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Stable isotopes and diet: You are what you eat

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Introduction

Archaeologists frequently use many different sources of information to reconstruct ancient diets, including faunal and macrobotanical remains, pollen and phytoliths, as well as pottery residues, coprolites, and indirect sources such as skeletal pathology, dental wear patterns, artistic depictions, and ethnographic observations. In most cases, however, the result is only the determination of the menu, and with the exception of animal foods, without any quantitative estimate of their importance. Furthermore, these data almost always pertain to groups of people and represent a time span of multiple generations if not a century or more.

In the late 1970s, though, a new area of dietary research developed based on the isotopic composition of human bone. This work began with the observation that radiocarbon dates on the remains of certain plants such as maize were offset from dates obtained on other remains from the same archaeological context. With the determination that this was due to maize having a different photosynthetic pathway than most plants, which results in a different relative quantity of carbon-14 (and carbon-13) in its tissues, it was realized that carbon isotope ratios in consumer tissues (*e.g.* bone) would also be affected, and therefore the measurement of carbon isotope ratios could be used to indicate the importance of maize in human diets [1, 2]. Following the observation that nitrogen isotope ratios also vary between different food sources, especially marine *vs.* terrestrial [3], the stable isotope analysis of human bone quickly became a widely applied technique, especially in areas where isotopically diverse food sources are known to have existed.

Analyses of human bone offer the specific advantage relative to faunal and floral

studies that the dietary results obtained are for individuals and not necessarily a larger group, meaning that comparisons can be made between individuals of different age, sex, and/or socioeconomic status, as well as between different sites and over multiple time periods. Reliable results have been obtained from samples millions of years old, and using modern instruments which require only tiny samples, it is also possible to study seasonal variation in incremental growth tissues such as hair and teeth, widening even further the subsistence questions which can be addressed by stable isotope analysis.

Principles of stable isotope analysis

Carbon and nitrogen isotope ratios in human bone may be used to reconstruct prehistoric diet because of differential fractionation, between certain plant groups, of atmospheric carbon dioxide during photosynthesis and of nitrogen during fixation or absorption (fig. 1). There are two stable isotopes each of carbon (^{12}C, ^{13}C) and nitrogen (^{14}N, ^{15}N), with ^{12}C and ^{14}N by far the most common in nature. Small differences in the ratios of these isotopes (^{12}C/^{13}C, ^{15}N/^{14}N) can be measured by isotope ratio mass spectrometry using samples smaller than 1 milligram. High-precision isotope measurements are reported using the delta notation (δ^{13}C, δ^{15}N) relative to internationally recognized standards and are expressed in parts per thousand or per mil (‰).

Experimental data have indicated that different bone tissues reflect different components of the diet [4,5]. In general, bone collagen is disproportionately produced from the protein portion of the diet, while bone carbonate and tooth enamel carbonate (both a calcium hydroxyphosphate, called apatite) are produced from a mixture of dietary protein, carbohydrates and fats. Stable isotope analysis of both bone collagen and apatite thus permits quantitative estimates of several dietary components. Both bone collagen and bone apatite are constantly being resorbed and replenished, so that their isotopic composition reflects dietary averages over at least the last several years of an individual's life, while the composition of tooth enamel will reflect diet during the age of crown formation.

Since teeth form at different ages (beginning in utero for deciduous teeth, and from 0 to 12 yrs of age for permanent teeth), the analysis of multiple teeth from the same individual may reveal dietary shifts caused by weaning (first the introduction of solid foods and later the cessation of breast feeding) [6], while significant differences between tooth (juvenile diet) and bone (adult diet) values may be the result of subsistence changes due to migration. Analysis of hair segments also may reveal short-term or seasonal dietary changes [7]. Overall, stable isotope analysis of multiple tissues can provide a quantifiable dietary life history of an individual [8]. While collagen is rarely preserved in bones predating the Upper Paleolithic, and is often badly degraded in hot and moist environments (*e.g.*, lowland Mesoamerica), bone apatite has provided reliable results for the Holocene, and tooth enamel for early hominids [9] and into the Miocene.

Typically, grasses originally native to hot, arid environments follow the C4 (Hatch-Slack) photosynthetic pathway, and will have δ^{13}C values averaging about -12.5‰; trees, shrubs, and grasses from temperate regions, which follow the C3 (Calvin-Benson)

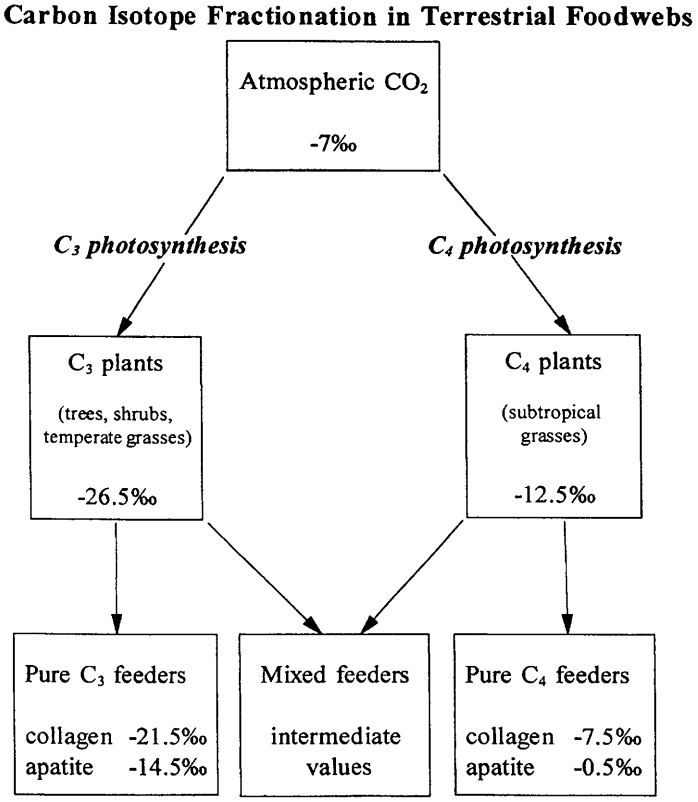

Fig. 1. – Carbon isotope fractionation in terrestrial foodwebs.

photosynthetic pathway, will have δ^{13}C values averaging about -26.5‰. In some forested areas, a canopy effect occurs due to incomplete atmospheric mixing, resulting in even more negative carbon isotope ratios [10]. Empirical data for large mammals combined with experimental data for rats and mice indicate that bone collagen is enriched about $+5$‰ relative to diet, although this value is affected by the proportion of protein in the total diet and differences in δ^{13}C values between protein and energy sources [11, 3, 4]. Experimental data on rats also convincingly demonstrates that bone apatite is enriched about $+9.5$‰ relative to the whole diet, regardless of the mixture or isotopic composition of the foods consumed [3], although empirical data for larger herbivores suggest the diet-apatite spacing is about $+12$‰. Stable carbon isotope analysis is particularly useful in New World dietary studies since maize is often the only C4 plant contributing significantly to human diets; its contribution to bone collagen and to bone apatite may be estimated by interpolation. Some caution is warranted, however, if succulent plants were present, since they utilize the alternative CAM (crassulacean acid metabolism) photosynthetic pathway which results in carbon isotope ratios similar to those of C4 plants. Nevertheless, CAM plants are unlikely to have been major sources of dietary protein, whether consumed

directly or indirectly through herbivorous faunal intermediaries.

The carbon isotope ratios of marine and freshwater organisms are more variable, depending on local ecological circumstances, and often overlap with those of terrestrial plants and their consumers. These foods typically have much higher nitrogen isotope values, however, and their high protein content will contribute much more carbon to bone collagen than will maize (about 10% protein) or other plant foods. The analysis of bone apatite, which is derived from all food groups, should allow the identification of just a few percent maize or other C4 resources in an otherwise C3-based diet.

The nitrogen isotope ratios for plants depend primarily on how they obtain their nitrogen —by symbiotic bacterial fixation or directly from soil nitrates— and these values are similarly passed along through the food chain accompanied by an approximately 2–3‰ positive shift for each trophic level, including between mother and nursing infant. Human consumers of terrestrial plants and animals typically have δ^{15}N values in bone collagen of about 6–10‰ whereas consumers of freshwater or marine fish, seals and sea lions may have δ^{15}N values of 15–20‰ [3]. While most plants follow either the C3 or C4 photosynthetic pathway and have similar carbon isotope ratios in most ecological settings, nitrogen isotope ratios vary according to rainfall, altitude and other factors [12], and both carbon and nitrogen isotope ratios vary considerably among marine organisms [13].

It is critical, therefore, to establish a site-relevant isotopic baseline for interpreting human skeletal data (fig. 2). Analyses of faunal remains provide a good estimate both of the animals themselves and the plants they consume. Establishing an isotopic baseline is particularly important in coastal areas where marine and riverine resources, as well as C4 and/or CAM plants may have been available for direct or indirect consumption by humans. Atmospheric carbon isotope ratios have become depleted by about 1.5‰ since the industrial revolution, so values obtained on modern terrestrial plants and animals must be adjusted accordingly for most archaeological studies.

Other surveys and summaries of stable isotope analysis in archaeology may be found in several recent publications [14-17].

Analytical methods

For the isotopic analysis of bone, it is first necessary to separate the specific tissue to be analyzed. For collagen, this involves demineralization of the bone using acid (with different laboratories using different acids, concentrations, and temperatures), and separation from any residual lipids. In my own laboratory at the University of South Florida, bone collagen is extracted using well-established laboratory procedures [18]. Whole bone is demineralized in 2% hydrochloric acid (72 h), base-soluble contaminants are removed using 0.1 M sodium hydroxide (24 h each before and after demineralization), and residual lipids are dissolved in a 2:1:0.8 mixture of methanol, chloroform, and water (24 h).

One milligram samples of the resulting collagen pseudomorphs —which make for a very useful visual indicator of sample integrity, which is often a problem for archaeological samples— are placed in tin capsules and analyzed for δ^{13}C and δ^{15}N in continuous flow mode using a CHN analyzer coupled with a Finnigan MAT stable isotope ratio mass

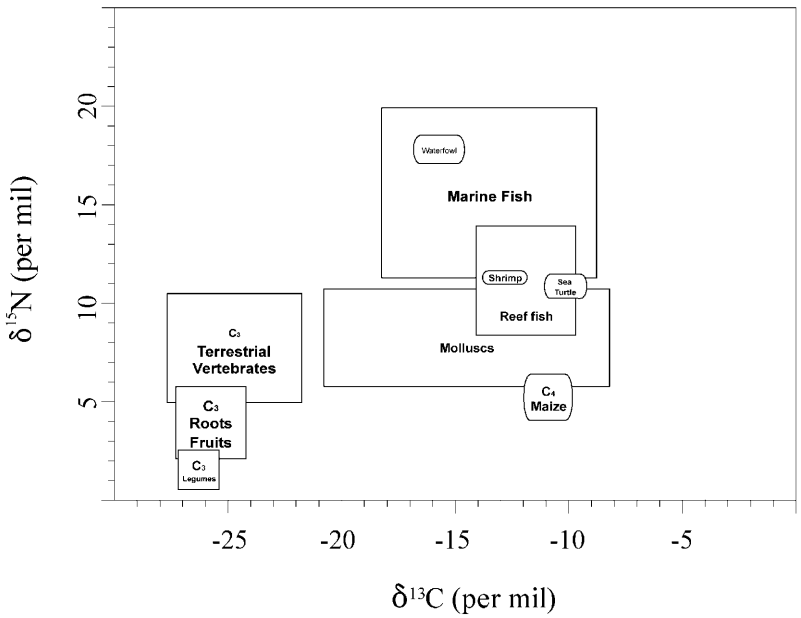

Fig. 2. – Carbon *vs.* nitrogen isotope ratios in common New World food groups.

spectrometer, also located at USF. Previously, it was necessary to convert collagen into CO_2 and N_2 offline, through high-temperature combustion in a vacuum-sealed quartz tube, which was followed by cryogenic distillation to separate these gases. Each gas sample was then introduced to the mass spectrometer using a manifold system. The CHN analyzer integrates the combustion step with separation in time of the resulting CO_2 and N_2 *en route* to the mass spectrometer, which is also able to switch quickly between the different mass ranges being measured (28 and 29 for N_2; 44, 45 and 46 for CO_2, with mass 46 mostly a result of a single oxygen-18 and its measurement allowing for a formulaic determination of how much of the mass 45 signal is from oxygen-17 and therefore how much from carbon-13). With either method of sample introduction, gas yields and C:N ratios are used to confirm the integrity of the collagen samples.

Bone apatite and tooth enamel samples are prepared using procedures designed to remove non-biogenic carbon without altering the biogenic carbon isotope values [19]. Powder samples are obtained by drilling from the center of carefully cleaned bone samples, or from tooth enamel after the surface layer has been removed. Approximately 10 mg of powder are immersed in 2% sodium hypochlorite to dissolve organic components (24 h for enamel, 72 h for bone apatite). Non-biogenic carbonates are then removed in 1.0 M buffered acetic acid for 24 hours. The integrity of apatite and enamel samples is assessed through yields obtained in each stage of the pretreatment process. Samples are analyzed on a second Finnigan MAT mass spectrometer equipped with a Kiel III individual acid bath carbonate system, which eliminates the need both for off-line production of CO_2 by reaction of the sample with acid in a vacuum-sealed glass tube, and the cryogenic

purification of the resulting gas sample.

In addition to reference gases against which samples are measured, several solid standard samples are analyzed at the beginning of each run and then after every six or seven archaeological samples to ensure reliability of all results. The analytical precision for stable isotope ratio mass spectrometry is typically 0.1‰ for δ^{13}C and 0.2‰ for δ^{15}N.

Recent applications

I have been involved in a number of collaborative research projects around the world using stable isotope or elemental analysis to assess ancient diets. Results have been presented at various conferences, some have been published, and others are in press or some stage of preparation for publication. Examples of some of these projects and the results obtained are given below.

Patagonia (Argentina and Chile). – In southernmost South America, ethnographic observations of historic groups of hunter-gatherers have revealed several discrete subsistence adaptations, including great dependence on marine foods for those living on the coasts and inlets of Tierra del Fuego. Growing archaeological evidence, however, has suggested at least that this historic pattern does not extend into antiquity. Isotopic analysis of bone collagen, bone apatite, and tooth enamel from a large number of sites in central and southern Patagonia, provided through collaborations with Julieta Gomez Otero, Juan Belardi, Ricardo Guichon and Luis Borrero, indicates that the coastal populations had a broad range of diets, in many cases depending more on terrestrial than marine foods (fig. 3). Some coastal groups clearly exploited high-trophic level marine resources such as sea lions and penguins, while others had more mixed diets which included fish, mollusks, guanaco, and land birds. The result appears to be that a continuum of subsistence combinations were employed, rather than the discrete selected diets proposed by ethnographic observations [20].

Highland Ecuador and Peru. – In highland Ecuador and Peru, collaborations with Richard Burger and J. Stephen Athens have provided the opportunity to examine the introduction and increasing importance of maize in the highland Andes. New collagen carbon and nitrogen isotope data from Pacopampa and La Chimba support earlier results based on collagen carbon from Huaricoto and Chavin de Huantar [21] which suggest modest importance of maize during the first millennium BC in the highland Andes. The apatite data, however, suggest that, while maize was a similar proportion of the whole diet in both regions during the 1st millennium BC, it was a greater contributor to dietary protein in highland Ecuador. In later periods maize became a dietary staple, with differential access (*e.g.* the consumption of maize beer) according to social status at sites such as La Florida [22]. Although there appears to be an increase in δ^{15}N values over time, which could be interpreted as due to greater consumption of animals (higher trophic level than plants) or the availability of small quantities of fish by trade with people living at lower elevations, this is most likely an effect of the differences in altitude of the sites in our study.

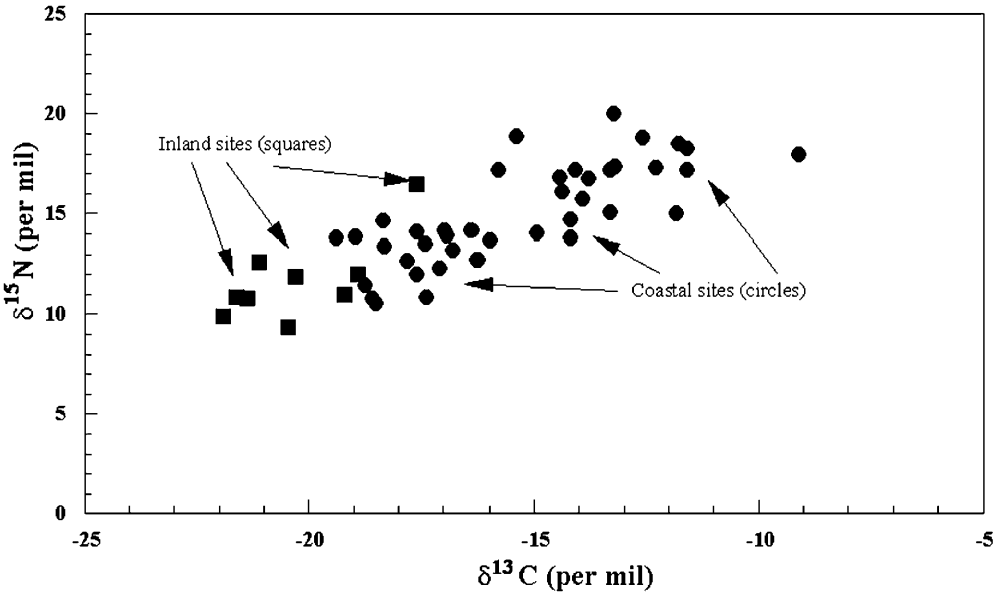

Fig. 3. – Carbon *vs.* nitrogen in human collagen from Patagonia, showing variation in importance of seafood among coastal populations.

Coastal Ecuador and Peru. – The diets associated with complex societies on the coasts of Ecuador and Peru have been the subject of great debate because of the conceived importance of agriculture —especially maize— as the basis for sedentary societies and the emergence of political hierarchies, and the reported presence of maize pollen and phytoliths in the region by 5000 BC. The precocious Valdivia culture on the coast of Ecuador has ceramics and ceremonial sites by 3000 BC, while monumental platform structures appear on the coasts of Peru in the 3rd millennium BC and ceramics shortly afterwards. Analyses of a time series of individuals from coastal Ecuador [23,24], however, suggest that maize is not noticeable in diets until the end of the Valdivia period (phases 7–8, about 2000 BC), long after the appearance of settled villages with ceramics and ceremonial architecture, and only became a dietary staple in the second half of the 1st millennium BC. In Peru, also, analyses of remains from the Lurin (Mina Perdida, Cardal, Tablada de Lurin) [25] and Viru [26] valleys suggest that maize was not a dietary staple until well into the Initial Period. For interpreting coastal diets, where a combination of riverine, marine, terrestrial C3 and C4 resources could have been consumed, it is important to obtain stable isotope data from both human bone collagen and apatite, as well as the available fauna and flora.

Belize and Guatemala. – Skeletal remains from the Preclassic site of Cuello, excavated by Norman Hammond of Boston University, have been analyzed for bone collagen, bone apatite, and tooth enamel [27], as have samples from Classic sites at La Milpa (Hammond

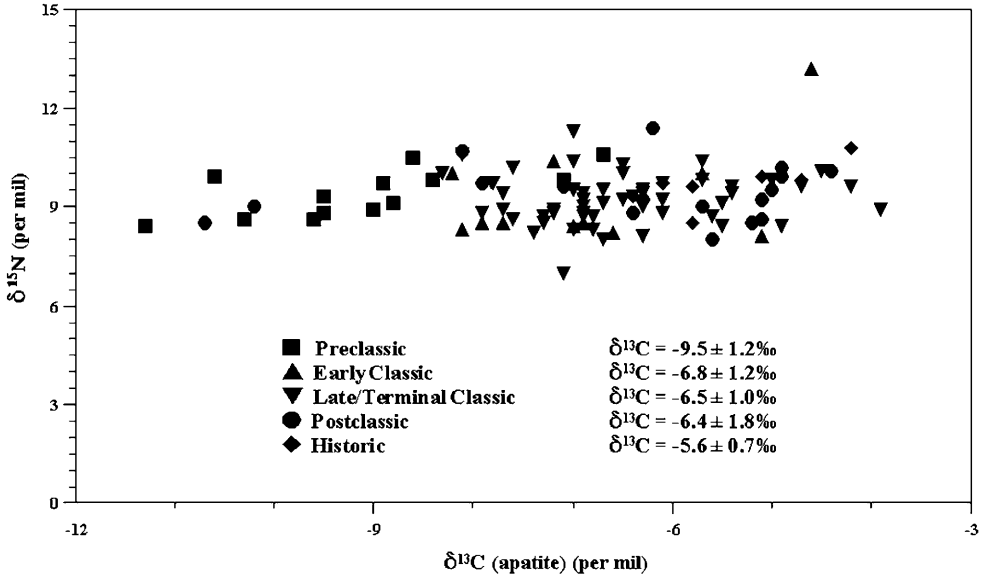

Fig. 4. – Bone apatite carbon *vs.* collagen nitrogen for Belize, suggesting a big increase in the importance of maize to the whole diet by the beginning of the Classic period.

excavations) and the Programme for Belize (Fred Valdez/Richard Adams excavations). In conjunction with data published by others, a chronological trend towards increasing maize reliance is evident both in bone collagen and bone apatite [28]. For whole diet as represented in bone apatite, however, the biggest shift in carbon isotope ratios is observed between the Preclassic and Early Classic, while only a statistically insignificant change is then observed through the Late, Terminal, and Postclassic periods (fig. 4). This is in contrast to the largest shift in carbon isotope ratios in bone collagen, which occurred between the Terminal and Postclassic periods. I interpret this to indicate that the real increase in the importance of maize to the whole diet came during the first millennium BC, when complex Maya society was first established, while changes in its importance as a protein source may be correlated with the collapse of Classic Maya society.

At Cuello, and probably other sites, some of the maize signature likely came from the consumption of maize-fed dogs. At La Milpa, diets also seem to vary based on social status and residence location. At Iximché in highland Guatemala, a number of individuals dating to the Late Postclassic period have been tested for bone collagen (by David Reed and Stephen Whittington) and tooth enamel. The results reveal an extremely high dependence on maize, more so than most other Guatemalan populations at least of the Late Classic period, and certainly more so than contemporary populations at Lamanai in Belize. This high dependence may be related to a combination of local ecological as well as socioeconomic factors. Within the group of individuals tested, most of whom were decapitated and may have formed a skull rack (*tzompantli*), several individuals stand out

as having significantly different diets. We suggest that they may have been warrior-elites from other communities who consumed different diets either because of differences in local ecology, and/or had access to different foods because of their high status [29].

Florida. – Stable carbon and nitrogen isotope analysis of human skeletal remains from several coastal, inland, and estuarine archaeological sites in Florida was performed to reconstruct indigenous subsistence adaptations prior to European contact, and to compare them with those associated with Mississippian and other cultural groups in the southeastern United States. The samples selected represent a time range from the Archaic Period up to the time of European contact about 1500 AD, and our preliminary results suggest that the local ecological setting was the prime factor in determining indigenous subsistence adaptations.

Native Americans living at inland settlements such as Cross Creek and Melton Mound have carbon isotope ratios in their collagen and apatite which are consistent with dependence entirely on C3 plants, wild animals that ate C3 plants, and freshwater fish, with the latter also suggested by their high nitrogen isotope ratios. In contrast, those tested from coastal sites such as Bay Pines and Bayshore Homes have the same nitrogen isotope ratios but significantly increased carbon isotope ratios in collagen and apatite, indicating the significant consumption of marine resources. While maize could have been consumed to a minor extent at these coastal sites, and not be apparent from the carbon isotope ratios, its clear absence in isotopic values for inland sites, and the lack of any archaeological evidence for maize consumption anywhere in central Florida, suggests that it had not yet been introduced to this region prior to European contact. This is particularly significant in that other sociopolitically complex Native American cultures were dependent on agriculture, but it appears that such cultures were able to develop in Florida based on hunting and gathering strategies that had been practiced for millennia [30].

Europe. – In Mediterranean Europe an important question is that of the importance of marine resources before, during, and after the introduction of domesticated plants and animals in the Neolithic. In this region, it is possible to quantify the contribution of fish and other marine resources to prehistoric diets, especially where C4 plants were not consumed directly by humans, or by animals as fodder. Surprisingly, analyses of many samples from coastal sites such as Grotta dell'Uzzo in Sicily, Arene Candide and other sites in peninsular Italy, and the islands of Malta and Crete, all have carbon isotope ratios which are not consistent with marine food consumption as a staple during the Mesolithic, Neolithic, or Bronze Age periods (fig. 5).

This is in contrast at least with work on Atlantic Europe by Michael Richards (Bradford University), who has documented heavy dependence on seafood in the Mesolithic, and a resurgence in its importance in the Roman period [31, 32]. Changes which do occur in Mediterranean Europe include dietary variation between elite and non-elite people during the Bronze Age, for example at sites like Mycenae, and the introduction of African millet (a C4 plant) in the 1st millennium BC, now documented at sites in Greece and Slovenia.

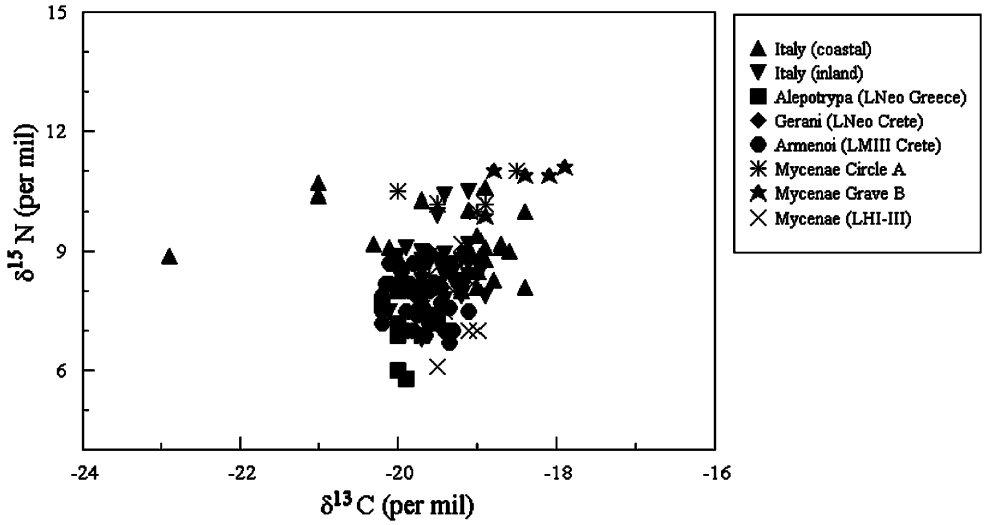

Fig. 5. – Carbon *vs.* nitrogen in human collagen for several Mediterranean inland, coastal, and island sites, with seafood only measurably important for some Mycenae elites.

China. – Current interpretations are that millet was domesticated in northern China and rice in southern China by the beginning of the neolithic period, and then spread to other regions of China and Asia. The chronology of this spread and the quantitative importance of these domesticates, however, is not well known. In collaboration with Anne Underhill at the Field Museum and colleagues in China, human bone and tooth samples were selected from a number of burials at the Longshan period (2600–1900 BC) site of Liangchengzhen in Shandong province site, and isotopically analyzed along with samples of flora and both terrestrial and aquatic fauna. Our preliminary results suggest that in northern China, millet was a staple crop used for domesticated animals, but to a far lesser degree for direct human consumption, probably because of the widespread use of rice by this period. Isotopic analyses of residues from two ceramic vessels in tombs were shown to be from fish, and support the general archaeological interpretation that a diversity of domesticated and wild foods were consumed.

Microsampling of teeth. – Like hair, teeth are incremental growth structures, which presents both advantages and limitations for isotopic analysis. In most cases, archaeologists attempt to be minimally destructive in their analyses, so the removal of a small sample of tooth enamel is required (with the tooth mostly preserved). The diet revealed by isotopic analysis of such a small sample, however, will be very short term, perhaps as little as a few weeks. A series of analyses of sequentially formed enamel layers may then be used to examine short-term or seasonal variations in diet, and even to correlate dietary changes with pathologies such as enamel hypoplasia. Preliminary results from our project in collaboration with Douglas Ubelaker show potentially large isotopic dif-

ferences within a single human tooth (4 per mil) [33]. On the other hand, "routine" studies in which comparisons are made of individuals (or of multiple teeth from a single individual) from which a single small enamel sample has been tested must allow for the possibility of seasonal dietary variation obscuring variability which otherwise would have been based on eruption age and weaning practices, migration, etc.

Strontium isotope analysis

Strontium isotope ratios ($^{87}Sr/^{86}Sr$) have been shown to be useful in identifying the geographic area in which people lived, since strontium from the local environment is incorporated into body tissues from the water, plants and animals consumed. In particular, tooth enamel samples are compared with bones from the same individuals to see if that person has moved between birth/childhood and adulthood. In central Europe, it has been shown that the Beaker People of the 3rd millennium BC were highly mobile, and thus probably directly responsible for the distribution of their widespread cultural attributes [34]. At the large site of Teotihuacan (Mexico), it has been demonstrated that many of the people living in the part of the city identified as a Oaxacan section actually were born in Monte Alban or another place in Oaxaca and later moved to Teotihuacan [35]. Current studies in my lab and elsewhere include early neolithic peoples and the spread of agriculture in Europe; seasonal movements and transhumance; and the origins of sacrificial victims found at sites in Mesoamerica and the southwest US.

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