

# The Biological Profile of Unidentified Human Remains in a Forensic Context

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## ABSTRACT

Estimation of the biological profile of unidentified human remains is a critical component of an anthropologic evaluation of unidentified human remains. The profile is used to search for missing persons that may match the decedent. The individual components of sex, ancestry, stature, and age at death require reliable methods to ensure accurate recording of these biological markers. This article showcases an unidentified skeleton that was misclassified as a female when the original evaluation was done in 1963. The reevaluation in 2004 quickly led to resolution of the identity. Methods used today to evaluate the components of the biological profile are reviewed along with a limited review of the historic literature. *Acad Forensic Pathol.* 2016 6(3): 370-390

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## INTRODUCTION

Unidentified human remains with a tentative identification are a common occurrence at most medical examiner's offices. Decomposition or other conditions may render a body unrecognizable. Modern forensic identification is accomplished by the comparison of antemortem records including fingerprints, radiographs, and DNA profiles with postmortem findings. Less common is an unidentified person with no tentative identification. In these circumstances, a biological profile is needed to lead the medicolegal investigation to a list of tentative persons. The biological profile includes the decedent's age at death, sex, ancestry, stature, and any individualizing traits that would be known to family and friends, such as the presence of braces on the teeth, healed or healing fractures, amputations, skeletal deformities, and other medical and anomalous conditions of the bones and teeth. An accurate biological profile provided by a trained forensic anthropologist is paramount for the investigation to reach an identification.

## DISCUSSION

Most forensic anthropologists are likely to have worked cases that have gone cold for various reasons. The anthropology lab at the Tarrant County Medical Examiner's Office (TCME) has investigated many past cases where the skeletal remains were initially examined by a nonanthropologist and a biological profile was generated at the time of examination. In 2004, the first author received a skeleton that had been housed in the Fort Worth Police Department Crime Laboratory Evidence Locker since 1963, along with a copy of the analyst's case file. At the time of discovery of the decomposed, mostly skeletal remains, the Tarrant County Medical Examiner's Office was not yet established. The duty of the examination of the remains fell to the Crime Laboratory Director of the Fort Worth Police Department. The Director, being a good scientist, turned to the literature and found the sentinel publication by Wilton Marion Krogman, "A Guide to the Identification of Human Skeletal Material," published in the Federal Bureau of Investigation Law Enforcement Bulletin in 1939 (1). In 1963, this

would have been the most comprehensive of a handful of published references (2-6) on the subject of human biological profile creation from skeletal remains. A review of the case file along with the remains showed that the Director collected some of the data outlined in Krogman's guide (1). The maximum diameters of the humeral and femoral heads were measured correctly and unambiguously fell into the male range. The femur, tibia, and humerus were measured in inches and used to estimate the stature. When the measurements and observations were repeated, it was found that each one had been correctly recorded. Krogman's guide (1) clearly placed the skeleton on the male side of the male/female continuum; however, for unknown reasons, the remains were labeled as female. In the bulletin published by the Fort Worth Police Department Crime Laboratory the narrative of the remains includes a description of a "green lady's jacket with gold lining and dark blue teen-age type socks." Furthermore, the pelvis was complete; thus, the indicators of sex from the pelvis could have been evaluated visually as recorded in Krogman (1). There is no notation in the file that these indicators were examined.

It is unknown why the results of metric analysis of a few traits in the skeleton were not taken into account. It could have been due to unfamiliarity with Krogman's methods and that the estimate of sex was based largely on the observations of the clothing. Contained within the case file is a copy of the Fort Worth Press from February 3, 1964, showing a facial approximation drawing of the decedent (**Image 1**). The chin has male characteristics and the overall appearance is of a male with a female hairstyle. Also contained in the file is correspondence between the Crime Laboratory Director and law enforcement agencies with missing females. Multiple comparisons with dental records of missing females are documented.

In 2004, following the anthropology analysis which included the male sex assessment, TCME commissioned a clay facial reconstruction and submitted the results to the news media. Within hours, the office received multiple calls regarding the case. All of the calls were about a possible match to the missing person, Kenneth Glaze. Mr. Glaze was a 35-year-old

male from Hillsboro, TX who lived in Fort Worth and was reported missing in August 1963. The facial reconstruction looked remarkably similar to Mr. Glaze (**Image 2**). In 1963 Hillsboro, TX was a small town that was served by a single dentist and we attempted to locate archived dental records to no avail. Fortunately, a maternal first cousin was located and was able to provide a sample for DNA analysis. The mitochondrial DNA confirmed the maternal relationship and the medical examiner classified the positive identification.

As the example above dramatically demonstrates, the standard components of the biological profile are critical to the successful resolution of many unidentified remains cases. The modern forensic anthropology lab-

**Image 1:** Facial approximation of the decedent, February 1964.



**Who Was She?**



**Image 2:** Clay facial reconstruction by Dr. Suzanne Baldon showing dentition compared to known photograph of decedent, 2005.

oratory uses macroscopic, microscopic, metric, and radiologic information to estimate the age at death, biological sex, ancestry, and stature of an individual from the skeleton. Modern methods are based on scientific research that provides quantified error and accuracy rates and meets the guidelines for admissibility of scientific evidence (7). Additionally, the continued development of the Forensic Anthropology Data Bank (FDB) (8) has provided forensic anthropology with a wider variety of comparative skeletal data from the contemporary period, thus allowing more meaningful studies to be conducted on temporally relevant data for the development of methods used in forensic contexts.

This paper will explore the processes by which the biological profile is estimated by the modern forensic anthropologist. There is a tremendous amount of published material that addresses different components of the biological profile. This paper will address the most salient methodology used today with a brief description of the most pertinent historic references.

### Sex/Ancestry Metric Evaluation

Similar metric methods are used to evaluate sex and ancestry and thus they are discussed together in this segment. Both sex and ancestry assessment rely on cranial and postcranial measurement data, Fordisc software, and multivariate discriminant function analysis (DFA). Morphological features are observed by the forensic anthropologist to form an initial opinion of the sex/ancestry for any skeleton, yet we rely on metric confirmation of our visual assessment. There has been a continual collection of measurement data for the past 30 years that is available for comparison to an unknown skeleton. Forensic anthropologists record spatial coordinates of anatomical landmarks on the cranium and measurement data of the mandible and postcranial skeleton. These data represent the size and shape of individual bones that allows for an estimation of sex and ancestry of unknown human remains. Forensic case data along with data from skeletal collections representing various genetic ancestries have been gathered by anthropologists into the FDB to build a database of groups commonly encountered in modern forensic casework. Sex and ancestry clas-

sifications are most commonly determined by using a computer program, Fordisc 3.0 (latest version: 3.1.307), that statistically compares measurements taken from unidentified cranial and postcranial remains to measurements from individuals of known sex and ancestry (9). Although this process appears to be uncomplicated, it is imperative to have an understanding of discriminant function analysis, the capabilities and limitations of the Fordisc software, and potential sources of error when applying these methods.

Current FDB cranial group sample sizes include adequate numbers of American whites (518 males/340 females) and blacks (156 males/96 females) and Hispanics of Mexican origin (227 males/62 females). The Chinese (n=79), Vietnamese (n=51), and Guatemalan (n=83) samples contain only males, and as with the Japanese (84 males/58 females), do not include many individuals who are American-born. Most individuals in the database are positively identified to sex and ancestry from antemortem information. All have 20th century birth years except the historical American Indian group (59 males/32 females). In this group, 15 males and five females were derived from forensic cases; however, the remainder have birth years from the mid to late 19th century (9, 10).

Recent technological tools, such as a digitizer, enable Cartesian coordinate (x, y, z) data point collection of cranial and postcranial anatomical landmarks that are used in sex and ancestry estimation. Software is available that converts the digitized coordinates into the cranial measurements used in Fordisc. The digitizer minimizes error as each landmark is recorded one time, as opposed to caliper measurements that necessitate the accurate location of many landmarks (e.g., basion) multiple times. The measurement is recorded by the instrument, reducing data entry errors. Measurement definitions are described by several authors (11-14) and proper understanding and application of measurement performance is essential to an accurate ancestry estimate. Because differences of a few millimeters may misclassify a cranium, and these missteps are common, measurement error should always be evaluated (9).

To use Fordisc, the operator inputs measurement data and selects sex/ancestry groups for the comparison. Multiple analytical, reporting, and graphing options are available. Recommended operational guidelines can be found in the “help” file (9, 10). It is important to realize that some of the populations included in Fordisc are underrepresented and that may cause classification errors. For example, Hispanic males frequently misclassify as female in Fordisc due to low sample sizes of Hispanic individuals (15). Thus, interpretation of the results is critical to a positive outcome. The lack of large sample sizes of varied populations is a considerable limitation; however, the numbers continue to grow with time (9).

Fordisc uses DFA to compare inputted cranial and postcranial metric data from an unidentified forensic case to a database of known populations within the FDB. Discriminant function analysis has been used for many years to estimate sex and ancestry from human skeletal remains using the skull (16-20) and the postcranial skeleton (15, 21-37). Most of the early studies were developed using the Terry and Hamann-Todd collections and American military war dead, and thus represent black and white males almost exclusively. More recently, DFA based studies for estimating sex have been expanded to include males and females of populations considered to be Hispanic (15,

35, 37, 38) and modern American blacks and whites (36). Other metric methods have been developed for use in the postcranial skeleton, including those using measurements of the femur (39-42). Within Fordisc, the discriminant score of an unknown individual is compared to the mean discriminant function score for each reference group. When multivariate analyses are run, the results are organized by Mahalanobis distance ( $d^2$ ) from the centroids of each group in the comparison (43, 44). Posterior probabilities and F, R, and Chi typicalities are reported for each test (**Image 3**). Higher probability and typicalities are more indicative of an assessment being correct. Low typicalities result from individual crania that are atypical of a group, don't belong to the group, or error, and should be considered with caution.

The estimation of sex from complete or nearly complete skeletal remains is straightforward in most cases when evaluated by a forensic anthropologist. Although it is commonly published that the reliability of sex assessment is best in the pelvis, second best in the cranium, and less accurate in the postcranial skeleton, recent research has shown that the latter two should be reversed in this statement. Recent studies looking at postcranial measurement data clearly shows that it outperforms the cranial measurement data for sex as-

Total Correct: 649 out of 1158 (56.0 %) \*\*\* CROSS-VALIDATED \*\*\*

Multigroup Classification of Current Case

Group	Classified into	Distance from	Probabilities			
			Posterior	Typ F	Typ Chi	Typ R
WM	<b>**WM**</b>	10.1	0.833	0.762	0.753	0.751 (122/485)
BM		14.9	0.076	0.406	0.385	0.442 (68/120)
HM		15.9	0.046	0.337	0.319	0.356 (146/225)
CHM		16.9	0.028	0.285	0.260	0.186 (58/70)
JM		19.3	0.009	0.174	0.155	0.094 (78/85)
AM		20.2	0.005	0.145	0.123	0.309 (39/55)
GTM		22.5	0.002	0.083	0.070	0.065 (73/77)
VM		23.7	0.001	0.063	<b>0.050</b>	<b>0.041 (48/49)</b>

Current Case is closest to WMs

**Image 3:** Fordisc 3.0 statistical output.

assessment (36). Recent statistical models using ordinal observation data are cited as having 83-90% correct sex classification for the cranium, compared to correct classification rates at 90% or higher for postcranial metric classifications of long bones (36).

Recently, Hispanic skeletal collections have received a large amount of attention and new DFA of the population has shown promise in assessing sex and ancestry. In a recent study by Spradley, DFA, similar to that used in the Fordisc software, resulted in high accuracy rates for estimating Hispanic ancestry from the cranium (85% correct for Guatemalans and 76% for Mexicans) (38). Postcranial measurements of Hispanic individuals developed on U.S.-Mexico border crossing decedents from an Arizona medical examiner combined with measurement data on Hispanic individuals from the FDB were used to create DFA formulae with a reported classification rate greater than 80% for sex estimation (15) and 70% for ancestry estimation (45). For sex estimation, single bone section points were reported for fragmentary or partially recovered remains, and the most accurate of these ranged from 85-87%, with the radius, humerus, and clavicle displaying the highest cross-validated classification rates. When compared to the data from American blacks and whites (36), population-specific differences in sexual dimorphism were apparent (15). The most accurate bones for Hispanic sex discrimination were the radius and humerus; for American whites, the humerus and ulna; and for American blacks, the humerus and clavicle. Univariate statistics compared among groups reported the top three bone measurements for correct classification between these populations (15). For ancestry estimation, DFA formulae for postcranial measurements were most accurate in American blacks (85%) and American whites (80%) and a bit less accurate in Hispanics (72%) (45). The lower accuracy rates for Hispanic populations are likely due to unavailability of large sample sizes of Hispanic remains with known provenience, but anthropologists hope to improve metric ancestry estimates of Hispanic individuals in the near future (45).

In addition to analysis performed using Fordisc software, many anthropologists are using geometric

morphometrics analysis (GM), a metric method that constructs a three-dimensional (3D) digital model of the bone in question by using Generalized Procrustes Analysis (GPA) to record digitized Cartesian coordinates of defined landmarks. The three-dimensional nature of GM data in its original state allows for the study of shape without size influences, which can incorporate sophisticated statistics into the formation of biological profiles. While GM can be used to test a variety of hypotheses (46, 47), several methods have been developed in recent years to estimate sex and ancestry from cranial, dental, and postcranial information with varying degrees of success (38, 48-59). The most holistic of these methods is 3D-ID, a computer program that acts in much the same way as Fordisc to produce sex and ancestry estimates of an unknown cranium (54). Another study used GM to assist in distinguishing between Hispanics and American blacks and whites, an ancestry estimation that has previously been difficult to perform accurately with traditional craniometrics (38). Geometric morphometric analysis has not yet been shown to be more accurate than traditional linear measurement methods, but these types of methods are still being refined (59, 60).

### Nonmetric Assessment of Sex

Skeletal variation attributed to sexual dimorphism can be visually evaluated and used to estimate the biological sex of the individual. Overall size and muscular development are good indicators of sexual dimorphism in adults because they are influenced by extrinsic mechanisms such as biomechanical load bearing and intrinsic mechanisms including sex hormone levels (61). Skeletal robusticity is assessed by bone diameter and length, while bone rugosity is judged by the extent of development of markers of muscular and connective tissue interface with the bone (62). Populations vary in the expression of sexual dimorphism and it is therefore important to use comparative data from an appropriate population when applying these techniques to forensic cases.

Although there is a great deal of interest in bioarchaeology and forensic contexts to assess sex of immature skeletal remains, it has been shown by multiple stud-

ies that there is insufficient discrimination to allow accurate estimations from unknown fetal, infant, or subadult material (63-67). Sex differences have been noted during fetal development in the ilium and pubis (68, 69), and postnatally in the os coxa (64, 65) and the mandible (70, 71). These explorations to separate the sexes of immature remains have shown that sexual dimorphism is not sufficiently developed in subadults and methods to estimate sex in subadult skeletons have low predictive value and high interobserver error.

In the forensic arena, the amelogenin locus as a biological sex marker is available in the forensic DNA short tandem repeat (STR) kit and is the most reliable method for confirming sex of immature remains. The commercial STR multiplex kits most commonly use a primer set that produces fragments of X and Y chromosomes. Amplification failure of the Y chromosome in male samples can cause misidentification of sex as female in these tests (72).

Sexual dimorphism in the adult human skeleton has been observed in the pelvis, skull, and postcranial skeletal elements. Nonmetric analysis of pelvic morphology (**Image 4**), along with metric methods, is reported to be the most reliable estimator of sex for adult skeletons (36, 73). Within the pelvis, the morphology of the pubic region is regarded by forensic anthropol-

ogists to contain the most reliable indicators for sex estimation. The presence or absence of the ventral arc (**Image 5**) and the subpubic concavity, and the morphology of the medial surface of the ischiopubic ramus were identified by Phenice as an accurate method for sex estimation (73). Recently, the three traits described by Phenice have been further explored by assigning five classifications to each trait (74). These revisions provide posterior probabilities and low estimated error rates in black and white males and females. The Klales method can be readily explored via a website with pictures and phase descriptions (<http://nonmetricpelvissexing.weebly.com/>) (74).

In addition to the features of the pubic region, the general robustness of the os coxa, particularly the diameter of the acetabulum, is a good indicator of sexual dimorphism. Studies reporting descriptions of morphology with scoring mechanism for sex estimation are available for the greater sciatic notch (63, 75), the auricular surface of the ilium (76), and pre- and postauricular sulci (63, 76). This anatomical area contains the thicker bone of the ilium that is likely to survive long-term burial and other destructive taphonomic processes more readily than the pubis.

Within the skull, nonmetric sex assessment relies mostly upon gradations in the robusticity and rugos-



**Image 4:** Comparison of female (left) and male (right) pelvises.

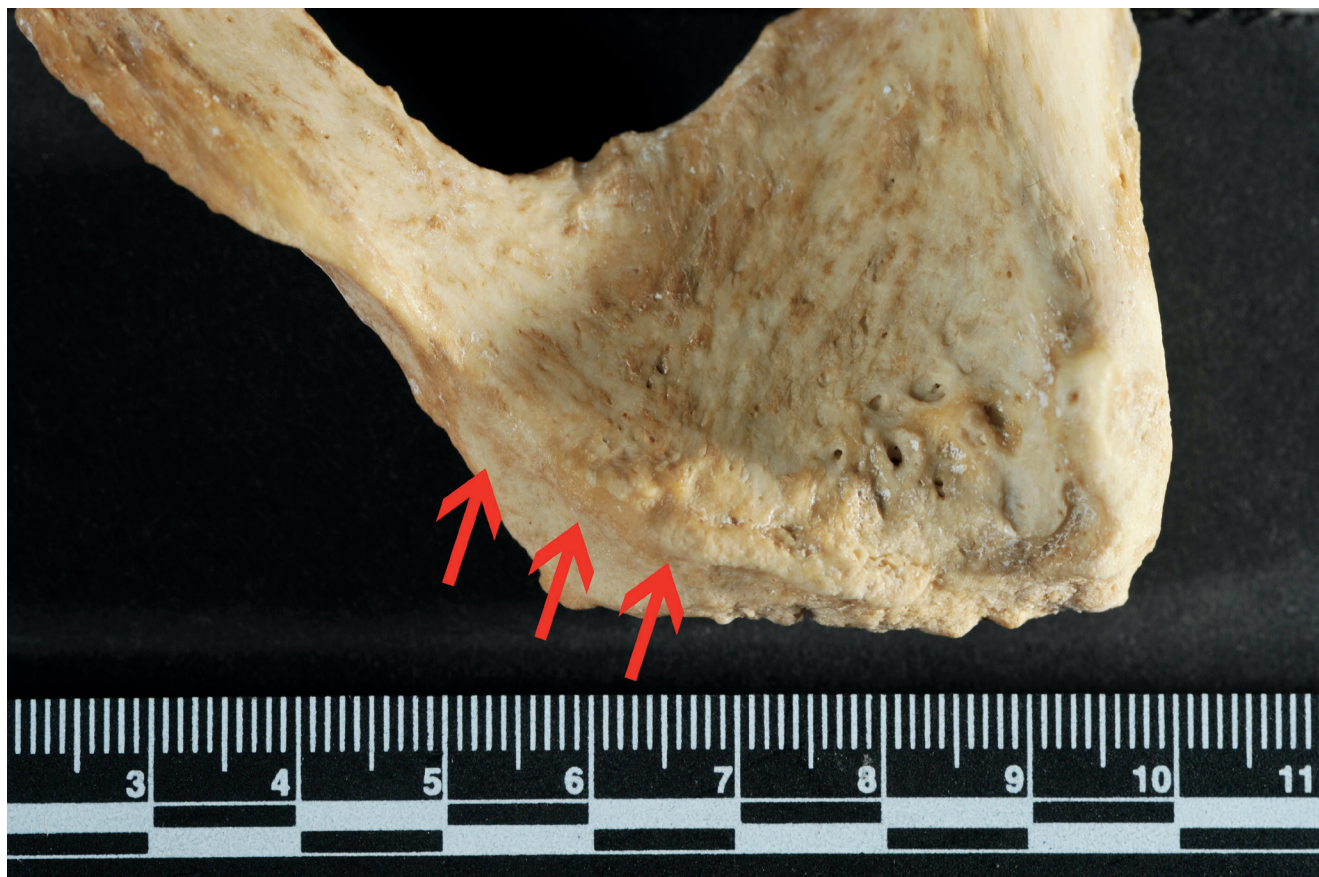
ity of the muscle attachment sites. Scoring methods for observations on the mental eminence, superior orbital margin, glabellar projection, nuchal crest development, and mastoid process volume are commonly used by the forensic anthropologist (63, 77). Recent evaluation has provided data on intra- and interobserver error and accuracy (77).

Nonmetric methods describing sexual dimorphism in other areas of the postcranial skeleton have been published recently on the morphology of the distal humerus (78, 79), rhomboid fossa of the clavicle (80), and bridging of the sacroiliac joint (81). Advanced valgus angulation of the female elbow is readily visible and is about 20-25° compared to 10-15° in males. Attempts to recognize the variation in morphology in the bony distal humerus have resulted in mildly accurate nonmetric techniques to distinguish sexual dimorphism

(78, 79). Observations of the bony marker of the syndesmosis of the costoclavicular ligament found that a fossa on the clavicle was a strong indication of male sex and more likely to be a young male 20-30 years of age (80). Bony bridging and ankylosis of the sacroiliac joint has been found to have dimorphic presentation; males more commonly manifest extra-articular bridging, defined as bony bridging from the ilium to the sacrum with a dome-like appearance over the superior portion of the sacroiliac joint, while females demonstrate intra-articular bridging defined as smooth, continuous fusion that can lead to ankylosis of the joint (81).

### Nonmetric Ancestry Assessment and Considerations

Morphoscopic ancestry estimation methods require visual assessment of skeletal traits that show popu-



**Image 5:** Female pubic bone. Ventral arc indicated by red arrows.



lation variation according to broad ancestral groups such as African, Asian, and European. Studies have examined the frequency of trait expression in skeletal samples of known ancestry and evaluated these traits in unidentified remains to estimate ancestry. Recent methods assign numerical values to the expression of a trait, allowing for the calculation of accuracy and error rates (82). This quantification has statistically proven that nonmetric methods perform equally well in ancestry estimation as metric methods such as Fordisc (44, 83).

The majority of nonmetric traits analyzed for ancestry estimations are found on the skull. Observed traits include: shape of the nasal aperture, root, spine, bridge, and sill (42, 62, 83-87); shape of the orbit and interorbital breadth (83, 85, 86); angle of the midfacial profile and zygomatics (42); shape of the zygomaticomaxillary suture (83, 85, 86); presence or absence of zygomatic tubercles,inion hook, wormian bones, postbregmatic depression, and canine fossa (42, 62, 83, 86); shape of the cranial vault and presence and complexity of its sutures (42, 86); palate shape and complexity of its sutures (83, 85, 86); and shape of the mandible border, rami, and gonial angles (86, 88). Dental traits can also be indicative of ancestry, including the presence or absence of Carabelli's cusp, crenulated molars, and shoveled incisors (86, 89, 90). Many of these nonmetric traits are expressions of evolutionary adaptations to differing climates, making it possible to categorize expressions into broad geographically-based ancestry groups. Traits are also examined in the postcranial skeleton with varying degrees of accuracy, including the rugosity of the gluteal ridge and tuberosities (91) and bifid spinous process expression of cervical vertebrae (92).

Recent statistical analysis has found that, in distinguishing between American blacks and whites, six cranial nonmetric traits are most accurate: anterior nasal spine, inferior nasal aperture, interorbital breadth, nasal aperture width, nasal bone structure, and postbregmatic depression (82). The analysis of these six traits together has been shown to be nearly 90% accurate in estimates of black and white ancestry (82). The development of ancestry estimations for populations

other than black and white has been delayed due to low representation of these groups in the Bass, Grant, Terry, and Hamann-Todd collections, the most readily available collections with known antemortem provenience. The most holistic collection currently available for study in the United States is the Forensic Anthropology Data Bank, developed at the University of Tennessee, which is based on traditional metric analysis of donated and forensically sourced remains from across the world (14, 43). The FDB includes Asian and Hispanic populations with limited sample sizes, thus representing less variation than may exist in the living populations. No Hispanic individuals in the FDB originate from the Caribbean, effectively eliminating the possibility of studying a large subset of the American Hispanic population (9, 49). While studies strive to establish methods for estimating Hispanic ancestry (38, 93-96), many in the field argue that borrowing the cultural definition of Hispanic as any persons hailing from Spanish-speaking countries is representative of far too much variation to be grouped into one biological ancestry (44, 49, 93, 96). Though Cuban and Mexican people would be considered Hispanic socially, those of Cuban ancestry have been found to display African morphology when evaluated geomorphometrically, while Mexican crania were found to appear more similar to Native Americans (49).

### Stature Estimation

Stature estimation methods use bone length measurement data and regression analysis to predict a stature range that are compared against antemortem reported height included in routine medical checkups and listed on government-issued identification and military records. Regression formulae have been developed for all long bones (97-102). Because limb length ratios vary between ancestry groups and are affected by sexual dimorphism, a population-specific set of equations should be used for each sex/ancestry group; in the most commonly used software, Fordisc, the available equations encompass male and female black and white Americans and male Hispanic Americans (43, 99). Fordisc also gives an option to use a combined "Any" sex/ancestry group, but the stature range produced is much larger to account for the increased error

when using these nonspecific equations and the use of this approach is suggested only when no population information is available (9). Based on original regression equations that estimated stature within a range of 3 cm (97-99), Fordisc calculates stature estimates for each long bone present as well as all possible combinations of bones and presents the stature range and error associated with each equation (9, 43). Final stature estimates are performed by choosing the equation with the lowest standard error and smallest range (9). Regression equations have also been developed using long bone measurements in non-American populations, including Eastern Europeans (103) and Guatemalans (104).

The most accurate adult stature calculations incorporate each bone that contributes to standing height and are deemed the “anatomical method.” In this method, measurements of the cranium, vertebrae, sacrum, talus and calcaneus, and bones of the lower limb are combined with given values representing soft tissues to form an estimate of complete stature within a range of approximately 4.5 cm (105, 106). While this method can be used regardless of the individual’s sex and ancestry, it does require all necessary bones to be present and complete, which can be difficult to achieve in the face of many taphonomic processes (105-107).

Total long bone length may be calculated from fragmentary long bones using linear regression that requires measurement data between anatomical landmarks (104, 108, 109). These equations are also sex- and population-specific and are currently available for American blacks and whites (108), Native Americans (109), and Guatemalans (104). Once calculated, the total long bone length may be used in stature estimation equations as substitutions for the actual measurements, but this method should be used with caution in a forensic setting as statistical error rates have not been established (104, 108, 109).

Just as in adults, subadult stature is estimated using long bone length equations that incorporate sex and ancestry (110-112). These age-specific regression equations are based on diaphyseal lengths taken from antemortem radiographic images (110, 111). Due to

heavy influences on children’s growth from a variety of environmental and nutritional differences, stature estimates in subadults are much more complex than those of adults and may be heavily influenced by socioeconomic status (110).

### Age

Age at death estimations of unidentified remains are dependent on growth and developmental indicators for immature remains and degenerative indicators for adult skeletons. Knowledge of skeletal and dental development is critical to identify processes that provide age-specific data. Endochondral and intramembranous bone formation, ossification center appearance and growth, epiphyseal ossification and fusion, dental formation and eruption patterns, and histomorphological changes in diaphyseal cortices from birth onward are processes that provide usable data when predicting age at death. A visual scan of the skeleton readily distinguishes adult and subadult individuals. “Subadult,” a broad term meaning “not yet adult,” is commonly used interchangeably in the literature with more narrowly defined age terms such as fetal, neonate, infant, child, juvenile, and young adult. A multivariate approach is more accurate than a single age method approach (113, 114) thus the more completely recovered skeleton has more data available to accurately estimate the age at death.

Gestational age has forensic significance in a variety of circumstances including maternal/stillborn death, illegal abortion investigations, unattended fetal demise cases, and infanticide. Crown-rump and crown-heel lengths are commonly used to estimate the age of fetal and neonate remains. Regression formulae for predicting crown-heel length (115) and age in weeks directly (67, 116) have been developed using radiographic measurements of long bone diaphyses. Regression formulae are available for dry bone measurement of most bones of the cranial and postcranial skeleton except for vertebral elements (69).

Histomorphometric methods of subadult bone analysis use a phase system to evaluate the amount and location of primary lamellar bone, the absence or pres-

ence and form of remodeling, the location of woven bone, and the thickness and composition of the cutaneous and pleural cortices in histological sections of subadult ribs (117).

Tooth development and eruption data are useful for estimating age until the early third decade (**Image 6**). Tooth mineralization has been found to correlate better with gestational age than skeletal development (67). Mineralization and cusp coalescence, crown formation, and root formation data for some deciduous and permanent teeth of males and females are available from radiological data (118, 119). Tooth formation/eruption charts are commonly used (63, 120); however, they combine sex data and present a wide age interval when the standard deviations of the mean are expanded to an appropriate range. Standards appropriate to the population and sex of the unidentified person should be applied when available. The London Atlas addresses tooth development and eruption for both sexes from 28 weeks *in utero* to 23 years. It can be downloaded and used free of charge (121, 122). The data in the atlas were compiled from cemetery populations and odontology collections and include English whites and Bangladeshi. Differences between dental and chronological ages have been evaluated and found to be minimal (123).

Early childhood age estimation is accomplished by comparison of skeletal development with radiographic atlases (124-126) and complimented by dental formation and eruption data. In the teenage and early adult years epiphyseal union together with permanent molar formation and eruption data (127-130), is most useful. Epiphyseal union data have been collected from various populations using dry bone evaluation (131-136) (**Images 7 and 8**) and medical imaging (137-141). Many of these studies document clinical norms of skeletal development for a particular chronological age. Forensic anthropologists work in reverse and estimate an age range corresponding to the physical expression of bone and tooth development.

Adult age estimates are generally much broader than those for immature remains due to the varied environmental and nutritional stresses experienced during the

lifespan and the discrepancy between biological and chronological age in many individuals. When fleshed unidentified individuals are evaluated, it is common for the anthropologist to pull bone samples for analysis to predict the age at death. The skeletal elements most commonly examined are the pubic symphysis (**Image 9**) and the sternal end of the right fourth rib that have shown predictable morphological change with age. Data sets comprised of hundreds of individuals of known age, sex, and ancestry have been examined to develop techniques for predicting an age range for an unknown individual (131, 142-148) and comparative testing between methods has been conducted (149).

Additional skeletal elements are used to supplement age estimation data obtained from the pubic sym-



**Image 6:** Maxillary dental arcade: neonate (top photo)/five-year-old (bottom photo).



Image 7: Medial clavicle epiphyses in the process of fusing.



Image 8: Humeral and radial epiphyses demonstrating fused elbow and ununited shoulder and wrist.



**Image 9:** Pubic symphysis morphology comparison: young adult (top photo)/middle years (middle photo)/ late years (bottom photo).

physis and rib. These methods are applied as needed and often depend upon the availability of skeletal elements for analysis. The auricular articular surface of the ilium undergoes a morphological change with age (150, 151). The first rib costal surface geometric shape and the texture of the rib's tubercle have been shown to undergo predictable changes with age. The study combines the expression of each of these traits in a component analysis that provides an age interval estimate (152). Cranial sutures of the vault (153, 154) and palate (155) provide broad age estimations and are often used when only a cranium is recovered. Remodeling of the occipital condyles due to osteoarthritis may help narrow this range to younger adult versus older adult. Tooth root translucency and periodontal regression (156, 157) may also provide information to narrow an age range when only a cranium is recovered.

Overall degenerative changes in the skeleton are often considered (**Image 10**), especially by a more experienced forensic anthropologist. A few scoring techniques exist to quantify age-related changes in joint surface morphology including the development of vertebral osteophytosis (39, 131, 158, 159). Marginal osteophytes develop at the site of the insertion of the anterior longitudinal ligament (**Image 11**) (160). In extreme cases, the marginal osteophytes of articulating vertebrae many exhibit bony union or form a joint-like space at the point where they meet (160). Studies have shown that, in general, regression analysis does not find a strong predictive value of age using the scoring methods on osteophyte and osteoarthritis development within the vertebral column (159). However, osteophyte development is often used to narrow an age at death estimate established by methods in other parts of the body. General guidelines indicate that individuals aged 45 and older always show some development of lipping of the vertebral bodies, although this will be dependent upon the population or subpopulation and the physical activity level (158, 160).

Histomorphometric methods may compliment an age profile generated by other methods such as those discussed above; however, these data may also replace morphological methods in cases that consist entirely



**Image 10:** Degenerative changes in the shoulder.



**Image 11:** Osteophytosis of the vertebral column.

of bone fragments or isolated long bones and ribs. An unstained bone section reveals a lifetime of bone turnover (161). Bone remodeling is responsible for mineral homeostasis, maintenance of gross structure within the stresses of biomechanics, and repair of damage caused by a multitude of insults. The aging process leads to an abundance of fragmentary osteons that result from creation of secondary osteons during bone remodeling. The quantification and ratio of these structures are the major predicting variables in most histological age estimation methods along with the amount of unremodeled bone (in subadults) and mean osteon size (161). The first published method observed and quantified complete and fragmentary osteons to develop regression formulae for the lower extremities (162). Modifications have included methods using less destructive, smaller bone samples and upper limb bones (163, 164). Rib and clavicle methods count complete and fragmentary osteons over the entire cortical surface of a section to estimate the osteon population density that is used in the regression formula to predict age (165, 166). The variability of these methods across various populations has also been explored (167).

In constructing a final age interval, the forensic anthropologist will assess the developmental and degenerative changes mentioned above that are most appropriate to the anticipated age of the individual. An age interval is constructed based on the data gathered from the assessment with appropriate caveats noted. The final age estimate is a matter of expert judgment after synthesis of all available information.

Statistical methods applied to age estimation are evolving in forensic anthropology. Many methods presented in this article use a phase system to evaluate morphological traits. Multiple observed traits are grouped together with the assumption that the bony morphology of all traits is related and based on age (168). In the phase system, an analyst must make a decision to value one variable over another in assigning the phase at times when an individual specimen does not completely match the description (168, 169). The sample size, mean age, standard deviation, and 95% range of age within a phase, referred to as a percent-

tile method, is not an optimal statistical strategy for comparison of an unknown sample. The 95% range is equivalent to listing the 2.5 and 97.5 percentiles of age. Percentile methods should include standard errors on the percentile because when the sample is subdivided into phases/stages, a single phase may only have a few cases to represent it and that stage may have substantial sampling variance (170).

An alternative approach that is gaining favor is to incorporate a component system that evaluates each trait independently and allows for that trait to have an individual course over the span of a lifetime (168). Component-based systems have been statistically evaluated using transition analysis, a parametric method for modeling the passage of individuals from a given developmental state to the next higher stage in an ordered sequence (170, 171). Transition analysis of component scores provides statistically robust age ranges that are less sensitive to problems resulting from developmental outliers and sample size constraints seen in the percentile method. A maximum likelihood estimate for the average age at which an individual is likely to transition from one phase to another is the result (136).

The recently released updated data collection protocols of the Forensic Data Bank reflects a movement from phase system aging methods to component-based systems evaluated with transition analysis (8). As described in the Data Collection Procedures manual, the pubic symphysis, iliac portion of the sacroiliac joint, and cranial sutures are examined. Defined components that undergo age-related morphological change are evaluated. Each component is scored by selecting one of the characteristic phase options presented with the system for each bone (8). Over time, the collection of these types of data will allow forensic anthropologists to test their accuracy and precision for estimating the age of adult skeletal remains.

## CONCLUSION

The case study presented in this article demonstrates how inaccurate assessment of any component within the biological profile may impede the successful

resolution of an unidentified remains investigation. Proper application and interpretation of anthropological methods produced an accurate biological profile 41 years later that resulted in a positive identification.

The biological profile comprised of sex, ancestry, stature, and age at death estimations is used to compare the data from unidentified remains to reported missing persons in order to generate leads in cases that have no tentative identification. The individual components are inputted into national databases such as NCIC and NamUs where they can be accessed by law enforcement agencies and private citizens concerned with resolving missing person cases.

This article has distilled a large body of literature consisting of methods for estimating the components of the biological profile in order to inform forensic pathologists of the practices used in modern forensic anthropology laboratories.

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