Research Article

Rapid Communications in Mass Spectrometry

Received: 30 August 2016 Revised: 3 January 2017 Accepted: 4 January 2017 Published online in Wiley Online Library

Rapid Commun. Mass Spectrom. **2017**, *31*, 583–589
(wileyonlinelibrary.com) DOI: 10.1002/rcm.7822

Reconstruction of travel history using coupled $\delta^{18}O$ and $^{87}Sr/^{86}Sr$ measurements of hair

Thuan H. Chau[1]*, Brett J. Tipple[1,2], Lihai Hu[3], Diego P. Fernandez[3], Thure E. Cerling[1,2,3], James R. Ehleringer[1,2] and Lesley A. Chesson[1]

[1]IsoForensics, Inc., 421 Wakara Way, Suite 100, Salt Lake City, UT 84108, USA
[2]Department of Biology, University of Utah, 257 S 1400 E, Salt Lake City, UT 84112, USA
[3]Department of Geology and Geophysics, University of Utah, 115 S 1460 E, Salt Lake City, UT 84112, USA

RATIONALE: Oxygen isotope ratios ($\delta^{18}O$ values) of hair largely reflect features of regional hydrology while strontium isotope ratios ($^{87}Sr/^{86}Sr$) are thought to reflect bedrock geology; combination of both isotope signatures may provide greater capacity for determining provenance and reconstructing travel history of an organism. To test this hypothesis, we compared the O-Sr isotope profiles of hair from domestic horses with known residency histories.
METHODS: Tail hairs were collected from a pair of horses pastured together for a period of 16 months, one of which lived in a different location for the 8 months prior. Hair samples were washed with solvents to remove external contaminants prior to sequential sampling for $\delta^{18}O$ and $^{87}Sr/^{86}Sr$ analysis via TC/EA-IRMS and MC-ICP-MS, respectively. Hair digests were concentrated and analyzed employing low-flow natural aspiration to measure $^{87}Sr/^{86}Sr$.
RESULTS: Tail hair from the control and transported horses had mean $\delta^{18}O$ values of 11.25 ± 1.62 ‰ and 10.96 ± 1.53 ‰, and mean $^{87}Sr/^{86}Sr$ of 0.7101 ± 0.0006 and 0.7109 ± 0.0020, respectively. The $\delta^{18}O$ and $^{87}Sr/^{86}Sr$ profiles for the control and transported horses were indistinguishable when they were pastured together. The $^{87}Sr/^{86}Sr$ profiles were significantly different during the period that the horses were living apart, while the $\delta^{18}O$ values were indistinguishable during that period.
CONCLUSIONS: By comparing the O-Sr isotope profiles of a control and transported horse, we investigated isotopic signal(s) potentially useful for reconstructing travel histories via high-resolution sequential sampling along single strands of tail hair. Improved analytical capabilities allowed for extremely low Sr abundance samples to be analyzed for $^{87}Sr/^{86}Sr$ and proved capable of resolving a horse's movement between distinct regions.

Analyses of stable isotope ratios in bone, tooth enamel, and hair have been widely utilized to identify region-of-origin of individuals in archaeological and forensic investigations. Individuals living in a particular region incorporate the geochemical fingerprints of that region into their tissues through ingestion of locally sourced foods and liquids.[1–3] Tissues also integrate regional geochemical fingerprints through exposure to the environment (via dust) and during bathing.[4–6] Thus, isotope analysis of these tissues can provide information on the provenance and/or travel movement history of individuals.

Studies have shown successful application of oxygen ($\delta^{18}O$ values) and strontium ($^{87}Sr/^{86}Sr$) isotope ratio analyses in determining region-of-origin of ancient and modern humans.[7–10] The O and Sr isotopic compositions of bone, tooth enamel, and the intestinal contents of the mummified Alpine Iceman, Ötzi, allowed investigators to reconstruct his migratory patterns.[7] In a more recent investigation, $\delta^{18}O$ analysis of bundled hair strands was used to reconstruct the travel history of Saltair Sally, an unidentified decedent, prior to her death. This information helped detectives focus on geographic areas of investigation and aided in her identification.[8,11,12] Additional studies by Font *et al.* and Vautour *et al.* measured the Sr isotopes of hair to reconstruct movements of individuals between international metropolises and found $^{87}Sr/^{86}Sr$ ratios to be a good recorder of travel.[9,10]

Oxygen isotope analysis is commonly applied in provenance studies because biological tissues readily incorporate environmental oxygen atoms from local water, which isotopically varies across landscapes in a predictable manner. Geographic characteristics such as latitude, longitude, and spatial distance from the ocean have been used to build prediction models for regional variations in the $\delta^{18}O$ values of water.[13–15] There have been similar efforts to build predictive models of Sr isotope ratios in the local ecosystem based on bedrock compositions.[16–18] Coupling O and Sr isotopes is ideal for region-of-origin investigations, because independent physical and chemical processes govern regional $\delta^{18}O$ and $^{87}Sr/^{86}Sr$ signatures. A coupled O-Sr geolocation model would possibly provide greater spatial specificity between regions with similar $\delta^{18}O$ values through comparison of the $^{87}Sr/^{86}Sr$ values, or vice versa.

Measurement of $^{87}Sr/^{86}Sr$ ratios along with a variety of other stable isotope ratios (e.g., $\delta^{2}H$, $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, and $\delta^{34}S$ values) can provide an additional layer of geographic

* *Correspondence to:* T. H. Chau, IsoForensics, Inc., 421 Wakara Way, Suite 100, Salt Lake City, UT 84108, USA.
E-mail: thuan@isoforensics.com

information that can be used to investigate an individual's region-of-origin.[19–21] However, analyses of many human tissues – such as bone or tooth enamel – only provide a "snapshot" of origin at particular phases of an individual's life cycle. Analysis of hair can be especially useful in contemporary provenance investigations because hair grows at a relatively constant rate and is continuously exposed to the environment, establishing an uninterrupted travel history record. The geographic timeline recorded in hair may span from months to years prior, depending on the length of hair.[22]

Here, we present a high-resolution, sequential analysis method to measure $^{87}Sr/^{86}Sr$ ratios at an analogous timeframe to $\delta^{18}O$ values along the length of hair. Hair samples used to assess the correspondence between and fidelity of $\delta^{18}O$ values and $^{87}Sr/^{86}Sr$ isotope records in this proof-of-concept study were acquired from domesticated horses; the tail hair provided sufficient material for stable isotope analysis using a single strand. An opportune collection of the hair provided a well-defined experimental design to monitor changes in $\delta^{18}O$ and $^{87}Sr/^{86}Sr$ signatures associated with a geographic movement, where two horses resided apart and then together for known intervals. Specifically, a resident horse provided an experimental control and another horse from a different continent was transported to the control horse's location. The two horses were then pastured together for approximately 1.25 years. This experimental design presented an opportunity to characterize $\delta^{18}O$ and $^{87}Sr/^{86}Sr$ signatures associated with a movement, but also establish an isotopic baseline for direct comparison. We hypothesized that the $\delta^{18}O$ and $^{87}Sr/^{86}Sr$ values of hair from the two horses would vary along the length of the strands, corresponding to geographic locations, with similar signatures observed when the horses were together in the same location, and distinct signatures when the horses were in different locations.

EXPERIMENTAL

Samples

Two strands of tail hair were collected from each of two healthy adult-male horses on 30 October 2007 in Salt Lake City, UT, USA. The hair strands were greater than 400 mm. One of the horses, designated as Control Horse (CH), had dark brown-black hair and was a local resident with no travel activity outside the Salt Lake Valley (SLV) from July 2005 to the date of hair collection. The other horse, designated as Transported Horse (TH), had dappled gray hair and was relocated to the SLV from Araçoiaba da Serra, Brazil (BR) in July of 2006 following a sale. Upon arrival to SLV, the TH was pastured in the same location as the CH. Samples of diet and drinking water were not available as hair collection was opportunistic following the TH's move.

One hair strand from the TH was analyzed for its $\delta^{18}O$ values, and the second hair strand was analyzed for $^{87}Sr/^{86}Sr$ ratios to evaluate the transition from BR to SLV. Similarly, one hair strand from the CH was analyzed for $\delta^{18}O$ values while the other was analyzed for $^{87}Sr/^{86}Sr$ ratios to establish baseline isotope values for comparison with the TH. Assuming a growth rate of 0.70 mm day^{-1} for all hair strands analyzed in this study,[23] we estimated that the geochemical signatures of the BR-SLV transition occurred at approximately 340 mm of hair length. To account for uncertainty regarding growth rate, as well as travel and quarantine time, hair lengths >360 mm are considered pre-transition while hair lengths <320 mm are considered post-transition. This uncertainty accounts for approximately 2 weeks around the date of the BR-SLV transition.

Chemicals

Ultrapure water used for sample cleaning treatments and acid dilutions was from a Milli-Q® Academic A10® system (EMD Millipore, Billerica, MA, USA) with a resistivity >18 MΩ. Chloroform (OmniSolv®, EMD Millipore), methanol (OmniSolv®, EMD Millipore), and ethanol (Decon™ Labs, King of Prussia, PA, USA) were used for sample cleaning treatments. Ultrapure HNO_3 (nitric acid) used for sample digestion was Aristar® Ultra (BDH® Chemicals, VWR, Radnor, PA, USA). Trace metal grade HNO_3 and HCl (hydrochloric acid) (Aristar® Plus, BDH® Chemicals) were used to prepare reagents for acid leaching of equipment and cleaning of digestion vessels.

Cleaning treatment

All sample cleaning treatments took place in a clean lab setting. Hair samples were wrapped in coffee filter paper and submerged in a glass beaker filled with a 2:1 solution of chloroform and methanol to remove lipids and surficial contaminants. Samples were allowed to soak in the wash solution for 5 min and occasionally agitated. The supernatant was discarded and the washing procedure was repeated three times. Washed hair was allowed to air-dry in a laminar flow hood. Dried samples were then stored in paper envelopes until further analysis-specific preparation procedures. Prior to sequential sampling, the selected hair strand was wiped with an ethanol-soaked Kimwipe™ to remove possible contaminants introduced from handling and storage post-cleaning.

Oxygen isotope analysis

Prior to weighing for oxygen isotope analysis, washed hair was allowed to equilibrate with ambient laboratory atmosphere for at least 48 h. A single strand of hair from each horse was sequentially cut with a metal razor blade into ~5 mm segments (representing *ca* 1 week), each enclosed in 3.5 × 5 mm silver capsule (Costech Analytical Technologies, Inc., Valencia, CA, USA). Every third segment from the distal end was selected for oxygen isotope analysis to achieve a temporal resolution of *ca* 1 month. Laboratory reference materials (keratin: DS, ORX, and POW), which had also equilibrated with the ambient laboratory atmosphere, were weighed into silver capsules at similar masses to the hair segments. Encapsulated samples and reference materials were placed in 96-well plastic trays and dried under vacuum for a minimum of 5 days prior to oxygen isotope analysis.

$\delta^{18}O$ measurements were performed at IsoForensics, Inc., in Salt Lake City, UT, USA, via isotope ratio mass spectrometry (IRMS) using a MAT 253 mass spectrometer (ThermoFinnigan, Bremen, Germany) attached to a high-temperature conversion elemental analyzer (TC/EA; ThermoFinnigan). A minimum of four sets of keratin reference materials were analyzed with

Rapid Communications in Mass Spectrometry

unknowns. Reference materials DS and ORX were used for normalization (assigned $\delta^{18}O$ = 6.02 ‰ and 25.09 ‰, respectively) while POW was used for quality assurance (long-term mean $\delta^{18}O$ = 12.44 ‰, 2σ = 0.54 ‰, n = 335).

Sample dissolution for strontium abundance and isotope analysis

The second hair strand from each horse was used for Sr abundance and isotopic measurements. Washed hair strands underwent a 10-min sonication (B3500-MT ultrasonic cleaner; VWR) with Milli-Q® water in an acid-leached 15-mL centrifuge tube to further remove deposited contaminants not incorporated into the hair structure.[24,25] Hair strands were wiped with a Kimwipe™ to remove excess water, and then dried using argon gas.

For Sr abundance and $^{87}Sr/^{86}Sr$ measurements, the strand of hair was cut with a metal razor into ~20 mm segments (representing *ca* 1 month), and each segment was placed in a 10-mL Teflon® digestion vessel. Segments were digested in 2 mL of 16 M HNO_3 using an Ethos EZ® SK-10 high-pressure rotor microwave digestion system (Milestone, Inc., Shelton, CT, USA). The digestion method was as follows: 13.3°C min^{-1} ramp to 200°C, followed by a 15-min isothermal period at 200°C, and a 60-min cool down to room temperature. Certified reference materials TORT-2 Lobster Hepatopancreas Reference Material for Trace Metals (National Research Council Canada, Ottawa, Ontario, Canada) and Human Hair No. 13 (National Institute for Environmental Studies, Tsukuba, Ibaraki, Japan) were digested with sample segments; method blanks were also included. Once cooled to room temperature, the digest solutions were transferred to 2-mL acid-leached centrifuge tubes for storage.

For Sr abundance analysis, a 200-µL aliquot of the primary digest was transferred to an acid-leached 15-mL centrifuge tube. 20 µL of a 1 mg kg^{-1} indium internal standard was added to the secondary aliquot and diluted to 2 mL with 2.4% HNO_3. The remaining primary digest solutions were reserved for Sr isotope analysis.

Strontium abundance analysis

Abundance measurements were performed at the ICP MS Metals Lab in the Department of Geology and Geophysics at the University of Utah, Salt Lake City, UT, USA. Sr was measured via inductively coupled plasma mass spectrometry (ICP-MS) using an Agilent 7500ce mass spectrometer with an octopole reaction system (Agilent Technologies; Santa Clara, CA, USA). The sample introduction system included a 0.1 mL min^{-1} perfluoroalkoxy fluorocarbon (PFA) nebulizer, double-pass quartz spray chamber, quartz torch, and nickel sampler and skimmer cones. The Sr abundance was calculated from a six-point calibration curve developed from measurement of solutions containing Sr prepared gravimetrically using single-element standard (Inorganic Ventures, Inc., Christiansburg, VA, USA). Standard reference solution T-205 (USGS, Reston, VA, USA) was measured as an external calibration standard at least five times within each analytical sequence to establish accuracy of measurement. The accuracy was within 10 % of the certified values.

Strontium isotope analysis

Strontium purification

Reserved primary digests were decanted into 22-mL round-interior Teflon® vials and evaporated to a minute droplet on a heating block inside a laminar flow hood that was evacuated to a fume hood. The evaporated digests were immediately removed from the heating block, rehydrated with 1 mL of 4 M HNO_3, capped, and stored at room temperature within the laminar flow hood.

Sr in the digest solutions was isolated by passing the solutions through a packed column of 100–150 µm crown ether Sr resin (Eichrom Technologies, Lisle, IL, USA). The columns were prepared in heat-shrunk Teflon® tubes (0.1″ i.d. after curing) with a frit inserted at the base of the column, leaving a ~2 mm gap from frit to column tip. Frit-columns were leached with a 3:1 HNO_3/HCl solution, then packed with 50 µL of resin (~1 cm in height). The packed columns were cleaned and conditioned with successive loading of 1-mL aliquots of Milli-Q® water, 4 M HNO_3, 0.1% v/v HNO_3, and 4 M HNO_3. Samples in 1 mL of 4 M HNO_3 were loaded onto the conditioned columns at 250-µL aliquots. 1 mL of 4 M HNO_3 was loaded into the columns to rinse the matrix. All solutions eluted from the aforementioned steps were collected into waste containers. The Sr fraction was eluted into a new round-interior Teflon® vial with 1 mL 0.1% v/v HNO_3. The collected Sr fractions were further evaporated to a minute droplet and rehydrated to 150 µL with 2.4% HNO_3, and then transferred to a 2-mL conical-interior Teflon® vial for Sr isotope analysis.

Analytical measurements

$^{87}Sr/^{86}Sr$ measurements were performed at the Strontium Isotope Geochemistry Laboratory in the Department of Geology and Geophysics at the University of Utah using a Thermo Scientific™ Neptune Plus™ high-resolution multi-collector inductively coupled plasma mass spectrometer (MC-ICP-MS; Thermo Fisher Scientific, Bremen, Germany). The sample introduction system used natural aspiration with a 20 µL min^{-1} autosampler probe, Teflon® PFA nebulizer, double-pass quartz spray chamber, quartz torch, and nickel sample and skimmer cones. The instrument was tuned using a standard Sr solution prior to the start of an analytical sequence for optimized signal sensitivity. Table 1 provides an example of the standard instrument settings and acquisition parameters.

Measurements of $^{87}Sr/^{86}Sr$ were made using a static multi-collector routine that consisted of one block of 72 cycles with an integration time of 4.194 s $cycle^{-1}$. The measured $^{87}Sr/^{86}Sr$ ratios were blank-corrected, interference-corrected, and normalized for instrumental mass discrimination using a defined $^{86}Sr/^{88}Sr$ value of 0.1194. Solutions of SRM®987 (National Institute of Standards and Technology, Gaithersburg, MD, USA) of 10 and/or 20 µg kg^{-1} to best match expected sample concentrations, with certified $^{87}Sr/^{86}Sr$ value of 0.71034 ± 0.00026, were analyzed before and after each set of three samples to verify measurement accuracy and precision. The reproducibility of the SRM®987 measurements (Supplementary Table S3, Supporting Information) through the life cycle of the study was 0.7103 ± 0.0003 (2σ, n = 41). The ^{88}Sr signal intensity and $^{87}Sr/^{86}Sr$ ranges were 0.266–0.844 volts (V) and 0.7101–0.7108, respectively.

Table 1. MC-ICP-MS instrument operational settings and acquisition parameters for $^{87}Sr/^{86}Sr$ measurement of 10 µg kg^{-1} solution. Argon was used as the cooling and auxiliary gas. Instrument settings and parameters were tuned prior to each analytical run for optimal blank and sensitivity

Instrument Settings	
RF Power	1198 W
Fore Vacuum	1.36e-3 mbar
High Vacuum	1.43e-7 mbar
Ion Getter Pressure	6.05e-8 mbar
Cooling Gas Flow Rate	16 L min^{-1}
Auxiliary Gas Flow Rate	0.98 L min^{-1}
Sample Gas Flow Rate	0.875 L min^{-1}
Blank	0.5 mV (Cup H2)
Sensitivity	800 mV (10 µg kg^{-1} Sr, Cup H2)
Cup Configuration	
L4	^{82}Kr
L3	^{83}Kr
L2	^{84}Sr
L1	^{85}Rb
C	^{86}Sr
H1	^{87}Sr
H2	^{88}Sr

Our typical [Sr] working limits for MC-ICP-MS were 50–100 µg kg^{-1}; this concentration range paired with a 20 µL min^{-1} autosampler probe typically provided an ^{88}Sr signal intensity between 2 and 4 (V) and individual $^{87}Sr/^{86}Sr$ measurement standard error of less than 1×10^{-5}. Subsequent tests determined that 10 µg kg^{-1} solutions provided acceptable $^{87}Sr/^{86}Sr$ ratios for SRM®987 with a measurement standard error less than 1×10^{-4}. Thus, with the prescribed 150-µL solution analytical method, the theoretical minimum amount of Sr needed for analysis was 1.5 ng. Initial concentration measurements of the digested horse-hair segments suggested there was sufficient [Sr] for $^{87}Sr/^{86}Sr$ measurement, with a range 0.7–15 ng of Sr.

Statistical analysis

Statistical analysis was performed using the Microsoft Excel® Data Analysis Package and GraphPad Prism 5.0c for the Mac OS. Data normality was determined using the D'Agostino-Pearson omnibus K2 test. For normally and non-normally distributed data, Student's unpaired *t*-test and *U*-test with $\alpha = 0.05$, respectively, were used to compare population averages.

RESULTS AND DISCUSSION

$\delta^{18}O$ values

The measured $\delta^{18}O$ values of serially sampled tail hair from the Control Horse (CH) and Transported Horse (TH) are presented in Fig. 1(a) and Supplementary Table S1 (Supporting Information). The mean $\delta^{18}O$ values ($\pm 1\sigma$) of the CH and TH for the entire hair strands were 11.25 ± 1.62 ‰ (n = 28) and 10.96 ± 1.53 ‰ (n = 31), respectively. The $\delta^{18}O$ values of hair from the CH and TH had ranges of 7.88 to 13.83 ‰ and 8.92 to 14.15 ‰, respectively. As hypothesized, during the interval of residency in the Salt Lake Valley (SLV; length < 320 mm), the $\delta^{18}O$ values of hair from CH and TH were similar (unpaired *t*-test, $t = 1.406$, df = 40, $p = 0.1673$). In contrast to our initial hypothesis, the $\delta^{18}O$ values of the hair from the TH during the interval of residency in Brazil (BR; length > 360 mm) were statistically indistinguishable (unpaired *t*-test, $t = 1.586$, df = 8, $p = 0.1514$) from those of the hair from the CH during the same period of time. The mean $\delta^{18}O$ values ($\pm 1\sigma$) of the CH and TH for the hair segments pre-transition (i.e., hair length > 360 mm) were 12.19 ± 2.48 ‰ (n = 5) and 13.96 ± 0.16 ‰ (n = 5), respectively.

In order to use the $\delta^{18}O$ signatures of hair to investigate travel history, we expected to obtain distinct isotopic signatures when the CH and TH were in different locations. However, the TH showed no significant differences from the CH when living in Brazil. This was intriguing and unexpected, given that prior research on animal and human hair has demonstrated that individuals record regional $\delta^{18}O$ signatures in hair.[26,27]

We assume that the $\delta^{18}O$ values of hair segments >360 mm from the TH reflected a pre-transition BR value; however, that signal was not statistically different ($p = 0.1514$) from the $\delta^{18}O$ values of the CH hair during the same period of time. As the $\delta^{18}O$ values of hair largely reflect drinking water isotopic composition,[26–29] isotopically similar water sources may provide an explanation for the lack of difference between the $\delta^{18}O$ values of the horses' hair during the time when they were living in different locations. The regions of Araçoiaba da Serra, Brazil and Salt Lake Valley, UT, USA have similar predicted $\delta^{18}O$ values for their precipitation, ranging from –9.2 to –3.5 ‰ and from –14.5 to –7.6 ‰, respectively[30] (Online Isotopes in Precipitation Calculator, version 2.2[31]). It was unforeseen that $\delta^{18}O$ values of hair from the CH and TH would not be distinct during the interval when they lived apart and the opportunistic sampling of tail hair from these horses prohibited us from explicitly targeting animals from regions with distinct water $\delta^{18}O$ values.

Interestingly, oscillations were observed post-transition (i.e., segments <320 mm) in the $\delta^{18}O$ profiles from the hair of both horses. Given that the CH had no known travel history outside the SLV region during the period of time represented by these hair segments, the variations are not related to changes in geographic location. The observed oscillations in the $\delta^{18}O$ values recorded by the hair strands are probably a consequence of seasonality and possibly related to variations in the isotopic compositions and proportions of drinking water and food water in the horses' diets at different times of the year.[32] Boner and Förstel observed similar trends when monitoring German cattle.[33] These authors found that $\delta^{18}O$ values in cattle tissue increased during the growing (warm) season when animals were fed fresh biomass that contained significant amounts of plant water, and decreased to a minimum value closely related to that of ground water during the winter (cool) season, when animal ate more dry feed and drank more water. This seasonal switch in feed type is a common practice in the Intermountain West of the United States.[34]

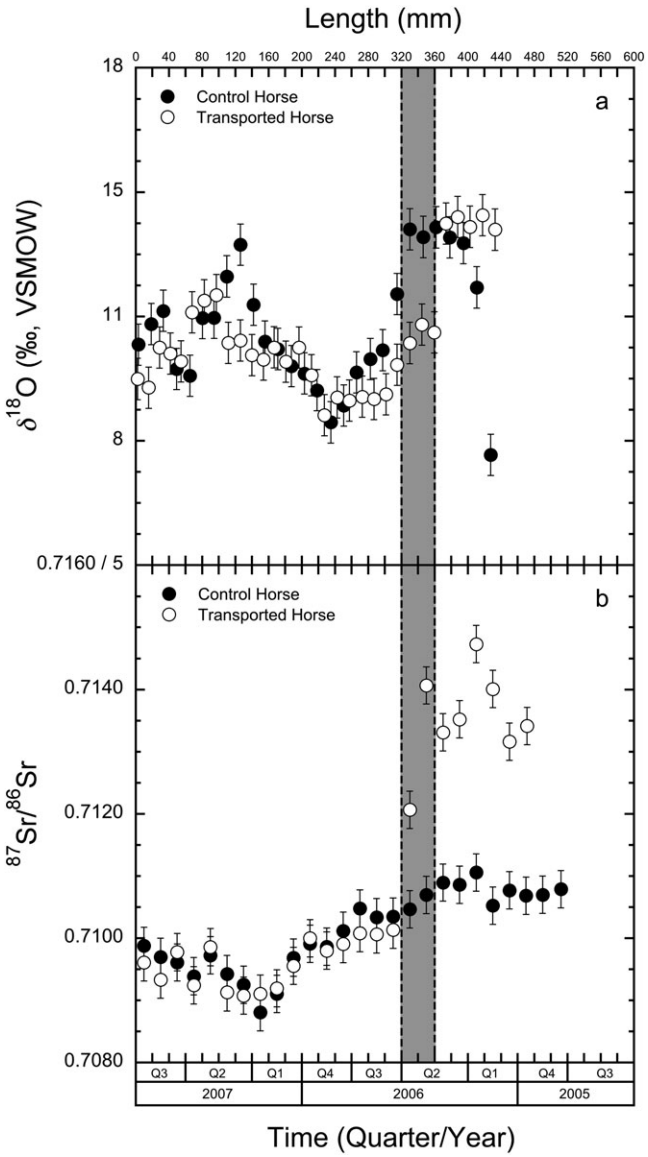

Figure 1. (a) $\delta^{18}O$ values of tail hair from the Control Horse (CH; solid circles) and Transported Horse (TH; open circles) across lengths ~430 mm. (b) $^{87}Sr/^{86}Sr$ of tail hair from the CH and TH across lengths ~510 mm (CH) and ~470 mm (TH). Lengths of hair represent a temporal period of *ca* 24 months (assuming a growth rate of 0.70 mm day^{-1}[23]). The move from Brazil to the United States of the TH is estimated to be at 340 mm; the gray box indicates uncertainty about that estimation due to variations in hair growth rate plus travel and quarantine time. Error bars for $\delta^{18}O$ and $^{87}Sr/^{86}Sr$ values correspond to 2σ of reference materials used in this study: 0.54 and 0.0003, respectively.

Sr abundance and $^{87}Sr/^{86}Sr$

Strontium abundance profiles of the hairs are shown in Fig. 2 and Supplementary Table S2 (Supporting Information). The CH and TH had significantly different mean Sr abundance values of 8.94 mg kg^{-1} and 2.48 mg kg^{-1}, respectively (Mann-Whitney *U*-test, $U = 31$, $p < 0.0001$). The difference in Sr abundances ([Sr]) between the hair strands from the two horses may be due to the differences in hair coloration. Previous research has shown that darker pigmentation leads to greater incorporation of many exogenous elements, including Sr.[35] Thus, the dappled nature of the TH's hair may be linked to the observed lower Sr abundance than in the darker hair of the CH.

Despite differences in absolute Sr abundance between the two horses, the trend in [Sr] over time was similar (Fig. 2), and thus the method of Sr incorporation into hair keratin appears to be similar. The exponential relationship in [Sr] versus segment length is probably related to combination of endogenous and exogenous Sr inputs to the hair. We expect that a solely endogenous (e.g., dietary, drinking water) input of Sr to the hair would be characterized by a relatively static [Sr] profile in the CH; a static profile would represent a non-dynamic Sr source for a horse that did not move or significantly change diet or drinking water. In contrast, the continual incorporation of exogenous Sr would lead to exponential increases in abundance over time; this is consistent with the pattern seen in Fig. 2, where [Sr] exponentially increases for both the CH ($R^2 = 0.89$) and the TH ($R^2 = 0.50$). Here, the oldest segments of the hair strands have the most elevated [Sr]. This pattern is also consistent with previously published [Sr] data from human hair studies.[5,9]

The $^{87}Sr/^{86}Sr$ ratios of hair from the TH reflected the move from BR to SLV at the predicted length/time in contrast to the $\delta^{18}O$ results (Fig. 1(b) and Supplementary Table S2, Supporting Information). The measured $^{87}Sr/^{86}Sr$ ratios of the horses' tail hair are presented in Fig. 1(b) to 4 significant figures and associated measurement uncertainty based on analyses of SRM®987. Based on the time needed for tail hair to reach steady ("equilibrium") $^{87}Sr/^{86}Sr$ ratios, it appears that the lag in response of the strontium isotope signal following the move of the TH was approximately 6 weeks.

The $^{87}Sr/^{86}Sr$ signatures of SLV, as represented by the values of hair from the CH, had a mean of 0.7101 ($1\sigma = 0.0006$ $n = 26$) for the entire hair length. In comparison, the mean $^{87}Sr/^{86}Sr$ of the hair from the TH was 0.7109 ($1\sigma = 0.0020$, $n = 24$) for the entire hair length and 0.7096 ($1\sigma = 0.0004$, $n = 16$) during its residency in SLV (e.g., <320 mm), which was not significantly different (unpaired *t*-test, $t = 0.7295$, df = 30, $p = 0.4713$) from the CH during the same time period. Conversely, $^{87}Sr/^{86}Sr$ ratios of the hair from the two horses were statistically different (unpaired *t*-test, $t = 13.57$, df = 12, $p < 0.0001$) for segment lengths >360 mm (Fig. 1(b)). The $^{87}Sr/^{86}Sr$ of hair from the TH had a mean of 0.7137 ($1\sigma = 0.0006$, $n = 6$) for hair lengths >360 mm.

While our understanding of the mechanism of Sr incorporation into hair is nascent, it appears that the initial Sr isotope signature from the TH's time in BR is conserved to some degree and distinguishable in the tail hair despite the horse's yearlong exposure to the new environment of SLV post-move. Given our findings and published evidence that Sr is continually incorporated into hair tissues from the environment,[9,10] the $^{87}Sr/^{86}Sr$ measured for the TH during its period of BR residency is probably not the "true" $^{87}Sr/^{86}Sr$ end-member value, but rather an overprinted signature. Without knowing the initial $^{87}Sr/^{86}Sr$ ratio of hair from the TH prior to the move, the degree to which the BR signature has been dampened by exogenous Sr in the longest/oldest segments of hair remains uncertain.

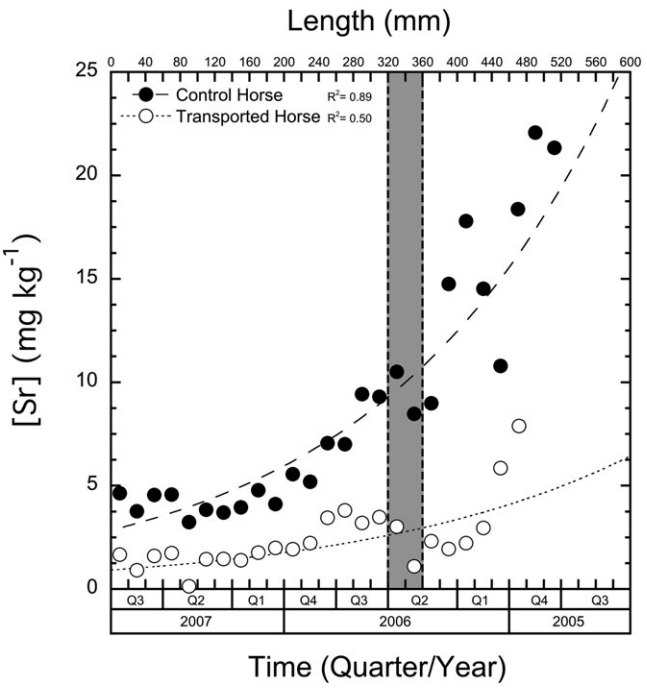

Figure 2. Sr concentrations of tail hair from the Control Horse (CH; solid circles) and Transported Horse (TH; open circles) across tail hair lengths ~510 mm (CH) and ~470 mm (TH). An exponential model best explained [Sr] along the lengths of hair with R^2 values of 0.89 and 0.50 for the CH and TH, respectively. The move from Brazil to the United States of the TH is estimated to be at 340 mm; the gray box indicates uncertainty about that estimation due to variations in hair growth rate plus travel and quarantine time.

CONCLUSIONS

In this study, we measured $\delta^{18}O$ and $^{87}Sr/^{86}Sr$ signatures at high resolution along the length of a tail hair from a pair of domesticated horses that resided apart and then together for known intervals. As the O and Sr isotope signatures of animal tissues are often individually applied to reconstruct geospatial movements, the goals of this study were to evaluate the correspondence between these isotope records along the length of a hair strand and to assess the fidelity of these two isotope systems to discriminate a major geospatial transition.

While measuring $\delta^{18}O$ values at high resolution along a single hair strand is straightforward, the sequential sampling for $^{87}Sr/^{86}Sr$ along hair is analytically challenging due to low Sr concentration in keratin. To overcome these hurdles, we applied novel analytical procedures to measure the $^{87}Sr/^{86}Sr$ ratios of samples with extremely low Sr abundance via multi-collector-ICP-MS, while maintaining acceptable measurement precision of SRM®987 at similar lowered concentration. The advances discussed here allowed for $\delta^{18}O$ and $^{87}Sr/^{86}Sr$ signatures to be measured at similar resolution along the length of single hair strands, and thus allowed for the direct comparison of these two isotope records.

In this proof-of-concept study, we found that $^{87}Sr/^{86}Sr$ ratios had greater utility than $\delta^{18}O$ values in reconstructing an individual's travel history through the isotope analysis of hair. This was surprising considering that the control and test animals used in this study were initially at locations nearly 10,000 km and 60° latitude apart; despite this, the $\delta^{18}O$ values of their hair were indistinguishable. While we did not have access to the animals' diets or drinking waters, we proposed possible explanations for this finding, including overlapping $\delta^{18}O$ values of precipitation at the two locations. We acknowledge that these findings might have been serendipitous in this particular case, but, nonetheless, they potentially suggest some caution when interpreting $\delta^{18}O$ signatures alone for travel movement investigations.

While the $^{87}Sr/^{86}Sr$ signal of movement was clearly evident in the horse tail hair, we discussed the potential for alteration of the Sr isotope signatures in the oldest segments of hair through continuous incorporation of exogenous Sr from the transported horse's "new" environment. Further investigation is necessary to determine the extent of dampening/amplification of the initial isotopic signature by new Sr source(s) through time. We acknowledge that the providential nature of this study did not allow for a complete investigation of this process and suggest that studies with the addition of better-defined endogenous and exogenous isotopic end-members would significantly expand on our findings.

Finally, we note that we used a model organism – the domestic horse – in this proof-of-concept study due to a well-defined movement history and significant [Sr] in segments of a single tail hair. While applications of $^{87}Sr/^{86}Sr$ measurement of human hair could be useful in provenance investigations,[6] we recognize that additional analytical advances will be needed to measure the Sr isotopic signatures of individual strands of human hair. Nonetheless, a similar analytical approach could be undertaken with current technologies by using bundled human hair strands. The bundling method is well established for $\delta^{18}O$ analysis of human hair with known uncertainties,[12] but little is known about the effect of hair bundling on the measured $^{87}Sr/^{86}Sr$ ratio.

Acknowledgements

This project was supported by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice (Award No. 2013-DN-BX-K009). The opinions, findings, and conclusions or recommendations expressed in this manuscript are those of the authors and do not reflect those of the Department of Justice. The authors thank D. Abbe for providing access to her horses. The authors are grateful to Luciano O. Valenzuela for his constructive input to this contribution, as well as two additional anonymous reviewers for their comments on the paper, all of which greatly improved the text.

REFERENCES

[1] K. J. Petzke, B. T. Fuller, C. C. Metges. Advances in natural stable isotope ratio analysis of human hair to determine nutritional and metabolic status. *Curr. Opin. Clin. Nutr. Metab. Care* **2010**, *13*, 532.
[2] J. R. Ehleringer, G. J. Bowen, L. A. Chesson, A. G. West, D. W. Podlesak, T. E. Cerling. Hydrogen and oxygen isotope ratios in human hair are related to geography. *Proc. Natl. Acad. Sci.* **2008**, *105*, 2788.

[3] T. E. Cerling, G. Wittemyer, J. R. Ehleringer, C. H. Remien, I. Douglas-Hamilton. History of animals using isotope records (HAIR): A 6-year dietary history of one family of African elephants. *Proc. Natl. Acad. Sci.* **2009**, *106*, 1275.
[4] I. M. Kempson, W. M. Skinner. ToF-SIMS analysis of elemental distributions in human hair. *Sci. Total Environ.* **2005**, *338*, 213.
[5] I. M. Kempson, W. M. Skinner, K. P. Kirkbride. Advanced analysis of metal distributions in human hair. *Environ. Sci. Technol.* **2006**, *40*, 3423.
[6] B. J. Tipple, T. H. Chau, L. A. Chesson, D. P. Fernandez, J. R. Ehleringer. Isolation of strontium pools and isotope ratios in modern human hair. *Anal. Chim. Acta* **2013**, *798*, 64.
[7] W. Müller, H. C. Fricke, A. N. Halliday, M. T. McCulloch, J.-A. Wartho. Origin and migration of the Alpine Iceman. *Science* **2003**, *302*, 862.
[8] C. D. Kennedy, G. J. Bowen, J. R. Ehleringer. Temporal variation of oxygen isotope ratios ($\delta^{18}O$) in drinking water: Implications for specifying location of origin with human scalp hair. *Forensic Sci. Int.* **2011**, *208*, 156.
[9] L. Font, G. van der Peijl, I. van Wetten, P. Vroon, B. van der Wagt, G. Davies. Strontium and lead isotope ratios in human hair: Investigating a potential tool for determining recent human geographical movements. *J. Anal. At. Spectrom.* **2012**, *27*, 719.
[10] G. Vautour, A. Poirier, D. Widory. Tracking mobility using human hair: What can we learn from lead and strontium isotopes? *Sci. Justice* **2015**, *55*, 63.
[11] J. R. Ehleringer, A. H. Thompson, D. W. Podlesak, G. J. Bowen, L. A. Chesson, T. E. Cerling, T. Park, P. Dostie, H. Schwarcz. A framework for the incorporation of isotopes and isoscapes in geospatial forensic investigations, in *Isocapes: Understanding Movement, Pattern, and Process on Earth Through Isotope Mapping*, (Eds: J. B. West, G. J. Bowen, T. E. Dawson, K. P. Tu). Springer, New York, **2010**, pp. 357–388.
[12] C. H. Remien, F. R. Adler, L. A. Chesson, L. O. Valenzuela, J. R. Ehleringer, T. E. Cerling. Deconvolution of isotope signals from bundles of multiple hairs. *Oecologia* **2014**, *175*, 781.
[13] G. J. Bowen, B. Wilkinson. Spatial distribution of $\delta^{18}O$ in meteoric precipitation. *Geology* **2002**, *30*, 315.
[14] G. J. Bowen, J. R. Ehleringer, L. A. Chesson, E. Stange, T. E. Cerling. Stable isotope ratios of tap water in the contiguous United States. *Water Resour. Res.* **2007**, *43*, 1.
[15] J. M. Landwehr, T. B. Coplen, D. W. Stewart. Spatial, seasonal, and source variability in the stable oxygen and hydrogen isotopic composition of tap waters throughout the USA. *Hydrol. Processes* **2014**, *28*, 5382.
[16] B. L. Beard, C. M. Johnson. Strontium isotope composition of skeletal material can determine the birth place and geographic mobility of humans and animals. *J. Forensic Sci.* **2000**, *45*, 1049.
[17] L. A. Chesson, B. J. Tipple, G. N. Mackey, S. A. Hynek, D. Fernandez, J. R. Ehleringer. Strontium isotopes in tap water from the coterminous USA. *Ecosphere* **2012**, *3*, 67.
[18] C. P. Bataille, G. J. Bowen. Mapping $^{87}Sr/^{86}Sr$ variations in bedrock and water for large scale provenance studies. *Chem. Geol.* **2012**, *304–305*, 39.
[19] K. J. Knudson, S. R. Williams, R. Osborn, K. Forgey, P. R. Williams. The geographic origins of Nasca trophy heads using strontium, oxygen, and carbon isotope data. *J. Anthropol. Archaeol.* **2009**, *28*, 244.
[20] L. Font, G. Jonker, P. A. van Aalderen, E. F. Schiltmans, G. R. Davies. Provenancing of unidentified World War II casualties: Application of strontium and oxygen isotope analysis in tooth enamel. *Sci. Justice* **2015**, *55*, 10.
[21] C. Lehn, A. Rossmann, M. Graw. Provenancing of unidentified corpses by stable isotope techniques – presentation of case studies. *Sci. Justice* **2015**, *55*, 72.
[22] A. H. Thompson, A. S. Wilson, J. R. Ehleringer. Hair as a geochemical recorder, in *Treatise on Geochemistry*, (Eds: T. E. Cerling, K. K. Turekian). Elsevier, **2014**, pp. 371–393.
[23] A. G. West, L. K. Ayliffe, T. E. Cerling, T. F. Robinson, B. Karren, M. D. Dearing, J. R. Ehleringer. Short-term diet changes revealed using stable carbon isotopes in horse tail-hair. *Funct. Ecol.* **2004**, *18*, 616.
[24] L. Hu. Spatial concentrations of trace elements in keratin. *Masters thesis*, University of Utah, **2016**.
[25] D. M. Ethier, C. J. Kyle, J. J. Nocera. Tracking animal movement by comparing trace element signatures in claws to spatial variability of elements in soils. *Sci. Total Environ.* **2014**, *468–469*, 699.
[26] J. R. Ehleringer, G. J. Bowen, L. A. Chesson, A. G. West, D. W. Podlesak, T. E. Cerling. Hydrogen and oxygen isotope ratios in human hair are related to geography. *Proc. Natl. Acad. Sci.* **2008**, *105*, 2788.
[27] D. W. Podlesak, A.-M. Torregrossa, J. R. Ehleringer, M. D. Dearing, B. H. Passey, T. E. Cerling. Turnover of oxygen and hydrogen isotopes in the body water, CO_2, hair, and enamel of a small mammal. *Geochim. Cosmochim. Acta* **2008**, *72*, 19.
[28] D. W. Podlesak, G. J. Bowen, S. P. O'Grady, T. E. Cerling, J. R. Ehleringer. δ^2H and $\delta^{18}O$ of human body water: A GIS model to distinguish residents from non-residents in the contiguous USA. *Isot. Environ. Health Stud.* **2012**, *48*, 259.
[29] S. P. O'Grady, L. O. Valenzuela, C. H. Remien, L. E. Enright, M. J. Jorgensen, J. R. Kaplan, J. D. Wagner, T. E. Cerling, J. R. Ehleringer. Hydrogen and oxygen isotope ratios in body water and hair: Modeling isotope dynamics in nonhuman primates. *Am. J. Primatol.* **2012**.
[30] G. J. Bowen, L. I. Wassenaar, K. A. Hobson. Global application of stable hydrogen and oxygen isotopes to wildlife forensics. *Oecologia* **2005**, *143*, 337.
[31] Available: waterisotopes.org (accessed October 27, 2016).
[32] Y. Jameel, S. Brewer, S. P. Good, B. J. Tipple, J. R. Ehleringer, G. J. Bowen. Tap water isotope ratios reflect urban water system structure and dynamics across a semiarid metropolitan area. *Water Resour. Res.* **2016**, *52*, 5891.
[33] M. Boner, H. Förstel. Stable isotope variation as a tool to trace the authenticity of beef. *Anal. Bioanal. Chem.* **2004**, *378*, 301.
[34] T. DelCurto, B. W. Hess, J. E. Huston, K. C. Olson. Optimum supplementation strategies for beef cattle consuming low-quality roughages in the western United States. *J. Anim. Sci.* **2000**, *77*, 1.
[35] I. M. Kempson, E. Lombi. Hair analysis as a biomonitor for toxicology, disease and health status. *Chem. Soc. Rev.* **2011**, *40*, 3915.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's website.

