

Ancestry Estimation: Advances and Limitations in Forensic Applications

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Abstract: Forensic anthropologists are faced with many challenges one of which is setting the grounds for the assessment of an unknown individual's population affinity, an essential element for building a biological profile that can lead to positive identification. A heavy body of literature is dealing with the topic of "race" – in early studies – or "ancestry/population affinity" – in more recent articles – in an effort to provide reliable tools for this challenging task. A great and ongoing debate exists on whether this type of classification should be done at all or whether it should be accepted as a "necessary evil". The over-simplistic categorization of people originating from vast geographical areas without taking into account micro-evolutionary and environmental factors, that are of great importance and influence population affinity, can greatly enhance social discrimination and stigma. On the other hand, it must be stressed that the point of assessing an individual's ancestry or geographic origin in a forensic context is not a matter of taxonomy or population labeling but rather an asset to the identification process, which can help narrow down possible matches from a missing persons' list. In that vein, the current paper offers an overview of different methodological approaches to the matter of ancestry estimation and aims to gather relevant information about the different approaches used, the current advances and the implications of the assessment regarding positive identification.

Keywords: ancestry, population affinity, biological variation, forensic anthropology, estimation

Introduction

One of the most important tasks for forensic anthropologists is to assist in the positive identification, for medicolegal purposes, of unknown individuals that are skeletonized or decomposed beyond recognition.¹ In order to assist in the identification process, the reconstruction of the deceased's biological profile must include demographic information related to sex, age-at-death, stature and ancestry, along with other identifiers such as pathology or unique biological markers.²⁻⁴ Ancestry estimation relates to the biogeographical origin of an individual, and not only constitutes one of the four main aspects for the reconstruction of the biological profile but it might also determine the subsequent methods when the expert considers the choice of the technique in relation to population-specific standards.⁵ Fundamental principles in which ancestry estimation is based are still questioned, more specifically in regards to population sampling and representation, validation studies, as well as applicability in day to day casework and ethical considerations.⁶⁻⁸ In 1992, Sauer⁹ summarized the controversy through a simple question: "If races don't exist, then why are forensic anthropologists so good at identifying them?". Ancestry is perhaps one of the most challenging aspects of biological profile as it constitutes a complex concept representing a bidirectional relationship between social constructs, environmental factors and biological characteristics.^{10,11}

The concept of race (or ancestry) constitutes a long-standing debate that can be tracked back to the 18th century. The work of the French taxonomist Carl Linnaeus (1707–1778) is probably one of the first attempts for human race classification.^{12,13} Around the 19th century, the theory of evolution gained ground and acceptance among the scientific community, and previously established ideas of fixed and static human variance were transformed into more dynamic approaches, taking into consideration new concepts such as gene flow and genetic variations associated with regional adaptation processes and demographic parameters.¹⁴ Other currents of thought such as Eugenics emerged in the 20th

century having an influence on social and scientific theories, and historical events.¹⁵ Through the evolution of both science and history, there has been a number of definitions assigned to the word “race”.¹⁶ The recent scientific advances, more notably in the field of population genetics, have provided the impulse to reject and expand beyond the boundaries of old typological approaches that can be misleading.⁵ Furthermore, social implications assigned to race have repeatedly lead to population oppression and phenomena of systematic racism in which biological and deterministic concepts are used to reinforce political views and maintain racial hierarchies, a prime example being the Apartheid legislation in South Africa.^{7,17,18}

The concept of ancestry in forensic anthropology was shaped from the discipline of physical anthropology. Anthropologists such as Aleš Hrdlička in the 20th century interlinked human variation with evolutionary theories, physical characteristics and other related factors such as environmental influences.¹⁵ In addition, molecular scientists found that there are more genetic similarities between populations compared to similarities noted within populations.¹⁹ Thus, a tendency of the eradication of the term “race” and a call for a throughout revision of both typology and classification approaches related to human variation is noted.^{20,21} In an attempt to minimize the heavy social load while maintaining the pragmatic concordance among geographic origin and skeletal traits, “ancestry” or “population affinity” have been proposed instead, and adopted in current forensic anthropology literature.^{5,20} Still, there is an excess of antiquated views of race in even the most current literature, mostly based on the simplistic trifecta of continental populations (European, Asian and African). Thus, even if the terminology has evolved over the years, the essence of it all remains doused in controversy, confusion and, on occasion, based on social stigma.²⁰ Moreover, terminology presented in the literature was found to be inconsistent making interpretation and cross-examination of data and results difficult.²² In a letter to the editor issued by Bethard and Digangi,^{7,23} the authors challenge the long-standing use of ancestry as part of the biological profile on the grounds of racial bias phenomena exhibited by law enforcement investigators that might hinder the identification processes. On the other hand, as vigorously supported by Stull and Bartelink,²⁴ the estimation of ancestry is an essential parameter of the biological profile that, in this modern era, aims to achieve identification through the assessment of variation patterns, the disassociation of social race and morphoscopic traits, the employment of analytical statistical analyses and through the employment of large, worldwide data pools to achieve reliable results. The authors conclude that the future trends in forensic anthropology should not revolve around abandoning the practice of ancestry estimation on account of racial bias possibilities, but focus on constantly educating all parties involved in the investigation instead.

In forensic anthropology practice, the estimation of ancestry generally refers to the classification of an individual into the most probable geographic origin group using statistical analyses and experience-based methods.^{25–27} Traditionally, that includes both metric and morphological approaches, most commonly performed on the cranium.¹⁵ Recent techniques make use of advanced statistical analyses, novel technologies and powerful computational systems, providing statistical robustness to the assessment. The present paper offers a discussion on the considerations, advantages and limitations of ancestry estimation reviewing traditional metric and morphoscopic skeletal methods as well as more sophisticated approaches such as geometric morphometrics and machine learning models. Laboratory methods such as population genetics and stable isotope analyses will not be addressed here but the reader should be aware of their potential for identification purposes.^{10,28–31}

Traditional Methods for Estimating Ancestry in Human Remains

Metric Assessment

The skull has shown to reflect a link to geographical origin due to a combination of evolutionary and genetic factors.³² Craniometric studies are of particular interest in exploring interpopulation biological variance perceiving the observed variation, in a large part, as a result of genetic factors.³³ The quantitative approach entails the collection of single measurements, indexes or ratios, with the most commonly used statistical analysis being linear discriminant function analysis (DFA).³⁴ The credit of the first craniometrics studies for ancestry estimation goes to Giles and Elliot³⁵ who provided equations for the classification of unknown crania in one of the three groups: Black, White or Indian. Several studies adopted this technique to develop ancestry estimation methodologies.^{36,37} Cranial metrics can achieve high rates of accuracy depending on the method used and the population under study, with factors such as sexual dimorphism and secular change having an impact on the accuracy rates.³⁸ Allocating an unknown individual to any geographical area in the world challenges the estimation of ancestry

and population representation must be considered when there is no assumption on the individual's origin. Yet, group assignment can be less complicated and more reliable in more restricted geographical regions, as shown for example by Stull et al³⁹ on a South African sample and by Kranioti et al⁴⁰ on Greek, Greek-Cypriot and Turkish samples.

When the skull is not available, the postcranial skeleton could be a potential alternative for ancestry estimation. Yet, the influence of environmental factors such as climate and its impact in body size and body proportions should be taken into account.^{41,42} The femur has been the focus of many studies due to its robusticity (eg).⁴³ Indexes such as the platymeric index on the femur have been proposed for ancestry estimation³⁷ with validation studies producing unreliable results^{44,45} and thus might not be recommended.⁵ Postcranial measurements of American and South African populations were also employed to estimate ancestry with acceptable results.^{41,46} In Europe, Kranioti et al⁴⁷ used metric parameters of the tibia to estimate population affinity on 6 Mediterranean populations, achieving the highest classification rates for the Greek sample (90%). Furthermore, the subpubic angle appeared to be a useful discriminator between European and black South Africans with differences between sexes and populations being highlighted.⁴⁸ A recent study⁴⁹ used vertebrae to estimate ancestry in individuals from distinct South African populations, achieving a classification accuracy rate of 92.3% excluding sex as a variable, with the thoracic vertebrae yielding the best results while the sacrum showed the lowest accuracy. In 2018, Bidmos et al⁵⁰ applied metric parameters from the talus to discriminate between Black and White South African individuals and demonstrated differences among the populations under study producing accuracy rates ranging from 80% to 95.5%.

A next step on the creation of discriminant functions for ancestry estimation was the development of automated custom-made computational systems that can produce estimation both easily and quickly. Two of the most well-known software are FORDISC⁵¹ and CRANID.⁵² FORDISC contains a set of 34 cranial and 39 postcranial measurements while CRANID only uses 29 cranial measurements. Both are based on large datasets which include, among others, Howells famous craniometrics database⁵³ and provide the opportunity to calculate posterior probabilities of correct classification for each estimate. Some studies have shown low accuracy rates regarding the use of these software, although expectations based on the target sample should be considered when interpreting their conclusions.^{8,54-57} On the other hand, other studies demonstrated a 91% accuracy when using FORDISC in forensic casework.⁵⁸ At this point, worth mentioning is another, freely distributed, web-based software, (hu) MANid (<https://anthropologyapps.shinyapps.io/humanid/>), that contains a large reference sample of mandibular data (26 geographic or chronological populations) and utilizes both metric and morphoscopic traits providing sex and ancestry classifications.^{59,60} (hu)MANid has been tested by its creators and was proven to function within the same efficacy rates as FORDISC.⁵⁹ A particularly useful feature of the application is the option of additional analysis methods, like multiple or linear DFA.

Morphological Traits

The qualitative, morphoscopic study of crania has been used extensively for ancestry estimation.⁶¹ The existing methodologies rely either on anthroposcopic or qualitative traits.⁶² Anthroposcopic traits are based on the variation and differences in the morphological appearance of specific cranial, facial or palatal regions through a present or absent scoring system (eg, nasal overgrowth) or through the identification of specific morphologies of a feature such as the inferior nasal margin.^{61,63} Morphological traits constitute a series of discrete skeletal and dental anomalies that seems to occur with higher frequencies in some populations (eg)⁶⁴ Methodologies have been improved over the years, with the earliest work being conducted by Rhine³⁷ who documented 45 cranial traits for the classification of American Black and White, Hispanic, and Amerindian groups. This approach appears to be a questionable method in the discipline as it lacks statistical standardization and is based on a very limited sample size exhibiting high occurrences of unexpected traits within groups.⁶⁵⁻⁶⁷ In order to tackle the deriving scientific inconsistencies, Hefner developed a standardized, statistics-based framework to assess macromorphoscopic traits.^{26,61,67,68} Brace and Hunt⁶⁹ even doubted the value of morphological traits in populations outside the borders of the Americas. This hypothesis was rejected with the study of L'Abbé et al⁶³ on South African, White, Black and Coloured groups, who confirmed the existence and relative value of these traits while noting a great variability in their expression.

Human teeth are highly preserved and commonly recovered, and thus, dental non-metric traits have also been assessed for purposes of identification and ancestry estimation.⁷⁰⁻⁷² Moreover, crown morphology is not altered, and so, dentition can be more resistant to environmental changes proving to be a valuable asset in ancestry estimation.⁷¹ Furthermore, dental variation can be of hereditary nature, thus allowing for tracing the origins of the individual back to a

specific geographically located group.⁷³ Among others, incisor shoveling, Carabelli's trait, canine nasal ridge and cusps are routinely examined.⁷⁴ Recently, an opportunity for an automated estimation appeared with an online application called *rASUDAS: a new Web-Based Application for Estimating Ancestry from Tooth Morphology*.⁷⁵ So far, no validation study has been published on the software performance. Nevertheless, morphological traits in dentition rely on experience and accurate identification is dependent on the sample under study and the population's degree of homogeneity.⁷⁰

New Age Methodologies and Novel Approaches

Recently, the evolution of population affinity estimation methodologies has been built on novel technologies and advanced statistical analyses reporting observer's bias, calculation of error rates and cross-validation of accuracy rates.^{76–78} Novel techniques such as Geometric Morphometrics and Machine Learning models have been applied in forensic anthropology offering a way forward to a “new age” of ancestry estimation.

Geometric Morphometrics (GMM)

Geometric Morphometrics is defined as the statistical analysis of shape variation and its covariation with other variables.⁷⁹ Since the 1990s, GMM has been gaining ground and establishing itself as a relatively novel, state-of-the-art method used in a number of scientific fields, including forensic anthropology.^{80–82} Essentially, GMM allows the separation of shape and size and their independent analyses.⁸³ Data pertaining to shape is encoded in the form of Cartesian coordinates of anatomical landmarks encompassing all geometric information, while also addressing the issues arising from the complexity of calculating the parameters of relative landmark positions (interlandmark distances, angles, etc).⁸⁴ The analysis of landmark coordinates is customarily performed through the so called Procrustes methodologies which are used to scale, translate and rotate the specimens under study for purposes of pair comparison in which the factor of size has been eliminated.^{39,72,84} One of the advantages of this fairly new methodology is that it utilizes different types of data in order to quantify shape variations, and thus, limits the effect of observer's bias.^{80,85,86} As a result, GMM has been proven to be extremely useful for highlighting quantifiable morphological differences between specimens, even in the case of nonmetric traits in which linear measurements cannot be easily employed.^{82,87} Finally, given the fact that osteological collections can be difficult to access, GMM provides great research opportunities by employing modalities in which the direct observation of skeletal remains is replaced by virtual data, such as Computed Tomography,^{88–92} Magnetic Resonance Imaging^{90,93} and Radiography, in addition to traditionally used 3D digitizers and surface scanners. Conducting retrospective studies and the use of clinical data from living individuals offers the possibility of acquiring samples representing the current population, avoiding the bias introduced by secular changes and other potential sources of error.

Applications of GMM can be found in various disciplines including bioarchaeology,^{94,95} physical^{81,96} and forensic anthropology^{89,91,97} for purposes of ancestry estimation. Based on the literature, most research conducted is based on adult samples, with few exceptions focusing on sub-adult individuals.^{98,99} As with the more traditional methods aforementioned, the skeletal element of choice for estimating ancestry has been the cranium with special focus on the mid-face region.^{11,18,39,88,100,101} Moreover, shape and size analysis have demonstrated the complex interaction between micro-evolutionary forces and environmental conditions with cranio-facial variation being modulated by geographical factors such as altitude and climate.¹⁰² Murphy and Gavin¹⁰³ assessed the cranial outline with the use of 2D GMM on African and European American populations reporting that the lateral cranial outline yields over 90% correct classification rates. The advantages of this method relate to the use of 2D images that can be extracted both from 3D scans as well as standardized photography, and the reduction in data collection time as opposed to the lengthy process of gathering a full set of 3D craniometric data. Musilova et al⁹¹ employed the use of CT on a sample of contemporary Czech and French individuals exploring the exocranial variation to discriminate between samples achieving a correct classification rate of 91.8% in the pooled sample and proving high population sensitivity for cranial shape. One observation made by the authors was that Central and Western Europeans are less diversified than other populations (e.g. Americans), thus more data from other closely related geographical samples needs to be included to further expand its application.

Aside from the degree of shape variation of the entire cranium, various morphological traits such as the nasal aperture shape and zygomatic morphology have been assessed through GMM in European and South African samples.^{11,18,88,97} Traditional morphological traits like for example orbital shape have also been used for ancestry estimation following a

GMM-centered technique on Europeans, Africans and Asians.^{100,101} Although sample size or sex bias present limitations, this approach offers a new possibility for 3D shape evaluation of traits traditionally assessed through simple macroscopic observation. Finally, worth noting is a GMM software developed by the North Carolina State University called *3D-ID*, which is freely distributed online¹ (<http://www.3d-id.org/>) and can be used for sex and ancestry estimation, providing that cranial data is obtained from a 3D digitizer.⁸⁵

Although most GMM studies focused on the mid-face region of the cranium, the cranial vault has also been explored. A 3D GMM study of the occipital bone was conducted by Zhang and Schepartz⁹² using micro-CT on a sample of European, African and Asian individuals confirming some variation between samples although not accurate enough to reliably discriminate between the populations. Additionally, Maass and Friedling¹⁰⁴ employed the use of GMM on the neurocranium for ancestry estimation on three distinct South African populations, producing 83% correct classification rate. This study demonstrates that reasonable accuracy rates can be achieved even with the use of a limited number of landmarks, and in cases where the mid-face regions are damaged or missing.

The mandible has not been extensively explored for ancestry estimation. Franklin et al⁸⁶ studied inter-population variation of the mandible in a sample consisting of South African Zulus and European Americans. Their results yielded accuracy rates exceeding 90%. They also observed that in the European American population, the incorporation of size notably improved classification accuracy, as was pointed out by other studies.⁹⁷ Buck et al⁹⁹ also examined shape variation on the mandible on a sub-adult sample achieving, however, lower accuracy rates (<70%), discouraging the employment of the method.

GMM-centered approaches focusing on the dentition have also been attempted, more specifically using the molars and premolars on American and Australian samples reporting acceptable degrees of accuracy.^{72,98} Notable limitations on the published studies include a limited age span of early adolescence (12–15 years old), to ascertain minimal tooth wear. Clark et al¹⁰⁵ assessed the maxillary dental arcade curvature for possible inter-population variance but showed low classification accuracy rates.

The postcranial skeleton has not been considered to be as reliable as the cranium for distinguishing different ancestral groups as it has accounts for higher plasticity as a result of environmental influences.⁴³ However, recent findings suggest that through the use of GMM, even postcranial elements (such as the humerus or fibula) can be valuable in distinguishing even closely related populations.^{17,106} In their work on the humerus morphology using 3D GMM, Maas et al¹⁷ reported accuracy rates higher than 84% in a South African sample consisting of 3 sub-groups (Black, White and Coloured). A more recent study of the same populations focused on the fibula producing lower classification rates.¹⁰⁶ These studies reported that bone morphology of the Coloured group shows unique and distinct traits, which is in opposition to historical view stating that the Coloured group accounts for an intermediate morphology between Black and White samples. These findings confirm the feasibility of ancestry estimation using GMM in the South African sample. They also highlighted the value of developing ancestry estimation methodologies based on long bones, such as the humerus, on account of their reasonably good survival and recovery rates in forensic contexts.^{68,107}

GMM applications using other skeletal elements such as the sacrum have been also explored. Rusk and Ousley¹⁰⁸ performed a 3D GMM study on African and European Americans that yielded varying classification accuracy rates, ranging from 60% to 98%. Their results showed that the dorso-ventral curvature, which has previously been used for sex assessment, is in fact more useful for ancestry estimation purposes. Additionally, they found that the component responsible for most variance among populations can be easily calculated using sliding calipers in a number of settings as well as on fragmented or incomplete sacra. GMM-based studies focusing on the pelvis for purposes of ancestry estimation were not widely encountered in the literature. In a study performed by Steyn et al using a sample of 115 adult South Africans (Black and White males and females) the utility of the greater sciatic notch as a discriminator of ancestry was tested and rejected due to the significant inter-population overlapping observed.¹⁰⁹

Machine Learning Models (ML)

Machine learning is the scientific discipline of how computers learn from data.⁷⁷ It is a term used to describe various algorithmic methodologies used for classification purposes, including artificial neural networks (aNNs), decision trees (DTs), random forest models (RFMs), support vector machines (SVMs) and cluster analysis. Classifications are made

through artificial intelligence techniques with the capacity to make predictions using mathematical models generated from training sample data.^{76,110,111} ML models are capable to rapidly and efficiently process extensive and complex sets of data, saving time and minimizing the possibility of human error. Over the past decades they have been incorporated into various scientific fields, forensics not excluded, proving their effectiveness. Their ability to predict outcomes from diverse parameters (both continuous and categorical) and to identify recurrent patterns makes this technique suitable when dealing with large datasets. Most of the limitations posed in the field of machine learning are associated with the availability and complexity of computational systems and the technical knowledge of their users.^{77,111}

Artificial neural networks (aNNs) are composed of interconnected neurons that employ the use of a search algorithm that imitates human brain processes in order to identify clusters in large data sets of predictor values. Decision trees (DTs) are created following a series of rules that are responsible for creating branches or “nodes” to the main sample (“root node”) until group membership is predicted when no further division is possible. Random forest models (RFMs) consist of multiple DTs, the ensemble of which is used to reach a consensus regarding the most effective classification rules. They are the evolution of DTs in the sense that they overcome the defects of overfitting and underfitting and are capable to simultaneously assess numerous variables while combing through vast compilations of combined categorical and continuous data. Support vector machines (SVMs) are algorithms used to separate a sample into categories in multi-dimensional spaces, dependent on the number of predictors used.^{18,76,77,112}

During the last few years, there has been a tendency to adopt ML classification models in forensic anthropology for purposes of sex and ancestry estimation.^{65,110,112,113} In the latter case, ancestry poses as the dependent variable whereas ancestral skeletal traits act as predictors. The results of suggest that morphoscopic traits can actually be used in a more scientific framework by being fed into ML algorithms, to successfully assess ancestry, while both eliminating observer’s bias and producing calculated error rates. Most of the research focuses on the use of cranial traits and metrics on American populations. Noteworthy is a study conducted by Hefner et al⁶⁵ focusing on Hispanic populations indicating that Southwest Hispanics commonly misidentify as European (White) Americans, whereas Guatemalan tend to misidentify as African (Black) Americans. These populations are frequently pertinent to forensic investigations conducted along the US – Mexico borders, a fact that strengthens the method’s pragmatic applicability.

Hefner and Ousley²⁶ compared various ML models to assess ancestry in a sample of African, European and Hispanic Americans using morphometric cranial traits as described in previous works by the first author.⁶¹ They concluded that aNN, SVM and RFM all showed mean classification accuracy rates of approximately 85%, with aNN exhibiting highest success (87.9% accuracy). Nikita and Nikitas⁷⁶ also examined the relative effectiveness of various models for predicting group membership in a diverse sample of six groups. They observed that SVM and LDA (Linear Discriminant Analysis) exhibit similar performance and the highest accuracies. On the other hand, DTs produced the poorest results while aNNs, even though mainly accurate, demanded a rather complicated tuning process. Finally, Spiros and Hefner⁶⁸ tested the accuracy of various ML approaches using a dataset of paired cranial and postcranial traits. Their results showed that when using combined traits, the classification accuracies generated are significantly improved by over 10%, with the lowest accuracy in combined traits (by RFMs) still exceeding the highest accuracies in either cranial or postcranial traits when used separately. They proceeded to suggest the use of aNNs as they were found to display the most relative stability (lowest classification bias). Along those lines, they provided a free web-based application, ComboMaMD Analytical, to facilitate and promote their use (*ComboMaMD Analytical v.0.1*).² (<https://macromorphoscopictraitanalysis.shinyapps.io/MaMDAnalytical/>)

On another front, Hefner et al¹¹⁰ tested the recommendation made by Feldesman⁷⁸ that DTs can be used as an alternative to LDA when predictor values are missing (in which case LDA is not applicable) and tested the efficacy of RFMs. A combined craniometric – morphoscopic dataset of a European – African – Hispanic American samples was used. Their research concluded in an RFM accuracy rate of almost 89.6%, outperforming LDA by approximately 4%. Furthermore, they proved that craniometric and morphoscopic data capture similar biological information, thus producing equivalent data on population affinity. Subsequently, the authors suggested the use of RFMs on account of their ability to combine categorical and continuous data to increase the accuracy. In 2014, Navega et al¹¹⁴ introduced their newly developed software called AncesTrees. The program is designed to receive craniometric input data (23 measurements) and estimate ancestry utilizing RFM algorithms. AncesTrees, when tested by its designers, demonstrated a generalization capacity, namely the ability to

operate satisfactory when fed with new samples, others than the ones used for its training. The authors concluded that the software, which is freely available online,³ (<http://lfa.uc.pt/ancestrees/>) shows great promise for future use and possible incorporation in a day-to-day forensic context. Today, the software prides itself of a database of almost 1734 individuals and is considered to be a major asset in the practice of forensic anthropology.⁵

Discussion: Further Considerations and Conclusions

The concept of ancestry has been unquestionably one of the most controversial and debated topics in physical and forensic anthropology.^{6,7,9,24,115} While it has been traditionally used for the reconstruction of the biological profile of unknown human remains, its legitimacy on both ethical and biological grounds has been questioned.⁶⁻⁸ The widely used European – Asian – African tripecta has long been used for purposes of ancestry description in old and new literature as pointed out by Ross and Williams.²⁰ However, the aforementioned practice is an over-simplistic categorization of people originating from vast geographical areas, not taking into account micro-evolutionary and cultural factors that are of great importance and influence population affinity.^{115,116} Naturally, such an approach comes with cultural and social implications, compromising the objective scope of scientific deduction. The traditionally used word *race* has nowadays been replaced by the “less provocative” term *ancestry* or *population affinity*, yet it has been argued to describe an underlying approach that could even be characterized as folk taxonomy.^{115,117} Furthermore, the use of inconsistent terms such as Black, White, Hispanic, etc, adds to the elusiveness of the concept while at the same time promotes phenomena of social stigma and injustice towards underrepresented populations.^{23,62,115,117,118} As a result, there are various misconceptions regarding the relationship between biological identity and the socially constructed concept of race or ancestry that can cause confusion during the process of a forensic investigation or even take part in marginalizing the living through the “racialization” of the deceased.^{6,8,115}

Population influxes, inter-group commingling and social constructs such as classes or modern day castes have an immediate effect on the gene flow that consequently affects biological identity.^{11,18,116} Furthermore, historical phenomena, such as armed conflicts, mass catastrophes and epidemics, as well as language or religious differences affecting interpersonal relationships, play a major role in shaping the micro-evolutionary forces that forge biological variance.¹¹⁶ A great example of the aforementioned social and historical forces at work is the Apartheid legislation in South Africa that significantly constricted the gene flow resulting in major inter-population differences.^{11,18,39,97,104} It becomes clear that under the influences of both intrinsic and extrinsic factors, biological traits become intertwined with social and historical events, and thus, the examination of ancestry is in fact more complicated than the typological approaches dictate. Along those lines, researchers^{21,115} suggest that the use of a more detailed, broad synthesis that is focused on regional evolutionary forces for the purpose of identifying the underlying patterns of human variation, would be more accurate and appropriate. That would constitute a full approach given the fact that people and populations do not exist in a social void, but rather within societies that grow, evolve and inevitably change over time along with those living and reproducing in and out of their confines. The use of typology and partially or even invalidated trait lists aiming to classify within the tri-continental racial frame, despite it being named ancestry estimation, is an outdated practice that needs to be abandoned as it ignores the social component of biological identity, and thus provides information that is unhelpful at best, misleading at worst.^{115,117} Finally, it ought to be stressed out that the return to racial categorizations is a dangerous step back that can amplify or give birth to phenomena of bigotry and social exclusion.

Another factor to consider besides ethical/scientific debates on the legitimacy of accessing or characterizing ancestry is the effectiveness of such an assessment in terms of identification. Forensic anthropology is an evolving discipline in which new methods are being developed, traditional methods revised and validated, and new approaches both in relation to methodologies and techniques’ applicability presented. Ancestry estimation methods have been also updated with the appearance of online software tools^{52,75,114,119} offering the practitioner an opportunity to access worldwide databases and conduct an assessment supported by a statistical output generated based on the case at hands and the method applied.

A reliable ancestry estimation will narrow down the most likely biogeographical origin of an individual which can be of crucial importance for the choice of subsequent methods for age-at-death, stature and sex estimation. In fact, traditional and more recently developed ancestry estimation methods often combine both ancestry and sex estimation (eg).³⁵ Sexual dimorphism presents different degrees of expression between populations, and both metric and morphological methods

have been developed, accounting for inter-population variation.^{120,121} However, sex estimation methods based on multi-population reference samples can also produce reliable accuracy rates.⁴⁷ If sex is required for the application of a particular ancestry estimation method, a decision should be made on how certain the sex estimation is having into account the 95% accuracy and reliability threshold for forensic case work. Moreover, if age and stature estimation are based on population-specific standards, the ancestry estimate should be carefully scrutinized to avoid misleading outcomes. Thus, the practitioner should always consider the case under scrutiny taking into account intrinsic factors such as age and sex as well as factors such as skeletal element available and taphonomic alterations, in order to decide the method to be applied.¹²² Moreover, available information on the case, reference data and statistical classification output as well as interpretation of posterior probabilities are highly recommended considerations when estimating ancestry with methods that automatically allocate the individual into one of the groups available.^{25,112}

Regarding data representation, appropriate reference populations should be considered for both morphoscopic and metric approaches, with complex groups and mixed ancestry individuals being more challenging to estimate.^{5,122} In their study on dry crania from a South African sample (Black, White and Coloured), Stull et al³⁹ refuted the claim that highly mixed populations cannot achieve high classification accuracy by producing classification rates of just over 80%. Moreover, in a validation study on Ancestrees performed by Fernandes et al,¹²³ a lower classification accuracy rate was obtained for a Brazilian sample likely due to the mixed ancestry of the individuals and the non-representation of this group in the software database. In addition, individuals from different parts within the same geographical areas (eg, Northern and Southern Mexican individuals) were shown to be associated to different reference samples when using *FORDISC.3* with Latin American individuals showing overall lower posterior probabilities than Whites and African Americans.⁵⁷ Thus, one of the challenges in ancestry estimation remains the identification of individuals for which representation is scarce or null. To solve this issue, future research should aim to investigate population variation regarding population affinity and focus on further exploring environmental, genetics and developmental influences supported by statistical approaches to improve our understanding of the assessment of ancestry on skeletal remains.²¹

Forensic Anthropology Society of Europe² and SWGANTH¹²² ultimately support ancestry estimation as long as methods follow certain requirements. SWGANTH highlights the need for appropriate sample size, representative of contemporary populations and the use of multifactorial and robust statistical approaches and suggests that assessment should be independent of suspected or presumptive identifications.¹²² A heavy body of literature including every single forensic anthropology textbook describes skeletal methods for metric and morphological assessment for ancestry estimation and most recent studies attempt to meet the aforementioned methodological requirements.^{1,5,124} Yet, problems such as biology versus typology, genetic admixture, and lack of population representation in the existing databases continue to pose problems. It is not surprising that certain recent articles on ancestry focus on whether this should be applied at all rather than the description of adequate methodology.^{7,8,20,21} On the other hand, a significant number of researchers of the field argue that ancestry is an essential part of the biological profile that nowadays interprets biological variation as the product of complex social and micro-evolutionary forces employing solid statistical analyses.^{5,24,32} Furthermore, they point out the lack of data-driven evidence in favor of the practice's negative ramifications within a forensic investigation (identification rates, case outcomes, etc).²⁴ Thus, their suggestion is to focus on scientifically sound research and thorough continuous education of practitioners and law enforcement personnel to avoid racial bias and social discrimination, rather than harmfully abandoning the practice completely in a "shortsighted act".^{32,39}

The current paper offers information on past and recent methods on ancestry estimation which are constantly revised in the view of the obvious problems of genetic admixture and infinite biological variation.^{5,21,62,115} There is value into the documentation and the analysis of populations' biological characteristics, although contextual information should be considered and interpreted with caution. For example, the application of Kranioti et al⁴⁰ craniometrics method can be suitable in an effort to discriminate Turkish-Cypriots from Greek-Cypriots in a mass grave where the deceased are believed to have died during the 1976 invasion of Cyprus. Contrary, the application of a similar metric method developed on tibia from Mediterranean European populations⁴⁷ would be limited in a context in which there is no information about the provenance of the remains, as the under-representation of other geographical groups limit the results. However, what must not be ignored is the factor of cognitive bias that stems from contextual information, such as non-medical information and circumstantial evidence provided by the investigative authorities.¹²⁵ Those can significantly influence the decision making process, regardless of the examiner's experience or expertise,

and even alter the course of an investigation and therefore it is recommended that they are viewed with caution.¹²⁶ Thus, ancestry estimation should be applied on a case by case basis, according to the case specifics, using adequate methodology and cautionary interpretation of the results, limiting speculations and room for cognitive bias, to a minimum.

Disclosure

The authors report no conflicts of interest in this work.

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