Chapter 16
Sorting and Identifying Commingled Remains of U.S. War Dead: The Collaborative Roles of JPAC and AFDIL

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Introduction

In 1995 the Defense Science Board Task Force was established with the aim of addressing "key issues arising from efforts to identify skeletal remains using new DNA testing technologies" (Lederberg et al. 1995). A report was issued in response to an inquiry regarding identification techniques for the remains of missing soldiers. The authors of that report summarized that DNA technology should continue to be used for identification of ancient remains repatriated to the United States. Mitochondrial DNA (mtDNA) analysis was emphasized as a tool to be used in conjunction with osteological, archaeological, and dental analyses.

This chapter demonstrates how ongoing collaboration among molecular biologists, archaeologists, physical anthropologists, and dentists is vital for achieving individuation from commingled remains. In this effort a combination of specialties is necessary, each controlling and validating the other and ensuring mitigation of potentially erroneous results. To this end, we discuss recent casework that highlights the combined efforts among professional staff at the Joint POW/MIA Accounting Command's Central Identification Laboratory (JPAC-CIL) and the Armed Forces DNA Identification Laboratory (AFDIL). In this combined effort, the identification of once-missing U.S. service members currently averages 100 individuals a year, nearly two persons a week; and over half of these cases involve DNA sequence data generated by AFDIL.

Joint POW-MIA Accounting Command (JPAC)

The JPAC is the agency responsible for recovery and identification of unaccounted-for U.S. service personnel from previous conflicts. Originating during World War II as Central Identification Points that were responsible for the consolidation and identification of war dead throughout Europe, the identification process of U.S. service members has evolved into JPAC. Today the JPAC headquarters and laboratory are located at Hickam Air Force Base, Hawaii. The mission of the JPAC is to search for, recover, and identify remains of U.S. service members associated

From: *Recovery, Analysis, and Identification of Commingled Human Remains*
Edited by: B. Adams and J. Byrd © Humana Press, Totowa, NJ

with World War I, World War II, the Korean War, the Cold War, and the Vietnam War. Excavations are conducted worldwide in order to recover skeletal remains and material evidence, such as dog tags and rank insignia. These items are repatriated to the United States and accessioned into the JPAC-CIL for analysis by the scientific staff, and the findings are reported to the CIL Scientific Director. The majority of the scientific staff are civilian anthropologists (forensic anthropologists and archaeologists). Part of this staff includes a small number of employees responsible for sampling remains for DNA analysis, maintaining communication with AFDIL, and providing external control for associating remains with DNA evidence. Finally, there are forensic odontologists (military dentists) who are responsible for analyzing teeth and for sampling them for DNA when appropriate. The Scientific Director bears the responsibility of compiling all lines of evidence including the results of skeletal, dental, artifact, and DNA analyses in order to make an identification.

Armed Forces DNA Identification Laboratory (AFDIL)

Located in Rockville, Maryland, the AFDIL is attached to the Armed Forces Institute of Pathology and the American Registry of Pathology; it falls under the command of the Armed Forces Medical Examiner System (AFMES). The laboratory was established in 1990 with the primary goal of working with the CIL and the AFMES to identify the remains of U.S. service members using the latest techniques in DNA analysis. There are two main sections of AFDIL performing casework, the Nuclear Section and the Mitochondrial Section. The Nuclear Section is involved with recent death investigations where remains and organic material tend to be well preserved. This section has assisted with the identification of victims from the September 11, 2001, attack on the Pentagon, the U.S. Embassy bombing in Nairobi, Kenya, and NTSB investigations of aircraft crashes including U.S. Airways Flight 427 and American Eagle Flight 4184. The largest section of the AFDIL is the mitochondrial group. This section is devoted to the analysis of mtDNA obtained from remains recovered by JPAC. Remains recovered by JPAC are in an historic context, often in harsh environments, and they are not as well preserved as those typically encountered by the Nuclear Section; thus, the mitochondria have been the standard source of genetic material for JPAC skeletal cases, and last year the AFDIL processed over 800 skeletal and dental samples for the CIL.

Between these two sections at AFDIL, there are three different types of DNA analysis being used in forensic casework. The first originates from the mitochondria, and the other two analyses originate from nuclear DNA. The different analyses include sequencing of the mitochondrial hypervariable regions, nuclear DNA autosomal STR profiling, and non-recombining Y-chromosome STR profiling. Y-chromosome analysis is currently under validation at AFDIL and not yet actively used, although the technology promises to be highly valuable in instances where there are no maternal relatives (e.g., mtDNA references) available. Given the

myriad of taphonomic processes that negatively affect preservation of skeletal material, mtDNA has proven to be the most reliable means for obtaining molecular data since there are many more mitochondrial genomes per cell than nuclear genomes. Recent improvements in nuclear DNA analysis such as the introduction of mini-STRs (Coble and Butler 2005) and research into low copy number (LCN) STR analysis, however, are finding a place alongside mtDNA in CIL casework.

MtDNA Sequencing

The circular mitochondrial genome consists of approximately 16,569 base pairs (bp) and is inherited as a single locus through the maternal line with no paternal recombination. There are multiple copies of the mitochondrial genome within each mitochondria and hundreds of copies per cell. The number of mitochondria per cell makes them a good target for difficult samples such as delaminated and friable skeletal samples that are often recovered by the JPAC-CIL.

Within the genome, the region of interest is an approximately 1,100-bp fragment called the control region (CR). This region is treated as a single locus with haplotype variants consisting of unique polymorphisms of point mutations, insertions, and deletions (Torroni et al. 1996, 1998). Within the control region, the two hypervariable regions (HV1 and HV2) are targeted because they tend to be more diagnostic (i.e., have more mutations) than the rest of the mtDNA genome. These regions, along with the rest of the CR, are noncoding and as such are not subject to recombination and natural selection. The remainder of the mitochondrial genome encodes various enzymes, transfer RNA, and ribosomal RNA genes.

In a survey of the samples in the AFDIL population database, 41.1% of the variation in the CR is found in HV1 and 33.7% in HV2 (Edson et al. 2004). Fig. 16.1 shows the distribution of polymorphic sites among HV1, HV2, and mini-variable regions one and two (mVR1 and mVR2).

After sequencing the mtDNA regions, AFDIL compares sequence data with the revised Cambridge Reference Sequence (rCRS) (Andrews et al. 1999) and records only the base-pair differences from the rCRS. These polymorphisms are then reported as the mtDNA profile. In order to distinguish one mtDNA profile from another, the base-pair polymorphisms are compared. If more than two differences are present between those being compared, the two sequences are excluded from one another, indicating that the sequences represent two individuals. AFDIL's current reporting protocol requires a minimum of two single nucleotide differences between sequences to exclude. Those differences must be in addition to any point heteroplasmies or length heteroplasmy resulting from variation in either of the two "c-stretch" regions. C-stretch regions are polycytosine stretches at base-pair positions 16182 to 16193 in HV1 and 302 to 315 in HV2. Amplification and sequencing of these repeat regions may be affected by strand slippage and a mixture of length variants within

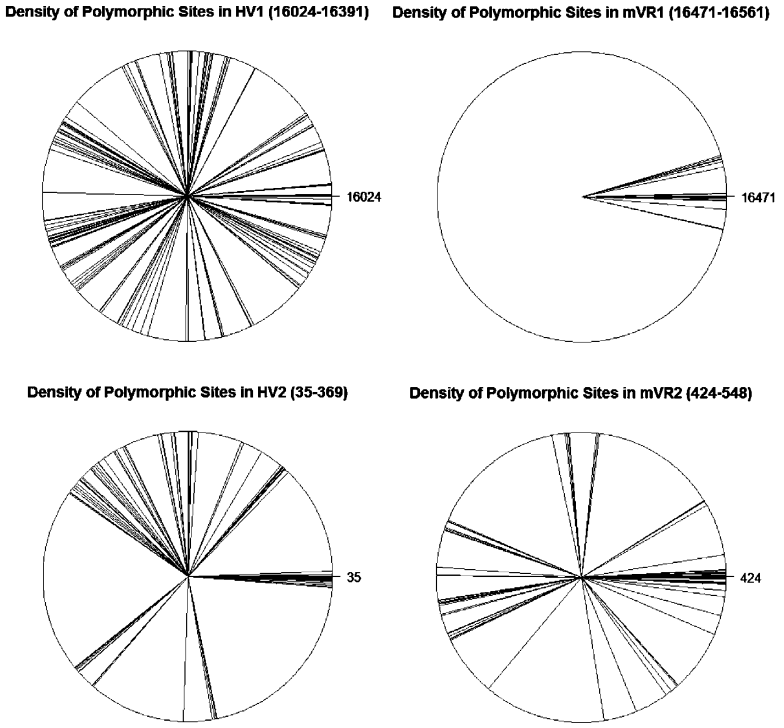

Fig. 16.1 Frequency plots of polymorphisms among the two hypervariable regions (HV1 and HV2) and the two mini-variable regions (mVR1 and mVR2) (redrawn from Edson et al. 2004). Each slice represents a specific polymorphic base-pair location, and each chart is read counter-clockwise from the listed base pair. Larger slices are interpreted as those polymorphic positions that appear with greater frequency in the database than the smaller slices, which indicate less common polymorphic sites ($n = 4021$). Note that while there are more polymorphic sites present in HV1, HV2 contains sites that are more common across all populations

an individual, thus generating a sequencing result of an indeterminate number of cytosine repeats in either HV1 or HV2 that cannot be accurately reported. Although typically a predominant length species can be determined, variability within these regions is not considered accurate enough for exclusionary purposes. Advances in mtDNA testing such as single nucleotide polymorphisms (mtSNP) that identify point mutations throughout the entire circular genome and additional sequencing in the mVRs have assisted in distinguishing similar HV1/HV2 sequences (i.e., by locating additional polymorphisms and thereby meeting current reporting protocol).

While the greatest amount of variation exists within the two hypervariable regions and sequences can be differentiated based only on two polymorphic positions, mtDNA profiles can still be quite common in certain populations. When samples are consistent with one another, there is a certain probability that the consistency resulted from a random match within the population at large. In order to determine how common a sequence is, the profile is always compared to a

population database of mtDNA sequences. In an analysis of mtDNA diversity from North American populations, Melton and Collegues (2001) reported that the most frequent mitotypes were observed in approximately 15% of the European-American population using single-stranded oligonucleotide typing. Drawing from the AFDIL database of HV1/HV2 sequences, the most common haplotype is present in just over 7% of individuals within the U.S. Caucasian population (Coble et al. 2004). Despite the disparity between these two findings, most likely the result of the use of different typing methods, the important point is that seemingly unrelated individuals could share the same mtDNA sequence across hypervariable regions (Fig. 16.2). However, the majority of mtDNA sequences are unique, having never been seen in the population database (represented by the long, low tail in Fig. 16.2).

Mitochondrial DNA data provide putative evidence for identification since the power of forensic mtDNA analysis is mainly through exclusion. Barring any other line of evidence, it is the non-individuating characteristic of mtDNA, or the possibility that two unrelated people share the same sequence, that prohibits mtDNA from being used as evidence of positive identification. When a consistency exists between two mtDNA sequences, this evidence is used in conjunction with other lines of forensic evidence, such as physical anthropology, archaeology, and odontology, thereby increasing the likelihood of individuation and identification as the independent lines of evidence are taken together.

Nuclear DNA STR Profiling

Short tandem repeats (STRs or microsatellites) are sections of nuclear DNA within genes that have repeating motifs of four to six nucleotides in length. Because STRs originate from nuclear DNA, an individual's genotype consists of pairs of alleles, one allele from each parent. Excluding amelogenin, which has only two alleles related to sex determination—X or Y—the number of alleles at each locus varies

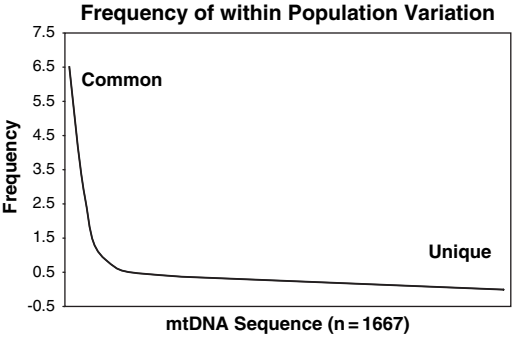

Fig. 16.2 Distribution of common mtDNA sequences within the Caucasian data set ($n = 1667$). There is a subset of individuals within the Caucasian database that has a similar sequence, approximately 7%. As single-event polymorphisms are introduced, the number of unique sequences increases dramatically (redrawn from Coble et al. 2004)

greatly; thus, the number of *possible* genotypes (combination of alleles) at a single locus is always greater than the number of alleles. When multiple loci are added, genetic variability increases and the number of possible genotypes increases dramatically. The majority of commonly tested loci are located on different chromosomes, and the repetitive elements that are passed from parent to offspring are inherited independently from those of the next locus. Because each locus is inherited independently, the multiplication rule of probability applies and as such a probabilistic statement can be calculated across all loci given the appropriate population frequency data (National Research Council 1996).

Recent death cases obtained from AFMES tend to have better tissue preservation than those cases received from the CIL, allowing AFDIL to perform STR testing. PowerPlex16® (Promega Corp., Madison, WI), a kit comprised of the 13 core CODIS (Combined DNA Index System) loci plus amelogenin, Penta E, and Penta D, can be used to generate an STR profile that identifies allelic variation at each of these specific heritable loci. By using these independently inherited alleles, consistency among all alleles evaluated between the unknown and the reference samples permits strong evidence for positive identification.

Y-Chromosome STR Profiling

Y-chromosome STR profiling is a relatively new type of DNA testing that observes heritable loci on the Y-chromosome. New heritable sites are constantly being identified, and a core set of testable loci has not yet been defined by the forensic DNA community. However, this type of testing is very useful for the identification of both recent and skeletal remains, as it provides yet another pool of references from which to draw. Y-DNA analysis is similar to STR testing in that it examines variation at specific loci; however, these loci are not independent of each other as they are all located on the same gene. Thus, there is a certain possible lack of independent assortment, although testing kits are being developed with specific loci to avoid this issue. Y-chromosome testing increases the pool of individuals that can be used as references, given that the variation within the Y is paternally inherited and remains fairly stable between generations. Like mtDNA testing, Y-STRs are a putative identification since multiple individuals can have the same profile. When combined with other types of DNA analysis and other evidence, Y-STRs will prove to be a strong analytical tool in the future.

Family Reference Samples

In addition to processing CIL skeletal samples where mtDNA is used most frequently, AFDIL processes mtDNA reference material collected from living relatives of the deceased for comparison with sequences derived from unknown skeletal samples. These reference materials are most often whole uncoagulated blood received in

potassium EDTA-treated tubes, but it is becoming more common to submit buccal swabs, a noninvasive collection method that causes little to no discomfort.

Since nuclear DNA is acquired from both parents and is recombinant, it is best to get a direct self-reference from the suspected unknown individual or from the presumed parents of the unknown decedent for comparison. For recent deaths of military personnel, nuclear DNA is a common means of identification. Today this is possible because all service members are required to submit a blood sample that is archived at the Armed Forces Repository of Specimen Samples for the Identification of Remains (AFRSSIR). In the event of a service member's death, DNA is extracted from their archived blood card, which is then used as a reference for comparison to DNA extracted from the decedent.

In contrast to nuclear DNA analysis, Y-STR profiles are from the non-recombining Y-chromosome; thus, the potential references are identified through the paternal line and include only male relatives. Mitochondrial DNA, on the other hand, is inherited strictly from the mother, as the father contributes no mitochondria to the child. In contrast to Y-chromosome inheritance, both males and females of the maternal line are appropriate references for mtDNA analysis.

Additional sources of reference material include items belonging to the victim (i.e., direct self-reference) or a relative. In the past, alternative references submitted to AFDIL for processing included hair from hairbrushes and razors; hats; sealed envelopes and stamps; nail clippings; clothing; or even archived medical biological material, such as paraffin blocks from a hospital. Positive interactions with the public and families can often produce items acceptable for analysis.

Skeletal Sample Selection

The process for selecting the best possible bone samples from commingled remains follows a few simple steps. First, remains from a single incident should be sorted as best as possible following anthropological techniques, which include evaluation of archaeological provenience and identification of duplicating elements, skeletal age indicators, size and shape differences among elements, articulating elements, and conjoining skeletal fragments. Metric sorting of skeletal elements is also applied (Byrd and Adams 2003). If two or more anatomically identical elements are present within a single assemblage, then sampling of those duplicated elements is preferred as they clearly represent distinct individuals and permit their possible identification. If several elements are associated by articulation, such as teeth to a mandible or a series of conjoining vertebrae, then only one element should be sampled. This not only minimizes the cost of analysis and destructive sampling, but it also identifies potential samples for additional testing should the first not produce a sequence.

If commingling of skeletal remains results in poorly associated cranial and postcranial remains, by either missing or poor articulation of the first cervical vertebra with the cranial base, then it is recommended to procure at least one cranial or dental sample and one sample from the postcranial remains. This strategy will

allow the majority of remains to be segregated from the grouped assemblage and reassociated into individuals, creating the potential for positive identification based on DNA and dental evidence.

Samples are also selected based on their potential for preserved endogenous DNA. Preservation depends highly on the environment from which the remains were recovered, and the persistence of DNA is correlated loosely with the same taphonomic processes that affect the micro- and macroscopic structure of skeletal material (Damann et al. 2002; Hagelberg et al. 1991; Herrmann and Hummel 1994; Parsons and Weedn 1997). Experience demonstrates that the best nondental samples are those from long bones with thick cortices, such as the femur, tibia, and humerus (Edson et al. 2005), whereas elements typically preferred when creating a biological profile such as the os coxae and crania are relatively poor DNA candidates due to their thin cortices (Fig. 16.3).

Generally, dense skeletal elements provide protection of endogenous DNA from deleterious taphonomic agents that destroy bone and its constituent parts. It should also be stated that the desire for samples with thick cortical tissue is due partly to pre-extraction laboratory processing that attempts to remove any exogenous contamination by sanding and washing. While destructive to the sample, it has been

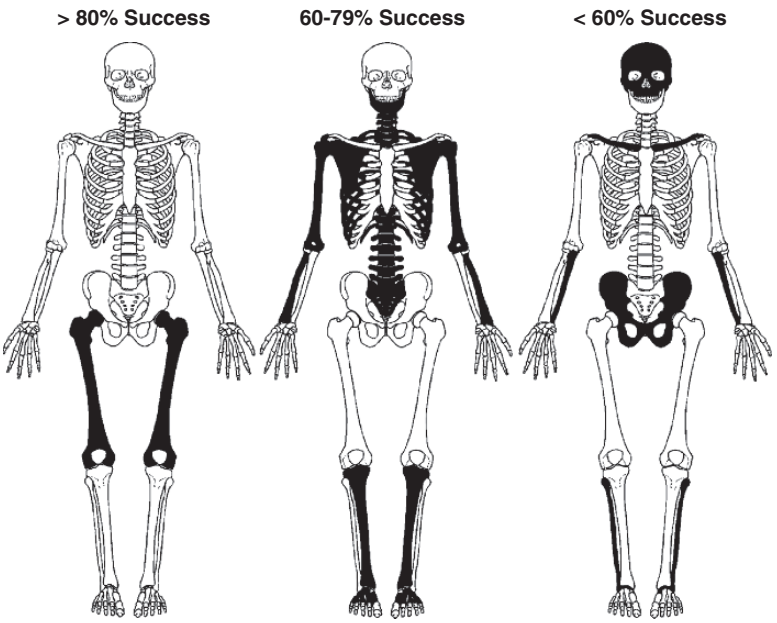

Fig. 16.3 Frequency distribution for the successful amplification and sequencing of mtDNA from human remains recovered by the CIL and processed by the AFDIL (redrawn from Edson et al. 2005). This distribution is based on 3,721 skeletal samples. Success is defined as a sample that yielded at least two identical sequences greater than 100 base pairs in length. Statistics pertain to elements shaded black. Elements with fewer than 30 samples such as the patella, sternal body, manubrium, carpals, and metacarpals are not included in this distribution

found that without these mitigating steps, many samples are unusable as sequences often contain mixed DNA sequences or the endogenous "ancient" target DNA is outcompeted by relatively new and more viable DNA from contaminating sources such as soil organisms from the area where the remains were recovered or analysts that handled the case.

When mtDNA results are obtained from multiple samples in a commingled case, matching sequences may be attributed to the same individual, they may represent more than one individual because of the non-unique properties of mtDNA, or they may represent an exogenous contaminate. In order to ensure the highest quality in the DNA sequence results, AFDIL analysts employ stringent protocols. This includes independent duplication of sequence results prior to reporting and their use of appropriate internal controls throughout the extraction, amplification, and sequencing processes.

Since the CIL submits samples to AFDIL free of provenience and incident-related information, the anthropologist must interpret the DNA results to determine if the mtDNA sequence originates from a single individual or if multiple people are represented by the same sequence. One easy way this is accomplished is by following a sound sampling strategy. If two samples are determined to have the same sequence, then the anthropologist must check to see if the shared sequence is from duplicating elements or elements that are excluded from one another based on differences in morphological and metric analyses.

Without the mitigating steps of the anthropologist's initial segregation and subsequent review of sample sequences and skeletal data, the potential exists for making erroneous skeletal associations based on accurate mtDNA sequence data. One recent case exemplifying this potential error occurred with an assemblage of remains recovered from a U.S. bomber crash in Papua New Guinea that was carrying a crew of nine service members. In late 1943, the crew took part in a night reconnaissance mission and an attack on Japanese ships in the vicinity of New Ireland. After reporting a successful attack, the aircraft and crew never returned to their airbase and were presumed lost at sea. Subsequent search efforts provided no useful information regarding the whereabouts of the bomber and crew. In 2002, a representative of the Morobe Provincial government provided information to the U.S. Embassy regarding the location of aircraft wreckage and remains. The following year JPAC-CIL deployed to the site and recovered remains and aircraft information correlating the recovery site to that of the bomber lost in late 1943. As part of the skeletal analysis, mtDNA sequences were obtained. A shared sequence between two individuals soon became evident to the anthropologist reviewing the case since the same sequence was reported for two duplicated fragments of right femur. With this information, the AFDIL analysts used the DNA extracts from the two femora fragments and obtained a partial nuclear STR profile for each sample. This additional information was sufficient to genetically distinguish the two individuals. Without the anthropologist's recognition that the two samples could not have originated from the same individual (duplication of skeletal elements), these bones might have been erroneously associated to the sample decedent. Following a well-planned sample selection process, the appropriate steps are in place to recognize potential problems of shared sequences.

A careful sampling strategy also prohibits unnecessary and excessive sampling that leads to stresses on the DNA laboratory workload and inflated costs to the submitting agency.

Kiska Island, Alaska

The following case example details the recovery and identification efforts of the JPAC-CIL and AFDIL for a World War II aircraft crash on Kiska Island, one of the largest islands in the Aleutian Island chain off the coast of Alaska.

Weinberg (1994) and Morison (2001) provide descriptions surrounding the events taking place in the North Pacific in June 1942. According to their descriptions, Japanese aircraft attacked islands of the Aleutian chain in order to draw U.S. attention away from the ongoing Battle of Midway and to keep the United States from staging an attack on Japan from the North Pacific. In this effort, the Japanese secured and deployed troops to Kiska and Attu Islands. Three days later, U.S. forces in the Pacific became aware of the Japanese movement into the Aleutian Islands and ordered U.S. Naval aircraft to attack. Over the next several days, U.S. aircraft continued bombing missions. On June 14, 1942, a U.S. Navy PBY-5, carrying a crew of seven, took part in the bombing of Japanese ships moored in Kiska Harbor, when "it [the PBY-5 aircraft] was last seen plunging into a cloud bank over Kiska Harbor" (Commander Fleet Wing Four to the Judge Advocate General 10 September 1943). Since the aircraft never returned to its unit, it was believed to have been shot down by enemy activity and subsequently lost in the vicinity of Kiska Island, Alaska.

After U.S. forces recaptured the island from the Japanese in August 1943, the bodies of seven crewmembers were found. A review of military records indicated that seven crewmembers of the PBY-5 were buried in a common grave on the northwest side of Kiska Volcano. At the head of the grave, a cross was placed with the words *Seven U.S.N. Airmen*. The bodies stayed at this location for nearly six decades before an attempt was made to recover and identify them.

In 2001, an associate professor of wildlife biology at the Memorial University of Newfoundland, Canada, was conducting research within the Alaska Maritime Wildlife Refuge where he encountered aircraft wreckage. In late 2002, the JPAC-CIL was made aware of the site and researchers at the CIL were able to correlate serial numbers on wreckage to the PBY-5 that was last seen in 1943. During the summer of 2003, a CIL archaeologist led a team of U.S. service members and civilians to recover the skeletal remains buried in a single mass grave on the side of Kiska Volcano. For several days the team lived on a ship in Kiska Harbor and flew from the ship to the site via helicopter. Once at the site, the team began their recovery effort by pedestrian reconnaissance on the northwest side of the volcano at 2,700 feet to locate the crash site. Miscellaneous wreckage was located in a number of erosional gullies along the downslope edge of a snowfield; however, the main wreckage field was discovered upslope at an elevation of 3,027 feet above sea level.

A small rock cairn with wood fragments was identified approximately three meters away from the main concentration of aircraft wreckage. Upon closer inspection, one wooden fragment had the letters "USN AIRMEN" carved on one side (Fig. 16.4).

The main wreckage field (approximately 15 by 15 meters in size) was excavated, including the rock cairn. Throughout most of the excavation, incident-sterile sediment was rather shallow given the erosional formation process of the site. The feature below the rock cairn was the only area where excavation extended in excess of 20 centimeters below ground surface. The rock cairn was mapped; after the initial level of boulders and cobbles was removed, a grave was located and excavated using trowels, brushes, and small bamboo sticks.

Human remains in the grave represented collections of skeletal elements, both articulated and disarticulated, some of which were wrapped in sheepskin flying garments. The commingling of the remains made it difficult, if not impossible, to sort individuals within the feature fill. Consequently, an effort was made to identify and excavate articulating elements (i.e., upper arm to forearm, upper leg to lower leg, etc.) and place the excavated articulations in individual bags. All sediments within the burial feature were screened through 1/4-inch wire mesh, and items recovered from the screen were bagged separately and labeled accordingly. All recovered skeletal, dental, and material evidence was then transported to the CIL for analysis.

Upon accessioning, skeletal analysis segregated seven potential individuals based on duplicated femora. Dental, osteology, and archaeological provenience data were

Fig. 16.4 Photograph of rock cairn with fragments of wooden cross at the site of the PBY-5 aircraft crash on the side of Kiska Volcano.

used during the initial sorting to identify a total of 14 clusters, consisting of seven groups of postcranial remains, six clusters of dental remains, and one group of unassociated remains. Separation was possible because provenience was maintained for elements articulating *in situ*; age and size differences were evaluated; bilateral nonmetric traits were recorded and compared; conjoining elements and fragments were refit; preservation factors such as bone deterioration, color, and integrity were evaluated; and metric sorting was applied (Byrd and Adams 2003). After all of the anthropological associations were completed, there were two main challenges: (1) linking the clusters to each other, in essence rebuilding people; and (2) positively identifying the seven airmen.

For this case, antemortem dental records were available for the entire crew from their archived medical records. The level of detail contained in the files, coupled with the diversity of dental treatment observed on the remains, made it relatively straightforward to identify most of the teeth and associated skeletal structures to the seven airmen. Although all of the crew could be identified, this would only pertain to a small amount of the total quantity of remains. As such, a decision was made to use mtDNA to reassociate the dental remains with the clusters of postcranial remains. A well-designed DNA sampling strategy was developed to test specific elements from the anthropologically sorted clusters. In addition to providing a solid means of reassociation, it also provided a confirmatory test of the gross sorting procedures used to group bones together.

Normally, family reference samples (maternal relatives) are needed when comparing mtDNA sequence data. Finding such references generations removed from this case proved difficult, which is becoming normal for cases examined nearly 60 years after the incident. Because this case involves the loss of U.S. Navy service members, correspondence with relatives, including finding the appropriate relatives for reference material, is the responsibility of the U.S. Navy Casualty Office. Given the antiquity of the case, the Navy Casualty Office had difficulty acquiring the maternal references and was only able to obtain one sample from the presumed decedents. Thus, in lieu of reference samples from living relatives, the CIL and AFDIL decided to use direct self-references for six of the seven sets of remains. This means that a decision was made to sample the identified teeth since they could be associated to specific crew members. By doing this, the need for collecting the other six references could be avoided. Congruent sequence matches between the positively identified teeth and the unassociated bone clusters would be conclusive evidence for linking these elements to the same person.

From the 14 groups of separated remains, a minimum of one sample from each of the seven postcranial clusters and six dental groups was taken. The anthropologist identified other elements for sampling in order to test the initial sorting hypotheses as well as associate those elements suitable for estimating a biological profile from the unassociated group. In all, the anthropologist selected 32 samples for mtDNA analysis, which included teeth from the grouped cranial remains and duplicated femora from the postcranial groupings.

Sampling of remains at the CIL always occurs in sterile hoods where a wedge of bone or a pulverized dental sample is packaged and then sent to AFDIL for analysis.

Of the 32 samples submitted to AFDIL, 29 generated full reportable sequences. From those 29 samples, seven distinguishable mtDNA profiles were obtained (i.e., there were at least two or more polymorphisms between any two sequences). The MNI as determined by osteology coincided with that determined by mtDNA. The seven separate mtDNA sequences supported the initial segregation into seven individuals; however, minimal rearrangement of a few elements among clusters was warranted. Due to some postmortem damage, some associations contained in the initial sort were considered to be tentative (i.e., they appeared to be more consistent with one set of remains than others, but the association was not to the exclusion of all other possibilities). The DNA sampling strategy was specifically designed to test some of these associations. Once the DNA results were available, it was found that there were some minor adjustments needed with some of the postcranial clusters. While the initial segregation needed more work, the well-thought-out sorting and sampling plan established by the anthropologist was able to resolve any discrepancies and make the appropriate associations.

The seven crewmembers aboard the U.S. Navy PBY-5 that crashed in early summer 1943 were able to receive an identification based on consistency with antemortem dental records, historical records documenting the reported loss location and flight manifest, archaeology, osteology, and mtDNA evidence. In this case, initial separation of commingled remains was accomplished using archaeological provenience and osteological techniques. Subsequent mtDNA sequence results allowed a confirmation of the initial sorting procedures (with minor adjustments) and were essential for linking the numerous clusters of bones and teeth into seven distinct individuals. The success of this case resolution was due in large part to the implementation of a systematic DNA sampling approach.

Conclusion

Applying DNA analysis to sort and identify commingled human remains requires the anthropologist to choose the type of DNA test that will best address their question. In so doing, it is important to understand the population dynamics and the inherent resolution power for the different types of DNA tests. When skeletal and soft-tissue preservation is good, nuclear STR data can be used. By generating full STR profiles, positive identification is possible because of the unique properties of nuclear DNA due to recombination. At the same time, STR profiles will allow for the segregation of remains based on observed differences in DNA profiles. For many anthropologists, mtDNA is an appropriate genetic tool given the relative ease for obtaining mtDNA sequences from cases with poor skeletal preservation. However, mtDNA only provides putative evidence due to the non-unique nature of mtDNA profiles in the population at large and, as such, requires additional information to support a positive identification. When there is a need to separate commingled remains, mtDNA is often sufficient, especially when an anthropologist institutes the

appropriate control mechanisms to her sampling strategy so that potentially shared sequences can be resolved.

Not only is it important to determine the type of DNA that would be most effective for testing, but it is also important to determine whether or not reference materials are available for the desired testing. DNA testing is generally only useful as long as there is a reference sequence to compare to the sequence from the unknown remains. For the individuals recovered from certain circumstances, such as Kiska Island, an mtDNA self-reference obtained from dental remains is appropriate because the anthropologists and dentists are able to group remains prior to DNA analysis.

When selecting samples for DNA analysis, the anthropologist's assessment of the skeletal assemblage and development of initial sorting into potential individuals is imperative to determine a thoughtful sample strategy and to prevent future errors of associating remains based solely on a shared profile. An appropriate strategy should consist of sampling duplicated elements and/or elements that can be associated through other techniques (e.g., articulation, pair-matching, etc.). Key elements to sample are also those that display characteristics useful in estimating a biological profile. By establishing testable hypotheses of those associations, an appropriate sampling strategy reduces the potentials for oversampling and unnecessarily increasing the cost of analysis.

Finally, it is imperative to develop and maintain rapport with the respective labs in order to reach identification and case resolution from commingled remains. Applying DNA analysis to the segregation of commingled remains can be an expensive and laborious process, but the process becomes efficient through collaboration among team members. Communication between the different groups of scientists on a regular basis is essential to the identification process. A successful relationship between laboratories will lead to rapid resolution of most casework issues, more targeted sampling of remains, and a more efficient identification process overall. Over the past 15 years, the combined efforts of the JPAC-CIL and AFDIL have identified hundreds of individuals from commingled settings, such as battlefield mass graves and aircraft crashes. The individuation and identification of the seven crewmembers recovered from a commingled grave on the slopes of Kiska Volcano is just one example of the interaction among anthropologists and molecular biologists that leads to the successful resolution of a complicated scenario.

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