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**Title:**

Letter to the editor: Enteseal changes and estimation of adult age-at-death

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**Main Text:**

In identified skeletal collections, age-at-death is the main variable explaining the distribution of entheseal changes (ECs) in adults: older individuals tend to display more ECs and more exuberant changes than younger ones (e.g. Mariotti, Facchini, & Belcastro, 2004; Milella, Belcastro, Zollikofer, & Mariotti, 2012; Shaibani, Workman, & Rothschild, 1993; Villotte, 2009). This has led to an attempt, published recently in *AJPA*, to estimate adult age-at-death from EC stages (Milella, Belcastro, Mariotti, & Nikita, 2020). In their article, Milella and colleagues used scores of “robusticity” (one type of EC) as predictors in multiple regression analyses with age as the dependent variable. The results are relatively disappointing: even with an age prediction interval of  $\pm 20$  years, the percentage of correctly classified individuals barely reaches 80% in their study. This poor performance is likely related to the expectation of an implicit linear relation between ECs and chronological age, whereas too many factors (e.g. genetic background, hormonal levels, physical activity levels) interact in EC development. As a result, the correlation between chronological age and ECs, although statistically significant, remains too low in our opinion to reliably and accurately estimate age-at-death using statistical approaches such as the one developed by Milella and collaborators. As we highly esteem the numerous and excellent studies carried on by our colleagues on various aspects of ECs (e.g. Bertsatos, Chovalopoulou, Boskovits, Garoufi, & Nikita, 2021; Mariotti et al., 2004; Milella et al., 2012), and as their recent article in *AJPA* motivated us to think about this issue, we would like to submit to them, and more broadly to the readers of the *AJPA*, a very simple idea that may create new avenues in the estimation of adult age-at-death using ECs.

If this correlation between age and ECs is well known, the underlying physio(patho)logical processes involved are not (Villotte & Knüsel, 2013). However, we do know that these processes likely differ, at least in part, for fibrocartilaginous and fibrous entheses (for this specific point, and for a presentation these two types of entheses in biological anthropology, see Villotte & Knüsel, 2013). Milella and colleagues (2020, p. 194) noted that “When fibrous and fibrocartilaginous entheses are treated separately, the former outperforms the latter [in age-at-death estimation]”. This is in agreement with a previous work that has shown a stronger correlation between chronological age-at-death and ECs at fibrous sites, compared to fibrocartilaginous ones (Villotte, 2009). However, careful data analyses indicate that 1) for both types of entheses this correlation remains too low for reliable age-at-death estimation

(Henderson, Mariotti, Santos, Villotte, & Wilczak, 2017; Villotte, 2009) and 2) for some fibrocartilaginous entheses, major ECs appear almost exclusively after the fourth decade of life and are usually seen in 50+ year-old individuals (Villotte, 2009). By “Major ECs” we mean exuberant changes (usually mineralized tissue formation associated with surface discontinuity) that affect the entire attachment site. Thus, even if the correlation of EC stages and chronological age-at-death is weaker for fibrocartilaginous entheses, major ECs for these attachment sites seem potentially good indicators of older individuals (a similar idea has been recently formulated (though not tested) by Bertatos and colleagues (2021)).

Major ECs could thus be relevant in the age-at-death estimation of adults, as they are likely “specific” to an age group. However, the contrary may be not true: healthy entheses and minor ECs can be seen in individuals of all ages (including older individuals), and we thus consider them as likely irrelevant for age estimation (Fig. 1). This type of reasoning may look surprising, but it is actually applied quasi systematically in bioarcheology and forensic anthropology for another indicator of age-at-death for adult skeletons, namely the fusion stage of the sternal extremity of the clavicle (Fig. 1). An unfused or partly fused epiphysis is extremely valuable to identify young adults. Conversely, a fused sternal extremity is near totally irrelevant for adult skeletons, as it indicates an individual older than 20 years (Schaefer, Black, & Scheuer, 2009).

In order to test this hypothesis, we used five identified skeletal collections (Table 1) for which ECs were recorded for 18 appendicular entheses (group 1 in Villotte, 2006). The method identifies three stages for each enthesis (Villotte, 2006): A (no change), B (minor EC) and C (major EC). All individuals with at least one stage recorded were included. Individuals with systemic diseases, such as diffuse idiopathic skeletal hyperostosis or spondyloarthropathies were excluded. Stages for bones displaying macrotrauma were not taken into account. Three of the collections (Bologna, Coimbra and Spitalfields) were used as a learning metapopulation sample and the last two collections were used as target samples. These later collections (Schoten and Sassari) were chosen as they differ dramatically from each other in terms of age-at-death distribution as well as in extent of preservation: older individuals and missing data are much more frequent in the Schoten collection than in the Sassari one (Table 1).

All the analytical process was carried out using R 4.0.5 (R Core Team, 2021); the whole R script along with complementary results being provided as Supporting Information online. The first

step was to provide some kind of reliable and generalizable estimate for the minimum age of individuals exhibiting stage(s) C, which allows us to distinguish between younger and older individuals (see figure 1). In order to do so, we represented the age distribution of the individuals from the learning metapopulation sample exhibiting at least one, two, and three stage(s) C, and computed in each case the associated 5% empirical quantiles (figure 2), i.e. the age threshold  $t$  such that 95% of individuals are older than  $t$  year-old. These quantiles were approximately equal to 41 years, 47 years, and 50 years old respectively. In a second step, we tested on the individuals of the Sassari and Schoten collections the decision rules empirically defined on the learning sample; i.e. that any individual with at least one stage C should be at least 41 year-old, and similarly for two stages C (47 year-old) and three stages C (50 year-old). Confusion matrices were computed to evaluate the accuracy of these decision rules (table 2). In a final step, we computed the percentage of misclassified observations: the number of individuals exhibiting stage(s) C with an age-at-death below the defined minimum age divided by the number of all individuals exhibiting stage(s) C.

Our approach seems promising, as it reliably identifies individuals over 40+ and/or 50+ year-old in the target skeletal samples with very few misclassified individuals ( $\leq 5.0\%$  (table 3), which was the expected error rate for these decision rules). This approach may thus be useful in forensic context, as reliable age-at-death estimation is crucial for identification. These broad, not clearly defined, age “categories” may not look particularly useful. However, one has to remember that the age-at-death of individuals from this age “group” is systematically underestimated by almost all methods (see for instance references in Milella et al., 2020). Another important point that needs to be stressed is that this approach deals very easily with missing data. For instance, age-at-death can be estimated for an individual with only three entheses scored (out of 18) but who displays a stage C. This approach may thus have bioarcheological and forensic applications, as it may be used on poorly preserved skeletons or on isolated bones. One of the limitations, though, is that this approach relies on one or very few major ECs that can also be produced by a traumatic event or a systemic condition. Such cases need to be carefully excluded as a consequence.

In our opinion, this approach is promising because there is great scope for improvement. For instance, one way to increase the minimum ages would be to compute them from a lower percentage of individuals with stage(s) C from the learning metapopulation sample. Reducing this threshold to 90% of individuals with at least one stage C leads to a minimum age of 48 years (but significantly increases the percentage of misclassified individuals, see SI). It seems also possible to exclude some entheses in the analyses in order to reduce the percentage of misclassified individuals and/or to increase the minimum age. Other ways to improve this approach may be to focus on one specific feature (such as major enthesophytes), or to use the extremes of a multi-stage method that distinguish a greater number of gradations than the one used here (e.g. 5 stages instead of 3). In any case, this approach seems promising, and we hope to see more articles, from Milella and colleagues and from other scholars, challenging and possibly improving it.

**Conflicts of interest:**

The authors have no conflicts of interest to declare.

**Data availability statement:**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Table 1. Summary statistics for the samples (for a presentation of the individual collections, see Milella, Belcastro, Mariotti, & Nikita, 2020; Orban, Eldridge, & Polet, 2011; Villotte, 2009). Ages are in years.

Collections	Sex		Age			Number of missing EC data per individual	
	N males	N females	Mean	SD	Min-Max	Median	Min-Max
Bologna	84	0	45.1	17.1	20-91	3	0-15
Coimbra	173	153	42.2	15.7	20-89	1	0-17
Spitalfields	83	95	53.4	15.7	21-87	5	0-17
Sassari	133	0	40.2	15.7	20-84	1	0-11
Schoten	25	20	61.2	21.7	19-94	7	1-15

Table 2. Confusion matrices for age classes (defined from figure 2) and number of enteses having reached stage "C" in target samples. "<1", "<2", "<3": individuals with less than, respectively, 1, 2, or 3 stage(s) C. "≥1", "≥2", "≥3": individuals with, respectively, at least 1, 2, or 3 stage(s) C.

	Sassari		Schoten		Both collections	
	[20,41)	[41,99)	[19,41)	[41,99)	[19,41)	[41,99)
<1	69	44	9	7	78	51
≥1	1	19	1	28	2	47
	[20,47)	[47,99)	[19,47)	[47,99)	[19,47)	[47,99)
<2	90	33	14	5	104	38
≥2	0	10	1	25	1	35
	[20,50)	[50,99)	[19,50)	[50,99)	[19,50)	[50,99)
<3	95	32	17	8	112	40
≥3	0	6	1	19	1	25

Table 3. Percentage of misclassified individuals for each collection.

	Sassari	Schoten	Both collections
≥1 stage C	5.0%	3.6%	4.1%
≥2 Stages C	0.0%	3.8%	2.8%
≥3 stages C	0.0%	5.0%	3.8%

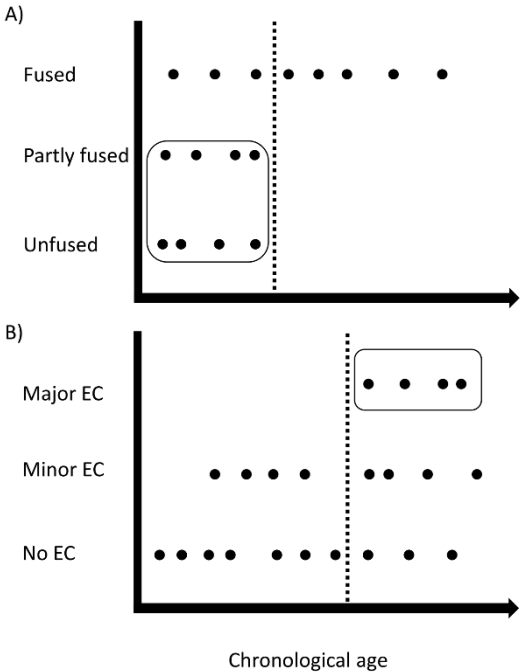


Figure 1. Schematic representations of approach of estimation of adult age-at-death using fusion stage of the sternal extremity of the clavicle (A), and major EC (B). Black dots: theoretical adult individuals. Dashed lines: maximum (A) or minimum (B) ages determined using these approaches. The rectangles indicate the individuals actually assessed for age using these approaches.

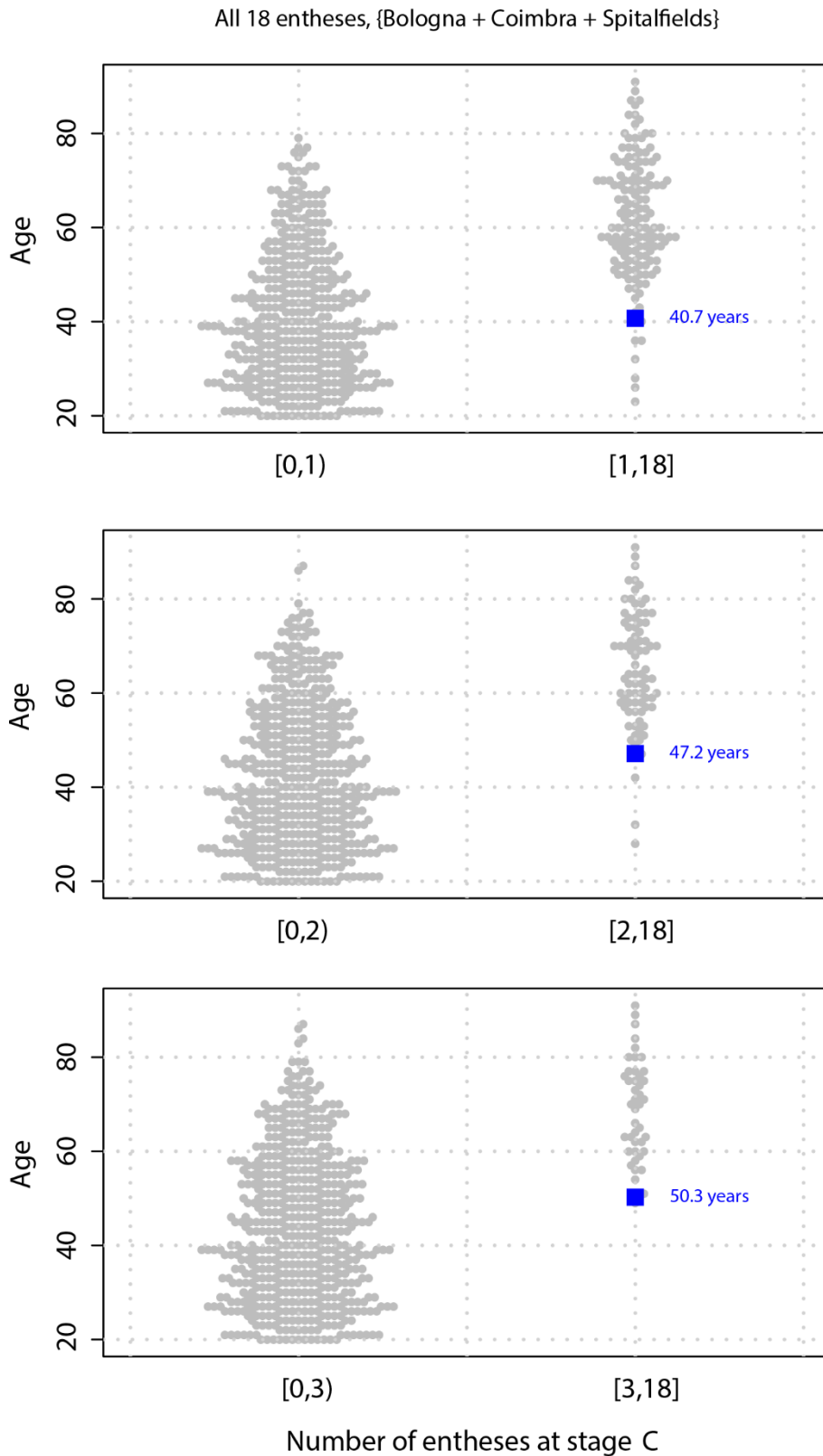


Figure 2. Age distributions of individuals from the learning metapopulation sample. A) Individuals with no stage C, and individuals with at least 1 stage C. B) Individuals with 0 or 1 stage C, and individuals with at least 2 stages C. C) Individuals with 0, 1 or 2 stage(s) C, and individuals with at least 3 stages C. The blue squares indicate the empirical quantiles of order 5% (i.e., 95% of individuals are older than this threshold).