CHAPTER 25
Spatial distribution of stable isotope values of human hair: Tools for region-of-origin and travel history assignment

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25.1 Introduction

Stable isotope values of human tissues contain information on the geographical location and population of origin of a person, at the time the tissue was synthesized, that can aid in the identification of unknown victims (Ehleringer and Matheson Jr, 2010; Ehleringer et al., 2008; Meier-Augenstein, 2010). Measurements of stable carbon (C), nitrogen (N), sulfur (S), oxygen (O) and hydrogen (H) isotope ratios can be used to test hypotheses related to the origin and recent travels of a person, potentially assigning or excluding a person to a specific population or geographical area (Ehleringer and Matheson Jr, 2010; Ehleringer et al., 2008; Meier-Augenstein, 2010). The spatial distribution of stable isotope values of human tissues is non-random and, importantly, predictable across regions and populations.

Of all human tissues, keratinous tissues such as hair (and fingernails) have some unique characteristics that make them valuable in forensic investigations (Ehleringer et al., 2008; Meier-Augenstein, 2010; Mützel Rauch et al., 2009; Thompson et al., 2014). Hair grows continuously, providing a timeline of a person's recent life, ranging from weeks to years. Hair can be sampled non-invasively, thus large reference datasets can be constructed. Finally, the high abundance of the five "light" elements (C, N, S, O and H) and resistance to weathering makes hair very suitable for stable isotope analyses. In this chapter we will lay out basic concepts related to hair and isotope incorporation, as well as known spatial patterns that provide the basis for human provenancing. We consider it is of uttermost importance that researchers considering these analyses understand how hair records the isotopic signals related to diet, drinking water and geographical environment. Therefore, effort has been placed into explaining these aspects.

25.2 Why hair?

Hair has been termed the "most sophisticated biological composite material" (Popescu and Höcker, 2007). Hair is a highly stable complex external secretion, with one of the lowest solubilities measured for a protein (Popescu and Höcker, 2007; Robbins, 2012). A thorough description of hair histochemistry, macro- and microscopic morphology, and the molecular and genetic factors regulating hair biosynthesis is beyond the scope of this chapter, and can be found in the provided references. However, it is important to describe a few important features of hair. Hair is 95% proteinaceous; this proteinaceous material is composed primarily of the fibrous protein α-keratin arranged as helicoidal microfilaments (Popescu and Höcker, 2007; Robbins, 2012; Swift, 1997). The hair shaft or fiber (visible above the skin) has a macroscopic structure comprised of a thin cuticle and a thicker cortex, and in some types of hair there is a central portion known as the medulla; externally the hair shaft can be described as a proteinaceous monofilament covered with scales.

The follicle, not visible above the skin, is made of epithelial cells and connective tissues, and it is where the hair fibers are "born" (Popescu and Höcker, 2007; Robbins, 2012). Five different zones are recognized in the follicle where the hair shaft is produced and extended: zone 1 – bulb zone (cell proliferation and differentiation); zone 2 – elongation (fibril formation); zone 3 – pre-keratinization (lateral aggregation); zone 4 – hardening (keratinization); and zone 5 – post-hardening (hard keratin).

Humans have three types of hair: lanugo, vellus and terminal (androgenic) (Robbins, 2012). Lanugo corresponds to the hair found covering the body of fetus and newborns; vellus is found in children and in some areas of adult women; while terminal hair is the most common hair type found in adults. Hair has a growth pattern with three phases followed by shedding (Popescu and Höcker, 2007; Robbins, 2012). Phase 1, the anagen, is the active growth phase of hair. During this phase the hair fiber grows about 0.8 to 1.4 cm every 30 days (Lehn et al., 2018; Robbins, 2012; Sachs, 1995). Phase 2, the catagen or transition stage, lasts only a few weeks (less than a month). During

catagen, the metabolic activity of the follicle slows down, and the base of the bulb migrates upward towards the skin pore, forming what is known as a club hair. Phase 3, the telogen or resting stage, lasts between 4 and 8 weeks. During this stage the bulb is completely atrophied and growth has stopped. At the end of the telogen the hair fiber is eventually shed, pushed out by the new hair that begins to grow beneath, and a new cycle starts. The main difference among terminal hair in different parts of the body corresponds to the duration of the different growth phases, particularly the anagen. Scalp hair stays in anagen for 2–6 years, although longer periods have been recorded. Usually 90% of scalp hair is at anagen at any given time, with the other 10% is in catagen or telogen.

In its natural, untreated state, hair is 95% proteinaceous, with the remaining 5% composed of lipids, glycoproteins, pigments (eumelanin and pheomelanin) and remnants of DNA (Robbins, 2012; Robbins and Kelly, 1970; Swift, 1997). Hair is composed of about 17 amino acids and amino acid derivatives (such as cysteic acid, Table 25.1: Petzke et al., 2005a; Robbins, 2012; Robbins and Kelly, 1970). Overall, hair is composed of 50 wt% (weight percent) C, 22 wt% O, 16 wt% N, 7 wt% H and 5 wt% S (Popescu and Höcker, 2007; Robbins, 2012). The high content of S, in comparison with other proteins, is due to the high abundance of cystine, the oxidized dimer of the amino acid cysteine. Cystine content in human hair is about 13 to 18% (Table 25.1). The cystine cross-links (S-S bonds) provide hair fibers with high temperature stability and very low solubility (Popescu and Höcker, 2007; Robbins, 2012). Also, these S cross-links are responsible for the curliness of hair and are the target of many hair treatments. Thus, the overall high content of C, N, S, O and H; the high stability that translates into high rates of preservation; and the continuous growth pattern of hair make it (and other keratinous tissues such as fingernails) a great candidate for stable isotope analysis.

Table 25.1 Amino acids contained in hair keratin.

Amino acid[*]	Molecular formula	% of amino acids
Alanine	$C_3H_7NO_2$	4.5
Arginine	$C_6H_{14}N_4O_2$	6.5
Aspartic acid	$C_4H_7NO_4$	5.1
Cysteine[**]	$C_3H_7NO_2S$	16.2
Glutamic acid	$C_5H_9NO_4$	13.0
Glycine	$C_2H_5NO_2$	5.8
Histidine	$C_6H_9N_3O_2$	0.9
Isoleucine	$C_6H_{13}NO_2$	2.6
Leucine	$C_6H_{13}NO_2$	6.2
Lysine	$C_6H_{14}N_2O_2$	2.6
Methionine	$C_5H_{11}NO_2S$	0.5
Phenylalanine	$C_9H_{11}NO_2$	1.6
Proline	$C_5H_9NO_2$	8.4
Serine	$C_3H_7NO_3$	11.5
Threonine	$C_4H_9NO_3$	7.0
Tyrosine	$C_9H_{11}NO_3$	2.1
Valine	$C_5H_{11}NO_2$	5.5

Percentages (%) are approximate and vary depending on hair type and methods of keratin digestion and amino acid separation (Robbins, 2012; Robbins and Kelly, 1970).

[*] Tryptophan and citrulline have been reported by some authors.

[**] Cysteine is also found as half-cystine and cystic acid.

25.3 Methods

Preparation of hair samples for stable isotope analysis (SIA) usually involves treatment with a chloroform:methanol (2:1) mixture to remove lipids and surface contaminants and is followed by drying in an oven or at room temperature (Bowen et al., 2005a; Ehleringer et al., 2008; Meier-Augenstein, 2010; O'Connell et al., 2001; Valenzuela et al., 2011). For bulk analyses, hair is ground to a fine powder. To reconstruct past travels and dietary changes, hair samples are sectioned into small segments; segment length depends on how many hair fibers are bundled together to reach a minimal mass per sample, but it could be between 0.25 and 1.0 cm. Powdered or sectioned hair is weighed, and then dried prior to SIA to remove sorbed water in cases of O and H isotope analyses (Bowen et al., 2005a; Meier-Augenstein, 2010). Stable isotope analyses are nowadays conducted in a continuous flow mode, coupling an elemental analyser (EA; for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) or a high-temperature conversion (TC/EA; for $\delta^{18}O$ and $\delta^{2}H$) to an isotope ratio mass spectrometry (IRMS) instrument.

For SIA of H, several days are needed for exchangeable H atoms to equilibrate with water vapor in the laboratory's local atmosphere before segmenting (Bowen et al., 2005a; Ehleringer et al., 2008; Meier-Augenstein, 2010). After analyses, the contribution of the exchangeable H atoms to the hair $\delta^{2}H$ values must be removed by comparison with appropriately calibrated laboratory hair standards with a known fraction of exchangeable atoms (Bowen et al., 2005a; Meier-Augenstein, 2010).

25.4 How is isotopic information incorporated into hair?

Although hair was previously described as a composite structure, during SIA the measured signal overwhelmingly corresponds to the amino acids of the keratin molecules (Figure 25.1). Stable isotope ratios of the elements C, N, S, O and H can be classified into two groups according to their source. C, N and S are solely incorporated into human tissues from food; as a consequence, sometimes they are referred to as "dietary isotopes." O and H are incorporated from a mix of sources (e.g. drinking water, food, atmospheric air, etc.) but are usually thought to reflect primarily locally available drinking water (Cerling et al., 2016; Chesson et al., 2014; Ehleringer et al., 2008, 2015; Fraser et al., 2006; Lehn et al., 2011; Meier-Augenstein, 2010).

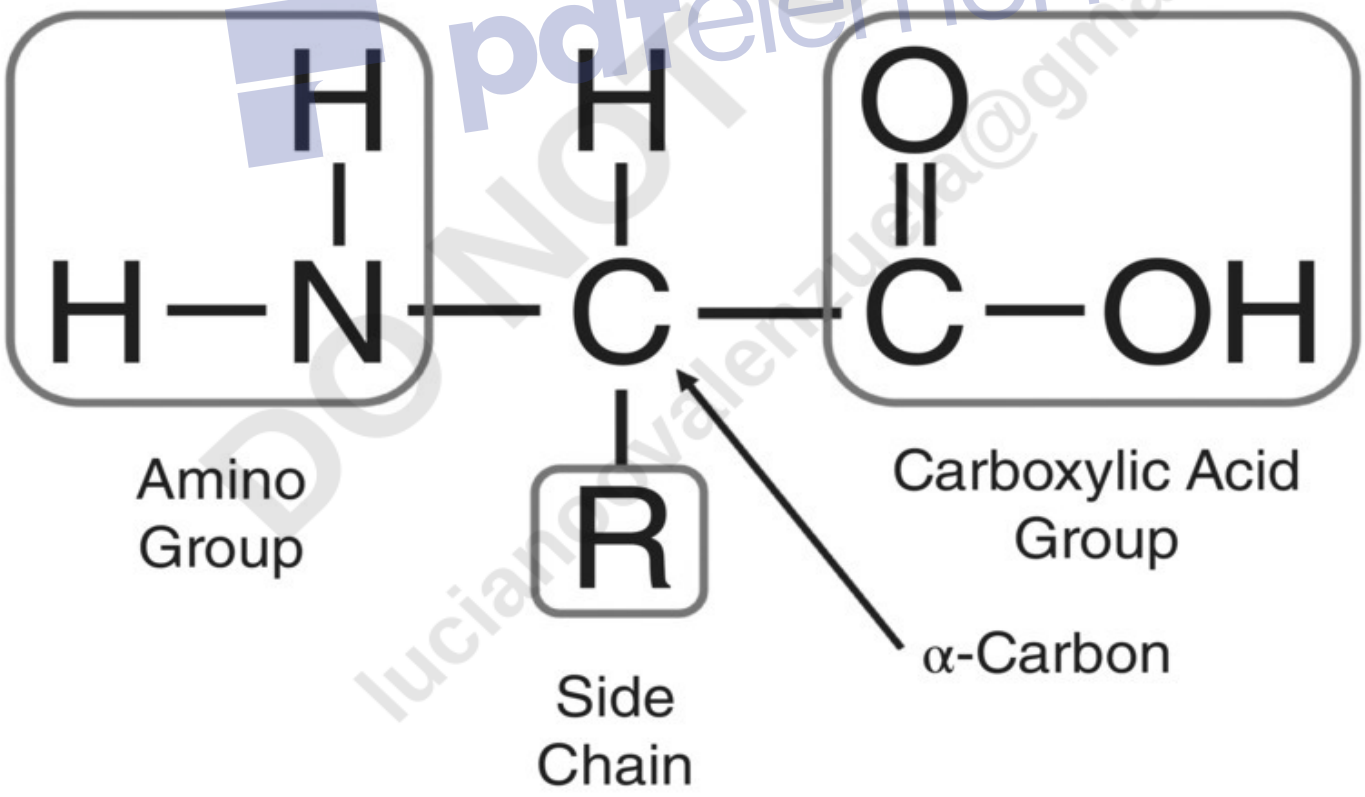

Figure 25.1 Basic structure of an amino acid. The general formula of an amino acid is composed of an α-carbon atom, a carboxyl group, an amino group and a side chain group (R). The side chain varies in each amino acid.

25.4.1 Carbon

The carbon atoms in amino acids of hair keratin are divided into different groups according to their origin and metabolic routing. The carbon skeletons of nine essential amino acids derive directly, without modification, from ingested dietary proteins, peptides and free amino acids (Brody, 1999). These are called essential amino acids due to the fact that humans lack the enzymatic pathways to synthesize them (Brody, 1999). On the other hand, the C skeletons from non-essential amino acids can be synthesized *de novo* using C derived from the catabolism of carbohydrates and fats (Brody, 1999). The amino acid tyrosine is derived directly from another amino acid, the essential phenylalanine. Non-essential amino acids can also be acquired directly from dietary protein or free amino acids. The proportion of *de novo* synthesis varies with many metabolic aspects and nutritional condition, as well as with diet quality (whether diet meets the demands of metabolism), which can change with growth, metabolic state and disease condition. Consequently, analyses of $\delta^{13}C$ values of individual amino acids reveal different values for essential amino acids and non-essential amino acids in hair (Petzke et al., 2005a). Ultimately the $\delta^{13}C$ values of hair represent a mixed signal, but always originating in the dietary carbon.

25.4.2 Nitrogen

The only source of N for the amino groups of proteins corresponds to the N of amino acids consumed as proteins or as free amino acids in the diet (Brody, 1999). All amino acids contain one atom of N in the amino group, and in hair only three amino acids contain N atoms in the side chain (Table 25.1). Regarding N isotope fractionation, amino acids can be divided into two groups (Table 25.2): those that are most frequently involved in transamination and deamination reactions (trophic amino acids), which are enzymatic reactions exchanging or removing N from amino acids; and those that seldom participate in such reactions (source amino acids) (Chikaraishi et al., 2009; McClelland and Montoya, 2002). The latter group will therefore undergo less enzymatic processing and less N isotopic fractionation, reflecting almost directly the isotopic composition of the source of the ingested amino acids. In contrast, during the deamination reactions that generate excretion products (urea), enzymes remove N atoms faster from ^{14}N-C bonds than from ^{15}N-C bonds, thus tissues become ^{15}N enriched (Fry, 2006; Kelly, 2000). Because of this, the trophic amino acids greatly increase their $\delta^{15}N$ values with each trophic step. Bulk hair analyses result in a mixed $\delta^{15}N$ signal with the end result of tissue $\delta^{15}N$ values being higher than the food by approximately 3–5‰.

Table 25.2 Amino acids classified as essential vs. non-essential according to the ability to synthesize the C backbone, and as source vs. trophic according to nitrogen isotope fractionation.

	Source	**Trophic**
	Phenylalanine	Isoleucine
Essential	Lysine	Leucine
	Threonine	Valine
	Serine	Aspartic acid
Non-essential	Glycine	Glutamic acid
	Tyrocine	Proline
		Alanine

25.4.3 Sulfur

Similar to N, the only sources of S for the side chain group of proteins are ingested amino acids and proteins – specifically, the ingestion of cysteine and methionine, both S-bearing amino acids (Brody, 1999). Cysteine is a non-essential amino acid; it can be obtained directly from food or synthesized *de novo*; however the tiol (or sulfhydryl) group (S-H) of newly synthesized cysteine is derived from the essential amino acid methionine (Brody, 1999). No large enzymatic fractionation is expected during S metabolism due to the low relative mass difference between the two isotopes measured, ^{34}S and ^{32}S (Fry, 2006). However, internal metabolic recycling of proteins appears to increase the $\delta^{34}S$ values of animal tissues under low quality diets (as based on protein content; Richards et al., 2003).

25.4.4 Body water

O and H isotope values of hair are closely associated with body water isotope ratios, because there is isotopic exchange between amino acids and body water (Ehleringer et al., 2008; O'Grady et al., 2012; Podlesak et al., 2008). Thus, hair amino acids reflect, to a certain degree, the body water $\delta^{18}O$ and $\delta^{2}H$ values. The body water pool defined as free water in the body, contained in the tissues and cells, is composed of a mixture of preformed drinking water

as free water in the body, contained in the tissues and cells, is composed of a mixture of preformed drinking water, inhaled water vapor, food water (free water contained in food, such as fruit juice) and metabolic water formed from the metabolism of organic molecules (sugars, fats and proteins), and inspired diatomic oxygen used in the oxidative phosphorylation (cellular respiration) in the mitochondria (Ehleringer et al., 2008; Kohn, 1996; Longinelli, 1984; O'Grady et al., 2012; Podlesak et al., 2008). Figure 25.2 shows a conceptual model of body water inputs and losses, as well as hair isotope values.

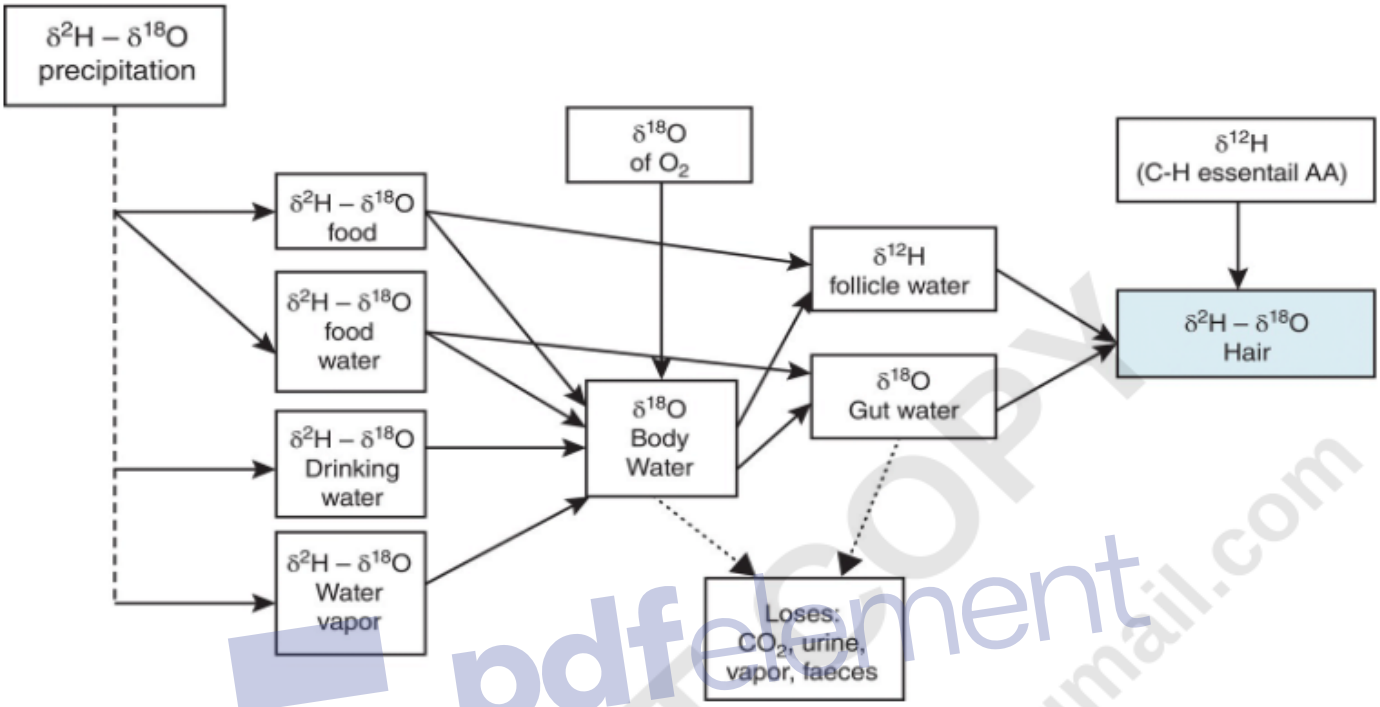

Figure 25.2 Conceptual illustration of Ehleringer et al. (2008) process-based model. The schematic represents the flow of O and H from the sources (drinking water, water vapor, food and food water, and atmospheric oxygen) through the intermediate pools (body water, gut water, and follicle water) and finally into hair keratin. Recent work adds a small contribution of O derived directly from dietary amino acids (Magozzi et al., under review).

25.4.5 Oxygen

Most of the O in proteins is associated with the carboxylic acid group. Only five amino acids have extra O atoms as part of their side chain (Table 25.1). At low pH the carboxyl O atoms are subject to isotopic exchange with the surrounding water (Ehleringer et al., 2008; Kohn, 1996; Podlesak et al., 2008). In the case of ingested foods, isotopic exchange takes place when amino acids are cleaved from proteins in the stomach (low pH). After absorption through the intestinal wall, subsequent isotopic exchange is low due to a more neutral pH. Thus, the O atoms in the carboxylic acid group during protein synthesis should largely reflect the isotopic composition of gut water. Gut water is mainly composed of gastric fluids, body water and drinking water (Figure 25.2). Human gut water isotope values are assumed to be in isotopic equilibrium with body water (Ehleringer et al., 2008). A fraction of the O atoms contained in amino acid side chains may be routed directly from dietary amino acids, though this fraction has not been estimated, and a recent estimate based on an inventory of O in essential and non-essential amino acids suggests that this comprises ~19% of all keratin O in human hair (Magozzi et al., under review).

25.4.6 Hydrogen

The H atoms in amino acids of hair can be classified into four groups according to their original source, their metabolic routing, and their exchangeability (Bowen et al., 2005a; Ehleringer et al., 2008; Fraser and Meier-Augenstein, 2007; Podlesak et al., 2008). First, H atoms involved in C-H bonds from both essential and non-essential amino acids routed directly from the diet do not exchange with body water, and thus their isotopic composition should be directly related to the isotopic composition of dietary protein (without fractionation or discrimination). Second, H atoms involved in C-H bonds from non-essential amino acids synthesized *de novo* should reflect the isotopic composition of body water at the time the H atoms were fixed. The degree of non-essential amino acids synthesized *de novo*, and thus the fraction of H atoms fixed *in vivo*, as mentioned before, likely depends on the amount of protein consumed, the amino acid composition of dietary intake and the nutritional condition of the person.

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Third, H atoms not involved in C-H bonds (i.e. H atoms in carboxyl, amide and sulfhydryl groups) can exchange with surrounding water and thus reflect body water isotopic composition. For these two groups (H atoms in C-H bonds of non-essential amino acids synthesized *de novo*, and H atoms not involved in C-H bonds) there is potential for H isotopic exchange with body water. Finally, a small fraction of H atoms (9–16%) in non-C-H bonds that freely exchange after hair production will reflect the local atmospheric water vapor, and this confounding effect should be removed or corrected during SIA and prior to interpretation of measured isotope data (Bowen et al., 2005a; Chesson et al., 2009; Coplen and Qi, 2012).

25.4.7 Integrated signal

Hair isotope values integrate a mixture of different inputs. Even in the seemingly simple case of S incorporation, where only two S-bearing amino acids are involved, the resulting $\delta^{34}S$ value of keratin responds to the different dietary sources of those amino acids. In more complex cases, like that of H incorporation, multiple exogenous and endogenous pools contribute to the hair $\delta^{2}H$ values. Therefore, variations in $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, $\delta^{18}O$ and $\delta^{2}H$ values of human hair among individuals are mainly determined by the isotope values of the different sources contributing to the isotope signal, and the proportional input or fraction (f) from those different sources. This can be expressed in terms of a simple linear mixing model by the following equations, using $\delta^{13}C$ values as an example:

$$\delta^{13}C_{hair} = f_1 \times \delta^{13}C_1 + f_2 \times \delta^{13}C_2 + \ldots + f_n \times \delta^{13}C_n \quad (25.1)$$

$$f_1 + f_2 + \ldots + f_n = 1 \quad (25.2)$$

This represents a system of n sources, where all proportional inputs (f) must add up to 1 (Equation 25.2). In Equation 25.1 it is assumed that all sources have been adjusted for isotope discrimination between source and keratin. In this system, differences among individuals or changes through time within an individual will be determined by differences or changes in any or all of the following:

a. the δ-values of the sources;

b. the proportional inputs (f) from the varied sources;

c. metabolic and physiological processes affecting discrimination factors.

Consequently, any differences such as geographical and cultural differences affecting the sources (δ-values or f) will translate into distinct hair isotope values, providing a tool to match individuals with regions and/or populations. In the following section we present some of the established isotope patterns that might be useful in provenancing studies. Later in the chapter we will discuss patterns at the individual level related to metabolic states that can change the expected isotope ratios and also serve as a tool aiding in individual identification.

25.5 Geographical and population patterns of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values

For years researchers have known that the isotope ratios of modern humans differ among some countries (Bol and Pflieger, 2002; Katzenberg and Krouse, 1989; McCullagh et al., 2005; Minagawa, 1992; Nakamura et al., 1982; O'Connell and Hedges, 1999; O'Connell et al., 2001; Schoeller et al., 1986), but it was not until recently that large datasets were developed and global, regional and even intra-city patterns of isotopic variation were revealed (Bol et al., 2007; Hülsemann et al., 2015; Lehn et al., 2011, 2018; Mützel Rauch et al., 2009; Nardoto et al., 2006; Valenzuela et al., 2011, 2012). The use of the "dietary isotopes" for region-of-origin assignment of humans is based on the principle that these isotopes reflect geographically distinct dietary patterns. The natural, and human-induced, variations in the isotopic composition of plants and animal tissues are reflected in the diet of modern humans (Hüelsemann et al., 2009; Nash et al., 2012; O'Connell and Hedges, 1999; Petzke et al., 2005a). Although there have not been extensive studies of modern human food isotope ratios (Carter and Chesson, 2017), some studies have revealed important patterns that influence human hair isotope values (Chesson, 2009; Chesson et al., 2008; Jahren and Kraft, 2008; Martinelli et al., 2011). Before describing hair isotope patterns, we describe a few important things about food isotope ratios.

Differences in the way plants use atmospheric ^{13}C during photosynthesis create the most basic level of variation in $\delta^{13}C$ values (Ehleringer and Cerling, 2002). C_3 plants (plants that make a 3-carbon compound as the first stable product of C fixation) are those that discriminate most against ^{13}C during photosynthesis, and thus their tissues have the lowest $\delta^{13}C$ values (average of −27‰; Farquhar et al., 1989). C_4 plants (CO_2 molecules are first incorporated into

the lowest δ^{13}C values (average of −27‰; Farquhar et al., 1989). C_4 plants (CO_2 molecules are first incorporated into a 4-carbon compound) incorporate more ^{13}C during photosynthesis and have higher δ^{13}C values (average of −13‰; Farquhar et al., 1989). Thus, C_3 and C_4 plants have distinct non-overlapping δ^{13}C values. This distinction is passed on to the δ^{13}C values of animal consumers. Corn (maize) and sugar cane are C_4 plants, while vegetables, fruits, and most grains (such as wheat, rice, oat, etc.) are C_3 plants. Consequently, δ^{13}C values can be used as markers of relative consumption of these two groups of plants or animals fed on their tissues.

The natural distribution of C_3 and C_4 plants is strongly influenced by temperature, thus there are latitudinal and elevation trends of δ^{13}C values (Ehleringer, 1978; Ehleringer and Monson, 1993; Sage et al., 2018). Martinelli et al. (2011) found that the δ^{13}C values of beef used to produce the McDonald's Big Mac vary across countries, and tend to be lower at higher latitudes, following the expected reduction of C_4 vegetation in colder regions. Furthermore, countries may deviate from these plant distribution patterns due to the practices of the modern industrialized agriculture and food industry. For example, in the USA, the $^{13}C/^{12}C$ isotopic ratios in beef and chicken reflect their consumption of corn silage or corn meal that comprise their feeds (Chesson, 2009; Jahren and Kraft, 2008). In contrast, European beef and lamb have lower carbon isotope ratios (−26 to −22‰; (Camin et al., 2007; Perini et al., 2009; Piasentier et al., 2003), reflecting a higher C_3 (e.g. wheat, rye, barley) component of the feed.

As mentioned above, the stable nitrogen isotope ratio in the tissues of a consumer is enriched in ^{15}N over the diet (by about 3 to 5‰; DeNiro and Epstein, 1978, 1981; Kelly, 2000; Peterson and Fry, 1987). In humans this increment with trophic position reflects the proportion of consumed animal proteins. While no major differences in $^{15}N/^{14}N$ isotopic ratios exist among different plant types, except for the N-fixing plants which tend to have lower isotope ratios (Peterson and Fry, 1987), the use of different agricultural practices may result in distinctive isotope ratios for crops. For example, the use of synthetic fertilizers with δ^{15}N values around 0.0‰ (Vitòria et al., 2004) produces crops with lower isotope ratios than crops fertilized with manure (Bahar et al., 2005; Masud et al., 1999). In modern industrialized agriculture, most synthetic fertilizers have δ^{15}N values around 0.0‰.

Sulfur isotope ratios vary significantly between marine and terrestrial environments (Peterson and Fry, 1987). Although terrestrial environments present large ranges of sulfur isotope ratios, plant δ^{34}S values average near +2 to +6‰ over large areas, and are distinct from the +17 to +21‰ values of marine phytoplankton and seaweeds (Fry, 2006). Very little sulfur isotope fractionation occurs during animal metabolic processes, thus the isotopic differences between terrestrial and marine primary producers are reflected in the consumer's tissues (Arneson and MacAvoy, 2005; Richards et al., 2003). Furthermore, atmospheric deposition (sea spray effect) generates soils, plants and animals with higher δ^{34}S values near the ocean, and lower values with increasing distance to the coast (Zazzo et al., 2011).

25.5.1 From continents to cities

The isotopic patterns of food are reflected in the hair isotope values of people. It has been shown that residents of different countries present distinctive keratin isotope ratios, primarily in $\delta^{13}C$ and $\delta^{34}S$. Carbon isotope values obtained from hair and fingernails have been used to distinguish US residents from western Europeans (Chapter 28; Bol and Pflieger, 2002; Bol et al., 2007; Mützel Rauch et al., 2009; Nardoto et al., 2006; Valenzuela et al., 2012). The difference between these two regions is so large that there is almost no overlap in the $\delta^{13}C$ values between the two distributions (Valenzuela et al., 2012). The US sample is characterized by values towards the C_4 spectrum (-17.2 ± 0.8, $n = 234$), while western Europe is characterized by values towards a more C_3 diet (-20.3 ± 0.8, $n = 126$) (Valenzuela et al., 2012). At a global scale, keratin $\delta^{13}C$ values present latitudinal trends, following C_3–C_4 plant distribution, with higher $\delta^{13}C$ values in tropical countries and lower values in more temperate and higher latitudes (Hülsemann et al., 2015; Lehn et al., 2011; Martinelli et al., 2011; Mützel Rauch et al., 2009). Even within continental regions this trend is observed, such as in western Europe and Asia (Hülsemann et al., 2015; Mützel Rauch et al., 2009; Thompson et al., 2010; Valenzuela et al., 2012).

In the case of nitrogen isotope ratios, some studies have detected significant differences among modern human tissue samples from different countries (Mützel Rauch et al., 2009; Nardoto et al., 2006; Valenzuela et al., 2012). Nardoto et al. (2006) found that fingernails from Brazilian subjects have higher $\delta^{15}N$ values than fingernails sampled from the USA and western Europe. Similar differences were detected for hair (Valenzuela et al., 2012). In addition, Mützel Rauch et al. (2009) found that hair samples from Russia and Denmark have much higher $\delta^{15}N$ values than hair samples from Pakistan, while hair samples from other European, Asian and Latin American countries have $\delta^{15}N$ values between these two extremes.

Considering sulfur isotope ratios, values have been found to be significantly lower in hair samples from inland regions (e.g. interior North America, Europe and Asia) than in samples from coastal locations (e.g. Chile, Australia: Katzenberg and Krouse, 1989; Mützel Rauch et al., 2009; Thompson et al., 2010). Furthermore, Valenzuela et al. (2012) discovered that Europeans and Americans do not overlap in $\delta^{34}S$ values, and they can be distinguished using this marker. Smaller-scale differences have been detected within the USA, with higher values towards the coastline (Valenzuela et al., 2011), as well as longitudinal trends within western Europe (Valenzuela et al., 2012).

Together, the dietary isotopes appear to be valuable at distinguishing populations and potentially detecting recent migrants or movements between countries or continents (Bol et al., 2007; Hülsemann et al., 2015; Meier-Augenstein, 2010; Mützel Rauch et al., 2009). However, more research and data are needed for undersampled regions such as South America, Africa and Asia.

25.5.2 From cities to individuals

Spatial patterns at smaller scales are more difficult to detect. However, within regions – and even within cities – cultural and socioeconomic differences among demographic groups exist, providing the basis for diverse dietary patterns. Although the differences might not be spatially structured, these groups could represent discrete subpopulations.

Marked differences in stable isotope ratios in hair have been demonstrated to be present among modern Europeans (UK and Germany) who follow omnivorous, ovo-lacto-vegetarian (OLV), and vegan diets (Bol and Pflieger, 2002; Nardoto et al., 2006; O'Connell and Hedges, 1999; Petzke et al., 2005a, 2005b). In the UK and Germany, people with omnivorous diets had higher hair $\delta^{15}N$ and $\delta^{13}C$ values than OLV and vegans, and OLV had higher isotope ratios than vegans, reflecting a positive trend in the amount of animal protein from vegans to omnivorous (Bol and Pflieger, 2002; O'Connell and Hedges, 1999; Petzke et al., 2005b). In the western US, Nardoto et al. (2006) detected that fingernails from OLV had $\delta^{15}N$ values significantly lower than omnivores, but no differences in $\delta^{13}C$ values. These studies demonstrate that these groups can be distinguished.

Sometimes keratin isotope patterns are related to socioeconomic factors and access to different food items rather than individual choices. For example, in the Colombian city of Cali, Bender et al. (2015) discovered that adult women with higher socioeconomic status (SES) had hair $\delta^{13}C$ and $\delta^{15}N$ values significantly higher than woman with lower SES. The authors did not find major differences between the groups using classic dietary surveys, but suggested that women in the higher SES group consumed more proteins from animal sources. Importantly, SES was defined in terms of residential address using a classification system in which each city block is classified into different strata on the

basis of the houses' external appearance, access to municipal services, and the condition of the streets (Bender et al., 2015). Consequently, this study revealed some degree of spatial heterogeneity in hair isotope values within the city of Cali, Colombia. Similarly, Gragnani et al. (2014) demonstrated that $\delta^{13}C$ values of fingernails from people of the city of Piracacaba, Brazil, rose with increasing income level. The authors propose that this change was due to a higher intake of beef, bread, soft drinks and dairy by the highest income group, together with a higher consumption of soybean oil, rice and sugar by the lowest income group.

Valenzuela et al. (2018) discovered that within Salt Lake City, USA, children and adolescents (9–16 years old) have substantially different hair isotope values depending on their ethnic background. Latino (Hispanic) children had higher $\delta^{13}C$ and $\delta^{15}N$ values than Caucasians (non-Hispanic white) children, while they did not differ in $\delta^{34}S$ values. Alongside the hair isotope values, the authors conducted dietary surveys, and found that non-Hispanic white children had a higher consumption of vegetables, grains and dairy products than Hispanic children; reported meat consumption by both groups was similar (Valenzuela et al., 2018). Furthermore, a few students from other ethnic backgrounds were sampled and had $\delta^{13}C$ and $\delta^{15}N$ values distinctly different from the Latino and Caucasian groups (Figure 25.3).

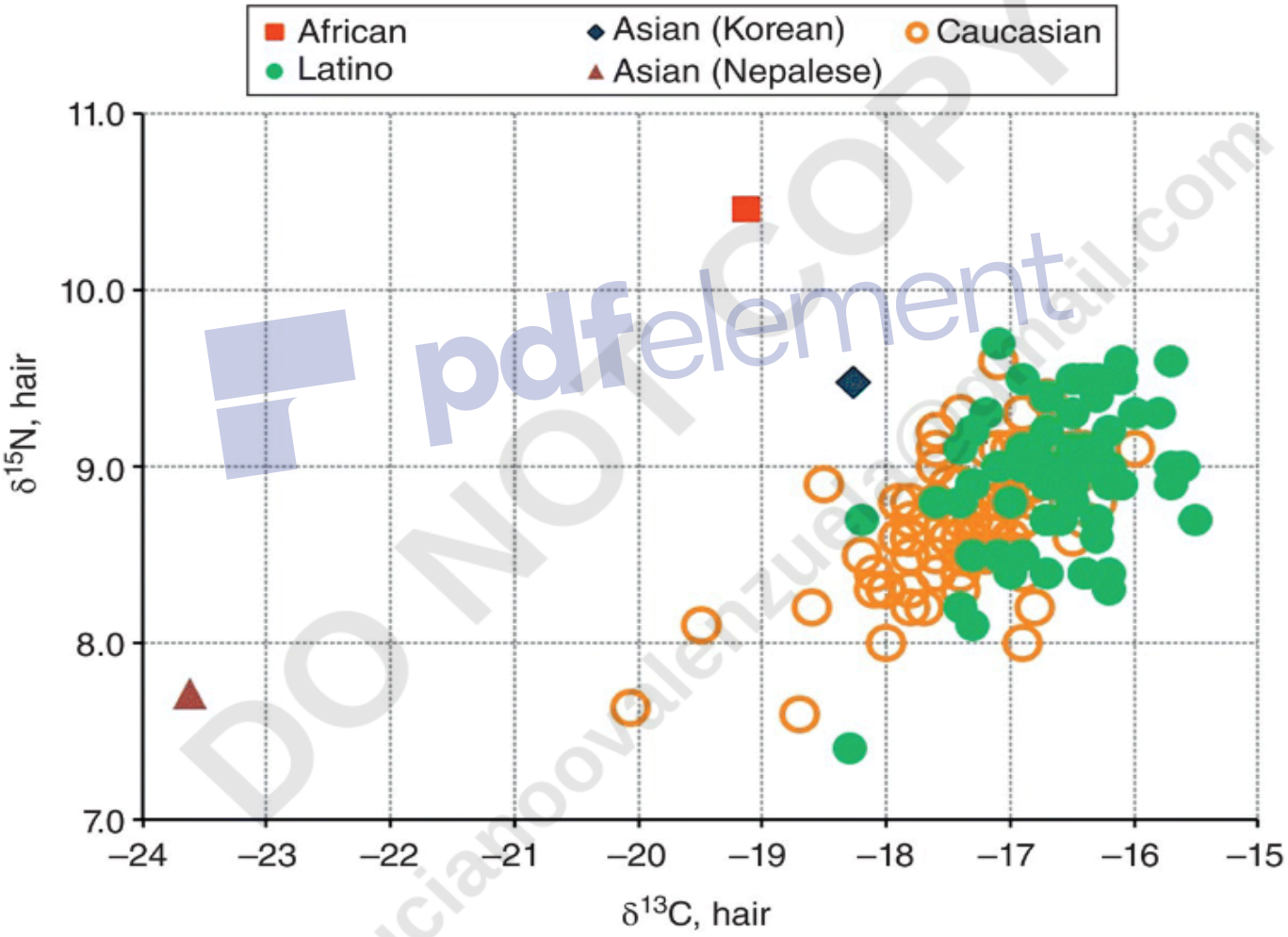


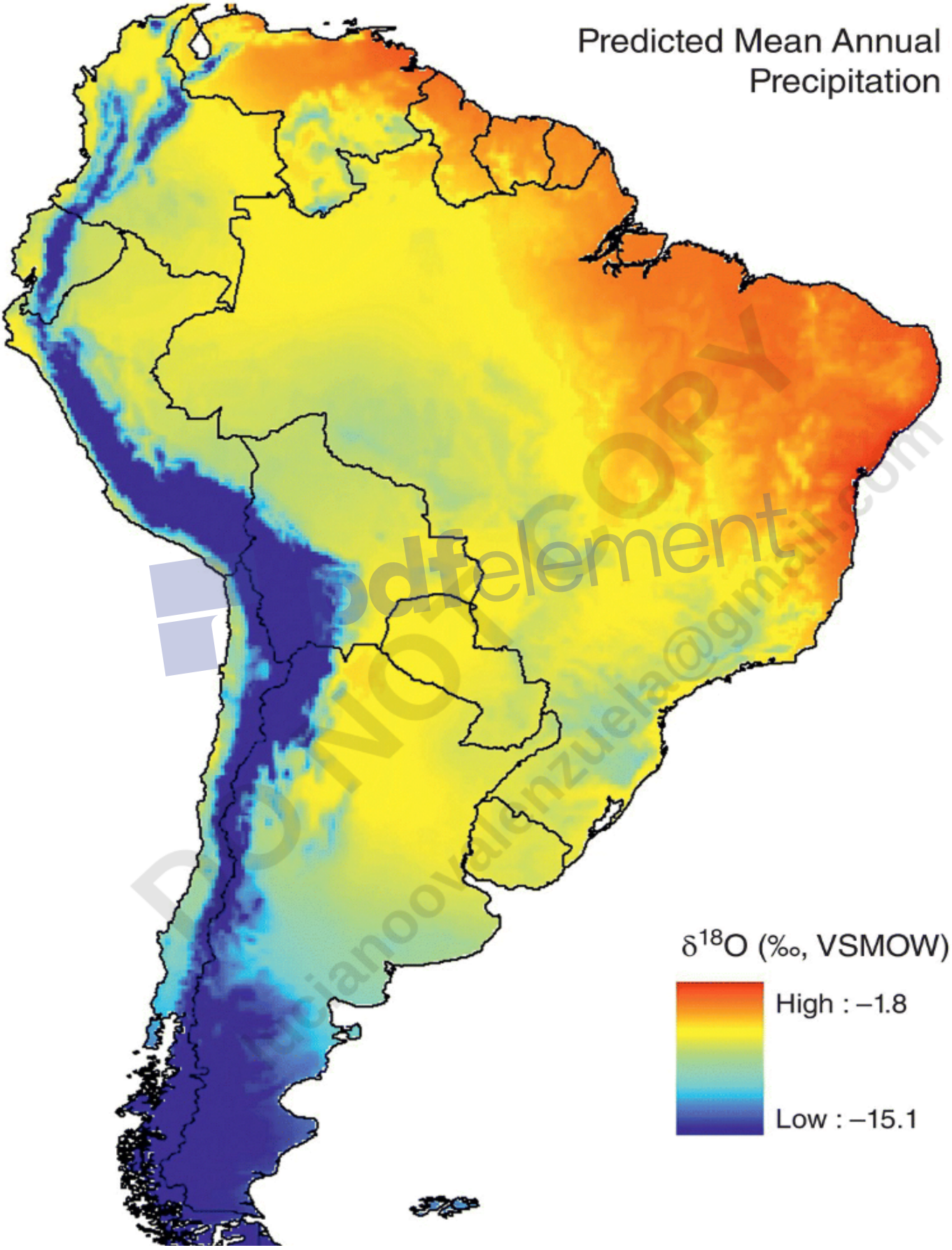
Figure 25.3 $\delta^{13}C$ and $\delta^{15}N$ values of hair from children sampled in three public schools in Salt Lake City, Utah, USA.

These studies show the importance of large datasets to detect differences among demographic groups within cities. These group distinctions could be used as an ancillary tool for individual assignment to a population of origin.

25.6 Geographical patterns of $\delta^{18}O$ and $\delta^{2}H$ values

The natural abundances of stable isotopes of O and H in precipitation (rain or snow) vary with geography (Bowen and Revenaugh, 2003; Bowen and Wilkinson, 2002; Craig, 1961; Dansgaard, 1954). The main reservoir of water and

the main source of rainwater (or snow) are the oceans, which by definition have $\delta^{18}O$ and $\delta^{2}H$ values equal to zero (Craig, 1961; Dansgaard, 1954; Fry, 2006). Evaporation from the oceans generates clouds with isotopic compositions lower than that of the ocean, determined by fractionation processes during that initial evaporation (Craig, 1961; Dansgaard, 1954). These clouds move inland, producing the first precipitations and depositing water with isotopic composition even lower than the original values of the oceans (Bowen and Revenaugh, 2003; Bowen and Wilkinson, 2002; Craig, 1961; Dansgaard, 1954). The vapor remaining in the clouds after precipitation, with isotopic values lower than that of the initial vapor, will continue traveling inland where, when condensing, water will precipitate with isotopic values even lower than the coastal precipitations (Craig, 1961; Dansgaard, 1954). Figure 25.4 presents a map of South America with predicted $\delta^{18}O$ values for precipitation (annual average). Because the fractionation processes depend, among other factors, on temperature and humidity, which globally are related to latitude and elevation, the overall trend is to have higher $\delta^{18}O$ and $\delta^{2}H$ values towards low latitudes, low elevation and coastal regions, and lower $\delta^{18}O$ and $\delta^{2}H$ values towards high latitudes, higher elevation and regions of the interior of the continents (Bowen, 2010a, 2010b; Bowen et al., 2013).



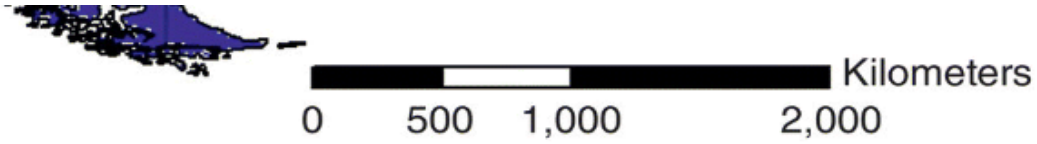


Figure 25.4 Visual representation of $\delta^{18}O$ values for predicted mean annual precipitation for South America. This spatial model, or isoscape, was generated by Bowen (2010a; Bowen et al., 2013) based on simple predictors such as latitude and elevation, plus an interpolation of residuals (obtained from the comparison of the model with global data from the Global Network of Isotopes in Precipitation, GNIP). The spatial distribution of predicted $\delta^{2}H$ values is similar to the $\delta^{18}O$ values.

The spatial variation of precipitation isotopes is translated into surface water, tap water and other drinking water sources such as bottled water, soda and alcoholic beverages (Bowen et al., 2007; Chesson et al., 2010). In the USA, the $\delta^{18}O$ and $\delta^{2}H$ values of tap water are strongly correlated with those of precipitation, although they are not equal due to different fractionation and mixing factors resulting from water management (Bowen et al., 2007; Good et al., 2014). There are certain regions where water isotopic compositions deviate from values predicted based on temperature and humidity; these correspond mainly to warm regions where water is stored in shallow reservoirs and open to evaporation, or in regions where the water source changes according to the intensity of consumption, moving from local water to water imported from other regions (Bowen et al., 2007; Good et al., 2014). Globally, and on average, it has been seen that the isotopic composition of bottled water tends to be similar to the composition of local water sources available naturally in the same region (Bowen et al., 2005b). The same happens with the isotope values of soda and beer purchased in different cities across the USA, which, despite slight variations, present a congruence with the isotopic composition of tap water of the places of purchase (Chesson et al., 2010).

As explained above, O and H atoms in human hair are directly acquired from drinking water, ambient water vapor, diet and atmospheric O_2. The spatial variation of the water $\delta^{18}O$ and $\delta^{2}H$ values is translated linearly to human tissues with a high correlation, because the isotopic signal of human body water is dominated by the liquid component of water directly imbibed or consumed through the cooking of food (Daux et al., 2008; Ehleringer et al., 2008; Fraser and Meier-Augenstein, 2007; Kohn, 1996; Longinelli, 1984; O'Brien and Wooller, 2007; Sharp et al., 2003). Water vapor acquired through respiration maintains the local geographical signal as it is the product of evaporation processes at the local or regional scale, but the isotopic composition of atmospheric O_2 is constant around the planet (Ehleringer et al., 2008; Kohn, 1996). The only source of isotopic variation with the potential to mask the local geographical signal is food imported from other regions (Bowen et al., 2009; Ehleringer et al., 2008). Even so, in cases where it is assumed that an isotopically homogeneous "continental supermarket" diet exists, for example, for modern human hair from the USA, the correlation coefficients between city average tap water and hair have been reported higher than 0.90 for both O and H isotope values (Ehleringer et al., 2008).

Ehleringer et al. (2008) presented a mechanistic model of incorporation of stable isotopes of water into human hair (Figure 25.2). This predictive model, which relates the $\delta^{18}O$ and $\delta^{2}H$ values of hair with those of water and therefore with geography, reached a congruence of approximately 86% when comparing the true region-of-origin of hair samples with the predicted origin within the USA. Later work made use of this model and improved it by incorporating additional parameters (Bowen et al., 2009; O'Grady et al., 2012). Bowen et al. (2009) worked with hair stored in museums of native populations of the mid-twentieth century from different regions of the world, and found higher slopes for the relationship between hair and water isotope values than those observed by Ehleringer et al. (2008) for modern residents of the USA. The interpretation of Bowen et al. (2009) is that for these populations the isotopic signal of the food is more strongly "linked" to that of precipitation because the great majority of the food was obtained locally, in contrast to the modern US diet described above. Bowen et al. (2009) modified Ehleringer's model by including two additional parameters, one that related and connected the isotope values of the food with the isotope values of local water, and another that varied the fraction of amino acids synthesized *de novo* in these populations, assuming different proportions of protein in their diet. Thompson et al. (2010) reported $\delta^{18}O$ and $\delta^{2}H$ values of hair from modern populations in Asia (China, India, Mongolia and Pakistan); the relationship between the isotopes of hair and water was intermediate between the US reported by Ehleringer et al. (2008) and the historical samples from native populations reported by Bowen et al. (2009). Thompson et al. (2010) suggested that for Asian populations there is a greater proportion of local food in the diet than for the US, but less than for the historical samples of indigenous populations. Furthermore, the slopes of the linear relationships between hair isotopes and water isotopes were lowest for the American samples (slope for $\delta^{18}O$ relationship was 0.35, slope of $\delta^{2}H$ was 0.27), intermediate for the Asian samples (for $\delta^{18}O$ it was 0.39 and for $\delta^{2}H$ it was 0.42) and highest for the historical samples (for $\delta^{18}O$ it was 0.70 and for $\delta^{2}H$ it was 0.78) (Bowen et al., 2009; Ehleringer et al., 2008; Thompson et al., 2010). It is worth

was 0.70 and for δ^2H it was 0.78) (Bowen et al., 2009; Ehleringer et al., 2008; Thompson et al., 2010). It is worth noting that in Ehleringer et al. (2008) and Thompson et al. (2010), water samples were collected alongside hair samples, while in Bowen et al. (2009) the local water isotopic composition was modeled.

25.7 Individual deviations from expected patterns

Different nutritional and metabolic conditions can alter the isotope ratios of a person's tissues, deviating the hair signal from the expected values according to their population of origin. This can obscure the interpretation of movements, but can also help in identifying an individual. In particular, nutritional stress and N imbalance, more specifically negative N balance, have been shown to affect the isotope ratios of hair. Fuller et al. (2005) showed in cases of nutritional stress, such as morning sickness during pregnancy, that the δ^{15}N values of hair increase. Similarly, patients with anorexia and/or bulimia nervosa have higher δ^{15}N values than clinically normal controls (Hatch et al., 2006; Mekota et al., 2006). In all these cases there are trends of increasing δ^{15}N values and weight loss under nutritional stress. One particular condition that was reported to produce lower δ^{15}N values in hair is cirrhosis (Petzke et al., 2006). It appears that in cirrhotic patients, the altered liver metabolism (deamination and transamination occurs in the liver) affects the nitrogen isotope values of the amino acids. Finally, Fuller et al. (2004) also reported that during gestation women show a decrease in δ^{15}N values but no change in δ^{13}C values, as a result of changes in the N balance. However, in most cases there was a concomitant dietary adjustment to deal with the aforementioned conditions, therefore it is not clear whether the changes in isotope ratios were due to the N imbalance or the new diet. Regardless, people with these conditions have been shown to have different isotope ratios.

Uncontrolled diabetes or any other metabolic condition affecting body water homeostasis and water flux has the potential to produce human tissues with markedly different isotope ratios. Although not in humans, O'Grady et al. (2010) showed that diabetic mice have different body water δ^2H and δ^{18}O values compared with control mice. This is because affected mice, as happens with diabetic humans, have a very high water flux and therefore their body water isotope values reflect closely this dominant pool.

25.8 Travel history

The sequential SIA of hair provides a longitudinal temporal record of recent past travels and diet of a person. Figure 25.5 represents a hypothetical analysis of a sample. In this case a conservative growth rate of 1 cm per month was used, and therefore the 16 cm length represents the reconstruction of approximately 16 months prior to death or sample collection. In this figure, some key features could be observed that provide information regarding habitat or diet change, as well as timing of the events. An investigator would look at these data and features, and immediately ask the following questions. Are the isotope values constant along the length of the hair? If there are changes along the hair, do they equilibrate to new values? Here the term "equilibrates" refers to the absence of change through time (or length). What is the direction and rate of change?

This graph shows an equilibrium period with isotope values of ~10‰ that corresponds to isotope region 1 (months 16 to 12 prior to sampling or death), followed by a move or change to isotope region 2, with higher values. In isotope region 2 the person spends enough time to reach a new equilibrium (at ~19‰). Then, after 5 to 6 months in isotope region 2, the person moved to a 3rd region as seen in the lower isotope values approximately 7 to 8 months prior to sampling or death, but did not stay in this region long enough to reach equilibrium. Finally he/she returned to isotope region 1 approximately 4 months prior to sampling or death.

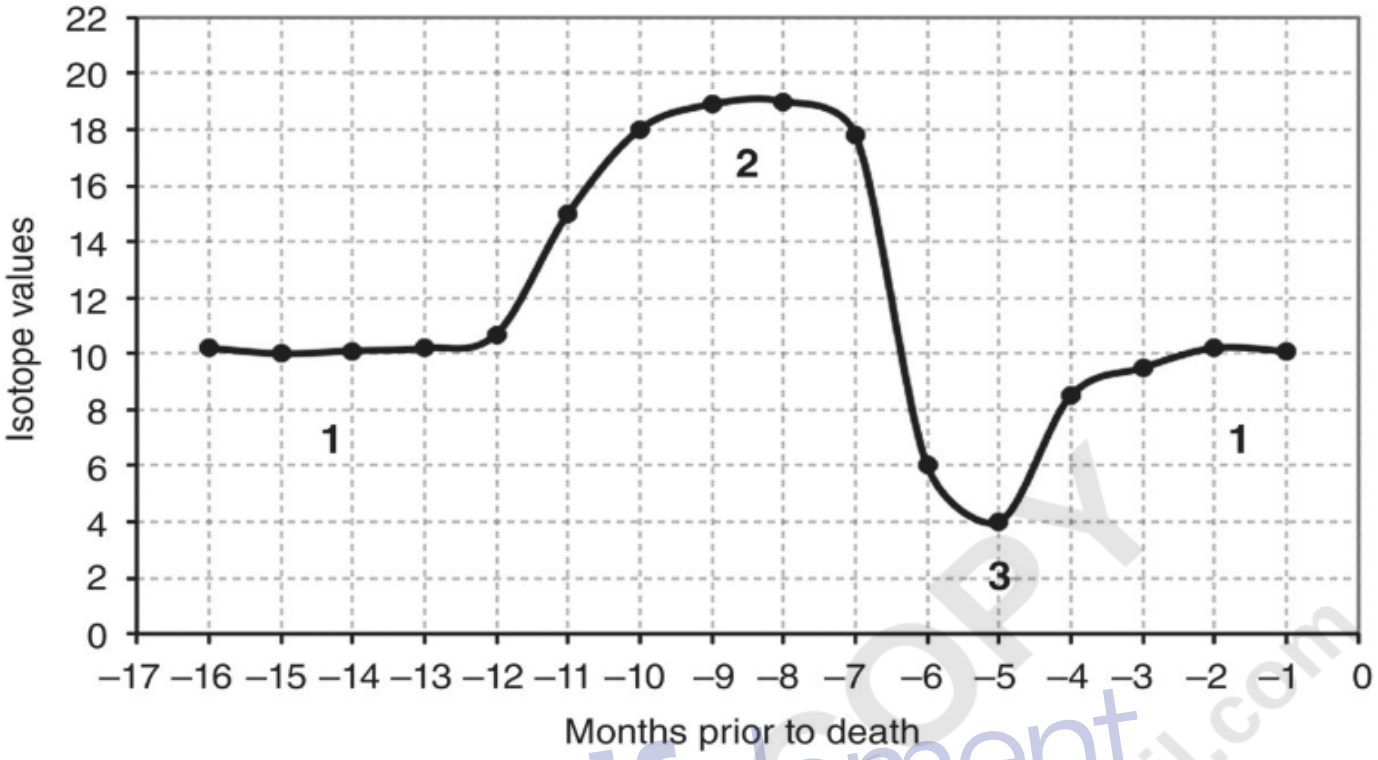


Figure 25.5 Hypothetical isotopic data showing different features that can be found in a sequential SIA of hair.

In this hypothetical example we see a few key features. First, it is possible to identify regions of equilibration, which are interpreted as lack of movement or a constant diet and observable as relatively consistent measured isotope values for sequential analyses of hair. The isotope values at equilibrium are used to define areas or population of residency. Second, there may be periods of time where no equilibrium was reached, such as isotope region 3. In this case, the actual isotope value of the visited region is unknown, but it must be lower or higher than the extreme value (in this example, lower than 4‰), thus defining a geographical boundary for the area. Finally, the defined values for the isotope regions are used in a mechanistic or statistical model to predict the geographical regions on an isoscape. Nowadays, a probabilistic assignment can be done using a Bayesian framework by incorporating the existing uncertainty in the isotope basemaps (for example, $\delta^{18}O$ of drinking water) and in the measured values of the hair sample (Wunder, 2010).

A change in signal caused by a movement or change in diet is not instantaneous. Several factors affect the speed at which the isotope values in hair reflect the new signal and reach equilibrium with changed inputs. Beard hair can show the influence of a new diet within a day (Lehn et al., 2018). Scalp hair might take up to a month to show the isotope values of a new location or diet (Hüelsemann et al., 2009; Petzke and Lemke, 2009). However, deviations from the previous equilibrated signal should occur in a short time (Lehn et al., 2018).

The rate at which a new signal is expressed in the isotope values of hair is not constant (for example, the change from region 1 to 2 is slower than from 2 to 3 in the example). Something to consider is the buffer capacity of the endogenous protein pool, which can slow down or accelerate the process of reaching equilibrium (Hüelsemann et al., 2009; Lehn et al., 2018; Petzke and Lemke, 2009). This has been modeled for $\delta^{13}C$ values using tail hair from horses; Ayliffe et al. (2004) proposed that the isotope values of hair represent a mixture of three different pools. The fast pool (exogenous dietary input) contributes approximately 40% of the signal and has a turnover rate of 0.5 days, while the slow pool (endogenous protein from slow metabolic tissues) contributes 44% of the signal and has a turnover rate of 140 days. The slow pool is characterized by having an isotope value equal or similar to the long-term average of the animal, and it has been interpreted as representing carbon molecules recycled primarily from tissues such as the skeletal muscle with minor contributions from connective tissue, collagen, brain and heart (Ayliffe et al., 2004). The remaining 16% corresponds to an intermediate pool (likely from some more active tissues) with an intermediate turnover rate (Ayliffe et al., 2004). Under this model, a dietary change away from the long-term average will be slower than a change that represents a return towards the long-term average. Thus, the equilibration to a new signal could take longer than the return to a previously equilibrated signal. Whether we can equate human scalp hair to

horse tail hair and all other isotopes to δ^{13}C values remains to be seen, but similar principles could apply.

25.9 Solved forensic investigations

A few solved cases in which investigators used isotope values of hair in conjunction with other tissues have been published (Cerling et al., 2016; Chesson et al., 2014; Ehleringer and Matheson Jr, 2010; Meier-Augenstein and Fraser, 2008; Rauch et al., 2007; Remien et al., 2014). In these cases the use of SIA was crucial in providing information on place of birth and past migration or movement events, which helped lead to the identification of the decedent and in some cases the conviction of their murderers. We will describe one case (Ehleringer et al., 2015; Remien et al., 2014).

In the year 2000, human remains (skull, hair) were found along with some fabrics in the region known as Saltair in the state of Utah, USA. These remains were identified as belonging to a young woman in her early 20s. At the time, no more information was obtained about her identity or cause of death, and the case became a *cold case* called "Saltair Sally." In 2009, Ehleringer and colleagues received samples of hair and teeth from "Saltair Sally." SIA of O in dental enamel and O and H in hair were performed. For the hair, short segments were analysed from the root to the distal end to reconstruct the last two years of the victim's life (the hair was approximately 23 cm long). In Figure 25.6 the arrow marks the place where the remains were discovered. The sequential hair analysis suggested that the victim had traveled several times in two years prior to death but always within the Western US, particularly in what is known as the "Inter-Mountain West" region (see regions 1 to 4 in Figure 25.6). The δ^{18}O values of dental enamel suggested that the Inter-Mountain West was the region of origin during childhood–adolescence. Equally important was that neither the enamel nor the hair data placed the victim in eastern USA. This information reduced the search area and temporally characterized the movements of the victim, allowing investigators at the Unified Police Department to refocus and concentrate their efforts. Finally, the woman was identified as Nikole Bakoles.

25.10 Final considerations

25.10.1 How fixed are the geographical patterns of δ^{13}C, δ^{15}N and δ^{34}S values?

Unless there are dramatic dietary transitions or modifications in the food industry, the patterns should hold for many years. However, there are a few examples where transitions have been detected Nardoto et al. (2011) sampled fingernails in several native populations living along the Solimoes River in Brazil. With increased urbanization, the authors detected a transition towards lower δ^{15}N values and higher δ^{13}C values as people abandoned their native customs with a high intake of freshwater fish, and consumed more imported food, particularly chicken from nearby cities. Another example arises from a small dataset from the Utah Study of Fertility, Longevity, and Aging (FLAG, unpublished data). As part of the FLAG study we were able to obtain six hair samples collected more than 70 years ago, prior to the widespread use of synthetic fertilizers and widespread consumption of corn-fed animals. The hair isotope values for these six samples were ~2.3‰ higher and ~1.6‰ lower than hair from modern children for δ^{15}N and δ^{13}C values, respectively. Thus, it is necessary to keep in mind potential dietary or agricultural changes when older, historical samples are analysed or compared with modern reference datasets.

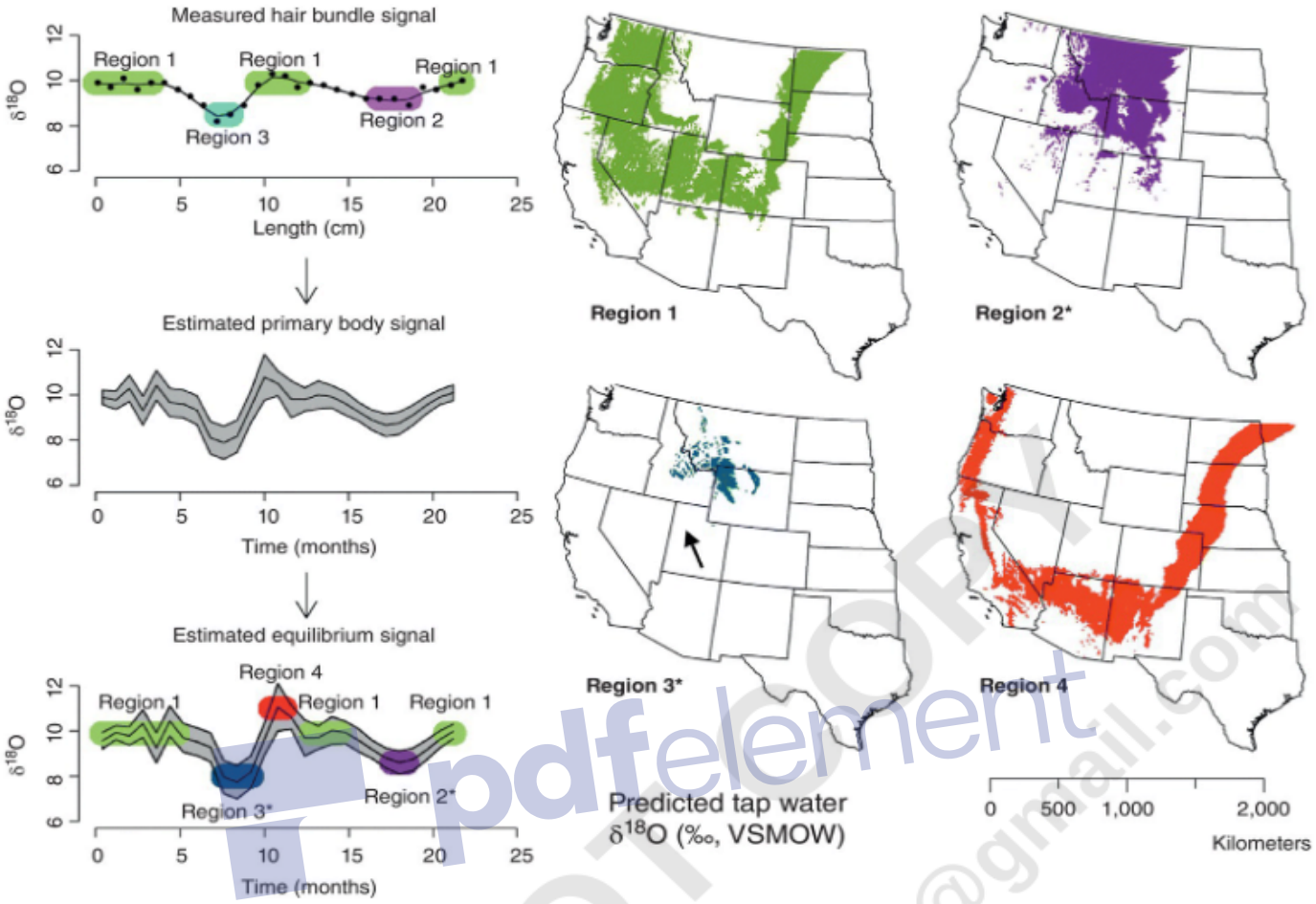


Figure 25.6 Isotope values measured and estimated equilibrium signal after Remien et al. (2014). Maps represent the areas determined by the model. The arrow on the map indicates where the remains were found.

25.10.2 Seasonal stability of drinking water $\delta^{18}O$ and $\delta^{2}H$ values

When using $\delta^{18}O$ and $\delta^{2}H$ values for provenancing, but particularly for reconstructing past travels and movements, we have the underlying assumption that the isotope ratios of local sources are constant over the period integrated in the hair. This assumption does not hold true for places where municipalities switch

water sources due to high demand at certain times of the year. This is certainly an area of active research currently due to the importance of characterizing how much tap water isotope ratios at a given site vary over season or other timescales (Kennedy et al., 2011).

25.10.3 Bundling and analysing very long hair

The analysis of a bundle of hair brings temporal and spatial interpretation issues, particularly with very long hair samples. Not all hair grows at the same rate and at the same time, and this can cause misalignment of the isotope values, producing attenuation of the signal towards the distal end of the hair (Remien et al., 2014). Furthermore, Lehn et al. (2018) showed that the photochemical degradation of cystine can cause loss of S and potentially an increase in $\delta^{34}S$ values. This pattern was seen in a lock of hair 54 cm in length, but not in shorter hairs. Therefore, caution must be exercised when interpreting the isotope values towards the tip of very long hair that has been exposed to the environment for several years and may have suffered weathering and structural alterations (Lehn et al., 2018; Remien et al., 2014).

25.10.4 Fingernails vs. hair

Although isotopically analogous to hair, fingernails have some subtle differences that need to be mentioned. The isotopic composition of fingernails differs from that of hair, and this does not appear to be related to different growth rates (Fraser and Meier-Augenstein, 2007; Fraser et al., 2006; Lehn et al., 2011; O'Connell et al., 2001). The difference appears to be related to the more complex synthesis of nail keratin than hair keratin (Fraser et al., 2006; Lehn et al., 2011). Some authors have argued that hair $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$ and $\delta^{2}H$ values are better recorders of their isotopic inputs than fingernails (Fraser and Meier-Augenstein, 2007; Lehn et al., 2011).

25.11 Conclusions

Hair isotope analyses can reveal important information on the recent past of an unidentified decedent. The combination of different isotopes, as well as the analyses of multiple tissues (hair/nail plus teeth, bones) when available, makes for a powerful tool to further develop the biological profile of a person (Cerling et al., 2016; Chesson et al., 2014; Ehleringer et al., 2015; Meier-Augenstein, 2010; Meier-Augenstein and Fraser, 2008; Rauch et al., 2007). The SIA of hair provides a means of aiding in human provenancing, inferring region-of-origin, but is equally important as an exclusionary tool.

Of the five light elements discussed in this chapter, stable isotope values of O and H represent the primary basis for region-of-origin assignment and reconstruction of past travels, but with the help of the dietary isotopes ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values) more information regarding the person's habits can be gained, for example, whether she/he was a vegan or vegetarian. Furthermore, although analyses have traditionally focused on the stable isotope ratios of these five elements, the analysis of isotopes of heavier elements such as strontium (Sr) and lead (Pb) can provide unique information about a sample that cannot be obtained using other techniques, particularly when the data are studied in combination (Cerling et al., 2016; Chesson et al., 2014; Font et al., 2012; Meier-Augenstein, 2010; Rauch et al., 2007; Tipple et al., 2018).

The future for the use of stable isotopes of hair for human provenancing is promising as new analytical tools are developed, such as SIA of individual amino acids (Fogel et al., 2016; McCullagh et al., 2005; Petzke et al., 2005a), larger and global comparison/reference datasets (Hülsemann et al., 2015; Nardoto et al., 2006; Valenzuela et al., 2012), and new models that generate a probability or likelihood of origin assignment (Kennedy et al., 2011; Wunder, 2010).

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