

Oral Histories: a simple method of assigning chronological age to isotopic values from human dentine collagen.

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Key terms: carbon and nitrogen isotope ratios, palaeodiet, incremental analysis, age assignment

Abstract:

Background: stable isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) in bone and dentine collagen have been used for over 30 years to estimate palaeodiet, subsistence strategy, breastfeeding duration and migration within burial populations. Recent developments in dentine microsampling allow improved temporal resolution for dietary patterns.

Aim: We propose a simple method which could be applied to human teeth to estimate chronological age represented by dentine microsamples in the direction of tooth growth, allowing comparison of dietary patterns between individuals and populations. The method is tested using profiles from permanent and deciduous teeth of two individuals.

Subjects and methods: using a diagrammatic representation of dentine development by approximate age for each human tooth (based on the Queen Mary University of London Atlas) (AlQahtani et al., 2010), we estimate the age represented by each dentine section. Two case studies are shown: comparison of M1 and M2 from a 19th century individual from London, England, and identification of an unknown tooth from an Iron Age female adult from Scotland.

Results and conclusions: The isotopic profiles demonstrate that variations in consecutively-forming teeth can be aligned using this method to extend the dietary history of an individual, or identify an unknown tooth by matching the profiles.

Introduction

The stable isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) in bone and dentine collagen have been used for over 30 years to estimate human palaeodiet. Lee-Thorp

(2008) summarised the pathway research has taken over the preceding 30 years leading to the established methods and interpretations of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in studies of human nutrition in the past. The advantage of analysing the skeletal and dental remains of humans is that “it reflects the foods actually eaten by an individual, or group of individuals” (Lee-Thorp, 2008). The method relies on fractionation of the isotopes, due to their different masses, during the incorporation of food into the body tissues: that means that values will rise by approximately 2-6‰ ($\delta^{15}\text{N}$) and 1-2‰ ($\delta^{13}\text{C}$) (Schoeninger and de Niro, 1984; Sealy et al., 1987) at each trophic level from the food ingested to the collagen and apatite of bone and tooth. By comparison with contemporary faunal bone collagen, and in the context of period and geographical location, we can estimate the diet, subsistence strategy and even migration of individuals in a given burial population. In particular the duration of breastfeeding and weaning has been investigated using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ from juvenile bone and dentine collagen tissues because there is an apparent trophic level shift in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ from mother to infant tissues while exclusively breastfed by the mother. This information can be used to determine the potential effect of prolonged breastfeeding on birth spacing and both maternal and infant health, as well as an indicator of cultural behaviour (e.g. Fuller et al., 2003; Jay et al., 2004; Howcroft, 2012). It is important to note that the changes in the nitrogen isotope ratios ($\delta^{15}\text{N}$) can reflect not only the diet of an individual, but also short-term changes due to growth, disease and nutritional stress: these have been demonstrated in modern individuals using the sequentially-forming tissues, hair and fingernail (e.g. Fuller et al., 2005, Mekota et al., 2006). The $\delta^{15}\text{N}$ values can rise during stress as a result of the recycling of body tissues (catabolism: Hatch 2006) which mimics the dietary trophic level shift. The

$\delta^{15}\text{N}$ values can also fall during growth as less nitrogen is excreted (anabolism: Waters-Rist and Katzenberg, 2010),

Recent developments in microsampling of dentine have allowed an improvement in the temporal resolution for infant, childhood and adolescent dietary patterns for an individual. Although the methods of sampling vary, it has been possible to estimate breastfeeding patterns (e.g. Eerkens et al., 2011; Henderson et al., 2014) and migration during childhood between geographical areas with differing diets (Beaumont et al., 2013). Beaumont et al., (in press) have also suggested the use of the earliest-forming dentine in deciduous teeth as a proxy for maternal $\delta^{15}\text{N}$ values of the mother during pregnancy, and hence a measure of the balance between healthy growth and nutritional stress in the mother. However, estimating the age which is represented by each increment of dentine, allowing for natural variation in developmental ages in a population, is an important factor: some of the increments taken from a deciduous tooth may represent as little as three months of life (Beaumont et al., 2014).

Here we present a diagrammatic representation designed to show the approximate age at which the development of dentine begins and ends in each human tooth, incorporating the age of crown completion. This allows the targeting of a particular period of life by choosing an appropriate tooth, or the age at death where a tooth is still forming. The assumption has been made that dentine develops at a regular rate throughout the formation of the tooth. Although it has been shown that there is some reduction in the rate of growth of the root length in the coronal and apical areas (Dean and Cole, 2013) the rate of tooth and root dentine secretion in a permanent tooth is relatively consistent at 4–6 mm per day throughout the permanent teeth (Dean and Scandrett, 1995). Because the sampling method takes regular-sized

sections along the direction of root growth there is some averaging as a result of including tissue formed at different times (see Beaumont et al., 2013, for a detailed discussion), thus the error introduced by the differing speed of dentine growth is not significant.

The diagrams produced for this study are based on the timings of tooth formation described in the Queen Mary University of London (QMUL) London atlas (AlQuahtani et al., 2010). In their review in 2009, Cunha et al. demonstrated the range of methods used to estimate age in juveniles, and the lack of standardization across forensic disciplines. It was decided that the London Atlas was an open-access resource which has been shown to be more accurate in modern individuals than two other widely-applied dental age estimation methods (AlQuahtani et al., 2014), allowing the proposed method in this paper to remain simple to understand and apply. It has been established that the average age at which human teeth develop is extremely similar regardless of period, geography or heritage, and is not affected significantly by the health or nutritional status of the individual (Cameriere et al., 2007; Dean et al., 2014; Elamin and Liversidge., 2014). The aim is to assign a putative chronological age to each tooth section using the same parameters from the London Atlas for each individual. Although it is well-known that there is a range of actual ages at which the stages of tooth formation are reached (AlQuahtani et al., 2010; Smith, 1991) and the use of median ages will introduce some errors (e.g. apparent differences in the known time for the three molar crowns to form: Reid and Dean 2006) using the same method for each tooth allows the comparison of the dietary and physiological values for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ at the same developmental stage in each individual. Until it is easier to sample smaller sections of tooth reliably in the

same direction as the incremental structures in the dentine, this is a pragmatic way of using the information which is currently available.

The application of this simple method is demonstrated by two case studies: comparison of dentine isotope profiles of a first permanent molar (M1) and second permanent molar (M2) from the same individual from 19th Century Lukin Street, London (Miles, 2013), and the evaluation of an in-situ tooth which was identified as either a retained deciduous or supernumerary tooth from an Iron Age female adult from High Pasture Cave, Scotland (Birch and Wildgoose, 2010).

Methods

A table was produced from the London atlas for each tooth type on the left side of the mouth showing the age at which they start to form, the age at which the crown is complete, and the apex complete (Table I). The London atlas quotes the ages for the mid-point of each stage. While there is little increase in the length of a root after the stage of root completion (Rc) the stage of apex closure (Ac) was used to ensure that the isotopic information obtained will include the whole period of life in which the tooth was forming.

An outline drawing was then produced for each individual human tooth showing the dentine from cusp/incisal tip to root apex, and coronal enamel as an outline over this.

A grid was used to represent age in years, and the drawing of each tooth was placed so that the earliest forming dentine is at the age at which the tooth starts to form (crown initiation Ci on the QMUL atlas). The drawing was then adjusted so that the most apical edge of the enamel outline coincides with the age at crown completion

(Crc on the QMUL atlas) and the apex of the root coincides with the age at which the apex is completed (Ac on the QMUL atlas) (e.g. Figure 1).

The completed drawing for each permanent tooth was then added to a diagram to show the relative ages of development for each tooth in the upper or lower left quadrant (Figure 2). The age of development for deciduous teeth is similar for maxilla and mandible, so only upper left quadrant drawings were produced (Figures 3 and 4). The validity of the diagrams was checked by comparing the median ages from the London Atlas with the age at which Rt1/2 appears on the diagram.

In order to assign an approximate chronological age for each tooth studied, the following method is used: as regular-sized sections (increments) have been taken from the tooth (Beaumont et al., 2013) it is assumed that each will represent the same period of growth. Thus the most coronal increment will represent the same age as the mid-point for Ci on the QMUL atlas. The same assumption is made for the most apical section, Ac. The time taken for the tooth to develop is the age at Ac minus the age at Ci. For example, the age at which the upper permanent canine starts to form is 0.6 years, the age at root completion is 14.5 years. The length of time the tooth takes to form is $14.5 - 0.6 = 13.9$ years. If this is then divided by the number of increments, say 10, the first increment will be assigned the age of 0.6 years, the second $0.6 + 1.39$ years, and so on, with the final increment being 14.5 years. If irregular increments have been used, it would be possible to vary the calculation to take this into account.

Results

Figure 5 shows the carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) dentine collagen profiles produced from the M1 and M2 teeth from the same individual from Lukin Street,

London, England (LUK 47). It demonstrates that where teeth are forming at the same time of life, by assigning an approximate chronological age to each section of each tooth using this method, the isotope ratio profiles show the same variations at the same age. This plot and the isotope data for the samples from LUK 47 were originally published in Beaumont et al. (2013).

Figure 6 is a photograph of the loose tooth (HPCS 100) recovered during the excavation of the Iron Age female mid-adult from the site of High Pasture Cave, Skye, Scotland. This was originally described as a retained deciduous canine tooth, and the crown removed for other isotopic analyses prior to the incremental sampling.

Figure 7 shows the $\delta^{15}\text{N}$ profiles for incremental sections of dentine collagen for both the loose tooth and M1 (HPCS 101) from the High Pasture Cave individual with approximate age in years on the x-axis, from data in Table II. The upper plot shows the M1 and the unknown tooth aged as though it was a deciduous canine. In the lower plot, the tooth is aged as a supernumerary permanent incisor. It can be seen that the plots for the $\delta^{15}\text{N}$ profiles against age for the two teeth are an extremely good match when the HPCS 100 tooth sections are assigned the chronological ages for a permanent supernumerary. There is sufficient variation in the values within the profiles to assume that this is a real dietary profile for both teeth from the same individual. This has allowed the identification of HPCS 100 as a permanent supernumerary tooth from this individual, rather than a retained deciduous tooth.

No dietary interpretations of the profiles have been included in this study.

Discussion

The diagrams and method proposed for the assignment of chronological ages to the sections of dentine taken from human teeth have been shown to allow the matching of co-forming teeth from the same individual. Whilst we accept that each individual will have their own biological clock, this means that we could potentially produce a complete lifeway for the diet of an individual from birth to the age of completion of the latest forming tooth which is present. If the individual dies during the formation of a tooth, isotope values at the time of death can also be established. Within a population, there may be variations in the timing of the growth of the dentition between individuals, but comparisons of the diet at similar developmental ages can still be made.

The diagrams can also help with the choice of a tooth to investigate a particular period of life. For example, if we wish to investigate whether there is a change in diet at a particular age, we can clearly see from the diagram which teeth will be forming at that period of life. This could be of use to researchers of childhood versus adult diet, breastfeeding and weaning, or changes in status at a known age such as marriage, entering a holy order, or to investigate migration between two areas with different dietary behaviour. One or more teeth may span the age at which the change has happened and the choice may then depend on availability and preservation of the teeth.

Conclusions

With the current interest in the analysis of incremental dentine, it would be of benefit if all researchers were to adopt a common method of chronological aging. The method proposed here is based on the use of simple and well-established parameters for the growth of the dentine in human teeth, and while it is accepted that

there are errors introduced by choosing the London Atlas as the source of aging data, these would be the same for all individuals. It is hoped that these charts will be of use for those who wish to compare their results with those of other researchers investigating varying periods and populations, and if a better method is developed in the future, this could then be applied to the same datasets.

Acknowledgements

The authors would like to thank Museum of London Archaeology and Steven Birch and Martin Wildgoose for access to the samples shown in this study. Grateful thanks to Jacqueline Towers and Andy Gledhill, University of Bradford for their advice and comments on the diagrams. We are indebted to the helpful comments of the reviewers.

Declaration of interest

The authors report no declarations of interest.

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Tooth	Age at initiation (in years)	Age at crown complete (in years)	Age at apex complete (in years)
U1	*0.5	4.5	9.5
U2	*0.9	5.5	10.5
U3	*0.6	5.5	14.5
U4	2.5	6.5	13.5
U5	3.5	6.5	14.5
U6	*0.3	3.5	9.5
U7	2.5	8.5	15.5
U8	8.5	14.5	21-23
L1	*0.6	3.5	8.5
L2	*0.6	3.5	8.5
L3	*0.9	5.5	13.0
L4	2.5	6.5	13.5
L5	2.5	6.5	14.5
L6	*0.3	3.5	10.0
L7	2.5	8.5	15.5
L8	8.5	14.5	22-23
DI 1	# -0.3	# 0.25	2.5
DI 2	# -0.3	# 0.3	2.5
DC	# -0.2	# 0.6	3.5
DM1	# -0.2	# 0.9	3.5
DM2	# -0.2	# 0.9	3.5

Table I: Age in years for Crown initiation (Ci), crown complete (Cc) and apex complete (Ac) for all human tooth types after AlQahtani et al. (2010). All values are approximate age in years $\pm$ 6 months except * which denotes $\pm$ 1.5 months and # denotes $\pm$ 0.5 month as quoted in the London Atlas.

Tooth	Age at initiation (in years)	Age at crown complete (in years)	Age at apex complete (in years)
U1	*0.5	4.5	9.5
U2	*0.9	5.5	10.5
U3	*0.6	5.5	14.5
U4	2.5	6.5	13.5

U5	3.5	6.5	14.5
U6	*0.3	3.5	9.5
U7	2.5	8.5	15.5
U8	8.5	14.5	21-23
L1	*0.6	3.5	8.5
L2	*0.6	3.5	8.5
L3	*0.9	5.5	13.0
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L5	2.5	6.5	14.5
L6	*0.3	3.5	10.0
L7	2.5	8.5	15.5
L8	8.5	14.5	22-23
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DI 2	# -0.3	# 0.3	2.5
DC	# -0.2	# 0.6	3.5
DM1	# -0.2	# 0.9	3.5
DM2	# -0.2	# 0.9	3.5

Table II: Carbon and nitrogen stable isotope ratio data and quality parameters for incremental dentine sections from teeth HPCS 100 and HPCS 101 from Iron Age mid-adult female individual from High Pasture Cave, Skye, Scotland. Approximate ages have been assigned to each increment using data for a retained deciduous canine and a permanent supernumerary incisor for HPCS 100, and for a first permanent molar for HPCS 101.

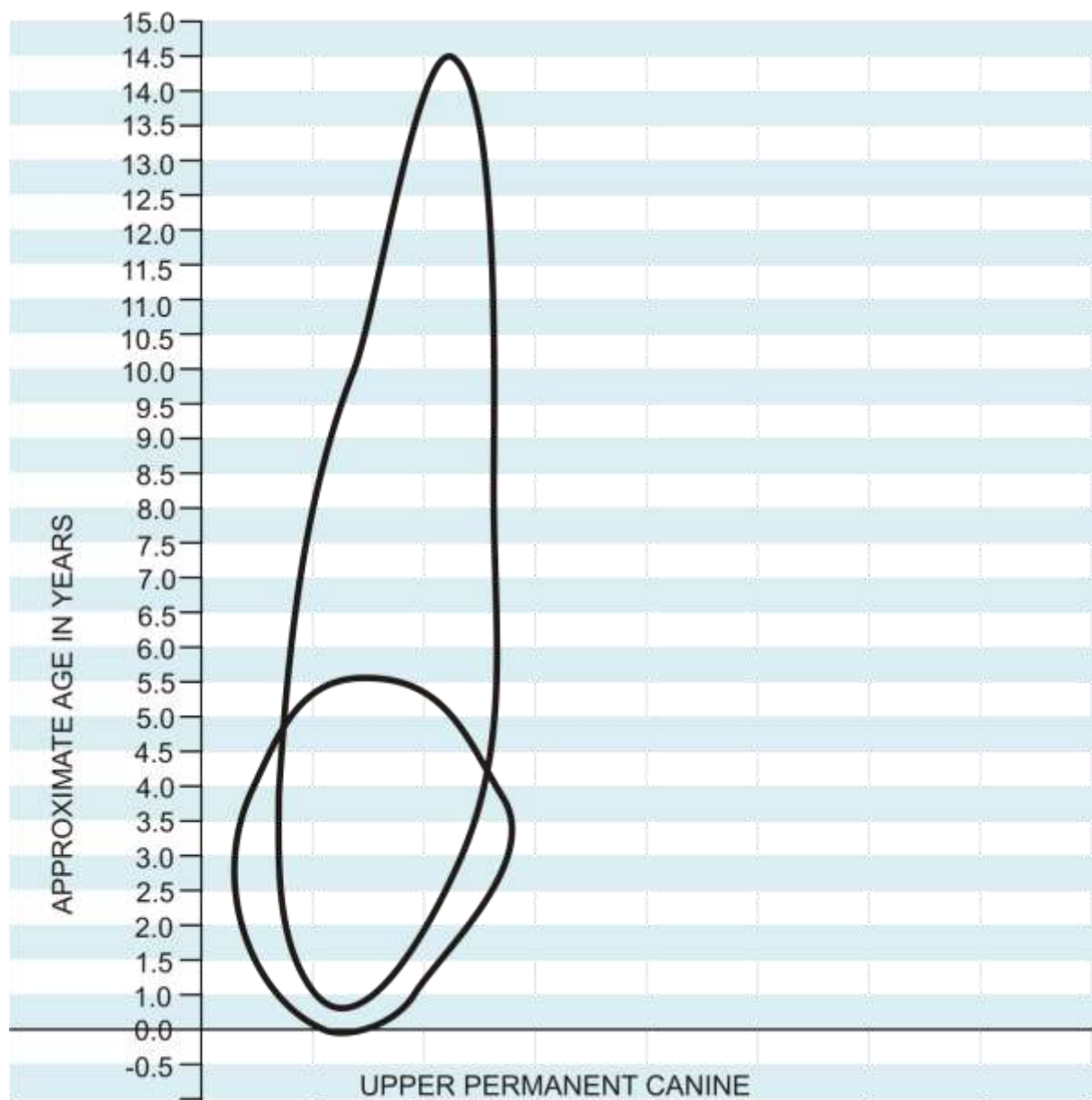


Figure 1: diagram to show the ages at which dentine development reaches Ci, Cc and Ac for an upper permanent human canine tooth. Ages taken from Table I



Figure 2: diagram to show the comparative developmental ages for all permanent upper tooth types. Ages taken from Table I

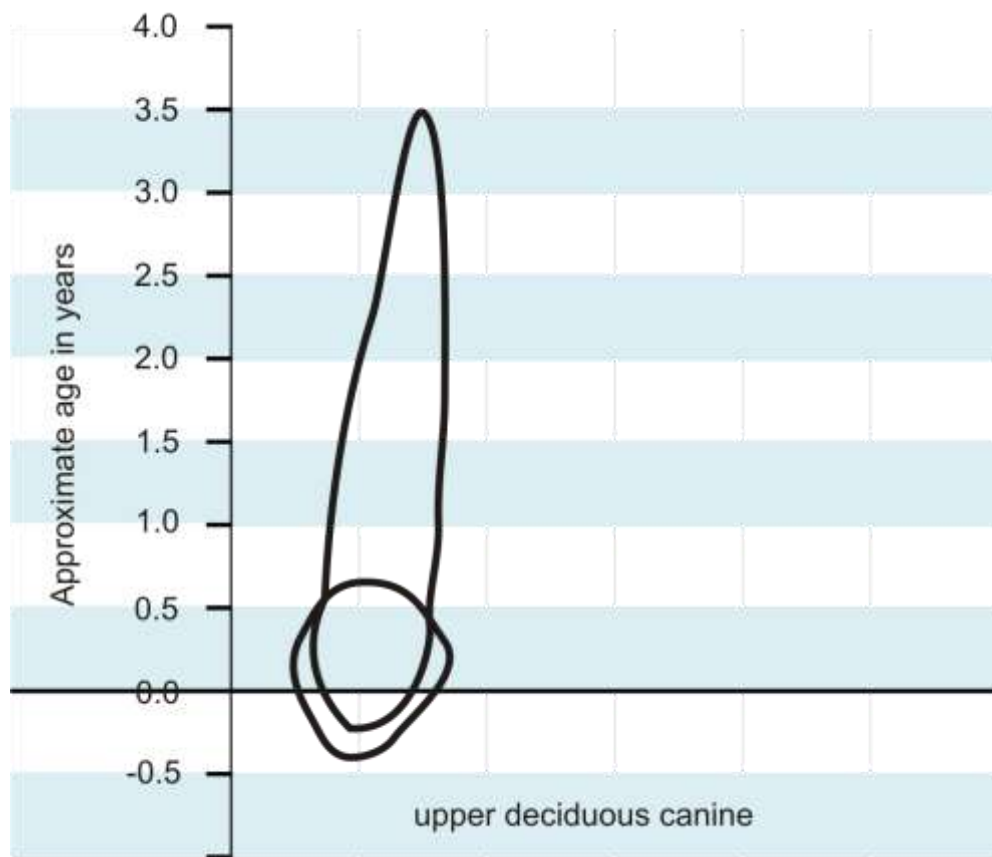


Figure 3: diagram to show the ages at which dentine development reaches Ci, Cc and Ac for a deciduous human canine tooth. Ages taken from Table I

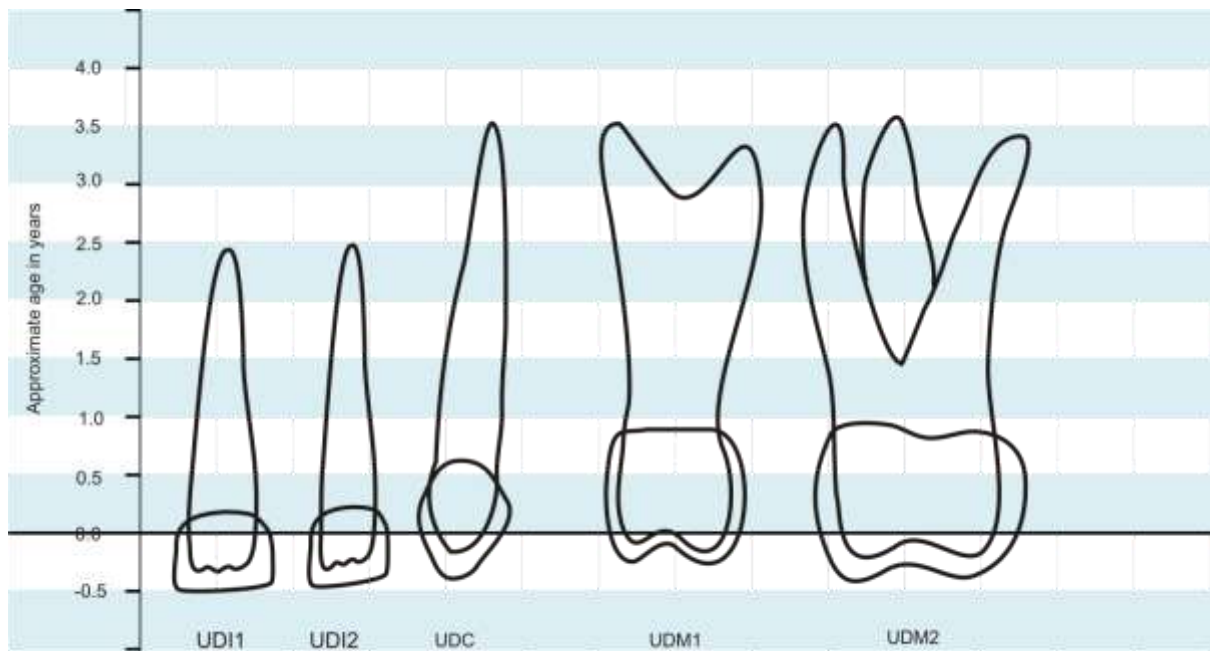


Figure 4: diagram to show the comparative developmental ages for all deciduous tooth types. Ages taken from Table I

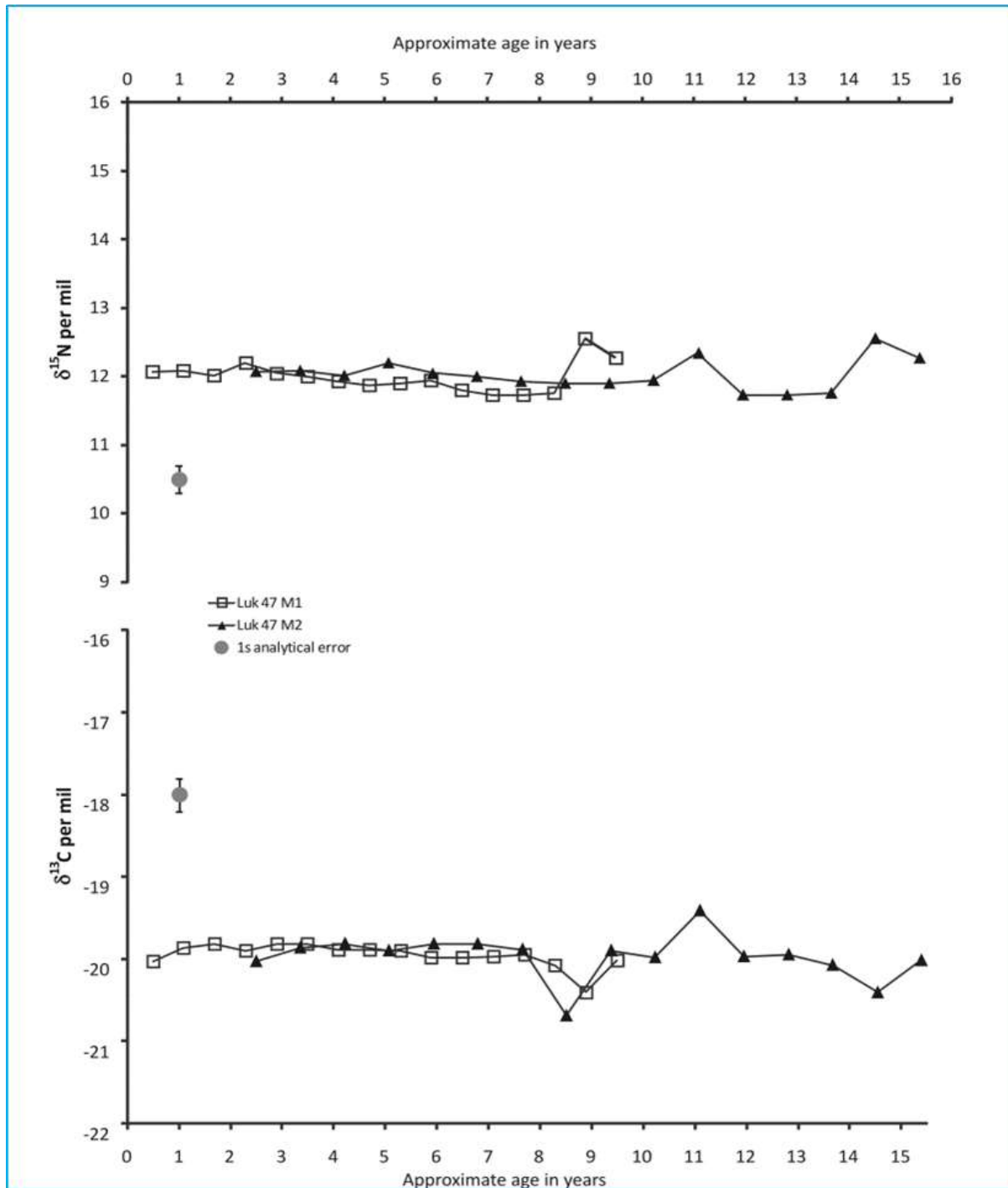


Figure 5: plot showing carbon and nitrogen stable isotope ratio dentine collagen profiles against approximate ages using data produced from the M1 and M2 teeth from the same individual from Lukin Street, London, England (LUK 47).



Figure 6: photograph of loose tooth HPCS 100 from Iron Age mid-adult female from High Pasture Cave, Skye, Scotland, prior to sampling.

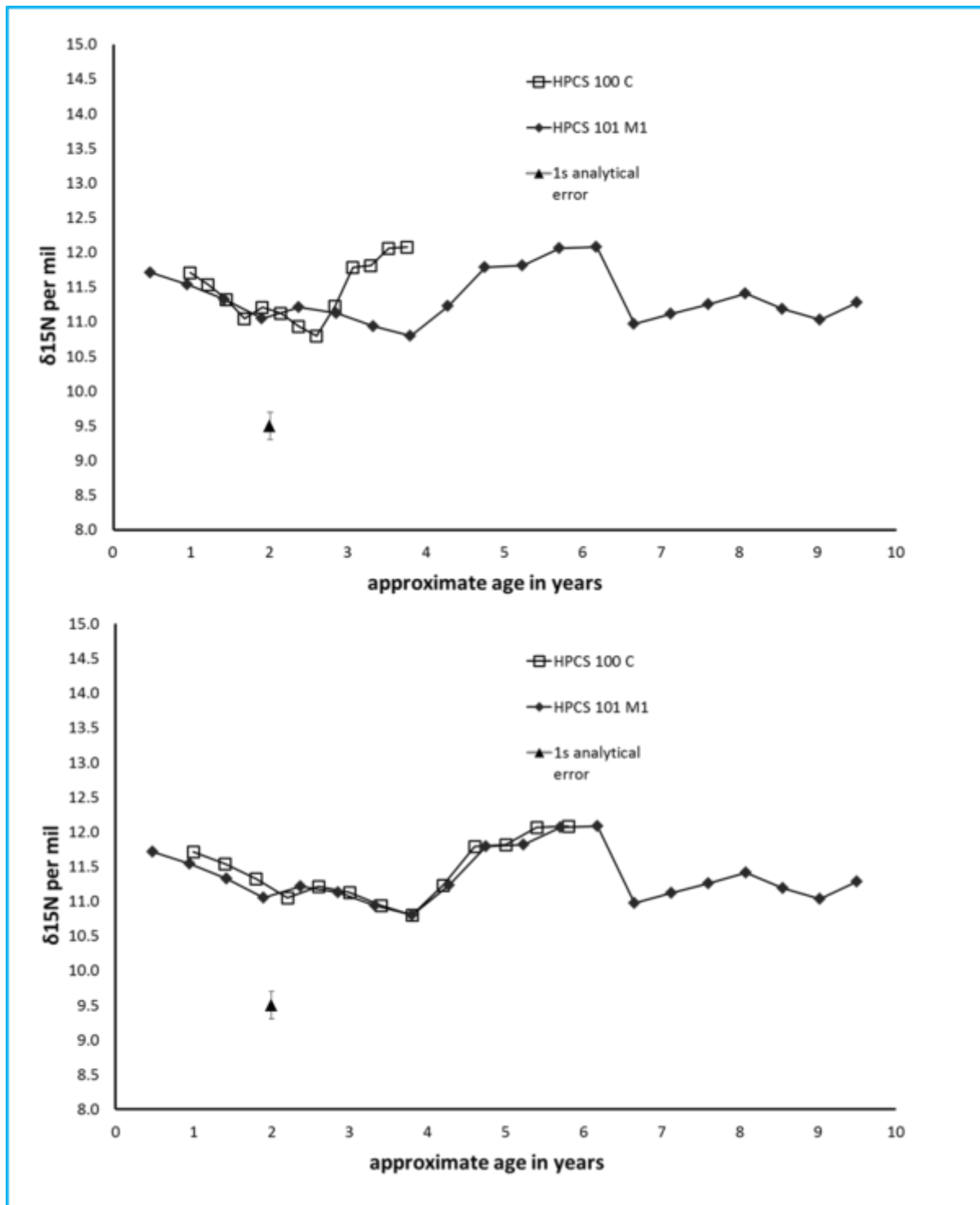


Figure 7: plot showing nitrogen stable isotope ratio dentine collagen profiles for HPCS 100 and 101. The upper plot shows HPCS 100 assigned ages for a deciduous canine, and the lower plot shows HPCS 100 assigned ages for a permanent supernumerary tooth.

SUPPLEMENTARY FIGURES:

