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Research paper

High-resolution serial sampling for nitrogen stable isotope analysis of archaeological mammal teeth

Eric J. Guiry*, Joseph C. Hepburn, Michael P. Richards

Department of Anthropology, University of British Columbia, Vancouver, BC V6T 1Z1, Canada

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ABSTRACT

We present the results of an archaeological application of a rapid method for high-resolution stable nitrogen isotope ($\delta^{15}N$) measurements of time-series samples of tooth dentine. Over 250 analyses of samples of untreated dentine powder taken at continuous millimeter intervals along the growth axis of archaeological pig tusks were compared to results from a subset of tandem $\delta^{15}N$ measurements of extracted and purified tooth collagen from the same teeth. Samples were also taken at 0.25 mm depth intervals to test the effect of depth with respect to temporal resolution of diet. Results show that $\delta^{15}N$ measurements of untreated dentine powder from well-preserved archaeological teeth provide: 1) broadly comparable $\delta^{15}N$ values to extracted and purified collagen, and 2) a rapid method of assessing dietary change over much shorter time intervals than is possible using extracted collagen. Analyses also show that large changes in $\delta^{15}N$ values can occur across the thickness of a tooth due to the inclusion of multiple growth layers and/or secondary dentine, which results in a significant time–averaging lag in dietary representation, as demonstrated by samples that analyze collagen from the full width of the tooth wall. This method will also be useful for initial prescreening of samples to select for specimens of interest before undertaking further, more rigorous, sample pre–treatment and measurement.

1. Introduction

In recent years there has been a significant increase in the use and development of stable carbon and nitrogen isotope analyses of tooth dentine as a means of investigating archaeological human diet at the level of the individual (e.g., Henderson et al., 2014; Makarewicz, 2014; Sandberg et al., 2014). This approach can provide diachronic insights into diet and mobility that address questions about changing resources regimes and weaning age patterns (e.g., Fuller et al., 2003; Fahy et al., 2014).

This paper presents the first archaeological application of a rapid and inexpensive method that is used in other fields (e.g. marine ecology, Ambrose et al., 2013; Borrell et al., 2013; Knox et al., 2014; and primatology, Fahy et al., 2014) for high-resolution stable nitrogen isotope ($\delta^{15}N$) measurements of time-series samples of modern mammal tooth dentine. We measured over 250 individual samples of untreated (i.e., not demineralized) dentine powder taken at continuous millimeter increments along the growth axes of archaeological pig tusks and compared these data to results from tandem $\delta^{15}N$ measurements of extracted and purified tooth collagen from the same teeth. We also compared a subset of samples taken at 0.25 mm depth intervals at the same locations on one tooth to test the effect of depth with respect to temporal resolution of diet in tooth dentine $\delta^{15}N$ values. This study demonstrates the archaeological potential of this high-resolution standalone method for showing seasonal dietary differences, and also as a method to initially screen samples for further more-rigorous sample pretreatment and measurement. While we test this method on pig teeth, as a simplified model, the technique should also be useful for time-series analyses of teeth from humans and other animals.

2. Stable isotope theory and tooth formation processes

Several reviews of stable carbon and nitrogen isotope ecology for archaeological analyses have been published (e.g., Lee-Thorp, 2008). This section briefly reviews factors of stable isotope theory and dentine formation processes that are relevant to this study. Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values are expressed in ‰ relative to VPDB and AIR standards, respectively. The $\delta^{13}C$ values of plants broadly differ based on: 1) whether a C_3 or C_4 photosynthetic pathway is used to fix carbon (O'Leary, 1981,

* Corresponding author.
E-mail address: eguriy@mun.ca (E.J. Guiry).

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1988), and 2) the marine versus terrestrial atmospheric origin of carbon that is used (Chisholm et al., 1982). Because $\delta^{13}C$ values do not change significantly as they are passed up the food chain they are useful for distinguishing between diets based on different kinds of plants and plant communities (DeNiro and Epstein, 1978) as well as between diets focusing on marine as opposed to terrestrial foods (Chisholm et al., 1982). Unlike $\delta^{13}C$, $\delta^{15}N$ values undergo a large change between different trophic levels in a food chain, becoming enriched in ^{15}N by roughly 3–5‰ at each ascending interval (DeNiro and Epstein, 1981). As marine ecosystems tend to have longer foods chains than terrestrial environments, this relationship makes $\delta^{15}N$ values a useful indicator of high trophic level marine dietary intake (Schoeninger et al., 1983). It is also important to note that there is a variety environmental and anthropogenic variables which can influence $\delta^{15}N$ isotope soil biogeochemistry in pant-soil systems at the base of a given food web (see Szpak, 2014 for review). Collagen forms the main organic component in bone and tooth dentine and has isotopic signatures that primarily reflect dietary protein intake (Ambrose and Norr, 1993; Tieszen and Fagre, 1993). In contrast to bone, which remodels over a lifetime, primary dentine is not thought to undergo turnover after it is laid down (Gage et al., 1989) and therefore preserves a record of isotopic dietary values over the course of its formation. Mammal dentine begins formation at the crown and growth generally proceeds in obliquely angled increments at a gradual pace down to the root where tooth formation is completed (for review see Hillson, 2005).

3. Overview of tooth and bone time-series analyses in archaeology and ecology

Recent publication trends show that isotope time-series analyses involving tooth dentine are becoming an important contributor to archaeological research (Fig. 1). There has been long-standing archaeological interest in obtaining dietary information for different periods of individual lives (e.g., Sealy et al., 1993). Such diachronic, intra-individual data provide an important means of addressing more-detailed questions about human and animal biology such as weaning age (e.g., Burt and Garvie-Lok, 2013; Fuller et al., 2003) as well as the interplay between individual life-histories and wider social and economic processes (e.g., Cox and Sealy, 1997; Szpak et al., 2014). Analyses of incrementally forming tissues such as hair and nails, when available, provide ideal sequential isotopic archives for human and animal diets (e.g., Fuller et al., 2006; Szpak et al., 2014). The most commonly preserved tissues in archaeological contexts are skeletons and, for this reason, most archaeological analyses have focused on bones and teeth. Fig. 2 provides an overview of methods for dentine time-series analyses in archaeology, ecology, and paleontology. The simplest approach for obtaining diachronic data at the intra-individual level has been analyses of bone materials with differing turnover rates (e.g., Cox and Sealy, 1997; Sealy et al., 1993; Sealy et al., 1995; White, 1993). These studies compare dietary values from collagen extracted from dense long bones (e.g., femora) and less robust bones (e.g., ribs) as representatives of relatively long- and short-term diets. Early archaeological and paleontological studies (Cox and Sealy, 1997; Drucker et al., 2001; Sealy et al., 1993; Sealy et al., 1995) moved this work a step further by comparing collagen stable isotope values from bones with those of whole teeth and reasoning that because dentine does not remodel after it is laid down, it will capture a record of an earlier time in the individual's life. A logical next step was to analyze collagen from a sequence of teeth formed at differing times in the life of an individual (e.g., Bocherens et al., 1994, 1995, 1997; Richards et al., 2002; Wright and Schwarcz, 1999). In subsequent work, researchers began to take multiple samples in series along the growth axis of an individual tooth to track diet change at a finer scale (e.g., Balasse and Tresset, 2002; Balasse et al., 2001; Koch et al., 1995; Fuller et al., 2003; Walker and Macko, 1999). These analyses usually proceed by partitioning a tooth at regular intervals either before or after it has been demineralized and have been widely used in archaeology, (e.g., Beaumont et al., 2013; Mongomery et al., 2013), paleontology (Fisher et al., 2014; Metcalfe et al., 2010; Rountrey et al., 2007), and ecology (Mendes et al. 2007a,b; Knoff et al., 2008; Newsome et al., 2009). More recent analyses in archaeology and especially ecology have further developed this serial-sectioning technique by taking into account histological observations during sampling (e.g., Ambrose et al., 2013; Burt and Garvie Lok, 2013; Elorriaga-Verplancken et al., 2013; Hanson et al., 2009; Newsome et al., 2006, 2007).

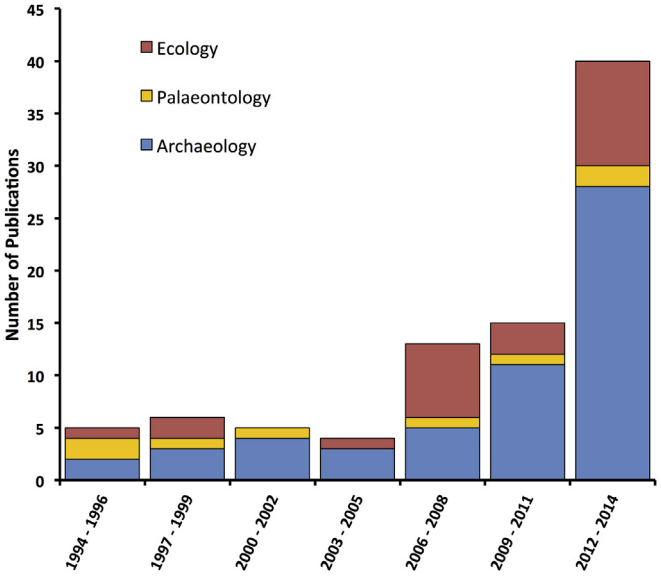

Fig. 1. Publication date versus number of papers (n = 90) that use intra-individual time-series information from dentine. These data were gathered through a systematic key word search ("dentine", "isotope", and "nitrogen") of over 150 archaeology, biology, and paleontology journals.

Despite these advances, archaeological studies (Fig. 1, 1994 to present, $n = 55$) have invariably focused on materials with relatively course temporal resolution and, for this reason, there remains significant potential for improvements in temporal control of dietary data. In particular, commonly used methods analyze collagen that has been extracted from dentine that spans multiple growth layer increments (e.g., Eerkens et al., 2011; Montgomery et al., 2013). This is due, in part, to the irregular contour followed by growth layer increments during the formation processes of most teeth. While it would be ideal to collect material only from individual growth layer increments, for most archaeologically relevant species (including humans), practical concerns with respect to tooth histology (i.e., a lack of visible annuli) and, more importantly, the need to collect sufficient sample material (usually at least 50–100 mg) have prevented previous archaeological work for tackling this issue.

High-resolution analyses have, however, been established in the field of ecology for almost two decades. Hobson and Sease (1998) conducted the first in-depth $\delta^{15}N$ measurements on untreated dentine samples taken from between annulated growth features in seal teeth. They were able to overcome the issue of sample size by omitting collagen extraction procedures and, instead, directly analyzing untreated dentine powder samples. They reasoned that, because the primary nitrogen-bearing material in tooth dentine