

**Student questions: Thure Cerling colloquium on “HAIR - History of Animals using Isotope Records”**

3/30/16

Question 1: What allows C3 and C4 plants to be identifiable in such a wide variety of different animals and tissues?

*This gets into the details of isotope incorporation. Both pathways use the enzyme “Rubisco” in the CO<sub>2</sub> uptake from the atmosphere. C<sub>3</sub> plants do this in an “open” system where the fractionation can be fully expressed; C<sub>4</sub> plants do this in a “closed” system whereby all carbon-atoms are incorporated into the plant tissues.*

Question 2: Apart from what was mentioned during the talk, what applications do you think isotope records could have in the future?

*The cross-section between the geological and ecological sciences is very fertile – I see a lot of increased interaction between these two disciplines that formerly did not “talk” to each other very much.*

Question 1: If you control the diet of an animal to only eat C4 meat, I'm assuming it would look like it was eating C4 plants, so how would you be able to tell if it was eating plants or meats during that time?

*We will be looking at other isotope systems that have a “trophic” effect. Usually people use the nitrogen isotope system ( $\delta^{15}N$ ), and that works very well when amino acids (collagen, hair) are preserved. For fossils, this is often not the case. People are exploring the use of calcium and magnesium isotopes in fossils to pursue this problem.*

Question 2: Is the claim that elephants have been historically (prior to 100,000 years ago) grazers substantiated by isotopes as well?

*Very much so – the isotope evidence is the strongest evidence for this.*

Question 1: You mentioned that a dean shut down one of your projects and it was a short-sighted move. What results were you expecting from that experiment?

*We were conducting “feeding trials” with large mammals – whereby we could change the diet from “C<sub>3</sub>-” to “C<sub>4</sub>-”. So we had to end the experiments pre-maturely. We wanted to see how the different digestive strategies compared (e.g., ruminants versus non-ruminants). We completed our first major trial and learned how to do it correctly; but then had to stop the project in the second feeding trial.*

Question 2: What data were you able to gather from the elephant hair growth rate? I didn't completely understand that section.

*The important thing was to establish the individual growth rates. Then we have an exact “time-line” for each hair and so can construct a detailed diet history. One thing we found out was that the growth rate for hairs varies by about 10%, but that the growth rate for each hair is constant over time. (Each hair is constant, but different hairs have different growth rates).*

Question 1: Could the air you breathe affect the isotopes in your hair? For example, would the amount of carbon dioxide in the air show in your hair?

*If the  $\delta^{13}\text{C}$  of the air would differ, then the  $\delta^{13}\text{C}$  of the plants would also change – and so your hair would reflect that difference. This is important because humans have changed the  $\delta^{13}\text{C}$  of the atmosphere by about 2‰ in the past 200 years. So that is a correction that we have to consider when compared modern to archaeological or fossil materials.*

Question 2: Can you measure isotopes related to consumption in dyed or bleached hair?

*The carbon concentration does not change due to bleaching or dying – so we are able to work with bleached and dyed hair. Oils or grease can make a difference, so we clean the samples prior to analysis.*

Question 1: Are there published papers on the mathematical methods you described in the presentation?

*Yes. Oecologia (Cerling et al., 2007) and Remien et al (2014).*

*Cerling, TE, LK Ayliffe, MD Dearing, JR Ehleringer, BH Passey, DW Podlesak, A–M. Torregrossa, AG West (2007) Determining biological tissue turnover using stable isotopes: the reaction progress variable. Oecologia 151:175–189.*

*Remien CH, Adler FR, Chesson LA, Valenzuela L, Ehleringer JR, Cerling TE (2014) Deconvolution of isotope signals from bundles of multiple hairs. Oecologia 175: 781-789. doi 10.1007/s00442-014-2945-3*

Question 2: How long ago did the change in elephants diets begin?

*Not sure when the major diet change took place. Probably in the past 100,000 years. This is a major research question that we are working on.*

Question 1: What is the method used for measuring and identifying the isotope in an object?

*Stable isotope mass spectrometry. Zach Sharp wrote a good book on this topic – Stable Isotope Geochemistry.*

Question 2: Are there any side effects when isotope consumption is changed by drinking water or eating different food? If yes, do they effect the individual directly or will affect the next generation of this individual?

*No. These are naturally occurring isotopes. The difference in isotope ratios is very small.*

Question 1: Do either C<sub>3</sub> or C<sub>4</sub> have any difference in health benefits or changes to the organism that eats them?

*Not if the chemical compounds are the same (e.g., sugar produced by a C<sub>3</sub> plant is identical in all respects, except the isotope ratio, to that produced by a C<sub>4</sub> plant).*

Question 2: It seems from the food examples that C<sub>4</sub> is less nutritious than C<sub>3</sub>, is that the case?

*It is true that fruits, bean, and vegetables are C<sub>3</sub> plants and that they are more nutritious. A purely C<sub>4</sub> diet, for humans, would be lacking in certain ways.*

Question 1: Do you think that the technology used in your work will become more affordable in the coming years so that police departments nationwide will be able to make it more commonplace in forensic investigations?

*It is still pretty affordable – certainly compared to the cost 20 years ago. This is a societal choice – should we invest properly in our teachers and police. We obviously value professional basketball players far more than teachers or police.*

Question 2: Was Lewis the Elephant stopped from his habit of agricultural night raids or was he allowed to continue out of respect for his intelligent way of doing so?

*He was killed by poachers when he was “too slow” on one of his trips.*

Question 1: Have you found any cases of a sample having beat your measurements where the results are completely inconclusive?

*That depends on the question – if you ask the wrong question you can often get inconclusive answers. Sometimes, based on what you learn, you need to then ask a different question.*

Question 2: Do you believe the rigor of this technique of assisting in cold cases can be ramped up to the point you know not only the region a person has been but the exact state or even city?

*No – but you may be able to eliminate large portions of the country and narrow it down to a manageable region.*

Question 1: Do you ever track smaller animals or is it always larger ones like elephants and horses? --like birds or rodents?

*We have done work on small animals – for example insects that have been in illegal shipments to try to determine where the containers may have been from originally (a different place than where it was claimed to be).*

Question 2: How does hair sampling differ from getting blood work done since both can detect things in your body and what you ate?

*Hair can be collected non-invasively and can be preserved for decades afterwards. Blood requires special handling and storage. For example, hair on a comb can be used for forensic studies.*

Question 1: Do any outside factors, such as pollution, hair products, UV radiation, affect the hair samples?

*The factors mentioned above do not affect the isotopes of C, H, N, O, S. But they do affect some isotopes such as  $^{87}\text{Sr}/^{86}\text{Sr}$  (dirt).*

Question 2: Without shaving and letting fresh hair to grow each day, do you know exactly which day a specific isotope was formed? Or do you just estimate using growth rate and length?

*You have to determine the growth rate. We discuss that in our paper in Chemical Geology and in PNAS (2009).*

*Cerling TE, G Wittemyer, JR Ehleringer, CH Remien, I Douglas–Hamilton (2009) History of Animals using Isotope Records (HAIR): A 6–year dietary history of one family of African elephants. Proceedings of the National Academy of Science 106: 8093–8100.*

*Wittemyer, G, TE Cerling, I Douglas–Hamilton (2009) Establishing longitudinal diet chronologies from isotopic profiles in serially collected animal tissues: An example using tail hairs from African elephants. Chemical Geology 267: 3–11.*

Question 1: During the talks you showed how your hair testing could be used for forensic police work; can it also be used for forensic anthropology or is there a time limit that the hair sample is viable?

*It has been used on Egyptian and Andean mummies, and mammoth hair back to more than 50,000 years ago. As long as the hair persists, it could be used.*

Question 2: Is it possible that any of the tests you do would be misread if someone eats food that comes from a source trucked all over the country? Like if someone were to only eat at a Dunkin' Donuts?

*Our geographic assignments are using  $\delta\text{D}$  and  $\delta^{18}\text{O}$  – these are derived primarily from drinking water. However, some of the water in humans is “metabolic water” – perhaps 20% and there is a slight effect that we see when comparing modern Americans to indigenous peoples (we used museum collections to see if there was a food globalization effect on the isotopes).*

*Thompson AH, LA Chesson, DW Podlesak, TE Cerling, JR Ehleringer (2010) Stable isotope analysis of modern human hair: An Asian geographic transect. American Journal of Physical Anthropology 141: 440–451*

*Bowen GJ, JR Ehleringer, L Chesson, A Thompson, D Podlesak, TE Cerling (2009) Dietary and physiological controls on the hydrogen and oxygen isotope ratios of hair from mid–20<sup>th</sup> century indigenous populations. American Jour. Phys. Anthro. 139: 494–504.*

*Ehleringer, JR, GJ Bowen, LA Chesson, AG West, DW Podlesak, TE Cerling (2008) Hydrogen and oxygen isotope ratios in human hair are related to geography. Proceedings of the National Academy of Sciences 105: 2788–1793.*

Question 1: During hair analysis, from my understanding the length of the hair gives a time line on where a person might be. Do you use the average growth rate for hair between male and females, or is the growth rate too varied to be reliable to estimate a proper timeline?

*There is a slight difference between male and females, but not enough to make a difference for the questions that we are pursuing.*

Question 2: Besides using isotopes and hair to find missing persons, are there any other useful applications that exist, or are being worked on?

*Many many! Last week there was a story in the papers about using stable isotopes to figure out where cocaine was coming from. That methodology was worked out at the University of Utah.*

---

Question 1: How far back in time have we gone on the history of animals using isotope records?

*At least 100 million years – but you have to ask questions that can be answered back then. One of the first studies of isotopes was determining the temperature of seawater, using marine animals known as ammonites.*

Question 2: We can see changes to animals in evolution from the past, but is there any way to see what evolution will do to some animals in the future? (what changes if any)

*That is difficult to answer without being more specific.*

---

Question 1: Are there plans to increase the sample database across the country or internationally?

*Yes. We are doing this at a variety of scales and questions – plants, animals, waters, etc. There are some interesting papers on many related topics. In the next Annual Reviews of Earth and Planetary Sciences, we will have a paper on stable isotope forensic applications.*

Question 2: Do the isotope signatures ever change at specific localities?

*Yes – seasonal changes are observed so you can tell summer rainfall from winter rainfall for  $\delta D$  and  $\delta^{18}O$ . Zach Sharps book on Stable Isotope Geochemistry discusses this.*

---

Question 1: What causes the different isotopes of hydrogen and oxygen to appear in the water from different locations?

*The principle controlling factor is temperature. See Zach Sharp's book on Stable Isotope Geochemistry.*

Question 2: Is it possible to trace human evolution as a function of diet using the isotope method?

*Yes – we did this on the long time scale in our PNAS paper.*

*Cerling TE, FK Manthi, E Mbua, LN Leakey, MG Leakey, RE Leakey, FH Brown, FE Grine, JA Hart, P Kaleme, H Roche, KT Uno, BA Wood (2013) Stable isotope-based diet reconstructions of Turkana Basin hominins. Proceedings of the National Academy of Sciences 110: 10501–10506.*

Question 1: During the Q& A at the end you said one of the oldest fossil records of hair was discovered here in Arizona at the age of 15,000 years old. What did this hair reveal about the person's diet and environment? Also, are samples of this age difficult to analyze or are they more inaccurate than younger samples?

*This was a giant sloth found in caves, not humans. Hair of this age is very rare.*

Question 2: I remember having a brief discussion about the migrational patterns of elephants and I learned that males are fairly solitary whereas the females remain consistently local to a specific area in order to rear their young. Are elephant families structured in a matrilineal way?

*Yes elephant families are comprised of elephant female adults – often sisters and cousin – with younger sub-adult males and females. The males often leave the families at about age 6 to 8 years old and make groups with other young males and learn about being “young males.”*

Question 1: If you use statistical methods can you localize a isotopic signature to more discrete areas?

*We are using statistics to get our “predicted regions”. Lesley Chesson has published a number of papers showing how this can be done for foods (e.g., milk, juice, hamburger).*

*Chesson LA, LO Valenzuela, GJ Bowen, TE Cerling, JR Ehleringer (2011) Consistent predictable patterns in the hydrogen and oxygen stable isotope ratios of animal proteins consumed by modern humans in the USA. Rapid Communications in Mass Spectrometry 25: 3713–3722. DOI: 10.1002/rcm.5283.*

*Chesson LA, Valenzuela L, O’Grady S, Cerling TE, Ehleringer JR (2010) Hydrogen and oxygen stable isotope ratios of milk in the USA. Journal of Agricultural and Food Chemistry 58: 2358–2363.*

Question 2: Are many of these region-identifying isotopic studies limited by a number of baseline analyses? Will more analyses lead to greater resolution in region?

*Yes – more baseline studies are needed. We also use plant and animal physiological models to improve predictions.*

Question 1: How long do you have to do controlled feeding experiments before having decent model parameters?

*That depends on the question – for some tissues like collagen, several years would be needed. On the other hand, you could eat a candy bar and see the effect of the metabolized sugars in a few hours – this is an experiment that can be done in a single afternoon.*

Question 2: How long on average does it take to reconstruct a diet for a certain individual?

*That depends on the detail of the question and the expected detail of the answer.*

Question 1: Could the isotope samples extracted from animal hairs be used to predict future migration patterns based on the availability of food sources?

*That would be a good application of this method.*

Question 2: How often are hair sample isotopes used in helping cold cases?

*This is becoming much more frequent than in the past, as the method gets more exposure and understanding.*



Question 1: Do you think there's a possibility to have the government fund a program to reopen unsolved cases for researchers to use hair as a method to try to solve them?

*There is a program that is on-going to work on remains from WW II, and also border crossers.*

Question 2: Why did your study choose elephant hair and not a different animal's hair?

*“Because it was there”. I was working with Kenya Wildlife Service and I sampled the elephants that were semi-wild and so we could get hair from the animals repeatedly (a year later) and this allowed us to establish the growth rates. We have worked with many other species of animals since then.*

Question 1: You mentioned shaving off beard hairs every day on your trip; are the isotope readings universal across an individual's hairs or do some work better than others?

*Each isotope ( $\delta D$ ,  $\delta^{18}O$ ,  $\delta^{15}N$ ,  $\delta^{13}C$ ) tells a different story. You need to match the isotope to the question it can answer.*

Question 2: Are there other similar tests hairs are good for, or is it just c3 and c4 isotopes?

*$\delta D$ ,  $\delta^{18}O$  are related to water sources;  $\delta^{15}N$  is related to meat intake.*

Question 1: In the talk today you spoke about the paper you had published about the Carbon isotopes found in the diets of the man eating lions. How was it possible to learn how many people the lions ate with the data collected, or was this information gotten from people in the area.

*This was based on statistical arguments using possible diets of prey items (humans,  $C_3$ -herbivores,  $C_4$ -herbivores). This uses isotope mixing models for possible combinations of these end-members.*

*Yeakel JD, Patterson BD, Fox–Dobbs K, Okumura MM, Cerling TE, Moore JW, Koch PL, Dominy NJ (2009) Cooperation and individuality among man–eating lions. *Proceedings of the National Academy of Science* 106: 19040–19043.*

Question 2: Do you have any theory on why elephants might have switched from grazers to browsers?

*No – that is a problem we are working on. A possibility is that the bovids “out-compete” them.*

Question 1: Do the isotopes within the hair samples vary by age? (are children follicles more/less susceptible to high/different isotopic concentration)

*If the diet changes with age, but not otherwise. This is important if the animal (for example, humans) is not weaned – this is seen in the nitrogen isotopes (it is essentially a trophic level effect).*

Question 2: Does the study take into consideration the traveling tendencies of the subjects? (would that distort the data or would one have to travel a lot to do so?)

*Travel history is something that we are interested in determining. So a lot of travel makes a complex story that we try to unravel. Usually we do not get unique solutions, but rather a family of solutions. Other information can be quite important in determining which answer is the correct one.*

Question 1: Why did you decide to test elephant hair? Is there a benefit to using elephants versus other animals?

*“Because it was there”. I was working with Kenya Wildlife Service and I sampled the elephants that were semi-wild and so we could get hair from the animals repeatedly (a year later) and this allowed us to establish the growth rates. We have worked with many other species of animals since then.*

*Elephant hair is particularly good because the hair is very thick and this means that we can get a very good time resolution.*

Question 2: What got you involved in so many different areas of science?

*Everything is interesting and interconnected. Isotopes also easily lend themselves to the study of almost any natural phenomena.*

Question 1: Would it be possible to observe NDVI trends for the Phoenix area? We have rainy seasons but I can't imagine the greenery changing that much.

*It definitely would work in the Phoenix area.*

Question 2: How difficult is it to keep hair segment/samples organized during analysis? With the sections of hair being cut very small it seems like it would be easy to have them get out of order or lost!

*That is a major challenge – we do the best we can (“Do all that you know and try all that you don't” – from the Hunting of the Snark). We use a lot of sample containers, sometimes!*