



# Oxygen Isotopes in Enamel Carbonate and their Ecological Significance

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Stable carbon isotope analysis of fossil tooth enamel carbonate, and oxygen isotope analysis of bone or enamel phosphate, are established tools for palaeodietary and palaeoclimatic reconstruction, respectively.  $^{13}\text{C}/^{12}\text{C}$  ratios provide evidence of an animal's diet and phosphate-based  $^{18}\text{O}/^{16}\text{O}$  values are used to establish palaeotemperature proxies. Recent studies of fossil enamel suggest that biogenic  $^{18}\text{O}/^{16}\text{O}$  signals are also retained in the carbonate compartment, despite assumptions that  $^{18}\text{O}/^{16}\text{O}$  ratios from apatite carbonate are highly susceptible to exchange during fossilization. Here, we re-examine existing enamel carbonate  $^{18}\text{O}/^{16}\text{O}$  data from the rich fossil assemblages of Swartkrans and Equus Cave. We find patterns that can be interpreted in terms of drinking behaviour, diet, and physiology. In general, herbivores that drink little are more enriched than those that drink frequently, while carnivores are depleted compared with herbivores. Thus, we can increase our knowledge of the ecology of fossil taxa by coupling carbon and oxygen isotope ratio data.

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## Introduction

Stable carbon isotope analysis of fossil tooth enamel is widely used to reconstruct ancient diets and habitats (e.g., Lee-Thorp & van der Merwe, 1987; Quade *et al.*, 1992; Kingston, Marino & Hill, 1994; Lee-Thorp, van der Merwe & Brain, 1994; Bocherens *et al.*, 1996; Cerling *et al.*, 1997). Carbon isotopes, however, provide a limited amount of information about an animal's ecological niche. They do not, for instance, distinguish between diets as different as frugivory and folivory which are both based on  $\text{C}_3$  plants. Moreover, they reveal little about other important aspects of an animal's ecology such as drinking behaviour. Therefore, if we hope to address relatively subtle, albeit important, questions about the interactions between fossil taxa and their environments, we must find ways to supplement carbon isotope ratio data. Oxygen isotope signals in fossil tooth enamel may prove useful in this regard.

The oxygen isotope composition of mammalian tooth enamel is directly related to that of body water, which reflects the isotopic composition of oxygen that enters and exits the body which, in turn, is a complex function of climate, diet, and physiology (Longinelli,

1984; Luz, Kolodny & Horowitz, 1984; Ayliffe & Chivas, 1990; Bryant, Luz & Froelich, 1994; Bocherens *et al.*, 1996; Kohn, 1996; Kohn, Schoeninger & Valley 1996). The body's main oxygen sources are atmospheric  $\text{O}_2$ , liquid water, and oxygen bound in food. Since atmospheric  $\text{O}_2$   $\delta^{18}\text{O}$  values are relatively constant (Dole *et al.*, 1954; Kroopnick & Craig, 1972) enamel oxygen isotope composition is controlled primarily by the composition of ingested water and, to a lesser extent, the composition of macronutrients in food. The isotopic composition of meteoric water (which is available to drink) is sensitive to climatic factors such as mean annual temperature and amount of local precipitation (Dansgaard, 1964). Mammals also ingest significant amounts of free water in plants. Indeed, many mammals obtain all of their water from food. Water in plant roots and stems is isotopically similar to meteoric water, but leaf water is relatively enriched in  $\text{H}_2^{18}\text{O}$  due to preferential evapotranspiration of the lighter  $\text{H}_2^{16}\text{O}$  molecule (Gonfiantini, Gratzu & Tongiorgi, 1965; Dongmann *et al.*, 1974; Epstein, Thompson & Yapp, 1977; Sternberg, 1989; Yakir, 1992). The isotopic composition of oxygen chemically bound in food is also variable. For instance, plant leaf cellulose has higher  $\delta^{18}\text{O}$  values than root

cellulose, which is generally enriched compared to animal foods (Epstein *et al.*, 1977; Sternberg, 1989; Yakir, 1992; Tredget *et al.*, 1993; Kohn, 1996).

While the effect of climate on mammalian oxygen isotope composition has been widely explored (Longinelli, 1984; Luz, Kolodny & Horowitz, 1984; Ayliffe & Chivas, 1990; Luz, Cormie & Schwarcz, 1990; Bryant *et al.*, 1994), relatively little is known about the effects of diet and physiology. However, recent studies of multiple modern taxa within limited areas (to control for climate) suggest that diet has strong and generally predictable effects on isotopic composition, causing sympatric herbivores to have  $\delta^{18}\text{O}$  values that differ by as much as 8–9‰ (Bocherens *et al.*, 1996; Kohn, Schoeninger & Valley, 1996; Sponheimer & Lee-Thorp, unpubl. data). Kohn, Schoeninger & Valley (1996) found that browsing and mixed-feeding herbivores tend to be enriched in  $^{18}\text{O}$  compared with grazing herbivores. Likewise, Cerling *et al.* (1997) found that browsing giraffes (*Giraffa camelopardalis*) are enriched in  $^{18}\text{O}$  compared with other herbivores in the local ecosystem. Conversely, Bocherens *et al.* (1996) found that browsing black rhinoceroses (*Diceros bicornis*) and mixed-feeding elephants (*Loxodonta africana*) are depleted in  $^{18}\text{O}$  compared with other local fauna. They also showed that hippopotamuses (*Hippopotamus amphibius*) are significantly depleted compared with other herbivores. Our studies demonstrate further that modern carnivores are depleted in  $^{18}\text{O}$  compared with herbivores in the same area (Sponheimer & Lee-Thorp, unpubl. data). We discuss the basis of these patterns below.

Although oxygen is present in both phosphate ( $\text{PO}_4^{3-}$ ) and carbonate ( $\text{CO}_3^{2-}$ ) ions in enamel apatite, and  $\delta^{18}\text{O}_{\text{phosphate}}$  and  $\delta^{18}\text{O}_{\text{carbonate}}$  are highly correlated ( $r^2 = 0.98$ ) (Bryant *et al.*, 1996; Iacumin *et al.*, 1996), most studies of mammalian oxygen isotope composition have focused on phosphate oxygen because the P-O chemical bond is much stronger than the C-O bond. Indeed, the P-O bond is so strong that lengthy and harsh chemical procedures are required to extract the oxygen and convert it to  $\text{CO}_2$  for isotopic measurement. This suggests that phosphate oxygen is less susceptible to diagenetic processes than carbonate oxygen. Furthermore, theoretical and empirical studies show that carbonate isotope signals are seriously altered in bone because it is poorly crystalline and highly porous (Lee-Thorp, 1989, in press; Wang & Cerling, 1994). Recent studies show, however, that biogenic oxygen signals can be retained in ancient enamel carbonate because enamel is much denser and more crystalline than bone. Bocherens *et al.* (1996) demonstrated that hippos from Early and Middle Pleistocene sites are depleted, and Cerling *et al.* (1997) found that Miocene giraffids are enriched in  $^{18}\text{O}$  compared with other herbivores. These are the same patterns that they found in the aforementioned modern studies. The persistence of this modern patterning in ancient enamel carbonate is particularly important

because carbonate oxygen isotope ratios are already conveniently obtained from evolved  $\text{CO}_2$  during analysis for carbon isotopes. Therefore, unlike analysis of phosphate oxygen, no additional work is necessary. We need only take a closer look at data which we have until now ignored. Our purpose here is two-fold. We return to data obtained for previous studies to (1) demonstrate that biogenic oxygen isotope ratios are reasonably preserved in enamel carbonate from the Pleistocene sites Swartkrans and Equus Cave and (2) discuss the possibility of using these data to derive information about the lifeways of fossil taxa.

## Sampling and Methods

### *The sites*

Swartkrans is located about 50 km west of Johannesburg, in Gauteng Province, South Africa. The cave's brecciated deposits are rich in fossils, the most noted of which are the hominids *Paranthropus robustus* and *Homo* sp. The Swartkrans breccias are currently divided into five Members, but for simplicity only Member 2 fauna are discussed here. Biostratigraphic analysis of Members 1–3 placed them between 1.8 and 1.0 Ma (Vrba, 1985; Watson, 1993), and thermoluminescence of quartz grains from Members 1 and 2 produced dates of 1.6 and 1.2 Ma, respectively (Vogel, 1985). While the age of Member 2 is still an open question, it is safe to assume that it is older than 1 Ma. Environmental reconstructions of Swartkrans during Member 2 times vary, but all agree that the area contained a mosaic of grassland and tree cover which was probably denser alongside the ancient Blaaubank stream (Vrba, 1985; Watson, 1993; Reed, 1996).

Equus Cave is located in the more arid, westerly part of the South African interior, about 130 km north of Kimberley in Northern Cape Province. The site is rich in fossil material, most of which was accumulated by hyaenas (Klein, Cruz-Urbe & Beaumont, 1991). Four depositional units have been identified which likely accumulated over the last 30,000 years (Lee-Thorp & Beaumont, 1995). The Equus Cave fauna is dominated by grazing taxa, which suggests that the area was dominated by open grassland in the Upper Pleistocene. This is somewhat mitigated by the presence of taxa that generally live in the vicinity of swamps, such as *Kobus leche* (Cruz-Urbe, 1991). Pollen from coprolites suggests that Equus Cave was cooler and possibly moister during the last glaciation (Scott, 1987). Carbon isotope studies of the grazing taxa show that there were at least two periods where  $\text{C}_3$  grasses appeared in small, yet significant numbers. These  $\text{C}_3$  spikes might reflect periods of lower rainfall seasonality within a predominantly summer rainfall regime (Lee-Thorp & Beaumont, 1995). For the purposes of this paper, we have grouped the fauna from all four units together, although we recognize that the two middle units span major climate events.

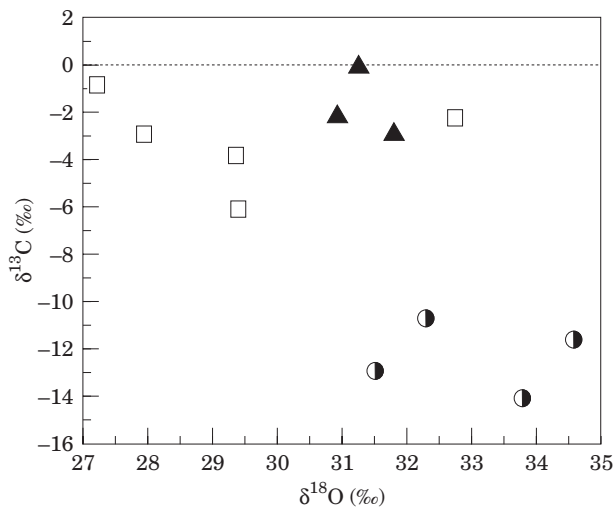


Figure 1.  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values of browsers (●) (*Oreotragus oreotragus*, *Antilocapra recki*, *A. australis*), grazers ▲ (*Equus capensis*, *E. burchelli*), and carnivores, (□) (*Panthera leo*, *P. pardus*) from Swartkrans Member 2.

### Methods

Isotopic analysis of the fauna from both sites was performed for earlier studies designed to glean dietary and environmental information from carbon isotopes alone. Consequently, samples were prepared as described in Lee-Thorp (1989), and not with our recently developed microsampling protocol (Lee-Thorp, Manning & Sponheimer, 1997; Sponheimer & Lee-Thorp, 1999). Enamel was manually separated from dentine and ground to a fine powder in a Spexmill. The powder was pre-treated in a 1.5% sodium hypochlorite solution to eliminate organic contaminants, and 1 M acetic acid to remove secondary carbonates.  $\text{CO}_2$  was obtained by acid hydrolysis using 100% phosphoric acid ( $\text{H}_3\text{PO}_4$ ) and collected by cryogenic distillation. Isotopic ratios were measured on a VG 602E dual inlet ratio mass spectrometer.  $^{18}\text{O}/^{16}\text{O}$  ratios are reported in  $\delta$  notation relative to the SMOW standard, and analytical precision is  $\pm 0.5\text{‰}$ . Although the reaction of carbonate apatite in phosphoric acid causes isotopic fractionation, we make no attempt to correct this for two reasons: (1) the isotope effect of  $\text{H}_3\text{PO}_4$  hydrolysis is unknown and (2) we are concerned with palaeoecology and not palaeotemperature. Hence, we are more concerned with the relationships between taxa than the  $\delta^{18}\text{O}$  values themselves. All data were downloaded to StatView for statistical analysis.

### Results and Discussion

The range of  $\delta^{18}\text{O}$  values within each site is similar to that found in modern herbivores from one area (Figures 1 & 2) (Bocherens *et al.*, 1996; Kohn, Schoeninger & Valley, 1996; Sponheimer & Lee-Thorp, unpubl. data). Intraspecific variation reaches 5‰ in

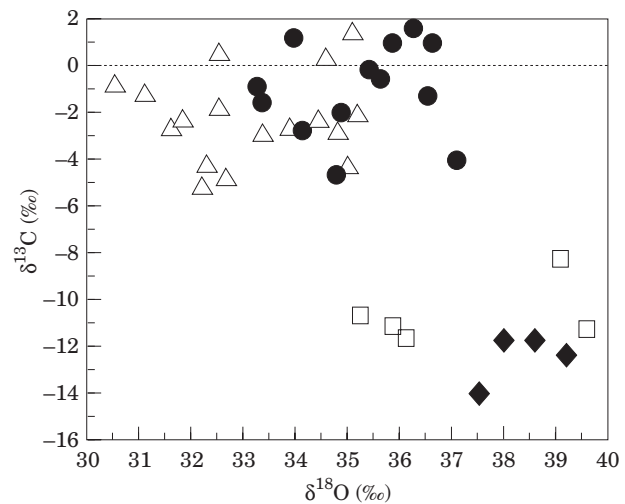


Figure 2.  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values of grazing (*A. bondi* (●), *E. burchelli* (△)) and browsing (*S. grimmia* (◆), *A. marsupialis* (□)) taxa from Equus Cave spanning the last 30,000 years.

some cases, which is greater than the variation reported in phosphate from domesticated horses and cattle from a single location ( $\sim 2.4\text{‰}$ ) (D'Angela & Longinelli, 1990; Bryant, 1995). However, intraspecific variation of 5‰ has been documented in some wild populations, even if infrequently (Bocherens *et al.*, 1996). A further factor to consider is that the fauna analysed here accumulated over thousands of years in different climatic regimes, making this variability even less surprising. What is most important for our purposes here, however, is that oxygen isotope patterning similar to that in modern assemblages is readily observed at both sites—even if biogenic  $\delta^{18}\text{O}$  values have been slightly altered in some instances. Swartkrans' browsing taxa ( $x=33.0\text{‰}$ , s.d.=1.4,  $n=4$ ) are enriched in  $^{18}\text{O}$  compared with grazing taxa ( $x=31.5\text{‰}$ , s.d.=0.6,  $n=3$ ) although the groups are not significantly different due to the small sample sizes. Equus Cave browsers ( $x=37.7\text{‰}$ , s.d.=1.6,  $n=9$ ) are also enriched compared with grazers ( $x=34.0\text{‰}$ , s.d.=1.7,  $n=30$ ) and the groups are strongly different ( $P<0.0001$ ). This pattern of browser enrichment has been reported in modern fauna by Kohn, Schoeninger & Valley (1996). The carnivores ( $x=29.3\text{‰}$ , s.d.=2.1,  $n=5$ ) from Swartkrans are depleted in  $^{18}\text{O}$  and significantly different from herbivores ( $P<0.05$ ), which is the same pattern we found in modern carnivores (Sponheimer & Lee-Thorp, unpubl. data). The larger sample sizes at Equus Cave also allow us to examine isotopic differences within the browsing and grazing groups. Among the browsing taxa *Sylvicapra grimmia* ( $x=38.4\text{‰}$ , s.d.=0.7,  $n=4$ ) is more enriched than *Antidorcas marsupialis* (which is a mixed-feeder today, but ate mostly browse in the past) ( $x=37.2\text{‰}$ , s.d.=2.0,  $n=5$ ), while the grazing *A. bondi* ( $x=35.2\text{‰}$ , s.d.=1.3,  $n=13$ ) has higher  $\delta^{18}\text{O}$  values than *Equus burchelli* ( $x=33.2\text{‰}$ , s.d.=1.5,  $n=17$ ).

Biogenic oxygen isotope patterns are still evident in enamel carbonate from these sites. For these patterns to be of diagenetic origin, diagenesis would have to affect browsers, grazers, and carnivores in different ways. There is no reason to believe that this is the case. The fidelity of enamel carbonate values is likely maintained because relatively little dissolution and reprecipitation or recrystallization occurs in fossil enamel (Sponheimer & Lee-Thorp, 1999). Therefore, isotopic alteration of enamel during fossilization probably results from very slow diffusion processes, as suggested previously (Lee-Thorp, 1989). We are not arguing, however, that absolutely no alteration of isotopic signals has occurred. Small changes should be expected. While this may be a problem when pursuing palaeothermometry indicators, it is of minimal concern here where our purpose is to extract information about the lifeways of fossil taxa. What is important is that the distinctions or relationships between taxa with different lifeways are maintained throughout fossilization. If alteration occurs, but the relationships are maintained, much can be learned from oxygen isotope ratios in fossil enamel carbonate. For example, if herbivores and carnivores are separated by 6‰ prior to fossilization but only 4‰ afterwards, trophic level information remains intact despite diagenesis.

#### Signal patterns

In our data set, browsers are enriched in  $^{18}\text{O}$  compared with grazers. Kohn, Schoeninger & Valley (1996) report similar results for modern fauna from Sibiloi National Park, Kenya, while data from Amboseli National Park, Kenya, seem to indicate the opposite (Bocherens *et al.*, 1996). These contradictory findings require discussion. Bocherens *et al.* (1996) postulate that the enrichment of grazers compared with browsers at Amboseli is due to stomatal closure in  $\text{C}_3$  plants under arid conditions to reduce water loss. Browsers ( $\text{C}_3$  consumers), they maintain, eat plants that undergo less evaporative enrichment than the  $\text{C}_4$  grasses eaten by grazers. A caveat is warranted here. Several researchers have observed that some  $\text{C}_4$  plant species are enriched in heavy isotopes (Sternberg, DeNiro & Johnson, 1984; Ziegler, 1989). However, this may not always be the case given the range of stomatal conductance in savanna plants and the complex water dynamics of the savanna ecosystem. One of the fundamental attributes of tropical and subtropical savannas is strong seasonality of rainfall. When the rainy season ends soils begin to dry out from the surface downward, and thus water is scarce first in the uppermost soil. Therefore, shallow-rooted  $\text{C}_4$  grasses experience water stress while water is still readily available for deeper rooted  $\text{C}_3$  trees. Indeed, evapotranspiration often continues unhindered during the dry season in trees, while it plummets in  $\text{C}_4$  grasses (Goldstein & Sarmiento, 1987). Thus, isotopic enrichment can continue in  $\text{C}_3$  trees while it slows in  $\text{C}_4$  grasses. Moreover,  $\text{C}_4$  plants

can photosynthesize at the same rate as  $\text{C}_3$  plants even with some stomatal restriction (Edwards & Walker, 1983). This also suggests that  $\text{C}_3$  plants might sometimes be enriched compared with  $\text{C}_4$  plants. Clearly, we should invoke  $\text{C}_3/\text{C}_4$  differences with caution when interpreting our mammalian signals until more is known about isotopic variation in plants within the savanna ecosystem.

Why did the Sibiloi and Amboseli studies produce contradictory results? We believe that the apparent contradiction arises because the browser/grazer isotopic distinction has less to do with diet *per se* than with water ingestion as it *relates* to diet. On the whole, grazing taxa drink more than browsers because the shrubs and trees that browsers feed on have roots capable of reaching down to moisture and producing green growth at times when grasses are parched (Goldstein & Sarmiento, 1987; Estes, 1991). Thus, browsers ingest more water with their food than grazers, and their reliance on open water sources is reduced. This is not to say that browsers ingest less water than grazers, but only that their primary water sources are distinct (but see Ambrose, 1991, for a discussion of diet and its relation to water conservation abilities among herbivores). As mentioned above, leaf water is enriched in  $^{18}\text{O}$  compared with meteoric water; therefore, browsers that obtain most of their water from leaves are relatively enriched. This might explain why Bocherens *et al.* (1996) observed no enrichment of their browsing taxa: they happened to sample only browsers/mixed-feeders that drink daily when possible (*Diceros bicornis* and *Loxodonta africana*).

Differences in the oxygen isotope composition of water sources offer plausible explanations for some of the more subtle differences evident within the grazing and browsing groups at Equus Cave. For example, *S. grimmia* seem enriched compared with *A. marsupialis*. Today, *S. grimmia* rarely if ever drinks and *A. marsupialis* drinks when water is available but can go without drinking if necessary. If we are correct, the same pattern prevailed in the past. Amongst the grazers at Equus Cave, equids are depleted compared with *A. bondi*. It is believed, on grounds of dentition, body size, and physiology, that *A. bondi* was primarily a fine, new grass feeder (Brink & Lee-Thorp, 1992). Since it fed on fresh new growth high in water content, it was probably less dependent on standing water than equids. The small extant antelope, *Ourebia ourebi*, is a fine-grass grazer which obtains enough water from its food to minimize its dependence on drinking water (Spinage, 1986; Estes, 1991). It is likely that *A. bondi* filled a similar niche in the past. The relative depletion of equids may also reflect their tendency to consume large quantities of grass stems, the water of which is depleted in heavy isotopes compared with grass leaves (Sternberg, 1989; Ziegler, 1989; Yakir, 1992).

The oxygen isotope composition of mammalian enamel is also affected by the isotopic composition

of oxygen chemically bound in food, i.e., metabolic oxygen. This best explains the low  $\delta^{18}\text{O}$  values of carnivores from Swartkrans. Although some carnivores drink regularly when water is available, their prey's body water can slake their thirst when necessary (Estes, 1991; Bailey, 1993). Therefore, carnivores should have  $\delta^{18}\text{O}$  values similar to grazers or higher. The Swartkrans carnivores, however, are significantly depleted compared with all herbivores. The simplest explanation for this is that animal foods are depleted in  $^{18}\text{O}$ , though evidence that this is the case is limited (but see Tredget *et al.*, 1993).

There are a number of other ecological and physiological factors that we have not discussed that should have important effects on oxygen isotope composition. For example, adaptations to heat stress will also influence oxygen isotope composition. Water vapour lost while panting is isotopically depleted compared with liquid water in sweat (Wong *et al.*, 1988). Consequently, if the oxygen inputs of two animals are isotopically equal, but one pants and the other sweats to lose heat, the former will have higher  $\delta^{18}\text{O}$  values because it loses depleted oxygen while panting. In addition, nocturnal herbivores obtain less enriched water from plant foods (since plant water  $\delta^{18}\text{O}$  peaks at midday), and do not have to cool their bodies significantly. Thus, *ceteris paribus*, nocturnal animals are expected to be depleted in  $^{18}\text{O}$  compared with their diurnal counterparts. Furthermore, some animals may drink frequently in certain environments, while their conspecifics in another area hardly drink at all (Spinage, 1986; Estes, 1991). Hence, oxygen isotope patterns may even vary from region to region. It is not our intention to provide an exhaustive list of all the variables that contribute to mammalian oxygen isotope composition. We believe that we have shown, however, that biology and ecology must be considered when interpreting oxygen isotope data, and conversely, that oxygen isotope data provide information on the biology and ecology of fossil species.

## Conclusion

Our re-examination of the Swartkrans and Equus Cave data reveals several points of interest. First, enamel carbonate  $\delta^{18}\text{O}$  values have not been obscured by diagenesis. This is evident because taxa with different eating/drinking behaviours remain isotopically discrete at both sites. Further, it is clear that drinking behaviour has a dominant effect on mammalian oxygen isotope composition. In addition, these data suggest that diet, too, has a strong affect on  $\delta^{18}\text{O}$  values inasmuch as it determines an animal's drinking behaviour, and because different food such as leaves, stems, and animal flesh are isotopically discrete. Together, these principles explain why some browsers are more enriched in  $^{18}\text{O}$  than grazers, while carnivores are depleted compared with herbivores.

As a result, we must be particularly aware of the scope of modern mammalian behavioural diversity if we are to reliably interpret fossil oxygen data. We should not simply sort fossil fauna into large, gross groupings of grazers, mixed-feeders, and browsers. Among these large divisions there exists an array of dietary and physiological variation. For instance, some grazers eat only grass leaves while others also crop stems; some exist on coarse grasses while others specialize in consuming fresh new growth; and some drink several times per day while others can scarcely be compelled to drink under the most arid conditions. We must bear this diversity in mind when interpreting oxygen isotope data in fossils, as these different behaviours all affect oxygen isotope compositions in different ways.

Such hurdles notwithstanding, it is clear that coupled carbon and oxygen isotope analysis is a promising means of addressing new questions about the lifeways of fossil taxa. This data source should enhance our ability to test hypotheses about competition and niche partitioning between hominid and non-hominid species. Appropriate and sensible application of this dual isotopic tool, however, requires further detailed study of oxygen isotope abundances in carefully chosen fauna from a variety of modern ecosystems.

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## References

- Ambrose, S. H. (1991). Effects of diet, climate and physiology on nitrogen isotope abundances in terrestrial foodwebs. *Journal of Archaeological Science* **18**, 293–217.
- Ayliffe, L. K. & Chivas, A. R. (1990). Oxygen isotope composition of the bone phosphate of Australian kangaroos: potential as a paleoenvironmental recorder. *Geochimica et Cosmochimica Acta* **54**, 2603–2609.
- Bailey, T. N. (1993). *The African Leopard: Ecology and Behavior of a Solitary Felid*. New York: Columbia University Press.
- Bocherens, H., Koch, P., Mariotti, A., Geraads, D. & Jaeger, J. (1996). Isotopic biogeochemistry ( $^{13}\text{C}$ ,  $^{18}\text{O}$ ) of mammalian enamel from African Pleistocene hominid sites. *Palaios* **11**, 306–318.
- Brink, J. S. & Lee-Thorp, J. A. (1992). The feeding niche of an extinct springbok, *Antidorcas bondi*, and its palaeoenvironmental meaning. *South African Journal of Science* **88**, 227–229.
- Bryant, J. D. (1995). *Oxygen isotope systematics in body water and in modern and fossil equid tooth enamel phosphate*. Ph.D. Thesis. Columbia University.

- Bryant, J. D., Luz, B. & Froelich, P. N. (1994). Oxygen isotopic composition of fossil horse tooth phosphate as a record of continental paleoclimate. *Palaeogeography, Palaeoclimatology, Palaeoecology* **107**, 303–316.
- Bryant, J. D., Koch, P. L., Froelich, P. N., Showers, W. J. & Genna, B. J. (1996). Oxygen isotope partitioning between phosphate and carbonate in mammalian apatite. *Geochimica et Cosmochimica Acta* **60**, 5145–5148.
- Cerling, T. E., Harris, J. M., Ambrose, S. H., Leakey, M. G. & Solounias, N. (1997). Dietary and environmental reconstruction with stable isotope analyses of herbivore tooth enamel from the Miocene locality of Fort Ternan, Kenya. *Journal of Human Evolution* **33**, 635–650.
- Cruz-Urbe, K. (1991). Distinguishing hyena from hominid bone accumulations. *Journal of Field Archaeology* **18**, 467–486.
- D'Angela, D. & Longinelli, A. (1990). Oxygen isotopes in living mammal's bone phosphate: further results. *Chemical Geology* **86**, 75–82.
- Dansgaard, W. (1964). Stable isotopes in precipitation. *Tellus* **16**, 436–468.
- Dole, M., Lange, G. A., Rudd, D. P. & Zaukelies, D. A. (1954). Isotopic composition of atmospheric oxygen and nitrogen. *Geochimica et Cosmochimica Acta* **6**, 65–78.
- Dongmann, G., Nurnberg, H. W., Forstel, H. & Wagener, K. (1974). On the enrichment of  $H_2^{18}O$  in the leaves of transpiring plants. *Radiation and Environmental Biophysics* **11**, 41–52.
- Edwards, G. & Walker, D. (1983). *C<sub>3</sub>, C<sub>4</sub>: Mechanisms, and Cellular and Environmental Regulation, of Photosynthesis*. Berkeley, CA: University of California Press.
- Epstein, S., Thompson, P. & Yapp, C. J. (1977). Oxygen and hydrogen isotopic ratios in plant cellulose. *Science* **198**, 1209–1215.
- Estes, R. D. (1991). *The Behavior Guide to African Mammals*. Berkeley: University of California Press.
- Goldstein, G. & Sarmiento, G. (1987). Water relations of trees and grasses and their consequences for the structure of savanna vegetation. In (B. H. Walker, Ed) *Determinants of Tropical Savannas*. Oxford: IRL Press Ltd, pp. 33–45.
- Gonfiantini, R., Gratziu, S. & Tongiorgi, E. (1965). Oxygen isotopic composition of water in leaves. In *Isotopes and Radiation in Soil-Plant-Nutrition studies*. Vienna: International Atomic Energy Commission, pp. 405–410.
- Iacumin, P., Bocherens, H., Mariotti, A. & Longinelli, A. (1996). Oxygen isotope analyses of co-existing carbonate and phosphate in biogenic apatite: a way to monitor diagenetic alteration of bone phosphate? *Earth and Planetary Science Letters* **142**, 1–6.
- Kingston, J. D., Marino, B. D. & Hill, A. (1994). Isotopic evidence for Neogene hominid paleoenvironments in the Kenya rift valley. *Science* **264**, 955–959.
- Klein, R. G., Cruz-Urbe, K. & Beaumont, P. B. (1991). Environmental, ecological, and paleoanthropological implications of the late Pleistocene mammalian fauna from Equus Cave, Northern Cape Province, South Africa. *Quaternary Research* **36**, 94–119.
- Kohn, M. J. (1996). Predicting animal  $\delta^{18}O$ : Accounting for diet and physiological adaptation. *Geochimica et Cosmochimica Acta* **60**, 4811–4829.
- Kohn, M. J., Schoeninger, M. J. & Valley, J. W. (1996). Herbivore tooth oxygen isotope compositions: effects of diet and physiology. *Geochimica et Cosmochimica Acta* **60**, 3889–3896.
- Kroopnick, P. & Craig, H. (1972). Atmospheric oxygen: isotopic composition and solubility fractionation. *Science* **175**, 54–55.
- Lee-Thorp, J. A. (1989). *Stable carbon isotopes in deep time: the diets of fossil fauna and hominids*. Ph.D. Thesis. University of Cape Town.
- Lee-Thorp, J. A. (in press). Preservation of biogenic carbon isotope signals in Plio-Pleistocene bone and tooth mineral. In (S. H. Ambrose & M. A. Katzenberg, Eds) *Close to the Bone: Biogeochemistry and Paleodietary Analysis*. NY: Plenum Press.
- Lee-Thorp, J. A. & van der Merwe, N. J. (1987). Carbon isotope analysis of fossil bone apatite. *South African Journal of Science* **83**, 712–715.
- Lee-Thorp, J. A. & Beaumont, P. B. (1995). Vegetation and seasonality shifts during the late Quaternary deduced from  $^{13}C/^{12}C$  ratios of grazers at Equus Cave, South Africa. *Quaternary Research* **43**, 426–432.
- Lee-Thorp, J. A., van der Merwe, N. J. & Brain, C. K. (1994). Diet of *Australopithecus robustus* at Swartkrans from stable carbon isotopic analysis. *Journal of Human Evolution* **27**, 361–372.
- Lee-Thorp, J. A., Manning, L. & Sponheimer, M. (1997). Exploring problems and opportunities offered by down-scaling sample sizes for carbon isotope analyses of fossils. *Bulletin de la Société Géologique de France* **168**, 767–773.
- Longinelli, A. (1984). Oxygen isotopes in mammal bone phosphate: a new tool for paleohydrological and paleoclimatological research? *Geochimica et Cosmochimica Acta* **48**, 385–390.
- Luz, B., Kolodny, Y. & Horowitz, M. (1984). Fractionation of oxygen isotopes between mammalian bone-phosphate and environmental water. *Geochimica et Cosmochimica Acta* **48**, 1689–1693.
- Luz, B., Cormie, A. B. & Schwarcz, H. P. (1990). Oxygen isotope variations in phosphate of deer bones. *Geochimica et Cosmochimica Acta* **54**, 1723–1728.
- Quade, J., Cerling, T. E., Barry, J. C., Morgan, M. E., Pilbeam, D. R., Chivas, A. R., Lee-Thorp, J. A. & van der Merwe, N. J. (1992). A 16-Ma record of paleodiet using carbon and oxygen isotopes in fossil teeth from Pakistan. *Chemical Geology (Isotope Geoscience Section)* **94**, 183–192.
- Reed, K. (1996). *The paleoecology of Makapansgat and other African Plio-Pleistocene hominid localities*. Ph.D. Thesis. State University of New York at Stony Brook.
- Scott, L. (1987). Pollen analysis of hyena coprolites and sediments from Equus Cave, Taung, Southern Kalahari (South Africa). *Quaternary Research* **28**, 144–156.
- Spinage, C. A. (1986). *The Natural History of Antelopes*. Beckenham: Croom Helm Publishers.
- Sponheimer, M. & Lee-Thorp, J. A. (1999). Alteration of enamel carbonate environments during fossilisation. *Journal of Archaeological Science* **26**, 143–150.
- Sternberg, L. S. L. (1989). Oxygen and hydrogen isotope ratios in plant cellulose: mechanisms and applications. In (P. W. Rundel, J. R. Ehleringer & K. A. Nagy, Eds) *Stable Isotopes in Ecological Research*. New York: Springer Verlag, pp. 124–143.
- Sternberg, L., DeNiro, M. J. & Johnson, H. B. (1984). Isotope ratios of cellulose from plants having different photosynthetic pathways. *Plant Physiology* **74**, 557–561.
- Tredget, E. E., Forsyth, N., Uji-Friendland, A., Chambers, M., Ghahary, A., Scott, P. G., Hogg, A. M. & Burke, J. F. (1993). Gas chromatography mass spectrometry determination of oxygen-18 in oxygen-18 labelled 4-hydroxyproline for measurement of collagen synthesis and intracellular degradation. *Journal of Chromatography-Biomedical Applications* **612**, 7–19.
- Vogel, J. (1985). Further attempts at dating the Taung tufas. In (E. Delson, Ed.) *Ancestors: The Hard Evidence*. New York: Alan R. Liss, pp. 189–194.
- Vrba, E. S. (1985). Ecological and adaptive changes associated with early hominid evolution. In (E. Delson, Ed.) *Ancestors: The Hard Evidence*. New York: Alan R. Liss, pp. 63–71.
- Wang, Y. & Cerling, T. (1994). A model of fossil tooth and bone diagenesis: implications for paleodiet reconstruction from stable isotopes. *Palaeogeography, Palaeoclimatology, Palaeoecology* **107**, 281–289.
- Watson, V. (1993). Composition of the Swartkrans bone accumulations, in terms of skeletal parts and animals represented. In (C. K. Brain, Ed.) *Swartkrans: A Cave's Chronicle of Early Man*. Pretoria: Transvaal Museum Monograph, pp. 35–74.
- Wong, W. W., Cochran, W. J., Klish, W. J., Smith, E. O., Lee, L. S. & Klein, P. D. (1988). In vivo isotope fractionation factors and the measurement of deuterium and oxygen-18-dilution spaces from plasma, urine, saliva, respiratory water vapor, and carbon dioxide. *American Journal of Clinical Nutrition* **47**, 1–6.
- Yakir, D. (1992). Variations in the natural abundances of oxygen-18 and deuterium in plant carbohydrates. *Plant, Cell, and Environment* **15**, 1005–1020.
- Ziegler, H. (1989). Hydrogen isotope fractionation in plant tissues. In (P. W. Rundel, J. R. Ehleringer & K. A. Nagy, Eds) *Stable Isotopes in Ecological Research*. New York: Springer Verlag, pp. 105–123.