Using stable isotopes as an additional tool to understand ancient human environments

Utilización de isótopos estables como una herramienta adicional para el conocimiento de ambientes humanos del pasado

Robert S. Feranec

INTRODUCTION

Ecological dynamics among fauna in ecosystems is contingent upon prior biotic and abiotic interaction. Therefore, to get a complete understanding of ecology in modern ecosystems as well as adequate predictions for ecology in future ecosystems, it is important to obtain an accurate idea of relationships among ancient animals and the environments in which they lived. Traditionally, paleontologists have focused on examining morphology and comparisons to modern analogs to address the ecology of ancient animals. One recent technique that has proven useful to examine ecology in ancient animals, including humans, is through examination of stable isotope values found in tooth enamel (BOCHERENS, 2003; BOCHERENS *et al.*, 1996; CERLING *et al.*, 1998; DRUCKER *et al.*, 2003; KOCH, 1998; KOHN *et al.*, 2005; QUADE *et al.*, 1992). Isotopic analyses can reveal information about resource use and partitioning among fauna, being able to determine diet and habitat use, for example. In this lecture I will present work done in collaboration with Nuria Garcia Garcia and others on Pleistocene sites in Spain. We have analyzed stable carbon and oxygen isotope values found in the tooth enamel of mammalian carnivores and herbivores with the overall aim of understanding the ecological context in which ancient humans lived during the Pleistocene in Spain. We will address: (1) whether stable isotope values permit identification of resource use and partitioning among taxa? (2) If resource use and partitioning are determined, do the results corroborate the ecology predicted by other methodologies (e.g., morphology)? While isotopic analyses generally have concentrated on examining herbivorous taxa where there is a mixture of plants using either the C_3 or C_4 photosynthetic pathways, recent studies, including my own at Yellowstone National Park, have shown resource use and partitioning in areas containing only C_3 plants (BOCHERENS, 2003; BOCHERENS *et al.*, 1996, 2004; DE NIRO & EPSTEIN, 1978; FERANEC, 2007; FERANEC & MACFADDEN, 2006; KOCH *et al.*, 1998, 2005; MACFADDEN & CERLING, 1996; VOGEL, 1978). The analysis of stable isotopes from ancient mammals provides another technique to determine the autecological and synecological relationships among fauna in ancient ecosystems enabling an accurate portrayal of the setting in which humans lived.

BACKGROUND

ISOTOPES IN PALEOECOLOGY

Isotopic results are expressed in the standard δ-notation: $X = [(R_{sample}/R_{standard}) - 1] \times 1000$, where X is the $\delta^{13}C$ or $\delta^{18}O$ value, and $R = {}^{13}C/{}^{12}C$ and ${}^{18}O/{}^{16}O$, respectively. The $\delta^{13}C$ and $\delta^{18}O$ values are reported relative to the VPDB standard. Animals that feed on dif-

---

Curator of Vertebrate Paleontology, New York State Museum, Albany, NY 12230

ferent kinds of plants will reflect the carbon isotope value of their food in their tissues (DE NIRO & EPSTEIN, 1978; TIESZEN *et al*., 1979; VOGEL, 1978). Studies utilizing differences in the carbon isotope values from mammals generally focus on communities containing a mixture of C_3 and C_4 plants, which enables taxa to be distinguished based on the predominant forage included in the diet, where grass feeders generally display more positive (C_4) $\delta^{13}C$ values, and browsers display more negative (C_3) $\delta^{13}C$ values (CERLING *et al*., 1998; KOCH *et al*., 1998; KOHN *et al*., 2005; MACFADDEND & CERLING, 1996; TIESZEN, 1994; VOGEL, 1978).

Based on previous studies and where Spain is geographically situated, it has been shown that the late Pleistocene and modern flora is dominated by C_3 plants (COLLINS & JONES, 1985; FLYNN *et al*., 1984; PAMLQVIST *et al*., 2003; SAGE *et al*., 1999). Different processes, besides photosynthetic pathway, such as variation in light intensity, temperature, nutrient availability, and water stress, can produce measurable variation in the $\delta^{13}C$ value in C_3 plants (BOCHERENS, 2003; EHLERINGER & MONSON, 1993; FARQUHAR *et al*., 1989; HEATON, 1999; KOCH, 1998; O'LEARY, 1988). C_3 plants typically have more negative values in closed, forested habitats; while in more open, drier habitats more positive isotope values are typical (BOCHERENS, 2003; CERLING *et al*., 2004; EHLERINGER & MONSON, 1993; FARQUHAR *et al*., 1989; HEATON, 1999; O'LEARY *et al*., 1992; VAN DER MERWE & MEDINA, 1991). Animals that eat these C_3 plants will reflect the carbon isotope ratio ingested. While herbivores do reflect the carbon isotope values of plants ingested, the $\delta^{13}C$ value of the tooth enamel is enriched by a consistent amount, about +14.0‰ for medium to large-bodied mammals (CERLING & HARRIS, 1999; PASSEY *et al*., 2005). Assuming a fractionation from plant material to tooth enamel at +14.0‰, extant taxa that feed solely on modern C_3 plants will display enamel carbon isotope values between 21.0‰ and 8.0‰. In Pleistocene taxa, a diet of pure C3 plants would range from 19.5‰ to –6.5‰. The diffe-

rence between Pleistocene and modern is due to fossil fuel burning over the last few hundred years. Isotope values more positive than –6.5‰ would imply incorporation of either C_4 or CAM plants into the diet (CERLING & HARRIS, 1999; CERLING *et al*., 2004; MACFADDEN & CERLING, 1996).

Similar to the herbivores, carnivore carbon isotope values within tooth enamel will reflect the isotopic value of prey. Based on the portion of prey ingested (e.g. muscle, organs), and the fractionation of isotopic values between the food and the tooth enamel, carnivores display similar carbon isotope values as is found in the tooth enamel of their prey (BOCHERENS *et al*., 1994; GROCKE, 1997; KOHN *et al*., 2005; LEE-THORP *et al*., 2000). For example, a specialist carnivore that consumes only herbivores whose tooth enamel $\delta^{13}C$ value is -10‰, will have a tooth enamel $\delta^{13}C$ value of 10‰.

OXYGEN ISOTOPES IN MAMMALS

The oxygen isotopes in mammal tooth enamel depends on the isotopic composition of ingested water, the consistent fractionation of oxygen isotopes between body water and the tooth enamel, and the metabolism of the particular animal (KOCH *et al*., 1989; KOHN, 1996; KOHN *et al*., 1996, 1998; LAND *et al*., 1980; LONGINELLI, 1984; LUZ & KOLODNY, 1985; LUZ *et al*., 1984). Mammals ingest water from two sources, either through drinking meteoric water or from what they consume. Meteoric water is affected by climatic influences such as temperature and humidity, such that $\delta^{18}O$ values are more positive where and when it is warmer and more negative where and when it is colder (DANSGAARD, 1964; DANSGAARD *et al*., 1982; FRICKE & O'NEIL, 1996; KOHN & WELKER, 2005; ROZANSKI *et al*., 1992, 1993).

METHODS

To collect tooth enamel, sampling involved drilling about 1020 mg of pristine enamel powder off the tooth along a non-occlusal surface parallel to the growth axis and across its

entire length using a 0.5 mm inverted cone carbide drill bit and a variable speed Dremel™ rotary tool. The powder was first collected and treated with 30% hydrogen peroxide for 24 hours to remove organics. The hydrogen peroxide was then decanted and the enamel powder was then washed with distilled water, and soaked in 0.1N acetic acid for another 24 hours to remove any diagenetic carbonate. The following day the acetic acid was decanted and the enamel powder was washed with distilled water and dried.

After treatment, the samples were analyzed using an ISOCARB automated carbonate preparation system attached to a Micromass Optima gas source mass spectrometer within the Geology Department at the University of California, Davis. The ~ 1 mg samples were dissolved in 100% phosphoric acid at 90ºC to create CO_2. Mean differences among species within localities were compared by ANOVA and post hoc Tukey HSD tests, which are similar to t-tests, but take into account multiple comparisons. Statistical analyses were run on JMP IN 5 for students, with significance set at $p < 0.05$. Precision was 0.1‰ for $\delta^{13}C$ and 0.1‰ for $\delta^{18}O$.

RESULTS AND DISCUSSION

So far we have analyzed three different ecosystems from the middle to late Pleistocene in Spain including: middle Pleistocene deposits from the Sierra de Atapuerca, the late Pleistocene deposits of Valdegoba Cave, and the late Pleistocene deposits of Pinilla del Valle. At each locality, tooth enamel from both carnivores and herbivores was analyzed. Results from each locality show that there are significant differences in $\delta^{13}C$ values implying the use of different resources within each guild.

Specifically, within the sampled deposits in the Sierra de Atapuerca, Fallow deer (*Dama dama*) isotope values are significantly different from Red deer (*Cervus elaphus*) and horses (*Equus* sp.) and show that this species likely did not eat in open environments. Red

deer and horses show similar feeding strategies with less negative carbon values implying use of open environments. For the carnivores, carbon isotope values for *Ursus deningeri* are significantly different from either lions (*Panthera leo*) or canids (*Vulpes vulpes*) and support the contention that this species is herbivorous. Special metabolic mechanisms involved in hibernation in *Ursus deningeri* might also have influenced its isotope values. The carbon isotope values of remaining carnivores were similar and suggest that each was an opportunistic carnivore, eating a wide variety of prey items.

At Valdegoba Cave, *Capra pyrenaica* displayed the most positive value for herbivores, while *Canis lupus* was the most positive carnivore. The most negative herbivore value was displayed by a grassland indicator species, *Stephanorhinus hemitoechus*, while the negative value for *S. hemitoechus* is not unusual for eating of a diet of C_3 grasses. *Ursus spelaeus* had the most negative values for the carnivores. All the carnivores at Valdegoba cave, except *Canis lupus*, are significantly more negative than the herbivores. This may result from the carnivores having rarely used the analyzed herbivores as primary prey, or isotopic fractionation differences based on the portion of food ingested. Species like *Vulpes vulpes* and *Lynx pardinus* likely consumed smaller mammals that were not sampled for this study. Further, the $\delta^{13}C$ values of *Ursus arctos* and *Ursus spelaeus* species imply resource partitioning, with more negative $\delta^{13}C$ values on cave bears, *U. spelaeus*. This is consistent with "classical" paleontological interpretations based on anatomical traits.

At Pinilla del Valle, similar to the other localities, individuals within a particular species generally group together. Fallow deer (*Dama dama*) displayed the most positive $\delta^{13}C$ values, while hyenas (*Crocuta crocuta*) displayed the most negative values. Brown bears (*Ursus arctos*) and pigs (*Sus scrofa*) show great variability in values suggesting ecological generalism or opportunism. Curiously, there are 7 individuals that show values indicative of consuming a large percen-

tage (up to 100%) of C_4 grasses. This result is unexpected, as the flora in Spain has been shown to be nearly 100% C_3 plants. Further analyses are currently underway to test these results to confirm or reject the presence of C_4 plants at Pinilla during the late Pleistocene.

CONCLUSIONS

The results gathered from the three localities show that it is possible to understand the ecology of taxa within ancient ecosystems through the examination of stable carbon and oxygen isotope values, even those ecosystems dominated by C_3 plants. In general, the isotopic results corroborate conclusions from previous studies that analyzed morphology or comparisons to modern analogs, but in some circumstances the isotopic results imply a different ecology than previous suggestions. Because of these analyses at the three different localities, a framework has been created to understand the ecosystems in which ancient humans lived over the Pleistocene in Spain.

REFERENCES

BOCHERENS, H. 2003. Isotopic biogeochemistry and the paleoecology of the mammoth steppe fauna. *Deinsea*, **9**: 57–76.

BOCHERENS, H., ARGANT, A., ARGANT, J., BILLIOU, D., CREGUT-BONNOURE, E., DONAT-AYACHE, B., PHILIPPE, M. & THINON, M. 2004. Diet reconstruction of ancient brown bears (*Ursus arctos*) from Mont Ventoux (France) using bone collagen stable isotope biogeochemistry. *Canadian Journal of Zoology*, **82**: 576–586.

BOCHERENS, H., FIZET, M. & MARIOTTI, A. 1994. Diet, physiology and ecology of fossil mammals as inferred from stable carbon and nitrogen isotope biogeochemistry: implications for Pleistocene bears. *Palaeogeography Palaeoclimatology Palaeoecology,* **107**: 213–225.

BOCHERENS, H., KOCH, P.L., MARIOTTI, A., GERAADS, D. & JAEGER, J.-J. 1996. Isotopic biogeochemistry (^{13}C, ^{18}O) of mammalian enamel from African Pleistocene hominid sites. *Palaios*, **11**: 306–318.

CERLING, T.E. & HARRIS, J.M. 1999. Carbon isotope fractionation between diet and bioapatite in ungulate mammals and implications for ecological and paleoecological studies. *Oecologia,* **120**: 347–363.

CERLING, T.E., HARRIS, J.M. & MACFADDEN, B.J. 1998. Carbon isotopes, diets of North American equids, and the evolution of North American C4 grasslands.

In: Stable Isotopes, GRIFFITHS, H. Ed. pp. 363–379. BIOS Scientific Publishers Ltd., Oxford.

CERLING, T.E., HART, J.A. & HART, T.B. 2004. Stable isotope ecology in the Ituri Forest. *Oecologia,* **138**: 5–12.

COLLINS, R.P. & JONES, M.B. 1985. The influence of climatic factors on the distribution of C_4 species in Europe. *Vegetatio*, **64**: 121–129.

DANSGAARD, W. 1964. Stable isotopes in precipitation. Tellus, **16**: 436–468.

DANSGAARD, W., CLAUSEN, H.B., GUNDESTRUP, N., HAMMER, C.U., JOHNSON, S.F., KRISTINSDOTTIR, P.M. & REEH, N. 1982. A new Greenland deep ice core. *Science*, **218**: 1273–1277.

DE NIRO, M.J. & EPSTEIN, S. 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta*, **42**: 495–506.

DRUCKER, D., BOCHERENS, H., BRIDAULT, A. & BILLIOU, D. 2003. Carbon and nitrogen isotopic composition of red deer (*Cervus elaphus*) collagen as a tool for tracking palaeoenvironmental change during the Late-Glacial and Early Holocene in the northern Jura (France). *Palaeogeography Palaeoclimatology Palaeoecology*, **195**: 375–388.

EHLERINGER, J.R. & MONSON, R.K. 1993. Evolutionary and ecological aspects of photosynthetic pathway variation. *Annual Review of Ecology and Systematics*, **24**: 411–39.

FARQUHAR, G.D., EHLERINGER, J.R. & HUBICK, K.T. 1989. Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology*, **40**: 503–37.

FERANEC, R.S. 2007. Stable carbon isotope values reveal evidence of resource partitioning among ungulates from modern C_3-dominated ecosystems in North America. *Palaeogeography Palaeoclimatology Palaeoecology*, **252**: 575–585.

FERANEC, R.S. & MACFADDEN, B.J. 2006. Isotopic discrimination of resource partitioning among ungulates in C_3-dominated communities from the Miocene of Florida and California. *Paleobiology*, **32**: 191–205.

FLYNN, J.J., MACFADDEN, B.J. & MCKENNA, M.C. 1984. Land-mammal ages, faunal heterochrony, and temporal resolution in Cenozoic terrestrial sequences. *Journal of Geology*, **92**: 687–705.

FRICKE, H.C. & O'NEIL, J.R. 1996. Inter- and intra-tooth variation in the oxygen isotope composition of mammalian tooth enamel phosphate: implications for palaeoclimatological and palaeobiological research. *Palaeogeography Palaeoclimatology Palaeoecology*, **126**: 9199.

GROCKE, D.R. 1997. Stable-isotope studies on the collagenic and hydroxylapatite components of fossils: palaeoecological implications. *Lethaia*, **30**: 65–78.

HEATON, T.H.E. 1999. Spatial, species, and temporal variations in the ^{13}C/^{12}C ratios of C_3 plants: implications for paleodiet studie. *Journal of Archaeological Science*, **26**: 637–649.

KOCH, P.L. 1998. Isotopic reconstruction of past continental environments. *Annual Review of Earth and Planetary Science*, **26**: 573–613.

KOCH, P.L., FISHER, D.C. & DETTMAN, D. 1989. Oxygen isotope variation in the tusks of extinct proboscide-

ans: A measure of season of death and seasonality. *Geology*, **17**: 515–519.

KOCH, P.L., HOPPE, K.A. & WEBB, S.D. 1998. The isotopic ecology of late Pleistocene mammals in North America Part 1. Florida. *Chemical Geology*, **152**: 119–138.

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. & WELKER, J. M. 2005. On the temperature correlation of $\delta^{18}O$ in modern precipitation. *Earth and Planetary Science Letters*, **231**: 87–96.

KOHN, M.J., MCKAY, M.P. & KNIGHT, J.L. 2005. Dining in the Pleistocene-Who's on the menu? *Geology*, **33**: 649–652.

KOHN, M.J., SCHOENINGER, M.J. & VALLEY, J.W. 1996. Herbivore tooth oxygen isotope compositions: Effect of diet and physiology. *Geochimica et Cosmochimica Acta*, **60**: 3889–3896.

— 1998. Variability on oxygen isotope compositions of herbivore teeth: Reflections of seasonality or developmental physiology. *Chemical Geology*, **152**: 97–112.

LAND, L.S., LUNDELIUS, E.L. & VALASTRO, S. 1980. Isotopic ecology of deer bones. *Palaeogeography, Palaeoclimatology, Palaeoecology,* **32**: 143–151.

LEE-THORP, J.A., THACKERAY, J.F. & VAN DER MERWE, N.A. 2000. The hunters and the hunted revisited. *Journal of Human Evolution*, **39**: 565–576.

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. 1985. Oxygen isotope variations in phosphate of biogenic apatites, IV. Mammal teeth and bones. *Earth and Planetary Science Letters*, **75**: 29–36.

LUZ, B., KOLODNY, Y. & HOROWITZ, M. 1984. Fractionation of oxygen isotopes between mammalian bone-phosphate and environmental drinking water. *Geochimica et Cosmochimica Acta*, **48**: 1689–1693.

MACFADDEN, B.J. & CERLING, T.E. 1996. Mammalian herbivore communities, ancient feeding ecology, and carbon isotopes; a 10 million-year sequence from the Neogene of Florida. *Journal of Vertebrate Paleontology*, **16**: 103–115.

O'LEARY, M.H. 1988. Carbon isotopes in photosynthesis. *BioScience,* **38**: 328–336.

O'LEARY, M.H., MADHAVAN, S. & PANETH, P. 1992. Physical and chemical basis of carbon isotope fractionation in plants. *Plant, Cell and Environment*, **15**: 1099–1104.

PAMLQVIST, P., GROCKE, D.R., ARRIBAS, A. & FARINA, R. A. 2003. Paleoecological reconstruction of a lower Pleistocene large mammal community using biogeochemical ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, Sr:Zn) and ecomorphological approaches. *Paleobiology*, **29**: 205–229.

PASSEY, B.H., ROBINSON, T.F., AYLIFFE, L.K., CERLING, T. E., SPONHEIMER, M., DEARING, M.-D., ROEDER, B.L. & EHLERINGER, J.R. 2005. Carbon isotope fractionation between diet, breath CO_2, and bioapatite in different mammals. *Journal of Archaeological Science*, **32**: 1459–1470.

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 Geosciences Section)*, **94**: 183–192.

ROZANSKI, K., ARAGUAS-ARAGUAS, L. & GONFIANTINI, R. 1992. Relation between long-term trends of Oxygen-18 isotope composition of precipitation and climate. *Science*, **258**: 9810–985.

— 1993. Isotopic patterns in modern global precipitation. *Geophysical Monographs*, **78**: 1–36.

SAGE, R.F., WEDIN, D.A. & LI, M. 1999. *The biogeography of C_4 photosynthesis: patterns and controlling factors*. Academic Press, New York.

TIESZEN, L.L. 1994. Stable isotopes on the plains: vegetation analyses and diet determinations. *In: Skeletal biology in the Great Plains: migration, warfare, health, and subsistence.* OWSLEY, D.W. & JANTZ, R.L. Eds. Smithsonian Institution Press, Washington D.C.

TIESZEN, L.L., SENYIMBA, M.M., IMBAMBA, S.K. & TROUGHTON, J.H. 1979. The distribution of C_3 and C_4 grasses and carbon isotope discrimination along an altitudinal and moisture gradient in Kenya. *Oecologia*, **37**: 337–350.

VAN DER MERWE, N.A. & MEDINA, E. 1991. The canopy effect, carbon isotope ratios and foodwebs in Amazonia. *Journal of Archaeological Science*, **18**: 249–259.

VOGEL, J.C. 1978. Isotopic assessment of the dietary habits of ungulates. *South African Journal of Science*, **74**: 298–301.