Chapter 17 A Framework for the Incorporation of Isotopes and Isoscapes in Geospatial Forensic Investigations

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17.1 Introduction

Stable isotope ratio analyses have been commonplace in the environmental, biological, and geological fields for many decades (Ehleringer et al. 1993; Griffiths 1998; Rundel et al. 1988, Sharp 2006; West et al. 2006). However, the extension and application of stable isotopes into forensic investigations is relatively new and rapidly expanding because of the many ways this analytical approach can help with law enforcement investigations (Benson et al. 2006; Ehleringer et al. 2007, 2008b;

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Meier-Augenstein and Fraser 2008; Meier-Augenstein and Liu 2004). As is clear from the contributions in this volume, stable isotope analyses complement other analytical approaches to chemical identification in an investigation (e.g., HPLC, GC/MS, LC/MS), because stable isotope analyses provide an additional "fingerprint" that further characterizes a piece of forensic evidence. Stable isotope analyses provide a means of relating or distinguishing between two pieces of evidence that have exactly the same chemical composition (e.g., drug specimen found at a crime scene and at the suspect's residence). The study of stable isotopes as a forensic tool is based on the analytical ability to precisely measure naturally-occurring differences in the amounts of the heavy-to-light stable isotopes in evidentiary material and to relate that composition to other specimens or other evidence. In this chapter, our interest is in comparisons involving specific consideration of geographic location of origin and isotopic "fingerprints" associated with specific locations. This is a new field and area of active research, applicable now to providing information that will help guide law-enforcement investigations. In the future as our ability to model geographic isotopic variation for substrates of forensic interest improves, these analytical tools should find greater impacts in law enforcement investigations and as scientific information in prosecution efforts. The forensic evidence under consideration can be in the form of specific compounds (e.g., cocaine), mixtures (e.g., heroin), and/or biological tissues (e.g., bird feathers, hair, teeth).

17.2 Breadth of Isotope Applications in Forensics

Stable isotope ratio analyses yield potentially unique information for forensic investigations, complementing other approaches by adding a "stable isotope signature" to compounds or materials that are identified as identical by other methods. Consider the three questions illustrated in Fig. 17.1.

First, consider the utility of isotope analyses in the comparative approach: do the isotope ratios of two or more specimens have indistinguishable or distinguishable isotope ratio values? Non-spatial applications of stable isotope analyses can involve comparing the stable isotope ratios of a specimen with that of other specimens associated with the forensic incident or comparing the stable isotope ratios of a specimen with specimens of known origin contained within a database (Fig. 17.1). With limited information about an evidence specimen, the main applications of stable isotope ratios here include sample matching within a database, sample processing information if fractionation effects are well known, and/or source location identification if there is an exhaustive database of samples of known origin. Consider several specimens of a material of interest (e.g., illicit drugs, counterfeit money, toxins) that may be obtained by investigators with the intent of identifying related groups, sources, or regions-of-origin of the material (Casale et al. 2006; Ehleringer et al. 2000). Biological samples obtained from the same location and time will have experienced the same environmental conditions and therefore should have similar stable isotope ratios. Chemical processing of materials can also result in distinctive stable isotope ratios, as can impurities left behind during the production

Inputs Framework for Approach Forensic Questions Is evidence Evidence: specimen biological, soil, consistent or not or water Database of observations with a set of relevant to evidence: known isotopes, element observations? environment at composition, etc. location where evidence specimen was seized What are predictions for Process-based specimens model seized in that Soils and location? elements database laver predicted Climate and spatial geography maps database layer What are the predicted inverse regions from GIS model spatial which evidence Water maps specimen could database layer have originated?

A Framework for Integrating Stable Isotopes into Geospatial Forensic Investigations

Fig. 17.1 A framework for the stable isotope ratio analysis of forensic samples. Three primary classes of information can be provided for seized evidence specimens based on stable isotope ratio data that allow investigators to address three questions (far right). Evidence specimens can be matched either to other evidence specimens seized, or to known authentics and previously acquired evidence specimens recorded in a database. If the database is sufficiently extensive or if other modeling has been completed (see text), then two additional questions can be addressed: (1) what are the expected stable isotope ratios of specimens from a given location and (2) given a specimen's stable isotope ratios, from where might that sample have originated?

of derived products (Benson et al. 2006; Toske et al. 2006). Because of this, "like" samples may be readily grouped based on their stable isotope ratio values. In addition to matching samples, forensic workers can compare individual samples to databases of stable isotope ratio information obtained from authentic samples (e.g., Casale et al. 2006). In this case, a sample of unknown origin could be assigned probable source location by comparison with an authentic stable isotope ratio database, or a previously made assignment of origin could be corroborated by database comparison. Although this can be considered a spatial application, it is limited in its utility because the approach has limited predictive power. In most cases, stable isotope observations will provide an insight into whether a specimen is "consistent with" or "not consistent with" another specimen(s) or locations(s).

A second class of questions and applications of stable isotopes in forensic studies are based on the predictive power of models, once the mechanisms and isotope effects influencing the isotopic composition of a material are understood for that material, compound, or organism. Alternatively, linear regression models may describe the relationships. Here a modeling approach may make a specific prediction of the stable isotope ratio expected for a material in a specific environment. By combining first-principles or process-based models of stable isotope fractionation in organisms with the spatial modeling capacity of Geographic Information Systems (GIS), spatial maps of predicted stable isotope ratios can be constructed (Bowen et al. 2005b, 2007; Ehleringer et al. 2008a; West et al. 2007). The results of stable isotope ratio analyses on a specimen can then be compared to the geographical predictions, and potential source locations identified. Climate conditions and other parameters needed to make these predictions are often not unique globally. Therefore, it is uncommon for models to uniquely predict a single region or origin for a specimen, because the climate drivers influencing the predicted stable isotope ratios of a material are not unique. Rather a set of stable isotope analyses often identifies one or more "geographic zones" or "geographic bands" from which a specimen could have originated.

These advances represent cutting edge applications of stable isotope ratios to forensics that will continue to develop over the coming years. As databases and fundamental understandings of stable isotope ratios grow, the process-based models can be refined, increasing accuracy. If so, then the combination of process-based models, verifiable databases, and GIS modeling will aid in forensic work. Here we expect new applications to develop that relate to both biological organisms and to materials synthesized or produced using local water or biological materials as starting materials. Examples related to biological materials, such as microbes, plants, and animals, are discussed further in this chapter. However not further discussed, because of page limitations, are synthetic and biosynthetic products, ranging from beeswaxes to hydrogen peroxide solutions, where aspects of the isotopic composition of the products are likely to be directly related to water-related isoscapes.

Lastly, two additional isotope approaches are being applied to the forensic sciences. First, isotope ratios of heavy elements exhibit detectable patterns across the landscape associated with geological features in some cases and anthropogenic activities in others. The abundances of isotopes in these heavy elements have become additional important tools in geolocating the region-of-origin of individuals. Heavy elements, such as lead, neodymium and strontium often exhibit distinct geographic patterns that can be used to provenance the origins of humans and biological materials (Beard and Johnson 2000; Crittenden et al. 2007; English et al. 2001; Ghazi and Millette 2004; Gulson et al. 1997; Pye and Croft 2004). Another useful technical approach is the measurement of ¹⁴C content of modern biological tissues to indicate the year of formation of a tissue, organ, and/or individual (e.g., Spalding et al. 2005). This technique, often referred to as carbon-14 bomb dating, capitalizes on the extensive aboveground nuclear testing in the early 1960s, ending with the adoption of the Nuclear Test Ban Treaty.

17.2.1 A Framework Based on Process-Based Models

During the past several decades, extensive theoretical and experimental progress has been made towards understanding the biochemical and physical basis of naturally occurring variations in the stable isotope abundances of different biological tissues and materials. These include many aspects of carbon, hydrogen, and oxygen stable isotopes in plant, animal, and microbe systems. For the forensic applications discussed in this chapter, it is the constructive combination of process-based models with the geospatial information associated with water isotopes that provides the basis of forensic geo-location applications (Fig. 17.1).

In the plant sciences, models have been developed to predict variations in the carbon (Ehleringer et al. 1993; Farquhar et al. 1989) and hydrogen and oxygen (Roden et al. 2000; Yakir and DeNiro 1990) isotope ratios of plant tissues (see also West et al. this volume). Similar progress has been made in understanding the basis for isotope ratio variations in microbes (Jarman et al. 2008; Kreuzer-Martin et al. 2003, 2004) and animals (Gretebeck et al. 1997; Kohn 1996; Podlesak et al. 2008). The chapter in this volume by Still and colleagues nicely shows how carbon isotope ratios of plants vary geographically and how this information might be useful in provenancing plant material. Here, of primary forensic interest is the application of hydrogen and oxygen isotope models to understanding how water and cellulose in plant tissues vary geographically, because both parameters are important components of food, wood, paper, and/or security documents. Yapp and Epstein (1982) established that there are strong correlations between the hydrogen isotopic composition of wood and environmental water; more recently Richter et al. (2008) have expanded on these observations. A first critical step in interpreting these patterns is an appreciation of the Craig-Gordon model that describes evaporative enrichment of a pool of water (Craig and Gordon 1965; Gat 1996). The second critical step is extending this theory to both the hydrogen and oxygen isotope ratios of plant tissue water (Dongmann et al. 1974; Flanagan et al. 1991; Lai et al. 2008) and eventually to the labile signal of water that might be recorded in plant organic matter (Richter et al. 2008; Roden et al. 2000). Understanding how environmental factors drive changes in the isotopic composition of plant-tissue water in stems, leaves, and fruits is essential to understanding hydrogen and oxygen isotope ratio variations in cellulose, but are also significant in their own right because product adulteration often occurs in fruit juices, wine, and other plant-based beverages (Breas et al. 1994; Pupin et al. 1998; Simpkins et al. 2000). Provenancing of wines and juices is already of significant forensic, commercial, and regulatory interest in the EU (Breas et al. 1994; Rossmann et al. 1999).

There are shared structural features that relate to the processes influencing the hydrogen and oxygen isotope ratios in biological organisms – insects, mammals, birds, plants, or microbes – that suggest forensic applications (Fig. 17.2). Understanding the processes occurring in biological systems that influence different pools of water within biological organisms is fundamental to determining the extent to which a biological signal might be useful in reconstructing aspects of geo-location information. By appreciating the common features of biological water within plants and animals, it is possible to examine how the same environmental processes and fractionation events might or might not influence the isotope ratios of an equivalent water pool.

Figure 17.2 shows the essential water pools of higher terrestrial organisms. Water is taken up in liquid form. That source water carries a geo-location signal and



Fig. 17.2 Biological organisms can be thought of as consisting of water pools that interact differently with their surrounding environment. As such, the four distinct but common pools of water within biological organisms influence how hydrogen and oxygen isotopes of water provide information that is useful in reconstructing aspects of geo-location

is transported to different internal tissues or to external tissues where an evaporative fractionation event might occur (i.e., lungs in animals or leaves in plants). Evaporative processes governed by humidity (another geo-location signal) affect the degree to which the hydrogen and oxygen isotopes of a water pool become evaporatively enriched. Here we see one distinction between plants and animals. Water that is isotopically enriched continues to circulate within animals (i.e., through blood or circulatory systems; sometimes referred to as "body water") and therefore should be isotopically enriched above the "input" drinking water, but the xylem of plants is effectively a one-way transport system. Thus, the isotopic composition of xylem conduits is not evaporatively enriched. The isotopic analyses of water in the transport tissues of animals and plants in a common environmental setting provide distinctly different but complementary isotopic information.

Internal plant and animal tissues not directly exposed to evaporative enrichment are not necessarily experiencing a water environment that is isotopically identical to that of the transport (plants) or circulatory system (animals; Fig. 17.2). Carbohydrate metabolism should tend to increase the oxygen isotope ratios of water pools within cells because the oxygen isotope ratio values of both diatomic oxygen and carbohydrates will always be higher than those of water in the existing pools. To the degree that water transport through aquaporins and/or water diffusion across plasma membranes is rapid in these tissues, the concentrations of metabolicallyenriched cellular water will be tend to be diluted by the net inward movement of surrounding circulatory water (e.g., body water in multi-cellular animals and aqueous media in microbes). However, if metabolically distinct water pools exist in plant, animal or microbe tissues, this implies that a 2-pool model should be applied to interpret the hydrogen and oxygen isotopic composition of fixed organic matter of tissues of forensic interest (e.g., actin-myosin or keratin in animals, cellulose in plants, or spore walls in microbes). The likelihood of a 2-pool versus a 1-pool water system in biological organisms should be a function of metabolic rate relative to aquaporin influences over water movement across the plasma membrane. Experimental evidence supports the possibility of two isotopically distinct pools in cells with high metabolic rates of both microbes (Kreuzer-Martin et al. 2005b, 2006) and animals (Podlesak et al. 2009b). No evidence is available to suggest that such gradients exist in cells with low metabolic rates (Kreuzer-Martin et al. 2005b).

As implied in Fig. 17.2, the hydrogen and oxygen isotope ratios of water in plants, animals and microbes or of the organic products produced by these organisms can contain information that is of forensic interest. It is important to recognize that the isotope ratio information in water pools is subject to evaporative influences unless measures are taken to ensure that subsequent evaporation or isotopic exchange do not occur following sample collection. These limitations do not necessarily apply to organic matter where there is a distinct likelihood that the hydrogen and oxygen atoms of the water pool environment have become recorded within the organic molecule.

17.3 Topics of Forensic Interest That Use Hydrogen and Oxygen Isotope Ratios

17.3.1 Hydrogen and Oxygen in Tapwater and Bottled Water

The majority of hydrogen and oxygen atoms in the body water pool of most organisms is derived from water taken up directly from their environment, and as a result the hydrogen and oxygen isotopic composition of environmental water can have a strong influence on the isotopic composition of body tissues. In some natural systems, this source water can be considered to be relatively "pristine" local meteoric water, and its isotopic composition estimated using geostatistical models for spatial distributions of precipitation water isotope ratios (Bowen, this volume). Within human systems, however, water can be derived from a complex blend of local and non-local, surface and subsurface systems that may contain waters with widely differing source locations, recharge ages, and residence times (and hence isotopic compositions). Human drinking water, for example, may include a blend of private, public, and commercial drinking waters and water-based beverages that each contributes to the total pool of hydrogen and oxygen atoms in human body water. In some cases, waters and beverages may be of forensic interest in and of themselves in cases where water rights, trademarks, or authenticity concerns are involved. Several studies have attempted to characterize the isotopic composition of water and beverage sources to human systems and investigate how variation within and among sources might be a useful or confounding factor in forensic investigations.

The complex blend of water sources exploited by humans introduces isotopic complexities that may be relevant to forensic interpretations of hydrogen and oxygen isotope data. Extensive research on groundwater systems has shown that fresh groundwater typically has an isotopic composition similar to the time-averaged composition of rainwater in recharge areas (Ingraham et al. 1991; Ingraham and Taylor 1991; Kortelainen and Karhu 2004; Smith et al. 2002). In many cases these isotope ratios may be similar to those predicted from precipitation sampling networks. However, in large aquifer systems where recharge and extraction sites are separated by considerable distance or elevation and fossil groundwater systems where recharge may have occurred under different climate regimes, modern precipitation isotope ratios can be very different from those of human water sources (Fritz et al. 1974; Sultan et al. 2007; Thiros 1995; Zuber et al. 2004). Surface water sources are fed by a combination of groundwater discharge and surface runoff, and may transport water across great distances encompassing large gradients in the isotopic composition of local precipitation. Both transport and evaporation from arid-region river, lake and reservoir systems can lead to large differences between surface water isotopic compositions and those of other water resources available at any particular geographic location (Dutton et al. 2005; Fekete and Gibson, this volume; Kendall and Coplen 2001). Additional complexity arises from the largescale diversion of water by humans, sometimes across large geographic distances, in arid climate regions. These diversions transport water across natural hydrological and isotopic barriers, and can contribute to regionally "anomalous" water resource isotopic compositions both through direct supply and through purposeful or accidental introduction to groundwater pools (Coplen et al. 1999; Williams 1997).

Despite this complexity, a survey of tap waters across the continental USA, which included data from a wide range of municipal water sources, does indicate the presence of strong, systematic, geographic gradients that mimic the general patterns characteristic of modern precipitation (Bowen et al. 2007; Fig. 17.3). The magnitude of these regional patterns relative to local isotopic heterogeneity in tap water can be assessed by examining the reduction of variance in the tap water data with a model that relates tap water isotope data to both spatial variation in incident precipitation isotope composition and to spatially coherent deviations between precipitation and tap water values. Although the complexities described above may be important factors in specific locations, this model explains 92–95% of the tap water isotopic variance, suggesting that on average they impart a relatively small uncertainty relative to the geographic gradients in North America at scales of thousands of kilometers. The implication for forensic studies is that generalized models of water resource isotope distributions, such as that presented by Bowen et al. (2007), may provide a reasonable baseline for large-scale geo-location of the origin of materials or humans, particularly in cases where the water consumed is a mixture of locally available water (e.g., for a person drinking water at home, work, and local restaurants). Still, significant improvements to the precision of such applications should be possible if the spatial distribution of specific water resource types can be incorporated into water isotope base maps in order to develop more sophisticated representations of the characteristic values and heterogeneity of local waters.

Significant attention has also been given to the stable isotopic composition of bottled waters and beverages, both as a source of water consumed by humans and as a commodity where authentication of provenance is of commercial interest. In the first regard, bottled beverages clearly represent a potential source of non-local hydrogen and oxygen that may be introduced to the human body and complicate



Fig. 17.3 Prediction maps showing estimated isotope ratios (a: δ^{2} H, b: δ^{18} O) and deuterium excess values (c) for tap water in the contiguous United States (Methods details are available in Bowen et al. 2008 and at http://waterisotopes.org). Fig. 17.3, see Appendix 1, Color Section

interpretations of the geographic movements of individuals. A broad survey of 234 bottled water samples representing at least 136 brand names, however, found that the isotopic composition of these waters was strongly correlated ($r^2 > 0.5$) with the composition of local meteoric water at the location of purchase (Bowen et al. 2005c). This likely reflects the widespread availability of bottled waters produced from filtered tap water and packaged regionally. Although "specialist" consumers of single-source bottled water brands may take in large quantities of water having atypical hydrogen and oxygen isotope ratios for their location of residence, this is not likely to be a significant factor for most bottled water consumers who consume primarily local or regional products. In the second regard, several studies have demonstrated that stable isotopes can be conservative tracers of the source of bottled waters and beverages (Bowen et al. 2005c; Brencic and Vreca 2006; Ingraham et al. 2004; West et al. 2007), although modification of isotope ratios during production (Brencic and Vreca 2006; Ingraham and Caldwell 1999) and storage (Spangenberg and Vennemann 2008) must be considered.

17.3.2 Hydrogen and Oxygen in the Body Water of Animals

The isotope composition of body water in animals is distinctly related to region of origin through drinking water sources, diet, and local influences of climate (Gretebeck et al. 1997; Kohn 1996; Longinelli and Padalino 1980; Podlesak et al. 2008). Recent modeling efforts have combined process-based models of the isotopic composition of human body water with GIS software to produce spatial maps of human body water isotopes (Podlesak et al. 2009a). These maps can be used forensically to identify region of origin for a specific sample, or as a parameter in predictive models of organic tissues such as hair and tooth enamel (see section on organic tissues).

Process-based models of body water isotopes are mass balance models based on the influx and efflux of hydrogen and oxygen through an organism (Kohn 1996; Luz et al. 1984; Schoeller et al. 1986). Detailed models of mammalian body water isotopes include all sources of hydrogen and oxygen such as drinking water, free water in food, hydrogen and oxygen bound in ingested organic molecules (food), atmospheric O2, and atmospheric water vapor. Models also include the effluxes of hydrogen and oxygen in the form of urine and sweat, water vapor loss associated with breathing and evaporation across the skin, and carbon dioxide loss. Evaporation across the skin and the lungs is influenced by temperature and humidity. Carbon dioxide loss, as well as metabolic water formation, is influenced by diet composition. Metabolic water and carbon dioxide are produced during the catabolism of energy (food) at the cellular level, influencing the isotope composition of the body water pool. The formation of metabolic water incorporates relatively ¹⁸O-enriched atmospheric O₂ and food oxygen into the body water pool, and the loss of oxygen as carbon dioxide will tend to deplete the body water pool in ¹⁸O (CO₂ is isotopically enriched relative to body water: $\alpha = 1.038$; Bottinga 1968; Brenninkmeijer et al. 1983). Detailed process-based models of body water isotopes include the

above variables as well as estimates for the isotopic composition of each influx and efflux, and estimates for the molar quantity of each influx and efflux.

Recently, Podlesak et al. (2009a) created a detailed process-based model for the isotopic composition of human body water. This model was created for an adult male that ate an average American diet. The authors used published studies on human energetics, metabolism, and water balance to estimate the molar quantities of each source of hydrogen and oxygen, and to also test the model. The model was subsequently input into ArcGIS 9.1 ModelBuilder© and combined with source layers for mean relative humidity, mean average temperature (CRU CL 2.0; 10' × 10'; www.cru.uea.ac.uk), δ^2 H and δ^{18} O values of drinking water, and δ^2 H and δ^{18} O values of human body water for the contiguous USA (Fig. 17.4).

Figure 17.4 displays the spatial map for equilibrium δ^2 H and δ^{18} O values of body water for an adult male. In this version of the model, the source layer for drinking water values was the spatial tap water map produced by Bowen et al. (2007) for the contiguous USA, and the δ^2 H and δ^{18} O values of food were linked to local drinking water. Values ranged from -147% to +4% for δ^2 H and from -16.3% to +1.4% for δ^{18} O (Fig. 17.4). The most enriched δ^{2} H and δ^{18} O values were in interior Texas and along the Gulf Coast and the most depleted values were in the higher latitudes and elevations of the interior West (Fig. 17.4). Drinking water supplies >55% of the hydrogen atoms and >40% of the oxygen atoms in the body water, and as a result, the isotopic composition of the body water values reflects the isotopic composition of tap water. However, the correlation between the isotope composition of drinking water and body water is not 1:1 due to the influence of climate through evaporation, the incorporation of food hydrogen and oxygen, and the incorporation of atmospheric O_2 into the body water pool. Atmospheric O_2 is responsible for >20% of the oxygen atoms in the body water. Thus, accurate spatial predictions of $\delta^2 H$ and $\delta^{18}O$ values of body water require detailed process models that incorporate all sources of hydrogen and oxygen and the influence of climate.

Podlesak et al. (2009a) also created a second parameterization of the model to determine the influence of drinking water source layer on body water isotope values. The first parameterization of the model used a spatial map of tap water values as the source for drinking water values. At this time, there are few areas of the world that have detailed spatial maps of tap water isotope values, and the isotopic composition of local tap water and local precipitation can be >40% and >7% different in hydrogen and oxygen isotope ratios, respectively (Bowen et al. 2007). The authors compared the δ^2 H and δ^{18} O values of body water created using a spatial map of tap water with the values created using a spatial map of precipitation. Body water isotope values were up to 40% and up to 5.5% different for hydrogen and oxygen isotope ratios, respectively, between the two spatial maps. In areas such as Texas, where tap water is more enriched than local precipitation, estimated δ^{2} H and δ^{18} O body water values were more enriched, and in areas where local tap water was more depleted than local precipitation, body water values were more depleted than those estimated using local precipitation values. Thus, reliable spatial maps of human body water and organic tissues will require detailed spatial maps of drinking water.



Fig. 17.4 Predicted average hydrogen and oxygen isotope ratios of body water across the USA (After Podlesak et al. in review), see Appendix 1, Color Section

Spatial maps of the isotopic composition of human body water have multiple uses in forensics. First, the spatial map can be used as an input to other models (such as a hair model, discussed later). Secondly, a spatial map of δ^2 H and δ^{18} O values of body water may be used to identify a local resident from a visitor. For example, the isotope composition of body water varies significantly across the USA (Fig. 17.4). A person traveling from one isotopically distinct region to another would be distinguishable from a local for a specific length of time, depending on the rate of body water turnover (half-life of body water ~6 days) and the isotopic difference between the regions.

17.3.3 Oxygen Isotopes in Inorganic Matter

The carbon isotopic composition of carbonate and phosphate in bioapatite found in mammalian tooth enamel and bone is linked to the isotopic composition of the diet (Longinelli 1984; Passey et al. 2007; Podlesak et al. 2008), and the oxygen isotopic composition is linked to the isotopic composition of the body water (Podlesak et al. 2009a). Carbon analysis of mammalian carbonates has been used to reconstruct diet in animals and humans (Ambrose and Norr 1993; Luz et al. 1990; Passey and Cerling 2002; Passey et al. 2005a, b; Stevens et al. 2006). The oxygen analysis of carbonates has been used to reconstruct climate and to identify region of origin for animals. In general, carbonate is enriched in 13 C between +9% and +15% relative to diet. The enrichment in ¹³C is likely the result of fractionation during the precipitation of bioapatite mineral from dissolved inorganic carbon within the blood. Similarly, carbonate is enriched in ¹⁸O also due to the precipitation of the mineral at normal mammalian body temperatures. As a result, the carbon isotope analysis of tooth enamel for humans can be used to identify geographic differences in diet related to the consumption of C_3 or C_4 crops. C_3 plants, such as wheat, are the basis of most agriculture in Europe and the Middle East, whereas, C₄ plants such as corn are the basis of much of the agriculture in North America. Similarly, latitudinal and elevational gradients in oxygen isotopes of precipitation are reflected in tooth enamel and the oxygen isotopic composition can be used to geo-locate samples.

As a first attempt at using carbonates in tooth enamel forensically to identify location of origin for modern human samples, we measured a group of teeth collected from across the contiguous USA. We measured teeth from California, North Carolina, New York, and Utah using methods described by Passey and colleagues. We used linear regression to compare oxygen isotopes of carbonate ($\delta^{18}O_c$) with oxygen isotopes of drinking water ($\delta^{18}O_{dw}$). The relationship was highly significant ($\delta^{18}O_c = 0.350\delta^{18}O_{dw} + 27.6\%c$, $r^2 = 0.85$, P < 0.0001; Fig. 17.5).

Next, we input the above regression relationship between local drinking water and tooth carbonate into ArcGIS 9.1 ModelBuilder© to produce a spatial representation of the δ^{18} O values of carbonate for the contiguous USA (Fig. 17.6). As expected, the distribution of carbonate δ^{18} O values closely reflected the variations in δ^{18} O values of tap water (Fig. 17.3). The regression in Fig. 17.5 compares well with previous observations for humans in Europe published earlier that were based on phosphate observations (D'Angela and Longinelli 1990; Longinelli 1984). At this initial stage of development, the exact slope and intercept are less important because these parameters are likely to change as the magnitude and diversity of the teeth observation, but a clear, linear pattern is suggested by the available data. We feel that the relevant take-home message here is that the same forces shaping the distributions of water isotopes across the USA are likely to be reflected in isoscapes of both hair and tooth isotope ratios.



Fig. 17.5 A plot of the relationship between oxygen isotope ratios of tap water in a region versus the oxygen isotope ratios of carbonate in human teeth from that region. Specimens were prepared as described by Passey et al. (2002, 2005a, b). The linear regression is y = 0.350x + 27.6% ($r^2 = 0.85$, P < 0.0001)



Fig. 17.6 The predicted distribution of oxygen isotope ratios of carbonates in enamel from human teeth across the contiguous USA, see Appendix 1, Color Section.

17.3.4 Hydrogen and Oxygen Isotopes in Organic Matter

Exchange processes during biosyntheses provide opportunities for the hydrogen and oxygen isotopes of cellular water to become incorporated into proteins, carbohydrates, and fatty acids. Each of these classes of biological compounds may have a forensic

application. Predicting spatial patterns to address a spatial forensic issue requires that at least some process-based understanding of isotope fractionation events is understood. Given the large variations seen in the hydrogen and oxygen isotopes of precipitation waters (Bowen this volume), there is an expectation that the isotopic composition of organic matter should, to some degree, reflect these broad spatial patterns if fractionation events during biosynthesis indeed incorporate a cellular water signal.

For several decades, supporting correlational data have been available. For example, the continental-scale correlations of hydrogen isotope ratios in tree-ring cellulose with local water by Yapp and Epstein (1982) provide evidence that the variations in hydrogen isotopes of cellulose are closely associated with changes in environmental waters. On the other hand, Roden et al. (2005) observed that cellulose isotope values in conifers did not follow a similar pattern along a climatic gradient from coastal to interior Oregon. The apparently conflicting patterns of both studies are resolved with the process-based model of Roden et al. (2000), which recognizes that both water source and humidity are important determinants of the final hydrogen isotope ratio values in cellulose. In some situations, the isotopic influences of humidity gradients and water sources result in a diminished correlation between water source and cellulose isotope ratios. It is our foundational premise that the spatial patterns of hydrogen and oxygen isotopes in biological systems are most consistently predictable by combining process-based models with spatially explicit climate data, which will both predict and explain apparent anomalies in correlational data.

Many of the key insights into how hydrogen and oxygen isotopes of the water environment become recorded into biological materials originate with the pioneering studies by DeNiro, Epstein, Fogel, Hoering, Sternberg, Yakir, and others from UCLA, Cal Tech, and the Carnegie Institution of Washington. It was the initial observations by DeNiro and Epstein (1977) that provided the mechanistic underpinnings of the fractionation processes associated with lipid synthesis. Experimental studies of plants by Sternberg et al. (1986) and Yakir and DeNiro (1990) helped define fractionation processes during synthesis of carbohydrates. Roden et al. (2000) later integrated many of the reactions to provide an overall model that linked source water, environment, fractionation processes, and carbohydrate movement among tissues to describe how the hydrogen and oxygen isotope ratios of cellulose reflected a combination of discrete steps where both source water and evaporated water could influence the isotope ratios of cellulose, the final carbohydrate product (Fig. 17.2). One relevant point with respect to Fig. 17.2 is that the leaf water and plant cellulose models do not use or require a 2-pool concept to predict hydrogen and oxygen isotope ratios of leaf water or of cellulose (or of carbohydrates in general). Two different tissues can be involved in carbohydrate synthesis, such as with sugar synthesis in autotrophic cells, transportation within the plant, and its subsequent incorporation into cellulose in heterotrophic cells. However, each of these are single pools that do not interact because water movement in xylem is unidirectional and therefore not a circulatory system.

Kreuzer-Martin et al. (2005a, 2003) extended the carbohydrate-model observations to show that microbial spores, a combination of protein and carbohydrates, would also reflect variations in the stable isotope ratios of water sources. Their interest was a forensic application: to source microbial spores, such as the anthrax spores mailed in letters in late 2001 (http://en.wikipedia.org/wiki/2001_anthrax_ attacks). Kreuzer-Martin and colleagues showed that the hydrogen and oxygen isotope ratios of spores record the isotopic composition of the culture medium and of the water in which these microbes were cultured, providing a potential geo-location signal. In *Bacillus* spores, approximately 74% of the oxygen atoms in a spore were derived from its source water. The utility of the stable isotope approach here is to allow investigators to both eliminate possible sources by showing that spore hydrogen and oxygen isotope values are not consistent with a region and to further guide investigations by indicating that stable isotope ratio values are consistent with particular geographical regions. Later studies by Kreuzer-Martin and Jarman (2007) showed that these relationships held, as expected, across *Bacillus* species.

The hair of humans and other mammals is composed predominately of the protein keratin, which in its isotope chemistry records information about food and water sources consumed by individuals. Perhaps most well documented are the patterns in the carbon, nitrogen, and sulfur isotope ratios of hair related to geographically distinct dietary preferences. European and Japanese hair is often distinct from USA hair, as is Chilean, Canadian, and German hair (Bol and Pflieger 2002; McCullagh et al. 2005). While these studies imply a geographical pattern, the pattern is based on culturally-related dietary preference and not fundamental climatic or environmental characteristics of a particular location.

The move to understand stable hydrogen and oxygen isotopes in proteins is a logical extension of the earlier plant cellulose and microbial studies. Human hair and fingernails should also record water source information, and in so doing provide geographic information for forensic studies. Several recent studies have suggested that isotope ratio differences in human hair exist and can be used to distinguish individuals of different geographic origin (Fraser et al. 2006; O'Connell and Hedges 1999; Sharp et al. 2003). A recent study of public interest involving stable isotopes was the case of the Ice Man discovered on the border between Austria and Italy (Hoogewerff et al. 2001; Macko et al. 1999).

Ehleringer et al. (2008a) developed a process-based model to explain hydrogen and oxygen isotope ratio values in proteins, assuming steady state conditions. Their particular application was to human hair and to the region-of-origin of that hair (Fig. 17.7), but it is a general model that is applicable to all proteins. Three predictions of that model are useful to point out here. First, essentially all oxygen atoms in a protein have exchanged with animal body water. Body water isotopes are influenced by drinking water, diatomic oxygen, and oxygen from energy sources. Second, a two-pool water model is required to explain water-environment patterns because water transport in animals is a circulatory system (not a one-way transport system as in plants). Third, the hydrogen atoms in R-groups do not interact with the aqueous media if the amino acid remains intact; thus they have the potential to provide food-source information. If the model adequately captures the essence of human biology, then this implies that oxygen isotope ratio analyses of protein can be used to infer geo-location information if body water isotopes can be adequately modeled. The model also suggests that simultaneous measurements of hydrogen and oxygen isotope ratio in protein can be used to extend the region-of-origin



Fig. 17.7 The predicted geographic distribution of the hydrogen (**a**) and oxygen (**b**) isotope ratios in human hair across the USA (From Ehleringer et al. 2008), see Appendix 1, Color Section

model to assess non-steady state conditions and to infer dietary conditions. While the Ehleringer et al. model was developed for steady state conditions, the multipool turnover concepts developed by Cerling et al. (2007) can be applied to extend this steady state model into more dynamic and realistic situations.

Four evaluations of the Ehleringer et al. (2008a) model have been made to date. Each independent set of observations provides support for the region-of-origin spatial model predictions. First, Ehleringer et al. (2008a) provide an evaluation based on random hair collections from 65 geographically distributed cities across the USA traversing a broad water-isotope gradient. The model accurately predicts the region-of-origin for 86% of the hair samples. Second, Chesson et al. (2008) observed hydrogen and oxygen isotope ratios of delipified hamburgers from fast-food meals collected throughout the USA. The correlations of the two isotopes in this protein are similar to those predicted by the Ehleringer et al. model. Third, Bowen et al. (2009) examined the hydrogen and oxygen isotope ratios of ancestral human populations in a diverse set of regions around the globe. The predictions of the model were in agreement with their observations, provided a local food component was added. Lastly, Thompson et al. (2009) examined hydrogen and oxygen isotope ratios along a geographical transect from southern India north through Mongolia. Their observations were fully predictable on the basis of parameters in the Ehleringer et al. model. Based on the model and the result of its four initial evaluations, we now apply this region-of-origin model to explore a forensic application – the geographical movements of unidentified murder victims prior to death.

17.4 Application of Stable Isotope Analysis to Unsolved Murder Investigations

In cases of unidentified human remains where traditional methods of establishing an individual's identity such as documentation, known samples of DNA and recognition by family members are not available, stable isotope analysis can provide useful information regarding an individual's recent and previous regions of residence. To investigators, reconstruction of movements across isotopic gradients can help to focus an investigation and enable the direction of valuable resources to the most promising geographic region. Isotopic evidence may answer a number of questions such as: Was this individual a resident in the region prior to death? Where was the childhood region of residence? Did the victim move prior to death? Was the diet of this individual unusual in any way? Two cases are detailed below that highlight the application of hydrogen and oxygen isotope ratio analyses to human hair and oxygen isotope ratio analysis to tooth enamel from unidentified human remains.

At this stage of the applications of stable isotope analyses to forensic applications, the focus is on patterns consistent with mean model predictions. Uncertainty in model projections and statistical patterns are unknown. Thus, none of the projections that follow should be construed as the definitive or ultimate patterns to be predicted. Instead they represent our best projections based on currently available models. To capture some of the uncertainty in model predictions, we have expanded the ranges of values used in model predictions by a factor of 2. Therefore, model predictions for where an individual may have traveled from represent broader geographic trends than predicted based on isotope measurement precision. Whether or not the model projections are sufficiently accurate to capture the actual trends will become known as the individual cases are solved now and in the future.

17.4.1 The Case of "Saltair Sally", Found in Utah

In October 2000, the unidentified remains of a young female, approximately 17–20 years of age, were discovered in a shallow grave in the desert close to Interstate Highway 80 at Saltair, west of Salt Lake City, Utah. The decomposed, scattered remains consisted only of incomplete skeletal elements, including the cranium and teeth, the victim's scalp and hair and a few distinctive personal effects. Physical examination provided an estimate of 12–24 months since death. An estimation of stature indicated a short individual with strikingly long hair. Despite efforts by police to establish the identity of this individual through the local media, no response from family or friends was elicited. A missing person report was not filed and the victim's identity remained unknown. The individual has been referred to as "Saltair Sally" and the case eventually became a cold case.

In 2007, the Utah State Medical Examiner released approximately 26 cm of hair for isotope ratio analysis along its length in order to provide information about the potential travel moments of this individual. Several hairs were aligned, sectioned, and analyzed for hydrogen and oxygen isotope ratios following established methods (Bowen et al. 2005a; Ehleringer et al. 2008a). Using an average growth rate of 0.4 mm/day (Saitoh et al. 1967), the hair length was estimated to represent approximately 22 months prior to death. The most recent proximal hair segment was also converted to CO_2 and sent for ¹⁴C analysis to establish a year of death using the "bomb-spike" signal, estimated as 1996, several years earlier than previously thought.

The oxygen isotope ratios from the hair showed periodic changes before death (Fig. 17.8). The oxygen isotope ratios demonstrated a cycle, reaching two peaks of approximately 10% at months 22 and 10 and a plateau of approximately 10% around 4 months prior to death.

It is estimated that oxygen isotope ratios measured from hair will attain complete equilibrium within 3 months (Ehleringer et al. 2008a). This corresponded well with the period between month 8 and month 4 prior to death, where the values increased until they reached a plateau and remained constant until death. Prior to this, the data indicated that the victim was not resident of a single location for long enough to reach complete isotopic equilibrium and moved across an isotopic gradient, possibly at least twice each year. The data suggested at least three movement events in the past 22 months followed by a period in one location just before death. These patterns suggest that this individual was mobile rather than being a long-term resident in one location.

While both hydrogen and oxygen isotope ratios should be correlated with the isotopes of water in the region, we feel that it is more appropriate to consider oxygen isotope ratios when inferring information about the geographical movements of an individual across isotopically distinct regions (isoscapes). This is because the Ehleringer et al. (2008a) model predicts complete oxygen isotope exchange of amino acids with the environment during protein hydrolysis, but only partial hydrogen isotope exchange (because of the non-exchangeable hydrogen on the R-groups



Fig. 17.8 Identification of locations on a plot of the oxygen isotope ratios measured from "Saltair Sally" as a function of time before the victim's death. Time was calculated based on an average growth rate of 0.4 mm/day. Data are 3-point running means. Three iso-regions are noted where it is suggested that the victim had resided for a period of time. Iso-region 1 is tentatively identified as corresponding to three points along the time line corresponding to the 4, 12 and 22 months prior to death. Iso-region 2 is tentatively identified as the minimum in the time line at months 19–17 prior to death. Iso-region 3 is tentatively identified as the minimum in the time line at months 12–8 prior to the victim's death

of amino acids). Five points along the oxygen isotope ratios measured along the length of the hair that was considered to represent three different geographical locations were chosen (Fig. 17.8). Predictions of the semi-mechanistic model of Ehleringer et al. (2008a) were inverted to predict the drinking water input that would produce the observed patterns of oxygen isotope ratios in hair. Using an isoscape of oxygen isotope ratios in drinking water (Bowen et al. 2007), with a margin of $\pm 0.5\%$ added to either side of the modeled value per location, maps of the geographic regions were produced to predict where this individual had traveled in the last 22 months of her life (Fig. 17.9a–c).

Iso-region 1 was consistent with three periods of time along the hair and suggested that at 22 months before death the victim could have been in an area consistent with the area where her remains were discovered (Fig. 17.9a). Immediately after this she moved to Iso-region 2, a smaller geographic area consistent with somewhat more depleted oxygen isotope values to the north of Iso-region 1 (Fig. 17.9b). Movement back to an area consistent with iso-region 1 occurred approximately 18 months prior to death, followed by movement to iso-region 3 approximately 6 months later. Iso-region 3 was consistent with a smaller geographical area further to the north of Iso-region 1, corresponding to significantly



Fig. 17.9 A Geographic Information System (GIS) representation of those geographic regions that are associated with iso-region 1 (**a**), iso-region 2 (**b**), and iso-region 3 (**c**). For these predictions, we used a range of δ^{18} O values that spans ±0.5% of the predicted mean δ^{18} O value for different regions

more depleted oxygen isotope values (Fig. 17.9c). Subsequently, the victim moved back to Iso-region 1 approximately 8 months before death and remained resident in this area until her death.

In order to establish whether Saltair Sally was local to the Intermountain West region of the USA during her childhood, tooth enamel from the upper left incisor and the upper left second molar was analyzed for oxygen isotope ratios. It was estimated that these teeth mineralized between 8 to 9 and 12 to 13 years of age, respectively, based on published measurement and modeling approaches (Nakamura et al. 2007; Spalding et al. 2005). The oxygen isotope values measured from both teeth were very similar (23.0% and 23.1%, respectively), suggesting that the victim did not move across an isotopic gradient between approximately 8 and 13 years of age. The relationship between oxygen isotopes in tooth enamel carbonate and oxygen isotope values in local water was used to calculate a predicted water oxygen isotope value and the corresponding geographic region of origin where the victim was likely resident while her teeth mineralized (Fig. 17.6). This region of residence was estimated to be consistent with a childhood in the Intermountain West, and is predicted to be the same region that she was a resident of just before and at the time of her death (as predicted from oxygen isotope analysis of her hair).

17.4.2 An Unidentified Victim from Mammoth Lakes, California

In May 2003, a hiker and his dog discovered a human skull in the Sierra Nevada near Mammoth Lakes, California and alerted the police. Soon afterwards a shallow grave was uncovered by police containing the fragmented remains of a female homicide victim and her clothing (Dostie 2007). The medical examiner reported that she was between 30 to 40 years of age, short in stature, slight of build and possibly of Southeast Asian origin. She had been buried for between 6 and 9 months under the snowpack since her death. An appeal to establish her identity was made by the police through the local media outlets. A US Forest Service employee responded and recalled a woman matching the victim's description had been in the area with a man thought to be her husband in the fall of 2002 and that she had confided she was afraid of him. Based on their description, a forensic artist produced a reconstruction of the victim that was circulated, however no family members contacted the police and a missing person report matching the victim was not filed. The investigation continued but the victim's identity remained unknown.

In this case, DNA analysis was applied to establish the victim's ethnic ancestry rather than as a comparative method to directly establish identity. The results suggested that she was of Native American rather than Asian origin, and this was further supported by a physical anthropologist who examined the remains. Further analysis of both mitochondrial and nuclear (HLA loci) DNA showed the victim's origins were likely to have been in the Oaxaca region of southern Mexico.

Carbon and oxygen isotope ratios were previously carried out on the victim's hair, tooth enamel and a bone sample (Schwarcz 2007). The ¹³C-enriched carbon isotope ratios suggested that the victim's diet contained significantly more maize derived carbon than the average US resident, supporting a Mexican or Central American origin. Oxygen isotope ratios of the tooth enamel and bone carbonate

showed values that corresponded to regions with highly enriched oxygen isotope values, indicative of a very warm climate and consistent with both a childhood and subsequent adulthood in southern Mexico.

In light of the above information, oxygen and carbon isotope ratios were measured along the length of the hair in order to reconstruct the victim's movements prior to death (Fig. 17.10). Almost 25 cm of hair was received for analysis, which corresponded to approximately the last 21 months of the victim's life. The oxygen isotope ratios showed relatively subtle but clear changes along the length of the hair that suggested movement across isotopic gradients by this individual (Fig. 17.10). The carbon isotope data were consistent with the values from previous analyses (Schwarcz 2004, 2008), and showed much more ¹³C enrichment along the whole length of the hair than is typically seen in USA residents (McCullagh et al. 2005; unpublished data).

When the oxygen isotope ratios were compared to the carbon isotope ratio data, both datasets showed corresponding depletions in values from approximately



Fig. 17.10 A plot of the carbon and oxygen isotope data measured from hair from the Sierra Nevada murder victim as a function of time prior to the victim's death with three regions marked. Time was calculated based on an average growth rate of 0.4 mm/day. Data are 3-point running means. Three regions are marked where it is suggested the victim resided for a period of time. Iso-region 1 is tentatively identified as corresponding to the plateau at months 22–12 prior to death; iso-region 2 is as the minimum in the time line at months 12–6 prior to death and iso-region 3 as the maximum in the time line immediately prior to the victim's death

month 12 to month 7 prior to death (Fig. 17.10). The hair carbon and oxygen isotope values for the period between 22 and 12 months prior to death were relatively steady, suggesting that the individual did not cross any significant isotopic gradients during this period. The data suggested that approximately a year before death the victim traveled from a warmer region, where a more ¹³C-enriched diet (likely due to maize) was also consumed, to a cooler region, where the ¹³C-enriched input to the diet decreased slightly. The depletion in hair oxygen isotope ratios was rapid but she did not remain in this region for long enough for oxygen isotope ratios to reach full equilibrium with local water-isotope values, evidenced by the lack of a plateau. About 6 months before death another travel event took place and both oxygen and carbon isotope ratios became more enriched again, suggesting movement to a warmer place and increased consumption of ¹³C enriched foods. Immediately prior to death, the isotopic values appeared to be in flux and were still increasing.

In order to assess what the expected oxygen isotope ratios of the hair of a Mammoth Lakes resident were, water from the site was collected, analyzed and used as the input to the Ehleringer et al. (2008a) model. The calculated oxygen isotope ratios predicted for hair were between $9.5\%_0$ and $10.1\%_0$. Even with an allowance of $\pm 0.5\%_0$, at no time did the values in the victim's hair reach these depleted values. The isotopic data therefore strongly suggested that this individual was a recent visitor to the Mammoth Lakes area and had not been a resident at any time in the last 2 years.

The shifts in isotope ratios suggested that the victim had moved across isotopic gradients at least twice in the last 2 years; 12 months before death, again at approximately 6 to 8 months before death and finally possibly 2 months prior to her death. Three points where shifts were seen in the oxygen isotope ratios measured from the hair were chosen and the oxygen isotope ratios were used to predict the corresponding geographic regions (Fig. 17.11). Predictions of regions-of-origin were made using the hair oxygen isotope ratios measured at these points as input to the inversion calculations in the Ehleringer et al. (2008a) model. Regions of the USA and Mexico with corresponding hair oxygen isotope ratios in drinking water and precipitation respectively were then selected as detailed above and maps were produced (Fig. 17.11a-c). These regions suggested geographical movements that may have involved crossing the USA-Mexican border. The supporting evidence from these and other analyses suggested that the victim was resident in or close to the Oaxaca to Sonora regions of Mexico in the 22-12 months before her death (Fig. 17.11a). She then moved north to a region that included northern Sonora and the USA side of the border, where she remained until 6 months prior to her death (Fig. 17.11b). At this point she may have traveled south again to the southern side of the USA-Mexico border, possibly as far as central Mexico (Fig. 17.11c), before her death and subsequent discovery in the Sierra Nevada of California.

Oxygen isotope ratios of carbonate within the tooth enamel of this individual have been analyzed by Schwarcz (2007). From its value of +22.0‰, the geo-location information for this individual is consistent with iso-region 2 in Fig. 17.11. This datum is consistent with an individual who may have grown up in Mexico before moving to the USA.



Fig. 17.11 A Geographic Information System (GIS) representation of those geographic regions that are associated with iso-region 1 (a), iso-region 2 (b), and iso-region 3 (c) for the Sierra Nevada murder victim. For these predictions, we used a range of δ^{18} O values that spans $\pm 0.5\%$ of the predicted mean δ^{18} O value for each region

Stable isotope analyses of hair and teeth have proved useful in other forensic investigations, confirming other forensic data and contributing to efforts that resulted in the identification of previous cold cases. However, at the time of submission of this publication, the identities of these two murder victims remain unknown.

17.5 Emerging Opportunities

The application of stable isotopes in forensic sciences is new (Meier-Augenstein and Liu 2004); the expansion into spatial forensic patterns is even newer (Ehleringer et al. 2008b; Meier-Augenstein and Fraser 2008). However, the need for stable isotope information that is relevant to broad spatial scales is already evident (Bowen et al. 2005b). As our understanding of both spatial patterns in water isotopes and of biological fractionation processes improves, it is expected that more applications will emerge where stable isotope analyses play an important role in guiding an investigation. When the use of both stable isotopes of light elements, reflecting biological process as described here, is combined with stable isotope analyses of heavy elements, reflecting soil parameters, finer-resolution provenancing capacities should be achievable. The heavy-element foundation in geosciences clearly establishes the forensic utility of strontium and neodymium isotopes in quantifying the regionof-origin of individuals and products of interest (Crittenden et al. 2007; Garcia-Ruiz et al. 2007; Pye and Croft 2004). In some cases, it is expected that stable isotope ratio data will provide key information that becomes part of the prosecution evidence introduced into the judicial court system. In other cases, the isotope data will serve to guide investigations, confirm suspected relationships, and reject others. Stable isotopes may well become an increasingly important component of food provenancing and its commercial and regulatory implications, as implied by the recent TRACE studies in the EU (http://www.trace.eu.org/) and by studies from Chesson et al. (2008). As our appreciation of natural, biosynthetic, and synthetic fractionation factors increases over time, the forensic applications of stable isotope are likely to play a greater role in forensic investigations and in criminal prosecution.

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