Kent Academic Repository

Augoyard, Mathilde, Zanolli, Clément, Santos, Frédéric, Oettlé, Anna C, L'Abbé, Ericka N, Le Luyer, Mona, Cazenave, Marine, Colard, Thomas, Hoffman, Jakobus, Profico, Antonio and and others (2023) *Evaluation of age, sex, and ancestry-related variation in cortical bone and dentine volumes in modern humans, and a preliminary assessment of cortical bone-dentine covariation in later Homo.* Journal of anthropological sciences = Rivista di antropologia : JASS, 100 . ISSN 2037-0644.

Downloaded from

https://kar.kent.ac.uk/102514/ The University of Kent's Academic Repository KAR

The version of record is available from

https://doi.org/10.4436/JASS.10019

This document version

Publisher pdf

DOI for this version

Licence for this version

CC BY-NC (Attribution-NonCommercial)

Additional information

Versions of research works

Versions of Record

If this version is the version of record, it is the same as the published version available on the publisher's web site. Cite as the published version.

Author Accepted Manuscripts

If this document is identified as the Author Accepted Manuscript it is the version after peer review but before type setting, copy editing or publisher branding. Cite as Surname, Initial. (Year) 'Title of article'. To be published in *Title of Journal* , Volume and issue numbers [peer-reviewed accepted version]. Available at: DOI or URL (Accessed: date).

Enquiries

If you have questions about this document contact ResearchSupport@kent.ac.uk. Please include the URL of the record in KAR. If you believe that your, or a third party's rights have been compromised through this document please see our Take Down policy (available from https://www.kent.ac.uk/guides/kar-the-kent-academic-repository#policies).

Evaluation of age, sex, and ancestry-related variation in cortical bone and dentine volumes in modern humans, and a preliminary assessment of cortical bone-dentine covariation in later *Homo*

Mathilde Augoyard[1], Clément Zanolli[1,2], Frédéric Santos[1], Anna C. Oettlé[3], Ericka N. L'Abbé[4], Mona Le Luyer[1,5,6], Marine Cazenave[7,8,9], Thomas Colard[1,10], Jakobus Hoffman[11], Antonio Profico[12] & Priscilla Bayle[1]

1) Univ. Bordeaux, CNRS, MCC, PACEA, UMR 5199, F-33600 Pessac, France
    email: mathilde.augoyard@u-bordeaux.fr, priscilla.bayle@u-bordeaux.fr

2) Department of Maxillofacial and Oral Surgery, Sefako Makgatho Health Sciences University, Ga-Rankuwa, Pretoria, South Africa

3) Department of Anatomy and Histology, Sefako Makgatho Health Sciences University, Ga-Rankuwa, Pretoria, South Africa

4) Forensic Anthropology Research Centre, Department of Anatomy, Faculty of Health Sciences, University of Pretoria, Pretoria 0031, South Africa

5) Center for Genomic Medicine, Massachusetts General Hospital, Boston, USA

6) Department of Psychiatry, Harvard Medical School, Boston, USA

7) Division of Anthropology, American Museum of Natural History, New York, NY, USA

8) Skeletal Biology Research Centre, School of Anthropology and Conservation, University of Kent, Canterbury, UK

9) Department of Anatomy, Faculty of Health Sciences, University of Pretoria, South Africa

10) Department of Oral Radiology, University of Lille, Lille University Hospital, Lille, France

11) South African Nuclear Energy Corporation SOC Ltd., Pelindaba, South Africa

12) Department of Biology, University of Pisa, Pisa, Italy

Summary - *Cortical bone and dentine share similarities in their embryological origin, development, and genetic background. Few analyses have combined the study of cortical bone and dentine to quantify their covariation relative to endogenous and exogenous factors. However, knowing how these tissues relate in individuals is of great importance to decipher the factors acting on their evolution, and ultimately to understand the mechanisms responsible for the different patterns of tissue proportions shown in hominins. The aims of this study are to examine age-, sex-, and ancestry-related variation in cortical bone and dentine volumes, and to preliminary assess the possible covariation between these tissues in modern humans and in five composite Neandertals. The modern analytical sample includes 12 immature individuals from France and 49 adults from France and South Africa. Three-dimensional tissue proportions were assessed from microtomographic records of radii and permanent maxillary canines. Results suggest ontogenic differences and a strong sexual dimorphism in cortical bone and dentine developments. The developmental pattern of dentine also seems to vary according to individual's ancestry. We measure a stronger covariation signal between cortical bone and dentine volumes than with any other dental tissue. A more complex covariation pattern is shown when splitting the modern sample by age, sex, and ancestry, as no signal is found in some subsamples while others show a covariation between cortical bone and either crown or radicular dentine. Finally, no difference in cortical bone volume is noticed between the modern young adults and the five young adult composite Neandertals from Marine Isotopic Stages (MIS) 5 and 3. Greater dentine*

volumes are measured in the MIS 5 chimeric Neandertals whereas a strong interpopulation variation in dentine thickness is noticed in the MIS 3 chimeric Neandertals. Further research on the cortical bone-dentine covariation will increase understanding of the impact of endogenous and exogenous factors on the development of the mineralized tissues.

Keywords – *Canine, Radius, Development, Sexual dimorphism, Bone/dentine covariation, Neandertal.*

Introduction

In recent years, the development of three-dimensional (3D) virtual imaging has enabled access to internal bony and tooth structures for the quantitative assessment of their endostructural organization (e.g., Bayle et al. 2010, 2011; Cazenave et al. 2017; Colombo et al. 2019; García-Campos et al. 2020; Genochio et al. 2019; Le Cabec et al. 2013; Puymerail 2017; Puymerail et al. 2012; Swan et al. 2020; Zanolli and Mazurier 2013; Zanolli et al. 2020). Functional adaptation is often put forward to explain differences in cortical bone and dental volumes, as well as in internal geometry of long bones and roots in hominins (Bondioli et al. 2010; Churchill 1998; Kupczik and Hublin 2010; Trinkaus and Ruff 2012). The mechanical-related adaptation of bone structure in response to different loadings has been widely described for decades (Pearson and Lieberman 2004; Ruff 2019; Shaw and Stock 2009; Wolff 1892) and the internal bone structure of hominin postcranial remains has been investigated for reconstructing joint position, joint loading, and ultimately past behaviors (see references in Cazenave and Kivell 2023; Kivell 2016). Regarding hominin roots, several studies have also highlighted a change of their volume to better sustain forces related to (para)masticatory activities (Kupczik and Hublin 2010; Le Cabec et al. 2013; Zanolli et al. 2018). However, phenotypic variation is multifactorial and must be seen as "a compromise between mechanical and other influences" (Ruff 2019, p.191). For example, many studies on extant and extinct humans have found consistent variation in stature, weight, and bone robustness due to different selection pressures depending on

the ecogeographic regions (Bernal et al. 2006; Cowgill et al. 2012; Foster and Collard 2013; Ruff 1994; Savell et al. 2016; Weisensee 2014), following Bergmann's and Allen's "rules" (Allen 1877; Bergmann 1847). Similarly, geographic variation in dental morphology among human groups has been the subject of numerous publications (Bernal et al. 2010; Irish 2016; rev. in Pilloud et al. 2016; rev. in Scott and Turner 1997), but none of them questioned the difference in dentine thickness depending on ancestry. Another factor that could lead to phenotypic variation in hominins is the morphological integration between anatomical structures linked together by functional and/or developmental similarities (Hallgrímsson et al. 2009; Rolian 2014). For example, previous studies have highlighted strong integration between dental elements that have an important role in occlusion and mastication (Gómez-Robles and Polly 2012). Many researchers have focused on developmental integration between mandible and lower dentition, two anatomical units that influence each other during development (Boughner and Hallgrímsson 2008; Coquerelle et al. 2012; Workman et al. 2002). In their study, Coquerelle and colleagues described interactions between mandibular form and dental mineralization in early development, highlighting a major influence of genetic background on both tooth and mandible structures during pre- and postnatal ontogeny. This mandible-dentition relationship would then decline over time, under the action of several exogenous factors, particularly functional ones, leading to an independence between mandible form and dental development (Coquerelle et al. 2010). Biological tissues whose formation is induced by the same signaling pathways and

development is controlled by the same genetic activation/inhibition ratio are therefore likely to covary (Boughner and Hallgrímsson 2008; Churchill 1998; Evans et al. 2016; Hallgrímsson et al. 2007).

Cortical bone and dentine tissues of modern humans share similarities in their composition, embryonic origin, and responsiveness to specific developmental signaling molecules, that can lead to coordinated variation of these two tissues. The mineralized fraction of cortical bone and dentine is composed of non-collagenous proteins that promote and control the deposition of hydroxyapatite on collagen fibers, and of an organic matrix that is mainly composed of type I collagen (Burr and Allen 2019; Dean 2017; Qin et al. 2004). Osteogenesis and dentinogenesis are driven by the same network of genes encoding signaling pathways that are regulated by epithelial-mesenchymal interactions (Brown et al. 2018; Jernvall and Thesleff 2012). Cortical bone amount is regulated by the activity of osteoblasts and osteoclasts responsible for the formation and resorption of cortical bone through the entire individual life, in response to biomechanical constraints (Burr and Allen 2019; Pearson and Lieberman 2004; Ruff et al. 2006). To a lesser extent, dentine may also be sensitive to functional loads induced by (para)masticatory activities: at the end of tooth formation, regular secondary dentine continues to form around the pulp chamber walls (Dean 2017) and irregular secondary dentine can be secreted by the odontoblasts in response to abrasion, attrition, erosion, or tooth pathologies (Dean 2017; Teaford et al. 2000). Some studies also highlighted direct correlation between tensile stress, occlusal wear, and dentine distribution pattern, suggesting that mechanical forces may have an impact on secondary dentine formation (Benazzi et al. 2014; Coon 1962; Kupczik and Hublin 2010; Oyama et al. 2007).

Cortical bone and dentine also share similar patterns of sexual dimorphism since their development responds to hormonal and chromosomal signals (Alvesalo 1997; Callewaert et al. 2010). Previous studies have shown a sexual dimorphism in the infra-cranial skeleton of modern humans with males having higher bone mineral density, periosteal circumference, and cortical bone area than females (Burr and Allen 2019; Leonard et al. 2010; Stuck et al. 2020). Differences in absolute and relative volumes of crown dentine (i.e., the proportion of the total crown volume constituted by dentine) were also assessed between males and females, with the former showing a greater volume of crown dentine (García-Campos et al. 2018; Saunders et al. 2007; Sorenti et al. 2019).

Despite these structural, biochemical, genetic, and developmental similarities, no study has investigated a possible correlation between the volumes of cortical bone and dentine among humans. Indeed, variations in dental and bone tissue proportions have been assessed separately among fossil hominins, but not together. For example, an extensive literature has shown greater overall cortical robustness in Neandertals than in modern humans (Churchill 1998; Genochio et al. 2019; Trinkaus and Ruff 2012; Trinkaus et al. 1999), reminiscent of that described for the crown and radicular dentine that is larger and much thicker in Neandertals (Buti et al. 2017; Kupczik and Hublin 2010; Le Cabec et al. 2013). The biomechanical history of the skeleton may explain a significant part of the differences in cortical bone thickness and internal diaphyseal geometry of long bones between adult Neandertals and modern humans (De Groote 2011; Puymerail et al. 2012; Trinkaus and Ruff 1989; Volpato et al. 2011). However, this pattern of postcranial and dental robustness may already be present in the early stages of development (Bayle 2008; Bayle et al. 2011; Maureille 2002; Weaver et al. 2016).

Bone and dentine tissue proportions act as a complex interaction between a host of factors including endogenous (e.g., genetic, physiologic), environmental (e.g., mechanical loadings), and evolutionary ones. Hence, there is a clear need for understanding how cortical bone and dentine thickness vary according to an individual's age, sex, and ancestry, and whether a systematic effect exists that affects both tissues, and that could lead to their coordinated variation during

development. Results from modern samples may provide an explanatory model for the more robust bone/dentine pattern observed in Neandertals.

Within this exploratory study carried out on a modern human ontogenic sample, we evaluated the variation in cortical bone and dentine volumes according to age, sex, and ancestry, and documented the degree of possible covariation between the volumes of these two tissues. More specifically, we tested the hypotheses that (i) the volumes of tissues vary according to individuals' age, (ii) sex, and (iii) ancestry, and that (iv) due to their structural, genetic, and developmental affinities, a coordinated variation in cortical bone and dentine volumes should be observed in immature individuals and should decline in adults due to the increasing impact of exogenous factors on tissue development. We used a sample of distal radii and permanent canine teeth to quantify the absolute and scaled volumes of cortical bone and dentine and to assess their degree of covariation in two skeletal samples of European- and African-derived immature and adult modern humans. The first investigation of five young adult composite Neandertals was also conducted to quantitatively compare the volume of cortical bone and dentine tissues and their potential covariation, and to give novel insights on the evolutionary mechanisms affecting both bone and dentine developments.

Materials and Methods

To quantitatively assess cortical bone and dentine (co)variation, we investigated bony and dental elements for which development is less dependent on the function. Finding bone portions for which mechanical factors have a negligible impact on tissue development is difficult. Minimal biomechanical variation of the midthoracic region has been observed among individuals, the ribs at levels 4-7 being less susceptible to mechanical loadings than appendicular skeleton (Crowder and Rosella 2007; Roberts and Chen 1972; Tommerup et al. 1993). However, identification of rib number can be challenging,

especially in the archaeological/palaeoanthropological record where the ribs are often fragmented or absent. To be able to further investigate this topic in the fossil record in the future, we thus decided to measure cortical bone volume from the distal radius, an element that is relatively frequent in the palaeoanthropological record (Pérez-Criado and Rosas 2017; Rodríguez et al. 2016; Zipfel et al. 2020). Moreover, although affected by mechanical constraints (Auerbach and Ruff 2006; Haapasalo et al. 2000) that lead to an asymmetry of the radius shaft (Hong et al. 2021), several authors highlighted a negligible side difference in anatomical measurements, cortical thickness, and mineral density in the distal portion of the radius compared to other long bones (Gray et al. 2019; Hildebrandt et al. 2016; Min et al. 2007). It should be noted that, because this study partly relies on archaeological specimens and/or on individuals where only the right or left arm was available, we could not test for their right vs. left handedness to select the non-dominant arm for analysis. Dentine volumes were measured from permanent upper canine teeth (UC). All the selected UCs show low wear degree (stages 1 to 3; Molnar 1971), so the apposition of secondary dentine in the pulp cavity, mainly induced by functional constraints (Dean 2017), would be negligible.

The studied modern sample consists of microcomputed tomography (microCT) scans of radii and UCs from a European sample composed of 12 immature and 17 adult modern humans. The sample comes from the French medieval cemeteries of Sains-en-Gohelle (Pas-de-Calais, France), and Jau-Dignac-et-Loirac (Gironde, France), dated from the High to the Low Middle Ages (Beauval et al. 2012; Cartron and Castex 2006). This European assemblage is supplemented with a South African sample composed of 32 adults of African and European ancestries from the contemporary identified osteological collection of Sefako Makgatho Health Sciences University (SMU, Gauteng Province, South Africa) and the Pretoria Bone Collection (PBC; Tab. S1). Individuals from these osteological collections are mostly migrant laborers from South

Africa or neighboring countries (i.e., Botswana, Mozambique, Zimbabwe, and Zambia), and reflect people from low socio-economic status (L'Abbé et al. 2021).

All individuals were selected under the condition that they each had one radius and UC well-preserved and free from observable pathology. The maturation stages of the UCs ranged from "root canal with parallel walls and apical end still partially open" to "root canal with apical end completely closed" (i.e., scores G to H of Demirjian et al. 1973). When necessary, reconstruction of the missing apical portion was done following Le Cabec et al. (2013) method. The age at death of the archaeological individuals has been assessed following Black and Scheuer (1996), Moorrees et al. (1963), Scheuer and Black (2000), and Schmitt (2005) methods. The sex of the adults from the archaeological samples has been determined following the DSP method based on hip-bone measurements (Murail et al. 2005). The immature individuals have been distributed between the age classes "Juvenile" 9–14 years ($n = 5$) and "Adolescence" 15–19 years ($n = 7$), according to Bogin (2003). The European adult sample is composed of 11 females and 7 males aged 20 to 49 years, whereas the South African sample consists of 14 females and 18 males aged 21 to 70 years (for a complete age distribution of the modern human sample, see Fig. S1).

Due to the scarcity of individuals having both their radius and canine preserved in association in the palaeoanthropological record, we created five young adult composite Neandertals from different archaeological sites (Tab. S1). To minimize phenotypic variation induced by evolutionary process, we combined bone and dental remains dated to either Marine Isotopic Stage (MIS) 5 or 3. To ensure optimum comparison with data obtained from modern humans, the selected radii were fully developed, and the canines showed low wear degree (stages 1 to 4; Molnar 1971). The three chimeric individuals from MIS 5 were created by combining the right radius from the Regourdou 1 individual (Regourdou, France) who has been described as a young adult (Volpato et al. 2012), with three UCs from

Krapina (Kr 36; Kr37, Kr76; Croatia). The first chimeric Neandertal from MIS 3 was represented by the association of the radius from the young adult Spy II (Shackelford 2014; Spy, Belgium) and the UC Vi 12.5 from Vindija (Croatia), whereas the second chimeric individual from MIS 3 was created by using the same radius in association with the UC Palomas 35 (La Sima de las Palomas del Cabezo Gordo, Spain). All the bone and dental elements, except for Palomas 35 (Bayle et al. 2017), were downloaded from the Digital Archive of the Neanderthal Museum (www.archiv.neanderthal.de).

Acquisitions parameters of the modern human and Neandertal samples are reported in Table S2. A semi-automatic threshold-based segmentation of bone and dental tissues was carried out with Watershed segmentation tool and manual corrections, using Avizo 7.0 software (FEI Visualization Sciences Groupe, VSG), and ImageJ 1.53. Surface rendering was performed using triangulation and constrained smoothing from the volumetric data (marching cube algorithm; Lorensen and Cline 1987).

Absolute and scaled volumes describing 3D tissue proportions were computed for one portion of the radius diaphysis, one portion of the UC root, and for the UC crown. Although epiphyses are under compressive articular loadings (Eschweiler et al. 2022; Rose 1993), some studies have found less bilateral asymmetry in surface area and size of upper limb bone articulations than in the midshaft (Lieberman et al. 2001; Ruff 2019; Trinkaus et al. 1994). A distal portion was therefore virtually cut between 20% and 30% of the total radius length (0% being at the distal end, and 100% at the proximal end), at the boundary between cortical and trabecular bone forming the radius distal end. Furthermore, a bigger portion between 20% and 80% of the total radius length, representing the entire cortical bone portion, was virtually extracted (Fig. 1A). As primary dentinogenesis begins from the future enamel-dentine junction towards the direction of the future pulp cavity (Dean 2017), and that crown formation of UC is completed around 5.5 years (AlQahtani et al. 2010), the
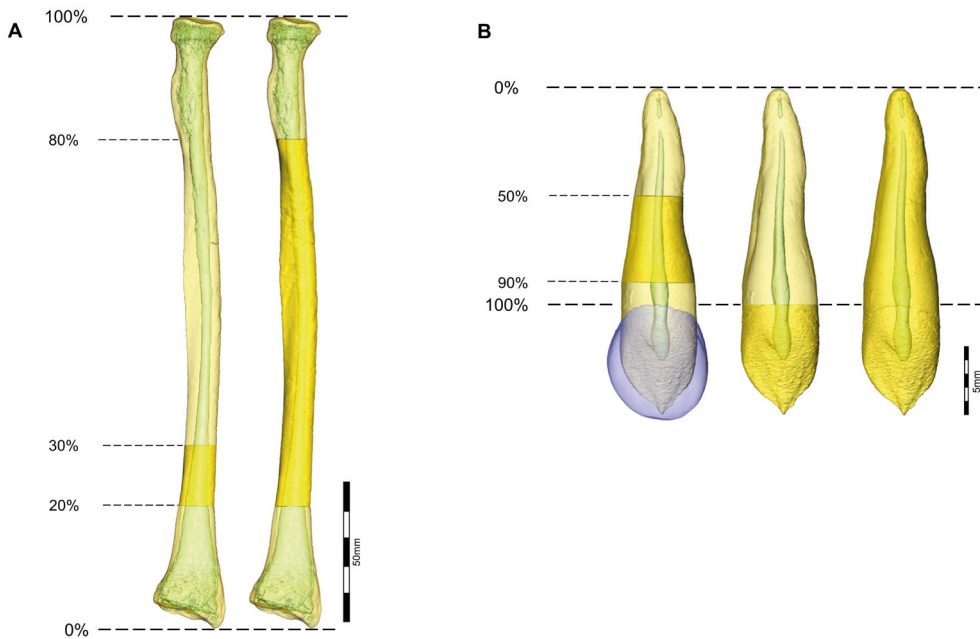
Fig. 1 - Portions (highlighted) used for the measurements of absolute and scaled volumes: A) portions of radius (anterior view) representing 10% to 60% of its total length; B) portions of upper canine (buccal view) representing 40% of the root length, the crown dentine, and the total dentine.

development of crown dentine may be strongly genetically controlled. Conversely, irregular secondary dentine can be secreted around the pulp cavity, and more specifically below the cementoenamel junction (Nudel et al. 2021), in response to environmental stimuli (Dean 2017; Teaford et al. 2000). Moreover, sex difference in the location of secondary dentine formation has been previously described (Zilberman and Smith 2001). It would appear that, within a single tooth, there are local variations in the degree of sensitivity of dental tissues to endogenous and exogenous factors. We therefore decided to select different portions of the UC to conduct this study: the radicular dentine portion between 50% and 90% of the total root length (0% being at the root apex, and 100% at the cervix), as well as the crown dentine portion, virtually extracted following the protocol described in Le Cabec et al. (2013) (Fig. 1B). Furthermore, the total dentine volume (i.e.,

crown and radicular dentine) was extracted (Fig. 1B). We hypothesize that, if there is a signal of cortical bone-dentine quantitative covariation, it should vary according to the dentine portion.

To avoid taking into account for an allometric consequence of change in individual body size in the measured tissue thickness variation, it was necessary to find the appropriate proxy to scale the volumes of cortical bone and dentine. The femoral head breadth, usually defined as the best proxy for body size estimation (McHenry 1992; Ruff et al. 1991), could not be measured in this study due to a lack of available femurs. OLS regression of base-10 logged variables and Spearman correlation tests were used to determine the relationship between tissue volumes and the total radius length (i.e., the maximum length parallel to the longitudinal axis of the radius, following Ruff 2002), and the total dentine length (i.e., the maximum length of the

dentine between the root and crown apices). The length measurement with the highest correlation with the cortical bone and dentine volumes was considered the best proxy for bone/dental size: the cortical bone volumes were therefore scaled to the total length of the radius ($r = 0.78$), and the dentine volumes were scaled to the total dentine length (r [0.73; 0.82]; Fig. S2).

The following variables were digitally measured or calculated using Avizo 7.0 software (Tab. S3, see also publicly available version on Zenodo; Augoyard et al. 2023): RL, the total radius length (mm); Vcor, the absolute volume of cortical bone for a specific bone portion (mm³); sVcor [= Vcor/RL], the scaled volume of cortical bone for a specific bone portion (mm²); DL, the total dentine length (mm); Ve, the volume of enamel (mm³); sVe [= Ve/DL], the scaled volume of enamel (mm²); Vp tot, the total volume of pulp (mm³); sVp tot [= Vp tot/DL], the scaled total volume of pulp (mm²); Vd tot, the total volume of dentine (mm³); sVd tot [= Vd tot/DL], the scaled total volume of dentine (mm²); Vd, the absolute volume of dentine for a specific dentine portion (mm³); sVd [= Vd/DL], the scaled volume of dentine for a specific dentine portion (mm²). The first author performed the analyses and Wilcoxon rank test for measurement accuracy revealed intra-observer differences less than 1% (see Tab. S4).

Due to small sample sizes, only nonparametric tests were performed. All plots and statistical analyses were computed in R 4.2.2 (R Core Team 2022; the R codes used in this study are available in Supporting Information). In this exploratory study, we interpreted *p*-values lesser than 0.05 as a substantial evidence against the null hypothesis, and *p*-values lesser than 0.10 as a weak to moderate evidence against the null hypothesis.

To compare cortical bone and dentine volumes between age group, sex, and ancestry, pairwise Wilcoxon rank sum tests were performed. These tests were supplemented by 95% confidence intervals (95% CIs) providing ranges of plausible values for the true difference between group means and obtained using a Bca bootstrap method (Zieffler et al. 2011). Boxplots

were computed to provide a visual representation of the median, standard deviation, minimum and maximum values of the variables for each subsample.

OLS regressions and Spearman correlation tests were first performed to get an overview of the relationship between the total volumes of cortical bone and the different dental tissues (i.e., enamel, dentine, pulp). Then, we focused our analyses on the distal part of the radius and on the crown and radicular dentine portions to provide a more thorough evaluation of the local variations in tissue thickness. To test the degree of cortical bone-dentine quantitative covariation through life in the modern sample, Spearman correlation tests were conducted for all immature individuals ($n = 12$) and for all adult regardless of sex and origin ($n = 49$). Then, to assess whether the degree of covariation can vary according to the sex, Spearman correlation tests were performed for female ($n = 25$) and male ($n = 25$) samples, separately. Finally, to evaluate if the ancestry can influence the degree of cortical bone-dentine covariation, we measured the Spearman correlation coefficients separately in the adults of African ($n = 30$) and European ($n = 19$) ancestry. The relationship between two variables was considered strong when their r value was larger than 0.7, moderate for r values between 0.5 and 0.7, and weak for r values between 0.3 and 0.5.

Cortical bone and dentine volumes of the five young adult composite Neandertals were first compared to the mean and to the limits of variation estimated in the entire sample of modern humans, using adjusted Z-score analysis. This test allows the comparison of parameters from small and unbalanced samples to a reference sample, using the inverse Student's t-test (Scolan et al. 2012). To ensure that the results obtained were not biased by the presence of modern individuals with different developmental stages (i.e., juvenile individuals, older adults etc.), the young adult composite Neandertals were secondly compared to chimeras created from modern adults aged 20-29 years. Results of the Wilcoxon rank sum test suggest that the

dentine/cortical bone ratios measured in modern young adult individuals (n = 12) are strictly comparable to those created by the random association of dentine and bone variables in the corresponding modern chimeras (n = 132; Fig. S3). We therefore assumed that the creation of composite individuals is a reliable way to preliminarily assess the bone-dentine relationship in a sparse palaeoanthropological record. The detailed composition of the modern young adult and Neandertal chimeric individuals and their associated cortical bone and dentine variables are presented in Table S5.

Results

Comparison of cortical bone and dentine volumes between age, sex, and ancestry

Descriptive statistics of all absolute volumes of cortical bone and dentine are presented in Table S6 and Figure S4, and compared between subsamples in Table S7. All absolute variables should be considered as indicative only. Descriptive statistics of scaled volumes of cortical bone and dentine are presented in Table 1. The results of the Wilcoxon rank test and the 95% CIs are provided in Table 2.

Statistically reliable differences in scaled volumes of cortical bone were measured between the 9–14 years and the 15–19 years subsamples (*p*-values = 0.01), while no significant difference in the scaled volumes of dentine is noticed between the two immature subsamples (Tab. 2, Fig. 2). The scaled volumes of cortical bone and dentine are statistically different between the 9–14 years individuals and the total adult sample (all *p*-values <0.01; Tab. 2). When splitting the adult sample by ancestry, only the scaled volumes of crown dentine are similar between individuals of age class [9–14y] and the European adults (Fig. 2). Except for the scaled radicular dentine, no statistical differences are noticed in the scaled volumes of cortical bone and crown dentine and their ratios between the 15–19 years and adult samples, when looking at the Wilcoxon *p*-value (Tab. 2, Figs. 2, 3). This difference can

be extended to the volume of crown dentine, according to the 95% CIs (Tab. 2). The slightly skewed distribution in the total adult sample and the presence of several outliers in the 15–19 years and adult samples may explain the H1 acceptance for the 95% CIs that are sensitive to skewness and extreme values, unlike Wilcoxon test. Crown dentine volumes were lower in 15–19y individuals compared to African adults (*p*-value = 0.05), whereas similar volumes are measured between age class [15–19y] and European adults (Fig. 2). All males and females statistically differ in their scaled volumes of cortical bone and dentine (Tab. 2, Fig. 2). To try to better discriminate the impact of sex relative to that of ancestry, we compare the volumes of tissues between males and females of the same ancestry. The results also indicate significant sexual dimorphism in all scaled volumes of cortical bone and dentine both between African males and females, and between European males and females (Tab. 2, Fig. 2). Compared to European adults, the adults of African ancestry have similar cortical bone volumes but higher dentine volumes, mostly due to African males having significantly higher volumes of radicular dentine than any other male and female individuals (Tab. 2, Fig. 2).

Correlation test performed on scaled volumes of cortical bone and dentine in the modern sample

The OLS regressions and Spearman correlation tests reveal a moderate but significant relationship between the volume of the entire cortical portion (i.e., sVcor 20-80%) and the total dentine volume in African adults (r = 0.68; r² = 0.49; Fig. 3A). For both the enamel and pulp volumes, correlations with cortical bone were significantly weaker, with values for r coefficients of 0.33 and 0.47 respectively, and r² coefficients of 0.12 (Figs. 3B, 3C).

The Spearman correlation coefficients computed between the cortical bone volume of the distal radius and the crown and radicular dentine volumes in modern human subsamples are provided in Table 3. Differences are observed in the signal of cortical bone-dentine quantitative covariation between immature and adult

Tab. 1 - Mean, range, and standard deviation of scaled volumes of cortical bone and dentine and their ratios, measured in modern humans of different age groups, sexes, and ancestries.

AGE GROUP	N	SEX	ANCESTRY		sVcor 20–30% (mm²)	sVd 50–90% (mm²)	sVd crown (mm²)	sVd 50–90% / sVcor 20–30%	sVd crown / sVcor 20–30%
9–14 y	5	u		mean	5.18	5.53	5.16	1.09	1.01
				range	4.43-6.22	4.94-6.16	4.56-5.63	0.79-1.33	0.78-1.27
				(s.d.)	(0.74)	(0.54)	(0.44)	(0.18)	(0.20)
15–19 y	7	6 u + 1 F		mean	7.83	5.59	5.86	0.74	0.77
				range	6-9.91	4.75-6.03	5.14-6.83	0.48-0.95	0.59-1.04
				(s.d.)	(1.44)	(0.46)	(0.64)	(0.15)	(0.15)
Adult	7	M	European	mean	10.33	6.99	7.19	0.68	0.70
				range	9.15-11.28	5.45-8.59	5.79-8.27	0.48-0.91	0.51-0.90
				(s.d.)	(0.88)	(1.16)	(0.84)	(0.14)	(0.12)
	12	F		mean	7.60	6.07	5.54	0.81	0.74
				range	6.34-9.10	5.18-7.66	4.81-6.46	0.59-1.01	0.56-0.93
				(s.d.)	(0.92)	(0.75)	(0.58)	(0.12)	(0.11)
	18	M	African	mean	9.55	8.62	7.90	0.91	0.84
				range	7.56-12.82	5.51-11.72	4.60-10.03	0.71-1.26	0.57-1.20
				(s.d.)	(1.31)	(1.26)	(1.37)	(0.17)	(0.20)
	12	F		mean	6.98	6.16	5.80	0.89	0.84
				range	6.05-8.66	5.27-7.16	4.94-6.56	0.74-0.98	0.65-1.04
				(s.d.)	(0.73)	(0.61)	(0.60)	(0.08)	(0.12)

Abbreviations: u, unknown; F, female; M, male; sVcor, the scaled volume of cortical bone for a specific bone portion (mm²); sVd, the scaled volume of dentine for a specific dentine portion (mm²); s.d., standard deviation.

individuals. The first ones present a weak positive correlation between the scaled volume of cortical bone and the scaled volume of crown dentine (r = 0.35, *p*-value = 0.27), whereas moderate positive correlations are observed in the total adult sample (r [0.56; 0.57], *p*-values < 0.01; Tab. 3).

When measuring the volumes of crown and radicular dentine relative to cortical bone volume (i.e., sVd 50-90% / sVcor 20-30% and sVd crown / sVcor 20-30% ratios), the values are significantly higher for the 9–14 years than for the 15–19 years and adult subsamples (Fig. 4).

Tab. 2 - *P-values of the Wilcoxon rank test and 95% CIs, allowing the comparison of scaled volumes of cortical bone and dentine and their ratios between modern samples of different age groups, sexes, and ancestries. A single asterisk indicates a moderately significant difference (0.01< p-values <0.05) and two asterisks indicate highly significant difference (p-values <0.01), according to the Wilcoxon test.*

SAMPLES PAIR COMPARISON	N		sVcor 20–30% (mm²)	sVd 50–90% (mm²)	sVd crown (mm²)	sVd 50–90% / sVcor 20–30%	sVd crown / sVcor 20–30%
9–14y vs. 15–19y	5/7	*p*	0.01*	1	0.20	0.01*	0.03*
		95% CI	[-3.75, -1.46]	[-0.55, 0.52]	[-1.27, -0.19]	[0.16, 0.54]	[0.07, 0.41]
9–14y vs. Adult	5/49	*p*	<0.01**	<0.01**	<0.01**	<0.01**	<0.01**
		95% CI	[-4.10, -2.65]	[-2.21, -1.03]	[-2.10, -1.06]	[0.07, 0.39]	[0.08, 0.36]
15–19y vs. Adult	7/49	*p*	0.31	<0.01**	0.15	0.11	0.63
		95% CI	[-1.84, 0.29]	[-2.13, -1.08]	[-1.41, -0.25]	[-0.23, -0.01]	[-0.13, 0.08]
Females vs. Males	25/25	*p*	<0.01**	<0.01**	<0.01**	0.70	0.73
		95% CI	[-3.12, -1.96]	[-2.66, -1.44]	[-2.54, -1.45]	[-0.09, 0.08]	[-0.10, 0.08]
Adults AF vs. Adults EU	30/19	*p*	0.85	0.01*	0.04*	<0.01**	0.02*
		95% CI	[-0.98, 0.87]	[0.51, 1.96]	[0.19, 1.64]	[0.07, 0.22]	[0.04, 0.20]
Males AF vs. Males EU	18/7	*p*	0.11	<0.01**	0.16	0.01*	0.16
		95% CI	[-0.17, 1.56]	[-2.55, -0.63]	[-1.54, 0.15]	[-0.37, -0.11]	[-0.26, -0.02]
Females AF vs. Females EU	12/13	*p*	0.23	0.41	0.31	0.16	0.13
		95% CI	[-1.12, 0.16]	[-0.43, 0.57]	[-0.22, 0.67]	[-0.003, 0.15]	[-0.03, 0.17]
Males AF vs. Females AF	18/12	*p*	<0.01**	<0.01**	<0.01**	0.95	0.82
		95% CI	[-3.32, -1.89]	[-3.12, -1.80]	[-2.78, -1.37]	[-0.12, 0.05]	[-0.12, 0.09]
Males EU vs. Females EU	7/13	*p*	<0.01**	0.05*	<0.01**	0.06	0.38
		95% CI	[-3.61, -2.06]	[-1.82, -0.05]	[-2.22, -0.92]	[0.01, 0.24]	[-0.05, 0.18]

Abbreviations: AF, African ancestry; EU, European ancestry; 95% CI, 95% bias-corrected and accelerated bootstrap confidence interval; sVcor, the scaled volume of cortical bone for a specific bone portion (mm²); sVd, the scaled volume of dentine for a specific dentine portion (mm²).

The results obtained from the correlation tests performed for male and female samples, show no clear signal of correlation (Tab. 3). Only a weak positive correlation between cortical bone and radicular dentine volumes and a weak negative correlation between cortical bone and crown dentine volumes are measured for females and males respectively, although none of the *p*-values
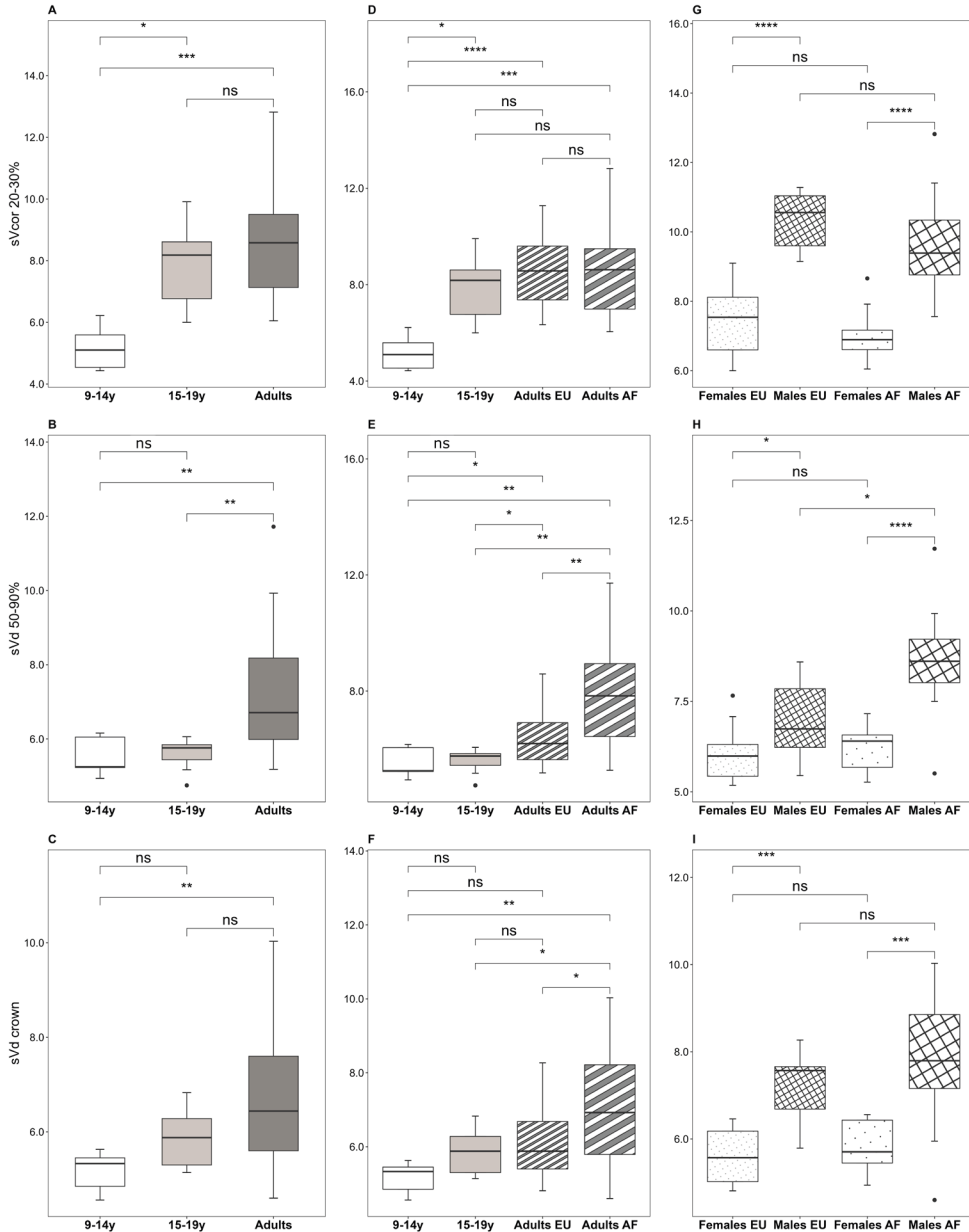
Fig. 2 *- Boxplots of the scaled volumes of cortical bone (1st row), radicular, and crown dentine (2nd and 3rd rows, respectively), measured in modern subsamples and compared between age groups (A, B, C), ancestries (D, E, F), and sexes (G, H, I). A single asterisk indicates a moderately significant difference (0.01< p-values <0.05) and two or more asterisks indicate highly significant difference (p-values <0.01), according to the Wilcoxon test. Abbreviations: AF, African ancestry; EU, European ancestry; sVcor, the scaled volume of cortical bone for a specific bone portion (mm²); sVd, the scaled volume of dentine for a specific dentine portion (mm²); ns, non-significant Wilcoxon test p-value.*

are significant. These results are different when comparing the cortical bone-dentine covariation signal between the African and European adult samples (Tab. 3). For the first one, there are significant strong and moderate positive correlations between cortical bone volume and radicular and crown dentine volumes, respectively (r [0.70; 0.56]). For the European sample, there are weak to moderate significant positive correlations between the three volumes of tissues (r = 0.44, between cortical bone and radicular dentine, and r = 0.60, between cortical bone and crown dentine; Tab. 3). When looking at the dentine-bone ratios, the adults of African ancestry have higher volumes of crown and radicular dentine relative to cortical bone compared with the European adults. These differences in ratio values are particularly significant between the males of the two ancestries (Fig. 4).

Comparison of absolute and scaled volumes of cortical bone and dentine and their ratios between the five composite Neandertals and modern humans

Adjusted Z-score analyses were first performed to provide an overall comparison of cortical bone and dentine volumes between the five Neandertal chimeric individuals and the modern variability (Tab. S8). The results show that the five Neandertal chimeric individuals are within the 95% of variation of the modern human sample for both the absolute and scaled volumes of cortical bone. Regarding the volumes of crown and radicular dentine and the dentine/bone ratios, almost all Neandertal chimeric individuals fall well beyond modern variability. Only the MIS 3 chimeric individual created by the combination of the Spy II radius and the Palomas35 UC fall entirely within the 95% of modern human variation (Tab. S8). Z-scores of cortical bone and dentine variables of the composite Neandertals compared to the modern young adult chimeras are similar to those obtained in comparison with the global modern variability, except for the same MIS 3 chimera for whom only the scaled volume of radicular dentine (sVd 50-90%) is slightly above the 95% upper limit of variation (Fig. 5).
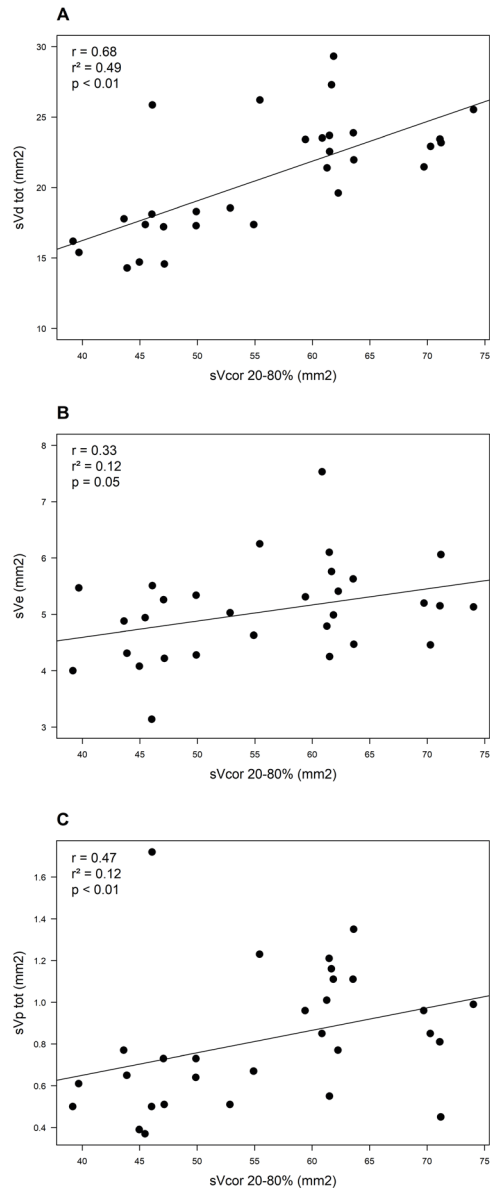

Fig. 3 - Regressions of the total scaled dentine (A), enamel (B), and total pulp (C) volumes against the scaled volume of cortical bone measured between 20% and 80% of the total radius length, among African adults (n = 30). Abbreviations: sVcor, the scaled volume of cortical bone for a specific bone portion (mm²): sVd tot, the scaled total volume of dentine (mm²); sVe, the scaled volume of enamel (mm²); sVp tot, the scaled total volume of pulp (mm²).
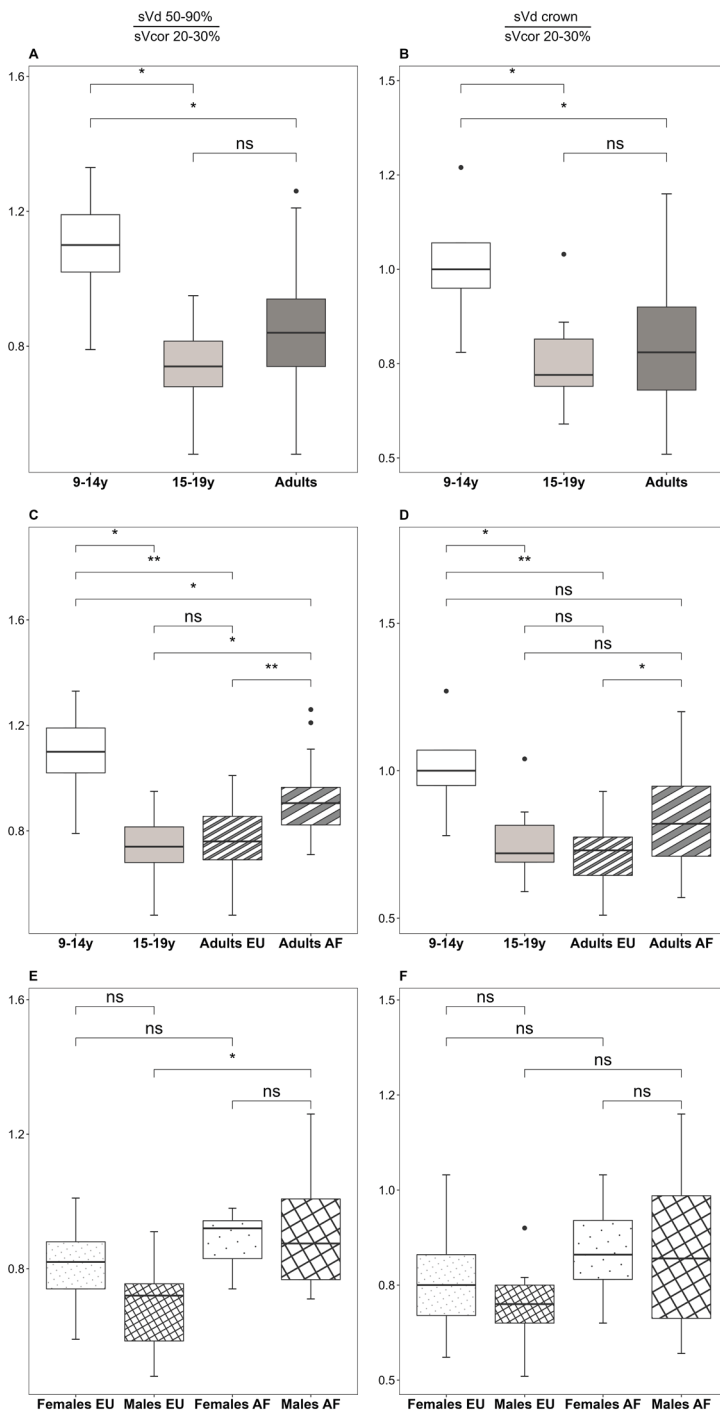
Fig. 4 - Boxplots of the radicular dentine-cortical bone and crown dentine-cortical bone ratios (1st and 2nd columns, respectively), measured in modern subsamples and compared between age groups (A, B), ancestries (C, D), and sexes (E, F). A single asterisk indicates a moderately significant difference (0.01< p-values <0.05) and two or more asterisks indicate highly significant difference (p-values <0.01), according to the Wilcoxon test. Abbreviations: sVcor, the scaled volume of cortical bone for a specific bone portion (mm²); sVd, the scaled volume of dentine for a specific dentine portion (mm²); ns, non-significant Wilcoxon test p-value.

Tab. 3 - Spearman correlation coefficients (r) and their associated p-values, performed on scaled volumes of cortical bone and dentine within modern human samples, according to the age group, sex, and ancestry.

SAMPLES	IMMATURES (N = 12)			ALL ADULTS (N = 49)		
3D variables	sVcor 20-30%	sVd 50-90%	sVd crown	sVcor 20-30%	sVd 50-90%	sVd crown
sVcor 20-30% r	1	-0.10	0.35	1	0.56	0.57
p-value		0.77	0.27		<0.01*	<0.01*
sVd 50-90%		1	-0.11		1	0.83
			0.73			<0.01*
sVd crown			1			1
SAMPLES	**ALL FEMALES (N = 25)**			**ALL MALES (N = 25)**		
3D variables	sVcor 20-30%	sVd 50-90%	sVd crown	sVcor 20-30%	sVd 50-90%	sVd crown
sVcor 20-30% r	1	0.36	-0.09	1	-0.22	-0.32
p-value		0.07	0.66		0.28	0.11
sVd 50-90%		1	0.42		1	0.69
			0.03*			<0.01*
sVd crown			1			1
SAMPLES	**ADULTS AF (N = 30)**			**ADULTS EU (N = 19)**		
3D variables	sVcor 20-30%	sVd 50-90%	sVd crown	sVcor 20-30%	sVd 50-90%	sVd crown
sVcor 20-30% r	1	0.70	0.56	1	0.44	0.60
p-value		<0.01*	<0.01*		0.06	<0.01*
sVd 50-90%		1	0.85		1	0.70
			<0.01*			<0.01*
sVd crown			1			1

Abbreviations: AF, African ancestry; EU, European ancestry; sVcor, the scaled volume of cortical bone for a specific bone portion (mm^2); sVd, the scaled volume of dentine for a specific dentine portion (mm^2).

Discussion

(Co)variation of cortical bone and dentine volumes over the lifetime

The results obtained from the comparison of cortical bone volumes between age groups highlight an age-related pattern in bone development.

The appositional bone growth in juveniles is characterized by a cortical bone deposition on the periosteal surface, simultaneously with its resorption in the endosteal cavity (Burr and Allen 2019). From adolescence to adult stages, cortical bone deposit takes place at both the endosteal and periosteal levels due to the drastic
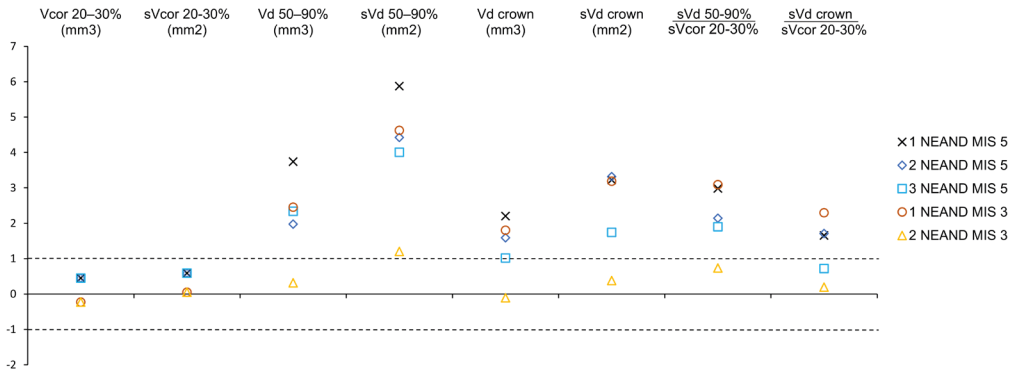
***Fig. 5** - Adjusted z-scores of the absolute and scaled volumes of cortical bone and dentine and their ratios calculated for the five chimeric Neandertals and compared with the variability of the chimeric modern young adults. Zone defined between dotted lines encompass 95% of modern variability, the zero line represents the mean. Abbreviations: Vcor, the volume of cortical bone for a specific bone portion (mm³); sVcor, the scaled volume of cortical bone for a specific bone portion (mm²); Vd, the volume of dentine for a specific dentine portion (mm³); sVd, the scaled volume of dentine for a specific dentine portion (mm²).*

increase in sex steroids (Frisancho et al. 1970). Smaller cortical bone areas were indeed measured in juveniles compared with adolescents and adults (Cambra-Moo et al. 2014; Frisancho et al. 1970). The results of our study support these 2D observations as significant differences in absolute and scaled cortical bone volumes are observed in the age group 9–14 years, compared with adolescents and adults, with values increasing throughout life. The difference in absolute bone volumes between age group 15–19 years and adults, and the similarities in their scaled counterparts may reflect a growth allometry, where differences in absolute bone growth can cover up similarities in relative growth (Buschang 1982).

Surprisingly, even if the upper canines selected for this study have comparable maturational stages (i.e., scores G to H of Demirjian et al. 1973) and low occlusal wear, variation in dentine volumes can be observed between age classes. The slow and regular rate of secondary dentine formation that proceeds at the end of tooth formation (Dean 2017) could be an explanation of this age-related pattern in dentine development: the dentine portions that first reach their dental maturation, as this is the case for crown dentine, will be first subjected to this secondary dentine deposition.

Therefore, we observe a gradual increase in crown dentine volumes between juveniles, adolescents, and adults. Radicular dentine volumes remain significantly lower in immature individuals compared to adults, and no dentine thickening is noted in the 15–19 years compared with 9–14 years age groups. This could suggest that secondary dentine preferentially forms first closer to the crown and does not affect much root dentine volumes between adolescence and early adulthood.

Cortical bone and dentine tissues share similarities in mineral composition, in genetic and hormonal affinities, and respond to the same signaling molecular pathways (Alvesalo 1997; Brown et al. 2018; Burr and Allen 2019; Callewaert et al. 2010; Dean 2017; Jernvall and Thesleff 2012), that are not shared with other dental tissues (i.e., enamel, pulp). We therefore hypothesized that endogenous factors could affect both cortical bone and dentine volumes and could lead to their coordinated variation during development. Especially, we expected to detect a stronger covariation signal in adolescents, for whom the distal epiphysis of the radius is no longer used for alternative locomotion, such as crawling (Colombo et al. 2019). Moreover, these individuals exhibit a shorter

history of exposure to biomechanical factors compared with adults since their radii and upper canines are still under development or have just reached their skeletal and dental maturity (ages for fusion of the distal radius epiphysis range from 14 to 20 years, rev. in Scheuer and Black 2000, and upper canine formation is completed around 15.5 years, AlQahtani et al. 2010). Thus, deviation from the genetic trajectory of tissue development, mainly induced by bone remodeling and secondary dentine deposition, would be negligible. However, the secretion of sex hormones leading to an acceleration in bone growth inherent to the adolescence stage (Bogin 2003) may confound the covariation signal.

The preliminary correlation test performed on an adult subsample confirms the existence of a significantly stronger covariation signal between cortical bone and dentine than between cortical bone and any other dental tissue. However, and contrary to our assumptions, Spearman correlation tests show weak covariation signals between scaled volumes of cortical bone and dentine in immature individuals. When splitting the immature sample by developmental stages, the dentine-bone ratios indicate that, relative to cortical bone, the volumes of crown and radicular dentine are significantly higher in juvenile individuals than in adolescents and adults. Previous studies have also shown a loss of synchronization between bone and dental developments in individuals younger than twelve years of age, resulting in differences in age estimation from bone or dental elements (Conceição and Cardoso 2011; Lewis 1991). Similar statements have been made from hominin juvenile remains (Cazenave et al. 2020; Dean and Smith 2009). This developmental disjunction could be the result of a great sensitivity of skeletal development to environmental insults, and notably to the socioeconomic condition of individuals (Cardoso 2007; Conceição and Cardoso 2011). It has also been shown that individuals may experience accelerated dental eruption in relation to early life stress (McDermott et al. 2021). In this study, adolescents and adults do not show significant differences in dentine-bone ratios, although

comparison tests highlighted significant differences in dentine volumes between the two subsamples, and especially between the adolescents and the African adults. This could be evidence of comparable dentine-bone relationship from the adolescent stage through adulthood. While the small size of the immature subsamples prevents any firm conclusions regarding covariation, the forty-nine adults show significant moderate correlations between the volumes of cortical bone at distal-radius level and crown and radicular dentine, suggesting a low impact of mechanical loading on the development of these portions, even in adulthood.

Influence of sex and ancestry on the volumes of cortical bone and dentine and their covariation

Significantly greater absolute and scaled volumes of cortical bone are measured in males compared with females. To what extent this difference is the result of sexual dimorphism solely and/or is linked to the impact of various functional loads on the distal arm due to sex-related activities remains to be understood. The distal part of the radius is sensitive to age-related osteopenia resulting in the increment of cortical porosity in response to estrogen deficiency at menopause (Milovanovic et al. 2015; Nishiyama et al. 2010). However, this physiological disturbance likely has a limited influence on our measurements since the female sample is mainly composed of premenopausal women. As previously measured (Feeney et al. 2010; García-Campos et al. 2018; Sorenti et al. 2019; Zilberman and Smith 2001), males have significantly greater area and absolute volumes of crown dentine than females. Our study confirms these observations and, at least for the upper permanent canine, extends them to the radicular dentine. Hormonal and chromosomal differences between sexes may explain the dimorphism in odontoblast activity, both at crown level (Alvesalo 1997; Zilberman and Smith 2001), but also at radicular level as supported by our results. These differences in both absolute and scaled volumes of dentine are particularly significant between females and males of African ancestry, where the distribution

of the former fell below the 25th percentile of the African male variation. These results highlight the importance of considering crown and radicular dentine volumes in sex determination (García-Campos et al. 2018; García-Campos et al. 2020; Sorenti et al. 2019), which may be particularly useful in forensic or archaeological context where human remains are fragmentary. Splitting the adult sample according to sex, no clear covariation signal is measured between scaled volumes of cortical bone and dentine. However, the similarity in dentine-bone ratios between females and males may therefore suggest a comparable relationship between these two tissues between sexes.

Unlike other research showing a correlation between variation in bone robustness and the ecogeographic regions (Bernal et al. 2006; Ruff 1994), we found no significant difference in absolute and relative volumes of cortical bone between the African and European samples. Direct comparison of activity level between the two adult subsamples is difficult as little information is available for the French sample. Even if the African males selected in this study were mostly migrant manual laborers (L'Abbé et al. 2021), we expected higher bone thickness in pre-industrial compared to post-industrial samples (French medieval vs. contemporary South African populations, respectively). The similarity in cortical bone volumes between samples could also highlight the lesser effect of mechanical loading on the distal portion of the radius.

Geographic variation in dental morphology has been widely described (Bernal et al. 2010; Irish 2016; rev. in Pilloud et al. 2016; rev. in Scott and Turner 1997). Especially, Esan and Schepartz (2018) showed advanced timing of tooth formation and dental emergence in children of African ancestry compared with European ones, which could lead to bigger tooth dimensions in African adults (Harris and Lease 2005; Pilloud et al. 2014). However, in the present study, the African adults have higher dentine volumes than the European ones only because of African males having much thicker dentine than any other subsample. This result is difficult to interpret since

it may reflect between-groups environmental differences (such as masticatory behavior), and/or genetic diversity. Unlike what we observed in the male and female subsamples, moderate to strong correlation coefficients were measured for adults of European and African ancestry, respectively. The latter also have higher values of dentine-bone ratios, due to statistically higher dentine volumes for comparable cortical bone volumes between the two adult subsamples. Hence, it seems that discriminating samples by ancestry rather than by sex is a better way to access the covariation signal observed in the overall adult sample.

Discussion on the first application of this study to evolutionary anthropology

Previous studies that have measured the dentine volume in Neandertals highlighted an overall thickening of this tissue compared to modern humans (Buti et al. 2017; Genochio et al. 2019; Kupczik and Hublin 2010; Le Cabec et al. 2013), variously interpreted as the influence of a host of endogenous and exogenous factors. This study partially confirmed these observations as greater absolute and scaled dentine volumes were measured in the three MIS 5 chimeric Neandertals and in the MIS 3 chimeric individual created by the combination of the Spy II radius and the Vi. 12.5 upper canine. For the SpyII/Palomas35 MIS 3 chimeric individual, however, dentine variables entirely fall within the modern variability. Although the permanent dentition of individuals from Palomas falls within Neandertal variability (Bayle et al. 2017), some of their mandible and dental characteristics extend the previously known ranges of variation observed in this taxon (Bayle et al. 2017; Walker et al. 2008). According to Walker et al. (2008), the morphological variability of the individuals from this site is much higher than what has been observed in other Neandertal populations. Previous studies support ecogeographical and chronological variability across Neandertal populations (Caramelli et al. 2006; Rosas et al. 2006; Walker et al. 2018). A strong interpopulation variation in dentine thickness between MIS 5 and MIS 3 Neandertals also emerges from our results.

Numerous studies showed greater cortical robustness in the long bones of Neandertals compared with modern humans (Churchill 1998; Trinkaus and Ruff 2012; Trinkaus et al. 1999), variously interpreted as a direct adaptation to cold climates (De Groote 2011; Trinkaus 1981), and/or the consequences of mechanical loading imposed by activity (Churchill 1998; Pearson 2000). In the present study, the absolute and scaled volumes of cortical bone measured on the radius were statistically identical between the modern young adult and the Neandertal chimeras. Pearson (2000) demonstrated higher robusticity indices of the lower limb in MIS 4 and MIS 3 Neandertals compared with recent humans, but significantly lower indices for the forelimb. Other studies confirmed the relative gracility of the radius in Neandertal that falls within the range of variation of recent human samples (Hambücken 1995; Vandermeersch and Trinkaus 1995), as supported by our results.

Analysis of the ratios of tissue volumes showed a similar bone-dentine relationship pattern between MIS 3 SpyII/Palomas35 Neandertal and modern humans, while MIS 5 chimeric individuals have higher dentine relative to cortical bone volumes. Comparing these tissue ratios between immature Neandertals where tissue development is more ontogenetically constrained and older adult specimens, would allow us to have a more precise information on the variation of this bone-dentine relationship through ontogeny in Neandertals. Indeed, the loss of synchronization of bone and tooth development observed in modern humans (Conceição and Cardoso 2011; Lewis 1991) and supported by our results, may also affect other hominin taxa (Cazenave et al. 2020; Dean and Smith 2009). The increasing number of discoveries associating hominin craniodental and postcranial remains, notably radii (e.g., Berger et al. 2010; Clarke 1998; Détroit et al. 2019; Morwood et al. 2005), will be of great relevance to question the timing of bone and dental developments in other hominin taxa than modern humans.

Conclusion

Through this exploratory study, we investigated the age-, sex-, and ancestry-related variation in cortical bone and dentine volumes in modern humans. Moreover, we questioned the relationship between these two tissues, both in modern humans and in Neandertals.

By quantifying absolute and scaled volumes of biological hard tissues measured on distal radius portions, likely to be less sensitive to mechanical loadings than other long bones, and on upper canines with a low wear degree, the objective was to better assess the genetic basis of tissue development. Our results first indicate ontogenic differences in cortical bone and dentine developments, as well as strong sexual dimorphism in tissue formation. This last result confirms the reliability of using dental and bony elements for sex determination in archaeological populations. The developmental pattern of dentine also seems to vary according to individual's ancestry. To what extent this population difference is the result of between-groups genetic diversity solely and/or can reflect differences in mechanical behaviors remains to be further investigated.

The preliminary results have outlined a covariation trend between volumes of cortical bone and dentine that does not exist with enamel nor pulp. However, this covariation pattern is more complex than anticipated as no covariation signal is measured in the immature sample and, only when splitting the adult sample by ancestry a strong covariation signal is found. The difference in covariation strength measured in the adult sample according to dentine portion suggested that, along the upper permanent canine, crown and radicular dentine tissues may not have the same degree of sensibility to environmental stimuli. Thus, accessing the genetic basis of morphological variation may be compromised by the impact of environmental factors, particularly mechanical ones, which affect tissue developments throughout life.

It is challenging to understand the phenotypic variation in humans. Another complementary approach to document the local variation

of cortical bone and dentine volumes along the bone shaft and the dental remains would be to investigate their local topographic distribution using morphometric maps (for previous works that studied bone and/or dentine distribution independently based on this approach, see Bayle et al. 2010; Bondioli et al. 2010; Cazenave et al. 2017; Profico et al. 2021; Puymerail et al. 2012; Zanolli et al. 2018).

The study of cortical bone-dentine covariation from modern humans of known biological (e.g., age, sex, pathology, etc.) and cultural (e.g., profession, sport practice, etc.) parameters, could shed light on the pattern of joint tissue thickening observed in the palaeoanthropological record, and especially in the majority of Neandertal specimens. Additional analyses must be conducted on a larger modern sample covering a wide range of developmental stages, ecogeographic origins, activity level, and composed of bones and teeth with different degrees of sensitivity to biomechanical forces, to be able to better decipher the (onto)genetic and functional factors acting on tissue development. Ultimately, it could allow the better understanding of the factors that rule the evolution of bone and dental tissues through the evolution of hominins.

Data sharing

The data that support the findings of this study are openly available in Zenodo at https://doi.org/10.5281/zenodo.6341546

Acknowledgements

Authors contribution

References

Allen JA (1877) The influence of physical conditions on the genesis of species. Radical review 1:108-140

AlQahtani SJ, Hector MP, Liversidge HM (2010) Brief communication: The London atlas of human tooth development and eruption. Am J Phys Anthropol 142:481–490. https://doi.org/10.1002/ajpa.21258

Alvesalo L (1997) Sex chromosomes and human growth. Human Genetics 101:1–5. https://doi.org/10.1007/s004390050575

Auerbach BM, Ruff CB (2006) Limb bone bilateral asymmetry: variability and commonality among modern humans. J Hum Evol 50:203–218. https://doi.org/10.1016/j.jhevol.2005.09.004

Augoyard M, Zanolli C, Santos F, et al (2023) Data file for Augoyard et al.'s article, "Evaluation of age, sex, and ancestry-related variation in cortical bone and dentine volumes in modern humans, and a preliminary assessment of cortical bone-dentine covariation in later *Homo*." [Dataset]. Zenodo. https://doi.org/10.5281/zenodo.6341546

Bayle P (2008) Proportions des tissus des dents déciduales chez deux individus de Dordogne (France): l'enfant néanderthalien du Roc de Marsal et le spécimen du Paléolithique supérieur final de La Madeleine. Bull Mem Soc Anthropol Paris 20:151–163. https://doi.org/10.4000/bmsap.6143

Bayle P, Bondioli L, Macchiarelli R, et al (2011) Three-dimensional imaging and quantitative characterization of human fossil remains. Examples from the NESPOS database. In: Macchiarelli R, Weniger GC (eds) Pleistocene Databases. Acquisition, Storing, Sharing, Mettman, Wissenschaftliche Schriften des Neanderthal-Museums 4, p. 29–46.

Bayle P, Macchiarelli R, Trinkaus E, et al (2010) Dental maturational sequence and dental tissue proportions in the early Upper Paleolithic child from Abrigo do Lagar Velho, Portugal. Proc Natl Acad Sci USA 107:1338–1342. https://doi.org/10.1073/pnas.0914202107

Bayle P, Le Luyer M, Brown KAR (2017) The Palomas Dental Remains. In: Trinkaus E, Walker MJ (eds) The people of Palomas: Neandertals from the Sima de las Palomas del Cabezo Gordo, Southeastern Spain, Texas A&M University Anthropology Series, p. 115–137.

Beauval C, Alonso L, Brunet M, et al (2012) Rapport d'opération de fouilles archéologiques—Sains-en-Gohelle "227 rue Lamartine," Vol. 1: Données administratives, scientifiques et techniques & présentation des résultats, Archéosphère, Bordeaux.

Benazzi S, Grosse IR, Gruppioni G, et al (2014) Comparison of occlusal loading conditions in a lower second premolar using three-dimensional finite element analysis. Clin Oral Investig 18:369–375. https://doi.org/10.1007/s00784-013-0973-8

Berger LR, de Ruiter DJ, Churchill SE, et al (2010) Australopithecus sediba: A new species of Homo-like australopith from South Africa. Science 328:195–204. https://doi.org/10.1126/science.1184944

Bergmann C (1847) Über die verhältnisse der wärmeökonomie der thiere zu ihrer grösse. Göttinger Studien 3:595–708.

Bernal V, Perez SI, Gonzalez PN (2006) Variation and causal factors of craniofacial robusticity in Patagonian hunter-gatherers from the late Holocene. Am J Hum Biol 18:748–765. https://doi.org/10.1002/ajhb.20546

Bernal V, Perez SI, Gonzalez PN, et al (2010) Spatial patterns and evolutionary processes in southern South America: A study of dental morphometric variation. Am J Phys Anthropol 142:95–104. https://doi.org/10.1002/ajpa.21206

Black S, Scheuer L (1996) Age changes in the clavicle: from the early neonatal period to skeletal maturity. Int J Osteoarchaeol 6:425–434. https://doi.org/10.1002/(SICI)1099-1212(199612)6:5<425::AID-OA287>3.0.CO;2-U

Bogin B (2003) The human pattern of growth and development in paleontological perspective. In: Thompson JL, Krovitz GE, Nelson AJ (eds) Patterns of Growth and Development in the Genus *Homo* (1st ed), Cambridge University Press, p. 15–44. https://doi.org/10.1017/CBO9780511542565.002

Bondioli L, Bayle P, Dean C, et al (2010) Technical note: Morphometric maps of long bone shafts and dental roots for imaging topographic thickness variation. Am J Phys Anthropol 142:328–334. https://doi.org/10.1002/ajpa.21271

Boughner JC, Hallgrímsson B (2008) Biological spacetime and the temporal integration of

functional modules: A case study of dento–gnathic developmental timing. Dev Dyn 237:1–17. https://doi.org/10.1002/dvdy.21383

Brown SDM, Holmes CC, Mallon AM, et al (2018) High-throughput mouse phenomics for characterizing mammalian gene function. Nat Rev Genet 19:357–370. https://doi.org/10.1038/s41576-018-0005-2

Burr DB, Allen MR (2019) Basic and Applied Bone Biology (2nd ed), Academic Press, Amsterdam, The Netherlands.

Buschang PH (1982) Differential long bone growth of children between two months and eleven years of age. Am J Phys Anthropol 58:291–295. https://doi.org/10.1002/ajpa.1330580307

Buti L, Le Cabec A, Panetta D, et al (2017) 3D enamel thickness in Neandertal and modern human permanent canines. J Hum Evol 113:162-172. https://doi.org/10.1016/j.jhevol.2017.08.009

Callewaert F, Venken K, Kopchick JJ, et al (2010) Sexual dimorphism in cortical bone size and strength but not density is determined by independent and time-specific actions of sex steroids and IGF-1: Evidence from pubertal mouse models. J Bone Miner Res 25:617–626. https://doi.org/10.1359/jbmr.090828

Cambra-Moo O, Nacarino Meneses C, Rodríguez Barbero MÁ, et al (2014) An approach to the histomorphological and histochemical variations of the humerus cortical bone through human ontogeny. J Anat 224:634–646. https://doi.org/10.1111/joa.12172

Caramelli D, Lalueza-Fox C, Condemi S, et al (2006) A highly divergent mtDNA sequence in a Neandertal individual from Italy. Curr Biol 16: R630–R632. https://doi.org/10.1016/j.cub.2006.07.043

Cardoso HFV (2007) Environmental effects on skeletal versus dental development: Using a documented subadult skeletal sample to test a basic assumption in human osteological research. Am J Phys Anthropol 132:223–233. https://doi.org/10.1002/ajpa.20482

Cartron I, Castex D (2006) L'occupation d'un ancien îlot de l'estuaire de la Gironde: du temple antique à la chapelle Saint-Siméon (Jau-Dignac-et-Loirac). Aquitania 22:253–282.

Cazenave M, Braga J, Oettlé A, et al (2017) Inner structural organization of the distal humerus in *Paranthropus* and *Homo*. C R Palevol 16:521–532. https://doi.org/10.1016/j.crpv.2017.06.002

Cazenave M, Dean C, Zanolli C, et al (2020) Reassessment of the TM 1517 odonto-postcranial assemblage from Kromdraai B, South Africa, and the maturational pattern of Paranthropus robustus. Am J Phys Anthropol 172:714–722. https://doi.org/10.1002/ajpa.24082

Cazenave M, Kivell TL (2023) Challenges and perspectives on functional interpretations of australopith postcrania and the reconstruction of hominin locomotion. J Hum Evol 175:103304. https://doi.org/10.1016/j.jhevol.2022.103304

Churchill SE (1998) Cold adaptation, heterochrony, and neandertals. Evol Anthropol 7:46–60. https://doi.org/10.1002/(SICI)1520-6505(1998)7:2<46::AID-EVAN2>3.0.CO;2-N

Clarke RJ (1998) First ever discovery of a well-preserved skull and associated skeleton of Australopithecus. S Afr J Sci 94:460–463.

Colombo A, Stephens NB, Tsegai ZJ, et al (2019) Trabecular Analysis of the Distal Radial Metaphysis during the Acquisition of Crawling and Bipedal Walking in Childhood: A Preliminary Study. Bull Mem Soc Anthropol Paris 31:43–51. https://doi.org/10.3166/bmsap-2018-0041

Conceição ELN, Cardoso HFV (2011) Environmental effects on skeletal versus dental development II: Further testing of a basic assumption in human osteological research. Am J Phys Anthropol 144:463–470. https://doi.org/10.1002/ajpa.21433

Coon CS (1962) The origin of races. Knopf, New York, NY, USA.

Coquerelle M, Bayle P, Bookstein FL, et al (2010) The association between dental mineralization and mandibular form: A study combining additive conjoint measurement and geometric morphometrics. J Anthropol Sci 88:129–150.

Coquerelle M, Prados-Frutos JC, Benazzi S, et al (2012) Infant growth patterns of the mandible in modern humans: a closer exploration of the developmental interactions between the symphyseal bone, the teeth, and the suprahyoid and tongue muscle insertion sites. J Anat 222:178–192. https://doi.org/10.1111/joa.12008

Cowgill LW, Eleazer CD, Auerbach BM, et al (2012) Developmental variation in ecogeographic body proportions. Am J Phys Anthropol 148:557–570. https://doi.org/10.1002/ajpa.22072

Crowder C, Rosella L (2007) Assessment of intra- and intercostal variation in rib histomorphometry: its impact on evidentiary examination. J Forensic Sci 52:271–276. https://doi.org/10.1111/j.1556-4029.2007.00388.x

Dean C (2017) How the microstructure of dentine can contribute to reconstructing developing dentitions and the lives of hominoids and hominins. C R Palevol 16:557–571. https://doi.org/10.1016/j.crpv.2016.10.006

Dean C, Smith BH (2009) Growth and Development of the Nariokotome Youth, KNM-WT 15000. In: Grine FE, Fleagle JG, Leakey RE (eds) The first humans – origin and early evolution of the genus *Homo*, Springer Netherlands, p. 101–120. https://doi.org/10.1007/978-1-4020-9980-9_10

De Groote I (2011) The Neanderthal lower arm. J Hum Evol 61:396–410. https://doi.org/10.1016/j.jhevol.2011.05.007

Demirjian A, Goldstein H, Tanner JM (1973) A New System of Dental Age Assessment. Hum Biol 45:211–227

Détroit F, Mijares AM, Corny J, et al (2019) A new species of Homo from the Late Pleistocene of the Philippines. Nature 568:181–186. https://doi.org/10.1038/s41586-019-1067-9

Esan TA, Schepartz LA (2018) The WITS Atlas: A Black Southern African dental atlas for permanent tooth formation and emergence. Am J Phys Anthropol 166:208–218. https://doi.org/10.1002/ajpa.23424

Eschweiler J, Li J, Quack V, et al (2022) Anatomy, biomechanics, and loads of the wrist joint. Life 12:188. https://doi.org/10.3390/life12020188

Evans AR, Daly ES, Catlett KK, et al (2016) A simple rule governs the evolution and development of hominin tooth size. Nature 530:477–480. https://doi.org/10.1038/nature16972

Feeney RNM, Zermeno JP, Reid DJ, et al (2010) Enamel thickness in Asian human canines and premolars. Anthropol Sci 118:191–198. https://doi.org/10.1537/ase.091006

Foster F, Collard M (2013) A reassessment of Bergmann's rule in modern humans. PLoS One 8:e72269. https://doi.org/10.1371/journal.pone.0072269

Frisancho AR, Garn SM, Ascoli W (1970) Ubperiosteal and endosteal bone apposition during adolescence. Hum Biol 42:639–664

García-Campos C, Martinón-Torres M, Martín-Francés L, et al (2018) Contribution of dental tissues to sex determination in modern human populations. Am J Phys Anthropol 166:459–472. https://doi.org/10.1002/ajpa.23447

García-Campos C, Modesto-Mata M, Martinón-Torres M, et al (2020) Sexual dimorphism of the enamel and dentine dimensions of the permanent canines of the Middle Pleistocene hominins from Sima de los Huesos (Burgos, Spain). J Hum Evol 144:102793. https://doi.org/10.1016/j.jhevol.2020.102793

Genochio L, Mazurier A, Dumoncel J, et al (2019) Inner structural organization of the mandibular corpus in the late Early Pleistocene human specimens Tighenif 1 and Tighenif 2. C R Palevol 18:1073–1082. https://doi.org/10.1016/j.crpv.2019.09.002

Gómez-Robles A, Polly PD (2012) Morphological integration in the Hominin dentition: Evolutionary, developmental and functional factors. Evolution 66:1024–1043. https://doi.org/10.1111/j.1558-5646.2011.01508.x

Gray RJ, Thom M, Riddle M, et al (2019) Image-based comparison between the bilateral symmetry of the distal radii through established measures. J Hand Surg Am 44:966–972. https://doi.org/10.1016/j.jhsa.2019.05.021

Haapasalo H, Kontulainen S, Sievänen H, et al (2000) Exercise-induced bone gain is due to enlargement in bone size without a change in volumetric bone density: a peripheral quantitative

computed tomography study of the upper arms of male tennis players. Bone 27:351–357. https://doi.org/10.1016/S8756-3282(00)00331-8

Hambücken A (1995) Étude du degré de robustesse des os longs du membre supérieur des Néandertaliens. Bull Mem Soc Anthropol Paris 7:37–47. https://doi.org/10.3406/bmsap.1995.2406

Hallgrímsson B, Jamniczky H, Young NM, et al (2009) Deciphering the palimpsest: studying the relationship between morphological integration and phenotypic covariation. Evol Biol 36:355–376. https://doi.org/10.1007/s11692-009-9076-5

Hallgrímsson B, Lieberman DE, Liu W, et al (2007) Epigenetic interactions and the structure of phenotypic variation in the cranium: Epigenetic interactions in the cranium. Evol Dev 9:76–91. https://doi.org/10.1111/j.1525-142X.2006.00139.x

Harris EF, Lease LR (2005) Mesiodistal tooth crown dimensions of the primary dentition: A worldwide survey. Am J Phys Anthropol 128:593–607. https://doi.org/10.1002/ajpa.20162

Hildebrandt EM, Manske SL, Hanley DA, et al (2016) Bilateral asymmetry of radius and tibia bone macroarchitecture and microarchitecture: a high-resolution peripheral quantitative computed tomography study. J Clin Densitom 19:250–254. https://doi.org/10.1016/j.jocd.2015.02.005

Hong E, Kwak DS, Kim IB (2021) Morphological symmetry of the radius and ulna—Can contralateral forearm bones utilize as a reliable template for the opposite side? PLoS One 16:e0258232. https://doi.org/10.1371/journal.pone.0258232

Irish JD (2016) Assessing dental nonmetric variation among populations. In: Irish JD, Scott GR (eds) A companion to dental anthropology, John Wiley & Sons Ltd, Chichester, UK, p. 265–286. https://doi.org/10.1002/9781118845486.ch18

Jernvall J, Thesleff I (2012) Tooth shape formation and tooth renewal: evolving with the same signals. Development 139:3487–3497. https://doi.org/10.1242/dev.085084

Kivell TL (2016) A review of trabecular bone functional adaptation: what have we learned from trabecular analyses in extant hominoids and what can we apply to fossils? J Anat 228:569–594. https://doi.org/10.1111/joa.12446

Kupczik K, Hublin JJ (2010) Mandibular molar root morphology in Neanderthals and Late Pleistocene and recent *Homo sapiens*. J Hum Evol 59:525–541. https://doi.org/10.1016/j.jhevol.2010.05.009

L'Abbé EN, Krüger GC, Theye CEG, et al (2021) The Pretoria bone collection: A 21st century skeletal collection in South Africa. Forensic Sci 1:220–227. https://doi.org/10.3390/forensicsci1030020

Le Cabec A, Gunz P, Kupczik K, et al (2013) Anterior tooth root morphology and size in Neanderthals: Taxonomic and functional implications. J Hum Evol 64:169–193. https://doi.org/10.1016/j.jhevol.2012.08.011

Leonard MB, Elmi A, Mostoufi-Moab S, et al (2010) Effects of sex, race, and puberty on cortical bone and the functional muscle bone unit in children, adolescents, and young adults. J Clin Endocrinol Metab 95:1681–1689. https://doi.org/10.1210/jc.2009-1913

Lewis AB (1991) Comparisons between dental and skeletal ages. Angle Orthod 61:87–92. https://doi.org/10.1043/0003-3219(1991)061<0087:CBDASA>2.0.CO;2

Lieberman DE, Devlin MJ, Pearson OM (2001) Articular area responses to mechanical loading: effects of exercise, age, and skeletal location. Am J Phys Anthropol 116:266–277. https://doi.org/10.1002/ajpa.1123

Lorensen WE, Cline HE (1987) Marching cubes: A high resolution 3D surface construction algorithm. Comput Graph (ACM) 21:163–169. https://doi.org/10.1145/37402.37422

Maureille B (2002) La redécouverte du nouveau-né néandertalien Le Moustier 2. Paléo 14:221–238. https://doi.org/10.4000/paleo.1458

McDermott CL, Hilton K, Park AT, et al (2021) Early life stress is associated with earlier emergence of permanent molars. Proc Natl Acad Sci USA 118:e2105304118. https://doi.org/10.1073/pnas.2105304118

Milovanovic P, Adamu U, Simon MJK, et al (2015) Age- and sex-specific bone structure patterns portend bone fragility in radii and tibiae in relation to osteodensitometry: a high-resolution peripheral quantitative computed tomography study in 385 individuals. J Gerontol A Biol Sci Med Sci 70:1269–1275. https://doi.org/10.1093/gerona/glv052

Min JY, Min KB, Paek D, et al (2007) side differences in the bone density of the distal radius and calcaneus in Koreans aged 4–86years. J Clin Densitom 10:184–188. https://doi.org/10.1016/j.jocd.2006.12.004

Molnar S (1971) Human tooth wear, tooth function and cultural variability. Am J Phys Anthropol 34:175–189. https://doi.org/10.1002/ajpa.1330340204

Moorrees CFA, Fanning EA, Hunt EE (1963) Age variation of formation stages for ten permanent teeth. J Dent Res 42:1490–1502. https://doi.org/10.1177/00220345630420062701

Morwood MJ, Brown P, Jatmiko, et al (2005) Further evidence for small-bodied hominins from the Late Pleistocene of Flores, Indonesia. Nature 437:1012–1017. https://doi.org/10.1038/nature04022

Murail P, Bruzek J, Houët F, et al (2005) DSP: A tool for probabilistic sex diagnosis using worldwide variability in hip-bone measurements. Bull Mem Soc Anthropol Paris 17:167–176. https://doi.org/10.4000/bmsap.1157

Nishiyama KK, Macdonald HM, Buie HR, et al (2010) Postmenopausal women with osteopenia have higher cortical porosity and thinner cortices at the distal radius and tibia than women with normal aBMD: an in vivo HR-pQCT study. J Bone Miner Res 25:882–890. https://doi.org/10.1359/jbmr.091020

Nudel I, Pokhojaev A, Bitterman Y, et al (2021) Secondary dentin formation mechanism: the effect of attrition. Int J Environ Res Public Health 18:9961. https://doi.org/10.3390/ijerph18199961

Oyama K, Motoyoshi M, Hirabayashi M, et al (2007) Effects of root morphology on stress distribution at the root apex. Eur J Orthod 29:113–117. https://doi.org/10.1093/ejo/cjl043

Pearson OM (2000) Activity, climate, and postcranial robusticity: Implications for modern human origins and scenarios of adaptive change. Curr Biol 41:R569–607. https://doi.org/10.1086/317382

Pearson OM, Lieberman DE (2004) The aging of Wolff's "law": Ontogeny and responses to mechanical loading in cortical bone. Am J Phys Anthropol 125:63–99. https://doi.org/10.1002/ajpa.20155

Pérez-Criado L, Rosas A (2017) Evolutionary anatomy of the Neandertal ulna and radius in the light of the new El Sidrón sample. J Hum Evol 106:38–53. https://doi.org/10.1016/j.jhevol.2017.01.016

Pilloud MA, Hefner JT, Hanihara T, et al (2014) The use of tooth crown measurements in the assessment of ancestry. J Forensic Sci 59:1493–1501. https://doi.org/10.1111/1556-4029.12540

Pilloud MA, Edgar HJH, George R, et al (2016) Dental morphology in biodistance analysis. In: Pilloud MA, Hefner JT (eds) Biological distance analysis: forensic and bioarchaeological perspectives, Academic Press, Amsterdam, The Netherlands, p. 109–133.

Profico A, Bondioli L, Raia P, et al (2021) morphomap: An R package for long bone landmarking, cortical thickness, and cross-sectional geometry mapping. Am J Phys Anthropol 174:129–139. https://doi.org/10.1002/ajpa.24140

Puymerail L (2017) The structural and mechanical properties of the Orrorin tugenensis femoral shaft and the assessment of bipedalism in early hominins. In: Macchiarelli R, Zanolli C (eds) Hominin biomechanics, virtual anatomy and inner structural morphology: From head to toe. A tribute to Laurent Puymerail. C.R. Palevol, Supplementary Information, p. 1–24

Puymerail L, Volpato V, Debénath A, et al (2012) A Neanderthal partial femoral diaphysis from the "grotte de la Tour", La Chaise-de-Vouthon (Charente, France): Outer morphology and endostructural organization. C.R. Palevol 11:581–593. https://doi.org/10.1016/j.crpv.2012.07.001

Qin C, Baba O, Butler WT (2004) Post-translational modifications of SIBLING proteins

and their roles in osteogenesis and dentinogenesis. Crit Rev Oral Biol Med 15:126–136. https://doi.org/10.1177/154411130401500302

R Core Team (2022). R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. URL https://www.R-project.org/

Roberts SB, Chen PH (1972) Global geometric characteristics of typical human ribs. J Biomech 5:191–201. https://doi.org/10.1016/0021-9290(72)90055-3

Rodríguez L, Carretero JM, García-González R, et al (2016) Fossil hominin radii from the Sima de los Huesos Middle Pleistocene site (Sierra de Atapuerca, Spain). J Hum Evol 90:55–73. https://doi.org/10.1016/j.jhevol.2015.09.010

Rolian C (2014) Genes, development, and evolvability in primate evolution. Evol Anthropol 23:93–104. https://doi.org/10.1002/evan.21409

Rosas A, Martínez-Maza C, Bastir M, et al (2006) Paleobiology and comparative morphology of a late Neandertal sample from El Sidrón, Asturias, Spain. Proc Natl Acad Sci USA 51:19266–19271. https://doi.org/10.1073/pnas.0609662104

Rose MD (1993) Functional anatomy of the elbow and forearm in primates. In: Gebo DL (ed) Postcranial adaptation in non-human primates, DeKalb, IL, Northern Illinois University Press, p. 70–95.

Ruff CB (1994) Morphological adaptation to climate in modern and fossil hominids. Am J Phys Anthropol 37:65–107. https://doi.org/10.1002/ajpa.1330370605

Ruff CB (2019) Biomechanical analyses of archaeological human skeletons. In: Katzenberg MA, Grauer AL (eds) Biological anthropology of the human skeleton (3rd ed), John Wiley & Sons Ltd, Chichester, UK, p. 189–224. https://doi.org/10.1002/9781119151647.ch6

Ruff CB (2002) Long bone articular and diaphyseal structure in old world monkeys and apes. I: Locomotor effects. Am J Phys Anthropol 119:305–342. https://doi.org/10.1002/ajpa.10117

Ruff CB, Holt B, Trinkaus E (2006) Who's afraid of the big bad Wolff?: "Wolff's law" and bone functional adaptation. Am J Phys Anthropol 129:484–498. https://doi.org/10.1002/ajpa.20371

Ruff CB, Scott WW, Liu AYC (1991) Articular and diaphyseal remodeling of the proximal femur with changes in body mass in adults. Am J Phys Anthropol 86:397–413. https://doi.org/10.1002/ajpa.1330860306

Saunders SR, Chan AHW, Kahlon B, et al (2007) Sexual dimorphism of the dental tissues in human permanent mandibular canines and third premolars. Am J Phys Anthropol 133:735–740. https://doi.org/10.1002/ajpa.20553

Savell KRR, Auerbach BM, Roseman CC (2016) Constraint, natural selection, and the evolution of human body form. Proc Natl Acad Sci USA 113:9492–9497. https://doi.org/10.1073/pnas.1603632113

Scheuer L, Black S (2000) Developmental juvenile osteology, Elsevier Academic Press, San Diego.

Schmitt A (2005) Une nouvelle méthode pour estimer l'âge au décès des adultes à partir de la surface sacro-pelvienne iliaque. Bull Mem Soc Anthropol Paris 17:89–101. https://doi.org/10.4000/bmsap.943

Scolan H, Santos F, Tillier A, et al (2012) Des nouveaux vestiges néanderthaliens à Las Pélénos (Monsempron-Libos, Lot-et-Garonne, France). Bull Mem Soc Anthropol Paris 24:69–95. https://doi.org/10.1007/s13219-011-0047-x

Scott GR, Turner CG (1997) Geographic variation in tooth crown and root morphology. In: Scott GR, Turner CG (eds) The anthropology of modern human teeth: Dental morphology and its variation in recent human populations (1st ed), Cambridge University Press, Cambridge, p. 165–242.

Shackelford LL (2014) Descriptive and comparative study of the upper limb. In: Rougier H, Semal P (eds) Spy cave. 125 years of multidisciplinary research at the Betche aux Rotches (Jemeppe-sur-Sambre, Province of Namur, Belgium), Volume II. Anthropologica et Praehistorica, Brussels, Royal Belgian Institute of Natural Sciences, Royal Belgian Society of Anthropology and Praehistory and NESPOS Society, p. 1–23.

Shaw CN, Stock JT (2009) Habitual throwing and swimming correspond with upper limb diaphyseal strength and shape in modern human athletes. Am J Phys Anthropol 140:160–172. https://doi.org/10.1002/ajpa.21063

Sorenti M, Martinón-Torres M, Martín-Francés L, et al (2019) Sexual dimorphism of dental tissues in modern human mandibular molars. Am J Phys Anthropol 169:332–340. https://doi.org/10.1002/ajpa.23822

Stuck AK, Schenk D, Zysset P, et al (2020) Reference values and clinical predictors of bone strength for HR-pQCT-based distal radius and tibia strength assessments in women and men. Osteoporos Int 31:1913–1923. https://doi.org/10.1007/s00198-020-05405-0

Swan KR, Ives R, et al (2020) Ontogenetic changes in femoral cross-sectional geometry during childhood locomotor development. Am J Biol Anthropol 173:80–95. https://doi.org/10.1002/ajpa.24080

Teaford MF, Smith MM, Ferguson MWJ (2000) Development, Function and Evolution of Teeth, Cambridge University Press, Cambridge.

Tommerup LJ, Raab DM, Crenshaw TD, et al (1993) Does weight-bearing exercise affect non-weight-bearing bone? J Bone Miner Res 8:1053–1058. https://doi.org/10.1002/jbmr.5650080905

Trinkaus E (1981) Neandertal limb proportions and cold adaptation. In: Stringer CB (eds) Aspects of human evolution, Taylor and Francis, London, p. 187–224.

Trinkaus E, Churchill ST, Ruff CB (1994) Postcranial robusticity in *Homo*. II: Humeral bilateral asymmetry and bone plasticity. Am J Biol Anthropol 93:1–34. https://doi.org/10.1002/ajpa.1330930102

Trinkaus E, Churchill ST, Ruff CB, et al (1999) Long bone shaft robusticity and body proportions of the Saint-Césaire 1 Châtelperronian Neanderthal. J Archaeol Sci 26:753–773. https://doi.org/10.1006/jasc.1998.0345

Trinkaus E, Ruff CB (1989) Diaphyseal cross-sectional morphology and biomechanics of the Fond-de-Forêt 1 femur and the Spy 2 femur and tibia. Anthropologie et Préhistoire 100:33–42.

Trinkaus E, Ruff CB (2012) Femoral and tibial diaphyseal cross-sectional geometry in Pleistocene Homo. Paleo Anthropol 2012:13–62. https://doi.org/10.4207/PA.2012.ART69

Vandermeersch B, Trinkaus E (1995) The postcranial remains of the Régourdou 1 Neandertal: the shoulder and arm remains. J Hum Evol 28:439–476. https://doi.org/10.1006/jhev.1995.1034

Volpato V, Couture C, Macchiarelli R, et al (2011) Endostructural characterisation of the Regourdou 1 Neanderthal proximal arm: bilateral asymmetry and handedness. In: Condemi S, Weniger GC (eds) Continuity and discontinuity in the peopling of Europe: One hundred fifty years of Neanderthal study, vertebrate paleobiology and paleoanthropology, Springer, New York, p. 175–178

Volpato V, Macchiarelli R, Guatelli-Steinberg D, et al (2012) Hand to mouth in a Neandertal: Right handedness in Regourdou 1. PLoS One 7:e43949. https://doi.org/10.1371/journal.pone.0043949

Walker MJ, Gibert J, López MV, et al (2008) Late Neandertals in Southeastern Iberia: Sima de las Palomas del Cabezo Gordo, Murcia, Spain. Proc Natl Acad Sci USA 105:20631–20636. https://doi.org/10.1073/pnas.0811213106

Weaver TD, Coqueugniot H, Golovanova LV, et al (2016) Neonatal postcrania from Mezmaiskaya, Russia, and Le Moustier, France, and the development of Neandertal body form. Proc Natl Acad Sci USA 113:6472–6477. https://doi.org/10.1073/pnas.1523677113

Weisensee KE (2014) Exploring the relative importance of spatial and environmental variation on the craniometrics of the modern Portuguese. Hum Biol 85:673–686. https://doi.org/10.3378/027.085.0502

Wolff J (1892) Das Gesetz der Transformation der Knochen, Verlag August Hirschwald, Berlin.

Workman MS, Leamy LJ, Routman EJ, et al (2002) Analysis of quantitative trait locus effects on the size and shape of mandibular molars in mice. Genetics 160:1573–1586. https://doi.org/10.1093/genetics/160.4.1573

Zanolli C, Martinón-Torres M, Bernardini F, et al (2018) The Middle Pleistocene (MIS

12) human dental remains from Fontana Ranuccio (Latium) and Visogliano (Friuli-Venezia Giulia), Italy. A comparative high resolution endostructural assessment. PLoS One 13:e0189773. https://doi.org/10.1371/journal.pone.0189773

Zanolli C, Mazurier A (2013) Endostructural characterization of the H. heidelbergensis dental remains from the early Middle Pleistocene site of Tighenif, Algeria. C R Palevol 12:293–304. https://doi.org/10.1016/j.crpv.2013.06.004

Zanolli C, Schillinger B, Kullmer O, et al (2020) When X-rays do not work. Characterizing the internal structure of fossil hominid dentognathic remains using high-resolution neutron microtomographic imaging. Front Ecol Evol 8:42. https://doi.org/10.3389/fevo.2020.00042

Zieffler AS, Harring JR, Long JD (2011) Comparing groups: randomization and bootstrap methods using R, John Wiley & Sons.

Zilberman U, Smith P (2001) Sex- and age-related differences in primary and secondary dentin formation. Adv Dent Res 15:42–45. https://doi.org/10.1177/08959374010150011101

Zipfel B, Richmond BG, Ward CV (2020) Hominin postcranial remains from Sterkfontein, South Africa, 1936-1995, Oxford University Press, Oxford.

Editor, Giovanni Destro Bisol