Spring 2013

Bones And Base Pairs: A Look Inside American G.i.'s Missing in Action

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Abstract
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Disciplines
Anthropology

BONES AND BASE PAIRS: A LOOK INSIDE AMERICAN G.I.'S MISSING IN ACTION

By

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In

Anthropology

Submitted to the
Department of Anthropology
University of Pennsylvania

Thesis Advisor: Dr. Janet Monge

2013

ABSTRACT

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PART I: BACKGROUND

INTRODUCTION

I began my project by reviewing cases in which osteological analysis was employed to identify the remains of soldiers missing in action. However, upon reading many reports, I realized that many of the bones were highly fragmented because many of the servicemen died in plane crashes or their remains rotted away in tropical environments. It for this reason that forensic anthropologists examining such remains resorted to mtDNA analysis to identify some of the remains. If two people have close matching or the same mtDNA, they share a common maternal ancestor, but one cannot be certain if that ancestor was recent or lived a long time ago" (Hill n.d.: 11). It is for this reason that mtDNA and osteology analyses can complement each other in forensic cases , questions left unanswered by one of the fields may be answered by the other. Each field brings a different piece of the puzzle to the table.

Thomas Dwight, known as the Father of Forensic Anthropology, published a paper in 1878 entitled, "The Identification of the Human Skeleton: A Medicolegal Study" (Pickering and Bachman. 2009: 1) from which forensic anthropology can trace its roots. However, forensic anthropology did not officially commence as a field of study until the 1970s and focused mainly on osteological identifications. This occurred not long after the structure of DNA was elucidated by James Watson and Francis Crick in 1953 (Watson & Crick 1953: 737-738), and mtDNA was discovered through the works of Margit M. K. Nass and Sylvan Nass in 1963 (Nass & Nass 1963: 593), and of Ellen Haslbrunner, Hans Tuppy and Gottfried Schatz in 1964 (Haslbrunner et al. 1964: 127). Knowledge of osteology as well as mtDNA has progressed a great deal over the last 50 years, and has been applied to a plethora of other fields, such as murder cases, genealogy projects, and studies of human evolution.

During the course of my research, I was unable to find clear descriptions of the mtDNA analysis used in forensic cases. I was fortunate enough to be given the opportunity to learn and perform the same techniques and processes utilized in government forensic labs, but with modern DNA samples in Dr. Theodore Schurr's Laboratory of Molecular Anthropology at the University of Pennsylvania. To borrow a quote from Aristotle, "what we learn, we learn by doing." My project focused on a Mexican population from the state of Veracruz known as the Popoluca.

Therefore, the first part of my thesis provides an introduction to and historical background of the process of searching for remains. The second part examines the use of osteology. The third part describes my analysis of Popoluca mtDNA variation. The fourth part provides case studies of how remains of soldiers were identified using osteology and mtDNA**.** The conclusion summarizes the project and demonstrates how each topic is related.

About the Central Identification Laboratory

Many American service personnel are still missing in action. They include 5,000 individuals from World War I; 78,000 from World War II; 8,100 from the Korean War; and almost 1,800 from the Vietnam War (Warren 2008: 47). As opposed to keeping the statuses of these as Prisoners of War (POW) or Missing in Action (MIA), military review boards decided to change each status to "presumed dead." As a result, these individuals remain "unaccounted for."

The Department of Defense, Joint POW/MIA Accounting Command (JPAC), on Hickam Air Force Base on Oahu, Hawaii, attempts to account for all these missing Americans as much as possible. The Central Identification Laboratory (CIL) is an important part of JPAC, as it "is charged with ensuring that remains recovery efforts and the subsequent forensic identification

are conducted with the highest and possible scientific standards" (Warren 2008: 48). The CIL developed out of the U.S. Army's Central Identification Laboratory in Hawaii. In 1947, the Army set up two laboratories to identify U.S. war dead on distant battlefields. One lab was situated in France, run by European staff and directed by the Curator of Physical Anthropology at the American Museum of Natural History in New York at the time, H.L. Shapiro (Warren 2008: 48). The other lab was located in Hawaii on the Army's Schofield Barracks. Americans staffed this lab, directed by a Professor of Anthropology at the University of Kentucky, Charles Snow. Snow did not work at the CIL very long, as he returned to teaching in 1948. He was replaced by Mildred Trotter, a Professor of Anatomy at Washington University at St. Louis (Warren 2008: 48).

Limited by technology and knowledge at the time, the CIL was deactivated in 1949. However, after the Korean War, the U.S. Army Central Identification Unit (CIU) formed in Kokura, Japan. After more than a thousand soldiers had been identified, the CIU approached the limits of time and technology and was deactivated (Warren 2008: 49).

The Vietnam War served as another stepping stone on the path to developing recovery and identification methods. During this war, the Army had two mortuaries, one at Tan Son Nhut Air Base near Saigon and the other in Da Nang that identified soldiers. After the fall of Saigon, the two mortuaries were combined in Thailand. However, something more along the lines of a forensic laboratory instead of an army mortuary was needed, especially because American recovery teams were not allowed back in Vietnam until 1986, which mean many of the remains of MIAs were skeletonized. This identification facility was built in 1976, with the establishment of the U.S. Army Central Identification Laboratory, Hawaii (CILHI), although unfortunately the

CILHI had improper funding and poor resources until the 1990s. In 2003, the Army surrendered its command of the CILHI so that it could become part of the JPAC (Warren 2008: 49).

The first step in returning the remains of fallen servicemen is organizing a recovery team, which consists of 12 to 14 people, both civilians and military personnel. The team is led by a civilian anthropologist (Recovery Leader), an Army or USMC captain or major (Team Leader) and a senior noncommissioned officer (Assistant Team Leader). Other members might include people specializing in photography, explosive ordnance disposal, aircraft wreckage analysis, as well as a linguist and a medic (Warren 2008: 54).

An anthropologist must determine if the remains are human or not before an identification effort is initiated. This can be accomplished by looking at differences in size and bone structure. The anthropologist then estimates the minimum number of individuals present in the remains that have been recovered. This is a crucial distinction because about half of all CIL cases involve commingled remains. The remains must be grouped individually before any further work is done. This is generally done by accounting for the number and types of bones present (Warren 2008: 110). For example, if two ulnas are found, one right and one left, then this could indicate the presence of a minimum of one individual. The presence of two left ulnas would indicate a minimum of two individuals.

Whereas the *preponderance of evidence* holds in most U.S. jurisdictions, the Department of Defense follows *clear and convincing* as the standard for the identification of U.S. servicemen. This criterion presents a challenge for the JPAC because many of the cases are over 50 years old, and the remains are not well preserved. While visual identification may suffice in deaths that occurred recently, this may not be adequate in identification cases, as many of the JPAC cases are old and the remains have decayed. In addition, there is a larger chance of DNA

contamination with older remains, thereby requiring the use of several methods to identify the remains "Historically, the CIL has drawn primarily from two areas of specialization: forensic anthropology and forensic odontology" (Warren 2008: 55).

When the CIL receives a case, an internal chain of custody document is processed (Warren 2008: 55). The evidence is photographed and assigned a case number. The evidence is triaged in "order to best route it through the analytical process." Two or more analysts work independently on "separate aspects of the case: forensic odontology, forensic anthropology, and material evidence" (Warren 2008: 55).

Some technological advances have been beneficial for the CIL. Dental radiographs enable forensic odontologists to make a positive identification with just a single tooth (Warren 2008: 56). Unfortunately, dental radiographs were not standard analytical techniques until the Vietnam era. In addition, while mtDNA is used routinely in forensic DNA analysis, Y-chromosomal and autosomal DNA testing will become increasingly available with advances in nuclear DNA technology.

Over 1,000 MIAs have been identified as of January 2002 via the CILHI (Mann et al. 2003: 115). Search and recovery teams are supervised on site by well-experienced forensic anthropologists and archaeologists. These supervisors are also trained in the conduct of investigations and handling of evidence. The teams utilize high-tech equipment and abide by strict legal and scientific principles to find and bring America's missing servicemen back home.

Basic Overview of Excavation

A visual search of the putative site of an MIA is conducted. If there are not many surface items (or none at all), a ground sweep with a metal detector or ground penetrating radar comes

next. Pin flags of different colors to designate different types of evidence are utilized to mark the spot where things are found/detected, and then a grid is set up using wooden stakes and string. The grid should be centered around the highest concentration of flags. The next step is to start digging (Mann and Williamson 2006: 220-221).

When human remains are discovered, teeth and bones are normally all that remain of a body after it has been buried for a long time because the soft tissue decays and/or is eaten. A disarticulated skeleton suggests that time allowed for the soft tissue decay before burial or it was removed, serving as a clue as to who buried the body. However, "most human burials on archaeological sites are found as articulated skeletons" (Mays 1998: 14). A trowel and hand-brush should be utilized to remove the fill of the grave of an articulated skeleton. Archaeologists use caution when the skeleton is being exposed so as not to disturb the positions of bones. As soil is removed from the upper surface of smaller fragile bones, one dental tools or a paintbrush should be utilized (Mays 1998: 18).

Once the skeleton is exposed, the next step is to photograph it and record its position on a context or skeleton sheet. The bones can then be hand-lifted from the site. Excavating sites by hand decreases the chances of overlooking small items associated with the remains. Sieving decreases such chances even further. Sieving especially aides in recovering bones of the extremities as well as "tapeworm cysts, kidney stones or material such as calcified blood vessel walls, lymph nodes or pleural plaques" (Mays 1998: 15).

PART II: OSTEOLOGY

Osteology Identification Techniques

Osteology is very important in forensic anthropology as a working knowledge of the human skeleton enables anthropologists to formulate of a biological profile based on skeletal

remains. The biological profile consists of "characteristics that an individual possessed during life, but which critically can also be determined from skeletonized remains after death. These characteristics consist of age, sex, stature, geographic ancestry, trauma and/or other conditions that were extant in life" (JPAC 2013: Web). The profile can be compared to military and/or medical records from the individual's lifetime. For the purpose of this study, I chose to focus mainly on assessing the age and height of the individual, since all of the MIA cases I've studied have been those of Caucasian males. To my knowledge, there are no American servicewomen that are or unaccounted for.

There are a number of techniques used to estimate the age of an adult skeleton, and these are listed below:

(1) Cranial Suture Closure: As a person ages, sutures in the cranial vault fuse progressively and disappear. (White & Folkens 2005: 369) & (Mays 1998: 59).

(2) Epiphysial Fusion: Fusion of different epiphyses corresponds to different age ranges. They are normally all fused by age 25 (White & Folkens 2005: 373).

(3) Rib-end Morphology: Rib ends, where the ribs join to the cartilage that connects them to the sternum, are flat in younger adults. Over time, a pit forms at the rib end that gets deeper and deeper. The rim of the pit will become more ragged and irregular with age. (Mays 1998: 60).

(4) Auricular Surface Morphology: Initially its surface is undulating but gets smoother as time goes on. Later in life it will become porous and show signs of bony lipping. (Mays 1998: 60-61).

(5) Pubic Symphysial Morphology: The pubic symphysis contains ridges and furrows that smoothen out as bone builds up around it. Eventually the bone breaks down and the symphysis forms pits and become porous. Degrees of morphology are associated with certain age ranges in

males and females. One disadvantage of this technique is that there is quite a bit overlap between different degrees/stages. (Mays 1998: 61).

(5) Investigation of Bone Microstructure: Slices of bone are ground into thin sections so that a microscope can be used to measure osteon frequency and various other characteristics. (Mays 1998: 61).

(6) The Complex Method: Loss of trabecular bone in the femoral and humeral heads is related to age. This loss can be studied via X-ray or sectioning and combined with cranial suture closure and pubic symphysis morphology to estimate an age. (Mays 1998: 61).

(7) Tooth Wear: Greater degrees of tooth wear are associated with older individuals. "Wear is generally most regular on the molar teeth, so it is these which are most often emphasized in aging studies" (Mays 1998: 71).

(8) In addition to tooth wear, tooth development is also used to estimate age. For example, the third molar begins to erupt between ages 18-21, and permanent dentition is completed around age 25. Dental development and calcification charts can also be compared to the state of the tooth/teeth in question, although maturation rates have been varying from generation to generation (White & Folkens 2005: 361, 366-368).

(9) Cementum Incremental Layers: The thickness of cementum layers increases with age. It is deposited in layers that might correspond to annual increments (Mays 1998: 61); and

(10) Dental Microstructure: Examining the transparency of a sectioned tooth under a microscope gives information about health and disease pathologies. Secondary dentine deposits in the pulp chamber and the cementum thickness are also considered in this technique. (Mays 1998: 61).

Metric variation can be used to determine a plethora of information about a skeleton. Measurements of long-bone lengths can be utilized in standardized formulas to estimate an individual's height. Craniometry,-measurements of the skull, can be utilized to determine a person's ethnic population/race. These measurements can be compared to standardized averages associated with a specific geographic population (Mays 1998: 91).

In addition to estimating the stature and ethnicity, measurements of certain features can be utilized in sexing the skeleton. In males, the sub-pubic angle is less than 90˚, whereas in females it is more than 90˚ (Mays 1998: 40-41). Although subject to the size of one's thumb, if one can wiggle one's thumb around the greater sciatic notch, then it is the notch of a female. If one cannot wiggle the thumb, then it is probably the pelvis of a male. Males have a flatter auricular surface than females, and also an oval shaped obturator foramen, while females have obturator foramen that is triangular shaped. Overall, females overall have wider pelvises (Mays 1998: 43).

If the pelvis is missing, one can study the skull. Studies based on more than a thousand individuals from eight different populations showed that female foreheads are more bossed, with male foreheads more sloping (Kuchment 2011: 73). Nuchal crests, glabellas, and supraorbital ridges are more prominent and robust in males as opposed to females (Shearer et al. 2012: 400e2-400e3). Females have more narrow and pointy chins than males, who have chins that are more rounded and bilobed. If the skull is also missing, one can look at the long bones, which are normally longer and thicker in males, due to the sexual dimorphism brought on by puberty.

Many of the standards such as height-calculation formulas, physiological features of different races, ages for different epiphysial fusions, and the like, were based on soldiers who lost their lives in the Korean War (White & Folkens 2005: 27). Army and medical records

enabled anthropologists to know precisely the age at death as well as the person's height and race. However, these standards from the 1950s may not accurately reflect standards of more recent skeletal remains. For example, there is an increasing trend in America in human development in which the wisdom teeth are erupting earlier and earlier in life, and children and maturing physically at a faster rate than in previous years (White & Folkens 2005: 361).

Interpreting Injuries

Traces of injury on the bone can provide the anthropologist with clues to how the person died. Burning bones can lead to splitting, cracking, discoloration, exfoliation, of the bones, as well as destruction of the subcutaneous surface of bones (White & Folkens 2005: 55). The amount of perimortem soft tissue covering bone correlates with the amount of damage done to the bone during burning. For example, molars are covered by more soft tissue than incisors. Thus, the enamel of the molars undergoes less exfoliation than that of the incisors during burning (Mays 1998: 38).

High-speed projectiles such as arrows have been modifying human skeletal remains for thousands of years (White & Folkens 2005: 62). Arrowheads are typically made of stone, whereas more recent and advanced projectiles are often some form of metal, such as bullets and shrapnel. A projectile wound is largely dependent on the velocity of the projectile upon contact. The amount of damage caused by a projectile correlates with the amount of kinetic energy possessed by the projectile. A blow from a blade is more likely to slice a bone as opposed to shattering it, owing to the bit of resilience of bone from the organic component (Mays 1998: 244-245).

One can distinguish perimortem from antemortem injuries by the degree (or lack thereof) of healing (Mays 1998: 240). A mark that a fatal blow leaves on bone tend to be somewhat polished in appearance and show the honeycomb structure of the trabecular, or spongy bone, with no sign of new bone formation. Blunt force trauma is another type of injury that leaves marks on bone. "When the skull vault in a living individual (or in a fresh corpse) is struck by a blunt weapon or object, a depressed fracture tends to result, where fragments of the bone are driven inwards towards the brain" (Mays 1998: 168). The resilience of living bone allows for most blunt injuries to have the bent-inward fragments still attached to the rest of the vault. Some blunt injuries may have fracture lines radiating from the center of impact.

With respect to the genetic analysis of forensic cases, each time a new bone sample is cut, anthropologists at the CILHI clean the entire lab with a 10-20% bleach solution, turning it into a sterile "wet" lab. "Samples of approximately 6-8 grams are ideal, and the harder, denser (cortical) bone is the target of sampling, since spongy (cancellous) bone does not produce results as frequently" (Webster & Shine 2003: 283). Forensic odontologists at the CILHI may also submit a dental sample in a powdered form.

DNA analysis must include results that can be repeated for confirmation. Unfortunately in many MIA cases, remains are too fragmented and deteriorated to have repeated tests done. mtDNA is ideal in this case since it is easier to obtain from skeletal remains. DNA reports consist of multiple pages of sequences of DNA nucleotide bases. Towards the end of the identification process, CILHI anthropologists do any necessary editing and submit an identification report for review by the Casualty Mortuary Affairs for the specific branch of service for a final approval. The report also needs the family's approval (Webster & Shine 2003: 287).

PART III: MITOCHONDRIAL DNA

The "power house" of the cell, mitochondria are vital organelles in most human cells. Mitochondria also contain their own DNA, known as mitochondrial DNA or mtDNA. The most probable theory to explain why mitochondria have their own DNA is the endosymbiotic theory, which follows that mitochondria were originally individual unicellular bacteria that were eaten by another cell, but instead of being digested, were incorporated into the cell's internal structure (Martin et al. 2012: 1). Since ubiquitin, a proteolytic chaperone, degrades paternal mtDNA in the sperm after fertilization (Sutovsky et al. 2004: 5), the mtDNA in the ova is the only type inherited, and thus mtDNA is inherited strictly through the maternal line. Thus, any direct maternal relative of an individual in question should have identical mtDNA (as long as the individual is not the one in about 33 generations that a mutation occurs (Parsons 1997: 363)). These facts facilitate the identification of soldiers MIA as it gives anthropologist a better idea of which type of relative to search for in order to obtain a matching sequence for positive identification. In addition, once an individual dies, due to the circular structure of mtDNA as well as its location in the cell, it is less prone to degradation over time and exposure to harsh elements, as opposed to nuclear DNA (Warren 2008: 57).

Yet another factor underlying the versatility of mitochondria is the abundance of their DNA. There are many more copies of mtDNA than copies of nuclear DNA in each cell (Mann and Williamson 2006: 115). There are thousands of copies of the mtDNA in every mitochondrion, and hundreds of mitochondria in each cell. That amounts to each cell possessing hundreds of thousands of copies of mtDNA, versus a mere 2 copies (1 set of chromosomes from each parent, so not exact copies) of nuclear DNA (Saferstein 2013: 385). The fact that mtDNA is more readily available makes it very useful when attempting to recover DNA in forensic cases.

Due to the difficulty I had while trying to learn more about how mtDNA is utilized in the identification process, I decided to analyze mtDNA variation. Learning the process myself enabled required me to perform a great deal of work in a relatively short amount of time. I would have needed to go through certain training and obtain special clearances to work with remains of soldiers and would not have been able to perform such work during the course of the school year. With these factors and time constraints in mind, I chose to work on the Popoluca samples that were already collected and waiting to be analyzed in the lab. In addition, there were only 21 Popoluca samples, ensuring that I would have enough time to perform techniques on and analysis of each one.

To analyze mtDNA variation in the Popoluca from Veracruz, Mexico, I used two different laboratory techniques, TaqMan assays and DNA sequencing. TaqMan assays allow one to determine the haplogroup (branch of mtDNA evolutionary tree) of a sample by screening them for coding region SNPs that identify basal portions of the mtDNA phylogeny. The samples can be assigned to a haplogroup once the SNP screening is complete. TaqMan assays work well even with DNA samples of low quality, as seen in the results section.

Direct sequencing requires DNA samples of higher quality, but it provides a better resolution of the DNA samples. The first step involving amplifying a template for sequencing through the process is known as the polymerase chain reaction (PCR). Sequencing allows us to read the HVS1 and HVS2 sequences for each sample and identify mutations by comparing it to the Cambridge Reference Sequence (rCRS: Anderson et al. 1981; Andrews et al. 1999). Not only can haplogroups be determined from sequencing, but also various subhaplogroups. In this

case, sequencing allowed me to confirm that three of the samples that were potentially from Haplogroup C (determined from the TaqMan results) were indeed from that haplogroup.

Background of the Popoluca

The Mixe-Zoquean language family ranges from the Isthmus of Tehuantepec to the Grijalva Depression (Coe & Koontz 2008: 16). Popoluca belongs to the Mixe-Zoquean language branch, which is divided into four non-mutually intelligible sublanguages described below in Table 1. Of those four sublanguages, Sierra Popoluca is spoken in Buena Vista, the county where twenty of the twenty-one samples are from, as well as Octal Chico, the county where the other sample was collected. Buena Vista and Octal Chico are located eight kilometers away from each other, are near the town of Soteapan in Veracruz (See Figures 1-3).

Table 1: Composition of Popoluca language, based on information from http://www.native,languages.org/popoluca.htm.

Sublanguage	Closer relation	Commonly spoken by:
Oluta Popoluca	Mixe	elders
Sayula Popoluca	Mixe	everyone
Sierra (Serrano) Popoluca	Zoque	everyone
Texistepec Popoluca	Zoque	elders

Figure 1: Map showing the location of Veracruz in Mexico. Map from http://maps.pickatrail.com/north_america/mexico/veracruz.html

Figure 2: Map showing where the Popoluca are located in Veracruz (towards bottom right). Map from www.inegi.org.

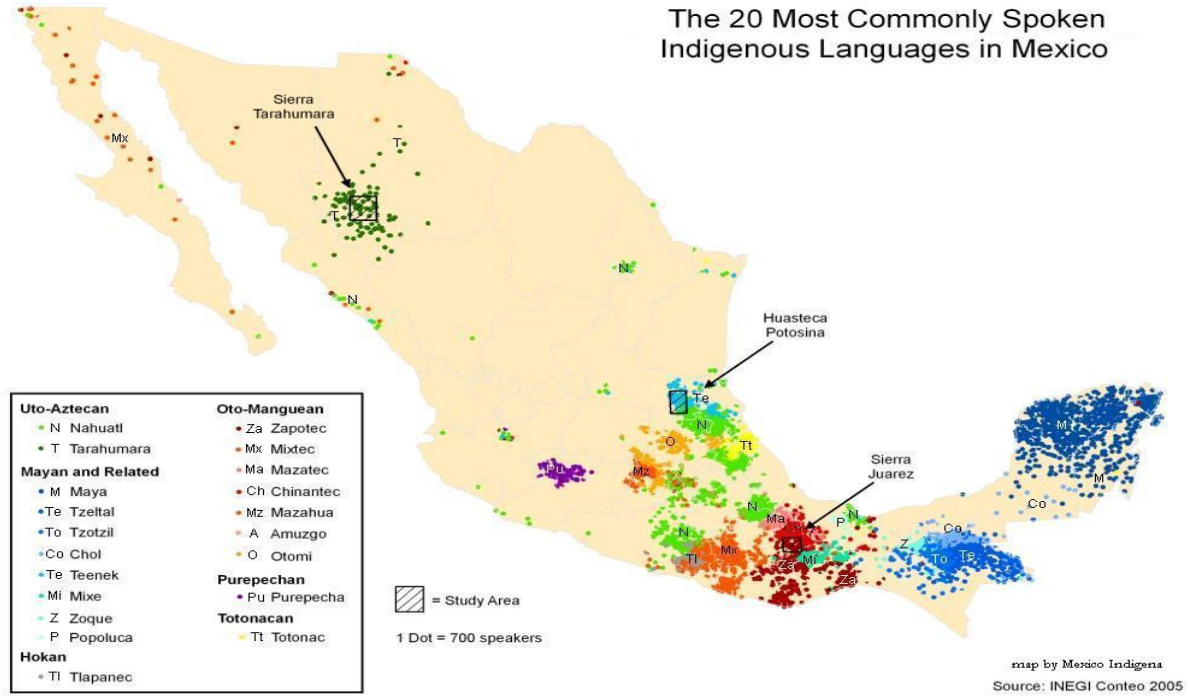
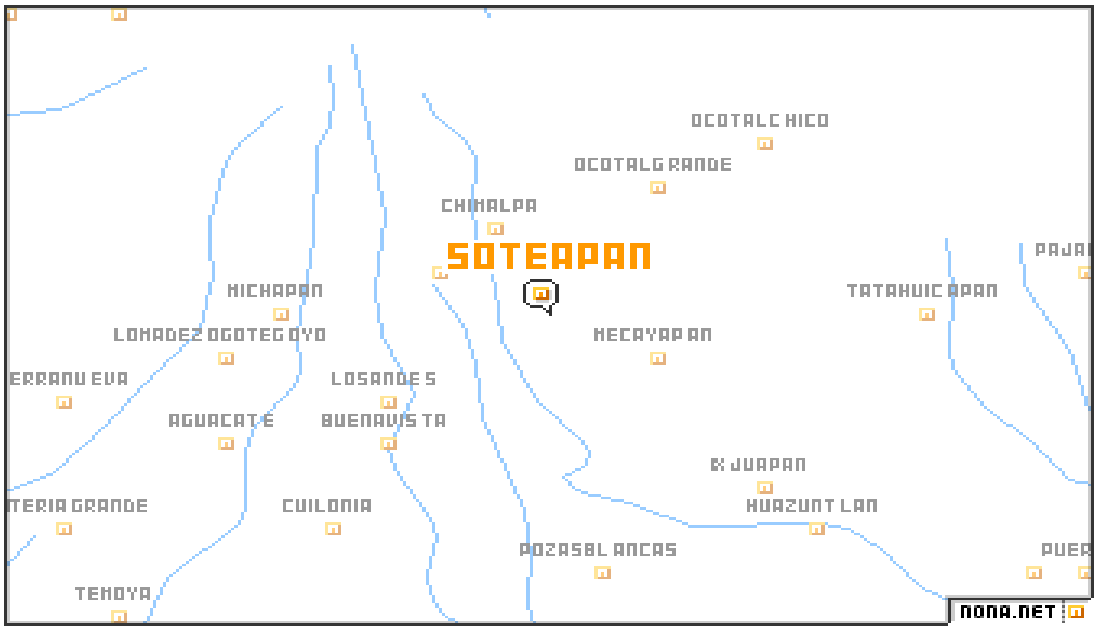


Figure 3: The relative locations of the two counties. The map was taken from http://nona.net/features/map/placedetail.1947046/Soteapan/.



The Sierra Popoluca live at elevations ranging from 100,800 meters. Savannas are common at lower elevations, while oak and pine forests frequent higher elevations. The Sierra Popoluca get a lot of precipitation, and this is perhaps reflected in their vast agricultural crops. They cultivate maize, beans, sesame, rice, and fruit trees (Melgar 1994: 17). Their lifestyle is relatively simple, and they earn money by growing and selling coffee. The Sierra Popoluca has had minimal contact with non-Popoluca people until the Mexican Revolution in the 20th century (Gámez Espinosa 2006: 28). The major religion among the Popoluca is Catholicism, introduced to them in the 16th Century by orders of Franciscans and Dominicans. However, Baptist and Jehovah's Witnesses are also common faiths there (Gámez Espinosa 2006: 46).

MATERIALS AND METHODS

Populations and Samples

In 2011, genealogical data and sample collection was carried out in the Popoluca communities of Buenavista and Ocotal Chico. Blood or mouthwash samples were obtained from 50 individuals, 21 of which were the focus of this study. Since 16 of the individuals were Jehovah's Witnesses, they were opposed to giving blood samples.

Approval for this study was granted from the University of Pennsylvania IRB #8 under protocol 803115, the Centro de Investigación y de Estudios del Instituto Politéchnico Nacional (CINVESTAV,IPN) [Center for Advanced Studies of the National Polytechnical Institute of the 15 United Mexican States], and La Comisión Nacional para el Desarrollo de los Pueblos Indígenas (CDI) [National Commission for the Rights of Indigenous Peoples of the United Mexican States]. All research participants gave their informed consent through written documents and oral interviews, using translators when necessary.

Laboratory Methods

All DNA samples were collected in the field as either blood or mouthwash samples. DNA was extracted from these samples using a Qiagen Puregene® Blood Core Kit B according to the manufacturer's protocol. Maternal genetic ancestry was elucidated through the analysis of mtDNA variation in 21 male and female participants. For each sample, the HVS1 and HVS2 of the control region was directly sequenced. For this analysis, HVS1 was amplified by polymerase chain reaction (PCR) using 0.25 uL of primers 15996FOR and 16401REV (10 pmol dilution), and combined with a PCR mix consisting of 1.25 uL 10x Taq Buffer, 0.25 uL dNTPs, 0.05 uL Taq polymerase, 0.75 uL $MgCl_2$, and 7.7 uL H_2O per sample. The PCR product was subsequently cleaned utilizing 0.1 uL of Exonuclease I, 0.1 ul of tSAP (thermosensitive Shrimp Alkaline Phosphatase), and 1.9 ul of ddH_2O per sample. The resulting 862 bp segment was primed for sequencing using 0.5 of primers 15977FOR and 269REV (3 pmol dilution), and a mixture of 0.5 uL of BigDye Terminator Pre,Mix v. 3.1, 2 uL Big Dye buffer, and 3 uL H_2O per sample. The sequencing product was then purified using a solution of 45 uL SAM and 10 uL X-terminator per sample. For some of the samples that did not work, sequencing of the HVS1 was attempted again utilizing primers 15838FOR and 16401REV. The HVS2 region was amplified using the same method with primers 1FOR and 725REV. Finally, primers 16028FOR and 269REV were utilized to overlap the region between HVS1 and HVS2.

Sequence Analysis

Sequences were read on an ABI 3130xl Gene Analyzer and aligned to the revised Cambridge Reference Sequence (rCRS: Anderson et al. 1981; Andrews et al. 1999) using the

SEQUENCHER 4.8 software tool. Mutations determined through comparison with the rCRS were confirmed for each sample by independently sequenced forward and reverse strands. Samples were assigned haplogroups and haplotypes based on PhyloTree.org mtDNA tree, Build 15 (Table 3) (van Oven & Kayser 2009).

Haplogroups determined from sequencing results were confirmed with Custom TaqMan assays that screened samples for phylogenetically informative single nucleotide polymorphisms (SNPs) that define major branches of the human mtDNA phylogeny (**Table 2**). All assays were read on an ABI 7900HT Fast Real-Time PCR System.

RESULTS

Out of a total of 21 individuals sampled, a haplogroup was successfully determined via TaqMan assays for 17 of them. With regards to the four samples that were not assigned to a haplogroup, three of the samples belong to Haplogroup C (as shown by the sequencing results). One sample from which neither TaqMan assays nor sequences could be obtained tested negative for Haplogroups A, B, and D, but we did not have a positive marker for Haplogroup C (**Table 2**).

Of the 14 samples that could be sequenced, 79% were determined to be from haplogroup A2. Two of these samples were further designated A2H1, and the other 21% were determined to be strictly from Haplogroup C1b (using what information?) (**Table 3**). Based on the results of the TaqMan assays, and since all of the individuals from haplogroup C being identified as C1b through sequencing, I assumed that the unknown sample also belonged to haplogroup C1b.

Overall, combing the results of the TaqMan assays and direct sequencing, haplogroups were assigned to 20 of the individuals. Among these, 81% of the individuals belonged to haplogroup A, 5% belonged to haplogroup B2, and 19% probably belonged to haplogroup C1b.

Table 2: TaqMan SNP Analysis Results

Sample	Hg	mt8701	mt8794	mt9540	mt12705	mt3010	mt14783	mt11177	mt1888	mt7256	Mt7697
VER42	C?	-	-		-	-	+		-	-	-
VER43	A	+	+		-					-	
VER44	A	+	+							-	
VER45	A	+	+							-	
VER46	A	+	+							-	
VER47	C?		-		-	-			-	-	
VER48	A	+	+							-	
VER49	A	+	+		-					-	
VER50	A	+	+							-	
VER51	A		+							-	
VER52	A	+	+		-					-	
VER53	B2		-		+			+		-	
VER54	A	+	+	+	-					-	
VER55	C?	-	-		-	-	+		-	-	-

VER56	?									-	
VER57	A	+	+							-	
VER58	C?		-	-	-	-	+		-	-	-
VER59	A	+	+							-	
VER60	A	+	+	+	-					-	
VER61	A	+	+		-					-	
VER62	A	+	+	+	-					-	

Derived SNPs and respective haplogroups:

8701 = A, B, F, H, J, K, N, P, R, T, U, V, X, W
8794 = A
9540 = A, B, F, H, J, K, N, P, R, T, U, V, X, W
12705 = B, F, H, J, K, P, R, T, U, V
3010 = J1
11177 = B2
1888 = C1c
14783 = D, E, C, G, M, Q, Z
7256 =L3
7697 = C1d

Table 3: mtDNA Control Region Sequences the Popoluca

Sample	Hg	HVS1 Mutations	HVS2 Mutations
VER 44	A2	C16223T,C16290T,G16319A,T16362C	C64T,A73G,T146C,A153G,C182T,A200G,A235G,A263G,315.1C
VER 46	A2	C16223T,C16290T,G16319A,T16362C	C64T,A73G,T146C,A153G,C182T,A235G,A263G,315.1C
VER 47	C1b	T16093C,C16223T,T16325C,C16327T	C64T,A73G,A153G
VER 50	A2	C16223T,C16290T,G16319A,T16362C	
VER 51	A2	C16111T,C16187T,C16223T,C16290T,G16319A, T16362C	C64T,A73G,A153G
VER 52	A2	C16111T,C16223T,C16256T,C16290T,G16319A, C16354T	C64T,A73G,T146C,A153G,A235G
VER 54	A2	C16111T,C16187T,C16223T,C16290T,G16319A, T16362C	C64T,A73G,T146C,A153G,A235G,A263G,:309.1C,:315.1C
VER 55	C1b	T16093C,C16223T,T16325C,C16327T	A73G,A249:,A263G,A290:,A291:,:351.1C
VER 56	A2	C16111T,C16187T,C16223T,C16290T,G16319A, T16362C	C64T,A73G,T146C,A153G,A235G,A263G, 309.1C,315.1C

VER 57	A2h 1	C16111T,T16126C,C16223T,C16290T,G16319A, A16335G	C64T,A73G,G143A,T146C,T152C,A153G,A235G
VER 58	C1b	T16093C,C16223T,C16287T,T16325C,C16327T	A73G,A249:,A263G,A290:,A291:,315.1C
VER 59	A2h 1	C16111T,T16126C,C16223T,C16290T,G16319A, A16335G	G143A,T146C,T152C,A153G,A235G, A263G,315.1C
VER 60	A2	C16223T,C16290T,G16319A,T16362C	C64T,A73G,T146C,A153G,C182T,A235G,A263G,315.1C
VER 62	A2	C16111T,C16187,C16223T,C16290T, G16319A,T16362C	C64T,A73G,T146C,A153G,A235G,A263G,309.1C,315.1C

The TaqMan assays proved quite useful in the determination of haplogroups in this study. Due to the failure of multiple sequence attempts for several of the samples, it is safe to say that DNA quality of some of the samples is poor. The TaqMan assays provided definite haplogroup identifications for 6 of the 7 samples that could not be successfully sequenced and a probable haplogroup identification for the other one.

The control region sequencing results demonstrated that there were at least six different haplotypes present in the Popoluca sample set. Within these haplogroups, several sequences were of interest. There were four A2 haplotypes that included a mutation at np 16187. This mutation also occurs in 9% of the samples from El Salvador, with four of the El Salvador samples having identical haplotypes to the Popoluca mtDNA that contained the 16187 mutation (Salas et al. 2009). The C16111T–C16187T–T16223C–C16290T–G16319A–T16362C haplotype was also seen in individual from Uruguay (Pagano et al. 2005: 2) and 19 individuals from the Ngöbé population in Panama (about 41% of that population) (Kolman et al. 1995). This haplotype was also found recently in Totonaca individuals from Veracruz (Schurr et al., unpublished data), as well as 11 Maya individuals, consisting of 7 Ch'orti' and 4 Poqomchi' (Justice et al. 2011: 93). Lastly, this haplotype was also found in 3 Rama samples from Nicaragua and 11 Abrojo-Guaymí samples from Costa Rica (Melton et al. 2013: 4). Based on this information, it is likely that the Popoluca share a common ancestor with several indigenous populations of Central America, or that a specific A2 haplotype is common among but limited to Mesoamerica.

The mutation at 16126 was present in both the A2h1 samples (C16111T–T16126C–C16223T–C16290T–G16319A–A16335G). This sequence was found in one Nashua sample

from Hidalgo (Schurr et al, unpublished data), as well was two Ch'orti' samples (Justice et al. 2011: 93).

Each of the samples from Haplogroup C1b has a back mutation at 16298, giving a haplotype of T16093C–C16223T–T16325C–C16327T, except for one that has the haplotype T16093C–C16223T– C16287T–T16325C–C16327T. This back mutation is very rare, and has never been reported on PhyloTree.org (van Oven & Kayser 2009). After a scan of more than 400 Mexican samples, it was found to occur in one Otomí sample and one Tepehua sample from the lab's data set. The back mutation is also present in one Maya sample, but the sample belongs to a slightly different haplotype. The 16298 back mutation alone is very rare, and this combined with the 16287 mutation is even rarer. I have not been able to find any other occurrences of these two mutations together, and perhaps this could be a haplotype limited to the Popoluca.

CHAPTER 4: CASE STUDIES

Now that the osteology and mtDNA analysis have been covered in detail, I will examine historical cases in which both fields were utilized to bring America's fallen heroes home.

The Case of Thomas Hembree

Thomas "Tommy" Hembree enlisted in the U.S. Navy when he was just seventeen. He served as an apprentice seaman on the seaplane tender USS *Curtiss*, stationed in berth X-ray 22 in the Middle Loch in Pearl Harbor, Hawaii (Mann and Williamson 2006: 162-163). On December 7, 1941, the Japanese attacked Pearl Harbor, and the USS *Curtiss* suffered a direct hit. Many crewmembers either died or were injured, and Hembree was one of three crewmembers unaccounted for after the attack; two were dead, one was missing. Not long before his death,

Hembree had written a letter to his mother. She didn't receive the letter until after the attack, so she thought that he had survived. Upon requesting more information, she found out that the two bodies were burned beyond the point of recognition and that her son was one of them.

The two bodies were buried in the Nuuanu Cemetery on Oahu on December 9, 1941. In 1947, the remains, labeled "Unknown X-24" and "Unknown X-25" were dug up and analyzed by Dr. Charles Snow, who identified "Unknown X-25 as Seaman Second Class Nikolas S. Ganas. This narrowed down the identity of X-24 to either Seaman First Class Wilson A. Rice or Apprentice Seaman Thomas Hembree. X-24 was reburied and remained at Nuuanu until 1949, when the Navy asked Hembree's family if they wanted his remains returned home or reburied in the National Memorial Cemetery of the Pacific in a volcanic crater referred to by the locals as the Punchbowl. The family decided to have the remains placed in the Punchbowl, so the remains of X-24 were wrapped in a cream-colored wool blanket with "US Navy" embellished on it, and buried.

A few years later, Hembree's sister, June Braiwood, arrived at the Punchbowl hoping to visit his grave, but was told by a cemetery attendant that he was buried at sea. During another visit in 1989, Braiwood asked a cemetery worker again. The worker did not know, so he called Ray Emory, a WWII vet and the man who catalogued all of the 18,093 WWII casualties buried in the Punchbowl. Emory knew about Hembree being unidentified and decided to aide in getting X-24 identified. Emory relied on advances in DNA technology and the help of Hawaii's Congresswoman Patsy Mink to exhume the remains of X,24 in January 2001. Unfortunately, June Braiwood died by that time, but her niece, Beth LaRosa, continued the mission (Mann and Williamson 2006: 159).

The law (Mann does not specify which one) required that a DNA sample from a relative be present before exhuming the remains (Mann and Williamson 2006: 159). Since mtDNA was specified and is passed down the maternal line, LaRosa, the daughter of Tommy's oldest brother, would not have the same mtDNA as Tommy. LaRosa inherited her mtDNA from her mother, the sister-in-law of Tommy. LaRosa contacted her cousin, Marion Price, the daughter of the only Hembree sister who had offspring, June Bailey. A blood sample was taken from Price in December 2000, and the remains of X-24 as well as on other WWII veteran and two from Korea were exhumed the next month.

The DNA analysis failed with X-24. No DNA could be sequenced. Scientists from the CILHI and AFDIL pondered the reason to why no DNA was yielded. The steel casket was waterproof and under fix feet of volcanic ground. Some of the guessed explanations were the volcanic soil, the fact that the remains were fluoroscoped, and the type of embalming powder that were placed on the remains. The anthropologists decided to give traditional osteology a second chance since the methods, techniques, and standards had advanced over time since Dr. Snow examined the remains in 1949.

Dr. Robert W. Mann, Ph.D. brought the remains out on a lab table and conducted his analysis. The biological profile he came up with matched perfectly that compiled by Dr. Snow. "Based on the development of the teeth and fusion of the long bone growth caps, I judged that the individual was between sixteen and nineteen years old when he died. The skull's narrow nasal opening, absence of alveolar prognathism (a forward jutting of the midface), and the shape of the upper palate were features most often found in Caucasoids. Using the left femur, I computed his stature as five feet nine inches tall. The features of his hips and skull, combined

with the overall size of his arm and leg bones, left no doubt that the bones were those of a man" (Mann and Williamson 2006: 161).

Dr. Mann did find something missing in Dr. Snow's assessment: there was a small indentation in his posterior left thigh bone, right above the knee. This bone scar was formed and healed a while before death and could have been caused by localized infection to the leg or an injury. The only signs of injury that might have happened during the Japanese attack were a broken hand bone and small scooped-out defect on the left femoral shaft.

Comparing the information obtained from the analysis to the records of Hembree, Dr. Mann was able to eliminate Rice, who was only five feet six inches, as the individual being examined. Thomas Hembree was a sailor, seventeen years old, white, five feet nine and three quarters inches tall, and had a scar on his left knee. The next step was for CIL dentist John Lewis to examine each of the 29 teeth of X-24. He compared his analysis to the dental records of Hembree, Rice, and Ganas. "The results were indisputable; the combination of fillings, cavities, and extracted teeth were consistent with Hembree and totally inconsistent with Rice and Ganas" (Mann and Williamson 2006: 162).

Hembree's family was notified but not before the last of his living sisters passed away. Hembree was given a full military funeral and reinterred at the Punchbowl, according to his family's wishes. He was buried in his dress blues with the same blanket and safety pins he was buried with in 1949 and in a coffin of the same color and style as before.

The Case of Captain Shine

On December 2, 1972, Air Force fighter pilot Captain Anthony C. Shine was declared Missing In Action. During a reconnaissance mission on a cloudy day, Captain Shine flew his A-

7D *Corsair* low to visually reconnoiter an area on the border of Laos and North Vietnam, and eventually was out of his wingman's sight. Other flyers saw a ground fire but after a Search and Rescue (SAR)team went to check it out, they determined it to be just a brush fire as no wreckage was found. After no contact had been made the next day, he was listed as MIA and his case given "REFNO 1950" as an official reference number (Bunch & Shine 1999: 279).

In 1987, American officials received a photograph of Shine's dog tag from a Laotian refugee in Thailand. Although the refugee provided no more information, a second source provided information on another dog tag and a photograph of what were thought to be Shine's skeletal remains. The remains were in the custody of a Laotian national based out of Xianghoang Province, Laos. In 1993, a joint investigation team searched an area associated with Shine, 15 km from the border of Laos. Pieces of wreckage and life support materials were collected and the team talked to villagers, including one witness who saw a plane break off from another and fly low over Route 7 to shoot at a supply convoy in either late 1971 or early 1972. He heard that it crashed and visited the site twice in a few weeks, and one the second visit, he ran into Vietnamese soldiers who said they were burying the body of the pilot. While the villager didn't know the exact location of the grave, he showed the team a pilot's helmet that he had recovered three years after the incident and led the team to the site.

Sometimes during an interview, while recalling an incident that took place a long time ago, a witness may not remember all of the details and mix facts up, withhold information or provide false information because he or she is intimidated by the officials from both America and Vietnam that are present at the interview. In other cases, he may exaggerate/fabricate a story to try to benefit monetarily by returning remains (Bunch & Shine 1999: 280).

Another joint U.S./S.R.V. went to the village to interview the witness and found that some details changed. This time, he said that he saw the body still in its ejection seat. He led the team to a garden area in the village where he thought the pilot's body lay. A probe was conducted and turned up remnants of personal effects, human skeletal remains, and pieces of a pilot's equipment. The grave was thought to be recently disturbed since green leaves were found below sterile soil. These facts indicated that "remains or other evidence may have been recently removed from the site" (Bunch & Shine 1999: 282). A joint forensic review was conducted at the CILHI by one of its forensic anthropologists and a Vietnamese counterpart. The remains were identified as human remains of European descent, so they selected for repatriations and Vietnamese officials turned them over to the U.S. in June 1994. The remains were sent to the CILHI and given an accession number for tracking, maintenance of privacy, and prevention of bias.

A forensic anthropologist with no previous knowledge of the case examined the remains. The minimum number of individuals was thought to be one. There were only a few skeletal remains present, limiting the analysis. "The remains were indeed human and appeared to be those of an adult. Brown staining on the surfaces of the bone fragments demonstrated that they had been in contact with soil for an extended period of time. No other determinations could be made" (Bunch & Shine 1999: 283). Since a personal identification could not be made, a bone sample was sent to the Armed Forces DNA Identification Laboratory (AFDIL) in November 1994 for mitochondrial DNA analysis.

A recovery team went back to the grave and crash sites in May 1995 to excavate both sites. The team faced heavy rain and extreme heat while in the triple canopy jungle but was able to get the job done. After setting up a grid system and documenting provenience of evidence,

dirt was sieved and more human remains and personal effects were found. Some of the recovered items were a dog tag with "SHINE, ANTHONY C." on it, pilot materials, and an aircraft engine. In June 1995, two bone samples from these excavations were sent to AFDIL for mtDNA analysis but proved inconclusive, as did the previous sample. In August, two more samples were submitted and conclusive results were obtained. The two sequences were identical in their respective sequenced regions (did not specify which part) and matched sequences taken from three of Shine's maternal relatives (Bunch & Shine 1999: 283).

In addition, the dog tag found *in situ*, witness story, pieces of wreckage with serial numbers specific to Shine's A,7D *Corsair*, and flight helmet with Shine's name written inside all served as evidence to support the identification of the remains as those of Shine. The Federal Bureau of Investigation examined the handwriting on the helmet and stated that it was "strongly correlated" with Shine's handwriting. The U.S. Secret Service Laboratory analyzed the ink used to write the name and concluded that it was an ink used "by the U.S. Government in the 1960s to indelibly mark metal surfaces" (Webster & Shine 1999: 287). Equipment personnel from Shine's base at the time of issue stated that the style of the flight helmet was the same type issued to pilots at that base and time.

An identification report was written up and sent outside the CILHI for peer review, for either approval (with suggested changes or as is) or disapproval. The report was approved by both the Casualty Mortuary Affairs and the family, and the remains were escorted to Arlington National Cemetery by Shine's brother for burial with full military honors. Four months after the burial, in February of 1997, half a mandible with four teeth all exhibiting restorations was found. CILHI odontologists compared the X-rays of the remains to antemortem X-rays and were able to positively identify the mandible as that of Shine's. A new case file was put together, submitted

for review, and approved. In July 1999, Shine's son escorted the remains to upstate New York for a private burial by the family.

The Case of Lieutenant Blassie

Michael Blassie was a lieutenant in the Air Force whose plane crashed in Vietnam. A recovery effort could not be carried out, since Blassie crashed behind enemy lines. Because a partner flying another plane witnessed the crash, "Blassie was classified Killed in Action, Body Not Recovered (KIA/BNR)." ARVN and American forces eventually took over the territory, and some of Blassie's bones were recovered: two right ribs, two left ribs, a pelvic bone, and the right humerus. In addition to the bones, some artifacts were found such as an ejection seat, one-man inflatable life raft, a flag, flight suit fabric and parachute fabric, holster, two compasses, and a wallet with Blassie's ID card. The wallet and ID were either stolen or lost, and without them, identifying the remains became much more difficult.

How could the lab be sure that the Vietnamese did not mix the bones from other recovery sites? Dr. Mann explained that "a single piece of evidence, no matter how convincing, is not sufficient" (Mann and Williamson 2006: 96). The Air Force had Blassie's medical records, and anthropologists were able to construct a biological profile (age, race, sex, height, blood type) from the remains for comparison. The lab results estimated the height to be shorter than the recorded height in the medical record. Dr. Mann indicated that the difference fell in the range of normal human variation from an averaged standard. The estimated age was older than Blassie's actual age. However, lifestyle greatly affects how old an individual's bones appear to be, and

years of strenuous physical activity, and drug/alcohol abuse could potential influence the appearance of the remains. The blood type determined from a leg hair found inside part of the flight suit was type O. Blassie was type A. It is possible that the "exposure aboveground in the harsh Vietnamese weather could have caused the loss or alteration of the antigens that serve as evidence of blood type, making any blood look like type O" (Mann and Williamson 2006: 98).

Several other pilots crashed in the same area, but only one was a possible alternative match to the remains – Captain Rodney Strobridge. However, his helicopter would not have had the life raft on board. In 1979, the analysis was to no avail, so the remains were put in a box and labeled X-26. This was the fourth box that was considered for the tomb of the Unknown Soldier. Since the remains could not be identified with the technology of the day, X-26 became the Unknown Soldier, despite attempts of the lab's commanding officer to have it rejected since it had been associated with the two pilots (Mann and Williamson 2006: 108).

After a magazine article and a *CBS News* broadcast in 1998 informed the public of a possible identification, authorities began to consider disinterring the remains and examining them with new techniques of DNA tests that did not exist at the time of burial. Secretary of Defense William Cohen decided to have the remains exhumed. Dr. Mann and another CIL anthropologist, Dave Rankin, flew to Washington, D.C. for the disinterment. Dr. Rankin oversaw that the coffin was sealed with evidence tape as soon as it was disinterred and that a proper chain of custody was established.

Drs. Mann and Rankin examined the bones independently. In addition to establishing biological profiles, they also searched for trauma caused by the crash. The estimated profiles were identical – about 5'8" tall and between 30-40 years old, and there was no sign of crash trauma. A wedge had been cut from each bone in the 1970s to identify the blood type, in such a

manner as to not destroy parts essential for age, sex, and height estimation. Dr. Rankin cut a fragment weighing between 5-7 grams from each bone with a Dremel tool and the fragments were placed in individual plastic bags, sealed with evidence tape, and labeled with preselected number to avoid any chance of bias during the laboratory investigation. The remains were also inventoried and photographed (Mann and Williamson 2006: 108).

DNA is easily contaminated or destroyed, especially in such circumstances as that of a fiery plane crash (Warren 2008: 209-210). MtDNA is useful because humans have much more mtDNA than nuclear DNA, and because mtDNA can be easily traced (and matched) using the maternal line, whereas nuclear DNA comes from both the mother and father. The results of the PCR and sequencing did not match those of any of the reference samples, but matched the samples of Blassie's sister. The scientific director of the CIL, Dr. Tom Holland declared that the remains were those of Blassie (Mann and Williamson 2006: 118). After the family received this news, the remains were given a proper military burial once again.

For these and related reasons, blood samples from all soldiers have been collected by a DNA registry since the late 1990s, with the hope that all MIA soldiers from Vietnam will be identified. However, Rodney Strobridge is still declared MIA.

The Case of Corporal Hammond

During the Vietnam War, there was program known at the Combined Action Program (CAP). CAP was designed to enhance the combat effectiveness of the South Vietnamese Popular Forces (SVPF) and gain respect for the government in Saigon by the Vietnamese. The CAP squads were composed of a group of marines and a navy medic. They lived in rural villages and learned the Vietnamese culture to obtain the trust of the Vietnamese. Marine

Corporal Denny Hammond was serving as a member of CAP Echo 2, a team stationed near the Danang Airbase. On February 8, 1968, just eight days before the end of his tour in Vietnam, Denny volunteered to join a reinforcement team for CAP Echo 4, a team that was under attack and running low on ammunition (Mann and Williamson 2006: 108). When a friend asked why he volunteered, Hammond said that this was his last chance to accomplish something in Vietnam since he had not done so yet in his two-year tour. An estimated 200-300 North Vietnamese soldiers ambushed the team sent out to rescue Echo 4. Twelve men were KIA, three (including Hammond) were captured, and two marines were able to escape the fight. Of the three POWs, one escaped later that day.

POWs endured physical and psychological torture and suffered from diseases "born of an inadequate and unfamiliar diet" (Mann and Williamson 2006: 83). POWs were moved from camp to camp so they would not be discovered and rescued by U.S. forces. POW camps were occasionally hit during bombing raids. Prisoners could be beaten, starved, or caged just for speaking with another prisoner.

Hammond and another marine attempted to escape on April 1st. One report said that the fellow escapee was shot on sight, and another stated that he was seen living with a woman in Vietnam in the early 1970s. Hammond was shot in the back of his lower leg, tied to a branch, and carried like a pig back to the camp. "He was beaten in front of the rest of the prisoners, put in stocks, and was fed a coffee cup of rotten rice daily" (Mann and Williamson 2006: 84). He was also forced to defecate into his hands and toss the dung as far away as possible. This punishment went on for two more weeks. In 1970, Hammond contracted dysentery. He died on March 7 or 8 and was buried by fellow POWs in the Quang Nam jungle. The marine captured

with Hammond died later that year and was buried near Hammond. His remains were recovered but repeated attempts to recover Hammond's remains were to no avail.

Dr. Mann was in downtown Danang waiting for authorization to dig at another aircraft crash site. "Such waits are our work. We can't simply go into an area and start digging; we have to get permission from representatives of the host country for everything we plan to do. In addition to the months of planning, coordination, and discussions before we arrive to dig a site, when we get there we have to meet with officials from the province, district, city, town, village, and ha. There we discuss topics such as how many local laborers we'll need assist us, how long it will take, how much wood or bamboo we'll need, and where we'll be staying" (Mann and Williamson 2006: 85).

Joint Task Force – Full Accounting (JTF-FA) was in Danang at the same time. JTF-FA was based out of Oahu and was responsible for investigating cases and supplying teams to find unmarked graves of U.S. servicemen and crash sites in Southeast Asia. In addition, they led oral history teams to study war records and historical documents in museums in Southeast Asia. The organization's Research and Investigation Team (RIT) went to Vietnam to search for a grave that if found would be excavated by the CIL. The CIL was requested by Chief Warrant Officer 2 Tony Banks (the leader of the RIT) to go with the team to provide technical advice. The teams were to help Command Sergeant Major Thomas J. Davis, a former POW who befriended Dennis Hammond while in the Quang Nam camp, find his friend. In 1995, Davis was only army sergeant major and former Vietnam POW still on active duty. He was liberated in 1973 and had recently helped a team find the remains of another of his friends.

The team rode in a helicopter to the Quang Nam Province, where a local man led them to the POW camp. Communication would not have been possible since the team's American

linguists were unable to understand the hill tribe dialect spoken by the villagers, but Vietnamese interpreters with the team were able to help. Five German missionaries became POWs at the camp. Three were eventually released but two women died in the camp. They were both repatriated to Germany but the remains of at least one of them passed through the CIL before going back to Germany.

The area where the camp once stood was now just an open field. No evidence of soldiers being the area existed except for a rusted bolt from a Chinese rifle. Photographs were taken of the area and the villagers who accompanied the men by the team, and video was taken by Davis. After discussing with Chief Warrant Officer Banks, the team decided to look in another location for Hammond's grave.

There are many dangers associated with excavating in the jungles. Sawgrass grows tall and can slice open one's skin. The subsequent cuts do not close quickly. In addition to sawgrass, there are biting ants, mosquitoes, and leeches. Foul smells are signs of leeches facilitating the decay of foliage. The leeches can wriggle their way onto any part of the body, so caution should be taken when dressing; pants should be tucked into socks and duct tape utilized to seal off the interface of the two. Leeches frequent moist ground and when they bite, they inject a chemical that numbs the area and prevents the blood from clotting. Leeches found in rice paddies are around the size of earthworms, but jungle leeches are out about the size of a toothpick. The thickness of jungle leeches grows to that of a pencil after they suck the blood of their prey. The search turned up no evidence, even after another hike to a temporary POW camp site. The RIT wrote up a report when they returned to Danang that day, but the search was not over. Two days later, Banks's team found a jackfruit tree with "Dennis Hammond" and an arrow

carved into its trunk while investigating another POW camp. Subsequent digs near the tree turned up no remains.

In October 2003 the CIL got a blind hit on an mtDNA analysis of some bones. "A blind hit is a match made by submitting a DNA sequence from a bone or tooth sample and comparing it against the entire DNA database without knowing to whom it belongs…Once we get a mitochondrial sequence, it can be compared with all the database's DNA sequences from Southeast Asia housed at the Armed Forces DNA Identification Laboratory (AFDIL) to see if it matches any of them" (Mann and Williamson 2006: 92). There is the possibility that an exact match could lead to the wrong person, but complementing mtDNA analysis with osteology minimizes this possibility. Dennis Hammond's sequence came up as a match. A biological profile was put together as the skeleton from which the mtDNA was taken as it was reexamined. The profile matched that of Hammond, and CIL forensic anthropologist Dr. Brad Adams superimposed the skull on a photograph of Hammond – it was a perfect fit. Hammond had a shaved head in the picture, facilitating the ability to see the shape of his skull. Hammond's remains were turned over to the U.S. by the Vietnamese in 1989 and had rested on a shelf in the CIL. No teeth came with the remains, and mtDNA technology was not yet advanced enough at the time. Dennis Hammond finally returned home to Detroit.

CONCLUSIONS

As demonstrated in the case studies above, the use of osteology or DNA analysis alone can be very useful in identifying remains, but employing both fields of study in repatriation identification attempts significantly increases the chance of success. Matches in mtDNA haplotypes from forensic remains and a relative of the missing person can eliminate possible

doubts about analysis of skeletal remains. In the cases of mtDNA in modern samples such as the Popoluca, patterns in genetic ancestry can help to determine evolutionary relationships between populations. The work on Popoluca enabled me to gain firsthand experience and knowledge about how mtDNA is analyzed. Despite the fact that population genetics and identifying remains of missing servicemen via DNA have different target goals, the means by which those goals are achieved are very similar. Although sequences were obtained for only 14 individuals, enough variation was observed to suggest that the Popoluca were distinct from any other Mexican group. Similar patterns of variation serve as tools when making identifications in the field of forensics.

As the war in Afghanistan progresses, the number of casualties continues to rise. Although there is only one MIA from Afghanistan (Rosie 2012) and one is missing from Iraq (Martinez 2012), it is simply a matter of time before another American goes missing. As mentioned earlier, the physical maturation process in humans has been occurring at faster rates than before (White & Folkens 2005: 361). One thing to consider in further studies would be the potential need for more modern standards that would yield more accurate estimations of age and stature for MIA soldiers killed in Iraq or Afghanistan.

These soldiers made the ultimate sacrifice for their country and deserve to be repatriated as opposed to remaining in an unknown and forgotten grave. Repatriation can provide the families with a sense of certainty and closure about their loved ones. As demonstrated in the case studies above, osteology and mtDNA analysis have played a major role in this.

ACKNOWLEDGMENTS

The author would like to thank Dr. Janet Monge for all of her guidance, advice, and support throughout the course of studies. Furthermore, I acknowledge and appreciate the Popoluca individuals from Veracruz, who made this study possible through their willingness to participate in the project. I would also like to thank Dr. Rocio Gomez for her efforts in data collection and project management in Mexico, and Dr. Marco Meraz from CINVESTAV for his intellectual and institutional support of the Mexico Genetic History Project, of which this study is part; Dr. Miguel Vilar for his much appreciated help with data, lab analyses, and comments; Akiva Sanders, Daniel Brooks, and Haleigh Zillges for their help in producing genetic results from the Nahua, Tepehua, Chichimeca, and Otomí populations; and Dr. Theodore Schurr for allowing me to work in the lab and all of his guidance and advice with the project. I further express my gratitude to the National Geographic Society, IBM, and the University of Pennsylvania for their support of the project, as well as the Waitt Family Foundation for its support in field research. Lastly, I would like to thank all of America's servicemen and women, both past and present, for putting their lives on the line to protect our country, keeping us free from oppression.

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