

## RESEARCH ARTICLE

# Strontium isotope ratios ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) of human fingernail clippings reveal multiple location signals

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**Rationale:** Strontium isotope ratios ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) in human fingernail keratin tissues have been underexplored for region of origin and travel history reconstruction studies. Here we investigated  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in fingernail keratin to establish baseline measurements in a resident group and to examine how  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios changed with relocation.

**Methods:** Fingernail clippings were collected from resident ( $n = 10$ ) and non-resident/traveler groups ( $n = 4$  and  $n = 4$ ) that were part of a larger study in Salt Lake City (UT, USA) from 2015 to 2016. Strontium abundance and  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios were determined via multicollector inductively coupled plasma mass spectrometry (MC-ICP-MS).  $^{87}\text{Sr}/^{86}\text{Sr}$  and oxygen ( $\delta^{18}\text{O}$ ) isotope ratios from the traveler participants were compared to examine temporal patterns.

**Results:** Strontium abundance and  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in fingernails from the resident group established a baseline against which we could evaluate potential differences in non-resident/traveler groups. Resident  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios remained constant over the study period and were consistent with previously measured tap waters for the area.  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio changes in non-resident/traveler groups were rapid and reflected the current location of the individual within 4–5 weeks of arrival. Lastly,  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of the same fingernail clippings did not exhibit similar temporal patterns, since fingernail  $\delta^{18}\text{O}$  ratios required more time to attain values characteristic of the new environment.

**Conclusions:** Our findings suggest that strontium is incorporated into fingernail tissues differently from hair and this could be advantageous to forensic investigations. We found that  $^{87}\text{Sr}/^{86}\text{Sr}$  and  $\delta^{18}\text{O}$  ratios of the same fingernail clippings revealed two different time points reflecting an individual's residence over short- (4–5 weeks,  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios) and long-term (3–5 months,  $\delta^{18}\text{O}$  values) time scales. It is likely that the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of fingernail clippings reflect exogenous signals that are incorporated through bathing waters and that these signals change rapidly with movement to a new location. Our results may aid future forensic studies in the determination of region of origin in unidentified remains.

## 1 | INTRODUCTION

Measurements of strontium isotope ratios ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) in human bone and tooth enamel have been well described and proven to be a useful tool to identify likely locations where individuals spent their early life and where they resided prior to death.<sup>1–3</sup>  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios relate to

the geology of a particular region with the age and type of bedrock influencing the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of the soils, and thus the composition of water, plants, and animals of that area.<sup>4–6</sup> These sources are then integrated into human bones, hair, and teeth through the foods and waters, leaving geographic markers that can be used for human provenance.

The integration of strontium into the human body has been explored and an estimated 2 mg of strontium per day is ingested through dietary and drinking water inputs. Nielsen<sup>7</sup> estimated that the majority of strontium inputs is excreted from the body through urine, feces, and perspiration, with the remaining portion being integrated into the bones and teeth. A very small fraction (0.2  $\mu\text{g}/\text{day}$ ) of strontium is integrated into hair and other keratin tissues, so the direct endogenous strontium signal in hair is minimal compared with that of bone and teeth. In addition, strontium recycling and exchange between bone and blood and later blood and soft tissue (keratin) may also impact the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of keratin tissues.<sup>7,8</sup> While the concentrations of strontium in hair are low, technological advances in mass spectrometry and the development of offline preparative column chemistry techniques have allowed  $^{87}\text{Sr}/^{86}\text{Sr}$  measurements in hair keratins to become more common.<sup>9–11</sup>

Over the past decade,  $^{87}\text{Sr}/^{86}\text{Sr}$  measurements in hair keratin have allowed investigators to explore patterns related to geolocation and travel history reconstruction. Font et al<sup>11</sup> and Vautour et al<sup>12</sup> demonstrated that  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of human scalp hair served as indicators of an individual's movement between international metropolitan regions. Chau et al<sup>9</sup> also demonstrated that animal hair (horse tail hair) could be used to explain specific movement patterns for an individual horse transported from Brazil to the United States. Most recently, Tipple et al<sup>13</sup> showed that  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of municipal waters in Salt Lake City (UT, USA) were sufficiently different that such measurements in hair were a useful tool to differentiate locations of individuals within an urban area even when hair oxygen isotope ratios ( $\delta^{18}\text{O}$ ) were similar. These studies have highlighted how strontium in combination with other isotope biomarkers (carbon, nitrogen, oxygen, hydrogen, and lead) can increase the spatial resolution for human provenance studies.<sup>9,12,13</sup> However, the precise source of the strontium signal in these studies is not clear. Both endogenous (dietary and drinking water) and exogenous (bathing water (i.e. showering, hand washing, swimming) and environmental deposition (dust)) sources can contribute to the strontium composition of hair, although recent studies suggest a predominant role for exogenous strontium.<sup>9,12,13</sup>

With these promising  $^{87}\text{Sr}/^{86}\text{Sr}$  studies with hair, it is surprising that human fingernail tissues have not been explored for similar purposes. Both hair and fingernail tissues are composed of the same  $\alpha$ -keratin proteins, contain similar amino acid profiles,<sup>14,15</sup> and grow at sufficiently constant rates that allow these tissues to serve as linear recorders of life events. Specifically, with light stable isotopes, once the proteins are formed the stable isotope signals are locked in and do not change with time, thus allowing for an assessment of past dietary inputs (food and water sources) that can be used to reconstruct travel history.<sup>16–18</sup> Despite similarities in the biochemical composition of hair and fingernails, the structures of these tissues differ significantly.<sup>19</sup> Hair strands are surrounded by cuticle scales that create the outer layer of the hair and these scales typically overlap with narrow margins.<sup>20</sup> Prior studies examining trace element contamination in hair strands have shown that elements on the surface of the hair can, with time, penetrate into the inner portions (medulla or cortex) of the hair strand through the edges of the cuticle scales.<sup>21–23</sup> On the other hand, fingernail tissues do not contain

an outer cuticle layer, and the cells are arranged in a lamellar or plate-like structure,<sup>24–26</sup> which may prevent integration of long-term exogenous inputs.

If bathing waters and atmospheric deposition are the primary contributors to the  $^{87}\text{Sr}/^{86}\text{Sr}$  composition in human hair, as suggested by Tipple et al<sup>13</sup> and Chau et al<sup>9</sup> perhaps the deep penetration of these exogenous inputs allows hair strands to maintain different  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios over time, i.e. the strontium signal is not completely “washed away” with new successive bathing events.<sup>9,13</sup> Thus, the  $^{87}\text{Sr}/^{86}\text{Sr}$  profile from the root to the tip of scalp hair contains information related to the geography of travel movements.<sup>9,11,12</sup> Similarly, if bathing waters strongly influence the keratin proteins of fingernail tissues, based on their structure, we hypothesize that  $^{87}\text{Sr}/^{86}\text{Sr}$  signals may be more superficial and as one moves from one location to the next, that the bathing waters may eliminate or override any prior  $^{87}\text{Sr}/^{86}\text{Sr}$  signal.

Here we examine  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio measurements using human fingernail clippings that were collected as part of a larger study of residents, recently moved to the region (non-residents), and travelers (individuals that moved away and returned) in Salt Lake City.<sup>16,27</sup> Based on the structure of fingernail tissues, we hypothesized that the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio would reflect an individual's most recent location and therefore provide only a short-term  $^{87}\text{Sr}/^{86}\text{Sr}$  signal for travel history reconstruction studies. To test this hypothesis, we examined  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in a resident sub-population within the Salt Lake City region, investigating if  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios would remain stable over time. Secondly, we measured  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in non-resident and traveler participants to test the possibility of using  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios as a tracer for travel history reconstruction. Lastly, we examined both  $\delta^{18}\text{O}$  values and  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in fingernail clippings from our traveler participants to compare geolocation signals between the two isotope markers.

## 2 | EXPERIMENTAL

### 2.1 | Participant recruitment

A subset of resident ( $n = 10$ ), non-resident ( $n = 4$ ), and traveler ( $n = 4$ ) participant fingernail clippings from our larger study in Salt Lake City (2015–2016) were included in this investigation (further details of these populations can be found in Mancuso and Ehleringer<sup>16,27</sup>). In short, residents were defined as individuals who lived consistently in the Salt Lake City region for a minimum of 9 months, non-residents were defined as individuals who had recently moved to the region (within 1 month of the start of this study), and travelers were residents of Salt Lake City who traveled outside the region for a period of time and then returned.

Fingernail clippings were collected for a period of 9–24 months, depending on the individual. In addition, demographic surveys (sex, age, travel history, and dietary questions) were obtained from volunteers throughout the study period to monitor changes in diet or travel. The University of Utah's Institutional Review Board approved this study (IRB protocol 00035524). All participants provided written consent to participate in the study and were free

to choose which sample(s) and demographic information to provide. These choice decisions account for the variations in sample sizes for analyses presented in this study.

## 2.2 | Fingernail samples

Fingernail clippings were collected from each individual and stored in the same manner as previously described until isotope analysis.<sup>16,27</sup> In brief, participants collected fingernail clippings for a period of 9–24 months (clipping their nails as they normally would) and stored them in labeled coin envelopes, where they recorded the date of clipping. Fingernail samples were stored in coin envelopes until they could be processed. Fingernail clippings from both hands were used in the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio analyses, as fingernail clippings were found to contain low [Sr] in proof-of-concept testing. Using clippings from both hands ensured enough material for isotope ratio analyses. In the subset of resident participants ( $n = 10$ ) we measured  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios four times for each participant that corresponded to the beginning (August/September 2015), middle (November 2015 and February 2016), and conclusion (April 2016) of the study. These values provided baseline  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios against which we could then compare non-resident and traveler values.

In the subset of non-resident ( $n = 4$ ) and traveler ( $n = 4$ ) populations, we initially measured the first and last sampling from each participant. If the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of these samples were identical, we did not proceed further to analyze additional samples. If differences were observed, we measured subsequent samples that would relate to their reported travel history. All sample processing, [Sr] measurements, and isotope analyses were conducted at the ICP-MS Metals and Strontium Isotope Geochemistry Laboratories at the University of Utah (Salt Lake City, UT, USA), where sampling, handling, and wet chemistry were performed in ultra-low particulate air laminar flow benches.

## 2.3 | Fingernail and water strontium abundance and isotope ratio analyses

### 2.3.1 | Sample cleaning

All fingernail samples underwent additional preparation in a clean laboratory as presented below to remove dirt and additional contaminants from the nail clippings. Ultrapure water that was used in sample preparation and chemistry was filtered through a Milli-Q Gradient point-of-use filtration unit (EMD Millipore, Billerica, MA, USA) with a resistivity  $> 18 \text{ M}\Omega$  (hereafter referred to as “18 M $\Omega$  water”). Fingernail clippings from both the left and the right hands for a particular sampling date were initially cleaned with 18 M $\Omega$  water to remove any visible surface dirt or material from them. Approximately 20–80 mg of fingernail material was placed into acid-leached 15 mL conical tubes. Fingernail samples were cleaned in a similar manner to that described by Tipple et al.<sup>28</sup> for human scalp hair. In brief, fingernail samples were cleaned with acetone (EMD Millipore, HPLC-grade), 18 M $\Omega$  water, acetone, and 0.1 M HCl washing process. Samples were sonicated for 10 min with each solution and the subsequent solutes were decanted into another labeled 15 mL conical tube. All decanted “leachate” solutes were collected into the same 15 mL tube for each

sample and stored for possible future residue analysis. Tubes containing fingernail samples were drained of any remaining liquid and placed uncapped in a laminar flow hood to dry at room temperature for a period of 72–120 h. Dried samples were then capped and stored in the clean room until further preparation.

### 2.3.2 | Sample digestion for strontium abundance and isotope analysis

Once the samples were completely dry they were digested using methods that have been previously described.<sup>9,28,29</sup> In brief, cleaned fingernail samples were placed in 10 mL Teflon digestion vessels, and digested in 2 mL of 16 M ultrapure nitric acid ( $\text{HNO}_3$ ) using an Ethos EZ SK-10 high-pressure Rotor microwave digestion system (Milestone, Inc., Sorisole, Italy). Details of the digestion method are described by Chau et al, Saul, and Tipple et al.<sup>9,28,29</sup> 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 fingernail samples, along with method blanks. Once samples had cooled to room temperature, the digest solutions were transferred to 2 mL acid leached micro-centrifuge tubes for storage.

The [Sr] was determined using a 100  $\mu\text{L}$  aliquot of the primary digest that was transferred to an acid-leached 15 mL conical tube. Then 25  $\mu\text{L}$  of a 1 mg  $\text{kg}^{-1}$  indium internal standard was added to the secondary aliquot and diluted to 2.5 mL with 2.4%  $\text{HNO}_3$ . The remaining primary digest solutions were reserved for  $^{87}\text{Sr}/^{86}\text{Sr}$  analyses. The [Sr] was measured via quadrupole inductively coupled plasma mass spectrometry (MC-ICP-MS) (Agilent 7500ce; Agilent Technologies, Santa Clara, CA, USA). The [Sr] was calculated using a six-point calibration curve that was developed from the measurement of solutions containing strontium that were prepared gravimetrically using single element standards (Inorganic Ventures, Christiansburg, VA, USA). In addition to the TORT-2 and Human Hair No. 13 reference materials, SRM 1643e (Trace elements in water, National Institute of Standards and Technology, Gaithersburg, MD, USA) was used as a quality control for the calibration curve.

### 2.3.3 | Sample purification and isotope analysis

The values of [Sr] in fingernail sample digests were very low; thus, the samples were concentrated and column chemistry purification was manually performed. The remaining primary digests (~1.8 mL) were decanted into a 22 mL rounded interior Teflon container and placed uncapped on a hot plate set to 200°C in a laminar flow hood. Sample digests were dried down to a minute droplet, which was immediately removed, and 400  $\mu\text{L}$  of a 4 M trace metal grade  $\text{HNO}_3$  (Aristar Plus, BDH Chemicals, VWR, Radnor, PA, USA) solution was added. Samples were capped and stored at room temperature within the laminar flow hood until purification could be completed. The strontium in the digests was isolated and purified using Teflon columns that were prepared as described by Chau et al<sup>9</sup> and packed with 100–150  $\mu\text{m}$  crown ether strontium resin (Eichrom Technologies, Lisle, IL, USA). Prior to strontium isolation, the columns were cleaned with 18 M $\Omega$  water and conditioned by loading eight column volumes (50  $\mu\text{L}$  each)

of 4 M trace metal grade  $\text{HNO}_3$  to the column, 100  $\mu\text{L}$  at a time. After column conditioning, sample digests (or procedural blanks of 4 M  $\text{HNO}_3$ ) were loaded onto the columns 100  $\mu\text{L}$  at a time, with a total of 400  $\mu\text{L}$  for each sample. Following sample loading, 300  $\mu\text{L}$  of 4 M trace metal grade  $\text{HNO}_3$  was added in 100  $\mu\text{L}$  volumes to clean the resin matrix. Strontium was eluted from the resin using two 200  $\mu\text{L}$  volumes of 0.1%  $\text{HNO}_3$  diluted with 18 M $\Omega$  water. The collected strontium sample was transferred to a 2 mL conical interior Teflon container and 16  $\mu\text{L}$  of concentrated trace metal grade  $\text{HNO}_3$  was added to acidify the solution (approximately 5%  $\text{HNO}_3$ ). In addition to fingernail samples, 2–3 procedural blanks were purified along with the fingernail samples to evaluate potential strontium contamination from this process. Columns were loaded with sample digests no more than two times (based on proof-of-concept testing of columns) and between samples the columns were cleaned by the above procedure, using 4 M  $\text{HNO}_3$  in place of the sample digest.

$^{87}\text{Sr}/^{86}\text{Sr}$  ratios were measured using high-resolution MC-ICP-MS (Neptune Plus, Thermo Scientific, Bremen, Germany). SRM 987 ( $\text{SrCO}_3$ , National Institute of Standards and Technology) was used to check the quality of the method. 200  $\mu\text{g kg}^{-1}$  and 50  $\mu\text{g kg}^{-1}$  solutions of SRM 987 were used to capture the expected range of sample concentrations and were analyzed before and after each set of three samples to verify measurement accuracy and precision. SRM 987 is certified with a  $^{87}\text{Sr}/^{86}\text{Sr}$  value of  $0.71034 \pm 0.00026$  and the long-term average  $\pm$  SD of the Strontium Isotope Geochemistry Laboratory at the University of Utah since 2010 was  $0.71028 \pm 0.000015$ .

The  $^{87}\text{Sr}/^{86}\text{Sr}$  values of SRM 987 measured in our study averaged  $0.71029 \pm 0.00001$  (mean  $\pm$  SD,  $n = 62$ ). Instrumental and procedural column blanks were also evaluated looking at intensity levels that were  $< 0.001$  V and which were below the lab threshold for strontium contamination ( $< 0.01$ ). Krypton and rubidium were also collected and used to correct for  $^{86}\text{Kr}$  interference at mass 86 and  $^{87}\text{Rb}$  interference at mass 87. The sample results were corrected and analyzed as previously described by Tipple et al.<sup>13</sup> and Chau et al.<sup>9</sup>

### 2.3.4 | Galapagos bathing water sample preparation and analysis

Participants who traveled to the Galapagos, Ecuador, returned with a sample of bathing water (water used for showering, dish washing, etc.) from the field station at which they were living for 4.5 months. The water sample was collected in a 125 mL low-density polyethylene (LDPE) bottle. Prior to collection, the water tap was opened and allowed to run for 10 s prior to filling the bottle. Samples were sealed with Parafilm and stored in the dark at 10°C prior to analysis. 10 mL of the water sample was poured into a 15 mL acid-leached conical tube for [Sr] analysis using the same protocol as previously listed above. Once the [Sr] had been determined, sample purification was completed using a second aliquot of the water sample containing 200 ng of strontium which was acidified (using trace metal grade  $\text{HNO}_3$ ) to a concentration of 2 M  $\text{HNO}_3$  and purified using an automated system for strontium purification (PrepFAST MC, Elemental Scientific, Omaha, NE, USA). The strontium fraction obtained after purification was in a 6 M  $\text{HNO}_3$  solution that was evaporated and re-dissolved in 1 mL of 5%  $\text{HNO}_3$  and the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios were measured as described in the previous section.

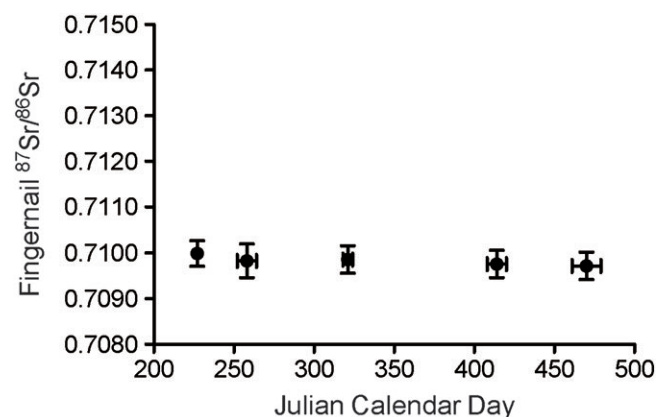
## 3 | STATISTICS

Statistical significance and graphic output were generated using Prism version 5.0 (GraphPad Software, San Diego, CA, USA). Data were summarized as mean  $\pm$  standard deviation (SD). Normality of distributions was assessed using the Shapiro-Wilks test. Strontium abundance [Sr] and isotope comparisons between sexes were determined using a Mann-Whitney or unpaired t-test (based on normality of data). Assessment of the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios over the study period in the resident sub-population was performed using a one-way analysis of variance (ANOVA) test with a Bonferroni multiple comparison post-test to look for significant differences between specific times. Differences were statistically significant at the  $p < 0.05$  level.

## 4 | RESULTS

Fingernail samples from the resident group ( $n = 10$ ) had [Sr] values that ranged from 0.011 to 0.110  $\mu\text{g g}^{-1}$  (Table S1, supporting information). The [Sr] values of fingernail clippings from male and female participants were  $0.027 \pm 0.014 \mu\text{g g}^{-1}$  ( $n = 5$ ) and  $0.038 \pm 0.028 \mu\text{g g}^{-1}$  ( $n = 5$ ), respectively, and were not significantly different ( $p = 0.0678$ ). The  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of fingernail clippings from the resident group ( $n = 10$ ) did not change significantly over the study period ( $p = 0.6312$ ) (Figure 1). The mean  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio value for the resident group was  $0.70982 \pm 0.0003$  and this was consistent with previously measured  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios from tap water samples from Salt Lake City (0.70840 to 0.71542).<sup>13,30</sup> The  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of males and females were identical ( $0.70980 \pm 0.0002$  and  $0.70980 \pm 0.0004$ , respectively) and not significantly different from each other ( $p = 0.8203$ ). These resident values served as baseline for comparisons with non-resident and traveler groups.

The [Sr] values of non-resident ( $n = 4$ ) and traveler ( $n = 4$ ) fingernail clippings ranged from 0.0084 to 0.101  $\mu\text{g g}^{-1}$  ( $n = 8$ ) and averaged  $0.033 \pm 0.022 \mu\text{g g}^{-1}$  (Tables S2 and S3, supporting information). The [Sr] did not differ significantly between males ( $n = 4$ ) and females ( $n = 4$ ) ( $0.37 \pm 0.019 \mu\text{g g}^{-1}$  and  $0.30 \pm 0.024 \mu\text{g g}^{-1}$ , respectively,  $p = 0.0817$ ). The initial and final  $^{87}\text{Sr}/^{86}\text{Sr}$  values from our non-resident

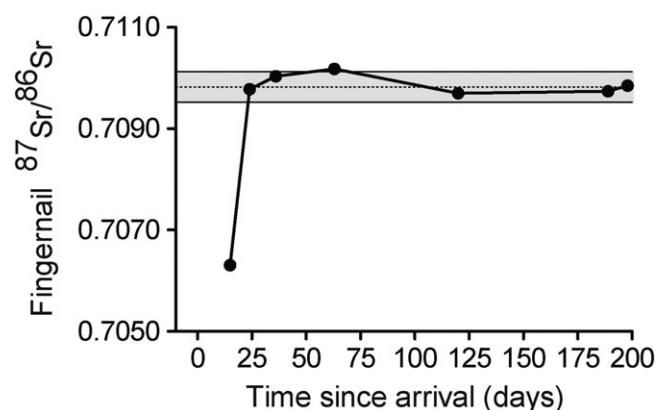


**FIGURE 1**  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of fingernail clipping from resident group ( $n = 10$ ) over the study period (August 2015 to April 2016). Values are listed as mean  $\pm$  SD for each time point. No significant differences were found over the study period ( $p = 0.631$ )

and traveler participants were measured to determine if the values had changed over time. We expected that  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios would be different based upon their location prior to Salt Lake City. However, several of the initial fingernail samples of our non-resident ( $n = 3$ ) and traveler ( $n = 1$ ) participants were within the resident isotope range and did not change by the time of the final sampling. Afterwards the number of days since their arrival was calculated for the initial sampling and it was found that these participants had been living in or returned to Salt Lake City for approximately 4–5 weeks (28–35 days) before their first clipping was collected.

With our limited subset of non-resident participants, only one individual had their initial sample collected within 5 to 20 days of arrival. Participant 19 moved to Salt Lake City from Baton Rouge (LA, USA); the first sample was collected ~15 days after arrival and the measured  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio (0.70631) was not within the Salt Lake City resident isotope range. This value was not consistent with the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of either Baton Rouge (values ranged from 0.709–0.711)<sup>31</sup> or Salt Lake City. We suspect that participant 19 may have traveled outside Baton Rouge for a period of time before arriving in Salt Lake City. We further analyzed fingernail clippings from participant 19 to see how their  $^{87}\text{Sr}/^{86}\text{Sr}$  profile would change over the study period (Figure 2). By the time of the second sampling, 24 days after arrival, the fingernail  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio was firmly within the resident group range (0.70997) and remained within this range throughout the remainder of the study period (~8 months). The fingernail  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio observations from the non-resident and traveler populations suggest that the fingernail  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio transitioned within a 4–5-week period after arrival. Using this time frame, we examined the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of the fingernail clippings of the traveler population for which we had already measured the fingernail  $\delta^{18}\text{O}$  values.<sup>16</sup>

Participants 5 and 6 were part of the group that traveled to the Galapagos, Ecuador, and subsequently returned.<sup>16</sup> Based on the geologic bedrock of the Galapagos (0.7024 to 0.7038),<sup>32,33</sup> the seawater (0.70921 to 0.70924),<sup>34</sup> and local bathing waters (0.70660), we expected that there would be differences in the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios between the two locations. The  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of fingernail clippings collected when our participants were physically in the Galapagos were



**FIGURE 2**  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio profile of non-resident participant 19 since time of arrival (days). Grey box represents the resident group strontium isotope ratio (mean  $\pm$  SD).  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio changes within ~24 days after arriving in Salt Lake City, UT

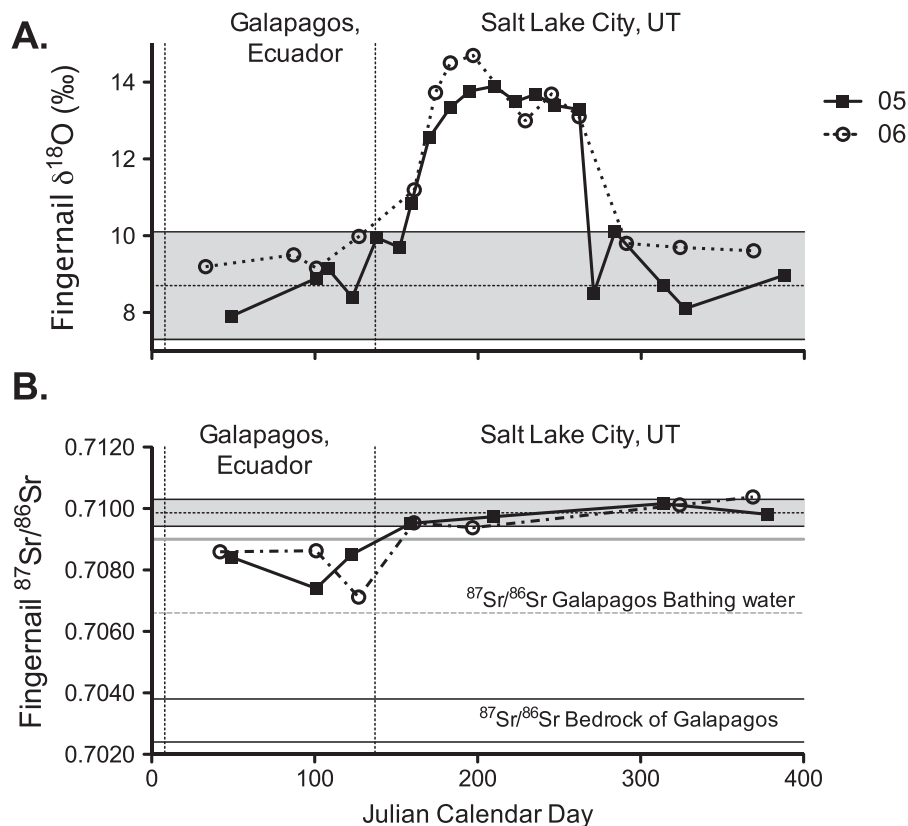
not within the range of  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of the Salt Lake City resident group (Figure 3B). The  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of the underlying bedrock was far below the measured fingernail  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios, suggesting that while participants were in the Galapagos a combination of seawater and local bathing water was influencing the fingernail  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios (Figure 3B). Upon returning from the Galapagos to Salt Lake City, the fingernail  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios quickly converged on the resident  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios (within 34–38 days after returning to Salt Lake City).

Participant 4 was also part of the traveler group and traveled to Panama and returned.<sup>16</sup> While we do not have any information on the bathing water source that was used while in Panama, we see similar patterns to those of the Galapagos participants. Specifically, the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of fingernail clippings collected when participant 4 was physically in Panama are not within the Salt Lake City resident range and when that participant returned the ratios converge on the resident values (Figure 4B). With the timing of samples, we are not able to make clear distinctions related to the change in  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios as the participant moved from one location to another. From the initial to the second sampling (~21 days), the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio changes as the participant traveled from Salt Lake City to Panama; however we do not have another sample until ~63 days later. Similarly, on that person's return to Salt Lake City, we do not have enough sample points to determine the earliest time that the ratio converged on the Salt Lake City resident signal.

It was interesting to note that temporal variations in fingernail  $\delta^{18}\text{O}$  values from the same Galapagos or Panama sampling times did not match the pattern of  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio values (Figures 3 and 4). The  $\delta^{18}\text{O}$  values of the fingernail clippings collected in the Galapagos were within the range expected for Salt Lake City residents because there had not been sufficient time for the Galapagos signal to reach the end of the fingernail (Figure 3A). This was not surprising, as fingernails that were developed in Salt Lake City months previously would take several months to grow to the point where they could be clipped as part of a normal grooming routine. The patterns of  $\delta^{18}\text{O}$  values and  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios were similar in participant 4 who made two trips to Panama and back. Similar to the Galapagos travelers, the  $\delta^{18}\text{O}$  values reflected the location where the fingernail had been developed (initial sample was developed while the participant was in Panama prior to the start of the study) and the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio reflected the physical location where the fingernail had been clipped (Figure 4).

## 5 | DISCUSSION

Our study focused on examining  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio profiles of human fingernail clippings in resident, non-resident, and traveler groups in Salt Lake City. This study is one of the first to describe  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in fingernail and our analyses suggest that  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios reflect an individual's most recent geographic location. When traveling or moving from one region to another, fingernail  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios change rapidly to reflect current environmental conditions. In addition, the combination of  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios and  $\delta^{18}\text{O}$  values of the same set of fingernail clippings provided a short- and long-term geolocation signal.



**FIGURE 3** (A) Oxygen isotope<sup>16</sup> and (B)  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of fingernail clippings collected from participants 5 and 6 throughout the study period. Location of where fingernails were clipped has been labeled. (A) Grey box represents the Salt Lake City, UT resident group oxygen isotope mean  $\pm$  SD<sup>27</sup> (B) Grey box represents the Salt Lake City, UT, resident sub-group  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio mean  $\pm$  SD. White box reflects the range of  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of bedrock from the Galapagos Islands,<sup>33</sup> solid grey line represents the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of modern seawater,<sup>34</sup> and grey dotted line represents the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of the bathing water at the Galapagos field station where the participants resided

## 5.1 | Resident strontium measurements

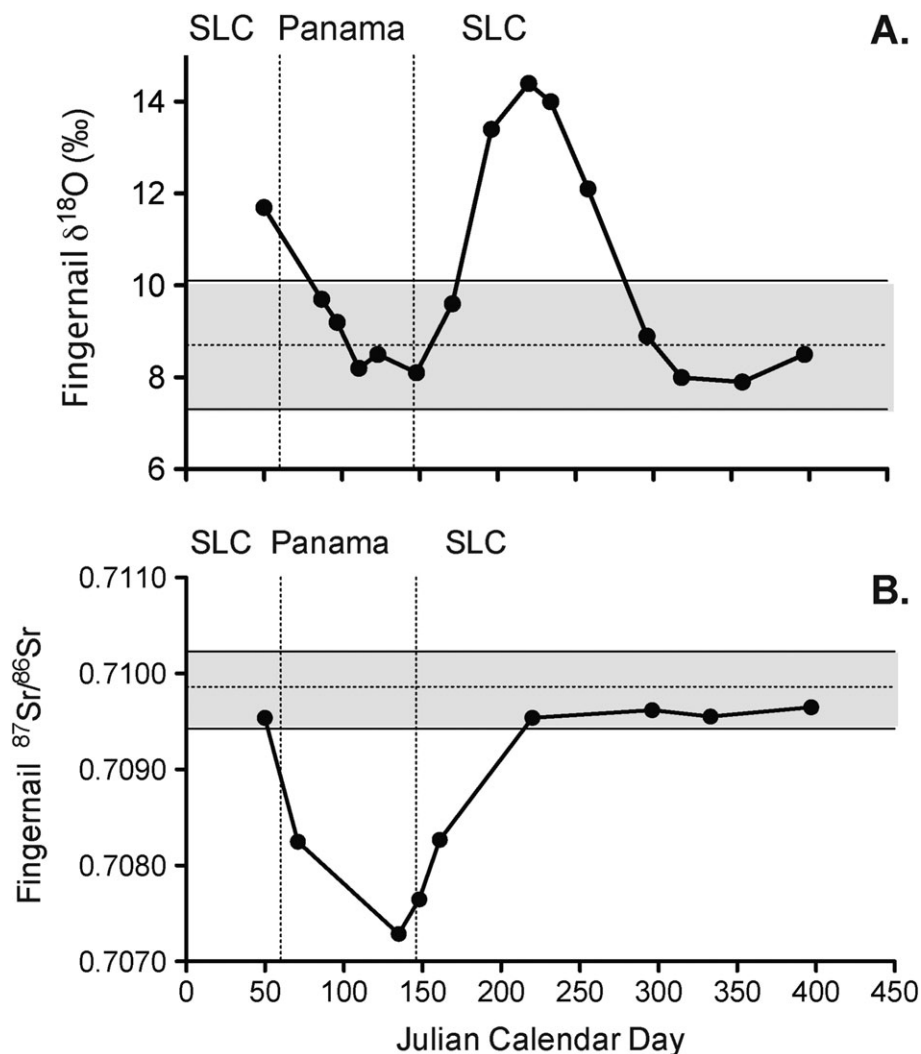
The [Sr] and  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio measurements served as a baseline marker from which we could make comparisons with the non-resident and traveler groups. As mentioned earlier, our study is perhaps the first to examine baseline  $^{87}\text{Sr}/^{86}\text{Sr}$  measurements of a resident group using fingernail keratin proteins. It is likely that few studies have been conducted earlier because of the low [Sr] within fingernail clippings. The range of [Sr] in our group was from 0.011 to 0.110  $\mu\text{g g}^{-1}$ , which was lower than the previously reported abundances in healthy adult fingernail clippings (0.54–1.0  $\mu\text{g g}^{-1}$ ).<sup>35,36</sup> The lower [Sr] in our study may have resulted from our cleaning method,<sup>28</sup> which was originally used for human scalp hair, and included an additional acetone and 0.1 M HCl wash that was not used in the other fingernail studies. It is possible that these extra washing steps could have reduced the [Sr] of the fingernail samples. Further analysis of the “leachate” composition from the fingernail cleaning could explain this effect, but this did not take place as part of this study.

The average [Sr] in fingernail clippings was lower than those previously reported from human scalp hair studies (0.8–27.5  $\mu\text{g g}^{-1}$ ) in the USA and Europe.<sup>13,28,37,38</sup> Tipple et al<sup>13</sup> speculated that the higher [Sr] in their study could be skewed because of the predominant female bias in their study group. Typically, women have longer hair and previous studies have shown that [Sr] increased distally from hair roots;<sup>9,10,12,22</sup> however, the relationship between hair length and sex

is dependent on the sampled culture, age group, and other factors. In our study, we did not observe sex-based differences in [Sr] and can expect that fingernail tissue in males and females would have similar [Sr], as the fingernail exposure time is similar among sexes. If there were sex-based differences, it is possible that females would have higher [Sr] due to slower sex-based growth rate. However, differences between male and female growth rates are minimal.<sup>14,39,40</sup>

There is also evidence that melanin in hair could contribute to differences in [Sr] between scalp hair and fingernails. Previous studies have shown that darker pigmentation in hair leads to greater incorporation of exogenous elements, including strontium.<sup>9,41</sup> For many individuals, their fingernail tissues do not contain melanin and none of the participants in our study had melanin pigmentation in their fingernail tissues. However, melanin bands or pigmentation are more likely to be found in individuals with darker skin pigmentation.<sup>14,40</sup> These individuals may have increased [Sr] abundance compared with those without. Blaurock-Bousch et al<sup>36</sup> found that [Sr] differed between healthy European and Punjabi adults; while they did not specify what could be driving this difference, the presence of melanin could play a role.

The  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in fingernails of residents did not show significant differences over the study period. The population-level variance was small (0.0003) and lower than previously published results for hair samples collected in the Salt Lake Valley, UT, USA (0.0014–0.005),<sup>13,28</sup> and from other sedentary populations (0.001).<sup>12</sup>



**FIGURE 4** (A) Oxygen isotope<sup>16</sup> and (B)  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of fingernail clippings collected from participant 4 over the study period. Location of where fingernails were clipped has been labeled. (A) Grey box represents the Salt Lake City, UT, resident population oxygen isotope mean  $\pm$  SD<sup>27</sup> (B) Grey box represents the Salt Lake City, UT, resident sub-population  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio mean  $\pm$  SD

The lack of variation among our resident group was not surprising, as participants did not move households during the study period and would therefore have consistent access to the same municipal water source throughout the study. The lower variation may be influenced by the limited sample size of our resident population ( $n = 10$ ) compared with these previous studies ( $n = 22\text{--}41$ ).<sup>13,28</sup> In addition, these studies included single time point samples, as opposed to repeated sampling from the same individuals in our study. Based on data from Tipple et al<sup>13</sup> and tap water samples collected by the Ehleringer Lab, the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of municipal waters (for both drinking and bathing) in Salt Lake City range from 0.70840 to 0.71542.<sup>30</sup> The resident fingernail samples were consistent with this range and we concluded that the limited variation over the study period suggests that our participants bathed in tap waters of a similar source.

## 5.2 | Non-resident and traveler population strontium measurements

The [Sr] values in the fingernail clippings from our non-resident and traveler groups were not significant different from those of the resident

group ( $p = 0.845$ ). We did not expect abundance differences as all samples were washed using the same treatment method and did not contain melanin pigmentation bands. The  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of the fingernail clippings did display unique patterns that suggest that the isotope ratio of fingernail clippings reflect an individual's most recent location and these changes occur rapidly (4–5 weeks). This pattern in fingernail keratin differs from some of the previous research examining  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in hair keratin. Font et al<sup>11</sup> suggest that endogenous signals (diet, drinking water, and strontium recycling as bone remodels itself) can have a lasting impact on the  $^{87}\text{Sr}/^{86}\text{Sr}$  hair profile of an individual even after that person has resided in a new location for ~18 months. Tipple et al<sup>13</sup> and Chau et al<sup>9</sup> have, however, suggested that exogenous inputs (bathing waters and aerosol deposition) are the dominant source of the strontium signal in hair. Although it remains unclear which source is primarily controlling the strontium signal in hair keratin, our findings support an exogenous input hypothesis.

The  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of fingernails clippings in this study suggest that the dominant geospatial control may be mediated by exogenous inputs that are integrated through bathing in local waters. It seems unlikely that the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios are derived from an endogenous

source, as they would be integrated into the fingernail tissue as it developed (Figures 3B and 4B). Specifically, among some of the non-resident and traveler participants the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of their initial fingernail clippings were consistent with the Salt Lake City resident signal; however, these fingernail samples were developed in locations outside Salt Lake City. Therefore, it is unlikely that the strontium source would be from an endogenous input. In addition, the rapid change and integration of the Salt Lake City resident signal are consistent with an exogenous input influencing the  $^{87}\text{Sr}/^{86}\text{Sr}$  composition. Specifically, the results from our traveler participants suggest that as an individual moves from one location to another, the strontium signal of the original location is "washed away" after a short period in a new location.

The rapid transition in  $^{87}\text{Sr}/^{86}\text{Sr}$  values as one moves to a new location is highlighted in the profiles of the Galapagos travelers (Figure 3). The bedrock of the region is composed of geologically younger formations that have lower  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios.<sup>33</sup> The region is also influenced by the proximity to the ocean, which has higher  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios.<sup>34</sup> The  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of the Galapagos bathing water was between the  $^{87}\text{Sr}/^{86}\text{Sr}$  values of the ocean and the bedrock, suggesting that this water was a mixture of the two sources. In addition, we know that our participants spent time swimming in the ocean and that could also have pushed their isotope ratios closer to the ocean  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios. While we do not know how often our participants bathed or swam during their time on the islands, the bathing water source remained constant throughout the study period. It seems likely that this source, along with the bathing waters, was the dominant input of strontium into the Galapagos travelers' fingernails.

Our results suggest that fingernail  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios reflect an individual's most recent location and that  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios change rapidly with a traveler's movement. The combination of  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios and  $\delta^{18}\text{O}$  values from the same set of fingernail clippings may be a powerful tool for travel history reconstruction studies.  $\delta^{18}\text{O}$  values are influenced by drinking water inputs that are integrated into the keratin tissue when the fingernails are developed and do not change as the nail grows and can be clipped.<sup>16,17,27</sup> Therefore, from a single fingernail clipping investigators can reveal two time periods: (1) past location ( $\delta^{18}\text{O}$  value), where an individual was 3–4 months previously and (2) the most recent location ( $^{87}\text{Sr}/^{86}\text{Sr}$  ratio), where an individual has been within the past 4–5 weeks. We have highlighted this application in our traveler participants (from the Galapagos and Panama) and, for this reason, measurements of  $\delta^{18}\text{O}$  values and  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in fingernail tissue should prove useful to law enforcement efforts directed at determining the residency status of antecedent human remains or determination of region of origin.

## 6 | CONCLUSIONS

Our study has highlighted the use of  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in human fingernail tissues to establish baseline values of a resident population and to examine the changes in  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios associated with individual travel histories. We have shown that  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in fingernails reflect an individual's most recent location and, when combined with  $\delta^{18}\text{O}$  values, constitute biomarkers that measure

recent and previous geographic locations that could be critical to forensic science studies. While this study was limited in the number of participants and sample frequency, our findings support the hypothesis that strontium in fingernail keratin is derived from exogenous waters that are used to wash hands, bathe, and recreationally swim. We speculate that the structural differences in the keratin tissues may influence the lack of variability of the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in fingernails compared with hair and that the frequency of washing or wetting an individual's hands, which is greater than that of hair, may also play a role. The findings from this study serve as a starting point for investigating the use of  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of human fingernail tissues for travel history reconstruction and residency determination studies. Future studies involving more detailed sampling of traveling participants and their environment would be useful in determining the time that it takes to change one's isotope ratios and converge on a resident signal. In addition, studies characterizing the influence and extent of exogenous water inputs in fingernail  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios would provide researchers with a clearer understanding of the strontium source.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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