Exploring the relationship between entheseal changes and physical activity: a multivariate study

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ABSTRACT

Analyses of entheseal changes (EC) in identified skeletal samples employ a common research strategy based on the comparison between occupations grouped on the basis of shared biomechanical and/or social characteristics. Results from this approach are often ambiguous, with some studies that point to differences in EC between occupational samples and others failing to provide evidence of behavioral effects on EC. Here we investigate patterns of EC among documented occupations by means of a multivariate analysis of robusticity scores in nine postcranial entheses from a large (N=372) contemporary skeletal sample including specimens from one Italian and two Portuguese identified collections. Data on entheseal robusticity, analyzed by pooled sides as well by separated sides and levels of asymmetry, are converted in binary scores and then analyzed through nonlinear principal component analysis and hierarchical cluster analysis. Results of these analyses are then used for the classification of occupations. Differences between occupational classes are tested by MANOVA and pairwise Hotelling's test. Results evidence three classes which separate occupations related to farming, physically demanding but generalized occupation, and physically undemanding occupations, with the more consistent differences between the first and the last classes. Our results are consistent with differences in biomechanical behavior between the occupations included in each class, and point to the physical and social specificity of farming activities. On the other hand, our study exemplifies the usefulness of alternative analytical protocols for the investigation of EC, and the value of research designs devoid of *a priori* assumptions for the test of biocultural hypotheses.

INTRODUCTION

The last decades have seen a resurgence of studies on entheseal changes (EC) and their reliability as "skeletal markers of activity" (Jurmain 1999; Jurmain et al. 2012 and authors therein). The analysis of identified human skeletal collections (IHSC) played a key role in this regard, allowing to test the effects of variables like age, sex and biomechanical stress on the expression of EC (Alves Cardoso and Henderson 2010; Belcastro et al. 2007; Mariotti et al. 2004; Milella et al. 2012; Niinimäki 2011; Niinimäki and Baiges Sotos 2013; Villotte et al. 2010). While some studies confirmed a correlation between EC and biomechanical stress (Niinimäki 2011; Villotte 2009; Villotte et al. 2010 – but only for fibrocartilaginous entheses), others highlighted the overruling role played by physiological features such as age and sex on EC variance (Alves Cardoso and Henderson 2010, 2013; Henderson et al. 2013; Milella et al. 2012; Perréard Lopreno et al. 2013; Villotte 2009 - but mainly for fibrous entheses). Contrasting results on the reliability of EC as skeletal markers of activity can be linked to methodological differences (e.g. inter-observer differences in scoring methods and/or in investigated sites) as well as to different approaches used for controlling age, sex, and other relevant factors (e.g. body size, body mass).

Most anthropological studies on IHSC classify occupation according to either socio-cultural or biomechanical criteria. A socio-cultural criterion – the division of tasks based on the concept of gender and their male and female categorization – was explicitly chosen by Alves Cardoso (2008) when testing the correlation between EC and degenerative joint changes (DJC) and known occupations. The assumption underlying this research was that the EC and DJC would reflect the sexual division of labor that would mirror gender constructs associated with sex-specific tasks performed by male and female individuals. The biological sex was therefore used as a proxy to gender, mediated by changes in the skeletons which were defined as markers of occupational stress: but gender and sex were always understood as separate categories. The socio-cultural criterion was chosen in order to allow inferences on possible gender differences in habitual activities in Portugal between the 19th and 20th centuries to explore this topic in a framework that allowed controlling variables capable of biasing the research, such as: sex, age at death and occupation.

To date, however, the more extensively used criterion is biomechanics, which classifies occupation according to the type and degree of associated biomechanical strain (Milella et al. 2012; Niinimäki and Baiges Sotos 2013; Villotte et al. 2010). Despite a shared theoretical background, biomechanical classifications vary largely among authors (Perréard Lopreno et al. 2013), and therefore prevent comparisons between different studies. In this sense, biomechanical criteria appear often affected by the same subjectivity biasing socio-cultural criteria. Another issue common to most studies on IHSC is the possible bias introduced by the adopted classification for the results of the study. The research design shared by most studies on EC consists in testing the consistency between patterns of EC and assumed social or biomechanical categories, by employing customary traditional parametric or nonparametric univariate and bivariate statistical protocols. However, the reliability of the chosen categories (i.e. their real consistency with the lifestyle and life history of the subject) is generally unverifiable, especially due to the inconsistent documentary information available for most of past occupations. Accordingly, this approach is prone to mask the real patterns underlying the data, leading to problems in the interpretation of the results. An alternative approach consists of classifying the various occupations based on the observed similarities between individuals without *a priori* established categories. Such a strategy would allow a subdivision of the sample that is much more consistent with the observed patterns of changes, therefore avoiding problems represented by both social and biomechanical classifications. From a statistical point of view, the use of eigenvector-based multivariate procedures (e.g. principal component analysis - PCA) seems particularly suited for an approach that aims to explore patterns of similarity/dissimilarity in a sample with regard to several variables without the need of *a priori* assumptions. Sperduti (1997) and Robb (1998) were the first to apply a multivariate approach to the study of EC. Other examples of alternative statistical approaches to the study of EC include the use of principal component analysis by Porčić and Stefanović (2009), Stefanović and Porčić (2013), and Takigawa (2014), and the use of generalized estimating equations by Villotte (2009) and Villotte and colleagues (2010).

In this study, we attempt to categorize occupations on the basis of a multivariate analysis of EC using a large (N=372) identified human skeletal sample. The aim of the study is to explore possible patterns of similarities in EC between occupations and to test if the same patterns represent a valid basis for classifying occupations.

To do so, we postulate the following hypotheses:

H1) Assuming that EC, when controlling for age, reflect biomechanical and social differences among occupations, we postulate that distinct groups of documented occupations should be reflected by specific patterns of EC;

H2) If H1 is confirmed, we postulate that the identified subgroups share consistencies from a biomechanical and/or social point of view, described through documented data obtained through bibliographic research.

MATERIAL AND METHODS

The sample consists of 372 male individuals with known occupation from one Italian identified human skeletal collection: the Frassetto collection of Sassari (Sardinia) (SISC), housed at the Museum of Anthropology of the University of Bologna (N= 136, Milella et al. 2012); and from two merged Portuguese collections (Table 1). The identified human skeletal collection of Coimbra (CISC), housed at the Department of Life Sciences in Coimbra University (Rocha, 1995) (former Department of Anthropology), and the Luis Lopes Collection (LLISC) (Cardoso 2006) housed at the Museum of Natural History in Lisbon (N = 236) were considered as a unique sample due to their similar chronological, historical, social and cultural settings (Alves Cardoso, 2008).

The major rationale for comparing these identified human skeletal collections (IHSC) is their chronological and cultural consistency. Both the Italian and the Portuguese collection represent pre-industrial (beginning of the 20th century) societies and are marked by an overlap regarding the documented occupations.

Only males were selected for analysis due to the scarce and ambiguous information on female's occupation. Female individuals in all considered collections are indeed mostly classified as "housewives", a term that, due to its generality, unfortunately hampers an exploration of the possible relationship between EC and occupation in females.

We expect that, even allowing for population differences, their individual signals should not be strong enough to consistently bias our analyses. Specimens were chosen on the basis of the following criteria: (i) age at death ≥ 20 years of age; (ii) absence of pathologies possibly linked to extra-spinal enthesopathy formation (e.g. DISH) (Freemont 2002; Jurmain 1999; Martin-Dupont et al. 2006; Rogers and Waldron 1995); and (iii) absence of skeletal changes possibly linked to altered body biomechanics (fractures, dislocations, and dysplasias). The cutoff point of 20 years of age-at-death was selected so that the sample would be representative of biologically mature adults, with almost all epiphyses fused.

Data on EC were originally collected by Milella and colleagues on SISC (Milella et al. 2012) and by Alves Cardoso on CISC and LLISC (Alves Cardoso 2008). In order to allow comparison between datasets, we considered only data on entheseal surface rugosity - "robusticity" - *sensu* Mariotti et al. (2007), and Hawkey and Merbs (1995). These changes were originally scored by Milella and colleagues (2012) applying five degrees (from 0 to 4), following Mariotti et al. (2007), and by Alves Cardoso (2008) applying four degrees (from 0 to 3) according to Hawkey and Merbs (1995). In a second step the original scores were then converted into binary data, applying specific criteria to each dataset according to their different theoretical backgrounds (Table S1). Data from Milella and colleagues (2012) were classified as absence (0) and presence (1). Absence would include the original grades 0 and 1, while presence the grades 2, 3, and 4. A different conversion was used for the data collected by Alves Cardoso (2008), which considered enthesis type (fibrous vs. fibrocartilaginous). Accordingly, absence (0) of a fibrous enthesis corresponds to the original grades 0 and 1, and in fibrocartilaginous entheses to the grade 0. Presence (1) in fibrous entheses corresponds to the grades 2 and 3, while in fibrocartilaginous entheses to the grades1, 2, and 3. The criterion underlying the different conversion of fibrous and fibrocartilaginous sites is based on their distinct skeletal morphology, which, in the first case, is represented by smooth areas, while in fibrocartilaginous sites by rough surfaces. The described strategy, which dichotomizes robusticity development, was chosen since it minimizes the bias introduced by differences in the used scoring methods, allowing therefore a better (though admittedly not perfect) comparability between observations.

Only sites analysed by both Milella and colleagues (2012) and Alves Cardoso (2008) were considered. This led to a total of nine postcranial entheses, analysed by considering the two sides separately (Table 2). In the case of the costoclavicular ligament, a distinction between fibrous vs. fibrocartilaginous histology is not possible. Accordingly, the authors decided to apply in this case the same criteria adopted for fibrocartilaginous sites. This choice was dictated by the morphological variability of this site, which, besides some obvious differences, can be compared with what is usually observed in fibrocartilaginous entheses. Note that, in order to allow a comparison between the two datasets, only variables recorded by both authors were considered in this study (i.e. entheseal robusticity, profession at death, and age at death). Accordingly, relevant factors (e.g. body size, body mass) were excluded from the analyses.

In order to check for the effect of asymmetry in robusticity, we also calculated an asymmetry index by subtracting the left side from the right. Accordingly, the index can assume the values 1, 0, and -1, reflecting right side dominance, lack of asymmetry, and left side dominance, respectively. Due to their nonmetric nature, our entheseal scores cannot be analysed through classical PCA. Accordingly, we used nonlinear principal component analysis (NLPCA - Gifi 1990), by specifying a number of dimensions equal to the number of variables (i.e. 18 for the full dataset, 9 for the asymmetry, left side, and right side datasets). NLPCA is computed as an extension of simple homogeneity analysis after setting rank constraints. Missing data are automatically treated according to the missing data passive option (Gifi 1990), which discards missing observations from the overall computation (for a full description see De Leeuw and Mair 2007, 2009). A hierarchical cluster analysis (by Ward's minimum variance method) was then used to explore possible patterns in the datasets. The obtained clusters were subsequently used as the basis for the classification of occupations, and differences between occupation classes tested by means of MANOVA and pairwise Hotelling's tests.

Due to the known effect of age on entheseal robusticity (Alves Cardoso and Henderson 2010, 2013; Mariotti et al. 2007; Milella et al. 2012), age-at-death deviations from normality were tested in all subsamples with the Shapiro-Wilk test. The latter was calculated in order to assess any possible bias of the sample with relation to age. Different statistical protocols are suggested in the literature to control for age. These include the use of age as continuous explanatory variable in generalized estimating equations (Villotte 2009; Villotte et al. 2010), subdivision of a sample in relatively small age classes (Milella et al. 2012), comparisons of residuals from age-skeletal features regressions (Pinhasi et al. 2014). In the present investigation, it was decided not to consider age categories due to the relatively small size of specific professional groups and for the possible bias represented by categories with different mean ages. On the other hand, both generalized estimating equations and residual analysis were excluded in order not to lose potentially useful information in the multivariate analyses. Consequently, a third strategy was adopted, which tests the correlation between age and each principal component (PC) after computing NLPCA, excluding in the follow-up analyses the PC(s) which are significantly correlated with age (choosing as threshold Pearson's r absolute values ≥ 0.2).

Given the possible bias introduced in our multivariate analyses by the small sample size of some occupations (Table 1), we repeated all procedures a second time, by considering only occupations with a sample size equal or superior than five.

NLPCA was computed with the package *homals* (De Leeuw and Mair 2007, 2009) in the software R version 3.0.2 (R Core Team 2014). Shapiro-Wilk test, Pearson's r test, and hierarchical cluster analysis were calculated with JMP®10.0 (SAS Institute Inc. 2012). MANOVA and pairwise Hotelling's test were computed in PAST (Hammer et al. 2001). For all tests, alpha was set at 95%.

RESULTS

Age: Age distribution deviates from the normal assumption in both the Italian and Portuguese samples (Table S2). Results demonstrate that for all datasets apart from that of asymmetry, the first PC (PC1) is the dimension characterized by the highest significant correlation with age (Table 3). In the full dataset, a significant correlation with age is also shown by PC7. Accordingly, in order to control for age in the subsequent analyses, we decided to exclude

PC1 from all analyses on the left, right and full datasets, together with PC7 for the latter. The reader should be aware of this when finding references throughout the text to analyses performed with "*all PCs*". Results obtained from a correlation test between age and PCs after excluding from the sample those occupations characterized by N lower than 5 (but nonetheless opting for the inclusion of the Portuguese farmers (N=3) in order to check for their positioning in this alternative dataset) are consistent with what was observed in the complete dataset. In this case, PCs showing a significant correlation with age and therefore excluded from the subsequent analyses are PC1 (left, right, both sides), PC3 (asymmetry), and PC6 (both sides).

Exploratory multivariate analysis: A cluster analysis performed on the PCs of the full dataset (after excluding PC1 and PC7 due to their significant correlation with age) fails to show a clear separation between the Italian and the Portuguese samples. Individuals from the two groups fail to form two different clusters (Figure1). This result confirms our initial hypothesis about a low population signal in the overall EC data, therefore justifying the pooling of the datasets from the various collections. Cluster analyses of all PCs (after excluding from each dataset the ones showing a significant correlation with age – see Methods section) highlights complex patterns in all datasets, consisting in several clusters grouping relatively highly diversified occupations. A straightforward interpretation of these results is accordingly difficult, given the apparent lack of social and/or biomechanical consistency in most of the single clusters. Nonetheless, when considering the overall clusters, it is possible to recognize a common pattern, represented by a relative closeness between occupations sharing the following basic features: 1) occupations related to farming activities and rural context (e.g. farmer, laborer); 2) occupations sharing relatively intense physical activity but not related to farming (e.g. tinsmith, shoemaker), nor to a rural context; 3) occupations not featuring manual or generalized physical tasks, not related to farming and at least in part referable to a more urban contexts (e.g. lawyer, bank clerk, shop assistant).

This pattern is consistent throughout the complete, left side, and right side datasets. On the other hand, cluster analyses of the asymmetry dataset do not evidence a specific distribution of occupations (Figures 2-5).

Test for differences between occupational categories: Results from the cluster analysis suggested the inclusion of occupations in three main classes: Class 1 (occupations related to farming), Class 2 (physically demanding occupations not related to farming), and Class 3 (physically undemanding occupations). Note that in this way the criterion used for grouping occupations is directly linked to EC patterns, therefore minimizing (though not eliminating, see discussion) the bias introduced by *a priori* biomechanical, social, and cultural criteria.

In order to test the consistency of such groupings, we then compared them by means of MANOVA and pairwise Hotelling's test, by using as variables in each dataset all principal components after excluding the ones significantly correlated with age (PC1 for left, right, both sides, PC3 for asymmetry, and PC6 for both sides).

Results reflect a complex scenario, with no differences between classes when considering asymmetry, a consistent significant difference between Classes 1 and 3 in the other datasets and a difference between Classes 2 and 3 when considering two sides together and the right side only (Table 4a).

Reduced dataset

Results from the multivariate analyses on the dataset after excluding occupations with $N < 5$ are largely overlapping what observed in the complete sample. The cluster analysis highlights indeed a distribution of occupations which is consistent with the criteria underlying Classes 1, 2, and 3 (Figures S1-4). This result is further confirmed by the MANOVA and Hotelling's tests. Also in this case no difference emerges between classes in the asymmetry dataset, whereas Classes 1 and 3 are different when considering the two sides together and left and right sides separately. Classes 1 and 2 differ only for the left side (Table 4b).

DISCUSSION AND CONCLUSION

Any attempt to explore and compare the impact of "specific" occupations in human skeletal remains based on the study of EC is considered a challenging endeavor. Theoretical, methodological and interpretative constraints are normally advanced as major sources of bias not only in the study of archaeological samples, but also in IHSC-based studies (Alves Cardoso 2008; Alves Cardoso and Henderson 2010; Milella 2010; Milella et al. 2012). In order to tackle these issues we explored the usefulness of an alternative approach for exploring differences among documented occupations on the basis of a multivariate study of EC patterns. Specifically, the aim of this work was to test the following hypotheses: H1) distinct groups of documented occupations should be reflected by specific patterns of EC; and H2) if H1 was confirmed, identified subgroups would share consistencies from a biomechanical and/or social point of view.

Our results confirmed these hypotheses only partially by showing a separation between individuals involved in farming activities (Class 1), subjects performing heavy physical tasks not related to farming (Class 2), and subjects theoretically featuring a more sedentary lifestyle (Class 3). Note however that such differences are not consistent throughout all datasets (sides separately, sides pooled, asymmetry). In particular, differences between Classes 2 and 3 and Classes 1 and 2 are found only in some instances.

It is interesting to note the lack of differences between classes when considering levels of bilateral asymmetry. Patterns of bilateral asymmetry of EC should indeed represent a good proxy of differences in physical activity (see e.g. Villotte and Knüsel 2014). In our case, it is possible that differences between occupations are masked by the type of EC considered in this study (robusticity), by the binary nature of our data (probably not able to capture subtle differences between the sides), and the chosen statistical procedure (which is based on the simultaneous analysis of asymmetry values from different attachment sites).

Regarding the differences between classes, the more consistent result is the contrast between Classes 1 and 3. Such result is consistent with previous works on EC (e.g. Niinimäki 2011; Villotte et al. 2010), as well as with historical and ethnographical data (see Alves Cardoso, 2008 for details).

The difference between Classes 1 and 3 can be related to both biomechanical as well as physiological factors. From a biomechanical point of view, occupations related to farming are likely to share high levels of generalized exposure to biomechanical stress and a prolonged (and probably precocious) involvement of the subject in the same occupation through time. In the Portuguese ethnographic literature, for example, farming activities performed by the digger/ditcher (*cavador*) are described as one of most physically demanding (Almeida and Martins, 2002). Furthermore, historical evidence supports that many farming activities started early in the individuals' lives. Statistical reports from the end of the 19th century state that juvenile work was common and occurred in higher frequencies in some Portuguese farming sectors. For instance, it is reported that between the years of 1870-1890 juvenile (and female) work increased by 700% in the Herdade de Palma – a farm dedicated to extensive agriculture (Southern Portugal), and for farming tasks such as "*monda*" (picking weeds in the rice fields) (Martins, 1997).

Moreover, farming activities are likely to share a relatively narrow range of biomechanical stimuli (i.e. daily physical tasks). Overall, these features would contrast with what one would expect from the wide range of occupations of the Class 3. Furthermore, it is possible to postulate for Classes 2 and 3 a higher occupational mobility (i.e. transition during life between different occupations) which would result in a more differentiated lifestyle, a point consistent with the lack of differences between Class 2 and 3. The only partial contrast evidenced between Class 1 and 2 can be interpreted as the byproduct of the high levels of biomechanical stress characterizing the occupations included in these groups, which would obscure more nuanced differences in terms of specialization of activity patterns (e.g. lateralization). On the other hand, the homogeneity characterizing the professions included in Class 1 (as opposed to the higher variance of professions included in Class 2) can be considered also in this case an important factor contributing to the observed differences.

An interesting example of the homogeneity apparently characterizing occupations of Class 1 is the proximity of farmers and workers in the Portuguese sample. Individuals ascribed to these categories would have been involved in a significant number of various activities during their lifetime, many of which sharing similar movements. They could, therefore be described as people performing a relatively narrow and uniform range of tasks. In a broad sense, farmers can be defined as subjects involved in working the land or more generally involved in agricultural activity. On the other hand, workers are described as people that would perform various tasks and activities related to farming, as well as conducting other types of work not only in rural but also in urban settings. However, these categories are not mutually exclusive. In Portugal, for example, many occupational categories fall into the farming group, such as the previously mentioned ditcher/digger (*cavador*). In contexts involving extensive farming economies, the ditcher belonged to the group of workers that performed any kind of job with the hoe and in any given season (Almeida and Martins

2002), while the daily-laborer/journey-man (*jornaleiro*) is described as a simple, wage-rural worker that performed any type of task related to farming, such as digging, sowing or reaping (Almeida 2002). Moreover, many farmers were also acting as workers (Alves Cardoso 2008). Hence, both farmers and workers would have been consistently exposed to hard physical labour during their lifetime, contrasting with other occupations that would either be specialized or featuring different physical demands.

Apart from biomechanical factors, it is worth considering the possible relevance of physiological factors on the expression of EC. These would include genetic, hormonal, and dietary factors. While genetic as well as hormonal variables are involved in the ontogeny, maintenance, and rate and type of degeneration of the musculo-skeletal system (Atteno et al. 2014; Karasik and Kiel 2010; Liang et al. 2009; Pocock et al. 1987; Smith et al. 1973; Smith and Smith 2002), such factors are unlikely to greatly influence the results of our study, due to the composition of our sample which include only one sex, no distinct pedigree-based clusters and the absence of subjects affected by (at least obvious) genetic or hormonal-based skeletal disorders. On the other hand, differences in diet, though not tested in this study, are worth to be considered as a possible factor influencing our results (see also Alves Cardoso and Henderson 2010; Milella 2010). Diet, especially regarding the relative intake of proteins, calcium, phosphorus and vitamin D, greatly influences variables like skeletal muscle mass, bone mass, bone mineral density and bone mineral content (e.g. Deutz et al. 2014; Dideriksen et al. 2013; Ilich et al. 2003; New 2002; Rosen 2002; Seibel 2007; Vicente-Rodriguez et al. 2008). Considering entheses as the interface between the muscular and skeletal system, we hypothesize that their variability could, at least in part, be influenced by differences in dietary regimes deriving from the socio-economic variability characterizing our sample. Note however that, for the moment, discussing an influence of different diet regimes on entheseal changes remains only an interesting working hypothesis.

Of particular relevance is the argument regarding the following challenges associated with the current investigation: 1) the inclusion in the same analyses of different types of entheses; 2) the analysis of only one type of EC (specifically robusticity), on the basis of data collected by different observers using different methodologies; and 3) the lack of specific sociocultural and biomechanical information on a large part of the occupations represented in our sample. The first two issues derived from the need to maximize the sample size of each occupation and the number of variables, at the same time trying to minimize the possible bias represented by the use of different methodologies. Accordingly, our results, though promising, can be interpreted only as a preliminary test of general entheseal changes (here robusticity). It is possible that more specific results would be obtained by using a larger number of attachment sites and controlling for their specific anatomy (e.g. by conducing multivariate analyses separately for fibrous and fibrocartilaginous sites), as well as by including in the analyses additional types of EC, ideally recorded by the same observer, or, at least, by different observers using the same scoring method. In particular, the inclusion in the analyses of data on enthesopathies would allow a discussion of results on the basis of data from clinical and anatomical studies. Concerning the third issue, the lack of detailed information on the life-style characterizing most of the occupations discussed in this study represent an obvious (and likely unavoidable) limit when discussing our results. Furthermore, due to the type of available documentation (which report the occupation performed by a subject around the time of death), factors like occupations and in general activities performed during the entire life course, as well as simultaneously to the documented one cannot be considered in the discussion of results. The type of available documentation on each profession is also a limit when trying to avoid *a priori* criteria in the classifications of occupations. It should be noted that, while our classification of subjects is primarily dictated by their relative proximity in the obtained clusters, the interpretation of the latter is also influenced by *a priori* biomechanical hypotheses. The resulting subjectivity could be one of the factors underlying the ambiguous pattern of differences between Classes 1, 2 and 3 evidenced by our analyses.

Despite such technical and theoretical considerations, the present work was nonetheless able to identify important basic patterns in our sample, possibly correlated to relevant biomechanical and sociocultural factors. On the other hand, it demonstrates the usefulness of a multivariate approach to the study of EC, and, more in general, the advantage of a research design not constrained by *a priori* assumptions in testing biocultural hypotheses.

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ABBREVIATIONS

IHSC = Identified human skeletal collections

SISC = Frassetto identified skeletal collection of Sassari

CISC = Identified human skeletal collection of

LLISC = Luis Lopes identified skeletal collection

NLPCA = Nonlinear principal component analysis

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Figure captions

FIGURE 1. Cluster representing mean PCs distance matrix of the full dataset. Note the lack of separation between the Italian and Portuguese samples (colored, respectively, in black and grey).

FIGURE 2. Complete dataset, both sides: a) Cluster representing mean PCs distance matrix (after excluding PC1 and PC7). Note the relative association between occupations related to farming (Class 1, light grey circles), physically demanding occupations (Class 2, dark grey triangles), and physically undemanding occupations (Class 3, black inverted triangles).b) Cluster representing mean PCs distance matrix by class.

FIGURE 3. Complete dataset, asymmetry scores: a) Cluster representing mean PCs distance matrix of the asymmetry dataset. Note the apparently random distribution of occupations. Class 1: light grey circles; Class 2: dark grey triangles; Class 3: black inverted triangles. b) Cluster representing mean PCs distance matrix by class.

FIGURE S1. Reduced dataset, both sides: a) Cluster representing mean PCs distance matrix (after excluding of PC1 and PC6) by including both sides. Note the relative association between occupations related to farming (Class 1, light grey circles), physically demanding occupations (Class 2, dark grey triangles), and physically undemanding occupations (Class 3, black inverted triangles). b) Cluster representing mean PCs distance matrix by class.

FIGURE S2. Reduced dataset, left side: a) Cluster representing mean PCs distance matrix (after excluding of PC1). Note the relative association between occupations related to farming (Class 1, light grey circles), physically demanding occupations (Class 2, dark grey triangles), and physically undemanding occupations (Class 3, black inverted triangles). b) Cluster representing mean PCs distance matrix by class.

FIGURE S3. Reduced dataset, right side: a) Cluster representing mean PCs distance matrix (after excluding of PC1). Note the relative association between occupations related to farming (Class 1, light grey circles), physically demanding occupations (Class 2, dark grey triangles), and physically undemanding occupations (Class 3, black inverted triangles). b) Cluster representing mean PCs distance matrix by class.

FIGURE S4. Reduced dataset, asymmetry scores: a) Cluster representing mean PCs distance matrix (after excluding of PC3). Note the apparently random distribution of occupations. Class 1: light grey circles; Class 2: dark grey triangles; Class 3: black inverted triangles. b) Cluster representing mean PCs distance matrix by class.