

STABLE ISOTOPES IN PALEONTOLOGY AND ARCHEOLOGY

INTRODUCTION

The isotopic composition of a given element in living tissue depends on: (1) the source of that element (e.g., atmospheric CO_2 versus dissolved CO_2 ; seawater O_2 vs. meteoric water O_2), (2) the processes involved in initially fixing the element in organic matter (e.g., C_3 vs. C_4 photosynthesis), (3) subsequent fractionations as the organic matter passes up the food web. Besides these factors, the isotopic composition of fossil material will depend on any isotopic changes associated with diagenesis, including microbial decomposition. In this lecture, we will see how this may be inverted to provide insights into the food sources of fossil organisms, including man. This, in turn, provides evidence about the environment in which these organisms lived.

ISOTOPES AND DIET: YOU ARE WHAT YOU EAT

In Lecture 27 we saw that isotope ratios of carbon and nitrogen are fractionated during primary production of organic matter. Terrestrial C_3 plants have $\delta^{13}\text{C}$ values between -23 and -34‰ , with an average of about -27‰ . The C_4 pathway involves a much smaller fractionation, so that C_4 plants have $\delta^{13}\text{C}$ between -9 and -17‰ , with an average of about -13‰ . Marine plants, which are all C_3 , can utilize dissolved bicarbonate as well as dissolved CO_2 . Seawater bicarbonate is about 8.5‰ heavier than atmospheric CO_2 ; as a result, marine plants average about 7.5‰ heavier than terrestrial C_3 plants. In contrast to the relatively (but not perfectly) uniform isotopic composition of atmospheric CO_2 , the carbon isotopic composition of seawater carbonate varies due to biological processes. Because the source of the carbon they fix is more variable, the isotopic composition of marine plants is also more variable. Finally, marine cyanobacteria (blue-green algae) tend to fractionate carbon isotopes less during photosynthesis than do true marine plants, so they tend to average 2 to 3 ‰ higher in $\delta^{13}\text{C}$.

Nitrogen isotopes are, as we saw, also fractionated during primary uptake. Based on their source of nitrogen, plants may also be divided into two types: those that can utilize N_2 directly, and those that utilize only "fixed" nitrogen as ammonia and nitrate. The former include the legumes (e.g., beans, peas, etc.) and marine cyanobacteria. The legumes, which are exclusively C_3 plants, utilize both N_2 and fixed nitrogen, and have an average $\delta^{15}\text{N}$ of $+1\text{‰}$, whereas modern nonleguminous plants average about $+3\text{‰}$. However, it seems likely that prehistoric nonleguminous plants were more positive, averaging perhaps $+9\text{‰}$, because the isotopic composition of present soil nitrogen has been affected by the use of chemical fertilizers. For both groups, there was probably a range in $\delta^{15}\text{N}$ of ± 4 or 5‰ , because the isotopic composition of soil nitrogen varies and there is some fractionation involved in uptake. Marine plants have $\delta^{15}\text{N}$ of $+7 \pm 5\text{‰}$, whereas marine cyanobacteria have $\delta^{15}\text{N}$ of $-1 \pm 3\text{‰}$. Figure 33.1 summarizes the isotopic composition of nitrogen and carbon in the various classes of photosynthetic organisms (autotrophs).

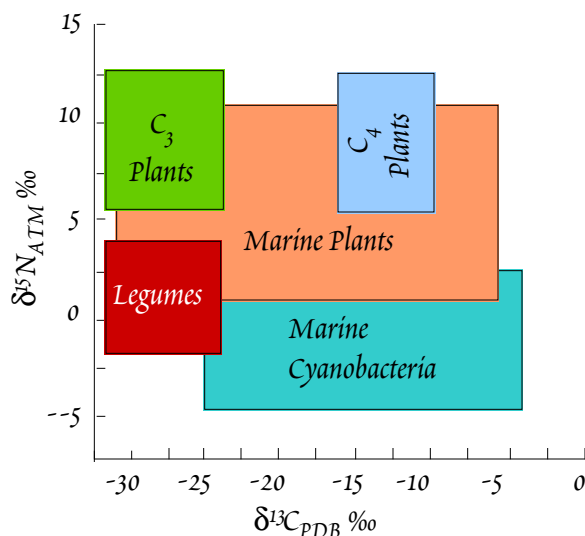


Figure 33.1. Relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ among the principal classes of autotrophs.

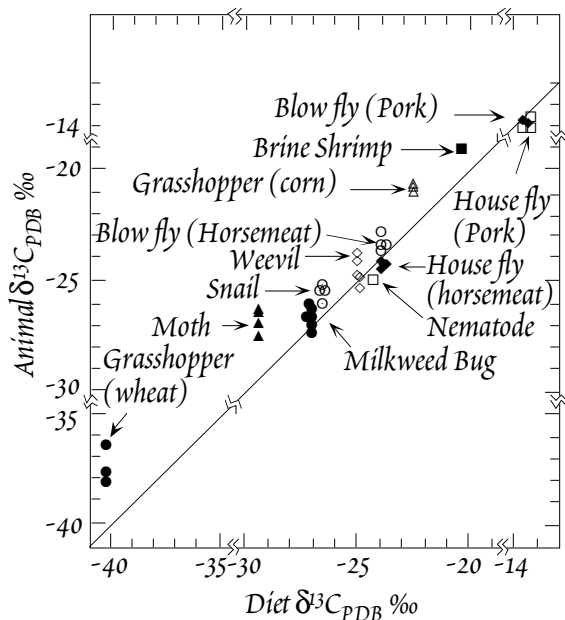


Figure 33.2. Relationship between $\delta^{13}\text{C}$ in animals and that of their diets. When a species was given more than one diet, that diet is shown in parentheses. After DeNiro and Epstein (1978).

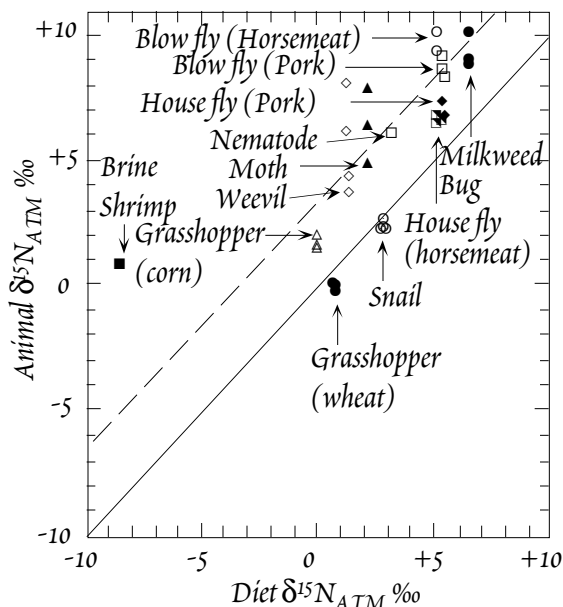


Figure 33.3. Relationship between $\delta^{15}\text{N}$ in animals and that of their diet. Animals typically have 3-4‰ heavier nitrogen than their diet. Dashed line is the isotopic composition plus 3.5‰. When a species was given more than one diet, that diet is shown in parentheses. After DeNiro and Epstein (1981).

DeNiro and Epstein (1978) studied the relationship between the carbon isotopic composition of animals and their diet. (Most of the animals in this study were perhaps of little direct paleontological interest, being small and soft-bodied. DeNiro and Epstein studied small animals for an important reason: it is not practical to dissolve up and analyze a large animal such as a horse.) Figure 33.2 shows that there is little further fractionation of carbon by animals and thus the carbon isotopic composition of animal tissue closely reflects that of their diet. Typically, carbon in animal tissue is about 1‰ heavier than their diet. Mice, although not analyzed whole and not shown in Figure 33.2 were also included in the study. Various tissues from mice had $\delta^{13}\text{C}$ within $\pm 2\text{‰}$ of their diet, so the relationships in Figure 33.2 extend to vertebrates as well. DeNiro and Epstein found that the same species has a different isotopic composition when fed a different diet. Conversely, different species had similar isotopic compositions when fed the same diet. Thus diet seems to be the primary control on the isotopic composition of animals.

The small fractionation between animal tissue and diet is a result of the slightly weaker bond formed by ^{12}C compared to $\delta^{13}\text{C}$. The weaker bonds are more readily broken during respiration, and, not surprisingly, the CO_2 respired by most animals investigated was slightly lighter than their diet. Thus only a small fractionation in carbon isotopes occurs as organic carbon passes up the food chain, about +1‰ at each step in the chain. Terrestrial food chains are usually not more than 3 trophic levels, implying a maximum further fractionation of +3‰; marine food chains can have up to 7 trophic levels, implying a maximum carbon isotope difference between primary producers and top predators of 7‰. These differences are smaller than the range observed in primary producers. In a similar study, DeNiro and Epstein (1981) found that $\delta^{15}\text{N}$ of animal tissue is related to the $\delta^{15}\text{N}$ of the animal's diet, but is typically 3 to 4‰ higher than that of the diet (Figure 33.3). Thus in contrast to carbon, significant fractionation of nitrogen isotopes will occur as nitrogen passes up the food chain.

Schoeninger and DeNiro (1984) studied the carbon and nitrogen isotopic composition of bone collagen in animals. Their findings reflected just the relationships expected from the work of DeNiro and Epstein (1978, 1981): in primary herbivores, carbon

Geol. 656 Isotope Geochemistry

Lecture 33

Spring 1998

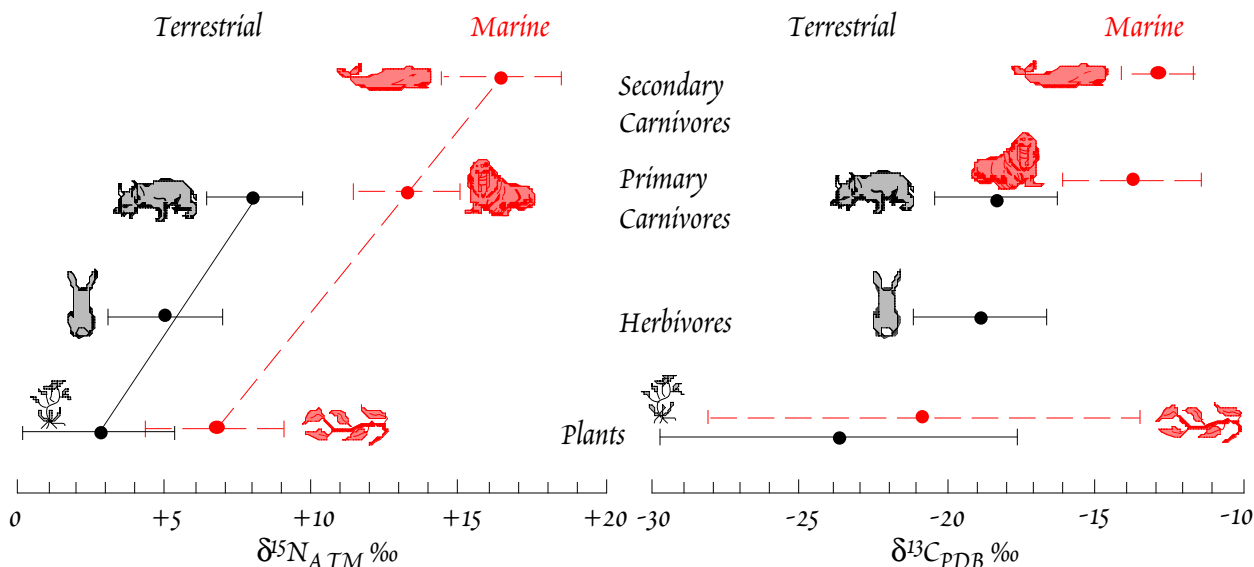


Figure 33.4. Values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in various marine and terrestrial organisms. From Schoeninger and DeNiro (1984).

in bone collagen was a few per mil heavier than the isotopic composition of plants, and $\delta^{15}\text{N}$ increased by about 3‰ at each trophic level. Marine vertebrates tend to have more positive $\delta^{15}\text{N}$ than do their terrestrial counterparts because they generally feed at a higher trophic level. The primary producers in the ocean are generally microscopic algae. Most herbivores are also microscopic (zooplankton); there are very few marine vertebrate herbivores (anchovies are an example). Most terrestrial food chains have only three levels: primary producers, herbivores, and predators. Marine food chains, by contrast, can have up to seven levels. Since nitrogen isotope fractionation occurs at each level, the top marine predators have more positive $\delta^{15}\text{N}$. These relationships are summarized in Figure 33.4.

Apatite in bone appears to undergo isotopic exchange with meteoric water once it is buried, but bone collagen and tooth enamel appear to be robust and retain their original isotopic compositions. Tooth enamel, in which carbon is present as carbonate, however, is systematically 12 to 15‰ heavier than carbon in organic tissue. (Such a fractionation is, of course, expected, and consistent with the observation that carbonate is always heavier than organic carbon.) Collagen typically has carbon about 4‰ heavier than diet. These results mean that the nitrogen and carbon isotopic composition of fossil bones and teeth can be used to reconstruct the diet of fossil animals.

CARBON ISOTOPES AND THE EVOLUTION OF HORSES AND GRASSLANDS

Horses (Family Equidae) have been around for 58 million years. Beginning in the early Miocene, a major radiation took place and the number of genera in North America increased from three at 25 Ma to twelve at 10 Ma. It subsequently fell at the end of the Miocene, and the last North American species became extinct in the Holocene. A major change in dental morphology, from low-crowned to high crowned, accompanied the Miocene radiation. For nearly 100 years, the standard textbook explanation of this dental change was that associated with a change in feeding from leaf browsing to grass grazing. Grasses contain enough silica to make them quite abrasive, thus a high crowned tooth would last longer in a grazing animal and would therefore be favored in horse's evolution as it switched food sources. The change in horse diet was thought to reflect the evolution of grassland ecosystems (or biomes). This line of reasoning led to the conclusion that grasslands first became important biomes in the Miocene.

Carbon isotope ratios provide the first opportunity to test this hypothesis. Grasses of tropical and temperate regions are almost exclusively C_4 plants. C_3 grasslands occur only in high latitude regions. In the North American prairie, for example, C_4 grasslands become important only north of the US-Canadian border (Figure 33.5). The appearance of grasslands inferred from the evolution of horse

Geol. 656 Isotope Geochemistry

Lecture 33

Spring 1998

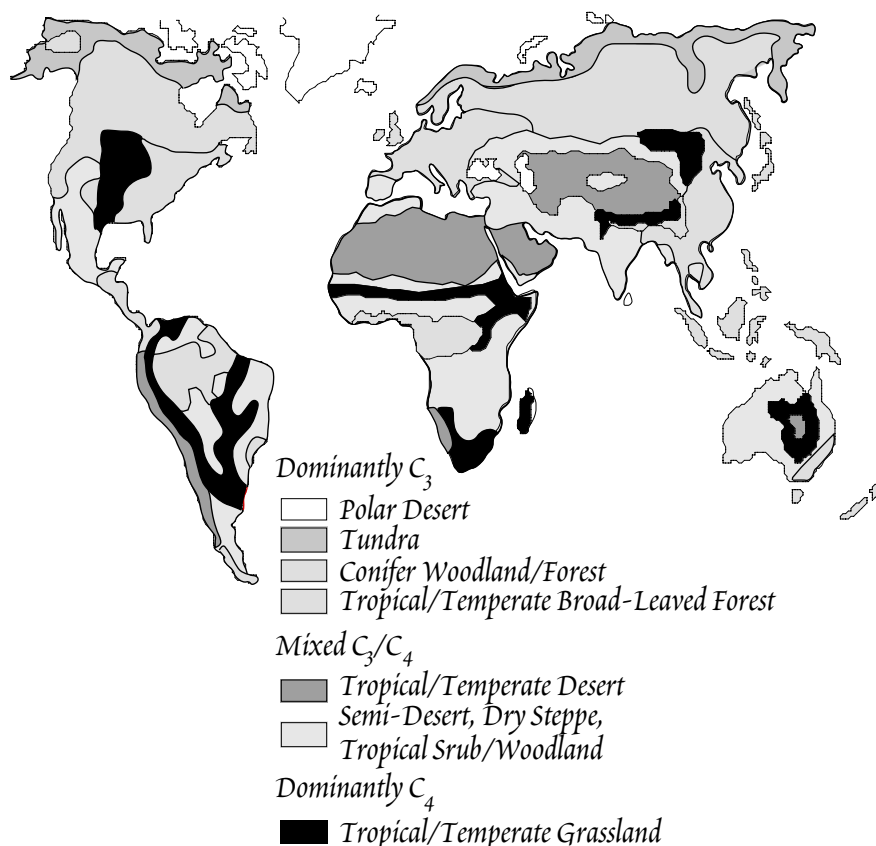


Figure 33.5. Present global distribution of C₃ and C₄ vegetation. From Cerling and Quade (1993).

teeth implies a change from a predominantly C₃ to a predominantly C₄ photosynthetic pathway and a corresponding shift in the $\delta^{13}\text{C}$ of the biomass in these regions. Since the carbon isotopic composition of animals reflects that of their diet, and since the $\delta^{13}\text{C}$ of dental enamel appears to record the $\delta^{13}\text{C}$ of the animal, the change in horse dentition should also be reflected in a change in the carbon isotopic composition of those teeth if the change in dentition were related to a change in diet.

Wang et al. (1994) carried out such a test by analyzing the carbon isotopic composition of dental enamel from fossil horse teeth of Eocene through Pleistocene age. They found a sharp shift in the isotopic composition of the teeth consistent with a change in diet from C₃ to C₄ vegetation, but it occurred later than the change in dental morphology (Figure 33.6). The change in dental morphology begins in the mid-Miocene (about 18 Ma), while shift in $\delta^{13}\text{C}$ occurs at around 7 Ma. This leads to an interesting dilemma. Which change, that in morphology or that in carbon isotopic composition, actually reflects the appearance of the grassland biome? It is possible that the change in dental morphology is unrelated to the evolution of grasslands? If that is the case, it is difficult to understand the change in dental morphology. Alternatively, grasslands may have appeared in mid-Miocene and only subsequently become dominated by C₄ grasses.

If the latter interpretation is correct, it raises the question of what evolutionary pressure caused the change from C₃ to C₄ photosynthesis in tropical and temperate grasses. An important observation in that respect is that C₄ grasslands appear to have become important in both North America and Asia at about the same time (7 Ma). Indeed, the first evidence for a shift from C₃ dominant to C₄ dominant ecosystems came from an observed change in the $\delta^{13}\text{C}$ of soil carbonate in Pakistan (Quade et al., 1989). Quade et al. (1989) first interpreted this as a response to the uplift of the Tibetan Plateau and the development of the Monsoon. However, other evidence, including oxygen isotope data from

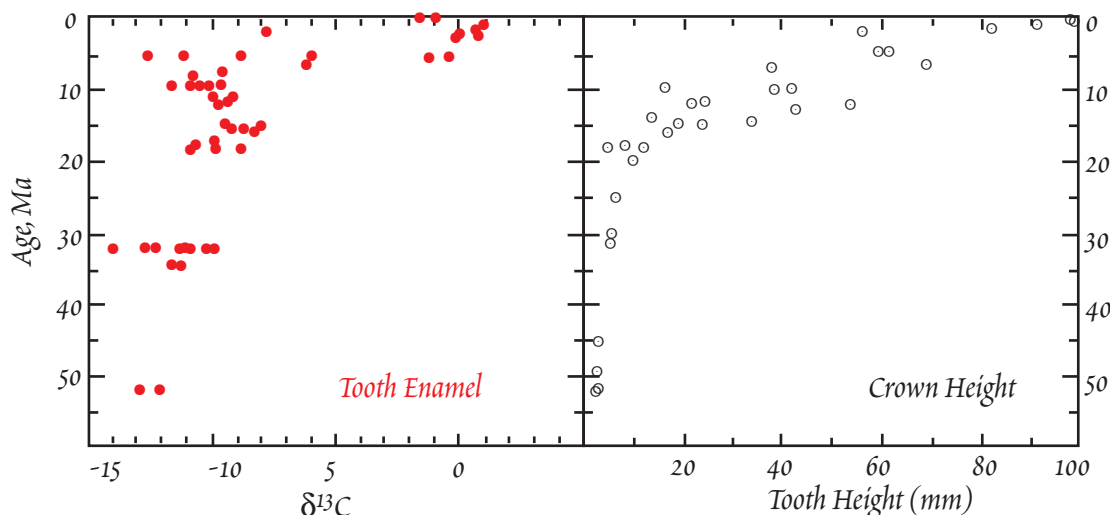


Figure 33.6. $\delta^{13}\text{C}$ and crown height in North American fossil horses as a function of age. From Wang et al. (1994).

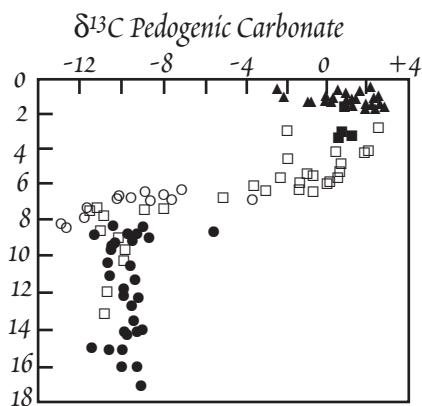


Figure 33.7. Comparison of variation in $\delta^{13}\text{C}$ in Pakistani soil carbonate and in horse teeth. From Cerling et al. (1993).

tion of plant enzymes and structures and it occurs in diverse, distantly related families. It may, therefore, have evolved independently in many families (Ehleringer, et al. 1991). This also suggests some global environmental change that favored C_4 photosynthesis.

Several groups have now suggested that the appearance of C_4 grasses reflects a drop in the concentration of atmospheric CO_2 in the Miocene. In the C_3 photosynthetic pathway, Rubisco can catalyze not only the fixation of carbon in phosphoglycerate, but also the reverse reaction where CO_2 is released, a process called photorespiration. When concentrations of CO_2 are high, the forward reaction is favored and the C_3 pathway is more efficient overall than the C_4 pathway. At low CO_2 concentrations, however, the C_4 pathway, in which CO_2 is

Pakistani soil carbonates, suggests the Monsoons developed about a million years earlier (about 8 Ma). Further, the synchronicity of the dominance of C_4 grasslands in Asia and North America (Figure 33.7) suggests a global cause, while the Monsoons are a regional phenomenon.

Though there has been some speculation that the C_4 photosynthetic pathway may have evolved as early as the Cretaceous, the oldest direct fossil evidence for C_4 plants (plants with enlarged bundle-sheath cells) is late Miocene; i.e., the same age as the observed $\delta^{13}\text{C}$ increase. Thus the isotopic shift may date the evolution of C_4 photosynthesis. C_4 photosynthesis involves only relatively minor modification

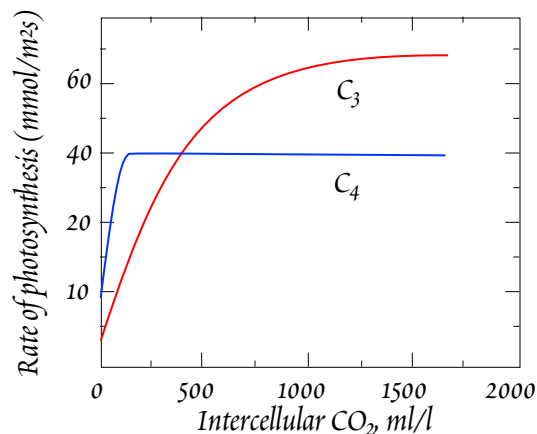


Figure 33.8. Rate of photosynthesis as a function of intercellular CO_2 concentrations in C_4 and C_3 plants. At concentrations of atmospheric CO_2 that prevailed before the Industrial Revolution, C_4 plants would have had a competitive advantage. At concentrations above the present level, C_3 plants are more efficient. From Ehleringer et al. (1991).

first transported into bundle-sheath cells, is more efficient, as the concentration in bundle-sheath cells is maintained at around 1000 ppm (Figure 33.8). Thus under present conditions, C_4 plants have a competitive advantage. At higher CO_2 conditions, C_3 plants are more efficient. There is some evidence that Eocene CO_2 concentrations were much higher than present (perhaps 800 ppm as opposed to 250 ppm pre-Industrial Revolution), and that concentrations dropped dramatically during the Miocene. Such a drop would give C_4 plants a competitive advantage. This would be particularly true in the warm climates where C_4 plants dominate because the rate of photorespiration is temperature dependent.

ISOTOPES AND PALEODIETS

The differences in nitrogen and carbon isotopic composition of various foodstuffs and the preservation of these isotope ratios in bone collagen provides a means of determining what ancient peoples ate. In the first investigation of bone collagen in human remains, DeNiro and Epstein (1981) concluded that Indians of the Tehuacan Valley in Mexico probably depended heavily on maize (a C_4 plant) as early as 4000 BC, whereas archeological investigations had concluded maize did not become important in their diet until perhaps 1500 BC (Figure 33.9a). In addition, there seemed to be steady increase in the dependence on legumes (probably beans) from 6000 BC to 1000 AD and a more marked increase in legumes in the diet after 1000 AD (Figure 33.9b).

Mashed grain and vegetable charred onto pot sherds provides an additional record of the diets of ancient peoples. DeNiro and Hasdorf (1985) found that vegetable matter subjected to conditions similar to burial in soil underwent large shifts in $\delta^{15}N$ and $\delta^{13}C$ but that vegetable matter that was burned or charred did not. The carbonization (charring, burning) process itself produced only small (2 or 3‰) fractionations. Since these fractionations are smaller than the range of isotopic compositions in various plant groups, they are of little significance. In the process of cooking, plant remains can become charred onto the pots in which they are cooked. Since pot sherds are among the most common artifacts recovered in archeological sites, this provides a second value means of reconstructing the diets of ancient peoples.

Figure 33.10 summarizes the results obtained in a number of studies of bone collagen and pot sherds (DeNiro, 1987). Studies of several historical populations, including Eskimos and the Tlinglit Indians of the Northwest US, were made as a control. The isotope data show that the diet of Neolithic

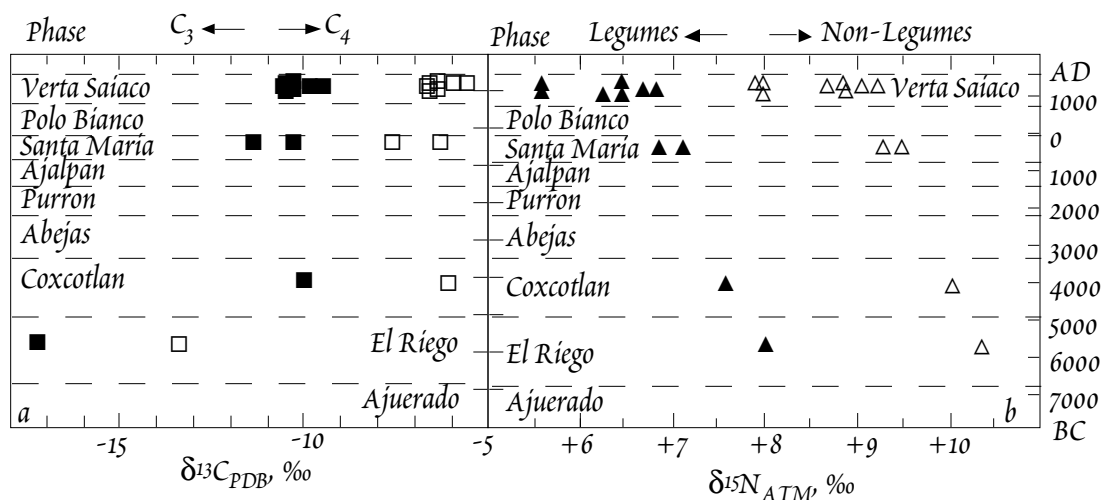


Figure 38.9. $\delta^{13}C$ and $\delta^{15}N$ in human bone collagen (open symbols) and calculated values in the diet (closed symbols) of the Tehuacan Indians as a function of age. The $\delta^{13}C$ data indicate a predominance of C_4 plants (probably maize) in all phases after the El Riego period. The $\delta^{15}N$ data indicate the importance of legumes (beans?) in the diet became increasingly important with time. After DeNiro and Epstein (1981).

Europeans consisted entirely of C_3 plants and herbivores feeding on C_3 plants, in contrast to the Tehuacan Indians, who depended mainly on C_4 plants. Prehistoric peoples of the Bahamas and Denmark depended both on fish and on agriculture. In the case of Mesolithic Denmark, other evidence indicates the crops were C_3 , and the isotope data bear this out. Although there is no corroborating evidence, the isotope data suggest the Bahamains also depended on C_3 rather than C_4 plants. The Bahamains had lower $\delta^{15}N$ because the marine component of their diet came mainly from coral reefs. Nitrogen fixation is particularly intense on coral reefs, which leads to ^{15}N depletion of the water, and consequently, of reef organisms.

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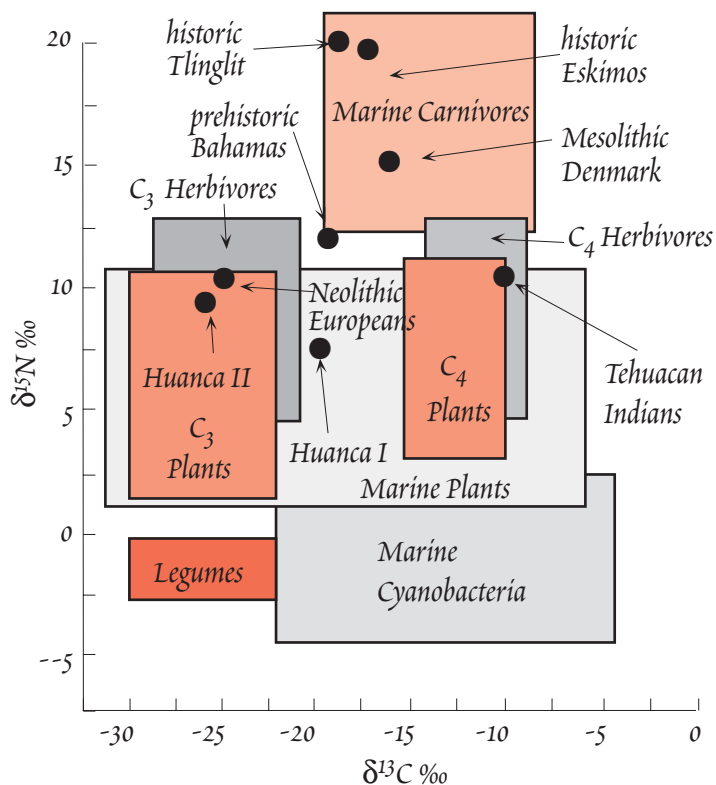


Figure 33.10. $\delta^{13}C$ and $\delta^{15}N$ of various food stuffs and of diets reconstructed from bone collagen and vegetable matter charred onto pots by DeNiro and colleagues. The Huanca people were from the Upper Mantaro Valley of Peru. Data from pot sherds of the Huanca I period (AD 1000-1200) suggest both C_3 and C_4 plants were cooked in pots, but on C_3 plants during the Huanca II period (AD 1200-1470).