Forensic isoscapes based on intra-individual temporal variation of $\delta^{18}$O and $^{206}$Pb/$^{207}$Pb in human teeth

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**ABSTRACT**

Isotopic signatures used in the georeferencing of human remains are largely fixed by spatially distinct geologic and environmental processes. However, location-dependent temporal changes in these isotope ratios should also be considered when determining an individual’s provenance and/or trajectory. Distributions of the relevant isotopes can be impacted by predictable external factors such as climate change, delocalisation of food and water sources and changes in sources and uses of metals. Using Multi-Collector Inductively-Coupled Plasma Mass Spectrometer (MC-ICP-MS) analyses of $^{206}$Pb/$^{207}$Pb in tooth enamel and dentin from a population of 21±1-year-old individuals born circa 1984 and isotope ratio mass spectrometry (IRMS) of $d^{18}$O in their enamel, we examined the expected influence of some of these factors. The resulting adjustments to the geographic distribution of isotope ratios (isoscapes) found in tooth enamel and dentin may contain additional useful information for forensic identification, but the shifts in values can also impact the uncertainty and usefulness of identifications if they are not taken into account.

**KEY POINTS**

- Isoscapes of $^{206}$Pb/$^{207}$Pb and $d^{18}$O used for geolocation are not static.
- Within a few years, the enamel and dentin of a person may exhibit measurable differences in $^{206}$Pb/$^{207}$Pb even without changing locations.
- Changes in climatic patterns tied to rising temperatures are more significant than the direct effect of increasing temperature on $d^{18}$O fixed in tooth bioapatite.
- Third molar (M3) enamel mineralisation includes material incorporated from before formal amelogenesis takes place.

**Introduction**

Isotopic analyses of human remains have proven valuable for forensic identification when more selective methods (e.g. dental records, fingerprinting or DNA analyses) cannot be used [1]. These analyses include light elements (e.g. carbon, nitrogen and oxygen: C, N and O) whose isotopes are fractionated due to their mass differences [2] and heavy elements (e.g. strontium and lead: Sr and Pb) that exhibit variation due to radiogenic differences.

Isoscapes (geographic distributions of isotope ratios plotted as topographies) for O and Pb measured in modern human teeth from United States Air Force Academy (USAFA) cadets (obtained from teeth collected by Regan in 2005/6 [3]), were recently developed by Keller et al. [4] for the conterminous USA. Empirical equations that related $d^{18}$O ($d$ defined below) and $^{206}$Pb/$^{207}$Pb in the teeth to local surface water and aeolian dust proxies, respectively, were also developed [4]. Furthermore, their analyses of the inter- and intra-tooth and geolocation variations in that population for $d^{13}$C, $d^{18}$O, $^{206}$Pb/$^{207}$Pb and $^{87}$Sr/$^{86}$Sr found only $d^{18}$O and $^{206}$Pb/$^{207}$Pb exhibited differences large enough for useful geolocation of individual cadets whose natal origins were within North America.

Small variations attributed to regional differences in wheat versus corn consumption were found in $d^{13}$C, and although $^{87}$Sr/$^{86}$Sr was correlated with local tap waters, only about 20% of the variation could be attributed to those waters [4]. Sr is incorporated from food and water, so the poor link between $^{87}$Sr/$^{86}$Sr and location in modern people’s teeth is likely due to delocalisation of civic water.
supplies post 1900, increasing use of bottled water and the rapid globalisation of food sources after World War II [5–7]. Isotopes of O and Pb that are incorporated in bioapatite are largely distributed by atmospheric processes, so broad regional patterns emerge in their isoscapes. For elements like C and Sr that are incorporated from food and water sources, high resolution or localized isoscapes are necessary in order to obtain useful geolocation information. Therefore, this study focuses only on O and Pb in modern individuals. Crown enamel and mid-root dentin Pb concentration and 206Pb/207Pb are mapped, and the increasing use of recycled Pb on these is examined. Variations in $\delta^{18}$O due to climate fluctuations recorded in the enamel are also explored and expected differences between the enamel and dentin, structural carbonate $\delta^{18}$O, values are modelled.

Ratios for light isotopes such as $^{18}$O/$^{16}$O are usually measured versus a standard such as the Vienna Pee Dee Belemnite (VPDB) and expressed in delta ($\delta$) notation in parts per thousand:

$$\delta^{18}\text{O}_{\text{VPDB}} \text{ sample} = \left\{ \frac{\text{[^{18}O/^{16}O]_{\text{sample}}}}{\text{[^{18}O/^{16}O]_{\text{VPDB}}}} - 1 \right\} \times 1000^\circ/\circ,$$

(1)

Conversion between VPDB and Vienna Standard Mean Ocean Water (VSMOW) $\delta^{18}\text{O}_w$ values can be made with a relationship such as Coplen et al.’s [8]:

$$\delta^{18}\text{O}_w(\text{VSMOW}) = 1.03092 \times \delta^{18}\text{O}_w(\text{VPDB}) + 30.92^\circ/\circ,$$

(2)

Temporal variations in $\delta^{18}$O and $^{206}$Pb/$^{207}$Pb based on sediment or fossil records that span centuries or millennia have been published [4,9–12], but recently attention has begun to focus on temporal changes in a given location that can impact assumed geolocation trajectories recorded within individuals. Kennedy et al. [13] measured the monthly variation of $\delta^{18}$O in ground and surface drinking waters in 2005 and 2006 and used the data to construct isoscapes for drinking water in the conterminous USA. Spatiotemporal changes in $\delta^{18}$O are explored by examining the variation in $\delta^{18}$O for an additional population of USAFA cadets who changed locations at different times. Adjustments to the temporal changes are modelled using National Oceanic and Atmospheric Administration/National Centres for Environmental Information (NOAA/NCEI) data for multi-year surface temperature anomalies converted to $\delta^{18}$O values [22]. Temporal changes in the cadets’ Pb and O tooth isotope ratios are compared to isoscapes for the mean year (1996) for enamel formation to determine how important these changes are to geolocation trajectories.

The mineral portion of the bioapatite in tooth enamel is a carbonate-substituted hydroxyapatite (Ca$_{10}$(PO$_4$)$_6$ - $x$(OH)$_2$ - $y$)(CO$_3$)$_x$ + $y$ that comprises more than 95% by mass of the enamel, with less carbonate at the surface [23]. Trace amounts of Pb (primarily ingested from aeolian-distributed dust deposition [3,24]) substitute for Ca in the bioapatite formed during amelogenesis (enamel formation). Most M3 enamel mineralisation begins after age 8 and is complete by age 16. It tends to be later (ca. 0.5 years) in males [25,26], but this difference is negligible compared to the age range. Primary dentin formation in M3 also fixes Pb, but the root mineralizes from the crown and reaches maturity between age 18 and 25 [27,28]. While enamel has virtually no natural turnover once amelogenesis is complete, Pb turnover rates of about 1%–3%/year have been
measured in dentin [24,29,30]. Thus, differences between enamel and mid-root dentin found in M3 extracted in 2005/6 from circa 21-year-old individuals born circa 1984 and who resided in single locations, should be useful for measuring integrated changes in local isotope ratios during the 6-year interval between enamel (age 12 ± 2) and mid-root dentin (age 18 ± 2) mineralisation [27,28].

As noted above, predicted differences in the isotope ratios for O and Pb between enamel and dentin arise for different reasons. Oxygen’s isotopes are incorporated in humans primarily from imbibed drinking water and beverages [31]. Water is fractionated during both evaporation (lighter molecules escape) and precipitation (heavier molecules are removed). These are temperature dependent processes, and to a first approximation, higher elevations and higher latitudes are more depleted (more negative δ18O) in the heavier 18O isotope relative to VSMOW. Average surface temperatures in the conterminous USA have been rising about 0.025 °C/year since 1980 [22], and these increases should shift δ18O towards more 18O (less negative δ18O values) by about 0.5%/°C, although these shifts will be different in different locations in response to changes in factors such as wind direction, water source and precipitation temperatures [32].

While O is taken up from natural sources, Pb is incorporated primarily from anthropogenic sources. 204Pb is constant while 206Pb, 207Pb and 208Pb accumulate from the decay of 235U, 235U and 232Th, respectively. Thus, geological ores develop characteristic isotopic ratios over millions of years and humans incorporate Pb during environmental exposure. Values for 206Pb/207Pb found in batteries, bullets, pigments, solder and gasoline additives vary from 1.04 in Northern Australia to 1.47 for some Upper Mississippi Valley Type (MVT) ores [33,34].

Most people incorporate Pb from ingestion of Pb distributed via aeolian dust, as Pb from the combustion of gasoline and fumes released from smelters oxidizes in the atmosphere and is removed by wet and dry deposition. Most of this removal takes place within tens to hundreds of kilometres depending on factors such as wind speed, direction, the nature of the surface, the size of the particles formed and precipitation levels [35]. However, very small particles are also transported globally [36]. Crawling children who lick their hands directly ingest dust from old paint, aeolian dust, etc., while adults incorporate Pb more from smoking, water and food sources [37].

Natural processes mix Pb released to the environment, but mixing of Pb from different sources through industrial recycling of the metal is likely a more important process in the USA for individuals born circa 1984. Secondary (recycled) domestic Pb production was near 28% in 1928, 55% in 1985, 86% in 2005 and has been near 100% since 2013 when the last primary Pb smelter in the USA was closed [38]. This amounts to an average growth rate of 1.5% recycled Pb/year during the period of this study. While the proportion of Pb from secondary sources has been increasing, total production has also been increasing and during the 1990s, the relative amount from the MVT sources in Missouri grew [38], suggesting that Pb released to the environment, especially in the Midwest and around the Great Lakes (downwind from Missouri), should exhibit a small increase in 206Pb/207Pb values relative to declines in the rest of the country. These changes are of interest for developing more nuanced forensic isoscapes.

**Methods**

Before collecting and using samples from live subjects, appropriate permissions were obtained. (Documentation may be found in Regan [3].) Only M3 (crown completion circa 1996–1999; root completion 1996–2005) extracted during the winter of 2005/6 from USAFA cadets born in 1984 ± 1 (1 s) were used in this study. Two outliers born in 1979 were included in the Pb study for statistical comparison. Thus, dentin remodelling was negligible. Molars used to develop the isoscapes in this study were from individuals who self-reported living in a single location from their year of birth until after 2000. The sex and race/ethnicity demographic profile of the samples used for the isoscapes were based on self-identification: 80% male, 20% female, 88% White, 6% Hispanic (any race), 4.5% Black/African and 1.5% Asian [3].

Regan’s analyses of the molars’ enamel for 206Pb/207Pb (n = 23) and δ18Oc (n = 158) were described previously [3,4]. Most of these individuals remained in a fixed location between ages 8 and 18. Excluding those born before 1981 or who lived in multiple locations, left n = 90 individuals for developing a fixed-in-time, 1996, δ18Oc isoscape. An additional 28 individuals who relocated once before age 8 were also identified. Their deviations from isoscape predictions were used to test the δ18Oc isoscape. (Regan’s δ18Oc enamel data are reproduced in Table S1 of the Supporting Information.)

All teeth used in Regan’s [3,4] and this study were first cleaned of organic matter and sterilized by soaking the teeth in 3% H2O2 for 2 days. Any adherent periodontal tissue was also removed. After sonicating in 18 MΩ water for 30 min and subsequent drying, interior enamel powder was obtained using a carbide drill bit. The powders were subsequently treated with 30% H2O2 to remove organic
residues and dilute acetic acid to remove any secondary carbonates before analysis [3].

In Regan’s study, the enamel powder analysed for Pb was dissolved overnight in 8 mol/L HNO₃ (triply distilled) in Teflon vials and taken to dryness. Pb bromide was formed by dissolving the residue with 1 mol/L HBr (Optima) and separated in a class-1000 clean lab using Dowex 1X-8 micro-columns following the method of Manhes et al. [39]. Analyses of the Pb isotope ratios were conducted using a Nu-Plasma, Multi-Collector Inductively-Coupled Plasma Mass Spectrometer (MC-ICP-MS). The enamel’s structural carbonate δ₁⁸O_c was measured in the enamel powder using a VG/Micromass PRISM Series II isotope ratio mass spectrometer (IRMS) with 90°C H₂PO₄ in an automatic Isocarb preparation device. (The H₂PO₄ is used to convert the enamel carbonates to CO₂(g). Note that different acid temperatures can create measurable isotope fractionations [40].)

To develop more detailed Pb isoscapes and to measure the circa 6-year difference between the cadets’ crown enamel and mid-root, primary dentin, n = 43 additional M3 not analysed in the previous study were prepared and analysed for the enamel’s Pb concentration and ²⁰⁶Pb/²⁰⁷Pb using a procedure based on Keller et al. [4], outlined below. Because of funding limitations, only a subset of these (n = 16) were ultimately analysed for their dentin’s Pb concentration and Pb isotopes. These were selected to maximise the coverage of the conterminous USA so that at least regional differences might be detected. Molars analysed for Pb were also selected that replicated sample locations in Regan’s [3] investigation in order to cross-validate the studies. Both this study and Regan’s [3] included analyses of laboratory blanks and NIST standards.

In this study, tooth surfaces were cleaned with a carbide burr and sonication in 18 MΩ H₂O that removed any remaining surface contamination and tissues. Crown enamel and mid-root dentin samples (10–20 mg) were removed inside of virgin polypropylene (PP) bags to avoid contamination. Dentin and enamel were manually selected under magnification. Following the method in Bower et al. [41] and Keller et al. [4], these samples were dissolved overnight in 1.0 mL of 16 mol/L triply distilled HNO₃ in capped, virgin PP vials on an orbital shaker at 200 rpm. The solutions were diluted to 5.0 mL with 18 MΩ H₂O, 20 mg of Eichrom Pb resin was added and the mixture was placed on an orbital shaker at 200 rpm for 1 h. Cleaning of the resin-bound Pb was performed by capturing the resin in acid-washed and rinsed PTFE columns followed by 3.0 mL of 2.0 mol/L HNO₃. Extraction of the Pb was achieved with 3.0 mL 18 MΩ H₂O and 1.0 mL 0.050 mol/L ammonium oxalate (Sigma, ≤0.01% trace metals) collected in PP vials which had 1.0 mL of 2.0 mol/L HNO₃ added to avoid subsequent Pb precipitation.

The Pb solutions were analysed for their Pb concentrations using a Thermo-Fisher iCAP Q ICP-MS. The data were collected with an instrumental precision of about 5% and a detection limit of 0.03 ppb. Pb isotope ratios were obtained using a Nu-Plasma High Resolution MC-ICP-MS. A Tl spike used for mass-bias correction was added to each sample just before analysis [33,42] and sample bracketing with NIST-981 was used to correct for any instrument drift.

We predicted temperature fluctuations over time would create deviations from the measured 1996 δ₁⁸O_c isoscape. These ∆δ¹⁸O_c offsets before and after 1996 were modelled using historic records of annual temperature anomalies [22]. These were used to estimate the magnitude and sign that changing locations should have on an individual’s enamel δ¹⁸O_c. These temperature changes were also used to calculate the offset expected between the median time of crown formation (1996) and mid-root formation (2002) [28].

To create an isoscape of these differences, the Δ°C anomalies for the NOAA/NCEI US Climate Divisions were used to estimate corresponding ∆δ¹⁸O_c values by subtracting the Bayesian-weighted data for 1996 from the data for 2002 in each climate division [43] and then using Equations (3) and (4) (see below) to convert Δ°C to ∆δ¹⁸O_c(VPDB). For simplicity, the δ¹⁸O_w to δ¹⁸O_c conversions were assumed the same for enamel and dentin and the Bayesian weights were assumed to follow a 5-year square distribution centred on 1996 and 2002, respectively. Because mineralisation occurs in episodic cycles that span multiple years, a square distribution was thought to provide as realistic a weighting of the fluctuations as a Gaussian distribution. Only interior enamel was used for the analyses, narrowing the time range [15,28].

\[
\Delta\delta^{18}O_w(VPDB) = z \times \Delta^°C
\]

\[
\Delta\delta^{18}O_c(VPDB) = 0.47 \times \Delta\delta^{18}O_w(VPDB)
\]

The Rayleigh equation for isotope fractionation at equilibrium produces a curved relationship with smaller slopes at higher temperatures [44]. However, temperature increments (Δ°C) of a few degrees may be fit with a linear model for the dependence of δ¹⁸O_w on surface temperature. Values for a non-equilibrium z in Equation (3) depend on factors such as the type of precipitation (snow vs. rain), the temperature of the source water, the condensation temperature and how much of the original water vapour has already precipitated out. Using global
mid-latitude data, Fricke and O’Neil [45] obtained an \( z = 0.578 \) for the winter and 0.417 for the summer. Liu et al. [46] measured a winter \( z = 0.64 \) and a summer \( z = 0.38 \) for 1992 and 0.63 and 0.56, respectively, for 1993 for the USA. Dansgaard [32] gave a number of different values for \( z \) around 20 °C ranging from 0.24 to 0.78 \( \delta^{18}O^\text{air} / C_{14} \) for various conditions. Thus, +0.5 \( \delta^{18}O^\text{air} / C_{14} \) was used for estimating the responsibility of \( \delta^{18}O^\text{w}(\text{VSMOW}) \) to temperature in Equation (3), as our method of sampling the enamel and dentin included multiple years that average the \( z \) extremes. Fast-growing tissues, such as hair [13], give a better analysis of seasonal variations [47], but species differences in the secretion and maturation cycles in tooth formation are still being determined [48]. Equation (4) (obtained by rearranging Equation (5) in Keller et al. [4]) was then used to estimate the conversion of \( \Delta \delta^{18}O^\text{w}(\text{VSMOW}) \) to \( \Delta \delta^{18}O^\text{c}(\text{VPDB}) \).

The data were statistically analysed using Microsoft Excel 2016 and Minitab ver.18.1 (Minitab, Inc., State College, PA, USA). Isoscapes for the Pb and O data were plotted using ArcGIS version 10.4.1 with Spatial Analyst Extension (Esri, ArcGIS, Redlands, CA, USA). Interpolation between points used an inverse square of the distance for the weighting of points, and contours were extended to the USA boundaries and then clipped.

**Results and discussion**

The Pb concentrations and isotope ratios from the 43 individuals measured during this study are presented in Table 1. Data from the 23 individuals previously analysed by Regan [3] are presented in Table 2. Instrument errors and laboratory blanks for the \( ^{206}\text{Pb} / ^{207}\text{Pb} \) data (Table 1) were negligible for these common-Pb samples. Figure 1 shows the enamel-based Pb concentration isoscape obtained from the individuals with a known and consistent geolocation during amelogenesis and dentinogenesis. Although the pattern is somewhat similar to that expected for oxygen (see below), the highest concentrations of Pb are primarily found downwind in an easterly direction from historic Pb-emission sites (e.g. smelters, urban traffic and coal burning) rather than being coupled to Pb-mine sources.

The data in Figure 2 illustrate the change in the spatial variation of the concentration of incorporated Pb (a \( \Delta \text{Pb} \) map, obtained by subtracting the enamel Pb from the dentin Pb) in the conterminous USA. In combination with Figure 1, the data in Figure 2 suggest individuals in the southern states started with lower levels of Pb, but these levels are increasing faster than in the Northern Plains and Rocky Mountain States. Some of this increase may be due to increasing USA Pb production, which went up by 37 500 tonnes/year during the 1980s and 1990s, a 79% increase between 1984 and 2005 [38]. The 43 %± 11% higher average in the dentin versus the enamel is readily explained by this growth in production. Furthermore, more secondary Pb smelters are located in the southern and eastern states and more were relocated to northern Mexico, which had much lower emission standards. These emissions are carried by seasonal winds into the southeastern states [49]. Although blood lead levels (BLLs) have been declining in the USA overall, lower income areas with older infrastructure and homes with decaying Pb paint as well as higher population densities have not experienced the same amount of improvement [50].

Figure 3 is an isoscape of \( ^{206}\text{Pb} / ^{207}\text{Pb} \) in the USAFA cadets’ tooth enamel. (Other ratios, such as \( ^{208}\text{Pb} / ^{206}\text{Pb} \), produce isoscapes with similar patterns: not shown.) A ratio of 1.19 for \( ^{206}\text{Pb} / ^{207}\text{Pb} \) is typical of background levels in soils, while values above 1.20 are indicative of more recently formed Pb ores. Gasoline made from Upper Mississippi Valley Type Pb exhibits ratios above 1.20 [51]. Lead ores imported from South America and Mexico as well as Pb mined in Idaho tend to have \( ^{206}\text{Pb} / ^{207}\text{Pb} \) values below 1.18 [41], and this may be seen in the lower ratios found along the west coast and in southern and western Texas where these ores were historically smelted. Furthermore, trans-Pacific airborne dust may also contribute, as Australia has been a major supplier of Pb to East Asia, and important ore sources there have \( ^{206}\text{Pb} / ^{207}\text{Pb} \) values circa 1.04 [33].

In Figure 4, the differences (\( \Delta ^{206}\text{Pb} / ^{207}\text{Pb} \)) in \( ^{206}\text{Pb} / ^{207}\text{Pb} \) in the cadets’ enamel and dentin are plotted. Isotopes \( ^{204}\text{Pb} \), \( ^{206}\text{Pb} \), \( ^{207}\text{Pb} \) and \( ^{208}\text{Pb} \) are too heavy to be fractionated by metabolic processes, so changes in the ratios indicate changes in the Pb sources between circa 1996 and 2002. Although leaded gasoline was largely phased out between 1976 and 1996 [52], the layer of dust it left as a legacy remains a source of background Pb that is slowly being mineralized and buried. Differences downwind from primary ore smelters in eastern Nebraska, Missouri and western Texas are probably more important than the background gasoline signature, as these locations also fit with the expected high and low isotope ratios, respectively. This can be seen in the (dentin–enamel) isoscape shown in Figure 4. Although more samples are needed to fill in the \( \Delta ^{206}\text{Pb} / ^{207}\text{Pb} \) isoscape and more work can be done using bi-plots of isotopic ratios (e.g. \( ^{208}\text{Pb} / ^{204}\text{Pb} \) vs. \( ^{206}\text{Pb} / ^{207}\text{Pb} \)) to better isolate the sources of Pb in specific areas, the differences are in keeping with expectations from wind patterns and source mixing. Furthermore, both the concentration
The magnitude of the errors depend on the distribution and number of points used to construct the plots. Still, the Δ plots contain useful information. They show that the regional dentin profiles mimic the enamel profiles, but with some additional, time-related differences.

Data for δ18Oc (VPDB) measured by Regan [3] (used without conversion to δ18Oc (VSMOW) or correction for any method offsets) were used to construct Figure 5. Assuming an average time for mineralisation during amelogenesis of 12 years and a 1984 ± 1 birth year, Figure 5 is a plot of the δ18Oc incorporated into the crown enamel by these individuals ca. 1994 – 1998. Keller et al. [4] plotted the δ18Oc enamel data for a larger group of the USAFA cadets that included multiple birth years and a few who had changed locations. That enamel data were significantly correlated with the circa 1984 surface...
water data from Coplen and Kendall (Pearsonian $R^2 = 0.81, P < 0.0001, df = 154$) [43]. The $\delta^{18}O_{\text{c(VPDB)}}$ data for the essentially same-location, same-age individuals in Figure 5 are significantly correlated with Bowen et al.’s circa 2005 tap water data [53]: $\delta^{18}O_{\text{c(VPDB)}} = -4.54 \pm 0.25 \pm 0.345 \pm 0.028 \times (\delta^{18}O_{\text{tap(SMOW)}}); R^2 = 0.75, P < 0.001, df = 50, \pm 1SE)$. Gaps in sample locations between the tooth and tap water data as well as disparities in tap water sources (ground, surface or managed) and residence times (0 to 100 years) explain some of the differences in the isoscapes [13].

The predicted $\Delta \delta^{18}O_c$ isoscape for dentin versus enamel based on the NOAA/NCEI Climate Division temperature anomalies with Bayesian weighting is shown in Figure 6. It shows the predicted spatial distribution of the shifts in the $\delta^{18}O_c$ values ($\Delta \delta^{18}O_c$) between the cadets’ crown enamel and mid-root dentin. It assumed a constant $+0.5 \pm 0.1^\circ C$ for the conversion of $\Delta \delta^{18}O_c$ values and ignored factors explored by Kennedy et al. [13], such as decoupling of imbibed water from local surface waters.

Figure 6 represents paired, predicted $\Delta \delta^{18}O_c$ data for the same teeth at the same location. It is important to note that the $\Delta \delta^{18}O_c$ variation in Figure 6 for mid-root dentin versus enamel is due only to climatic fluctuations. The average year-to-year variations ($\pm 0.66^\circ C$) in temperature anomalies for the contiguous USA due to shifting climatic patterns over the time of this study are more important than the

### Table 2. Original enamel data from Regan [3] used with the data in Table 1 to construct the $^{206}\text{Pb}/^{207}\text{Pb}$ isoscape. The $^{206}\text{Pb}/^{207}\text{Pb}$ was obtained by dividing the $^{206}\text{Pb}/^{204}\text{Pb}$ data by the $^{207}\text{Pb}/^{204}\text{Pb}$ data.

| Identity | City          | State | 208/204  | 207/204  | 206/204 | Pb (ppm) |
|----------|---------------|-------|----------|----------|---------|----------|
| AFA-004  | Clarksville   | TN    | 38.4127  | 15.6477  | 18.8067 | 0.06     |
| AFA-006  | Laguna Niguel | CA    | 38.3241  | 15.6381  | 18.6474 | 0.04     |
| AFA-017  | Phoenix       | AZ    | 38.4275  | 15.6492  | 18.8189 | 0.09     |
| AFA-021  | Goodyear      | AZ    | 38.3369  | 15.6418  | 18.6627 | 0.11     |
| AFA-031  | Denver        | CO    | 38.3722  | 15.6484  | 18.8110 | 0.43     |
| AFA-032  | Sprucegrove   | Alberta | 38.2715 | 15.6369  | 18.6870 | 0.15     |
| AFA-051  | Avada         | CO    | 38.3673  | 15.6436  | 18.7709 | 0.14     |
| AFA-056  | Chaska        | MN    | 38.3927  | 15.6595  | 18.7845 | 0.02     |
| AFA-060  | Kearney       | NE    | 38.6165  | 15.6749  | 19.0493 | 0.08     |
| AFA-078  | Burlington    | VT    | 38.3427  | 15.6374  | 18.6884 | 0.09     |
| AFA-088  | Torrington    | CT    | 38.2804  | 15.6291  | 18.5967 | 0.17     |
| AFA-096  | Orlando       | FL    | 38.3107  | 15.6324  | 18.6480 | 0.24     |
| AFA-106  | Bland         | VA    | 38.3654  | 15.6432  | 18.7485 | 0.11     |
| AFA-103  | Golden        | CO    | 38.2570  | 15.6279  | 18.6099 | 0.32     |
| AFA-111  | Old Shasta    | CA    | 38.2731  | 15.6260  | 18.6253 | 0.11     |
| AFA-133  | Scottsboro    | AL    | 38.2865  | 15.6256  | 18.6444 | 0.05     |
| AFA-134  | Southaven     | MS    | 38.4202  | 15.6564  | 18.8606 | 0.05     |
| AFA-146  | Burley        | ID    | 38.2489  | 15.6265  | 18.5676 | 0.06     |
| AFA-163  | Houston       | TX    | 38.4059  | 15.6306  | 18.6732 | 0.12     |
| AFA-164  | Washington    | GA    | 38.3386  | 15.6357  | 18.7481 | 0.09     |
| AFA-173  | Vacaville     | CA    | 38.1825  | 15.6251  | 18.4710 | 0.14     |
| AFA-174  | Elizabethtown | KY    | 38.3703  | 15.6463  | 18.7275 | 0.2      |
| AFA-176  | Tallahassee   | FL    | 38.3703  | 15.6431  | 18.7920 | 0.09     |
| Blank    | NIST-981      |       | 36.695   | 15.490   | 16.937  | <0.01    |
|         | 981s          |       | 0.009    | 0.003    | 0.004   |          |
small change in $\delta^{18}O_c$ predicted by the 0.04°C/year rise in temperatures between 1980 and 2005, even with the 5-year integration intervals used for amelogenesis and dentinogenesis.

Using 5-year intervals for integrating the climatic anomalies gives a $\Delta$C for the 1996 to 2002 interval of $0.27 \pm 0.40 \, ^\circ\text{C}$ (1 s), which yields a $\Delta \delta^{18}O_c = 0.063 \pm 0.094\%/\text{o}$ (1 s) using Equations (3) and (4). This means the differences are insignificant compared to interpolation (ca. ±1.5%/o) and sampling (±0.5%/o) errors. However, if a large population of individuals are averaged together (decreasing the standard error), or if the 5-year integration interval is narrowed to a half year (e.g. to the seasonal variation recorded in scalp hair), the $\Delta$C contribution to $\delta^{18}O_c$ may become significant.

Relocating and temperature fluctuations are not expected to impact $\delta^{18}O_c$ values until after age 8 when M3 amelogenesis begins. Surprisingly, a significant relationship was found between the measured and estimated $\Delta \delta^{18}O_c$ values (Supporting Information Table S2) for the 26 cadets who had relocated by age 8: $\Delta \delta^{18}O_c = 0.18(\pm 0.13) + 0.076(\pm 0.025) \times \Delta \delta^{18}O_{oc} + 0.04(\pm 0.31) \times \Delta C \ (R^2 = 0.26; P = 0.019; df = 26; \pm 1\text{SE})$. This implies M3 mineralisation includes material incorporated or remobilised from before amelogenesis begins, blurring the tooth development times often used to calculate life trajectories. To a lesser extent due to its lower mobility, this also occurs with Pb [37]. Because the change in locations was significant, it may be that shifts in regional climatic patterns may also be significant, although it is expected to be a much smaller effect. The complex modelling required to separate these variables, given the multiple natal and relocation years, locations and climatic patterns, was not justified for the limited data set available for this study.

To put the magnitude of the predicted $\Delta \delta^{18}O_c$ isoscape for the USAFA cadet population in Figure 6 in perspective, recently Chesson et al. [40]
conducted matched analyses of 30 of the USAFA cadet teeth and found almost constant offsets of up to 0.05%/year (relative to their range of 0.02%−0.05%/year (relative to their range of values), possibly due to a redistribution of dust globally as well as the increasing production of recycled Pb in secondary smelters. Tooth bioapatite tracks these environmental changes.

Lastly, the Δ plots, when coupled with the primary isoscapes, can improve the usefulness of the isoscapes, especially for fast-growing tissues. They may also shed light on the source of any measured differences in tissues formed at different times.

Finally, the Δ isoscapes demonstrate that O and Pb bioapatite isoscapes are not static, as climatic patterns, changes in metal sources, urbanisation of water sources and globalisation of food sources are tracked in the plotted values. This is apparent in the average decrease (ca. 0.1% of the range over the 6-year interval) found for the 206Pb/207Pb values in the USAFA cadet population compared to a similar increase found in the 1990s for crown enamel versus mid-root dentine in teeth from Scotland by Farmer et al. [54]. The difference in direction of the trends is likely due to Pb with 206Pb/207Pb = 1.04 from Australia being used in the UK versus 206Pb/207Pb = 1.22 from Missouri being used in the USA. Both are trending towards the world average (ca. 1.19 [11]). While these Δ isoscapes are small compared to the geolocation variation, they provide insight into the stasis and uncertainties of these isotope ratios as well as additional forensic information that may be helpful with geolocation of individuals.

Conclusion

Firstly, the Δ plots of Pb highlight spatial changes in Pb over time. In addition to their use for determining life trajectories, they are useful for seeing the impact of environmental remediation and changes in Pb exposure in different region of the USA.

Secondly, a combination of isoscapes can help detect outliers that sometimes arise in one, but not multiple, isoscapes. They also provide additional information that may be used for narrowing the geolocation of individuals or the sourcing of contaminants.

Thirdly, the Pb and O tooth bioapatite isoscapes are not static. Based on long-term data in Keller et al. [4], environmental Pb isoscapes have been exponentially trending to global means in the USA at a rate of about 0.02%−0.05%/year (relative to their range of values), possibly due to a redistribution of dust globally as well as the increasing production of recycled Pb in secondary smelters. Tooth bioapatite tracks these environmental changes.

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Authors’ contributions

Nathan W. Bower, Samuel J. Brown and Laura A. Regan conceived the project, prepared samples, conducted measurements and drafted the paper; Gideon E. Bartov and Craig C. Lundstrom were responsible for the new concentration and lead isotope measurements, respectively; Matthew D. Cooney was responsible for the GIS analyses and isoscapes. All authors contributed to the final text and approved it.

Compliance with ethical standards

The protocol was first approved (July 22, 2005) by the US Department of the Air Force (Protocol FAC2005026H). Subsequently (October 11, 2005), the Institutional Review Board (IRB) at the University of Florida, Gainesville, FL,
USA approved the research (document IRB #474-2005), as did the US Department of Veteran’s Affairs (October 19, 2005, VA#0001).

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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