

Sydney Harrison*, **Chance Hannold****, **Yang Wang PhD****
Florida State University*, **National High Magnetic Field Laboratory****

INTRODUCTION

I. Equid Coexistence in the Early Pliocene (4.8-5.3 mya)

- o Understanding extinct species' diets provides insights into ecological interactions, habitat preferences, and coexistence mechanisms.
- o Multiple equine species coexisted in Central Mexico, raising questions about resource partitioning.
- o One hypothesis suggests niche partitioning (dietary and/or habitat-use differences), particularly in C4 grass consumption, facilitated coexistence and minimized competition.

II. Stable Isotope Analysis

- o Chemical composition of tooth enamel records carbon from blood dissolved inorganic carbon (DIC) and oxygen from body water. DIC derives most carbon from dietary items. Body water includes oxygen primarily from ingested water, and dietary items; however, ingested water is the largest source in drought intolerant species.
- o Carbon isotopes ($\delta^{13}C$) from carbonate substitutions in enamel can be used to reconstruct diet (C3 vs C4 plant consumption).
- o Oxygen isotopes ($\delta^{18}O$) from carbonate or phosphate in enamel can be used to compare water sources, reconstruct air temperature, and check for seasonality.

III. Study Objectives

- o Use stable isotope analyses of enamel carbonate to assess dietary overlap and environmental conditions among Hemphillian equids (*Neohipparion eurystyle*, *Dinohippus mexicanus*, *Nannippus aztecus*, *Astrohippus stockii*, and unidentified Equidae) in Central Mexico.
- o Use statistical analyses to evaluate niche overlap and/or separation of sampled horse species.
- o Compare findings with the limited niche partitioning found in Great Plains equids by Parker et al. (2018).

METHODS

I. Sample Collection and Preparation

- o Fossil teeth from *Neohipparion eurystyle*, *Dinohippus mexicanus*, *Nannippus aztecus*, *Astrohippus stockii*, and unidentified Equidae were drilled with a diamond tipped burr to collect enamel powder.
- o Researchers followed established protocols for preparation and stable isotope analysis of fossil tooth enamel (i.e., McCrea, 1950; Bowman et al., 2017).
- o Powders were:
 - Treated with 5% sodium hypochlorite to remove organic contaminants and then rinsed with distilled water
 - Treated with 1M acetic acid to remove secondary carbonates and then rinsed with distilled water
 - Freeze dried to remove water
 - Reacted with phosphoric acid to produce carbon-dioxide in a sealed tube filled with helium

II. Stable Isotope Analysis

- o Carbon and oxygen isotope ratios were measured using a stable isotope ratio mass spectrometer with a connected gas bench.

III. Stable Isotope Interpretation and Statistical Analyses

- o $\delta^{13}C$ values were used to estimate C4 plant consumption (C4%) via a two-source mixing model (Wang & Cerling, 1994).
- o $\delta^{18}O$ values were used to:
 - Identify seasonal fluctuation or lack thereof in water sources based on oscillation or stability of intra-tooth $\delta^{18}O$ values, respectively.
 - Reconstruct temperature based on modern relationships between rainwater $\delta^{18}O$ values and air temperature.
- o ANOVA and post-hoc pairwise comparisons were conducted to test for significant differences in C4% among species ($\alpha=0.05$).
- o Statistically significant differences would suggest resource partitioning, while no significant difference would suggest lack of partitioning.

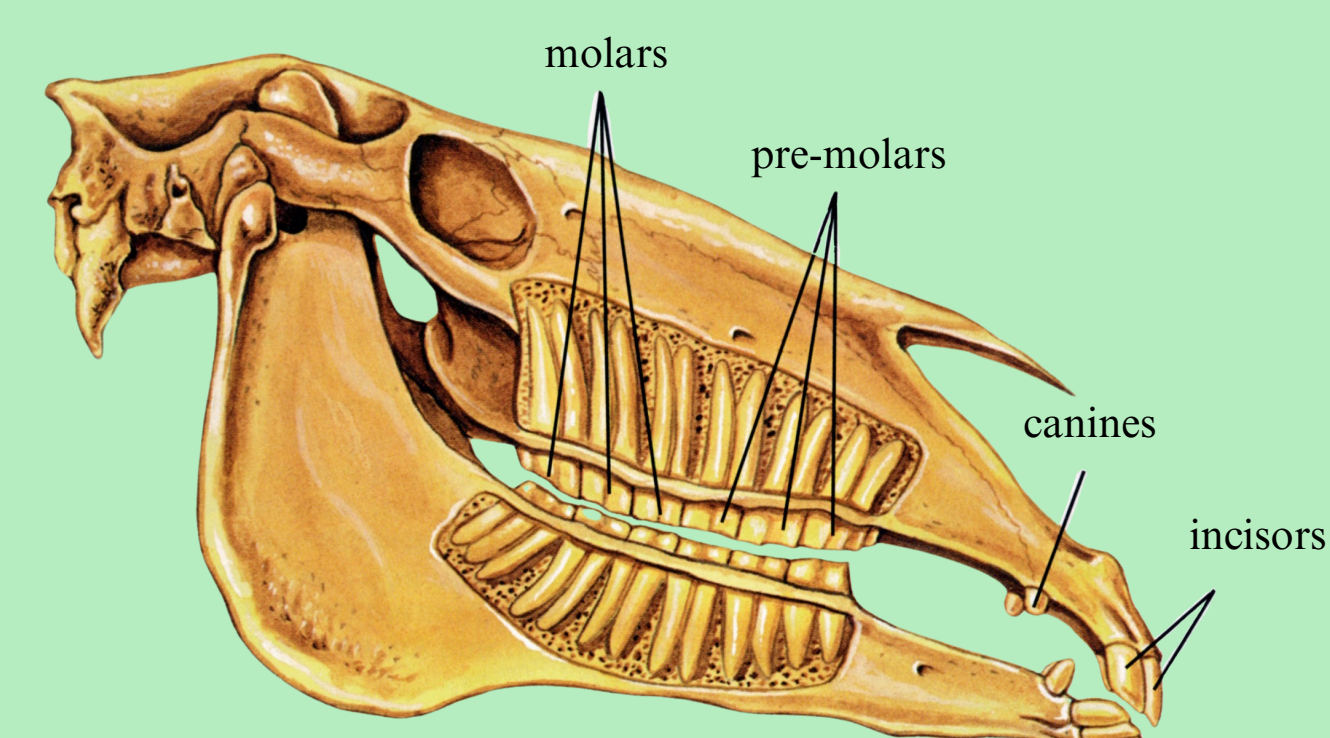


Image 1. Equine dental anatomy



Image 2. Serial sampling

RESULTS

I. Environmental Reconstruction

- o High C4% in diet suggests a warm, arid grassland or savanna.
- o Average $\delta^{18}O$ suggests a mean annual temperature of $\sim 18^{\circ}C$.
- o Minimal intra-tooth $\delta^{18}O$ variation in some teeth suggests access to stable water sources (lakes or perennial rivers) or seasonal migration.

II. Diet Reconstruction

- o C4% in diet was similar across species suggesting no niche partitioning.



Fig 1. Serial sample data from six individuals show intra-tooth $\delta^{13}C$ and $\delta^{18}O$ variations, reflecting seasonal changes in diet and environmental conditions.

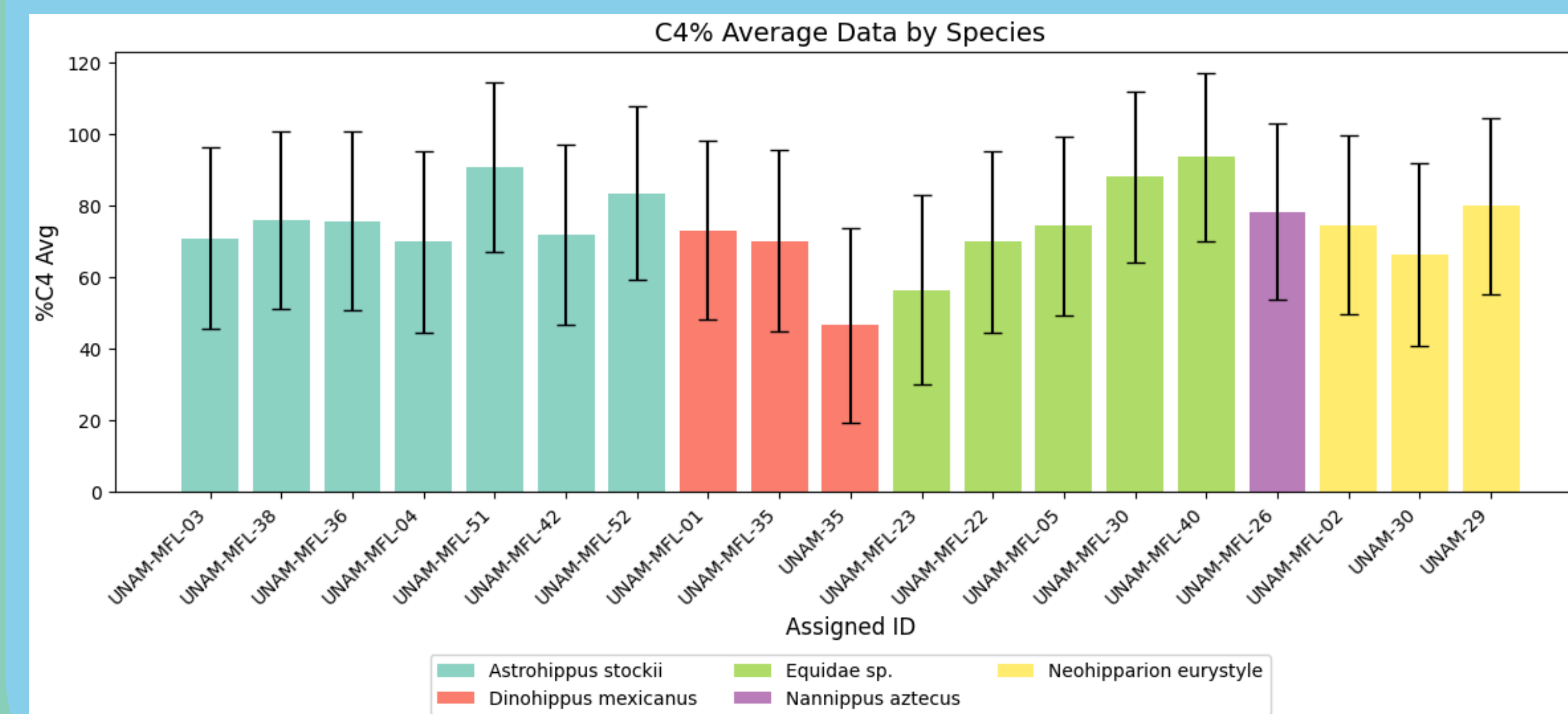


Fig 2. Average C4% data collected via serial and bulk methods across nineteen samples of similar region.

CONCLUSION

- I. There is no evidence for dietary niche partitioning recorded in the isotopic composition of fossil equid enamel from Central Mexico in agreement with previous findings in Great Plains fossil equids (Parker et al., 2018).
- II. Enamel $\delta^{13}C$ and $\delta^{18}O$ values suggest high amounts of C4 plants in the diet of fossil horses and warm air temperature ($\sim 18^{\circ}C$).
- III. These reconstructions suggest a warm, arid, and open environment in Central Mexico during the early Pliocene.
- IV. Migration or resource sharing, rather than dietary specialization between species, likely facilitated coexistence of several equid species in this environment.

DISCUSSION

I. Dietary Overlap and Niche Partitioning

- o Stable isotope results indicate no significant differences in C4 plant consumption among early Pliocene equids in Central Mexico.
 - Challenges the hypothesis that niche specialization mitigated competition and allowed coexistence of various horse species.
 - Supports Parker et al. (2018), which found similar dietary overlap among North American fossil equids.
- o Species likely exhibited flexible foraging strategies or coexisted through alternative ecological mechanisms.
 - Migration and behavioral flexibility likely played key roles in equid survival.
- o Supports previous work (Cerling et al., 1993; Parker et al., 2018) indicating that many extinct herbivores were generalists, adapting to diverse environmental conditions without strict dietary specialization.
- o Future studies using microwear and mesowear analyses may detect subtle dietary differences not identified in isotopic analysis (e.g., different parts of the same plant).

II. Environmental, Climatic, and Ecological Implications

- o The warm, arid, open environment dominated by C4 grasses suggested by $\delta^{13}C$ and $\delta^{18}O$ values is consistent with the global late Miocene grassland expansion (Cerling et al., 1993).
- o Minimal intra-tooth $\delta^{13}C$ variation indicates a stable diet without seasonal shifts between C3 and C4 plants in the diet.
- o Low intra-tooth $\delta^{18}O$ variability implies either continuous access to stable water sources (e.g., groundwater, lakes) or seasonal migration.

III. Alternative Strategies for Coexistence

- o Seasonal or spatial migration may have reduced direct competition for food and water.
- o Species may have occupied different areas at different times of the year or followed separate migratory routes.
- o Behavioral differences, such as herding dynamics or reproductive timing, could have structured resource use.
- o Competition with other large herbivores (e.g., proboscideans, artiodactyls) may have influenced equid dietary strategies.
- o Future studies incorporating broader faunal analyses could further clarify community-level ecological dynamics.

REFERENCES

Bowman, J. L., et al. (2017). Insights into land plant evolution garnered from the Marchantia polymorpha genome. *Cell*, 171(2), 287–304.e15.

Cerling, T. E., et al. (1993). Expansion of C4 ecosystems as an indicator of global ecological change in the late Miocene. *Nature*, 361(6410), 344–345.

McCrea, J. M. (1950). On the isotopic chemistry of carbonates and a paleotemperature scale. *The Journal of Chemical Physics*, 18(6), 849–857.

McIlwraith, C. W., et al. (2010). Lateral view of skull showing roots of cheek teeth. In *Illustrated atlas of clinical equine anatomy and common disorders of the horse* (p. 47). Teton NewMedia.

Parker, A. K., et al. (2018). Niche modeling reveals lack of broad-scale habitat partitioning in extinct horses of North America. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 511, 103–118.

Wang, Y., Cerling, T. E. (1994) ** A model of fossil tooth $\delta^{18}O$ and $\delta^{13}C$ as a seasonal signal and predictor of diet and environment. *Geochimica et Cosmochimica Acta*, 58*(4), 849–865.

