaron un molar de cada una de las especies de équidos antes mencionadas, que fueron recolectados en la localidad de Santa María, de la cuenca de Tecolotlán, Jalisco. Los molares fueron analizados por isótopos estables de carbono y oxígeno presentes en el esmalte dental, los cuales revelan que en ambas localidades, N. eurystyle poseía una dieta mixta C_3/C_4 *y habitaba en zonas de vegetación abierta (*δ^{13}*C: -3.1‰ y -1.3‰;* δ^{18}*O: -4.9‰ y -6.4‰). En cambio, D. mexicanus de Rancho El Ocote se alimentó de plantas* C_4 *y vivió en zonas abiertas (*δ^{13}*C: -1.3‰;* δ^{18}*O: -4.9‰), mientras que el ejemplar de Santa María poseía una dieta mixta* C_3/C_4 *con un importante consumo de plantas* C_3 *(*δ^{13}*C: -7.7‰;* δ^{18}*O: -6.4‰), lo que es contrario a otras propuestas basadas en isótopos estables que indican que los D. mexicanus de México sólo se alimentaban de plantas* C_4*, lo cual contribuye a comprender que los hábitos alimentarios de las especies de équidos que habitaron durante el Plioceno el centro de México variaba de animales con consumo exclusivo de plantas* C_4 *a aquellos con dietas mixtas* C_3/C_4*.*

Palabras clave: Dinohippus mexicanus; Neohipparion eurystyle; Henfiliano tardío; isótopos estables; México.

INTRODUCTION

Taxonomic and biostatigraphic research in the Mexican Late Tertiary sedimentary basins has shown a highly diversified mammal fauna. Among the best known and important local faunas are those from Rancho El Ocote in the San Miguel Allende basin, Guanajuato, and from Santa María in the Tecolotlán basin, Jalisco. The faunal complex known for those late Hemphillian localities includes the following: equids, *Nannippus aztecus*, *Neohipparion eurystyle*, *Astrohippus stockii* and *Dinohippus mexicanus*; rhinoceros, *Teleoceras fossiger*; carnivores, *Agriotherium schneideri*, *Borophagus secundus*, *Canis ferox*, and *Machairodus* cf. *M. coloradensis*; camelids, *Hemiauchenia vera*, *Megatylopus matthewi*, and *Alforjas* sp.; rodents, *Spermophilus* sp., *Ammospermophilus* sp., *Paenemarmota* sp., *Perognathus* sp., ?*Pliogeomys* sp., *Calomys* sp., *Baiomys* sp., *Prosigmodon* sp. and *Neotoma* sp.; the xenarthran *Megalonyx* sp.; lagomorphs, *Hypolagus mexicanus* and *Notolagus velox;* and the proboscidean *Rhynchotherium* sp. (Miller

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and Carranza-Castañeda, 1984, 1998; Carranza-Castañeda, 2006; Jiménez-Hidalgo and Carranza-Castañeda, 2010; Carranza-Castañeda *et al.*, 2013). These faunas constitute the basis for the late-Hemphillian biostratigraphy of central Mexico.

Carbon isotope values from dental enamel record the photosynthetic pathways of plants consumed by the animal in an early life stage when the molars were being formed (Koch, 2007). On the other hand, the mesowear method is based on the attrition (tooth-on-tooth contact) and abrasion (tooth-on-food contact) of the teeth (largely their cusps), regardless of the photosynthetic pathway used by the plant that generates it (Kaiser and Solounias, 2003). This method assesses the dental wear caused by the animal's diet during months to years of its life span, *i.e.*, the long-term alteration of the enamel (Fortelius and Solounias, 2000). The abrasion produced by the intake of abiotic material (*e.g.* sand, dust, or soil) could be many times more severe than the effect of enamel attrition (Sanson *et al.,* 2007).

Analysis of carbon and oxygen stable isotopes in fossil molars of the equids *Astrohippus stocki*, *Dinohippus mexicanus*, *Nannipus aztecus,* and *Neohipparion eurystyle* from Yepómera, Chihuahua, and Rancho El Ocote, Guanajuato, suggested that these species fed predominantly on C_4 plants, such as grasses or other herbaceous plants (MacFadden, 2008). On the other hand, a mesowear analysis of molars from *D. mexicanus* and *N. eurystyle* from the Tecolotlán basin (Jalisco) indicated that *N. eurystyle* had specialized in feeding on abrasive material (such as grass or external grit), whereas *D. mexicanus* was a mixed feeder (Barrón-Ortiz and Guzmán, 2008). A combination of mesowear, microwear and stable isotope analyses (Bravo-Cuevas *et al.*, 2015) suggested that *A. stocki* from Rancho El Ocote had a C_3/C_4 mixed diet; most C_3 plants are shrubs and trees, with a few C_3 grasses. Unfortunately, those authors did not provide the stratigraphic context of their specimens. Carbon and oxygen stable isotopes in molars of *D. mexicanus* and *N. eurystyle* from the Tecolotlán basin have not previously been analyzed. In the present study, enamel from the molar of an individual from each species from Tecolotlán basin was analyzed and the data were compared with those from the molar of an individual of each species from Rancho El Ocote. The objective was to detect differences and similarities in diet of the two species in comparison with the results recorded by MacFadden (2008).

$\delta^{13}C$ and $\delta^{18}O$ biogeochemical markers

Since 1980, the application of carbon and oxygen stable isotopes analysis has been important in inferring diet and habitat of Cenozoic herbivorous mammals and birds (Barrick, 1998; Koch, 1998; MacFadden and Higgins, 2004; Angst *et al.*, 2014). Carbon is incorporated into animals by three plant photosynthetic pathways. The C_3 pathway has $\delta^{13}C$ from -22‰ to -35‰ and is found in trees and shrubs, as well as in some cold-zone grasses (Smith and Epstein, 1971; O'Leary, 1981; Medina *et al.*, 1986). The C_4 pathway has $\delta^{13}C$ values from -10‰ to -12‰ and is typical for grasses, and also for some dry-zone trees and shrubs (Stowe and Teeri, 1978; Cerling, 1999; Cerling and Ehleringer, 2000). Finally, the Crassulacean Acid Metabolism (CAM) pathway has $\delta^{13}C$ values from -35‰ to -12‰ and is found in succulent plants such as cacti and some orchids (Gröcker, 1997; Andrade *et al.*, 2007).

Herbivores incorporate the carbon of the plants on which they feed into their organs and tissues, so they have carbon isotope values reflecting those of the plants they feed upon. In the case of tooth enamel, the tissue is enriched by 14.1‰ relative to the plant value (Cerling and Harris, 1999). Animals that feed on C_3 plants have carbon isotopic values from -19‰ to -9‰, while herbivores that have consumed C_4 plants have carbon isotopic values from -2‰ to +2‰. The C_3/C_4 mixed feeders show values from -9‰ to -2‰ (MacFadden and Cerling, 1996).

On the other hand, oxygen is incorporated into animals by inhalation, from water in food, and mainly by ingested water. Such oxygen is in equilibrium with what is lost through CO_2 exhalation, feces, urine and sweat (Koch *et al.*, 1994; Sánchez, 2005). Given that ingested water comes from meteoric water, its composition is affected primarily by temperature, but also by altitude, latitude and the amount of rain fall in a zone (Dansgaard, 1964). The oxygen composition of dental enamel from mammals is used mainly for inferring the paleoclimatic conditions that prevailed in a locality in the past (Longinelli and Nuti, 1973; Kohn, 1996; Kohn *et al.*, 1998; Schoeninger *et al.*, 2000; Mahboubi *et al.*, 2014).

STUDY LOCALITIES

Rancho El Ocote is located at 21º05´28"N and 100º41´01"W in the San Miguel Allende basin, Guanajuato. The ashes found in the stratigraphic sequence were dated by fission tracks, and provided ages of 4.8 Ma for the lower sediments (the Rhino layer) formed during the late Hemphillian, and 4.7 Ma for the upper ash from the early Blancan (Kowallis *et al.*, 1998). These ages provide the limits of the North American Land Mammal Ages (NALMA), late Hemphillian and early Blancan (Figures 1 and 2) (Flynn *et al.*, 2005; Carranza-Castañeda, 2006; Cohen *et al.*, 2013). Four equid species, *D. mexicanus*, *N. eurystyle*, *A. stockii*, and *N. aztecus* occur in the Rhino level, with lithology and sedimentation rates showing a more humid environment than those of the overlying Blanco level, where only *D. mexicanus* and *N. neurystyle* are found (Carranza-Castañeda, 2006).

The molars from Rancho El Ocote were collected at the GTO-2A locality. Carranza-Castañeda *et al.* (2013) recorded that *Dinohippus mexicanus* and *Neohipparion eurystyle* were found in the Rhino level, and to Hh3 in the Mammalian Chronology proposed by Tedford *et al.* (2004). On the other hand, *N. eurystyle* was not found above the Rhino level. This implies that the level above the Rhino level represents the final portion of the late Hemphillian (Hh4) (Figure 2).

The Tecolotlán basin is 100 km southeast of Guadalajara, in the state of Jalisco (Figure 1; the late Hemphillian stratigraphic sequence is divided by lacustrine sands). The best faunal representation is from the Santa María locality and the studied molars were collected at this site (Figure 3). Lower ash has been dated by $^{40}Ar/^{39}Ar$ at 4.89 Ma (Kowallis *et al.*, 1998). Carranza-Castañeda *et al.* (2013) have shown that the Santa María area belongs to Hh3. As an erosional discordance, the late Blancan-Irvingtonian, Buenaventura sequence contains gravel and sands, with an internal ash dated at 2.6 Ma (Kowallis *et al.*, 1998). Santa María has a stratigraphic sequence in the Tecolotlán basin that terminates at the bottom of the lacustrine sands. Lithology and sedimentation rates at Santa María indicate the presence of a humid environment, containing remains of *D. mexicanus*, *N. eurystyle*, *A. stockii* and *N. aztecus*. The last two species are not found in the San José stratigraphic sequence that is overlying the lacustrine sands, with lithology and sedimentation rates typical for a dry environment (Carranza-Castañeda *et al.,* 2013).

MATERIALS AND METHODS

Sample extraction, preparation and statistical analysis of the results

A bulk sample was taken from isolated teeth from Rancho El Ocote: MPGJ-2056 (*Dinohippus mexicanus*), MPGJ-2026 (*Neohipparion eurystyle*) and Tecolotlán Basin: MPGJ-2956 (*D. mexicanus*), MPGJ-2959 (*N. eurystyle*), each of them representing a single individual. The samples were taken at the crown-root joint following the protocol of

Figure 1. Late Hemphillian fossiliferous localities in the Tecolotlán basin (1) and Rancho El Ocote (2), Mexico.

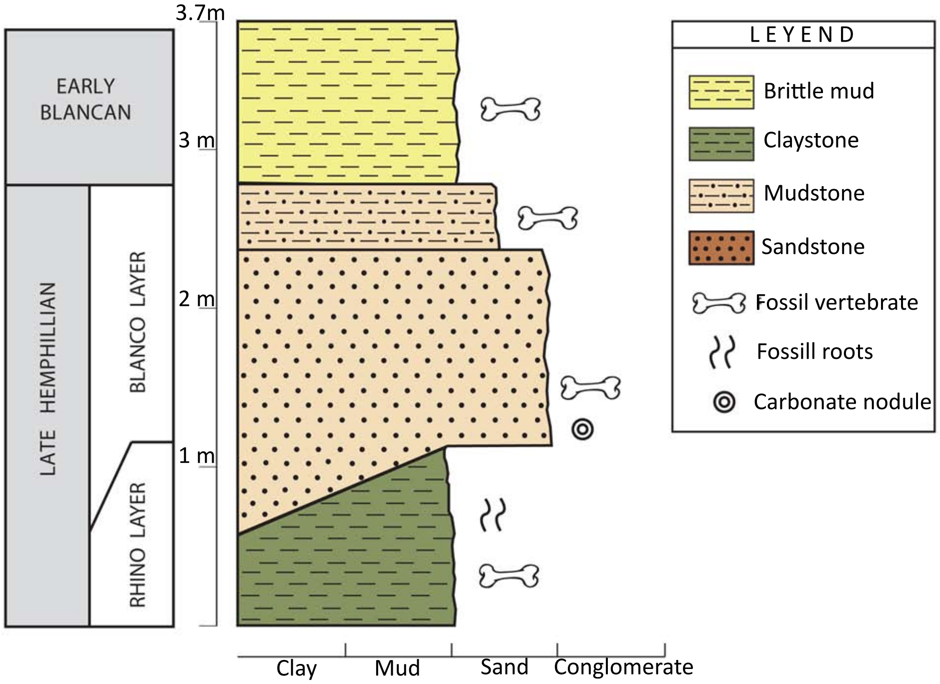

Figure 2. Stratigraphic column for Rancho El Ocote (GTO 2A). Investigated specimens came from the Rhino layer. Taken from Pacheco-Castro and Carranza-Castañeda (2015).

Feranec and MacFadden (2000) and belong to the Palaeontological Collection of the *Centro de Geociencias* of the *Universidad Nacional Autónoma de México* (UNAM), *Campus Juriquilla, Querétaro*.

Samples were processed in the Stable Isotope Laboratory at the *Instituto de Geología*, UNAM, with the method proposed by Koch *et al.* (1997). First, 20 mg of enamel was ground and sieved (125 µm mesh) to obtain a fine and uniform powder. Then 10 ml of hydrogen peroxide at 30 % was added to eliminate the organic matter. After 2 h, the samples were centrifuged and the hydrogen peroxide decanted and washed again three times with water type I (grade HPLC 18.2 MΩ). Once the washing was finished, 5 ml of a buffer solution, $Ca(CH_3CO_2)_2$-CH_3COOH 1.0 M, pH 4.75, was added and allowed to rest for 9 h. The buffer solution was decanted and the samples were washed again three times with water type I. Finally, to eliminate any remaining water, ethanol was added, and the solution was left for 20 h in an oven at 90 °C. Isotopic ratios were determined with a Finnigan MAT 253 mass spectrometer with a dual inlet system and auxiliary Gas Bench equipment with a GC Pal autosampler with a temperature-controlled aluminum plate adjoined to the mass spectrometer (Révész and Landwehr, 2002). Results were reported as $\delta^{18}O_{VPDB}$ and $\delta^{13}C_{VPDB}$, and they were normalized using NBS-19, NBS-18 and LSVEC to the Vienna Pee Dee Belemnite (VPDB) scale in accordance with the corrections described by Coplen (1988), Werner and Brand (2001) and Coplen *et al.* (2006). For this technique, the standard deviation was 0.2‰ for oxygen and carbon.

RESULTS

Carbon and oxygen isotopic values for *D. mexicanus* from Rancho El Ocote are more positive than those from Tecolotlán (Table 1, Figure 4). The ^{13}C values for specimens of *N. eurystyle* from the two sites are similar to each other, although somewhat higher from Rancho El Ocote than from Tecolotlán (Table 1, Figure 4).

DISCUSSION

Diet

The carbon isotope value ($\delta^{13}C$: -1.3‰) for *Dinohippus mexicanus* (MPGJ-2056) from Rancho El Ocote implies that this individual was a specialist feeding on C_4 plants such as grasses or other herbs (Table 1), also observed by MacFadden (2008) for Rancho El Ocote and Yepómera locations. In contrast, individuals found in Florida and Texas were mixed C_3/C_4 feeders (MacFadden, 2008), but consumed an important amount of C_3 plants, such as C_3 grasses, tree leaves and shrubs. On this basis, MacFadden (2008) assumed that, during the late Hemphillian, C_4 plants were abundant in Mexican ecosystems, whereas C_3 plants were abundant further north. However, the present results with specimen MGPJ-2956 from Santa María (Tecolotlán basin) differ in that they show a $\delta^{13}C$ value that characterize it as a C_3/C_4 mixed feeder, but with an important component of C_3 plants (Table 1).

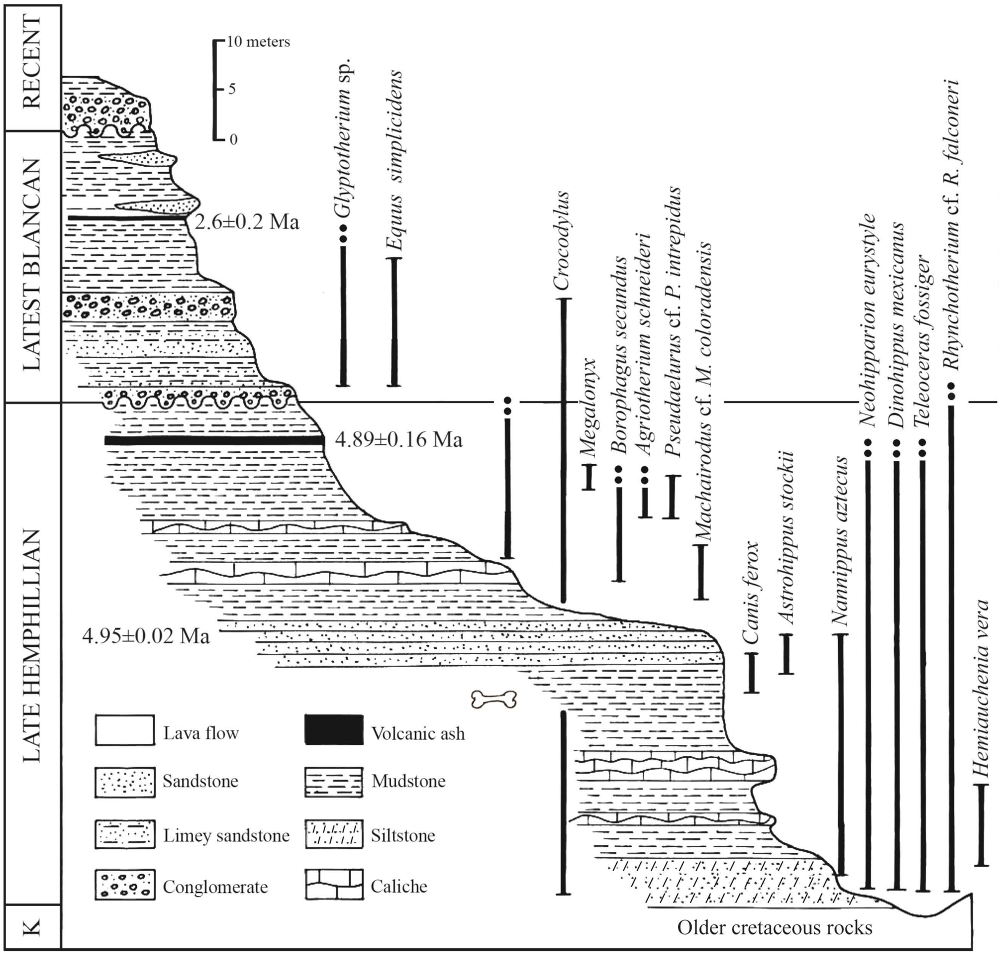

Figure 3. Statigraphic column for the Tecolotlán basin (Santa María). Investigated specimens came from the bone layer. B: Bone layer, K: Cretaceous. Taken from Carranza-Castañeda (2006).

Table 1. Carbon and oxygen isotopic values of molar enamel from fossil equids in Mexico.

Catalogue number	Species	$\delta^{13}C_{V\text{-}PDB‰}$	$\delta^{18}O_{V\text{-}PDB‰}$	Locality	Diet suggested by MacFadden (2008)	Present study
MPGJ-2056	*Dinohippus mexicanus*	-1.3	-3.3	Rancho El Ocote	C_4 feeder	C_4 feeder
MPGJ-2956	*Dinohippus mexicanus*	-7.7	-6.4	Tecolotlán	C_4 feeder	C_3/C_4 feeder
MPGJ-2026	*Neohipparion eurystyle*	-3.1	-4.8	Rancho El Ocote	C_4 feeder	C_3/C_4 feeder
MPGJ-2959	*Neohipparion eurystyle*	-4.9	-6.4	Tecolotlán	C_4 feeder	C_3/C_4 feeder

Mesowear studies on specimens of *D. mexicanus* from the Tecolotlán basin (Barrón-Ortiz and Guzmán, 2008) found both browsers and mixed feeders.

The present isotope analysis has suggested that specimen MPGJ-2956, pertaining to *D. mexicanus* from Tecolotlán, may have fed on trees, shrubs and C_3 grasses, a diet similar to that proposed by Barrón-Ortiz and Guzmán (2008) for other Tecolotlán specimens. MPGJ-2956 was collected in the lower stratigraphic sequence of the Tecolotlán basin (Carranza-Castañeda, 2006), whereas Barrón-Ortiz and Guzmán (2008) assumed that their specimens likely came from the upper part of the sequence. Their lack of stratigraphic data precludes direct comparison of the two studies; it is uncertain whether the individuals represented by the different specimens were contemporaneous and whether the diet of this species remained constant through time. In Costa Rica, *D. mexicanus* seems to have had a generalist, not a specialized, diet based on morphological ground (Laurito and Valerio, 2010), similar to that suggested by the isotopes in specimens from Texas, Yepómera and Rancho El Ocote (MacFadden, 2008).

In the case of *N. eurystyle*, carbon isotope values for both MPGJ-2026 from Rancho El Ocote and MPGJ-2959 from the Tecolotlán basin show that these animals were C_3/C_4 mixed feeders, with in the case of MPGJ-2026, an emphasis on the intake of C_4 plants (Table 1). This pattern is different from what was found by MacFadden (2008) with specimens from Rancho El Ocote and Yepómera, which fed exclusively on C_4 plants. Mesowear analyses (Barrón-Ortiz and Guzmán, 2008) suggested that *N. eurystyle* from the Tecolotlán basin was a grazer, but the lack of stratigraphic control makes it difficult to ascertain whether the diet variation between individuals was due to different locations from the same time period, or whether the differences were due to environmental change.

Since the individuals of *N. eurystyle* analyzed here appear to have been C_3/C_4 mixed feeders like some individuals in Florida (MacFadden, 2008), both *N. eurystyle* and *D. mexicanus* from the Mexican late Hemphillian appear to have been generalists in their diet.

This plasticity in equid diets may be explained by different factors. For example, in desert areas in the southern USA and northern Mexico, C_3 plants are more abundant in winter, the rainy season, whereas C_4 plants are more abundant in summer (Ehleringer and Monson, 1993). In temperate areas in general, the two kinds of plants co-inhabit year-around, but in microhabitats that differ in temperature and humidity. Isotope analysis has shown that present-day feral horses in southern New Mexico feed on leaves and grasses that differ in proportion according to seasonal availability (Hansen, 1976; Smith *et al.,* 1998). Furthermore, factors such as soil salinity, light intensity and soil nutrients may influence the carbon isotope composition of plants (Bocherens, 2003) and hence the composition of the dental enamel of herbivores.

Competition with other herbivore species may in part explain the wide feeding spectra shown by these equids; Late-Pleistocene bison and horses at Rancho La Brea, California, fed mainly on C_3 plants, but bison more frequently preferred C_4 plants (Feranec *et al.*, 2009), suggesting the presence of resource partitioning. The small sample size in the present study precludes further speculation.

Habitat

The carbon and oxygen isotope values of both specimens from Rancho El Ocote suggest a preference for open vegetation zones (Figure 4). In the case of the specimens from the Tecolotlán basin, *N. eurystyle* lived in grassland or savannas, whereas *D. mexicanus* preferred areas with major tree cover. In Florida, *N. eurystyle* seems to have inhabited open zones, whereas *D. mexicanus* could live in either open regions or areas with some tree cover (MacFadden *et al.*,1999a; MacFadden, 2008). Hence, *D. mexicanus* may have been more flexible in its habitat and feeding habits according to local conditions.

Although *D. mexicanus* had a mixed feeding habit associated with environments of mixed vegetation and high humidity (Laurito and Valerio, 2010), some individuals were grazers living in herbaceous grassland and arid areas. There is a latitudinal gradient: above 35 °-40 °N, C_3 plants such as grasses, shrubs and trees are abundant, with diminishing C_4 grasses (MacFadden *et al.*, 1999b); this is reflected in the differences in diet between specimens from the USA and those from Mexico (MacFadden, 2008).

The results of mesowear analyses of *D. mexicanus* and *N. eurystyle* from the Tecolotlán basin suggest that this basin developed a heterogeneous vegetation of trees and grasses fostered by a seasonal climate (Barrón-Ortiz and Guzmán, 2008); dry seasons were favorable for C_4 plant growth, and wet seasons were favorable for more abundant C_3 plants. In the Santa María locality, lacustrine sediments and paleochannels indicate the presence of water bodies such as rivers,

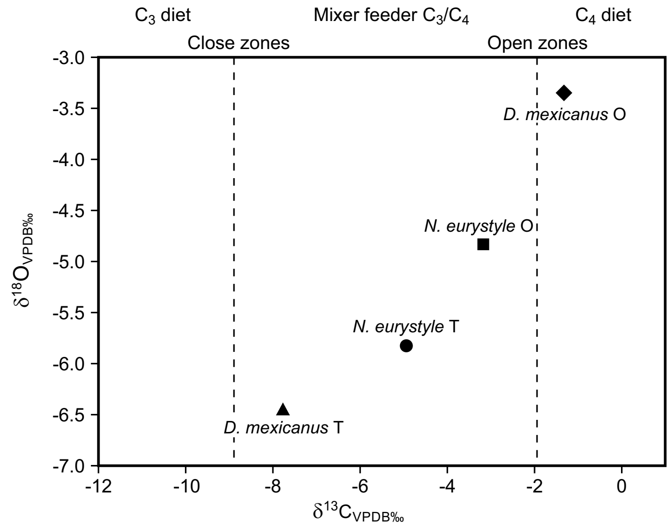

Figure 4. $\delta^{13}C$ *vs* $\delta^{18}O$ for equid molars from Rancho El Ocote (GTO 2A) and the Tecolotlán basin (Santa María). O: Rancho El Ocote; T: Tecolotlán.

lakes and water channels (Carranza-Castañeda, 2006). Fossil freshwater gastropods, teeth of *Crocodylus* sp., all aquatic animals, and remains of the rhinoceros *Teleoceras fossiger*, have been found there (Carranza-Castañeda *et al*., 2005). Although *T. fossinger* has been defined as a browser, isotopic analyses of specimens from Florida showed its diet to be more generalist (MacFadden, 1998), so its presence would not necessarily indicate the existence of C_3 plants in the locality. Nevertheless, the wet environment would have been favorable to C_3 plants (Ehleringer and Monson, 1993). Abundance of C_3 plants at Santa María did not depend on the presence of a latitudinal gradient, but on specific conditions at each locality.

The conclusions above are supported by the oxygen isotope values: *D. mexicanus* from Santa María shows a $\delta^{18}O$ value of -6.4‰, whereas from Rancho El Ocote it is -3.3‰. For *N. eurystyle* from Santa María it is -6.4‰, whereas from Rancho El Ocote it is -4.8‰. With increasing amounts of rain, oxygen isotope values become more negative (Dansgaard , 1964). Because Santa María and Rancho El Ocote are at different altitudes and latitudes, differences in $\delta^{18}O$ values are to be expected. Additionally, the location of Santa María on the foothills of the Sierra Madre Occidental implies that it received more precipitation, creating more humidity favorable for the establishment of C_3 plants, as reflected by the lacustrine sediments (Carranza-Castañeda, 2006). Another factor, however, could explain the different oxygen isotope values. Both *D. mexicanus* and *N. eurystyle* may have migrated from place to place in search of food (MacFadden, 2008). They drank water from different feeding areas, and hence the isotopic composition could differ, as found for bison in the eastern Great Plains (Widga *et al.*, 2010).

Carbon isotope analyses on the two other equid species found at Rancho El Ocote, *A. stocki* and *N. aztecus,* showed that some of these individuals were C_3/C_4 mixed feeders, suggesting that this area was drier than at the Florida and Texas localities, and favored the presence of C_4 plants (MacFadden, 2008). However, no inference could be made regarding whether during the late Hemphillian grassland with some trees or shrubs existed.

On the basis of the diversity of fossil herbivorous species found at Rancho El Ocote or in the Tecolotlán basin, other species should be included in subsequent studies in order to draw more precise inferences regarding the vegetation type and environmental conditions that existed at each locality during the late Hemphillian.

CONCLUSIONS

At Rancho El Ocote, the diet of the *Dinohippus mexicanus* specimen was primarily based on C_4 plants, and this animal preferred open zones, such as grasslands or savannas. In contrast, the *Neohipparion eurystyle* was a C_3/C_4 mixer feeder with an emphasis on C_4 plants, living in open zones.

In the Tecolotlán basin, the *Dinohippus mexicanus* and *Neohipparion eurystyle* were C_3/C_4 mixed feeders and lived in areas with some tree coverage; this suggests that conditions here were more favorable for C_3 plants than at Rancho El Ocote.

These preliminary conclusions require substantiation by analyses of more specimens and from a wider range of species.

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