**Project IDENTIFICATION: Developing Accurate Identification Criteria for Hispanic Individuals**

1. The source of potential subjects for Project IDENTIFICATION is the Pima County Office of the Medical Examiner (PCOME) in Tucson, Arizona. The PCOME receives approximately 250 U.S. / Mexico border-crossing fatalities per year with approximately 40 – 50% comprising skeletal remains. Because the PCOME has a 70% identification rate, the projected sample size for this project is approximately 175 positively identified individuals over a two year period. These individuals will be predominantly from Mexico, however some come from other areas in Latin America. While this particular project will only use data from positively identified adult individuals, their identity (name, identification number, residential address) will remain anonymous. Data will be collected as individuals are brought to the PCOME. At the time of data collection their identity will not be know, however at a later date, either through dental analysis or DNA anlaysis, some will be positively identified. It is the positively identified individuals that will be used in the proposed research. Only the individuals sex, age, and the geographic location (country, state, city or town) will be revealed to me for analyses in the proposed research.
2. The subjects that will be used in the proposed research are U.S. / Mexico border crossing fatalities brought to the PCOME by U.S. Border Patrol. No consent forms will be utilized as the subjects are deceased and their identity will not be know at the time of data collection. Further, their personal identity will not be revealed to me.
3. The goal of data collection for this project is to create a data base of cranial and post-cranial data that will provide accurate sex and ancestry criteria. Further, data will be archived and made publically available for subsequent use by anthropologists for additional research. Craniometric data will be collected using a Microscribe® G2X digitizer in conjunction with the program Threeskull (Ousley 2004). The Microscribe® G2X digitizer collects landmark or coordinate data and 3Skull allows for the archival of the data. The end result is a database containing inter-landmark distances (or traditional craniometric data) and the landmark data. The traditional craniometric data archived includes all the standard craniometric data and additional craniometric data defined by Howells (1973).

Most forensic anthropologists utilize traditional, standard craniometric data (Buikstra and Ubelaker 1994; Moore-Jansen et al. 1994). The most popular application of traditional craniometric data by forensic anthropologists is FORDISC 3.0, used for estimation of sex, ancestry, and stature from unknown forensic cases. Although the cranial data that will be collected is coordinate data, the traditional craniometric data is automatically computed by 3Skull and will be archived in both formats. Therefore, all traditional craniometric will be input into FORDISC 3.0 for use by forensic anthropologists all over the country.

Standard postcranial metric data will also be collected (Buikstra and Ubelaker 1994; Moore-Jansen et al. 1994), however additional postcranial metric data will be collected following the measurements outlined in Zobeck (1983). These additional postcranial measurements have shown to differentiate population groups better than the standard measurements (Zobeck 1983). The standard postcranial measurements will be used to develop sex estimation criteria, thus providing familiar methods easily used by all forensic anthropologists. However, the additional Zobeck measurements will be archived and used in subsequent research to determine if these measurements provide better estimates of sex. If additional measurements do provide more accurate criteria, results and measurement definitions will be published.

Because inter-observer variation can be an important source of error in multivariate analysis of metric data (Jamison and Zegura 1974), one person, experienced in data collection will collect all craniometric data. Mr. Francisco Baires, a graduate student in the Department of Anthropology at the University of Arizona, will collect cranial and postcranial data. Mr. Baires has extensive training in craniometric data collection. The PI of this proposal personally trained Mr. Baires during the Spring and Summer of 2005. Mr. Biares repeatedly digitized (using the Microscribe® digitizer) crania from the William M. Bass skeletal collection at the University of Tennessee. Mr. Biares’ measurements were compared against the PI of this proposal and Dr. Richard Jantz for accuracy. Further, intra-observer variation was rigorously tested by digitizing the same set of crania at least three times in order to detect any significant deviations.

Craniometric data will be collected at the PCOME in Tucson, Arizona using a three-dimensional Microscribe® digitizer and laptop. Postcranial data will be collected using standard anthropometric equipment including sliding and spreading calipers and an osteometric board (available at the PCOME). All data collected will then be submitted to the Forensic Anthropology Data Bank (FDB) and to FORDISC 3.0 for widespread use by forensic anthropologists.

Both univariate and multivariate methods will be used to develop sex estimation criteria. Previous studies have shown high classification rates using univariate data (France 1998; Spradley and Jantz 2003). Univariate methods will include taking all of the 44 standard postcranial measurements (Buikstra and Ubelaker 1994; Moore-Jansen et al. 1994) from the left side, substituting the right side only when measurements from the left side are missing. Mahalanobis distances, sectioning points, and expected classification results will be produced, using SAS 9.1 (SAS 2002-2003). Sectioning points and associated classification results, especially for standard measurements, are easy to use and interpret and will published for each individual measurement. The same statistical procedures will be used on the additional Zobeck measurements (described in ***Data Collection***) in order to ascertain if any other measurements provide higher classification rates for sex estimation. If any Zobeck measurements provide better results, they will also be published along with the measurement definitions.

Multivariate analysis of sex estimation will be assessed via Discriminant Function Analysis (DFA). First, a stepwise DFA will be performed in SAS 9.1 in order to find the best subset of variables for discrimination of sex. A stepwise DFA will be run per bone, and then again for all possible combinations of measurements from all bones. The most significant subset of variables, respectively, will then be run using a DFA in SAS 9.1 in order to arrive at the best combination of variables for that discriminate sex.

Ancestry estimation criteria will be assessed using traditional morphometric and geometric morphometric methods. DFAs using traditional craniometric data are widely used and more familiar to the majority of practicing forensic anthropologists (Barrio et al. 2006; Case and Ross 2007; Karaman 2006; Kim et al. 2006; Spradley et al. 2008; Wescott 2006). However, geometric morphometric methods are gaining popularity in forensic anthropology (Christensen 2005; Franklin and Cardini 2007; Franklin et al. 2007; Kimmerle et al. 2008; Ross et al. 1998; Ross et al. 2004). In a geometric morphometric analysis, landmark data, or coordinate data, is "pre-processed," for example, by a General Procrustes Analysis (GPA) or a Thin Plate Spline (TPS) that provides principal component scores or partial warp scores. These scores are then used in a traditional multivariate analysis (i.e. DFA) for group discrimination.

The added advantage of using landmark data is that the geometric properties of shape are preserved and the ultimately shape differences are visually observed (Figure 3). In addition to its ability to discriminate via traditional multivariate methods, the visualization process is what makes geometric morphometrics a useful tool in forensic anthropology. For forensic anthropologists in areas other than the West and Southwest, the visualization of differences in cranial morphology between individuals considered Hispanic and other population groups will provide a heuristic device.

Most forensic anthropologists are familiar with 24 standard cranial measurements (Buikstra and Ubelaker 1994; Jantz and Ousley 2005; Moore-Jansen et al. 1994). Therefore, these measurements will be used to assess the degree of ancestry estimation between the proposed reference sample and other reference groups. Reference groups used in all analyses for ancestry estimation will include indigenous Guatemalans, American Blacks, and American

Whites. These reference groups, obtained from the FDB, contain large sample sizes and represent recent forensic cases. A stepwise DFA will be used to determine which linear combination of measurements best differentiate between the proposed reference sample and other population groups.

A multi-variate analysis of variance (MANOVA) will also be included to test for significant differences between group means. Mahalanobis distances will be used to explore relationships between groups. Cross-validation classification rates will be used to assess the overall classification accuracy. Depending on the size of the proposed reference sample, presumably Mexican, a subset of this sample will be left out of the DFA to be used as a test sample in order to test the accuracy of the DFA. Further, data collected from individuals that originate from countries other than Mexico will also be compared to the reference sample using DFA and Mahalanobis distances to assess how they relate to the reference sample.

Previous research indicates that these 24 measurements provide poor classification rates, (**45%**, cross-validated) for individuals considered Hispanic (Spradley and Algee-Hewit 2007; Spradley et al. 2008), and that classification rates increase (**87.5%**, cross-validated) using measurements published in Howells (1973). Therefore, these additional measurements will also be subjected to the same multivariate tests described above to determine if they provide better classification results for ancestry.

Geometric morphometric analysis will include using landmark data to test for mean shape differences in groups, pre-processing landmark data for use multivariate analyses, and visualization of the shape differences between references groups. First, a General Procrustes analysis in Morpheus et al. (Slice 1998) will scale, rotate and translate all the landmark data into the same shape space so that mean shape differences can be tested for significance differences. Next, a General Procrustes Analysis will be performed in Morphologika 2.5 (O'Higgins and Jones 2006), followed by a Principle Components Analysis (PCA). The principle coordinates will then be subjected to the same multivariate methods (DFA) as the traditional craniometric methods in SAS 9.1. The purpose of repeating the same analysis with the PC scores from the landmark data is that geometric morphometric methods have been shown to discriminate better among closely related groups (Adams et al. 2004). Shape differences will be produced for visualization and described.

1. There are no potential risks to the subject participants in Project IDENTIFICATION.
2. There is little to no risk for subject participants. Subject participants are deceased and their personal identity will not be revealed to me.
3. No immediate benefits will be gained by the subject participants. However, a benefit to society does exist. Individuals considered Hispanic in the United States are now the largest minority population group. There are no population specific and reliable criteria for sex and ancestry determination. The formulae used by forensic anthropologists are only as good as the data that are used to derive them. The new formulae, for sex and ancestry estimation, derived from the data collected by this project will aid forensic anthropologists, especially in areas outside of the West and Southwest, in recognizing individuals, primarily of Mexican origin. For a forensic anthropologist, recognizing sex and ancestry of an individual is crucial in trying to positively identify an unknown individual. Being able to recognize that individual is of Mexican origin, or that the individual may have entered the country via the shared border with Mexico, will undoubtedly lead to more positive identifications.
4. There is no compensation offered to study participants.
5. There are no risks to subject participants, only benefits to society (see item #6).
6. All data will come from the PCOME (see attached letter of support).
7. N/A
8. N/A
9. N/A
10. The dissemination plan for the results from the proposed project include, but are not limited to, presentation of the findings at the American Academy of Forensic Sciences and publication of new sex and ancestry estimation formulae in the Journal of Forensic Sciences.