

Estimation of Sex in the Portuguese Identified Collections

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Contributor: Francisco Curate

The estimation of biological sex, a parameter of critical importance in the identification of unidentified skeletal remains both in contemporary forensic contexts and bioarcheological studies of past societies. Sex pertains to the biological and/or genetic attributes of an individual, and according to which it is classified as female, male or intersex. The conventional anthropological workflow for the evaluation of a biological profile—i.e., sex, ancestry, age at death and stature—often begins with sex assessment, as the analyses of age at death and stature are sex-contingent. The estimation of sex in skeletal remains depends on the identification and evaluation of the phenotypic differences between the skeletons of males and females. Differences in size and shape are unequally expressed throughout the skeleton, and the pelvis is generally considered the most dimorphic skeletal region.

Keywords: biological profile ; skeletal sex ; Portugal ; forensic anthropology

1. Introduction

In humans, pelvic sexual dimorphism is deeply associated with the selective pressures of bipedal locomotion and parturition but adaptative dissimilarities between sexes also stem from sexual selection ^{[1][2][3][4]}. It has been also suggested that hormonal mediation of the female pelvis is partially dependent on developmental plasticity ^[5]. Pelvic bones are fragile, particularly the pubic region ^[6], and when they are absent or extensively fragmented other skeletal elements are used to estimate sex ^[7]. Within the conventional frameworks of analysis, the cranium is usually the following skeletal region to be considered when estimating sex, but long bones seem to provide a better prediction performance ^[8].

Molecular methods, namely proteomic and genomic analyses, show high accuracy in the estimation of skeletal sex, but they are costly, technically challenging and reliant on data quality ^{[9][10]}. Thus, methods for sex assessment classically fall in two comprehensive categories, the morphoscopic (i.e., morphological, or visual) and metric approaches ^{[11][7]}. Decision making with morphoscopic methods is inherently more subjective as these approaches are observer-dependent and biased. Metric approaches are more reliable and interpretable, with a less steep learning curve ^{[7][12]}.

Excepting pelvic-centered methods, both morphological and metric techniques are highly affected by population-specific factors ^{[13][14]}, including robusticity or body shape and size. Populations also differ regarding the morphological expressions of sexual dimorphism ^[15] and secular change ^{[15][16]}—the regional diversity of the skeletal system obviously constrains the wide-ranging application of sex estimation methods, substantiating the need for geographic-specific methodologies and databases.

Significantly, most of the seminal methods for the assessment of sex are historically rooted in the 20th century North-American academic milieu, within an emerging scientific genealogy that was nurtured by institutional support, interested researchers and the availability of documented skeletal collections ^[17]. The global development of forensic and bioarcheological research agendas, combined with the establishment of new reference collections around the world, abetted the research of skeletal sexual dimorphism in different populations and a growing number of regional-based methods for the estimation of sex is now available, e.g., ^{[18][19][20][21][22][23][24][25][26][27][28]}. Likewise, a plethora of standardized recommendations that can be used to estimate sex from unknown skeletal remains have been developed and/or tested in Portuguese documented collections. These methods hail from a long research tradition within an earlier landscape of typological and descriptive skeletal anatomy, and, of course, from the availability of different skeletal reference collections in the country.

2. The Perplexing Bones of Sex—Achievements and Opportunities

The previous account of sex estimation techniques created in the Portuguese identified skeletal collections intends to be more than a sequence of events, experiences, and results: it provides a constellational depiction of the most relevant projects committed to the protocols and conventions of sexing human remains. A cumulative inventory such as this reveals an underlying structure in the scientific gaze towards the body, and the pervasive motifs that define and restrict the mapping of the skeletal differences between sexes. The Portuguese reference collections represent, to an extent, the empirical tradition within biological anthropology that boosted a methodological concern with the development of more accurate, sample-specific methods for sexing human remains. Notwithstanding, the historical consciousness of the technical apparatuses intertwined in these collections reveals how they are an imprecise form of knowledge.

The Coimbra Identified Skeletal Collection is omnipresent in studies concerning adult sex estimation in Portugal. Samples garnered from this collection appear in 88.9% of the methods created and/or tested in Portuguese reference collections. The collection is on par—or almost—with other famous identified skeletal assemblages, such as the Hamman–Todd or Terry collections, sustaining several research works in different areas within anthropology ^{[29][30]}. The CISC is composed of individuals that were born between 1822 and 1921, and died between 1904 and 1936; thus, it is important to consider the short-term effects of environment on skeletal anatomy and the potential consequences of secular change (i.e., biological modifications that occur over decades or generations, apparently ascribable to environmental factors) in the accuracy of sexing methods when employed in medico-legal contexts. Secular changes affect adult skeletal morphology, including height, cranial size and shape, and long bone length and proportions ^[16]. As metric variation in the skeleton presents diachronic modifications, there are implications for the assessment of sex from skeletal dimensions ^{[16][31]}, namely an increased bias in sex-specific accuracy. Some studies have suggested that methods fitted in chronologically older samples—such as the CISC—perform worse in more recent collections, with a decrease in accuracy rates and increased bias ^{[32][31]}. This is frequently related to a higher misclassification of females ^[33]: in general, the linear dimensions of the long bones are larger for both sexes in more recent samples, but the increase is relatively larger in females ^[32]. Incorrect evaluations imply the collapse of evidence and expert testimonies, particularly affecting forensic cases involving recent deceased individuals. As the overwhelming majority of adult sex estimation methods in Portugal derive from the CISC, this poses the challenge to test and update long-standing methodologies with appropriate reference samples, particularly those that originate in chronologically more recent collections such as the BoneMedLeg collection ^[34] or the 21st Century Identified Skeletal Collection ^{[35][36]}.

In the last few years, the CEI21 has been increasingly employed to create and test sexing methods, but the collection shows demographic characteristics that can affect the creation of new methodologies, namely the fact that it is mostly composed of elderly individuals ^[36]. This issue can be envisioned as a caveat or as an opportunity. Research has suggested that some skeletal changes occurring after adulthood tend to obfuscate sexual dimorphism ^[11]; hence, this collection provides an excellent opportunity to map dimorphism in aging individuals, and also to evaluate and develop methods that are more reliable in older age classes.

The «Portuguese» techniques for assessing sex in non-adults mostly rely in the Lisbon Collection, with a somewhat limited number of skeletons available for study, and mostly composed of individuals that died before 1960 ^{[37][38]}. The limitations of existing reference collections, including the dearth of non-adults and of contemporary (21st century) individuals, can be partially surmounted with the aid of medical imaging techniques ^[39]. Conversely, medical imaging approaches, such as computer tomography, conventional radiography, or bone densitometry, have been widely used to address the prediction of sex in cranial and postcranial bones ^[40], but with a restricted usage in Portugal, at least partially related to funding constraints. Alongside this chronological and demographic benefit, the use of medical imaging data to develop new methods would also have an effect on the curation of identified skeletal collections, by decelerating the bone damage caused by frequent handling. Basic ethical tenets, such as dignity and respect, will only be upheld if the unjustified physical disturbance of human skeletal remains ceases ^[41].

The described methods for the assessment of sex are at times insular, with a narrow geographical scope—they were created in Portuguese collections to be employed in the Portuguese medico-legal or archeological contexts. Thus, the evaluation of these methods in samples of different geographical origins would certainly feature an interesting research path in the research of region-specific sexual dimorphism.

The spatialization and patterning of biological sex throughout the skeleton requires an anthropological gaze that is also (although not only) a statistical perception of reality. Statistical analyses are of particular significance for forensic anthropology, and efforts to implement both groundbreaking and trustworthy analytical statistics in forensic research are becoming ubiquitous ^{[42][43]}. The recent explosion of statistical plurality is noticeable in the diachronic narrative of sexing

methods created in Portuguese collections: linear discriminant analysis (LDA) reigned unchallenged for years, but during the second decade of the 21st century the use of different statistical approaches became routine. LDA, the oldest classifier still in use, identifies a linear combination of predictor variables that optimally separates mutually exclusive groups ^[40]. The resulting discriminant function provides a cutoff point that induces a strict dichotomic estimation of sex ^[44]. The utilization of sectioning points is, in this regard, similar to LDA.

Sexual dimorphism in the skeleton, however, is expressed along a continuum of phenotypic variation with a substantial overlap in dimorphic trait manifestations ^{[44][45]}. In fact, skeletal morphologies have a range of variation and the dimorphic traits based on shape and size show both variation between and within the sex groups; hence, human skeletal variation is the norm, and the conflation between the morphologies of different a priori categories (such as sex) will often occur ^[45]. Visual standards ^[46] for sexing human remains recognize the overlap and the uncertainty associated with it by using a scale of five categories, «hyperfeminine», «feminine», «sex indeterminate», «masculine», and «hypermasculine». Another classical statistical algorithm, logistic regression, allows a probabilistic assessment of sex that is both consentaneous with the clinal expression of sexual dimorphism and the standards enacted in the wake of the *Daubert v Merrell Dow* ruling ^[44] ^[47]. Thus, the non-binary spectrum of sex variation should always be acknowledged by presenting the uncertain or unclear sex attributions that can simply reverberate biological features ^[48].

The more sophisticated machine-learning classifiers can lower the error rate but should be used cautiously, particularly artificial neural networks, which present less than desirable interpretability ^[43]. Moreover, the results of some studies focusing on sex estimation in Portuguese collections suggest that the type of statistical classifier—classical or machine-learning—does not impact accuracy and bias in a substantial way ^{[49][50][32]}. Even when the performance of the machine-learning algorithms is better, the difference is not overwhelming ^[40] and further practical research indicates that complex machine-learning models do not necessarily attain better performance metrics ^[51]. Again, the flexibility, elegance and simplicity of logistic regression accommodates much of the most advantageous characteristics in a statistical technique that aims to estimate the sex of unidentified skeletal remains ^[44].

Fittingly, in recent years, the prediction of sex has mostly become a probability statement. However, beyond the technicalities that inform much of the contemporary statistical research, the estimation of skeletal sex is still somewhat typological, and well within the confines of sex as a binary dichotomy. Constructionist arguments contend that, as sex can be fluid, the binary partition of sex into males and females is a product of discourse ^[45]. During the long 18th century (1688–1815), a fundamental change in the scientific discourse solidified the notion of masculine and feminine as diametrically opposed, rather than contiguous and hierarchical ^[52]. Anthropologists must be conscious about the possibility that this binary rift was peripheral in many societies who recognized human variation in ways that did not fall into a precise two-sex dichotomy ^{[53][54]}. Even chromosomal sex is multifarious and does not map clearly onto two bodily morphologies ^[53].

The anthropological responsibility to dismantle the rigid binary sex categorization stems, among other factors, from the fact that transgender and gender non-conforming people experience higher rates of violence ^{[55][56]}. Moreover, a distinction between sex and gender is almost never presented (regrettably, this is generalized), and the conflation of the two concepts is thus acritically acknowledged. Although gender—the expected social roles grounded in the sex of the person (gender role) or personal identification of one's own gender based on a personal awareness (gender identity)—cannot be directly observed in the skeleton, there are skeletal expressions of gender delimited by skeletal plasticity ^{[45][53]}. This thorny legacy inherited by contemporary anthropologists needs to be recognized, pondered, and contested. A first step to address the embodiment of gender in the skeleton pertains, for example, the evidence of gender-based division of labor: patterns of musculoskeletal stress markers or of degenerative joint disease have been used in bioarcheology to convey the potential disparity of sex and gender. Activity reconstruction, mortuary analysis (including the research of material culture associated with the dead body), intentional body modification, among others, are being referenced and mapped in gender-based bioarcheological analyses ^{[54][57]}.

An interesting trend observed in the methods created in Portuguese identified collections pertains to the expanding relevance of proper goodness-of-fit metrics (measures of goodness-of-fit classically synopsise the disagreement between observed values and the values anticipated under the proposed model), including accuracy under cross-validation and in independent test samples. The majority of the early published methods merely displayed resubstitution classification errors, recognized for being optimistically biased. Cross-validation is probably the most common method to evaluate prediction error, but often appropriately assesses only the expected prediction error. Thus, the generalization error of any classification model should be evaluated with an independent test sample ^[58]—and that is increasingly the case in sex estimation techniques created in the Portuguese identified collections.

Web-based decision support systems and data sharing are contingent to the ongoing use of imaging methods and cutting-edge statistical approaches in forensic research. Online applications of interest for skeletal sex estimation are usually straightforward responsive interfaces that offer comprehensive documentation about methods, and include data and graphical analyses as well as classification outputs. Additionally, most of the available web-based applications—particularly those that feature models generated in Portuguese collections—bestow an educational diorama focused on human skeletal variation and statistical analyses ^[43]. Data sharing encourages networked collaboration models, global learning opportunities and diversity of research. The sharing of raw data also allows the iteration of research, enabling the validation of the original project or the development of new avenues of study ^{[59][60]}. Interestingly, some early studies concerning sexual dimorphism in Portuguese reference collections provided a wealth of raw data, e.g., ^{[61][62]}, a valuable practice that has not been pursued in more recent research. Even though there are clear advantages in data sharing and online archiving, some ethical plights need to be addressed, including, but not limited to, the protection of identities by fully anonymizing the data, the consultation with living or descendant communities, and the clarification of data ownership rights by academic institutions or museums ^{[59][60]}.

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