

CASE REPORT**CRIMINALISTICS; ANTHROPOLOGY**

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Integrated DNA and Fingerprint Analyses in the Identification of 60-Year-Old Mummified Human Remains Discovered in an Alaskan Glacier

ABSTRACT: This report describes the identification of a merchant mariner who perished in 1948 when Northwest Airlines Flight 4422, a DC-4 carrying 24 seamen and six crew members crashed into Mount Sanford, Alaska. Fifty-one years later, a human forearm and hand were found close by the wreckage of the plane, prompting identification efforts using DNA and fingerprints. There were significant challenges to both the fingerprint and DNA analyses. The hand was badly desiccated, making fingerprint friction-ridge detail almost invisible and the remains had been embalmed upon discovery, making DNA amplification difficult. We present the results of an interdisciplinary approach that successfully addressed these challenges and ultimately led to the identification of the remains. These efforts relied on efficient fingerprint rejuvenation and imaging techniques that improved print resolution, as well as new DNA extraction techniques optimized for aggressively embalmed remains.

KEYWORDS: forensic science, fingerprint analysis, low copy number STR, mitochondrial DNA, ancient DNA, embalming, interdisciplinary, forensic genealogy, merchant mariners

At 9:14 P.M. on March 12, 1948, Northwest Airlines Charter Flight 4422, en route from Shanghai to New York City, crashed in the Wrangell Mountains of Alaska. The Douglas DC-4 was carrying six crew members and 24 seamen returning home after a mission on the oil tanker *SS Sunset* of the Overseas Tankship Corp. After a fuel stop, the plane took off from Anchorage, Alaska at 8:12 P.M. At 9:03 P.M., the plane was spotted flying at 11,000 feet above the Gulkana Civil Aeronautics Administration range station, 159 miles northeast of Anchorage. Eleven minutes later, the plane impacted Mount Sanford, a 16,237-foot peak located 200 miles northeast of Anchorage. The next day, aeronautic officials flew over the crash site. From the air, they observed the wreckage lying at an elevation of 8500 feet on the west slope of the mountain, below a section of smoke-blackened snow and ice at 11,000 feet that likely corresponded to the point of impact. A rescue operation

was ruled impossible because the scene of the accident was inaccessible by air. No ground search was initiated as it was determined that the active glacier would bury the wreckage in a matter of days. However, a positive identification of the wreckage as that of Flight 4422 was made by the Northwest Airlines insignia on the plane's vertical tailfin observed from the air.

Fifty-one years later, the wreckage was rediscovered by Kevin McGregor and Marc Millican, two former United States Air Force pilots. In 1997, after three unsuccessful expeditions, the two men finally located pieces of the plane, including a radial engine and a propeller at an altitude of 6200 feet. Two years later, they returned to the site and discovered a frozen human left forearm and hand amid the wreckage (1,2).

Alaskan State Troopers recovered the remains and delivered them to the Office of the Medical Examiner in Anchorage, Alaska. X-rays of the arm showed extensive damage to the bones (Fig. 1). Fingerprint images were also taken of the hand and were compared to the fingerprints of 22 of the 24 merchant mariners. The mariners' fingerprints had been collected at the time of their enlistment and archived at the National Maritime Center in Arlington, VA. Unfortunately, in 1999 no match was obtained, most likely because of the difficulty of resolving details from the fingerprints of the damaged remains in combination with the poor quality of the enlistee fingerprint cards. Following these examinations, the remains were aggressively embalmed by submersion for 5 days in a solution composed of Metasyn Normal (20 index), Metaflow, Restorative (Dodge Chemical Company, Cambridge, MA), and water (Fig. 2).

The effort to identify the flight 4422 remains was first revived in 2002 when the hand and forearm were sent to a commercial

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FIG. 1—X-rays of the human forearm recovered from Mount Sanford.



FIG. 2—Embalmed left forearm and hand found on Mount Sanford, Alaska, in 1999.

paternity laboratory for autosomal short tandem repeat (STR) testing. Again, however, the identification efforts were unsuccessful. The DNA extraction from the sample yielded no quantifiable genetic material so DNA testing was not pursued at the time.

Recent advances in fingerprint and DNA technologies prompted renewed identification efforts. We describe here the DNA and the fingerprint analyses that led to the identification of the hand and forearm as belonging to Seaman Francis Joseph van Zandt.

Material and Methods

DNA Extraction and Amplification from the Embalmed Arm and Hand

To ensure independent replication of the results, mitochondrial DNA (mtDNA) analysis of the remains was performed in two separate laboratories exclusively devoted to pre-amplification procedures of ancient and/or degraded remains.

All samples were treated prior to extraction to remove possible surface contamination. In the ancient DNA laboratory of Simon Fraser University (SFU-aDNA, Burnaby, Canada), the bone samples were soaked in a 100% commercial bleach solution for 7–10 min, decontaminated via immersion in 1 N HCl solution for 30–60 sec, and finally immersed in 1N NaOH for 30–60 sec. The samples were rinsed twice in ultra-pure water and UV irradiated in a cross-linker for 30 min on two sides. At the Armed Forces DNA Identification Laboratory (AFDIL, Rockville, MD), the soft tissue was removed and then the bone was sanded with

TABLE 1—Change in DNA concentration when bone powder is washed with phosphate-buffered saline pH 7.4 solution (PBS).*

Treatment	Amount of Powder (mg)	qPCR (RU)
No wash-1	100	66.49
No wash-2	200	75.25
No wash-3	200	79.86
Wash with PBS buffer-1	100	124.05
Wash with PBS buffer-2	200	197.47
Wash with PBS buffer-3	200	193.61

RU, Relative unit.

*Preliminary data.

an aluminum oxide sanding stone attached to a dremel tool (Dremel, Racine, WI). The bone fragments were sonicated first with diluted bleach, then water and finally absolute ethanol. Following decontamination, samples were powdered separately with a sterilized stainless steel Waring MC2 blender cup (Waring, Torrington, CT), a vise, or a cryogenic impact grinder (CertiPrep 6750 Freezer Mill, Spex, Metuchen, NJ). At AFDIL, we have observed that the presence of oxidized formaldehyde (paraformaldehyde) in bone powder reduces extracted DNA yields by up to 50%; however, if the powdered sample is washed in a phosphate-buffered saline pH 7.4 solution (PBS), our preliminary data suggest that DNA yields in the final extract tend to increase significantly (Table 1). We have also observed that when washed with PBS, the powdered bone sinks to the bottom of the tube while the oxidized formaldehyde floats to the surface of the buffer. For this reason, most of the DNA extractions that we performed incorporated a preliminary PBS wash of the powdered sample that included careful removal of the floating aldehyde from the solution. After the removal of PBS, the powder was incubated at 56°C overnight in 3–15 mL of extraction buffer (EDTA 0.5 M, 0.5% Sodium N-lauroylsarcosinate and 100 µL proteinase K at 20 mg/mL) to obtain a complete decalcification as described in (3,4). In contrast to the method recently published (3), this extraction protocol does not require any organic solvents and produces inhibitor-free DNA extracts yet still results in the complete dissolution of the bone powder and produces comparable DNA yields. After an overnight incubation, the tubes were briefly centrifuged and the buffer was transferred directly into an Amicon Ultra-4 or an Ultra-15 Centrifugal Filter Unit, fitted with an Ultracel-PL 30-kDa Membrane (Millipore Corp., Bedford, MA), then concentrated until the volume had decreased to ≤100 µL. The buffer solution was then purified using either the MinElute™ PCR Purification kit (Qiagen, Valencia, CA) at AFDIL or the QIAquick columns (Qiagen) at the SFU-aDNA. The final volume varied between 25 and 100 µL.

In both laboratories, the amplifications of mtDNA were carried out using 1 µL of template DNA with primers and thermal cycling conditions as described in (5). Only small fragments ranging between 126 and 170 bp in size were targeted, as severe DNA degradation was expected in a sample subjected to a high concentration of formaldehyde. One extraction blank and two PCR negative controls were carried out for each PCR experiment. Five microliters of PCR products were visualized on a gel, and the remainder was purified using either exonuclease I (EXO) and shrimp alkaline phosphatase (SAP; USB Corporation, Cleveland, OH) in a ratio of 2 U EXO to 0.1 U SAP for each µL of PCR product (AFDIL) or the Qiagen MinElute™ purification kit (SFU-aDNA). Purified PCR products were then sequenced with the Big Dye Terminator Cycle sequencing kit v1.1 (Applied Biosystems, Foster City, CA). Sequencing reactions were analyzed on a 3100 or 3130xl Genetic

Analyzer (Applied Biosystems) following purification with AGTC columns (Edge Biosystems, Gaithersburg, MD). The multiple sequences were aligned using either ChromasPro (Technelysium Pty Ltd., Tewantin, Australia) or Sequencher v4.7 (GeneCodes, Ann Arbor, MI). The finalized sequence from each laboratory was then compared to the sequences of the available family references and to the SWGDAM Caucasian database of 1655 individuals.

At AFDIL, Y chromosome short tandem repeat (Y-STR) profiles were obtained using the AmpF/STR[®]Yfiler[™] PCR Amplification kit (Applied Biosystems). However, for each amplification, twice the manufacturer's recommended AmpliTaq Gold concentration and six additional PCR cycles were necessary (6,7). PCR products were separated on a 3130xl Genetic Analyzer (Applied Biosystems) and analyzed using Genemapper[®] v3.2 (Applied Biosystems). Y haplotypes were compared to two family reference profiles and to two Y-STR databases.

References for DNA Comparison: Localization and DNA Analysis

To pursue the identification of the remains using mtDNA, forensic genealogical techniques were used to locate living maternal relatives of the 24 seamen and six crew members aboard Flight 4422. Telephone directories, obituaries, and vital records were among the resources used to find a suitable reference for mtDNA testing.

Saliva or blood was collected using a Bode Buccal DNA Collector at AFDIL (The Bode Technology Group Inc., Springfield, VA) or an IsoCode STIX at Genesis Genomics (Schleicher & Schuell, Keene, NH). DNA was extracted with the QiaAmp DNA Mini kit (Qiagen). Amplifications and sequencing of mtDNA were performed as in (8,9). Two paternal relatives were tested for Y-STRs using the AmpF/STR[®]Yfiler[™] PCR Amplification kit following the manufacturer's instructions.

After several months of intensive research, it became evident that DNA references were unlikely to be obtained for all 30 people onboard the plane. As a result, and although the 1999 fingerprint analysis had been unsuccessful, another fingerprint study was initiated with new experts in an effort to obtain a complete set of exclusionary references.

Fingerprint Analysis

To attempt the identification of the mummified hand by fingerprint analysis, tissue rejuvenation was initiated. To produce better ridge detail, the complete hand was first immersed in a rehydration solution (KDL; Evident, Union Hall, VA). The fingers were checked hourly and, as this solution is nontoxic and nonvolatile (as opposed to other available buffers) the temperature was eventually elevated to 50°C to expedite rehydration. After 8 h of soaking, the fingertips were photographed and cast with two different brands of silicone rubber (Mikrosil[™] Casting Putty and AccuTrans; Evident). With details permanently preserved, further improvement of the friction-ridge detail was attempted by removing the finger pad tissue and re-soaking the pads in the heated KDL solution. After another day of soaking, the finger pads were again photographed and coated with silicone rubber. These silicone rubber molds were used to make castings with Castone[®] Dental Stone to create a positive impression of each fingertip. All these hard casts were dusted lightly with black fingerprint powder to bring out the friction-ridge detail for easier comparison. Finally, to make the dermal papillae even more apparent, the rubber casts of the pads were scanned with a flatbed scanner and the scans were processed in Adobe Photoshop[®] (Adobe Systems Inc., San Jose, CA) to enhance detail. All

of the photographs along with the silicone rubber and Castone casts were turned over to an experienced latent print examiner to be compared with the fingerprint records of the merchant mariners archived at the National Maritime Center.

Examination of the Merchant Mariners' Fingerprint Cards

Since the original examination of the hand in 1999, six sets of the merchant mariner's fingerprint cards had been misplaced and were not available for the re-examination. This was in addition to two sets that had never been located. Fingerprint references for the flight crew were sought in collaboration with the National Military Repository in St. Louis, MO, since the members of the crew had previous military experience. Unfortunately, these efforts were unsuccessful. In the end, fingerprint cards from eight seamen and all six crew members were unavailable. Thus, the new fingerprint analysis was conducted by comparing the 16 available merchant mariner fingerprint cards with the fingerprint casts and photographs produced from the mummified hand. The identification was based on the recommended ACE-V (analysis, comparison, evaluation, and verification) methodology.

Results

The search for family DNA references proved extremely challenging for several candidates. Although mtDNA references were sought for all missing individuals, as the number of candidates dwindled as a result of exclusion by fingerprint and DNA comparisons, it became increasingly difficult to locate family members. In many cases, locating a suitable DNA reference was difficult because of the long time period that had elapsed since the accident. Some of the older victims of the crash were born in the late 1800s, some were unmarried or had no siblings, and some had only loose family ties. Furthermore, some maternal relatives simply refused to give a DNA sample.

In total, maternal relatives representing 19 of the 30 persons on Flight 4422 were located for mtDNA testing and paternal relatives were found for two individuals. For one of these victims, a crew member, the paternal reference was the only DNA reference that could be located. In total, a DNA reference was available for 20 candidates. With fingerprint cards covering the other ten, reference material was obtained for all 30 victims onboard Flight 4422 (Table 2). For six passengers, both DNA and a fingerprint card references were available.

DNA Analysis of the Remains

The mtDNA control region profile for the Flight 4422 remains, generated from multiple short amplicons, resulted in a 697 base profile at AFDIL (HVI: 16,009–16,391; HVII: 57–369). The sequence generated at SFU-aDNA resulted in a 681 base profile that was identical to the one obtained at AFDIL: C16192T C16256T C16270T C16291T A73G A263G 315.1C (mtDNA haplogroup U5; see ref [10]). This sequence was observed five times among 1655 individuals in the Caucasian SWGDAM database (0.3%; see ref [11]), was seen twice among 1148 individuals in the African American SWGDAM database (0.17%), and was unique in the 2036 individuals in the Native American or Asiatic SWGDAM databases. It was observed nine times in the EMPOP West Eurasia database of 3830 individuals (0.23%; <http://www.empop.org> [accessed April 30, 2009]). This profile was identical to the sequence obtained from Francis van Zandt's maternal relative, a second cousin twice removed living in Ireland. The mtDNA profiles from the remaining

TABLE 2—Crew and passengers of Northwest Airlines flight 4422.

		Exclusion with DNA	Exclusion with Fingerprints
Seamen			
Adler	Eugene	X	
Beswick	Wilfred	X	
Brooks	Morris	X	
Comshick	John		X
Davidson	Howard	X	
Delaney	Robert	X	
Eilertsen	Arthur	X	X
Elkins	John		X
Foote	Eugene		X
Jacobsen	Olav	X	
Jamele	John	X	
Jenkins	Everett	X	X
Koistinen	August		X
Lampman	James		X
Marushak	Michael		X
McCall	Travis		X
Mooney	James		X
Mustra	Edwin	X	X
Rabich	Robert		X
Rapchinski	John		X
Rice	Daniel	X	X
Sigmund	Carl	X	X
van Zandt	Francis		
Wilkowski	Stanley	X	
Crew			
Haslett	Robert	X	
Petry	Robert	X	
Rector	Donald	X	
Stickel	Jehu	X	
van Cleef	James	X	
Worsley	Wayne	X	

18 references differed from the arm's sequence at five or more positions, clearly excluding the missing individuals represented by these references as the source of the remains.

As neither a fingerprint nor a maternal reference was available for one of the crew members, the crash remains and the paternal reference obtained through the genealogy search were typed for Y-STRs. We also obtained a paternal reference for van Zandt as a possible confirmation of the mtDNA results. Multiple amplifications of the DNA from the remains using three independent extracts resulted in concordant Y-STR profiles and replicated alleles at 13 Y loci (Table 3). This haplotype differed at 10 loci from the paternal reference for the Flight 4422 crew member, clearly excluding him as the source of the remains. However, the composite profile was consistent with the van Zandt reference haplotype at every locus. A search of the US Y-STR database of 7075 haplotypes (<http://usystrdatabase.org> [accessed April 30, 2009]) and the YHRD database of 15,956 haplotypes (<http://www.yhrd.org> [accessed April 30, 2009]) indicated that the complete Y-STR profile obtained from Francis van Zandt's paternal relative, as well as the partial profile recovered from the remains, has never been previously observed. The combined mtDNA and Y-STR analyses not only permitted the exclusion of 18 victims but also dramatically increased the probability that the remains belonged to van Zandt because of the rarity of both haplotypes.

Fingerprint Analysis

Photographs of two silicone rubber molds obtained from the rehydrated fingertips are shown in Fig. 3. The most straightforward way to compare fingerprints is to determine the basic pattern on the ridge of a finger and compare it to a reference. The three

TABLE 3—Y chromosome short tandem repeat genotypes for the arm and the LDR.

Markers	LDR	Hand 1	Hand 2	Hand 3	Hand 4	Hand 5
DYS456	15	15	15	15	15	15
DYS389I	13	13	13		13	13
DYS390	22	22			22	22
DYS389II	29					29
DYS458	15	15	15	15	15	15
DYS19	14	14		14		14
DYS385a/b	13,16	13,16	13,16	13,16	13,16	13,16
DYS393	13	13	13	13	13	13
DYS391	10	10	10	10	10	10
DYS439	10			10	10	10
DYS635	20		20			
DYS392	13					
GATA H4	11	11		11	11	11
DYS437	14			14	14	
DYS438	9	9	9	9	9	9
DYS448	19					

LDR, living DNA reference.

reference patterns are as follows: loops where the ridges enter and exit from the same side, forming a loop on the fingertip; arches where the ridges enter from one side and exit on the opposite side, exhibiting a rise as they cross the fingertip; and whorls where the ridges are circular and completely contained on the fingertip. In the case of the left hand recovered in Alaska, the pictures and casts clearly showed a loop pattern on each of the five fingertips. Comparison of these images to the 16 available fingerprint cards revealed that ten individuals possessed whorls or arches on at least one of their left fingertips. Thus, these ten individuals could be excluded as a match. Further detailed visual examination of the fingerprints resulted in the exclusion of four additional merchant mariners. Only two candidates remained on the list: Francis J. van Zandt and John V. Elkins. The file of seaman van Zandt contained an original rolled ten-print card including all ten fingers, as well as four separate index cards with an impression of his left thumb. Based on 22 Galton details (i.e., points of identity) from a composite of his fingerprint cards, the hand from the glacier was positively identified as belonging to mariner Francis Joseph van Zandt.

Discussion

The conclusive identification of the arm as that of Francis Joseph van Zandt, via mtDNA analysis, Y-STR analysis, and fingerprint analysis, demonstrates the benefits of a combined interdisciplinary approach to missing person identification efforts, especially in cases for which one particular line of evidence or source of data may be compromised or unavailable.

The circumstantial evidence linking the remains found in the glacier to a passenger or a crew member of Flight 4422 was essential. The location at which the remains were found was extremely remote and in the immediate proximity of debris and personal effects from Flight 4422. In addition, X-rays of the arm showed many fractures consistent with the type of violent impact that occurred when Flight 4422 struck Mount Sanford. Although this information allowed us to limit our investigation to the known crash victims, a number of challenges still had to be overcome to conclusively identify the remains.

Fingerprints from mummified remains have been obtained in the past for forensic identification purposes (12,13). The case reported here, however, stands out because of the age of the remains. These 60-year-old remains represent the oldest ever identified by fingerprint analysis. This success was possible because of a number of unique

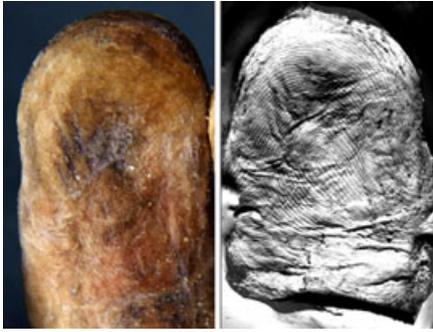


FIG. 3—On the left, image of the middle finger before treatment. On the right, mirror image of the cast of the rejuvenated fingertip, demonstrating improvement in ridge detail. Pictures of the other casts are available upon request.

circumstances. First, the fact that the remains could be linked to Flight 4422 limited the number of potential candidates and permitted a highly targeted approach for reference sample acquisition. Second, the individuals on board were mariners and thus fingerprint cards were available as reference material. Although the quality of the old fingerprint cards was generally poor, under almost any other circumstances, reference fingerprint cards would not have been available at all. Third, and most importantly, as a result of the cold climate, glacial ice, and embalming, the 60-year-old hand was in a fairly good condition for fingerprint analysis. The cold climate preserved the arm for the first 50 years, and following the arm's discovery, the embalming process preserved the soft tissue. If the remains had not been embalmed, it is very possible that the soft tissue of the hand would have degraded substantially over the additional 8 years, potentially precluding any sort of fingerprint analysis.

Ironically, the preservation techniques that benefited the fingerprint analysis were a detriment to the initial DNA identification efforts in 2002. When an organism dies, its DNA becomes degraded by endogenous and microorganismal nucleases. In this particular case, the relatively good state of preservation of the human forearm and hand suggested that the remains had been trapped in ice for many years and resurfaced shortly before being discovered so that most of the destructive action of enzymes had been limited by low temperature. Even so, because the remains were found lying on rocks, for at least a short time before they were found, they had lost the protection provided by the ice entombment and therefore the nucleic acids were subjected to new degradation factors such as oxygen and radiation. Furthermore, shortly after discovery, the remains were fixed to prevent continued physical degradation. Embalming solutions such as the one used on the remains, although beneficial in terms of preserving the appearance of soft tissue, are extremely destructive to DNA. Formaldehyde denatures, cross-links, and damages nucleic acids, making access to high-quality templates required for DNA profiling purposes very difficult. In their review, Schander and Halanych explain that the time from the collection of a sample to its fixation greatly influences the possibility of extracting useful DNA (14). If the nucleic acids are already damaged when fixed, the action of formaldehyde is even more detrimental to the extraction of amplifiable DNA. Thus, the standard autosomal STR tests conducted by the commercial laboratory in 2002 and intended for pristine DNA samples were unlikely to produce usable data from a specimen like the arm.

Our DNA typing success primarily resulted from knowledge of the effects of formaldehyde on DNA acquired during AFDIL's efforts to identify embalmed remains of unidentified casualties of

the Korean War interred in the National Cemetery of the Pacific in Honolulu, HI (15). It can also be attributed to our optimized DNA extraction protocol using a complete decalcification of the bone matrix (which likely resulted in access to more amplifiable templates), small DNA target amplicons (5), and aggressive amplification protocols for nuclear STRs (6,7,16,17).

Ultimately, the identification of Francis van Zandt reflects not only the successful, independent applications of improved fingerprint and DNA technologies, but also—and for us more importantly—the successful integration of two disciplines, which generally overlap very little in practice but often address the same questions. Without both investigations, a complete set of exclusionary references would not have been obtained and, ultimately, it was the complete picture offered by these complementary perspectives that made this identification irrefutable.

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