Title: Is enamel the only reliable hard tissue for sex metric estimation of burned skeletal remains in biological anthropology?

Short title: Is enamel reliable for odontometric sex estimation of burned remains?

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Abstract

Sex diagnosis is a crucial element in the analysis of skeletal remains from forensic and archaeological contexts. Thus, researchers have developed several methods using different anatomical regions to estimate sex. Despite such variety of methods, sexing of collective cremated human skeletal remains is still challenging due to heat-induced size changes and fragmentation, along with the typical commingling of collective contexts. This study aims to examine the potential of burned tooth crowns for odontometric sex estimation. To that end, heat-induced size changes were quantified in experimentally burned teeth. Then, odontometric sex estimation was performed in a set of theoretical samples of pre and post-burned tooth crowns. Results show burned tooth crowns undergo variable but consistent and statistically significant expansion, which is due to micro-fracturing. Such heat induced size changes are of sufficient magnitude to impact odontometric sex diagnosis and sex ratios of the theoretical samples. Yet, sexing using burned tooth crowns may still be useful to estimate the minimum number of females in a given sample. Further, the effect of heat-induced size changes may be calculated and removed using μCT scanning.

Keywords

Odontometry; Teeth; Archaeology; Sex diagnosis

1 Introduction

Sex diagnosis is crucial for the analysis of human skeletal remains (Milner, et al., 2008). In forensic contexts it is critical to assess the biological profile because it may assist in the positive identification of victims (Bruzek and Murail, 2006, Christensen, et al., 2013). In archaeological contexts, sex estimation allows analysis of, among others, palaeodemographic profiles of populations (Chamberlain, 2006, Séguy and Buchet, 2014), gender-based funerary differences (Baine, 2014, Härke and Belinskij, 2014, Kurila, 2015), and differences in diet between sexes (Pearson, et al., 2013). Thus, to estimate sex and gauge any of such variables, researchers commonly use the most sexually dimorphic skeletal elements, the os coxae and the skull (Bruzek, 2002, Murail, et al., 2005). However, such regions are frequently absent or fragmented in archaeological contexts and so other bones that also present some degree of sexual dimorphism are frequently used as well (Spradley and Jantz, 2011).

Although often less reliable, methods of sexing have been developed using, for example, the hyoid (Kindschuh, et al., 2010), scapula (Dabbs, 2010, Dabbs and Moore-Jansen, 2010, Macaluso, 2011, Özer, et al., 2006, Papaioannou, et al., 2012, Prescher and Klümpen, 1995), clavicle (Papaioannou, et al., 2012), humerus (Albanese, et al., 2005, Mall, et al., 2001), radius (Barrier and L'Abbé, 2008, Mall, et al., 2001), ulna (Barrier and L'Abbé, 2008, Cowal and Pastor, 2008, Mall, et al., 2001), carpals (Sulzmann, et al., 2008), metacarpals (Barrio, et al., 2006, Case and Ross, 2007, Manolis, et al., 2009), femur (du Jardin, et al., 2009, Frutos, 2003, İşcan and Shihai, 1995), metatarsals (Case and Ross, 2007, Mountrakis, et al., 2010), tarsals (Gualdi-Russo, 2007, Harris and Case, 2012), and teeth (Acharya and Mainali, 2007, Aris, et al., 2018, Ateş, et al., 2006, Cardoso, 2008, Gouveia, et al., 2017, İşcan and Kedici, 2003).

Despite such variety of methods, sex estimation is often inconclusive and especially difficult in collective cremation funerary contexts. This is due to the usual high fragmentation and commingling of burned skeletal remains (Godinho et al, submitted), and heat-induced size changes bones undergo during burning (Buikstra and Swegle, 1989, Shipman, et al., 1984, Thompson, 2005). To overcome such difficulties, which impair systematic examinations of sexually dimorphic morphognostic features of the skeleton, studies focusing specifically on the impact of burning on sex diagnosis have targeted different anatomical regions. Specifically, the joints of the humerus and femur, the talus, calcaneus (Gonçalves, 2011, Gonçalves, et al., 2013), the pars petrosal and the auditory canal (Gonçalves, et al., 2015, Masotti, et al., 2013, Schutkowski, 1983, Schutkowski and Herrmann, 1983, Wahl, 1981), cranium (Gejvall, 1969, Holland, 1989) and tooth roots (Gouveia, et al., 2017).

On the other hand, tooth crowns have not been examined yet. This is likely because tooth crowns often fracture during burning (Beach, et al., 2008, Schmidt, 2008), precluding odontometric analysis. Yet, some studies of both modern and archaeological cremated remains report the survival of tooth crowns (Garriga, et al., 2016, Rubio, et al., 2018, Rubio, et al., 2016, Sandholzer, et al., 2013, Schmidt, 2008). Thus, it is of interest to examine the impact of burning on tooth crown size and its potential for sex estimation.

Regarding the impact of experimental burning on tooth crown size, our working hypothesis was that the tooth crown reacts differently to burning relative to other dental hard tissues, which tend to both expand or shrink upon burning (Gouveia, et al., 2017). Specifically, we hypothesized that, when complete, the enamel cap does not undergo significant heat-induced size changes and odontometric sex estimation is therefore not affected by this phenomenon. This was hypothesized because, by mass, enamel is 96% mineral, 1% organic material and 2% water, in contrast with dentin (the largest constituent in teeth) which is 70% mineral, 20% organic material and 10% water (Currey, 2006). As a result, enamel is harder but more brittle (Currey, 2006, Lucas, 2004), and so it is possible that when burned tooth crowns are complete, no significant heat-induced size changes occurred. If significant heat-induced size changes had taken place, then that would likely cause fragmentation of tooth crowns due to brittleness of enamel.

Given our working hypothesis, the objective of this paper was to assess if heat exposure causes significant metric changes to dental crowns that impact its potential for sex estimation. For that purpose, we assessed heat-induced metric changes both at the macroscopic and microscopic levels, micro CT (μCT) being used for the latter. The potential impact of these changes on odontometric sex estimation of a simulated sample of teeth was then investigated.

2 Materials and methods

This study was based on a sample of 34 human posterior teeth that were donated to the University of Coimbra by patients followed at dental offices and who have agreed to do so after signing an informed consent (Ref. 108-CE-2014). Twenty-two teeth did not suffer extensive burning-related fragmentation and were thus used in the study (Table 1). The donated teeth were cleaned and all remaining soft tissues and dental calculus removed. Mesiodistal and buccolingual crown diameters were measured with a Mitutoyo Digimatic caliper (0.01 mm precision) before and after experimental burning at 900 ^{o}C for 135 minutes to assess if heat led to differences in such measurements. This temperature was chosen since heat-induced size changes are more pronounced at such very high temperatures. Experimental burning was carried out by using an electric muffle (Barracha K-3 three-phase 14A). The furnace temperature was measured with a type K probe (negative: nickel-aluminum, positive: nickel-chrome) complying with norm IEC 60584–2. A *t*-test for paired samples was performed to examine if mean differences in size before and after burning were statistically significant.

Seven teeth were µCT scanned before and after experimental burning to further examine, at a µmetric scale, the heat-induced size changes dental crowns underwent. Only two teeth presented undamaged crowns and so were included in this study. The teeth were scanned using a compact desktop high resolution microtomograph, Skyscan 1172 scanner. TheX-ray microtomograph operation-scanning procedure was optimized to produce the best images by reducing artifacts such as beam hardening, ring, star and line artifacts as much as possible (Cnudde, et al., 2004, Ketcham and Carlson, 2001, Maurício, et al., 2013), with an isometric resolution of 24.53 µm. DataViewer (version 1.5.6.2) was used to visualize and quantify the impact of heat induced size changes. Three cross sections per tooth were measured in the µCT scans of the two teeth before and after burning. Line probes were used in DataViewer to extract attenuation values (Hounsfield Units) along those probes. The values were then exported as *.csv files that were later imported into Microsoft Excel (2007). In Excel, the length of the fractures present in the enamel was measured (Figure 1) and subtracted to the length of the full cross-sections of the burned teeth. This procedure allowed examining if eventual pre and post-burning size differences were due to heat-induced fractures in the crowns of teeth.

Table 1: Composition of the sample used in this study (only includes teeth that survived experimental burning).

	Not µCT scanned	µCT scanned
M2	1	
M3	5	

2M	1	1
3M	5	
2M	1	1
3M	4	
M3	2	
M3 (indetermined quadrant)	1	
Total	20	2

The possible impact of heat-induced dental crown size changes in the correct sex classification of a given sample was assessed on 10 random samples composed of 100 teeth each. Given that it would be impracticable to experimentally burn 1000 teeth for this purpose, we chose to compute such samples by using the norminv function in Excel. This function allows to randomly generate values based on a mean and a standard deviation provided by the user. For this purpose, we used the descriptive statistics reported by Zorba et al. (2011) who studied crown diameters of the human dentition on a sample from the Greek population. We choose the buccolingual diameter as standard measurement because several studies report it is more sexually dimorphic and thus reliable for sex estimation than the mesiodistal diameter (Ateş, et al., 2006, Cardoso, 2008, Prabhu and Acharya, 2009, Zorba, et al., 2011). As a result, each of the 10 randomized samples was composed of the upper second molars (M^2) of 50 males (mean = 11.47 mm; SD = 0.70) and 50 females (mean = 10.47 mm; SD = 0.86). The M^2 was chosen due to its availability for experimental burnings and also because it is not as morphologically variable as the M^3 (the other tooth in our experimental burning sample). These samples recreated 1000 unburned teeth.

To replicate the effects of burning in the randomized samples, the descriptive statistics of the metric changes observed in our experimental burning were also randomly applied to each crown buccolingual diameter using the norminv Excel function. This allowed us to mathematically calculate plausible post-burning crown diameters. We then performed odontometric sex estimation on both samples, applying the cut-off point obtained on the simulated pre-burning samples, to illustrate the impact of heat-induced tooth crown size changes on odontometric sex estimation.

After generating the theoretical pre- and post-burning buccolingual diameter samples, we sexed the pre-burned teeth according to the sample specific method of Albanese et al. (2005), which was applied to teeth in an identified skeletal sample by Cardoso (2008) and in a Neolithic sample by Gonçalves et al. (2014). This method requires that some pre-requisites are met, such as a balanced sex ratio and the availability of more than 40 specimens (Albanese et al., 2005). Basically, the sample specific cut-off point, corresponding to the mean of the sample, is used to score individuals as males if presenting features larger than the reference cut-off point and as females if features are smaller than the reference. The pre-burning cut-off points of each of our sets of samples were then used as reference to estimate sex of the individuals composing their theoretically burned counterparts. Sex estimation and sex ratios between the pre- and post-burned sets of samples were then compared to illustrate the expectable impact of burning in odontometric sexing.

3 Results

Both mesodistal and buccolingual crown diameters increased after burning in all cases, although with varying magnitudes (Table 2). The *t*-test showed the pre and post burning size differences in both mesodistal ($t=-10.74$, $p<0.00001$) and buccolingual ($t=-13.07$, mean $p<0.00001$) diameters were statistically significant.

Table 2: Descriptive statistics of the heat-induced size changes (post-burning diameters / pre-burning diameters X 100).

	Diameter	
	Mesodistal	Buccolingual
Mean	7.73%	8.34%
SD	2.80%	2.72%
Min	1.56%	2.62%
Max	13.35%	12.87%

Consistently with caliper measurements, post-burning measurements using the µCTs were larger than pre-burning measurements (Table 3). Since heat-induced fractures in the crown may inflate its diameter, fracture length along cross-sections was measured and subtracted from the crown diameters to determine if actual metric change occurred (Figure 1). The fractures subtraction demonstrated that the post-burning buccolingual diameters were almost exactly similar to the pre-burning diameters when the length of the fractures is removed (Table 3). Thus, heat-induced fracturing of the enamel explained most pre- and post-burning size differences of crown diameters in our sample.

Table 3: Pre- and post-burning cross-section measurements (three per tooth) in the two µCT scanned teeth (A66 and AE2). The final column expresses the ratio of the post-burning diameters (minus the heat-induced micro-fractures) divided by the pre-burning diameters.

	µm				
	Pre-burned diameter	Post-burned diameter	Σ fractures	Post-burning - Σ fractures	(Post-burning - fractures)/Pre-burning
AG6_1	10523.48	11639.20	1117.75	10521.45	1.00
AG6_2	9140.87	9650.22	340.31	9309.91	1.02
AG6_3	8898.47	10086.85	947.92	9138.93	1.03
AE2_1	10226.21	11324.35	790.07	10534.28	1.03
AE2_2	9174.53	10034.86	790.33	9244.52	1.01
AE2_3	9079.12	10202.03	1077.68	9124.35	1.00

Figure 1: Comparison of cross-section measurements before (A) and after (B) burning. Points 1 and 2 in Fig. 1B are located at heat-induced fractures. In the top, fractures are visualised in a slice of the µCT stack. In the bottom, fractures correspond to negative peaks in the plots of extracted Hounsfield Units along line probes.

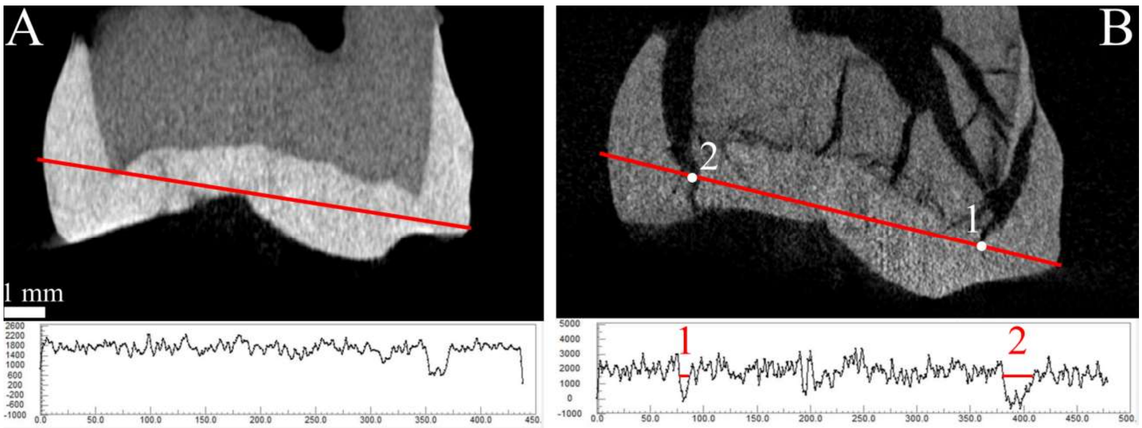

Table 4 shows that the pre-burning buccolingual diameters (mean and SD) simulated on Microsoft Excel were, as expectable, similar to those of Zorba et al. (2011). Simulated post-burning diameters were, as predicted, significantly larger than pre-burning diameters in all simulated sets of samples (mean $t=-30.31$, mean $p=<0.00001$; Table 4). Odontometric sex estimation based on these theoretical data showed that post-burning sexing overestimated the number of males and underestimated the number of females, thus providing biased sex ratios (Table 4).

Table 4: Mean descriptive statistics of the pre- and post-burning buccolingual crown diameter in the 10 simulations, along with mean sex estimation based on those simulations (see text for details).

	Pre-burning (BL diameter)	Post-burning (BL diameter)
Mean	10.97	11.88
SD	0.93	1.05
Min	8.56	9.21
Max	13.00	14.28
♀	48.00	18.40
♂	52.00	81.60
Sex ratio (♀/♂)	0.93	0.23

4 Discussion

In our experiment, burning at high temperatures caused an expansion in tooth crowns. This was observed for all crowns composing our sample which contrasts with what has been reported for other hard tissues exposed to heat, as is the case for dental roots (Gouveia, et al., 2017, Sandholzer, et al., 2013) and bones (Shipman, et al., 1984, Thompson, 2005). Normally bones experience shrinkage, although bone expansion has been reported as well. Our µCT-based measurement in teeth showed most of tooth crown expansion was in fact related to heat-induced micro-fracturing. Nonetheless, a minor part of it may also have been related to (i) heat-induced effects other than micro-fracturing, (ii) difficulty in locating equivalent pre and post-burning landmarks in tooth crowns for measurement and (iii) normal error involving measurements in the µCTs. Most probably, the latter two explain most of the small variability in our results. Regardless, the resulting effect is negligible (Table 3) and our results showed heat-induced micro-fracturing explains size differences between pre and burned teeth almost completely. This supports our research hypothesis, that enamel does not undergo significant heat-induced size changes thus maintaining its metric sex estimation potential whenever preserved.

Our simulation of post-burning sex estimation resulted in the overestimation of the frequency of males and underestimation of females, thus leading to biased sex ratios. As a result, crown odontometric sex estimation based on references for unburned teeth is limited by heat-induced size changes. The metric accounting of heat-induced fractures through µCT seems to be the only mean to override the bias caused by crown expansion. However, it is an expensive and time consuming resource that is not widely available to biological anthropologists, and so it is difficult to use in large samples.

Yet, crown metrics are presumably not entirely devoid of potential for the sex estimation of burned teeth. Most crown measurements below reference sectioning points discriminating males from females are attributable to women because if they are below the sectioning point despite heat-induced expansion then they were necessarily below that sectioning point prior to heat exposure. Conversely, the same reasoning does not apply to values above the sectioning point. Diameters above the sectioning point could originally be below that value meaning that feminine metrics could have been 'masculinized', i.e. expanded due to heat exposure. This caveat could be overcome by applying correction factors for size changes to affected teeth. However, our experimental burning results show expansion varying between 1.56% and 13.35%, and so the range is too large to allow the use of reliable correction factors. Thus, we argue that odontometric sex estimation based on burned tooth crowns can be used to estimate the minimum number of women present in a given sample, but not to provide relatively precise sex ratios. This approach can be combined with metric approaches on bones which usually experience shrinkage when exposed to high temperatures (e.g. Shipman et al., 1984; Buikstra and Swegle, 1989; Thompson et al., 2005). In such cases, most masculine burned bone measurements are theoretically attributable to males following the reverse logic explained above for teeth. As a result, a more comprehensive analysis of sex distribution can be attained through the combination of the two procedures.

Our investigation demonstrated that enamel is the only skeletal tissue almost entirely immune to metric heat-induced changes since the latter seem to be mainly the result of fracturing and not of actual size alterations. This is a major step forward for the anthropological examination of burned skeletal remains which has so far been unable to devise metric procedures able to tackle the obstacle imposed by such changes.

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Declaration of interest statement

The authors declare no competing interests.

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