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AGE AT DEATH DETERMINATION FROM MORPHOLOGICAL CHANGES IN THE CLAVICLE

FROM ADOLESCENCE TO ADULTHOOD - THE IMPACT OF ENVIRONMENTAL FACTORS

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Preliminary note

The Literature cited follows the guidelines of the *American Journal of Physical Anthropology*

Resumo

A determinação da idade à morte é um dos principais desafios que se apresentam numa identificação individual a partir de restos humanos ósseos. Obter a idade que o indivíduo tinha aquando da morte é uma tarefa complicada e que vai aumentando de grau de dificuldade à medida que a idade do indivíduo aumenta. Até ao final da sua formação e erupção, os dentes representam indicadores muito precisos da idade (Hillson, 1996). Durante a infância e adolescência, o comprimento dos ossos longos e a fusão epifisária aparecem como as formas mais fiáveis da determinação da idade. A partir dos 18 anos, o terceiro molar está muitas vezes já erupcionado, e muitas das principais epifises começam a estar fundidas à medida que o corpo deixa de crescer (Cardoso, 2008).

A partir desta altura a precisão dos métodos começa a decair drasticamente e após os 30 anos os indicadores usados para determinar a idade em indivíduos adultos prendem-se com degeneração dos ossos, degeneração esta que está muito menos dependente de factores genéticos e muito mais dependente de factores ambientais, como a nutrição, o clima e principalmente a actividade física (Scheuer e Black, 2000).

Existem vários estudos que estabelecem um padrão de fusão para a epífise esternal da clavícula para indivíduos adolescentes e jovens adultos, através da análise de restos ósseos (*e.g.* Stevenson, 1924; Todd e D'Errico, 1928; MacLaughlin, 1990; Black e Scheuer, 1996; Veschi e Facchini, 2002; Coqueugniot e Weaver, 2007; Shirley e Jantz, 2010),

Com este trabalho pretendeu-se desenvolver uma metodologia que permita de uma forma tão precisa quanto possível, determinar a idade à morte de esqueletos (provenientes de contexto arqueológico ou forense) através da análise da clavícula, nomeadamente do processo de fusão da epífise esternal.

Foram estudados esqueletos de três colecções osteológicas distintas. A colecção de esqueletos identificados do Museu Bocage em Lisboa, que contem indivíduos nascidos em meados do século 20, e representa uma população eminentemente urbana (Cardoso, 2006); a colecção do Museu Antropológico da Universidade de Coimbra, com indivíduos nascidos no inicio do século 20 provenientes de uma população rural (Fernandes, 1985); foi ainda estudada uma colecção de esqueletos conservada no Museu de História Natural de Londres, a colecção de Spitalfields, mais antiga que as anteriores, pois contém esqueletos que viveram em meados do século 18, numa zona muito industrializada de Londres (Molleson *et al.,* 1993).

No total foram estudados 246 esqueletos (125 do sexo masculino e 121 do sexo feminino), divididos da seguinte forma pelas 3 colecções: 138 de Coimbra (74 do sexo masculino e 64 do sexo feminino), 87 de Lisboa (40 do sexo masculino e 47 do sexo feminino) e 21 da colecção inglesa (11 do sexo masculino e 10 do sexo feminino).

A comparação de idade entre as colecções revelou diferenças significativas entre Lisboa e as restantes duas, sendo os indivíduos de Lisboa mais jovens. Não se encontraram diferenças entre Coimbra e Spitalfields. Uma análise análoga para a robustez, apenas revelou diferenças entre Lisboa e Coimbra, com os indivíduos de Lisboa a serem em média menos robustos.

Os dados recolhidos, foram o comprimento máximo da diafise e o perímetro ao meio de modo a obter o índice de robustez. As clavículas foram classificadas quanto ao seu grau de fusão da epifise com 3 escalas diferentes:

a) 3 Fases, que é a divisão mais frequentemente usada, e o mais simples de aplicar, pois apenas diferencia o desenvolvimento da epifise em : **não fundido (Fase 1),** ou seja sem floco epifisario; **em fusão (Fase 2)**, isto é com floco visível e **fundido (Fase 3)** quando este está completamente fundido e não se vislumbra qualquer linha de fusão;

b) 4 Fases, que é mais complexo que o anterior: não fundido (Fase 1); **se o floco cobre menos de 50% da superfície de epífise (Fase 2), se cobre 50% ou mais da epifise (Fase 3)** (Figura 4) e fase 4 se a fusão é completa.

c) O método de 5 Fases divide a fase não fundido das outras escalas em duas fases: **não fundido com *billowing* marcado (Fase 1)** e **não fundido com *billowing* pouco profundo (Fase 2)**; as restantes fases (3,4 e 5) são iguais ao método de 4 estádios.

Várias técnicas estatísticas foram utilizadas, para comparar as colecções (ANOVA, Kruskal-Wallis e Mann-Withney-Wilcoxon), e para caracterizar as passagens de grau e comparar os graus entre si (análise de transição e analise de sobrevivência por Kaplan-Meier), e por último usada a regressão Ordinal Logística para modelar os dados e obter as probabilidades de estimação da idade. Todo o trabalho estatístico foi efectuado com recurso a softwares específicos, nomeadamente STATA e R. De referir que todos os pressupostos dos testes foram estudados e respeitados, embora não se mostrem detalhes neste trabalho.

A análise de transição revelou que como se esperava os indivíduos do sexo feminino passam para um grau de fusão superior numa idade, em média, inferior aos do sexo masculino. Ou seja, a fusão ocorre mais cedo. A mesma analise mostra que os indivíduos de Lisboa maturam mais rapidamente que os das outras duas colecções.

A utilização das técnicas de análise de sobrevivência de Kaplan-Meier, revelaram diferenças significativas entre todos os graus dos métodos de 3 e 4 etapas. No método de 5 etapas não foi possível separar os graus mais baixos, sendo pois aconselhável a sua junção.

A modelação por regressão ordinal logística forneceu vários modelos significativos, com uma boa capacidade explicativa, usando a idade categorizada em 7 classes (15-17; 18-20; 21-23; 24-26;27-29; 30-32; 33-35), e a variável grau de fusão (Stage) do método em 3 e 4 estados. Estes modelos procuraram aumentar a flexibilidade de aplicação do método. Como exemplo mostra-se em detalhe o modelo com o grau em 3 estados e as outras variáveis no modelo (sexo e robustez).

Antes de proceder à obtenção de probabilidade de estimação da idade, introduziu-se a variável colecção para tentar confirmar a tendência de maturação mais cedo dos indivíduos da colecção de Lisboa (de referir que esta variável não pode fazer parte do modelo final sob pena de o modelo apenas poder predizer idades dentro destas três colecções). A variável colecção mostrou-se significativa, e mostrou que um indivíduo com o mesmo grau de fusão, mesma robustez e mesmo sexo, tem uma possibilidade maior de ser mais velho se pertencer a uma colecção que não a de Lisboa.

Ficamos então com um modelo que se traduz em 6 equações:

$$G(y \leq 1) = 1.8001 - (1.5599 * Stage2 + 3.4719 * Stage3 + 0.0911 * Robusticity - 0.1701 * Sex)$$

$$G(y \leq 2) = 2.9810 - (1.5599 * Stage2 + 3.4719 * Stage3 + 0.0911 * Robusticity - 0.1701 * Sex)$$

$$G(y \leq 3) = 4.0323 - (1.5599 * Stage2 + 3.4719 * Stage3 + 0.0911 * Robusticity - 0.1701 * Sex)$$

$$G(y \leq 4) = 4.9407 - (1.5599 * Stage2 + 3.4719 * Stage3 + 0.0911 * Robusticity - 0.1701 * Sex)$$

$$G(y \leq 5) = 5.6435 - (1.5599 * Stage2 + 3.4719 * Stage3 + 0.0911 * Robusticity - 0.1701 * Sex)$$

$$G(y \leq 6) = 6.4021 - (1.5599 * Stage2 + 3.4719 * Stage3 + 0.0911 * Robusticity - 0.1701 * Sex)$$

As variáveis Stage2, Stage3, robustez e sexo, devem ser substituídas pelos valores do indivíduo. Por exemplo se tivermos um indivíduo do sexo masculino (0), com grau de fusão 2, e robustez 22, devemos substituir estes valores nas equações (é necessário ter em conta que o facto de a variável Stage ser ordinal com 3 categorias, torna necessário a sua transformação em variável *dummy*, pelo que neste caso teríamos que substituir nas equações por Stage2=1, Stage3=0).

Cada uma das equações dá-nos um valor que nos permite obter a probabilidade cumulativa do indivíduo estar nessa classe de idade ou abaixo dela. Por exemplo ao substituir na equação G ($y \le 3$) obtemos o valor de 0.4673, do qual temos que obter a probabilidade. Podemos fazê-lo de duas formas: ou com o comando do R *pnorm*(valor) ou com a formula do Excel *dist.normp* (valor). Neste caso pnorm(0.4673)=0.6798 que significa que o nosso desconhecido tem uma probabilidade de 68% de estar incluído ou abaixo da classe 3, ou seja abaixo dos 23 anos.

Uma vez que estamos a trabalhar com probabilidades cumulativas, para calcular a probabilidade de estar numa determinada classe temos que subtrair as probabilidades anteriores. Assim, voltando ao nosso exemplo, para obter a P ($y=3$) temos que subtrair P ($y \le 2$) à já calculada P ($y \le 3$). Ficamos então com P ($y=3$) =0.68-0.28=0.40. portanto o nosso desconhecido tem assim cerca de 40% de probabilidades de ter entre 21 e 23 anos.

Os resultados apontam para uma tendência secular de aceleração do desenvolvimento, havendo uma diferença significativa entre a maturação dos indivíduos de Lisboa e os das outras duas colecções. Os indivíduos de Lisboa apresentam uma maturação mais cedo e mais rapidamente, podendo esta diferença ser devida às melhores condições de vida da Lisboa de meados do século 20, relativamente a zonas rurais de Coimbra no inicio do século 20, ou mesmo da Londres do século 18.

Este tipo de tendências seculares no desenvolvimento tem sido documentado (Lin, *et al,* 2006; Ahmed and Warner, 2007), e ligado à mudança de condições de vida. De facto, Hawley e colaboradores (2009) estabeleceram uma ligação estatisticamente significativa na maturação, entre crianças da África do Sul num período de 40 anos. O estudo atribui a aceleração da maturação às importantes melhorias das condições de vida ocorridas no seio daquela população da cidade de Pretoria, naquele período.

Shirley e Jantz (2010) também documentaram esta tendência na maturação esquelética, ao compararem as colecções McCormick (final do século 20) e Hamman-Todd (inicio do século 20), nomeadamente nos indivíduos do sexo masculino.

A estratégia de modelação adoptada, permitiu atingir o principal objectivo proposto que foi o de obter um novo método, que fosse estatisticamente robusto para estimar a idade à morte.

Este trabalho apresenta resultados que apontam para uma tendência secular de aceleração na maturação esquelética. É demonstrado que indivíduos mais recentes, com melhores condições de vida, tem um desenvolvimento mais cedo e mais acelerado.

Os métodos apresentados são estatisticamente significativos, mas acima de tudo são flexíveis e fáceis de utilizar quer sejam em contexto arqueológico ou forense.

Palavras-Chave: Determinação da idade à morte, tendência secular, clavícula, Regressão logística Ordinal

Abstract

This paper describes a new method for age at death estimation using the epiphyseal union at the medial end of the clavicle. Individuals from 3 human skeletal collections with ages between 15 and 35 were used in a total of 246 specimens – 125 males and 121 females. The Spitalfields collection represents a 1750's sample from industrial London, whereas the Coimbra's specimens represent a largely rural population and range from late nineteen to early twentieth century, and the Lisbon collection is the most recent (mid to late twentieth century) and representative of a more typical urban population. Clavicles were scored according to three classification schemes, a 3 stage scheme (1-unfused, 2-fusing and 3-fused), a 4stage scheme(1-unfused; 2-fusing with flake covering under 50% of epiphysis, 3-fusing with flake covering 50% or more of epiphysis, 4-fused), and 5 stage scheme(1-unfused with marked billowing; 2-unfused with shallow billowing; 3-fusing with flake covering under 50% of epiphysis; 4-fusing with flake covering 50% or more of epiphysis; 5-fused). Maximum length and perimeter at the middle of the shaft were also measured to establish the robustcity index. Collections were compared by age and robusticuty, and transition analysis and survival analysis were used to compare mean age of attainment for maturation/fusion stages. Ordinal Logistic Regression was used to establish several probabilistic models for age ate death estimation.
Results show that there is a secular tendency for the development to be earlier in more recent individuals (Lisbon) when compared to the other collections. Robusticity was found to significantly influence the fusion of the epiphyses, unlike sex. Modeling techniques demonstrated that a 5 degree method is not suitable to our data, since there are no differences between the first 2 stages. The extracted models with 3 and 4 stages provide reliable probabilities for age at death given a certain stage of fusion. Modeling techniques proved to a very useful tool as they allow introducing several factors that influence the variation that we are trying to describe.

Keywords: Age at death determination, secular trend, clavicle, Ordinal Logistic Regression

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Table of Contents:

List of Tables

List of Figures

1. Introduction

Estimating age at death is one of the most important challenges in individual identification, in both the living and the dead, either in archeological or forensic contexts. The question is: How old was the individual when he/she died? In general terms, the younger the individual the more accurate the existing methods of age estimation are, because they are based on very rapid and well established changes associated with growth and development. Developing teeth are very accurate age indicators (Hillson, 1996), as well as long bones length and fusion of primary and secondary centers of ossification (Scheuer and Black, 2000). But accuracy of methods based on these indicators tends to decrease as the individual approaches late adolescence. At the age of 18, the third molar is erupted in many cases, while its formation is still progressing, and almost all bone epiphysis are fused, as the body begins to cease its growth and approaches full maturation (Scheuer and Black, 2000). From this point on, the accuracy of age estimation methods begins to drop further and after 30 years of age the commonly used age indicators show a very loose association with age. This disagreement results from the fact that age is now estimated from degenerative changes in bone and teeth and these probably show an increasingly greater environmental component, which vary widely among individuals. This environmental component is related to factors which change the rate at which bone degenerates and include diet, climate, physical activity, and overall health status (Scheuer and Black, 2000).

Although the clavicle has been used for developing age estimation methods, little has been explored with respect to its potential. The clavicle is a peculiar bone since it is the first to attain its adult configuration (around the 11th week of gestation), and the last to end its development around 30 years of age (Black and Scheuer, 2000). In 1920 Stevenson published an extensive article on the epyphiseal union of several bones of the body, with the clavicle among them. Later, Todd and D'Errico (1924), in response to Stevenson's paper published their work on the clavicular epiphysis.

More recently Webb and Suchey (1985) used a large sample from the Chief Medical Examiner-Coroner in Los Angeles to establish four degrees of fusion: non-fused (up to 25 yo – males; 23 yo - females); non-fused with separate epiphysis (16 to 22 yo – males; 16 to 21 females); partial fusion (17 to 30 - males; 16 to 33 – females) and complete fusion (100% of sample after 31yo – males; 34yo – females). Although Webb and Suchey's method attempts to capture the a wider range of variation in age-related changes in the medial end of the clavicle, stage number 2 (non-fused with separate epiphysis) is hard to determine, because in most occasions the separate epyphiseal flake is never found with the shaft (Scheuer and Black, 2000), so it is not very practical. Also, if we look at the results we see that stage number 2 is completely within the age interval of stage 3.

Other authors developed a 5 state scale in two works (McLaughlin, 1990 and Black and Scheuer, 1996), using a Portuguese sample from the National Museum of Natural History in Lisbon, Portugal in the first, and a mix of this same sample and two British ones (Spitalfields, also used in present paper and St. Brides). Although a good effort to distinguish 2 more intermediate states the samples was taken they are too small in order to achieve a statistical meaning (35 males and 31 females total). More studies have been published (e.*g*. Veschi and Facchini, 2002; Coqueugniot and Weaver, 2007; Shirley and Jantz, 2010) in order to achieve more accurate estimates for age at death, all having some kind of limitation.

The limitations range from the difficulty on applying the same stages (Webb and Suchey, 1985), the small size of the samples (McLaughlin, 1990 and Black and Scheuer, 1996), or the truncation of the samples (Veschi and Facchini, 2002; Coqueugniot and Weaver, 2007), in this case truncation refers to the maximum age of the individuals in the sample (25 yo in Veschi and Facchini, and 29 in the case of Coqueugniot and Weaver). Since these studies were not designed specifically for the clavicle, the specimens are not old enough so that at maximum age all specimens had attained complete fusion of the epiphysis. Shirley and Jantz (2010) work is a good attempt to obtain statistically significant result, but it lacks two main things: on the one hand it does not have a probabilistic approach and on the other, it relies only on development of the epiphysis to determine age at death. That is, it does wishes to look further in what factors can affect the rate of epiphyseal fusion.

The present study attempts to take a step further, as it uses: 1) a significantly large sample; 2) a wide age range(15-35 years of age) that documents the full variation in fusion of epiphysis; 3) methods that are easy to score(3 and 4 stages) and that have little inter-observer rating issues; 4) a solid statistical methodology (Ordinal Logistic Regression), that not only assigns the probability of an unknown subject belonging to a certain age class, but also takes into account other factors like the bone’s robusticity, that proved to be significant to establish an accurate estimate for age at death.

2. Materials and Methods

2.1 Sample Description

The sample was composed of all the skeletons with age at death between 15 and 35 years of age, from three different collections of known-age skeletons, namely the Lisbon Collection, the Coimbra Collection, and the Spitalfields Collection. Clavicles with signs of gross pathology or taphonomic damage were excluded. The final sample included 246 individuals, 125 males and 121 females.

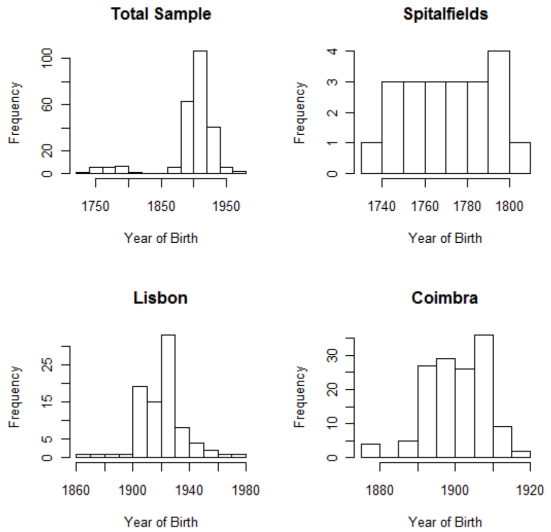

Figure 1. Distribution of individuals by year of birth. Separated by collections.

Figure 1 shows the distribution of the specimens by year of birth. As we can see the total sample has a range of roughly 200 years, being the earliest specimens born in mid-18th century and the latest in the 1980's. The distribution by collection is not even, individuals from the Spitalfields collection were born almost a century before the individuals in the rest of the sample. There is some overlapping in the Portuguese collections, but Coimbra individuals are generally from earlier decades of the 20th century. These differences, as well as the differences in socio-

Table1. Sample specimens by sex.

Sex	Collection			
	Coimbra	Lisbon	Spitalfields	Total
Males	74	40	11	125
Females	64	47	10	121
Total	138	87	21	246

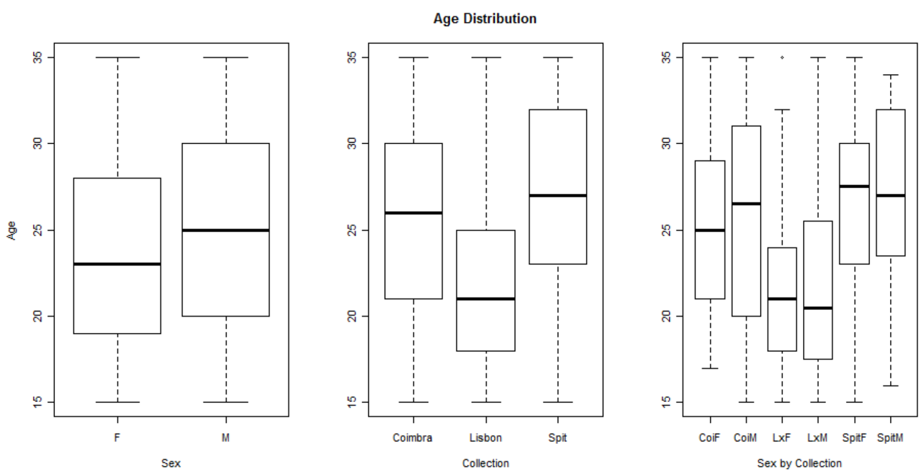

Figure 2. Age distribution of the sample. Between (from left to right) sex, collection, and sex inside collection.

economic background will be taken into account later.Sex is more evenly distributed among the 3 collections studied (Table 1).

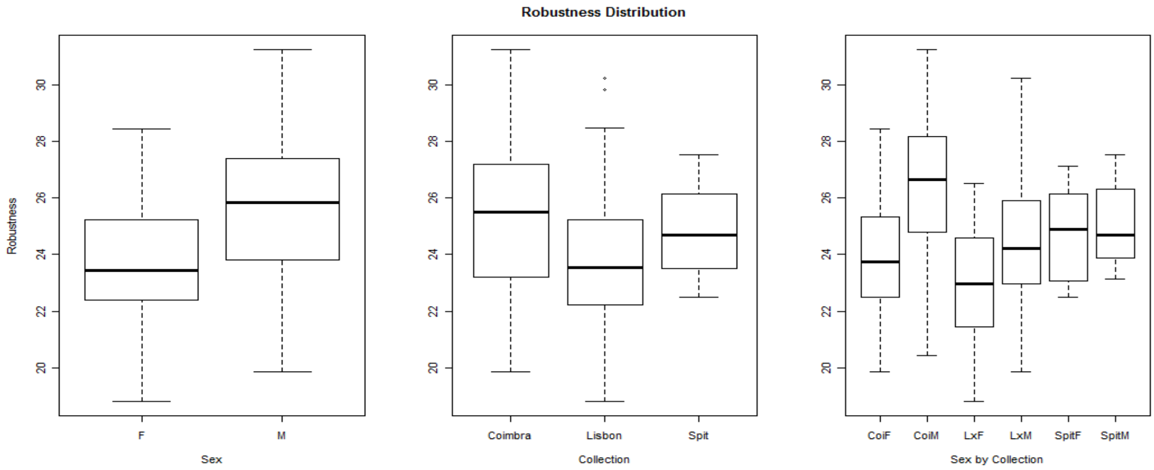

Figure 3. Robusticity distribution of the sample. Between (from left to right) sex, collection, and sex inside collection.

Figures 2 and 3 show results for age and robusticity. The most striking information that arises from both figures is the Lisbon Collection sample includes a greater proportion of younger and less robust individuals than the other 2 collections. As expected females are less robust than males, although this feature is less evident in the Spitalfields sample, in which the sex difference is much smaller. When comparing samples, the Spitalfield females are more robust than the Lisbon males.

2.1.1Variables

Besides sex, the influence on age of two other variables will be determined. This two variables were obtained through data collection: the Robusticity index (calculated by the expression: $Rob = \frac{maximum\ length}{perimeter\ in\ the\ middle\ of\ the\ \ shaft} x100$), and 3 different classification scales for degrees of fusion (stages) or fusion progression were recorded:

a) 3 Stages, that is the most commonly used, and the simplest to apply, as it only differentiates the development of the epiphysis in **unfused (stage 1)**, when the epiphyseal flake is unfused; **fusion/partial fusion (stage 2),** when the flake is present and only partially fused; and **fused (stage 3)**if the epiphyseal flake is completely and no fusion line is visible;

b) 4 Stages, that ads a new stage to the previous scheme: unfused (stage 1)**); epiphyseal flake is fusing and covers less than 50%** of the epiphysis (stage 2); epiphyseal flake is fusing and **covers 50% or more**(stage 3), and **complete fusion (stage 4)** (Figure 4);

c) 5 Stages, adds yet another stage to the previous scheme by dividing the unfused stage in two: **unfused with marked billowing** (stage 1) and **unfused with shallow billowing** (stage 2); the remaining 3 stages are the same as stages 2, 3 and 4 of the 4 stages scale.

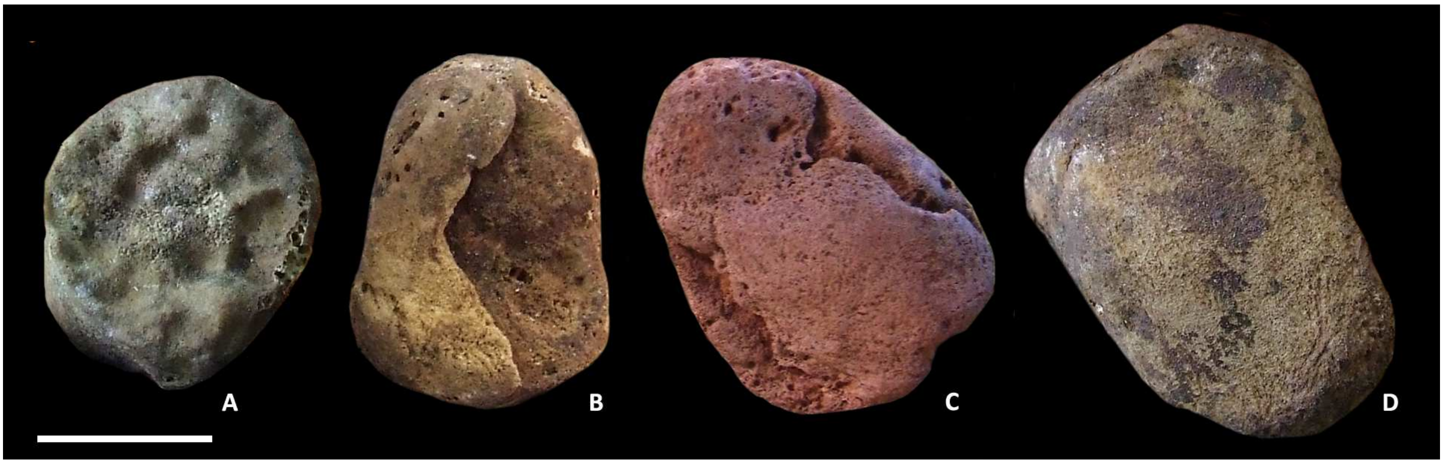

Figure 4. Four sternal epiphysis, illustrating the 4 degrees of Fusion.
A – stage1 (Lisbon female, 15yo); B – stage2 (Lisbon female, 21yo); C – stage3 (Coimbra male, 28yo); D – stage4 (Spitalfields male, 34yo). Scale:1 cm

2.2. Statistical Methodology

2.2.1 Descriptive analysis

Comparisons were conducted between the three collections in order to characterize more accurately our sample. Age and robusticity were the variables used to compare the collections at this point. Tests were chosen according to the power of the test, and the fulfilling of the assumptions. Therefore One Way ANOVA was performed to find differences between the robusticity of the three collections (normality and homoscedasticity were tested, details not shown). Once these differences were found a post hoc test (Scheffé) established where the differences were.

When we were unable to perform an ANOVA, the non-parametric alternative was used, namely the Kruskal-Wallis (K-W) test. When differences were found by this test, since there are no genuine post-hoc tests for K-W we resorted to performing Mann-Whitney-Wilcoxon tests 2 by 2. Because this test is not designed to be a multiple comparison test, it is necessary to implement Bonferroni correction in order to be able to use it for this purpose. So we divided the initial significance level (0.05) by the number of comparisons (3 – Lisbon vs Coimbra; Lisbon vs Spitalfields and Coimbra vs Spitalfields) obtaining a 0.0167 significance level to compare to the obtained p-values. Obviously this correction makes rejecting the null hypothesis (that there are no differences between the samples) more demanding.

Cohen's K analysis was performed in order to evaluate concordance in application of both 3 and 4 stages methods. 20 specimens of Lisbon collection were assigned a second time in these 2 methods and results were compared.

2.2.2 Transition analysis

This methodology was presented in 2002 by Boldsen and coworkers as an effort to establish a new approach for age estimation in both forensic and archaeological context, as it uses statistical modeling to determine the mean age for the transition between stages of development. Whether it is a 10 stage method for the pubic synphisis (Brooks and Suchey, 1990) or a 3 or 4 stage method as presented in this paper. Its statistical basis is to transform the general linear model equation presented by McCullagh and Nelder (1989), by converting the intercept and slope of the equation in the mean and standard deviation for a logistic distribution of the age at each transition of stage. In this early work (Boldsen *et al.*, 2002) a logit link function is used, but the software nphases2 developed by Lyle Konigsberg, uses a cumulative probit model in order to obtain the transition analysis parameters. This is the software that we use in order to compare transition ages and thus maturation timing between 3, 4 and 5 degrees of fusion for all collections individually and combined, as well as for males and females and sexes combined. This kind of analysis does not carry a significance level with it, meaning that any differences found cannot be assumed to be statistically relevant. Nevertheless they are without a doubt an invaluable indicator of differences between the compared groups. It is available for download at http://konig.la.utk.edu/nphases.exe.

2.2.3 Kaplan Meier

Survival analysis is a set of statistical methods, extensively used in biomedical research, that allow us to model time until one event of interest occur (Hosmer and Lemeshow, 1999), and takes into account the individuals that did not reach that event – censored data. One of the most commonly used methods in survival analysis is Kaplan-Meier's non-parametric method of estimation of the survivorship function (Kaplan and Meier, 1958). This function is the probability of observing a survival time (T) greater than or equal to some time (*t*): $S(t) = P(T \geq t)$.At any given moment S(t) is expressed as: $S(t) = \prod_{t_{(i)} \leq t} \frac{n_i - d_i}{n_i}$; where*n* is the number of subjects at risk of dying, and *d* is the number of deaths (events) (Hosmer and Lemeshow, 1999).

In this paper the event of interest was the attainment of a certain stage of fusion/maturation, so we will not have any censored data, since all our individuals have a degree of fusion assigned. All individuals will attain a certain stage of maturation. We will use it to compare the 3 different scales of degree of fusion used to see if the stages are separable and also it will allow us to further describe our sample, since it attributes a probability that a certain individual with a given degree of fusion has a determined age.

2.2.4 Ordinal Logistic Regression (OLR)

The ordinal logistic regression is part of a large family of statistical models called General Linear Models (GLM) (McCullagh and Nelder, 1989). Under this enormous array of methods we can find very well known tests such as ANOVA with continuous data as response, or the not less widely used logistic regression for binomial responses.

The OLR is widely used for example in surveys; in which responses can range from *totally disagree* to *totally agree*. There are several kinds of ordinal regression, but the most commonly used is the proportional odds model, with the logit as link function (Hosmer and Lemeshow, 2000). In this paper we use the cumulative probit, that is similar to the aforementioned, but it uses the function probit as link function. This model is less demanding in what the proportional odds assumption is concerned, because the nature of the link function does not allow a direct interpretation of the odds ratio, and since this interpretation was not the goal of our model, but obtaining the probabilities, all modeling described concerns a Cumulative Probit Model. It can be, parameterized such that:$G(x) = \theta_k - x'\beta$ (Hosmer and Lemeshow, 2000), where our θ_k are each one of the intercepts (1 for each of the equations provided by the model) given by the model output, and $x'\beta$ is the multiplication of the coefficient of each variable by its value.

The output of the model gives us one coefficient for each variable (categorical variables with more than two states will have 1 coefficient for each comparison with the baseline category, meaning that if one variable has 3 stages the model will provide us with 2 coefficients, one that compares stage 2 with stage 1 and the other that compare stage 3 with stage one) and k-1 intercept values, with k being the number of classes in the response variable.

The 5 stages method showed that not all categories were significant, so it was immediately set aside. Both 3 and 4 degrees method had all stages with statistical significance. Statistical significance between stages 2 and 3 of the 4 stage method was tested, and found (likelihood ratio test p value=0.0027). So, all models were constructed with 3 and 4 stages scheme.

We used this technique with two main purposes: first to investigate if the Collection influenced age estimation in order to corroborate our hypothesis of secular tendency for individuals to maturate earlier. Second we obtained several models to determine age at death, with more than one combination of variables in order to achieve maximum flexibility in the method´s application.

In order to adjust an ordinal logistic regression model, our response variable was categorized into classes. Several attempts were made to achieve a good balance between the width of the classes (as narrow as possible) and the goodness of the model in terms of explanatory ability. The final variable was a categorization into 7 classes:

1 – 15 to 17 years;
2 – 18 to 20 years;
3 – 21 to 23 years;
4 – 24 to 26 years;
5 – 27 to 29 years;
6– 30 to 32 years;
7– 33 to 35 years.

3. Results

3.1 Cohen´s K

Cohen´s K test (Table 2) shows that both classification schemes reveal a high level of concordance between the two observations, which makes them easy to apply, with consistent results.

Table 2. Results for the inter-observer error by Cohen´s K.

Method	Agreement	Expected Agreement	Kappa	Std. Err	Z	Prob>Z
3 Stages	94,74%	39,61%	0,9128	0,1756	5,2	< 0,0001
4 Stages	94,74%	36,01%	0,9177	0,1575	5,83	< 0,0001

3.2 Comparisons

The comparisons made, for robusticity, revealed that the only difference found was between Lisbon and Coimbra (Table 3). As shown earlier Coimbra individuals are more robust than Lisbon ones, and the difference in the means (23.70 – Lisbon and 25.32 – Coimbra) is highly significant (p-value <0.001).

Differences were found in the variable age between Lisbon collection and the other two. Figure 2 showed that Lisbon has the younger individuals, and it is statistically significant.

Table 3. Results for the comparison (between collections) tests performed for age and Robusticity.

			post-hoc			
Variable	Test	p-value	Test	Lx vs Coim	Lx vs Spit	Coim vs Spit
Age	Kruskal- Wallis	**0,001**	Mann-Whitney -Wilcoxon (with Bonferroni correction*)	**0,001**	**0,001**	0,318
Robusticity	ANOVA	**< 0,0001**	Scheffé	**< 0,0001**	0,219	0,804

3.3 Transition Analysis

Table 4 displays the results obtained with the help of nphases2 software (results for 3 stages are illustrated in figure 5). As we can see the age at transition for females is systematically lower than males, which indicates, as expected, that female have a faster maturation, attaining maturation earlier than males.

Table 4. Transition analysis parameters for 3, 4 and 5 degrees methods, obtained with nphases2 software.

Number of Stages	Transitions	All sample	Males	Females	Lisbon	Coimbra	Spitalfields
3	Stage 1-Stage 2	21,465	22,053	20,932	20,084	22,421	23,764
	Stage 2-Stage 3	24,698	26,041	23,897	23,545	25,801	25,646
	Std Dev	2,549	2,972	2,172	2,158	2,597	1,406
4	Stage 1-Stage 2	21,263	21,871	20,704	19,519	22,613	NA
	Stage 2-Stage 3	22,333	22,989	21,658	20,871	23,285	
	Stage 3-Stage 4	25,055	26,524	23,903	22,998	26,227	
	Std Dev	3,360	3,766	2,686	2,311	2,390	
5	Stage 1-Stage 2	14,305	14,213	14,716	11,913	16,342	NA
	Stage 2-Stage 3	20,992	21,540	20,488	19,525	22,327	
	Stage 3-Stage 4	22,174	22,762	21,567	20,866	23,052	
	Stage 4-Stage 5	25,113	26,547	24,002	22,981	26,194	
	Std Dev	3,919	4,366	3,218	2,281	3,883	

In what the collections are concerned there are obvious differences between the collections, especially when comparing Lisbon Collection with the other two, we found that Lisbon individuals maturate earlier than the others.

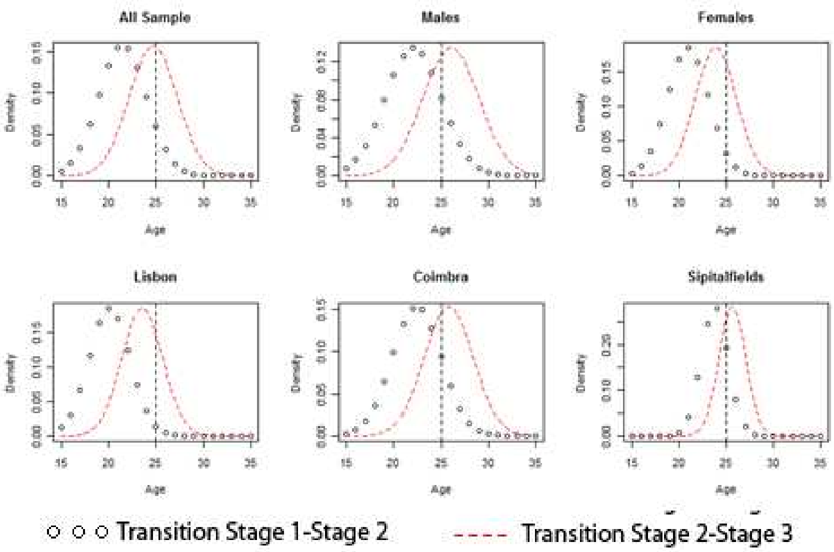

Figure 5. Age at transition plot for the 3 stages method. Vertical dashed line represents 25yo.

3.4 Kaplan-Meier

Figure 6 shows the survivorship function obtained by Kaplan-Meier, and the respective probabilities. As an example we can look at the horizontal dashed line in figure 6 and see that for example in the 3 degrees method:

- an individual in stage=1 has a 0.8 probability of having 16 years;
- an individual in stage=2 has P=0.8 of having 20 years;
- an individual in stage=3 has 0.8 probability of having 27 years.

Table 5. Results for the K-M curves comparison.

Number of stages	Comparison	p-value
3	Stage 1-Stage 2	<0,0001
	Stage 2-Stage 3	<0,0001
4	Stage 1-Stage 2	0,028
	Stage 2-Stage 3	<0,0001
	Stage 3-Stage 4	<0,0001
5	Stage 1-Stage 2	0,134
	Stage 2-Stage 3	0,0709
	Stage 3-Stage 4	<0,0001
	Stage 4-Stage 5	<0,0001

Comparison of the curves showed that all Kaplan-Meier curves are statistically different for both 3 and 4 degrees method. For 5 degrees the tests show that only stages 3, 4 and 5 are different (Table 5).

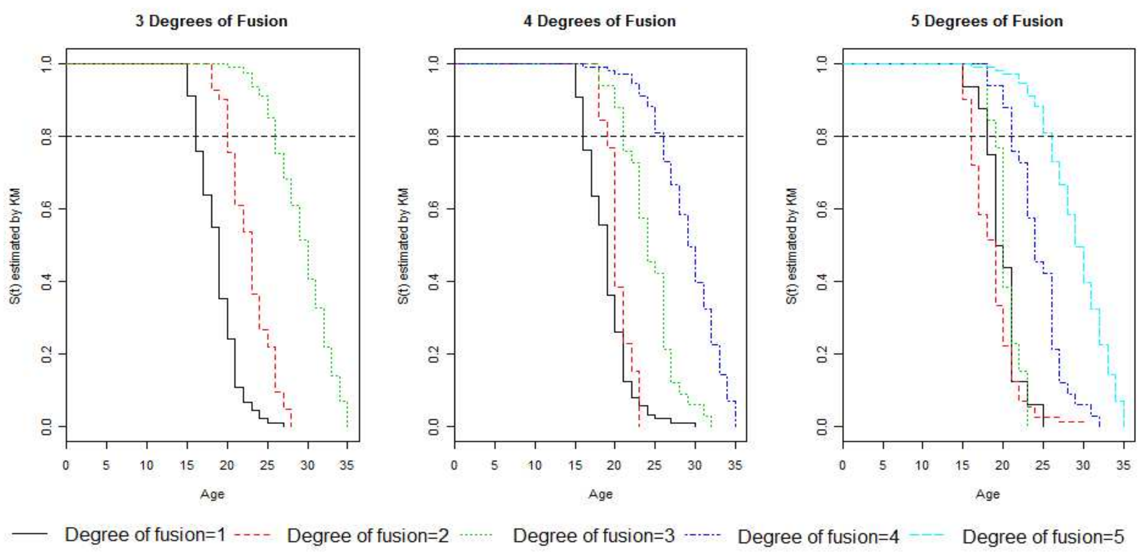

Figure 6. Kaplan-Meier survivorship functions for each method (3, 4 and 5 Degrees of fusion). the dashed horizontal line represent a 0.8 probability, that correspond to an age in the horizontal axis.

3.5 OLR Model:

Several models were constructed to achieve maximum flexibility in the applicability of the method. The following description is of a model with variable degree of fusion in 3 categories, shown as an example of the modeling technique. Other probability tables will be shown.

Univariate analysis showed that Degree of fusion was highly significant (p value< 0.0001), unlike robusticity (p value =0.180) and sex (p value =0.451). Hosmer and Lemeshow (2000) guidelines state that after univariate analysis, the variables with p values < 0.25 must be modeled together. When degree of fusion and robusticity were run together they both were significant (p value <0.0001 and 0.001 respectively). At this point sex was introduced in the model to see if its behavior changed. Although the p value decreased (0.301) it was still not significant. It was decided to keep it in the model, because it can be an important factor in development.

Complete residues analysis was conducted (details not shown), showing a few points that could be outliers and/or influential observations. After carefully reviewing the individuals, it was decided to remove specimen #77 from the Coimbra collection since there was a suspicion concerning the accuracy of the biographical information of this individual. The records in this collection are handwritten, and a transcription error may have resulted in an inaccurate age record. Specimen #314 from the Lisbon collection was re-assessed as it was unclear from the photograph which degree of fusion this individual should be assigned to. After a re-assessment it was decided to re-assign the individual to stage2 instead of the stage 3. We then obtained a final model without interactions (Table 6).

Table 6. Output for the Ordinal Logistic Regression Model (Cumulative Probit).

		Value	Std. Error	Wald Z	p value
Coefficients	Stage 2	1,5599	0,2392	6,5200	< 0,0001
	Stage 3	3,4719	0,2684	12,9400	< 0,0001
	rob	0,0911	0,0321	2,8400	0,0050
	sex	-0,1701	0,1646	-1,0300	0,3010
Intercepts	1|2	1,8001	0,8377		
	2|3	2,9810	0,8485		
	3|4	4,0323	0,8695		
	4|5	4,9407	0,8909		
	5|6	5,6435	0,9034		
	6|7	6,4021	0,9113		

Before obtaining the probabilities from this model, the variable collection was inserted in the model to interpret its behavior, knowing that it could not enter in the final model otherwise the model could only make predictions within these collections. Nevertheless this exercise allowed us to evaluate whether the collections are significant, and the positive coefficient that was obtained is interpreted in this way: an individual with the same robusticity, sex and degree of fusion has a higher possibility of being in an upper age class if it belongs to a collection other than Lisbon.

From table 6 and the probit model presented earlier we have:

$$x'\beta = 1.5599 * Stage2 + 3.4719 * Stage3 + 0.0911 * Robusticity - 0.1701 * Sex$$

So our 6 equations are:

$$G(y \leq 1) = 1.8001 - (1.5599 * Stage2 + 3.4719 * Stage3 + 0.0911 * Robusticity - 0.1701 * Sex)$$

$$G(y \leq 2) = 2.9810 - (1.5599 * Stage2 + 3.4719 * Stage3 + 0.0911 * Robusticity - 0.1701 * Sex)$$

$$G(y \leq 3) = 4.0323 - (1.5599 * Stage2 + 3.4719 * Stage3 + 0.0911 * Robusticity - 0.1701 * Sex)$$

$$G(y \leq 4) = 4.9407 - (1.5599 * Stage2 + 3.4719 * Stage3 + 0.0911 * Robusticity - 0.1701 * Sex)$$

$$G(y \leq 5) = 5.6435 - (1.5599 * Stage2 + 3.4719 * Stage3 + 0.0911 * Robusticity - 0.1701 * Sex)$$

$$G(y \leq 6) = 6.4021 - (1.5599 * Stage2 + 3.4719 * Stage3 + 0.0911 * Robusticity - 0.1701 * Sex)$$

After getting the model it is possible to obtain the probability of an individual with a given profile being in a certain age class. Before providing an example of how to use the equations, a preliminary note is needed. The variable degree of fusion has 3 categories, so software computation transforms it into a dummy variable in order to be able to work with the binary code. Consequently, Values have to be inserted as shown in table 7.

Table 7. Transformation of variable degree of fusion in dummy variables.

Degree of Fusion	Value on the model equation	
	Stage2	Stage3
1	0	0
2	1	0
3	0	1

3.5.1. Example

If we want to determine age at death from a skeleton using this method a few steps must be made:

Step 1

Define the profile. For example if we have a John Doe: male, robusticity of 22 and degree of fusion 2. This means that the values to replace in the equations are: Stage2 = 1; Stage3 =0; Robusticity=22; Sex=0;

Step 2

Replace values in all equations (values in bold):

$$G(y \leq 1) = 1.8001 - (1.5599 * \mathbf{1} + 3.4719 * \mathbf{0} + 0.0911 * \mathbf{22} - 0.1701 * \mathbf{0}) = -1.7650$$

$$G(y \leq 2) = 2.9810 - (1.5599 * \mathbf{1} + 3.4719 * \mathbf{0} + 0.0911 * \mathbf{22} - 0.1701 * \mathbf{0}) = -0.5841$$

$$G(y \leq 3) = 4.0323 - (1.5599 * \mathbf{1} + 3.4719 * \mathbf{0} + 0.0911 * \mathbf{22} - 0.1701 * \mathbf{0}) = 0.4673$$

$$G(y \leq 4) = 4.9407 - (1.5599 * \mathbf{1} + 3.4719 * \mathbf{0} + 0.0911 * \mathbf{22} - 0.1701 * \mathbf{0}) = 1.3756$$

$$G(y \leq 5) = 5.6435 - (1.5599 * \mathbf{1} + 3.4719 * \mathbf{0} + 0.0911 * \mathbf{22} - 0.1701 * \mathbf{0}) = 2.0785$$

$$G(y \leq 6) = 6.4021 - (1.5599 * \mathbf{1} + 3.4719 * \mathbf{0} + 0.0911 * \mathbf{22} - 0.1701 * \mathbf{0}) = 2.8371$$

Step 3

Obtain probability associated with each value obtained. This can be achieved easily with for example R with the code: *pnorm(value)*or in EXCEL with the function *normsdist(value)*. In our case we have:

$$P(y \leq 1) = pnorm(-1.7650) = 0.0388$$

$$P(y \leq 2) = pnorm(-0.5841) = 0.2796$$

$$P(y \leq 3) = pnorm(0.4673) = 0.6798$$

$$P(y \leq 4) = pnorm(1.3756) = 0.9155$$

$$P(y \leq 5) = pnorm(2.0785) = 0.9812$$

$$P(y \leq 6) = pnorm(2.8371) = 0.9977$$

$$P(y \leq 7) = 1$$

Note that there is a 7th equation (P≤7), because we are talking about cumulative probabilities, so the probability of a individual being in the class 7 or lower is always 1.

At this point we can say that our John Doe has about 68% ((P(y≤3)=0.6798) chance of being less than 23 years old, but only 4% chance of being less than 17 years of age (P(y≤1)).

Step 4

Because we obtained cumulative probabilities, in order to get exact ones we have to subtract the previous ones:

$$P(y = 1) = P(y \leq 1) = 0.0388$$

$$P(y = 2) = P(y \leq 2) - P(y \leq 1) = 0.2796 - 0.0388 = 0.2408$$

$$P(y = 3) = P(y \leq 3) - P(y \leq 2) = 0.6798 - 0.2796 = 0.4003$$

$$P(y = 4) = P(y \leq 4) - P(y \leq 3) = 0.9155 - 0.6798 = 0.2357$$

$$P(y = 5) = P(y \leq 5) - P(y \leq 4) = 0.9812 - 0.9155 = 0.0656$$

$$P(y = 6) = P(y \leq 6) - P(y \leq 5) = 0.9977 - 0.9812 = 0.0166$$

$$P(y = 7) = P(y \leq 7) - P(y \leq 6) = 1 - 0.9977 = 0.0023$$

So we can say that John Doe has a 0.4003 probability (40%) of being in class 3 of age (21-23 years), or that it has 0.6411 (0.2408+0.4003) probability of being between 18 and 23 years of age at the time of his death.

All this steps can be made rather quickly by using an Excel worksheet that has been constructed and made available in the annex CD. Then all you need is to input the values on the profile and obtain immediately the desired probabilities.

The previous description was an example. Three more models were constructed under the same guidelines. One with degree of fusion in 3 stages, but without sex, and the same for the 4 stages scheme (With and without sex). All the models probabilities can be obtained using the attached excel file. Tables 8 and 9show posterior probabilities calculated for different profiles for 3 and 4 Stages method, namely all stages of fusion, quartiles of robusticity, for males and females. It is obvious that it does not cover the entire range of robusticity since it is a continuous variable, but it gives a good idea on the behavior of the probabilities.

Table 8. Probabilities calculated for different profiles for the 3 stages method

		Males							Females						
Robusticity	Degree of fusion	15-17	18-20	21-23	24-26	27-29	30-32	33-35	15-17	18-20	21-23	24-26	27-29	30-32	33-35
18,8	1	0,5345	0,3630	0,0923	0,0096	0,0006	0,0000	0,0000	0,6013	0,3234	0,0689	0,0061	0,0003	0,0000	0,0000
	2	0,0703	0,3147	0,3911	0,1762	0,0388	0,0080	0,0009	0,0963	0,3551	0,3722	0,1434	0,0275	0,0051	0,0005
	3	0,0004	0,0134	0,1107	0,2789	0,2732	0,2116	0,1119	0,0007	0,0203	0,1419	0,3075	0,2648	0,1822	0,0827
22,9	1	0,3870	0,4273	0,1598	0,0237	0,0020	0,0002	0,0000	0,4535	0,4029	0,1265	0,0160	0,0012	0,0001	0,0000
	2	0,0324	0,2203	0,3973	0,2521	0,0750	0,0200	0,0029	0,0468	0,2632	0,4007	0,2177	0,0565	0,0134	0,0017
	3	0,0001	0,0049	0,0584	0,2047	0,2655	0,2668	0,1996	0,0002	0,0079	0,0794	0,2395	0,2735	0,2440	0,1555
24,44	1	0,3345	0,4399	0,1900	0,0322	0,0030	0,0003	0,0000	0,3985	0,4237	0,1537	0,0222	0,0018	0,0002	0,0000
	2	0,0234	0,1866	0,3867	0,2789	0,0927	0,0273	0,0045	0,0346	0,2277	0,3986	0,2462	0,0715	0,0187	0,0027
	3	0,0000	0,0032	0,0445	0,1763	0,2537	0,2812	0,2411	0,0001	0,0053	0,0618	0,2109	0,2674	0,2632	0,1914
26,52	1	0,2686	0,4450	0,2333	0,0473	0,0052	0,0006	0,0000	0,3275	0,4411	0,1944	0,0336	0,0032	0,0003	0,0000
	2	0,0147	0,1449	0,3624	0,3103	0,1198	0,0402	0,0077	0,0224	0,1821	0,3847	0,2824	0,0953	0,0284	0,0047
	3	0,0000	0,0018	0,0299	0,1398	0,2316	0,2930	0,3039	0,0000	0,0030	0,0428	0,1724	0,2517	0,2828	0,2472
31,25	1	0,1473	0,4055	0,3290	0,1000	0,0156	0,0024	0,0002	0,1900	0,4291	0,2931	0,0760	0,0103	0,0014	0,0001
	2	0,0046	0,0722	0,2768	0,3493	0,1888	0,0853	0,0231	0,0074	0,0970	0,3142	0,3404	0,1612	0,0647	0,0152
	3	0,0000	0,0004	0,0107	0,0728	0,1654	0,2833	0,4673	0,0000	0,0008	0,0163	0,0962	0,1930	0,2933	0,4005

Table 9. Probabilities calculated for different profiles, for the 4 stages method

		Males							Females						
Robusticity	Degree of fusion	15-17	18-20	21-23	24-26	27-29	30-32	33-35	15-17	18-20	21-23	24-26	27-29	30-32	33-35
18,8	1	0,5127	0,3586	0,1096	0,0170	0,0018	0,0002	0,0000	0,5816	0,3227	0,0834	0,0111	0,0010	0,0001	0,0000
	2	0,2555	0,4157	0,2456	0,0687	0,0121	0,0021	0,0002	0,3145	0,4171	0,2089	0,0504	0,0078	0,0012	0,0001
	3	0,0371	0,2097	0,3544	0,2539	0,1006	0,0369	0,0074	0,0536	0,2513	0,3618	0,2245	0,0786	0,0258	0,0045
	4	0,0011	0,0236	0,1281	0,2592	0,2513	0,2126	0,1240	0,0019	0,0347	0,1610	0,2832	0,2436	0,1838	0,0919
22,9	1	0,3909	0,4040	0,1663	0,0337	0,0045	0,0006	0,0000	0,4591	0,3818	0,1329	0,0232	0,0027	0,0003	0,0000
	2	0,1670	0,3865	0,3055	0,1108	0,0244	0,0053	0,0006	0,2142	0,4070	0,2731	0,0856	0,0166	0,0032	0,0003
	3	0,0181	0,1421	0,3189	0,2942	0,1450	0,0650	0,0167	0,0274	0,1789	0,3422	0,2738	0,1193	0,0478	0,0107
	4	0,0004	0,0111	0,0797	0,2064	0,2472	0,2564	0,1987	0,0007	0,0172	0,1053	0,2373	0,2524	0,2333	0,1538
24,44	1	0,3472	0,4133	0,1900	0,0425	0,0061	0,0009	0,0001	0,4134	0,3977	0,1548	0,0298	0,0038	0,0005	0,0000
	2	0,1396	0,3678	0,3240	0,1296	0,0310	0,0072	0,0008	0,1819	0,3944	0,2952	0,1020	0,0215	0,0045	0,0005
	3	0,0135	0,1200	0,2995	0,3037	0,1625	0,0785	0,0222	0,0209	0,1539	0,3275	0,2881	0,1363	0,0589	0,0144
	4	0,0002	0,0082	0,0652	0,1851	0,2400	0,2687	0,2326	0,0005	0,0129	0,0878	0,2169	0,2498	0,2493	0,1829
26,52	1	0,2913	0,4179	0,2230	0,0569	0,0092	0,0015	0,0001	0,3536	0,4122	0,1864	0,0410	0,0059	0,0009	0,0001
	2	0,1077	0,3373	0,3439	0,1568	0,0420	0,0108	0,0014	0,1435	0,3708	0,3214	0,1267	0,0299	0,0069	0,0008
	3	0,0090	0,0937	0,2698	0,3108	0,1855	0,0993	0,0318	0,0142	0,1232	0,3026	0,3025	0,1598	0,0764	0,0213
	4	0,0001	0,0053	0,0487	0,1566	0,2257	0,2804	0,2831	0,0003	0,0086	0,0673	0,1883	0,2412	0,2670	0,2273
31,25	1	0,1825	0,3947	0,2948	0,1017	0,0214	0,0045	0,0005	0,2321	0,4117	0,2610	0,0778	0,0144	0,0027	0,0002
	2	0,0553	0,2551	0,3619	0,2217	0,0767	0,0249	0,0043	0,0776	0,2967	0,3583	0,1900	0,0580	0,0168	0,0026
	3	0,0032	0,0491	0,1954	0,3002	0,2297	0,1552	0,0671	0,0054	0,0683	0,2322	0,3099	0,2102	0,1267	0,0472
	4	0,0000	0,0018	0,0230	0,0981	0,1798	0,2832	0,4140	0,0001	0,0031	0,0337	0,1252	0,2041	0,2861	0,3478

If we find a clavicle, that it is not complete, and/or we cannot determine the sex or calculate the robusticity index, the previous models are quite useless, so in addition we provide other tables with the degree of fusion as only variable, and with age divided into 1 year intervals. This time all possible values are given, so it is just a matter of checking table 10 in order to obtain the posterior probability of a subject with a given degree of fusion having a certain age. As expected probabilities in 1 year age intervals are much smaller, so it is the choice of the user to build age intervals as needed, one just have to sum the probabilities of the ages one wants. For example, and individual of undetermined sex and Stage 1 has 0.1480 probability of having 16 years old, but if we broaden the age interval to 16 to 18 for example we find a 0.3743 probability (0.1480+0.1163+0.1100 in bold). We have to advise that obviously this model has a much lower adjustment to the data, once the response variable has 20 categories. It is shown here only as an alternative if the investigator is unable to use the complete model.

Table 10. Probabilities calculated for a model without robusticity, and with age in 1 year intervals.

Stage 1	Stage 2	Stage 3	Age	Stage 1	Stage 2	Stage 3	Stage 4
0,0846	0,0021	0,0000	15	0,0930	0,0240	0,0007	0,0000
0,1480	0,0111	0,0000	16	0,1545	0,0665	0,0047	0,0001
0,1163	0,0170	0,0000	17	0,1126	0,0649	0,0077	0,0002
0,1100	0,0254	0,0001	18	0,1060	0,0741	0,0125	0,0005
0,1762	0,0707	0,0006	19	0,1659	0,1457	0,0379	0,0022
0,1379	0,1032	0,0020	20	0,1292	0,1467	0,0610	0,0057
0,1155	0,1639	0,0076	21	0,1124	0,1655	0,1116	0,0170
0,0432	0,1065	0,0101	22	0,0431	0,0794	0,0799	0,0181
0,0374	0,1477	0,0274	23	0,0400	0,0886	0,1255	0,0405
0,0175	0,1184	0,0457	24	0,0167	0,0444	0,0878	0,0395
0,0051	0,0519	0,0341	25	0,0112	0,0344	0,0890	0,0527
0,0055	0,0833	0,0971	26	0,0088	0,0324	0,1163	0,0967
0,0015	0,0368	0,0817	27	0,0030	0,0133	0,0668	0,0776
0,0007	0,0234	0,0845	28	0,0016	0,0078	0,0507	0,0761
0,0003	0,0166	0,0971	29	0,0009	0,0054	0,0443	0,0858
0,0001	0,0107	0,1059	30	0,0005	0,0034	0,0370	0,0947
0,0000	0,0049	0,0794	31	0,0002	0,0015	0,0215	0,0720
0,0000	0,0039	0,1059	32	0,0001	0,0012	0,0219	0,0987
0,0000	0,0015	0,0795	33	0,0000	0,0004	0,0117	0,0752
0,0000	0,0007	0,0706	34	0,0000	0,0002	0,0071	0,0668
0,0000	0,0002	0,0706	35	0,0000	0,0001	0,0042	0,0801

4. Discussion

Descriptive statistical analysis showed differences between the collections studied, especially between Lisbon collection and the other two, and these results were corroborated when we introduced the variable “collection” in the model, and it was found to be significant in both 3 and 4 stage classification models. There seems to be an earlier maturation in Lisbon individuals, compared to the other two collections. Because Lisbon collection is more recent we can infer that the results reveal a secular trend towards the acceleration of maturation. This tendency has been documented in hand-wrist maturation (Lin, et al 2006; Ahmed and Warner 2007), and it has been linked to improvements in life conditions. Hawley and co-workers (2009) documented secular trends in black South African urban children in a period of only 40 years. They attribute this secular increase in skeletal maturity to the improvement of life conditions for black children in Pretoria from the 1960’s to the 21st century. As said earlier the Lisbon collection consists mostly of elements of an urban environment (Cardoso, 2006) while Coimbra collection is not only form an earlier period but also a rural population (Fernandes, 1985)(Spitalfields will not be discussed concerning secular trend because the sample consisted of only 21 individuals). The statistical differences found between Lisbon and Coimbra collections are due to the differences in living conditions experienced by the individuals of these two collections.

Shirley and Jantz (2010) also documented a secular acceleration in skeletal maturation when comparing the individuals in the McCormick collection (from late 20th century) and those of the Hamman-Todd Collection (from early 20th century). They found statistical differences for males of these two collections. Although they have differences that are statistically significant, a closer look to the transition ages for McCormick ads Hamman-Todd males show that the difference is mainly in the first transition (almost 4.5 years), because the last transition occurs at a more closer age, and mainly the range of development between the two collections point in a different direction. Meaning that in McCormick collection the difference between the first and last transition is of 10 years (26.09-16.19) and in Hamman-Todd is only 7 years (27.94-20.59). This is an odd result, because a more accelerated maturation would mean not only an earlier beginning, but also a faster development. This apparent paradox was not discussed by the authors. In this matter our results show consistency. One look at table 4 reveals that comparing Lisbon and Coimbra ages of transition, not only Lisbon begins earlier but ends faster than Coimbra showing a consistent acceleration in maturation. When comparing our transition ages with the ones found by Shirley and Jantz (2010), we see that the age of the first transition is similar in Lisbon and Todd collection. Lisbon sample revealed an age for the first transition (3 stage method, sexes combined) of 20.084 years while Todd males exhibit 20.500 and females 19.240. The main difference is again in the time of maturation, because Lisbon specimens take only 3.5 years to reach the 2nd transition, while Todd collection individuals need at least twice that time (6 years for females, and 7 for males). The conclusion seems that Lisbon individuals have in fact a more accelerated maturation than Todd collection, which strengthens our hypothesis of secular acceleration.

Results show that Black and Scheuer (1996) 5 stage method is not easily applicable, as no statistical differences were found between stages 1 and 2 in our data, and these stages are often hard and subjective to distinguish. Because they have a small sample it is not easy to fairly compare results, but it is curious that they find that Spitalfields has earlier and faster maturation that Lisbon individuals. At the time of their work only a few individuals within these age ranges were available in the Lisbon collection (87 are used in the present study), and this can be one of the reasons for this result.

Studies on the clavicle provide valuable information on age estimation, especially after the age of 20 years. Studies have shown that most epiphysis are fused at the age of 18 (Cardoso, 2008), and our results show that at this age the clavicle epiphysis did not begin its formation in many cases. For this reason, the studies conducted on clavicles are important as they provide information in the ages between early 20's and early 30´s, a decade that lacks both the developmental factors in sub-adult studies and the degenerative indicators in which adult studies are based.

The combination of several collections of different times and backgrounds provide an increase in the variability of the sample and consequently in the application of the method, because the background of an unknown subject whether it is in forensic or archeological context can be very variable. It is not correct to say that all individuals that live today have an earlier and faster maturation than individuals that lived one century ago, because it depends on more factors like the socio-economic background that is very variable within the same time frame. Living conditions is several developing nations are similar to that of Lisbon in the early 20th century, and it is in these populations that this method may also work best. We believe that this increase in variability is in fact a positive factor in the applicability of the provided method.

The modeling strategy undertaken, led us to strong probabilistic estimates for age at death, and introduced the variable robusticity as significant for age determination. The results show that a higher robusticity index tends to lead to an older individual when comparing with other that have the same sex and degree of fusion. Thus increased robusticity is associated with delayed maturation. The full extent of this association between robusticity and development was not completely clear, but since Lisbon specimens are less robust and maturate faster than the other collections, maybe the higher robusticity in Coimbra and Spitalfields associated with a larger number of specimens (138 Coimbra+21 Spitalfields against 87 in Lisbon) is producing an artificial association with maturation.

On the other hand sex was found not to be significant. This can be linked to the late development of the clavicle, whose epiphysis begins to fuse after menarche age, and thus diluting the effect of sex on development.

Another advantage of our method are the narrower age intervals used, that combined with the probabilistic approach leads to a higher level of accuracy, that is especially important in forensic context, in which a narrow interval can dictate the difference between a positive identification and a negative one. But also in archaeological context, where many time a small piece of bone is all that is found of a single specimen.

5. Conclusion

This paper presents results that point to a secular trend in skeletal maturation. It is shown that more recent individuals especially that have improved life conditions have an earlier and faster maturation.

The methods proved in this study are easy to apply as the several models constructed provides a high degree of flexibility, whether we have a complete clavicle from a complete skeleton or just the medial half of a single clavicle. However, further studies should be conducted in order to confirm these results and other models with more variable should be constructed, in order to increase accuracy of the model estimated probabilities.

The presented methods are statistically significant, but mainly they are flexible and easy to apply whether it is in forensic and archeological context.

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